Letter

Discovery of PARP1-Sparing Inhibitors for Protein ADP-Ribosylation

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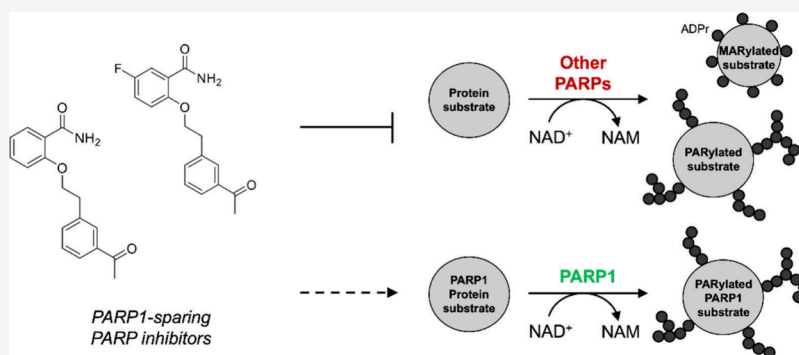
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ABSTRACT: Poly-ADP-ribose polymerases (PARPs) that catalyze cellular ADP-ribosylation play important roles in human health. PARP inhibitors have found success in the clinic for cancer treatment. However, isoform-specific inhibitors are needed for improved safety. Here, we report the unexpected discovery of nicotinamide mimics that block non-PARP1-catalyzed ADP-ribosylation at micromolar concentrations. These PARP1-sparing PARP inhibitors represent first-in-class probes for ADP-ribosylation, shedding light on the selective inhibition of PARPs.

KEYWORDS: ADP-ribosylation, PARP1, inhibitor, PARP, enzyme

Poly-ADP-ribose polymerases (PARPs) catalyze the post-translational transfer of ADP-ribose (ADPr) from β-nicotinamide adenine dinucleotide (NAD$^+$) onto substrate proteins.[1−5] In this 17-member family, PARP1, PARP2, PARP5a, and PARP5b synthesize ADPr polymers, whereas others make mono-ADP-ribosylations.[6−15] PARPs are involved in transcriptional regulation, DNA damage repair, immune responses, and many other processes and are heavily implicated in human diseases.[16−23] To date, four PARP inhibitors that compete for binding in the NAD$^+$ pocket have been approved by the Food and Drug Administration (FDA) for cancer treatment.[24−26] Despite their clinical successes, significant toxicities due to lack of selectivity for particular PARP isoforms are common.[27−29] Thus, the development of PARP inhibitors with high specificity attracts considerable interest.

In this study, we designed a series of nicotinamide-derived analogues on the basis of X-ray crystal structures of human PARP1 and PARP2 for generating selective PARP inhibitors (Figure 1).[30] Surprisingly, our study revealed several compounds with micromolar inhibition activities against human PARPs, except PARP1. These synthetic molecules offer valuable tools for studying PARP isoforms and insights into the selective inhibition of PARP enzymes.

Results. The general lack of selectivity of current PARP inhibitors can be largely attributed to high levels of sequence and structure similarities in the catalytic domains of PARP family enzymes.[5] Structural analysis of the NAD$^+$ binding sites of human PARP1 and PARP2 identified notably different residues in the α-helix 5 (α5) of the helical domain,[30] including D766 versus E335, E763 versus Q332, Q759 versus S328, and A755 versus Q324 (Figure 1). Neighboring the pocket of the nicotinamide moiety of NAD$^+$, amino acid residues in α5 of PARP1 and PARP2 could possibly form distinct interactions with ligand candidates occupying this cavity, thereby leading to differential inhibition activity. On the basis of these findings, we explored the generation of new PARP1- or PARP2-selective inhibitors by designing nicotinamide mimics.

To this end, benzamide as an analogue of nicotinamide was chosen to capture interactions surrounding the nicotinamide-binding pocket. Furthermore, acetophenone was utilized to create potential hydrophobic and hydrogen bond interactions with residues adjacent to and/or in α5 via the benzene ring
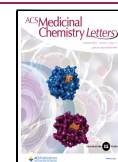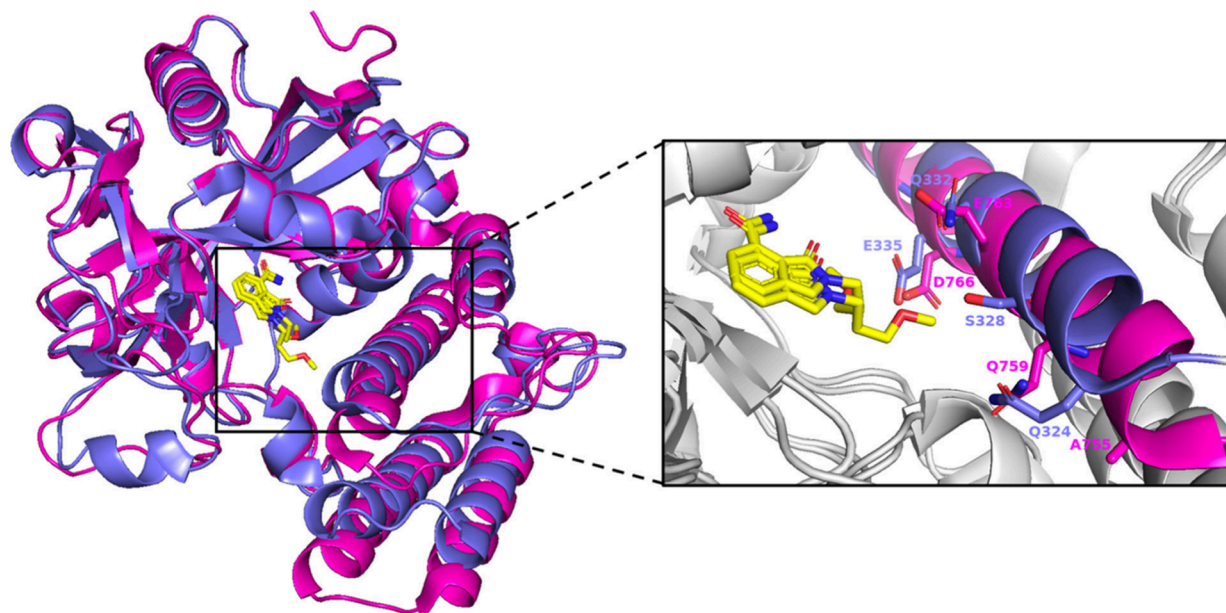
Figure 1. Superimposed crystal structures of human PARP1 (PDB ID: 4ZZZ) (pink) and human PARP2 (PDB ID: 4ZZX) (purple) in complex with 2-(3-methoxypropyl)-3-oxo-2,3-dihydro-1H-isoindole-4-carboxamide (yellow). Right panel: the helical domains α5 in the NAD$^+$ binding pockets.

and carbonyl bond, respectively. The two aromatic groups were connected by amine-, amide-, and ether-based linkers to facilitate reaching the desired interacting residues for each group, which afforded compounds **1−3** (Figure 2a). To evaluate their inhibition activities, full-length human PARP1 and PARP2 were recombinantly expressed and purified from bacteria.[12,31,32] Auto-poly-ADP-ribosylation (PARylation) of PARP1 and PARP2 was performed in the absence and presence of 100 μM **1−3**. Immunoblot analysis of auto-modified PARP1 and PARP2 using an anti-poly-ADPr (PAR) antibody indicated that none of the compounds have inhibition activities for PARP1 at this concentration, but **3** functions as an inhibitor for PARP2 (Figure 2b).

To improve inhibition activity of **3**, we then designed its derivatives by adding a methyl group in the linker region, introducing a fluorine substituent into the benzene ring, and/ or extending the linker (Figure 2a). The resulting compounds **4−9**, along with **3**, were examined for inhibitory activities with purified PARP1 and PARP2 through immunoblots as detected by the anti-PAR antibody. Like **3**, compounds **6−8** can significantly inhibit PARP2 enzymatic activity but not PARP1 at concentrations of 100 μM (Figure 2c). According to quantified PARylated signals, **6−8** showed increased potency for PARP2 in comparison with **3**. In contrast, **4**, **5**, and **9** featuring a methylated or more elongated linker displayed reduced inhibition activities against PARP2.

Next, lead compounds **3** and **6−8** were evaluated for inhibiting ADP-ribosylation catalyzed by other PARPs. In addition to PARP1 and PARP2, PARP5a and PARP5b, also known as tankyrases, are enzymes responsible for protein PARylation.[18] PARP5a, along with PARP10, a mono-ADP-ribosyltransferase,[14] were selected as representative PARPs for the inhibition study. Catalytic domains of PARP5a and PARP10 were recombinantly expressed and purified from bacteria.[10,12] Auto-ADP-ribosylation of PARP5a and PARP10 catalytic domains were carried out without and with 100 μM **3** and **6−8**. PARylation by PARP5a and mono-ADP-ribosylation

(MARylation) by PARP10 were assessed by immunoblots using a macrodomain of AF1521, a pan-ADPr binding reagent that can recognize both ADPr and PAR covalently attached to protein substrates.[33] To our surprise, incubations of PARP5a and PARP10 with NAD$^+$ in the presence of lead compounds **3** and **6−8** resulted in significantly decreased levels of ADP-ribosylation (Figure 2d). Taken together, these results indicate that **3** and **6−8** can inhibit ADP-ribosylation catalyzed by PARP2, PARP5a, and PARP10 but not by PARP1.

To determine whether **3** and **6−8** are PARP1-sparing PARP inhibitors, their activities were analyzed using lysates of human HAP1 wild-type (WT) and human HAP1/PARP1-knockout (KO) cells.[7] HAP1 cells have been found to express nearly all PARPs except PARP15.[34] Levels of ADP-ribosylated proteins in cell lysates were examined by immunoblotting using the AF1521 macrodomain-based pan-ADPr binding reagent. Consistent with the above results of recombinant PARPs, all four lead compounds displayed significant inhibition activities for protein ADP-ribosylation in both HAP1-WT and HAP1/PARP1-KO cell lysates (Figure 2e). Importantly, the levels of ADP-ribosylated proteins for HAP1-WT samples with **3** and **6−8** were significantly higher than those of olaparib-treated ones, indicating residual PARylation activities in these groups (Figure 2e). By contrast, the ADP-ribosylation signals for HAP1/PARP1-KO samples incubated with **3** and **6−8** revealed no significant differences to those of olaparib-treated ones and non-modified controls, thereby supporting full inhibition of PARP enzymatic activities in these lysates (Figure 2e). Collectively, these results indicate that **3** and **6−8** act as PARP inhibitors sparing PARP1.

The IC$_{50}$ values of **3** and **6−8** for PARylation by PARP2 and MARylation by PARP10 were then measured. In contrast to compound **3** with moderately selective inhibition for PARP10 (IC$_{50}$: 102.3 μM for PARP2 and 15.3 μM for PARP10), **6−8** displayed comparable inhibitory activities against both enzymes (IC$_{50}$: 36−60 μM), as characterized by improved
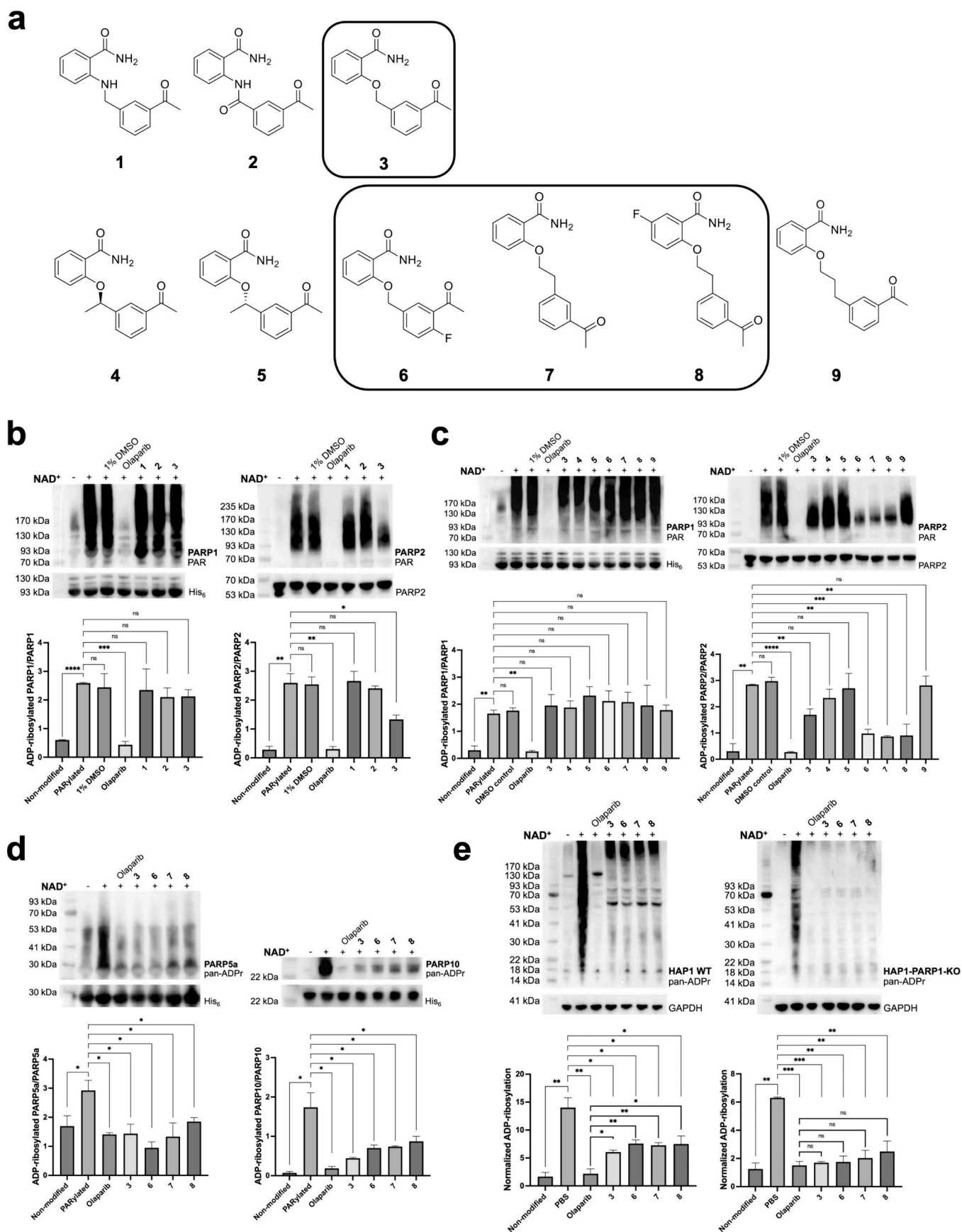
Figure 2. Synthetic molecules as PARP inhibitors sparing PARP1. (a) Chemical structure of **1–9**. Compounds of interest are outlined. (b,c) Inhibition activities of **1–3** (b) and **3–9** (c) for PARP1- (left) and PARP2-catalyzed (right) auto-modification. (d,e) Inhibition activities of **3** and **6–8** for PARP5a- (left) and PARP10-catalyzed (right) auto-ADP-ribosylation (d) and protein ADP-ribosylation in cell lysates of HAP1 wild-type

Figure 2. continued

(left) and HAP1-PARP1-KO (right) (e). Compounds at 100 μM were tested for their inhibition activities. Immunoblots were analyzed with an anti-PAR antibody for PARP1 and PARP2 reactions and a pan-ADPr binding reagent for PARP5a, PARP10, and cell lysate reactions. Loading controls were detected by an anti-His$_6$ antibody for PARP1, PARP5a, and PARP10; an anti-PARP2 antibody for PARP2; and an anti-GAPDH antibody for cell lysates. Bottom panels: densitometric analysis of protein ADP-ribosylation normalized to loading controls. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$; ns, not significant.

Table 1. IC$_{50}$ Values of 3 and 6–8 for Human PARP2 and PARP10

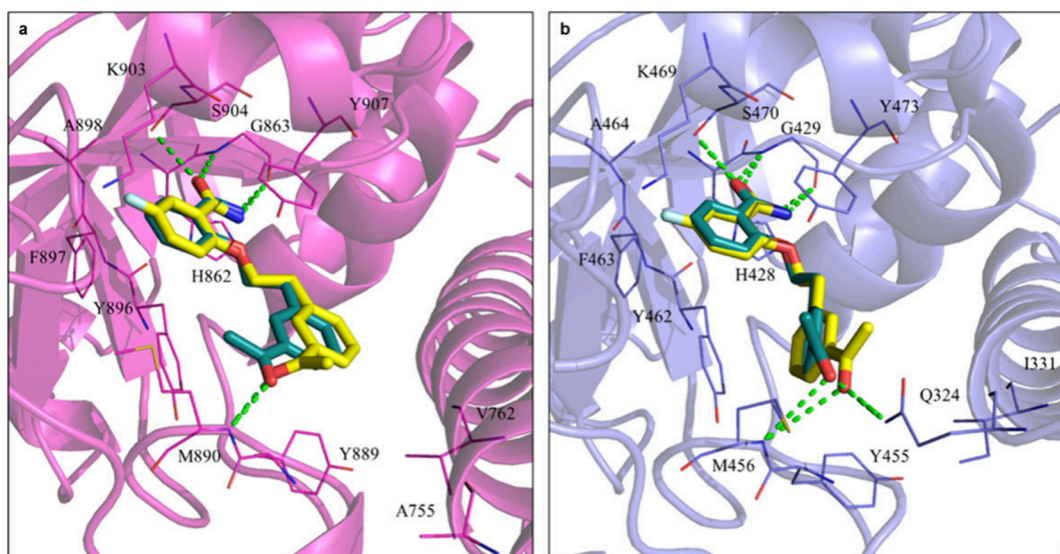|	IC$_{50}$ (μM)			
	3	**6**	**7**	**8**
PARP2	102.3 ± 14.3	50.1 ± 11.8	59.7 ± 10.6	51.5 ± 9.4
PARP10	15.3 ± 13.4	39.4 ± 2.4	48.3 ± 14.9	36.9 ± 17.4

Figure 3. Analysis of potential interactions of compounds **7** and **8** with human PARP1 and PARP2. Compounds **7** (teal) and **8** (yellow) were virtually docked into active sites of (a) PARP1 (pink) and (b) PARP2 (purple). Hydrogen bonds are shown in green dashed lines.

potency for PARP2 and decreased inhibitory effects on PARP10 (Table 1).

To gain insight into the selective inhibition activities, *in silico* docking of compounds **7** and **8** into active sites of human PARP1 and PARP2 was performed. It was shown that the amide groups are involved in three hydrogen bonds with residues G863 and S904 of PARP1 and G429 and S470 of PARP2 and that acetyl groups participate in a hydrogen bond with residues M890 of PARP1 and M456 of PARP2 (Figure 3). The benzamide moieties are stacked with side chains of residues Y907 of PARP1 and Y473 of PARP2. Notably, docking analysis indicated that in contrast to PARP1 lacking hydrogen bonding interactions between the ligand and α5, PARP2 features an additional hydrogen bond with **7** and **8** through the side chain of Q324 in α5 (Figure 3), suggesting a key role of α5 of PARP1 and PARP2 in differential binding to the inhibitors.

To demonstrate their utility, compounds **7** and **8** were applied to examine PARP1 activity in living cells. HeLa cells stimulated by H$_2$O$_2$ were treated without and with **7** or **8** (100 μM). Cellular ADP-ribosylation was then visualized by confocal microscopy using the AF1521 macrodomain-based pan-ADPr binding reagent (Figure 4a). In addition, cell lysates were collected, and levels of ADP-ribosylated proteins were analyzed by immunoblots using the same pan-ADPr binding

reagent (Figure 4b). As anticipated, transient stimulation with H$_2$O$_2$ resulted in significantly increased cellular levels of ADP-ribosylation, which were largely suppressed by olaparib (Figure 4). Interestingly, treatment with **7** or **8** gave rise to no significant impact on the overall ADP-ribosylation signals triggered by H$_2$O$_2$ in HeLa cells. Considering the PARP1-sparing nature of **7** and **8** inhibitors, these results suggest that the majority of ADP-ribosylation activities detected in H$_2$O$_2$-treated HeLa cells could be attributed to PARP1, which is consistent with previous reports regarding abundant PARP1 in HeLa cell nuclei.[35−37]

Discussion. On the basis of structural analysis, nicotinamide analogues were designed for potential interactions with nonconserved active site residues of PARP1 or PARP2. Inhibition activity and specificity tests with representative PARPs and cell lysates without and with PARP1 KO indicate unexpected PARP1-sparing characteristics for the generated PARP inhibitors **3** and **6−8**. Compared with other PARPs, PARP1 is more resistant to inhibition by these compounds. Analysis of initial compounds **1−3** reveals the ether linker as a key determinant for inhibition activities. These results suggest the possible formation of an intramolecular hydrogen bond for **3** between the aryl C-2 oxygen and the benzamide amine group, resulting in a constrained conformation of the compound for favorable interactions with residues at the
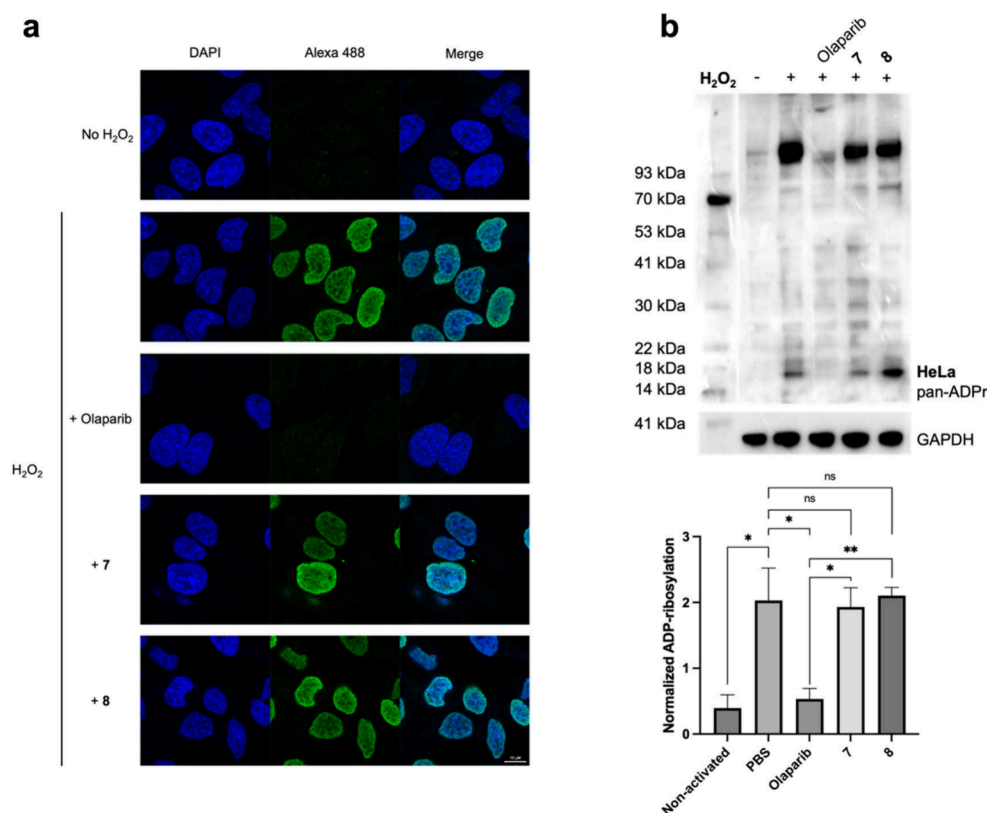
Figure 4. Inhibition activities of **7** and **8** in cellular contexts. (a) Confocal microscopic analysis of cellular ADP-ribosylation in HeLa cells stimulated with H_2O_2 in the absence and presence of 100 μM inhibitors. (b) Immunoblot analysis of lysates of treated HeLa cells using a pan-ADPr binding reagent. Loading controls were detected by an anti-GAPDH antibody. Bottom panels: densitometric analysis of protein ADP-ribosylation normalized to loading controls. *, $p < 0.05$; **, $p < 0.01$; ns, not significant.

catalytic site. In addition, activity studies of the derivatives of **3** suggest the enhancement of interactions with PARPs through a fluorine substitution or one-carbon extension of the linker. Potency optimization guided by experimental and/or computational analysis is needed for these compounds before in-depth studies of binding modes at molecular levels.

The unique inhibition profiles of **3** and **6−8** can possibly enable delineation of PARP1-associated PARylation and related functions and roles in cells and tissues by exclusively inhibiting other ADP-ribosylation activities. Furthermore, these analogues may inform the development of therapeutic candidates for diseases relevant to other PARPs beyond PARP1.

In conclusion, PARP1-sparing PARP inhibitors with potency at micromolar levels were generated, which represent new probes for studying and modulating cellular ADP-ribosylation.

■ ASSOCIATED CONTENT

Data Availability Statement

Data supporting the findings of this study are available from the corresponding author upon request.

ⓢ Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.4c00395.

Supplementary results and methods for chemical synthesis (PDF)

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

ADPr, ADP-ribose; FDA, Food and Drug Administration; KO, knockout; MAR, mono-ADP-ribose; MARylation, mono-ADP-ribosylation; NAD$^+$, nicotinamide adenine dinucleotide; PAR, poly-ADP-ribose; PARP, poly-ADP-ribose polymerase; PARylation, poly-ADP-ribosylation; WT, wild-type

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This paper was originally published October 30, 2024. Due to a production error, Table 1 was overlapping some text in the PDF version. The corrected version reposted on October 30, 2024.