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Rational Design of Cell-Active Inhibitors of PARP10

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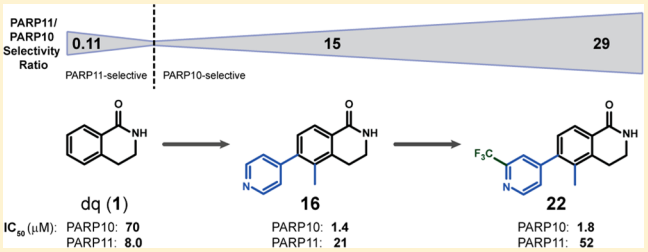
Supporting Information

ABSTRACT: Poly-ADP-ribose polymerases (PARPs 1−16) have emerged as major regulators of diverse cellular processes. PARPs can be subclassified based on their ability to catalyze poly-ADP-ribosylation (PARylation) or mono-ADP-ribosylation (MARylation). While much is known about the cellular roles of PARPs that catalyze PARylation (e.g., PARP1), the function of PARPs that catalyze MARylation (e.g., PARP10) is substantially less understood. This is due in large part to the lack of small-molecule inhibitors that are selective for individual PARP family members that catalyze MARylation. Herein, we describe the rational design and synthesis of selective inhibitors of PARP10. Using structure-based design, we targeted a hydrophobic subpocket within the nicotinamide-binding site of PARP10. We synthesized a series of small molecules based on a 3,4-dihydroisoquinolin-1(2*H*)-one (dq, **1**) scaffold that contain various substituents at the C-5 and C-6 positions designed to exploit this hydrophobic subpocket. We found a dq analogue (**22**) that contains a methyl group at the C-5 position and a substituted pyridine at the C-6 position that exhibits >10-fold selectivity for PARP10 over a large subset of other PARP family members. The results of this study will serve as a platform for future small-molecule probe development for PARP10 and other PARP family members that catalyze MARylation.



KEYWORDS: *PARPs, ADP-ribosylation, MARylation, PARylation, NAD*$^+$*, Rational design*

The poly(ADP-ribose) polymerase (PARP) family of enzymes (17 in humans; also known as ADP-ribosyltransferases or ARTDs) catalyzes the transfer of ADP-ribose (ADPr) onto amino acids of protein substrates from donor nicotinamide adenine dinucleotide (NAD^+) in a process known as ADP-ribosylation. In recent years, PARPs have emerged as major regulators of cellular processes ranging from chromatin modulation, transcription, RNA biology, DNA repair, stress responses, and metabolism.[1] PARPs can be divided into two subfamilies based on their differences in catalytic activity: those that catalyze poly-ADP-ribosylation (PARylation; PARPs 1, 2, 5a,b) and those that catalyze mono-ADP-ribosylation (MARylation; PARPs 3, 4, 6−8, 10−12, 14−16).[2] The PARP family can also be further subcategorized based upon on a conserved catalytic triad motif: His-Tyr-Glu/Φ (where Φ = a hydrophobic residue).[3] While the histidine and tyrosine residues are required for proper orientation of NAD^+ within the active site, the third residue varies across the PARP family. A glutamate is found in PARPs 1−5a,b (H−Y−E PARPs), while the remaining active PARPs 6−8, 10−12, 14−16 (H−Y−Φ PARPs) contain a hydrophobic residue (isoleucine, leucine, or tyrosine) at the third position of the triad.

Much progress has been made in understanding the functions of PARPs that catalyze PARylation. This is due in large part to the development of selective inhibitors for members of this subfamily such as PARP1/PARP2 (e.g., veliparib/ABT-888)[4] and the tankyrases (PARPs 5a/5b).[5−7] However, a comprehensive understanding of the functions of the remaining PARPs that catalyze MARylation has been hindered due to the lack of selective inhibitors.[8] Progress in the design of selective inhibitors of both PARP10 and PARP14 is preliminary, as many of the reported inhibitors display modest to no selectivity, or the full PARP family selectivity profile has not been analyzed.[9−14] The PARP field would benefit greatly from a toolbox of selective inhibitors targeting specific members of PARPs that catalyze MARylation.

PARP10 (ARTD10) is a member of the subfamily of PARPs that catalyze MARylation and has been implicated in various biological pathways involving DNA repair and inflammation,[15,16] yet a connection of PARP catalytic activity to the regulation of these pathways is still not clear. A selective small-molecule inhibitor of PARP10 would facilitate detailed mechanistic studies of PARP10-mediated biology and, furthermore, validate PARP10 as a therapeutic target for diseases such as cancer.[17−20]

A recent study described a small-molecule chemical probe known as OUL35 (Figure 1a) as a potent and selective inhibitor of PARP10, exhibiting >12-fold selectivity over other

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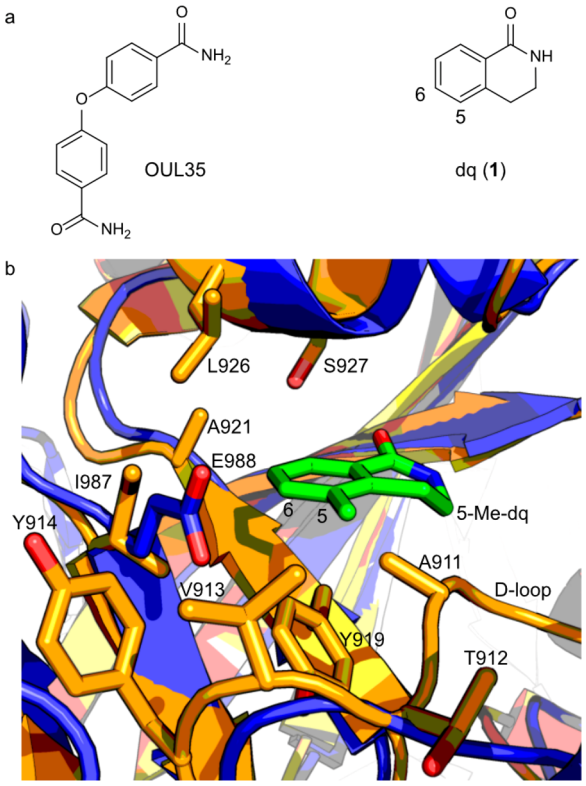

Figure 1. Rational design of a selective inhibitor of PARP10 based on a 3,4-dihydroisoquinolin-1(2*H*)-one scaffold. (a) Structure of previously reported PARP10-selective inhibitor OUL35[12] and 3,4-dihydroisoquinolin-1(2*H*)-one (dq, **1**) scaffold with the C-5 and C-6 positions indicated. (b) Model of PARP10 (orange, PDB 3HKV) overlaid with the cocrystal structure of PARP1 and 5-methyl-dq (**3**) (blue, PDB 1PAX).

PARP family members examined.[12] Of the PARPs tested in the study, OUL35 was not assayed against PARP11. We therefore profiled OUL35 against full-length PARP11 and the catalytic domain of PARP10 (PARP10$_{cat}$) using a 96-well plate assay that monitors MARylation of the promiscuous PARP substrate SRSF protein kinase 2 (SRPK2)[21,22] using NAD$^+$ at 100 μM (MARylation is detected using an ADP-ribose binding reagent; see Supporting Information (SI)).[23] In our hands, OUL35 exhibited less than 2-fold selectivity for PARP10 over PARP11 (Table 1). This result motivated us to develop a small-molecule inhibitor of PARP10 that exhibits greater selectivity across the PARP family. A selective inhibitor of PARP10 would enable mechanistic studies into PARP10-mediated MARylation without confounding results from inhibition of other PARP family members.

The 3,4-dihydroisoquinolin-1(2*H*)-one (dq, **1**; Figure 1a) scaffold has been used for the development of inhibitors of PARP1.[24,25] We showed previously that **1** can be modified to inhibit PARP10 using a chemical genetics strategy[26] and reasoned that **1** would be a good starting point for optimizing an inhibitor with increased selectivity for PARP10 over PARP11. We analyzed the structure of PARP10 overlaid with the cocrystal structure of PARP1 and 5-methyl-dq (**3**) and hypothesized that substituents at the C-5 and C-6 positions of the dq scaffold could interact with a hydrophobic pocket in PARP10 formed by Ile987 and D-loop amino acids: Tyr914, Val913, and Ala911 (Figure 1b). Given that the D-loop is highly variable across the PARP family,[27] we proposed that targeting this region for inhibitor development could impart selectivity for PARP10 over PARP11 and perhaps other PARP family members.

We synthesized a series of dq compounds substituted at both the C-5 and C-6 positions for structure−activity relationship (SAR) studies (Scheme 1 and SI). The synthesis of the dq scaffold was conducted using either a Schmidt reaction of substituted 1-indanones a−c or an acid-catalyzed cyclization of carbamoyl salicylates of substituted phenethylamines d or **27**.[28] To introduce aryl modifications on the dq scaffold, a Suzuki reaction of bromo-modified dq compounds **2**, **5**, or **25** was performed using the corresponding aryltrifluoroborate or arylboronic acid species.

The dq analogues were initially screened against PARP10$_{cat}$ and PARP11 using the plate assay described above. Unsubstituted **1** is 9-fold more selective for PARP11 than PARP10 (IC_{50} = 8 μM versus 70 μM) (Table 1). Substitutions at the C-5 position of **1** increased potency for both PARP10 and PARP11, yet selectivity for PARP11 was still retained. Compound **4** with a phenyl group at the C-5 position was the most potent (1.2 μM) and selective (30-fold) for PARP11 among the set of compounds tested. When compared to unsubstituted **1**, C-6-position modifications increased potency for PARP10 but had no effect or decreased potency for PARP11. In particular, the addition of a phenyl group at the C-6 position (compound **8**) increased potency for PARP10 (28-fold) compared to **1**. Compound **8** was also 4-fold more selective for PARP10 over PARP11, highlighting **8** as a starting point to further increase selectivity for PARP10.

We proposed that modifications on the phenyl ring of **8** might further increase the selectivity for PARP10 over PARP11. We began by introducing small substituents at the *ortho*-, *meta*-, or *para*-position. Substituents at the *para*-position of the phenyl ring had little effect on the potency of the compounds for both PARP10 and PARP11, while substituents at the *ortho*-position greatly reduced potency compared to **8** (data not shown). However, modifications at the *meta*-position of the phenyl ring had modest increases in potency for both PARP10 and PARP11 compared to **8** (Table 1), with the exception of the trifluoromethyl-modified **12**, which showed a 2-fold decrease in potency for PARP11 compared to **8**. Compound **12**, in turn, exhibited the greatest selectivity for PARP10 over PARP11 (8-fold), a 2-fold improvement from parent **8**. We sought to further increase this observed selectivity for PARP10 over PARP11.

We hypothesized that combining the most promising modifications at the C-5 and C-6 positions of the dq scaffold could have a synergistic effect on increasing both potency and selectivity for PARP10. Compound **3**, with a methyl group at the C-5 position, resulted in an 8-fold increase in potency for PARP10 compared to unsubstituted **1** (8.6 vs 70 μM) (Table 1). Compound **14**, with a methyl and phenyl group at the C-5 and C-6 positions, respectively, resulted in a further 5-fold increase in potency for PARP10 compared to **3** (1.6 vs 8.6 μM) (Table 1). To our surprise, the selectivity of **14** for PARP10 over PARP11 (17-fold) compared to the selectivity of **3** for PARP10 over PARP11 (0.24-fold) is improved 70-fold by the combination of both the 5-methyl and 6-phenyl modifications in **14**. Compound **14** exhibited increased potency and selectivity for PARP10 over PARP11 compared to **3**; however, we encountered aqueous solubility issues at higher concentrations when assaying **14**. We sought modifications at the C-6 position that could maintain the

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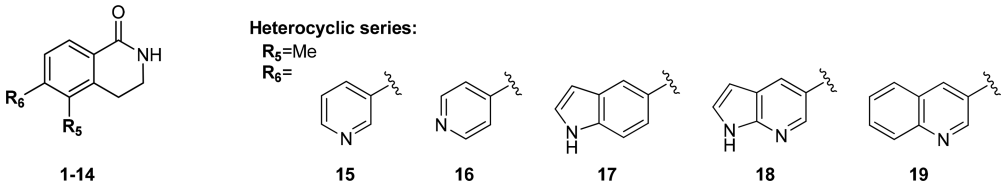
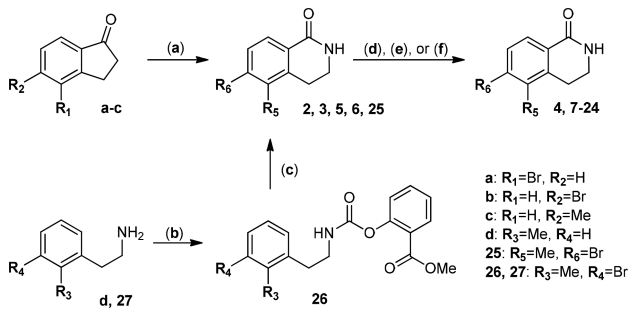
Table 1. In Vitro IC_{50} Values for Selected C-5- and C-6-Substituted dq Compounds against $PARP10_{cat}$ and PARP11[a]



ID	-R_5	-R_6	$PARP10_{cat}$		PARP11		PARP11/ PARP10
			IC_{50} (μM)	pIC_{50} ± SEM	IC_{50} (μM)	pIC_{50} ± SEM	
OUL35	-	-	1.3	5.88 ± 0.02	2.1	5.68 ± 0.03	1.6
1	H	H	70.3	4.15 ± 0.03	8.0	5.10 ± 0.04	0.11
2	Br	H	10.1	4.99 ± 0.04	2.9	5.54 ± 0.04	0.29
3	Me	H	8.6	5.01 ± 0.03	2.1	5.67 ± 0.05	0.24
4	Ph	H	35.0	4.46 ± 0.04	1.2	5.93 ± 0.04	0.03
z5	H	Br	17.1	4.77 ± 0.03	13.5	4.87 ± 0.03	0.79
6	H	Me	33.1	4.48 ± 0.04	34.1	4.47 ± 0.06	1.0
7	H	Bn	>50	-	16.7	4.78 ± 0.03	<0.33
8	H	Ph	2.5	5.61 ± 0.03	8.7	5.06 ± 0.03	3.5
9	H	3-F-Ph	1.4	5.85 ± 0.03	6.1	5.22 ± 0.04	4.4
10	H	3-Cl-Ph	1.1	5.95 ± 0.02	5.8	5.24 ± 0.03	5.3
11	H	3-Me-Ph	2.1	5.67 ± 0.02	4.6	5.34 ± 0.03	2.2
12	H	3-CF_3-Ph	2.3	5.65 ± 0.03	17.4	4.76 ± 0.04	7.6
13	H	3-Ac-Ph	1.6	5.81 ± 0.04	4.9	5.31 ± 0.05	3.1
14	Me	Ph	1.6	5.80 ± 0.03	26.4	4.58 ± 0.07	17
15	Me	pyridin-3-yl	2.5	5.60 ± 0.04	30.4	4.52 ± 0.06	12
16	Me	pyridin-4-yl	1.4	5.84 ± 0.03	21.2	4.67 ± 0.04	15
17	Me	1*H*-indol-5-yl	1.1	5.96 ± 0.03	4.2	5.38 ± 0.04	3.8
18	Me	1*H*-pyrrolo[2,3-*b*]pyridin-5-yl	0.71	6.15 ± 0.03	12.0	4.92 ± 0.05	17
19	Me	quinolin-3-yl	1.8	5.75 ± 0.02	14.0	4.86 ± 0.04	7.8

[a]SEM from three representative dose−response experiments.

Scheme 1. Synthesis of 3,4-Dihydroisoquinolin-1(2*H*)-ones with Modifications at the C-5 and C-6 Positions[a]



[a]Reagents and conditions: (a) NaN_3 (1.5 equiv), DCM/MsOH (2:1), 0 °C to rt, 18 h; (b) **28** (1.05 equiv), THF, rt, 0.5 h; (c) TfOH (10 equiv), DCM, 0 °C to rt over 3 h; (d) R-BF_3K (1.5 equiv), $Pd(OAc)_2$ (5 mol %), RuPhos (10 mol %), Cs_2CO_3 (3 equiv), toluene/H_2O (3:1), 95 °C, 3 h; (e) R-$B(OH)_2$, $Pd(OAc)_2$ (1 mol %), TBAB (1 equiv), K_2CO_3 (10 equiv), H_2O, 95 °C, 3 h; (f) R-$B(OH)_2$, $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$ (5 mol %), aq K_2CO_3 (6 equiv), toluene/EtOH (3:1), 95 °C, 3 h.

increased selectivity observed with **14** while mitigating any solubility issues.

We therefore synthesized compounds with heterocyclic modifications at the C-6 position for increasing aqueous solubility. In addition to the various heterocyclic modifications at the C-6 position, these compounds also contain a methyl group at the C-5 position. We found that compounds containing a pyridin-3-yl (**15**), a pyridin-4-yl (**16**), or a 1*H*-pyrrolo[2,3-*b*]pyridin-5-yl (**18**) modification at the C-6 position exhibited a similar PARP11/PARP10 selectivity ratio as **14** (12-, 15-, and 17-fold, respectively, versus 17-fold) (Table 1). Both 1*H*-indol-5-yl (**17**) and quinolin-3-yl (**19**) modifications at the C-6 position resulted in decreased selectivity compared to **14** (4- and 8-fold vs 17-fold) (Table 1). Compounds **15** and **16** with pyridinyl modifications at the C-6 position exhibited increased solubility compared to **14** and were taken forward for further optimization.

We first focused our attention on generating a set of compounds based on **16** with halogen modifications at the *meta*-position of the pyridinyl ring given our results from Table 1. We introduced fluoro, chloro, or trifluoromethyl groups at the 2-position of the pyridin-4-yl substituent. Overall, the

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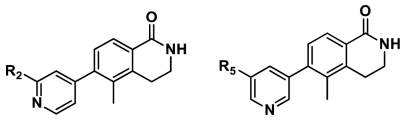
Table 2. In Vitro IC_{50} Values for 20−24 against $PARP10_{cat}$ and PARP11[a]



			PARP10$_{cat}$		PARP11		
ID	$-R_2$	$-R_5$	IC_{50} (μM)	pIC_{50} ± SEM[a]	IC_{50} (μM)	pIC_{50} ± SEM[a]	PARP11/PARP10
16	H		1.4	5.84 ± 0.03	21.2	4.67 ± 0.04	15
20	F		1.9	5.71 ± 0.03	44.7	4.35 ± 0.05	24
21	Cl		1.8	5.76 ± 0.03	27.0	4.57 ± 0.02	15
22	CF_3		1.8	5.74 ± 0.02	51.7	4.29 ± 0.04	29
15		H	2.5	5.60 ± 0.02	30.4	4.52 ± 0.06	12
23		F	2.2	5.66 ± 0.02	54.7	4.23 ± 0.03	25
24		CF_3	2.7	5.57 ± 0.03	92.1	4.04 ± 0.03	34

[a]SEM from three representative dose−response experiments.

introduction of a fluoro group (**20**) or a trifluoromethyl group (**22**) increased selectivity almost 2-fold further for PARP10 over PARP11 compared to **16** (24- or 29-fold vs 15-fold) (Table 2). A chloro substitutent (**21**) retained similar selectivity for PARP10 over PARP11 (Table 2). Similar selectivity trends were observed for **23** (−F) and **24** ($-CF_3$) containing the pyridin-3-yl group, increasing selectivity >2-fold for PARP10 over PARP11 compared to **15** (25- or 34-fold vs 12-fold) (Table 2). Compound **24** exhibited the greatest selectivity overall for PARP10 over PARP11 (34-fold); however, compound **22** was slightly more potent for PARP10 overall (1.8 vs 2.7 μM).

We have shown that **22** is 29-fold more selective for $PARP10_{cat}$ over PARP11 (Table 2). We next wanted to determine the selectivity profile for **22** against all other active PARP family members. We screened **22** against PARP family members in a plate assay that measures MARylation of SRPK2 using an N^6-alkyne tagged NAD^+ (6-a-NAD^+), a clickable NAD^+ analogue (Table 3).[29,30] Compound **22** did not inhibit H−Y−E PARPs (PARPs 1−4, 5b) up to 100 μM, exhibiting >37-fold selectivity for PARP10 (Table 3 and SI, Figure 1a). The observed selectivity is most likely due to differences in the last position of the H−Y−E catalytic triad of these PARPs versus the H−Y−I triad found in PARP10.[3] The glutamate (E988 in PARP1) in PARPs 1−4 and PARP5b most likely clashes with the pyridiny-4-yl group at the 6-position of **22**. PARP10 contains an isoleucine (I978) at this position that could potentially form hydrophobic interactions with this group (Figure 1b).

Table 3. Selectivity Profile of 22 against PARP Family Members

enzyme	IC_{50} (μM)	pIC_{50} ± SEM[a]	PARP10 SR[d]
PARP1	≫100		≫37
PARP2	>100		>37
PARP3	≫100		≫37
PARP4$_{brct-cat}$	>100		>37
PARP5b$_{cat}$	≫100		≫37
PARP6$_s$[e]	>30[b]		>10
PARP6$_L$[f]	>30[b]		>10
PARP7	19	4.72	7.0
PARP8	>30[b]		>10
PARP10$_{cat}$	2.7	5.56 ± 0.06	1.0
PARP11	63	4.20 ± 0.06	23
PARP12	>30[b]		>10
PARP14$_{cat-wwe}$	61	4.22 ± 0.04	22
PARP15$_{cat}$	63	4.20 ± 0.05	23
PARP16$_{\Delta TM}$	6.7	5.17 ± 0.08	2.5

[a]SEM from two representative dose−response experiments. [b]Measured using IP-auto-MARylation assay. [d]Selectivity ratio = IC_{50} PARPX/IC_{50} PARP10. [e]Short isoform. [f]Long isoform.

Compound **22** is >20-fold selective for $PARP10_{cat}$ compared to H−Y−Φ PARPs PARP11, 14, 15 (Table 3 and SI, Figure 1b). Compound **22** exhibits a 7-fold selectivity for PARP10 over PARP7 (IC_{50} = 19.1 vs 2.7 μM) (Table 3 and SI, Figure 1b). Compound **22** is 2.5-fold more potent for PARP10 compared to PARP16 (IC_{50} = 6.7 vs 2.7 μM) (Table 3 and SI, Figure 1b).

Some family members (PARP6, PARP8, and PARP12) are most active when expressed in mammalian cells and were assayed against **22** using an immunoprecipitation (IP)-auto-MARylation assay[2,26] using 6-a-NAD^+. Using this assay, a dose-dependent inhibition of PARP10 is observed (~80% inhibition at 30 μM), whereas H−Y−Φ PARPs PARP6 (short and long isoforms), PARP8, and PARP12 are not inhibited by **22** up to 30 μM (Table 3 and SI, Figure 1c). Taken together, these experiments demonstrate that **22** is >37-fold selective for PARP10 against the H−Y−E subfamily of PARPs and >10-fold selective for PARP10 against the H−Y−Φ subfamily of PARPs with the exception of PARP7 (7-fold selective) and PARP16 (2.5-fold selective).

We next determined if **22** could inhibit PARP10-dependent auto-MARylation in cells. PARP10 is one of the better characterized H−Y−Φ PARPs and is known to exhibit robust auto-MARylation in cells.[22,26] Human embryonic kidney (HEK) 293T cells expressing full-length PARP10 were treated with increasing concentrations of **22** (1−100 μM). Auto-MARylation of PARP10 was monitored by Western blot using an ADP-ribose binding reagent.[23] We found that **22** inhibited auto-MARylation of PARP10 in addition to PARP10-mediated MARylation of high molecular weight (HMW) targets in a dose-dependent manner (Figure 2a). The EC_{50} value varies depending on the species quantified. The EC_{50} for inhibition of PARP10-mediated MARylation of HMW targets by **22** was lower compared to inhibition of auto-MARylation of PARP10 itself (2.3 vs 11 μM) (Figure 2b), which could be due to the relative stoichiometry differences between the levels of endogenous PARP10 targets and overexpressed PARP10. Both EC_{50} values are in agreement with the in vitro calculated

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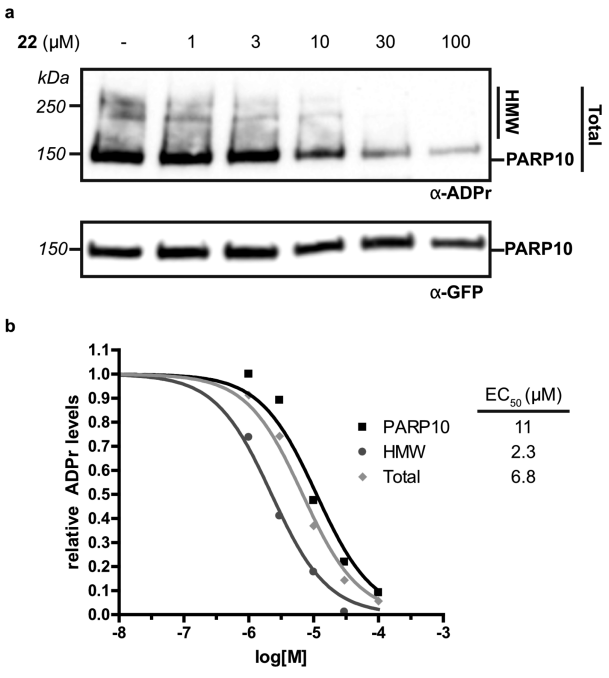

Figure 2. Compound **22** inhibits PARP10-dependent MARylation in cells. (a) Dose-dependent inhibition of PARP10-mediated MARylation by **22** in cells. HEK293T cells were transfected with GFP-PARP10 and incubated with increasing concentrations of **22** (1−100 μM) for 3 h. Lysates were prepared and auto-MARylation of PARP10, and its high molecular weight (HMW) targets were analyzed with an ADP-ribose binding reagent (α-ADPr). (b) Quantification of data shown in (a).

IC_{50} value for **22** against $PARP10_{cat}$ (1.8 μM). These results demonstrate that **22** is membrane permeable and inhibits MARylation of both full-length PARP10 itself and PARP10 targets in cells.

This study provides key insight into the development of selective inhibitors targeting the H−Y−Φ PARP subfamily of PARPs. On the basis of previous studies, we aimed to develop an inhibitor for PARP10 with increased selectivity over PARP11. SAR studies using the dq scaffold revealed that combined C-5- and C-6-position modifications provided increased selectivity for PARP10 over PARP11. Compound **22**, containing a 5-methyl and a 6-(2-trifluoromethyl-pyridin-4-yl) modification, was both potent (1.8 μM) and selective (29-fold) for PARP10 over PARP11. When screened against other PARP family members, **22** was >10-fold selective for PARP10 over most members of the PARP family. When tested in cells, **22** inhibited both auto-MARylation of PARP10 and MARylation of endogenous PARP10 targets.

A major hurdle in the PARP field is the design of selective inhibitors for individual PARP family members. While selectivity of PARP10 over PARP11 was obtained with **22**, the selectivity of **22** for PARP10 over both PARP7 and PARP16 was <10-fold. Future studies will be focused on obtaining structural information on **22** and other dq compounds in complex with PARP10 and other PARP family members to rationalize selectivity differences and guide future inhibitor optimization. Compound **22** will be a useful probe to study PARP10 biology when combined with other PARP10-selective probes with different selectivity profiles (e.g., OUL35 and its derivatives[31]) to rule out potential off-target inhibition of other PARP family members. The results of this study will be important for guiding future development of selective inhibitors, especially optimizing inhibitors for members of the less understood H−Y−Φ PARP subfamily.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.8b00429.

Selectivity profile of **22** across PARP family; supporting experimental procedures (PDF)

■ AUTHOR INFORMATION

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Author Contributions

This study was designed by R.K.M. and M.S.C. R.K.M. synthesized the compounds and performed most of the experiments described in the manuscript. I.T.K. and A.V.-S. screened compound **22** across the PARP family. K.R. synthesized 6-a-NAD^+ used in these studies. The manuscript was written by R.K.M. and M.S.C. All authors have given approval to the final version of the manuscript.

Notes

The authors declare the following competing financial interest(s): R.K.M. and M.S.C. are co-inventors on a provisional patent describing the inhibitors in this study.

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■ ABBREVIATIONS

PARP, poly-ADP-ribose-polymerase; NAD^+, nicotinamide adenine dinucleotide; dq, 3,4-dihydroisoquinolin-1(2*H*)-one; DCM, dichloromethane; RuPhos, 2-dicyclohexylphosphino-2′,6′-diisopropoxybiphenyl; TBAB, tetrabutylammonium bromide; dppf, 1,1′-bis(diphenylphosphino)ferrocene.

■ REFERENCES

(1) Gupte, R.; Liu, Z.; Kraus, W. L. PARPs and ADP-ribosylation: Recent Advances Linking Molecular Functions to Biological Outcomes. *Genes Dev.* **2017**, *31*, 101−126.

(2) Vyas, S.; Matic, I.; Uchima, L.; Rood, J.; Zaja, R.; Hay, R. T.; Ahel, I.; Chang, P. Family-wide Analysis of poly(ADP-ribose) Polymerase Activity. *Nat. Commun.* **2014**, *5*, 4426.

(3) Hottiger, M. O.; Hassa, P. O.; Lüscher, B.; Schüler, H.; Koch-Nolte, F. Toward a Unified Nomenclature for Mammalian ADP-ribosyltransferases. *Trends Biochem. Sci.* **2010**, *35*, 208−219.

(4) Donawho, C. K.; Luo, Y.; Luo, Y.; Penning, T. D.; Bauch, J. L.; Bouska, J. J.; Bontcheva-Diaz, V. D.; Cox, B. F.; DeWeese, T. L.; Dillehay, L. E.; Ferguson, D. C.; Ghoreishi-Haack, N. S.; Grimm, D. R.; Guan, R.; Han, E. K.; Holley-Shanks, R. R.; Hristov, B.; Idler, K. B.; Jarvis, K.; Johnson, E. F.; Kleinberg, L. R.; Klinghofer, V.; Lasko, L. M.; Liu, X.; Marsh, K. C.; McGonigal, T. P.; Meulbroek, J. A.; Olson, A. M.; Palma, J. P.; Rodriguez, L. E.; Shi, Y.; Stavropoulos, J. A.; Tsurutani, A. C.; Zhu, G.-D.; Rosenberg, S. H.; Giranda, V. L.; Frost,

DOI: 10.1021/acsmedchemlett.8b00429
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

D. J. ABT-888, an Orally Active poly(ADP-ribose) Polymerase Inhibitor That Potentiates DNA-damaging Agents in Preclinical Tumor Models. *Clin. Cancer Res.* **2007**, *13*, 2728−2737.
(5) Chen, B.; Dodge, M. E.; Tang, W.; Lu, J.; Ma, Z.; Fan, C.-W.; Wei, S.; Hao, W.; Kilgore, J.; Williams, N. S.; Roth, M. G.; Amatruda, J. F.; Chen, C.; Lum, L. Small Molecule-mediated Disruption of Wnt-dependent Signaling in Tissue Regeneration and Cancer. *Nat. Chem. Biol.* **2009**, *5*, 100−107.
(6) Lau, T.; Chan, E.; Callow, M.; Waaler, J.; Boggs, J.; Blake, R. A.; Magnuson, S.; Sambrone, A.; Schutten, M.; Firestein, R.; Machon, O.; Korinek, V.; Choo, E.; Diaz, D.; Merchant, M.; Polakis, P.; Holsworth, D. D.; Krauss, S.; Costa, M. A Novel Tankyrase Small-molecule Inhibitor Suppresses APC Mutation-driven Colorectal Tumor Growth. *Cancer Res.* **2013**, *73*, 3132−3144.
(7) Johannes, J. W.; Almeida, L.; Barlaam, B.; Boriack-Sjodin, P. A.; Casella, R.; Croft, R. A.; Dishington, A. P.; Gingipalli, L.; Gu, C.; Hawkins, J. L.; Holmes, J. L.; Howard, T.; Huang, J.; Ioannidis, S.; Kazmirski, S.; Lamb, M. L.; McGuire, T. M.; Moore, J. E.; Ogg, D.; Patel, A.; Pike, K. G.; Pontz, T.; Robb, G. R.; Su, N.; Wang, H.; Wu, X.; Zhang, H.-J.; Zhang, Y.; Zheng, X.; Wang, T. Pyrimidinone Nicotinamide Mimetics as Selective Tankyrase and Wnt Pathway Inhibitors Suitable for in Vivo Pharmacology. *ACS Med. Chem. Lett.* **2015**, *6*, 254−259.
(8) Kirby, I. T.; Cohen, M. S. Small-Molecule Inhibitors of PARPs: From Tools for Investigating ADP-Ribosylation to Therapeutics. *Current Topics in Microbioogy and Immunology*; Springer: Berlin, Heidelberg, 2018; DOI: 10.1007/82_2018_137
(9) Peng, B.; Thorsell, A.-G.; Karlberg, T.; Schüler, H.; Yao, S. Q. Small Molecule Microarray Based Discovery of PARP14 Inhibitors. *Angew. Chem., Int. Ed.* **2017**, *56*, 248−253.
(10) Yoneyama-Hirozane, M.; Matsumoto, S.-I.; Toyoda, Y.; Saikatendu, K. S.; Zama, Y.; Yonemori, K.; Oonishi, M.; Ishii, T.; Kawamoto, T. Identification of PARP14 Inhibitors Using Novel Methods for Detecting Auto-ribosylation. *Biochem. Biophys. Res. Commun.* **2017**, *486*, 626−631.
(11) Ekblad, T.; Lindgren, A. E. G.; Andersson, C. D.; Caraballo, R.; Thorsell, A.-G.; Karlberg, T.; Spjut, S.; Linusson, A.; Schüler, H.; Elofsson, M. Towards Small Molecule Inhibitors of mono-ADP-ribosyltransferases. *Eur. J. Med. Chem.* **2015**, *95*, 546−551.
(12) Venkannagari, H.; Verheugd, P.; Koivunen, J.; Haikarainen, T.; Obaji, E.; Ashok, Y.; Narwal, M.; Pihlajaniemi, T.; Lüscher, B.; Lehtiö, L. Small-Molecule Chemical Probe Rescues Cells from Mono-ADP-Ribosyltransferase ARTD10/PARP10-Induced Apoptosis and Sensitizes Cancer Cells to DNA Damage. *Cell Chem. Biol.* **2016**, *23*, 1251−1260.
(13) Upton, K.; Meyers, M.; Thorsell, A.-G.; Karlberg, T.; Holechek, J.; Lease, R.; Schey, G.; Wolf, E.; Lucente, A.; Schüler, H.; Ferraris, D. Design and Synthesis of Potent Inhibitors of the mono(ADP-ribosyl)transferase, PARP14. *Bioorg. Med. Chem. Lett.* **2017**, *27*, 2907−2911.
(14) Holechek, J.; Lease, R.; Thorsell, A.-G.; Karlberg, T.; McCadden, C.; Grant, R.; Keen, A.; Callahan, E.; Schüler, H.; Ferraris, D. Design, Synthesis and Evaluation of Potent and Selective Inhibitors of mono-(ADP-ribosyl)transferases PARP10 and PARP14. *Bioorg. Med. Chem. Lett.* **2018**, *28*, 2050−2054.
(15) Nicolae, C. M.; Aho, E. R.; Vlahos, A. H. S.; Choe, K. N.; De, S.; Karras, G. I.; Moldovan, G.-L. The ADP-ribosyltransferase PARP10/ARTD10 Interacts with Proliferating Cell Nuclear Antigen (PCNA) and Is Required for DNA Damage Tolerance. *J. Biol. Chem.* **2014**, *289*, 13627−13637.
(16) Verheugd, P.; Forst, A. H.; Milke, L.; Herzog, N.; Feijs, K. L. H.; Kremmer, E.; Kleine, H.; Lüscher, B. Regulation of NF-κB Signalling by the mono-ADP-ribosyltransferase ARTD10. *Nat. Commun.* **2013**, *4*, 1683.
(17) Vyas, S.; Chang, P. New PARP Targets for Cancer Therapy. *Nat. Rev. Cancer* **2014**, *14*, 502−509.
(18) Schleicher, E. M.; Galvan, A. M.; Imamura-Kawasawa, Y.; Moldovan, G.-L.; Nicolae, C. M. PARP10 Promotes Cellular Proliferation and Tumorigenesis by Alleviating Replication Stress. *Nucleic Acids Res.* **2018**, *46*, 8908−8916.
(19) Yu, M.; Schreek, S.; Cerni, C.; Schamberger, C.; Lesniewicz, K.; Poreba, E.; Vervoorts, J.; Walsemann, G.; Grötzinger, J.; Kremmer, E.; Mehraein, Y.; Mertsching, J.; Kraft, R.; Austen, M.; Lüscher-Firzlaff, J.; Lüscher, B. PARP-10, a Novel Myc-interacting Protein with poly(ADP-ribose) Polymerase Activity, Inhibits Transformation. *Oncogene* **2005**, *24*, 1982−1993.
(20) Zhao, Y.; Hu, X.; Wei, L.; Song, D.; Wang, J.; You, L.; Saiyin, H.; Li, Z.; Yu, W.; Yu, L.; Ding, J.; Wu, J. PARP10 Suppresses Tumor Metastasis through Regulation of Aurora A Activity. *Oncogene* **2018**, *37*, 2921−2935.
(21) Venkannagari, H.; Fallarero, A.; Feijs, K. L. H.; Lüscher, B.; Lehtiö, L. Activity-based Assay for Human mono-ADP-ribosyltransferases ARTD7/PARP15 and ARTD10/PARP10 Aimed at Screening and Profiling Inhibitors. *Eur. J. Pharm. Sci.* **2013**, *49*, 148−156.
(22) Morgan, R. K.; Cohen, M. S. A Clickable Aminooxy Probe for Monitoring Cellular ADP-Ribosylation. *ACS Chem. Biol.* **2015**, *10*, 1778−1784.
(23) Gibson, B. A.; Zhang, Y.; Jiang, H.; Hussey, K. M.; Shrimp, J. H.; Lin, H.; Schwede, F.; Yu, Y.; Kraus, W. L. Chemical Genetic Discovery of PARP Targets Reveals a Role for PARP-1 in Transcription Elongation. *Science* **2016**, *353*, 45−50.
(24) Suto, M. J.; Turner, W. R.; Arundel-Suto, C. M.; Werbel, L. M.; Sebolt-Leopold, J. S. Dihydroisoquinolinones: The Design and Synthesis of a New Series of Potent Inhibitors of poly(ADP-ribose) Polymerase. *Anti-Cancer Drug Des.* **1991**, *6*, 107−117.
(25) Ferraris, D. V. Evolution of poly(ADP-ribose) Polymerase-1 (PARP-1) Inhibitors. From Concept to Clinic. *J. Med. Chem.* **2010**, *53*, 4561−4584.
(26) Morgan, R. K.; Carter-O'Connell, I.; Cohen, M. S. Selective Inhibition of PARP10 Using a Chemical Genetics Strategy. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 4770−4773.
(27) Pinto, A. F.; Schüler, H. Comparative Structural Analysis of the Putative mono-ADP-ribosyltransferases of the ARTD/PARP Family. *Curr. Top. Microbiol. Immunol.* **2014**, *384*, 153−166.
(28) Kurouchi, H.; Sumita, A.; Otani, Y.; Ohwada, T. Protonation Switching to the Least-basic Heteroatom of Carbamate through Cationic Hydrogen Bonding Promotes the Formation of Isocyanate Cations. *Chem. - Eur. J.* **2014**, *20*, 8682−8690.
(29) Jiang, H.; Kim, J. H.; Frizzell, K. M.; Kraus, W. L.; Lin, H. Clickable NAD Analogues for Labeling Substrate Proteins of poly(ADP-ribose) Polymerases. *J. Am. Chem. Soc.* **2010**, *132*, 9363−9372.
(30) Carter-O'Connell, I.; Jin, H.; Morgan, R. K.; David, L. L.; Cohen, M. S. Engineering the Substrate Specificity of ADP-ribosyltransferases for Identifying Direct Protein Targets. *J. Am. Chem. Soc.* **2014**, *136*, 5201−5204.
(31) Murthy, S.; Desantis, J.; Verheugd, P.; Maksimainen, M. M.; Venkannagari, H.; Massari, S.; Ashok, Y.; Obaji, E.; Nkizinkinko, Y.; Lüscher, B.; Tabarrini, O.; Lehtiö, L. 4-(Phenoxy) and 4-(benzyloxy)-benzamides as Potent and Selective Inhibitors of mono-ADP-ribosyltransferase PARP10/ARTD10. *Eur. J. Med. Chem.* **2018**, *156*, 93−102.

DOI: 10.1021/acsmedchemlett.8b00429
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