Supporting Information

Evaluation and Structural Basis for the Inhibition of Tankyrases by PARP inhibitors

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Content:

EXPERIMENTAL PROCEDURES

Chemical collection. Tested compounds were purchased form Alexis Biochemicals, Sigma-Aldrich, Calbiochem, JS Research Chemicals Trading, Maybridge and Selleck Biochemicals. Details of the tested compounds are provided in Supplementary Table 1. The purity of all the compounds was ≥ 95% based on GC, HPLC, NMR or TLC analysis by the suppliers. When such information was not available, the compounds were identified and the purity was verified with HPLC.

Protein Expression. The expression and purification of ARTD5 (residues 1030-1317) and ARTD6 (residues 873-1161) for biochemical assays and ARTD6 (residues 946-1162) for crystallography was carried out as previously reported.[1–3]

Activity assay. Enzymatic assays were conducted as reported earlier using automodification reaction of tankyrases with 500 nM NAD^+ as a substrate.[1] Compounds were screened at 10 µM and 500 nM concentrations in triplicate and the best compounds were selected for further studies. The dose response curves were measured for the selected compounds. The observed fluorescence values were used for fitting or when intrinsic compound fluorescence was observed at high concentrations the fluorescence was normalized to a conversion percentage using the controls. The data points were measured in quadruplicate and all IC_{50} values were measured at least three times and fitted individually.

Crystallization, Data collection and Refinement. The ARTD6 catalytic domain was crystallized as previously reported.[2] The crystals were soaked in the precipitant solution supplemented with 250 mM NaCl and the inhibitors (**16**, **18:** 100 µM, **10**: 1 mM, **17**: 5 mM or **29**: 10 mM). Crystals were incubated for 24 hours (**16**, **17**, **18**) or 90 days (**10**, **29**) and were dipped into a cryosolution containing additional 20% glycerol and 250 mM NaCl before flash cooling in liquid nitrogen. Data and refinement statistics are shown in Supplementary Table 2.

Supplementary Table 1. PARP inhibitor like compounds tested.

No.	Compound names	SMILES	Supplier
1[4]	3AB	NC(=O)c1cccc(N)c1	Alexis Biochemicals
2[5]	Niacinamide, Nicotinamide	NC(=O)c1cccnc1	Alexis Biochemicals
3[4]	Benzamide	NC(=O)c1ccccc1	Alexis Biochemicals
4[6]	Veliparib, ABT-888	C[C@@]1(CCCN1)c1nc2c(cccc2[nH]1)C(N)=O	Alexis Biochemicals
5[7]	5-aminoisoquinolinone, 5-AIQ	Nc1cccc2c1cc[nH]c2=O	Alexis Biochemicals
6*	3-methyl-5-aminoisoquinolinone, 3-methyl-5-AIQ	Cc1cc2c(N)cccc2c(=O)[nH]1	Alexis Biochemicals
7[5]	4-amino-1,8-naphthalimide, 4-ANI	Nc1ccc2C(=O)NC(=O)c3cccc1c23	Alexis Biochemicals
8[7]	DPQ	O=C1NCCc2c(OCCCCN3CCCCC3)cccc12	Alexis Biochemicals
9[8]	DR2313	Cc1nc(=O)c2CSCCc2[nH]1	Alexis Biochemicals
10[9]	EB-47	Nc1ncnc2n(cnc12)[C@@H]1O[C@@H]([C@@H](O)[C@H]1O)C(=O)N1CCN(CC(=O)Nc2cccc3C(=O)NCc23)CC1	Alexis Biochemicals
11[5]	4-Hydroxyquinazoline, 4-HQN	O=c1nc[nH]c2ccccc12	Alexis Biochemicals
12[10]	INH2BP	Nc1ccc2oc(=O)ccc2c1I	Alexis Biochemicals
13[5]	1,5-Isoquinolinediol, DHQ	Oc1cccc2c1cc[nH]c2=O	Alexis Biochemicals
14[11]	Minocin, Minocycline	CN(C)[C@H]1[C@@H]2C[C@@H]3Cc4c(ccc(O)c4C(O)=C3C(=O)[C@]2(O)C(=O)\C(=C(/N)O)C1=O)N(C)C	Alexis Biochemicals
15[12]	NU1025	Cc1nc(=O)c2cccc(O)c2[nH]1	Alexis Biochemicals
16[5]	Phenanthridinone	O=c1[nH]c2ccccc2c2ccccc12	Alexis Biochemicals
17[13]	PJ-34	CN(C)CC(=O)Nc1ccc2[nH]c(=O)c3ccccc3c2c1	Alexis Biochemicals
18[14]	TIQ-A	O=c1[nH]c2sccc2c2ccccc12	Alexis Biochemicals
19[15]	Paraxanthine, 1,7-dimethylxanthine	Cn1cnc2[nH]c(=O)n(C)c(=O)c12	Sigma-Aldrich
20[16]	CNQ	NC(=O)c1cccc2ncc(nc12)-c1ccc(Cl)cc1	Calbiochem

No.	Compound names	SMILES	Supplier
21[17]	Olaparib, KU-0059436, AZD-2281	Fc1ccc(Cc2n[nH]c(=O)c3ccccc23)cc1C(=O)N1CCN(CC1)C(=O)C1CC1	JS Research Chemicals Trading
22[18]	BYK204165	Cn1cccc1\C=C1/C(=O)NC(=O)c2ccccc12	Sigma-Aldrich
23[19]	XAV939	FC(F)(F)c1ccc(cc1)-c1nc(=O)c2CSCCc2[nH]1	Maybridge
24[$]	RF03877	Oc1nc(nc2CCSCc12)-c1ccccn1	Maybridge
25[20]	RF03876	Oc1nc(nc2CCSCc12)C1CC1	Maybridge
26[21]	IWR-1	O=C(Nc1cccc2cccnc12)c1ccc(cc1)N1C(=O)[C@H]2[C@@H]3C[C@@H](C=C3)[C@H]2C1=O	Sigma-Aldrich
27[21]	IWP-2	Cc1ccc2nc(NC(=O)CSc3nc4CCSc4c(=O)n3-c3ccccc3)sc2c1	Sigma-Aldrich
28[22]	Iniparib, BSI-201	NC(=O)c1ccc(I)c(c1)N(=O)=O	Selleck Biochemicals
29[23]	AG014699, Rucaparib	CNCc1ccc(cc1)-c1[nH]c2cc(F)cc3C(=O)NCCc1c23	Selleck Biochemicals
30[24]	UPF1035	O=C(Oc1cccc2c1cc[nH]c2=O)c1ccccc1	Alexis Biochemicals
31[24]	UPF1069	O=C(COc1cccc2c1cc[nH]c2=O)c1ccccc1	Alexis Biochemicals
32[5]	Flavone	O=c1cc(oc2ccccc12)-c1ccccc1	Sigma-Aldrich

Supplementary Table 2. Data and refinement statistics.

Compound	**10**	**16**	**17**	**18**	**29**
PDB code	4BJ9	4AVU	4BJB	4AVW	4BJC
Data quality					
Beamline	Diamond I04-1	ESRF ID14-1	ESRF ID23-2	ESRF ID14-1	Diamond I04-1
Wavelength (Å)	0.92	0.93340	0.87260	0.93340	0.92
Space group	$C222_1$	$C222_1$	$P\,4_12_12$	$C222_1$	$P\,4_12_12$
Cell dimensions a, b, c (Å)	90.56, 97.78, 120.03	93.37, 96.69, 116.33	66.33, 66.33, 121.39	91.57, 97.99, 117.95	65.30, 65.30, 122.91
Resolution (Å)	30-2.05 (2.10-2.05)	67- 2.40 (2.46-2.40)	30-2.30 (2.36-2.30)	67- 2.15 (2.21-2.15)	30-2.20 (2.26-2-20)
R_{merge}	0.091 (0.742)	0.126 (0.587)	0.123 (1.032)	0.115 (0.579)	0.111 (0.879)
I / σI	13.1 (2.3)	9.7 (2.4)	18.0 (3.0)	10.2 (2.4)	15.6 (2.6)
Completeness (%)	99.8 (99.8)	99.7 (99.7)	99.9 (100.0)	99.2 (98.9)	98.6 (99.4)
Redundancy	6.4 (6.0)	3.7 (3.7)	10.2 (10.5)	3.7 (3.7)	12.6 (12.0)
CC1/2 (%)	99.8 (75.4)	99.1 (71.7)	99.8 (86.1)	99.3 (73.3)	99.9 (83.4)
Refinement					
Reflections	32035	19819	12007	27534	13240
R_{work} / R_{free}	0.171 / 0.197	0.182 / 0.244	0.199 / 0.255	0.187 / 0.228	0.195 / 0.247
B-factors					
Protein	35.2	20.8	39.9	20.6	43.3
Compound	54.9	16.7	53.0	13.9	41.4
Zn	32.7	20.3	41.0	19.9	42.0
SO_4	45.0	31.8	49.9	33.5	69.6
Water	40.6	18.8	37.7	21.6	44.5
Glycerol	37.4	35.7	46.3	24.4	-
PEG	-	45.9	-	43.4	-
R.m.s.d.					
Bond lengths (Å)	0.010	0.012	0.005	0.011	0.005
Bond angles (°)	1.4	1.5	1.0	1.5	1.0
Ramachandran plot					
Most favorable regions (%)	98.0	98.0	96.4	98.0	97.4
Additionally allowed regions (%)	2.0	2.0	3.6	2.0	2.6

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$ This study