Supplementary Information

Pre-clinical lead optimization of a 1,2,4-triazole based tankyrase inhibitor

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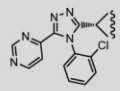
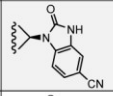
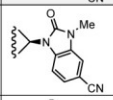
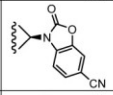
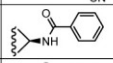
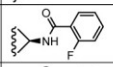
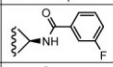
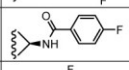
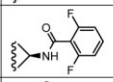
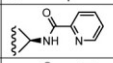
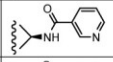
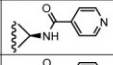
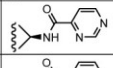
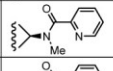
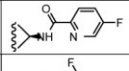
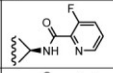
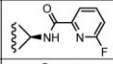
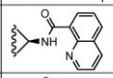
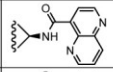
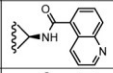
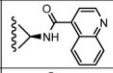
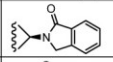
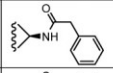
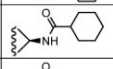
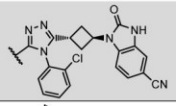
Contents

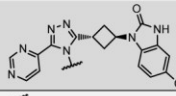
a)

East variation		Biochemical IC_{50} TNKS2 (nM)	Cellular IC_{50} HEK293 (nM)
1		6.3	19
17		34	116
18		48	274
36		5.5	3
37		6.4	30
38		6.7	40
39		4.1	30
40		1700	7880
20		4.9	1.7
41		18	50
42		57	70
43		3.5	4
44		25000	>100000
45		2.7	2.4
46		37	57
47		3.0	6.7
48		5.4	1
21		2.6	0.048
49		350	750
50		680	1300
51		6.6	32
52		6300	>10000
53		1700	2260
54		2300	8900

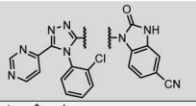
b)

West variation		Biochemical IC_{50} TNKS2 (nM)	Cellular IC_{50} HEK293 (nM)
1		6.3	19
55		3.9	40
56		8.4	330
57		18	43
58		5.7	51
22		9.3	21
59		2.3	140
60		23	290
61		30	400
62		120	6400
63		220000	>10000
64		2600	8410

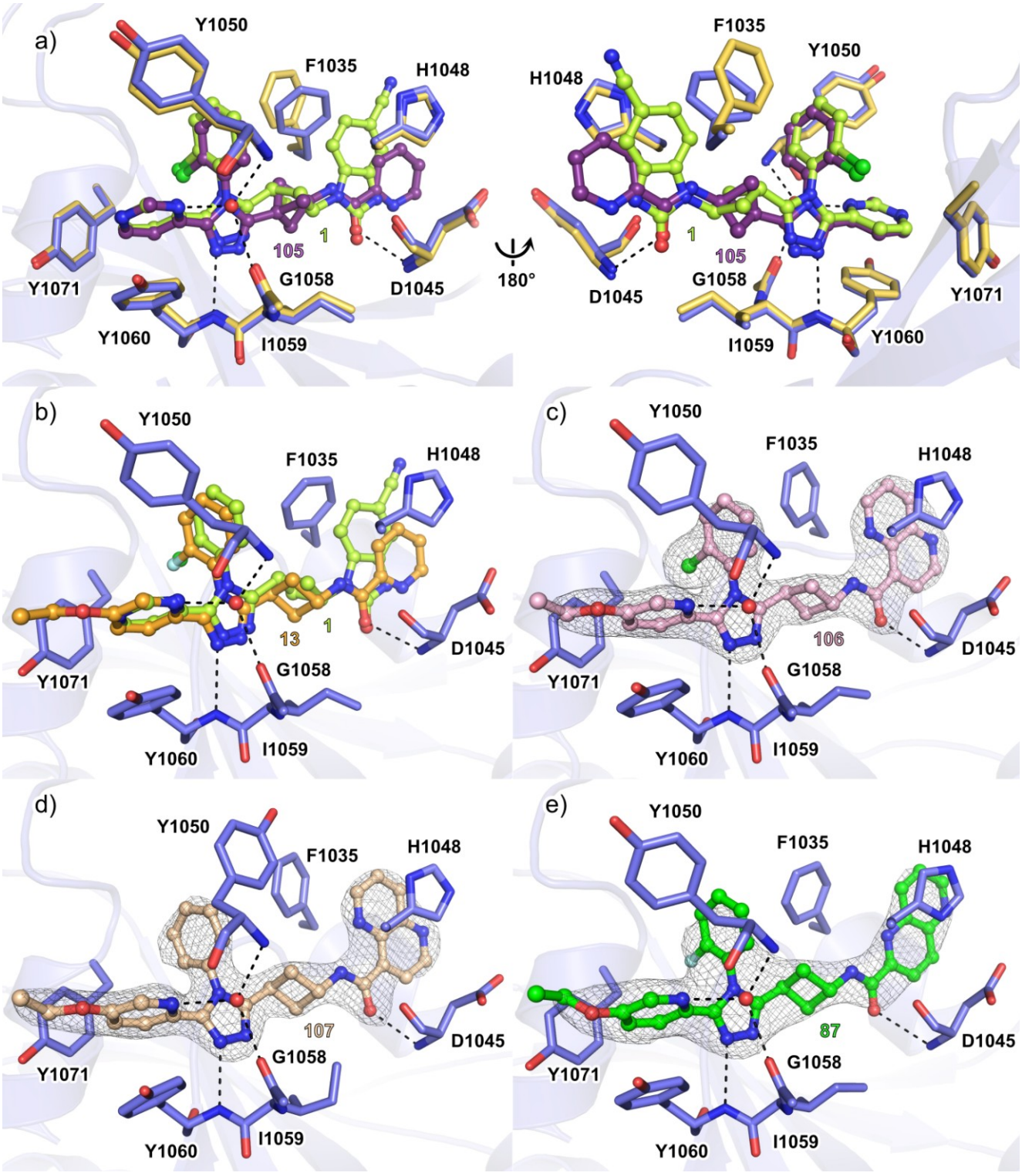
c)

South variation		Biochemical IC_{50} TNKS2 (nM)	Cellular IC_{50} HEK293 (nM)
1		6.3	19
65		15	200
66		5.4	30
67		19	260
23		30	142
68		23	310
69		50	590
70		25	290
71		49	460
72		170	300
73		1700	1050
74		5600	>10000

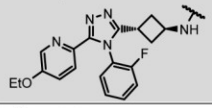
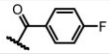
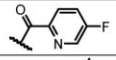
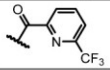
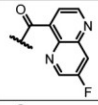
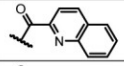
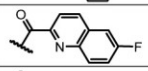
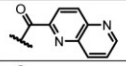
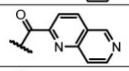
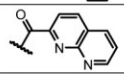
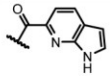
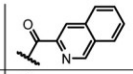
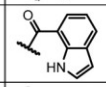
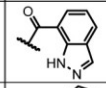
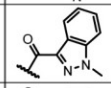
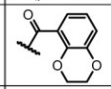
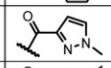
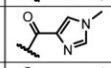
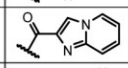
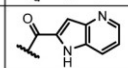
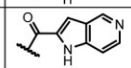
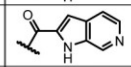
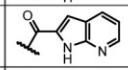
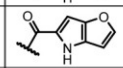
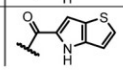
d)

Linker variation		Biochemical IC_{50} TNKS2 (nM)	Cellular IC_{50} HEK293 (nM)
1		6.3	19
75		44	220
19		3.6	4.7

Supplementary Table 1. (a) East, (b) West, (c) South and Linker (d) variations.



Supplementary Figure 1. Co-crystal structures of TNKS2 with inhibitors. (a) Superposition of **105** and **1** co-crystal structures (PDB codes 6TKN, 5NOB) showing the compounds and TNKS2 proteins for the TNKS2-**105** co-crystal structure (blue) and TNKS2- **1** co-crystal structure (yellow). Hydrogen bonds (dashed lines) and a water molecule (red sphere) are shown for the TNKS2-**105** co-crystal structure. (b) Superposition of **13** and **1** co-crystal structures showing the compounds and TNKS2 protein from the TNKS2- **13** co-crystal structure (PDB codes 6TG4, 5NOB). (c) Binding mode of **106** with TNKS2 catalytic domain (PDB code 6TKP). (d) Binding mode of **107** with TNKS2 catalytic domain (PDB code 6TKQ). (e) Binding mode of **87** with TNKS2 catalytic domain (PDB code 6TKR). The σ_A weighted $2F_o-F_c$ electron density maps around the ligands are contoured at 1.4-1.7σ. Crystal structures were solved with molecular replacement using the structure of TNKS2 (PDB code: 5NOB) as a starting model.

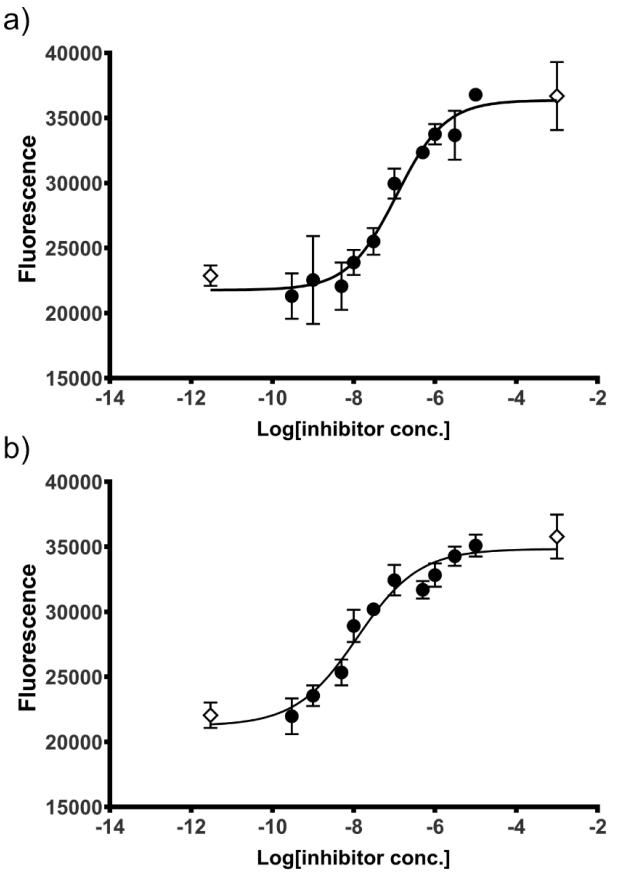
ID	East point mutations 	Biochemical IC_{50} TNKS2 (nM)	Cellular IC_{50} HEK293 (nM) **Solubility** (μM)
76		9.5	73
77		10	62
13		14	19 (>80)
78		4.2	14
79		4.5	25
80		190	919
81		5.0	39
82		28	70
83		2.1	1.1 (3.4)
10		4.3	0.63 (>80)
84		2	0.17 (3.1)
85		6.4	14 (14)
86		9.1	12
87		1.5	5.0 (<2)
88		2.8	7.5 (>80)
89		19	78 (<2)
90		4.9	14 (<2)
91		5.4	18 (13)
92		10	57
93		21	67
94		13	36
95		4.5	47
96		18	65
97		87	550
98		12	100
99		5.0	8.0
100		4.4	34
101		4.1	27
102		19	144
103		14	25
104		7.4	27

Supplementary Table 2. East-side variations of compound **13**.

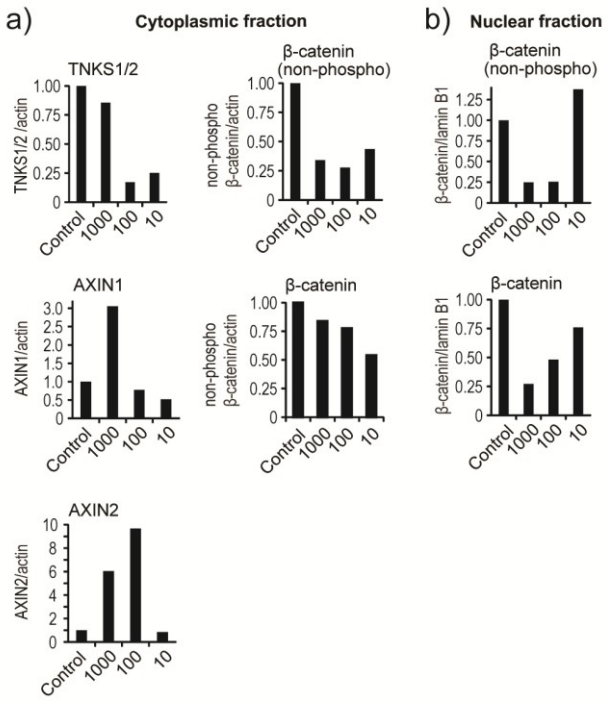
	1**	13
ARTD1/PARP1 (μM)	>100	29
ARTD2/PARP2 (μM)	>100	26
ARTD3/PARP3 (μM)	>100	75
ARTD4/PARP4 (μM)	>100	>100
ARTD5/TNKS1 (nM)	29 (7.54±0.007)	127 (6.90±0.05)
ARTD6/TNKS2 (nM)	6.3 (8.20±0.03)	14 (7.85±0.04)
ARTD7/PARP15$^{\Delta}$(μM)	> 10	>>10
ARTD8/PARP14 (μM)	> 10	>100
ARTD10/PARP10 (μM)	> 10	>> 10
ARTD12/PARP12$^{\Delta}$(μM)	> 10	>>10

>> no inhibition detected
> below 50% inhibition
Δ concentration limited by DMSO tolerance
** *reference 11*

Supplementary Table 3. Inhibition of ARTDs/PARPs (IC_{50} [pIC_{50}±SEM]).

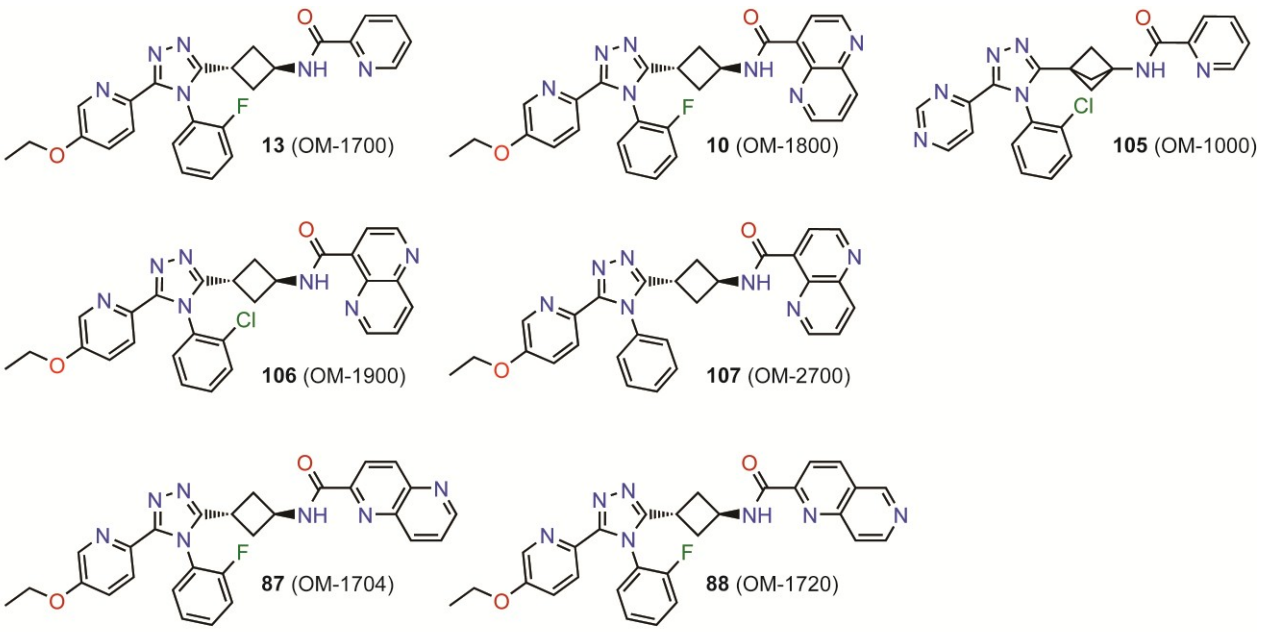


Supplementary Figure 2. Exemplary dose-response measurements of **13** with TNKS1 (a) and TNKS2 (b). The measurements were fitted using 4-parameters with GraphPad Prism. As compound showed no fluorescence interference, raw values were used for the fit. Controls were placed 2-logarithm units below or above the highest, and lowest compound concentrations (open diamonds).



Supplementary Figure 3. Quantification of immunoblots (protein/actin or lamin B1 loading controls) relative to controls (0.01% DMSO = 1) shown in Fig. 5a. (a) Cytoplasmic TNKS1/2, AXIN1, AXIN2, transcriptionally active β-catenin (non-phospho) and β-catenin. (b) Nuclear active β-catenin and β-catenin.

a)



b)

Compound	13	10	105	106	107	87	88
PDB code	**6TG4**	**6TKM**	**6TKN**	**6TKP**	**6TKQ**	**6TKR**	**6TKS**
Beam line	ESRF ID30B	DLS I04	ESRF ID30B	ESRF ID23-1	ESRF ID23-1	DLS I04	ESRF ID30A-1
Wavelength (Å)	0.9677	0.9795	0.97625	1.03285	0.97625	0.9795	0.966
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions	42.68,	42.32,	41.73,	42.01,	42.12,	41.99,	41.60,
a, b, c (Å)	77.9,	77.41,	76.76,	77.08,	76.84,	76.76,	76.42,
	149.52	148.68	147.91	148.36	148.8	148.12	148.19
Resolution (Å)	37 - 2.76	29.7 - 2.7	38.4 - 2.5	41.6 - 2.4	41.7 - 2.5	41.5 - 2.75	42 - 2.50
	(2.86-2.76)	(2.80- 2.7)	(2.59- 2.5)	(2.49- 2.4)	(2.59- 2.5)	(2.85- 2.75)	(2.56-2.50)
R_{merge}	29.0 (153.7)	24.4 (114.4)	22.0 (151.7)	12.1 (87.4)	19.7 (85.1)	30.1 (200.1)	10.1 (72.5)
I / σI	5.33 (1.06)	5.09 (1.21)	7.64 (1.10)	11.03 (1.93)	8.37 (2.04)	5.66 (1.12)	9.91 (2.03)
Completeness (%)	99.3 (99.6)	98.4 (99.9)	99.8 (99.6)	99.8 (99.9)	99.7 (99.5)	98.3 (99.5)	99.3 (98.7)
Redundancy	6.2 (5.8)	3.4 (3.5)	6.3 (6.5)	6.5 (6.3)	6.5 (6.7)	6.4 (6.5)	5.4 (5.7)
Refinement							
R_{work} / R_{free}	0.226/0.270	0.237/0.265	0.201/ 0.244	0.202/ 0.231	0.188/0.229	0.228/0.282	0.214/0.259
B-factors							
Protein	49	42	45	44	35	55	58
Inhibitor	42	31	38	36	24	41	51
R.m.s.d.							
Bond lengths (Å)	0.013	0.013	0.013	0.013	0.013	0.013	0.013
Bond angles (°)	1.72	1.64	1.72	1.64	1.79	1.72	1.66
Ramachandran plot (%)							
Favored regions	97.22	97.24	96.03	99.5	99	96.02	97.01
Additionally allowed regions	2.78	2.76	3.97	0.5	1	3.98	2.99

Supplementary Table 4. (a) Structures for compounds shown in (b). (b) Data collection and refinement statistics for the crystal structures.