

# Design and discovery of 3-aryl-5-substituted-isoquinolin-1-ones as potent tankyrase inhibitors

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## Experimental section

### Homology model

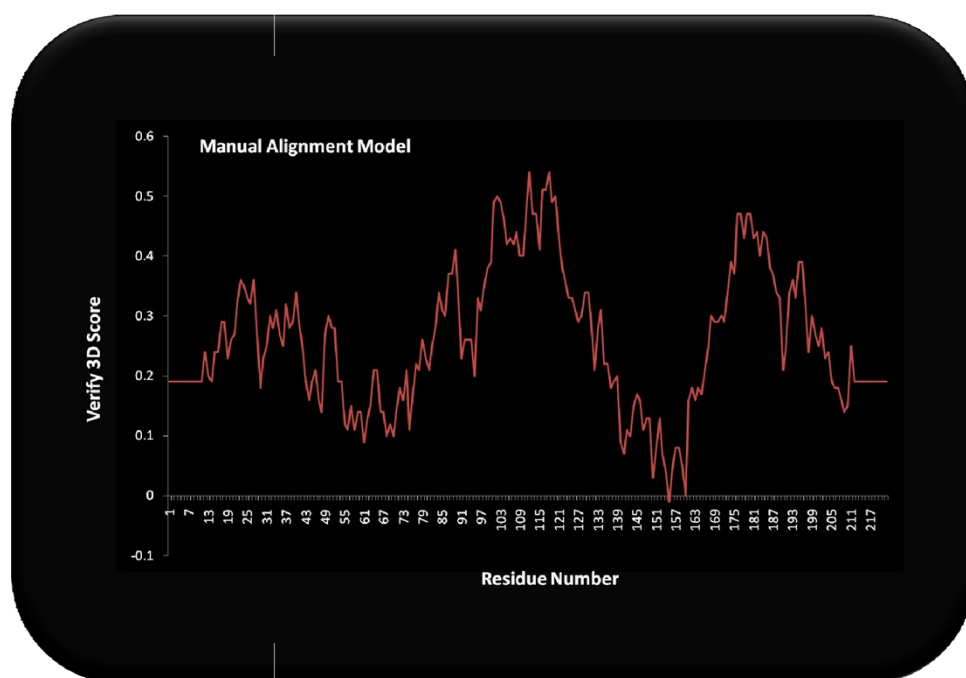
An amino acid sequence alignment between the TNKS “PARP domain” and the truncated PARP1 structure was produced (see below), using the structural superimposition of the TNKS “closed” form (2RF5) and the truncated PARP1 structure (“2RCW\_truncated”) as a guide.

2RF5	PDBID CHAIN S	QGTILLDLAPEDKEYQSVEEEMQSTIREHRDGGNAGGIFNRYNVIRIQKV
2RCW	PDBID CHAIN S	-KTDIKVVDRDSEAEIIRKYVKNTTHATTHNAYD-----LEVIDIFKI
2RF5	PDBID CHAIN S	VNKKLRERFCHRQKEVSEENHNHHNERMLFHGSP--FINAIIHKGFDERH
2RCW	PDBID CHAIN S	EREGECQRYKPFKQL-----HNRRLWHSRRTTNFAGILSQGLRIAP
2RF5	PDBID CHAIN S	AYIGGMFG-----AGIYFAENSSKSNQYVYGIGGGTGCPHDKDRSCYIC
2RCW	PDBID CHAIN S	IAPPEAPVTGYMFGKGIYFADMVSKSANYCHTS-----QGD
2RF5	PDBID CHAIN S	HRQMLFCRVTLGKSFLQFST-IKMAHAPPGHHSVIGRP-----
2RCW	PDBID CHAIN S	PIGLILLGEVALGNMYELKHASHISKLPKGKHSVKGLGKTTPDP SANI SL
2RF5	PDBID CHAIN S	-----SVNGLAYAEYVIYRGEQAYPEYLITYQIMKPEAPS
2RCW	PDBID CHAIN S	DGVDVPLGTGISSGVNDTSLLYNEYIVYDIAQVNLKYLLKLKFNFKT---
2RF5	PDBID CHAIN S	QTATAAEQ
2RCW	PDBID CHAIN S	-----

The sequence identity between the PARP1 and TNKS structures when aligned in this manner was 18%, but given the high level of structural correlation between the two proteins this was not deemed problematic.

This manual alignment was then used in conjunction with the truncated PARP1 structure to produce a comparative model of the “open” form of the TNKS structure using the MODELLER software.

The quality of the model was assessed via the Verify 3D software and all but one of the Verify 3D scores for the residues in the model were greater than zero:



The residue which failed to achieve a positive score (Lys155) is found on a loop which is remote from the active site, and as such the impact of the poor score for this residue is unlikely to affect the topology of the nicotinamide-binding active site.

The model produced via the amino-acid sequence alignment shows that the loop adjacent to the active site has moved to the desired “open” form, which would facilitate binding of nicotinamide.

### ***In silico* screening**

The *in silico* work to identify initial hits was performed using a two-stage process. The first stage took 140,000 commercially available compounds which contained an aryl-CONH sub-structure, and queried these against a four-point pharmacophore that also approximately defined the shape of the nicotinamide-binding site. The pharmacophore work was undertaken using Accelrys' Pipeline Pilot (7.5) protocols, which were set-up through the graphical environment of Discovery Studio 2.0. This is essentially a newer version of the company's “Catalyst” program. The ligand from the 2RCW crystal structure was placed into the TNKS homology model (which was based on the on the PARP1 structure in 2RCW) and this was used to help define the positions of the pharmacophore points. A hydrogen-bond acceptor and donor feature were placed in the active site to extract compounds that had the same hydrogen-bonding features that could interact between the enzyme and the amide group as found in known nicotinamide-containing binders. A flat-ring feature requirement was also defined at the centre of the benzamide ring. Finally, a second hydrogen-bond donor feature was defined in order to be placed near the catalytic glutamate side-chain (Glu327 in 2RCW). These feature requirements were augmented by exclusion spheres, whose centroids were defined by the backbone carbon atoms of the homology model in the vicinity of the 2RCW ligand. No ligand atoms were permitted within these spheres. Known PARP ligands – including PJ34 and 3-aminobenzamide – were queried against the pharmacophore to check that they were recognised as hits. The known PARP ligands were successfully recognised by the pharmacophore. The 8,200 hits resulting from the virtual-screen query against the pharmacophore all had equivalent (or better) FitValue scores than the known PARP ligands.

The second stage was to take these 8,200 hits and dock them into the homology-model binding-site using the FLEXX program. The dockings required bi-dentate interaction with Gly202 (in 2RCW) as well as an aromatic requirement defined by the reference ligand. All dockings had to satisfy these requirements

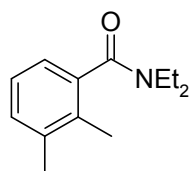
before being considered successful. Using known PARP ligands (as above), the docking set-up was tested and these ligands were successfully docked using this procedure. We also identified the lowest docking scores obtained by a correctly docked compound and rejected any compounds from the virtual hits which scored below this.

## Chemical synthesis

All materials were obtained from commercial suppliers and used without further purification unless otherwise noted. Anhydrous solvents were either obtained from Aldrich or Fisher Scientific. All reactions involving air- or moisture-sensitive reagents were performed under a nitrogen atmosphere. Routine analytical thin layer chromatography was performed on pre-coated plates (Alugram®, SILG/UV254). Reaction analyses and purity were determined by reverse-phase LC-MS using an analytical C18 column (Phenomenex Luna C18 (2) 150 x 4.6 mm, 5 µm for 4 minute methods or Phenomenex Gemini C18 150 x 4.6 mm, 5 µm for 13 minute methods), using a diode array detector and an A:B gradient starting from 95% A: 5% B at a flow rate of 2.25 mL/min or 1.5 mL/min, where eluent A was 0.1% formic acid/H<sub>2</sub>O and eluent B was 0.1% formic acid/MeOH or eluent A was 10 mM NH<sub>4</sub>HCO<sub>3</sub> (aq) and eluent B: MeOH. Silica gel chromatography was performed with pre-packed silica gel Biotage® SNAP (KP-Sil) cartridges. Ion exchange chromatography was performed using Isolute® Flash SCX-2 cartridges. Reverse-phase preparative HPLC was carried out on a Waters ZQ instrument using mass-directed purification on a preparative C18 column (Phenomenex Luna C18 (2), 100 x 21.2 mm, 5 µm). Depending upon the retention time and the degree of separation of the desired compound from any impurities, an A:B gradient was employed starting from high %A/low %B at a flow rate of 20 mL/min. The following combinations of A and B were typically used: A = H<sub>2</sub>O + 0.1% formic acid; B = MeOH + 0.1% formic acid or A = 10 mM NH<sub>4</sub>HCO<sub>3</sub> (aq); B = methanol. <sup>1</sup>H and <sup>13</sup>C spectra were measured with a Bruker DRZ 400 MHz spectrometer. All observed protons are reported as parts per million (ppm) and are aligned to the residual solvent peak e.g. for DMSO-d<sub>6</sub> at δ<sub>H</sub> 2.50 and δ<sub>C</sub> 39.5 and for CDCl<sub>3</sub> at δ<sub>H</sub> 7.26. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, br = broad, m = multiplet), coupling constants (*J*) recorded in Hz, and a number of protons. Low-resolution mass spectrometry data were determined on Waters ZQ4000 single quadrupole or Micromass Ultima triple quadrupole mass spectrometers. High-resolution mass spectrometry was determined using Positive Ion Electrospray on the Orbitrap. All compounds tested (bioassays) were determined to be at least 95% pure by LC-MS unless otherwise stated.

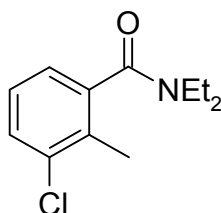
### General Preparation of *N,N*-diethyl-benzamide analogues using TBTU

#### *N,N*-Diethyl-2,3-dimethyl-benzamide (7d):



The following is a typical procedure used to synthesize the *N,N*-diethyl-benzamide analogues *via* a coupling method. To a stirred solution of 2,3-dimethyl-benzoic acid **6d** (1.52 g, 10.1 mmol) in DCM/DMF (118 mL/ 12 mL) was added DIPEA (1.76 mL, 10.1 mmol) and TBTU (3.25 g, 10.1 mmol) and the reaction mixture stirred at rt for 50 min. *N,N*-diethylamine (1.58 mL, 15.2 mmol) was added and the reaction mixture stirred for 18 h. The reaction mixture was washed with 10% Na<sub>2</sub>CO<sub>3</sub> solution (2 x 100 mL) and concentrated *in vacuo*. The crude material was purified by silica gel column chromatography, eluting with *iso*-hexane and increasing the polarity to 30% EtOAc/*iso*-hexane to obtain *N,N*-diethyl-2,3-dimethyl-benzamide **7d** (1.48 g, 72%) as a colourless liquid. <sup>1</sup>H NMR (400MHz, DMSO-*d*<sub>6</sub>): δ 7.20-7.10 (m, 2H), 6.90 (d, *J* = 8 Hz, 1H), 3.70-3.55 (m, 1H), 3.35-3.20 (m, 1H), 3.15-2.90 (m, 2H), 2.25 (s, 3H), 2.07 (s, 3H), 1.17 (t, *J* = 7 Hz, 3H), 0.95 (t, *J* = 7 Hz, 3H); LCMS (ESI<sup>+</sup>) *m/z* 206 [M + H]<sup>+</sup>, *R*<sub>t</sub> = 2.75 min.

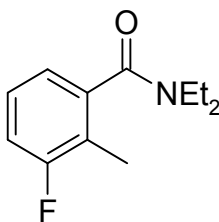
### 3-Chloro-*N,N*-diethyl-2-methylbenzamide (**7b**)



Compound **7b** was prepared as a colourless oil in 77% yield (10 g) from compound **6b** using a similar procedure to that of **7d**. <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>): δ 7.18 (dd, *J* = 8.1, 1.3 Hz, 1H), 7.30-7.26 (m, 1H), 7.15-7.13 (m, 1H) 3.64-3.59 (m, 2H), 3.12-3.08 (m, 2H), 3.01-2.95 (m, 1H), 2.20 (s, 3H), 1.16 (t, *J* = 7.1 Hz, 3H), 0.96 (t, *J* = 7.1 Hz, 3H); LCMS (ESI<sup>+</sup>) *m/z* 226 [M + H]<sup>+</sup>, *R*<sub>t</sub> = 2.84 min.

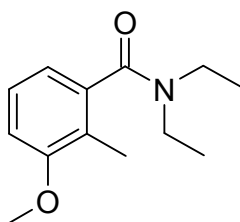
## General Preparation of *N,N*-diethyl-benzamide analogues *via* acid chloride preparation

### *N,N*-Diethyl-3-fluoro-2-methylbenzamide (**7c**)



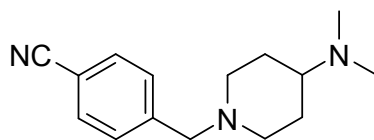
The following is a typical procedure used to synthesize the *N,N*-diethyl-benzamide analogues *via* acid chloride preparation. To a stirred solution of 3-fluoro-2-methylbenzoic acid **6c** (1.00 g, 6.49 mmol) in DCM (13 mL) and DMF (0.13 mL) was added oxalyl chloride (0.66 mL, 7.79 mmol) and DMF (0.1 mL) at 0°C under nitrogen and the resulting mixture was allowed to warm to rt over 2 h. The reaction mixture was concentrated *in vacuo* and further co-evaporated with toluene (10 mL) to afford 3-fluoro-2-methylbenzoyl chloride as an oil (10 g) which was used without further purification. To a stirred solution of the acid chloride in DCM (10 mL) was added diethylamine (2.02 mL, 19.5 mol) under nitrogen at 0°C and the mixture stirred at rt for 18 h. The reaction mixture was diluted with DCM (80 mL) and washed with saturated aqueous NaHCO<sub>3</sub> solution (100 mL) and brine solution (100 mL). The organic layer was passed through a phase separation cartridge and the crude material was purified by silica gel column chromatography, eluting with *iso*-hexane and increasing the polarity to 30% EtOAc/*iso*-hexane to obtain *N,N*-diethyl-3-fluoro-2-methylbenzamide (**7c**) (1.15 g, 86%) as a colourless oil. <sup>1</sup>H NMR (400MHz, DMSO-*d*<sub>6</sub>): δ 7.31-7.26 (m, 1H), 7.20-7.15 (m, 1H), 7.00 (dd, *J* = 7.6, 1.0 Hz, 1H), 3.59 (br s, 1H), 3.32 (br s, 1H), 3.04 (br s, 1H), 2.09 (d, *J* = 2.3 Hz, 3H), 1.15 (t, *J* = 7.1 Hz, 3H), 0.96 (t, *J* = 7.1 Hz, 3H); LCMS (ESI<sup>+</sup>) *m/z* 210 [M + H]<sup>+</sup>, *R*<sub>t</sub> = 2.63 min.

### *N,N*-Dimethyl-3-methoxy-2-methylbenzamide (**7a**)



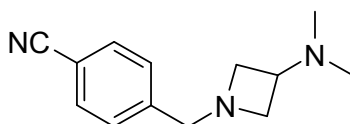
Compound **7a** was prepared as a pale yellow oil in 75% yield (10 g) from compound **6a** using a similar procedure to that of **7c**. *R*<sub>f</sub>: 0.6 (10% EtOAc/pet ether); <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>): δ 7.18 (t, *J* = 7.9 Hz, 1H), 6.83-6.76 (m, 2H), 3.83 (s, 3H), 3.78 (m, 1H), 3.40-3.36 (m, 1H), 3.15-3.09 (m, 2H), 2.13 (s, 3H), 1.26 (t, *J* = 7 Hz, 3H), 1.02 (t, *J* = 7.3 Hz, 3H). LCMS (ESI<sup>+</sup>) *m/z* 222 [M + H]<sup>+</sup>, *R*<sub>t</sub> = 2.88 min.

#### 4-(4-Dimethylamino-piperidin-1-ylmethyl)-benzonitrile (**9a**)



4-Bromomethylbenzonitrile (3.00 g, 15.3 mmol), 4-dimethylaminopiperidine (2.90 g, 23.0 mmol) and triethylamine (3.2 mL, 23.0 mmol) in THF (50 mL) were heated at 50 °C for 62 h. The reaction mixture was concentrated *in vacuo* and the crude material was purified by silica gel column chromatography, eluting with DCM and increasing the polarity to 30% MeOH/DCM to afford 4-(4-dimethylamino-piperidin-1-ylmethyl)-benzonitrile (1.4g, 56%) as a white solid. LCMS (ESI<sup>+</sup>) *m/z* 244 [M + H]<sup>+</sup>, *R*<sub>t</sub> = 2.40 min.

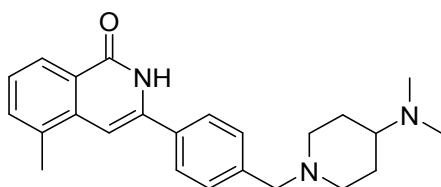
#### 4-(3-Dimethylaminoazetidin-1-ylmethyl)benzonitrile (**9b**)



To a stirred solution of 4-bromomethyl benzonitrile (660 mg, 3.37 mmol) and K<sub>2</sub>CO<sub>3</sub> (1.40 g, 10.1 mmol) in CH<sub>3</sub>CN (22 mL) at rt under nitrogen, was added *N,N*-di-Me-3-azetidinamine hydrochloride (700 mg, 4.04 mmol) and DIPEA (1.41 mL, 8.08 mmol) in CH<sub>3</sub>CN (3 mL). The reaction mixture was stirred for 18 h and then evaporated to remove most of the solvent, suspended in DCM and washed with water. The organic phase was separated, passed through a phase separation cartridge and concentrated *in vacuo*. The crude material was purified by silica gel column chromatography, eluting with DCM and increasing the polarity to 10% MeOH/DCM to obtain 4-(3-dimethylaminoazetidin-1-ylmethyl)benzonitrile (**9b**) (469 mg, 65%) as a bright yellow oil. <sup>1</sup>H NMR (400MHz, DMSO-*d*<sub>6</sub>): δ 7.76 (d, *J* = 8.3 Hz, 2H), 7.45 (d, *J* = 8.3 Hz, 2H), 3.63 (s, 2H), 3.36-3.33 (m, 2H), 2.82-2.74 (m, 3H), 1.98 (s, 6H); LCMS (ESI<sup>+</sup>) *m/z* 230 [M + H]<sup>++</sup>, *R*<sub>t</sub> = 0.39 min.

#### General Preparation of 2H-Isoquinolin-1-one analogues

##### 3-[4-(3-Dimethylaminopiperidin-1-ylmethyl)phenyl]-5-methyl-2H-isoquinolin-1-one (**21**)

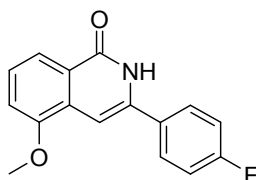


The following is a typical procedure used to synthesize the 2H-isoquinolin-1-one analogues. *N,N*-Diethyl-2,3-dimethyl-benzamide (**7d**) (2.75 g, 13.4 mmol) was dissolved in anhydrous THF (15 mL) under N<sub>2</sub> and cooled to -78 °C. *n*-BuLi (2.5M in *n*-hexanes, 11.2 mL, 28.1 mmol) was added dropwise and the reaction mixture was stirred at -78 °C for 30 minutes. 4-(4-Dimethylamino-piperidin-1-

ylmethyl)-benzonitrile (**9a**) (3.26 g, 28.1 mmol) in anhydrous THF (40 mL) was added dropwise to the reaction mixture and the reaction mixture stirred at this temperature for a further 3 h. The reaction mixture was poured into cold water, allowed to warm to rt and extracted with EtOAc (3 x 100 mL). The combined organic extracts were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated *in vacuo*. The crude solid was dissolved in MeOH/DCM and passed through a SCX-2 cartridge (50 g) eluting with MeOH followed by elution with 0.5M NH<sub>3</sub>/MeOH. The crude material was purified by silica gel chromatography eluting with DCM and increasing the polarity to 50% MeOH/DCM followed by trituration with *iso*-hexane followed by *iso*-hexane/EtOAc (2:1) to afford 3-[4-(3-dimethylaminoazetidin-1-ylmethyl)phenyl]-5-methyl-2H-isoquinolin-1-one (**21**) (2.23 g, 44% yield) as a white solid. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 11.54 (s, 1H), 8.08-8.05 (m, 1H), 7.77 (d, *J* = 8.3 Hz, 2H), 7.57-7.54 (m, 1H), 7.40 (d, *J* = 8.3 Hz, 2H), 7.36 (t, *J* = 7.7 Hz, 1H), 6.85 (s, 1H), 3.49 (s, 2H), 2.85-2.82 (m, 2H), 2.55 (s, 3H), 2.15 (s, 6H), 2.06-1.98 (m, 1H), 1.97-1.90 (m, 2H), 1.72-1.67 (m, 2H), 1.42-1.32 (m, 2H); <sup>13</sup>C NMR (101MHz, DMSO-*d*<sub>6</sub>) 162.94, 140.05, 139.76, 136.65, 133.68, 133.18, 132.70, 128.97, 126.64, 125.80, 124.90, 124.56, 99.74, 61.64, 61.58, 52.45, 41.50, 28.06, 18.77; LCMS (ESI<sup>+</sup>) *m/z* 376 [M + H]<sup>++</sup>, *R*<sub>t</sub> = 4.09 min; HRMS (ESI<sup>+</sup>) *m/z* [M + H]<sup>+</sup>, calcd for C<sub>24</sub>H<sub>30</sub>ON<sub>3</sub>: 376.2383 Found 376.2378.

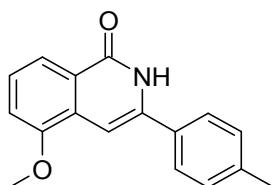
The following 2H-isoquinolin-1-one analogues were prepared using a similar procedure to that of (**21**), unless otherwise stated.

### 3-(4-Fluorophenyl)-5-methoxy-2H-isoquinolin-1-one (**10**)



The crude material was purified by trituration with cyclohexane/EtOAc (2:1) to afford the title compound (**10**) as a white solid in 31% yield (84 mg). <sup>1</sup>H NMR (400MHz, DMSO-*d*<sub>6</sub>): δ 11.63 (br s, 1H), 7.84-7.76 (m, 3H), 7.43 (t, *J* = 8.1 Hz, 1H), 7.35-7.26 (m, 3H), 6.90 (s, 1H), 3.94 (s, 3H); LCMS (ESI<sup>+</sup>) *m/z* 270 [M + H]<sup>++</sup>, *R*<sub>t</sub> = 8.23 min.

### 5-methoxy-3-*p*-tolyl-2H-isoquinolin-1-one (**11**)

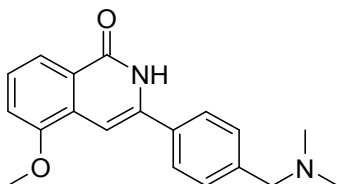


The crude material was purified by trituration with *iso*-hexane/Et<sub>2</sub>O (1:1) to afford the title compound (**11**) as a pale yellow solid in 31% yield (80 mg). <sup>1</sup>H NMR (400MHz, DMSO-*d*<sub>6</sub>): δ 11.56 (br s, 1H), 7.80-7.76 (m, 1H), 7.66 (d, *J* = 8.1 Hz, 2H), 7.41 (t, *J* = 8.0 Hz, 1H), 7.29 (d, *J* = 8.1 Hz, 2H), 7.26



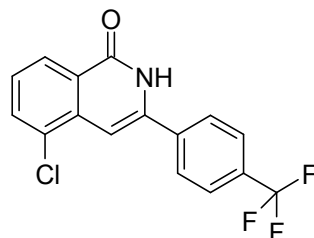
(dd,  $J = 8.0, 1.0$  Hz, 1H), 6.92 (s, 1H), 3.94 (s, 3H), 2.36 (s, 3H);  $^{13}\text{C}$  NMR (101MHz,  $\text{DMSO-}d_6$ ) 162.48, 154.26, 139.57, 138.82, 131.16, 129.35, 128.45, 126.59, 126.46, 125.61, 118.17, 112.15, 96.28, 55.86, 20.77; LCMS ( $\text{ESI}^+$ )  $m/z$  266  $[\text{M} + \text{H}]^{++}$ ,  $R_t = 8.55$  min. HRMS ( $\text{ESI}^+$ )  $m/z$   $[\text{M} + \text{H}]^{++}$ , calcd for  $\text{C}_{17}\text{H}_{16}\text{O}_2\text{N}$ : 266.1176; found 266.1169.

### 3-(4-dimethylaminomethylphenyl)-5-fluoro-2H-isoquinolin-1-one (12)



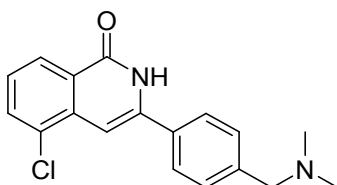
The title compound (**12**) was isolated as a yellow solid in 58% yield (123 mg) by concentration of the reaction mixture under reduced pressure followed by filtration of the resulting solid, which was washed with water and then methanol.  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO-}d_6$ ):  $\delta$  11.65 (s, 1H), 11.00-10.70 (br s, formic acid  $\text{CHO}$ , 0.7H), 7.86 (d,  $J = 8.3$  Hz, 2H), 7.80-7.77 (m, 1H), 7.67 (d,  $J = 8.3$  Hz, 2H), 7.45 (t,  $J = 8.1$  Hz, 1H), 7.29 (dd,  $J = 8.1, 1.0$  Hz, 1H), 7.01 (s, 1H), 4.27 (s, 2H), 3.95 (s, 3H), 2.67 (s, 6H); LCMS ( $\text{ESI}^+$ )  $m/z$  309  $[\text{M} + \text{H}]^{++}$ ,  $R_t = 5.05$  min; HRMS ( $\text{ESI}^+$ )  $m/z$   $[\text{M} + \text{H}]^+$ , calcd for  $\text{C}_{19}\text{H}_{21}\text{O}_2\text{N}_2$ : 309.1598; found 309.1590.

### 5-Chloro-3-(4-trifluoromethylphenyl)-2H-isoquinolin-1-one (13)



The title compound (**13**) was isolated as a brown solid in 52% yield (895 mg) by concentration of the reaction mixture under reduced pressure followed by filtration of the resulting solid, which was washed with EtOAc.  $^1\text{H}$  NMR (400MHz,  $\text{DMSO-}d_6$ ):  $\delta$  12.01 (br s, 1H), 8.20 (d,  $J = 7.8$  Hz, 1H), 8.00 (d,  $J = 8.1$  Hz, 2H), 7.90-7.85 (m, 3H), 7.51 (t,  $J = 7.8$  Hz, 1H), 6.98 (s, 1H); LCMS ( $\text{ESI}^+$ )  $m/z$  323  $[\text{M} + \text{H}]^{++}$ ,  $R_t = 8.89$  min.

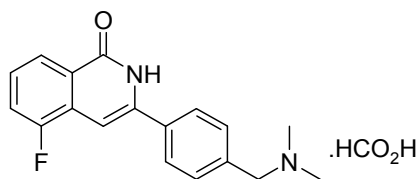
### 5-Chloro-3-(4-dimethylaminomethylphenyl)-2H-isoquinolin-1-one (14)



The crude material was purified by reverse phase preparative HPLC-MS to afford the title compound (**14**) as a cream solid in 67% yield (140 mg).  $^1\text{H}$  NMR (400MHz,  $\text{CDCl}_3$ ):  $\delta$  10.06 (br s, 1H), 8.35-

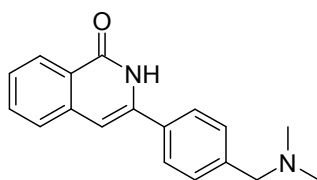
8.31 (m, 1H), 7.75-7.71 (m, 3H), 7.49 (d,  $J = 8.3$  Hz, 2H), 7.39 (t,  $J = 7.8$  Hz, 1H), 7.16 (s, 1H), 3.52 (s, 2H), 2.29 (s, 6H); LCMS (ESI<sup>+</sup>)  $m/z$  313  $[M + H]^{++}$ ,  $R_t = 5.44$  min.

**3-(4-Dimethylaminomethylphenyl)-5-fluoro-2H-isoquinolin-1-one (15); formic acid salt**



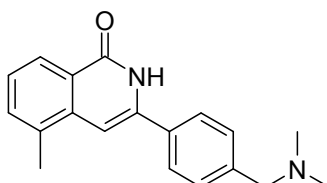
The crude material was purified by reverse phase preparative HPLC-MS to afford the title compound (**15**) as a cream solid in 91% yield (192 mg). <sup>1</sup>H NMR (400MHz, DMSO-*d*<sub>6</sub>):  $\delta$  11.75 (s, 1H), 8.17 (s, formic acid  $\text{CHO}$ , 1H), 8.06-8.03 (m, 1H), 7.79 (d,  $J = 8.3$  Hz, 2H), 7.60 (ddd,  $J = 10.4, 8.1, 1.3$  Hz, 1H), 7.49 (td,  $J = 8.1, 5.3$  Hz, 1H), 7.43 (d,  $J = 8.3$  Hz, 2H), 6.86 (s, 1H), 2.20 (s, 6H); LCMS (ESI<sup>+</sup>)  $m/z$  297  $[M + H]^{++}$ ,  $R_t = 4.90$  min.

**3-(4-Dimethylaminomethylphenyl)-2H-isoquinolin-1-one (16)**



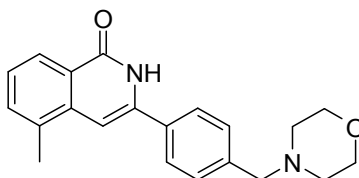
The title compound (**16**) was isolated as a pale yellow solid in 66% yield (144 mg) by concentration of the reaction mixture under reduced pressure followed by filtration of the resulting solid, which was washed with water and then methanol. <sup>1</sup>H NMR (400MHz, DMSO-*d*<sub>6</sub>):  $\delta$  11.52 (s, 1H), 8.20 (d,  $J = 8.1$  Hz, 1H), 7.79 (d,  $J = 8.3$  Hz, 2H), 7.73-7.70 (m, 2H), 7.52-7.43 (m, 3H), 6.95 (s, 1H), 3.64 (s, 2H), 2.29 (s, 6H); LCMS (ESI<sup>+</sup>)  $m/z$  279  $[M + H]^{++}$ ,  $R_t = 4.78$  min.

**3-(4-Dimethylaminomethylphenyl)-5-methyl-2H-isoquinolin-1-one (17)**



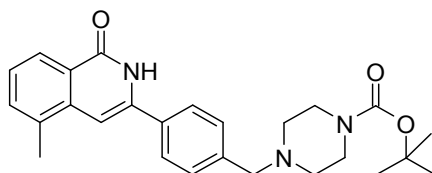
The crude material was purified by trituration with iso-hexane/EtOAc (2:1) to afford the title compound (**17**) as a pale yellow solid in 58% yield (133 mg). <sup>1</sup>H NMR (400MHz, DMSO-*d*<sub>6</sub>):  $\delta$  11.56 (s, 1H), 8.08-8.05 (m, 1H), 7.78 (d,  $J = 8.6$  Hz, 2H), 7.57-7.54 (m, 1H), 7.40 (d,  $J = 8.3$  Hz, 2H), 7.36 (t,  $J = 7.7$  Hz, 1H), 6.85 (s, 1H), 3.44 (s, 2H), 2.56 (s, 3H), 2.17 (s, 6H); LCMS (ESI<sup>+</sup>)  $m/z$  293  $[M + H]^{++}$ ,  $R_t = 4.94$  min.

**5-Methyl-3-(4-morpholin-4-ylmethylphenyl)-2H-isoquinolin-1-one (18)**



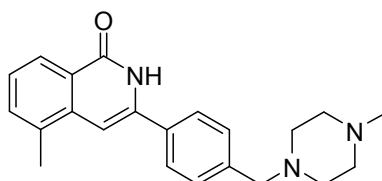
The crude material was purified by trituration with *iso*-hexane/EtOAc (2:1) to afford the title compound (**18**) as a white solid in 19% yield (51 mg). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 11.56 (s, 1H), 8.08-8.05 (m, 1H), 7.78 (d, *J* = 8.3 Hz, 2H), 7.57-7.54 (m, 1H), 7.43 (d, *J* = 8.3 Hz, 2H), 7.36 (t, *J* = 7.7 Hz, 1H), 6.85 (s, 1H), 3.60-3.57 (m, 4H), 3.52 (s, 2H), 2.55 (s, 3H), 2.38 (br s, 4H); LCMS (ESI<sup>+</sup>) *m/z* 335 [M + H]<sup>+</sup>, *R*<sub>t</sub> = 5.11 min.

**4-[4-(5-Methyl-1-oxo-1,2-dihydroisoquinolin-3-yl)benzyl]piperazine-1-carboxylic acid *tert*-butyl ester (19)**

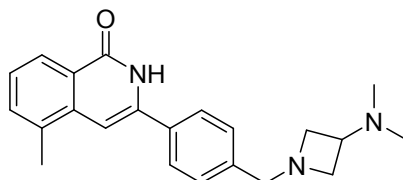


The crude material was purified by trituration with Et<sub>2</sub>O to afford the title compound (**19**) as a white solid in 46% yield (395 mg). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 11.51 (s, 1H), 8.09-8.05 (m, 1H), 7.79 (d, *J* = 8.3 Hz, 2H), 7.57-7.54 (m, 1H), 7.42 (d, *J* = 8.3 Hz, 2H), 7.36 (t, *J* = 7.8 Hz, 1H), 6.85 (s, 1H), 3.54 (s, 2H), 3.35-3.31 (m, 4H, masked by H<sub>2</sub>O peak), 2.56 (s, 3H), 2.35-2.33 (m, 4H), 1.39 (s, 9H); LCMS (ESI<sup>+</sup>) *m/z* 434 [M + H]<sup>+</sup>, *R*<sub>t</sub> = 6.41 min.

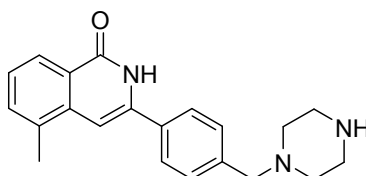
**5-Methyl-3-[4-(4-methylpiperazin-1-ylmethyl)phenyl]-2H-isoquinolin-1-one (20)**



The crude material was purified by trituration with *iso*-hexane/EtOAc (2:1) to afford the title compound (**20**) as a white solid in 59% yield (615 mg). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 11.55 (br s, 1H), 8.08-8.05 (m, 1H), 7.77 (d, *J* = 8.3 Hz, 2H), 7.57-7.54 (m, 1H), 7.41 (d, *J* = 8.3 Hz, 2H), 7.36 (t, *J* = 7.6 Hz, 1H), 6.84 (s, 1H), 3.50 (s, 2H), 2.55 (s, 3H), 2.46-2.22 (br s, 8H), 2.15 (s, 3H); LCMS (ESI<sup>+</sup>) *m/z* 348 [M + H]<sup>+</sup>, *R*<sub>t</sub> = 5.47 min.

**3-[4-(3-Dimethylaminoazetidin-1-ylmethyl)phenyl]-5-methyl-2H-isoquinolin-1-one (22)**

The crude material was dissolved in MeOH and passed through a SCX-2 cartridge (5 g) eluting with MeOH followed by elution with 0.5M NH<sub>3</sub>/MeOH. The desired product was then purified by reverse phase preparative HPLC-MS to afford the title compound (**22**) as an off-white solid in 43% yield (120 mg). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 11.54 (s, 1H), 8.08-8.05 (m, 1H), 7.78 (d, *J* = 8.3 Hz, 2H), 7.76 (d, *J* = 8.3 Hz, 2H), 7.56-7.53 (m, 1H), 7.39-7.34 (m, 3H), 6.83 (s, 1H), 3.60 (s, 2H), 3.39-3.31 (m, 2H, masked by H<sub>2</sub>O peak), 2.82-2.76 (m, 3H), 2.55 (s, 3H), 2.00 (s, 6H); LCMS (ESI<sup>+</sup>) *m/z* 348 [M + H]<sup>++</sup>, *R*<sub>t</sub> = 4.77 min.

**5-Methyl-3-(4-piperazin-1-ylmethylphenyl)-2H-isoquinolin-1-one (23)**

To a stirred solution of 4-[4-(5-Methyl-1-oxo-1,2-dihydroisoquinolin-3-yl)benzyl]piperazine-1-carboxylic acid *tert*-butyl ester (170 mg, 0.35 mmol) in DCM (2 mL) was added TFA (1 mL) and the reaction mixture stirred at rt for 2 h. The reaction mixture was concentrated under reduced pressure and further co-evaporated with DCM. The resulting solid was dissolved in MeOH/DCM and a drop of 4 M NH<sub>3</sub>/MeOH and passed through an SCX-2 cartridge (5 g) eluting with MeOH. The desired product was eluted from the column with 4M NH<sub>3</sub>/MeOH and the solvent removed *in vacuo* to afford 5-methyl-3-(4-piperazin-1-ylmethylphenyl)-2H-isoquinolin-1-one (**23**) (107 mg, 38%) as a white solid.

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.08-8.05 (m, 1H), 7.77 (d, *J* = 8.3 Hz, 2H), 7.57-7.54 (m, 1H), 7.41 (d, *J* = 8.3 Hz, 2H), 7.36 (t, *J* = 7.7 Hz, 2H), 6.84 (s, 1H), 3.48 (s, 2H), 2.70-2.68 (m, 4H), 2.34-2.27 (m, 4H) (NH peaks are not observed); <sup>13</sup>C NMR (101MHz, DMSO-*d*<sub>6</sub>) 162.95, 139.76, 139.65, 136.65, 133.69, 133.19, 132.72, 129.13, 126.61, 125.81, 124.89, 124.55, 99.76, 62.34, 54.04, 45.53, 18.77; LCMS (ESI<sup>+</sup>) *m/z* 334 [M + H]<sup>++</sup>, *R*<sub>t</sub> = 5.36 min; HRMS (ESI<sup>+</sup>) *m/z* [M + H]<sup>+</sup>, calcd for C<sub>21</sub>H<sub>24</sub>ON<sub>3</sub>: 334.1914; found 334.1906.

**TNKS/PARP Biochemical assay**

Tankyrase activity was assayed using a 96-well format HT Universal Chemiluminescent PARP Assay Kit (Trevigen, Inc, cat. no. 4676-096-K) according to the manufacturer's instructions. In short, tankyrase/PARP activity is quantified by the incorporation of biotinylated nicotinamide adenine dinucleotide (biotin-NAD<sup>+</sup>) onto the immobilised pseudo substrate, Histone. The extent of

poly(Biotin-ADP)ribosylation (PARylation) in the presence of increasing dose of inhibitor is then quantified by binding of streptavidin conjugated horse radish peroxidase (strep-HRP) followed by chemiluminescent detection. The published  $K_m$  of TNKS1 is 1.5 mM<sup>9</sup> and the final NAD<sup>+</sup> concentration in the reaction mixture is 25  $\mu$ M. The assays were carried out using human recombinant full length PARP1 (provided in the PARP assay kit, Trevigen, Inc, cat. no. 4676-096-K) and the PARP domain of human recombinant TNKS1 (see below).

Prior to assay initiation, inhibitor stocks were prepared in aqueous DMSO (10 % (v/v)) from 5 mM stock in 100% DMSO (Sigma Aldrich, cat. no. 265855) as 10x concentrations (10-0  $\mu$ M final concentration). The assay was initiated by the addition of 10x inhibitor (5  $\mu$ L) or 10% aqueous DMSO (5  $\mu$ L) to triplicate wells. Twenty microlitres of diluted TNKS1 protein (200 nM final conc.) in PARP buffer (Trevigen, Inc, cat. no. 4671-096-02) was added to each histone coated well, which was previously hydrated with PARP buffer. Triplicate wells with 1% DMSO/buffer alone (no enzyme) were also added as a measure of assay 'noise'. Positive control for PARP inhibition included the addition of 4-amino-1,8-naphthalimide (Sigma Aldrich, cat. no A0966) in corresponding doses.

The mixture was incubated for 10 minutes at room temperature and the PARylation reaction initiated by the addition of PARP cocktail (25  $\mu$ L, Trevigen, Inc) containing biotin-NAD<sup>+</sup> (Trevigen, Inc, cat. no. 4670-500-01), activated DNA (Trevigen, Inc, cat. no. 4671-096-06) and PARP buffer. The reaction was incubated for 1.5 hours (for TNKS1) or 1 hour (for PARP1) at room temperature. The reaction mixture was then removed by aspiration and the wells washed (3 x 200  $\mu$ L) with phosphate buffered saline containing Triton X-100 (0.1% (v/v), Sigma Aldrich cat. no. T8787). The wells were then washed (3 x 200  $\mu$ L) with phosphate buffered saline and then incubated with strep-HRP (50  $\mu$ L, Trevigen, Inc, cat. no. 4800-30-06) in strep-diluent (1:500 dilution, Trevigen Inc, cat. no. 4671-096-04) for 1 hour at room temperature. The Strep-HRP mixture was then aspirated and the wells washed (3 x 200  $\mu$ L) with phosphate buffered saline containing Triton X-100 (0.1% (v/v)) followed by phosphate buffered saline (3 x 200  $\mu$ L) and then incubated with PeroxyGlow<sup>TM</sup> reagent (100  $\mu$ L, Trevigen, Inc, cat. nos. 4675-096-01, 4675-096-02, room temperature, mixed 1:1).

The amount of light emitted as a result of the peroxidase-chemiluminescent reagent reaction was in proportion to the extent of poly(Biotin-ADP)ribosylation and was immediately measured with a Victor<sup>x5</sup> plate reader (Perkin Elmer, luminescence detection assay, luminescent units described as 'Counts Per Second' (CPS)). The data were normalized to the DMSO control after subtraction of 'noise' and was expressed as % PARP activity as a function of inhibitor dose. Dose response curves used to determine IC<sub>50</sub> values were Log transformed and analyzed by non-linear regression analysis (variable slope) using Prism (GraphPad Software, Inc).

## Wnt-Luciferase Reporter Assay

### *Generation of reporter cell lines:*

A Wnt dependent cell line (i.e., DLD1 colorectal adenocarcinoma cell line) was transduced with replication incompetent VSV-g pseudotyped lentiviral particles expressing the firefly luciferase gene under the control of minimal cytomegalovirus (mCMV) promoter and tandem repeats of the TCF/LEF transcriptional response element (Qiagen, cat no. CLS-018L). Post-transduction selection using puromycin (Sigma Aldrich, cat. no. P8833, 1.5 microgrammes per millilitre ( $\mu\text{g/mL}$ )) was carried out until the corresponding, non-infected, control cells were devoid of colonies. A polyclonal cell population (DLD1-Wnt-Luc cells) was expanded and collected for minimal passage and stored in liquid nitrogen.

### *Wnt-reporter assay:*

DLD1-Wnt-Luc cells were seeded (5000 cells/well) in a 96-well plate (Greiner Bio-One, cat. no. 655098) in Dubelco's Modified Eagle Medium (DMEM, SFBCO/Invitrogen, cat no. 41965-039) supplemented with Fetal Bovine Serum (FBS, 10%, SFBCO/Invitrogen, cat no. 10108-165). After overnight incubation, the media was replaced with OptiMEM (SFBCO/Invitrogen, cat no. 11058-021) supplemented with FBS (0.5%) and non-essential amino acids (1%, SFBCO/Invitrogen, cat no. 11140-035) and the appropriate putative TNKS inhibitor at a final concentration of 10, 3, 1, 0.30, 0.10, 0.030, 0.010 and 0  $\mu\text{M}$  with 1% (v/v) final DMSO in double-triplicate wells. Positive control includes the use of XAV-939 (Maybridge, FisherScientific, 3,5,7,8-tetrahydro-2-[4-(trifluoromethyl)phenyl]-4*H*-thiopyrano[4,3-*d*]pyrimidin-4-one, cat. no. RF03920, see: Huang *et al.*, Nature, 2009, Vol. 461, pp. 614-620). Cells were incubated for 20-22 hours before assaying for luciferase (first set of triplicates: Wnt activation) and viability (second set of triplicates: cell survival for data normalisation vs Wnt-activation) using ONE-Glo (Promega, cat. no. E6110) and CellTiter-Glo (Promega, cat. no. G7570) reagents consecutively. The assay was measured using a Victor<sup>2</sup> plate reader. The data were normalized to the DMSO control and were expressed as % Wnt activity as a function of inhibitor dose. Dose response curves used to determine  $\text{IC}_{50}$  values were Log transformed and analyzed by non-linear regression analysis (variable slope) using Prism (GraphPad Software, Inc).

## Tumour Cell Inhibition in Colorectal Cancer Cells

### *96-well Assay*

Mycoplasma-free cell lines were obtained from the American Tissue Culture Collection or the Health Protection Agency (HT55, Colo320DM) and incubated in the appropriate media at 37 °C and 5% CO<sub>2</sub>. Cells were seeded at 500 cells/well in a 96-well plate followed by continuous dosing of cells over a five-day period. Cells were maintained in MEM (HT55, LS174T), RPMI (COLO320DM, RKO, HCT15), DMEM (SW620, SW48), McCoy's (HCT116, HT29) or HAM/F12 (LoVo) supplemented with FBS. Cells were treated with the appropriate TNKS inhibitor in 10 concentrations ranging from

0 $\mu$ M to 10 $\mu$ M at 1% final DMSO concentration in appropriate media supplemented with 10% or 0.5% FBS. Dosages were carried out in triplicate. Termination of the assay and measurement of cell viability was achieved by the use of Cell Titre Glo reagent and luminescence measurement on a Victor<sup>x5</sup> plate reader. Dose response curves used to determine SF<sub>50</sub> values were normalized to untreated wells, Log transformed and analysed by non-linear regression analysis (variable slope) using Prism (GraphPad Software, Inc).

#### *Clonogenic Assay*

DLD1 cells were seeded at 500 cells/well in 6-well dishes in DMEM supplemented with 10% FBS. After 24 hours, the cells were dosed in triplicate at 10, 3, 1, 0.3, 0.1 and 0  $\mu$ M in DMEM supplemented with 0.5% FBS. Media and compound dosage was refreshed every 24 hrs for 14 days and the assay was terminated by the addition an equivalent volume of trichloroacetic acid (10% (v/v)) followed by incubation for 1 hour at 4 °C. The fixed cells were then washed with deionised water and stained with sulforhodamine B (0.057% (w/v) in 1% acetic acid (v/v)) for a minimum of 30 minutes at room temperature. The cells were then washed copiously with acetic acid (1% (v/v)) to remove all excess sulforhodamine B. The immobilised sulforhodamine B was then dissolved in 1mL of 10 mM Tris pH 10.5 and absorbance (A<sub>560</sub> nm) read on a Victor<sup>x5</sup> plate reader. Dose response curves used to determine SF<sub>50</sub> values were normalized to untreated wells, Log transformed and analysed by non-linear regression analysis (variable slope) using Prism (GraphPad Software, Inc).

#### **Western Blotting and PAR assay**

SW480 cells were seeded at a density of 5 X10<sup>4</sup> cells/well in a six-well dish in DMEM supplemented with 10% FBS. After overnight incubation, the cells were dosed as indicated in DMEM supplemented with 0.5% FBS and at a final DMSO concentration of 1% (v/v). The cells were incubated for 24 hours before being lysed in RIPA buffer (50 mM Tris pH 8, 150 mM NaCl, 1% NP-40 (Igepal CA-630, Sigma), 0.5% sodium deoxycholate and 0.1% sodium dodecyl sulfate). Fifty micrograms of total cell lysate per well was loaded onto a 4-12% Bis-Tris polyacrylamide gradient gel (Invitrogen). After electrophoresis, proteins were electro-blotted onto nitrocellulose membrane followed by primary antibody incubation in 5% skim milk/TBS-Tween 20 (0.1%). Anti-TNKS1/2 antibody was obtained from Santa Cruz (cat no. sc-8337), where AXIN2 (cat no. 2151) and beta-catenin (cat no. 9581) were obtained from Cell Signalling Technology. For the PAR western blot, SW480 cells were treated with compound **21** at the indicated concentrations. After 24 hrs, DNA damage was induced by subjecting the cells to 10 Gy ionising radiation. The cells were then lysed in RIPA buffer containing the PARG inhibitor (ADP-HPD, 1 $\mu$ M, Calbiochem) and proteins separated by 4-12% SDS-PAGE followed by electro-blotting onto nitrocellulose. Primary anti-bodies used included mouse anti-PAR (Enzo, 804-220-R100) and  $\alpha$ -tubulin (Sigma, T-6074). Protein detection was carried out by incubation with the appropriate secondary antibody conjugated to HRP followed by chemiluminescent reagent exposure.

## Expression and purification

We obtained the expression construct pNIC-Bsa4-TNKS1 from the Structural Genomics Consortium, Oxford, UK. This encodes a N-terminal hexahistidine affinity tag, with integrated TEV protease cleavage site, followed by amino acids 1091-1325 of human TNKS (Uniprot Accession Code: TNKS1\_HUMAN). Please see <http://www.thesgc.org/structures/2rf5> for additional details.

The expression vector was transformed into E.coli strain Rosetta2(DE3) [Merck-Millipore, Watford, UK]. A 'starter culture' was prepared by inoculating 50 ml of Turbo-broth [Molecular Dimensions, Newmarket, UK] supplemented with 100 µg/ml Kanamycin and 34 µg/ml Chloramphenicol. This was then incubated at 37 °C, in an orbital shaking incubator set at 220 rpm, until the absorbance at a wavelength of 600nm ( $A_{600}$ ) reached 1.0. The culture then was stored overnight at 4 °C. The following day, 10 ml of the starter culture was used to inoculate 1 litre of Turbo-broth in a 2 litre Erlenmeyer flask (supplemented as before with antibiotics). Again, the culture was incubated at 37 °C, in an orbital shaking incubator set at 220 rpm, until the  $A_{600}$  reached 2.0. The Erlenmeyer flasks were then placed on ice, and the temperature of the incubator reduced to 16 °C. When this temperature was attained, the flasks were placed back into the incubator, and recombinant protein expression induced by the addition of IPTG (isopropyl β-D-1-thiogalactopyranoside) to a final concentration of 0.5mM. The cultures were then incubated for a further period of ~16 hours. Cells were then harvested by centrifugation (5000 x g, 10 minutes, 4 °C) and the resultant pellet stored at -20 °C until required.

The frozen cells were re-suspended in 2 ml / gram of cell pellet of Buffer A: 50 mM HEPES.NaOH pH 7.5, 300 mM NaCl, 0.5 mM Tris(2-carboxyethyl)phosphine hydrochloride (TCEP), 30 mM imidazole supplemented with protease inhibitor tablets [Roche Diagnostics, Burgess Hill, UK] and 30-40 µl of Benzonase [Merck Millipore]. Cells were disrupted by sonication, then insoluble material and debris removed by centrifugation (38000 x g, 30 minutes, 4 °C).

The resulting supernatant was applied to a 5 ml HisTrap FF column (GE Healthcare, Little Chalfont, UK). Unbound material was removed by washing the column with 5 column volumes (CV) of Buffer A. Any retained protein was eluted with the stepwise application of 40 % Buffer B, followed by 100% Buffer B: 50 mM HEPES.NaOH pH 7.5, 300 mM NaCl, 0.5 mM TCEP, 300 mM imidazole]. The majority of recombinant TNKS1 eluted at the second step. Fractions containing TNKS1 were pooled, concentrated using centrifugal filter concentrators, and then applied to a HiLoad 26/60 Superdex 75 size exclusion column (GE Healthcare), pre-equilibrated in Buffer C: 20 mM HEPES.NaOH pH 7.5, 300 mM NaCl, 0.5 mM TCEP). Fractions containing purified TNKS1 were identified by SDS-PAGE, pooled and concentrated (as before) to a final concentration of 19 mg/ml, then stored at -80 °C until required.



## Crystallisation

Compounds were initially dissolved in 100% v/v dimethyl sulfoxide (DMSO) or 1,3-dimethyl-2-imidazolidinone (DMI), followed by dilution in Buffer C to a final working concentration of ~ 20 mM and between 10-20% v/v DMSO or DMI.

TNKS1 + **12**: Protein was mixed with 20 mM **12** at a 1:1 molar ratio, then incubated on ice for 30 minutes. Hanging drop vapour diffusion experiments, at 20 °C, against 0.1 M MES pH 6.0, 0.1 M ammonium tartrate, 12% w/v PEG 3350, produced crystals after a period of approximately 1 week. Successive swipes through buffers containing increasing amounts of glycerol, to a final concentration of 30% v/v, were sufficient for cryoprotection.

TNKS1 + **23**, **21**: Crystals of the apo-form of the protein, generated by hanging drop vapour diffusion experiments at 20 °C, against 0.1 M MES pH 6.0, 0.1 M ammonium tartrate, 12-16% v/w PEG 3350, were soaked in cryoprotectant containing **23** or **21** at a final concentration of between 1 – 2 mM and a final DMI concentration of between 10 – 20 % v/v.

## Data collection, model building and refinement

All diffraction data were collected at the Diamond Light Source, on beamlines I02 for TNKS1 + **12**, I04 for **23** and I03 for **21**. Data were processed and scaled using either the CCP4 suite of crystallographic software<sup>1</sup> with the Xia2 pipeline<sup>2</sup> using XDS<sup>3</sup> and Aimless<sup>4</sup>, or directly with XDS / XDSGUI, followed by Aimless. Each structure was solved by molecular replacement using PHASER<sup>5</sup> and PDB: 2RF5 as a search model. Iterative cycles of manual building in Coot<sup>6</sup> and refinement with BUSTER<sup>7</sup> or PHENIX<sup>8</sup> produced the final models deposited in the Protein Data Bank with accession codes 4UW1, 4UUH, 4U6A for TNKS1 + **12**, TNKS1 + **23** and TNKS1 + **21** respectively.

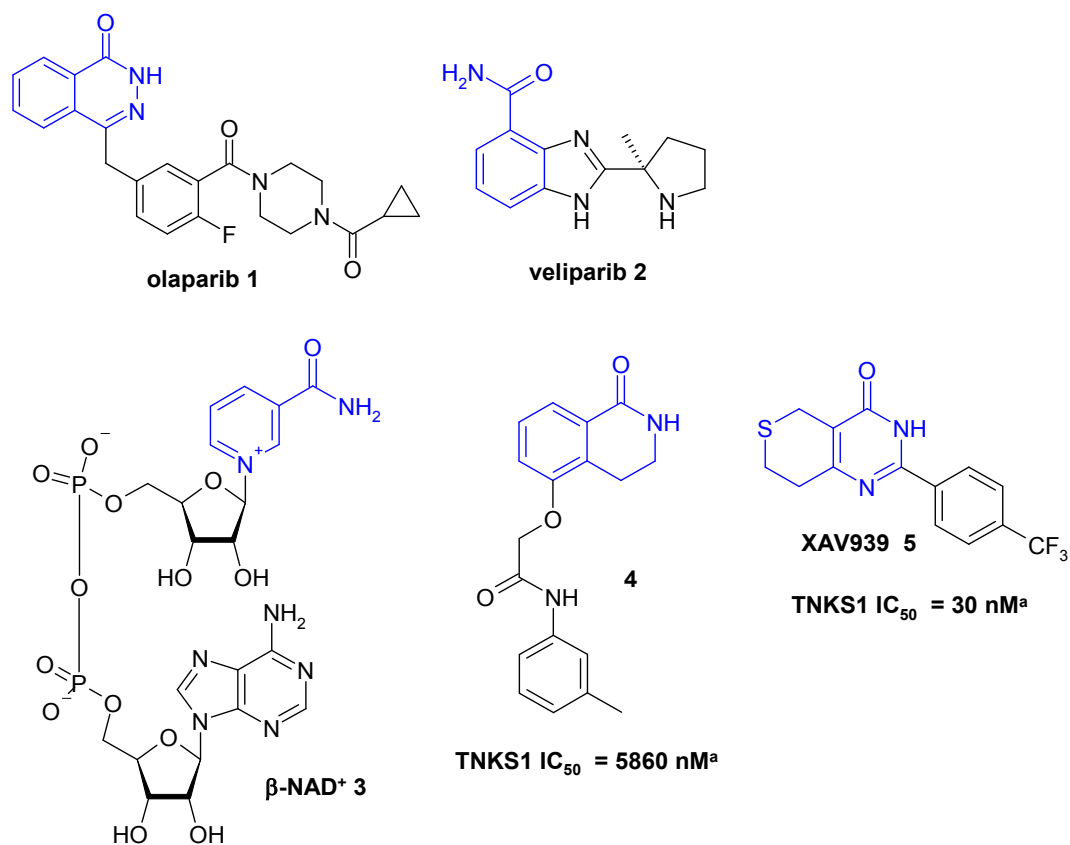
**Table 1:** Data collection, phasing and refinement statistics

	TNKS1 + 12	TNKS1 + 21	TNKS1 + 23
<b>Data Collection</b>			
Beamline	DLS, I02	DLS, I03	DLS, I04
Spacegroup	P1	I2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	I2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>
Cell dimensions a, b, c (Å)	80.59, 82.32, 86.73	81.38, 80.98, 84.02	81.32, 80.89, 83.70
Cell angles α, β, γ (°)	71.38, 67.32, 89.51	90, 90, 90	90, 90, 90
Resolution (Å)	46.92-3.37 (3.57-3.37)*	58.31-2.37 (2.43-2.37)	33.80-2.52 (2.62-2.52)
Mn(I)/(σI)	3.5 (1.3)	8.4 (1.9)	11.6 (2.0)
Mn(I) half-set correlation CC(1/2)	0.916 (0.633)	0.990 (0.650)	0.995 (0.598)
Completeness (%)	99.1 (98.6)	98.3 (96.8)	98.4 (99.5)
Multiplicity (fold)	3.2 (3.2)	4.0 (4.1)	2.9 (2.9)
<b>Refinement</b>			
Resolution (Å)	46.92 - 3.37	58.31 - 2.37	33.80 - 2.52
Reflections (work/free)	27022 / 2714	11373 / 581	9364 / 478
R <sub>work</sub> / R <sub>free</sub>	0.27 / 0.32	0.19 / 0.25	0.17 / 0.22
Number of atoms / B-factor			
Protein	12744 / 46.3	1645 / 45.4	1642 / 40.6
Heterogen (inc. ligand)	216 / 22.1	60 / 46.4	168 / 51.4
Solvent (water)	N/A	82 / 50.5	101 / 44.8
R.m.s.d. values			
Bond lengths (Å)	0.002	0.010	0.010
Bond angles (°)	0.47	1.04	1.08
<b>Molprobability Score</b> (v4.1-537)			
Ramachandran Plot			
Favoured / Allowed / Outliers (%)	94.4 / 4.93 / 0.67	98.5 / 1.49 / 0.00	97.5 / 2.49 / 0.00
	5.6	2.4	2.4
Clashscore (all atoms)	100% percentile N=37 3.00 – 9999 Å	100 <sup>th</sup> percentile N=334 2.37 Å ± 0.25	100 <sup>th</sup> percentile N=262 2.52 Å ± 0.25
	1.69	1.53	1.53
Molprobability Score	100 <sup>th</sup> percentile N=646 3.00 – 9999 Å	100 <sup>th</sup> percentile N=8020 2.37 Å ± 0.25	99 <sup>th</sup> percentile N=6707 2.52 Å ± 0.25

\* Highest resolution shell is shown in parenthesis

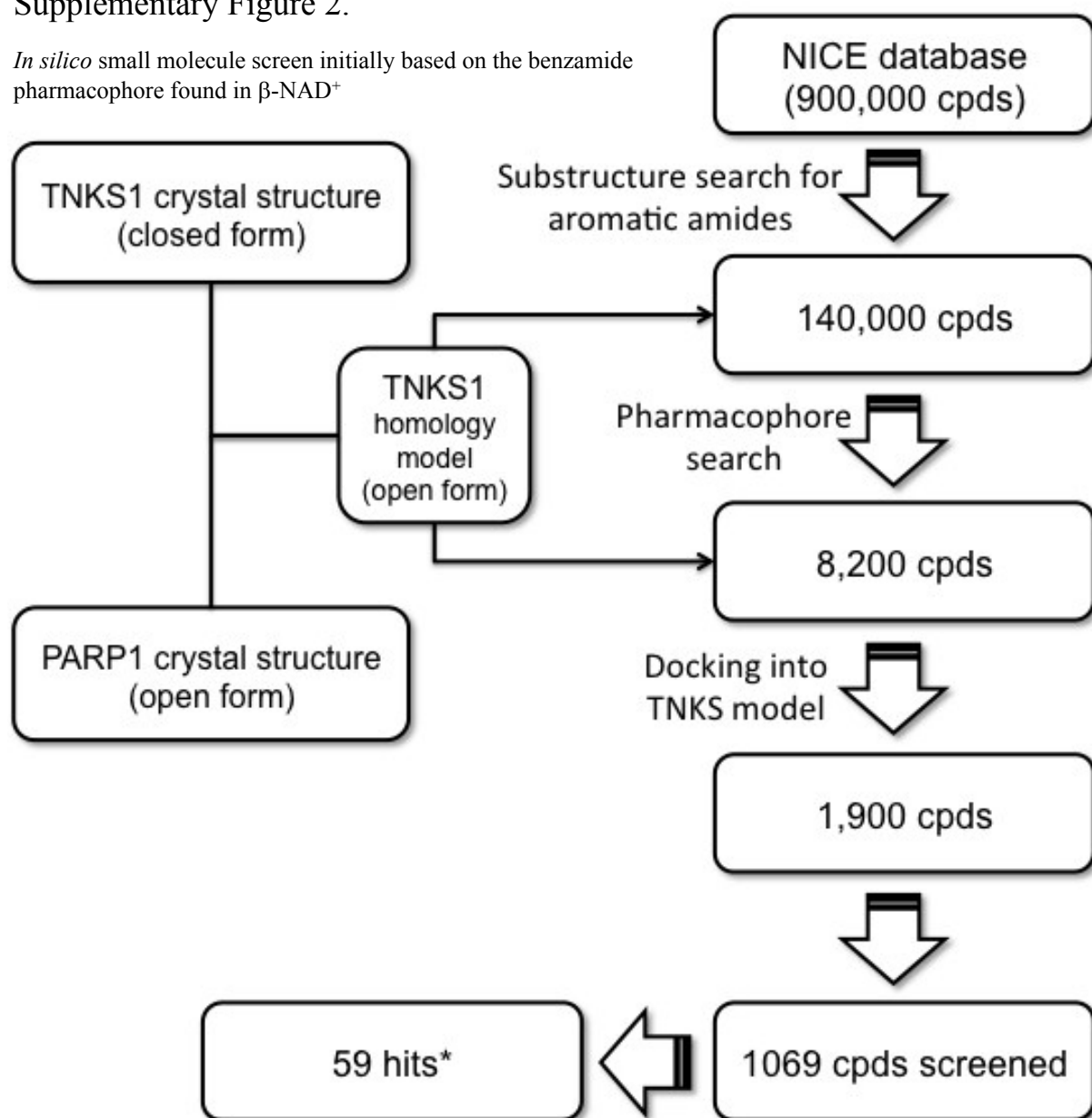
## Supplementary Figure 1.

Chemical structures of PARP inhibitors olaparib **1** and veliparib **2**, of  $\beta$ -NAD<sup>+</sup> **3**, and of tankyrase inhibitors **4**, and XAV939 **5**. The nicotinamide group of  $\beta$ -NAD<sup>+</sup> and nicotinamide-mimetics of **1**, **2**, **4** and **5** are highlighted in blue. <sup>a</sup>The indicated IC<sub>50</sub> values were determined by measuring inhibition of PARylation activity for recombinant TNKS protein.



## Supplementary Figure 2.

*In silico* small molecule screen initially based on the benzamide pharmacophore found in  $\beta$ -NAD<sup>+</sup>



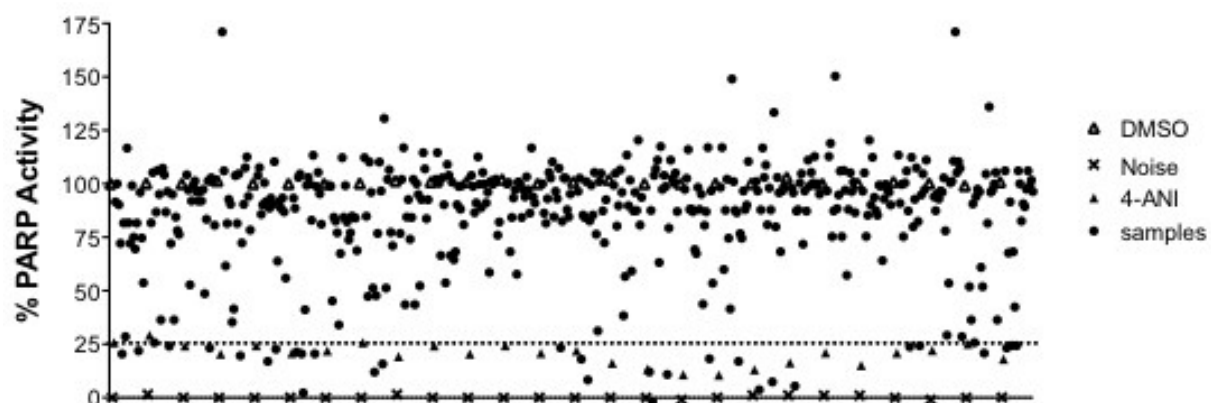
\* > 75% inhibition against TNKS1

- 630 additional compounds screened
- \*72 hits (including similarity searches)

## Supplementary Figure 3.

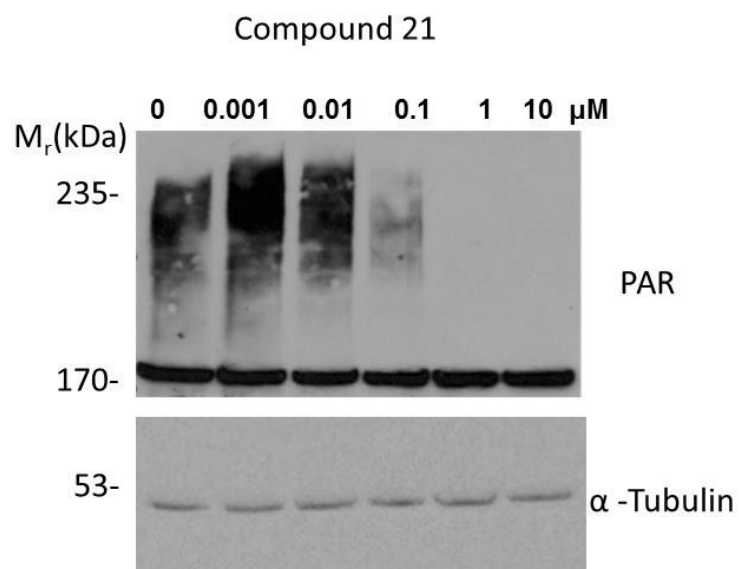
Representative screening set of 528 compounds, 10 $\mu$ M dose (528 compounds including controls; mean values of Normalised % Activity to DMSO control (100% activity) and assay noise (0% activity)).

Positive control for inhibition: 10 $\mu$ M 4-aminonaphthalimide (4-ANI).

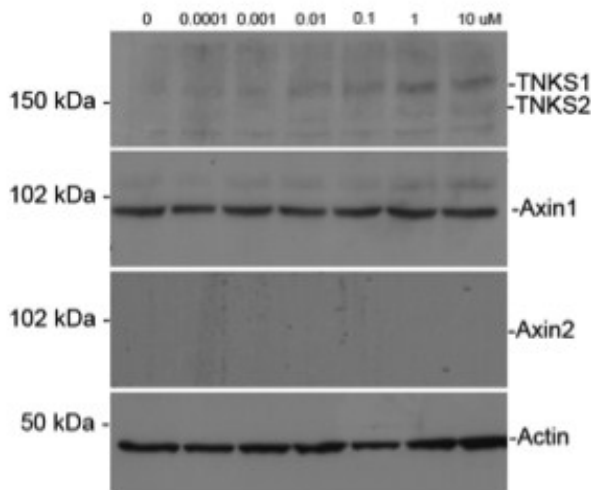


## Supplementary Figure 4.

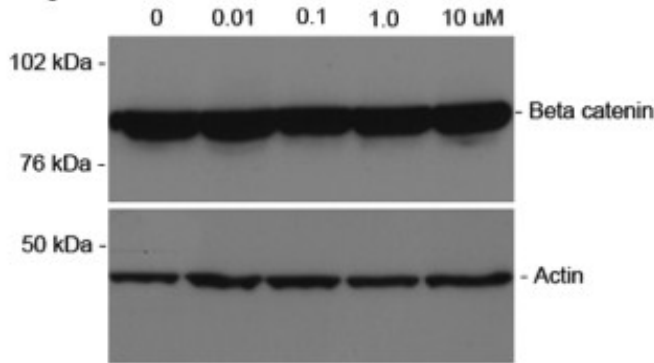
PAR Western blot indicating the effect of Compound **21** on DNA damage induced PARylation.



A. Olaparib cannot stabilize TNKS (except at very high dose) or Axin1/2



B. Olaparib cannot cause destabilization of beta-catenin



## References

- 1 M. D. Winn, C. C. Ballard, K. D. Cowtan, E. J. Dodson, P. Emsley, P. R. Evans, R. M. Keegan, E. B. Krissinel, A. G. W. Leslie, A. McCoy, S. J. McNicholas, G. N. Murshudov, N. S. Pannu, E. A. Potterton, H. R. Powell, R. J. Read, A. Vagin and K. S. Wilson, *Acta Crystallographica Section D-Biological Crystallography*, 2011, **67**, 235-242.
- 2 (a) G. Winter, *Journal of Applied Crystallography*, 2010, **43**, 186-190; (b) G. Winter, C. M. C. Lobley and S. M. Prince, *Acta Crystallographica Section D-Biological Crystallography*, 2013, **69**, 1260-1273.
- 3 W. Kabsch, *Acta Crystallographica Section D-Biological Crystallography*, 2010, **66**, 125-132.
- 4 P. R. Evans and G. N. Murshudov, *Acta Crystallographica Section D-Biological Crystallography*, 2013, **69**, 1204-1214.
- 5 A. J. McCoy, R. W. Grosse-Kunstleve, P. D. Adams, M. D. Winn, L. C. Storoni and R. J. Read, *Journal of Applied Crystallography*, 2007, **40**, 658-674.
- 6 (a) P. Emsley and K. Cowtan, *Acta Crystallographica Section D-Biological Crystallography*, 2004, **60**, 2126-2132; (b) P. Emsley, B. Lohkamp, W. G. Scott and K. Cowtan, *Acta Crystallographica Section D-Biological Crystallography*, 2010, **66**, 486-501.
- 7 (a) O. S. Smart, T. O. Womack, C. Flensburg, P. Keller, W. Paciorek, A. Sharff, C. Vonnrhein and G. Bricogne, *Acta Crystallographica Section D-Biological Crystallography*, 2012, **68**, 368-380; (b) B. E. Bricogne G., Brandl M., Flensburg C., Keller P., Paciorek W., Roversi P, S. A., Smart O.S., Vonnrhein C., Womack 2011.
- 8 P. D. Adams, P. V. Afonine, G. Bunkoczi, V. B. Chen, I. W. Davis, N. Echols, J. J. Headd, L. W. Hung, G. J. Kapral, R. W. Grosse-Kunstleve, A. J. McCoy, N. W. Moriarty, R. Oeffner, R. J. Read, D. C. Richardson, J. S. Richardson, T. C. Terwilliger and P. H. Zwart, *Acta Crystallographica Section D-Biological Crystallography*, 2010, **66**, 213-221.
- 9 J. F. Rippmann, K. Damm and A. Schnapp, *J. Mol. Biol.*, 2002, **323**, 217-224.