Citation for published version:

DOI:
10.1016/j.bmc.2015.05.005

Publication date:
2015

Document Version
Early version, also known as pre-print

Link to publication

University of Bath

Alternative formats
If you require this document in an alternative format, please contact: openaccess@bath.ac.uk

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Download date: 15. Mar. 2022
SUPPLEMENTARY INFORMATION

Structure-based design, synthesis and evaluation in vitro of arylnaphthyridinones, arylpyridopyrimidinones and their tetrahydro derivatives as inhibitors of the tankyrases

Katerina Kumpan,a Amit Nathubhai,a Chenlu Zhang,a,b Pauline J. Wood,a, Matthew D. Lloyd,a Andrew S. Thompson,a Teemu Haikarainen,c Lari Lehtiöc and Michael D. Threadgill*a

a Medicinal Chemistry, Department of Pharmacy & Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY, UK
b Department of Pharmacy, Shandong University, Jinan 250100, China
c Biocenter Oulu and Faculty of Biochemistry and Molecular Medicine, University of Oulu, Oulu, Finland

Contents

Chemistry experimental 2
Data collection and refinement statistics for the tankyrase-2 crystal structures 9
Examples of concentration-inhibition graphs for inhibition of TNKS-1 10
Examples of concentration-inhibition graphs for inhibition of TNKS-2 12
Examples of concentration-inhibition graphs for inhibition of TNKS-1 14
References for Supplementary Information 15
Chemistry experimental

1-Iodo-4-phenylmethoxybenzene (19h). BnBr (389 mg, 4.7 mmol) was added to 19i (1.00 g, 4.6 mmol) and Cs₂CO₃ (1.50 g, 4.6 mmol) in dry DMF (10 mL) and the mixture was stirred for 90 min. The mixture was diluted with water (10 mL) and extracted thrice with EtOAc. The combined extracts were washed (water, brine). Drying and chromatography (petroleum ether / EtOAc, 99:1) gave 19h (1.20 g, 82%) as white crystals: mp 59-61°C (lit.° mp 56.5-57°C); IR νₘₐₓ 1582 cm⁻¹; ¹H NMR (CDCl₃) δ 4.99 (2 H, s, CH₂), 6.76 (2 H, d, J = 9.0 Hz, 3,5-H₂), 7.31-7.43 (5 H, m, Ph 2,3,4,5,6-H₅), 7.56 (2 H, d, J = 9.0 Hz, 2,6-H₂); ¹³C NMR (CDCl₃) δ 70.06 (CH₂), 83.01 (4-C), 117.29 (2,6-C₂), 127.37 (Ph 3,5-C₂), 128.06 (Ph 4-C), 128.59 (Ph 2,6-C₂), 130.50 (Ph 1-C), 138.21 (3,5-C₂), 158.61 (1-C).

4-Methoxy-1-trimethylsilylethynylbenzene (20c). Et₃N (10 mL) and THF (10 mL) were added to 4-methoxyiodobenzene 19c (1.05 g, 4.5 mmol), CuI (87 mg, 450 μmol), (Ph₃P)₂PdCl₂ (158 mg, 225 μmol) and Na ascorbate (89 mg, 450 μmol) under Ar. The mixture was stirred at 40°C for 30 min. Me₃Si=CH (1.03 g, 10.5 mmol) was added and the mixture was stirred at 40°C for 16 h. The evaporation residue, in CH₂Cl₂, was washed (water, 2 ×) and dried. Chromatography (petroleum ether / EtOAc 99:1) gave 20c (909 mg, 99%) as an oil (lit.° oil): ¹H NMR (CDCl₃) δ 0.23 (9 H, s, SiMe₃), 3.79 (3 H, s, OMe), 6.80 (2 H, d, J = 6.8 Hz, 3,5-H₂), 7.40 (2 H, d, J = 4.9 Hz, 2,6-H₂); ¹³C NMR (CDCl₃) δ -0.02 (SiMe₃), 55.17 (OMe), 92.34 (ethyne 1-C), 105.15 (ethyne 2-C), 113.75 (2,6-C₂), 115.25 (4-C'), 133.39 (3,5-C₂), 159.70 (1-C); MS m/z 205.1037 (M + H)⁺ (C₁₂H₁₇OSi requires 205.1049).

4-Trifluoromethyl-1-trimethylsilylethynylbenzene (20d). 4-Trifluoromethyl-1-iodobenzene 19d was treated with CuI, (Ph₃P)₂PdCl₂, Na ascorbate and Me₃Si=CH in THF and Et₃N, as for the synthesis of 20c, to give 20d (92%) as a colourless oil (lit.° oil): IR νₘₐₓ 2161, 2063, 1614, 1511, 1323 cm⁻¹; ¹H NMR (CDCl₃) δ 0.27 (9 H, s, SiMe₃), 7.54 (4 H, s, 2,3,5,6-Ha); ¹³C NMR (CDCl₃) δ -0.02 (SiMe₃), 88.07 (ethyne 2-C), 97.13 (ethyne 1-C), 123.90 (q, J = 272 Hz, CF₃), 125.13 (q, J = 3.9 Hz, 3,5-C₂), 126.99 (q, J = 1.4 Hz, 1-C), 130.19 (q, J = 32.7 Hz, 4-C), 132.17 (2,6-C₂), 19°F NMR (CDCl₃) δ -62.94 (s, CF₃).

4-Chloro-1-trimethylsilylethynylbenzene (20e). 4-Chloroiodobenzene 19e was treated with CuI, (Ph₃P)₂PdCl₂, Na ascorbate and Me₃Si=CH in THF and Et₃N, as for the synthesis of 20c, to give 20e (96%) as a pale buff powder: mp 42-45°C (lit.° mp 43-45°C); IR νₘₐₓ 2157, 1643, 1588, 825, 756 cm⁻¹; ¹H NMR (CDCl₃) δ 0.24 (9 H, s, SiMe₃), 7.26 (2 H, d, J = 8.8 Hz, 2,6-C₂), 7.37 (2 H, d, J = 8.9 Hz, 3,5-C₂); ¹³C NMR (CDCl₃) δ 0.00 (SiMe₃), 95.47 (ethyne 2-C), 121.78 (ethyne 1-C), 115.08 (4-C), 128.65 (2,6-C₂), 133.29 (3,5-C₂), 134.62 (1-C).

1-Amino-4-trimethylsilylethynylbenzene (20g). 4-Iodoaniline 19g was treated with CuI, (Ph₃P)₂PdCl₂, Na ascorbate and Me₃Si=CH in THF and Et₃N, as for the synthesis of 20c, to give 20g (74%) as an off-white solid: mp 94-98°C (lit.° mp 93-95°C); IR νₘₐₓ 3467, 2158, 1622, 1510 cm⁻¹; ¹H NMR (CDCl₃) δ 0.22 (9 H, s, SiMe₃), 3.77 (2 H, br, NH₂), 6.56 (2 H, d, J = 8.6 Hz, 2,6-H₂), 7.27 (2 H, d, J = 8.2 Hz, 3,5-H₂); ¹³C NMR (CDCl₃) δ -0.002 (SiMe₃), 91.22 (ethyne 2-C), 105.86 (ethyne 1-C), 112.45 (4-C), 114.38 (2,6-C₂), 133.24 (3,5-C₂), 146.63 (1-C).

(4-Phenylmethoxyoxyphenylethynyl)trimethylsilane (20h). Compound 19h was treated with CuI, (Ph₃P)₂PdCl₂, Na ascorbate and Me₃Si=CH in THF and Et₃N, as for the synthesis of 20c, to give 20h (92%) as a white solid: mp 45-47°C (lit.° mp 50°C); IR νₘₐₓ 2157, 1602
4-(Trimethylsilylethynyl)pyridine (20)). Cul (95 mg, 0.50 mmol), (Ph₃P)₂PdCl₂ 3 (174 mg, 0.25 mmol), Na ascorbate (98 mg, 0.50 mmol) and 4-bromopyridine hydrochloride 22 (970 mg, 5.0 mmol) were placed in a flask, which was degassed and filled with Ar. Pr₂NHz (10 mL) and THF (10 mL) were added. The mixture was stirred at 40°C for 30 min. Me₂SiC=CH (532 mg, 5.0 mmol) was added and the mixture was stirred at 40°C for 10 h. Evaporation and chromatography (petroleum ether / EtOAc 5:1 → 3:1) gave 20l (767 mg, 89%) as a pale buff oil (lit.⁹ yellow liquid); ¹H NMR (CDCl₃) δ 2.97 (1 H, s, C≡CH), 7.29 (2 H, d, J = 4.4 Hz, 3,5-H₂) 8.55 (2 H, d, J = 4.4 Hz, 2,6-H₂); ¹³C NMR (CDCl₃) δ -0.002 (SiMe₃), 100.24 (ethylene 2-C), 102.31 (ethylene 1-C), 126.16 (3,5-C₂), 131.52 (4-C), 150.03 (2,6-C₂).

1-Ethynyl-4-methoxybenzene (21c). Compound 20c (1.00 g, 4.9 mmol) in THF (1.0 mL) was stirred with Bu₄NF in THF (1.0 M, 10 mL) for 16 h. Saturated aq. NaHCO₃ was added and the mixture was extracted (Et₂O, 3 ×). Drying and chromatography (petroleum ether / EtOAc 19:1) gave 21c (595 mg, 92%) as a pale yellow oil (lit.⁸ oil): IR νmax 2158, 1607, 1570, 1507 cm⁻¹; ¹H NMR (CDCl₃) δ 2.98 (1 H, s, C≡CH), 3.80 (3 H, s, Me), 6.83 (2 H, d, J = 9.5 Hz, 3,5-H₂), 7.42 (2 H, d, J = 9.4 Hz, 2,6-H₂); ¹³C NMR (CDCl₃) δ 55.20 (OMe), 67.89 (ethylene 2-C), 83.60 (ethylene 1-C), 113.88 (2,6-C₂), 114.14 (4-C), 133.52 (3,5-C₂), 159.90 (1-C).

1-Ethynyl-4-trifluoromethylbenzene (21d). Compound 20d was treated with Bu₄NF, as for the synthesis of 21c, to give 21d (66%) as a colourless oil (lit.⁹ oil): IR νmax 1519, 1323 cm⁻¹; ¹H NMR (CDCl₃) δ 3.18 (1 H, s, C≡CH), 7.58 (4 H, s, 2,3,5,6-H₄), ¹³C NMR (CDCl₃) δ 79.51 (ethylene 2-C), 82.13 (ethylene 1-C), 123.81 (q, J = 288 Hz, CF₃), 125.24 (2,6-C₂), 125.88 (4-C), 130.57 (q, J = 33 Hz, 4-C), 132.33 (3,5-C₂), 19F NMR (CDCl₃) δ -62.96 (s, CF₃).

1-Chloro-4-ethylbenzene (21e). Compound 20e was treated with Bu₄NF, as for the synthesis of 21c, to give 21e (57%) as a pale amber powder: mp 41-44°C (lit.¹⁰ mp 43-45.5°C): IR νmax 2154, 1645, 1589, 1488, 756 cm⁻¹; ¹H NMR (CDCl₃) δ 3.09 (1 H, s, C≡CH), 7.29 (2 H, d, J = 8.8 Hz, 3,5-H₂), 7.41 (2 H, d, J = 8.9 Hz, 2,6-H₂); ¹³C NMR (CDCl₃) δ 78.07 (ethylene 2-C), 85.45 (ethylene 1-C), 120.55 (4-C), 128.61 (3,5-C₂), 133.28 (2,6-C₂), 134.86 (1-C).

1-Amino-4-ethylbenzene (21g). Compound 20g (423 mg, 2.2 mmol) in THF (100 mL) was stirred with Bu₄NF in THF (1.0 M, 6.7 mL) for 16 h at 35°C. Saturated aq. NaHCO₃ was added. The mixture was extracted (EtO₂O, 3 ×). Drying and chromatography (petroleum ether / EtOAc 1:3) gave 21g (230 mg, 81%) as a pale green powder: mp 81-85°C (lit.¹¹ mp 88-90°C): IR νmax 3486, 3388, 2095, 1619, 1513 cm⁻¹; ¹H NMR (CDCl₃) δ 2.95 (1 H, s, C≡CH), 3.79 (2 H, s, NH₂), 6.58 (2 H, d, J = 7.6 Hz, 2,6-H₂), 7.28 (2 H, d, J = 7.7 Hz, 3,5-H₂); ¹³C NMR (CDCl₃) δ 74.79 (ethylene 2-C), 84.32 (ethylene 1-C), 111.34 (4-C), 114.52 (2,6-C₂), 133.41 (3,5-C₂), 146.95 (1-C).

4-Ethynylpyridine (21l). Compound 20l (2.30 g, 13.1 mmol) in THF (30 mL) was stirred at 40°C with Bu₄NF in THF (1.0 M, 30 mL) for 10 h. Sat. aq. NaHCO₃ was added and the mixture was extracted (EtO₂O, 3 ×). Drying, evaporation and chromatography (petroleum ether / EtOAc 3:1) gave 21l (550 mg, 42%) as an off-white powder with a strong unpleasant
odour: mp 51-54°C (lit.12 62-63°C); IR νmax 3378, 1641, 1594 cm⁻¹; ¹H NMR (CDCl₃) δ 3.28 (1 H, s, C≡CH), 7.33 (2 H, d, J = 5.8 Hz, 3,5-H₂), 8.59 (2 H, m, 2,6-H₂); ¹³C NMR (CDCl₃) δ 60.29 (ethyne 1-C), 80.88 (ethyne 2-C), 126.16 (3,5-C₂), 130.25 (4-C), 149.74 (2,6-C₂).

2-Bromo-3-cyanopyridine (24). 2-Chloro-3-cyanopyridine 23 (3.68 mg, 26 mmol) was boiled under reflux with AcOH (100 mL) for 12 h. The evaporation residue, in THF (80 mL) and H₂O (20 mL), was boiled under reflux for 4 h. Evaporation gave 3-cyanopyridin-2-one (3.12 g, quant.) as white crystals: mp 110-114°C (lit.13 117-117.5°C); ¹H NMR δ 6.47 (1 H, t, J = 6.6 Hz, 5-H); 7.91 (1 H, dd, J = 6.5, 2.1 Hz, 4-H), 8.14 (1 H, dd, J = 7.0, 2.1 Hz, 6-H), 11.48 (1 H, s, 1-H); ¹³C NMR δ 105.80 (3-C), 124.05 (5-C), 141.87 (CN), 144.50 (6-H), 150.02 (4-C), 154.20 (2-C); MS m/z 143.0214 (M + Na) (C₆H₄N₂NaO requires 143.0222). Bu₄NBr (8.55 g, 26 mmol) and P₂O₅ (3.67 g, 26 mmol) were heated in PhMe (250 mL) at 80°C for 30 min. The above 3-cyanopyridin-2-one (1.59 g, 13 mmol) was added and the mixture was boiled under reflux for 12 h. The mixture was cooled, poured into cold water and extracted (EtOAc, 2 ×). Drying and evaporation gave 24 (2.24 g, 94%) as off-white crystals: mp 114-118°C (lit.14 mp 105°C); IR νmax 2236, 1574, 1550, 1472 cm⁻¹; ¹H NMR (CDCl₃) δ 7.42 (1 H, dd, J = 7.7, 4.8 Hz, 5-H), 8.01 (1 H, dd, J = 7.8, 2.0 Hz, 4-H), 8.57 (1 H, dd, J = 4.9, 2.0 Hz, 6-H); ¹³C NMR δ 114.27 (3-C), 115.63 (CN), 122.37 (5-C), 142.53 (4-C), 143.84 (2-C), 152.92 (6-C).

3-Bromopyridine-4-carboxylic acid (31). 3-Bromopyridine 30 (164 mg, 1.0 mmol) was added to LiNPr₃ in dry THF (1.0 M, 15 mL, 1.5 mmol) and the mixture was stirred at -78°C for 30 min under Ar. Crushed solid CO₂ was added under Ar, and the cooling was removed. The mixture was stirred until reaching 20°C. Water (10 mL) was added. The organic solvents were evaporated. The solution was washed thrice with Et₂O. Aq. HCl (9 M) was added to pH 3. The mixture was stirred for 1 h and extracted (EtOAc, 3 ×). The combined extracts were washed (brine) and dried. Evaporation gave 31 (20 mg, 10%) as white needles: mp 175-176°C (decomp.) (lit.15 mp 240°C); ¹H NMR δ 7.71 (1 H, d, J = 4.8 Hz, 6-H), 8.70 (1 H, d, J = 4.9 Hz, 5-H), 8.91 (1 H, s, 2-H) 12.56 (1 H, br, OH); ¹³C NMR (CDCl₃) δ 117.26 (4-C), 123.77 (6-C), 148.94 (5-C), 152.61 (2-C), 165.00 (C=O), 166.07 (3-C); MS m/z 202 / 200 (M – H⁻).

3-Bromopyridine-4-carboxamide (32) and ethyl 3-bromopyridine-4-carboxylate (33). EtO₂CCl (0.58 g, 5.4 mmol) was added dropwise to an ice-cold mixture of 31 (1.00 g, 4.6 mmol), dry THF (15 mL) and dry Et₃N (1.0 mL). The mixture was stirred for 1 h at 0°C, then NH₃ was bubbled through the suspension for 15 min. The mixture was filtered. The solids were washed with hot Me₂CO. The solvent was evaporated from the combined filtrate and washings. Recrystallisation (EtOH) gave 32 as an off-white powder (777 mg, 78%): mp 149-150°C; ¹H NMR (CDCl₃) δ 7.48 (1 H, d, J = 4.8 Hz, 5-H), 7.88 (1 H, br, NH), 8.11 (1 H, br, NH), 8.65 (1 H, d, J = 4.8 Hz, 6-H), 8.84 (1 H, s, 2-H); ¹³C NMR (CDCl₃) δ 116.82 (3-C), 122.73 (5-C), 146.02 (4-C), 148.58 (6-C), 151.68 (2-C), 167.03 (C=O); MS m/z 224.9469 (M + Na⁺) (C₆H₅BrN₂NaO requires 224.9457), 222.9485 (M + Na⁺) (C₆H₅BrN₂NaO requires 222.9477).
3-Bromo-4-cyanopyridine (34). Compound 32 (687 mg, 3.4 mmol) was stirred at reflux with POCl₃ (5.0 mL) for 2 h. The mixture was cooled, poured onto ice (100 g) and neutralised with aq. NaOH (5 M, 60 mL). The mixture was washed and filtered. Evaporation and recrystallisation (petroleum ether) gave 34 as an off-white powder (292 mg, 42%); mp 79-81°C (lit. mp 96.6-98.2°C); ¹H NMR (CDCl₃) δ 7.53 (1 H, d, J = 4.9 Hz, 5-H), 8.69 (1 H, d, J = 4.9 Hz, 6-H), 8.92 (1 H, s, 2-H); ¹³C NMR (CDCl₃) δ 114.79 (C≡N), 122.14 (4-C), 124.17 (3-C), 126.75 (5-C), 148.76 (6-C), 152.69 (2-C); MS m/z 182.9541 (M + H)⁺ (C₆H₄N₂Br requires 182.9552).

1,3-Bis(4-methylphenyl)propane-1,3-dione (37b). NaH (2.00 g, 50 mmol, 60% in oil) was washed free from oil with dry hexane (10 mL) at 0°C under Ar. Dry THF (30 mL) was added, followed by methyl 4-methylbenzoate (3.30 g, 22 mmol) in dry THF (10 mL) and 4-methyl-1-acetylenylbenzene (2.66 g, 20 mmol) in dry THF (10 mL) at 0°C under Ar. The suspension was stirred under reflux for 16 h. The mixture was cooled and filtered (Celite®). The solid was washed with EtOH (20 mL). The combined filtrates were poured into Et₂O (20 mL) and aq. HCl (1 M, 20 mL). The aq. layer was extracted with Et₂O (2 ×). The combined extracts were washed (brine, 3 ×) and dried. Evaporation and recrystallisation (EtOH) gave 37b (2.50 g, 50%) as yellow needles: mp 112-113°C (lit. 17 117-118°C); ¹H NMR (CDCl₃) (enol) δ 2.46 (6 H, s, 2 × Me), 6.84 (1 H, s, CH), 7.31 (4 H, d, J = 8.0 Hz, 2 × Ph 3,5-H₂), 7.91 (4 H, d, J = 8.5 Hz, 2 × Ph 2,6-H₂); ¹³C NMR (CDCl₃) δ 21.65 (2 × Me), 92.50 (CH), 127.18 (2 × Ph 2,6-C₂), 129.39 (2 × Ph 3,5-C₂), 132.94 (2 × Ph 1-C), 143.11 (2 × Ph 4-C), 185.50 (C=O, C-OH); MS m/z 527.2193 (2 M + Na)⁺ (C₆H₃CN₂O₄ requires 527.2188), 275.1055 (M + Na)⁺ (C₁₇H₁₆Na₂O₄ requires 275.1043).

1,3-Bis(4-methoxyphenyl)propane-1,3-dione (37c). Methyl 4-methoxybenzoate was treated with 4-methoxy-1-acetylenylbenzene and NaH, as for the synthesis of 37b, to give 37c (46%) as an off-white powder: mp 110-111°C (lit. 18 111.5-113.5°C); ¹H NMR (CDCl₃) (enol form) δ 3.90 (6 H, s, 2 × Me), 6.76 (1 H, s, =CH), 6.99 (4 H, d, J = 8.5 Hz, 2 × Ph 3,5-H₂), 8.01 (4 H, d, J = 9.0 Hz, 2 × Ph 2,6-H₂); ¹³C NMR (CDCl₃) δ 55.49 (2 × Me), 91.50 (=CH), 113.95 (2 × Ph 3,5-C₂), 128.23 (2 × Ph 1-C), 128.09 (2 × Ph 2,6-C₂), 163.03 (2 × Ph 4-C), 184.63 (C=O, C-OH); MS m/z 591.1950 (2 M + Na)⁺ (C₆H₃BrNaO₈ requires 591.1984), 307.0933 (M + Na)⁺ (C₁₇H₁₂NaO₄ requires 307.0941).

1,3-Bis(4-trifluoromethyl)propene-1,3-dione (37d). Methyl 4-trifluoromethylbenzoate was treated with 4-trifluoromethyl-1-acetylenylbenzene and NaH, as for the synthesis of 37b, to give 37d (57%) as an off-white powder: mp 116-117°C (lit. 19 130°C); ¹H NMR (CDCl₃) (enol) δ 6.91 (1 H, s, CH), 7.80 (4 H, s, 2 × Ph 3,5-H₂), 8.12 (4 H, s, 2 × Ph 2,6-H₂); ¹³C NMR (CDCl₃) δ 94.15 (CH), 122.53 (2 × Ph 1-C), 124.66 (q, J = 270.8 Hz, 2 × CF₃), 125.81 (q, J = 3.5 Hz, 2 × Ph 3,5-C₂), 127.62 (2 × Ph 2,6-C₂), 134.26 (q, J = 32.8 Hz, 2 × Ph 4-C), 184.70 (C=O, C-OH); MS m/z 383.0495 (M + Na)⁺ (C₁₇H₁₀F₆NaO₂ requires 383.0477).

1,3-Bis(4-chlorophenyl)propane-1,3-dione (37e). Methyl 4-chlorobenzoate was treated with 4-chloro-1-acetylenylbenzene and NaH, as for the synthesis of 37b, to give 37e (68%) as a pale pink plates: mp 130-131°C (lit. 20 158-159°C); ¹H NMR (CDCl₃) (enol) δ 6.70 (1 H, s, CH), 7.40 (4 H, d, J = 8.5 Hz, 2 × Ph 3,5-H₂), 7.85 (4 H, d, J = 8.5 Hz, 2 × Ph 2,6-H₂); ¹³C NMR (CDCl₃) δ 92.84 (CH), 128.49 (2 × Ph 2,6-C₂), 129.01 (2 × Ph 3,5-C₂), 133.69 (2 × Ph 1-C), 138.87 (2 × Ph 4-C), 184.71 (C=O, C-OH); MS m/z 316.9863 (M + Na)⁺ (C₁₅H₁₀Cl³⁷ClNaO₂ requires 316.9923), 314.9948 (M + Na)⁺ (C₁₅H₁₀⁵⁷Cl₂NaO₂ requires 314.9950).
1,3-Bis(4-bromophenyl)propane-1,3-dione (37f). Methyl 4-bromobenzoate was treated with 4-bromo-1-acetylbenezene and NaH, as for the synthesis of 37b, to give 37f (30%) as an off-white powder: mp 198-200°C (lit.21 197-198.5°C); 1H NMR (CDCl3) (enol) δ 6.70 (1 H, s, CH), 7.55 (4 H, d, J = 9.0 Hz, 2 × Ph 3,5-H2), 7.77 (4 H, d, J = 8.5 Hz, 2 × Ph 2,6-H2); 13C NMR (CDCl3) δ 92.50 (CH), 126.80 (2 × Ph 4-H), 156.00 (C=O, C=O), 111.39 (C≡N), 118.53 (NCPh 1-2), 9.01 (4 H, d, J = 5.6 Hz, 2 × pyridine 3,5-H2); 13C NMR δ 97.50 (CH), 121.84 (2 × pyridine 3,5-C2), 144.00 (2 × pyridine 4-C), 184.00 (C=O, C=O); MS m/z 406.8946 (M + Na)+ (C15H10Br2NaO2 requires 406.8901), 404.8966 (M + Na)+ (C15H1079Br3BrNaO2 requires 404.8920), 402.8960 (M + Na)+ (C15H1079Br2NaO2).

1,3-Di(pyridin-4-yl)propane-1,3-dione (37l). NaH (1.00 g, 25 mmol, 60% in oil) was washed free from oil with dry hexane (10 mL) at 0°C under Ar. Dry THF (30 mL) was added. Methyl pyridine-4-carboxylate (1.51 g, 11 mmol) in dry THF (10 mL) and 4-acetylpyridine (1.21 g, 10 mmol) in dry THF (10 mL) at 0°C under Ar were added. The suspension was stirred under reflux for 16 h. The cooled mixture was filtered (Celite®). The filtrate was poured into Et2O and water. Aq. HCl (9 M) was added to pH 4. The precipitate was collected and washed (petroleum ether) to give 37l (960 mg, 42%) as pale buff needles: mp 158-159°C (lit.22 156-157°C); 1H NMR (CDCl3) (enol) δ 7.77 (1 H, s, CH), 8.34 (4 H, d, J = 5.0 Hz, 2 × pyridine 3,5-H2), 7.01 (4 H, d, J = 5.6 Hz, 2 × pyridine 2,6-H2); 13C NMR δ 97.50 (CH), 121.84 (2 × pyridine 3,5-C2), 144.00 (2 × pyridine 4-C), 184.00 (C=O, C=O); MS m/z 227.0826 (M + H)+ (C13H11N2O2 requires 227.0815).

4-Phenylethynylbenzonitrile (42j). Cul (52 mg, 0.3 mmol), (Ph3P)2Pd (162 mg, 0.14 mmol), Ns ascorbate (33 mg, 0.16 mmol) and 42f (500 mg, 2.75 mmol) were placed in a flask, which was degassed and filled with Ar. Pr2NH (5 mL) and THF (10 mL) were added. The mixture was stirred at 40°C for 30 min. PhC≡CH 21a (281 mg, 2.75 mmol) was added and the mixture was stirred at 40°C for 16 h. Evaporation and chromatography (petroleum ether / EtOAc 199:1 → 99:1) gave 42j (480 mg, 87%) as an off-white powder: mp 84-86°C (lit.2 157-159°C); 1H NMR (CDCl3) δ 7.33-7.41 (3 H, m, Ph 3,4,5-H3), 7.41 (3 H, m, Ph 3,4,5-H3), 7.65 (NCPh 2,3-4), 7.76 (2 H, d, J = 8.5 Hz, 2 × Ph 2,6-H2); 13C NMR δ 131.99 (2 H, d, J = 8.5 Hz, 2 × Ph 2,6-H2); 13C NMR δ 122.15 (Ph 1-2), 184.71 (C=O, C=O); MS m/z 215.9508 (M + Na)+ (C14H8N2 requires 215.9489), 213.9531 (M + Na)+ (C14H8N2BrNaO2 requires 213.9508), 211.9554 (M + Na)+ (C14H8N2Cl2 requires 211.9527).

N-Methoxy-4-methylbenzimidamide (43b). 4-Methylbenzonitrile 42b (234 mg, 2.0 mmol) in MeOH (10 mL) was stirred with MeONH2.HCl (167 mg, 2.0 mmol) and Na2CO3 (212 mg, 2.0 mmol) in water (5 mL) for 24 h. MeONH2.HCl (83.5 mg, 1.0 mmol) was added and the mixture was stirred for 2 d. The mixture was filtered. The evaporation residue, in CH2Cl2, was dried. Evaporation and recrystallisation (CHCl3 / petroleum ether) gave 43b (10 mg, 3%) as a white powder: mp 89-91°C (lit.23 85°C); 1H NMR (CD2OD) δ 2.43 (3 H, s, PhMe), 3.89 (3 H, s, OMe), 7.27 (2 H, d, J = 8.2 Hz, Ph 3,5-H2), 7.59 (2 H, d, J = 8.2 Hz, Ph 2,6-H2); 13C NMR (CDCl3) δ 21.82 (PhMe), 61.39 (OMe), 125.71 (2,6-C2), 129.28 (3,5-C2); MS m/z 165.1036 (M + H)+ (C9H12N2O requires 165.1028).

N-Methoxy-4-trifluoromethylbenzimidamide (43d). Compound 42d (342 mg, 2.0 mmol) in MeOH (5 mL) was added to MeONH2.HCl (167 mg, 2.0 mmol) and Na2CO3 (212 mg, 2.0 mmol) in water (5 mL). The mixture was sonicated at 55°C for 30 min, then stirring continued at 20°C for 20 h. MeONH2.HCl (83.5 mg, 1.0 mmol) was added and the mixture was stirred at 25°C for 24 h. The mixture was filtered. The evaporation residue, in CH2Cl2, was dried. Evaporation and recrystallisation (CHCl3 / petroleum ether) gave 43d (85 mg, 20%) as a white powder: mp 92-94°C; 1H NMR (CD2OD) δ 3.95 (3 H, s, Me), 7.76 (2 H, d, J = 8.7 Hz, Ph 3,5-H2), 7.90 (2 H, d, J = 8.8 Hz, Ph 2,6-H2), 13.99 (2 H, br, NH2); 13C NMR (CDCl3) δ...
N-Hydroxy-4-methylbenzimidamide (44b). 4-Methylbenzonitrile 42b (940 mg, 8.0 mmol) in EtOH (30 mL) was added to NH₂OH.HCl (3.34 g, 48 mmol) and NaHCO₃ (2.54 g, 24 mmol) in water (30 mL) and the mixture was boiled under reflux for 3 h. The EtOH was evaporated and the residue was poured into water. The precipitate was collected, washed (water) and dried to give 44b (940 mg, 78%) as a white powder: mp 138-139°C (lit.²⁴ 136-137°C); ¹H NMR δ 2.37 (3 H, s, Me), 5.77 (2 H, br, NH₂), 7.22 (2H, d, J = 8.0 Hz, Ph 3,5-H₂), 7.61 (2 H, d, J = 8.0 Hz, Ph 2,6-H₂), 9.55 (1 H, s, OH); ¹³C NMR δ 20.78 (Me), 125.24 (2,6-C₂), 128.60 (3,5-C₂), 130.55 (1-C), 138.21 (4-C), 150.74 (C=N); MS m/z 219.0730 (M + H)⁺ (C₈H₁₀FN₂O requires 219.0745).

N-Hydroxy-4-trifluoromethylbenzimidamide (44d). Compound 44d was prepared as previously described.²⁵

4-Chloro-N-hydroxybenzimidamide (44e). Compound 44e was prepared as previously described.²⁵

4-Bromo-N-hydroxybenzimidamide (44f). 4-Bromobenzonitrile 42f was treated with NH₂OH.HCl and NaHCO₃, as for the synthesis of 44b, to give 44f (90%) as a white powder: mp 140-141°C (lit.²⁶ 139-140°C); ¹H NMR δ 5.90 (2 H, br, NH₂), 7.60 (2 H, d, J = 8.8 Hz, Ph 3,5-H₂), 7.67 (2 H, d, J = 8.8 Hz, Ph 2,6-H₂), 9.77 (1 H, s, OH); ¹³C NMR (HSQC / HMBC) δ 122.06 (4-C), 127.39 (3,5-C₂), 130.55 (1-C), 149.97 (C=N); MS m/z 216.9792 (M + H)⁺ (C₇H₅BrN₂O requires 216.9780), 214.9814 (M + H)⁺ (C₇H₆BrN₂O requires 214.9820).

4-Amino-N-hydroxybenzimidamide (44g). 4-Aminobenzonitrile 42g (472 mg, 4.0 mmol) in EtOH (15 mL) was added to NH₂OH.HCl (1.67 g, 24 mmol) and NaHCO₃ (1.27 g, 12 mmol) in water (15 mL). The mixture boiled under reflux for 8 h and cooled. The EtOH was evaporated. The residue was poured into water and extracted (EtOAc, 3 ×). The combined extracts were dried. Evaporation gave 44g (90 mg, 15%) as a white powder: mp 157-158°C (lit.²⁷ 166-168°C); ¹H NMR δ 5.30 (2 H, br, NH₂), 5.62 (2 H, br, NH₂), 6.57 (2 H, d, J = 8.6 Hz, Ph 3,5-H₂), 7.38 (2 H, d, J = 8.6 Hz, Ph 2,6-H₂), 9.25 (1 H, s, OH); ¹³C NMR (CDCl₃) δ 113.04 (3,5-C₂), 120.36 (1-C), 126.31 (2,6-C₂), 149.57 (4-C), 151.49 (C=N); MS m/z 174.0649 (M + Na)⁺ (C₇H₆N₃NaO requires 174.0643), 152.0853 (M + H)⁺ (C₇H₁₀N₂O requires 152.0824).

N-Hydroxy-4-nitrobenzimidamide (44k). 4-Nitrobenzonitrile 42k was treated with NH₂OH.HCl and NaHCO₃, as for the synthesis of 44b, to give 44k (97%) as a white powder: mp 174-176°C (lit.²⁸ 176°C); ¹H NMR δ 4.62 (2 H, br, NH₂), 7.95 (2 H, d, J = 8.7 Hz, Ph 2,6-H₂), 8.32 (2 H, d, J = 8.7 Hz, Ph 3,5-H₂); ¹³C NMR δ 124.53 (3,5-C₂), 128.19 (2,6-C₂), 137.00 (1-C), 149.00 (4-C), 163.00 (C=N); MS m/z 182.0562 (M + H)⁺ (C₆H₆N₃O requires 182.0566).

N-Hydroxypyridine-4-carboximidamide (44l). 4-Cyanopyridine 42l was treated with NH₂OH.HCl and NaHCO₃, as for the synthesis of 44b, to give 44l (83%) as a white powder: mp 181-184°C (lit.²⁹ 178-179°C); ¹H NMR (CDCl₃) δ 6.04 (2 H, br, NH₂), 7.69 (2 H, d, J = 4.6 Hz, 3,5-H₂), 8.62 (2 H, d, J = 4.8 Hz, 2,6-H₂), 10.08 (1 H, s, OH); ¹³C NMR (CDCl₃) δ 121.9 (3,5-C₂), 143.07 (4-C), 150.37 (2,6-C₂), 152.22 (C=N); MS m/z 138.0683 (M + H)⁺ (C₆H₆N₃O requires 138.0667).
4-Methylbenzimidamide (45b). Compound 44b (150 mg, 1.0 mmol) was boiled under reflux with HCOONH₄ (403 mg, 6.3 mmol) and Pd/C (10%, 150 mg) in AcOH (5 mL) for 4 d under Ar. The cooled mixture was filtered (Celite®). Aq. NaOH (1.0 M, 20 mL) was added to the evaporation residue and the mixture was extracted (EtOAc, 3 ×). Drying and evaporation gave 44b (82 mg, 61%) as a white powder: mp 53-55°C (lit.30 68°C); ¹H NMR δ 7.51 (2 H, br, NH₂), 7.53 (2 H, d, J = 7.6 Hz, 3,5-H₂), 7.79 (2 H, d, J = 7.2 Hz, 2,6-H₂), 7.93 (1 H, br, NH); ¹³C NMR δ 126.73 (3,5-C₂), 128.22 (2,6-C₂), 129.59 (4-C), 131.20 (1-C), 162.57 (C=N); MS m/z 135.1018 (M + H)⁺ (C₆H₅N₂ requires 135.0922).

4-Trifluoromethylbenzimidamide (45d). Compound 45d was prepared as previously described.²⁵

4-Chlorobenzimidamide (45e). Compound 45e was prepared as previously described.²⁵

4-Bromobenzimidamide (45f). Compound 44f was treated with HCOONH₄ and Pt/C, as for the synthesis of 45e, to give 45f (94 mg, 68%) as a white powder: mp 257-259°C (lit.3¹ 258.5-259.5°C); ¹H NMR δ 7.51 (2 H, br, NH₂), 7.53 (2 H, d, J = 7.6 Hz, 3,5-H₂), 7.79 (2 H, d, J = 7.2 Hz, 2,6-H₂), 7.93 (1 H, br, NH); ¹³C NMR δ 126.73 (3,5-C₂), 128.22 (2,6-C₂), 129.59 (4-C), 131.20 (1-C), 166.90 (C=N); MS m/z 200.9851 (M + H)⁺ (C₇H₈BrN₂ requires 200.9851), 198.9877 (M + H)⁺ (C₇H₇BrN₂ requires 198.9871).

4-Aminobenzimidamide (45g). Compound 44g (151 mg, 1.0 mmol) was stirred under reflux in AcOH (5 mL) with Pd/C (1%, 362 mg) and HCOONH₄ (800 mg, 12.6 mmol) for 4 d, before being cooled and filtered (Celite®). The evaporation residue was basified with aq. NaOH (5 M, 20 mL) and extracted thrice with EtOAc. Drying and evaporation gave 45g (80 mg, 59%) as a white powder: mp 200-201°C (lit.3² 171°C); ¹H NMR δ 6.70 (3 H, br, NH & NH₂), 7.35 (2 H, br, NH₂), 7.66 (2 H, d, J = 8.6 Hz, Ph 3,5-H₂), 7.73 (2 H, d, J = 8.6 Hz, Ph 2,6-H₂); ¹³C NMR δ 117.80 (3,5-C₂), 127.21 (2,6-C₂), 133.0 (1-C), 140.0 (4-C), 152.0 (C=N); MS m/z 136.0898 (M + H)⁺ (C₇H₈N₂ requires 136.0875).

4-Phenylethynylbenzimidamide (45j). NaOMe (134 mg, 2.5 mmol) was stirred under reflux for 2 h with 44j (480 mg, 2.36 mmol) in dry MeOH (15 mL). NH₄Cl (278 mg, 5.2 mmol) was added and stirring under reflux continued for 2 h. Evaporation and recrystallisation (water) gave 45j (355 mg, 68%) as an off-white powder: mp 90-91°C; ¹H NMR d 7.51-7.54 (3 H, m, Ph 3,4,5-H₃), 7.67 (2 H, m, Ph 2,6-H₂), 7.81 (2 H, m, amidine Ph 2,6-H₂), 7.89 (1 H, br, NH), 7.97 (2 H, m, amidine-Ph 3,5-H₂), 9.06 (1 H, br, NH), 9.45 (1 H, br, NH); ¹³C NMR d 88.30 (ethyne 1-C), 93.60 (ethyne 2-C), 111.05 (amidine Ph 1-C)118.44 (amidine Ph 4-C), 121.39 (Ph 1-C), 128.88 (Ph 3,4,5-C₃), 129.55 (Ph 4-C), 131.63 (Ph 2,6-C₂), 132.15 (amidine Ph 3,5-C₂), 132.62 (amidine Ph 2,6-C₂); MS m/z 242 (M + Na)⁺, 221.1090 (M + H)⁺ (C₁₃H₁₃N₂ requires 221.1073).

Pyridine-4-carboximidamide (45i). Compound 44i (137 mg, 1.0 mmol) was boiled under reflux with Pd/C (1%, 150 mg) and HCOONH₄ (400 mg, 6.3 mmol) in AcOH (5 mL) for 4 d. The suspension was filtered (Celite®). The evaporation residue was basified with aq. NaOH (5 M, 20 mL) and extracted (EtOAc, 3 ×). Evaporation and drying gave 45i (30.5 mg, 25%) as a white powder: mp >200°C (decomp.) (lit.3³ 235-238°C); ¹H NMR δ 7.78 (2 H, d, J = 5.5 Hz, 3,5-H₂), 8.87 (2 H, d, J = 5.0 Hz, 2,6-H₂), 9.52 (3 H, br, NH and NH₂); ¹³C NMR δ 121.39 (3,5-C₂), 141.24 (4-C), 150.24 (2,6-C₂), 166.38 (C=N); MS m/z 122.0710 (M + H)⁺ (C₈H₆N₂ requires 122.0718).
### Data collection and refinement statistics for the tankyrase-2 crystal structures

<table>
<thead>
<tr>
<th>Compound</th>
<th>17a</th>
<th>12b</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDB code</td>
<td>4W5I</td>
<td>4UX4</td>
</tr>
<tr>
<td><strong>Data</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beam line</td>
<td>Diamond I04-1</td>
<td>Diamond I04-1</td>
</tr>
<tr>
<td>Wavelength (Å)</td>
<td>0.92000</td>
<td>0.92000</td>
</tr>
<tr>
<td>Space group</td>
<td>C222₁</td>
<td>C222₁</td>
</tr>
<tr>
<td><strong>Cell dimensions</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a, b, c (Å)</td>
<td>91.55, 98.12, 119.22</td>
<td>91.56, 97.89, 118.47</td>
</tr>
<tr>
<td>Resolution (Å)</td>
<td>30-1.95 (2.00-1.95)</td>
<td>50-1.80 (1.85-1.80)</td>
</tr>
<tr>
<td>R&lt;sub&gt;merge&lt;/sub&gt;</td>
<td>13.2 (86.4)</td>
<td>6.3 (83.6)</td>
</tr>
<tr>
<td>I / σI</td>
<td>9.87 (2.15)</td>
<td>18.80 (2.17)</td>
</tr>
<tr>
<td>Completeness (%)</td>
<td>99.8 (100)</td>
<td>99.9 (100)</td>
</tr>
<tr>
<td>Redundancy</td>
<td>6.7 (6.9)</td>
<td>6.8 (7.0)</td>
</tr>
<tr>
<td><strong>Refinement</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R&lt;sub&gt;work&lt;/sub&gt; / R&lt;sub&gt;free&lt;/sub&gt;</td>
<td>0.162/0.200</td>
<td>0.162/0.198</td>
</tr>
<tr>
<td><strong>B-factors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>27.2</td>
<td>28.3</td>
</tr>
<tr>
<td>Inhibitor</td>
<td>18.1</td>
<td>34.3</td>
</tr>
<tr>
<td><strong>R.m.s.d.</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bond lengths (Å)</td>
<td>0.011</td>
<td>0.012</td>
</tr>
<tr>
<td>Bond angles (°)</td>
<td>1.4</td>
<td>1.5</td>
</tr>
<tr>
<td><strong>Ramachandran plot (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Favoured regions</td>
<td>99.3</td>
<td>99.3</td>
</tr>
<tr>
<td>Additionally allowed regions</td>
<td>0.7</td>
<td>0.7</td>
</tr>
</tbody>
</table>
Examples of concentration-inhibition graphs for inhibition of TNKS-1

![Graph 1](image1.png)

![Graph 2](image2.png)

![Graph 3](image3.png)

![Graph 4](image4.png)

![Graph 5](image5.png)
Examples of concentration-inhibition graphs for inhibition of TNKS-2
Examples of concentration-inhibition graphs for inhibition of PARP-1
References for Supplementary Information


