Fragment-Based Drug Design of Novel Pyranopyridones as Cell Active and Orally Bioavailable Tankyrase Inhibitors

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[1]Discovery Chemistry, [2]Non-clinical Safety, [3]Discovery Technologies, [4]Discovery Oncology

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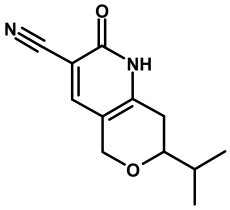
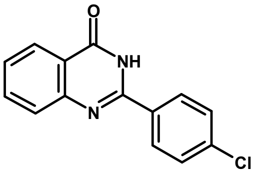
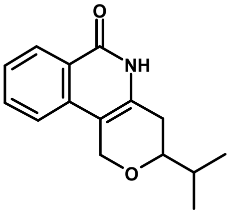
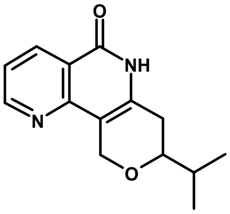
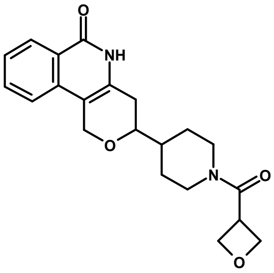
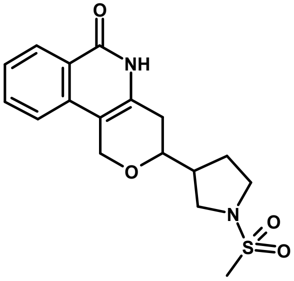
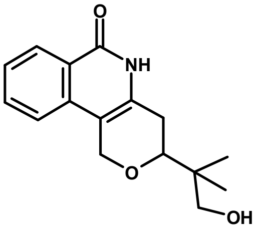
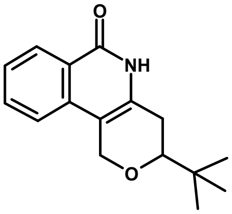
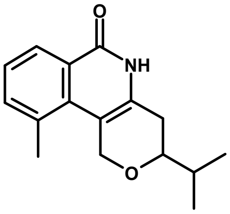
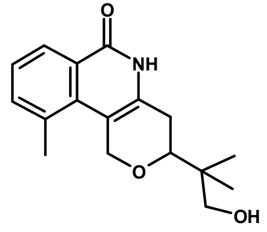
Table S1. Structures and biochemical activity against TNKS1, and TNKS2.

Compound	IC_{50} TNKS1 (µM)	IC_{50} TNKS2 (µM)
1 Pyrimidinopyrimidone *(racemic)* 	1	1.7

2 Pyranopyridone 	0.14	0.2
3 *(racemic)* 	< 0.06	< 0.06
4 *(racemic)* 	0.061	0.093
5 *(racemic)* 	< 0.06	< 0.06
6 *(racemic)*	< 0.06	< 0.06

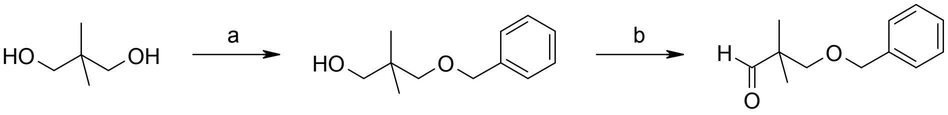
		
7 *(racemic)* 	< 0.06	< 0.06
8 *(racemic)* 	< 0.06	< 0.06
9 *(racemic)* 	< 0.06	< 0.06
10 *(racemic)*	< 0.06	< 0.06

S3. Chemistry data and characterization

General Methods. All reagents and solvents were purchased from commercial sources and used without further purification. ^{1}H NMR spectra were measured using a Bruker NMR Avance 400MHz or 300MHz spectrometer, and chemical shifts were expressed in δ (ppm) units using tetramethylsilane as an internal standard (in ^{1}H NMR description, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad peak). All coupling constants (*J*) are reported in hertz. Mass spectra were recorded on an Agilent AG1 Single Quad G6140A or an Agilent 6110A spectrometer. HPLC were obtained on a SHIMADZU-LC2010A with an UV detector, or an Agilent-1100 with a DAD detector. Purity values for all tested compounds were found to be above 95% from the high-performance liquid chromatography (HPLC) analyses.

Scheme S1. Synthetic route to 3-benzyloxy-2,2-dimethylpropionaldehyde.



19 **20** **12c**

^{a}Reagents, conditions and yields: (a) KO^{t}Bu, benzyl bromide, dioxane, 0°C to 90°C, 51% (b) PCC, SiO$_2$, CH$_2$Cl$_2$, rt, 73%

2-Isopropyl-tetrahydro-pyran-4-one (**13a**). A solution of **11** (1.0 g, 13.9 mmol) and **12a** (2.00 g, 27.7 mmol) in methylene chloride (62 ml) was cooled to 0°C. Trifluoroacetic acid (25 ml) was added slowly under nitrogen atmosphere. The reaction mixture was allowed to warm to room temperature and stirred for 18 h. The resulting mixture was carefully washed with a saturated sodium bicarbonate solution until the aqueous layer remained basic. The organic layer was concentrated *in vacuo* and the residue was taken up with methanol (100 mL). The reaction mixture was treated with potassium carbonate (5 g, 13.9 mmol) and allowed to stir for 30 min. at which time all starting material had been consumed. The resulting mixture was filtered and concentrated *in vacuo*. The residue was diluted with water and extracted with methylene chloride. The combined organic layer was dried over sodium sulfate, filtered, and concentrated *in vacuo* to give 2-isopropyl-tetrahydro-pyran-4-ol as a crude oil (1.37 g, 68%) which was taken on without further purification. ^{1}H NMR (CHLOROFORM-d) δ: 4.02 (ddd, J = 11.7, 4.9, 1.5 Hz, 1H), 3.76 (tt, J = 11.0, 4.7 Hz, 1H), 3.37 (td, J = 12.1, 1.9 Hz, 1H), 2.98 (ddd, J = 11.2, 6.1, 1.9 Hz, 1H), 1.96 (ddt, J = 12.1, 4.4, 2.1 Hz, 1H), 1.88 (ddq, J = 12.3, 4.4, 2.1 Hz, 1H), 1.71 (sxt, J = 6.8 Hz, 1H), 1.38 - 1.56 (m, 1H), 1.19 (q, J = 11.5 Hz, 1H), 0.94 (d, J = 6.8 Hz, 3H), 0.91 (d, J = 6.8 Hz, 3H). A solution of 2-isopropyl-tetrahydro-pyran-4-ol (14.9 g, 103 mmol), silica gel (60 mL) and PCC (28.9 g, 134 mmol) was stirred at room temperature overnight. The resulting dark mixture was filtered through a pad of silica and concentrated *in vacuo* to give 2-isopropyl-tetrahydro-pyran-4-one **13a** as a yellow oil (12.24 g, 83%). This material was taken on to the next step without further purification. ^{1}H NMR (CHLOROFORM-d) δ: 4.31 (ddd, J = 11.4, 7.5, 1.1 Hz, 1H), 3.63 (td, J = 11.9, 3.0 Hz, 1H), 3.30 (ddd, J = 11.1, 6.0, 2.8 Hz, 1H), 2.59 (ddd, J = 13.6, 12.5, 7.6 Hz, 1H), 2.32 - 2.46 (m, 2H), 2.25 - 2.36 (m, 1H), 1.81 (dq, J = 13.3, 6.7 Hz, 1H), 0.98 (d, J = 6.8 Hz, 3H), 0.93 (d, J = 6.8 Hz, 3H)

2-tert-Butyl-tetrahydro-pyran-4-one (**13b**). The title compound was prepared according to the procedure previously described for **13a** starting from **11** and **12b**. Flash chromatography (SiO_2, 0% to 50% ethyl acetate in heptanes) afforded 2-tert-butyl-tetrahydro-pyran-4-ol as a slow forming solid (6.65 g, 76%). ^{1}H NMR (CHLOROFORM-d) δ: 4.04 (ddd, J = 11.7, 4.9, 1.9 Hz, 1H), 3.77 (tt, J = 10.9, 4.6 Hz, 1H), 3.35 (td, J = 12.1, 1.9 Hz, 1H), 2.87 (dd, J = 11.3, 1.9 Hz, 1H), 1.96 (ddt, J = 12.1, 4.3, 2.0 Hz, 1H), 1.87 (ddq, J = 12.5, 4.4, 2.0 Hz, 1H), 1.37 - 1.54 (m, 1H), 1.20 (q, J = 11.2 Hz, 1H), 0.91 (s, 10H). From 2-tert-butyl-tetrahydro-pyran-4-ol, 2-tert-butyl-tetrahydro-pyran-4-one **13b** was obtained as a light brown crude oil (5.21 g, 73%). This material was taken on to the next step without further purification. ^{1}H NMR (CHLOROFORM-d) δ: 4.32 (ddd, J = 11.4, 7.6, 0.9 Hz, 1H), 3.60 (ddd, J = 12.5, 11.5, 2.8 Hz, 1H), 3.19 (dd, J = 11.0, 3.0 Hz, 1H), 2.49 - 2.66 (m, 1H), 2.23 - 2.46 (m, 3H), 0.94 (s, 9H)

3-Benzyloxy-2,2-dimethyl-propan-1-ol (**20**). A solution of **19** (25.85 g, 248 mmol) in dioxane (400 mL) was cooled to 0°C. Potassium tert-butoxide (30 g, 267 mmol) was added portionwise to the cooled solution. The resulting mixture was stirred at room temperature for 1 h. Benzyl bromide (29.5 mL, 248 mmol) was added dropwise via addition funnel. The mixture was heated to 90°C and stirred for 4 h. The resulting mixture was concentrated *in vacuo*. The resulting residue was partitioned between water (200 mL) and ethyl acetate (200 mL) and extracted with ethyl acetate (3 × 200 mL). The organics were combined, washed with brine (100 mL), dried on sodium sulfate, and concentrated *in vacuo*. The crude material was purified by Flash chromatography (SiO_2, 5% to 60% ethyl acetate in heptane) to afford 3-benzyloxy-2,2-dimethyl-propan-1-ol **20** as a yellow oil (24.84 g, 51%). ^{1}H NMR (CHLOROFORM-d) δ: 7.28 - 7.42 (m, 5H), 4.52 (s, 2H), 3.47 (s, 2H), 3.34 (s, 2H), 0.94 (s, 6H). LC-MS: 195 $[M+H]^+$, R_t = 2.558 min.

3-Benzyloxy-2,2-dimethyl-propionaldehyde (**12c**). A solution of **20** (23.8g, 123 mmol), silica gel (25 g) and PCC (26.5 g, 123 mmol) was stirred at room temperature overnight. The resulting dark mixture was filtered through a pad of silica and concentrated *in vacuo* to give 3-benzyloxy-2,2-dimethyl-propionaldehyde **12c** as a pale yellow oil (17.25 g, 73%). This material was taken on to the next step without further purification. ^{1}H NMR (CHLOROFORM-d) δ: 9.58 (s, 1H), 7.28 - 7.40 (m, 5H), 4.52 (s, 2H), 3.46 (s, 2H), 1.10 (s, 6H)

2-(2-Benzyloxy-1,1-dimethyl-ethyl)-tetrahydro-pyran-4-one (**13c**). The title compound was prepared according to the procedure previously described for **13a** starting from **11** and **12c**. Flash chromatography (SiO_2, 0% to 40% ethyl acetate in heptanes) afforded 2-(2-benzyloxy-1,1-dimethyl-ethyl)-tetrahydro-pyran-4-ol as a crude viscous yellow oil (3.76 g, 68%). ^{1}H NMR (CHLOROFORM-d) δ: 7.28 - 7.45 (m, 5H), 4.50 (s, 2H), 4.00 (ddd, J = 11.6, 4.8, 1.7 Hz, 1H), 3.68 - 3.86 (m, 1H), 3.34 (d, J = 8.7 Hz, 2H), 3.35 (td, J = 12.2, 2.1 Hz, 1H), 3.19 (d, J = 8.7 Hz, 2H), 3.23 (dd, J = 11.3, 1.5 Hz, 1H), 1.81 - 1.95 (m, 2H), 1.37 - 1.54 (m, 1H), 1.14 - 1.32 (m, 2H), 0.93 (s, 3H), 0.91 (s, 3H). From 2-(2-benzyloxy-1,1-dimethyl-ethyl)-tetrahydro-pyran-4-ol the crude material was purified by flash chromatography (SiO_2, 0% to 40% ethyl acetate in heptane) to afford 2-(2-benzyloxy-1,1-dimethyl-ethyl)-tetrahydro-pyran-4-one **13c** as a colorless oil (3.54 g, 94%). ^{1}H NMR (CHLOROFORM-d) δ: 7.27 - 7.40 (m, 5H), 4.49 (d, J = 2.6 Hz, 2H), 4.27 (dd, J = 11.3, 7.6 Hz, 1H), 3.50 - 3.66 (m, 2H), 3.35 (d, J = 8.7 Hz, 1H), 3.22 (d, J = 8.7 Hz, 1H), 2.56 (ddd, J = 14.5, 12.5, 7.7 Hz, 1H), 2.34 - 2.42 (m, 2H), 2.25 - 2.34 (m, 1H), 0.98 (s, 3H), 0.92 (s, 3H)

4-(4-Oxo-tetrahydro-pyran-2-yl)-piperidine-1-carboxylic acid *tert*-butyl ester (**13d**). A solution of **11** (1.60 g, 22.2 mmol) and **12d** (9.46 g, 44.4 mmol) in methylene chloride (500 ml) was cooled to 0°C. Trifluoroacetic acid (200 mL) was added slowly under nitrogen atmosphere. The reaction mixture was allowed to warm to room temperature and stirred for 18 h. The resulting mixture was concentrated *in vacuo*. The crude material was dissolved in methylene chloride (200 mL) and treated with di-*tert*-butyl dicarbonate (10 g, 45.8 mmol) and triethylamine (46.4 ml, 333 mmol). The resulting mixture was stirred at room temperature overnight and subsequently concentrated *in vacuo*. The crude material was dissolved in methanol (500 mL) and treated with potassium carbonate (312 g, 22.2 mmol). The reaction mixture was stirred at room temperature for 3h. The resulting mixture was diluted with water (250 mL) and extracted with methylene chloride. The organic layers were dried over magnesium sulfate and concentrated *in vacuo*. The crude residue was purified by flash chromatography (SiO_2, 10% to 80% ethyl acetate in hexanes) to afford 4-(4-hydroxy-tetrahydro-pyran-2-yl)-piperidine-1-carboxylic acid *tert*-butyl ester as a colorless oil (5.00 g, 79%). ^{1}H NMR (CHLOROFORM-d) δ: 4.07 - 4.21 (m, 2H), 4.02 (ddd, J = 11.7, 4.9, 1.5 Hz, 1H), 3.77 (tt, J = 11.0, 4.7 Hz, 1H), 3.36 (td, J = 12.2, 2.1 Hz, 1H), 3.03 (ddd, J = 11.1, 6.6, 1.5 Hz, 1H), 2.66 (tdd, J = 13.2, 4.5, 3.0 Hz, 2H), 1.77 - 2.03 (m, 3H), 1.48 - 1.68 (m, 3H), 1.45 (s, 9H), 1.09 - 1.31 (m, 3H). A solution of 4-(4-hydroxy-tetrahydro-pyran-2-yl)-piperidine-1-carboxylic acid *tert*-butyl ester (5.5 g, 19.3 mmol,), silica gel (15 g) and PCC (4.99 g, 23.1 mmol) in methylene chloride was stirred at room temperature overnight. The resulting reaction mixture was filtered through a pad of celite and silica. The filtrate was concentrated *in vacuo* to give 4-(4-oxo-tetrahydro-pyran-2-yl)-piperidine-1-carboxylic acid *tert*-butyl ester **13d** as a pale yellow crude solid (3.8 g, 70%). This material was taken on to the next step without further purification. ^{1}H NMR (CHLOROFORM-d) δ: 4.30 (dd, J = 11.5, 6.6 Hz, 1H), 4.16 (br. d, J = 11.0 Hz, 2H), 3.56 - 3.68 (m, 1H), 3.35 (ddd, J = 11.0, 6.3, 2.6 Hz, 1H), 2.49 - 2.77 (m, 3H), 2.22 - 2.48 (m, 3H), 1.88 (d, J = 12.8 Hz, 1H), 1.55 - 1.69 (m, 2H), 1.46 (s, 9H), 1.11 - 1.33 (m, 2H).

3-(4-Oxo-tetrahydro-pyran-2-yl)-pyrrolidine-1-carboxylic acid *tert*-butyl ester (**13e**). The title compound was prepared according to the procedure previously described for **13d** starting from **11** and **12e**. 3-(4-Hydroxy-tetrahydro-pyran-2-yl)-pyrrolidine-1-carboxylic acid *tert*-butyl ester was obtained as an oil (2.38 g, 63%). ^{1}H NMR (CHLOROFORM-d) δ: 3.95 - 4.07 (m, 1H), 3.70 - 3.84 (m, 1H), 2.93 - 3.62 (m, 7H), 2.13 - 2.30 (m, 2H), 1.51 - 1.95 (m, 5H), 1.45 (s, 9H). From 3-(4-hydroxy-tetrahydro-pyran-2-yl)-pyrrolidine-1-carboxylic acid *tert*-butyl ester, 3-(4-oxo-tetrahydro-pyran-2-yl)-pyrrolidine-1-carboxylic acid *tert*-butyl ester **13e** was obtained as a viscous oil (1.90 g, 76%). ^{1}H NMR (CHLOROFORM-d) δ: 4.18 - 4.38 (m, 1H), 3.56 - 3.75 (m, 2H), 3.37 - 3.56 (m, 2H), 3.28 (d, J = 7.2 Hz, 1H), 2.94 - 3.11 (m, 1H), 2.53 - 2.71 (m, 1H), 2.41 - 2.53 (m, 1H), 2.23 - 2.41 (m, 3H), 1.71 - 1.97 (m, 1H), 1.54 - 1.69 (m, 1H), 1.47 (s, 9H).

2-Isopropyl-1,2,4,10-tetrahydro-3-oxa-10-aza-phenanthren-9-one (**3**). A mixture of **13a** (0.38 g, 2.7 mmol), xantphos (31 mg, 53.4 μmol) and **14a** (0.70 g, 2.7 mmol), cesium carbonate (1.91 g, 5.9 mmol), and tris(dibenzylideneacetone)dipalladium(0) (24 mg, 27 μmol), in dioxane (2.0 mL) was placed under argon atmosphere and stirred at 80-90°C for 15 h. The reaction mixture was pre-absorbed onto silica and purified by flash chromatography (SiO_2, 0% to 50% EtOAc in hexanes) to afford a crude oil (50 mg). The crude oil was dissolved in a solution of ammonia in methanol (7M, 2 mL). The reaction mixture was heated at 140°C in a microwave reactor for 1h. The precipitate was filtered and dried *in vacuo* to afford 2-isopropyl-1,2,4,10-tetrahydro-3-oxa-10-aza-phenanthren-9-one **3** as an off-white solid (25 mg, 4%). ^{1}H NMR (METHANOL-d_4) δ: 8.32 (d, J = 8.3 Hz, 1H), 7.69 - 7.78 (m, 1H), 7.41 - 7.57 (m, 2H), 5.02 (d, J = 14.4 Hz, 1H), 4.75 (ddd, J = 14.4, 5.3, 2.5 Hz, 1H), 3.39 - 3.50 (m, 1H), 2.55 - 2.63 (m, 2H), 1.84 (dqd, J = 13.6, 6.7, 3.2 Hz, 1H), 1.09 (dd, J = 6.8, 3.0 Hz, 3H), 1.03 (dd, J = 6.8, 3.0 Hz, 4H). LC-MS: 244 $[M+H]^+$, R_t = 2.666 min

7-Isopropyl-5,7,8,9-tetrahydro-6-oxa-3,9-diaza-phenanthren-10-one (**4**). The title compound was prepared according to the procedure previously described for **3** starting from **13a** and **14b**. The crude material was purified by flash chromatography (SiO_2, 80% to 100% EtOAc in heptane). 7-Isopropyl-5,7,8,9-tetrahydro-6-oxa-3,9-diaza-phenanthren-10-one **4** was obtained as a pale yellow solid (28 mg, 5%). ^{1}H NMR (DMSO-d_6) δ: 11.40 (br. s., 1H), 8.88 (s, 1H), 8.61 (d, J = 5.3 Hz, 1H), 7.98 (d, J = 5.3 Hz, 1H), 5.06 (d, J = 14.7 Hz, 1H), 4.67 (dt, J = 14.6, 2.3 Hz, 1H), 3.39 (q, J = 6.5 Hz, 1H), 1.77 (dq, J = 13.6, 6.8 Hz, 1H), 0.98 (d, J = 6.4 Hz, 3H), 0.93 (d, J = 6.8 Hz, 3H). LC-MS: 245 $[M+H]^+$, R_t = 1.757 min.

4-(9-Oxo-1,4,9,10-tetrahydro-2H-3-oxa-10-aza-phenanthren-2-yl)-piperidine-1-carboxylic acid tert-butyl ester (**15**). The title compound was prepared according to the procedure previously described for **3** starting from **13d** and **14a**. 4-(9-Oxo-1,4,9,10-tetrahydro-2H-3-oxa-10-aza-phenanthren-2-yl)-piperidine-1-carboxylic acid *tert*-butyl ester **15** was obtained as a light yellow solid (357 mg, 11%). ^{1}H NMR (DMSO-d_6) δ: 11.24 (br. s., 1H), 8.18 (dd, J = 7.9, 1.1 Hz, 1H), 7.68 (td, J = 8.0, 1.0 Hz, 1H), 7.45 (t, J = 7.7 Hz, 1H), 7.40 (d, J = 8.3 Hz, 1H), 4.90 (d, J = 14.4 Hz, 1H), 4.59 (d, J = 14.7 Hz, 1H), 3.98 (d, J = 12.8 Hz, 2H), 3.39 - 3.52 (m, 1H), 2.69 (br. s., 2H), 1.90 (d, J = 15.1 Hz, 1H), 1.64 (d, J = 7.2 Hz, 1H), 1.57 (d, J = 13.6 Hz, 1H), 1.39 (s, 9H), 1.06 - 1.26 (m, 2H). LC-MS: 239 [M – *t*Butyl +H]$^+$, R_t = 2.556 min.

2-[1-(Oxetane-3-carbonyl)-piperidin-4-yl]-1,2,4,10-tetrahydro-3-oxa-10-aza-phenanthren-9-one (**5**). A mixture of **15** (210 mg, 546 μmol), trifluoroacetic acid (3.7 g, 2.5mL, 32.4 mmol) , and dichloromethane (2.5 mL) was stirred for 20 min at room temperature and then concentrated *in vacuo*. The residue was re-evaporated twice from acetonitrile, twice from acetonitrile containing 5 drops 0.5 N HCl and once from acetonitrile to give 4-(9-oxo-1,4,9,10-tetrahydro-2H-3-oxa-10-aza-phenanthren-2-yl)-piperidinium chloride 290 mg of as a sticky, light brown solid. LC-MS: 285.2 $[M+H]^+$, R_t = 1.345 min. A mixture of 4-(6-oxo-3,4,5,6-tetrahydro-1H-pyrano[4,3-c]isoquinolin-3-yl)piperidinium chloride (31.4 mg, 110 μmol), DIPEA (42.7 mg, 57.7 μl, 330 μmol) and oxetane-3-carboxylic acid (16.5 mg, 165 μmol, Eq: 1.50) were combined in DMF (1.00 mL). 1-(3-Dimethylaminopropyl-3-ethylcarbodiimide hydrochloride (31.6 mg, 165 μmol) was added and the mixture was stirred at room temperature overnight. The reaction mixture was distributed into 50 mL ethyl acetate, washed with 10 mL 0.2 N hydrochloric acid, water and saturated sodium bicarbonate. The aqueous layers were back-extracted with 2 x 20 mL ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered and evaporated to a white solid. The crude material was pre-absorbed onto silica and purified by flash chromatography (SiO_2, 0% to 100% of 5% MeOH/EtOAc in hexanes) to afford 2-[1-(oxetane-3-carbonyl)-piperidin-4-yl]-1,2,4,10-tetrahydro-3-oxa-10-aza-phenanthren-9-one **5** (18.6 mg, 46%) as a white solid. ^{1}H NMR (DMSO-d6) δ: 11.20 (br. s., 1H), 8.19 (dd, J = 7.9, 1.1 Hz, 1H), 7.64 (td, J = 8.0, 1.0 Hz, 1H), 7.42 (t, J = 7.7 Hz, 1H), 7.38 (d, J = 8.3 Hz, 1H), 5.20 (m, 2H), 4.85 (m, 1H), 4.45 (m, 3H), 3.80 (m, 4H), 3.46 (m, 1H), 1.90 (m, 1H), 1.76 (m, 4H), 1.57 (m, 1H), 1.48 - 1.32 (m, 2H). LC-MS: 369.0 $[M+H]^+$, R_t = 1.833 min.

3-(9-Oxo-1,4,9,10-tetrahydro-2H-3-oxa-10-aza-phenanthren-2-yl)-pyrrolidine-1-carboxylic acid *tert*-butyl ester (**16**). The title compound was prepared according to the procedure previously described for **3** starting from **13e** and **14a**. 3-(9-Oxo-1,4,9,10-tetrahydro-2H-3-oxa-10-aza-phenanthren-2-yl)-pyrrolidine-1-carboxylic acid *tert*-butyl ester **16** was isolated via filtration of the reaction mixture as an off-white solid. The filtrate was purified by flash chromatography (SiO_2, 0% to 100% EtOAC in heptane) to afford additional material which was combined with the precipitate. The combined material (721 mg, 40%) was taken on to the next step without further purification. ^{1}H NMR (DMSO-d_6) δ: 11.24 (s, 1H), 8.18 (dd, J = 7.9, 1.1 Hz, 1H), 7.69 (t, J = 7.9 Hz, 1H), 7.40 (d, J = 7.9 Hz, 1H), 7.45 (t, J = 7.7 Hz, 1H), 4.90 (dd, J = 14.4, 4.5 Hz, 1H), 4.63 (d, J = 14.7 Hz, 1H), 3.44 - 3.68 (m, 2H), 3.27 - 3.44 (m, 2H), 2.98 - 3.26 (m, 2H), 2.30 - 2.43 (m, 1H), 1.82 - 2.12 (m, 1H), 1.54 - 1.77 (m, 1H), 1.39 (s, 9H). LC-MS: 315 $[M - \text{tert-butyl} + H]^+$, R_t = 2.068 min., 2.121 min.

2-(1-Methanesulfonyl-pyrrolidin-3-yl)-1,2,4,10-tetrahydro-3-oxa-10-aza-phenanthren-9-one (**6**). Acetyl chloride (200 uL, 2.81 mmol) was added to methanol (3 mL) at 0 °C. The reaction was allowed to stir at room temperature for 5 min. The freshly prepared hydrochloric acid in methanol was combined with a mixture of **16** in methanol (3 mL). The reaction mixture was stirred at room temperature for 72 hr and then concentrated *in vacuo*. 2-Pyrrolidin-3-yl-1,2,4,10-tetrahydro-3-oxa-10-aza-phenanthren-29-one hydrochloride (123 mg, 99%) was taken on to the next reaction without purification. LC-MS: 271 $[M + H]^+$, R_t = 0.786 min. Methanesulfonyl chloride (31 µl, 399 µmol) was added to a solution of 2-pyrrolidin-3-yl-1,2,4,10-tetrahydro-3-

oxa-10-aza-phenanthren-29-one hydrochloride (102 mg, 332 μmol) and diisopropylethyl amine (116 ul, 665 μmol) in NMP (1.00 mL). The mixture was allowed to stir at room temperature for 18 h. The resulting mixture was poured into 5 mL ethyl acetate and washed with 1 M sodium hydroxide. The organics was dried on sodium sulfate and concentrated *in vacuo* to afford **6** as a white solid (50 mg, 43%). ^{1}H NMR (DMSO-d_6) δ: 11.26 (d, J = 4.5 Hz, 1H), 8.19 (dd, J = 8.3, 1.1 Hz, 1H), 7.69 (td, J = 7.6, 1.5 Hz, 1H), 7.41 (d, J = 7.9 Hz, 1H), 7.45 (t, J = 7.6 Hz, 1H), 4.91 (dd, J = 14.2, 4.0 Hz, 1H), 4.64 (d, J = 14.4 Hz, 1H), 3.53 - 3.74 (m, 1H), 3.46 (dd, J = 10.2, 7.9 Hz, 1H), 3.01 - 3.28 (m, 2H), 2.90 (d, J = 3.0 Hz, 3H), 1.67 - 2.22 (m, 2H). LC-MS: 313 $[M+H]^+$, R_t = 2.091 min., 2.155 min.

2-(2-Benzyloxy-1,1-dimethyl-ethyl)-1,2,4,10-tetrahydro-3-oxa-10-aza-phenanthren-9-one (17). The title compound was prepared according to the procedure previously described for **3** starting from **12c** and **14a**. 2-(2-benzyloxy-1,1-dimethyl-ethyl)-1,2,4,10-tetrahydro-3-oxa-10-aza-phenanthren-9-one **17** was obtained as an off-white solid (620 mg, 30%). The material was taken to the next step without further purification. ^{1}H NMR (DMSO-d_6) δ: 11.19 (s, 1H), 8.18 (dd, J = 8.3, 1.1 Hz, 1H), 7.68 (td, J = 8.3, 1.5 Hz, 1H), 7.44 (t, J = 7.2 Hz, 1H), 7.39 (d, J = 8.3 Hz, 1H), 7.23 - 7.36 (m, 5H), 4.92 (d, J = 14.4 Hz, 1H), 4.55 (d, J = 15.5 Hz, 1H), 4.48 (d, J = 3.0 Hz, 2H), 3.60 (dd, J = 11.1, 3.2 Hz, 1H), 3.35 (d, J = 8.7 Hz, 1H), 3.27 (d, J = 8.7 Hz, 1H), 2.62 (dd, J = 17.0, 11.0 Hz, 1H), 2.39 (d, J = 16.6 Hz, 1H), 0.96 (s, 3H), 0.92 (s, 3H). LC-MS: 364 $[M+H]^+$, R_t = 2.755 min.

2-(2-Hydroxy-1,1-dimethyl-ethyl)-1,2,4,10-tetrahydro-3-oxa-10-aza-phenanthren-9-one (7). A mixture of **17** (100 mg, 0.275 mmol), Pearlman's catalyst (50 mg), and concentrated hydrochloric acid (1 drop) in 1:1 ethanol/dioxane (10 mL) was placed under a hydrogen atmosphere (3 bars) and reacted at room temperature for 8 h. The mixture was filtered through a pad of celite and the filtrate concentrated *in vacuo*. The crude material was purified by flash chromatography (SiO_2, 0% to 100% ethyl acetate in heptanes) to afford 2-(2-hydroxy-1,1-dimethyl-ethyl)-1,2,4,10-tetrahydro-3-oxa-10-aza-phenanthren-9-one **7** as off-white solid (18 mg, 24%). ^{1}H NMR (DMSO-d_6) δ: 11.20 (s, 1H), 8.18 (dd, J = 7.8, 1.1 Hz, 1H), 7.68 (td, J = 7.8, 1.0 Hz, 1H), 7.39 (d, J = 7.9 Hz, 1H), 7.44 (t, J = 7.6 Hz, 1H), 4.91 (d, J = 14.0 Hz, 1H), 4.57 (t, J = 5.3 Hz, 1H), 4.58 (d, J = 14.0 Hz, 1H), 3.54 (dd, J = 11.0, 3.0 Hz, 1H), 3.20 - 3.27 (m, 1H), 2.53 - 2.66 (m, 1H), 2.33 - 2.43 (m, 1H), 0.91 (s, 3H), 0.83 (s, 3H). LC-MS: 274 $[M+H]^+$, R_t = 2.264 min.

2-tert-butyl-1,2,4,10-tetrahydro-3-oxa-10-aza-phenanthren-9-one (**8**). The title compound was prepared according to the procedure previously described for **3** starting from **13b** and **14c**. 2-*tert*-Butyl-1,2,4,10-tetrahydro-3-oxa-10-aza-phenanthren-9-one **8** was obtained as a white solid (34 mg, 7%). ^{1}H NMR (DMSO-d_6) δ: 11.19 (br. s, 1H), 8.18 (dd, J = 7.9, 1.1 Hz, 1H), 7.61 - 7.77 (m, 1H), 7.40 (d, J = 7.9 Hz, 1H), 7.45 (d, J = 7.6 Hz, 1H), 4.94 (d, J = 14.0 Hz, 1H), 4.59 (dt, J = 14.2, 2.2 Hz, 1H), 3.30 - 3.37 (m, 1H), 2.36 - 2.55 (m, 2H), 0.94 (s, 9H). LC-MS: 362 $[M+H]^+$, R_t = 2.421 min.

2-Isopropyl-5-methyl-1,2,4,10-tetrahydro-3-oxa-10-aza-phenanthren-9-one (**9**). The title compound was prepared according to the procedure previously described for **3** starting from **13a** and **14c**. 2-Isopropyl-5-methyl-1,2,4,10-tetrahydro-3-oxa-10-aza-phenanthren-9-one **9** was obtained as a colorless crystalline solid (72 mg, 13%). ^{1}H NMR (DMSO-d_6) δ: 11.17 (br. s., 1H), 8.11 (dd, J = 7.7, 1.3 Hz, 1H), 7.45 (d, J = 6.8 Hz, 1H), 7.29 (t, J = 7.7 Hz, 1H), 5.12 (d, J = 14.0 Hz, 1H), 4.89 (dt, J = 14.0, 2.3 Hz, 1H), 3.28-3.34 (m, 1H), 2.58 (s, 3H), 1.72 (sxt, J = 6.7 Hz, 1H), 0.97 (d, J = 6.8 Hz, 3H), 0.92 (d, J = 6.8 Hz, 3H). LC-MS: 258 $[M+H]^+$, R_t = 2.375 min.

2-(2-Benzyloxy-1,1-dimethyl-ethyl)- 5-methyl-1,2,4,10-tetrahydro-3-oxa-10-aza-phenanthren-9-one (**18**). The title compound was prepared according to the procedure previously described for **3** starting from **12c** and **14c.** The crude material was purified by flash chromatography (SiO_2, 5% to 60% ethyl acetate in heptanes) to afford 2-(2-benzyloxy-1,1-dimethyl-ethyl)-5-methyl-1,2,4,10-tetrahydro-3-oxa-10-aza-phenanthren-9-one **18** as a white solid (77 mg, 5%). ^{1}H NMR (DMSO-d_6) δ: 11.14 (s, 1H), 8.10 (d, J = 7.9 Hz, 1H), 7.45 (d, J = 6.8 Hz, 1H), 7.24 - 7.38 (m, 6H), 5.12 (d, J = 14.0 Hz, 1H), 4.83 (d, J = 14.7 Hz, 1H), 4.51 (d, J = 12.5 Hz, 1H), 4.46 (d, J = 12.5 Hz, 1H), 3.55 (dd, J = 12.5, 1.9 Hz, 1H), 3.23 - 3.28 (m, 1H), 2.57 (s, 3H), 2.53 - 2.78 (m, 2H), 0.91 (s, 3H), 0.95 (s, 3H). LC-MS: 378 $[M+H]^+$, R_t = 2.892 min.

2-(2-hydroxy-1,1-dimethyl-ethyl)-5-methyl-1,2,4,10-tetrahydro-3-oxa-10-aza-phenanthren-9-one (**10**). A mixture of **18** (35 mg, 0.092 mmol), Pearlman's catalyst (50 mg), and HCl (1 drop) in dioxane (5 mL) was placed under a hydrogen atmosphere (3 bars) and reacted at room temperature for 28 h. The mixture was filtered through a pad of celite and the filtrate concentrated *in vacuo* to afford 2-(2-hydroxy-1,1-dimethyl-ethyl)-5-methyl-1,2,4,10-tetrahydro-3-oxa-10-aza-phenanthren-9-one as an off-white solid (24 mg, 92%). ^{1}H NMR (DMSO-d_6) δ: 11.15 (s, 1H), 8.10 (d, J = 7.6 Hz, 1H), 7.45 (d, J = 7.2 Hz, 1H), 7.28 (t, J = 7.6 Hz, 1H), 5.13 (d, J = 13.6 Hz, 1H), 4.88 (d, J = 14.0 Hz, 1H), 4.57 (t, J = 5.3 Hz, 1H), 3.51 (dd, J = 11.3, 3.0 Hz, 1H), 3.16 - 3.30 (m, 2H), 2.57 (s, 3H), 2.36 - 2.45 (m, 1H), 0.89 (s, 3H), 0.82 (s, 3H). LC-MS: 288 $[M +H]^+$, R_t = 2.072 min.

TNKS/IWR2 Binding Assay and fragment screen

Test compounds (600 µM stock in DMSO) were diluted 3 folds in series and 1.5 µl per well was transferred into black polypropylene 384-well microtiter plates (Thermal Scientific). Two microliters of 1.14 µM biotinylated-IWR2 [1] in Assay Buffer (50 mM Tris-HCl, pH 7.4, 100 mM NaCl, 1 mM MgCl2, 1 mM DTT and 0.2 mg/ml BSA, 0.025% NP40) containing 12.5 % DMSO and 25 µL of 91 nM Tankyrase 1 or Tankyrase 2 in Assay Buffer were added. Plates were centrifuged for 1 minute at 1200 rpm (Eppendorf) and incubated at room temperature for 30 minutes. An aliquot (9.5 µl per well) of detection solution containing 2.8 nM europium (Eu) conjugated Streptavidin (PerkinElmer) and 40 nM allophycocyanin (APC) conjugated anti-6His antibody (Columbia Biosciences) in Assay Buffer (without DTT) was added. After the samples were incubated on a plate shaker for 60 minutes at room temperature, the assay signals were monitored by reading excitation at 340 nm and emission fluorescence at 665 nm and 615 nm on an Envision reader. IC50 was calculated using Assay Analyzer and Condoseo software (GeneData). The lowest detectable IC50 value in this assay is at 30 nM due to assay sensitivity.

TCF-luciferase assay

DLD1-TCFluc cells were plated in 24 well plates at a concentration of 4×10^4 cells/well one day before compounds treatment. On the following day, serially diluted compounds were added to the cells in 1ml media and incubated for 24 hours. The cells were harvested and assayed for luciferase activity as follows: the media were aspirated from the plates and 600 ul diluted ONE-Glo Luciferase Assay solution (1:1 dilution with 1 x PBS) (Promega, Cat# E6120) were added. The plates were then incubated in the dark with shaking for 10 minutes. 100 µl of lysates from each well were aliquoted in triplicate into a 96 well black tissue culture plate. The luciferase activity was read with a luminometer. The EC50 of each compound was calculated using XLfit software.

Western blot analysis

DLD1 cells were plated in 6 well plates at a concentration of 7×10^5 cells/well one day before treatment. On the following day, the compounds were added to the cells and incubated for 24 hrs. Cells were harvested in lysis buffer (2.5% SDS, 20 uM Tris-HCL, 1 mM EDTA) and heated at 95°C in a heating block for 10 minutes. The lysates were then passed through Qiashredders (Qiagene, Cat# 79656) by centrifuging for 15 seconds. Gel electrophoresis and Western blot analysis of proteins were carried out with standard laboratory techniques. The Axin2 antibody is from Cell signaling (Cat# 2151). β-actin antibody is from Sigma Aldrich (Catalog# A2228).

RNA isolation and qRT-PCR

DLD1 cells were plated in 24 well plates at a concentration of 1 x 10^4 cells/well one day before compounds treatment one day before compounds treatment. On the following day, the compounds were added to the cells and incubated for 72 hrs. Cells were harvested in RNA lysis buffer (Qiagen). RNA isolation, and reverse transcription-PCR (RT-PCR) were conducted using standard laboratory techniques. The catalog numbers for each probe set were: Axin2 (Hs01063168_m1), TNFRSF19 (00218634_m1) and 18S (4319413E).

Tankyrase-2 cloning, expression, purification, crystallization, data collection and refinement

Cloning, expression, and purification of Tankyrase-2(His6-946-1162) was carried out following the protocol described by the Structural Genomics Consortium. [2] Briefly, Tankyrase-2(His6-946-1162) was cloned into vector pET25, and expressed in *Escherichia coli* Rosetta 2 DE3. The protein was purified using Ni^{+2} chelating chromatography followed by gel filtration. α-Chymotrypsin at 1:100 w/w was added to the final sample, and the protein was concentrated to >10 mg/ml in 500mM NaCl, 20mM HEPES pH 7.5, 2.0mM TCEP and 10% glcyerol.

Crystals were grown at 4°C by vapor diffusion in sitting drops against 30-40% PEG3350, 5% saturated ammonium sulfate, 0.1M Tris pH 8.5. Crystals grew for several days, after which they were soaked for 24 hours with compound, then flash frozen in 80% mother liquor and 20% glycerol. X-ray data for all three complexes were collected at SLS Beamline X10SA on a Pilatus 6M pixel array detector. Data was processed with XDS, scaled and reduced with Scala/Truncate using ccp4. [3, 4] The structure was solved by molecular replacement using structure 3KR8 as a search model. Subsequent refinement was carried out using CNX. [5] Details of data collection and structure refinement can be found in Table S2.

Table S2. Crystallographic data collection and refinement statistics. All datasets were collected on SLS Beamline X10SA using a Pilatus 6M pixel detector. Number in parentheses denotes value for highest shell.

Parameters	Compound **1** (5C5R)	Compound **9** (5C5P)	Compound **7** (5C5Q)
Space group	C2221	C2221	C2221
Cell dimensions (Å)	91.2, 98.1, 119.33	90.6, 99.2, 118.0	90.8, 98.5, 117.3
Resolution (Å)	1.55	2.0	1.75
Completeness	99.8 (99.8)	99.9 (99.9)	100.0 (100.0)
Multiplicity	6.4 (6.3)	6.5 (6.8)	6.6 (6.7)
R_{sym}	0.063 (0.371)	0.093(0.435)	0.045 (0.445)
Total reflections in refinement	76896 (12007)	36148 (5652)	53201 (8344)
Reflections in R_{free}	3851 (669)	1791 (303)	2635 (448)
R_{factor}/R_{free}	0.188/0.202	0.192/0.220	0.181/0.202
Average B			
Protein	20.3	31.1	26.4
Ligand	14.1	22.4	21.0
Water/Ions	32.9/37.1	41.6/46.9	39.1/42.6
RMS deviations			
Bond lengths (Å)	0.375	0.371	0.381
Bond angles (deg)	1.9	1.6	1.8

Determination of stability in hepatic microsomes.

Microsome preparations prepared from male and female rat or human livers were obtained from BD Biosciences (Woburn, MA). Test compounds were diluted from 10 mM stock solutions in DMSO; incubations contained <1% residual DMSO. Incubations were performed in 96-well deep-well plates with a final incubation volume of 600 µL. Incubations contained 1-2 µM test compound, 0.5 mg/mL liver microsomes and NADPH regenerating system. Aliquots of 50 µL were removed at timepoints from 1 to 45 minutes, quenched in acetonitrile containing internal standard, and cooled prior to centrifugation and analysis by LC-MS/MS. The natural log of the ratio of the test compound peak area to the internal standard peak area was plotted again incubation time and the slope of a linear fit of the initial rate of compound disappearance was used to calculate the intrinsic clearance.

Cl_{int} (µL/min/mg = -slope (min^{-1}) * 1000 / [protein concentration (mg/mL)]

Caco-2 permeability assay.

Caco-2 cells were seeded in collagen coated 24-transwell plates at a density of 4.55 x 105 cells/cm2 and maintained in medium for 7-9 days. The pH values in the apical and basolateral wells were 6.5 and 7.4, respectively. Compounds were added to the donor wells at a target concentration of 50 µM. Concentrations in the donor and receiver wells were determined by LC/MS/MS after 60 minutes. The apparent permeability (Papp) was calculated using the following equation where *C* is the receiver drug concentration, *t* is time in seconds, *V* is the volume of the receiver chamber in cm3, *Co* is the initial drug concentration in the donor chamber and *A* is the surface area of the filter in cm^2:

P_{app} (μL/min/mg) = (dC/dt)* V / (C * A)

Pharmacokinetic studies.

All studies were conducted under IACUC-approved protocols and animals were allowed free access to food and water. Male Wistar/Han (CRL:WI) rats (Charles River Laboratories, Hollister, CA or Raleigh, NC) typically weighed between 180 and 300 g and were surgically implanted with cannulas in the jugular and/or femoral veins. Single intravenous bolus doses were administered into the jugular cannula of dual-cannulated animals; the femoral cannulas were used for blood collection. Compounds in screening IV studies were typically administered to two rats per study as cocktails containing four test compounds plus a control compound with known PK; doses in these studies were 0.5 mg/kg. The lead compound 10 was administered individually to two rats at a dose of 1 mg/kg.

Male C57BL/6J mice (The Jackson Laboratory, Bar Harobor, ME) typically weighed 25 ± 10 g. Two animals were used per timepoint in a composite design; with blood collected twice from each animal via the retro-orbital sinus under isoflurane/O_2 anesthesia. Single iv doses were administered via the tail vein and oral doses were administered by gavage.

For iv studies, blood was collected into tubes containing K2-EDTA at typically 2 (cocktail studies only), 5, 15, and 30 minutes, and 1, 2, 4, 7 and 24 h after dosing into tubes containing EDTA and plasma was prepared by centrifugation. The 2 and 5 minute time points were omitted for po studies. Plasma concentrations were determined by a liquid chromatography/tandem mass spectrometry method (LC/MS/MS) following protein precipitation with acetonitrile. Non-compartmental pharmacokinetic analysis was done on mean plasma data using Watson Lims Software.

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