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**From PARP1 to TNKS2 Inhibition: A Structure-Based Approach**

# SUPPORTING INFORMATION

## Crystallographic Studies

### Recombinant protein (PARP1) expression and purification

Human hPARP1c (residue 663-1014) was expressed with an *N*-terminal His6-SUMO tag in *E. coli* codon+ in LB medium at 37°C until an OD600 of 0.8 was reached. After a 30 min cold shock on ice, expression was induced with 0.5 mM IPTG and the cells were grown overnight at 20°C. Harvested cells were disrupted in lysis buffer (50 mM TRIS pH 7.5 200 mM NaCl) by micro fluidization. Cleared lysate was subjected to affinity chromatography on His-Tag purification resin (Roche), eluted protein dialysed against lysis buffer during Senp2-cleavage of the His6-SUMO tag, and the tag removed through reverse affinity chromatography. Wash buffer for affinity chromatography included 500 mM NaCl and 10 mM imidazole, and the elution buffer 250 mM imidazole. The protein was then passed over a Superdex 200 gel filtration column (GE Healthcare) in 25 mM TRIS pH 7.5, 150 mM NaCl. Eluted protein was concentrated and stored at -80 °C.

### hPARP1c/MC2050 complex crystallization and structure determination

hPARP1c stock (500 µM) was supplemented with MC2050 (20 mM in DMSO) in a 2-fold molar excess and crystallized at 20°C in sitting drops mixed from 0.3/0.3 µL protein/reservoir solution (0.1 M TRIS pH 8.5, 20% (w/v) PEG 2.000 MME and 0.01 M $NiCl_2$). 25% (v/v) glycerol was added to reservoir for cryoprotection and crystals flash frozen in liquid nitrogen. Diffraction data were collected at 100 K at a wavelength of 1.00002 Å on a PILATUS 2M-F detector at beamline X06DA of the Swiss Light Source at Paul Scherrer Institute (Villigen, Switzerland). Data were processed using XDS[1] and phases determined by "molecular replacement" with Phaser-MR[2] and a PARP1 structure (PDB ID 4ZZZ)[3] as a search model. Manual rebuilding was done in Coot[4] and model refinement with phenix.refine.[5] Structure figures were created using PyMOL (https://pymol.org/2/).

**Table S1.** Diffraction data and refinement statistics.

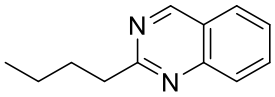
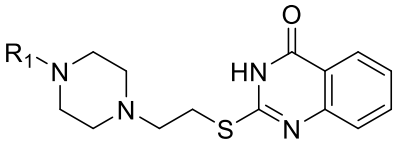
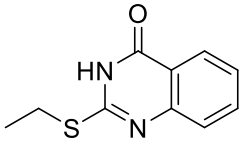
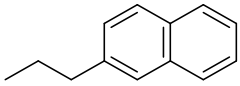
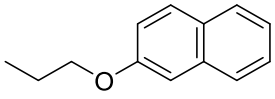
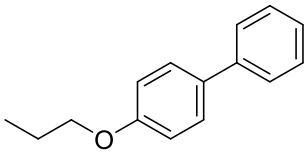
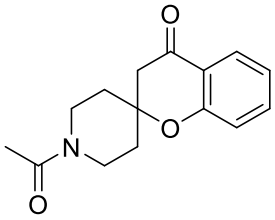
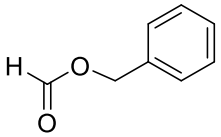
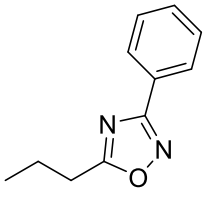
| hPARP1c/MC2050 | | | |
|---|---|---|---|
| **Diffraction Data** | | **Refinement** | |
| wavelength [Å] | 1.0000 | $R_{work}$ [%] | 22.6 (37.8) |
| resolution range [Å][a] | 71.73 - 2.00 (2.07 - 2.00) | $R_{free}$ [%] | 26.5 (40.1) |
| space group | P $2_1$ $2_1$ $2_1$ | rmsd (bonds) [Å] | 0.004 |
| unit cell [Å] | 68.17 86.73 127.64 | rmsd (angles) [°] | 0.64 |
| total reflections | 224 981 (21 188) | Ramachandran favored [%] | 98.7 |
| unique reflections | 96 382 (4 967) | Ramachandran outliers [%] | 0.00 |
| completeness [%] | 99.3 (97.3) | non-H-atoms | 5 916 |
| multiplicity | 2.3 (2.3) | protein | 5 466 |
| mean I/σ(I) | 7.7 (0.5) | ligand (MC2050) | 52 |
| Wilson B-factor | 42.9 | solvent (except water) | 38 |
| $R_{meas}$ [%] | 9.3 (203.5) | solvent (water) | 360 |
| $CC_{1/2}$ [%] | 99.8 (19.1) | average B-factor [$Å^2$] | 52.0 |
| | | protein [$Å^2$] | 52.0 |
| | | ligand (MC2050) [$Å^2$] | 37.7 |
| | | solvent (except water) [$Å^2$] | 70.3 |
| | | solvent (water) [$Å^2$] | 51.9 |

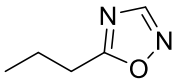
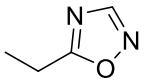
[a]Numbers in parentheses belong to the highest resolution shell.

**Computational Studies**

Dockings into the active site of PARP1 and TNKS2 (PDB: 1UK0)[6] were carried out by means of Molegro Virtual Docker (MVD) software (CLCbio).[7] To this end, the obtained three-dimensional (3D) structure of PARP1 was prepared by automatically assigning bond orders and hybridization and adding explicit hydrogens, charges, and Tripos atom types. A search space with a 15 Å radius, centred on the active-site cleft, was used for docking. The grid-based MolDock score with a grid resolution of 0.20 Å was used as a scoring function, and MolDock SE was used as a docking algorithm. 100 runs were defined. The retrieved poses were ranked according to their score (obtained with the scoring function "Rerank Score" that is implemented in MVD). Compounds were also re-docked with AutoDock v.4.2.5.1.[8] To this end, the Lamarckian genetic algorithm (LGA) implemented in AutoDock was used, using the following values: number of individuals in population of 150, maximum number of energy evaluations of 2,500,000, maximum number of generations of 27 × 103, and rate of gene mutation of 0.02. All other parameters were kept at their default values. Only those poses showing a similar (root mean square deviation [RMSD] of <2.0 Å) conformation, as assessed by MVD and AutoDock, were kept, and the first-ranking one was chosen for subsequent analysis. Scaffold hopping was carried out using the MOE2009 software (©CCG). To this end, the "Scaffold Replacement" application was used. Starting from MC2050, the 2-mercapto-quinazolinone and ethylene-piperazinyl moieties were kept fixed, while the pyridine ring was defined as a changeable ($R_1$-) group (Table S2). The "Add Group to Ligand", which binds new fragments to a given fixed scaffold molecule, was selected. No default filters (usually a default set of three descriptors are applied: molecular weight, SlogP and TPSA) were used for searching an in-house database of fragments, derived from the default MOE.mdb database. "Minimize Generated Structures" and "London dG" as scoring functions were selected. All the other parameters were kept at their default values. The obtained scaffolds were redocked with MVD as previously detailed and ranked according to the re-rank score to select among the results. Finally, the best 10 compounds according to the score were considered for further investigation and among them the best 5 different chemotypes were selected for synthesis.

**Table S2. Scaffold hopping approach**. The fixed scaffold and the final 10 $R_1$ groups that were chosen for further analysis are displayed.

| **$R_1$** | | | **Fixed Scaffold** |
|---|---|---|---|
| **IUPAC Name** | **Scaffold** | **MVD RS** | |
| 2-butylquinazoline |  | -137 |  2-((2-(Piperazin-1-yl)ethyl)thio)quinazolin-4(3H)-one |
| 2-(ethylthio)quinazolin-4(3*H*)-one |  | -189 | |
| 2-propylnaphthalene |  | -98 | |
| 2-propoxynaphthalene |  | -118 | |
| 4-propoxy-1,1'-biphenyl |  | -157 | |
| 1'-acetylspiro[chromane-2,4'-piperidin]-4-one |  | -162 | |
| benzyl formate |  | -123 | |
| 3-phenyl-5-propyl-1,2,4-oxadiazole |  | -160 | |

| | | | |
|---|---|---|---|
| 5-propyl-1,2,4-oxadiazole |  | -141 | |
| 5-ethyl-1,2,4-oxadiazole |  | -122 | |

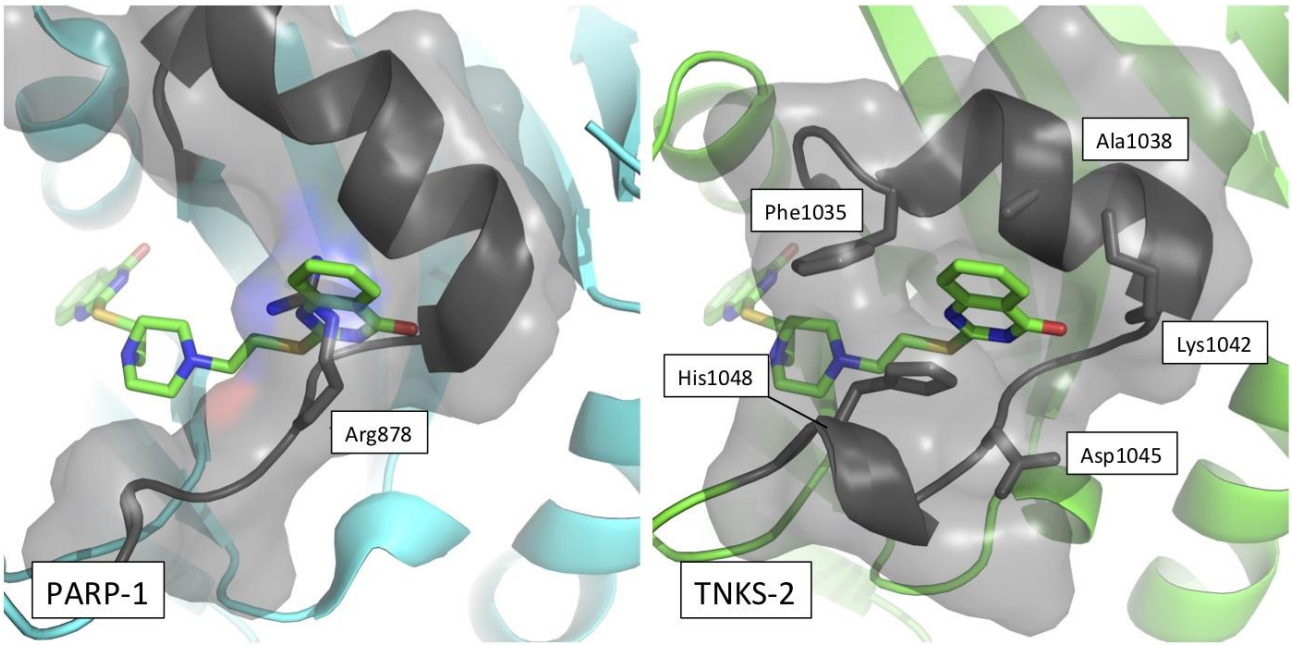



**Figure S1.** Structural comparison between the predicted binding mode of **5** (green) in the active sites of PARP1 (left) and TNKS2 (right). The D-loop is shown in grey ribbons. In PARP1, Arg878 hampers an energetically favorable binding mode of compound **5**. Conversely, in TNKS2 the same conformation is accommodated in a pocket formed by residues Phe1035, Ala1038, Lys1042, Asp1045 and His 1048.

## EXPERIMENTAL SECTION

**Chemistry**. Melting points were determined on a Stuart melting point apparatus SMP10. $^{1}$H-NMR and $^{13}$C-NMR spectra were recorded at 400 MHz and 100 MHz, respectively, by using a Bruker AC 400 spectrometer; chemical shifts are reported in δ (ppm) units relative to the internal reference tetramethylsilane ($Me_4Si$). Microwave-assisted reactions were performed with a Biotage Initiator (Uppsala, Sweden) high frequency microwave synthesizer working at 2.45 GHz, fitted with magnetic stirrer and sample processor; reaction vessels were Biotage microwave glass vials sealed with applicable cap; temperature was controlled through the internal IR sensor of the microwave apparatus. Mass spectra were recorded on an API-TOF Mariner by Perspective Biosystem (Stratford, Texas, USA), and samples were injected by a Harvard pump using a flow rate of 5-10 μL/min, infused in the Electrospray system. All compounds were routinely checked by TLC and $^{1}$H-NMR. Final compounds **1-6** have been also checked by $^{13}$C-NMR. TLC was performed on aluminum-backed silica gel plates (Merck DC, Alufolien Kieselgel 60 F254) with spots visualized by UV light or using a KMnO4 alkaline solution. All solvents were reagent grade and, when necessary, were purified and dried by standard methods. Concentration of solutions after reactions and extractions involved the use of a rotary evaporator operating at reduced pressure of ~ 20 Torr. Organic solutions were dried over anhydrous sodium sulfate. Elemental analysis has been used to determine purity of the final compounds **1-6** that is > 95%. Analytical results are within ± 0.40% of the theoretical values. All chemicals were purchased from Sigma Aldrich srl, Milan (Italy) or from TCI Europe NV, Zwijndrecht (Belgium), and were of the highest purity. As a rule, samples prepared for physical and biological studies were dried in high vacuum over phosphorus pentoxide for 20 h at temperatures ranging from 25 to 40 °C, depending on the sample melting point.

**Synthesis of 2-((2-(4-(3-([1,1'-biphenyl]-4-yloxy)propyl)piperazin-1-yl)ethyl)thio)quinazolin-4(3*H*)-one (1).** A microwave vial was charged with a solution of **8** (102 mg, 0.3 mmol, 1 equiv) and commercial 2-mercaptoquinazolin-4(3*H*)-one (53 mg, 0.3 mmol, 1 equiv) in anhydrous DMF (1 mL).

The reaction was stirred at 0 °C under inert atmosphere for 5 min, then a 1M solution of trimethylphosphine in toluene (660 µL, 0.66 mmol, 2.2 equiv) and DIAD (118 µL, 0.6 mmol, 2 equiv) were added and the obtained mixture was heated in a microwave reactor at 40 °C for 30 min. The reaction was quenched with brine (10 mL) and extracted with ethyl acetate (4 x 10 mL). The combined organic layers were washed with brine (10 mL), dried over anhydrous $Na_2SO_4$, filtered and the organic solvents removed under reduced pressure to afford a pale yellow crude which was purified by silica gel column chromatography (chloroform/methanol 95:5 v/v) to afford 73 mg (0.147 mmol, 49%) of the product as an off-white solid. $^1$H-NMR (400 MHz, $CDCl_3$) δ ppm: 13.95 (bs, 1H, CON*H*), 8.23 (d, 1H, C*H* quinazolinone ring), 7.72 (t, 1H, C*H* quinazolinone ring), 7.61-7.53 (m, 5H, C*H* quinazolinone and biphenyl rings), 7.46-7.39 (m, 3H, C*H* biphenyl ring), 7.34-7.30 (m, 1H, C*H* biphenyl ring), 7.00 (d, 2H, C*H* biphenyl ring), 4.11 (t, 2H, $CH_2CH_2CH_2O$), 3.21-3.19 (m, 2H, $SCH_2CH_2$), 2.98-2.95 (m, 2H, $SCH_2CH_2$), 2.90-2.73 (m, 10H, $CH_2CH_2CH_2O$, 4x$CH_2$ piperazine ring), 2.07 (q, 2H, $CH_2CH_2CH_2O$). $^{13}$C-NMR (100 MHz, $CDCl_3$) δ ppm: 26.9, 29.8, 51.6 (2C), 54.1 (2C), 54.8, 60.9, 66.3, 114.8 (2C), 120.6, 125.9, 126.4, 126.5, 126.6, 126.7 (2C), 128.1 (2C), 128.7 (2C), 133.7, 134.6, 140.9, 149.1, 155.6, 159.6, 163.1. Anal. ($C_{29}H_{32}N_4O_2S$) Calcd (%): C, 69.57; H, 6.44; N, 11.19; S, 6.40. Found (%): C, 69.77; H, 6.46; N, 11.05; S, 6.35. MS (ESI-MS): Calculated: 501.7 for $C_{29}H_{33}N_4O_2S$ $[M+H]^+$, Found: 501.2. Mp: 182-184 °C.

**Synthesis of 2-((2-(4-(3-(3-phenyl-1,2,4-oxadiazol-5-yl)propyl)piperazin-1-yl)ethyl)thio)quinazolin-4(3*H*)-one (2).** A microwave vial was charged with a solution of **10** (95 mg, 0.3 mmol, 1 equiv) and commercial 2-mercaptoquinazolin-4(3*H*)-one (53 mg, 0.3 mmol, 1 equiv) in anhydrous DMF (1 mL). The reaction was stirred at 0 °C under inert atmosphere for 5 min, when a 1M solution of trimethylphosphine in toluene (660 µL, 0.66 mmol, 2.2 equiv) and DIAD (118 µL, 0.6 mmol, 2 equiv) were added and the so obtained mixture was heated in a microwave reactor at 40 °C for 30 min. The reaction was quenched with brine (12 mL) and extracted with ethyl acetate (4 x 10 mL). The combined organic layers were washed with brine (10 mL), dried over anhydrous $Na_2SO_4$,

filtered and organic solvents removed under reduced pressure to afford a pale yellow oil which was purified by silica gel column chromatography (chloroform/methanol 95:5 v/v) to afford 61 mg (0.129 mmol, 43%) of the product as pale grey-yellow solid. $^{1}$H-NMR (400 MHz, $CDCl_3$) δ ppm: 13.94 (bs, 1H, CON*H*), 8.21 (d, 1H, C*H* quinazolinone ring), 8.11-8.20 (d, 2H, C*H* benzene ring), 8.10 (d, 1H, C*H* quinazolinone ring), 7.58 (d, 1H, C*H* quinazolinone ring), 7.52-7.50 (m, 3H, C*H* benzene ring), 7.40 (t, 1H, C*H* quinazolinone ring), 3.16-3.15 (m, 2H, SC*H*$_2$CH$_2$), 3.07-3.03 (m, 2H, CH$_2$CH$_2$C*H*$_2$-oxadiazole), 2.62-2.91 (m, 12H, SCH$_2$C*H*$_2$, C*H*$_2$CH$_2$CH$_2$-oxadiazole, and 4xC*H*$_2$ piperazine ring), 2.16-2.10 (m, 2H, CH$_2$C*H*$_2$CH$_2$-oxadiazole). $^{13}$C-NMR (100 MHz, $CDCl_3$) δ ppm: 23.9, 24.8, 29.8, 51.3 (2C), 54.1 (2C) , 56.9, 60.9, 120.6, 125.9, 126.4, 126.5 (2C), 127.0 (2C), 127.4, 128.9, 131.1, 134.6, 149.1, 155.7, 163.1, 168.2, 180.0. Anal. ($C_{25}H_{28}N_6O_2S$) Calcd (%): C, 63.00; H, 5.92; N, 17.63; S, 6.73. Found (%): C, 63.18; H, 5.94; N, 17.51; S, 6.65. MS (ESI-MS): Calculated: 477.2 for $C_{25}H_{29}N_6O_2S$ $[M+H]^+$, Found: 477.2. Mp: 117-119 °C.

**Synthesis of 1'-(2-(4-(2-((4-oxo-3,4-dihydroquinazolin-2-yl)thio)ethyl)piperazin-1-yl)acetyl)spiro[chromane -2,4'-piperidin]-4-one (3).** A microwave vial was charged with a solution of **13** (124 mg, 0.32 mmol, 1 equiv) and commercial 2-mercaptoquinazolin-4(3*H*)-one (57 mg, 0.32 mmol, 1 equiv) in anhydrous DMF (1.2 mL). The reaction was stirred at 0 °C under inert atmosphere for 5 min, then a 1M solution of trimethylphosphine in toluene (700 µL, 0.70 mmol, 2.2 equiv) and DIAD (125 µL, 0.64 mmol, 2 equiv) were added and the resulting mixture was heated in a microwave reactor at 40 °C for 30 min. The reaction was quenched with brine (12 mL) and extracted with ethyl acetate (4x15 mL). The combined organic layers were washed with brine (12 mL), dried over anhydrous $Na_2SO_4$, filtered and volatiles removed under reduced pressure to afford a pale yellow oil which was purified by silica gel column chromatography (chloroform/methanol 96:4 v/v) to afford 108 mg (0.2 mmol, 62%) of the product as a pale yellow solid. $^{1}$H-NMR (400 MHz, $CDCl_3$) δ ppm: 13.96 (bs, 1H, CON*H*), 8.20 (d, 1H, C*H* quinazolinone ring), 7.90 (d, 1H, C*H* chromanone ring), 7.72 (t, 1H, C*H* quinazolinone ring), 7.59 (d, 1H, C*H* quinazolinone ring), 7.54 (t, 1H, C*H* quinazolinone

ring), 7.41 (t, 1H, C*H* chromanone ring), 7.08-7.02 (m, 2H, C*H* chromanone ring), 4.41 (m, 1H, CH*H*-N(piperidine)-CHH), 3.82 (m, 1H, C*H*H-N(piperidine)-CHH), 3.54-3.48 (m, 2H, $CH_2CH_2$-N(piperidine)-C*H*$_2$CH$_2$), 3.40-3.36 (m, 2H, SC*H*$_2$CH$_2$), 3.22-3.11 (m, 3H, SCH$_2$C*H*$_2$, COCH*H*-piperazine), 2.97-2.80 (m, 9H, 4x$CH_2$ piperazine, COC*H*H-piperazine), 2.75 (d, 2H, COC*H*$_2$-C(spiro)), 2.17-2.10 (m, 2H, C*H*HCH$_2$-N(piperidine)-CH$_2$CH*H*), 1.66-1.77 (m, 2H, CH*H*CH$_2$-N(piperidine)-CH$_2$C*H*H). $^{13}$C-NMR (100 MHz, $CDCl_3$) δ ppm: 29.5, 34.1. 34.6, 37.3, 40.8, 48.1 (2C), 51.7 (2C), 53.8, 59.9, 60.5, 77.7, 118.3, 120.5, 120.7, 121.5, 126.0, 126.4, 126.5, 126.7, 134.7, 136.5, 149.1, 155.5, 158.9, 163.0, 167.7, 191.4. Anal. ($C_{29}H_{33}N_5O_4S$) Calcd (%): C, 63.60; H, 6.07; N, 12.79; S, 5.85. Found (%): C, 63.79; H, 6.09; N, 12.64; S, 5.78. MS (ESI-MS): Calculated: 548.2 for $C_{29}H_{34}N_5O_4S$ $[M+H]^+$, Found: 548.2. Mp: 147-149 °C.

**Synthesis of benzyl 4-(2-((4-oxo-3,4-dihydroquinazolin-2-yl)thio)ethyl)piperazine-1-carboxylate (4).** 2-Mercaptoquinazolin-4(3*H*)-one (672 mg, 3.77 mmol, 1 equiv) and **15** (1.48 g, 4.50 mmol, 1.2 equiv) were dissolved in anhydrous DMF (8 mL) in a round bottom flask, then $K_2CO_3$ (677 mg, 4.90 mmol, 1.3 equiv) and sodium iodide (678 mg, 4.50 mmol, 1.2 equiv) were added and the resulting suspension was stirred at rt overnight. The reaction was quenched with water (80 mL) and extracted with ethyl acetate (4x80 mL). The organic layers were combined, washed with brine (30 ml), dried over anhydrous $Na_2SO_4$, filtered and volatiles removed under reduced pressure. The obtained yellow oil was purified by silica gel column chromatography (petroleum ether/ethyl acetate 70:30 v/v) to afford 795 mg (1.89 mmol, 50%) of the product as an off white solid. $^1$H-NMR (400 MHz, $CDCl_3$) δ ppm: 13.36 (bs, 1H, CON*H*), 8.20 (d, 1H, C*H* quinazolinone ring), 7.74-7.70 (m, 1H, C*H* quinazolinone ring), 7.60 (d, 1H, C*H* quinazolinone ring), 7.44-7.34 (m, 6H, C*H* quinazolinone and benzene rings), 5.17 (s, 2H, C*H*$_2$Ph), 3.84 (m, 4H, 2x$CH_2$ piperazine ring), 3.26-3.23 (m, 2H, SC*H*$_2$CH$_2$), 2.96-2.94 (m, 2H, SCH$_2$C*H*$_2$), 2.72-2.70 (m, 4H, 2x$CH_2$ piperazine ring). $^{13}$C-NMR (100 MHz, $CDCl_3$) δ ppm: 29.4, 42.8 (2C), 53.6 (2C), 60.4, 67.3, 120.4, 126.1, 126.4, 126.5, 127.9 (2C), 128.1 (2C), 128.5, 134.8, 136.6, 149.1, 155.1, 155.2, 163.1. Anal. ($C_{22}H_{24}N_4O_3S$) % Calcd: C, 62.25;

H, 5.70; N, 13.20; S, 7.55. Found (%): C, 62.45; H, 5.72; N, 13.15; S, 7.44. MS (ESI-MS): Calculated: 425.2 for $C_{22}H_{25}N_4O_3S$ $[M+H]^+$, Found: 425.2. Mp:109-112 °C.

**Synthesis of 2,2'-((piperazine-1,4-diylbis(ethane-2,1-diyl))bis(sulfanediyl))bis(quinazolin-4(3*H*)-one) (5).** To a solution of **16** (53 mg, 0.30 mmol) in anhydrous DMF (2 mL), commercial 2-mercaptoquinazolin-4(3*H*)-one (103 mg, 0.60 mmol, 2 equiv) was added. The reaction was stirred at 0 °C under inert atmosphere for 5 min, then a 1M solution of trimethylphosphine in toluene (1.32 mL, 1.32 mmol, 4.4 equiv) and DIAD (240 µL, 1.20 mmol, 4 equiv) were added. The mixture was heated in a microwave reactor at 40 °C for 30 min. The reaction was quenched with brine (20 mL) and extracted with ethyl acetate (4x20 mL). The combined organic layers were washed with brine (20 mL), dried over anhydrous $Na_2SO_4$ and volatiles removed under reduced pressure to afford a crude which was purified by silica gel column chromatography (chloroform/methanol 98:2 v/v) to afford 93 mg (0.189 mml, 63%) of the desired product as a white solid. $^1$H-NMR (400 MHz, $CDCl_3$) δ ppm: 11.5 (bs, 2H, 2 x CON*H*), 8.22-8.20 (m, 2H, C*H* quinazolinone rings), 7.75-7.71 (m, 2H, C*H* quinazolinone rings), 7.63-7.61 (m, 2H, C*H* quinazolinone rings), 7.44-7.40 (m, 2H, C*H* quinazolinone rings), 3.23-3.18 (m, 16H, 2 x S$CH_2CH_2$-piperazine, and 4xC$H_2$ piperazine ring). $^{13}$C-NMR (100 MHz, $CDCl_3$). δ ppm: 29.8 (2C), 52.2 (4C), 60.9 (2C), 120.5 (2C), 126.0 (2C), 126.4 (2C), 126.6 (2C), 134.7 (2C), 149.2 (2C), 155.8 (2C), 163.5 (2C). Anal. ($C_{24}H_{26}N_6O_2S_2$) Calcd (%): C, 58.28; H, 5.30; N, 16.99; S, 12.96. Found (%): C, 58.47; H, 5.32; N, 16.85; S, 12.88. MS (ESI-MS): Calculated: 495.2 for $C_{24}H_{27}N_6O_2S_2$ $[M+H]^+$, Found: 495.2. Mp: 234-236 °C (with decomposition).

**Synthesis of 1'-(3-((4-oxo-3,4-dihydroquinazolin-2-yl)thio)propanoyl)-1*H*-spiro[benzo[*g*]quinazoline-2,4'-piperidin]-4(3*H*)-one (6).** Intermediates **18** (80 mg, 0.3 mmol, 1 equiv) and **20** (75 mg, 0.30 mmol, 1 equiv) were placed in a round bottom flask and dissolved in anhydrous DMF (2 mL). Afterwards, *N*-(3-dimethylaminopropyl)-*N′*-ethyl-carbodiimide hydrochloride (75 mg, 0.39 mmol, 1.3 equiv), 1*H*-benzotriazol-1-ol (51 mg, 0.33 mmol, 1.1 equiv) and triethylamine (104 µL, 0.75 mmol, 2.5 equiv) were added and the resulting mixture stirred at rt

overnight under inert atmosphere. Upon completion the reaction mixture was quenched with $NaHCO_3$ s.s. (10 mL) and stirred for 1 h. The solid formed was filtered, washed with $NaHCO_3$s.s. and then dried in a laboratory oven to give 83 mg (0.17 mmol, 55%) of the title compound as a yellow solid. $^1$H-NMR (400 MHz, DMSO-*d*$_6$) δ ppm: 12.98 (bs, 1H, CON*H*-quinazolinone ring), 8.55 (s, 1H, C*H* dihydrobenzoquinazolinone ring), 8.28 (m, 1H, C*H* quinazolinone ring), 7.97 (d, 1H, C*H* dihydrobenzoquinazolinone ring), 7.83 (d, 1H, C*H* dihydrobenzoquinazolinone ring), 7.75 (t, 1H, C*H* quinazolinone ring), 7.62 (m, 1H, C*H* dihydrobenzoquinazolinone ring), 7.43-7.33 (m, 3H, C*H* quinazolinone and dihydrobenzoquinazolinone rings), 7.23-7.19 (m, 1H, C*H* quinazolinone ring), 7.10 (s, 1H, CON*H*-dihydrobenzoquinazolinone ring), 6.94 (s, 1H, N*H*-dihydrobenzoquinazolinone ring), 4.60 (m, 2H, spiropiperidine ring), 3.78-3.53 (m, 4H, spiropiperidine ring and SC*H*$_2$CH$_2$CO), 2.79-2.75 (t, 2H, COC*H*$_2$CH$_2$S), 1.84-1.80 (m, 4H, 2xCH$_2$ spiropiperidine ring). $^{13}$C-NMR (100 MHz, DMSO-*d*$_6$) δ ppm: 30.2, 37.3 (2C), 38.2, 42.9 (2C), 67.2, 108.7, 116.1, 116.3, 118.3, 123.0, 124.9, 125.9, 127.0, 127.7, 128.5, 128.9, 129.7, 135.9, 137.1, 139.8, 143.6, 159.8, 163.2, 168.8, 175.3. Anal. ($C_{27}H_{25}N_5O_3S$) Calcd (%): C, 64.91; H, 5.04; N, 14.02; S, 6.42. Found (%): C, 65.09; H, 5.06; N, 13.89; S, 6.34. MS (ESI-MS): Calculated: 500.6 for $C_{27}H_{26}N_5O_3S$ $[M+H]^+$, Found: 500.2. Mp: > 250 °C (with decomposition).

**Synthesis of 4-(3-bromopropoxy)-1,1'-biphenyl (7).** A round bottom flask was charged with [1,1'-biphenyl]-4-ol (1.5 g, 8.81 mmol, 1 equiv) and $K_2CO_3$ (2.4 g, 17.6 mmol, 2 equiv) and the reactants suspended in anhydrous acetonitrile (45 ml), then 1,3-dibromopropane (4.5 mL, 44.1 mmol, 5 equiv) was added and the mixture was stirred at 95 °C for 3 h under inert atmosphere. The organic solvent was evaporated, and the resulting mixture transferred into a separating funnel with ethyl acetate (40 mL) and $H_2O$ (40 mL). The aqueous phase was extracted with organic solvent portions (4x40 mL) and the combined layers washed with brine (20 mL). The organic phase was collected, dried over anhydrous $Na_2SO_4$, filtered and volatiles removed under reduced pressure to afford a crude which was purified by silica gel column chromatography (hexane/ethyl acetate 280:1 v/v) to give 1293 mg

(4.47 mmol, 76%) of the compound as a white solid. $^1$H-NMR (400 MHz, $CDCl_3$) δ ppm: 7.58-7.54 (m, 4H, C*H* biphenyl ring), 7.46-7.42 (m, 2H, C*H* biphenyl ring), 7.33 (t, 1H, C*H* biphenyl ring), 7.02-7.00 (m, 2H, C*H* biphenyl ring), 4.18 (t, 2H, $BrCH_2CH_2CH_2O$), 3.66 (t, 2H, $BrCH_2CH_2CH_2O$), 2.37 (m, 2H, $BrCH_2CH_2CH_2O$). MS (ESI-MS): Calculated: 291.0 for $C_{15}H_{16}BrO$ $[M+H]^+$, Found: 291.0. Mp: 70-71 °C.

**Synthesis of 2-(4-(3-([1,1'-biphenyl]-4-yloxy)propyl)piperazin-1-yl)ethan-1-ol (8).** Compound **7** (469 mg, 1.61 mmol, 1 equiv), sodium iodide (266 mg, 1.77 mmol, 1.1 equiv) and $K_2CO_3$ (334 mg, 2.47 mmol, 1.5 equiv) were placed in a round bottom flask under inert atmosphere and suspended in anhydrous DCM (4.5 mL). After five min of stirring, a solution of 2-(piperazin-1-yl)ethan-1-ol (1050 mg, 8.06 mmol, 5 equiv) in anhydrous DCM (4.5 mL) was added dropwise and the reaction allowed to stir at 60 °C for one hour. Afterwards, the reaction was cooled down and then quenched with brine (90 mL). The mixture was transferred in a separating funnel, the aqueous solution extracted with ethyl acetate (4x100 mL) and the combined organic phases washed with brine (30 mL). The organic layer was collected, dried over anhydrous $Na_2SO_4$, filtered and volatiles removed under reduced pressure to give a crude which was purified by silica gel column chromatography (chloroform/methanol 12:1 v/v) to afford 470 mg of the product (1.38 mmol, 86%) as a white solid. $^1$H-NMR (400 MHz, $CDCl_3$) δ ppm: 7.58-7.53 (m, 4H, C*H* biphenyl ring), 7.45-7.42 (m, 2H, C*H* biphenyl ring), 7.32 (t, 1H, C*H* biphenyl ring), 7.01-6.98 (m, 2H, C*H* biphenyl ring), 4.12-4.07 (m, 2H, $CH_2CH_2CH_2O$-biphenyl), 3.64 (t, 2H, $HOCH_2CH_2$), 2.59-2.56 (m, 12H, $CH_2CH_2CH_2O$-biphenyl, 4x$CH_2$ piperazine ring, $HOCH_2CH_2$-piperazine ), 2.05-2.00 (m, 2H, $CH_2CH_2CH_2O$-biphenyl). MS (ESI-MS): Calculated: 341.5 for $C_{21}H_{29}N_2O_2$ $[M+H]^+$, Found: 341.2.

**Synthesis of 5-(3-bromopropyl)-3-phenyl-1,2,4-oxadiazole (9).** In a round bottom flask *N*'-hydroxybenzimidamide (1 g, 7.35 mmol, 1 equiv) and diisopropylethylamine (1.84 mL, 13.2 mmol, 1.8 equiv) were dissolved in anhydrous DCM (30 mL) and then cooled down at 0 °C on an ice bath. 4-Bromobutanoyl chloride (1.27 mL, 11.0 mmol, 1.5 equiv) was dissolved in anhydrous DCM (15

mL) and the so obtained solution dropped into the reaction mixture over 20 min by dropping funnel. After one hour the reaction was complete, the solvent was evaporated to dryness under reduced pressure and water (65 mL) was added. The so obtained white suspension was stirred for 30 min before it was filtered and dried under reduced pressure. The solid was then transferred into round bottom flask, anhydrous toluene (60 mL) was added and the suspension heated up to reflux for 6 h. The solvent was then removed in a rotary evaporator and the crude mixture purified by silica gel column chromatography (hexane/ethyl acetate 80:1 v/v) to give 1275 mg (4.77 mmol, 65%) of the title compound as a colourless oil. $^{1}$H-NMR (400 MHz; $CDCl_3$) δ ppm: 8.08-8.10 (m, 2H, C*H* benzene ring), 7.49-7.51 (m, 3H, C*H* benzene ring), 3.58-3.61 (t, 2H, $CH_2CH_2CH_2Br$), 3.14-3.19 (t, 2H, $CH_2CH_2CH_2Br$), 2.44-2.50 (m, 2H, $CH_2CH_2CH_2Br$). MS (ESI-MS): Calculated: 267.0 for $C_{11}H_{12}BrN_2O$ $[M+H]^+$, Found: 267.0.

**Synthesis of 2-(4-(3-(3-phenyl-1,2,4-oxadiazol-5-yl)propyl)piperazin-1-yl)ethan-1-ol (10).** A round bottom flask was charged with **9** (590 mg, 2.2 mmol, 1 equiv), sodium iodide (364 mg, 2.43 mmol, 1.1 equiv) and $K_2CO_3$ (458 mg, 3.31 mmol, 1.5 equiv) and the mixture suspended in anhydrous DMF (6 mL) under vigorous stirring. Then a solution of 2-(piperazin-1-yl)ethan-1-ol (1.36 mL, 11.05 mmol, 5 equiv) in anhydrous DMF (6 mL) was added dropwise and the resulting mixture stirred at 60 °C. After 1 h the reaction was completed and allowed to cool down at rt, before being quenched with 120 mL of brine. The aqueous solution was extracted with ethyl acetate (4x120 mL) and the combined organic phases were washed with 40 mL of brine. The organic phase was dried over anhydrous $Na_2SO_4$, filtered and organic solvents removed *in vacuo* to give a crude which was purified by silica gel column chromatography (chloroform/methanol 15:1 v/v) affording 650 mg of the product (2.05 mmol, 93%) as a white solid. $^{1}$H-NMR (400 MHz; $CDCl_3$) δ ppm: 8.07-8.10 (m, 2H, C*H* benzene ring), 7.49-7.51 (m, 3H, C*H* benzene ring), 3.59-3.62 (t, 2H, $CH_2CH_2OH$), 3.00-3.04 (t, 2H, $CH_2CH_2CH_2N$), 3.28-3.30 (m, 10H, 4 × $CH_2$ piperazine ring and $CH_2CH_2CH_2N$), 2.04-2.11 (m, 2H,

C*H*$_2$CH$_2$OH), 1.75 (m, 2H, CH$_2$C*H*$_2$CH$_2$N). MS (ESI-MS): Calculated: 317.2 for $C_{17}H_{25}N_4O_2$ $[M+H]^+$, Found: 317.2. Mp: 74-75 °C.

**Synthesis of spiro[chromane-2,4'-piperidin]-4-one hydrochloride (11).** 2-Hydroxyphenone (1 g, 7.35 mmol, 1 equiv), pyrrolidine (287 mg, 4.04 mmol, 0.55 equiv) and *tert*-butyl 4-oxopiperidine-1-carboxylate (1.54 g, 7.7 mmol, 1.05 equiv) were dissolved in anhydrous methanol (10 mL) and heated to reflux under inert atmosphere. After 24 h the organic solvent was evaporated and the so obtained thick orange liquid was purified by silica gel column chromatography (hexane/ethyl acetate 12:1 v/v) to afford 1.95 g (6.14 mmol, 84%) of the desired product as an off-white solid. $^1$H-NMR (400MHz, $CDCl_3$) δ 7.89 (d, 1H, C*H* chromanone ring), 7.51 (t, 1H, C*H* chromanone ring), 7.00 (m, 2H, C*H* chromanone ring), 3.90 (m, 2H, CH*H*-N(piperidine)-CH*H*), 3.24 (m, 2H, C*H*H-N(piperidine)-C*H*H), 2.74 (s, 2H, -(C=O)C*H*$_2$), 2.05 (d, 2H, C*H*H-C(spiro)-C*H*H), 1.64 (m, 2H, CH*H*-C(spiro)-CH*H*), 1.48 (s, 9H, Boc). MS (ESI-MS): Calculated: 318.2 for $C_{18}H_{24}NO_4$ $[M+H]^+$, Found: 318.2. Mp: 90-92 °C.

The *N*-Boc protected intermediate (970 mg, 3.06 mmol, 1 equiv) was dissolved in dry THF (43 mL) and placed at 0 °C in an ice bath, then a 4 M solution of HCl in 1,4-dioxane (45.8 mL) was added dropwise and the mixture was stirred at rt overnight. The obtained suspension was filtered and the resulting white solid washed over filter with dry THF and $Et_2O$. The filtrate was evaporated under reduced pressure and the resulting solid triturated with dry $Et_2O$ (35 mL) for 2 h. The new solid over filter was combined with the previously obtained one to afford 707 mg (2.78 mmol, 91%) of the product as an off-white crystalline solid. $^1$H-NMR (400 MHz, DMSO-*d*$_6$) δ ppm: 9.08 (bs, 2H, N*H*·*H*Cl), 7.75 (d, 1H, C*H* chromanone ring), 7.62 (t, 1H, C*H* chromanone ring), 7.15-7.08 (m, 2H, C*H* chromanone ring), 3.21-3.17 (m, 2H, C*H* piperidine ring), 3.12-3.07 (m, 2H, C*H* piperidine ring), 2.92 (s, 2H, (C=O)C*H*$_2$), 2.13-2.09 (m, 2H, C*H* piperidine ring), 1.97-1.90 (m, 2H, C*H* piperidine ring). MS (ESI-MS): Calculated: 218.1 for $C_{13}H_{16}NO_2$ $[M+H]^+$, Found: 218.1. Mp: 206-207 °C.

**Synthesis of 1'-(2-bromoacetyl)spiro[chromane-2,4'-piperidin]-4-one (12).** To a solution of **11** (200 mg, 0.79 mmol, 1 equiv) and triethylamine (0.39 mL, 2.76 mmol, 3.5 equiv) in anhydrous DCM

(3 mL), a solution of 2-bromoacetyl chloride (0.01 mL, 1.18 mmol, 1.5 equiv) in anhydrous DCM was added dropwise at 0 °C, and the resulting mixture was stirred at 0 °C for 30 min and then at rt for 22 h. Upon completion the reaction mixture was diluted with 10 mL of DCM and then the organic layers were washed in sequence with HCl 2N (2x5 mL), $NaHCO_3$s.s. (2x5 mL), and brine (1x5 mL), dried over anhydrous $Na_2SO_4$, filtered and concentrated *in vacuo* to give a crude which was purified by silica gel column chromatography (ethyl acetate/hexane 1:1.5 v/v) affording 174 mg of the product (0.52 mmol, 85%) as a white solid. $^1$H-NMR (400 MHz, $CDCl_3$) δ ppm: 7.90 (d, 1H, C*H* chromanone ring), 7.52 (t, 1H, C*H* chromanone ring), 7.08-7.01 (m, 2H, C*H* chromanone ring), 4.21 (d, 1H, COCH*H*Br), 4.16-4.09 (m, 2H, COC*H*HBr and C*H* piperidine ring), 3.71 (d, 1H, C*H* piperidine ring), 3.62 (m, 1H, C*H* piperidine ring), 3.17 (m, 1H, C*H* piperidine ring), 2.77 (s, 2H, -(C=O)C$H_2$), 2.17 (m, 2H, C*H* piperidine ring), 1.76 (m, 1H, C*H* piperidine ring), 1.66 (m, 1H, C*H* piperidine ring). MS (ESI-MS): Calculated: 338.0 for $C_{15}H_{17}BrNO_3$ $[M+H]^+$, Found: 338.0.

**Synthesis of 1'-(2-(4-(2-hydroxyethyl)piperazin-1-yl)acetyl)spiro[chromane-2,4'-piperidin]-4-one (13).** A round bottom flask was charged with **12** (203 mg, 0.6 mmol, 1 equiv) and 1-(hydroxyethyl)-piperazine (391 mg, 3.0 mmol, 5 equiv) and anhydrous DMF (3 mL) was added. After the reactants were completely dissolved $K_2CO_3$ (124 mg, 0.9 mmol, 1.5 equiv) and sodium iodide (99 mg, 0.66 mmol, 1.1 equiv) were added and the mixture was heated at 60 °C for 2 h. The reaction was cooled down and then quenched with 40 mL of brine. The solution was transferred into a separating funnel and then extracted with ethyl acetate (4x30 mL). The combined organic layers were washed with a further amount of brine (30 mL), then dried over anhydrous $Na_2SO_4$, filtered and volatiles evaporated under reduced pressure. The crude was purified by silica gel column chromatography (chloroform/methanol 10:1 v/v) to afford 230 mg (0.59 mmol, 99%) of the product as an off-white solid. $^1$H-NMR (400 MHz, $CDCl_3$) δ ppm: 7.90 (d, 1H, C*H* chromanone ring), 7.53 (t, 1H, C*H* chromanone ring), 7.07-7.01 (m, 2H, C*H* chromanone ring), 4.39 (d, 1H, C*H* piperidine ring)-C*H*H), 3.92 (d, 1H, C*H* piperidine ring), 3.65 (m, 2H, HOC$H_2$C$H_2$), 3.48 (m, 1H, C*H* piperidine ring), 3.29

(d, 1H, C*H* piperidine ring), 3.17-3.12 (m, 2H, (CO)$CH_2$), 2.75 (m, 2H, COC*$H_2$*-piperazine), 2.60 (m, 8H, 4xC*$H_2$* piperazine ring), 2.15-2.10 (m, 2H, HOC$H_2$C*$H_2$*), 1.70-1.60 (m, 4H, C*H* piperidine ring). MS (ESI-MS): Calculated: 388.2 for $C_{21}H_{30}N_3O_4$ $[M+H]^+$, Found: 388.2.

**Synthesis of benzyl 4-(2-hydroxyethyl)piperazine-1-carboxylate (14).** The commercially available 2-(piperazin-1-yl)ethan-1-ol (1 g, 7.68 mmol, 1.1. equiv) was dissolved in anhydrous THF (45 mL) along with triethylamine (1071 µL, 7.68 mmol, 1.1 equiv) in a round bottom flask and placed at 0 °C in an ice bath. A solution of commercial benzyloxycarbonyl chloride (983 µL, 6.98 mmol, 1 equiv) in anhydrous THF (6 mL) was added under inert atmosphere by a dropping funnel within 10 min and then allowed to stir at rt. After 4 h the solvent was removed under reduced pressure and the mixture partitioned between a saturated solution of $NaHCO_3$ (50 mL) and ethyl acetate. The aqueous phase was extracted with ethyl acetate (3x60 mL) and the combined organic layers were washed with brine (15 mL). Organic phase was dried over anhydrous $Na_2SO_4$, filtered and volatiles removed under reduced pressure to afford a thick oil (2250 mg) which was purified by silica gel column chromatography (dichloromethane/methanol 25:1 v/v) to give 1495 mg (5.66 mmol, 81%) of the product as a pale yellow oil. $^1$H-NMR (400 MHz, $CDCl_3$) δ ppm: 7.28-7.39 (m, 5H, C*H* benzene ring), 5.16 (s, 2H, COOC*$H_2$*Ph), 3.66-3.64 (m, 2H, $CH_2$C*$H_2$*OH), 3.57-3.54 (m, 4H, 2xC*$H_2$* piperazine ring), 2.59-2.57 (m, 2H, C*$H_2$*$CH_2$OH), 2.52-2.49 (m, 4H, 2xC*$H_2$* piperazine ring). MS (ESI-MS): Calculated: 265.2 for $C_{14}H_{21}N_2O_3$ $[M+H]^+$, Found: 265.1.

**Synthesis of benzyl 4-(2-bromoethyl)piperazine-1-carboxylate (15).** Compound **14** (1.66 g, 6.28 mmol, 1 equiv) and tetrabromomethane (2.5 g, 7.54 mmol, 1.2 equiv) were dissolved in anhydrous THF (12.6 mL) in a round bottom flask. Afterwards, a solution of triphenylphosphine (1977 mg, 7.54 mmol, 1.2 equiv) in anhydrous THF (25 mL) was added by a dropping funnel at rt and the so obtained mixture was stirred 4 h at rt. Upon completion hexane (15 mL) was added and the resulting suspension was additionally stirred for 1 h at rt. Afterwards the suspension was filtered and the solid over filter washed with a mixture THF/hexane 1:1 v/v (3x5 mL). Then the filtrate was evaporated, re-dissolved

in ethyl acetate (40 mL) and washed in sequence with a saturated solution of $NaHCO_3$ (2x30 ml) and brine (15 mL). The organic phase was dried over anhydrous $Na_2SO_4$, filtered and volatiles removed under reduced pressure. The so obtained crude and the previous solid were combined and purified by silica gel column chromatography (petroleum ether/ethyl acetate 7:3 v/v) to afford 1.5 g (4.58 mmol, 73%) of the product as a colourless oil. $^1$H-NMR (400 MHz, $CDCl_3$) δ ppm: 7.28-7.38 (m, 5H, C*H* benzene ring), 5.15 (s, 2H, COOC*H*$_2$Ph), 3.57-3.54 (m, 4H, 2xC*H*$_2$ piperazine ring), 3.46-3.42 (m, 2H, $CH_2$C*H*$_2$Br), 2.84-2.81 (m, 2H, C*H*$_2$$CH_2$Br), 2.52-2.49 (m, 4H, 2xC*H*$_2$ piperazine ring). MS (ESI-MS): Calculated: 327.1 for $C_{14}H_{20}BrN_2O_2$ $[M+H]^+$, Found: 327.1.

**Synthesis of 2,2'-(piperazine-1,4-diyl)bis(ethan-1-ol) (16).** 2-(Piperazin-1-yl)ethan-1-ol (1 g, 7.68 mmol, 1 equiv) and 2-bromoethan-1-ol (813 µL, 11.5 mmol, 1.5 equiv) were dissolved in anhydrous acetonitrile (25 mL), then $K_2CO_3$ (3.18 g, 23.0 mmol, 3 equiv) was added and the resulting suspension was refluxed under argon atmosphere for 18 h. Upon completion the reaction mixture was cooled down, the resulting solid was filtered and washed with acetonitrile. The filtrate was evaporated under reduced pressure, triturated in DCM (40 mL) and filtered again. The collected solids were combined (1.9 g) and purified by silica gel column chromatography (chloroform/methanol 12:1 v/v + 1% $NH_3$) to obtain 600 mg (3.46 mmol, 45%) of the product as a white solid. $^1$H-NMR (400 MHz, $CDCl_3$) δ ppm: 3.64 (t, 4H, 2x$CH_2$C*H*$_2$OH), 2.57-2.47 (m, 12H, 2xC*H*$_2$$CH_2$OH, 4xC*H*$_2$ piperazine ring). MS (ESI-MS): Calculated: 175.1 for $C_8H_{19}N_2O_2$ $[M+H]^+$, Found: 175.1. Mp: 135-136 °C.

**Synthesis of 3-amino-2-naphthamide (17).** 3-Amino-2-naphthoic acid (1 g, 5.34 mmol, 1 equiv), *N*-(3-dimethylaminopropyl)-*N*′-ethyl-carbodiimide hydrochloride (1.13 g, 5.88 mmol, 1.1 equiv), 4-methylmorpholine (646 µL, 5.88 mmol, 1.1 equiv) and 1*H*-benzotriazol-1-ol (849 mg, 5.34 mmol, 1 equiv) were placed in a round bottom flask and then suspended in anhydrous THF (32 mL) at rt and under inert atmosphere. After 30 min, a 30 % (w/w) ammonia solution (1.35 mL, 21.4 mmol, 4 equiv) was added dropwise and the resulting mixture stirred at rt for 22 h. The mixture was quenched with brine (15 mL) and the organic solvent evaporated under reduced pressure. The aqueous solution was

transferred into a separator funnel and extracted with ethyl acetate (4x100 mL), the organic layers were combined and washed in sequence with $NaHCO_3$s.s. (100 mL) and brine (50 mL). The organic phase was collected, dried over anhydrous $Na_2SO_4$, filtered and volatiles removed under reduced pressure to give a crude (1040 mg) which was triturated twice with chloroform (30 mL each) and then filtered to afford 746 mg (4.0 mmol, 75%) of the title compound as a yellow solid $^1$H-NMR (400 MHz, DMSO-$d_6$) δ ppm: 8.13-8.06 (m, 2H, C*H* naphthalene ring), 7.67 (t, 1H, C*H* naphthalene ring), 7.52 (t, 1H, C*H* naphthalene ring), 7.38-7.35 (m, 2H, C*H* naphthalene ring), 7.17-7.13 (m, 1H, CON*H*H), 6.97-6.94 (m, 1H, CONH*H*), 6.27-6.23 (m, 2H, N$H_2$). MS (ESI-MS): Calculated: 187.1 for $C_{11}H_{11}N_2O$ $[M+H]^+$, Found: 187.1. Mp: 235-236 °C.

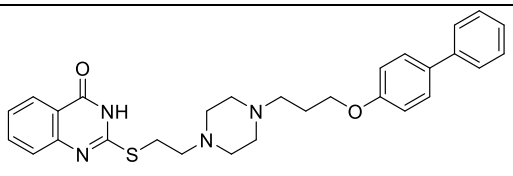
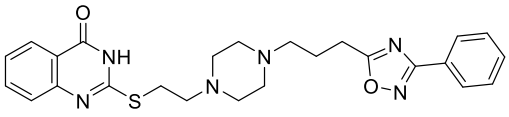
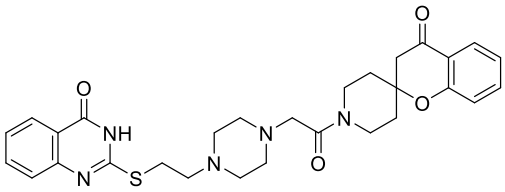
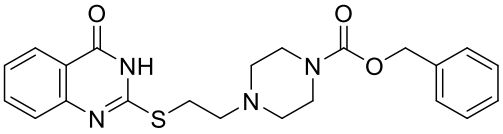
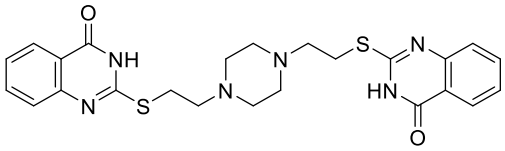
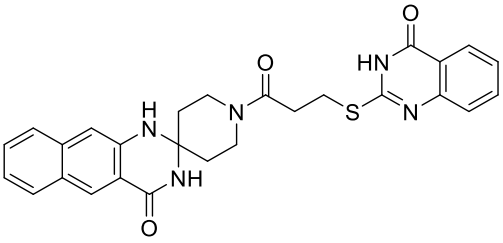
**Synthesis of 1*H*-spiro[benzo[*g*]quinazoline-2,4'-piperidin]-4(3*H*)-one (18).** A round bottom flask was charged with compound **17** (200 mg, 1.07 mmol, 1 equiv) and piperidin-4-one hydrochloride (184 mg, 1,07 mmol, 1 equiv). These reactants were dissolved in acetic acid (5 mL) in the presence of a catalytic amount of concentrated sulphuric acid and then stirred at rt overnight. The reaction was quenched with water (5 mL), placed in an ice bath and alkalinized with a 1 M solution of NaOH. The basic aqueous solution was transferred in a separator funnel, extracted with ethyl acetate (4x50 mL) and the combined organic layers washed with brine (10 ml). The organic phase was collected, dried over anhydrous $Na_2SO_4$, filtered and volatiles removed under reduced pressure to give 201 mg (0.75 mmol, 70%) of the compound as a pale yellow solid. $^1$H-NMR (400 MHz, DMSO-$d_6$) δ ppm: 8.40-8.38 (m, 1H, C*H* dihydrobenzoquinazolinone ring), 8.27-8.22 (m, 1H, C*H* dihydrobenzoquinazolinone ring), 7.82-7.80 (m, 1H, C*H* dihydrobenzoquinazolinone ring), 7.60-7.57 (m, 1H, N*H*), 7.42-7.40 (m, 1H, C*H* dihydrobenzoquinazolinone ring), 7.18-7.12 (m, 2H, C*H* dihydrobenzoquinazolinone ring), 6.82-6.80 (m, 1H, CON*H*), 2.80 (bs, 4H, 2xC$H_2$ piperidine ring), 1.72 (bs, 4H, 2xC$H_2$ piperidine ring). MS (ESI-MS): Calculated: 268.2 for $C_{16}H_{18}N_3O$ $[M+H]^+$, Found: 268.1. Mp: 240-241 °C.

**Synthesis of ethyl 3-((4-oxo-3,4-dihydroquinazolin-2-yl)thio)propanoate (19).** A round bottom flask was charged with 2-mercaptoquinazolin-4(3*H*)-one (1 g, 5.6 mmol, 1 equiv), sodium iodide (967 mg, 6.5 mmol, 1.15 equiv) and $K_2CO_3$ (931 mg, 5.7 mmol, 1.02 equiv) and the mixture was suspended in anhydrous DMF (6 mL). Afterwards, a solution of ethyl 3-bromopropanoate (827 µL, 6.5 mmol, 1.15 equiv) in anhydrous DMF (3 mL) was added under inert atmosphere and the so obtained mixture was stirred at rt. After 3 h the reaction was heated at 90 °C and stirred at this temperature overnight. The day after, an aliquot of ethyl 3-bromopropanoate (108 µL, 0.84 mmol, 0.15 equiv) and $K_2CO_3$ (116 mg, 0.84 mmol, 0.15 equiv) were added and the mixture was stirred at the afore mentioned conditions for further 6 h. The reaction was cooled down at rt and then 100 mL of water were added. A white suspension that was formed was stirred at rt for 2 h before it was filtered. The white solid was dried in a laboratory oven and the powder recrystallized from acetonitrile to afford 210 mg (0.76 mmol, 13%) of the title compound as a white crystalline solid. $^1$H-NMR (400 MHz, DMSO-$d_6$) δ ppm: 12.97 (bs, 1H, CON*H*), 7.96 (d, 1H, C*H* quinazolinone ring), 7.76 (d, 1H, C*H* quinazolinone ring), 7.33-7.39 (m, 2H, C*H* quinazolinone ring), 4.64 (t, 2H, COOC$H_2$CH$_3$), 4.06 (q, 2H, SC$H_2$CH$_2$), 2.72 (t, 2H, SCH$_2$C$H_2$), 1.17 (t, 3H, COOCH$_2$C$H_3$). MS (ESI-MS): Calculated: 279.3 for $C_{13}H_{15}N_2O_3S$ $[M+H]^+$, Found: 279.1. Mp: 212-213 °C.

**Synthesis of 3-((4-oxo-3,4-dihydroquinazolin-2-yl)thio)propanoic acid (20).** Compound **19** (170 mg, 0.61 mmol) was dissolved in ethanol (3 mL) in a round bottom flask and placed in an ice bath when a 2 M solution of KOH (3 mL) was added dropwise under vigorous stirring. The reaction mixture was then allowed to warm at rt and stirred overnight. Upon completion the organic solvent was evaporated under reduced pressure and the resulting aqueous solution was acidified with HCl aq. 2 M until a white solid started to form. This was filtered, washed over filter with water and then dried in a laboratory oven to provide 130 mg (0.52 mmol, 85%) of the compound as a white solid. $^1$H-NMR (400 MHz, DMSO-$d_6$) δ ppm: 12.98 (bs, 1H, COO*H*), 12.40 (bs, 1H, CON*H*), 7.96 (d, 1H, C*H* quinazolinone ring), 7.74 (d, 1H, C*H* quinazolinone ring), 7.42-7.37 (m, 2H, C*H* quinazolinone ring),

4.60 (t, 2H, SC*H*$_2$CH$_2$COOH), 2.65 (t, 2H, SCH$_2$C*H*$_2$COOH). MS (ESI-MS): Calculated: 249.0 for $C_{11}H_9N_2O_3S$ $[M-H]^-$, Found: 249.0. Mp: 227-229 °C.

**Table S3.** Chemical and physical data of final compounds **1-6**.

| Cpd | Structure | Mp | Yield (%) | Crystallization system[a] |
|---|---|---|---|---|
| **1** |  | 182-184 °C | 49 | A |
| **2** |  | 117-119 °C | 43 | B |
| **3** |  | 147-149 °C | 62 | C |
| **4** |  | 109-112 °C | 50 | B |
| **5** |  | 234-236 °C (with decomp.) | 63 | A |
| **6** |  | >250 °C (with decomp.) | 55 | D |

[a]A: acetonitrile/methanol; B: cyclohexane; C: acetonitrile; D: methanol.

**PARPs and TNKSs Inhibition Assays**

Inhibitory activity of test and reference compounds was evaluated by the means of a filter binding assay where incorporation of the radioisotope-labelled $NAD^+$ into the substrate captured on filter was detected after washout free $NAD^+$. The enzymes employed were full-length hPARP1 (GenBank Accession No. NM_001618.3, aa-1014, MW = 114.8 kDa, *C*-term His-tag expressed in Sf9 cells via baculovirus expression system) and hPARP2 (GenBank Accession No. NM_005484.3, aa2-583, MW = 93 kDa, *N*-term GST-tag expressed in Sf9 cells via baculovirus expression system.), and ADP-ribosyltransferase (ART) catalytic domains of hTNKS1 (GenBank Accession No. NM_003747, a.a. 1001-1327(end), with *N*-terminal GST-tag, MW=62.5 kDa, expressed in Sf9 cells via a baculovirus expression system) and hTNKS2 (GenBank Accession No. NM_025235, aa849-1166, MW = 62 kDa, *N*-term GST-tag expressed in Sf9 cells via baculovirus expression system). Briefly, test or reference inhibitors were incubated with PARP or TNKS proteins, corresponding substrates and radioisotope-labelled $^{32}P$-$NAD^+$. The reaction mixtures were then spotted onto a filter paper, which binds the radioisotope-labelled catalytic reaction product, while the unreacted $^{32}P$-$NAD^+$ is washed out from the solid support. The different grade of adsorbed $^{32}P$-labelled product on the filter paper was then detected using a scintillation counter. Inhibition assays were carried out using different substrates depending on the specific PARP enzyme: core histones from chicken (0.01 mg/ml; RBC Cat. No. HMT-35-435) for hPARP1, histone H3.3 (conc. 20 µM; RBC Cat. No. HMT-11-134) for hPARP2 and human histone H2A (conc. 20 µM; GenBank Accession No. NM_033445) for hTNKS1 and hTNKS2. The substrates were dissolved in freshly prepared reaction buffer (50 mM Tris-HCl (pH 8.0), 50 mM NaCl, 10 mM $MgCl_2$, 0.02% Brij 35, 1 mM DTT, 1% DMSO, and 20 µg/mL activated DNA), then PARP proteins were delivered into the proper substrate solutions (2.5 nM for PARP1; 10 nM for PARP2; 1.5 nM for TNKS1 and 0.5 nM for TNKS2) and gently mixed. Afterwards, test and reference compounds at different concentrations in DMSO (final concentration in the reaction mixture ≤ 1%) were delivered in the reaction mixture by using Acoustic Technology (Echo 550, LabCyte Inc. Sunnyvale, CA) in nanoliter range and incubated 20 min at rt. Subsequently, $^{32}P$-$NAD^+$ co-substrate (10 µM, PerkinElmer) was added to the reaction mixture and incubated for 1h at rt. Finally, the mixture was transferred onto a filter paper and this washed with phosphoric acid (0.75% w/w) to remove unreacted $^{32}P$-labelled co-substrate. Residual radioactivity on filter paper was detected by a liquid scintillation counter and obtained data fitted for $IC_{50}$ curves determination using Excel and GraphPad Prism version 8.0. All experiments were done in triplicate and repeated at least two times.

**DLD-1 Colony Formation Assay**

DLD-1 cells obtained from ATCC (American Type Culture Collection, Manassas, VA, USA) were maintained in RPMI medium (EurocloneSpA Milan Italy) supplemented with fetal bovine serum to a final concentration of 10%, *L*-Glutamine at 2 mM, penicillin at 100 units/mL and streptomycin at 100 µg/mL (EurocloneSpA Milan Italy). Cells were plated in 6-well plates at a density of $5\times10^2$ cells each well in 6ml of media and incubated at 37°C with 5% $CO_2$ for 12 days. The test compounds **2**, **5** and IWR-1 were then added to the fresh media at various concentrations from 0.1 to 10 µM, while the positive control IWR-1 has been used only at 1 µM. After incubation for 12 days, cells were fixed with 70% ethanol and subsequently stained by 0.2% crystal violet solution. The images were obtained and the colonies were counted.

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