Highly potent and isoform-selective dual-site-binding tankyrase/Wnt signaling inhibitors that increase cellular glucose uptake and have anti-proliferative activity.

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KEYWORDS: Tankyrase, PARP, Glucose uptake, Wnt signaling, Crystal structure, Insulin.

ABSTRACT: Compounds 13 and 14 were evaluated against eleven PARP isoforms to reveal that both 13 and 14 were more potent and isoform-selective towards inhibiting tankyrases (TNKSs) than the “standard” inhibitor 1 (XAV939)5, i.e. IC50 = 100 pM vs. TNKS2 and IC50 = 6.5 μM vs. PARP1 for 14. In cellular assays, 13 and 14 inhibited Wnt-signaling, enhanced insulin-stimulated glucose uptake and inhibited the proliferation of DLD-1 colorectal adenocarcinoma cells to a greater extent than 1.

INTRODUCTION

Tankyrase (TNKSs) are members of the poly(ADP-ribose)-polymerase (PARP) family of seventeen enzymes that use NAD+ as a substrate to transfer ADP-ribose units to target proteins.1 There are two human isoforms, TNKS1 and TNKS2. Target proteins include telomere repeating binding factor-1 (TRFI), 2,3 nuclear mitotic apparatus protein (NuMA)4 (essential for the resolution of chromatids during mitosis) and axin, the limiting component of Wnt/β-catenin signaling.5 Increased Wnt signaling correlates with the overexpression of TNKSs in several human cancers.6,9 Inhibition of TNKSs is reported to lead to stabilization of axin and decreased nuclear β-catenin-driven proliferation of cancer cells.10 Epidemiological studies show that patients with Type-2 diabetes are at higher risk of developing site-specific cancers11 but the precise mechanisms that link diabetes to cancer remain unclear. The axin-TNKS-KIF3A complex is required for insulin-stimulated translocation of GLUT4 to the cell membrane.12 Insulin-regulated aminopeptidase (IRAP) is also a binding partner and target protein of TNKSs.13 Together, IRAP and TNKSs can enhance insulin-stimulated exocytosis of GLUT4, which could result in increased uptake of glucose.13 TNKS-knockout mice display increased sensitivity to insulin and reduced adiposity and pan-PARP inhibitors have been used to investigate the role of TNKSs in studies on the translocation of GLUT4.14 Inhibitor 1 (XAV939)5 (Fig. 1) has been extensively used as a tool to inhibit TNKSs and of Wnt/β-catenin signaling5 but lacks the required isoform-selectivity to avoid off-target effects. Related inhibitors of TNKSs include flavones,15 2-arylnicotinolin-4-16,17 isoquinolin-1-ones18 and aryltetrahydro-naphthyridinones, which maintain a classical binding mode in the nicotinamide-binding site.19 Compound 3 (IWR-1)20 (Fig. 1) is an inhibitor of the Wnt signaling cascade through inhibiting TNKSs, binding only to the adenosine-binding site.20 The norbornane of 3 forces a conformational change of Tyr5250 (TNKS2 numbering), allowing the quinoline of 3 to π-stack with His5038 within this site.21 Our previous reports of novel inhibitors include 2-aryl-8-methylquinazolin-4-ones with 4’-large or electron-withdrawing substituents (e.g. 2) to provide inhibition of TNKSs (IC50 in the low nM range) and of Wnt/β-catenin signaling.16,17 Some known inhibitors bind in both the nicotinamide-binding site and the adenosine-binding domain,22,23 although the increases in potency vs. 1 were modest.

Figure 1. Examples of previously studied inhibitors of TNKSs 1, 2 (nicotinamide-site binders) and 3 (adenosine-site binder). Inhibitor 2 is shown with locant numbers.

Here, we report the rational design and evaluation of advanced inhibitors (13, 14) which maintain the 8-methylquinazolin-4-
RESULTS AND DISCUSSION

Modeling. Structural alignment of the co-crystal structures of 2 (PDB 4UFU) and 3 (PDB 3UA9) with TNKS2 provided initial insights towards the design of 13 and 14. The quinazolone of 2 binds in the nicotinamide-binding site, making the expected H-bonds with Ser1068 and Gly1072 and π-stack with Tyr1203 (TNKS2 numbering). The quinoline of 3 is located in the adenosine-binding site, making a π-stack with His1048 (TNKS2 numbering). Features binding at each site were linked to create chimeric compounds 13 and 14, which combine important H-bonds and stacking interactions at both binding sites. Modeling of 13 and 14 into the active site of TNKS1 (PDB 4I91) predicted that the designed compounds could bind to the pockets in the intended way and that the length and nature of the linker were appropriate (Fig. 2). The quinazolin-4-one core could bind in the nicotinamide-binding site to make the classical H-bond and π-stacking interactions. Compounds 1 and 2 contain a 2-aryl group, which is shown to occupy a hydrophobic cavity. Modeling of 13 into TNKS1 suggests that the chosen linker could allow Tyr1203 to move towards the nicotinamide-binding site and decrease the volume of the hydrophobic cavity. However, this shrinkage of the pocket could still allow the panamide linker to thread through. The modeling study reveals that the linker could interact further with the protein, in that the C=O of the panamide linker is appositely located to H-bond with the backbone NH of Tyr1213. Moreover, the central benzene ring and the quinoline of 13 are predicted to cause movement of His1048 of the adenosine-binding site to place its imidazole appropriately for π-stacking with the quinoline. We have previously reported that an 8-Me group enhances inhibitory activity in the simple 2-aryquinazoliones and related 3-arylisoquinolinones, we also incorporated the 8-Me in 14, to test if it would still make a contribution in these advanced dual-site-binding compounds (Fig. 2).

Figure 2. Model of 13 docked into the structure of TNKS1 (PDB code 4I91). The protein is shown in pink and 13 in cyan. Key H-bonds shown as grey dashed lines.

Chemical Synthesis. The synthesis of target compounds (Sch. 1) began by acylation of 8-aminoquinoline 4 with 4-nitrobenzoyl chloride to give 5, followed by transfer hydrogenation to provide amine 6. Anthranilamides 7 and 8 were acylated with ethyl 4-chloro-4-oxobutanoate to provide 9 and 10. One-pot cyclisation and hydrolysis in aqueous base led to the quinazolonepropanoic acids 11 and 12. Careful optimization of the coupling conditions was required to join 11 / 12 with 6; simultaneous addition of the activating agent (CDI) and quinazolones 11 / 12 to 6 was essential to provide the candidate dual-site-binding inhibitors 13 and 14 in modest yields.

Scheme 1. Synthesis of 13 and 14. Reagents and conditions: i) nitrobenzoyl chloride, pyridine, THF, 16 h, Ar; ii) NH2, HCO2-. 10% Pd/C, DMF/MeOH (2:1), 2 h, Ar; iii) EtOC2CH2CH2COCl, pyridine, THF, 16 h; iv) eq. NaOH (0.5 M), 60°C; v) 6, DMF, Pr3NEt, carbonyldiimidazole, 72 h, Ar.

Biochemical evaluation. The target compounds 13 and 14 were evaluated in vitro for inhibition of TNKS1 and TNKS2 and counterscreened against eleven PARP isozymes, including the major isofrom PARP1 (Table 1). Known inhibitors 1 and 3 were also examined as standards against PARP1, PARP2, TNKS1 and TNKS2. The nicotinamide-site binder 1 showed IC50 values in the low nanomolar range for inhibition of TNKS1 and TNKS2, which were similar to those reported by Huang et al. However, although 1 showed reasonable selectivity for TNKS2 vs. PARP1 (131-fold), other comparisons called into question the use of this agent as a selective inhibitor (TNKS1 vs. PARP1 25-fold; TNKS1 vs. PARP2 4.1-fold). Compound 3, which binds in the adenosine-binding site, had moderate activity against TNKS1 and TNKS2 (IC50 = 343 nM and 31 nM, respectively) and did not inhibit PARP1 or PARP2 up to 10 μM. These data are again consistent with earlier evaluations of the selectivity of this agent. Huang et al. reported IC50 (TNKS2) = 56 nM and selectivity vs. PARP1 and PARP2 > 300-fold, while Narwal et al. confirmed the selectivity of 3 with IC50 = ca. 100 μM (PARP1) and IC50 = ca. 35 μM (PARP2). Thus binding at the adenosine site has potential for greater selectivity for TNKSs vs. other PARPs, although this may not give high potency as the sole binding site.

Intermediate 6, containing the putative adenosine-site-binding benzamidoquinoline, was evaluated for ability to bind in the absence of the anchoring quinazolin-4-one but it failed to inhibit any of the isozymes. This shows that the norbornane of 3 is essential, interacting with Tyr1203 to modify the geometry of the adenosine-binding site to accept the benzamidoquinoline.

The candidate dual-site inhibitors 13 and 14 were shown to be extremely potent and isoform-selective inhibitors of TNKS1s, inhibiting TNKS2 in the pM range and thus they are both...
considerably more potent than the “standard” inhibitor 1 and the adenosine-site binder 3. This increase in potency of inhibition of the TNKSs for 13 and 14 was not accompanied by an increase in activity against other members of the PARP family (Table 1). In particular, 13 and 14 showed very weak activity against PARP1 and PARP2 (IC$_{50}$ > 6 µM), leading to exquisite isoform-selectivity. Inhibitor 13 is 4.1 × 10$^4$-fold selective for TNKS2 vs. PARP1 and 3.5 × 10$^4$-fold selective for TNKS2 vs. PARP2. Similarly, 14 is 6.5 × 10$^4$-fold selective for TNKS2 vs. PARP1 and 11.6 × 10$^4$-fold selective for TNKS2 vs. PARP2. The somewhat weaker potency of these agents against TNKS1 corresponds to selectivities for TNKS1 vs. PARP1 and PARP2 in the range 1-2.3 × 10$^3$-fold. Thus exploiting dual-site binding with an appropriate linker makes a step-change in potency and provides isoform-selectivity with this potency. Compound 14 is approximately twice as potent as 13 against both isoforms, confirming the moderate advantage of the 8-Me on the quinazoline core.$^{16,18,17}$ Intermediates 11 and 12, carrying only a propanoic acid at the quinazoline 2-position, were evaluated to explore the contribution of the quinazoline-CH$_2$CH$_2$CO unit towards potency and selectivity; both showed no inhibition of TNKSs. We have previously shown that a 2-aryl group is required for potent inhibition of TNKSs; this requirement is thereby reinforced for inhibitors that bind only as mimics of nicotinamide.$^{16-19}$ However, 11 and 12 do inhibit PARP1 and PARP2. Previously, we showed that polar groups at the 4'-position of 2-arylquinoxalin-4-ones decreased selectivity for TNKSs, in that inhibition of PARP1 was enhanced.$^{16,18}$ Therefore, the 2-propanoic acid of 11 and 12 is well suited to interact with the corresponding region of protein of PARP1 that contains hydrophilic residues. Furthermore, 11 and 12 displayed moderately potent and selective inhibition of PARP2 (54- and 6.9-fold, respectively, vs. PARP1), results which place 11 amongst the most PARP2-selective agents known.$^{25}$ Therefore, 11 and 12 provide a new scaffold towards the development of novel PARP2-selective inhibitors.

**X-ray crystallography.** To rationalize the potency and iso-

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**Table 1.** IC$_{50}$ (pIC$_{50}$ ± standard error) values for inhibition of TNKS1, TNKS2 and PARP isoforms in biochemical assays and for inhibition of Wnt signaling in a cellular assay.

<table>
<thead>
<tr>
<th>Compound</th>
<th>1</th>
<th>3</th>
<th>6</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>PARP1</td>
<td>854 nM (6.07 ± 0.13)</td>
<td>&gt;100000 nM a</td>
<td>&gt;100000 nM a</td>
<td>6700 nM (5.18 ± 0.17)</td>
<td>1000 nM (5.18 ± 0.17)</td>
<td>8230 nM (5.08 ± 0.12)</td>
<td>6500 nM (5.19 ± 0.06)</td>
</tr>
<tr>
<td>PARP2</td>
<td>141 nM (6.85 ± 0.08)</td>
<td>&gt;100000 nM a</td>
<td>- b</td>
<td>125 nM (6.90 ± 0.04)</td>
<td>145 nM (6.84 ± 0.12)</td>
<td>6900 nM (5.16 ± 0.12)</td>
<td>11600 nM (4.94 ± 0.05)</td>
</tr>
<tr>
<td>PARP3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>40000 nM (4.40 ± 0.14)</td>
<td>45000 nM (4.34 ± 0.10)</td>
</tr>
<tr>
<td>PARP4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>69000 nM (4.16 ± 0.10)</td>
<td>65000 nM (4.19 ± 0.07)</td>
</tr>
<tr>
<td>TNKS1</td>
<td>34 nM (7.47 ± 0.23)</td>
<td>343 nM (6.46 ± 0.09)</td>
<td>&gt;100000 nM a</td>
<td>&gt;100000 nM a</td>
<td>&gt;100000 nM a</td>
<td>9.1 nM (8.04 ± 0.05)</td>
<td>5.1 nM (8.29 ± 0.27)</td>
</tr>
<tr>
<td>TNKS2</td>
<td>6.5 nM (8.19 ± 0.09)</td>
<td>31 nM (7.51 ± 0.10)</td>
<td>&gt;100000 nM a</td>
<td>&gt;100000 nM a</td>
<td>&gt;100000 nM a</td>
<td>0.20 nM (6.89 ± 0.17)</td>
<td>0.10 nM (1.0 ± 0.05)</td>
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<tr>
<td>PARP10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&gt;100000 nM a</td>
<td>&gt;100000 nM a</td>
<td></td>
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<tr>
<td>PARP12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&gt;100000 nM a</td>
<td>&gt;100000 nM a</td>
<td></td>
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<tr>
<td>PARP14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&gt;100000 nM a</td>
<td>&gt;100000 nM a</td>
<td></td>
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<tr>
<td>PARP15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&gt;100000 nM a</td>
<td>&gt;100000 nM a</td>
<td></td>
</tr>
<tr>
<td>PARP16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&gt;100000 nM a</td>
<td>&gt;100000 nM a</td>
<td></td>
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<tr>
<td>Wnt</td>
<td>220nM (6.66 ± 0.10)</td>
<td>136 nM</td>
<td></td>
<td></td>
<td></td>
<td>29 nM (7.54 ± 0.05)</td>
<td>37 nM (7.43 ± 0.06)</td>
</tr>
</tbody>
</table>

- a Limited by solubility, b Empty cells indicate not determined. c Determined previously.$^{23}$

The extreme charged residues present in PARP correspond to the nicotinamide moiety of the protein of PARP1 that contains hydrophilic residues. Furthermore, 11 and 12 displayed moderately potent and selective inhibition of PARP2 (54- and 6.9-fold, respectively, vs. PARP1), results which place 11 amongst the most PARP2-selective agents known.$^{25}$ Therefore, 11 and 12 provide a new scaffold towards the development of novel PARP2-selective inhibitors.
Inhibition of Wnt signaling in TCF/LEF reporter-HEK293 cells. The intracellular activity and potency of 13 and 14 was evaluated in a functional assay using a TCF/LEF Reporter-HEK293 cell line (BPS Bioscience, catalog #60501). Upon extracellular Wnt signals, β-catenin is stabilized and associates with TCF/LEF transcription factors, activating transcription. TNKSs control the stability of the β-catenin destruction complex and inhibition is expected to stabilize the destruction complex and thus lower the levels of β-catenin. The TCF/LEF Reporter-HEK293 cell line is used to measure this interference with this pathway. Both 13 (IC_{50} = 29 nM) and 14 (IC_{50} = 37 nM) were shown to be extremely effective and potent cellular inhibitors of Wnt/β-catenin signaling, confirming their uptake into cells and their activity therein (Table 1). Thus 13 and 14 are ca. 10× more potent in cells than the standard inhibitor 1, reflecting the greater potency of the dual-site inhibitors in vitro. Cell viability was monitored with a light microscope; 13 and 14 were not cytotoxic in this assay.

Anti-proliferative activity towards DLD-1 human colon carcinoma cells. Aberrant Wnt signaling is found in > 90% of colorectal cancers, owing to mutation in the APC protein which is a component of the β-catenin destruction complex. Compound 1 has been reported to have anti-proliferative activity against DLD-1 human colon cancer cells but only under serum-deprived conditions. Using a colony-forming assay, 13 and 14 were shown to inhibit formation of colonies of DLD-1 cells even under normal-serum conditions (Fig. 4) at 100 nM and 1.0 µM, conditions under which 1 was inactive. This observation again reflects the greater potency of 13 and 14 in vitro and in cells.

Insulin-stimulated glucose uptake. The axin-TNKS-KIF3A complex is stabilized through inhibition of TNKS. Ablation of expression of TNKSs has been reported to upregulate GLUT4 at the post-transcriptional level, potentially increasing uptake of glucose into adipocytes. To address this pharmacologically, 13 and 14 were examined for their ability to enhance insulin-stimulated uptake of glucose. 3T3-L1 Adipocytes were treated with 13 and 14 in the presence of insulin (100 nM). Compound 1 was used for comparison as the current “standard” TNKS inhibitor. The insulin-stimulated uptake of radiolabeled 2-deoxy-D-glucose into the cells was measured. The weaker TNKS inhibitor 1 had no effect on insulin-stimulated glucose uptake in comparison to the insulin-only control. The slight decrease in non-stimulated glucose uptake in presence of 1 (1.0 µM) was not significantly different from control. Non-stimulated glucose uptake in presence of 13 and 14 (1.0 µM) was not significantly different from control. In comparison to insulin only and with

Figure 3. Crystal structure of A) TNKS2-13 (PDB code 5FPF) and B) TNKS2-14 (PDB code 5FPG). Hydrogen bonds are shown in dashed lines and the electron density (2mFo-DFc) is contoured at 1 Å around the ligand.

Figure 4. Upper: Exemplary image of DLD-1 colony forming assay (1000 cells/well) with 1, 3, 13 and 14 (0 µM (control with 1% DMSO v/v only), 1.0 µM and 100 nM). Lower: Histogram of data from assay. 1, 3, 13 and 14 (n = 3).
1, both 13 and 14 significantly further increased insulin-stimulated glucose uptake. Glucose uptake increased by ca. 40% and 20% using 100 nM and 1.0 µM of 13, respectively. Insulin-stimulated uptake of glucose increased by ca. 20% by 14 at 100 nM and 1.0 µM in comparison to insulin treatment only (Fig. 5). This confirms potent intracellular activity of 13 and 14.

Figure 5. Effect of 1, 13 and 14 on % insulin-stimulated glucose uptake in 3T3-L1 adipocytes. Basal levels (B) with or without compound contain no insulin (I). (I with and without compound) administered at final concentration 100 nM. Results are mean and SEM relative to I only (n = 3 for 1 and n = 5 for 13 and 14). I + 100 nM 13 vs. I p = 0.02; I + 1.0 µM 13 vs. I p = 0.02; I + 100 nM 14 vs. I p = 0.07; I + 1.0 µM 14 vs. I p = 0.01; B vs. B + 13 p = 0.98; B vs. B + 14 p = 0.94; B vs. B + 1 p = 0.21.

CONCLUSION
In this paper, we disclose the design, synthesis and evaluation of two new highly potent and isoform-selective TNKS inhibitors, 13 and 14. Crystal structures of these compounds bound into the catalytic domain of TNKS2 confirmed that, as designed, the quinazolin-4-one moiety occupied the nicotinamide-binding site, setting up the linker so that the quinoline moiety interacts with the adenosine-binding region. This dual-site design has led to the most potent and isoform-selective inhibitors reported to date, with IC50 = 100 pM for inhibition of TNKS2 by 14 and 1.2 × 103-fold selectivity for inhibition of TNKS2 vs. the major PARP isoform, PARP1. Cellular uptake of these agents was demonstrated by their potent inhibition of Wnt / β-catenin signaling in the low nM range. Significant anti-proliferative activity was demonstrated in DLD-1 human colon carcinoma cells at 100 nM under normal serum conditions. For the first time, truly potent and selective TNKS inhibitors are shown to increase insulin-stimulated glucose uptake, whereas previous studies have used non-selective pan-PARP inhibitors;13 this provides further evidence of a role of TNKSs in insulin-stimulated glucose transport and further studies will elucidate the mechanisms of this activation, presumably through the axin-TNKS-KIF3A complex.12 In comparative studies in vitro, the widely-used TNKS inhibitor 1 has been shown to be markedly less potent than 13 and 14 and to lack the exquisite isoform-selectivity of these new agents. The improved potency and isoform-selectivity of 13 and 14 is manifest in three comparative cellular functional assays, where they show useful activity in situations where 1 lacks potency or activity.

Compounds 13 and 14 are now available as potent and isoform-selective TNKS inhibitors which inhibit the Wnt response pharmacologically in cells. They will have applications as molecular tools in studies on Wnt and related systems. These results also indicate that inhibition of TNKSs can increase sensitivity of cells to insulin, with potential application in Type-2 diabetes, as high doses of insulin can cause dysregulation of various signaling cascades (PI3K / Akt / mTOR).30 Antiproliferative activity has also been demonstrated in human colon cancer cells under normal serum conditions, supporting TNKS as a therapeutic target in the many cancers with aberrant Wnt signaling. A new structural scaffold (11, 12) for selective inhibitors of PARP2 has also been identified.

EXPERIMENTAL SECTION
Chemistry
The purity of target compounds were >95% as determined by 1H and 13C NMR, high resolution mass spectrometry using electrospray ionization and HPLC analysis at two different wavelengths (see supplementary information for general experimental, spectra of all compounds and synthetic procedures of intermediate compounds).

Synthesis of target compounds 13 and 14.

2-(3-Oxo-3-(4-((quinolin-8-yl)aminocarbonyl)phenylamino)propyl)quinazolin-4-one (13). Compound 6 (548 mg, 2.08 mmol) in dry DMF (50 mL) was treated with Pr2NEt (2.96 g, 22.9 mmol), then carbonyldimidazole (371 mg, 2.29 mmol), followed by addition of 11 (500 mg, 2.29 mmol). The mixture was stirred for 72 h under Ar. The solvent was evaporated and the residue was dissolved in EtOAc/MeOH (1:2, 70 mL). The organic solution was washed with water (3 × 30 mL) and brine (3 × 30 mL), then dried. Evaporation and chromatography (1:9 EtOAc / CH2Cl2 → 1:9 MeOH / EtOAc) gave a mixture containing 13. Further chromatography (EtOAc) gave 13 (74 mg, 8%) as a pale purple solid: mp 265-268°C 1H NMR ((CD3)2SO) δ 2.95-2.98 (4 H, m), 7.47 (1 H, t, J = 7.0 Hz), 7.63 (1 H, d, J = 8.0 Hz), 7.64-7.69 (2 H, m), 7.72-7.83 (2 H, m), 7.82 (2 H, d, J = 8.5 Hz), 8.00 (2 H, d, J = 8.5 Hz), 8.08 (1 H, d, J = 8.0 Hz), 8.46 (1 H, dd, J = 8.0, 1.5 Hz), 8.72 (1 H, d, J = 7.5 Hz), 8.97 (1 H, dd, J = 4.0, 1.5 Hz), 10.45 (1 H, s), 10.59 (1 H, s), 12.27 (1 H, s); 13C NMR ((CD3)2SO) δ 29.09, 32.34, 116.44, 118.70, 120.93, 122.13, 122.38, 125.77, 126.04, 126.76, 127.11, 128.36, 128.44, 131.16, 133.15, 136.81, 138.26, 142.76, 148.74, 149.18, 156.52, 161.64, 163.96, 170.73; MS m/z 464.1722 [M + H]+ (C27H22N5O3 requires 464.1723).

8-Methyl-2-(3-oxo-3-(4-((quinolin-8-yl)aminocarbonyl)phenylamino)propyl)quinazolin-4-one (14). Compound 6 (514 mg, 1.95 mmol) in dry DMF (50 mL) was treated with Pr2NEt (2.78 g, 21.5 mmol), then carbonyldimidazole (348 mg, 2.15 mmol), followed by addition of 12 (300 mg, 2.15 mmol). The mixture was stirred for 72 h under Ar. The solvent was evaporated and the residue was dissolved in EtOAc/MeOH (1:2, 70 mL). The solution was washed with water (3 × 30 mL) and brine (3 × 30 mL), then dried. Evaporation and chromatography (1:9 EtOAc / CH2Cl2 → EtOAc) gave 14 (119 mg, 21%) as a beige solid: mp 280-282°C; 1H NMR ((CD3)2SO) δ 2.44 (3H, s), 2.93
(2 H, t, J = 6.0 Hz), 3.01 (2 H, t, J = 7.0 Hz), 7.31 (1 H, t, J = 7.5 Hz), 7.58 (1 H, m), 7.63-7.69 (2 H, m), 7.70 (1 H, dd, J = 8.5, 1.0 Hz), 7.83 (2 H, d, J = 8.5 Hz), 7.91 (1 H, dd, J = 8.0, 1.0 Hz), 8.00 (2 H, d, J = 8.5 Hz), 8.46 (1 H, dd, J = 8.5, 2.0 Hz), 8.72 (1 H, dd, J = 7.5, 1.5 Hz), 8.97 (1 H, dd, J = 4.5, 2.0 Hz), 10.76 (1 H, s), 10.59 (1 H, s), 12.25 (1 H, s); 13C NMR ((CD3)2SO) δ 17.06, 28.99, 32.08, 116.45, 118.67, 120.76, 122.11, 122.38, 123.36, 125.44, 127.11, 127.86, 128.09, 128.34, 134.17, 134.59, 134.84, 136.81, 138.26, 142.91, 147.07, 149.17, 155.12, 161.94, 163.98, 170.98; MS m/z 478.1876 [M + H]+; C20H14N4O requires 478.1879.

Counter-screening against PARP3, PARP4, PARP10, PARP12, PARP14, PARP15 and PARP16. Compounds 13 and 14 were counter-screened for inhibition of these isoforms using methods described previously.31 32 The new inhibitors show no structural alerts for PAINS and are colorless and non-fluorescent.

Insulin-stimulated glucose uptake. 3T3-L1 fibroblasts (from the American Type Culture Collection), were cultured in DMEM and differentiated to adipocytes by treatment with insulin, dexamethasone and isobutylmethylxanthine, as described previously.33 On the day of the experiment, 10-12 d post-differentiation, the cells were incubated with serum-free DMEM for 2 h at 37°C. Cells in the treatment group were treated with increasing concentrations of 13 or 14 for 1 h. At the end of the incubation period, the cells were washed thrice with Krebs-Ringer-HEPES (KRH) buffer (140 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl2, 1.25 mM MgSO4, 2.5 mM NaH2PO4, 10 mM HEPES, (pH 7.4)) and incubated for 30 min with 13 or 14 and in either the absence or presence of insulin (100 nM) at 37°C. After the 30 min incubation period, 2-deoxy-D-[2-6,2H]glucose (final concentration 50 μM, 0.1 μCi ml−1) was added for 5 min and the cells were washed four times with ice-cold KRH buffer. Nonspecific uptake of 2-deoxy-D-glucose was measured in the presence of 10 μM cytochalasin B. The cells were lysed in aq. NaOH (0.1 M) and radioactivity was counted in a TriCarb Packard scintillation counter (Perkin-Elmer). The concentrations of proteins were measured using BCA protein assay kit (Thermo Fisher Scientific). Results were calculated as nmol of 2-deoxy-D-glucose min−1 (mg protein)−1 and are expressed as % of insulin-only control. Statistical analysis; results were analyzed using two-tailed paired t tests.

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Author Contributions

The manuscript was written through contributions of all authors. The authors declare no competing financial interest.

Accession codes

Coordinates and structure factors are deposited at the Protein Data Bank with codes 5PPF and 5PFG. Authors will release the atomic coordinates and experimental data upon article publication.

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ABBREVIATIONS

NAD+, Nicotinamide Adenosine Dinucleotide; ADP, Adenosine diphosphate; GLUT4, Glucose Transporter Type-4; CDI, N,N’-Carbonyldiimidazole; IC50, half-maximal inhibitory concentration; PDB, Protein Data Bank; TCF, T-cell factor; LEF, Lymphoid Enhancer-binding Factor; DMEM, Dulbecco’s Modified Eagle Medium; BCA, Bicinchoninic acid.

ASSOCIATED CONTENT

Supporting Information. Synthetic details, 1H NMR, 13C NMR, HRMS, HPLC, TNKS1 enzyme assay method, TNKS1 IC50 graphs, TNKS2 enzyme assay method, TNKS2 IC50 graphs, PARP1 enzyme assay method, PARP1 IC50 graphs, PARP2 enzyme assay method, PARP2 IC50 graphs, Wnt signaling inhibition cellular assay method, Wnt signaling inhibition IC50 graphs, X-ray crystallography refinement data, colony-forming cellular assays experimental and Molecular Formula Strings. This material is available free of charge via the Internet at http://pubs.acs.org.

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