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Development and structural analysis of adenosine site binding tankyrase inhibitors

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ABSTRACT

Tankyrases 1 and 2, the specialized members of the ARTD protein family, are druggable biotargets whose inhibition may have therapeutic potential against cancer, metabolic disease, fibrotic disease, fibrotic wound healing and HSV viral infections. We have previously identified a novel tankyrase inhibitor scaffold, JW55, and showed that it reduces mouse colon adenoma formation in vivo. Here we expanded the scaffold and profiled the selectivity of the compounds against a panel of human ARTDs. The scaffold also enables a fine modulation of selectivity towards either tankyrase 1 or tankyrase 2. In order to get insight about the binding mode of the inhibitors, we solved crystal structures of the compounds in complex with tankyrase 2. The compounds bind to the adenosine pocket of the catalytic domain and cause changes in the protein structure that are modulated by the chemical modifications of the compounds. The structural analysis allows further rational development of this compound class as a potent and selective tankyrase inhibitor.

Tankyrase 1 (TNKS1/PARP-5a/ARTD5) and tankyrase 2 (TNKS2/PARP-5b/ARTD6) are protein modifying enzymes, which have recently been in the focus of intense search for specific inhibitors. Tankyrases are composed of multiple protein domains and share a catalytic ARTD domain responsible for poly(ADP-ribosyl)ation of target proteins, a SAM domain involved in tankyrase oligomerization, and ankyrin repeats which mediate interactions with other proteins. Tankyrases regulate the stability of target proteins by binding to a specific motif and attaching poly(ADP-ribose) chains to the target protein. The poly(ADP-ribose) chain serves as a recognition site for the E3 ubiquitin ligase RNF146 that induces ubiquitination leading to proteasomal degradation.[1,2]

The most extensively studied pathways in which tankyrases have been shown to elicit a regulatory function are centriole elongation and mitotic spindle formation, telomere cohesion, exocytosis of IRAP and GLUT4 containing trans-Golgi vesicles and the WNT/β-catenin signaling pathway.[3–16]

Here we studied a recently identified tankyrase inhibitor JW55 (**1**)[15] and its analogs. We used a cell-based SuperTOP-Luciferase/Renilla (ST-Luc/Ren) reporter assay to screen compounds for their inhibition of canonical WNT/β-catenin signaling. With a biochemical assay we confirmed that the analogs specifically inhibit tankyrases and profiled the selectivity of the compounds against eight other human ARTDs. To provide structural insights on their inhibitory mechanism, we determined crystal structures of the most potent analogs and the parent compound **1** in complex with TNKS2 and show that the inhibitors anchor to the adenosine subsite of the donor NAD^+ binding groove. The structures explain the high selectivity of the scaffold and provide further routes for enhancing compound potency.

The primary hit **1** moderately inhibited tankyrases and WNT/β-catenin signaling.[15] A selection of JW55 (**1**) analogs (Table 1) were tested for their ability to inhibit β-catenin-mediated canonical WNT signaling at various compound concentrations. This ST-Luc/Ren assay was performed using a WNT3a-induced and stably transfected HEK293 cell line (ST-Luc/Ren HEK293), as reported earlier.[14,17] In vitro inhibition, as measured by biochemical assay complemented the ST-Luc/Ren assay, showed moderate potency of **1** towards tankyrases (TNKS1 IC_{50} 1.80 μM, TNKS2 IC_{50} 2.01 μM, ST-Luc/Ren IC_{50} 1.23 μM), which is in agreement with a previously measured value by an independent biochemical activity assay.[15]

Compounds based on the core structure **1** with substitutions at the furan ring exhibited modest improvements on inhibiting canonical WNT/β-catenin signaling as compared to **1** (Table 1).

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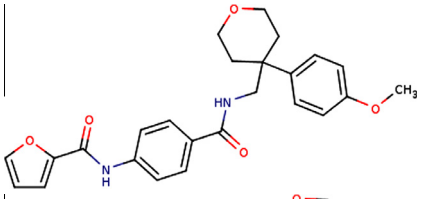
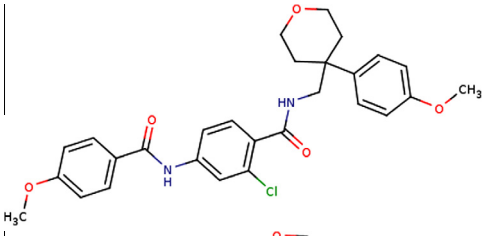
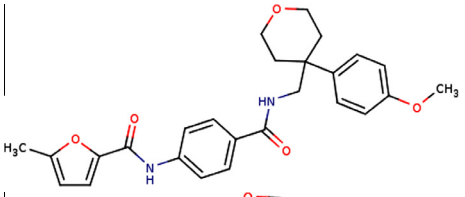
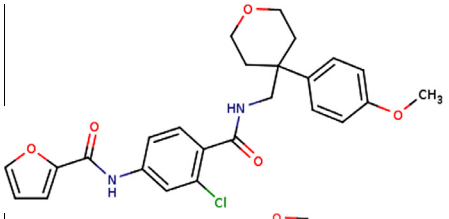
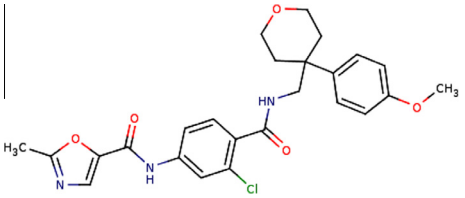
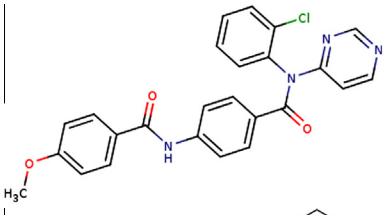
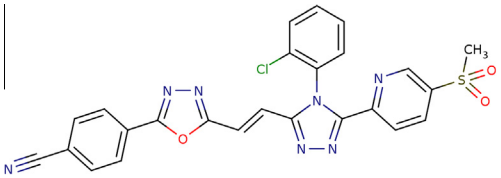
Substituting the furan group with a 4-methoxyphenyl group led to **2** and improved the cellular IC_{50} to 0.79 μM. Interestingly, **2** is a selective inhibitor of TNKS2 with IC_{50} 0.26 μM but has poor potency towards TNKS1 (IC_{50} 11 μM). Despite this isoform selectivity **2** stabilized AXIN1 and destabilized non-phoshorylated β-catenin moderately better than **1** in both HEK293 cells and SW480 cells (Fig. 1). Substituting the furan group with 2-methylfuran led to **3** and gave an improved cellular IC_{50} of 0.38 μM (TNKS1 IC_{50} 0.69 μM; TNKS2 IC_{50} 2.30 μM). Altering the central *p*-phenylene ring of **1** to a 2-chloro-*p*-phenylene led to **4** and showed a cellular IC_{50} of 0.38 μM (TNKS1 IC_{50} 0.57 μM; TNKS2 IC_{50} 2.15 μM). Substituting the furan group in **4** with a 2-methyl oxazole gave **5** but did

Table 1
JW55 analogs as tankyrase inhibitors

	Compound	IC_{50} (pIC_{50} ± SEM)		ST-Luc/Ren IC_{50} ± SDM (μM)	PDB code
		TNKS1	TNKS2		
1		1.80 μM (5.74 ± 0.10)	2.01 μM (5.70 ± 0.12)	1.23 ± 0.60	5ADQ
2		11.08 μM (4.96 ± 0.05)	0.26 μM (6.59 ± 0.19)	0.79 ± 0.05	ND
3		0.69 μM (6.16 ± 0.02)	2.30 μM (5.64 ± 0.21)	0.38 ± 0.20	5ADR
4		0.57 μM (6.25 ± 0.03)	2.15 μM (5.67 ± 0.07)	0.36 ± 0.10	5ADS
5		1.13 μM (5.95 ± 0.18)	2.23 μM (5.65 ± 0.09)	0.69 ± 0.04	5ADT
6		0.55 μM (6.26 ± 0.09)	0.037 μM (7.43 ± 0.08)	1.91 ± 0.10	5AEH
7		0.046 μM ± 0.001	0.025 μM ± 0.006	0.05 ± 0.02	4HYF

Biochemical IC_{50} and corresponding pIC_{50} ± SEM (n = 3) and ST-Luc/Ren IC_{50} ± SDM are reported. Data for **7** is from Voronkov and co-workers and ± SDM is shown for both biochemical and ST-Luc/Ren data.[17]

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