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Discovery of potent and selective nonplanar tankyrase inhibiting nicotinamide mimics

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ABSTRACT

Diphtheria toxin-like ADP-ribosyltransferases catalyse a posttranslational modification, ADP-ribosylation and form a protein family of 17 members in humans. Two of the family members, tankyrases 1 and 2, are involved in several cellular processes including mitosis and Wnt/β-catenin signalling pathway. They are often over-expressed in cancer cells and have been linked with the survival of cancer cells making them potential therapeutic targets. In this study, we identified nine tankyrase inhibitors through virtual and in vitro screening. Crystal structures of tankyrase 2 with the compounds showed that they bind to the nicotinamide binding site of the catalytic domain. Based on the co-crystal structures we designed and synthesized a series of tetrahydroquinazolin-4-one and pyridopyrimidin-4-one analogs and were subsequently able to improve the potency of a hit compound almost 100-fold (from 11 μM to 150 nM). The most potent compounds were selective towards tankyrases over a panel of other human ARTD enzymes. They also inhibited Wnt/β-catenin pathway in a cell-based reporter assay demonstrating the potential usefulness of the identified new scaffolds for further development.

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1. Introduction

Tankyrases belong to the diphtheria toxin-like ADP-ribosyltransferase (ARTD) protein superfamily also known as poly(ADP-ribosyl)polymerases (PARPs) (EC 2.4.2.30).[1] Human tankyrase 1 (TNKS1/ARTD5/PARP5a) and tankyrase 2 (TNKS2/ARTD6/PARP5b) have a C-terminal catalytic ARTD domain, which is conserved in the protein family and is responsible for modifying target proteins by adding one or more ADP-ribose units to specific residues. TNKS1 and TNKS2 are homologous with 82% sequence identity and have overlapping functions.[2] In addition to the ARTD domain, tankyrases have sterile alpha motifs (SAM) responsible for their oligomerization and five ankyrin repeat clusters (ARC) responsible for recognizing and binding target proteins.[3]

The first indication of the therapeutic potential of tankyrase inhibition arose from the observation that tankyrases control the length of human telomeres by poly-ADP-ribosylating (PARsylating) a shelterin protein complex component TRF1. Shelterin protects telomeres by preventing the access of telomerase to telomeres.[4] PARsylation of TRF1 by tankyrases releases TRF1 from the telomeres and allows telomerase to extend the DNA ends. This system is over-activated in cancer cells leading to an uncontrolled telomere extension.[5] Recently many different functions for tankyrases has been discovered[2,6,7] and these have caused an increasing interest in developing tankyrase inhibitors. Regarding cancer two functions of tankyrases are of special interest. TNKS1 was found in spindle poles during mitosis and it is believed to facilitate the formation of normal spindle structure and function.[8] Disrupting this process might be a way to disturb rapidly dividing cancer cells. Tankyrases also control the Wnt signaling pathway, which is a key survival pathway in many cancer cells. Tankyrases PARsylate Axin, which is an essential protein for the formation of the β-catenin destruction complex: a multiprotein complex controlling β-catenin stability through phosphorylation.[9] The PARsylation of axin destabilizes the destruction complex,

Abbreviations: ADE, adenosine subsite; ARC, ankyrin repeat cluster; ARTD, ADP-ribosyltransferase with diphtheria toxin homology; NI, nicotinamide subsite; PAR, poly(ADP-ribose); PARP, poly(adp-ribose) polymerase; SAM, sterile alpha motif; TNKS, tankyrase; TRF1, telomeric repeat binding factor 1.

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stabilizes β-catenin and leads to the activation of the Wnt signaling pathway.[10] Inhibition of tankyrases, therefore, increases cellular levels of Axin and decreases the levels of β-catenin, which ultimately decreases the oncogenic expression mediated by β-catenin and leads to the inhibition of tumorigenesis.[9,11]

Crystal structures of the catalytic domains of both human tankyrases have been solved,[12,13] which enables rational design of tankyrase inhibitors. Protein crystallography has also helped to rationalize the observed selectivity of some of the inhibitors[14–16] and it has been utilized in the development of several TNKS inhibitor scaffolds.[10,17] The donor NAD^+ binding groove of the ARTD domain has two sub-sites, namely the nicotinamide (NI) and the adenosine (ADE) sites, which have been targeted by inhibitors.[7] The known TNKS inhibitors such as **1**–**4** (Fig. 1a) bind to the NI subsite whereas some inhibitors bind to the ADE subsite.[18,19] Also dual binders interacting with both of the subsites have recently been developed.[20,21] The hit compounds identified in this study bind to the NI subsite similarly to several other previously characterized ARTD inhibitors, such as **1** (XAV939). We utilized the available structural data in structure-based virtual screening approach with an aim to identify new tankyrase inhibitor scaffolds. The initial hit compounds were further developed using the existing structural knowledge as a guide for compound synthesis. Structure–activity relationship and tankyrase selectivity was rationalized with the help of protein crystallography. We demonstrate the selectivity of the new inhibitors and show that the compounds are active in a cell-based reporter assay.

2. Materials and methods

2.1. Virtual screening and docking

Energy-based pharmacophore modelling (E-pharmacophore) is a combined effort of pharmacophore perception and protein-ligand interaction energies. E-pharmacophore is computed by docking simulation and the pharmacophore features are then ranked based on their site scores. Here, the E-pharmacophore model was generated from the co-crystal of **1** complexed with TNKS2 and it was subjected to re-docking studies (PDB code 3KR8). This structure was selected as it is a high resolution complex structure of a highly potent compound (XAV939) with TNKS2, which is also used in the biochemical testing of the compounds. The RMSD between the docked pose and crystal conformation is 0.95 Å with glide score of −9.52. It indicates the docking reliability in terms of reproducing the experimentally observed binding mode. The detailed methodology of E-pharmacophore modelling, docking, and ROCS modelling is discussed in Supporting information.

Glide XP (extra precision) module of Schrödinger 9.2 (Glide, version 5.7, Schrödinger, LLC, New York, NY, 81 2011) was utilized for docking. TNKS2–**1** complex structure (PDB: 3KR8) was used for docking of compounds. The protein was prepared using protein preparation wizard and glide energy grids were generated for the prepared protein complex. The binding site was defined by a rectangular box surrounding the ligand (**1**). The ligand was refined using the 'Refine' option in Glide, and the option 70 to output Glide XP descriptor information was chosen (Glide 71 v5.7, Schrodinger, LLC, New York, NY). For the refinement and docking calculations, the default settings as available in the software package were used. The results from the re-docking studies were used for E-pharmacophore modelling.

The generated E-pharmacophore model was further validated by enrichment factor (EF) studies in screening a database. A small library, consisting of 250 tankyrase inhibitors were divided into three bins based on activity range, either highly active (<1 μM), moderately active (1–10 μM) or inactive (>10 μM). Database screening was done by using the pharmacophore model to validate the predictive power of the model. The results were analyzed using a set of parameters such as hit list (Ht), number of active percent of

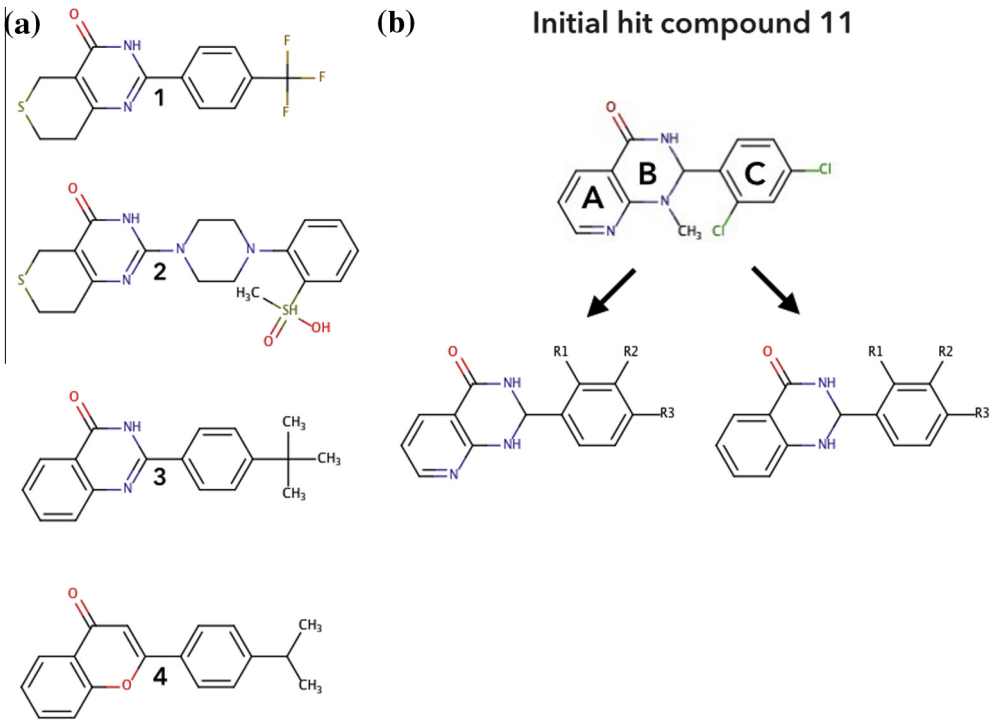

Figure 1. (a) Chemical structure of known tankyrase inhibitors binding to the NI site: **1** XAV939,[13] **2** G244-LM,[38] **3** 2(*tert*-butylphenyl)-3,4-dihydroquinazolin-4-one[15] and **4** 2-4-(propan-2-yl)phenyl-3,4-dihydro-2*H*-1,3-benzoxazin-4-one.[14,30] (b) Schematic representation of **11** and the scaffolds of synthesized pyridopyrimidinone and dihydroquinazolines analogs. The R1, R2 and R3 represent the *ortho*-, *meta*- and *para*-position, respectively, where substitutions were made.

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