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Virtual screening approach of sirtuin inhibitors results in two new scaffolds

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ABSTRACT

Sirtuins (SIRT1–SIRT7) are NAD dependent deacetylases and intriguing drug targets in for example neurodegenerative diseases and cancer. Virtual screening has been shown to be a fast and efficient method of discovering new sirtuin inhibitors. In this study, a new putative binding site on the zinc binding domain of sirtuins was utilized to screen the ZINC database virtually in order to discover new sirtuin inhibiting scaffolds. Altogether 26 compounds were tested *in vitro* and initially 15 inhibitors displayed >30% SIRT3 inhibition. However, the evaluation of raw data from *in vitro* assay revealed that many of the compounds had intrinsic property to interfere with the fluorescence signal at the assay wavelengths resulting in false positives. All compounds with over 30% SIRT3 inhibition were studied more closely for their behavior in the assay and eventually, three compounds were identified as novel sirtuin inhibitors. They displayed 32–40% SIRT3 and 21–60% SIRT2 inhibition. The inhibitors display two new scaffolds, the smaller of which can be considered as a promising fragment, which offers a base for structural optimization.

1. Introduction

The seven human sirtuins (SIRT1–7) are class III histone deacetylases that control the activity of their target proteins by deacetylating the acetylated lysine residues in the substrate sequence. The unique NAD-dependent mechanism of sirtuins links them directly to the nutritional status of cells. The nuclear SIRT1 is a well-studied epigenetic regulator of cellular stress response (Chang and Guarente, 2014). SIRT2 is mainly cytosolic and controls cell division for example with deacetylation of transcription factors and α-tubulin (Jing et al., 2007; North et al., 2003). SIRT3 is the main mitochondrial deacetylase and it plays an important role in the energy homeostasis by controlling the activity of more than 30% of energy production related proteins (Anderson and Hirschey, 2012). Sirtuins generally have a role in keeping cells alive during environmental stress and maintaining their genetic integrity, even though in some cases they have been shown to function as tumor promoters (Wu et al., 2014; Yuan et al., 2013). The inhibition of sirtuins has been shown both to induce cancer cell death and sensitize them to anticancer agents, such as tamoxifen (Cheon et al., 2014; Disch et al., 2013; Li et al., 2013; Mahajan et al., 2014; Peck et al., 2010; Rotili et al., 2012; Verma et al., 2013; Zhang et al., 2013). Other proposed clinical uses of sirtuin inhibitors include the prevention of paracetamol induced liver injuries and neurodegenerative diseases, such as Huntington's and Parkinson's (Di Fruscia et al., 2014; Lu et al., 2011; Sussmuth et al., 2014).

Virtual screening is a widely-used, fast and cheap method for finding new inhibitors for enzymes. If the 3D structure of the enzyme is known, like that of SIRT3, millions of molecules can be docked to the structure to see if they could potentially bind (Jin et al., 2009). This speeds up drug discovery process by reducing the need for *in vitro* experiments, as only the most promising compounds can be chosen for testing. Previously, we have shown that the virtual screening using SIRT3 crystal structure can be utilized to identification of new sirtuin inhibitors (Salo et al., 2013). The screening based on the substrate binding region produced two new scaffolds with up to 71% inhibition.

In the present study our aim was to search potential small molecule sirtuin inhibitors targeting putative allosteric binding sites. We used the crystal structure of SIRT3 bound with a stalled reaction intermediate (PDB ID: 3GLT) (Jin et al., 2009). This structure was chosen as it reveals high-resolution structure of a sirtuin catalytic domain in the active conformation during the deacetylation reaction. By using the SiteMap v. 2.6 feature a potential binding groove was found on the zinc binding domain between residues Phe186, Gln260, Asp290 and Glu296 (Fig. 1) (Halgren, 2009). This putative binding region was used as the target in our virtual screening.

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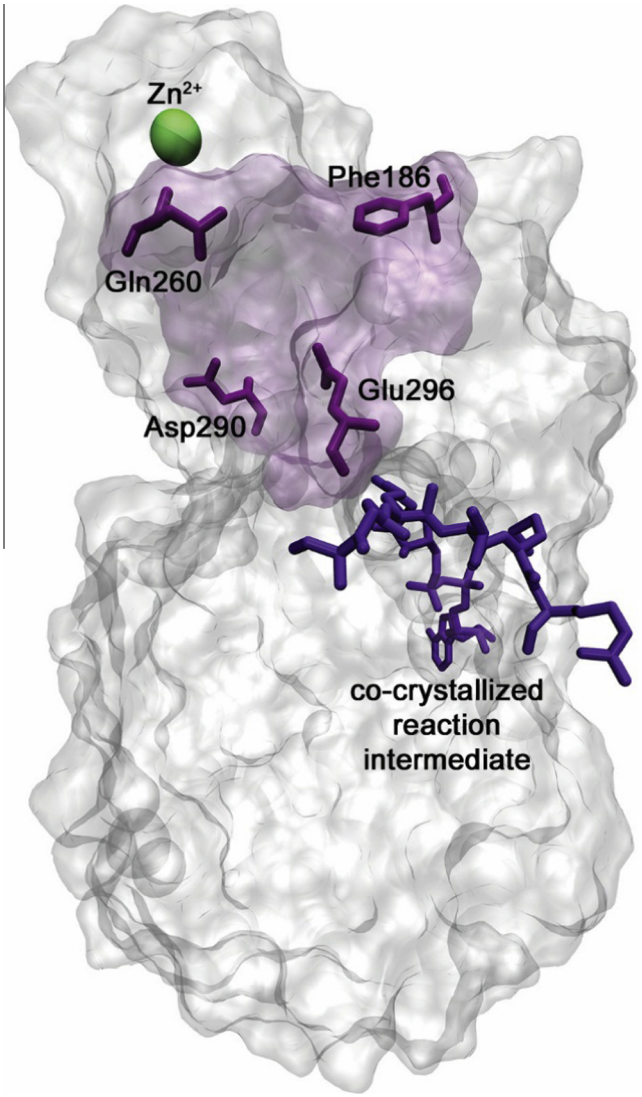

Fig. 1. The crystal structure of SIRT3 bound with a stalled reaction intermediate formed from a peptide substrate with a thioacetyllysine residue and NAD (PDB ID: 3GLT) (Jin et al., 2009). The hypothesized allosteric binding site used in the initial screening is shown in magenta. The residues surrounding the putative binding site and used in the Grid generation are also shown. The image was created with Visual Molecular Dynamics (VMD) v. 1.8.7. (Humphrey et al., 1996). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2. Materials and methods

2.1. Virtual screening

The overview of virtual screening procedure is presented in Fig. 2. For the virtual screening, the protein structure (PDB ID: 3GLT) (Jin et al., 2009) was preprocessed with Protein Preparation Wizard of Schrödinger (Maestro version 9.3, 2012) using standard settings (add hydrogens, assign bond orders, create zero order bonds to metals and disulfide bonds and delete waters beyond 5 Å from heteroatoms). The hydrogen bonds were assigned and the prepared structure was minimized using OPLS_2005 force field and restrained minimization (heavy atom converging RMSD 0.30 Å). The Glide grid was constructed for ligands ⩽20 Å in length and the center of the grid was defined with residues Phe186, Gln260, Asp290 and Glu296 (Fig. 1). The rotation of hydroxyl groups was not allowed.

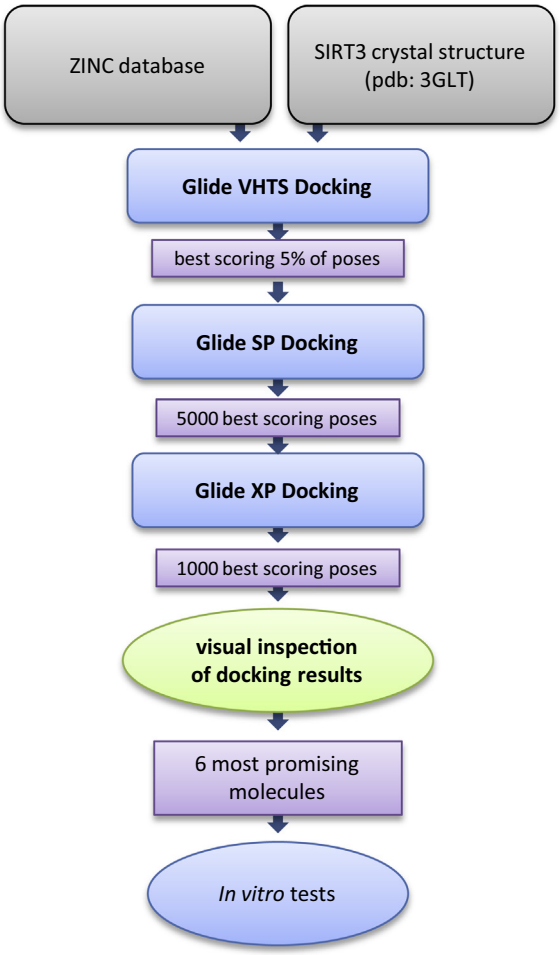

Fig. 2. The overview of the virtual screening procedure.

ZINC database (downloaded on February 8th, 2012) was preprocessed with (LigPrep version 2.5, 2011): the molecules were desalted, the tautomers were determined at pH 7 ± 2 using Epik and all possible enantiomers (or a maximum of 32) were created if chirality was not present in the original structures. We used Glide version 5.8 VHTS docking for the initial screening of the whole ZINC database of over 8 million compounds with standard settings. The best scoring 5% of poses from the VTHS docking were re-docked with Glide version 5.8 SP with standard settings. The 5000 best scoring poses from the SP run were re-docked again with Glide version 5.8, 2012 XP with standard settings to further refine the accuracy of the docking. The 1000 best scoring poses of the XP docking run were visually inspected.

2.2. Inhibition assay

The sirtuin inhibition of the compounds was determined at 200 μM as previously described with Fluor de Lys SIRT1, SIRT2 and SIRT3 assays using commercial assay components (Enzo Life Sciences) (Salo et al., 2013).

2.3. Signal interference

The deacetylated standard (BML-KI142) was used to study the signal interference of the compounds in the assay. The standard curves were determined for the inhibitors and solvent using different deacetylated standard concentrations in the assay buffer. The deacetylated standard was pipetted to wells with inhibitor in