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Quantitative insights for the design of substrate-based SIRT1 inhibitors

Piia Kokkonen, Paolo Mellini, Olli Nyrhilä, Minna Rahnasto-Rilla, Tiina Suuronen, Päivi Kiviranta, Tero Huhtiniemi, Antti Poso, Elina Jarho *, Maija Lahtela-Kakkonen *

School of Pharmacy, Faculty of Health Sciences, University of Eastern Finland, P.O. Box 1627, 70211 Kuopio, Finland

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ABSTRACT

Sirtuin 1 (SIRT1) is the most studied human sirtuin and it catalyzes the deacetylation reaction of acetylated lysine residues of its target proteins, for example histones. It is a promising drug target in the treatment of age-related diseases, such as neurodegenerative diseases and cancer. In this study, a series of known substrate-based sirtuin inhibitors was analyzed with comparative molecular field analysis (CoMFA), which is a three-dimensional quantitative structure–activity relationships (3D-QSAR) technique. The CoMFA model was validated both internally and externally, producing the statistical values concordance correlation coefficient (CCC) of 0.88, the mean value r^2_m of 0.66 and Q^2_{F3} of 0.89. Based on the CoMFA interaction contours, 13 new potential inhibitors with high predicted activity were designed, and the activities were verified by *in vitro* measurements. This work proposes an effective approach for the design and activity prediction of new potential substrate-based SIRT1 inhibitors.

1. Introduction

Histone deacetylases (HDACs) are considered attractive epigenetic drug targets for metabolic and aging-related diseases. They catalyze the deacetylation reaction of N^{ε}-acetylated lysine residues of histones and other proteins with either zinc dependent (classes I, II and IV) or NAD dependent mechanism (class III, sirtuins). Two inhibitors of zinc dependent HDACs, vorinostat and romidepsin, have been approved for the treatment of lymphoma in the USA, which validates zinc dependent HDACs as anticancer targets. Expectations in cancer drug research are also loaded on sirtuins, and their unique NAD dependent mechanism of action makes it possible to specifically target them independently from other classes of HDACs (Balcerczyk and Pirola, 2010).

Among the seven human sirtuins (SIRT1–7), SIRT1 is the most studied. At the cellular level it is localized to the nucleus and the cytoplasm controlling mitochondrial energy production, circadian cycle, autophagy, and cell adaptation to environmental stress (Chalkiadaki and Guarente, 2012; Haigis and Sinclair, 2010). SIRT1 has been associated with aging and age-related diseases, including Alzheimer's disease and type 2 diabetes (Sebastian et al., 2012; Wang et al., 2012). The inhibition of SIRT1 offers possibilities in treatment of human immunodeficiency virus infection and cancer (Chen, 2011; Pagans et al., 2005). So far, the lack of structural information has hindered the use of modern drug design methods in the search for SIRT1 inhibitors. Recently, the SIRT1 crystal structure with bound inhibitor revealed the canonical sirtuin structure (Zhao et al., 2013).

Sirtuins have a conserved catalytic domain with the large Rossmann fold domain and the small zinc binding domain (Zhao et al., 2013). The conserved groove between these two domains is the binding site for the acetylated lysine residue of a protein substrate (Jin et al., 2009). In the active conformation of SIRT1, the substrate binding induces a shift in the linking loop toward the large domain and consequently a rigid body rotation of the small domain relative to the large domain. In apo conformation of sirtuins, the linking loop is further away and therefore the cleft between small and large domain is more open preventing the substrate binding (Yuan and Marmorstein, 2012). We have studied structure–activity relationship of substrate-based sirtuin inhibitors, which use this same binding site. Our studies were initially triggered by the work of Fatkins et al. (2006), when they reported the inhibition of SIRT1 by N^{ε}-thioacetyllysine containing peptides. Later on, the mechanism of inhibition was revealed to be due to a formation of a stalled S-alkylamidate intermediate (Hawse et al., 2008). We have previously reported SIRT1 selective peptidic inhibitors, studied different lysine N^{ε}-modifications and developed many N^{ε}-thioacetylated inhibitors, some of them with antiproliferative properties in breast and lung cancer cell lines (Huhtiniemi et al., 2010, 2011; Kiviranta et al., 2009; Mellini et al., 2013).

* Corresponding authors. Tel.: +358 40 355 2460 (E. Jarho). Tel.: +358 40 355 3692 (M. Lahtela-Kakkonen).
E-mail addresses: Elina.Jarho@uef.fi (E. Jarho), Maija.Lahtela-Kakkonen@uef.fi (M. Lahtela-Kakkonen).

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The large number of developed substrate-based inhibitors prompted us to conduct a 3D quantitative structure–activity relationship (QSAR) study that can be used in the prediction of inhibitory activity of new inhibitors. 3D QSAR connects the activity with the properties of the whole molecule instead of individual substituents, which gives a better understanding of the chemical and structural features affecting the inhibitory activity. One of the most used techniques in 3D QSAR is comparative molecular field analysis (CoMFA), where compounds are studied by their Lennard–Jones and electrostatic interaction energies in a grid lattice (Cramer et al., 1988). CoMFA requires compounds to be aligned in a 3D grid lattice, preferably in their biologically active conformation. The interaction energies are calculated individually for each compound in every point of a typically 2 Å spaced lattice using an sp^3-hybridized carbon with +1 charge as a probe. Partial least squares (PLS) algorithm is used to find the connection between the interaction contours and the biological activities, resulting in a multidimensional QSAR equation. Finally, the activity of a new compound can be predicted based on the interaction energies calculated in an identical lattice using the obtained PLS equation.

In this study, we used the SIRT1 crystal structure to align 79 previously reported substrate-based SIRT1 inhibitors (Fig. 1) and built a CoMFA model validating it with an external test set (Huhtiniemi et al., 2010, 2011; Kiviranta et al., 2009; Mellini et al., 2013). The inhibitory activities of these compounds have been previously determined with the same procedure in our laboratory, which makes the comparison of the activities reliable. Also, 13 new SIRT1 inhibitors were synthesized in order to test the predictivity of this first reported 3D QSAR model of substrate-based SIRT1 inhibitors.

2. Methods

2.1. Computational methods

The inhibitors were exported from our ChemBioFinder Ultra v. 12.0 database and converted to 3D structures using XConcord v. 6.3.1 (Pearlman) with default settings and limits: max rotatable bonds 100, max heavy atoms 200 and max ring size 10. The chirality of molecules was manually checked and corrected if necessary. The tautomers were prepared using LigPrep version 2.5, 2011, ionizer at pH 7 ± 1 with force field OPLS_2005, while the chiralities were not allowed to be changed. The synthetized new inhibitors were sketched with Maestro version 9.2, 2011, and prepared in a similar manner as the rest of the inhibitors.

The crystal structure of SIRT1 (PDB: 4I5I (Zhao et al., 2013)) was preprocessed: bond orders were assigned, hydrogens and zero order bonds to metals added and waters >5 Å from heteroatoms deleted. Missing side chains were added to crystal structure using Prime version 3.0 (2011), and hydrogen bonds were refined with exhaustive sampling keeping sampling water orientations at neutral pH. Impref minimization was performed using OPLS_2005 force field for the protein (heavy atom convergence 0.30 Å). After the minimization of protein structure, the ligands (NAD and (*S*)-EX-527) were removed. For docking run Glide version 5.7 (2011) grid was generated for protein within 20 Å from the point in the middle of the residues His363, Phe414, Asn417 and Arg446. Grid constraints with Val412 carbonyl oxygen, Arg446 carbonyl oxygen and Gly415 amide hydrogen were created to guide the substrate mimicking inhibitors to their assumed binding pose (Jin et al., 2009). The rotation of all OH-groups was allowed.

Molecular docking was performed in order to align the inhibitors in their biologically active conformation. The substrate-based sirtuin inhibitors are assumed to mimic substrate binding so that their lysine forms hydrogen bonds with Val412, Gly415 and Glu416 (Fig. 2) (Huhtiniemi et al., 2011). Glide SP (Glide version 5.7, 2011) docking was done for all inhibitors with all grid constraints, 500 poses for minimization and a maximum of 50 poses were returned for each tautomer. The highest scoring docking pose displaying the hydrogen bonds of the binding hypothesis was collected for the CoMFA analysis. The compounds for which the docking did not produce the substrate-mimicking binding pose, were aligned with a docked compound (Fig. 2) using the Align flexible ligands option in Maestro version 9.2 (2011).

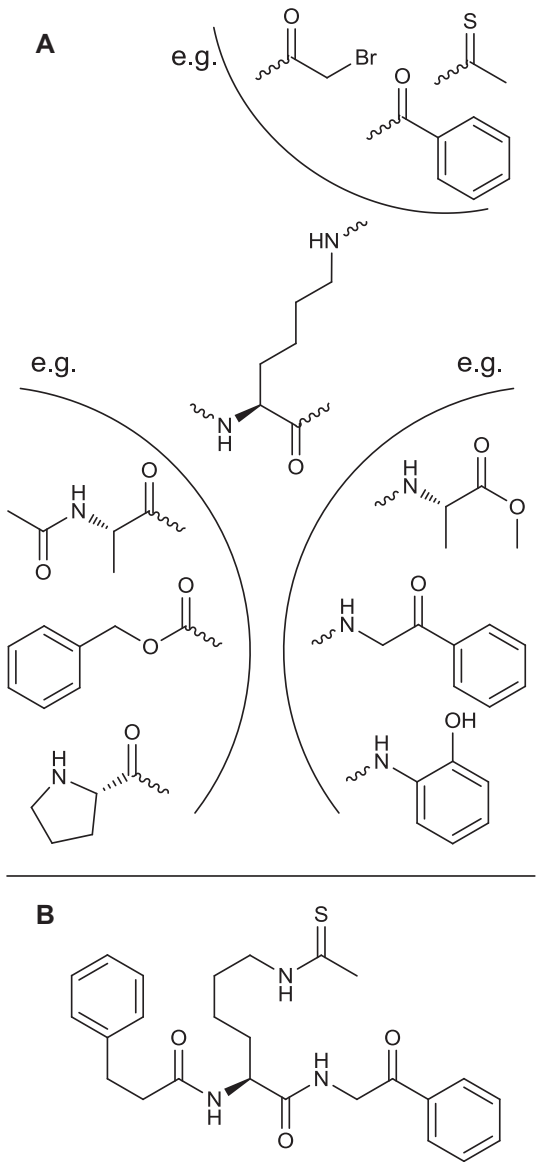

Fig. 1. (A) General structure of the studied peptidic and pseudopeptidic SIRT1 inhibitors. The inhibitors contain a lysine residue with varying C- and N-terminals and N^{ε}-modifications, examples of which are shown. (B) An example of studied inhibitors (**S37**).

The compounds were imported to SYBYL-X 2.0 (2011), for CoMFA analysis. The possible charges of nitrogen and oxygen atoms were manually removed. This was done to prevent the errors caused by the similar structures having different tautomeric forms and thus differing interaction contours. The molecules were divided to training (65 compounds) and test sets (14 compounds, 17% of inhibitors), so that the test set contained compounds with highly variable activities and structures. As the semi-empirical charges have been shown to produce predictive CoMFA models,