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Ligand and structure-based approaches for the identification of SIRT1 activators

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ABSTRACT

SIRT1 is a NAD^+-dependent deacetylase that involved in various important metabolic pathways. Combined ligand and structure-based approach was utilized for identification of SIRT1 activators. Pharmacophore models were developed using DISCOtech and refined with GASP module of Sybyl X software. Pharmacophore models were composed of two hydrogen bond acceptor (HBA) atoms, two hydrogen bond donor (HBD) sites and one hydrophobic (HY) feature. The pharmacophore models were validated through receiver operating characteristic (ROC) and Güner–Henry (GH) scoring methods. Model-2 was selected as best model among the model 1–3, based on ROC and GH score value, and found reliable in identification of SIRT1 activators. Model-2 (3D search query) was searched against Zinc database. Several compounds with different chemical scaffold were retrieved as hits. Currently, there is no experimental SIRT1 3D structure available, therefore, we modeled SIRT1 protein structure using homology modeling. Compounds with Q_{fit} value of more than 86 were selected for docking study into the SIRT1 homology model to explore the binding mode of retrieved hits in the active allosteric site. Finally, *in silico* ADMET prediction study was performed with two best docked compounds. Combination of ligand and structure-based modeling methods identified active hits, which may be good lead compounds to develop novel SIRT1 activators.

1. Introduction

Sirtuins are a family of deacetylase enzymes that require a cofactor nicotinamide adenine dinucleotide (NAD^+), and catalyze deacetylation reaction in two steps. In the first step, sirtuins cleaves NAD and produce nicotinamide (NAM), and in second step acetyl group is transferred from substrate to ADP-ribose moiety of NAD to generate O-acetyl-ADP ribose and the deacetylated substrate [1]. They originated as conspicuous regulator of several aspects of cell survival, stress resistance, and metabolism [2]. Sirtuins consists of a large gene family of primordial origins with homology existing from yeast to humans [3]. Sirtuins regulates gene expression, which is based upon energetic state of cell, sensed through NAD^+ level that deacetylates histones as well as transcription factors and co-regulators [4]. Sirtuins also control circadian clocks and mitochondrial biogenesis. The first sirtuin, SIR2 (silent information regulator 2) was originally found to promote longevity in yeast. Subsequent research resulted into seven mammalian SIR2 homologs proteins (SIRT1–7). SIRTs have different specific substrates and biological functions, and are found in various cell compartments. SIRT1 is one of seven *human* sirtuins (SIRT1–7) of 747 residues that in mammals, which share a catalytic domain of approximately 277 amino acids with other sirtuins and require NAD^+ to mediate deacetylation of histone and non-histone proteins [5]. Human SIRT1 plays a role in wide range of cellular process like transcription, aging, apoptosis, endocrine signaling, inflammation [6] and stress resistance, as well as energy efficiency and alertness during low-calorie situations [7]. SIRT1 consist of four different structural regions: N-terminal region, the allosteric site made of 4 helices, catalytic core domain and C-terminal region. To date, experimental structure of human SIRT-1 has not yet been reported. 3D structure of SIRT1 catalytic core domain is available on the protein data bank (PDB ID: 4I5I [8], 4IF6, 4KXQ [9]). SIRT1 catalytic core domain composed of a large NAD^+-binding subdomain and a small subdomain made up of a helical module and a Zn^{2+}-binding module. In literature, it is reported that allosteric site is responsible for the activation of SIRT1 [10–12]. Resveratrol, a phenolic compound is the first found allosteric activator of SIRT1 [13]. A nuclear protein AROS increases SIRT-1 activity by interacting with the

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allosteric site, is an endogenous activator [14]. Recent data showed that SIRT1 activation is beneficial in the treatment of certain metabolic disorders. SIRT1 regulates several metabolic process that allows cell for adapting nutrient stress, and has a crucial role in age-related metabolic diseases. SIRT1 protects the organism from metabolic diseases by leading to confine energy consumption and utilization in the physiological state [15]. Several studies showed that SIRT1 mediates stress responses, including inflammation, hypoxic stress, heat shock and genotoxic stress, and inflammation. These are important cause of aging and age-related diseases and disorders. SIRT1 suppress inflammation through its effect on nuclear factor-kB (NF-kB) [16,17]. SIRT1 is also responsible for age-dependent overall transcriptional changes through chromatin modification [18]. In pancreatic β cells, SIRT1 regulates glucose-stimulated insulin secretion through the synthesis of uncoupling protein 2 (UCP2) [19,20]. Cardiovascular diseases increase with aging, and are also closely affected by the cellular metabolism. Several evidence showed that SIRT1 has pivotal role in cardiovascular functions [11,21]. Role of SIRT1 in tumor progression is controversial as it might have dual function, as an oncogene and tumor suppressor. SIRT1 deacetylate lysine residue (LYS383) of p53, thereby repressing its transcriptional activity and function as an oncogene [22,23]. However, several studies showed that SIRT1 has potential tumor suppressor effect [11,24]. SIRT1 play an important role in neuronal physiology and pathology related to Alzheimer's disease [25]. Expression of SIRT1 changes in several physiological conditions, resulting in induction during low energy state and repression during excess energy state [26]. SIRT1 activators are useful agents for prevention or treatment of various metabolic disorders. Activation of SIRT1 is thought to be beneficial not only for diseases related to metabolism, such as type-2 diabetes and obesity, but also for neurodegenerative diseases like Alzheimer's disease and Parkinson's disease [27].

Combination of structure and ligand-based approach along with ADMET prediction study is a best way to identify lead compounds in the drug discovery process [28–31]. Recently, Sakkiah et al., identified potent compounds as SIRT1 activator [32]. In this work, the authors developed bayesian and pharmacophore model and applied density functional theory (DFT) to identify the fragments and critical chemical features of small molecules as SIRT1 activator. In pharmacophore modeling six known SIRT1 activator were used to generate six feature based hypothesis. Overall, ligand-based 2D and 3D approaches were utilized and a total of 16 compounds were selected as leads molecules. In our work, we utilized combination of ligand and structure-based methods as a rational tool for the identification of novel hits with diverse chemical scaffolds as SIRT1 activators. Ligand-based pharmacophore models were prepared using ten known SIRT1 activator and validated by ROC and GH scoring methods. The best pharmacophore model as a 3D search query was screened against Zinc database. Several compounds with different chemical scaffolds were retrieved as hits. Because of the lack of details on experimental 3D structure of SIRT1 and allosteric site, therefore, in this work we modeled human SIRT1 protein of 747 residues and performed molecular docking study with retrieved hits to known the interactions of the molecules with the active site. Finally, two best docked compounds were selected for *in silico* ADMET prediction study.

2. Materials and methods

2.1. Dataset and computational details

A data set of known SIRT1 activators were obtained from the published literature [32–37] and used for generation of pharmacophore models. Molecular modeling, and all *in silico* simulations were carried out on windows platform with core i3 processor. Pharmacophore mapping, database searching and molecular docking studies were performed using the SYBYL X 1.2 software from Tripos Inc., St. Louis, MO, USA [38]. The multiple sequence alignment for homology modeling was performed using CLUSTALW program [39]. I-TASSER program was used for the protein homology modeling [40,41]. PROCHECK was used for structure validation study [42]. Retrieved hits for docking study were sketched using SKETCH function of SYBYL. Partial atomic charges were calculated by the Gasteiger–Huckel method and energy minimizations were performed using the Tripos force field [43] with a distance-dependent dielectric and the Powell conjugate gradient algorithm convergence criterion of 0.01 kcal/mol Å [44].

2.2. Generation of pharmacophore models

Ten molecules (**A**–**J**) (Fig. 1) were selected form the data set to generate 6 pharmacophore models using DISCOtech module, considering the structure and activity diversity. The genetic algorithm similarity program (GASP) was used to refine the generated models, which results in 3 pharmacophore models (1–3 models). All the parameters were kept as default, except population size (125), mutation weight (96), fitness increment (0.02) and number of alignment (04).

2.3. Validation of the pharmacophore model

3D pharmacophore models (models 1–3) were validated using receiver operating characteristic (ROC) and Güner–Henry (GH) scoring methods.

2.3.1. Receiver operating characteristic (ROC) analysis

ROC analysis [28,45] was performed to validate pharmacophore models 1–3 to selectively capture diverse SIRT1 activators from a large list of decoys database (Directory of Useful Decoys) [46]. A testing set was prepared, which contains decoy compounds in combination with diverse SIRT1 activators. Conformers of the testing set compounds were generated using a genetic algorithm-based global optimizer to find the low energy conformations. ROC curve is a function of Sp (1-specificity) versus the Se (sensitivity), and the area under curve (AUC) value. ROC analysis is the important way of measuring the performance of the test.

2.3.2. Güner–Henry (GH) scoring method

The GH scoring method was applied to quantify model selectivity (best model), accuracy of hits and the recall of actives from a molecule dataset consisting of known actives and inactives [28,47,48]. The GH score ranges from 0 (null model) to 1 (ideal model), it should be greater than 0.7 for a reliable model. The GH scoring method takes into account both the percent yield of actives in a database (%Y, recall) and the percent ratio of actives in the hit list (%A, precision). The GH scoring method was applied to 30 known SIRT1 activators and the decoy dataset (200 molecules) to validate the pharmacophore models.

2.4. Virtual screening

After 3D pharmacophore model generation and validation, the best pharmacophore model (model-2) was used as a 3D search query for retrieving potent hit molecules from the Zinc database. The hits identified from Zinc database were filtered by applying Lipinski's rule-of-five [49] that sets the criteria for drug-like properties. Those molecules which satisfied all the features of the pharmacophore model were retrieved as hit molecules.

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