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ORIGINAL ARTICLE

In Silico Docking Analysis of Marine Actinobacteria Derived Antibiotic, Grincamycin against the Epigenetic Enzyme Coactivator-Associated Arginine Methyltransferase1 (CARM1)

Sasirekha Sivakumar[1,2], Jerrine Joseph[3], Radhakrishnan Manikkam[3], Kishore Narayanan[1] and Balagurunathan Ramasamy[2]*

[1]Aurigene Discovery Technologies Limited, 39-40, KIADB Industrial Area, Phase II Electronic City, Hosur Road, Bangalore-560100 Karnataka, India.
[2]Department of Microbiology, Periyar University, Periyar Palkalai Nagar, Salem 636011, Tamil Nadu, India.
[3]Centre for Drug Discovery and Development, Sathyabama Institute of Science and Technology, Chennai 600 119, Tamil Nadu, India
*Corresponding Author Email: actinobalaguru@gmail.com

ABSTRACT

Cancer is a heterogeneous disease that is managed with drugs that have diverse modes of action. The disease's complexity necessitates the evaluation of additional inhibitors to be developed as promising drug candidates. The role of epigenetic modifiers in cancer, as well as inhibitors targeting epigenetic modifiers, has recently received considerable attention. Many secondary metabolites derived from marine actinobacteria that have been reported to have anti-cancer activity must be investigated to determine their mode of action. Grincamycins, which are angucycline glycosides isolated from the marine Streptomyces strain, have shown to have anti-cancer activity. Cresset flare software was used in this study to perform in silico simulations of several Grincamycin derivatives with coactivator-associated arginine methyl transferase belonging to epigenetic writer class of enzymes. The docking scores for the best compounds' selected poses ranged from -5.808 to -7.438. Among the tested derivatives, the Grincamycin derivative B with a ligand fitscore of -9.735 had the best pose and docking score. According to the findings, Grincamycin B could be a potential inhibitor of coactivator-associated arginine methyltransferase, which warrants further in-vitro evaluation on the cancer target.

Keywords: *Marine actinobacteria, Grincamycin, epigenetic target, anti-cancer, CARM1, in silico.*

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INTRODUCTION

Cancer is a disease characterized by uncontrolled growth of cells. The integrity of the cell growth and maintenance is controlled by well-orchestrated network of signaling pathways. Abnormalities in genetic and epigenetic mechanisms cause uncontrolled cell growth. Genetics is a blueprint that is present in every cell and cannot be changed. While epigenetics is the modifications carried out in the nucleosome by epigenetic modifiers which are reversible and classified as readers, writers, and erasers. Epigenetic modifiers are being investigated as therapeutic targets, potentially leading to the development of Epidrugs [1, 2]. Tazemetostat, an inhibitor of epigenetic enzyme, enhancer of zeste homolog 2, was recently approved for clinical trials [3].

Protein arginine methyl transferases (PRMTs) belongs to the writer class of epigenetic modifiers which adds methyl groups to arginine in substrates which are histone and non-histone proteins. Coactivator-associated arginine methyl transferase (CARM1) alias PRMT4 is a type 1 protein arginine methyl transferase, co-ordinates transcriptional activities and associates with other transcription factors to regulate target gene expression. CARM1 is known to be oncogenic and high levels are reported in breast [4], prostate, colon [5] and lung [6] cancers. CARM1's oncogenic role in multiple cancer indications has

positioned it to be investigated as a potential therapeutic target, and synthetic inhibitors to inhibit its activity are being developed.

Actinobacteria have been a rich source of secondary metabolites with anticancer activity. Grincamycins belonging to the class of angucycline glycosides isolated from marine derived *Streptomyces lucitanus* strain are known to possess anti-tumor activity [7]. Several types of Grincamycins are identified and they are reported to have cytotoxic activity in breast, liver, lung, pancreatic, colon, and cervical cancer cell lines [8]. In a recent study, it has been reported that Grincamycin B functions as a potent inhibitor for glioblastoma stem cell via targeting RHOA and PI3K/AKT axis [9].

In this study, *in silico* experiments were carried out to determine if CARM1 could be a potential target of the Grincamycin class of compounds, with the goal of identifying potential inhibitors as anticancer leads targeting CARM1 for further wet lab validation.

MATERIAL AND METHODS

Target preparation

Based on a review of the literature, the enzyme CARM1 (Co-activator associated arginine methyl transferase) was chosen as a protein target [10]. Protein Data Bank was used to obtain its crystal structure (PDB ID: 6D2L) from the web sitehttps://www.rcsb.org/structure/. The co-crystallized ligand was removed from the target using the Cresset Flare software by protein preparation module.

Ligand preparation

The 3D structures of Grincamycin derivative ligands B, C, D, E, F, G, and H were obtained from the PubChem database and saved in SDF format. For the docking studies, the structures of these ligands were imported into Cresset Flare software.

Docking

The docking grid box was defined based on the co-crystallized structure of the enzyme bound with ligand. The docking calculations were performed using Cresset Flare software (Flare, version, Cresset®, Litlington, Cambridgeshire, UK) in normal mode and default settings [11].The obtained results included parameters such as Lipinski's rule-of-five violations, rank score, Virtual Screening (VS) score, and binding energy (dG). The above values were generated as a table and ranked according to the poses and binding energy scores. The PDB structure was stripped of all water molecules and co-crystallized ligands. Ligands were docked using a standard rigid receptor-flexible ligand docking method that employs five potential energy maps. The maps were created in a rectangular box with 0.5A^{o} grid spacing that was centered on the ligand-binding site. Each molecule was first conformationally analyzed outside of the protein pocket, and a stack of low energy conformations were collected and used as the starting geometries for grid docking. Ligand binding modes were scored according to the quality of the complex, and a user-defined number of the top-scoring poses were re-ranked using the scoring function. The weighted sum of the set of parameters was used to calculate the predicted score. The parameters considered were ligand-target Van der Waals interactions and internal force field energy of the ligand, free energy changes due to conformational energy loss upon ligand binding, hydrogen bonding interactions, hydrogen bond donor-acceptor desolvation energy, solvation electrostatic energy upon ligand binding, hydrophobic free energy gain, and a size correction term proportional to the number of ligand atoms. Docking in Flare™ makes use of Lead Finder™ to provide accurate pose prediction. Using a ligand template to seed docking of multiple ligands with a common substructure will result in better docking results [12].

RESULTS AND DISCUSSION

Molecular docking is a computer-aided technique used for low-cost and swift identification of small compounds that bind to specific targets. Since docking involves the binding site of specific targets, promising ligands with potential binding affinity against the target can be selected for biological testing. The virtual docking method was used to identify novel inhibitors because it plays an important role in the identification of new compounds for the inhibition of protein targets. In this study, molecular docking was performed for Grincamycin B, C, D, E, F, G, and H using bioinformatics docking software Flare, version, Cresset®, Litlington, Cambridgeshire, UK, and Spark version against the CARM1protein.

Table 1.The Grincamycin derivatives docking results based on the Ligand Fit rank scoring.

Compound Name	Poses	Ligand Fit Rank Score
Grincamycin B	8	-9.735
Grincamycin C	7	-2.899
Grincamycin D	9	-3.377
Grincamycin E	8	-3.527
Grincamycin F	9	-4.289
Grincamycin G	4	-3.617
Grincamycin H	9	-4.043

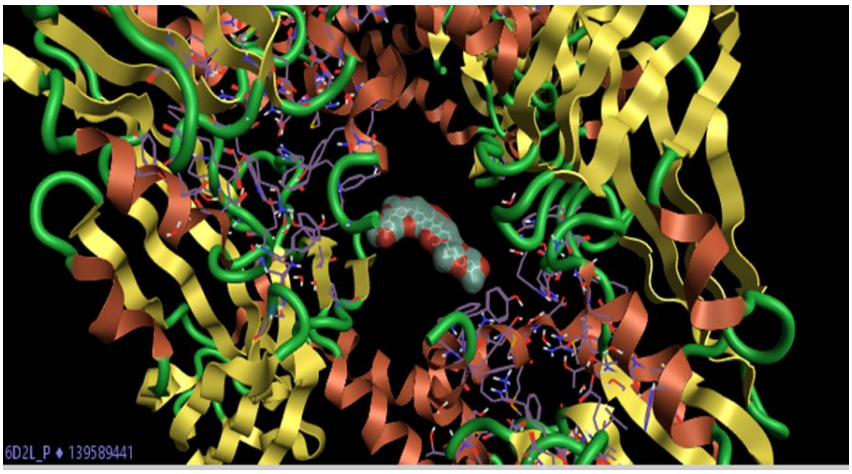

Figure 1.The docking results of the CARM1protein and Grincamycin B ligand

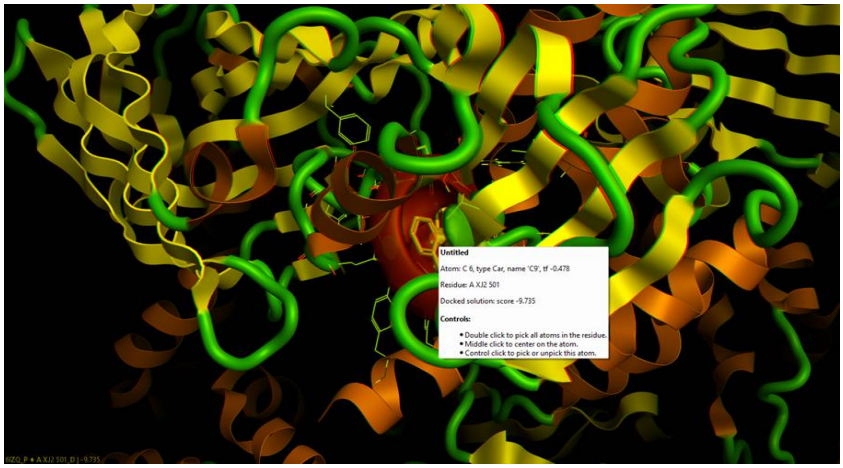

Figure 2. Best docked Grincamycin B molecule with LF score of -9.735

The energy score for docked images of the ligand into the binding site is tabulated and shown in Table 1. The docked image of the ligand into the binding site is depicted in Figure 1. All the produced binding poses were manually checked for accurate positioning inside the binding pocket, paying special attention to the interactions of the ligand moieties with the amino acid residues that are important for inhibitory activity. Residues such as Ser 145, Ala 146, Val 147, Gln 148, Tyr 149, Phe 150, Glu 151, Phe 152, Tyr153, and Gly 154 play an important role in the interactions of the protein with inhibitors which is depicted in Figure 2. These residues were used as a filter to discard the incorrect poses derived from the docking. Moreover, the compounds were ranked by a docking score. The scoring function was employed to predict the biological activity by scrutinizing the interactions between the compound and the potential target. The docking scores for the selected poses of the best compounds were in the range of −5.808 to −7.438. Among the tested derivatives, the Grincamycin B derivative had the best pose and docking score, with a Ligand fit (LF score) of -9.735; the higher the negative score, the more stabilised the docked ligand inside its pocket with best-fit parameters of binding affinity properties [13].

CONCLUSION

The screening of new potential inhibitors with a scaffold based on Grincamycin was evaluated in the current study using the cresset flare software. The docking score function results obtained based on the algorithm of its force fields, energy minimization and binding affinity show that Grincamycin B derivative has the best ligand fit score. This study suggests that Grincamycin B could be a promising inhibitor for CARM1 thereby qualifying for further *in vitro* evaluation in cancers which are dependent on CARM1 activity.

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