Arenosclerin E Derivatives as Inhibitors for Mycothiol-S-conjugate Amidase

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Abstract - Background and Objective: ***Mycobacterium tuberculosis*** **is the causative agent of tuberculosis (TB) and accounts for latently infected one-third of the world population. Increasing incidence of multidrug-resistant and extensively drug-resistant TB is a serious concern. Therefore, it is urgent to identify effective and inexpensive anti-tubercular drugs. Materials and Methods: In this regards, 3D model structure of Mycothiol-S-conjugate amidase (Mca) was previously built by comparative homology modeling program in our work. Arenosclerin E was a marine sponge derived antibacterial agent and inhibitor of Mca protein reported in our previous work. In this work, twelve Arenosclerin E derivatives were screened from pubchem compound database were docked with Mca model structure using AutoDock4.2.The docked complex structures were optimized by molecular dynamics simulation for 5 ps with the CHARMM-22 force field using NAMD incorporated in VMD 1.9.2 and then evaluating the stability of complex structures by calculating RMSD. In silico ADME/Tox of best predicted derivatives of Mca were evaluated. Results: From docked compound, we got two best compound CID 102080317 and CID 16215157 with optimal binding energy -13.54 kcal/mol and -13.72 kcal/mol, which were lower than Arenosclerin E (-13.11 kcal/mol). Compounds have HIA in the range of well absorbed compounds (HIA: 70 ~ 100 %) and P_{CaCO2} value in standard range (P_{CaCO2} >70 nm/sec). The cell permeability in MDCK of compounds was in mean range 25 to 500 nm/s and Skin permeability showed negative values. Derivatives bind strongly to plasma proteins and belonging to active compound range (BBB >1). The Ames test showed compounds were non-mutagen and carcinogenicity in mouse and rat showed negative value. Conclusions: Molecular dynamics simulations of docked complex and in silico ADME and Toxicological properties of predicted compounds CID 102080317 and CID 16215157 showed stable and satisfactory results and could be promising inhibitors for Mca protein as drug target.**

Keywords: Mycothiol-S-conjugate amidase; *Mycobacterium tuberculosis*; Permeability; Arenosclerin E; Carcinogenicity

I. INTRODUCTION

Tuberculosis (TB) had been considered as one of the most fatal diseases of human and remains a major world-wide health hazard. From the beginning of seventeenth century until World War-II, several parts of Europe, America and Japan suffered from the TB epidemic with the consequence of death of several million people[1]. Approximately, one third of the world's population is currently infected with TB. WHO in March 2017 estimated that six countries account for 60% of the total, with India leading the count, followed by Indonesia, China, Nigeria, Pakistan and South Africa[2]. *Mycobacterium tuberculosis* is the causative agent of TB poses a challenge for modern medicine. Presently available tuberculosis treatment has many problems such as (i). D*uration* and complexity of treatment result in multidimensional health care problems. (ii) Increasing incidence of multidrug-resistant and extensively drug-resistant TB is a serious concern. Second-line drugs such as para-aminosalicylate (PAS), fluoroquinolones, kanamycin, ethionamide, cycloserine and capreomycin used for drug-resistant TB are not available everywhere, more toxic with serious side effects and require longer use than first-line drugs[3]. (iii) Co-infection of TB and HIV is a problem by itself causes 1.5 to 2 million deaths per year[4]. Combined treatment of TB and HIV involves a high pill count with associated adherence problems, overlapping toxicity profiles of the antiviral and anti-TB drugs and drug interactions. WHO has developed the DOTS strategy to optimize response and adherence to TB treatment. However, DOTS (directly observed treatment, short-course) is labor-intensive and expensive. It causes a high burden on public health programs, especially in developing countries with limited human resources. The continuing emergence

 DOI : 10.35291/2454-9150.2019.0272

of these problems novel, effective and inexpensive anti-tubercular drugs is an urgent priority.

To do this, we have target sulfur metabolic pathways that are absent in humans and present in *M. tuberculosis*. These pathways represent unique targets for therapeutic intervention. Mycothiol regulates cellular redox status and is essential for *M. tuberculosis* survival[5]. Mycothiol (MSH) is the functional equivalent of glutathione in mycobacteria[6, 7] and protect of *M. tuberculosis* from toxic oxidants and antibiotics[8]. Mca enzyme involved in MSH metabolism and detoxification and plays a critical role in mycobacterial detoxification of antibiotics[5]. Therefore, inhibitors of Mca could enhance the sensitivity of MSH-producing bacteria to antibiotics, establishing Mca enzyme as a promising new drug target.

Marine Sponges had efficient defense mechanisms against foreign attackers such as bacteria, viruses or eukaryotic organisms. They are richest sources of pharmacologically-active chemicals. Marine sponge derived Arenosclerin E was an antibacterial agent and inhibitor of Mca, already reported in our previous published work[9]. In this work, we have screen Arenosclerin E derivatives from pubchem compound database[10] using similar compound, score >=95 and docked with model Mca protein. Docked complexes stability was evaluated by molecular dynamics simulation. In this study, in silico absorption, distribution, metabolism, and excretion (ADME) and toxicological properties of best predicted Arenosclerin E derivatives of Mca were evaluated.

II. MATERIALS AND METHODS

Inhibitors dataset

We have screened Arenosclerin E derivatives from pubchem compound database[10] using similar compounds, score>=95. The 3D structures of screen 12 derivatives were downloaded in .sdf format from pubchem compound database. They were later converted in .pdb format with the help of open babel tool[11].

Molecular Docking

Homology model of Mycothiol-S-conjugate Amidase (Mca) was retrieve from our previous work[9]. Docking of 12 derivatives of Arenosclerin E against Mycothiol-S-conjugate Amidase (Mca) structure was done using molecular docking program AutoDock4.2[12]. Gasteiger charges are added to the ligand and maximum 6 numbers of active torsions are given to the lead compounds using AutoDock tool[13]. Kollman charges and the solvation term were added to the protein structure. The Lamarckian genetic algorithm implemented in Autodock was used for docking.

Molecular dynamics simulations

Molecular dynamics simulations were done using the NAMD[14] graphical interface module incorporated in VMD[15]. The protein-ligand complex was immersed in the center of a 50 Å box of water molecules where all water molecule atoms were closer than 1.5 Å and a CHARMM22 parameter file for proteins and lipids was used in the force field for complexes. The psf was created from the initial pdb and topology files using psfgen package of VMD. After running psfgen, two new files were generated protein.pdb and protein.psf and by accessing PSF and PDB files; NAMD generated the trajectory DCD file.

ADME and Toxicological properties of inhibitors

Absorption, distribution, metabolism, and excretion (ADME) and toxicological properties were essential for pharmacological/clinical applications of identified inhibitors. Therefore, The predicted inhibitors were evaluated for key physicochemical properties like molecular weight, Hydrogen Bond Donor Count, Hydrogen Bond Acceptor Count, XLogP and ADME properties like percentage of human intestinal absorption (HIA), cell permeability (P_{Caco-2}), cell permeability Maden Darby Canine Kidney (MDCK), skin permeability, Plasma protein binding (PPB), blood brain barrier (BBB) using PreADMET[16] tool. Toxicological properties of mutagenicity and carcinogenicity were also evaluated using PreADMET tool.

III. RESULTS AND DISCUSSION

Molecular Docking

Docking studies predicted the interaction of ligands with protein. For such interaction studies, the most important requirement was the proper orientation and conformation of ligand which fitted to the enzyme binding site appropriately and formed protein-ligand complex. Therefore, optimal interactions and the best AutoDock score were used as criteria to interpret the best conformation among the 10 conformations, generated by AutoDock program. The docking results of 12 compounds with Mca model was shown in Table 1. Among the above docked compounds CID 102080317 and CID 16215157 had the lower binding energy -13.54 kcal/mol and -13.72 kcal/mol, even lower than Arenosclerin E (-13.11 kcal/mol) with Mca protein. Docking poses of the best conformation of CID 102080317 and CID 16215157 with Mca protein model were shown in fig. 1 & 2.

Similar work was performed Watty et al[17], they conducted in silico screening using AutoDock and Vina to obtain potential marine fungi bioactive compounds as EGFR-tyrosine kinase inhibitors. They concluded that the three marine fungi compounds with the lowest binding free energy, Fiscalin A, Aspergiolide B and Sporothrix A have great potential as inhibitors of EGFR-tyrosine kinase inhibitors.

Khamkar et al[18] studies the interaction of antibiotic squalamine and LAQ824 with selected anticancer drug target enzymes using in silico molecular docking approach.

 DOI : 10.35291/2454-9150.2019.0272

The ligand squalamine showed minimum binding energy with promyleocytic leukemia and estrogen related receptor α. Similarly, the compound LAQ824 showed minimum binding energy with BRCA2. They concluded that squalamine and LAQ824 ligand would be of potent drugs to treat various cancers.

Molecular dynamics simulation

After Molecular dynamics simulation, the results were analyzed in VMD by calculating the Root mean square deviation (RMSD) of the complex using rmsd.tcl source file from the Tk console and finally rmsd.dat was saved and accessed in Microsoft office excel. RSMD, a crucial parameter to analyze the equilibration of MD trajectories, is estimated for backbone atoms of the compounds CID 102080317 and CID 16215157 with Mca protein complex (shown in fig. 3 & 4). Measurements of the backbone RMSD for the complex provided insights into the conformational stability.

ADME and Toxicological properties of best predicted Arenosclerin E derivatives

In analyzing the parameters of the best predicted compounds was observed that all had values within Lipinski parameters, except XlogP to evaluate oral absorption (table2). It was observed that the compounds CID 102080317 and CID 16215157 have human intestinal absorption (HIA) values 98.155672 and 98.141383, respectively (table3). These compounds are categories as in the range of well absorbed compounds (HIA: 70 ~ 100 %)[19]. The absorption processes are related to the permeation of compounds through biological membranes under the influence of physicochemical characteristics. The cell permeability in vitro Caco-2 is an important test to assess intestinal absorption of drugs. It was found that the P_{CaCO2} (nm/s) value were 56.0907 nm/s and 56.0531 nm/s for compound CID 102080317 and CID 16215157, respectively (table 2). P_{CaCO2} value of compounds were in standard range, i.e. P_{CaCO2} >70 nm/sec[20] . The cell permeability in vitro in MDCK system is used as a tool for the rapid analysis of permeability. These derivatives had 69.7924 nm/s and 73.2333 nm/s as MDCK, respectively (table 2). The cell permeability in MDCK of compounds was in mean range 25 to 500 nm/s[21]. Skin permeability parameter is used in the pharmaceutical industry to assess the risk chemical products in case there is accidental contact with skin[22]. Predicted derivatives CID 102080317 and CID 16215157 showed negative permeability values (table3). The binding of drug to blood and plasma proteins can alter the half-life of the drug in the body of the individual[23, 24]. It is verified that derivatives bind strongly to plasma proteins, being 86.485044 and 84.754945 (table 3). The blood-brain barrier (BBB) has an importance in the pharmacology of drugs, because the compounds are classified as inactive and active compounds. Identified derivatives were belonging to active compound range (BBB >1)[25]. The Ames test[26] assesses mutagenicity of the compounds. Compounds CID 102080317 and CID 16215157 submitted to this test showed negative prediction, i.e., were predicted as a non-mutagen (table 5). Carcinogenicity is a toxicity that causes cancer in body. On analyzing carcinogenicity in mouse and rat both compounds CID 102080317 and CID 16215157 showed negative value (table 3). Similar work was performed by Stella and Meena[27], they identifying the activity of compounds using flexible docking isolated from the marine sponges - Diplastrella sp, genus Lendenfeldia, Ancorina sp. and Coscinaderma sp., and to identify their critical chemical features, with reliable geometric constraints that can be used as an effective drug. They evaluated compounds for antiviral activities. Compounds fulfill the Lipinski's rule of five and have showed optimum ADMET properties that are desirable as a drug.

IV. CONCLUSION

Two best predicted compounds CID 102080317 and CID 16215157 observed having lower binding energy even lower than Arenosclerin E. Molecular dynamics simulations showed that predicted compounds were stable. In silico ADME and Toxicological properties of predicted compounds showed satisfactory results. Therefore it is predicted that Compounds CID 102080317 and CID 16215157 could be promising inhibitor for Mca protein as drug target yet experimental studies have to confirm it.

ACKNOWLEDGEMENT

This article is a part of Ph.D. studies carried out by the author registered at IFTM University, Moradabad, India. The authors are grateful to Prof. R. M. Dubey, Vice Chancellor, IFTM University, Moradabad, India for providing an institutional research promotion program and their generous help and encouragement to proceed with the studies in the School of Biotechnology IFTM University, Moradabad, India.

CONFLICT OF INTEREST

The authors have no conflict of interest regarding the publication of this paper.

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Table 1: Docking result of Arenosclerin E derivatives with Mca protein.

Derivative	PubChem CID	BE	IME	IE	TorE	VdwE	EE
	Arenosclerin E (44421344)	-13.11	-13.41	0.13	0.3	-10.26	-3.14
1	16215036	-11.4	-11.7	0.18	0.3	-9.19	-2.5
2	10254693	-11.84	-12.14	0.15	0.3	-10.38	-1.76
3	10413003	-11.7	-12.0	0.2	0.3	-8.94	-3.06
4	10624661	-12.12	-12.42	0.22	0.3	-9.75	-2.67
5	16215157	-13.72	-14.02	0.18	0.3	-10.68	-3.34
6	102006431	-11.81	-12.1	0.17	0.3	-9.34	-2.76
7	102006432	-12.35	-12.65	0.21	0.3	-10.22	-2.43
8	102006433	-12.53	-12.82	0.19	0.3	-10.33	-2.49
9	102006434	-12.66	-12.96	0.19	0.3	-10.75	-2.22
10	102080317	-13.54	-13.84	0.23	0.3	-10.51	-3.33
11	102080319	-11.84	-12.14	0.23	0.3	-9.53	-2.61
12	102080320	-12.37	-12.67	0.25	0.3	-10.18	-2.49

BE: Binding Energy (Kcal/mol); IME: Intermolecular Energy (Kcal/mol); IE: Internal Energy (Kcal/mol); TorE: Torsional Energy (Kcal/mol); VdwE: vdW + Hbond + desolv Energy (Kcal/mol); EE: Electrostatic Energy (Kcal/mol).

 DOI : 10.35291/2454-9150.2019.0272

Table 2: Physicochemical and absorption properties of best predicted compounds**.**

Pubchem CID	Mol. Wt. (g/mol)	HBD	HBA	XLogP	Absorption			
					HIA (%)[a]	P_{Caco-2} (nm/sec)[b]	MDCK[c]	Skin Permeability[d]
102080317	482.797	1	3	8.4	98.155672	56.0907	69.7924	-1.60764
16215157	482.797	1	3	8.7	98.14138	56.0531	73.2333	-1.85837

Mol. Wt.: Molecular Weight; HBD: Hydrogen Bond Donor Count; HBA: Hydrogen Bond Acceptor Count; [a]: percentage of human intestinal absorption; [b:] cell permeability (Caco-2 in nm/s); [c]: cell permeability Maden Darby Canine Kidney (MDCK) in nm/s; [d] skin permeability.

Table 3: Distribution properties in percentages of PPB, penetration of the blood brain barrier, toxicological properties of mutagenicity (Ames test) and carcinogenicity (mouse and rat) for best predicted compounds.

PubChem CID	Distribution		Ames Test	Carcinogenicity	
	PPB (%)	BBB		Mouse	Rat
102080317	86.485044	18.11	non-mutagen	negative	negative
16215157	84.754945	16.6651	non-mutagen	negative	negative

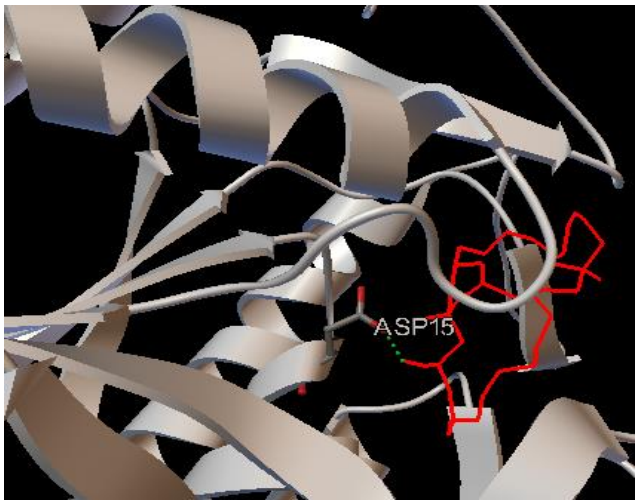

Figure 1: Docking orientation of compound CID 102080317 with Mca protein. Complex depicting compound formed one H-bond with ASP15 of protein, which is represented by green dotted sphere. ASP15 is represented by sticks and balls and colored by atom type. Compound is represented by red lines.

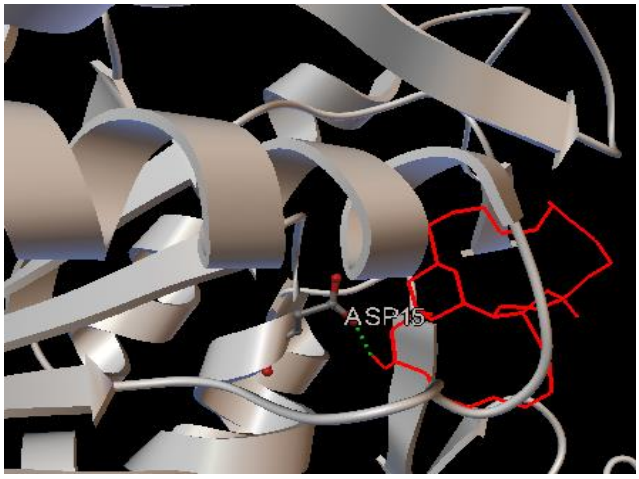

Figure 2: Docking orientation of compound CID 16215157 with Mca protein. Complex depicting compound formed one H-bond with ASP15 of protein, which is represented by green dotted sphere. ASP15 is represented by sticks and balls and colored by atom type. Compound is represented by red lines.

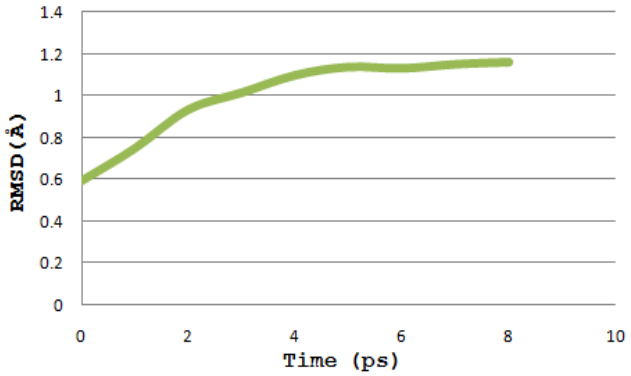

Figure 3: Graph displaying RMSD of the backbone atoms of docked complex (CID 102080317 - Mca protein) versus time at 310 K, resulted in highest peak at 1.15 Å.

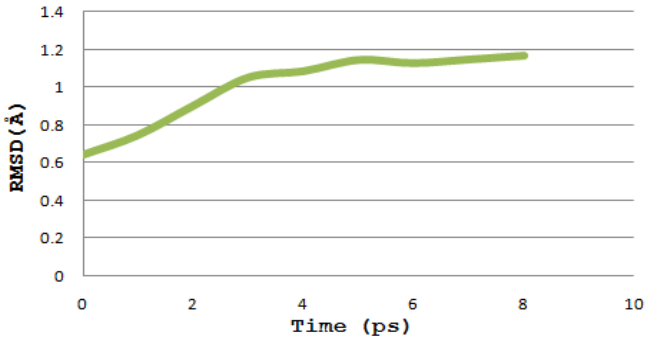

Figure 4: Graph displaying RMSD of the backbone atoms of docked complex (CID 16215157 - Mca protein) versus time at 310 K, resulted in highest peak at 1.16 Å.

 DOI : 10.35291/2454-9150.2019.0272