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MOLECULAR DOCKING OF SALMONELLA TYPHI QUORUM SENSING REGULATED TRANSCRIPTION FACTOR SDIA

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ABSTRACT
Quorum sensing (QS) regulated transcription factor SdiA responds to a broad range of AHLs and vitally involved in multiple gene expression. SdiA positively regulate the genes, responsible for MDR Salmonella typhi leads to serious potential public health problem. Aims of antimicrobial therapies are inhibiting cell growth (bacteriostatic) or causing cell death (bacteriocidal). Amongst these, therapies that promising strategies are development of challenging inhibitors against quorum sensing siganlling pathways causing multi drug resistant pathogens. In the present study, SdiA docked with inhibitors Coumarin-3-Carboxylic acid, Esculetin, Resveratrol, Ginkgolic acid, 7-Hydroxy indole, C6-HSL, Daphnetin, 4-Tetradecanol. The esculetin compound has the good G-score better binding affinity with SdiA. This study conclude Esculetin may potentially inhibit SdiA activity and their role in and MDR.

INTRODUCTION
Salmonella species are facultative and motile, non-spore forming, and are piece of the normal flora in the gut of vertebrates. Utiization of contaminated chicken, beef, pork, sausage, meat paste, unpasteurized cheese, and lettuce is a major cause of food-borne salmonellosis (Quinn et al.,2006). There are two types of *Salmonella* infections: the clearly known typhoidal *Salmonella* (caused by serotypes *S.* Typhi and *S.* Paratyphi A, B or C) and the less serious and generally self-limiting non-typhoidal *Salmonella* (caused by serotypes other than *S.* Typhi and *S.* Paratyphi). Both types of *Salmonella* infections are procured by the fecal-oral transmission mode, but typhoid fever is closely associated with incidences direct human contamination of foods or sewage contamination of crops, meats, and water and humans are the only known reservoirs of typhoidal *Salmonella*. Affected humans will be ill for about a day and very rarely have serious complications other than dehydration associated with their symptoms of diarrhea and vomiting. There is an impressive array of virulence factors contributing to the pathogenesis of *S. aureus*, summarized by Murray, *et al. (2013)*

The LuxR protein, which regulates the transcription of several genes, including the ones that lead to bioluminescence (Engebrecht *et al*., 1983). Numerous bacteria contain LuxR homologues, the *E. coli* LuxR homologue, SdiA (suppressor of division inhibition), is regulated in a quorum-sensing-dependent manner (Sitnikov *et al*., 1996) and has been shown to regulate the *ftsQAZ* cell division genes (Wang *et al*., 1991). In Gram-negative bacteria, multiple distinct classes of quorum-sensing signals have been discovered thus far, and additional signals probably exist. The *N*-acyl homoserine lactone (AHL) family functions in the LuxR–LuxI pathway (Fuqua and Greenberg, 1998) *Escherichia coli* and *Salmonella enterica* serovar Typhimurium encode a single *luxR* homolog named *SdiA* (Ahmer, 1998, Wang, 1991). The genomic organizations of the *SdiA* region are identical in the two species (Bindhu Michael, 2001)Upstream of *SdiA* is an uncharacterized open reading frame (ORF) named *yecC* which is like to the ATP binding component of ABC transporters (Ahmer, 1998, Blattner 1997). Downstream of *SdiA* is a gene named *uvrY* in *E. coli* and *sirA* in *Salmonella* serovar Typhimurium (Johnston, C 1996, Kahn, 1991). Further downstream is the *uvrC* gene, which encodes a DNA repair enzyme. Despite the name, *uvrY* plays no role in DNA repair but instead encodes a transcription factor of the FixJ family that controls virulence functions in all g-proteobacterial pathogens examined to date (Goodier, 2001). The *SdiA* gene was expressed under the control of the *araBAD* promoter on a multicopy plasmid (pJVR2) and placed in a *Salmonella* serovar Typhimurium *SdiA* mutant strain so that expression of *SdiA* was dependent on the presence of arabinose. Random

lacZY transcriptional fusions (MudJ transposon insertions) were created in this strain and screened for a difference in expression on plates containing glucose versus arabinose. Ten MudJ fusions that react to plasmid-borne *SdiA* but not to a vector control were recognized. Although these fusions were responsive to *SdiA* overexpression, they were not active when *SdiA* was expressed from its natural position in the chromosome (Ahmer, 1998). This suggested that any putative ligand detected by SdiA was absent in pure cultures of *Salmonella* serovar Typhimurium and that overexpression of *SdiA* somehow bypasses the requirement for a ligand (Ahmer, 1998). A biofilm is an assemblage of microbial cells that is irreversibly related (not removed by gentle rinsing) with a surface and enclosed in a matrix of primarily polysaccharide material. This property is important because it permits association of divalent cations such as calcium and magnesium, which have been shown to cross-link with the polymer strands and provide greater binding force in a developed biofilm. The importance from a public health perspective is the role of biofilm in antimicrobial drug resistance. The resistance of microbes residing in the biofilms towards various types of antimicrobial agents poses a serious threat to the pharmaceutical industries.

MATERIALS AND METHODS

Sequence and Compound retrieval

The aminoacid sequence of SdiA from *Salmonella typhi* retrieved from UniProtKB database (http://www.uniprot.org/). Three dimensional structure of Coumarin-3-Carboxylic acid, Esculetin, Resveratrol, Ginkgolic acid, 7-Hydroxy indole, C6-HSL, Daphnetin, 4-Tetradecanol, compounds retrieved in SDF format from Pubchem (http://www.ncbi.nlm.nih.gov/pccompound) for comparative docking studies on their efficacy towards Quorum sensing regulator SdiA. SDF format compound were converted into PDB format by using Open Babel GUI.

Homology Modelling

There being no reported X-ray crystal structure available for *S.typhi* SdiA protein. So, the sequence identity of targeted protein was compared against Protein Data Bank using blastP. SdiA had 72% identity with *E.coli* SdiA structure 4Y15 (PDB ID) respectively. So homology modeling of SdiA from Salmonella *typhi* against template SdiA protein of *E.coli* (PDB ID: 4Y15) carried out using MODELLER 9.11V. Structure validation was carried out using online server PROCHECK.

Docking

Docking of SdiA protein with retrieved compounds has been carried out using schrodinger glide module. Protein structure refinement, optimization, energy minimization, and partial atomic charges were done using protein preparation wizard. Retrieved compounds were prepared using ligprep. For each compounds tautamers were generated and optimized. Grid box were generated for all the residues. All the prepared compounds were then subjected to docking against the molecular target SdiA using Glide extra-precision method. Glide-xp (Extra Precision) mode determines all reasonable conformations for each low-energy conformer in the designated binding site. In the process torsional degrees of each ligand are relaxed, though the protein conformation is fixed. The glide scoring function (G-Score) was used to select the best interaction for each ligand.

RESULTS AND DISCUSSION

SdiA Sequence and compound structure retrieval

The SdiA sequence retrieved from UniprotKB database. Accession number, no of amino acid sequences and Molecular weight of the SdiA Sequences are Q8Z5T1, 240 amino acids, 28.14 KDa. Retrieved compound details are shown in Table 1.

Protein structure prediction and validation

S.typhi SdiA protein structure was theoretically modeled using Modeller9.11V. The modeled structure of SdiA protein is shown in **Fig 1**. Modelled structure validated by Ramachandran plot analysis using PDBSum PROCHECK. Ramachandran plot for Modelled SdiA protein are shown in **Fig 2**. The sequence alignment of the S.typhi SdiA with template protein sequence are shown in **Fig 3**. The Ramachandran plot is probably the most powerful determinant of the quality of protein (Laskowski RA et a.,l 1993, Hooft RW et al.,1996). When Ramachandran plot quality of the model is comparatively worse than that of the template, then it is likely that error took place in backbone modeling. 204 amino acids (93.2 %) are present in allowed region and 15 amino

acids (6.8%) are located in additional allowed regions of Ramachandran plot. No amino acids located in disallowed regions. This suggest the modeled protein are suitable for Drug designing.

Molecular docking

Molecular docking used to predict the structure of the intermolecular complex formed between two or more molecules. The most interesting application in medicine is protein ligand interactions (Sharma *et al*., 2010). Molecular docking used to predict the binding orientation of a small molecule (ligand) to the receptor that means drug receptor interactions. This will give the theoretical knowledge in binding affinity of the small molecule towards receptor. The docking of receptor SdiA with esculetin ligand exhibited well established bonds with two amino acids TYR 71 and SER 134. We have docked with HSL molecule which is helpful for Quorum sensing and upregulate the expression of the MDR pump (Rahmati *et al*., 2002). The esculetin compound and C6-HSL compound interact with SER 134 but the esculetin have good glide score -6.99 (Kcal/mol). The interaction of Esculetin and C6-HSL are shown in **Fig 4 & 5**. This suggests esculetin may occupy the AHL interaction with SdiA and inhibit the SdiA activity through which it can control MDR expression.

Table 1: Name of the selected compounds and its pubchem ID

S.No	Compound Name	G-Score (Kcal/mol)	No of H.Bonds	Interactive residues
1	Esculetin	-6.99	2	TYR 71 SER 134
2	Daphnetin	-6.33	1	SER 134
3	Resveratrol	-6.09	2	TYR 71 (2)
4	C6-HSL	-6.37	2	SER 34 ASP 80
5	7-Hydroxy Indole	-5.86	3	TYR 63 TRP 67 PHE 100
6	Coumarin	-5.72	2	SER 134 TRP 95
7	Tetra decanol	-5.61	2	TYR 63 TRP 67
8	Ginkgolic acid	-3.73	1	ARG 60

Table 2: Molecular Docking of selected compounds, Hydrogen bonding interaction against S.typhi SdiA.

S.No	Pubchem ID	Compound Name
1	10752	Coumarin-3-Carboxylic acid
2	688505	C6-HSL
3	5281416	Esculetin
4	445154	Resveratrol
5	5281858	Ginkgolic acid
6	2737651	7-Hydroxy indole
7	5280569	Daphnetin
8	33740	4-Tetradecanol

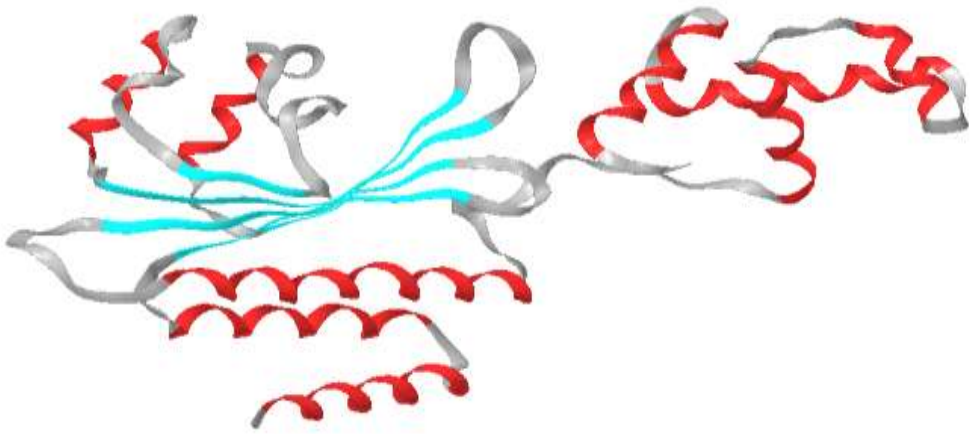

Fig 1: Theoretically modeled structure of S,typhi SdiA by selecting E.Coli SdiA (PDB ID: 4Y15) as template using Modeller 9.11v.

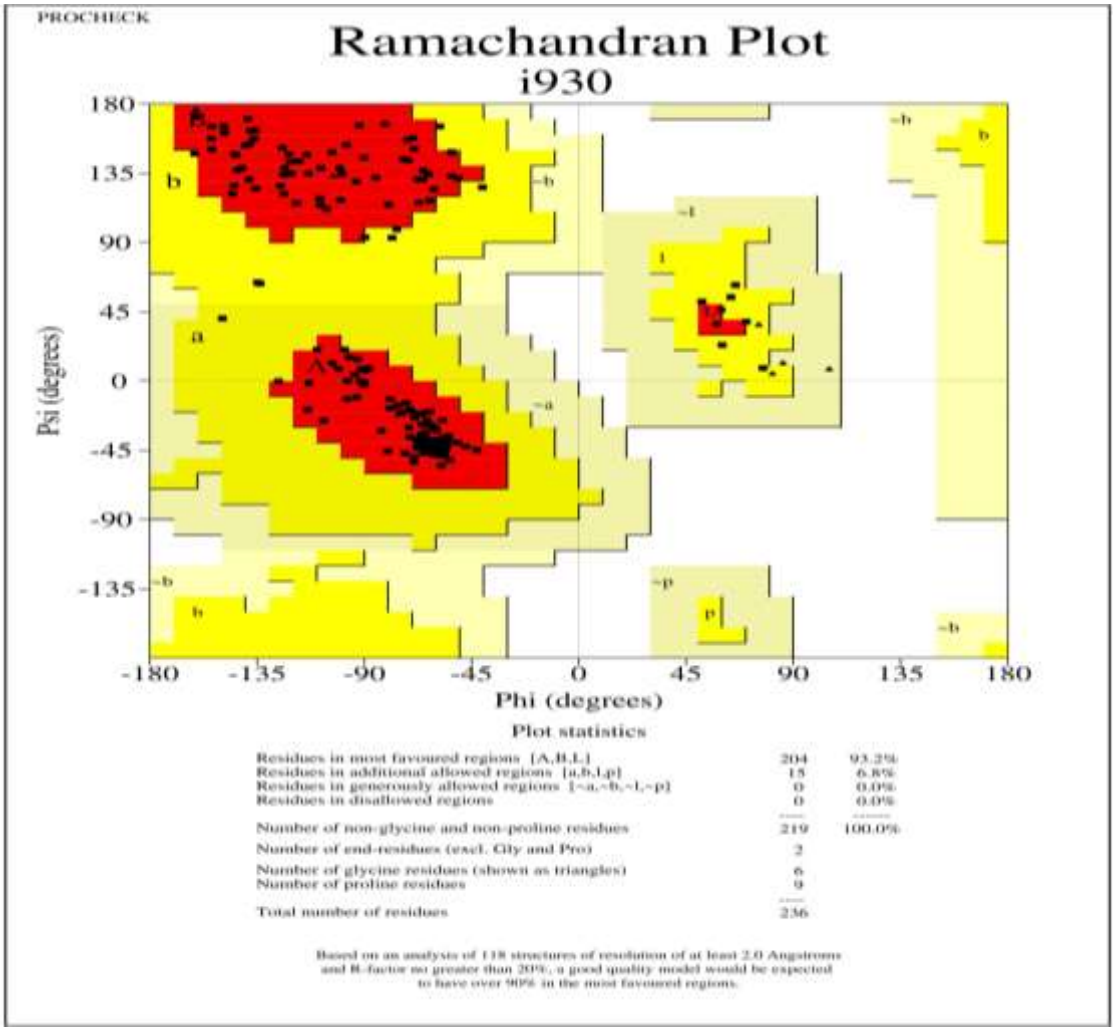

Fig 2: Ramachandran plot for theoretically modeled SdiA protein structure

Fig 3: Sequence allignment of SdiA protein sequence against E.Coli SdiA protein sequence

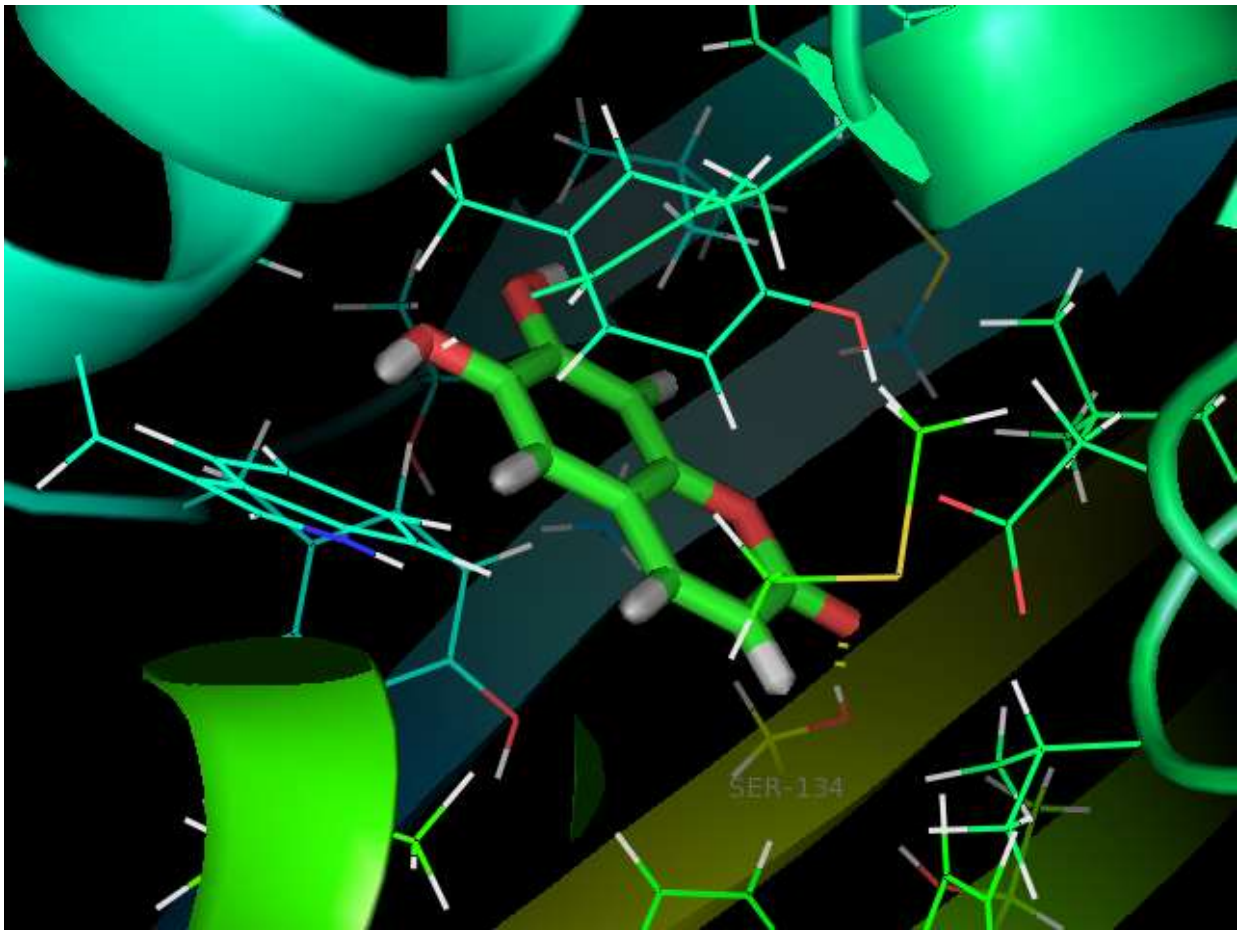

Fig 4: Esculetin interact with SdiA TYR 71 and SER 134 residues. glide score of the interaction are -6.99 Kcal/mol.

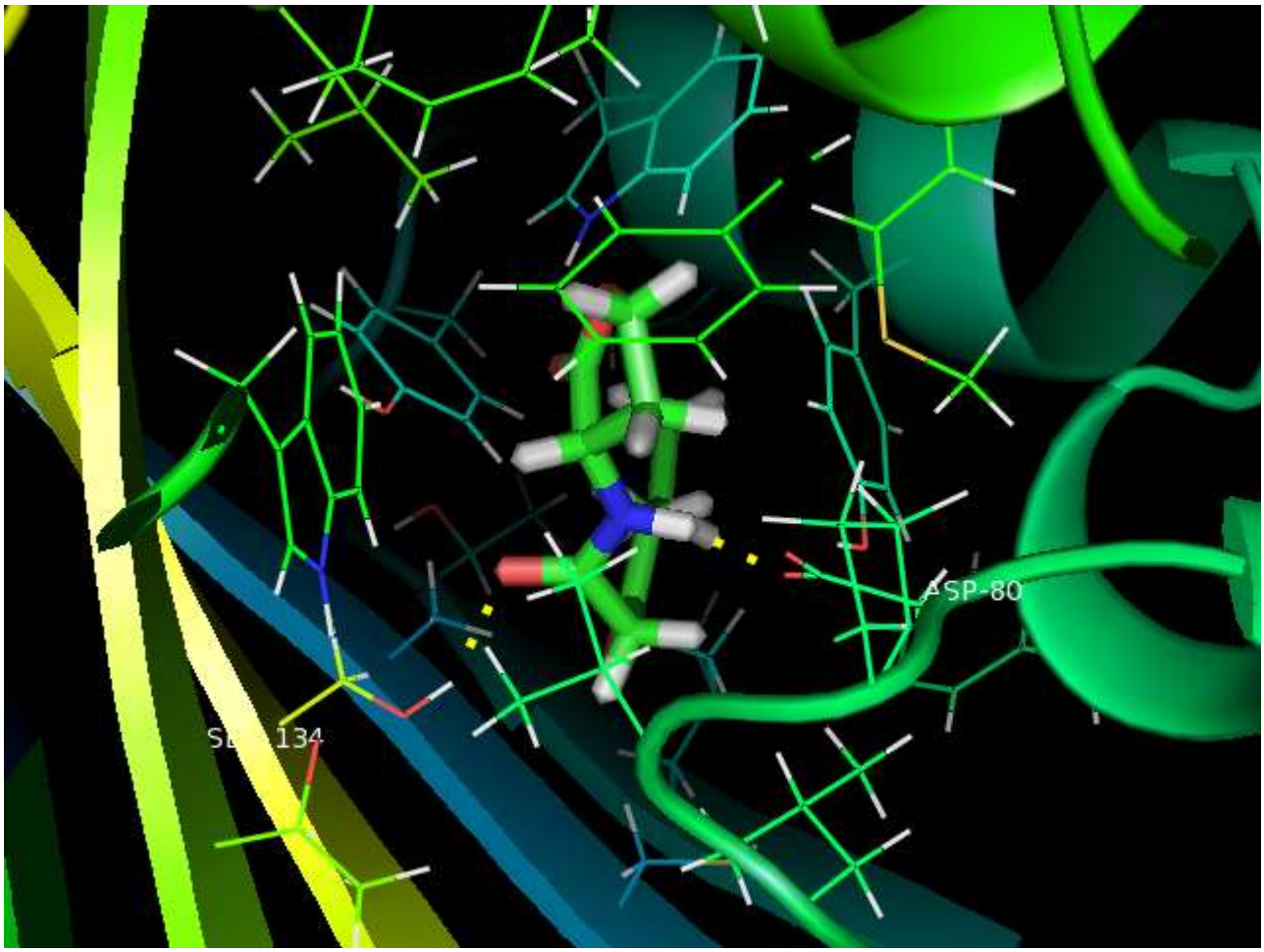

Fig 5: C6-HSL interact with SER 134,ASP 80 residues of SdiA with the glide score -6.37 Kcal/mol

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