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Research Article

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Homology modeling of S-adenosylmethionine synthase present in *E. maximum* as a drug target and its docking with 1-monocaproin

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ABSTRACT

The parasitic infection becomes more common nowadays, mortality due to coccidiosis in poultry birds are increasing day by day. This kind of infection will affect the production and economy of poultry farms, mostly this infection are caused by parasites of Genus Eimeria affects birds such as poultry and mammals such as cattle and rabbits. Hence, it is necessary to control the death of the birds through a novel approach. This study is mainly investigated on the targeting the crucial enzyme S-Adenosylmethionine synthase present in E.maximum. The 3D structure of the S-Adenosylmethionine synthase is modeled using homology modeling techniques and docking with 1-monocaproin, found the binding energy as -2.85kcal/mol. The interaction is observed in Gly and Phe amino acid present in the S-Adenosylmethionine synthase. Hence in future, passing out the trails it can be used as one drug candidate for treating this poultry disease caused by E.maximum.

Keywords: Coccidiosis, *E.maximum*, S-Adenosylmethionine synthase, homology modeling, poultry disease

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INTRODUCTION

The parasite infection is common in bird caused by coccidian protozoa. Infection will spread quickly from one bird or animal to another via infected feces or ingestion of infected tissue. This kind of infection will affect the production and economy of poultry farms, mostly this infection are caused by parasites of Genus *Eimeria* affects birds such as poultry and mammals such as cattle and rabbits[1]. The species *E. acervulina, E. maxima and E. tenella* are most important poultry coccidian parasite[2]. Tyzzer, 1929 described E. maxima was the first avian coccidial parasites, usually large oocysts, distinctive size of *E. maxima* is used in a diagnosis of the disease and differentiation from other species of *Eimeria*[3]. An *E.maximum* is commonly used for experiments of an immunological nature, it is considered to be highly immunogenetic and capable to re-infection even with small numbers of parasites[4]. The life cycle of *E. maxima* is more complex than other parasites, there are three distant phases namely sporogony, schizogony and a sexual phase, gamogony, so far there are four vaccines preferred to prevent the poultry birds from this disease[5]. Anticoccidials are given in the feed to prevent disease and the economic loss often associated with subacute infection. Prophylactic use is preferred, because most of the damage occurs before signs become apparent and because drugs cannot completely stop an outbreak. Continuous use of anticoccidial drugs promotes the emergence of drug-resistant strains of coccidia. Various programs are used in attempts to slow or stop selection of resistance. For instance, producers may use one anticoccidial continuously through succeeding flocks, change to alternative anticoccidials every 4–6 mo, or change anticoccidials during a single growout (ie, a shuttle program). While there is little cross-resistance to anticoccidials with different modes of action, there is widespread resistance to most drugs. “Shuttle programs,” in which one group of chickens is treated sequentially with different drugs

(usually a change between the starter and grower rations), are common practice and offer some benefit in slowing the emergence of resistance.

The effects of anticoccidial drugs may be coccidiostatic, in which growth of intracellular coccidia is arrested but development may continue after drug withdrawal, or coccidiocidal, in which coccidia are killed during their development. Some anticoccidial drugs may be coccidiostatic when given short-term but coccidiocidal when given longterm. Most anticoccidials currently used in poultry production are coccidiocidal[6]. Our approach is to evaluate 1-monocaproin as a prophylactic by adding in the feed as feed additive to prevent and control the coccidiosis.

In this current study, we approached the new techniques using computational biology to attack the important enzyme that present in the parasite *E.maximum*. S-Adenosylmethionine synthase is a key enzyme in the synthesis of polyamines in mammals, plants, and many other species[7]. This enzyme plays an important role in gene transcription and cell proliferation and hence in this research study it is considered as crucial drug target protein[8]. The X-ray or NMR 3D structure are not available for this enzyme present in the *E.maximum*. Hence computational homology modeling method is used to build the protein model of this structure, subsequently it is validated in structure verification server to confirm its quality of the model. Finally, it is docked with naturally isolated compound 1-monocaproin and its interaction and binding energy is studied using Auto dock 4.0v in Linux environment.

EXPERIMENTAL SECTION

(i) Retrieval of target protein sequence from database

The target sequence of the Eimeria maxima is identified using the UniProt database (http://www.uniprot.org) S-adenosylmethionine synthase is a kind of enzyme involved in various biological process such as production of secondary metabolites, gene transcription and cell proliferation and it is fast growing drug target enzyme in many diseases. Eimeria maxima is a parasite which as no 3D structure for this particular drug target enzyme, so it is chosen as definite target for theoretical modeling or homology modeling of proteins.

(ii) Template identification and alignment

Template selection is the most crucial process in homology modeling it was done with blast program(Blast.ncbi.nlm.nih.gov) search in PDB database provided with scoring parameters of matrix BLOSUM62, match and misact scores of 1,-2 along with gap cost of existence:11 ,extension:1 and adjusted with conditional compositional score matrix adjustment. After selection of template, it was the aligned in two means of template sequence with target sequence (seq-seq alignment) and template structure with query sequence (stru-seq alignment)

(iii) Modeling of protein and its validation

Models are built based on the target-template alignment using Promod-II(ProMod Version 3.70). This program is inbuilt version of swiss model workspace; it built the model based on the conserved amino acid between the target and the template. The most conserved amino acid are copies directly in the form of structure from template to query, again remodeled using the fragment library by considering insertion, deletions and gaps. Finally, the side chains of each amino acid in the modeled protein are rebuilt, it was then regularized by using a force field. The final modeled protein is validated through online tool with ramachandran plot to confirm the quality of the structure is same as of the experimental structure.

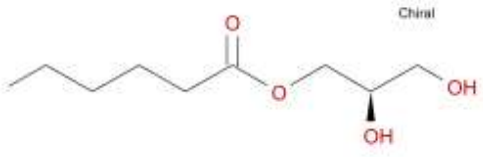
(iv) Modeled protein and ligand preparation

The modeled protein with negative energy implies it was stable structure, that optimized structure was taken for further docking studies. On the same manner, the target ligands were initially drawn using chemsketch tool, the chemical language format is converted into protein data bank format for the docking, and the force field is applied initially to avoid bad clashes and streic problem.

(v) Structure based docking

In this current study, docking is carried out in Linux environment with software of auto dock 4.02v free ware. Structure based drug designing is the method of performing the docking using the known protein docking with known ligands.

Table 1: Molecular properties of ligand

Structure	No Of Atoms	Molecular Composition	Molecular Formula	Molecular weight
 1-monocaproin	13	C: 0.568, H: 0.095, O: 0.336	C9 H18 O4	190.243

It is common method of analyzing the receptor-ligand interaction between the active site of the protein and the ligands molecules[9]. In the current study, the modeled protein is docked with ligand 1-monocaproin, the molecular properties of ligand is shown in the Table 1.

RESULTS AND DISCUSSION

1. Target sequence for modeling

S-adenosylmethionine synthase sequence from *Eimeria maxima* of Uniprot id: U6M1Q6 was retrieved from Uniprot database with and amino acid sequence length of 369 and a mass of 40,311 daltons. The .fasta format of the sequence is shown below

>tr|U6M1Q6|U6M1Q6_EIMMA S-adenosylmethionine synthase OS=Eimeria maxima GN=EMWEY_00053530 PE=3 SV=1
MSTRPIVTTPGRFLFTSESVNEGHPDKLCDQVSDAILDACLREDPNSKVACETCTKTGMVMIFGEITTNASV
DYEKIIRETCRDIGYDDESKGLDYKTMKVVVAIEDQSPEIAHAVHINKNIEEIGAGDQGHMFGYACDETPEL
MPLSHSLATSLGKRLTDVRKTGLLPYIRPDGKTQVTVEYDNSSGVPIPIRVHTIVISTQHSPEVDNNKLREDII
NKVIKPVVPSNYLDKDTIYLLNPSGRFVIGGPHGDAGLTGRKIIIDTYGGWGAHGGGAFSGKDSTKVDRSA
AYAARWAAKSLVANHFCKRCLVQVSYSIGVATPLSLYVDSYGTAAEGYTDDCLLKIVADNFDFRPGVIQR
DLRLKVNPK

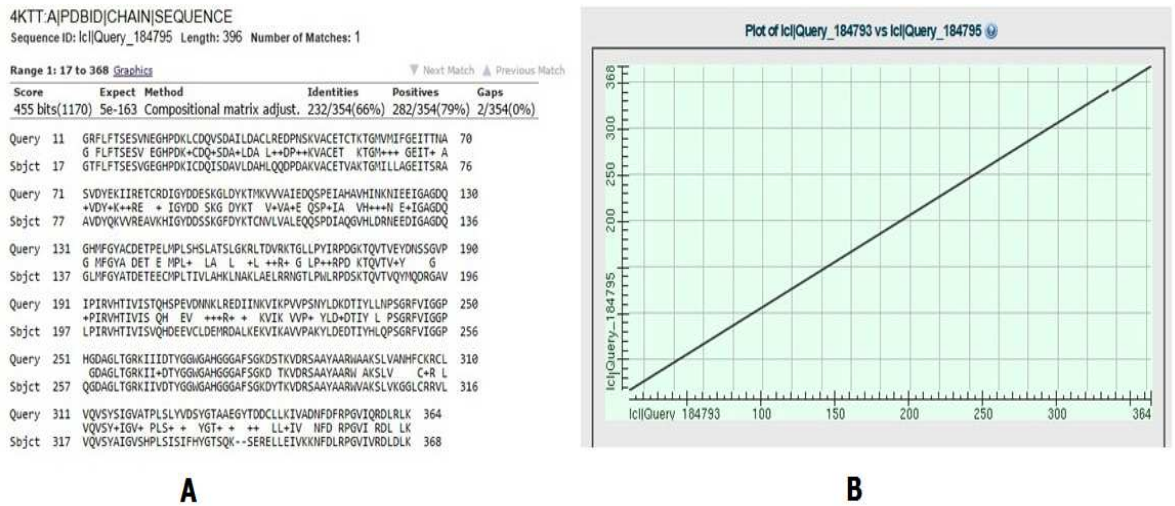


Figure 1: Sequence to sequence alignment

2. Theoretical modeling and alignment

The 84 templates were found to match the target sequence using PDB blast available in BLAST tool. The top five templates (Table 2) with good query coverage and similarity, sequence identity between the target sequence and template sequence must be chosen as appropriate template for homology modeling. Alignment is done in two ways initially with sequence-to-sequence alignment and structure -to-sequence. In sequence-to-sequence alignment using

composition matrix adjust shows 66% identities zero gaps and 79% positives(figure1 A) with dot matrix is generated to show the relation and common amino acid sharing in between the target and query sequence(figure1 B). For Structure to sequence alignment of the template protein retrieved from Protein data bank (www.rcsb.org) ID: 4ktt: A chain with query sequence shows 65.91 sequence identity with 95% query coverage (from below figure 2 C&D) also with 50% of similarity was chosen for the homology modeling in this study .

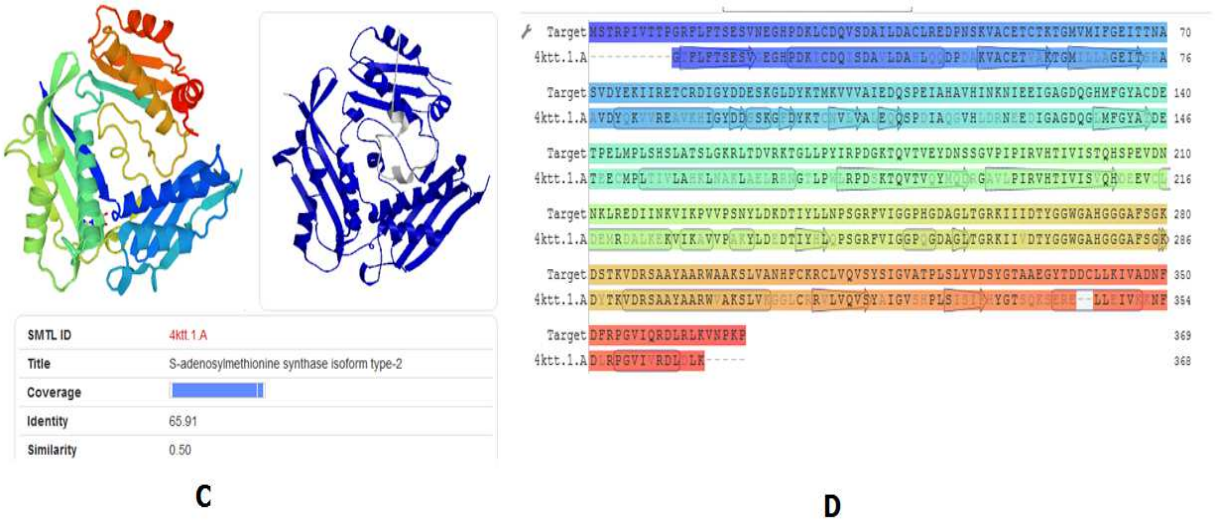


Figure 2: Structure to sequence alignment

Table 2: Query with template details

Template	Chain	Sequence Identity	Oligo State	Description
4KTT	A	65.17	hetero-oligomer	S-adenosylmethionine synthase isoform type-2
4ODJ	A	63.87	homo-dimer	S-adenosylmethionine synthase
2OBV	A	63.56	homo-tetramer	Methionine Adenosyltransferase, Alpha Form
3SO4	A	60.74	homo-tetramer	Methionine-adenosyltransferase
3TDE	A	54.89	homo-tetramer	S-adenosylmethionine synthase

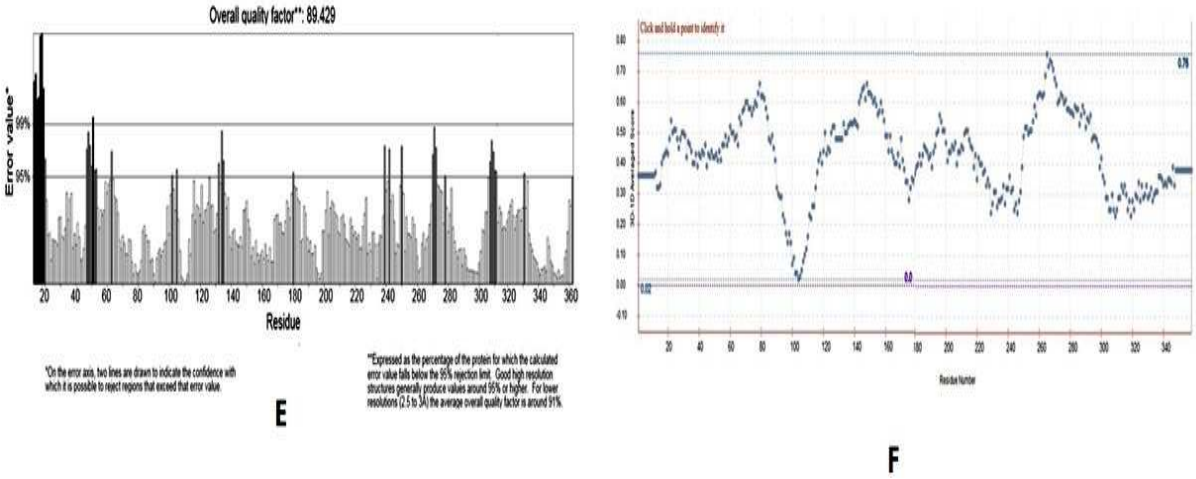


Figure 3 : Errat and Verify 3D for modeled protein

Finally, the structure is modeled with two types of aligement, the modeled structure is verified using various quality check program that available in saves. The Structure Analysis and Verification Server, is used to analyzed the quality of the homology modeled protein through Errat [10] and verify 3D[11,12]. The results shows errat overall quality factor is 89.429 with error value in two confidence levels 95% and 97% respectively (Figure 3 E),where as verify

3D shows the overall quality of the protein as satisfactory and nearly 80% of the amino acids have scored >= 0.2 in the 3D/1D profile (Figure 3 F).

Similarly, ramachandran plot [13] (figure 4)shows 87% of aminoacid in the most favoured region and 12.3% in additional allowed regions and two residues in generously allowed region, the role of glycine and proline is not considered in ramachandran plot .none of the aminoacid are observed in disallowed region.

3. Receptor-Ligand interaction

After the protein passes in the entire quality test, it has been calculated for energy to confirm it stability and it was found to be as -11774.983KJ/mol. The polar hydrogens were added along with Kollmann charges and the respective drug target protein was saved in current mode of protein data bank for docking studies. Initially grid is calculated and then docking was performed, during grid calculation, the grid maps were centered on the ligand's binding site and were of dimension 40× 40 × 40 points. The grid spacing was 0.359Å yielding a receptor model with grid co-ordinates of 28.168 × -14.842 ×96.946 as binding site center. The default parameter settings generated by the program genetic algorithm were used for docking. The initial population was set to 150 individuals; maximum number of energy evaluations was 2.5×10^5; maximum number of generations was 27,000 for each lead compounds program was performed individually. The predicated ligand and the parasite, drug target proteins are docked using Autodock4.1 in Linux environment and the interaction results are shown in below figure 5 and table 3.Table 4 describes about, a role of energy parameters in binding energy.

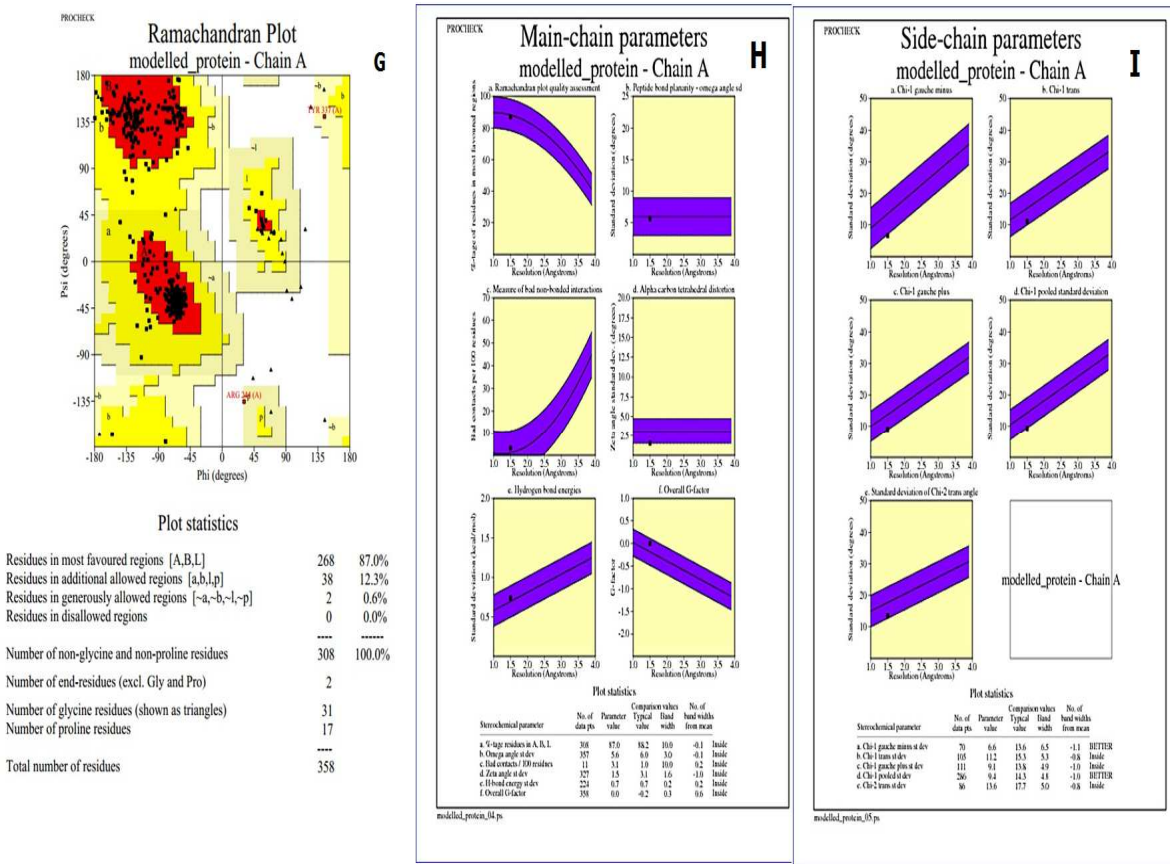


Figure 4: Ramachandran plot details of modeled protein

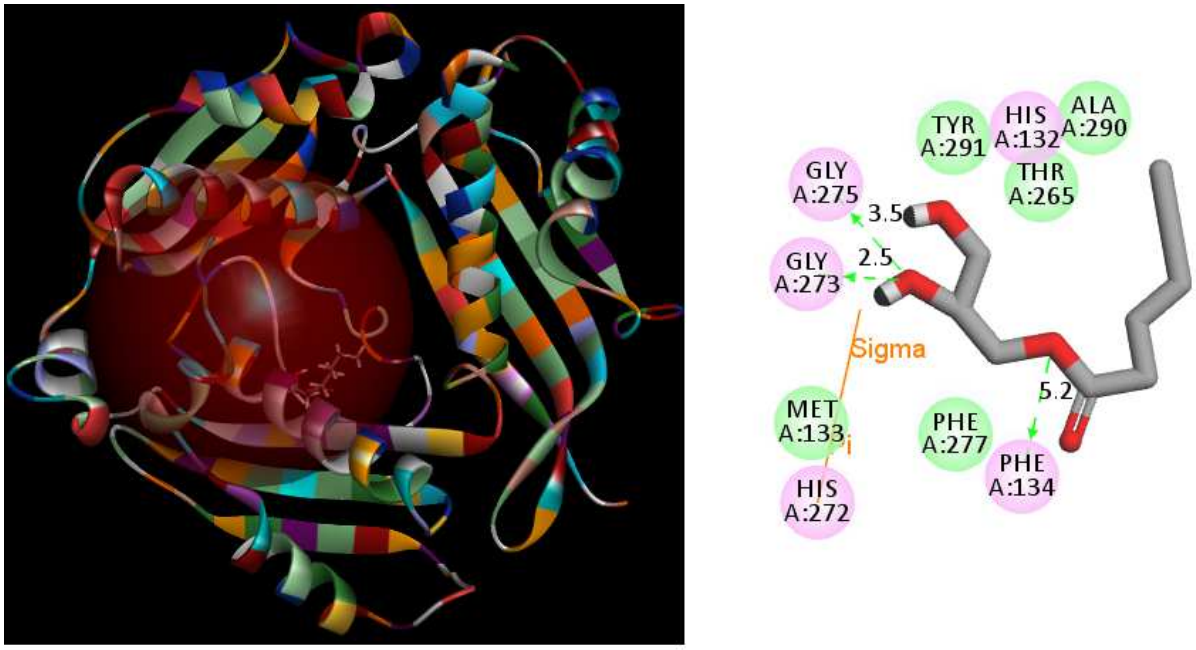


Figure 5: Binding of 1-monocaproin to active site of modeled protein(S-adenosylmethionine synthase) with amino acid interactions

Table 3: Binding energy of ligand to the active site/binding site of the protein molecule (receptor)

RECEPTOR and LIGAND	Amino acid Binding	Distance in Å	Free Energy of Binding kcal/mol
Modeled protein With 1-monocaproin	GLY275	3.5	-2.85
	GLY273	2.5	
	PHE134	5.2	

Table 4: Role of energy parameters in docking

Parameters	1-monocaproin
Estimated Inhibition Constant, Ki at 298.15 K	8.15 mM (millimolar)
Estimated Free Energy of Binding	-2.85 kcal/mol
Final Intermolecular Energy	-3.61 kcal/mol
vdW + Hbond + desolv Energy	-3.39 kcal/mol
Electrostatic Energy	-0.22 kcal/mol
Final Total Internal Energy	-0.07 kcal/mol
Torsional Free Energy	+0.82 kcal/mol
Unbound System's Energy	+0.00 kcal/mol

CONCLUSION

Thus, in modeled protein shows nearly 97% amino acid is in allowed and favorable region of Ramachandran plot. Other structure analysis tools also show good quality of modeling. Hence, this protein can further taken for structure-based drug designing and molecular docking. 1-monocaproin and Modeled protein, It shows three interaction with estimated free energy of binding of -2.85 kcal/mol Gly and Phe residues present in the active site of the drug target protein favors, the binding of 1-monocaproin.Also, sigma–pi stack interaction is observed between the compound and HIS 272 amino acid. Estimated Inhibition Constant, Ki of 1-monocaproin acid is 8.15 mM (millimolar) at Temperature 298.15 K. Hence, this compound 1-monocaproin is drug candidate for parasite infection treatment of drug target protein S-adenosylmethionine synthase.

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