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# *In silico* designing of derivatives of Diethyl carbamazine for treatment of Filariasis targeting *Wolbachia Pipientis* (Wmel)

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## ABSTRACT

*Wolbachia*, which are found in a variety of invertebrate species, are of great interest due to their diverse interactions with distinctive hosts, which range from many forms of reproductive parasitism to mutualistic symbiosis. Analysis of the *Wolbachia pipientis* (wMel) genome, in particular phylogenomic comparisons with other intracellular bacteria, has revealed many insights into the biology and evolution of wMel and *Wolbachia* in general. *W. pipientis* has been the subject of a growing number of studies in recent decades, because of the remarkable effects it has on its arthropod hosts, its potential as a tool for biological control of arthropods of agricultural and medical importance and its use as a target for treatment of filariasis. In the present study subtractive genomics approach was used for the identification of novel antimicrobial target in *W. pipientis*. This target protein was then modelled by Swiss model and Phyre 2 and the structure was validated by different validation servers. The best selected target was docked by Diethylcarbamazine, an anthelmintic drug used for the treatment of filariasis and with seven different analogs or derivatives of this drug designed by ChemDraw Ultra 7.0. The results revealed that the analog $C_{11}H_{22}N_2O$ had best docking energy than diethylcarbamazine and showed three hydrogen bond interaction with Tyr 352, Lys 347 and Thr 317. It was concluded that this analog could replace Diethylcarbamazine as it possess adverse drug effects in filarial patients.

**Keywords:** *Wolbachia pipientis,* Diethylcarbamazine, filariasis, anthelmintic drug.

## 1. INTRODUCTION

Parasitic diseases are widespread throughout the developing world and are associated with a heavy burden of morbidity and mortality. Lymphatic filariasis (LF) is one of the oldest and most debilitating diseases throughout the world and millions of people suffer from this disease. In 1995, the World Health Organization (WHO) ranked LF as the second-leading cause of long-term chronic disability worldwide. Human filarial nematodes, transmitted by arthropod vectors, are endemic in tropical and sub-tropical regions around the world [1]. Human lymphatic filariasis is a debilitating parasitic disease characterized by down regulation of the host's immune response in asymptomatic carriers along with profound hyperactivity in chronic patients apart from putatively immune endemic normals. Lymphatic filariasis is a mosquito-borne parasitic disease caused by nematode worms of the genus *Brugia* and *Wuchereria.* The disease represents a wide spectrum of clinical manifestations with varying immune responses ranging from putative immunity through asymptomatic microfilaremic infection to chronic pathology. Chronic infection is a quintessential feature of lymphatic filariasis and results from the ability of a parasite to modulate the host's immune system [2].

A major breakthrough in filarial research was the discovery of an endosymbiont *Wolbachia* in the filarial worms that is essential for worm fertility and survival. *Wolbachia* is a Gram-negative bacterial endosymbiont of filarial nematodes [3]. These parasites infect more

than 150 million people in tropical areas and cause diseases of eyes, skin and the lymphatic system, such as river blindness and elephantiasis. *Wolbachia* are abundant in all developmental stages of filarial nematodes, including the lateral cords of both sexes and the reproductive apparatus of adult females [4]. Furthermore, the obligatory relationship between filariae and their abundant endobacteria has been exploited to develop a novel chemotherapeutic approach using tetra-cycline derivates that cause developmental block, long-term worm sterility and adult worm death [5,6].

In filarial nematodes and a wasp species, *Asobara tabida*, *Wolbachia* bacteria are required for host biology [7]. The ability of *Wolbachia* to control host reproduction and development has promoted applied research dedicated to its use to restrain pest invertebrates [8, 9]. However, a rational use of *Wolbachia* for pest control requires a comprehensive analysis of the genetic and molecular basis of *Wolbachia* host interactions.

The genome of *Wolbachia* (*B. malayi*) has recently been deduced and a few bacterial proteins have been ascribed to be essential in nematode biology, while others may act at the nematode–mammal interface [10, 11]. Despite numerous reports indicative of *Wolbachia* as antifilarial drug target, not much effort has been made to investigate the role of *Wolbachia* in nematode and vertebrate host biology.

## 2. MATERIALS AND METHODS

### 2.1 Target sequence retrieval

The complete list of proteome present in the genome of the *Wolbachia pipientis* (wMel) was retrieved from NCBI database [12]. These proteome sequences were subjected to two different screening processes. Primary screening included removal of all unnamed, putative and hypothetical sequences. Then in secondary screening process using BLASTP [13] subtractive proteomic approach against Homo sapiens (taxid: 9606) proteome was done. The enzyme having least similarity with Homo sapiens was selected for further study.

### 2.2 Homology modeling and structure validation

After the screening processes the primary structure of the target protein was subjected to homology modelling. The three dimensional structure of the protein was determined using SWISS-MODEL [14] and Phyre 2 [15]. SWISS-MODEL is an automated system for 3D structure modeling of a protein from its amino acid sequence using homology modeling techniques whereas Phyre2 is a suite of tools available on the web to predict and analyze protein structure, function and mutations. SWISS-MODEL provides three possible predicted 3D models of the protein. All the models obtained were visualized in pymol **(Figure 1)** and validated based on their stability. The stability analysis was done using *in silico* tools such as RAMPAGE, PROSA and PROQ.

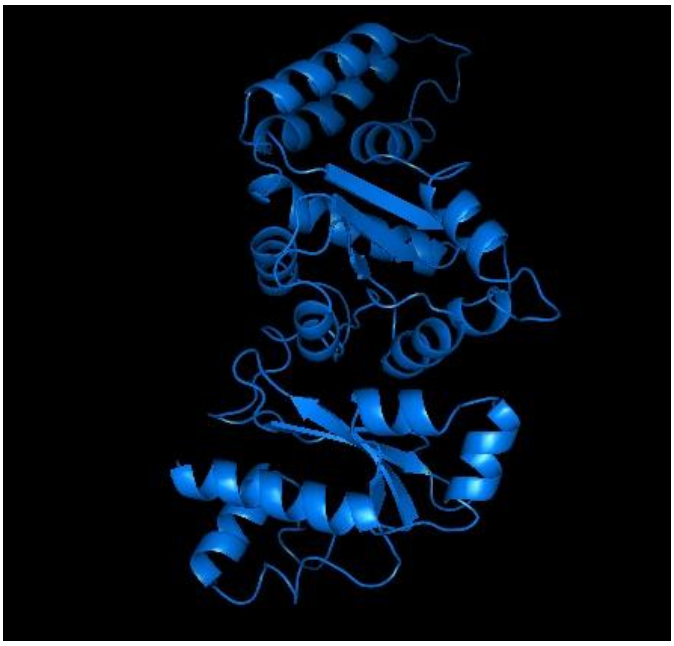


**Figure 1:** Homology model of DNA primase (dnaG) of *Wolbachia pipientis* (wMel) obtained by Phyre 2

### 2.3 Retrieval of ligand molecule

Diethylcarbamazine, a synthetic organic compound which is considered to be an anthelmintic drug was selected as the primary ligand molecule for docking with the target molecule. Diethylcarbamazine is primarily used as the citrate in the treatment of filariasis, particularly infestations with Wucheria bancrofti or Loa loa. It is reported to alter glucose uptake and inhibits phosphoenol pyruvate carboxykinase, fumarate reductase and succinate

dehydrogenase. The 3D structure of diethylcarbamazine (CID 3052) was retrieved from PubChem database [16]. Then using ChemDraw taking diethylcarbamazine as primary ligand seven other derivatives were designed keeping structural requirements of pharmacophore in view. The analog designing was based strictly on Lipinski Rule of 5. This rule describes molecular properties important for a drug's pharmacokinetics in the human body, including their absorption, distribution, metabolism, and excretion.

### 2.4 Molecular docking simulation

Docking simulation study was carried by Autodock vina using standard protocols [17]. Target protein and the ligands were prepared using Autodock 4.2 program [18]. Preparation process of target protein involved addition of polar hydrogen atoms and addition of gasteiger and kollman charges whereas for ligands all the bonds were made rotatable. Molegro Virtual Docker was also used for docking and H-bond interaction analysis [19].

## 3. RESULTS AND DISCUSSION

After an extensive screening process the sequence "WD_0348 DNA primase (dnaG)" was found suitable to act as the target protein for it's *in silico* drug designing process. The results of Blastp screening revealed that 3 proteins showed max similarity with a max score of 32.7 with three different proteins of Homo sapiens having accession no. AAA35606.1, NP_001714.1 and BAG61741.1.

### 3.1 Molecular modeling and validation

The 3D structure of the target protein was predicted using online tools Swiss Model and Phyre2. Structure prediction using Swiss Model gave three possible models of the protein and phyre2 gave one model. All the four models were then screened based on their stability. Screening of these predicted models indicated that model formed using Phyre2 is best among all four models having 95.5% of residues in favoured region which was highest as compared to other three models obtained by Swiss Model which showed number of residues in favoured region to be 92.3%, 93.6% and 88.7% based on Ramachandran Plot analysis. Based on the results of ProQ the model obtained by Phyre2 was predicted as "extremely good model" with LG score of 4.788 and "very good model" with Maxsub 0.460. Also the Z-value of -9.07 and local model quality predicted by ProSA validated the structure to be beyond gratification.

### 3.2 Ligand and analog preparation

Diethylcarbamazine was used as the primary lead molecule which is an anthelmintic drug used in generic drug used for the treatment of filariasis. The 3D structure of Diethylcarbamazine (CID 3052) was obtained from PDB database in .sdf format. However, .sdf format was converted in .pdb format for *in silico* analysis using pymol. Further 7 derivatives or analog structures of the lead molecule were prepared using chemDraw tool, in a process to predict a suitable active compound which could help in treatment of filariasis more effectively than Diethylcarbamazine. The analogs were made by altering the chemical structure of the lead molecule using the strategy such that its effectivity is not disturbed keeping Lipinski rule of 5 in consideration (Table 2).

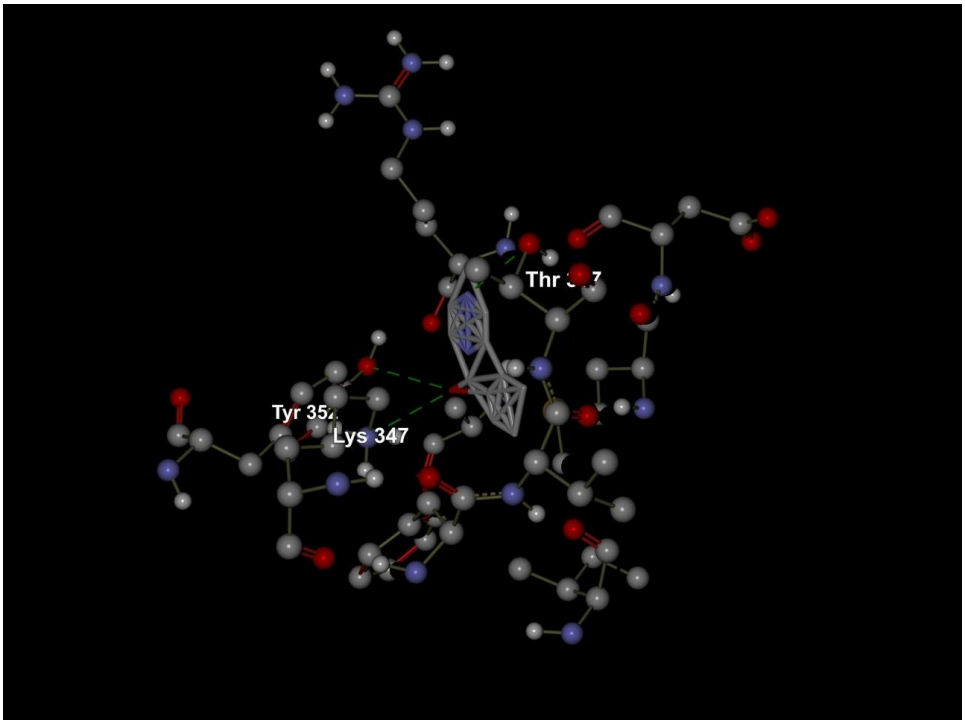
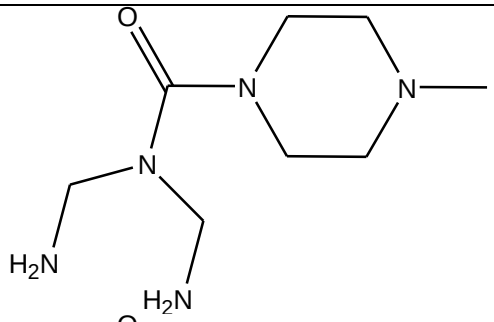
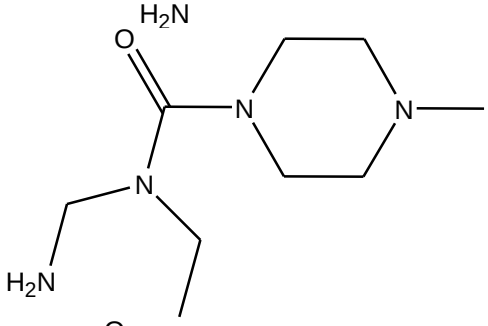
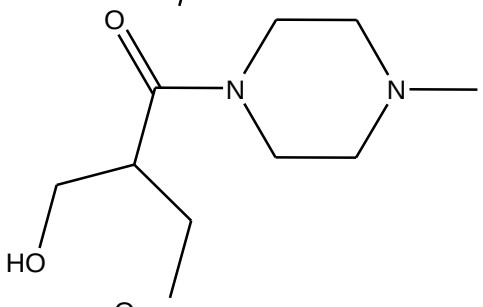
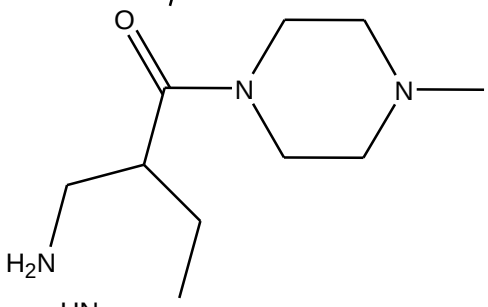
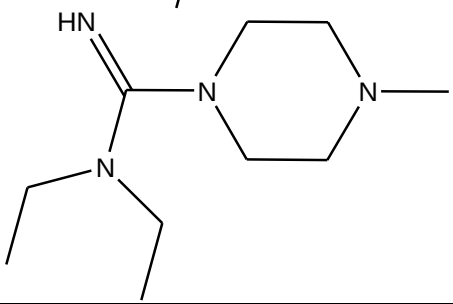


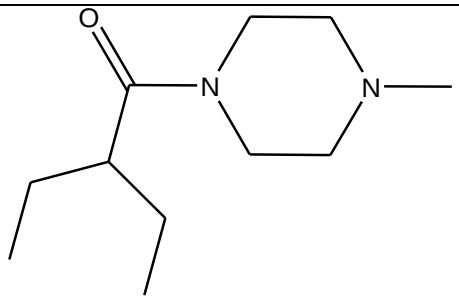
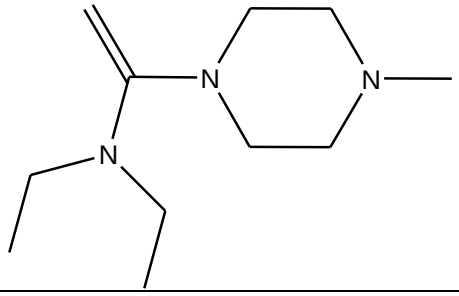

**Figure 2:** H-bond prediction of analog 6 using Molagro where 3 H-bonds was detected having interaction with amino acids Tyr 352, Lys 347 and Thr 317

**Table 1:** Molecular docking of Diethylcarbamazine and its seven analogs using Autodock vina rendering ANALOG 6 (C11H22N2O) as lead molecule.

| Ligand | Energy |
|---|---|
| DIETHYLCARBAMAZINE | -5.1 |
| ANALOG 1 ($C_8H_{19}N_5O$) | -7.0 |
| ANALOG 2 ($C_9H_{20}N_4O$) | -6.1 |
| ANALOG 3 ($C_{10}H_{20}N_2O_2$) | -6.6 |
| ANALOG 4 ($C_{10}H_{21}N_3O$) | -6.4 |
| ANALOG 5 ($C_{10}H_{22}N_4$) | -6.5 |
| ANALOG 6 ($C_{11}H_{22}N_2O$) | -7.2 |
| ANALOG 7 ($C_{11}H_{23}N_3$) | -6.0 |

**Table 2:** Chemical structures of analogs and their pharmacophores.

| ANALOG | CHEMICAL FORMULA | STRUCTURE | Log P | MOLECULAR Weight | Molar refractivity |
|---|---|---|---|---|---|
| ANALOG 1 | $C_8H_{19}N_5O$ |  | -1.83 | 201.16 | 42.104294 |
| ANALOG 2 | $C_9H_{20}N_4O$ |  | -0.87 | 200.16 | 45.631893 |
| ANALOG 3 | $C_{10}H_{20}N_2O_2$ |  | -0.15 | 200.15 | 46.499992 |
| ANALOG 4 | $C_{10}H_{21}N_3O$ |  | -0.53 | 199.17 | 53.656590 |
| ANALOG 5 | $C_{10}H_{22}N_4$ |  | 1.19 | 198.18 | 51.724991 |

| | | | | | |
|---|---|---|---|---|---|
| ANALOG 6 | $C_{11}H_{22}N_2O$ |  | 1.32 | 198.17 | 47.226494 |
| ANALOG 7 | $C_{11}H_{23}N_3$ |  | 1.60 | 197.19 | 53.984989 |

### 3.3 Docking

Molecular docking was carried out in a semi flexible manner using Autodock vina tool. Firstly, Diethylcarbamazine was docked which showed a binding affinity of -5.1 Kcal/mol. The interaction analysis using Autodock reveilled that the docking results had one hydrogen bond between ASN 210 and HD21. Then using Autodock the derivatives were docked keeping the parameters such as grid sizes same. The results showed that two derivatives analog 6 ($C_{11}H_{22}N_2O$) and analog 1 ($C_8H_{19}N_5O$) had better binding affinity as compared to the primary lead molecule. The docking energy of analog 6 ($C_{11}H_{22}N_2O$) was -7.2 Kcal/mol and that of analog 1 ($C_8H_{19}N_5O$) was -7.0 Kcal/mol Table 1. In order to study about the interaction between the derivatives and the target molecule, these derivatives were also docked using Molegro Virtual Docker. These studies suggested that the derivative $C_8H_{19}N_5O$ had one hydrogen bond interaction with Thr 317 whereas the derivative $C_{11}H_{22}N_2O$ showed three hydrogen bond interaction with Tyr 352, Lys 347 and Thr 317 (Figure 2).

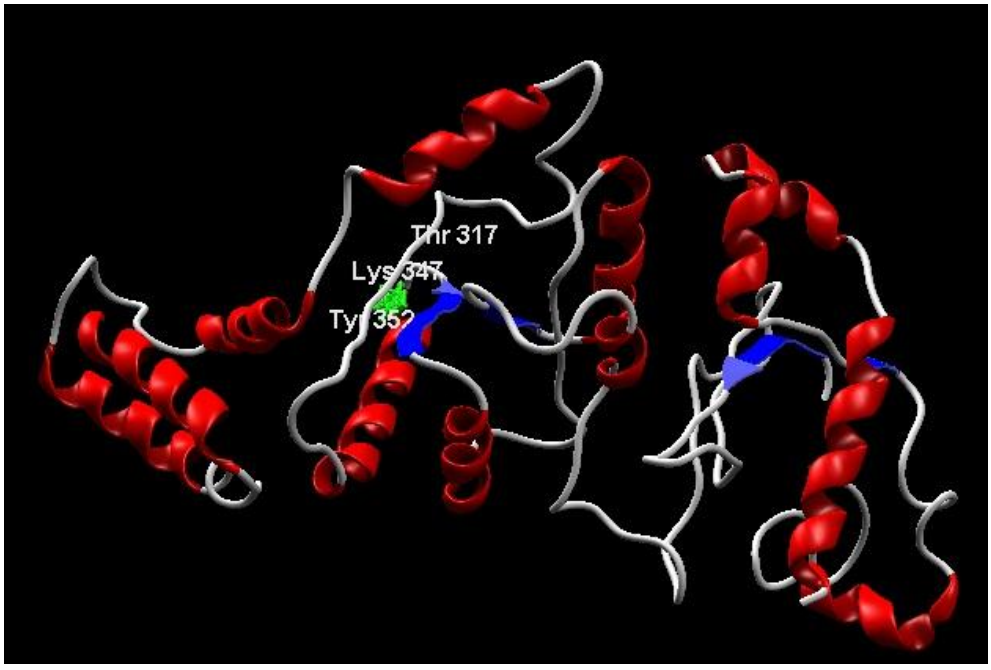



**Figure 3:** Secondary structure complex of protein ligand complex showing analog 6 and amino acids taking part in H-bonding

## 4. CONCLUSION

The present study was aimed to predict the possible potent analog of diethylcarbamazine which is currently used for the treatment of filariasis. Here, subtractive proteomic approach was used for selecting the target protein. Molecular modeling was done by Swiss model

and Phyre 2 out of which model formed by Phyre 2 was selected for further studies as it was best validated by several validations tools. Then molecular docking process was carried out by Autodock. Diethylcarbamazine and its seven analogs prepared by Chemdraw were docked with the target protein. Diethylcarbamazine is indicated as a primary agent in the treatment of Bancroft's filariasis caused by *Wuchereria bancrofti.* Keeping its adverse drug reactions in view the analogs or derivatives were prepared. These *in silico* study reviled that the analog 6 ($C_{11}H_{22}N_2O$) could be a possible alternative of Diethylcarbamazine for treatment of filariasis. The docking analysis showed that analog 6 had best docking energy than other and had three hydrogen bond interactions with Tyr 352, Lys 347 and Thr 317 **(**Figure 3). Also the ligand analog 6 statisfied well the Lipinski Rule of 5. It had LogP value of 1.32, molecular weight as 198.17 daltons and molecular refractivity as 47.226494 which confirms its druglikeness. This work needs to be further analyzed by wet lab experiments but however; to our knowledge this is the first *in silico* work carried out so far.

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