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Molecular Docking Studies of *Wolbachia* Endosymbiont of *Brugia Malayi*'s Carbonic Anhydrase Using Coumarin-chromene Derivatives Towards Designing Anti-filarial Agents

P. Malathy[1†], G. Jagadeesan[2], K. Gunasekaran[1], and S. Aravindhan[2]

Abstract

Filariasis causing nematode *Brugia malayi* is shown to harbor *wolbachia* bacteria as symbionts. The sequenced genome of the *wolbachia* endosymbiont from *B.malayi* (*wBm*) offers an unprecedented opportunity to identify new *wolbachia* drug targets. Hence the enzyme carbonic anhydrase from *wolbachia endosymbiont of Brugia malayi* (*wBm*) which is responsible for the reversible interconversion of carbon dioxide and water to bicarbonate and protons (or vice versa) is chosen as the drug target for filariasis. This enzyme is thought to play critical functions in bacteria by involving in various steps of their life cycle which are important for survival, The 3D structure of *wBm* carbonic anhydrase is predicted by selecting a suitable template using the similarity search tool, BLAST. The BLAST results shows a hexapeptide transferase family protein from *Anaplasma phagocytophilum* (PDB ID: 3IXC) having 77% similarity and 54% identity with *wBm* carbonic anhydrase. Hence the above enzyme is chosen as the template and the 3D structure of carbonic anhydrase is predicted by the tool Modeller9v7. Since the three dimensional structure of carbonic anhydrase from *wolbachia* endosymbiont of *Brugia malayi* has not yet solved, attempts were made to predict this protein. The predicted structure is validated and also molecular docking studies are carried out with the suitable inhibitors that have been solved experimentally.

Keywords: Carbonic Anhydrase, Filariasis, *Wolbachia* of *Brugia Malayi* (*wBm*), Induced Fit Docking, Endosymbiotic.

Abbreviations: OPLSAA, optimized liquid solution all atoms; *wBm*, *Wolbachia Brugia malayi.* BLAST; (Basic Local Alignment Search Tool).

1. Introduction

Wolbachia are alpha-proteobacteria that infects a range of arthropods and nematodes, possibly the most common endosymbiotic bacteria on the planet. This bacterium is present in most of the filarial nematode species including *Brugia malayi, Onchocerca volvulus and Wuchereria bancrofti.* The above said parasites are the causative agent for lymphatic filariasis in humans leading to medical conditions called elephantiasis or onchocerciasis (African river blindness). Lymphatic filariasis is caused predominantly by *Wuchereria bancrofti* and *Brugia malayi* and affects 120 million individuals while onchocerciasis, caused by *Onchocerca volvulus,* affects 18 million people of whom 500,000 have visual impairment and 270,000 are blind. It is estimated that nearly 1 billion people in more than 90 countries are at risk from filarial nematode infections[1], and 150 million people are infected with this parasites.

It is also evidenced that the depletion of wolbachia filarial parasites is observed in both laboratory and human trials by treatment with antibiotics like doxcycline and tetracycline which kills adult worms in addition to affecting the embryogenesis, microfilarial output and worm development of the parasite[2]. Also the vertically transmitted *wolbachia* endosymbionts are indispensible for filarial hosts and represent a promising therapeutic strategy for filariasis control.

The current anti-filarial treatments[3] include Diethylcarbamazine (DEC), albendazole and Ivermectin which works predominantly by killing the microfilaria showing no activity against the adult worms. However DEC and albendazole shows macrofilaricidal activity only

[1]CAS in Crystallography and Biophysics, University of Madras, Chennai-600025, India.
[2]Department of Physics, Presidency College, Chennai 600005, India
[†]Corresponding author : bioinfomalathi@gmail.com
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after repeated rounds of mass drug administration (MDA). Since the above treatments have to be administered annually on a community-wide basis for many years to break the infection cycle, drug resistance may be emerging and there is an urgent need to develop novel drugs particularly macrofilaricidal.

Hence the enzyme carbonic anhydrase from *wolbachia endosymbiont of Brugia malayi* (*wBm*) which is responsible for the reversible interconversion[4] of carbon dioxide and water to bicarbonate and protons (or vice versa) is chosen as the drug target for molecular docking studies.

This enzyme is thought to play critical functions in bacteria by involving in various steps of their life cycle which are important for survival, colonization, acquisition of nutrients for growth and proliferation, facilitation of dissemination, invasion and pathogenicity. So far three distinct classes α, β and γ-class of carbonic anhydrase has been indentified that has evolved independently having no sequence or structural homology with one another. All of the mammalian species including all the 10 isoforms belongs to the α-class. The beta class comprises the enzymes from the chloroplasts of both monocotyledonous and dicotyledonous plants as well as enzymes from phylogenetically diverse species from the Archaea and Bacteria domains. The only gamma class carbonic anhydrase[5] that has thus far been isolated and characterized is from the methanoarchaeon, *Methanosarcina thermophila*.

Interestingly, some prokaryotes contain multiple genes encoding carbonic anhydrases from different class. The presence of multiple carbonic anhydrase genes within a species underscores the importance of this enzyme in prokaryotic physiology; however, the roles of this enzyme are still unknown. Even though most of the information known about the function of carbonic anhydrase primarily relates to its role in cyanobacterial CO_2 fixation, the prokaryotic enzyme has also been shown to function in cyanate degradation and the survival of intracellular pathogens within their host.

Since the three dimensional structure of carbonic anhydrase from *wolbachia* endosymbiont of *Brugia malayi* has not yet solved, attempts were made to predict the structure. The predicted structure is validated and also molecular docking studies were carried out with the coumarin and chromene derivatives that have been analyzed in our lab.

2. Material and Methods

2.1. Sequence Analysis

The complete amino acid sequence of carbonic anhydrase (accession No: YP_198120) from *wolbachia endosymbiont of Brugia malayi* is retrieved from the biological database of NCBI. The three-dimensional structure of *wBm* carbonic anhydrase is not available in Protein Data Bank (PDB); hence the present study of developing the 3D model of the *wBm carbonic anhydrase* is undertaken. BLASTP (protein query- protein database) search is performed against PDB with the default parameters to find suitable templates for homology modeling. Multiple sequence alignment is done using CLUSTAL W[6] program to analyze the similarities of *wBm* carbonic anhydrase enzyme from various organisms to find the conserved with the functionally important residues.

2.2. Homology Modeling

The academic version of Modeller9v6[7] is used for 3D structure generation based on the information obtained from sequence alignment. The Modeller software employs probability density functions (PDFs) as the spatial restraints rather than energy. The 3D model of a protein is obtained by optimization of the molecular pdf such that the model violates the input restraints as little as possible. The molecular pdf was derived as a combination of pdfs restraining individual spatial features of the whole molecule. Out of 10 models generated by Modeller, the one with the best DOPE score and G-score was subjected to Energy minimization using OPLS-AA force field of Schrodinger's GLIDE. The predicted model was also validated using Procheck program[8]. Once the structure was modelled, it is submitted to Dali server[9] for structural comparison via structure database searching. Geometrical comparison of protein structures may reveal biologically interesting similarities that are not detectable by sequence comparison. The Dali server is routinely used to compare newly elucidated structures against those in Protein Data Bank and to compare *ab initio* predicted structures to the experimentally determined similar structures.

2.3. Binding Site Analysis

Pocket Finder[10], a program for identifying and characterizing protein active sites, binding sites and func-

tional residues located on protein surface is used to identify binding pockets of *wBm carbonic anhydrase*. Pocket-Finder works by scanning probe radius 1.6 Å along all gridlines of grid resolution 0.9 Å surrounding the protein. Cubic diagonals were also scanned by using this probe. Grid points are defined to be a part of a site when the probe is within range of protein atoms followed by free space followed by protein atoms.

2.4. Docking

Docking is carried out using Schrödinger's Glide. Glide (Grid based LIgand Docking with Energetics)[11] uses a hierarchical series of filters to search for possible locations of the ligand in the active site region of the receptor. The prepared protein is docked using Induced-fit protocol. Initially softened potential filter (van der Waals radii scaling) was used to retain 20 geometrically possible poses per ligand and then it is evaluated for the coulombic-vdW scores. The structures whose coulombic-vdW scores less than 100 and H-bond score less than -0.005 were retained. The retained structures enter one round of prime side chain prediction for each of the protein-ligand complex with the given distance of 5 Å. Poses that pass these initial screens enter the final stage of the algorithm, which involves evaluation and minimization of a grid approximation to the OPLS-AA non-bonded ligand-receptor interaction energy. Each of the protein-ligand complexes were specified with the lowest energy (30 Kcal/mol) and then the ligand was docked with the receptor molecule. Final scoring is then carried out on the energy-minimized poses. Schrödinger's proprietary GLIDE Score multi ligand scoring function is used to score the poses.

3. Results and Discussion

3.1. Sequence Analysis

The amino acid sequence of *wBm* carbonic anhydrase containing 175 amino acids has been retrieved from NCBI database. The 3D structure of *wBm* carbonic anhydrase is predicted by selecting a suitable template using the similarity search tool, BLAST (available in NCBI database). The BLAST results shows a hexapeptide transferase family protein from *Anaplasma phagocytophilum* (PDB ID: 3IXC) having 77% similarity and 54% identity with *wBm* carbonic anhydrase. Hence the above enzyme was chosen as the template and the 3D structure was predicted by the tool Modeller9v7 which run on the python script at the backend of the software. Multiple sequence alignment with various other carbonic anhydrases shows that the *wBm* carbonic anhydrase has retained most of its conserved residues for the metal and substrate binding to carry out its catalytic function.

3.2. 3D Structure Prediction and Validation

The three dimensional structure of *wBm* carbonic anhydrase is predicted using the comparative modeling program, Modeller. Based on the results obtained from

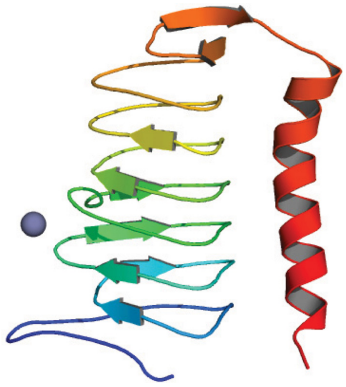

Fig. 1. Predicted structure of *Wolbachia endosymbiont of Brugia malayi* carbonic anhydrase by Modeller9v7.

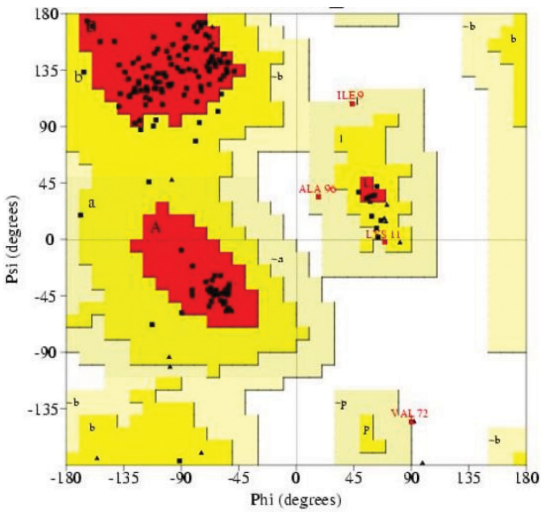

Fig. 2. Ramachandran map of the predicted *wBm* carbonic anhydrase structure (95% of amino acids falls on the allowed regions).

sequence analysis, suitable templates were chosen for the prediction. The predicted structure of *wBm* carbonic anhydrase (Fig. 1) is validated using Ramachandran plot analysis which shows 95% of the residues fall on the allowed regions of the map (Fig. 2). The above results are also validated using ERRAT program whose graph shows that the predicted structure has a reasonable good quality.

3.3 Active Site Prediction

The active sites of *wBm* carbonic anhydrase are predicted by structural comparison with the other homologous structures available in Protein Data Bank which shows the catalytic His triad namely His 71, His 95, His 106 and Arg 50. The above analysis is also cross checked and confirmed by the tool; q-site finder. The program q-site finder also suggests the same pocket or the surface accessible area with the volume of 150Units. The above said active site residues, is also predicted by superposition of homologues structures. These results were also confirmed by multiple sequence alignment of *wBm* carbonic anhydrase with various other carbonic anhydrases which also shows the conserved catalytic triad. Since the metal ion and the catalytic water molecules are necessary for the activity of the enzyme; the metal ion, $Zn^{2+}$and the water molecules are fixed manually in the predicted model of *wBm* carbonic anhydrase.

3.4 Docking Analysis

Molecular docking analysis (using Schrodinger's Glide) for the predicted structure is carried out using

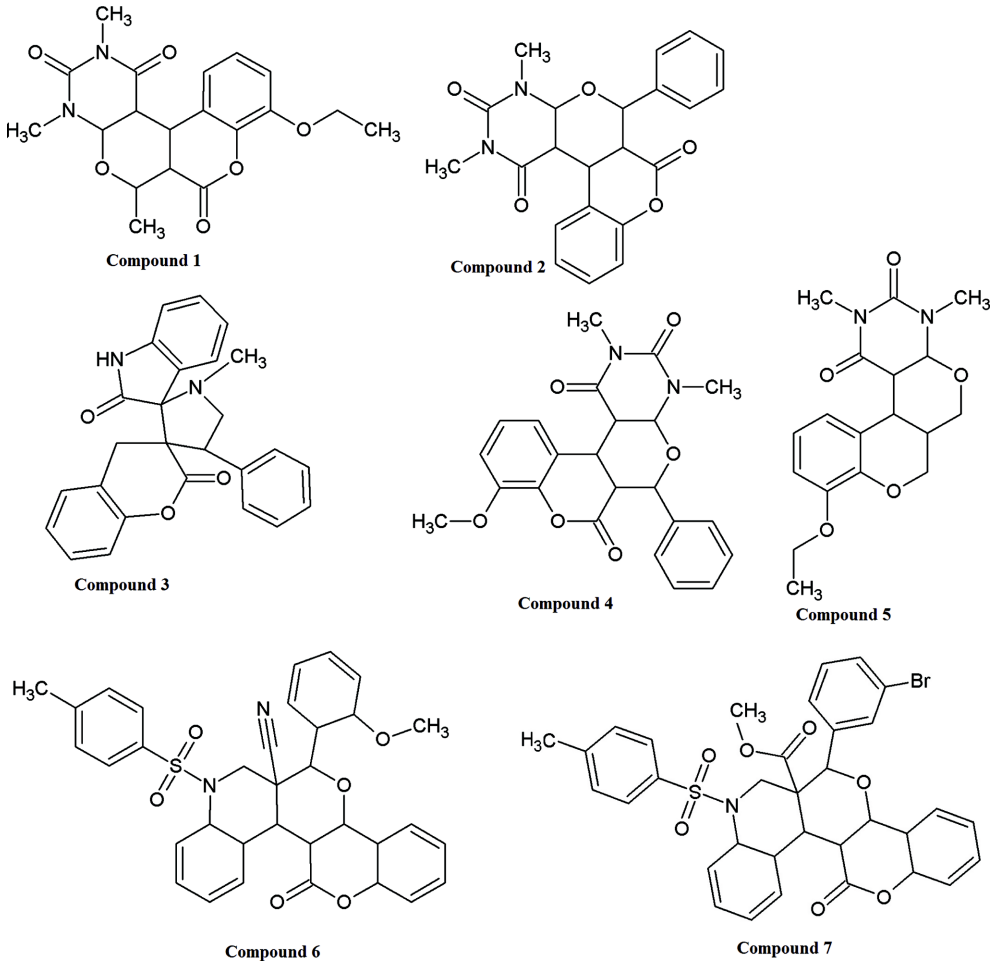

Fig. 3. Schematic representation of coumarin derivates.

benzopyrone family (ex., coumarin & chromene) derivatives as the ligand molecules that have been X-ray Crystallographically[12-17]. The schematic diagrams of the compounds are given in Fig. 3. In order to understand the mechanism of inhibition and binding interaction of ligand molecules with the target protein, the already established inhibitor for carbonic anhydrase, Acetazolamide is included in our docking analysis. The above resulted enzyme-inhibitor complex is taken as a model for explaining the binding interaction of coumarin derivatives with the target protein. Also it has been demonstrated that the use of benzopyrones greatly reduces the attack of infections that is common in lymphedema through which it improves the patient mobility, ability to work and general quality of life[18]. Hence we selected the benzopyrones as a inhibitor molecules against *wBm* carbonic anhydrase.

Out of the seven compounds, the compound 5 and 6 shows better interaction similar to that of the known enzyme-inhibitor complex. The O3 atom of compound 5 forms bifurcated hydrogen bond with amino group (NH1) of Arg 50 and oxygen (OW180) atom of catalytic water molecule

Forming a D-H…A distance of 3.0 Å and 2.8 Å respectively. Similarly the ε-2 nitrogen atom of His 71 forms bifurcated hydrogen bond (D…A of 3.1Å each) with the oxygen atom of water molecules (HOH 179 and HOH 180). The oxygen atom (O3) of compound 6 forms hydrogen bond with NH1 atom of Arg 50 forming a D…A distance of 3.0 Å. Similarly the amino group (NH2) of Arg 50 forms the hydrogen bond with O4 atom of the compound 6 forming a D…A distance of 3.1 Å. The hydrogen bond interactions for the best three compounds were shown in Fig. 4. The interaction of metal ions with the active site residues of *wBm* carbonic anhydrase is given in Fig. 5. The Glide energy

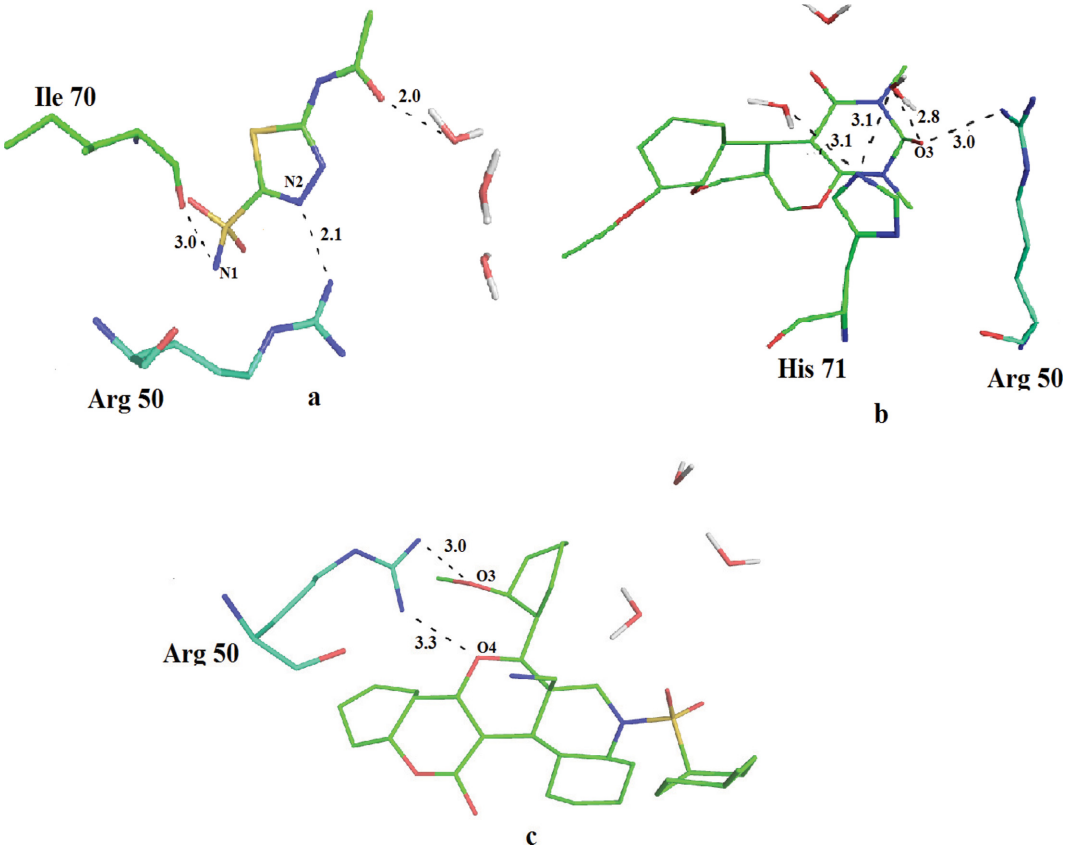

Fig. 4. Interaction of the coumarin / chromene derivatives with the active site residues of *wBm* carbonic anhydrase (a) already established inhibitor, acetazolamide, (b) Compound 5 and (c) compound 6.

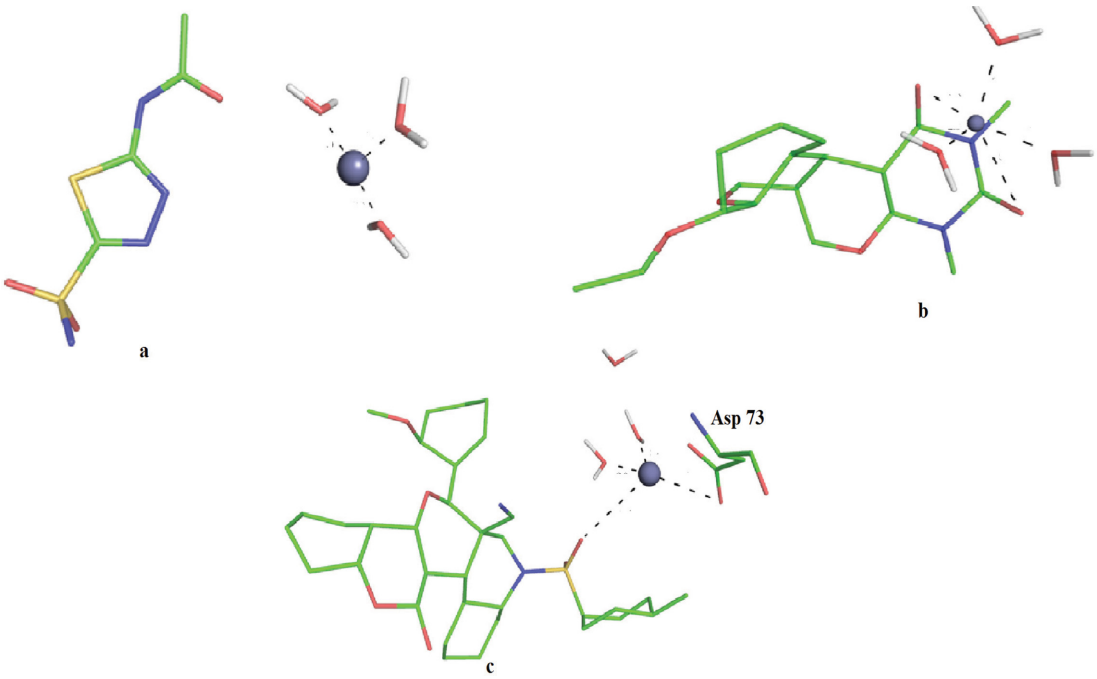

Fig. 5. Interaction of the coumarin / chromene metal ion (Zn^{2+}) with the active site residues of *wBm* Carbonic anhydrase (a) already established inhibitor, acetazolamide, (b) compound 5 and (c) compound 6

Table 1. Interaction of ligands with *w*Bm carbonic anhydrase: Docking score and hydrogen bond interactions

Ligand Name	Glide E-model	Glide Energy	Distance (D…A) (Å)	Interaction
Acetazolamide (Inhibitor)	-38.427	-30.77	3.0 2.1	N(1)-H…O Ile 70 Arg 50N-H(2) …N2
Compound 5	-36.077	-31.064	2.8	Arg 50 N-H (1) …O3
Compound 6	-34.813	-30.423	3.3 3.0	Arg 50 N-H (1)…O4 Arg 50 N-H (2)…O3

and its score along with their hydrogen bond interaction are given in Table 1.

4. Conclusion

In this chapter we propose X-ray crystallographically solved compounds that can possibly inhibit the *wolbachia* enzyme, carbonic anhydrase by which this work may helpful in designing anti-filarial agents. This enzyme is thought to play critical functions in bacteria by involving in various steps of their life cycle. Extensive literature studies are carried out to carbonic anhydrase as the potential drug target. In the absence of crystal structure, we used homology modeling protocol to predict the three dimensional structure of the protein with the suitable template. Docking studies were carried for targeting *wBm* carbonic anhydrase using Schrödinger's Glide which shows better interaction similar to that of the known enzyme-inhibitor complex. With the above study and from obtained results we conclude that the proposed chemical compounds could provide prime lead for effective drug designing. Any way this needs to be tested experimentally.

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