International Journal of Drug Research and Technology

Available online at http://www.ijdrt.com

Research Article

IDENTIFICATION OF POTENT INHIBITORS OF UDP-GALACTOPYRANOSE MUTASE FOR FILARIAL NEMATODES USING AN *IN SILICO* APPROACH

Karthik L[1], Wubliker Dessie Lemma[2], **Gunasekaran K***[1]

[1]CAS In Crystallography & Biophysics, University of Madras, Guindy Campus, Chennai 600 025, Tamil Nadu, India

[2]Department of Bioinformatics, University of Madras, Guindy Campus, Chennai 600 025, Tamil Nadu, India

ABSTRACT

Lymphatic filariasis, commonly known as elephantiasis, is a neglected tropical disease. Infection occurs when filarial parasites are transmitted to humans through mosquitoes. In its most obvious manifestations, lymphatic filariasis causes enlargement of the entire leg or arm, the genitals, vulva and breasts. It has been identified by the World Health Organization (WHO) as the second leading cause of permanent and long-term disability (WHO 1997a, b). Current filariasis control strategies are not entirely successful and filarial infections are on the rise. Therefore novel chemotherapeutics and vaccines are urgently needed. UDP-galactopyranose mutase (UGM) is a flavoenzyme that catalyzes the conversion of UDP-galactopyranose to UDP-galactofuranose, which is a central reaction in galactofuranose biosynthesis. The importance of UGM for the viability of many pathogens and its absence in humans makes UGM a potential drug target. In this study, UGM from the parasitic nematode *Brugia malayi* has been considered as a target during *in silico* drug design of potent filarial inhibitor. As there are no crystal structures are available for *Brugia malayi's* UGM, homology modelling approach has been employed for determining the 3D structure. A library of 55 compounds has been screened against the target structure using rigid docking. Based on the various parameters from the initial docking results about 5 compounds were filtered out and were further subjected for induced fit docking and the detailed results are reported. In addition to this, the existing anti-filarial drugs have also been subjected for induced fit docking with the *Brugia malayi's* UGM. The results show that the reported compounds have not only exhibited greater docking score and energy but also possessed reliable hydrogen bonding interactions with the active site key residues. As no anti-filarial

compounds are available, compounds reported in this study can be optimized further and can be developed into potent drugs in combating lymphatic filariasis.

Keywords: Lymphatic Filariasis; Brugia Malayi, UDP Galactopyranose Mutase; Virtual Screening; Induced Fit Docking.

INTRODUCTION

Parasitic infections of one kind or the other have been estimated to affect about 3 billion people in the world; of which about 250 million people are infected with filarial parasites. The majority of these infections occur in resource limited tropical and subtropical developing countries of the world, where over half of the population may harbour infections. Of the various helminthic infections in man, those caused by filarial parasites are particularly important because of the huge loss of man-hours they cause. Lymphatic filariasis is caused by the mosquito borne, lymphatic-dwelling nematodes *Wuchereria bancrofti*, *Brugia malayi*, or *Brugia timori*. Lymphatic filariasis has been identified by the World Health Organization (WHO) as the second leading cause of permanent and long-term disability (WHO 1997a, b). A WHO initiated Global Program for Elimination of Lymphatic Filariasis (GPELF) is ongoing and the target year is 2020.

A clear understanding of the mode of action of antifilarials awaits greater knowledge of the biochemical pathways operating in filarial parasites (Gupta, *et al.*, 2005). The classical drugs used against onchocerciasis and lymphatic filariasis are diethylcarbamazine (DEC), ivermectin (IVM) and albendazole (ALB). For the past two decades they have been used as the major mode of intervention for filarial disease in successful mass drug administration (MDA) programmes (Rahman, *et al.*, 2007 and Palumbo, *et al.*, 2008). The drugs are mainly microfilaricidal (Gupta, *et al.*, 2005; Hoerauf, *et al.*, 2008; Mathew, *et al.*, 2008; Bhullar, *et al.*, 2010 and Murthy, *et al.*, 2011) that is, killing the first larval stage, the microfilariae. None of these is effective in killing the adult worms, which can live in the host for several years and the treatments are therefore aimed solely at reducing transmission and pathology.

The cell wall of pathogenic microorganisms has been a target for antimicrobial drugs for many years. Since this highly complex and species-specific structure has a number of components not found in humans and other higher eukaryotes, the biosynthesis of the cell wall and its individual components continues to be an attractive target for development of novel antimicrobial drugs. One such component is D-galactofuranose (Gal*f*1), the 5-membered-ring form of galactose. Gal*f* residues are found as components of the cell walls of pathogenic bacteria such as *Escherichia coli*, *Klebsiella pneumoniae* and *Mycobacterium tuberculosis* (Crowther, *et al.*, 2010). It is also found in the cell walls of fungi such as *Aspergillus fumigatus* and in cell-surface structures of protozoa such as *Trypanosoma cruzi* and *Leishmania* species.

UDP-GALACTOPYRANOSE MUTASE AS A DRUG TARGET

In this study, UDP-Galactopyranose Mutase of *Brugia malayi* was considered as a target during *in silico* drug design of potent filarial inhibitor. *Brugia malayi* is a nematode (roundworm), one of the three causative agents of lymphatic filariasis in humans. The principle mosquito vectors that transmit *Brugia malayi* include *Mansonia, Anopheles,* and *Aedes m*osquitoes (Karthik, *et al.,* 2011 and Palayam, *et al.,* 2012). And it is the first parasitic nematode genome to be sequenced (Ghedin, *et al.,* 2007) that ranges approximately 90-95 mega bases in size.

UDP-galactopyranose mutase (UGM) is a flavoenzyme that catalyzes the conversion of UDP-galactopyranose (UDP-Gal*p*) to UDP-galactofuranose (UDP-Gal*f*), the precursor of galactofuranose (Gal*f*). This reaction is essential for many pathogenic species of bacteria, protozoa and fungi, because UDP-Gal*f* serves as the activated Gal*f* donor during cell wall biosynthesis in these organisms (Beverley, *et al.,* 2005) UGM is a homodimeric enzyme, which binds one equivalent of flavin adenine dinucleotide (FAD) per monomer, and catalyzes the reversible conversion of UDP-Gal*p* to UDP-Gal*f*. The identification of UGM as an FAD-containing enzyme prompted questions about the role of its cofactor. A number of different roles have been proposed for the flavin, including nucleophilic attack, single electron transfer, hydride transfer, charge stabilization, and a structural role. Indeed, mechanistic studies implicate a catalytic role for the flavin cofactor. Specifically, a reduced flavin is required for catalytic activity; the oxidized flavoenzyme is inactive (Gruber, *et al.,* 2009 and Gruber, *et al.,* 2009).

The mechanism for the reaction catalyzed by UGM has been the subject of much discussion and several possibilities have been proposed. The mechanistic hypothesis proposed by (Soltero-Higgin, *et al.,* 2004) involves direct nucleophilic addition of the reduced flavin to C1 of the sugar substrate, based on the trapping of the coenzyme-substrate adduct during the interconversion. UGM must be reduced for activity, yet there are no candidate reducible functional groups on its substrates, UDP-Gal*p* or UDP-Gal*f*. It is known, however, that during turnover the UDP group is released transiently from galactose. The UGM flavin acts as a nucleophile, and, in a substitution reaction, captures the anomeric carbon position of the substrate at the reactive N5 position of flavin (Figure 1) (Oppenheimer, *et al.,* 2012). Formation of iminium ion allows opening of the sugar ring, which can then close to the furanose form. The inability of 5-deaza-FAD to promote conversion (Gruber, *et al.,* 2009) is consistent with the proposed nucleophilic role of N5.

The emergence of multi-drug resistant strains of human pathogens is a major concern. The prevalence of these resistant strains has compelled researchers to investigate new targets/ approaches for antimicrobial drug design. The presence of UGM in several

prokaryotic and eukaryotic microbial pathogens, the important roles played by Gal*f*-containing glycans in these organisms and the absence of this biosynthetic pathway in higher eukaryotes have led to the proposal of Gal*f* metabolism as a target for broad-spectrum chemotherapeutic intervention (Pedersen, *et al.,* 2003). To finalize, Galactofuranose has never been found in humans but is an essential building block of the cell wall and extracellular matrix of many bacteria, fungi, and protozoa. The importance of UGM for the viability of many pathogens and its absence in humans makes UGM a potential drug target.

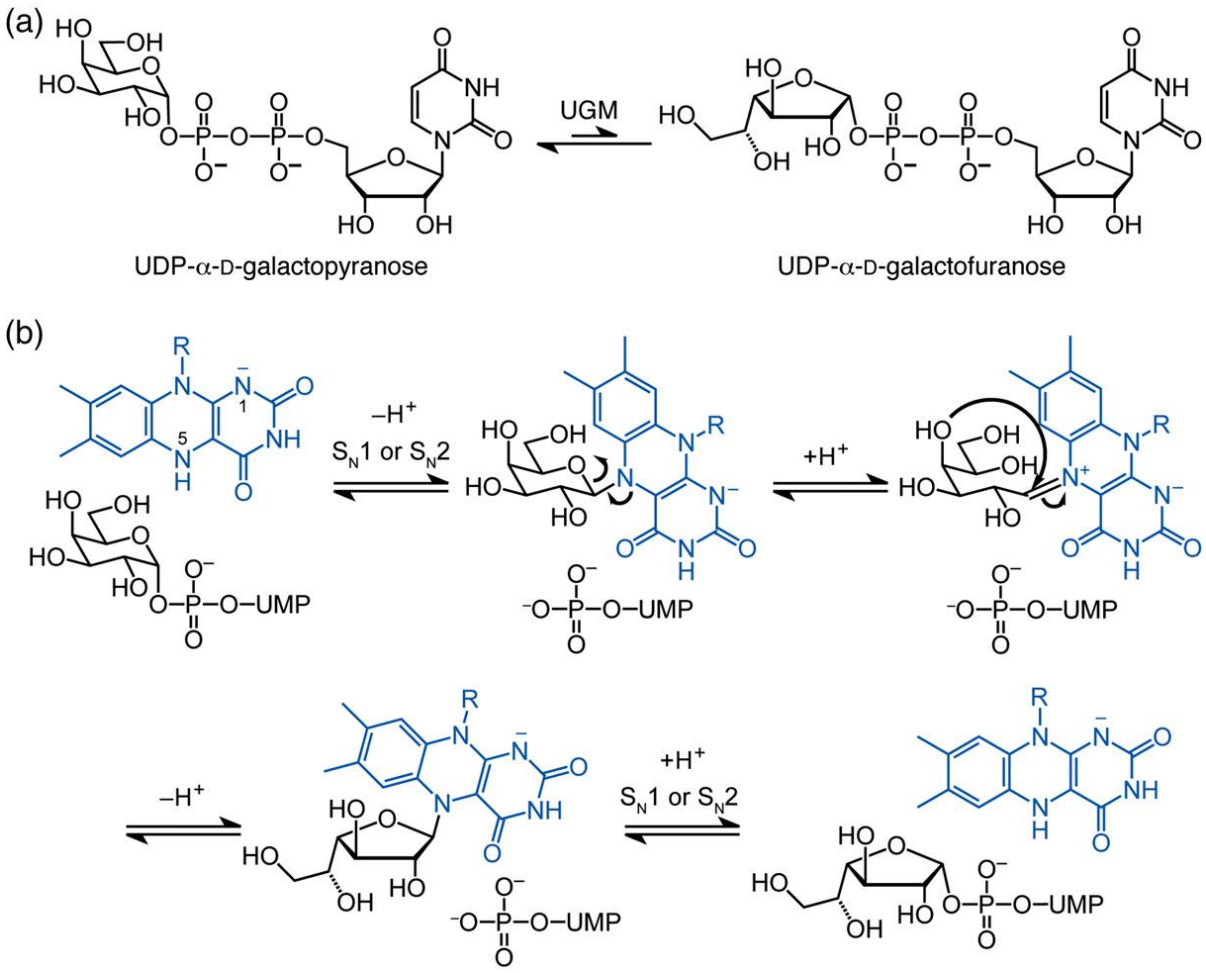


Figure 1: Interconversion of UDP-Gal*p* to UDP-Gal*f* catalyzed by UGM.
(a) The reaction catalyzed by UGM. (b)Nucleophilic mechanism for catalysis by UGM; the reaction could proceed by either SN1 or SN2.

Since the existence of Gal*f* in nematodes has not been established, the role of UGM homolog glf-1 in worm development of the *Caenorhabditis elegans* (*C. elegans*) was investigated (Novelli, *et al.,* 2009). Glf-1 mutants display significant late embryonic and larval lethality; other phenotypes indicative of defective surface coat synthesis, the glycan-

rich outermost layer of the nematode cuticle. Overall the results of this study demonstrate the presence of Gal*f* in *C. elegans* and reveal its pivotal role in synthesis of the surface coat, a particularly important structure in nematodes, as it represents the interface between the worm and its environment, and likely important for survival of free-living and parasitic species. According to this study, this structure has a role in completion of embryogenesis, late larval development and survival of the nematode in an adverse environment.

Sanjay Kumar and his colleagues reported results from the first genome-wide application of a rational drug target selection methodology to a metazoan pathogen genome, the completed draft sequence of *Brugia malayi*, a parasitic nematode responsible for human lymphatic filariasis. Their study results show that UDP-galactopyranose mutase (*B. malayi* pub locus Bm1_34455) is one of the predicted drug targets (Kumar, *et al.*, 2007). Jacopo F. Novelli *et al* analyzed *C. elegans* nucleotide sugar pools to confirm the presence of Gal*f* in *C. elegans*. UDP-Gal*f* was detected in wild-type animals while absent in glf-1 deletion mutants. Their data indicate that Gal*f* likely has a pivotal role in maintenance of surface integrity in nematodes, supporting investigation of UGM as a drug target in parasitic species.

STRUCTURE OF UGM

In general, mutase monomers can be divided into three domains. Domain 1 binds the FAD molecule and consists of a central four-membered parallel β-sheet that includes the αβα Rossmann fold motif. Domain 2 is primarily a five-helix bundle that is connected to the first domain by domain 3, which is a six-stranded and seven-stranded antiparallel β-sheet in *E.coli* and *A. fumigatus* respectively (Dhatwalia, *et al.*, 2012). This domain participates in substrate binding.

UGM is a flavin-dependent enzyme. Mechanistic studies suggest that its noncovalently associated flavin adenine dinucleotide (FAD) plays an important role in catalysis. Specifically, UGM is active only when the cofactor is reduced (Gruber, *et al.*, 2009 and Sanders, *et al.*, 2001). UGM has been isolated from several bacterial sources and all have been found to contain a flavin adenine dinucleotide (FAD) in the active site. Unlike flavin-dependent oxidoreductases, the redox state of the flavin in UGM is unchanged upon product formation (Sanders, *et al.*, 2001). Although it is known that the FAD must be reduced for maximal catalytic activity, the precise role that the flavin plays in catalysis remains controversial.

MATERIAL AND METHODS

Homology Modeling

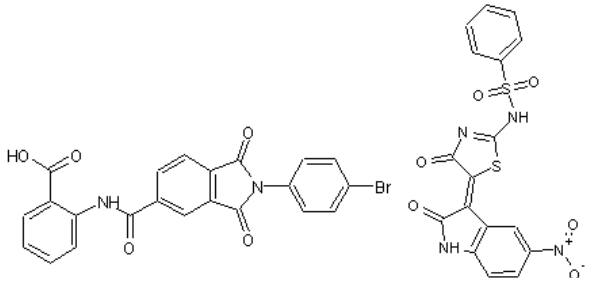
The target protein sequence (476 amino acids) of UDP Galactopyranose Mutase from *Brugia malayi* was retrieved from the Entrez database of NCBI (Accession No: ACZ05047.1). As there is no experimentally determined structure available, the 3D structure of UGM was predicted by homology modeling using CPH models 3.2 Server (Nielsen, *et al.*, 2010). An UGM from *Aspergillus fumigatus* (PDB ID: 3UTE) was used as a template (Dhatwalia, *et al.*, 2012). The template recognition is based on profile-profile alignment guided by secondary structure and exposure predictions. The backbone conformation for the predicted model was evaluated by PROCHECK using Structural Analysis and Verification Server (SAVES) - NIH MBI Laboratory for Structural Genomics and Proteomics (Laskowski, *et al.*, 1993). This enables to check the stereochemical quality of a protein structure by analyzing residue-by-residue geometry and overall structure geometry.

Active Site Prediction

The active site residues of substrate (UDP-Gal*p*) and co-factor (FAD) were predicted by superimposing the predicted model and its corresponding template structure using PYMOL (Schrodinger, *et al.*, 2010). First, the active site residues of UDP-Gal*p* and FAD were assessed from the crystal structure of *A. fumigatus by* the help of PYMOL and crossed checked with literature (Dhatwalia, *et al.*, 2012). Then, the model and template were superimposed to find out the corresponding active site residues for the model.

Screening of Inhibitors

From the literature studies, it was found that few compounds were tested for their inhibitory activity against UGM's of various pathogens (Tu, *et al.*, 1995; Lipton, *et al.*, 2002 and Patch, *et al.*, 2005). Therefore, attempts have been made to screen each of the specified compounds in the PubChem database. For this, the 2D structure of all the compounds were first drawn with the help of ChemSketch (www.acdlabs.com) and searched against the database. In this process two compounds were found with the PubChem Ids CID_1271230 and CID_5762794 (Figure 2). These compounds, in turn, were then screened for related structures and thus gave considerable number of similar compounds (about 44 and 11, respectively) which were finally taken for the docking studies. Therefore, two libraries of inhibitors were created, the first library with 44 compounds and the second with 11.



a) 2-({[2-(4-bromophenyl)-1,3-dioxo-2,3-dihydro-1H-isoindol-5-yl]carbonyl}amino)benzoic acid

b) N-[(5Z)-5-(5-nitro-2-oxo-1H-indol-3-ylidene)-4-oxo-1,3-thiazol-2-yl]benzene sulfonamide

Figure 2: a) Starting compound for library 1. b) Starting compound for library 2.

Ligand Structure Preparation

The two libraries of inhibitors, substrate and cofactor structure were downloaded from PubChem database. By using the LigPrep (2.3) module (LigPrep, 2009), the substrate and co-factor were geometry optimized by using the Optimized Potentials for Liquid Simulations-2005 (OPLS-2005) force field with the Steepest Descent followed by Conjugate gradient protocol for further Induced Fit Docking (IFD). All the other compounds were constructed using the fragment dictionary of Schrodinger's graphical user interface. Maestro provided LigPrep process to perform conversions, apply corrections to the structures, generate variations on the structures, eliminate unwanted structures, and optimize the structures. Many of the steps are optional, and are controlled by selecting options in the LigPrep panel or specifying command-line options.

Protein Structure Preparation

The 3D structure of the B. malayi UGM was modelled using CPH models 3.2 Server. Schrödinger offers a Protein Preparation facility for use with **G**rid based **L**igand **D**ocking with **E**nergetics (GLIDE) that is designed to ensure chemical correctness and to optimize protein structures (Friesner, *et al.*, 2004 and Halgren, *et al.*, 2004). The protein is pre-processed, optimised and then minimised using protein preparation wizard of Schrodinger suite. In most cases, the full preparation of a protein can be done with the Protein Preparation Wizard in Glide.

Molecular Docking Studies

Molecular docking studies were done for the substrate, cofactor, two libraries of inhibitors and two already existing filarial drugs.

Induced Fit Docking (IFD) for the Cofactor and Substrate

In standard virtual docking studies, ligands are docked into the binding site of a receptor where the receptor is held rigid and the ligand is free to move. However, the assumption of a rigid receptor can give misleading results, since in reality many proteins undergo side-chain or backbone movements, or both, upon ligand binding. These changes allow the receptor to alter its binding site so that it more closely conforms to the shape and binding mode of the ligand. This is often referred to as "induced fit" and is one of the main complicating factors in structure based drug design. IFD were done for the cofactor and the substrate to ensure whether they could bind to the respective active residues of the modelled protein.

Rigid Docking

The 44 inhibitors from the first and 11 inhibitors from the second libraries were screened against the parent compound using SP (Standard Precision) and XP (Extra Precision) modules (rigid docking) in the Glide software. During this screening some criteria like Docking score, Glide Energy, Glide Emodel and non-bonded interactions of the inhibitors with the active site residues were subjected for docking analysis. From the SP and XP results, best possible inhibitors were finally selected for Induced Fit Docking (Flexible docking) studies.

Induced Fit Docking of Inhibitors

Induced Fit Docking was carried out using Schrodinger's GLIDE where ligand and protein were held flexible and protein side chains goes conformational changes based on the ligand binding mode in the active site. IFD uses a hierarchical series of filters to search for possible locations of the ligand in the active site region of the receptor. The shape and properties of the receptor are represented on a grid by several different sets of fields that provide progressively more accurate scoring of the ligand poses. 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. Final scoring is then carried out on the energy-minimized poses where the best one was chosen among the screened conformational poses based on Docking score, Glide energy, and Glide emodel and hydrogen bond interactions.

Induced Fit Docking of Existing Filarial Drugs

IFD of two popular existing filarial drugs were done to compare with the newly screened drugs. These widely used filarial drugs taken for IFD were diethylcarbamazine (DEC) and levamisole.

RESULTS AND DISCUSSION

Model Prediction

Table 1: Active site residues of the template and target structures for substrate binding (UDP-Gal*p*) and cofactor (FAD). Abbreviations: AF- *Aspergillus fumigates*, BM-*Brugia malayi***.**

UDP-Gal*p*		**FAD**	
AF	***BM***	***AF***	***BM***
GLN-107	GLN-113	THR-18	THR-11
ASN-163	THR-169	ASP-38	GLU-39
TRP-167	TRP-173	LEU-46	LEU-47
ARG-182	ARG-188	HIS-63	HIS-64
ASN-207	LEU-203	SER-39	LYS-40
TYR-317	TYR-326	VAL-242	VAL-251
ARG-327	ARG-336	GLU-373	GLU-359
TYR-419	TYR-405	HIS-417	TYR-403
TYR-453	TYR-439	TYR-453	TYR-439
ASN-457	ASN-443	GLY-456	SER-442
		GLN-458	GLN-444
		SER-461	CYS-447

The target UGM from Brugia malayi was predicted using a template UGM from A. *fumigatus* (PDB Id: 3UTE). The model was generated using CPH models 3.2 Server (Figure 3). On superimposition, the backbone Cα atoms of the predicted model and the template structure have not only yielded a minimum rms deviation but also have located the active site residues of FAD and UDP-Gal*p* (Table 1).

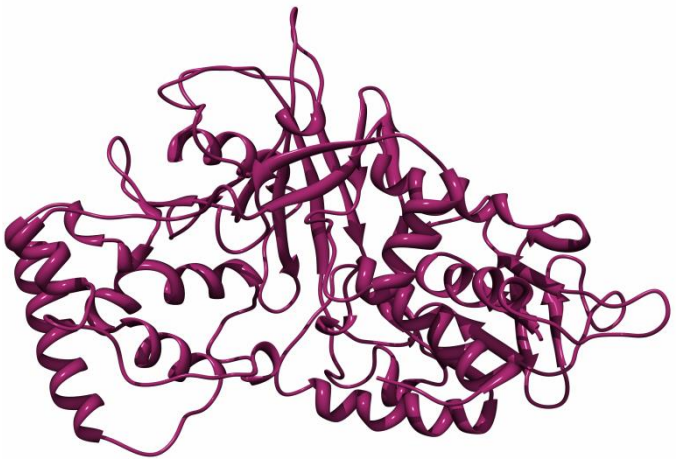


Figure 3: Predicted 3D model of *B. malayi* UGM

IFD of Substrate and Cofactor

Table 2: IFD result of the substrate and cofactor with *Brugia malayi's* UGM

	UDP-Gal*p*		FAD	
Pose	Docking Score	Glide Energy (Kcal/mol)	Docking Score	Glide Energy (Kcal/mol)
1	-13.12	-60.24	-13.55	-101.05
2	-12.68	-63.68	-12.48	-77.99
3	-12.24	-64.18	-12.39	-87.81
4	-12.81	-63.55	-12	-82.44
5	-11.75	-62.06	-12.03	-96.21
6	-12.51	-55.24	-11.15	-65.2
7	-11.16	-53.92		
8	-9.85	-54.71		
9	-8.85	-54.16		

UDP-D-galactofuranose (UDP-Gal*f*), which is essential for both cell growth and virulence in many pathogenic microorganisms, is converted from UDP-D-galactopyranose (UDP-Gal*p*) by the flavin adenine dinucleotide (FAD) dependent enzyme UDP-galactopyranose mutase (UGM). Analysis of docking score, glide energy and hydrogen bond interactions proves that the substrate UDP-Gal*p* and the cofactor FAD can bind tightly with the predicted model of *Brugia malayi's* UGM with good affinity and maximum energy (Table 2).

It is known that the protein is active when the FAD cofactor binds to it and exists in the reduced state. Studies on the enzyme's mechanism reveals that flavin N5 is perched for attack at the anomeric C1 atom of UDP-Gal*p* with the UDP moiety positioned to serve as a leaving group. Moreover, the distance separating nucleophilic flavin N5 and the anomeric position is 3.6 Å (Figure 4).

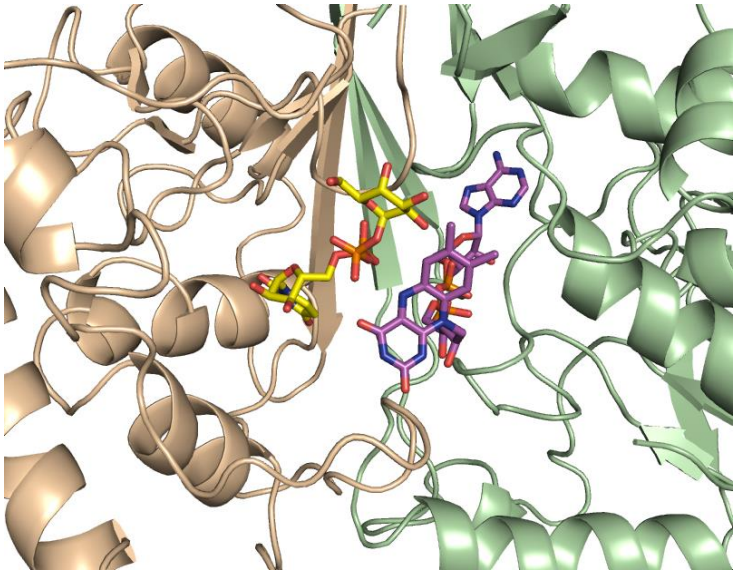


Figure 4: Superimposition of the substrate UDP - Gal*p* (Yellow Stick) bound UGM with FAD (Magenta Stick) bound UGM. The IFD complex of UGM – substrate has been coloured Tint whereas the IFD complex of UGM – FAD complex has been coloured Green. For better resolution, in the UGM – substrate complex FAD binding domain has been removed and likewise in the UGM – FAD complex substrate binding domain has been removed.

Validation of the docking protocol

The most straightforward method of evaluating the accuracy of a docking procedure is to determine how closely the lowest energy pose (binding conformation) predicted by the object scoring function. For the prediction of these results mainly three parameters are

considered viz., Docking score, Glide energy, and H-bonds interactions. On the basis of these parameters the binding affinity of ligand towards receptor are discussed.

The more negative value of Docking-score indicates good binding affinity of the ligand with receptor. The minimum energy for the formation of complex between ligand and receptor indicates good binding affinity. In this study and most literatures, Glide energy is considered as the primary criteria to select the best ligands (inhibitors) against the receptor. More H-bonds in the structure shows ligand having good binding mode to the receptor. H-bond interaction also relates to antagonist and agonist action of ligand with receptor. The docking results of the five best inhibitors of UGM are shown in the Table 3 and Figure 5

Inhibitor Docking

Two libraries of inhibitors were prepared, each with 44 and 11 compounds. Rigid docking (XP and SP) was done to screen out these compounds. Then 11 compounds from the first library and 5 compounds from the second library were selected after analysing their docking score, glide energy and hydrogen bond interactions with the active site residues of the target enzyme. These compounds were taken for further flexible docking studies. Out of 17, finally, 5 compounds were suggested as best inhibitors of UGM of filarial nematodes based on their IFD results (Table 3).

Three ligands from the first library (CID_2865563, CID_4081902 and CID_5762793) and two from the second library (CID_6795517 and CID_40453907) were found to posses reliable docking score and binding energy of -7.52, -11.11, -11.56, -6.17, -7.80 and -63.34 kcal/mol, -58.59 kcal/mol, -56.82 kcal/mol, -56.65 kcal/mol, -56.77 kcal/mol respectively. Ligands from the first library show better scores than the second library.

where, vdW = van der Waal energy; Coul = Coulomb energy; Lipo = lipophilic contact term; Hbond = hydrogen-bonding term; Metal = metal-binding term; BuryP = penalty for buried polar groups; RotB = penalty for freezing rotatable bonds; Site = polar interactions at the active site; the coefficients of vdW a = 0.065 and Coul b = 0.130.

Table 3: IFD results of five best UGM inhibitors.

Compound	Glide Score *	Glide Energy (Kcal/mol)	H-Bond Interaction (D-H…A)	Distance (Å)
LIBRARY 1				
1	-7.52	-63.34	O-H…O Thr 169 Gln 93 N-H…O Trp 173 N-H…O Arg 188 N-H…O Tyr 439 O-H…O Asn 443 N-H…O	2.9 2.6 2.8 2.9 2.8 2.7
2	-11.11	-58.59	Tyr 168 O-H…O O-H…O Ile 185 Arg 336 N-H…O Tyr 439 O-H…O	2.9 2.8 3.2 3.3
3	-11.56	-56.82	Gln 93 N-H…O O-H…O Ile 185 Tyr 326 O-H…O Tyr 439 O-H…O	2.7 2.8 2.9 2.9
LIBRARY 2				
4	-6.17	-56.65	Tyr 110 O-H…O Tyr 168 O-H…O Tyr 439 O-H…N Asn 443 N-H…O	3.4 2.8 3 2.9
5	-7.8	-56.77	N-H…O Ile 185 Tyr 168 O-H…O N-H…O Tyr 439	2.8 3.5 2.9

* Glide Score = a * vdW + b * Coul + Lipo + Hbond + Metal + BuryP + RotB + Site

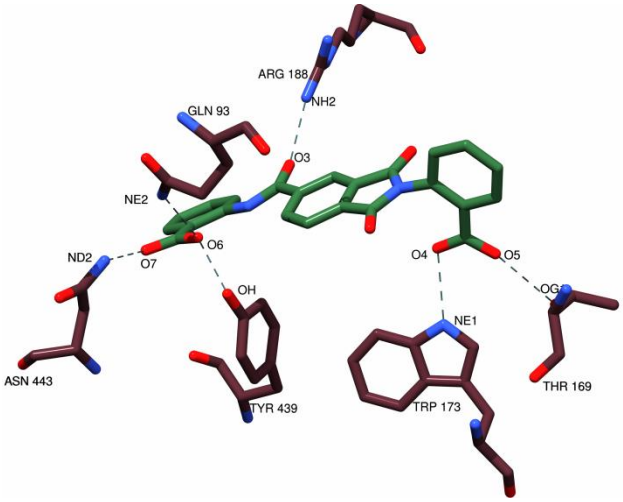


Figure 5a: H-Bond Interactions of Compound 1 With the Active Site Residues of *Brugia Malayi's* UGM

The IFD of compound 1 with the target UGM has exhibited a dock score of -7.52 and glide energy -63.34 (Table 3). A total of six hydrogen bond formations (with all being hydrogen acceptors) were observed between the inhibitor and enzyme. Four h-bond interactions were formed between the side chain nitrogen of Gln 93 (NE2), Trp 173 (NE1), Arg 188 (NH2) and Asn 443 (ND2) with oxygen O6, O4, O3 and O7 inhibitor atoms respectively (Figure 5a). The remaining two interactions were found between side chain oxygen of Thr 169 (OG1) and Tyr 439 (OH) with O5 and O6 respectively of the inhibitor atoms. Of the six interactions, a bifurcated hydrogen bond formation has been observed between the ligand and active site residues through Gln 93 and Tyr 439 (Figure 5a).

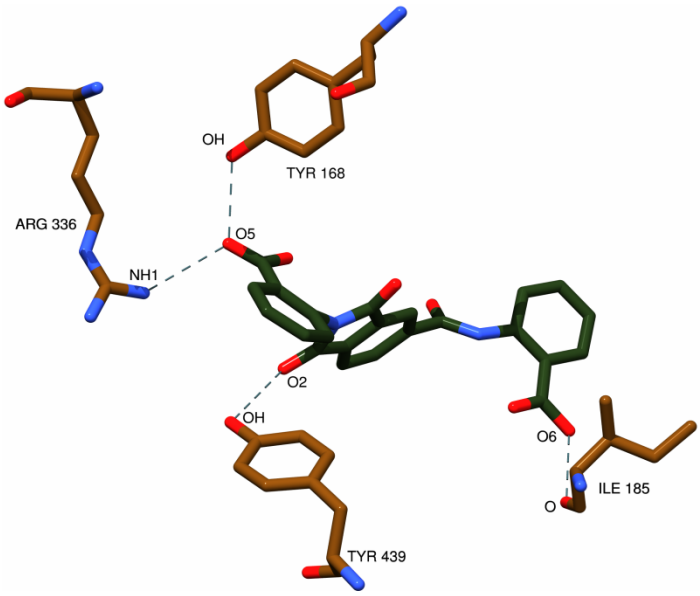


Figure 5b: H-Bond Interactions of Compound 2 With the Active Site Residues of *Brugia Malayi's* UGM

The binding of compound 2 with the target UGM has possessed a dock score of -11.11 and glide energy -58.59 (Table 3). Four hydrogen bond formations (with 3 being hydrogen acceptors) were observed between the side chain oxygen of Tyr 168 and Tyr 439 (OH) with oxygen O5 and O2 respectively; as well as between side chain nitrogen of Arg 336 (NH1) with O5. The remaining interaction (hydrogen donor) was between O6 of the inhibitor atom with back bone oxygen of Ile 185 (O). A bifurcated hydrogen bond formation was observed between the ligand and active site residues through Arg 336 and Tyr 168 (Figure 5b).

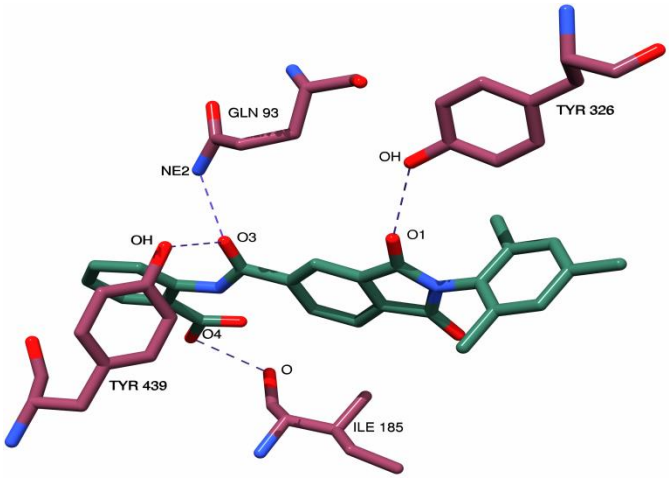


Figure 5c: H-Bond Interactions of Compound 3 With the Active Site Residues of *Brugia Malayi's* UGM

The binding of compound 3 with the target UGM has possessed a dock score of -11.56 and glide energy -56.82 (Table 3). A total of four hydrogen bond formations were observed in this enzyme – inhibitor complex. While the side chain oxygen of Tyr 326 (OH), Tyr 439 (OH) and side chain nitrogen of Gln 93 (NE2) were deprotonated by the inhibitor atoms O1 and O3 respectively (hydrogen acceptors), the back bone oxygen of Ile 185 (O) was abstracting the oxygen atom O4 (hydrogen donor) of the inhibitor (Figure 5c). As observed in Compound 1 and 2, the binding of compound 3 with the target UGM has also exhibited a bifurcated hydrogen bond formation between the ligand and active site residues through Gln 93 and Tyr 439 (Figure 5c).

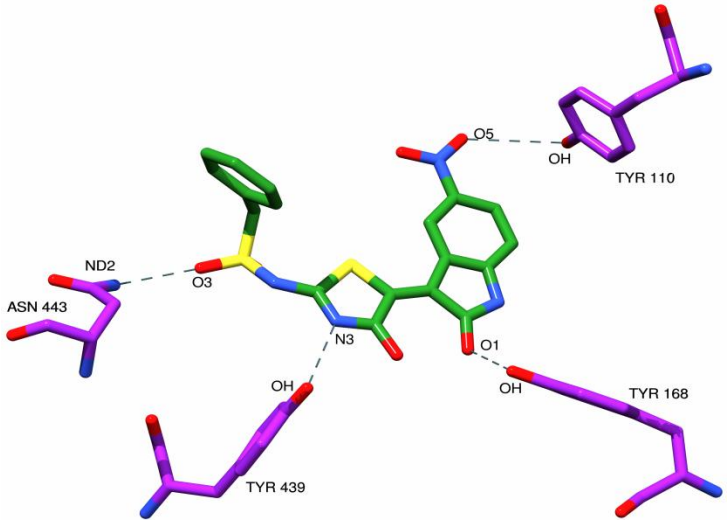


Figure 5d: H-Bond Interactions of Compound 4 With the Active Site Residues of *Brugia Malayi's* UGM

The IFD of compound 4 with the target UGM has possessed a dock score of -6.17 and glide energy -56.65 (Table 3). A total of four hydrogen bond interactions (hydrogen acceptors) were observed in this enzyme – inhibitor complex. This was felicitated through the oxygen atom O3 from the sulphate moiety and the nitrogen atom N3 from the five membered ring of the inhibitor abstracting hydrogen atom from the side chain nitrogen of Asn 443 (N) and side chain oxygen of Tyr 439 (O) respectively (Figure 5d). Similarly the oxygen atom O1 from the imidazole ring and another oxygen atom O5 from the benzene ring of the inhibitor have deprotonated the side chain oxygen of Tyr 168 (O) and Tyr 110 (O) respectively (Figure 5d).

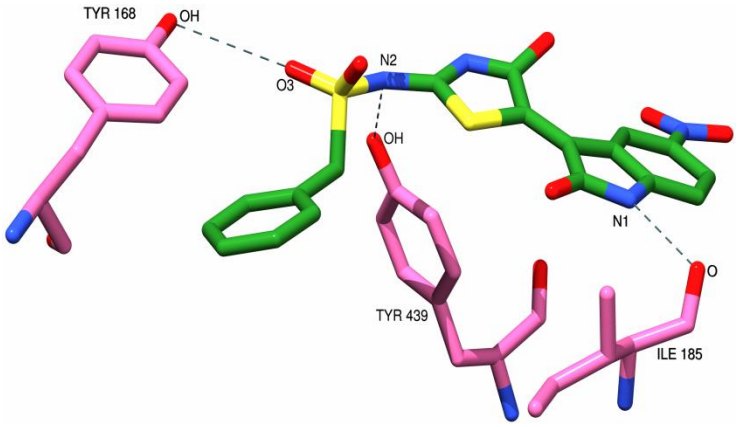


Figure 5e: H-Bond Interactions of Compound 5 With the Active Site Residues of *Brugia Malayi's* UGM

The binding of compound 5 with the target UGM has possessed a dock score of -7.80 and glide energy -56.77 (Table 3). Three reliable hydrogen bond interactions were obtained in this enzyme – inhibitor complex. While the side chain oxygen of Tyr 168 (OH) was deprotonated by the inhibitor atom O3 (hydrogen acceptor), the back bone oxygen of Ile 185 (O) and side chain oxygen of Tyr 439 (O) were abstracting the nitrogen atom N1 and N2 (hydrogen donor) of the inhibitor (Figure 5e).

IFD of Existing Drugs

IFD was done for the two commonly used filarial drugs against *B. malayi's* UGM. The IFD result shows that these drugs are not effective to inhibit the enzyme activity.

Table 4: IFD result of commonly used filarial drugs

	Diethylcarbamazine		**Levamisole**	
Pose	Docking Score	Glide Energy (Kcal/mol)	Docking Score	Glide Energy (Kcal/mol)
1	-5.1	-30.31	-7.13	-36.55
2	-5.56	-30.17	-6.5	-31.91
3	-2.59	-29.7	-6.63	-31.45
4	-2.49	-26.78	-6.3	-30.94
5	-5.25	-26.02	-6.45	-30.71
6	-3.32	-24.41	-6.14	-30.34
7	-4.55	-24.41	-6.22	-30.04
8	-4.29	-22.57	-6.96	-29.35
9	-2.91	-22.32	-5.96	-28.68
10	-4.03	-21.05	-6.54	-28.3
11	-4.2	-19.51	-6.83	-28.09
12	-3.31	-19.29	-6.1	-27.74
13	-3.75	-1733	-6.37	-25.61

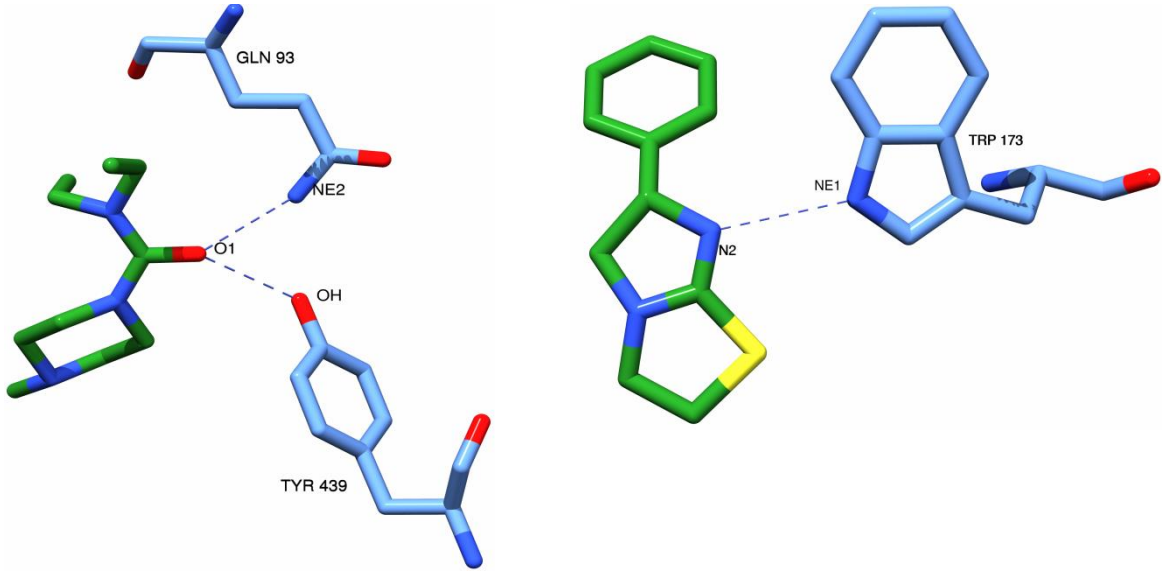


Figure 6: Structure of commonly used filarial drugs showing interactions with the active site residues of the protein. Pictures are generated using PYMOL.

Qikprop Results

ADME properties influence the drug level and kinetics of the drug exposure to the tissues and hence influence the pharmacology activity of the compound as drug. The ligands were prepared from 2Dgeometries using Ligprep. Glide package uses Qikprop applications to calculate the descriptors. More than 36 descriptors are calculated using Qikprop for all the 5 best UGM inhibitors. Potent compounds are selected after screening and checked whether they satisfy Lipinski's rule of five. Calculated descriptors for selected 5 compounds are shown in the table 5.

CONCLUSION

The Protein-Ligand interaction plays a significant role in structural based drug designing. In this study a docking model for UDP-galactopyranose mutase of *Brugia malayi* inhibitor was developed. To the best of our knowledge, this is the first report on molecular docking of the above mentioned ligands with UGM. Analysis of this work reveals that UGM of *B. malayi* can bind FAD cofactor and catalyse the conversion of UDP-Gal*p* into UDP-Gal*f*. Besides, the docking results signify that the screened five compounds have a potential to inhibit UGM. The binding energy, docking score and hydrogen bonding of the ligand-protein interactions also confirmed that the ligand tightly fit to the protein. These inhibitors show interaction with the active site residues THR-169, TRP-173, ARG-188, TYR-326, ARG-336, TYR-439, ASN-443 and others.

Table 5: ADME studies of compounds using Qikprop (GLIDE)

Compounds	Mol.Wt (130 to 725)	HB Donor (0 to 6)	HB Acceptor (2 to 20)	Qplogpw (4.0 to 45.0)	Qplogs (-6.5 to 0.5)	Rule of five (max. 4)
CID_40453907	430.37	2	8.5	16.45	-5.05	0
CID_4081902	430.37	2	8.5	16.46	-5.07	0
CID_2865563	428.44	1	6.5	12.07	-6.44	0
CID_6795517	444.43	2	11.5	18.59	-3.79	0
CID_5762793	444.43	2	11.5	18.53	-3.76	0

Table 6: Comparison the docking result of UGM inhibitors with known filarial drugs.

	Screened UGM Inhibitors					Known Filarial Drugs	
	Cmp 1	Cmp 2	Cmp 3	Cmp 4	Cmp 5	DEC	Levamisole
Docking Score	-7.52	-11.11	-11.56	-6.17	-7.8	-5.1	-7.13
Glide Energy (kcal/mol)	-63.34	-58.59	-56.82	-56.65	-56.77	-30.31	-36.55
H-bonds	6	4	4	4	3	2	1

The overall results of this study will help to use and develop potent antifilarial drugs targeting UGM of parasitic nematodes. Further detail studies are required to understand the mechanism and role of the enzyme (UGM) in nematodes.

ACKNOWLEDGEMENTS

The authors acknowledge Bioinformatics Infrastructure Facility at University of Madras. LK acknowledges UGC, India for funding in the from of UGC-BSR Research Fellowship in Sciences.

REFERENCES

1. Beverley, SM; Owens, KL; Showalter, M; Griffith, CL; Doering, TL; Jones, VC and McNeil, MR (2005) Eukaryotic UDP Galactopyranose Mutase (*GLF* Gene) in Microbial and Metazoal Pathogens. ***Eukaryot Cell*** *4:* 1147-1154.
2. Bhullar, N and Maikere, J (2010) Challenges in mass drug administration for treating lymphatic filariasis in Papua, Indonesia. ***Parasites & Vectors*** *3*: 70-76.
3. Crowther, GJ; Shanmugam, D; Carmona, SJ; Doyle, MA; Hertz-Fowler, C; Berriman, M; Nwaka, S; Ralph, SA; Roos, DS; Van Voorhis, WC. and Agüero, F (2010) Identification of Attractive Drug Targets in Neglected-Disease Pathogens Using an In Silico Approach. ***PLoS Negl Trop Dis*** *4:* 1-18.
4. Dhatwalia, R; Singh, H; Oppenheimer, M; Karr, DB; Nix, JC; Sobrado, P and Tanner, JJ (2012) Crystal structures and small-angle X-ray scattering analysis of UDP-galactopyranose mutase from the pathogenic fungus Aspergillus fumigates. ***J Biol Chem*** *287:* 9041-9051.
5. Friesner, RA; Banks, JL; Murphy, RB; Halgren, TA; Klicic, JJ; Mainz, DT; Repasky, MP; Knoll, EH; Shelley, M; Perry, JK and Shaw, DE (2004) Glide: A new Approach for Rapid, Accurate Docking and Scoring. 1. Methods and Assessment of docking Accuracy. ***J Med Chem*** *47*, 1739-1749.
6. Ghedin, E; Wang, S; Spiro, D; Caler, E; Zhao, Q; Crabtree, J; Allen, JE; Delcher, AL; Guiliano, DB; Miranda-Saavedra, D and Angiuoli, SV (2007) Draft Genome of the Filarial Nematode Parasite Brugia malayi. ***Science*** *317*: 1756-1760.
7. Gruber, TD; Westler, WM; Kiessling, LL and Forest, KT (2009) Forest. X-ray Crystallography Reveals a Reduced Substrate Complex of UDP Galactopyranose Mutase Poised for Covalent Catalysis by Flavin. ***Biochemistry*** *48:* 9171–9173.
8. Gruber, TD; Westler, WM; Kiessling, LL and Forest, KT (2009) Ligand Binding and Substrate Discrimination by UDP-Galactopyranose Mutase. ***J Mol Biol*** *391*: 327–340.
9. Gupta, S and Srivastava, AK (2005) Biochemical targets in filarial worms for selective antifilarial drug design. ***Acta Parasitologica*** 50: 1–18.
10. Halgren, TA; Murphy, RB; Friesner, RA; Beard, HS; Frye, LL; Pollard, WT and Banks, JL (2004) Glide: a new approach for rapid, accurate docking and scoring. 2. Enrichment factors in database screening. ***J Med Chem*** *47:* 1750–1759.

11. Hoerauf, A (2008) Filariasis: new drugs and new opportunities for lymphatic filariasis and onchocerciasis. ***Curr Opin Infect Dis*** *21*: 673–681.
12. Karthik, L; Malathy, P; Trinitta, A and Gunasekaran, K (2011) Molecular Docking with Trehalose- 6 Phosphate Phosphatase: A Potential Drug Target of Filarial Parasite 'Brugia Malayi'. ***Letters in Drug Design & Discovery*** *8:* 363-370.
13. Kumar, S; Chaudhary, K; Foster, JM; Novelli, JF and Zhang, Y (2007) Mining Predicted Essential Genes of *Brugia malayi* for Nematode Drug Targets. ***Plos One*** *2*: 1-9.
14. Laskowski, RA; MacArthur, MW; Moss, DS and Thornton, JM (1993) PROCHECK - a program to check the stereochemical quality of protein structures. ***J App Cryst*** 26: 283-291.
15. LigPrep (2009) version 2.3, Schrödinger, LLC, New York, NY.
16. Lipton, SA and Bossy-Wetzel, E (2002) Dueling activities of AIF in cell death versus survival: DNA binding and redox activity. ***Cell*** *111*: 147-150.
17. Mathew, N; Misra-Bhattacharya, S; Perumal, V. and Muthuswamy, K; (2008) Antifilarial Lead Molecules Isolated from Trachyspermum ammi. ***Molecules*** *13:* 2156-2168.
18. Murthy, PK and Joseph, SK (2011) Plant products in the treatment and control of filariasis and other helminthic infections and assay systems for antifilarial/antihelminthic activity. ***Planta Med*** *77*: 647-661.
19. Nielsen, M; Lundegaard, C; Lund, O and Petersen, TN (2010) CPH models-3.0 - Remote homology modeling using structure guided sequence profiles. ***Nucleic Acids Research*** *38*: 576-581.
20. Novelli, JF; Chaudhary, K; Canovas, J; Benner, JS; Madinger, CL; Kelly, P; Hodgkin, J and Carlow, CK (2009) Characterization of the Caenorhabditis elegans UDP-galactopyranose mutase homolog glf-1 reveals an essential role for galactofuranose metabolism in nematode surface coat synthesis. ***Developmental Biology*** *335:* 340–355.
21. Oppenheimer, M; Valenciano, AL; Kizjakina, K; Qi, J and Sobrado, P (2012) Chemical Mechanism of UDP-Galactopyranose Mutase from Trypanosoma cruzi: A Potential Drug Target against Chagas' disease. ***Plos One*** *7:* 1-11.
22. Palayam, M; Lakshminarayanan, K; Radhakrishnan, M and Krishnaswamy, G (2012) Preliminary analysis to target pyruvate phosphate dikinase from *wolbachia* endosymbiont of *Brugia malayi* for designing anti-filarial agents. ***Interdisciplinary Sciences: Computational Life Sciences*** *4*: 74-82.
23. Palumbo, E (2008) Filariasis: diagnosis, treatment and prevention. ***Acta Biomed*** *79:* 106-109.
24. Patch, CL and Sancar, A (2005) Photochemistry and photobiology of crypto chrome blue-light photopigments: the search for a photocycle. ***Photochem. Photobiol*** *81:* 1291-1304.

25. Pedersen, LL and Turco, SJ (2003) Galactofuranose metabolism: a potential target for antimicrobial chemotherapy. ***Cell Mol Life Sci*** *60:* 259-266.
26. Rahman, RA; Hwen-Yee, C and Noordin, R (2007) Pan LF-ELISA using BmR1 and BmSXP recombinant antigens for detection of lymphatic filariasis. ***Filaria Journal*** *6:* 1-7.
27. Sanders, DA; Staines, AG; McMahon, SA; McNeil, MR; Whitfield, C and Naismith, JH (2001) UDP-galactopyranose mutase has a novel structure and mechanism. ***Nat Struct Biol*** *8*: 858-863.
28. Schrodinger, LLC (2010) The PyMOL Molecular Graphics System, Version 1.3.
29. Soltero-Higgin, M; Carlson, EE; Gruber, TD and Kiessling, LL (2004) A unique catalytic mechanism for UDP-galactopyranose mutase. ***Nat Struct Mol Biol*** *11:* 539-543.
30. Tu, SC and Mager, HI (1995) Biochemistry of bacterial bioluminescence. ***Photochem Photobiol*** *62*: 615-624.
31. www.acdlabs.com

Correspondence Author:

Gunasekaran K*

CAS In Crystallography & Biophysics, University of Madras, Guindy Campus, Chennai 600 025, Tamil Nadu, India

Contact No: +91-9841737043

E mail id: gunaunom@gmail.com

Cite This Article: Karthik, L; Lemma, WD and Gunasekaran, K (2017) "Identification of Potent Inhibitors of UDP-galactopyranose Mutase for Filarial Nematodes Using an In Silico Approach", ***International Journal of Drug Research and Technology***, Vol. 7 (3), 134-156.

INTERNATIONAL JOURNAL OF DRUG RESEARCH AND TECHNOLOGY