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# Ligand Docking Based Identification of Novel Drug Analog for an Effective Treatment against Filaria

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**Original Research Article**

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**Abstract:** Filariasis (Philariasis) is a parasitic and infectious tropical disease that is caused by thread-like filarial nematode worms and their larvae. Filariasis is rarely fatal and it is the second leading cause of permanent and long-term disability in the world. The larvae transmit the disease to humans through a mosquito bite. Considering the Tubulin protein as drug target, which is the principle component of the Microtubular system in the filarial nematodes, the drugs were found by using the Drug Bank and their analogs by using NCI Enhanced Database Browser. Benzimidazole carbamates, a class of microtubule depolymerizing drugs was found to act by disrupting the assembly of tubulin dimers into microtubules. Thus the filarial nematode muscular system gets severely affected. The Filariasis can be prevented and eradicated by administrating one of the drugs belonging to benzimidazole carbamates class. Using Discovery Studio Visualisation tool, the analogs of this class of drugs were docked with the tubulin protein and novel drug was identified for an effective treatment against Filaria.
**Keywords:** Filarial drugs, Brugia malayi, microtubular system, tubulin protein, Lipinski's rule.

## INTRODUCTION

Filariasis (Philariasis) is a parasitic and infectious tropical disease, which is caused by thread-like filarial nematode worms and their larvae. The larvae transmit the disease to humans through a mosquito bite.

Filariasis is characterized by fever, chills, headache, and skin lesions in the early stages and, if untreated, can progress to include gross enlargement of the limbs and genitalia, a condition called Elephantiasis.

Approximately 170 million people in the tropical and subtropical areas of Southeast Asia, South America, Africa, and the islands of the Pacific are affected by this debilitating parasitic disease. While filariasis is rarely fatal, it is the second leading cause of permanent and long-term disability in the world. The World Health Organization (WHO) has named filariasis one of only six "potentially eradicable" infectious diseases and has embarked upon a 20-year campaign to eradicate the disease.

Lymphatic Filariasis is thought to have affected humans since approximately 4000 years ago. Artifacts from ancient Egypt (2000 BC) and the Nok civilization in West Africa (500 BC) show possible elephantiasis symptoms. The first clear reference to the disease occurs in ancient Greek literature, where scholars differentiated the often similar symptoms of lymphatic filariasis from those of leprosy. In 1866, Timothy Lewis, building on the work of Jean-Nicolas Demarquay and Otto Henry Wucherer, made the connection between microfilaria and elephantiasis, establishing the course of research that would ultimately explain the disease. In 1876, Joseph Bancroft discovered the adult form of the worm. In 1877, the life cycle involving an arthropod vector was theorized by Patrick Manson, who proceeded to demonstrate the presence of the worms in mosquitoes. In 1900, George Carmichael Low determined the actual transmission method by discovering the presence of the worm in the proboscis of the mosquito vector. In all cases, a mosquito first bites an infected individual then bites another uninfected individual, transferring some of the worm larvae to the new host. Once within the body, the

larvae migrate to a particular part of the body and mature to adult Worms.

Filariasis is classified into three distinct types according to the part of the body that becomes infected: lymphatic filariasis affects the circulatory system that moves tissue fluid and immune cells (lymphatic system); subcutaneous filariasis infects the areas beneath the skin and whites of the eye; and serous cavity filariasis infects body cavities but does not cause disease. The two most common types of the disease are Bancroftian and Malayan filariasis, both forms of lymphatic filariasis. The Bancroftian variety is found throughout Africa, southern and southeastern Asia, the Pacific islands, and the tropical and subtropical regions of South America and the Caribbean. Malayan filariasis occurs only in southern and southeastern Asia. Filariasis is occasionally found in the United States, especially among immigrants from the Caribbean and Pacific islands (Table-1).

Tubulin is of particular interest in parasitic nematodes, since it is the target of benzimidazole carbamates, a class of microtubule depolymerizing drugs [1]. The 13-tubulin chains are of special interest because benzimidazole resistant strains of Physarum polycephalum and C.elegans have altered β-tubulins [2, 3]. Brugia nematodes express β-tubulin molecules that contain binding sites for the benzimidazole carbamates [4].These drugs act by disrupting the assembly of tubulin dimers into microtubules [5]. Each antifilarial agent has the possible sites of action (Table-2).

**Table-1: Types of Filariasis, Parasites and the Vector Causing the Disease**

| Disease | Parasite | Vector |
|---|---|---|
| Onchocerciasis | O volvulus | Blackflies: Simulium species |
| Bancroftion filariasis | W bancrofti | Mosquitos: Anopheles, Aedes, Culex and Mansonia species |
| Malayan filariasis | B malayi and B timori | Mosquitos: Anopheles, Aedes, Culex and Mansonia species |
| Loiasis | L loa | Red files: Chrysops species |
| Mansonelliasis | M streptocerca | Midges: Culicoides species |
| Dirofilariasis | Dirofilaria species | Mosquitos: Culex species |

**Table-2: Ant filarial Agent and the Possible Sites of Action**

| Anti filarial agent | Metabolism / System effected |
|---|---|
| Diethylcarbamazine | Neuromuscular system, carbohydrate and folate metabolism |
| Ivermectin | Neuromuscular system |
| Suramin | Carbohydrate and folate metabolism |
| Benzmidazoles | Microtubular system and carbohydrate metabolism |
| Isothiocyanates | Carbohydrate,nucleic acid and protein metabolism |
| Levamisole | Neuromuscular system,carbohydrate metabolism |
| Arsenicals | Carbohydrate and Gluthathione metabolism |
| Antimonials | Carbohydrate metabolism |

The symbiosis of filarial nematodes and intracellular Wolbachia bacteria has recently been exploited as a target for antibiotic therapy of filariasis. Antibiotic treatment of filarial nematodes results in sterility and inhibits larval development and adult worm viability. In the first trial on human onchocerciasis depletion of bacteria following treatment with doxycycline resulted in a complete and long-term block of embryogenesis. Bacteria are unable to repopulate nematode tissues up to 18 months after depletion, suggesting these effects may be permanent. Following ivermectin treatment, individuals given antibiotic therapy showed sustained reductions in skin microfilariae, with the majority of people remaining microfilarial negative 12-18 months after treatment. Since Wolbachia also contribute to the inflammatory pathogenesis of filarial disease, antibiotic therapy could, in addition to effects on worm fertility or viability, prevent the onset or development of filarial pathology [6].

Tubulin is of particular interest in parasitic nematodes,since it is the target of benzimidazole carbamates,a class of micro-tubule depolymerizing drugs which was stated by Lacey in 1988.The Beta Tubulin chains are of special interest because benzimidazole resistant strains of Physarum polycephalum and C.elegans have altered Beta tubulins in the stydy done by Foster *et al*., in 1987, and Driscoll et al., in 1989.Brugia nematodes express Beta Tubuli Molecules that contain binding sites for the Benzimidazole carbamates [4]. These drugs act by disrupting the assembly of tubulin dimers into microtubules [5, 7].

Tubulin was identified in the filarial nematodes Brugia malayi and B. pahangi by several approaches. Initially, a monoclonal antibody (6D8) was selected for its unusual binding to B. malayi microfilariae in indirect immunofluorescence assays: 6D8 showed granular, heterogeneously dispersed fluorescence on fixed parasites but did not bind to unfixed microfilariae. The microfilarial sheath did not bind 6D8, although it did bind fluoresceinated wheatgerm agglutinin. By Western blotting against microfilarial sonicate, 6D8 reacted with a 50000-55000 mol. wt protein, and also bound to purified chicken brain 13—tubulin. Additionally, this monoclonal antibody reacted with a recombinant fusion protein expressed by a clone (Bpa-7) originally isolated from an adult B. pahangi cDNA expression library by its reaction with chronic human filariasis serum. This clone encodes a small 40 amino acid C—terminal segment corresponding to residues 409-449 of 13—tubulin, and shows complete amino acid sequence homology with vertebrate 13—tubulin from 409 to 430 but 55% divergence (six amino acid substitutions, four insertions and one deletion) from human and chicken (3—tubulin over positions 431-449 at the C terminus. Antibody to both parasite and vertebrate (chicken) tubulin was found in filarial infection sera, with higher levels of autoreactive antibody apparent in amicrofilaraemic individuals. Immunogold electron microscopy was then used to localize (3—tubulin in B. malayi microfilariae and adult worms. Tubulin was shown not to be exposed on the microfilarial sheath or in the cuticle of either stage, but was found to be abundant in the somatic tissues. In microfilariae, 6D8 bound myofibril structures under the hypodermal layer, and also bound within cell nuclei. In the adult stage, tubulin was associated with muscle blocks, as well as the intestinal brush border and the embryonic uterine microfilariae [8].

A new approach to therapy targets endosymbiotic Wolbachia bacteria. In 2000, a landmark study first showed that doxycycline cleared Wolbachia bacterial endosymbionts from the endodermis and uteri of adult female worms, leading to unusually extensive worm sterility not seen in other antifilarial treatments. In a nonrandomized, placebo-controlled trial involving humans , doxycycline (100 mg per day for 6 weeks), followed by a single 150-fig/kg dose of ivermectin, resulted in up to 19 months of amicrofilaridermia, as well as 100% elimination of Wolbachia species from worms that were isolated and tested immunohistologically. The effect on microfilaridermia is thought to result from a complete block of embryogenesis for at least 18 months. In contrast, ivermectin only works against late-stage developing microfilariae still in the uterus, and it has little or no effect on early-stage embryos. The authors suggest that infected patients who permanently leave areas of endemicity Should be offered, in addition to ivermectin, a 4-6-week course of doxycycline (100-200 mg Per day) to achieve long-term a microfilaridermia. PCR-detectable presence of Wolbachia species may remain and could signify the presence of dormant but viable bacteria, but these bacteria appear unable to repopulate the worms up to 18 months after treatment . More research is needed to secure this conclusion [9].

Lymphatic filarial nematodes are infected with endosymbiotic Wolbachia bacteria. Lipopolysaccharide from these bacteria is the major activator of innate inflammatory responses induced directly by the parasite. Here, we propose a mechanism by which Wolbachia initiates acute inflammatory responses associated with death of parasites, leading to acute filarial lymphangitis and adverse reactions to antifilarial chemotherapy. We also speculate that repeated exposure to acute inflammatory responses and the chronic release of bacteria, results in damage to infected lymphatics and desensitization of the innate immune system. These events will result in an increased susceptibility to opportunistic infections, which cause acute dermatolymphangitis associated with lymphoedema and elephantiasis. The recognition of the contribution of endosymbiotic bacteria to filarial disease could be exploited for clinical intervention by the targeting of bacteria with antibiotics in an attempt to reduce the development of filarial pathology [10].

Female filariae of the species Brugia malayi and Litomosoides carinii were investigated by means of electron microscopy after in vivo treatment with flubendazole. The earliest fine-structure alteration in both species was the disappearance of microtubuli from the intestinal cells as soon as 6 h after treatment. There was no further disintegration of intestinal cells for several days. Microtubuli disappeared from the outer zone of the hypodermal cytoplasm 24 h after treatment. At this time, marked alterations were also observed in the oogonia and in the embryonic cells. Many of these were swollen; their nuclear envelope was partly resolved and the chromatin was condensed, but no spindle apparatus was formed. The early fine-structure alterations observed after in vivo treatment with flubendazole consisted of the disappearance of microtubuli from various tissues. This led to the interruption of cell division in oogonia and embryonic cells and, subsequently, to the disintegration of most other filarial tissues. These morphological alterations differed considerably from those observed after treatment with benzothiazole derivatives, which do not affect the microtubuli of the filariae [11].

The pathogenesis of filarial disease is characterized by acute and chronic inflammation. Inflammatory responses are thought to be generated by either the parasite, the immune response, or opportunistic infection. We show that soluble extracts

of the human filarial parasite Brugia malayi can induce potent inflammatory responses, including tumor necrosis factor (TNF)-r, interleukin (IL)-1R, and nitric oxide (NO) from macrophages. The active component is heat stable, reacts positively in the Limulus amebocyte lysate assay, and can be inhibited by polymyxin B. INF-1, IL-1R, and NO responses were not induced in macrophages from lipopolysaccharide (LPS)-nonresponsive C3H/HeJ mice. The production of TNF-ri after chemotherapy of microfilariae was also only detected in LPS-responsive C3H/HeN mice, suggesting that signalling through the Toll-like receptor 4 (TLR4) is necessary for these responses. We also show that CD14 is required for optimal TNF-, responses at low concentrations. Together, these results suggest that extracts of B. malayi contain bacterial LPS. Extracts from the rodent filaria, Acanthocheilonema viteae, which is not infected with the endosymbiotic Wolbachia bacteria found in the majority of filarial parasites, failed to induce any inflammatory responses from macrophages, suggesting that the source of bacterial LPS in extracts of B. malayi is the Wolbachia endosymbiont. Wolbachia extracts derived from a mosquito cell line induced similar LPS-dependent TNF-, and NO responses from C3H/HeN macrophages, which were eliminated after tetracycline treatment of the bacteria. Thus, Wolbachia LPS may be one of the major mediators of inflammatory pathogenesis in filarial nematode disease [12].

Arthropod-transmitted (filarial) nematodes are important causes of disease in humans in tropical countries, yet no safe drug appropriate for mass delivery kills the adult Worms. However, most filarial nematodes contain rickettsia-like bacteria of the genus Wolbachia, and related bacteria also occur in insects. There is increasing evidence that these bacteria have significant functions in the biology of filarial nematodes. They are thus important targets in the search for antifilarial drugs and experiments in animals and humans have suggested that antibiotic therapy has potential in treating filarial infections. To optimize future clinical trials there is a need for a fast and simple in vitro drug screen to compare drug efficacies against Wolbachia. In the absence of Wolbachia-infected nematode cell lines, we have utilized an Aedes albopictus insect cell line, naturally infected with Wolbachia, to test the activity of antimicrobial agents. Of the five antibiotics tested, doxycycline, oxytetracycline and rifampicin showed good activity (MICs of 0.0625, 4 and 0.0625 mg/L, respectively) whereas ciprofloxacin and penicillin were shown to have no effect [13].

Lymphatic filariasis (LF) is a disease targeted for elimination. The global strategy is a once-yearly, single-dose, two-drug regimen utilised by communities at risk for LF, with the goal of reaching 80% population coverage yearly, for at least 5years, in order to interrupt transmission of LF. Where onchocerciasis is co-endemic, the regimen is ivermectin 200 — 400 pig/kg plus albendazole 400mg; elsewhere, the regimen should be diethylcarbamazine 6mg/kg plus albendazole 400mg. This paper reviews in detail the evidence for the efficacy and safety of these two-drug regimens underpinning the global strategy and makes recommendations for future developments in chemotherapy for LF, focusing on unresolved issues. These include optimal frequency, duration and end point of treatment, tools for monitoring successful therapy and means for detecting the potential development of resistance to any of the three antifilarial drugs on which the Global Programme to Eliminate LF depends [14].

Lymphatic filariasis is the most widespread of human filarial infections, a group of vector-borne infestations. After the discovery of diethylcarbamazine (DEC), little advance was made in the development of new chemotherapeutic agents for the treatment of lymphatic filariasis until 1985. Since then, several new initiatives have occurred as the result of a global effort by the World Bank/UNDP/WHO Special Programme on Tropical Diseases and the Onchocerciasis Control Programme. Some of these global research initiatives are reviewed in this paper. Recent observations throw a new light on the rational use of DEC including its deployment as a medicated salt. Ivermectin, an established drug for the treatment of river-blindness is examined for its potential use in the treatment of lymphatic filariasis. Experimental results from two novel compounds out of several being developed by the WHO/OCP Macrofil project are considered in respect to their potential macrofilaricidal activity, particularly in relation to lymphatic filarial infections [15].

Citing earlier advances in the treatment of lymphatic filariasis [particularly the effectiveness of single-dose diethylcarbamazine (DEC) in reducing microfilaraemia and its enhanced effectiveness when co-administered with single-dose ivermectin], Eric Ottesen, Mahroof Ismail and John Horton consider recent studies on the antifilarial activity of albendazole that have led to the current recommendations for its use in single-dose regimens in conjunction with either DEC or ivermectin for large-scale control/elimination programmes. Furthermore, the potential of albendazole as a macrofilaricide for treating individual patients with lymphatic filarial infections is emphasized as one of a number of important research questions that remain to be explored [16].

Wolbachia are one of the most abundant groups of bacterial endosymbionts in the biosphere. Interest in these heritable microbes has expanded with the discovery of wider genetic diversity in

undersampled host species. Here, we report on the putative discovery of a new genetic lineage, denoted supergroup H, which infects the Isopteran species *Zootermopsis angusticollis* and *Z. nevadensis*. Evidence for this novel supergroup is based on portions of new Wolbachia gene sequences from each species spanning 3.5 kilobases of DNA and the following genes: 16S rDNA, dnaA, gltA, groEL, and ftsZ. Single-gene and concatenated maximum likelihood phylogenies establish this new supergroup and validate the positioning of the other Wolbachia supergroups. This discovery is the first example of a termite Wolbachia that is highly divergent from the Isopteran Wolbachia previously described in supergroup F. This study highlights the importance of multilocus approaches to resolving Wolbachia supergroup relationships. it also suggests that surveys of Wolbachia in more earlier-originating (and undersampled) groups of arthropods are more apt to reveal novel genetic diversity [17].

The discovery of the endosymbiont Wolbachia, which has a mutualistic relationship with filarial nematodes, and its importance in filarial parasite biology has provided a lead for developing novel chemotherapeutic agents against human filariasis. Wolbachia also appears to be involved in immunopathological responses as well as adverse reactions after antifilarial therapy. The aim of the present study was to explore the potential of administering anti-Wolbachial therapy before antifilarial treatment to improve the filaricidal efficacy of the present-day filaricide diethylcarbamazine. An additional objective was to minimize host inflammatory reactions using a rodent model Mastornys concha and Meriones unguiculatus infected with human lymphatic filariid Brugia malayi. We observed: (1) a 40-day treatment schedule of tetracycline alone resulted in delayed reduction in microfilaraemia and a low degree of macrofilaricidal efficacy; (2) tetracycline therapy followed by 100 mg/kg diethylcarbamazine (DEC) x5 days led to marked reduction in microfilaraemia from day 48 onward after initiation of treatment. The combination treatment also brought about 70% death of adult B. malayi and sterilization of 82.3% of the surviving female worms, thus exhibiting remarkable enhancement in the antifilarial activity of DEC; (3) tissue inflammatory reactions and pathogenesis were significantly reduced as observed by histopathology, and peritoneal macrophage mediated oxidative burst shown by florescence-activated cell sorting (FAGS) analysis using dichlorofluorescein diacetate (DCF-DA); and (4) the characteristic filarial antigen-specific and mitogen-specific cellular unresponsiveness was significantly reversed, possibly due to marked Clearance of microfilaraemia. It is therefore advisable to give an anti-Wolbachial antibiotic trial before starting antifilarial therapy to achieve maximum benefits [18].

Filarial nematodes are parasitic worms that cause some of the most devastating of all tropical diseases such as elephantiasis and river blindness. Studies on the inflammatory pathogenesis of filarial disease have shown that endotoxin-like activity derived from endosymbiotic Wolbachia bacteria is the major inflammatory stimulus of filarial nematodes. Wolbachia appear to have evolved as essential symbionts of their filarial nematode hosts. Antibiotic depletion of bacteria shows that they are required for normal fertility and development of the worm and may even protect the parasites from host immunity. In addition to the uncovering of a fascinating symbiotic relationship, this discovery means we can now consider using antibiotics as a new approach to the treatment of filarial diseases [19].

A project to study the genome of the lymphatic filarial parasite Brugia malayi was initiated in 1995. This project has been funded by the World Health Organization and the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) with the ultimate objective of identifying new vaccine candidates and drug targets for filariasis. Because fewer than 60 Brugia genes had been cloned by the end of 1994, it was determined that the first goal of the project would be the identification of thousands of new genes. These genes have been identified by randomly selecting clones for DNA sequence analysis (ESTs) from cDNA libraries that have been constructed from all life cycle stages of B. malayi. To date, over 22,000 Brugia ESTs have been entered into the National Center for Biotechnology Information's dbEST database and about 8000 new genes have been identified (estimated to be about 40% of the complete set of B. malayi genes). In addition to new gene discovery, the 22,000 ESTs can be used to identify genes that are most highly expressed at each stage of development. Such analyses can provide insights into the biology of the organism and can suggest new molecules for study as drug targets and vaccine candidates [20].

The purpose of this study is to identify a novel drug that could affect the micro tubular system of the filarial nematodes for an effective treatment against Filaria.

## MATERIALS

### Protein Information Resource

The Protein Information Resource (PIR) located at Georgetown University Medical Center (GUMC), is an integrated public bioinformatics resource to support genomic and proteomic research, and scientific studies.

PIR was established in 1984 by the National Biomedical Research Foundation (NBRF) as a resource

to assist researchers in the identification and interpretation of protein sequence information. For four decades, PIR has provided many protein databases and analysis tools freely accessible to the scientific community, including the Protein Sequence Database (PSD), the first international database (see PIR-International), which grew out of Atlas of Protein Sequence and Structure.

In 2002, PIR along with its international partners, EBI (European Bioinformatics Institute) and SIB (Swiss Institute of Bioinformatics), were awarded a grant from NIH to create UniProt, a single worldwide database of protein sequence and function, by unifying the PIR-PSD, Swiss-Prot, and TrEMBL databases.

**NCI Enhanced Database Browser**

Enhanced NCI Database Browser used to search the 250,000-compound Open NCI Database. This database is the publicly available part of the half-million structure collection assembled by the NCI's Developmental Therapeutics Programme during the program's 45 years of screening compounds against cancer and more recently AIDS. In collaboration with the researchers at the Computer Chemistry Centre of the University of Erlangen-Neuremberg, we have implemented a Web-based graphical user interface for searching the structure and data in the Open NCI Database. This interface offers the user powerful tools for searching, analyzing and displaying search results. With this interface in place it is now easier to add large amounts of additional, mostly calculated data to the pool of searchable information. ln collaboration with a group at the Russian Academy of Medical Sciences in Moscow, predictions are now included for more than 500 different types of biological activities for most of the quarter-million structures in the database. A three-dimensional (3D) pharmacophore search feature has also been implemented. Furthermore, hyperlinks to additional services allow users immediate access to further processing of individual structures or hit sets in a wide variety of ways. The CADD group's home page is found at http://cactus.nci.nih.gov; the NCI Database Browser service is at http://cactus.nci.nih.gov/ncibd2/. We hope this tool will be useful in drug design for researchers both inside and outside the NCI.

**Drug Bank**

The Drug Bank database is a unique bioinformatics and cheminformatics resource that combines detailed drug (i.e. chemical, pharmacological and pharmaceutical) data with comprehensive drug target (i.e. sequence, structure, and pathway) information. The database contains nearly 4800 drug entries including >1,350 FDA-approved small molecule drugs, 123 FDA-approved biotech (protein/peptide) drugs, 71 nutraceuticals and >3,243 experimental drugs. Additionally, more than 2,500 non-redundant protein (i.e. drug target) sequences are linked to these FDA approved drug entries. Each Drug Card entry contains more than 100 data fields with half of the information being devoted to drug/chemical data and the other half devoted to drug target or protein data.

Drug Bank is supported by David Wishart, Departments of Computing Science & Biological Sciences, University of Alberta. Users may query Drug Bank in any number of ways. The simple text query (above) supports general text queries of the entire textual component of the database. Clicking on the Browse button (on the Drug Bank navigation panel above) generates a tabular synopsis of Drug Bank's content. Clicking on a given Drug Card button brings up the full data content for the corresponding drug. The Pharma Browse button allows users to browse through drugs as grouped by their indication. This is particularly useful for pharmacists and physicians, but also for pharmaceutical researchers looking for potential drug leads. The ChemQuery button allows users to draw (using Marvin Sketch applet or a ChemSketch applet) or write (SMILES string) a chemical compound and to search Drug Bank for chemicals similar or identical to the query compound. The Text Query button supports a more sophisticated text search (partial word matches, case sensitive, misspellings, etc.) of the text portion of Drug Bank. The Seq Search button allows users to conduct BLASTP (protein) sequence searches of the 18,000 sequences contained in Drug Bank. Both single and multiple sequence (i.e. whole proteorne) BLAST queries are supported. The Data Extractor button opens an easy-t (3-use relational query search tool that allows users to select or search over various combinations of subfields. The Data Extractor is the most sophisticated search tool for Drug Bank.

**Discovery Studio Visualisation**

DS Visualizer, one can visualize and share molecular information in a clear and consistent way, and in a wide variety of industry-standard formats. We can also create high quality graphics. DS Visualizer runs on Windows 2000, XP and Vista, Red Hat Enterprise Linux versions 3, 4 & 5 and SUSE Enterprise Linux 10.

**ADMET**

ADMET refers to the absorption, distribution, metabolism, excretion, and toxicity properties of a molecule within an organism.

**ADMET Descriptors in Discovery Studio 2.0 includes**

- ADMET Absorption: Predicts Human Intestinal Absorption (HIA) after oral administration
- ADMET Aqueous Solubility: Predicts the solubility of each compound in water at 25°C

- ADMET Blood Brain Barrier: Predicts the blood brain barrier penetration of a molecule
- ADMET Plasma Protein Binding: Predicts likelihood that a compound will be highly bound to carrier proteins in the blood ADMET CYP2D6 Binding: Predicts cytochrome P450 2D6 enzyme inhibition

ADMET Hepatotoxicity: Predicts the occurrence of dose-dependent human hepatotoxicity

**Predictive Toxicology-DS TOPKAT**

A QSAR – based system generates and validates accurate ,rapid assessments of chemical toxicity solely from a chemical's molecular structure .DS TOPKAT is fast ,cost effective and proven.

**DS TOPKAT Models include**

Rodent Carcinogenicity
Weight of evidence Rodent Carcinogenicity
Rat Maximum Tolerated Dose
Aerobic Biodegradability
Eye Irritancy
Log P
Rabbit Skin Irritancy
Rat Inhalation Toxicity LC50
Rat Maximum Tolerated Dose
Ames Mutagenicity
Rat Oral LD50
Rat Chronic LOAEL
Developmental Toxicity Potential
Skin Sensitization
Fathead Minnow LC50
DaphniaMagnaEC50

**Phamacophore Modelling**

Pharmacophore modelling is powerful method to rapidly identify new potential drugs. For the numerous therapeutically relevant drug targets with underdetermined active site geometrics, pharmacophore modelling provides an effective mechanism for virtual screening.

**Pharmacophore Modeling in Discovery Studio 2.0 includes**

Experimentally accurate pharmacophore model generation methods include advanced analysis tools that allow you to cluster features into interactive dendrograms Fast and reliable 3D database building and searching Top-ranked conformational generation algorithms optimized for various classes of small molecules Information-rich geometric descriptors to accelerate and add chemical diversity to queries Cutting-edge methods for fragment-based design and structure-based pharmacophore generation.

**Protein Modelling and Sequence Analysis**

Protein Modelling on the other hand enables access to sensible structural models within a matter of hours or even minutes. Protein Modelling and Sequence Analysis solutions in Discovery Studio provide the necessary set of tools for the construction of molecular structures, as well as macromolecular docking.

**Protein Modelling and Sequence Analysis in Discovery Studio 2.0 includes**

Access to BLAST and PSI-BLAST algorithms to search for homolog using sequence similarity. Automated CDR identification and annotation for antibody modelling. Renowned algorithms for the generation of high quality homology models %. Advanced methods to optimize and validate protein models. The fastest and most accurate algorithms, ZDOCK and RDOCK, for protein-protein docking Macromolecular pK prediction based on the G-Born model for charge distribution

**QSAR**

With appropriate molecular descriptors, the large quantity of relatively easily available data inherent in chemical libraries can be mined, analyzed and used to select compounds that can become drug leads.

**QSAR in Discovery Studio 2.0 includes:**

Extensive set of proven descriptors to effectively capture critical properties.
Advanced modelling tools for easy analysis of complex data.
Powerful, customizable, and easily accessible SAR tools.
Methods to easily design targeted chemical libraries

**Simulations**

Computational simulation of bio-molecular systems helps in the understanding of these processes by providing a visual representation of the molecular geometries, spatial alignments, and energetics that contribute to molecular interactions.

**Simulations in Discovery Studio 2.0 includes**

Fast and accurate protein ionization and pK estimation
Entropy estimation for accurate MM-PBSA / MM-GBSA scoring
CHARMm based methods for docking and scoring
Realistic, rational flexible docking
A complete simulation package for macromolecules
Industry-standard force fields

**Structure-Based Design**

Structure Based Design is a powerful method for rapidly identifying new lead compounds when a receptor structure is available.

**Structure-Based Design in Discovery Studio 2.0 includes:**

A rational approach to flexible docking
Fast and accurate protein ionization and pK Estimation
Docking tools optimized for vHTS applications
Industry-validated de novo ligand generation and optimization
A comprehensive collection of ligand scoring tools

**Visualization**

Molecular visualization is a key aspect of data analysis that can provide understanding about the implications of molecule's structure on certain interactions and biochemical reactions.

**Visualization in Discovery Studio 2.0 includes:**

Multiple perspectives to let you simultaneously view 3D structures; hierarchy of groups; and sequences of peptides, proteins and nucleic acids.Support for a multitude of file formats for 3D structures, SMILES, sequences and graphics.Convenient tools for structure building, editing and analyzing.

**Methods**

Using Protein Information Resource, the information of the protein Beta Tubulin, which is the principle component of the microtubular system of the Filarial nematodes, has been retrieved.

It was known that there are five isoforms of beta tubulin in the filarial worms. From the review articles and journals the drugs that acts on the tubulin protein was found to be the benzimidazole carbamates, a class of microtubule depolymerising drugs. These drugs were known to act by disrupting the assembly of tubulin dimers into microtubules. Mebendazole, Thiabendazole, Albendazole were some of the examples of this class of drugs. Using Drug Bank, the molecular information and the SMILES notation of Mebendazole, Thiabendazole, Albendazole was retrieved and the analogs of Mebendazole, Thiabendazole, Albendazole and their structure data has been retrieved from NCI Enhanced Database Browser. The drug card showing the information of Thiabendazole is shown in figure 1.

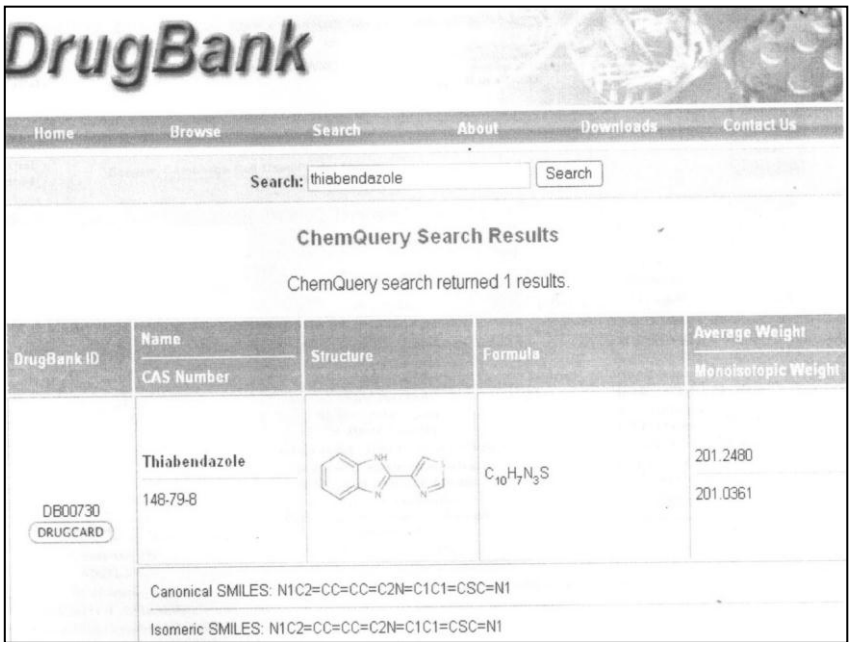



**Fig-1: Drug Bank Card Showing the Information of Thiabendazole**

The structure and properties of Ethyl-7-Amino-4-Hydroxy [1, 8] Naphthyridine-3-Carboxylate, an analog of Mebendazole using NCI Enhanced browser is shown in figure 2.

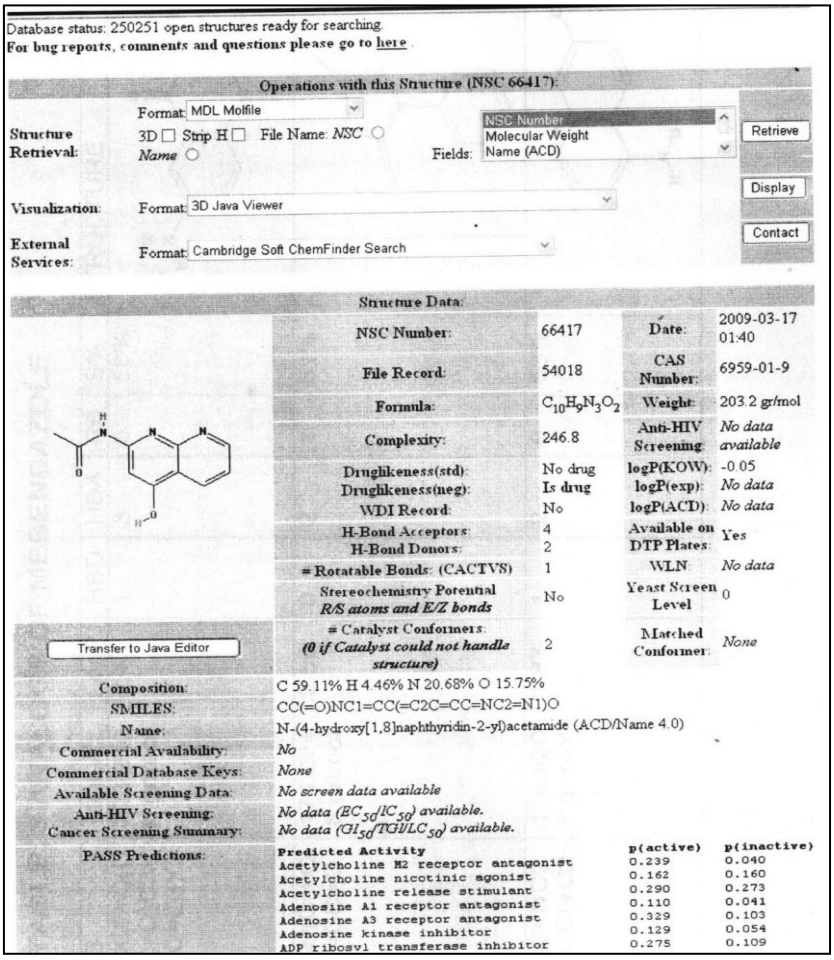



**Fig-2: NCI Enhanced Browser showing the structure and properties of Ethyl-7-Amino-4 Hydroxy [1,8] Naphthyridine-3-Carboxylate-An analog of Mebendazole.**

The names, formula, SMILES, Mol Wt, Log P value, Hydrogen bond Donor (HBD),Hydrogen Bond Accepter (HBA), Receptor Binding (RB), percentage of sites being binded (S%), structures of the three analogs each of Mebendazole, Thiabendazole, Albendazole are shown in the tables 3,4,5 respectively.

**Table-3: Analogs of Mebendazole**

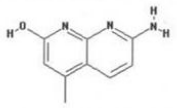
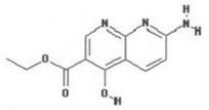
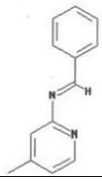
| NAME | FORMULA | SMILES | MOL Wt | Log P | HBD | HBA | RB | S% | STRUCTURE |
|---|---|---|---|---|---|---|---|---|---|
| 7-amino-4-methyl[1,8]naphthyridin-2-ol | $C_9H_9N_3O$ | CC1=C2C=CC(=NC2=NC(=C1)O)N | 175.1896 gr/mol | 1.66 | 2 | 4 | 0 | 56% |  |
| **ethyl 7-amino-4-hydroxy[1,8]naphthyridine-3-carboxylate** | **$C_{11}H_{11}N_3O_3$** | **CCOC(=O)C1=CN=C2N=C(N)C=CC2=C1O** | **233.2262 gr/mol** | **1.94** | **2** | **6** | **3** | **57%** |  |
| N-benzylidene-4-methyl-2-pyridinamine | $C_{13}H_{12}N_2$ | CC1=CC(=NC=C1)N=CC2=CC=CC=C2 | 196.2512 gr/mol | 2.56 | 0 | 2 | 2 | 57% |  |

**Table-4: Analogs of Thiabendazole**

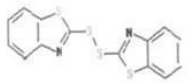
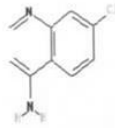
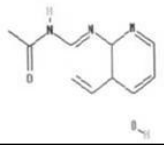
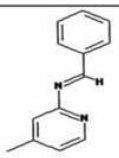
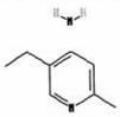
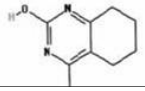
| NAME | FORMULA | SMILES | MOL Wt | Log P | HBD | HBA | RB | S% | STRUCTURE |
|---|---|---|---|---|---|---|---|---|---|
| 2-(1,3-benzothiazol-2-yldithio)-1,3-benzothiazole | $C_{14}H_8N_2S_4$ | S(SC1=NC2=CC=CC=C2S1)C3=NC4=C(S3)C=CC=C4 | 332.470 gr/mol | 2.08 | 0 | 2 | 3 | 72% |  |
| 7-chloro-4-quinolinamine | $C_9H_7ClN_2$ | NC1=CC=NC2=C1C=CC(=C2)Cl | 178.620gr/mol | 1.87 | 1 | 2 | 0 | 56% |  |
| N-(5-hydroxy[1,8]naphthyridin-2-yl)acetamide | $C_{10}H_9N_3O_2$ | CC(=O)NC1=NC2=C(C=C1)C(=CC=N2)O | 203.2 gr/mol | 0.30 | 2 | 4 | 1 | 53% |  |

**Table- 5: Analogs of Albendazole**

| NAME | FORMULA | SMILES | MOL Wt | Log P | HBD | HBA | RB | S% | STRUCTURE |
|---|---|---|---|---|---|---|---|---|---|
| N-benzylidene-4-methyl-2-pyridinamine | $C_{13}H_{12}N_2$ | CC1=CC(=NC=C1)N=CC2=CC=CC=C2 | 196.2512 gr/mol | 2.56 | 0 | 2 | 2 | 55% |  |
| **5-ethyl-2-methyl-4-pyridinamine** | **$C_8H_{12}N_2$** | **CCC1=C(N)C=C(C)N=C1** | **136.1962 gr/mol** | **1.47** | **1** | **2** | **1** | **52%** |  |
| 4-methyl-5,6,7,8-tetrahydro-2-quinazolinol | $C_9H_{12}N_2O$ | CC1=C2CCCCC2=NC(=N1)O | 164.2066 gr/mol | 2.72 | 1 | 3 | 0 | 56% |  |

The analogs were further analyzed for drug like properties based on the Lipinski's Rule, and those passing these rules were sorted out. The rules are as follows:

1. Number of H-bond donor < 5
2. Number of H-bond Acceptor < 10
3. Molecular weight < 500
4. Log P value <5

Using Discovery Studio Visualisation tool, taking tubulin protein as receptor and the sorted analogs as ligands, the Receptor-Ligand Docking was performed. The docking results were analyzed.The one best analog in each of the three drugs was selected. They were namely-

- Ethyl 7- Amino -4- Hydroxy [1,8] Naphthyridine-3-Carboxylate
- N-(5 Hydroxy [1,8 ] Naphthyridine -2-YL) Acetamide
- 5-Ethyl -2-Methyl -4 - Pyridinamine

Then the diverse conformation and the Common feature pharmacophore was generated for these three drugs and the reports were analyzed and the best is suggested based on its dock score.The research protocol was approved by the Institutional Ethical Committee

## RESULTS AND DISCUSSIONS

The results of this study, reveals that Tubulin protein was the principle component of the filarial nematode microtubular system (Table 2). By considering Tubulin as the drug target, the benzimidazole carbamates, a microtubule depolymerising drugs namely Mebendazole, Thiabendazole, Albendazole were found by using Drug bank. The analogs of these drugs were collected by using NCI Enhanced Browser. These analogs were tested for the drug like properties by following Lipinski's Rule and those passed were sorted out.

Using Discovery Studio Visualisation tool, taking tubulin protein as receptor and the sorted analogs as ligands, the Receptor-Ligand Docking was performed (figure 3).

The docking of Ethyl 7- Amino-4- Hydroxy [1, 8] Naphthyridine-3-Carboxylate was shown in the figure-4.

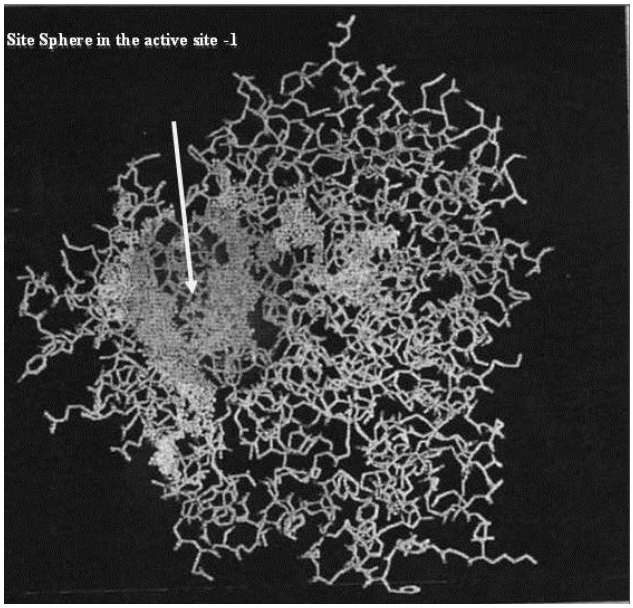



**Fig-3: Discovery Studio Visualization showing the site sphere in the active site-1 of Tubulin protein.**

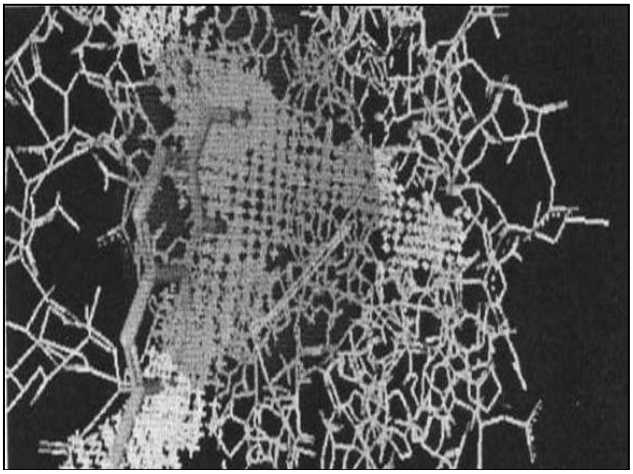


**Fig-4: The docking of Tubulin protein with the Ligand Ethyl 7- Amino-4- Hydroxy [1,8] Naphthyridine-3-Carboxylate.**

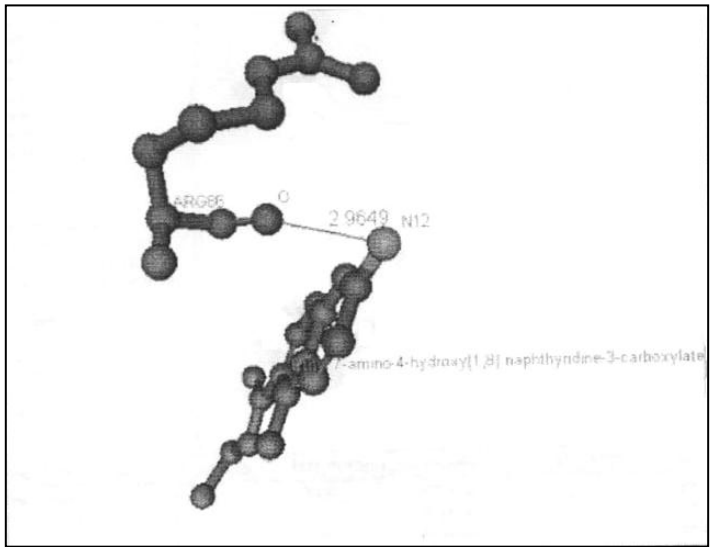



**Fig-5: Hydrogen bond monitor of Tubulin protein and the ligand Ethyl 7- Amino-4- Hydroxy [1, 8] Naphthyridine-3-Carboxylate**

The hydrogen bond monitor of tubulin protein and the ligand Ethyl-7-Amino-4-Hydroxy [1, 8] Naphthyridine-3-Carboxylate is shown in the figure 5.

Results of the diverse conformation and common feature Pharmacophore of the Ethyl-7-Amino-4-Hydroxy [1, 8] Naphthyridine-3-Carboxylate are analyzed (figure 6,7).

| | Structure | Name | Index | AbsoluteEnergy | ConfNumber | RelativeEnergy | MolNumber |
|---|---|---|---|---|---|---|---|
| 1 | | Molecule-1 | 1 | 46.483 | 1 | 0 | 1 |
| 2 | | Molecule-1 | 2 | 48.327 | 2 | 1.844 | 1 |
| 3 | | Molecule-1 | 3 | 50.399 | 3 | 3.916 | 1 |
| 4 | | Molecule-1 | 4 | 49.573 | 4 | 3.09 | 1 |
| 5 | | Molecule-1 | 5 | 51.935 | 5 | 5.452 | 1 |
| 6 | | Molecule-1 | 6 | 51.769 | 6 | 5.286 | 1 |
| 7 | | Molecule-1 | 7 | 51.93 | 7 | 5.446 | 1 |
| 8 | | Molecule-1 | 8 | 56.095 | 8 | 9.612 | 1 |
| 9 | | Molecule-1 | 9 | 54.875 | 9 | 8.392 | 1 |
| 10 | | Molecule-1 | 10 | 57.543 | 10 | 11.06 | 1 |
| 11 | | Molecule-1 | 11 | 57.884 | 11 | 11.401 | 1 |
| 12 | | Molecule-1 | 12 | 58.687 | 12 | 12.204 | 1 |
| 13 | | Molecule-1 | 13 | 59.913 | 13 | 13.43 | 1 |

**Fig-6: Result Page for diverse Confirmation of the Ethyl 7- Amino-4- Hydroxy [1, 8] Naphthyridine-3-Carboxylate**

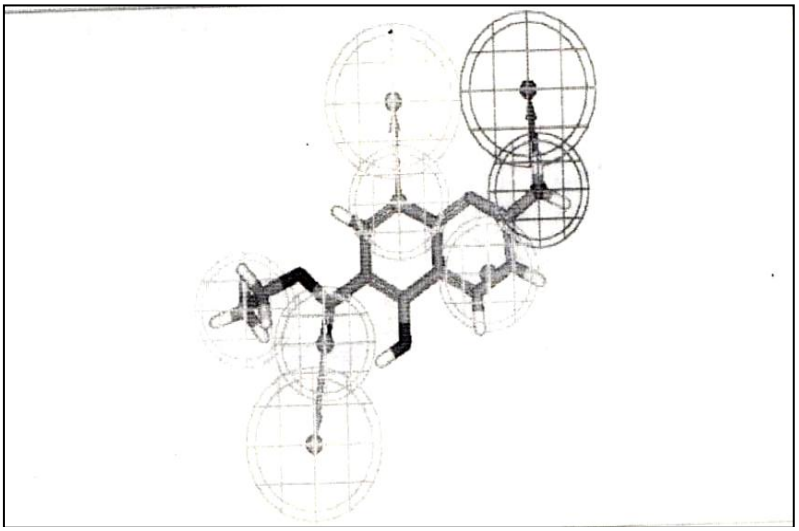


**Fig-7: Result Page of Common feature Pharmacophore of Ethyl 7- Amino-4- Hydroxy [1, 8] Naphthyridine-3-Carboxylate**

The one best analog in each of the three drugs was selected. They were namely- Ethyl 7- Amino -4- Hydroxy [1, 8] Naphthyridine-3-Carboxylate, N-(5 Hydroxy [1,8 ] Naphthyridine -2-YL) Acetamide, 5-Ethyl -2-Methyl -4 – Pyridinamine.

Then the diverse confirmation and the Common feature pharmacophore was generated for these three drugs and the reports were analyzed and the best is suggested based on its dock score (table 6) which was found to be Ethyl 7- Amino -4- Hydroxy [1, 8] Naphthyridine-3-Carboxylate in the binding site 5 of the Tubulin protein present in the Filarial nematodes.

**Table-6: Dock score results**

| SL.NO. | NAME OF THE DRUG | DOCK SCORE | DOCK SITE | POSES | DIVERSE CONFORMATION | COMMON FEATURE PHARMACOPHORE |
|---|---|---|---|---|---|---|
| 1. | Ethyl 7-amino-4-hydroxy[1,8]naphthyridine-3-carboxylate | 48.963 | $5^{th}$ /11 | 10 | 13 | 10 |
| 2. | N-(5-Hydroxy[1,8]naphthyridin-2-yl) acetamide | 45.871 | $5^{th}$ /11 | 10 | 2 | 10 |
| 3. | 5-Ethyl-2-methyl-4-pyridinamine | 28.464 | $11^{th}$ /11 | 10 | 3 | 3 |

## CONCLUSION

Tubulin protein is the principle component of the filarial nematode microtubular system, which was likely to be the target, the analogs of three drugs were chosen to dock with the Tubulin protein. Among them "Ethyl 7-Amino-4-Hydroxy [1, 8] Naphthyridine-3-Carboxylate" was found to be the best drug based on its docking score and so it can be suggested as the novel drug for an effective treatment against Filaria (Brugia malayi).

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## REFERENCES

1. Lacey, E. (1988). The role of the cytoskeletal protein, tubulin, in the mode of action and mechanism of drug resistance to benzimidazoles. *International journal for parasitology*, *18*(7), 885-936.
2. Monteiro, M. J., & Cox, R. A. (1987). Primary structure of an α-tubulin gene of Physarum polycephalum. *Journal of molecular biology*, *193*(3), 427-438.
3. Driscoll, M., Dean, E., Reilly, E., Bergholz, E., & Chalfie, M. (1989). Genetic and molecular analysis of a Caenorhabditis elegans beta-tubulin that conveys benzimidazole sensitivity. *The Journal of cell biology*, *109*(6), 2993-3003.
4. Tang, L., & Prichard, R. K. (1989). Characterization of tubulin from Brugia malayi and

Brugia pahangi. *Molecular and biochemical parasitology*, *32*(2-3), 145-152.

5. Lacey, E., & Prichard, R. K. (1986). Interactions of benzimidazoles (BZ) with tubulin from BZ-sensitive and BZ-resistant isolates of Haemonchus contortus. *Molecular and biochemical parasitology*, *19*(2), 171-181.
6. Taylor, M. J., & Hoerauf, A. (2001). A new approach to the treatment of filariasis. *Current opinion in infectious diseases*, *14*(6), 727-731.
7. Gupta, S., & Srivastava, A. K. (2005). Biochemical targets in filarial worms for selective antifilarial drug design. *Acta Parasitologica*, *50*(1), 1-18.
8. Helm, R., Selkirk, M. E., Bradley, J. E., Burns, R. G., Hamilton, A. J., Croft, S., & Maizels, R. M. (1989). Localization and immunogenicity of tubulin in the filarial nematodes Brugia malayi and B. pahangi. *Parasite immunology*, *11*(5), 479-502.
9. Udall, D. N. (2007). Recent updates on onchocerciasis: diagnosis and treatment. *Clinical infectious diseases*, *44*(1), 53-60.
10. Taylor, M. J., Cross, H. F., Ford, L., Makunde, W. H., Prasad, G. B. K. S., & Bilo, K. (2001). Wolbachia bacteria in filarial immunity and disease. *Parasite immunology*, *23*(7), 401-409.
11. Franz, M., Zahner, H., & Benten, P. (1990). Fine-structure alterations in femaleBrugia malayi andLitomosoides carinii after in vivo treatment with flubendazole. *Parasitology research*, *76*(5), 401-405.
12. Taylor, M. J., Cross, H. F., & Bilo, K. (2000). Inflammatory responses induced by the filarial nematode Brugia malayi are mediated by lipopolysaccharide-like activity from endosymbiotic Wolbachia bacteria. *Journal of Experimental Medicine*, *191*(8), 1429-1436.
13. Hermans, P. G., Hart, C. A., & Trees, A. J. (2001). In vitro activity of antimicrobial agents against the endosymbiont Wolbachia pipientis. *Journal of Antimicrobial Chemotherapy*, *47*(5), 659-663.
14. Gyapong, J. O., Kumaraswami, V., Biswas, G., & Ottesen, E. A. (2005). Treatment strategies underpinning the global programme to eliminate lymphatic filariasis. *Expert opinion on pharmacotherapy*, *6*(2), 179-200.
15. Navaratnam, V. (1993). Recent advances in the chemotherapy of lymphatic filariasis. *The Southeast Asian journal of tropical medicine and public health*, *24*, 51-54.
16. Ottesen, E. A., Ismail, M. M., & Horton, J. (1999). The role of albendazole in programmes to eliminate lymphatic filariasis. *Parasitology today*, *15*(9), 382-386.
17. Bordenstein, S., & Rosengaus, R. B. (2005). Discovery of a novel Wolbachia supergroup in Isoptera. *Current microbiology*, *51*(6), 393-398.
18. Shakya, S., Bajpai, P., Sharma, S., & Misra-Bhattacharya, S. (2008). Prior killing of intracellular bacteria Wolbachia reduces inflammatory reactions and improves antifilarial efficacy of diethylcarbamazine in rodent model of Brugia malayi. *Parasitology research*, *102*(5), 963-972.
19. Taylor, M. J. (2002). Wolbachia endosymbiotic bacteria of filarial nematodes. A new insight into disease pathogenesis and control. *Archives of medical research*, *33*(4), 422-424.
20. Williams, S. A., Laney, S. J., Lizotte-Waniewski, M., Bierwert, L. A., & Unnasch, T. R. (2002). The river blindness genome project. *TRENDS in Parasitology*, *18*(2), 86-90.