In silico Virtual Screening for Novel Ligands Against ATP-Phosphoribosyltransferase of *Mycobacterium paratuberculosis*

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Abstract

Mycobacterium paratuberculosis, a multi-host mycobacterial pathogen has been proved to cause chronic inflammation of digestive system called Crohn's disease in human. The bacterium that is the major cause of John's disease in cattle is transmitted to the human through the cattle food and contaminated water. Crohn's disease also increases the risk of cancer in the area of inflammation. To date, treatment involves microcline antibodies. A combination of antibiotics such as Rifabutin and a macrolide such as Clarithromycin. Treatment regimes can last years. Modeling of the 3D structure of ATP-PRTase (P60805) and predicting its binding site and hence docking with commercially available anti-Map drugs and antibiotics that are used to cure Crohn's disease. Modeling and Drug Docking for protein ATP-Phosphoribosyltransferase (ATP-PRTase) in the Mycobacterium ATP-Phosphoribosyltransferase is performed using Accelry's DISCOVERY STUDIO 2.1. Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. The ADMET and Pharmacophore properties of the docked drugs are studied. The project is extended to discover the chemical compounds that are analogous of anti-Map drugs that dock with the protein. Aim of the project is to find the chemical compounds that are non-toxic, non-carcinogenic and more efficient in inactivating the protein than the commercially available drugs.

Key words: Clarithromycin, ATP-Phosphoribosyltransferase, Phosphoribosyltransferase

Introduction

Bioinformatics is the field of science in which biology, computer science and information technology merges to form a single discipline. It focuses more on hypothesis testing and discovery in the biological domain. The tasks used in bioinformatics range from the creation and maintenance of databases of biological information to the analysis of sequence information. The wide range of application of bioinformatics include molecular medicine, gene therapy, drug development, waste cleanup, forensic analysis of microbes, evolutionary studies, comparative studies etc.

Molecular modeling is a collective term that refers to theoretical methods and computational techniques to model or mimic the behavior of molecules. In bioinformatics the three dimensional architecture of the biological molecules is interpreted, visually represented, manipulated in order to determine their molecular properties. The prediction of the molecule's three-dimensional shape can be estimated from sequence data of previously identified molecular shapes. The benefit of molecular modeling is that it reduces the complexity of the system, allowing many more particles (atoms) to be considered during simulations. The types of biological activity that have been investigated using molecular modeling include protein folding, enzyme catalysis, protein stability, conformational changes associated with bimolecular function, and molecular recognition of proteins, DNA , and membrane complexes. Popular software for molecular modeling are Millsian, BALL View, Cerius2, InsightII, Sybyl, MOE, Ghemical, MMTK, Molsoft ICM, PyMOL, VMD, SPARTAN etc.

In the field of molecular modeling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. The aim of molecular docking is to achieve an optimized conformation for both the protein and ligand and relative orientation between protein and ligand such that the free energy of the overall system is minimized.

ADMET stands for administration, distribution, metabolism, excretion and toxicity. It is not sufficient for a drug molecule to bind tightly to its biological target in an invitro assay. It must also be able to reach its site of action in vitro. The molecule must remain in the body for an appropriate period of time to give the desired effect but must also be ultimately removed from the body by metabolism, excretion or other pathways. Moreover, neither the drug nor its metabolism must be toxic. The acronym ADMET is often used to refer to such aspects of drug discovery. The prediction of absorption, distribution, metabolism, and excretion

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(ADME) properties has become increasingly important as failures late in the drug discovery process become more costly. Rather than providing "the correct answer", modeling provides a means of "stacking the deck" in favor of the medicinal chemistry effort, increasing the likelihood that a given compound will show the desired effect *in vitro* or *in vivo.*

Comparative sequence analysis reveals several features of the K-10 genome including: a relative paucity of the PE/PPE family of sequences that are implicated as virulence factors and known to be immunostimulatory during Mtb infection; truncation in the EntE domain of a salicyl-AMP ligases (MbtA), the first gene in the mycobactin biosynthesis gene cluster, providing a possible explanation for mycobactin dependence of Map; and Map-specific sequences that are likely to serve as potential targets for sensitive and specific molecular and immunologic diagnostic tests.(Li L, Bannantine JP, *et al.* 2005). Presence of *Mycobacterium paratuberculosis* DNA in Crohn's disease tissue. The presence of it in two-third of Crohn's disease tissues but in less than 5% of ulcerative colitis tissues is consistent with an etiological role for *M.paratuberculosis* in crohn's disease.(Sanderson JD, Moss MT, *et al.* 1992) MAP in Crohn's disease is present in a protease-resistant nonbacillary form, can evade immune recognition and probably causes an immune dysregulation. The problems caused by MAP constitute a public health issue of tragic proportions for which a range of remedial measures are urgently needed. (Hermon-Taylor J, Bull TJ, *et al.* 2000) In situ identification of mycobacteria in Crohn's disease patient tissue using Confocal Scanning Laser Microscopy (CSLM). In situ hybridization performed on full thickness tissue using rabbit anti-MAP polyclonal antibody that was absorbed with *E.coli* protein extracts detected in the microvillus region in tissue specimen from CD patients. (Naser SA, Shafran I, *et al.* 2002)

M. avium subsp paratuberculosis, adherent-invasive *E. coli* and Candida are good candidates for an infectious etiology of Crohn's disease on the basis of genetic susceptibility, which relates to impaired function in the defence against intracellular bacteria. (Pineton de Chambrun G, Colombel JF, *et al.* 2008). Considerable evidence supports the presence of *M. paratuberculosis* in the intestinal tissues of many patients with Crohn's disease including culture, detection of homologous mycobacterial DNA, detection of the mycobacterial insertion sequence IS900 by both PCR and in situ hybridization in tissues, and a serologic immune response to recombinant *M. paratuberculosis* antigens. (El-Zaatari FA, *et al.* 2002) Presence of Ziehl Neelsen positive MAP in the stool of attendants working with MAP-infected animals was unique to humans. ELISA based on antigens derived from indigenous MAP 'bison type' genotype of goat origin was most sensitive modality for screening Crohn's disease patients. (Singh AV, Singh SV, *et al.* 2008)The epidemiological models of disease causation, the major philosophical doctrines about causation, the established epidemiological criteria for causation, and the currently known epidemiological shows evidence of *M. avium* sub sp. paratuberculosis as a possible cause of Crohn's disease. (Uzoigwe JC, Khaitsa ML, *et al.*)

Investigations emphasize the role of contaminated food and water in human infection around the world and determine the possible zoonotic role of M. avium subsp. paratuberculosis. (Cirone K, Morsella C, *et al.* 2007 Jan) Presence of Mycobacterium avium subspecies paratuberculosis in locally and commercially pasteurized cow's milk in the Czech Republic shows that humans are being exposed to this chronic enteric pathogen by this route. (Ayele WY, Svastova P, *et al.* 2005) Members of the homologous PRT family are catalytic and regulatory proteins involved in nucleotide synthesis and salvage. New crystal structures have revealed key elements of PRT protein function, as well as glimpses of how the fold has evolved to perform both catalytic and regulatory functions. (Sinha SC, Smith JL. 2001) Multivariate analysis showed that meat consumption is significantly increased risk of Crohn's disease. (Abubakar I, Myhill DJ, *et al.* 2007) The unique genome sequence incorporated into an antigen discovery project may allow reliable detection of the bacterium in antigen-based diagnostic tests for addressing food borne issues of *M.paratuberculosis*. (Bannantine JP, Barletta RG, *et al.* 2004 Spring) Diagnostic tests for paratuberculosis are also used in Crohn's disease, a chronic human ileitis mimicking Johne's disease, in which isolates identified as *M. paratuberculosis* have been found. (Cocito C, Gilot P, *et al.* 1994) Polymerase chain reaction was used to detect the presence of IS900 DNA sequence specific to *M.paratuberculosis* genomes in biopsies and resection from children's appears to support the hypothesis that *M.paratuberculosis* is involved in the pathogenesis of Crohn's disease. (Dell'Isola B, Poyart C, *et al.*1994) Growing evidence suggests that prolonged antimycobacterial combination therapy can improve Crohn's disease in some patients. (Borody TJ, Leis S, *et al.* 2002).In Sep 1988, *Mycobacterium paratuberculosis* cervical lymphadenitis, followed five years later by terminal ileitis similar to Crohn's disease resolved on prolonged treatment with a combination of rifabutin and clarithromycin, leaving a healed ileal scar which required excision. (John Hermon-Taylor, *et al* 1998). *M. paratuberculosis* may survive high-temperature, short-time pasteurization when the initial organism concentration is greater than 101 cells/ml. (Nackmoon Sung and Michael T. Collins 15, 1997).

Materials and methods

Mycobacterium paratuberculosis is a gram-positive, rod prokaryote (dividing) organism Phenotyically it grows much slowly, requires an iron-transport chemical known as mycobactin for *in vitro* growth, forms rough colonies on solid agar media, and infects mammals instead of birds. MAP can grow only inside animal cells where it assimilates iron from its host's cells, most often immune cells called *macrophages. Paratuberculosis* has the capacity to thrive inside macrophages

Mycobacterium paratuberculosis occurs in two forms,. the bacillary form and the spheroplast form. Many paratuberculosis bacteria of the bacillary form may be required to cause clinical disease. In contrast, only a few paratuberculosis bacteria of the spheroplast form will cause disease. This difference between diseases caused by the two forms of paratuberculosis is a result of the infected host's immune reaction. *M.paratuberculosis* has been recognized in many species of animals, in both its bacillary and spheroplast forms. The bacillary form is easily detected in animals, since it grows in large numbers and is identifiable by a simple chemical test. In contrast, the spheroplast form was not detected until the advent of modern genetic testing techniques.

MAP is recognized as a multi-host mycobacterial pathogen with a proven specific ability to initiate and maintain systemic infection and chronic inflammation of the intestine of a range of histopathological types in many animal species including primates. Map causes Johne's disease in cattle and other ruminants, and it has long been suspected as a

causative agent in Crohn's disease in humans; this connection is controversial. Recent studies have shown that Map present in milk can survive pasteurization, which has raised human health concerns due to the widespread nature of Map in modern dairy herds. Map is heat resistant and it is capable of sequestering itself inside white blood cells, which may contribute to its persistence in milk. It has also been reported to survive chlorination in municipal water supplies. Even though Map is hardy, it is slow growing and fastidious, which means it is difficult to culture. Map, like most mycobacteria, is difficult to treat. It is not susceptible to anti-tuberculosis drugs (which can generally kill Mycobacterium tuberculosis), but can only be treated with a combination of antibiotics such as Rifabutin and a macrolide such as Clarithromycin. Treatment regimes can last years.

rohn's disease is an inflammatory disease of the digestive system which may affect any part of the gastrointestinal tract from mouth to anus. As a result, the symptoms of Crohn's disease can vary significantly among afflicted individuals. Crohn's disease is classified as an inflammatory bowel disease. The disease has also been called regional ileitis or regional enteritis and also granulomatous colitis There is no known drug based or surgical cure for Crohn's disease. Treatment options are restricted to controlling symptoms, putting and keeping the disease in remission and preventing relapse.

A common recurrent theory is that a specific species of Mycobacterium, Mycobacterium avium subspecies paratuberculosis is responsible for Crohn's disease, and modern industrial farming practices have led to the spread of Mycobacterium avium subspecies paatuberculosis. Crohn's disease is caused by a combination of environmental and genetic factors. Many environmental factors have also been hypothesized as causes or risk factors. Proven environmental risk factors include living in an industrialized country, smoking, and living in an urban area. Diets high in sweet, fatty or refined foods may also play a role. Smoking has been shown to increase the risk of the return of active disease, or "flares". Abnormalities in the immune system have often been invoked as being causes of Crohn's disease.

Map in Food like Meat, milk and other products from animals infected with Map are being continually entering the human food chain. There is a wealth of evidence which appears to indicate that Map is capable of surviving the food processing methods that we employ to protect us from disease, such as cooking and pasteurization.

Map in Water is the another possible route of transmission of Map from cattle to humans is via contaminated water supplies. Map is shed onto pastures and will be washed off into ground and river waters. Where such water is piped to households for human consumption, it may enable Map to infect people by this route as well. While it is possible for individuals to eliminate milk/dairy/beef products from their diet in an attempt to limit Map exposure, it appears to be nearly impossible to avoid drinking Map contaminated water from public water supplies. It is equally as difficult to avoid foods that have potentially been infected with Map via irrigation methods using infected water.

Gastrointestinal Symptoms: Abdominal pain, diarrhea may be the initial symptom of Crohn's disease. Visible bleeding in the feces is less common in Crohn's disease. Flatulence and bloating may also add to the intestinal discomfort. Perianal discomfort may also be prominent in Crohn's disease.

Systemic Symptoms: Among children, growth failure is common. Among older individuals, Crohn's disease may manifest as weight loss.

Extra intestinal Symptoms:

Inflammation of one or more joints i.e. arthritis or muscle insertions i.e. enthesitis.

Skin manifestation like erythema nodosum, presents as red nodules usually appearing on the skins.

Increases the risk of blood clots. Painful swelling of the lower legs can be a sign of deep venous thrombosis.Crohn's disease can also cause seizures, strokes, myopathy, peripheral neuropathy, headache and depression.

Crohn's disease also increases the risk of cancer in the area of inflammation. Crohn's disease can lead to several mechanical complications within the intestines, including obstruction; fistulae and abscesses.Individuals are at risk of malnutrition, problematic pregnancy.etc.

Diagnosis and Treatment

Immune reaction: By taking a sample of the patient's blood, and testing it for the presence of antibodies and/or other immune reactions to MAP in blood or tissue. Tissue Sample: By taking a tissue sample, by biopsy, from the patient and testing it for the presence of MAP.Fecal sample: By taking a sample of the patient's feces and testing it for the presence of MAP.To date, treatment involves macrolide antibodies. A combination of antibiotics such as Rifabutin and a macrolide such as Clarithromycin. Treatment regimes can last years.

Software used (Discovery Studio 2.1)

Discovery Studio was developed by Accelrys Company Inc. Accelrys provide targeted scientific software product throughout the drug discovery and drug development process, along with platform technologies that let you integrate best-of-breed solutions an that aid the flow of data information and knowledge through the process. Discovery Studio is a single unified, easy to use, graphical interface for powerful drug design and protein modeling research.

BLAST Search (NCBI server)

Searches protein database at the NCBI with a query sequence using BLAST. It is a statistically driven search method that finds region of similarity between a query sequence and database sequences and produces gapped or ungapped alignment of these regions.

Databases Used

The Protein Information Resource (PIR), located at Georgetown University Medical Center (GUMC), established in 1984 by the National Biomedical Research Foundation (NBRF) serves as a resource to assit researchers in the identification and interpretation of protein sequence information.

PDB archive contains information about experimentally-determined structures of proteins, nucleic acids, and complex assemblies. The RCSB PDB also provides a variety of tools and resources. Users can perform simple and advanced searchers based on annotation relating to sequences, structure and function.

Established in 1988 as a national resource for molecular biology information, NCBI creates public databases, conducts research in computational biology, develops software tools for analyzing genome data, and disseminates biomedical information for the better understanding of molecular processes affecting human health and disease. The NCBI houses genome sequencing data in GenBank and an index of biomedical research articles in Pub Med Central and Pub Med, as well as other information relevant to biotechnology.

The Drug Bank database is a unique bioinformatics and chemo informatics 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 more than 4800 entries.

NCBI's Conserved Domain Database is a collection of multiple sequence alignments for ancient domains and full-length proteins. The CD-Search services may be used to identify the conserved domains present in a protein query sequence. KEGG PATHWAY is a collection of manually drawn pathway maps representing our knowledge on the molecular interaction and reaction for: Metabolism, Genetic Information Processing, Environmental Information Processing, Cellular Processes, Human Diseases and Drug Development. Structural analysis and verification server (SAVS) checks the stereo chemical quality of a protein structure by analyzing residue-by-residue geometry and overall structure geometry. Determines the compatibility of an atomic model (3D) with its own amino acid sequence by assigned a structural class based on its location and environment and comparing its results to good structures.

Results and Discussions

ATP-PRTase was modeled using Accelry's Discovery Studio 2.1. The active sites were predicted and certain anti-MAP drugs were selected and attempted for STRUCTURE-BASED DRUG DOCKING with the protein. The drugs that docked, namely Amino salicylic acid, Azathioprine, Cycloserine, Ethionamide and Isoniazide were subjected to ADMET, TOPKAT and pharmacophore prediction to analyze the toxicity, solubility, absorption carcinogenicity etc. Thus Amino salicylic acid, Azathioprine, Cycloserine, Ethionamide and Isoniazide have been found to inhibit the pathogen activity , it is found to cause cause harmful effects in human by analyzing their pharmaco kinetic and pharmacodynamic properties of drugs. Thus, to discover better drugs for the inactivation of ATP-PRTase consequently safe for human, the project is extended to LIGAND–BASED DRUG DOCKING. For this, Amino salicylic acid, Ethanolamine and Isoserine were considered. Cycloserine crosses the Blood-Brian Barrier, solubility level and Azathioprine had zero hip-hop hypotheses in the pharmacophore analysis so they were not taken for the drug discovery.

The figures show the docking of the chemical compound to the specific site of the protein and their CDOCK Interaction values. Of the 23 ligands only 7 ligands docked with the receptor proteinThe figures show the ADMET Descriptor analysis of the seven compounds that have docked with the protein. All the seven compounds did not cross the Blood-Brain barrier. Hence they are safe to use..

The above table shows the ADMET analysis value for solubility, BBB, absorption, CYP2D6 and hepatotoxicity all the seven compounds. The absorption is below 6.1261 implying that they have good absorption. The hepotoxicity value must be zero to be non-toxic. It is clear that 2-methyl-2-phenylpanamide, Diethyl-2-(benzoylamino) manalate, Ethyl7-amino-4-hydroxy[1,8]naphthyridine 3-carboxylateand N-(5-hydroxyl[1,8]naphthyridine-2-yl)acetamide are non-toxic to the body. The too low values of CYP2D6 shows that all the drugs are non-inhibitors. Diethyl-2-(benzoylamino) manalate is an inhibitor. The BBB penetration values are too low so they do not cross the blood-brain barrier. The solubility values show that these chemical compounds have good and optional solubility.

TOPKAT predicts the level of toxicity and the reaction of the drug with the body. When the probability of carcinogenicity is $X < 0.3$ - non-carcinogenic, $X > 0.7$ – carcinogenic and $0.3 > x > 0.7$ – intermediate The

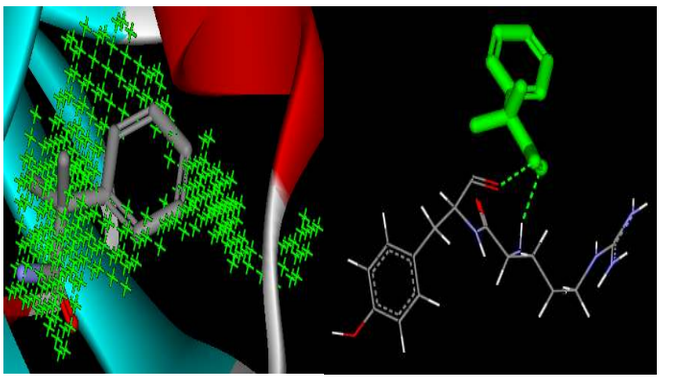

Figure 1. 2-methyl-2-phenylpropanamide docked at site 3

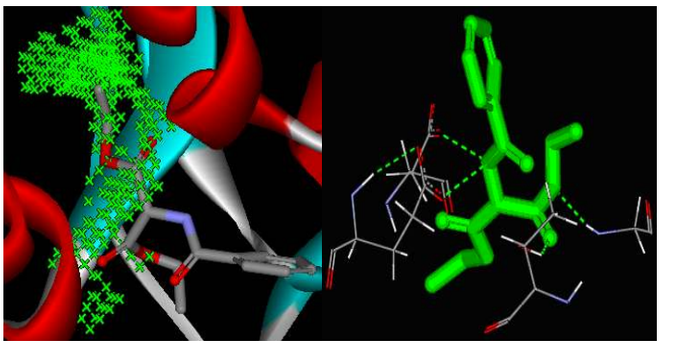

Figure 2. Diethyl 2-benzoyl amino malonate docked at site 3

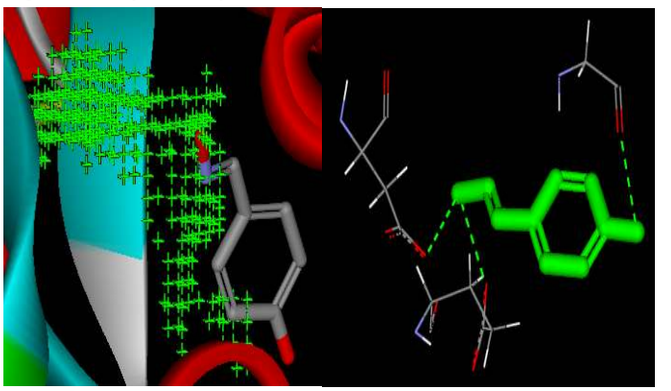

Figure.3. 4-hydroxybenzaldehyde oxime docked at site 3

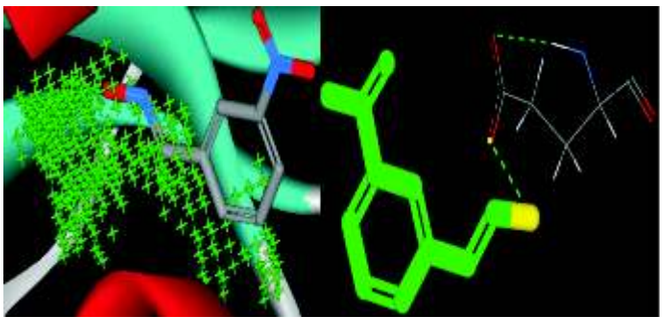

Figure 4. 3-(hydroxy (oxido) amino) benzaldehyde oxime docked site3

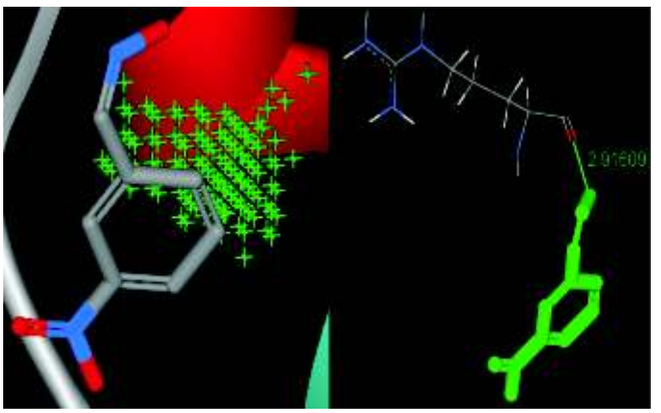

Figure.5. 3-(hydroxy (oxido) amino) benzaldehyde oxime docked at site 10

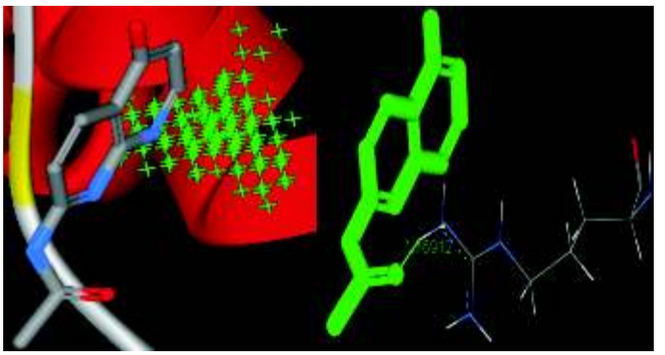

Figure.6. N-(5-hydroxy [1,8]naphthyridin-2-yl)acetamide docked at site 10

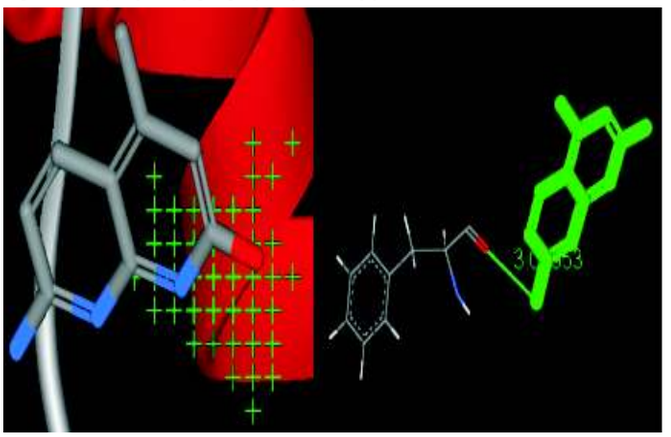

Figure.7. 7-amino-4-methyl [1, 8] naphthyridin-2-ol docked at site 10

above table shows that Diethyl-2-(benzoylamino) manalate is non-carcinogenic, N-(5-hydroxyl [1, 8] naphthyridine-2-yl) acetamide is intermediate.

S.No	CHEMICAL COMPOUND
	DOCKED AT SITE 3
1	2-methyl-2-phenylpropanamide
2	Diethyl 2-(benzoylamino)malonate
3	Ethyl 7-amino-4-hydroxy[1,8]naphthyridine-3-carboxylate
4	4-hydroxybenzaldehyde oxime
	DOCKED AT SITE 10
5	N-(5-hydroxy[1,8]naphthyridin-2-yl)acetamide
6	7-amino-4-methyl[1,8]naphthyridin-2-ol
	DOCKED AT SITE 3 & 10
7	3-(hydroxy(oxido)amino)benzaldehydeoxime

Table 1. The table shows the name of the chemical compound that docked with the protein and its structure.

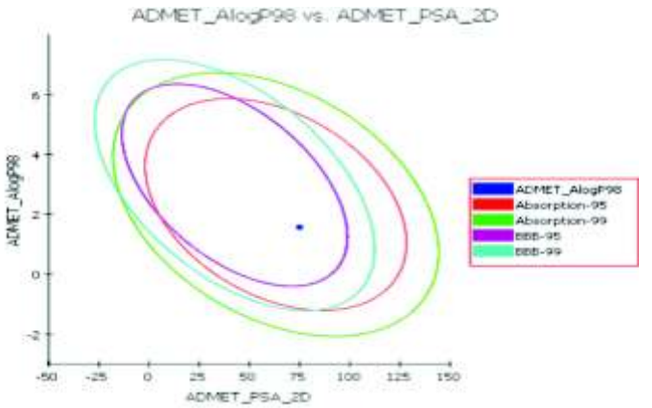

Figure.8. ADMET Descriptor of 3(hydroxy(oxido)amino)benzaldehyde oxime

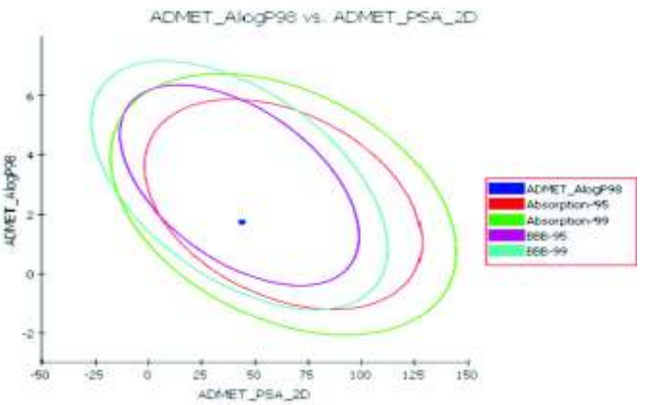

Figure.9. ADMET Descriptor of 2-methyl-2-phenylpanamide

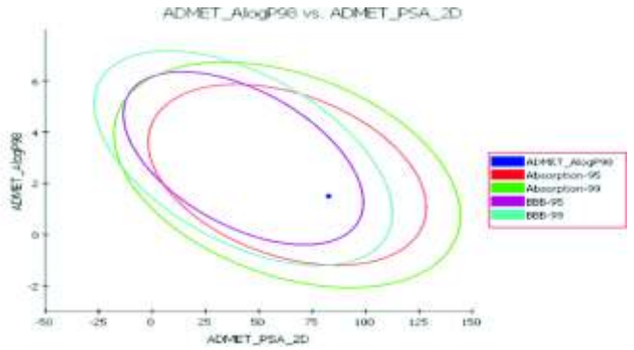

Figure.10. ADMET Descriptor of Diethyl-2-(benzoylamino)manalate

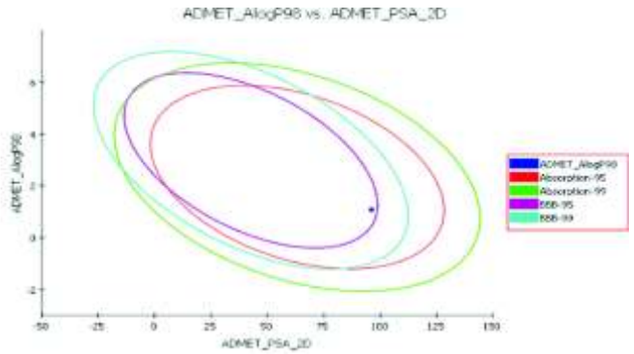

Figure.11. ADMET Descriptor of Ethyl 7-amino-4-hydroxy [1, 8] naphthyridine 3-carboxylate

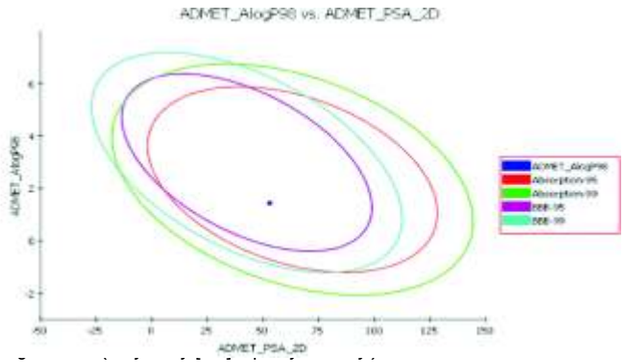

Figure 12. ADMET Descriptor of 4-hydroxybenzaldehyde oxime

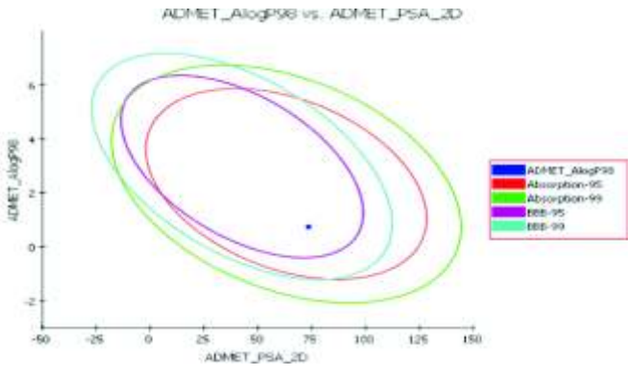

Figure13. ADMET Descriptor of N-(5-hydroxyl [1, 8] naphthyridin-2-yl) acetamide

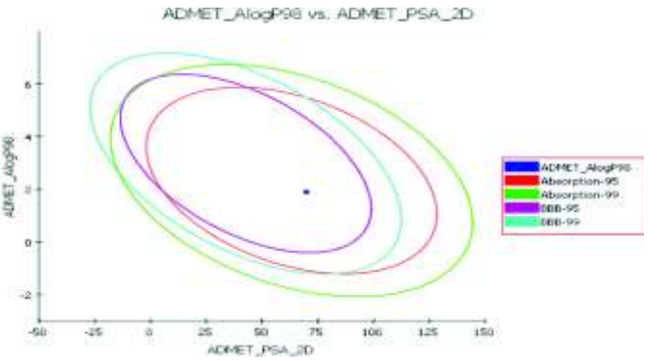

Figure 14. ADMET Descriptor of 7-amino-4-methyl [1, 8] naphthyridin-2-ol

Conclusion

Mycobacterium paratuberculosis, a multi-host mycobacterial pathogen has been proved to cause chronic inflammation of digestive system

LIGAND	BBB	ABSORPTION LEVEL
3(hydroxy(oxido)amino)benzaldehyde oxime	-0.855	0
2-methyl-2-phenylpanamide	-0.308	0
Diethyl-2-(benzoylamino)manalate	-0.997	0
Ethyl7-amino-4-hydroxy[1,8] naphthyridine 3-carboxylate	-1.342	0
4-hydroxybenzaldehyde oxime	-0.549	0
N-(5-hydroxyl[1,8]naphthyridine-2-yl) acetamide	-1.088	0
7-amino-4-methyl[1,8]napthyridine-2-ol	-0.673	0

SOLUBILITY	CYP2D6	HEPATOTOXICITY
-1.945	0	1
-2.177	0	0
-2.164	1	0
-2.379	0	0
-1.092	0	0.741
-1.715	0	0
-2.733	0	1

Table2. ADMET Descriptor Predicted Values for Docked Chemical Compounds
BBB – Blood Brain Barrier; CYP2D6 – It's a vector

called Crohn's disease in human. The bacterium that is the major cause of John's disease in cattle, is transmitted to the human through the cattle food and contaminated water.ATP-Phosphoribosyltransferase present in the MAP has been found to play an important role in the causation of the Crohn's disease. By inactivating this protein using commercially available drugs the activity of the pathogen can be reduced. In Structure-Based Drug Docking the drugs that docked with the protein ATP-PRTase are namely Aminosalicylic acid, Azathioprine, Cycloserine, Ethionamide and Isoniazide. But these drugs were found to be toxic and

carcinogenic to the humans on doing the ADMET and TOPKAT analysis. Hence Ligand-Based Drug Docking was performed. The analogs were selected based on the similarity in A:D:H ratios of the

LIGAND	PROBABILITY OF CARCINOGENICITY		
	CMR	CRF	FRC
3(hydroxy(oxido)amino)benzaldehyde oxime	0.916	1	0.183
2-methyl-2-phenylpanamide	0	0.986	1
Diethyl-2-(benzoylamino)manalate	0.015	0.002	0.04
Ethyl7-amino-4-hydroxy[1,8]naphthyridine3-carboxylate	0.85	0	0
4-hydroxybenzaldehyde oxime	0.883	0.669	-
N-(5-hydroxyl[1,8]naphthyridine-2-yl)acetamide	0.007	0	0.506
7-amino-4-methyl[1,8]napthyridine-2-ol	0.276	-	0.996

Table 2. TOPKAT Prediction for Docked Chemical Compounds
CMR – NTP Carcinogenicity Call (Male Rat), CRF – NTP Carcinogenicity Call (Female Rat), FRC – FDA Carcinogenicity Female Rat Non Vs Carc

chemical compound and the binding sites of the protein. Among the selected analogs, chemical compounds like 3(hydroxy(oxido)amino)benzaldehyde oxime, 2-methyl-2-phenylpanamide, Diethyl-2-(benzoylamino)manalate, Ethyl7-amino-4-hydroxy[1,8] naphthyridine3-carboxylate, 4-hydroxybenzaldehyde oxime, N-(5-hydroxyl[1,8]naphthyridine-2-yl)acetamide and 7-amino-4-methyl[1,8]napthyridine-2-ol docked with the protein. From the ADMET and TOPKAT analysis they are non-toxic as well as non-carcinogenic. Hence these chemical compounds are suggested for the clinical trials to inactivate ATP-PRTase.

References

Abubakar I, Myhill DJ, et al. 2007 Apr. 'A case-control study of drinking water and dairy products in Crohn's Disease--further investigation of the possible role of Mycobacterium avium paratuberculosis". *Am J Epiderminol* **165(7);** 776-81.

Ayele WJ, Svastora P, et al. 2005 Mar. 'Mycobacterium avium subspecies paratuberculosis cultured from locally and commercially pasteurized cow's milk in the Czech Republic". *Appl Environ Microbiol*, **71(3)**; 1210-4.

Balfour Sartor R. 2002 Mar. "Bacteria in Crohn's disease: mechanisms of inflammation and therapeutic implications". *J Clin Gastroentero*l **41(6);** 637.

Bannantine JP, Barletta RG, *et al.* 2004 Spring. "Application of the genome sequence to address concerns that Mycobacterium avium subspecies paratuberculosis might be a foodborne pathogen".

Borody TJ, Leis S, *et al*. 2002 Jan. "Treatment of severe Crohn's disease using antimycobacterial triple therapy--approaching a cure?" Dig Liver Dis, **34(1);** 29-38.

Bull TJ, McMinn EJ, *et al.* 2003 Jul. "Detection and verification of Mycobacterium avium subsp. paratuberculosis in fresh ileocolonic mucosal biopsy specimens from individuals with and without Crohn's disease". *J.Clin Microbiol*, **41(7);** 2915-23.

Chamberlin W, Graham DJ, *et al.* "Review article: Mycobacterium avium subsp. paratuberculosis as one cause of Crohn's disease".

Cirone K, Morsella C, *et al.* 2007 Jan. "Mycobacterium avium subsp. paratuberculosis in food and its relationship with Crohn's disease". *Rev Argent Microbiol*, **39(1);** 57-68.

Cocitol C, Gilot P, *et al*. 1994 Jul. "Paratuberculosis".*Clin Microbiol* Rev, **7(3);** 328-45.

Dell'Isola B, Poyart C, *et al.* 1994 Feb. "Detection of Mycobacterium paratuberculosis by polymerase chain reaction in children with Crohn's disease".*J Infect Dis*, **169(2);** 449-51.

El-Zaatari FA, Osato Ms, *et al.* 1988. *"Etiology of Crohn's disease*: the role of *Mycobacterium avium paratuberculosis"*.

Hermon-Taylor J, Bull TJ, *et al.* 2000 Jan. "Causation of Crohn's disease by Mycobacterium avium subspecies paratuberculosis". *Can J Gastroenterol*, **14(6);** 521-39.

John Hermonn-Taylor, *et al.* "Grand Round: Mycobacterium paratuberculosis cervical lymphadenitis, followed five years later by terminal ileitis similar to Crohn's disease".

Lil, Bannantine JP, *et al.* 2005 Aug. "The complete genome sequence of Mycobacterium avium subspecies paratuberculosis".Proc *Natl Acad Sci USA*, **102(35);** 1244-9.

Nackmoon Sung & Michael T Collins. 1998 Mar. "Thermal Tolerance of Mycobacterium paratuberculosis". *Appl environ microbial* **64(3);** 999-1005.

Naser SA, Shafran I, *et al.* 2002 Feb. "In situ identification of mycobacteria in Crohn's disease patient tissue using confocal scanning laser microscopy". *Mol Cell Probes*, **16(1);** 41-8.

Pineton de Chambrun G, colombel JF, et al. 2008 Jul. "Pathogenic agents in inflammatory bowel diseases". *Curr Opin Gasteroenterol*, **24(4);** 440-7.

Sanderson JD, Moss MT, *et al*. 1992 Jul. "Mycobacterium paratuberculosis DNA in Crohn's disease tissue". *Gut*, **33(7)**; 890-6.

Shafran I, Kugler L, *et al.* 2003 Jan. "Open clinical trial of rifabutin and clarithromycin therapy in Crohn's disease". *Dig Liver Dis* **34(1);** 22-8

Singh AV, Singh SV, *et al.* 2008 Mar. "Presence and characterization of Mycobacterium avium subspecies paratuberculosis from clinical and suspected cases of Crohn's disease and in the healthy human population in India". *Int J Infect Dis*, **12(2);** 190-7.