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DRUGS DESIGN FOR TUBERCULOSIS: A REVIEW

[1]Ranjan Kumar Dora, [2] Akankshya Purohit,[3]Udaya Nath Rath

[1]Student of Panchayat Samiti Degree Mahavidyalaya, Deogaon, [2]Lecturer In Botany Panchayat Samiti Degree Mahavidyalaya, Deogaon,[3]Lecturer in Chemistry, Panchayat Samiti Degree Mahavidyalaya, Deogaon
[1]Department Of Science,
[1]Panchayat Samiti Degree Mahavidyalaya, Deogaon, Balangir, Odisha, India

Abstract:

RmlC (dTDP-6-deoxyD-xylo-4-hexulase 3, 5 epimerase) is an enzyme. Its main component to produce L-rhamnosyl through the pathway of L-rhamnosyl biosynthetic. The RmlC enzyme is the highly essential enzyme for *Mycobacterium tuberculosis*. Which main function is cell wall biosynthesis. If it is absent in then the cell wall of *Mycobacterium tuberculosis bacterium* then the cell wall is Brust and the bacterium will be died. So, it is a valid target for drug design. In this study is an *in-silico* method study which is helps to know the Potential inhibitor for RmlC**.** RmlC enzyme**.** Present day DOTS therapy uses large numbers of drugs for addressing the Tuberculosis problem. These drugs are still a higher rate of incidence and deaths because of this disease. The Tuberculosis bacterium developed it is resistant power, it is the long treatment courses and emergence of cancer from the current medicine of this disease. So, has choose for the identification and isolation of new drugs against Tuberculosis. There are many pathways for the sake of drug inhibitors. The dTDP-6-deoxyD-xylo-4-hexulase 3, 5 epimerase (RmlC) enzyme is an important component to produce L-rhamnosyl through L-rhamnosyl biosynthetic pathway. The RmlC enzyme is one of the four enzymes in the cascade reaction. For the identification and isolation of a potential drug, five molecules were virtually screened onto the enzyme from the Naturally occurring Plant-based Anti-cancerous Compound -Activity- Target database. From this, the molecule 12- deoxy phorbol 13- (9, 10- methylene) undecanoate or NPACT00038 has been selected as a potential drug for the enzyme and may be used as a drug against tuberculosis. However, wet-lab experiment will complement the result more profoundly.

Key words: Tuberculosis, RmlC, *Mycobacterium tuberculosis*, bacterium

I. INTRODUCTION

Tuberculosis is a deadly disease worldwide. which is caused by *Mycobacterium tuberculosis*. Which is spread through the air from an infected person to a healthy person[1 12 14]. It is the second top most deadly disease in worldwide. Which is an infectious disease after COVID-19 Pandemics[1 13 12 14]. Around 1.3 million of peoples were death due to Tuberculosis, in 2017[2].All over the world several drugs are discovered to prevent the disease Tuberculosis, but the duration of treatment for the disease is very high and the *Mycobacterium tuberculosis* bacterium developed the body into Multi-Drug Resistant (MDR)[3].

In 2018, there were an estimate 10million people fell from TB[14].In 2017 there were estimated 10.4 million people been sick and ill from TB[2] . In 2021, most people WHO infected TB were in the WHO regions of South-East Asia is 45%, Africa is 23% and the Western Pacific is 18%, with smaller proportions in the Eastern Mediterranean is 8.1%, the Americas is 2.9% and Europe is 2.2%. The 30 high TB burden countries accounted for 87% of all estimated incident cases worldwide, and eight of these countries accounted for more than two thirds of the global total India is 28%, Indonesia is 9.2%, China is 7.4%, the Philippines is 7.0%, Pakistan is 5.8%, Nigeria is 4.4%, Bangladesh is 3.6% and the Democratic Republic of the Congo 2.9%[1].

To find tuberculosis infection in a person by the help of tuberculin skin test and TB blood test[4], if symptoms are not showing for latent TUBERCULOSIS infection, then to find out through the Mantoux test or Mendel- Mantoux test is needed. When the host body immunity system is week than the *Mycobacterium tuberculosis* bacterium will be replicate and causes some symptoms like cough, chest pain, fatigue, and unexplained weight loss etc. which is eventually leads to death[5]. Due to infectious nature, complex immunological response, chronic progression and the need for long-term treatment, tuberculosis has always been a major health burden. The evolution of multi-drug resistant tuberculosis (MDR-TB) and the TB-HIV epidemic has become much of a bigger concern for the human kind.

The disease tuberculosis had been recorded as late back to the Egyptian era. There are many cases that were found even in the ancient times and Middle Ages.

The enzymes from RmlA to RmlD work one by one to form the product out of the substrates. To block the L-rhamnosyl pathway, we have gone with checking the working of the third enzyme involved in the production of the L-rhamnose residues, the dTDP-6-deoxy-D-xylo-4-hexulose 3,5 epimerase (RmlC). Out of the four enzymes, RmlB and RmlC are reported to be essential for mycobacterial survival (Le *et. al*., 2006). Out of these two, we chose RmlC over the other enzyme because of its specificity towards its substrate and structural uniqueness. There is not involvement of any cofactors so there is no tension of getting the docking wrong[9].

The dTDP-6-deoxy-D-xylo-4-hexulase 3, 5 epimerase (RmlC) is translated from the RmlC gene of the bacterium. RmlC epimerises theC3' and C5' positions of dTDP-6-deoxy-Dxylo-4-hexulose making dTDP-6-deoxy-L-lyxo-4-hexulose. The atomic mass of the RmlC enzyme is 22 kDa and does not require any cofactors. There are currently four reports of RmlC structures and co-complexes have been obtained with dTDP-phenol[10], dTDP (Crystal structure of dTDP-4-keto-6-deoxy-D-hexulose 3,5-epimerase from

Methanobacterium thermoautotrophicum complexed with dTDP), dTDP-D-glucose and dTDP-D-xylose (High-resolution structures of RmlC from Streptococcus suis in complex with substrate analogs locate the active site of this class of enzyme.)[11]. The co-complexes of RmlC from Streptococcus suis with dTDPD-glucose and dTDP-D-xylose respectively, provided experimental evidence for the location of the active site (High-resolution structures of RmlC from Streptococcus suis in complex Review of Literature 21 with substrate analogs locate the active site of this class of enzyme[11] [12]. Four chains consist of protein, chain A to chain D where chain A and B are the inverted copies of the other two chains, chain C and D.

The *In-silico* drug designing concentrates on docking molecules which are thought to be a potential drug into the target site to check it's action and so a drug is prepared. The drug designing process in traditional wet lab method would take long time in identifying the drug targets first, then to list and extract the potential inhibitors and then to react them and wait to find out the results. The computer science and information technology in combination with biology and biotechnology made the whole process simple and easy as the whole part of finding drug target and inhibitors and reaction part is carried out in computer programs and the last part would be the testing part which has to be done in the wet lab till today. Moreover, the modern in silico method provide ease for screening of large number of molecules into a single target site and determining the best inhibitor out of them.

The early diagnosis is required for the proper treatment of the patients. The first step for any tuberculosis diagnosis is the find out TUBERCULOSIS infection in a person by the help of tuberculin skin test and TB blood test. As mentioned in the earlier section[17], if there are no such symptoms for latent tuberculosis infection; the only way to find out if somebody got the infection is to check for the above-mentioned test results to be positive. The most commonly used screening and diagnosis for tuberculosis is the Mantoux test or Mendel- Mantoux test (also known as Mantoux screening test, tuberculin sensitivity test, Pirquet test, or PPD test for purified protein derivative)[18]. It includes a standard dose of 5 tuberculin units injected between the layer of skin. The injected area is inspected after 48 to 72 hours. If it shows a swelling, it is a classic example of the reaction of the protein with those of the bacterial proteins.

Tuberculin is a glycerol extract of the tubercle bacillus. Purified protein derivative (PPD) tuberculin is a precipitate of specific-nonspecific molecules obtained from filtrates of sterilized, concentrated cultures. The tuberculin reaction was first described by the German physician Felix Mendel in 1908 and named after Charles Mantoux[19].

Other diagnosis tests include blood test namely, Interferon Gemma Release Assay(IGRA).This reaction measures the immune relativity to *Mycobacterium tuberculosis* bacterium. With blood cells from the infected person are reacted with Interferon-gamma and Observed for the results of the test. Other tests like chest radiography are used for diagnosis of tuberculosis disease[20].

The sputum smear test helps for the diagnosis of tuberculosis by the production of smear from sputum by the application of acid-fast microscopy [21] [22]. But this diagnosis technique is inaccurate as not all the acid-fast bacteria in the human body are caused by *Mycobacterium tuberculosis* bacterium.

TB these days can be treated taking medication of about 6-9 months. There are 10 drugs that are approved by the Food and Drug Administration (FDA). Out of the approved drugs, the first-line anti-TB agent that forms the core of treatment are Isoniazid, Rifampin, Ethambutol, Pyrazinamide, etc.

There are many problems seen in the present-day drugs in use for the treatment of tuberculosis. Firstly, the duration of treatment of this disease is too long, which in turn can lead to the initiation of drug-resistant tuberculosis, like multiple-drug resistant TB (MDR TB) or extensively drug-resistant TB (XDR TB)[23]. Second, adverse events in response to anti-TB drugs are common and contribute to the problem of non-adherence[24]. Third, the increasing rate of multi drug resistant (MDR; resistance to at least rifampin and isoniazid) and extensively drug-resistant (XDR; MDR resistance plus resistance to a fluoroquinolone and an aminoglycoside) TB is a serious concern[3]. Fourth, co-infection of TB and HIV is a problem by itself. Combined treatment of TB and HIV involves a high pill count with associated adherence problems, overlapping toxicity profiles of the antiretroviral and anti-TB drugs, drug interactions between rifampin and the antiretroviral protease inhibitors, and the risk of immune reconstitution syndrome[25]. Fifth, prophylactic therapy of latent TB (TB infection without symptoms) with isoniazid is also associated with problems Introduction 9 of nonadherence[26] TB can be successfully be cured by the application of DOTS which takes a time period of 6 to 18 months but the concern is the initiation of drug-resistant TB due to the long treatment period.

TB can affect anyone, regardless any age or sex. The highest infected person is the adult men, who accounted for 56.5% of all TB cases in 2021; by comparison, adult women accounted for 32.5% and children for 11% of cases. The higher share of TB cases among men is consistent with evidence from national TB Prevalence surveys, which show that TB disease affects men more than women, and that gaps in case detection and reporting are higher among men[1].

In 2021all incident cases of TB, 6.7% were people living with HIV; this proportion has been steadily declining for several years. The proportion of people with a new episode of TB who were coinfected with HIV was highest in countries in the WHO African countries Region, exceeding 50% in parts of southern Africa countries region[1].

II. MATERIALS

Target enzyme structure

Three-dimensional structure of target protein is recovered from Protein Data Bank (PDB) with its substrate analogue dTDP-rhamnose. It is an online database, which is provide three-dimensional structure of enzyme or protein. Here the target protein or enzyme is RmlC which PDB id- 2IXC[7].

Structure of the Ligand Molecule

The ligand molecules are retrieved from NPACT database (Naturally Occurring Plant Anti-Cancerous Compound -Activity-Target). Which is an online database. There are several ligands molecules are present which are essential for Breast cancer. Out of these we have taken five ligands molecules[8].

Docking software

We use ArgusLab 4.0.1. which is an offline software. This software is used for molecular docking and identifying the active site of the protein. It is free in nature.

III. Methods

At first the retrieved RmlC put in the ArgusLab4.0.1 software and remove the water molecule. After the water molecule remove chain-C and Chain-D are also remove because Chain-C and Chain-D are the inverted copies of chain A and Chain-B. After that the substrate analogue dTDP-rhamnose was then removed from the target site. After that select the binding site then make a group.

Then we have selected one by one ligand molecule and select them and make a group.

After the grouping process is completed docking process will be start. The active site and the ligands are docked in the GADock engine. The docking process start between the Active site and one by one ligand molecule. After that we noted the binding energy of the all-ligand groups. The lowest binding energy of the ligand molecule is potential inhibitor.

Preparation of Target enzyme

The dTDP-6-deoxy-zD-xylo-4-hexulose 3′, 5′-epimerase (RmlC)is an enzyme which is involved in the production of the dTDP-β-L-rhamnose, which is a sugar molecule. This sugar molecule acts as a binding agent in the cell wall of the Mycobacterium tuberculosis bacteria.

Section hit molecule

The Amino acid residues are selected from RmlC enzyme chain A and Chain B. Amino acid residues that were selected for chain A- GLN47, ASN49, SER51, ARG59, GLY60, HIS62, LYS72, HIS119, GLY120, PHE121, TYR132, TYR138, GLU143, SER168, ARG170, ASP171 and for chain Bare-HIS19, ASP21, PHE26, GLU28.

Preparation of ligand molecule

The ligand molecules were then retrieved from the NPACT database. The NPACT or Naturally occurring Plant based Anti-cancerous Compounds- Activity- Target database is an online database which packs naturally occurring plant-based compounds which show anti-cancerous properties. Five ligand molecules were retrieved from the breast cancer section of the NPACT database.

The ligand molecules that are retrieved from the online database are then imported into ArgusLab one by one in the same window as the enzyme molecule. The molecule is then selected and marked as the ligand molecule.

The docking process

After both the molecules being ready in the ArgusLab, the docking process is started. The active site and the ligands are docked in the GADock engine. The docking axes are then set to twenty-five so that the docking is carried out smoothly. The docking process is carried out one by one and the affinity of each molecule is recorded. The affinity of the ligand molecules towards the active site of the enzyme signifies the binding energy of the molecules. The lesser the binding energy, the more stable will be the docked enzyme-inhibitor complex.

Section hit molecule

From the five molecules that are docked, top one molecules is selected based on their binding energy. The lower the binding energy, the better will be the inhibitor for the protein.

IV. Result and Discussion

Preparation of ligand molecules

The ligand molecules are retrieved from the NPACT database. The molecules which were retrieved are naturally obtained and are currently used as drugs for breast cancer. The molecules which are retrieved from NPACT database. Those NPACT id are NPACT00004, NPACT00026, NPACT00035, NPACT00036, NPACT00037. The ligand molecules were then imported one by one into ArgusLab 4.0.1 where the enzyme molecule was kept ready for docking. The ligand molecules were then grouped and marked and the docking process was initiated.

Docking process

When the enzymes and ligands being ready for the docking, the process is started. The docking engine is selected as GADock. The docking is carried out one by one on each ligand. The binding energy of ligand-enzyme complex are noted and the top one hit molecules selected.

SI.NO	NPACT ID	BINDING ENERGY (Kcal/Mole)
1.	NPACT00004	-12.44
2.	NPACT00026	-10.312
3.	NPACT00035	-13.142
4.	NPACT00036	-10.852
5.	NPACT00037	-13.663

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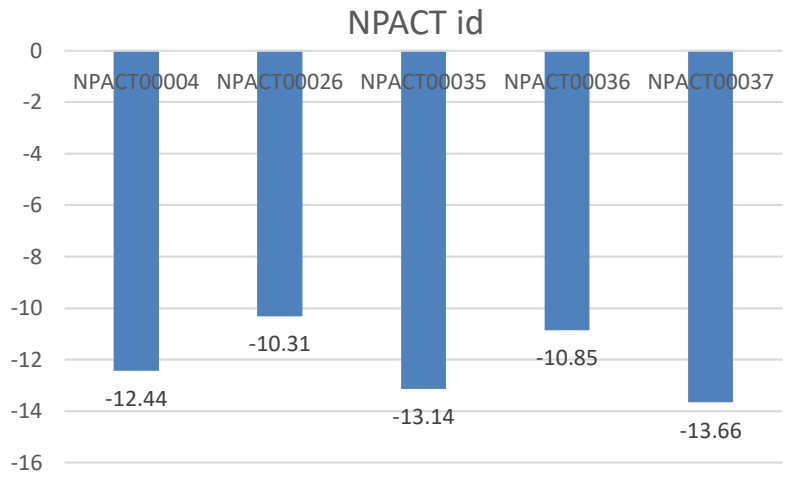
Binding energy of the compound

Graphical representation of binding energy



Top most hit molecule

When the ligand molecules which are Chosen as the potential inhibitors for the target i.e. (RmlC protein of *Mycobacterium tuberculosis*) are the drugs that are currently in use for the treatment of breast cancer. After the docking process is completed, the top one hit molecules are selected based on their binding energy with the target protein. The top one molecule is NPACT00038 Selected from out of the Five molecules.

V. Conclusion

There are several issues relating the modern structure of treatment of tuberculosis. Some new drugs have some side-effect of some drugs of TUBERCULOSIS leads to cancer like breast cancer and lungs Cancer, and other issues is the bacterium *Mycobacterium tuberculosis* resistant his body from new drugs. The duration of the treatment of the disease varies from 6- 18 months. To reduce the duration of treatment the new drugs will be discovered. There is serious need of invention of novel drug for the treatment of tuberculosis. By this project, I am concentrating on the RmlC protein of Mycobacterium tuberculosis which play a vital role in the production of the L-rhamnosyl residues in the cell wall of the bacterium. By checking the RmlC protein, the L-rhamnosyl production will be affected greatly and hence, leading to the death of the bacteria. The inhibitor of the RmlC protein would have a great impact on the life cycle of the bacterium. Hence, the molecule 12- deoxyphorbol 13- (9, 10- methylene) undecanoate emerges as the inhibitor for the l-rhamnosyl pathway by blocking the RmlC enzyme and thus can be used as a potential drug against tuberculosis

REFERENCES

[1] World Health Organization, Global Tuberculosis Report, 2022.webpage-https://www.who.int/teams/global-tuberculosis-programme/tuberculosis-reports/global-tuberculosis-report-2022
[2] World Health Organization, Global Tuberculosis Report, 2018.webpage-https://www.who.int/publications/i/item/9789241565646
[3] World Health Organization. 2006. Guidelines for the programmatic management of drug-resistant tuberculosis. Web page - Guidelines for the programmatic management of drug-resistant tuberculosis - 2011 update (who.int).
[4] Metcalfe JZ, Everett CK, Steingart KR, et al. (November 2011). webpage- https://pubmed.ncbi.nlm.nih.gov/22403663/
[5] JEAN-PAUL ILUNGA MUSASA, 2011.Webpage- https://www.researchgate.net/publication/335128528_TUBERCULOSIS_thesis_pdf
[6] PDB database. Webpage - https://www.rcsb.org/
[7] NPACT Database. Webpage- https://webs.iiitd.edu.in/raghava/npact/index.html
[8] ArgusLab Software. Web address- http://www.arguslab.com/arguslab.com/ArgusLab.html
[9] Le *et. al*., 2006
[10] Giraud MF, Leonard GA, Field RA, Berlind C, Naismith JH; Nat Struct Biol. 2000 May; 7(5):398-402
[11] Dong C, Major LL, Allen A, Blankenfeldt W, Maskell D, Naismith JH; Structure. 2003 Jun; 11(6):715-23
[12] Supercomputing Facility for Bioinformatics &Computational Biology, IIT Delhi
[13] World Health organization, Global tuberculosis report,2017
[14] World health Organization, Global tuberculosis report,2019
[15] World Health Organization, Global tuberculosis report,2020
[16] World Health Organization, Global tuberculosis report,2021
[17] Metcalfe JZ, Everett CK, Steingart KR, et al. (November 2011)
[18] TUBERCULOSIS Elimination - Tuberculin Skin Testing" (PDF). CDC.gov. CDC - National Canter for HIV/AIDS, Viral Hepatitis, STD, and TUBERCULOSIS Prevention - Division of Tuberculosis Elimination. October 2011
[19] F. Mendel. Therapeutische Monat Shefte, Berlin, 1903, 16:177. Die von Pirquet'she Hautreaktion and die intravenous Tuberkulinbehandlung.Medizinische Klinik, München, 1908, 4:402-404
[20] Ferrara G, Losi M, D'Amico R, Roversi P, Piro R, Meacci M, et al. (April 2006)
[21] Kumar, Vinay; Abbas, Abul K.; Fausto, Nelson; & Mitchell, Richard N. (2007). Robbins Basic Pathology (8th Ed)
[22] Robbins Basic Pathology (8th Ed.). Saunders Elsevier. pp. 516-522
[23] Volmink, J., and P. Garner. 2007. Directly observed therapy for treating tuberculosis. Cochrane Database Syst.
[24] Chan, E. D., and M. D. Iseman. 2002. Current medical treatment for tuberculosis. BMJ 325:1282-1286; Volmink, J., and P. Garner. 2007. Directly observed therapy for treating tuberculosis. Cochrane Database Syst.

[25] Narita, M., D. Ashkin, E. S. Hollender, and A. E. Pitchenik. 1998. Paradoxical worsening of tuberculosis following antiretroviral therapy in patients with AIDS. Am. J. Respir. Crit. Care Med. 158:157-161.
[26] Zhang, Y. 2004. Persistent and dormant tubercle bacilli and latent tuberculosis. Front. Biosci. 9:1136-1156