# A MOLECULAR DOCKING AND HIGH THROUGHPUT SCREENING OF POTENT SMALL INHIBITOR TO NLRP3 IN TUBERCULOSIS THROUGH DRUG DESIGNING STUDIES

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## ABSTRACT

Tuberculosis disease caused by *Mycobacterium tuberculosis* (Mtb) and one of the major bacterial infections in the worldwide. Host defense against Mtb is mediated by a combination of innate and adaptive immune responses. Toll-like receptors (TLRs) have been revealed to be critical for the recognition of pathogenic microorganism including *Mycobacterium*; the present study revealed that NOD-like receptors and C-type lectin receptors are responsible for the TLR-independent recognition of *Mycobacterium*. The mode of action of NLRP3 protein plays a critical role for causing the tuberculosis disease. The inactive NLRP3 is activated by CARD like ASC protein. This complex is carried out in two processes, one is inflammation process and another one pyronecrosis (cell death) leads causing the tuberculosis. The main objective of this paper  is to design a small potent drug to block the second pathway (pyronecrosis) through 'Molecular Docking approach' by using the In silico tools.

**Keywords:** Tuberculosis, *Mycobacterium tuberculosis,* NLRP3, 3UN9, Insilco tools

## INTRODUCTION

Tuberculosis*, Mtb*, or *tb* (short for *tubercle bacillus*) is a common, lethal, infectious disease caused by various strains of *Mycobacterium,* usually *Mycobacterium tuberculosis*[1]. Tuberculosis typically attacks the lungs, but also affects other parts of the body. It spreads through the air when people who have an active TB infection cough, sneeze, or otherwise transmit their saliva through the air[2]. Most infections are asymptomatic and latent, but about one in ten latent infections eventually progresses to active disease which, if left untreated, kills more than 50% of those so infected.

Tuberculosis a leading cause of death worldwide due to infectious disease, killing around 2 million people annually, in developing countries. About one third of world population was infected with TB (*Tubercles bacillus),* approximately 8 million new cases per every year. Now –a- days TB is increasing gradually day by day.

The causative agent of tuberculosis disease is *Mycobacterium tuberculosis* (Mtb), which is a

gram positive bacterium, is obligate aerobes whose sole host is humans[3]. The *Mycobacterium tuberculosis* bacteria are identified by Robert Koch in 1882 bacteria causes' T*uberculosis*. Despite the existence of effective chemotherapies, no new drugs have come to the market for more than 40 years[4]. In addition, the rise drug resistance among *Mycobacterium tuberculosis* strain is becoming a server threat to public health. A better understanding of the biology of *Tubercle bacillus*, with the goal of unveiling and validating new therapeutic targets, is an imperative need to improve the control and treatment of *tuberculosis*[5].

About 5–10% of those without HIV, infected with tuberculosis, develop active disease during their lifetimes[6]. In contrast, 30% of those confected with HIV develops active disease. Tuberculosis may infect any part of the body, but most commonly occurs in the lungs[7]. Extra pulmonary TB occurs when tuberculosis develops outside of the lungs. Extra pulmonary TB may coexist with pulmonary TB as well[8]. General signs and symptoms include fever, chills, night sweats, loss of appetite, weight loss, and fatigue, and significant finger clubbing may also occur[9]. In 15–20% of active cases, the infection spreads outside the respiratory organs, causing other kinds of TB[10]. These are collectively denoted as "extra pulmonary tuberculosis". Extra pulmonary TB occurs more commonly in immunosuppressed persons and young children. In those with HIV, this occurs in more than 50% of cases. Notable extra pulmonary infection sites include the pleura (in tuberculoses pleurisy), the central nervous system (in tuberculosis meningitis), the lymphatic system (in scrofula of the neck), the genitourinary system and the bones and joints among others. When it spreads to the bones, it is also known as "osseous tuberculosis"[11.] An ulcer originating from nearby infected lymph nodes are painless, slowly enlarging and has an appearance of "wash leather"[12].

The classic symptoms of active TB infection are a chronic cough with blood-tinged sputum, fever, night sweats, and weight loss. Infection of other organs causes a wide range of symptoms. Diagnosis of active TB relies on radiology (commonly chest X-rays), as well as microscopic examination and microbiological culture of body fluids[13]. Diagnosis of latent TB relies on the tuberculin skin test (TST) and/or blood tests. Treatment is difficult and requires administration of multiple antibiotics over a long period of time. Social contacts are also screened and treated if necessary[14]. Antibiotic resistance is a growing problem in multiple drug-resistant tuberculosis (MDR-TB) infections.
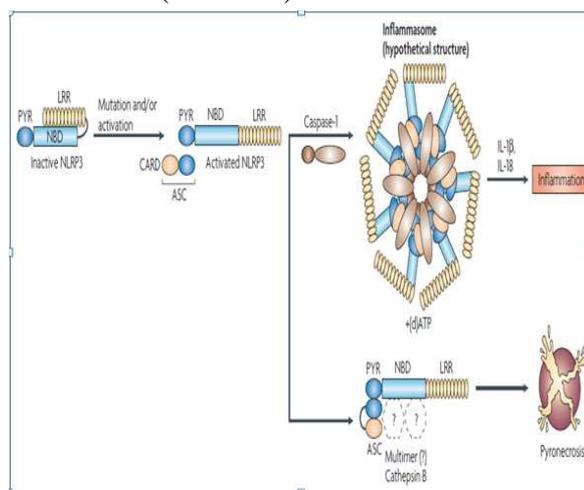


**Fig 1:** Major pathway for Pyronecrosis

NLRP3 activation or disease-associated NLRP3 mutations might weaken putative inhibitory intramolecular interactions between the NLRP3 nucleotide binding domain (NBD) and its C‑terminal leucine rich repeats (LRRs). Using the adaptors ASC (apoptosis-associated speck-like protein containing a CARD) and CARDINAL/TUCAN activated NLRP3 aggregates caspase‑1 molecules through the formation of a macromolecular complex[15]. Complex formation potentiates subsequent caspase‑1 cleavage and activation. Based on studies of the NLRP1 inflammasome, the complex is probably comprised of 5–7 subunits, each with the ability to recruit pro-caspase‑1 molecules. Interleukin-1β (IL‑1β) is activated by caspase‑1-mediated cleavage of the inactive pro-IL‑1β precursor molecule. However, formation of the inflammasome is not the only function of NLRP3. Activated NLRP3 also initiates pyronecrosis a molecular pathway of

necrotic cell death that is dependent on ASC and proceeds through cathepsin B[16]. This cell-death pathway does not rely on caspase‑1 or IL‑1β, and therefore is independent of inflammasome function. The term '(d) ATP' is used to reflect that both dATP and ATP may be involved in regulating complex formation and activation.

## MATERIALS AND METHODS

The protein NLRP 3 was responsible for the disease. The NLRP3 sequence was retrieved from the NCBI (National Centre for Biotechnology Information) database with accession number AAl43360.1, which is having a length of 1016 amino acids.

### Structural Analysis Prediction

Primary, secondary and Tertiary structure analysis of NLRP3 protein to know about the physico-chemical properties, helix and coils and pdb ids on the basis of the least e-value and identities by the tools and databases.

### Prediction of Protein Tertiary Structure

In first step tertiary structure of NLRP3 protein was predicted through CPH Model, HH Pred and Geno 3D. Identification of common PDB ID through these results. CPH Model (Comparative Protein Homology Modelling) is a protein homology modelling server. It was used to identify the common PDB ID on the basis of its score and it's E-value (http://www.cbs.dtu.dk/services/CPHmodels/).

HH Pred is a free protein function and protein structure prediction server that is based on the HH search and HH bits (http://toolkit.tuebingen.mpg.de/hhpred).

Geno3D is an automated web server for protein molecular modelling (http://geno3d-pbil.ibcp.fr/cgi-bin/geno3d_automat.pl?page=/GENO3D/geno3d_home.html). By comparing these results 3UN9 was the common PDB ID for the activated NLRP3 causing pyronecrosis.

### Preparation of Drugs

The drugs were prepared from the natural compounds. Hence there are various sources for selecting the natural compound such as Natural Products, Plant sources, Antimicrobial sources and Marine sources among all these Gliotoxin was selected from Antimicrobial sources for treatment of tuberculosis. The CID files of the Ligands were downloaded from PUBCHEM. It is a Database of chemical molecules and their activities against biological assays. 50 ligands were prepared in CHEMSKETCH software.

### Screening of Drugs

From the library 50 drugs were screened and obtained the molecular properties such as Molecular weight, Molecular formula, Hydrogen acceptors, Hydrogen Donors and Aromatic Rings with the help of Accelyrs Discovery Studio in which all properties should be follow the Lipinski's Rule of 5.

The drugs were screened on the following basis

1. Lipinski rule of 5
2. ADME/TOPKAT
3. Energy Minimization

50 Drugs were selected to inhibit the pyronecrosis pathway, all the drugs were passed in molecular property test according to the Lipinski's rule of 5. Out of it 25 drugs were screened by (Toxicity prediction test). High throughput screenings which are non-carcinogenic and these are passed through ADMET. From these 13 drugs were selected as non-toxic according to their TOPKAT and ADMET parameters.

### Molecular Minimization

The molecular minimization studies were carried out both 3UN9 receptor and 13 successful drugs. This step was mainly for the stability of the receptor and ligand molecules. The molecular minimization step was done in ADS (Accelrys Discovery Studio) software.

### Docking studies

In the present study the nature of interactions, binding mode and selectivity of NLRP3 protein with the ligands docking was carried out with ADS, HEX and Ligand scout software.

### ADS 2.5

Discovery Studio is a well-known suite of software for simulating small molecule and macromolecule systems. It is developed and distributed by Accelrys. It is typically used in the development of novel therapeutic medicines, including small molecule drugs, therapeutic

antibodies, vaccines, synthetic enzymes, and areas such as consumer products. It consists of 17-33 software. Molecular minimization studies, High through screening studies and docking studies of NLRP3 was carried out by this software.

**Hex 6.3**

HEX is an interactive protein docking and molecular superposition program. Hex understands NLRP3 protein and DNA structures in PDB format, and it can also read small-molecule SDF files.

**Ligand Scout 2.0**

LIGANDSCOUT is a software tool that allows to model 3D pharmacophore models from structural data of macromolecule/ligand complexes or from training and test sets of organic molecules. It incorporates a complete definition of three-dimensional chemical features (such as hydrogen bond donars, acceptors, lipophilic areas, positively and negatively ionizable chemical groups) that describe the interaction of a bound small organic molecule (ligand) and the surrounding binding site of the macromolecule. These pharmacophores can be overlaid and superimposed using a pattern- matching based alignment algorithm that is solely based on pharmacophoric feature points instead of chemical structure. From such on overlay, shared features can be interpolated to create a so-called shared-feature pharmacophore that shares all common interactions of several binding sites/ligands or extended to create a so-called merged-feature pharmacophore. The software has been successfully used to predict new lead structures in drug design.

## RESULTS

**Prediction of Protein Tertiary Structure**
The results were obtained from CPH model, Geno3D and HHpred is shown as follows (fig 2) By comparing these results to identify the common PDB ID was 3UN9.

**CPH MODEL**
Round 0. Hits better than threshold: 0.000010:
entry: 3UN9 chain: A score: 400 E: 1e-111

entry: 3UN9 chain: B score: 400 E: 1e-111
entry: 3UN9 chain: C score: 396 E: 1e-110

**RASMOL**

Rasmol is a computer program written for molecular graphics visualization intended and used primarily for the depiction and exploriration of biological macromolecular structures, such as those found in the Protein Data Bank, by given command in the command window. To identify the regions of Helix, sheets, various amino acids.
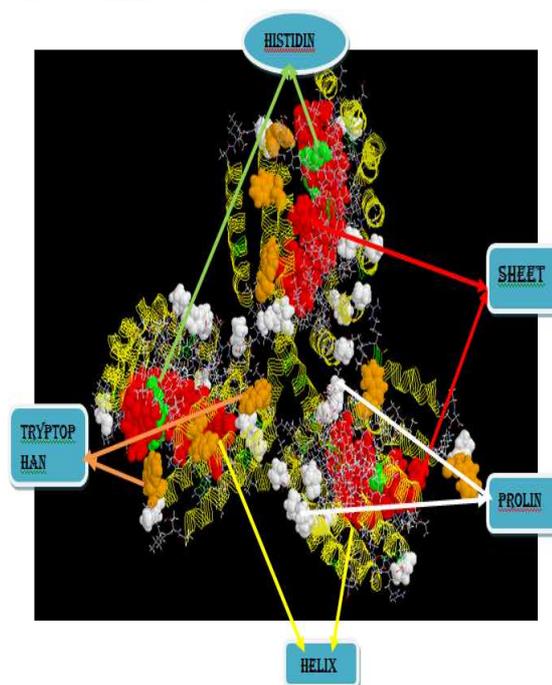


**Fig 3:** Various regions of NLRP3 protein in RASMOL DOCKING in ADS

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. Docking is frequently used to predict the binding orientation of drug candidates to our protein target (receptor) in order to in turn predict the affinity and activity of the drug molecule. After the minimization of all drugs and receptor, docked TOPKAT and ADMET filtered drugs with our receptor- 3UN9 to find orientation with Receptor Binding Pocket with the help of Discovery Studio. 10 poses were obtained and found the lowest Cdocker energy.
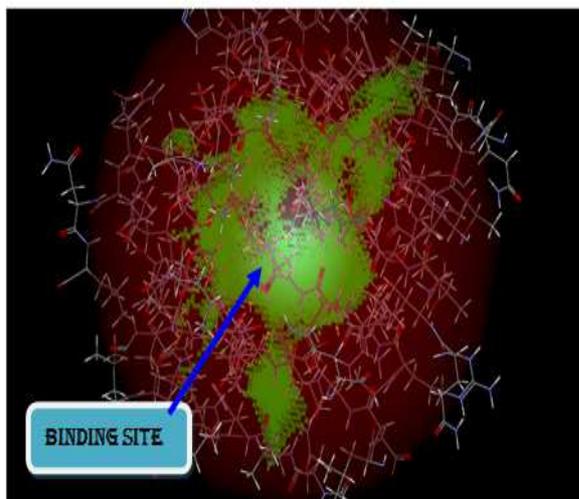
**Fig 4: Docking between 3UN9 recetor and ligand**
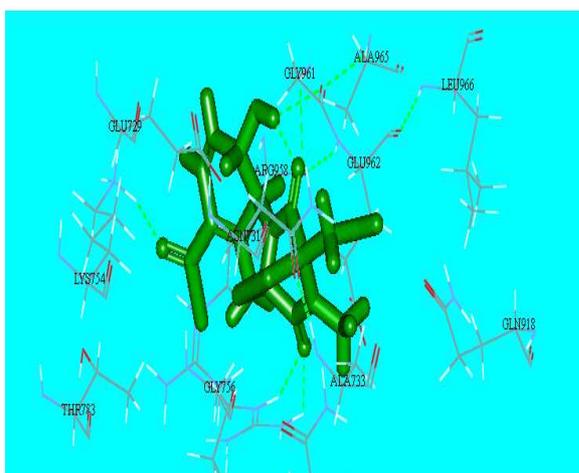


**Fig 5: C Docker complexes with amino acids**

### DOCKING IN HEX

Docked the best drug molecule ((1R,7S,8S,11R)-7-hydroxy-11-(hydroxymethyl)-15- methyl-10,14-dioxo-12,13-dithia-9,15-diazatetracyclo[9.2.2.0^{1,9}.0^{3,8}]pentadeca - 3,5-diene-4-carbonyl chloride), which having a least Cdocker energy in Hex, to study the Pharmacophore Analysis. In Hex, E.min and E.max energy was found.

### Fig 6:  Hex docking

**a) intermolecular axis**

**b) Harmonic surface Pharmacophore Analysis :** From the docked complex of pharmacophore the hydrophobic, aromatic, hydrogen bond receptor, a hydrogen donor, a cation, or an anion.

### Fig 7: a) Pharmacophore
### b) Drug in receptor cavity

On the basis of assays, best molecule was found, having a lowest binding energy. The best molecule was identified and further evaluated by molecular dynamics simulation of protein-ligand complex. Minimized energy of receptor is 55.08625 kcal/mol. The docked ligand with the receptor and obtained 10 poses and their Cdocker energy, the drug  (1R,7S,8S,11R)-7-hydroxy-11-(hydroxymethyl)-15-methyl-10,14-dioxo-12,13-dithiadiazatetracyclo [9.2.2.0^{1,9}.0^{3,8}]  pentadeca-3,5-diene-4-carbonylchloride was found which have lowest Cdocker energy -31.2239 Kcal/mol at 10th pose, then other drugs.

### CONCLUSION

The protein NLRP 3 was responsible for the disease. The important activated NLRP3 protein initiates pyronecrosis, a molecular pathway of necrotic cell death that was dependent on ASC and proceeds through cathepsin B. This protein was more responsible to cause the disease and death. Drugs were designed to block the pyronecrosis pathway and it was concluded that the (1R,7S,8S,11R)-7- hydroxy-11-(hydroxymethyl)-15- methyl-10, 14-dioxo-12,13-dithia-9,15-diazatetracyclo [9.2.2.0^{1,9}.0^{3,8}]  pentadeca-3,5-diene-4-carbonyl chloride .

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## Geno 3D

| | TEMPLATE | E- VALUE | FIRST | LAST | ID | ALIGNEMENT | COMMENT | NPSA link |
|---|---|---|---|---|---|---|---|---|
| ☐ -0 | pdb3un9A | 2.000000e-42 | 667 | 870 | 84.000000 | see alignment | IMMUNE SYSTEM 15-NOV-11 3UN9 CROSS_PDB | NPSA |
| ☐ -0 | pdb3un9B | 2.000000e-42 | 667 | 870 | 84.000000 | see alignment | IMMUNE SYSTEM 15-NOV-11 3UN9 CROSS_PDB | NPSA |
| ☐ -0 | pdb3un9C | 3.000000e-42 | 670 | 870 | 84.000000 | see alignment | IMMUNE SYSTEM 15-NOV-11 3UN9 CROSS_PDB | NPSA |

## HHpred

No Hit Prob E-value P-value Score SS Cols Query HMM Template HMM

☐ **1 3un9_A** NLR family member X1; l 100.0 4.7E-30 1.6E-34 286.5 25.6 252 625-876 1-252 (372)

☐ **2 3un9_A** NLR family member X1; l 99.8 4.3E-20 1.5E-24 205.8 15.5 209 663-876 64-282 (372)

☐ **3 2ca6_A** RAN GTPase-activating p 99.8 1.7E-18 5.9E-23 194.8 13.5 237 626-877 24-288 (386)

☐ **4 3goz_A** Leucine-rich repeat-con 99.8 1.4E-18 4.8E-23 193.6 10.6 207 671-877 22-239 (362)

☐ **5 2ca6_A** RAN GTPase-activating p 99.7 2.5E-17 8.7E-22 185.2 18.0 242 626-878 52-318 (386)

**Fig 2:** NLRP3 tertiary structure prediction by using a) CPH model b) Geno3d c) HH pred

**Table 1**: Lowest C docker energy of drugs

| S. No | Name of Drugs | Lowest CDocker Energy (Kcal/mol) | Number of poses |
|---|---|---|---|
| 1 | (1R,7S,8S,11R)-7-hydroxy-11-(hydroxymethyl)-15- methyl-10,14-dioxo-12,13-dithia-9,15-diazatetracyclo[9.2.2.0^{1,9}.0^{3,8}]pentadeca- 3,5-diene-4- | -31.2239 | 10 |

|  | carbonyl chloride |  |  |
|---|---|---|---|
| 2 | (1R,7S,8S,11R)-7-hydroxy-11-(hydroxymethyl)-15-methyl-5-(prop-2-en-1-yl)-12,13-dithia-9,15-diazatetracyclo[9.2.2.0^{1,9}.0^{3,8}]pentadeca-3,5-diene-10,14-dione | -4.62417 | 10 |
| 3 | (1R,7S,8S,11R)-7-hydroxy-11-(hydroxymethyl)-15-methyl-10,14-dioxo-12,13-dithia-9,15-diazatetracyclo[9.2.2.0^{1,9}.0^{3,8}]pentadeca-3,5-diene-4-carbaldehyde | -28.8263 | 10 |
| 4 | (1R,7S,8S,11R)-7-hydroxy-11-(hydroxymethyl)-15-methyl-10,14-dioxo-12,13-dithia-9,15-diazatetracyclo[9.2.2.0^{1,9}.0^{3,8}]pentadeca-3,5-diene-5-carbaldehyde | -19.6437 | 10 |
| 5 | (1R,2S,7S,8S,11R)-2-ethenyl-7-hydroxy-11-(hydroxymethyl)-15-methyl-12,13-dithia-9,15-diazatetracyclo[9.2.2.0^{1,9}.0^{3,8}]pentadeca-3,5-diene-10,14-dione | -10.5364 | 10 |
| 6 | (1R,2S,7S,8S,11R)-7-hydroxy-11-(hydroxymethyl)-15-methyl-2-(prop-2-en-1-yl)-12,13-dithia-9,15-diazatetracyclo[9.2.2.0^{1,9}.0^{3,8}]pentadeca-3,5-diene-10,14-dione | -14.6329 | 10 |
| 7 | (1R,7S,8S,11R)-7-hydroxy-11-(hydroxymethyl)-15-methyl-4-(prop-2-en-1-yl)-12,13-dithia-9,15-diazatetracyclo[9.2.2.0^{1,9}.0^{3,8}]pentadeca-3,5-diene-10,14-dione | -25.998 | -10 |
| 8 | (1R,2R,7S,8S,11R)-2-acetyl-7-hydroxy-11-(hydroxymethyl)-15-methyl-12,13-dithia-9,15-diazatetracyclo[9.2.2.0^{1,9}.0^{3,8}]pentadeca-3,5-diene-10,14-dione | -27.9227 | 10 |
| 9 | (1R,7S,8S,11R)-4-acetyl-7-hydroxy-11-(hydroxymethyl)-15-methyl-12,13-dithia-9,15-diazatetracyclo[9.2.2.0^{1,9}.0^{3,8}]pentadeca-3,5-diene-10,14-dione | -12.5365 | 10 |
| 10 | (1R,7S,8S,11R)-5-acetyl-7-hydroxy-11-(hydroxymethyl)-15-methyl-12,13-dithia-9,15-diazatetracyclo[9.2.2.0^{1,9}.0^{3,8}]pentadeca-3,5-diene-10,14-dione | -9.1193 | 10 |
| 11 | (1R,7S,8S,11R)-7-hydroxy-11-(hydroxymethyl)-15-methyl-4-nitroso-12,13-dithia-9,15-diazatetracyclo[9.2.2.0^{1,9}.0^{3,8}]pentadeca-3,5-diene-10,14-dione | -11.6439 | 10 |
| 12 | N-[(1R,7S,8S,11R)-7-hydroxy-11-(hydroxymethyl)-15-methyl-10,14-dioxo-12,13-dithia-9,15-diazatetracyclo[9.2.2.0^{1,9}.0^{3,8}]pentadeca-3,5-dien-4-yl]acetamide | -13.24905 | 10 |
| 13 | (S,1R,7S,8S,11R)-7-hydroxy-11-(hydroxymethyl)-15-methyl-10,14-dioxo-12,13-dithia-9,15-diazatetracyclo[9.2.2.0^{1,9}.0^{3,8}]pentadeca-3,5-diene-4-sulfinic acid | -16.6257 | 10 |

The table contains 13 ligands having lowest Cdocker energy, in which one ligand (1R, 7S, 8S, 11R)-7-hydroxy-11-(hydroxy methyl)-15- methyl-10,14-dioxo-12,13-dithia-9,15 diazatetracyclo [9.2.2.0^{1,9}.0^{3,8}] pentadeca- 3,5-diene-4-carbonyl chloride) having least Cdocker energy -31.2239 Kcal/mol was the best drug molecule, in comparative with other ligands.