

## **A Comprehensive Investigation of the Interactions between Proteins and Ligands in the Crystal Structures of Mycobacterium Tuberculosis**

### **ABSTRACT**

Mycobacterium tuberculosis (MTB), an acid-fast aerobic bacterium that may grow on gram stain as either a gram-positive or gram-negative bacterium, is the disease-causing agent of tuberculosis (TB). Rifampin, isoniazid, pyrazinamide, and ethambutol, the first-line anti-tubercular drugs, can all have hepatotoxic side effects. The new medicine needs to work through a novel mode of action or to a novel target, be more active than presently available treatments, and shorten the course of treatment for the MDR-TB and XDR-TB. And active against both active and latent bacteria, and not interact with antiretroviral medications because many TB patients also have HIV. Additionally, it must work well with other anti-TB medications **in order to form** at least an effective three-drug regimen. This article discusses the analysis of few FDA approved anti-tubercular medications and their binding locations with respective targeted proteins. This mainly focuses on the amino acids of the proteins which are responsible for the formation of interactions with a drug molecule. **So**, researchers can modify the existing drugs or its derivatives or can construct a new molecule according to the binding sites of enzymes corresponding to mycobacterium tuberculosis.

**Keywords:** *Anti-tubercular drugs; mycobacterium tuberculosis; MDR-TB; XDR-TB.*

## 1. INTRODUCTION

TB is still among the deadliest infectious diseases in the world. Nearly 30,000 people become ill with this preventable and treatable disease every day, and close to 4400 people lose their lives to TB. Access to TB diagnosis and treatment as well as the burden of TB disease continue to be negatively impacted by the COVID-19 epidemic. Global TB objectives are off track and advancement made in the years leading up to 2019 has slowed, stopped, or reversed. The number of people who infected TB was expected to be 10.6 million (95% UI: 9.9-11 million) in 2021, an increase of 4.5% from 10.1 million (95% UI: 9.5-10.7 million) in 2020. The number of new cases of tuberculosis (TB) per 100,000 people increased by 3.6% between 2020 and 2021, reversing annual decreases of roughly 2% for the most of the preceding two decades. The net decrease from 2015 to 2021 was 10%, which is barely halfway to the first End TB Strategy milestone [1]. Reductions in number of new TB cases in 2020 and 2021 indicate that there are more people with the disease who are undiagnosed and untreated, which first leads to more TB deaths and community transmission of the infection before, after a lag, increasing the number of new cases of TB. We anticipate that the impact of COVID-19 will vary depending on the context. For instance, nations with high rates of tuberculosis (TB), like India and Viet Nam, have seen substantially disparate COVID-19 occurrence [2, 3]. The Mycobacterium tuberculosis infections medications approved by the FDA include Rifampin, Isoniazid, Pyrazinamide, and Ethambutol [4]. Approximately 13 new compounds have been recently discovered, including Bedaquiline, Delamanid, Pretomanid, Contezolid, Delpazolid, Sutezolid, GSK-3036656, Macozinone, OPC-167832, Q203, SQ109, TBA-7371, and TBI-166. This occurs following almost 40 years of active research and development work without any novel approved TB drugs. Based on their phase II clinical trials, two of these

drugs bedaquiline and delamanid have been given accelerated or conditional regulatory approval [5-7]. Linezolid, Levofloxacin, Moxifloxacin, and Nitrazoxanide are the additional substances. For better TB illness therapy, more research is required. Although the TB typically assaults the lungs, they can also affect the kidney, spine, and brain [8]. Droplets from speaking, coughing, and sneezing propagate the illness. Microbacterial cultures, chest X-rays, and tuberculin skin tests are used to make the diagnosis [9]. The appearance of tuberculosis varies, and based on clinical manifestation, it is categorized into pulmonary TB (PTB) and extrapulmonary TB (EPTB) [10]. Because extrapulmonary TB (EPTB) accounts for more than 50% of all TB diagnoses in HIV-positive individuals, the situation is made more difficult by the HIV pandemic [11]. EPTB frequently presents a significant challenge for early diagnosis due to its heterogeneity of presentation. Constitutional symptoms including fever, anorexia, weight loss, malaise, and exhaustion may be present [12]. Drug resistance is a recurring issue in the treatment of TB. For instance, in 2016, it was reported that there were 490,000 instances of multidrug-resistant tuberculosis (MDR-TB) and an additional 110,000 cases of TB that was susceptible to isoniazid but resistant to rifampicin (RR-TB), the best first-line anti-TB medication [13]. MDR-TB is resistant to two most potent anti-TB medications, rifampicin, and isoniazid. Extensively drug-resistant tuberculosis (XDR-TB), also known as MDR-TB and is resistant to at least one fluoroquinolone plus a second-line injectable medication (kanamycin, capreomycin, or amikacin), is a different form of resistant TB. In the intensive phase, which lasts for 6 to 12 months, all XDR-TB cases are treated with injections of Capreomycin, Moxifloxacin (MIFX), PAS high dose Isoniazid, clofazimine, Linezolid, and Co-amoxiclav. The rest of the drugs, ~~with the exception of~~ **except for** the injections, are continued for 18 months in the continuation phase. The following obstacles must be overcome by researchers working in the field of TB treatment, among others: the newer drug must be more effective than currently available drugs in order to shorten the length of treatment; it

must also be active against both active and latent bacteria; it must act by a novel mechanism of action or to a novel target, particularly for the treatment of MDR-TB and XDR-TB; and it must not interact with antiretroviral drugs because many TB patients also take these drugs[14,15]. In this review, 14-FDA approved Anti-tubercular drugs obtained from different literatures and their protein-ligand interactions are discussed.

## 2. FDA approved drugs used in TB

### 2.1 Bedaquiline

For the treatment of tuberculosis, bedaquiline, a diarylquinoline, is offered under the trade name Sirturo. Both the FDA and the EMA authorised bedaquiline for the treatment of MDR-TB in December 2012 and March 2014, respectively[16-20].

**Mechanism of Action:** Bedaquiline is extremely effective and works through a novel mechanism that involves inhibition of the proton pump of mycobacterial ATP synthase [21].

**Binding Pockets:** In their Nucleic Acid Research publication, Kailu Yang and Junjie Zhang reported that bedaquiline has hydrophobic connections with PHE-691, LEU-721, PRO-172, VAL-176, GLU-654, ILE-705, ALA-669, and TYR-681[22].

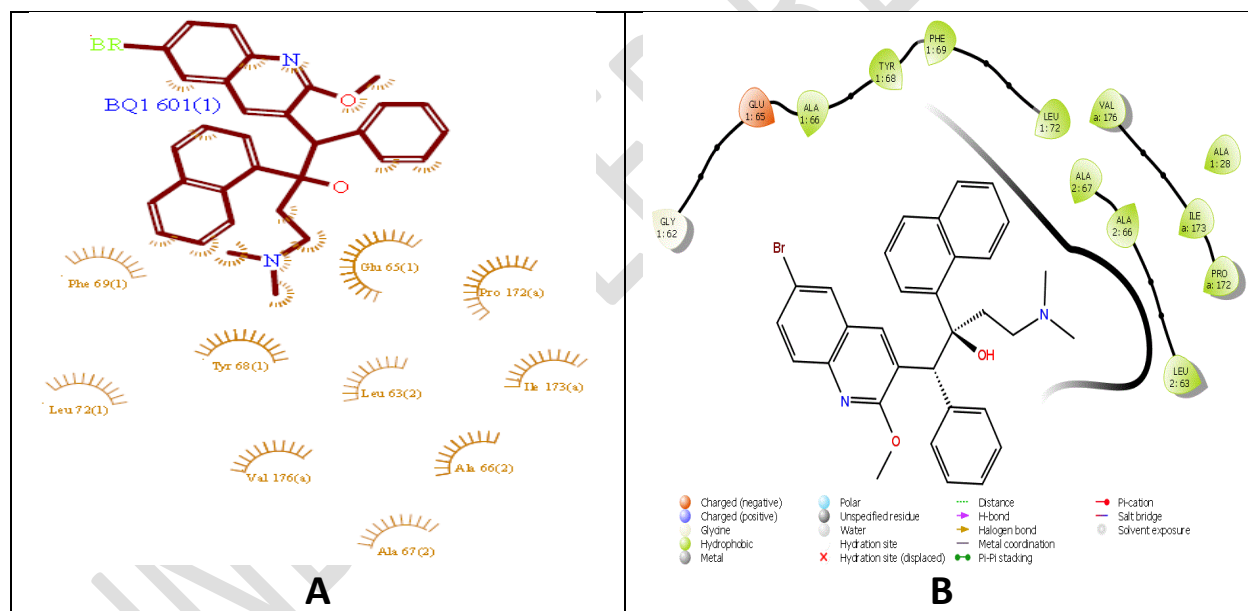
### 2.2 Delamanid

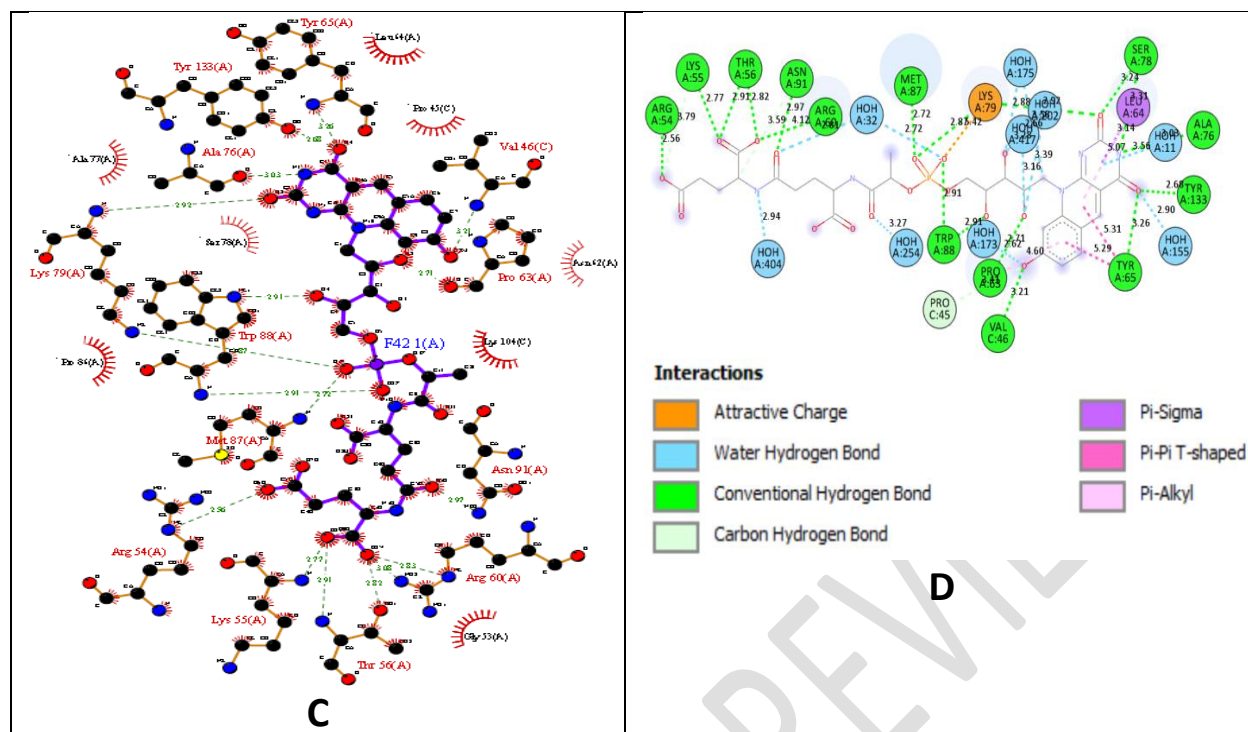
Delamanid, also marketed as Deltyba, is a derivative of nitroimidazooxazole (OPC-67683). On April 28, 2014, the EMA granted delamanid marketing authorization for use.

**Mechanism of Action:** Delamanid works by preventing the mycolic acid synthesis. A Keto-mycolic and methoxy-mycolic acid, two crucial

elements of *M. tuberculosis* cell wall, is inhibited, which results in bacterial cell death [23].

**Binding pockets:** Cellitti S.E. came to the conclusion in their study that the Delamanid exhibits hydrophobic and other interactions with LYS-55A, ARG-60A, PRO-63A, LEU-64A, and MET-87A, VAL-46A, LYS-55A, THR-56A, ASN-62A, PRO-63A, TYR-65A, ALA-76A, LYS-79A, SER-78A, MET-87A, TRP-88A, and A5N-91A are other molecules with which the delamanid formed H-bonds. Delamanid also creates the TYR-65A that stacks. Delamanid joins forces with GLY-53A, TYR-133A, LYS-104A, ARG-54A, and PRO-86A to form hydrophobic and other connections [24]. Liglot and 2D complexes of bedaquiline and delamanid shown in figure 1.





**Figure 1. A) Ligplot and B) 2D Complex of Bedaquiline with 7JGCC) Ligplot and D) 2D Complex of Delamanid with 3R5R**

### 2.3 Pretomanid

Pretomanid, also known as PA-824, is a nitroimidazole medication that is presently undergoing clinical studies to see whether it has any effect on both drug-susceptible and drug-resistant TB. Pretomanid, shorten the duration of TB treatment, surpassed rifampicin, and pyrazinamide in terms of its ability to kill non-replicating Mycobacterium tuberculosis [25, 26].

**Mechanism of Action:** The suppression of lipid and protein synthesis is its mode of action. It prevents the formation of the mycolates found in mycobacterial cell walls [27]. A recent investigation on *M. smegmatis*, a species that is genetically related to MTB, however, revealed that pretomanid may operate instead by interfering with bacterial sugar phosphate metabolism and causing the bacteria to produce the deadly substance methylglyoxal [28].

**Binding pockets:** Barry C. Finzel and Courtney C. Aldrich in the Journal of Medicinal Chemistry ~~came to the conclusion~~ concluded that pretomanid exhibits hydrophobic interactions with PRO-24A and TYR-25A. Pretomanid had the ability to stack with TRP-64A at various distances and TYR-157A. Additionally, GLY-156A forms halogen bonds with it. Together with ILE-256A, ALA-226A, GLY-227A, PRO-24A, MET-91A, THR-318B and PH-92B, it also forms the other bonds[29].

#### **2.4 Contezolid:**

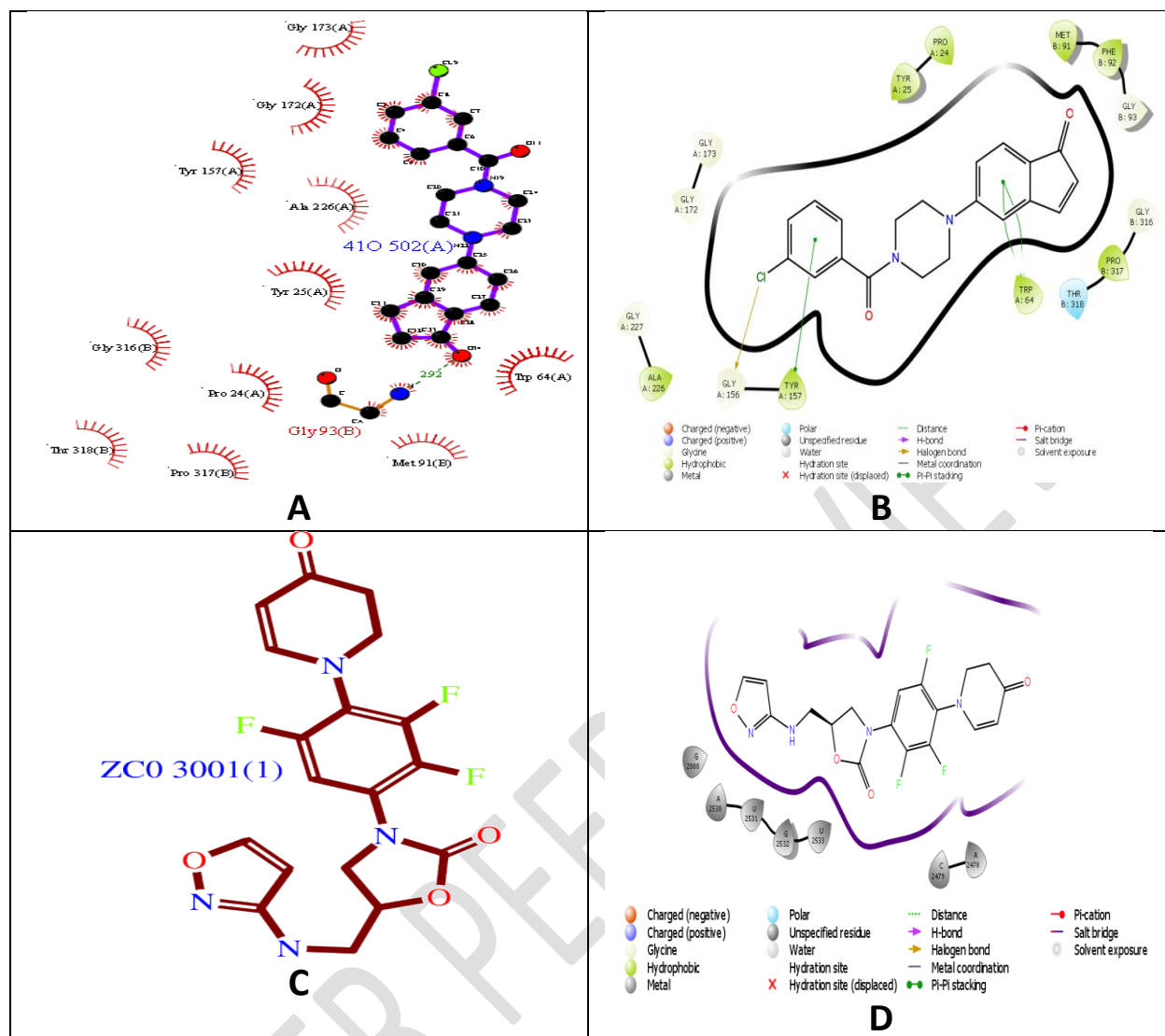
The first oxazolidinone antibiotic to show potential for use in the management of infections driven on by Gram-positive pathogens was contezolid or MRX-I[30].

**Mechanism of Action:** In general, oxyazolidinones work by preventing the production of new proteins. It was believed that they accomplish this by obstructing translational integrity[31, 32]. Figure 2 depicts the Liglot and 2D complexes of pretomanid and contezolid.

#### **2.5 Sutezolid**

An oxazolidinone derivative of linezolid called sutezolid (PNU-100480 or U-100480) is now being tested in clinical studies to treat tuberculosis. In a murine model, sutezolid was found to be more effective than linezolid, and its combination with I and II-line anti-TB medications demonstrated excellent bactericidal action with the ability to accelerate therapy.

**Mechanism of Action:** Sutezolid inhibits protein synthesis[33-35].



**Figure 2. A) Ligplot and B) 2D Complex of Pretomanid with 4XJOC) Ligplot and D) 2D Complex of Contezolid with 6WQN**

**Binding Pockets:** According to the research paper by Mendes, V. Sutezolid formed hydrophobic and other interactions with ASN-179A, TRP-103, PHE-110-GLU-180, LEU-183, PHE-114, MET-142, TRP-145, TRY-148, THR-149, TRP-138, ILE107, GLY-106, and LEU-87[36].

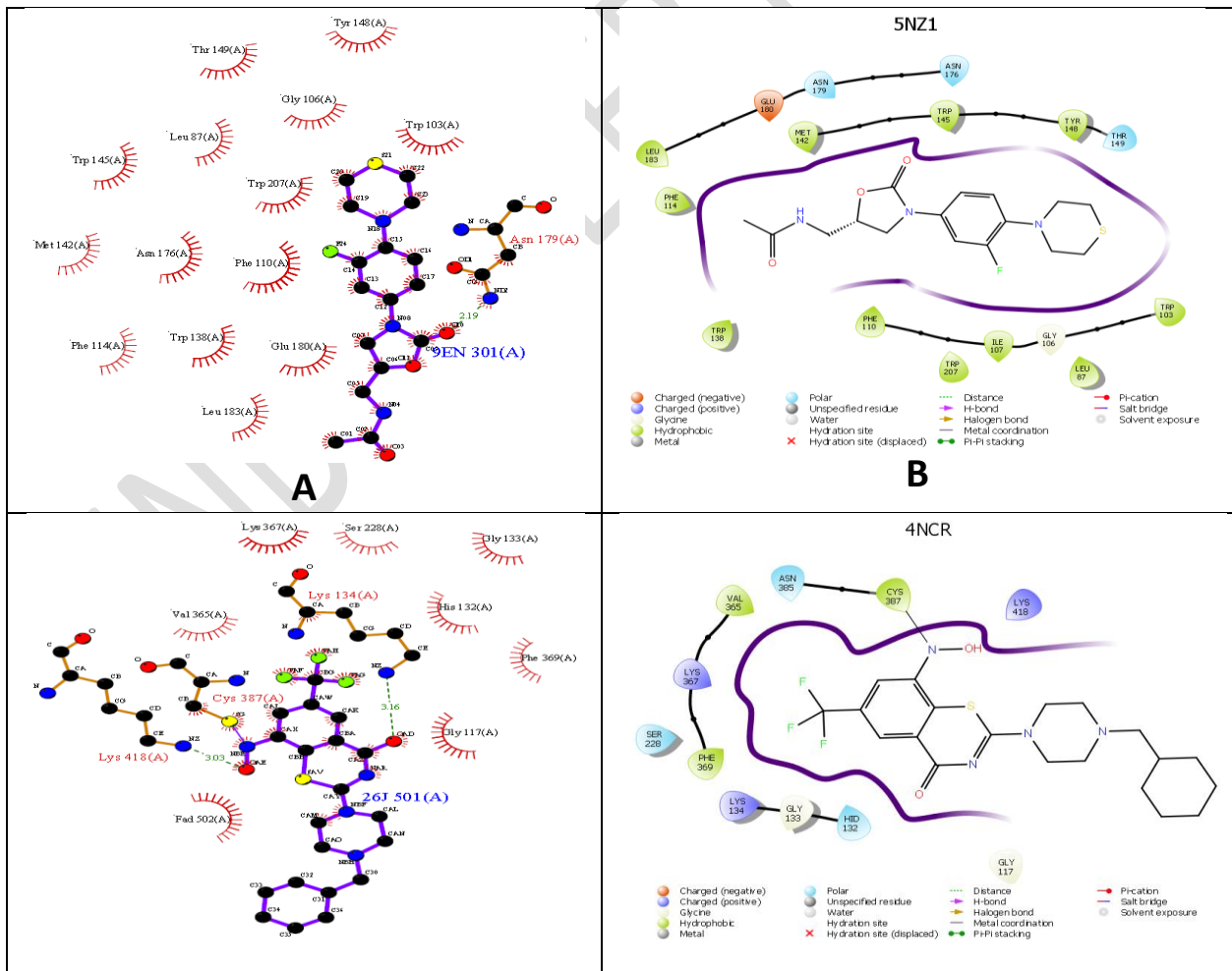
## 2.6 Macozinone

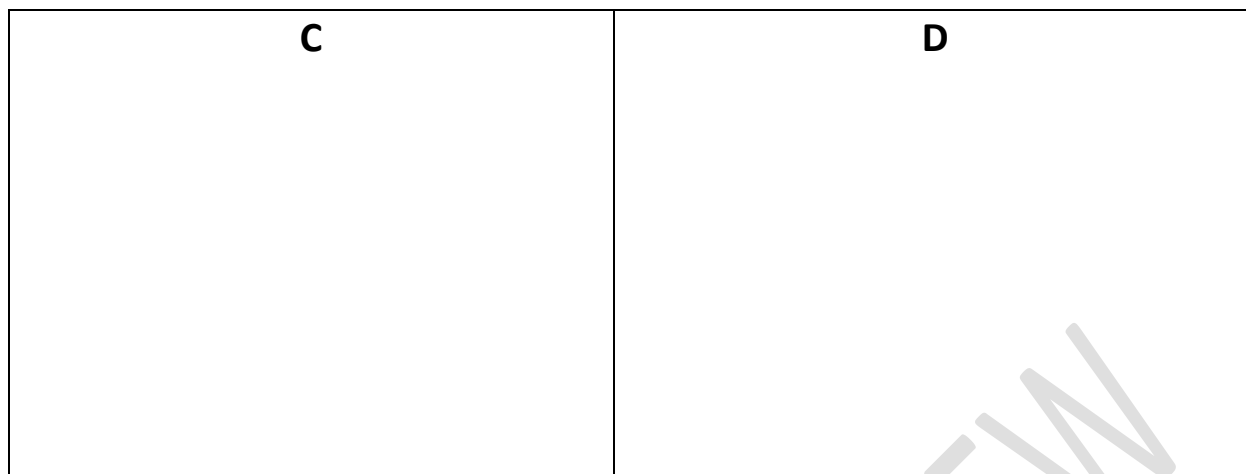
Macozinone did not interact negatively or favourably with I-line or II-line drugs in a trial conducted by Lupine and colleagues. Macozinone

had improved bactericidal action in the lungs and spleen that was comparable to isoniazid when tested in a chronic TB murine model.

**Mechanism of Action:**By binding covalently to a cysteine residue in the active site of decaprenylphosphoryl-b-D-ribose 2'-epimerase (DprE1), inhibits the formation of cell wall[37].

**Binding pockets:** According to the research paper by Vadim Makaro and Stewart T. Cole, Macozinone exhibits hydrophobic interactions with VAL-365A. Macozinone joins LYS-134A and LYS-418A to produce H-bonds. It also displays ASN-385A halogen bonding. Additionally, it displays interactions with SER-228A, GLY-133A, HIS-132A, PHE-369A, GLY-117A, and CYS-387A as well as additional interactions [38].Figure 3 depicts the Liglot and 2D complexes of sutezolid and macozinone.





**Figure 3. A) Ligplot and B) 2D Complex of Sutezolid with 5ZN1C) Ligplot and D) 2D Complex of Macozinone with 4NCR**

## 2.7 Linezolid

Linezolid exhibits significant action against M.tuberculosis that is rapidly developing, slowly growing, and incapable of reproducing itself, demonstrating its potential to be used in TB therapy regimens that are shorter in duration[39].

**Mechanism of Action:** An oxazolidinone antibiotic called linezolid works to suppress translation by attaching to the ribosome's 50S subunit and competing with the cell's natural substrates[40-41].

**Binding pockets:** According to the research paper by Mendis V., Linezolid exhibits hydrophobic and other interactions with ASN-179, ASN-176, VAL-152, THR-149, TYR-148, TRP-145, MET-142, LEU-183, MET-102, TRP-103, TRP-207, ILE-107 and PHE-110[38].

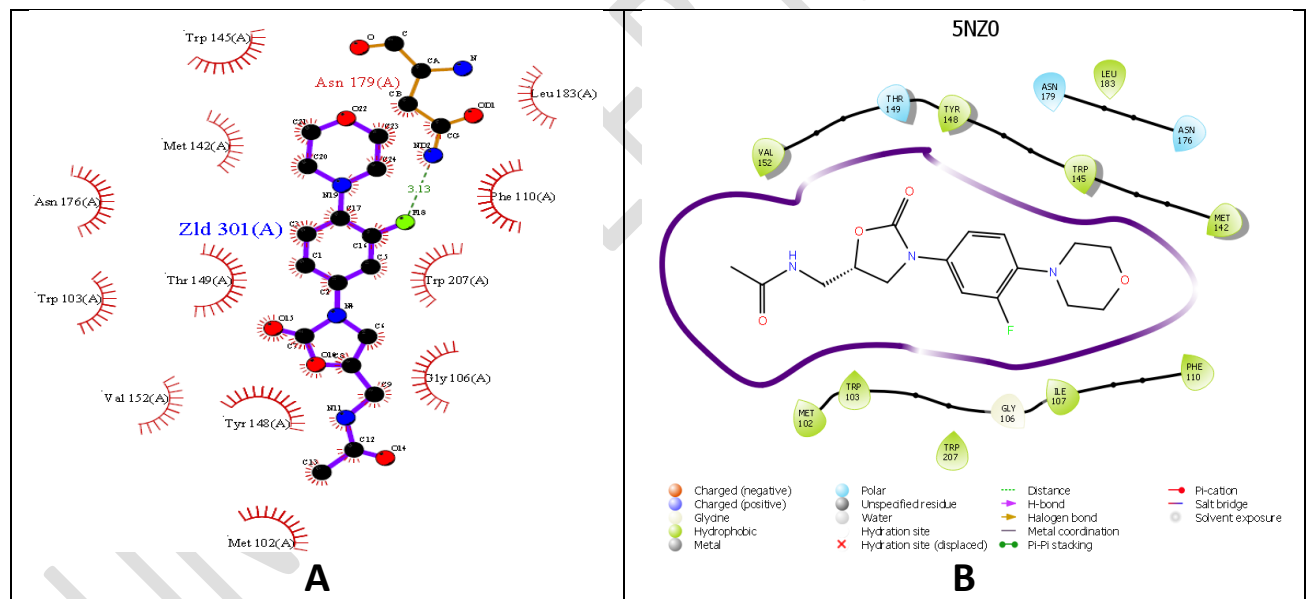
## 2.8 Levofloxacin

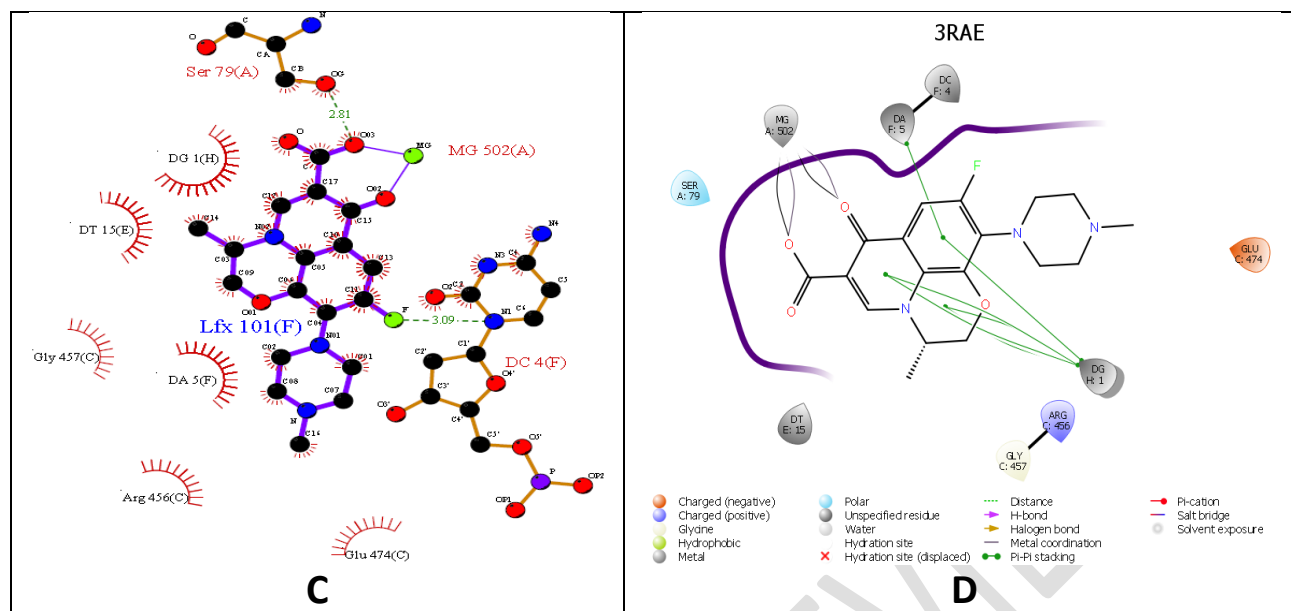
The L-isomer of the D, L-racemate ofloxacin, levofloxacin is an oral fluoroquinolone antibiotic that was created in Japan. Levofloxacin has a tolerability profile that is comparable to other oral fluoroquinolones, with gastrointestinal and central nervous system side effects being the

most frequent. The pharmacokinetics of levofloxacin is not influenced by food, age, or gender.

**Mechanism of Action:** Modifications to the cell membrane porin channels or the DNA gyrase A or B subunits are two examples of chromosomal abnormalities that might cause bacterial resistance to levofloxacin[42-46].

**Binding pockets:** Levofloxacin is used in the research work by L. Mark Fisher and Mark R. Sanderson, and they discover that it exhibits hydrophobic interactions with ILE-170A. Along with ARG-117A, GLY-173A, ALA-175A, ASN-326A, SER-436D, LYS-458C, ASN-461C, and ASN-473, it also forms H-bonds with them [47]. Figure 4 displays the linezolid and levofloxacin liglot and 2D complexes.





**Figure 4. A) Ligplot and B) 2D Complex of Linezolid with 5N2C) Ligplot and D) 2D Complex of Levofloxacin with 3RAE**

## 2.9 Moxifloxacin

A fluoroquinolone antibiotic that exhibits a broad spectrum of activity, moxifloxacin is used to treat endocarditis, sinusitis, pneumonia, and TB [48, 49].

**Mechanism of Action:** Moxifloxacin inhibits the synthesis of bacterial DNA [50-52].

**Binding pockets:** In their article, Pan F. Chan, Velupillai Srikannathan, and Jianzhong Huang ~~came to the conclusion~~ concluded that moxifloxacin exhibits hydrophobic interactions as well as others with ARG-122A, GLY-459B, SER-84A, and GLY-473B [53].

## 2.10 Rifampicin

Rifampicin, also known as rifampin, was developed in 1968 and is still one of the main medications used to treat tuberculosis (TB) because of its sterilizing properties and capacity to accelerate recovery times when used at high doses [54-58]. ~~In order~~ To treat patients with pulmonary TB

who were chronically resistant to treatment, rifampicin was initially used in clinical settings in the late 1960s [59-61].

**Mechanism of Action:**The enzyme responsible for DNA transcription in bacteria, RNA polymerase, is inhibited by rifampicin[62].

**Binding pockets:**Gerard D. Wright and his research team demonstrated in their study, "Rifamycin Resistance Enzyme with an Unprecedented Mechanism of Action," that rifampicin interacts hydrophobically with LEU-176A, PHE-257A, and PRO-284A. The H-bonds with GLY-9A, THR-285A, ARG-213A, MET-205, ALA-204, VAL-215, ARG-201, MET-342, GLY-286, GLY-287, PHE-74, VAL-93, GLY-44, GLN-43, VAL-69 and ARG-196 are also shown [63].

### 2.11 Isoniazide

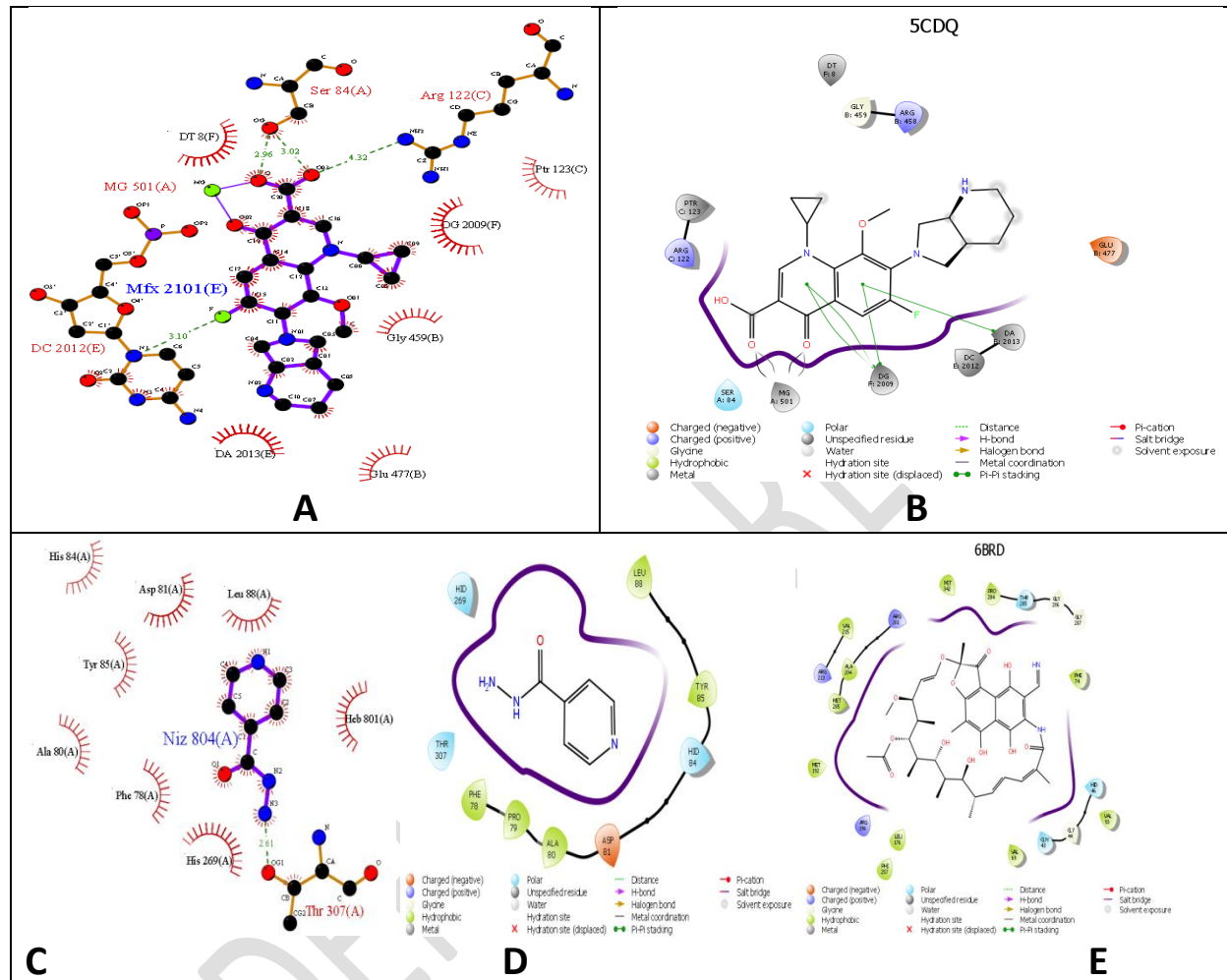
This synthetic drug was first designated by Meller and Melley in the year 1912. The antibiotic isoniazid, also referred to as isonicotinic acid hydrazide, is used to treat tuberculosis. It is often combined with rifampicin, pyrazinamide, and either streptomycin or ethambutol to treat active tuberculosis. It is frequently used alone to treat latent tuberculosis.

**Mechanism of Action:**Isoniazideinhibits mycolic acid biosynthesis[64].

**Binding pockets:** According to Gegia M. study, the isoniazide interacts hydrophobically with PHE-78, PRO-79, ALA-80, LEU-88 and TYR-85A. Instead, it displays an H-bond with the amino acid THR-307A. It Displays other interactions with THR307, HIS-269, and HIS-84.<sup>[65]</sup>Figure 5 displays the moxifloxacin, rifampicin and isoniazide liglot and 2D complexes.

### 2.12Pyrazinamide

Because of its unique method of action (interference with ATP synthesis), pyrazinamide will probably continue to play a significant role for treatment of drug-susceptible and multidrug-resistant TB (MDR-TB).



**Figure 5. A) Ligplot and B) 2D Complex of Moxifloxacin with 5CDQC) Ligplot of Isoniazidecomplexed with 4PAE D& E) 2D Complex of Isoniazide with 4PAE&Rifampicin with 6BRD**

**Mechanism of Action:**Pyrazinamide inhibits the growth of bacteria[66-67].

**Binding pockets:**According to Petrella, S. study published in the PLoS One journal, pyrazinamide exhibits interactions with the following

amino acids: ARG-255A, HIS-109, GLN-105A, ASP-108A, GLU-258 and PRA-598[68].

### 2.13 Ethambutol

The main purpose of the drug ethambutol is to cure tuberculosis. It is typically administered along with other anti-tuberculosis drugs such as isoniazid, rifampicin, and pyrazinamide. Mycobacterium avium complex and Mycobacterium kansasii may also be treated with it.

**Mechanism of Action:** The bacteriostatic medication ethambutol prevents bacilli from reproducing by interfering with the formation of arabinogalactan in the cell wall [69].

**Binding pockets:** In their article, Zhang, L. demonstrated that ethambutol has hydrophobic and other interactions with VAL-1004A, ASP-279A, TRP-965, TRP-572, TYR-282, ILE-283, TYR-314 and ARG-383[70].

### 2.14 Para Amino Salicylic Acid

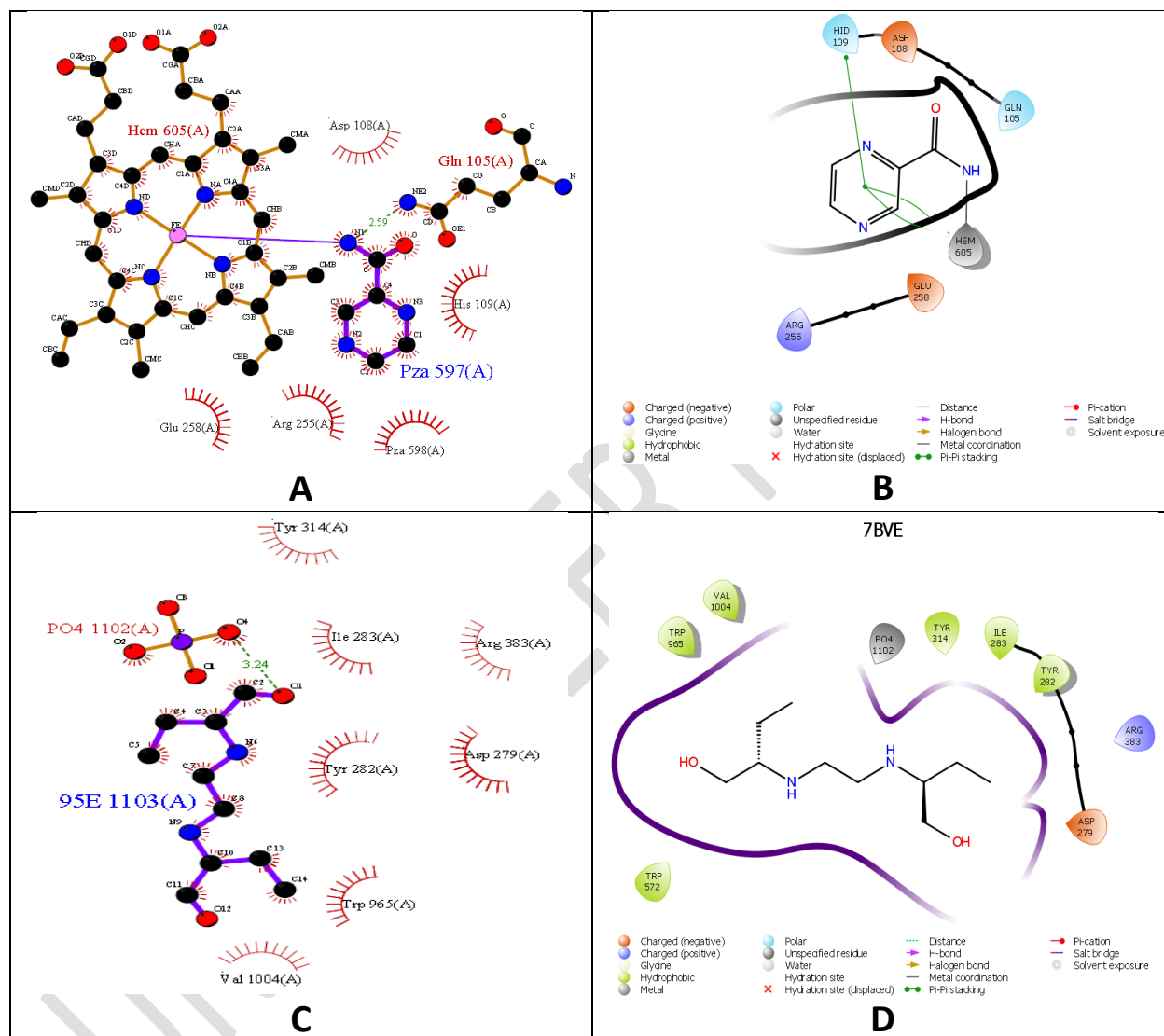
In the 1940s, it was discovered that para-amino salicylic acid (PAS), a second-line anti-TB medication (SLD), was useful for treating tuberculosis.

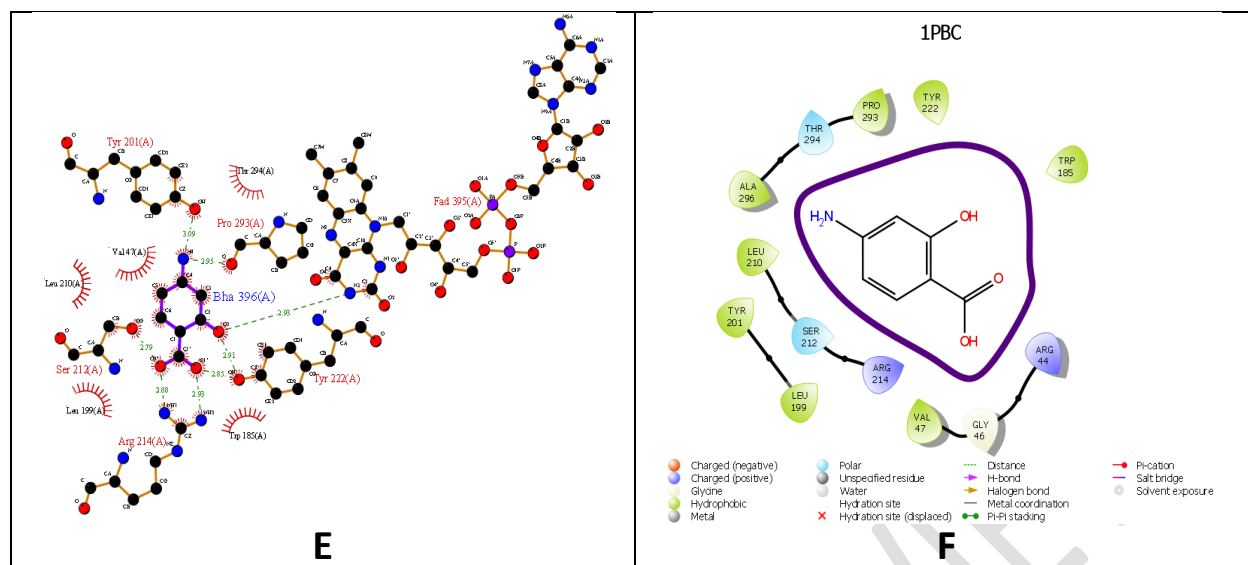
**Mechanism of Action:** Inhibition of folic acid synthesis[71].

**Binding pockets:** In their article, Schreuder, H.A. demonstrated that the para-aminosalicylic acid exhibits interactions with TRP-185, TYR-222, PRO293, ALA-296, LEU-210, TYR-201, LEU-199, VAL-47, ARG-214, SER-212, THR-296 and ARG-44 that are hydrophobic and others[72]. Figure 6 displays the pyrazinamide, ethambutol, and para-amino salicylic acid liglot and 2D complexes.

## 3. RESULTS AND DISCUSSION

The 3D crystal structures of all the enzymes were retrieved from the protein data bank-RCSB (<http://www.rcsb.org>). For interpretation and visualization of interactions of protein-ligand complex, Mastero and Biovia discovery studio visualizer and freely accessible server PDBsum (<http://www.ebi.ac.uk/pdbsum>) for Ligplot were used[73].





**Figure 6. A) Ligplot and B) 2D Complex of Pyrazinamide with 3PL1 C) Ligplot and D) 2D Complex of Ethambutol with 7BVE E) Ligplot and D) 2D Complex of PAS with 1PBC**

Bedaquiline forms non-bonded contacts with amino acids of 7JGC protein. The oxygen and carbons of amino acid proline-172A forms non bonded contacts with carbon and oxygen atoms of bedaquiline with varying distances (3.05-3.65Å). Isoleucine-173A forms two non bonded contacts with O and C atoms of bedaquiline with distance 3.05-3.24Å. Valine-176A formed two interactions with carbons having distance 3.51 and 3.35A. Five different number carbons of Glutamic acid-65 interacts with C and N atoms of bedaquiline and oxygen atoms of seven different positions of Glutamic acid-65 forms interactions with C and O atoms of the molecule BQ with varying distances. The Tyrosine-68 residue forms non bonded contacts with different C and N atoms of bedaquiline. Also, C and O atoms of Alanine-66 and 67, Leucine-63 and 72 and Phenylalanine-69 residue creates interactions with C atoms of BQ (distances ranges from 3.10 to 3.90 Å).

The drug delamanid formed 18 (hydrogen bonds) bonded and 140 non-bonded interactions with 3R5R.O and N atoms of amino acids from A chain Arginine-54 and 60, Lysine-55 and 79, Thyronine-56, Proline-63, Tyrosine-65 and 133, Alanine-76, Methionine-87, Treptophan-88, Asparagine-91 and Valine-46 formed hydrogen bonds (having distance

ranges from 2.56 to 3.26Å) with different number of O and N atoms of delamanid molecule. Pretomanid forms one hydrogen bond and 62 non-bonded interactions with protein 4XJO. Nitrogen atom of Glycine-93B formed hydrogen bond with oxygen-34 atom of pretomanid molecule with distance 2.92Å. In non-bonded interactions of Proline-24A and 317B, Tyrosine-25A, tryptopan-64A, Tyrosine-157A, Glycine-172A, 316B and 93, Methionine-91, Threonine-318B amino acids were involved. Sutezolid forms one hydrogen bond and 67 non-bonded interactions with protein 5NZ1. Nitrogen atom of Asparagine-179A formed hydrogen bond with oxygen-10 of sutezolid molecule with distance 2.19Å. Macozinone molecule forms 3-(hydrogen bonds) bonded and 33 non-bonded contacts with protein 4NCR. Nitrogen atom of Lysine-134A and 418A interacts with oxygen of molecule with distance 3.16Å and 3.03 respectively. S atom of Cystine-187A formed hydrogen bond with N atom with distance 1.66 Å.

Linezolid forms single hydrogen bond and 53 non-bonded interactions with protein 5NZ0. Nitrogen atom of Asparagine-179A formed hydrogen bond with C-18 atom of linezolid molecule with distance 3.13Å. MET-102A, TRP-103A, GLY -106A, ILE-107A, PHE-110A, TRP-145A, TYR-148A, THR-149A, ASN-176A and 179A and TRP-207A residues contributes into non-bonded contacts. Levofloxacin forms a hydrogen bond and 87 non-bonded interactions with protein 3RAE. Oxygen G atom of Serine-79A formed hydrogen bond with O-18 atom of levofloxacin molecule with distance 2.81Å. Moxifloxacin molecule forms 3-(H-bond) bonded and 87 non-bonded contacts with protein 5CDQ. Nitrogen atom of Lysine-134A and 418A interacts with oxygen of molecule with distance 3.16 and 3.03Å respectively. Rifampicin formed 2 hydrogen bonds and 52 non-bonded interactions with protein 6BRD. Nitrogen from Arginine-196 and 216A binds with oxygen atom no.8 and 2 of rifampicin molecule with distance 2.67 and 3.18Å respectively. Isoniazide formed one hydrogen bond and 53 non-bonded contacts with protein 4PAE. Amino acid Thyronine-307A binds with N-3 of Isoniazide molecule with distance 2.61Å.

Pyrazinamide formed 1 hydrogen bond and 5 non-bonded contacts with protein 3PL1. Amino acid Glanine-105A formed hydrogen bond with N of pyrazinamide molecule with distance 2.59Å. Ethambutol formed 1 hydrogen bond and 20 non-bonded interactions with the protein 7BVE. Aspartic acid-279A formed hydrogen bond with nitrogen of ethambutol with distance 5.14Å. Para-amino salicylic acid formed 10 hydrogen and 42 non-bonded contacts with the protein 1PBC. The residues involved in the hydrogen bonds are Tyrosine-201 and 222A, Serine-212A, Arginine-214 and Proline-293A. The information about crystal structures of the entire protein-ligand complexes were summarized in table 1.

**Table 1. FDA Approved Antitubercular drugs MOA and their PDB details:**

S. N.	Drug	PDB Code	Classification	Organism	Mutation	Resolution	Mechanism of Action
1	Bedaquiline	7JG C	Hydrolase	M. Smegmatis	No	3.40 Å	Inhibits the Proton pump ATP Synthase
2	Delamanid	3R5 R	Oxidoreductase	M. tuberculosis	No	2-10 Å	Inhibits mycolic acid biosynthesis.
3	Pretomanid	4XJ O	Transferase inhibitor	M. tuberculosis	No	1.50 Å	Inhibition of mycolic acid synthesis
4	Contezolid	6W QN	Ribosome	S. aureus	Yes	2.90 Å	Inhibits protein synthesis.
5	Sutezolid	5NZ 1	Transcription	M. Tuberculosis	No	2.33 Å	Inhibits protein synthesis.
6	Macozinone	4NC R	Oxidoreductase	M. Tuberculosis	No	1.88 Å	By forming a covalent bond

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				is			with a cystine residue, it prevents the synthesis of cell wall.
7	Linezolid	5NZ0	Transcription	M. tuberculosis	No	1.82 Å	Inhibits translocation by binding to the 50s subunits of ribosomes.
8	Levofloxacin	3RAE	Isomerase / DNA/Antibiotic	S. pneumoniae	No	2.90 Å°	Inhibits DNA gyrase subunit (Gyrase A & Gyrase B) topoisomerase iv subunit (Part C & Part E).
9	Moxifloxacin	5CDQ	Hydrolase	S. aureus subaureus	No	2.95 Å°	Inhibits DNA gyrase subunit (Gyrase A & Gyrase B) topoisomerase iv subunit (Part C & Part E).
10	Rifampicin	6BRD	Oxidoreductase	S. venezuelig e	No	3.32 Å°	Inhibits RNA polymerase.
11	Isoniazide	4PAE	Oxidoreductase	Synechococcus elongatus	Yes	3.21 Å	Inhibits synthesis of mycolic acid.
12	Pyrazinamide	3PL1	Oxidoreductase	A. flavus	Yes	1.68 Å°	Inhibits the growth of bacteria.

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13	Ethambutol	7BE	Transferase	M. Smegmatis	No	2.81 Å	Inhibition of arabinosyl transferase.
14	PAS	1PC	Oxidoreductase	Pseudomonas fluorescens	No	2.80 Å	Inhibition of folic acid synthesis.

#### 4. CONCLUSION

Collectively, our research offers a structural basis for understanding the significance of binding sites of amino acids of a particular protein. It serves as a useful tool for understanding the relationship between structure and function. ~~On the basis of~~ Based on significant binding pockets, including donors and acceptors of amino acid residues, the researchers can investigate the newer molecular architectures for treating M. tuberculosis. The main finding is that future research using combined ligands and receptor-based design will generate a new class of anti-tubercular drugs that will benefit millions of patients.

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