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Design, Synthesis, Molecular Docking, Characterization of Aniline Derivatives and Their Evaluation of Anti-Tubercular Activity

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ABSTRACT

A series of novel Aniline derivatives have been synthesized in multiple steps with the suitable reactions and then characterized by various analytical techniques. All the synthesized compounds were evaluated for their biological evaluation of Anti-tubercular activity. Mycobacterium originated more than 150 million years ago. The German microbiologist Robert Koch in 1882 conformed the rod shaped organism caused TB. The discovery of the Bacillus-Calmette Guerin (BCG) vaccine in 1908 and anti-tuberculosis drugs starting in 1943, offered hope for the eradication of this disease[5]. Mortality rates decreased significantly from the early to mid-20th century. The Directly Observed Treatment Short-Course (DOTS) program was introduced in 1993. In 1998 the DOTS-plus program was introduced to address multidrug resistant (MDR) TB.

The Food and Drug Administration (FDA), on 28 December 2012, granted accelerated approval to SIRTURO™ (bedaquiline) Tablets as a part of combination therapy in adults with multidrug- resistant TB (MDR-TB). It is the first new anti-TB drug to be approved after 1998 (rifapentine was approved in 1998) and the first anti-TB drug with a novel mechanism of action to be approved after 40 years (rifampicin was approved in 1974). It is also the first to be introduced specifically for the treatment of MDR-TB in combination with other drugs.

KEYWORDS: Aniline derivatives, Tuberculosis, Mycobacteria, Tubercular drugs, Biological evaluation of anti tubercular activity.

I. INTRODUCTION

Medicinal chemistry is a chemistry based discipline, also involving aspects of biological, medical and pharmaceutical science. It is concerned with the invention discovery, design, identification and preparation of biologically active compound, the study of their metabolism, interpretation of their mode of action at the molecular level and construction of structural activity relationship. The synthesis of new derivatives has being an important part and aimed at modifying the action of drugs, particularly to reduce the side effects and to potentiate the drug action. Today more than 60% drugs used in practices are synthesized derivatives and day by day the scope of synthetic medicinal chemistry is broadening.[1]

Tuberculosis

Tuberculosis is an infectious disease usually caused by the bacteria Mycobacterium tuberculosis. Tuberculosis may infect any part of the body, but most commonly occur in the lungs (known as pulmonary tuberculosis). Extra pulmonary TB occurs when tuberculosis develops outside of the lungs. Extra pulmonary TB may co exist with pulmonary TB. The classic symptoms of active TB are chronic cough with blood-tinged sputum, fever, night sweat and weight loss.

Tuberculosis is spread through the air when people who have active TB in their lungs cough, spit, speak or sneeze. Active infection occurs more often in people with HIV/AIDS and in those who smoke.[2]

Tuberculosis is a chronic granulomatous disease which is a major health problem in developing countries. About one third of the world's population is infected with Mycobacterium tuberculosis.[3]

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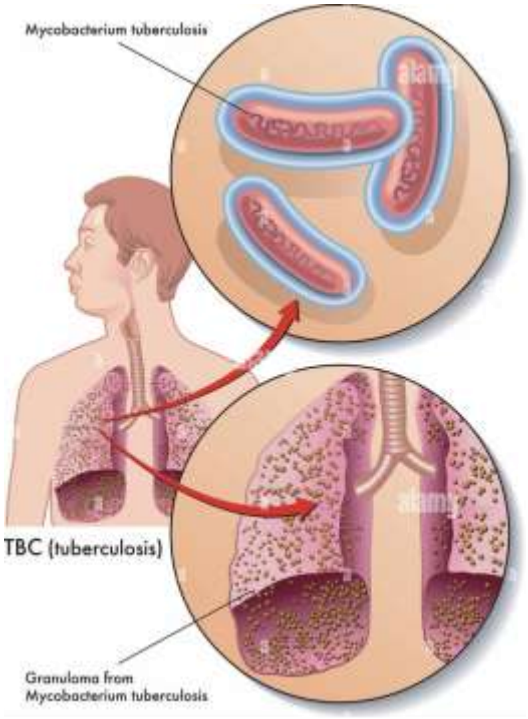

Types of tuberculosis:

Tuberculosis is a contagious disease; it affects almost all the important organs of the body and are generally considered to be,[4]

1.Pulmonary tuberculosis,

2.Extra pulmonary tuberculosis.

According to WHO global tuberculosis report

The World Health Organization (WHO) has published a global TB report every year since 2021. The purpose of the report is to provide a comprehensive and up-to-date assessment of the status of the TB epidemic, and of progress in the response to the epidemic – at global, regional and country levels – in the context of global commitments and strategies.

The report is based primarily on data gathered by WHO in annual rounds of data collection. In 2020, data were reported by 198 countries and territories that accounted for more than 99% of the world's population and estimated number of TB cases.[5]

TB burden in India

Each year 12 lakh (1,200,000) Indians are notified (that is reported to the RNTCP) as having newly diagnosed TB. In addition at least 2.7 lakh (270,000) Indians die. Some estimates calculate the deaths as being twice as high.[6]

Mycobacteria

The main cause of TB is Mycobacterium tuberculosis which is a rod shaped, small, aerobic, non motile bacillus. The high lipid content of this pathogen accounts for many of its unique clinical characteristics.

It divides every 16 to 20 hours .Which is an extremely slow rate compared with other bacteria. Mycobacterium have an outer membrane lipid bilayer. If a gram stain is perform, MTB either stains very weekly gram – positive or does not retain dye as a result of the high lipid and mycolic acid content of its cell wall.[7]

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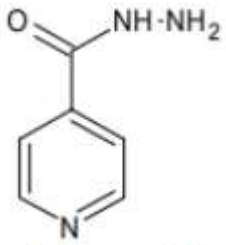
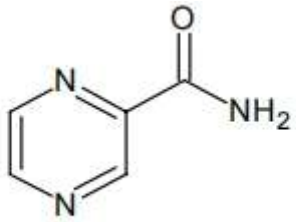
Fig. 3 Mycobacterium Tuberculosis.

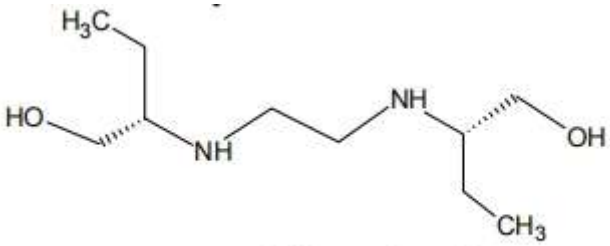
Chemical structures of Anti-tubercular drugs:



Isoniazid



Pyrazinamide



Ethambutol

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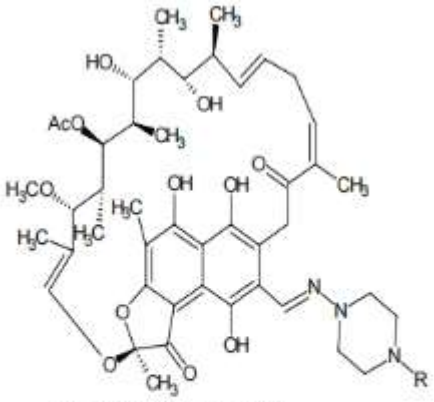
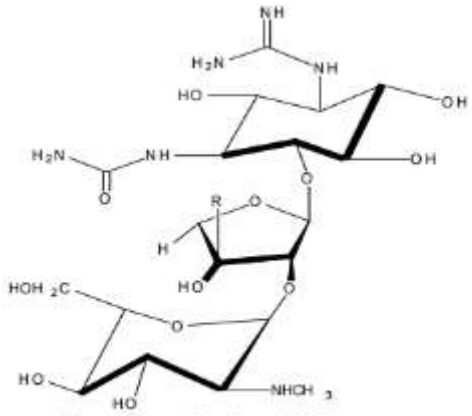


Rifampicin R = CH_3
Rifabutin R = $CH_2CH(CH_3)_2$
Rifapentine R =



Streptomycin R = CHO
R = CH_2OH

Classification of anti tubercular drugs[8]

First line drugs:

- Isoniazid (H)
- Rifampin (R)
- Pyrazinamide (Z)
- Ethambutol (E)
- Streptomycin (S)

Second line drugs:

1.Fluoroquinolones

- Ofloxacin
- Levofloxacin
- Moxifloxacin
- Ciprofloxacin

2.Other oral drugs

- Ethionamide
- Prothinomide
- Cycloserine
- Para amino salicylic acid (pas)
- Rifabutin

3.Injectable drugs

- Kanamycin
- Amikacin
- Capreomycin

II. MATERIALS AND METHODS

Molecular docking by drug design

Argus lab 4.0® is freely available software for windows platform distributed by Plannaria® software. It is an introductory molecular modeling package for academics. Docking involves the fitting of a ligand into the binding site with most favorable interaction. Docking allows the medicinal chemist to virtually screen a set of compounds and predict the strongest binding capacity based on various scoring function. It explores ways in which two molecules such as ligand and receptor (protein) fit together and docks to each other well. The molecule binding to a receptor inhibits its function and thus acts as drug.

Working with AutoDock 4® includes 3 steps:

1. Preparation of receptor & ligand files.
2. Calculation of affinity maps by using a 3D grid around the receptor & ligand.
3. Defining the docking parameters and running the docking simulation.[9]

Insilico screening of drug likeness

The designed and docked molecules were screened using **Molinspiration® cheminformatics software** to evaluate for their drug likeness. Molinspiration helps in the calculation of important molecular properties such as logP, polar surface area, number of hydrogen bond donors and acceptors, as well as prediction of bioactivity score for the most important drug targets like GPCR ligands, kinase inhibitors, ion channel modulators and nuclear receptors.[10]

Synthetic methodology

Procedure: Synthesis of Schiff bases

Aromatic primary amines (0.01mol) were dissolved in 30ml ethanol containing few drops of glacial acetic acid. The appropriate aromatic aldehyde (0.01mol) was added to reaction mixture. It was refluxed for 3 to 5hrs, cooled and poured in

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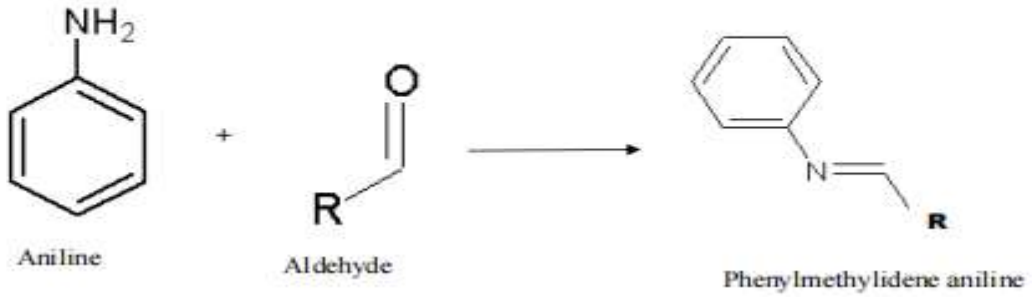
to beaker contain crushed ice. The solid obtained was filtered, washed with water and crystallized using ethanol.



COMPOUND 01- R = Benzaldehyde,
COMPOUND 02 R = Vannilin,
COMPOUND 03-R = Salicylaldehyde,
COMPOUND 04- R = Cinnamaldehyde.

CHARACTERIZATION

Melting point :

The melting point of the synthesized compounds is determined by capillary tube method .

TLC :

Thin layer chromatography used to determine the purity of the compounds using readymade silica gel plate and spots were visualized using iodine chamber. The solvent system used chloroform & ethanol **(6:4).**

IR spectroscopy : [11]

Infrared (IR) spectrometry is one of the most common spectroscopic techniques used by organic chemists for detection of functional compounds and mixtures and for compound comparison. The spectrum obtained in minutes using a few mg of the compound which can also be recovered. IR spectroscopy is an important and popular tool for structural elucidation and compound identification. Infrared spectrum shows per cent transmittance versus frequency expressed as wave numbers.

IR spectrum were recorded by absorption of infrared radiation it causes changes in vibrational energy in the ground state. Using the KBr pellet press method and determined the functional group present in the compounds .

NMR spectroscopy : [12]

Nuclear magnetic resonance (NMR) spectroscopy is the important analytical technique available for organic chemist. It involves the interaction of the electromagnetic radiation and the hydrogen of the nucleus when placed in an external static magnetic field. NMR spectra will provide detailed information about a molecule's structure and will prove what the compound really is. NMR is a nondestructive technique. The NMR spectra were recorded on 300 MHz BRUKER Advance III NMR spectrometer. DMSO was used as a solvent .

Nuclear magnetic resonance spectroscopy is a technique that permits the transition of a molecule at the level of the individual atom and giving information about the environment of that atom.(beckett and stanlake)

Biological activity

MABA Procedure

The anti-mycobacterial activity of compounds were assessed against M. tuberculosis using micro plate Alamar Blue assay (MABA).Briefly, 200µl of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation.The 96 wells plate received 100 µl of the Middlebrooks 7H9 broth and serial dilution of compounds were made directly on plate. The final drug concentrations tested were 100-0.2 µg/ml.Plates were covered and sealed with Para film and incubated at 37ºC for five days.After this time, 25µl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink.[11]

III. RESULT AND DISCUSSION

In our present research work, we have synthesized Schiff base derivatives. The

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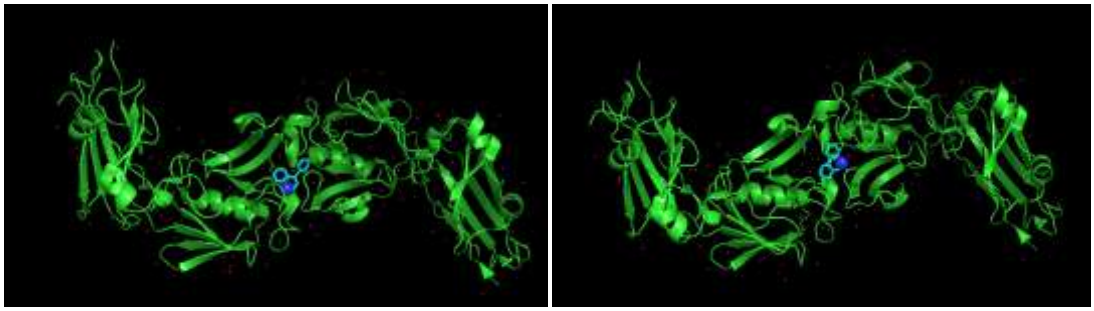
physicochemical properties of derivatives were calculated from computational tools and characterization of compounds carried out by spectral methods. The pure compounds were screened for anti-tubercular activity.

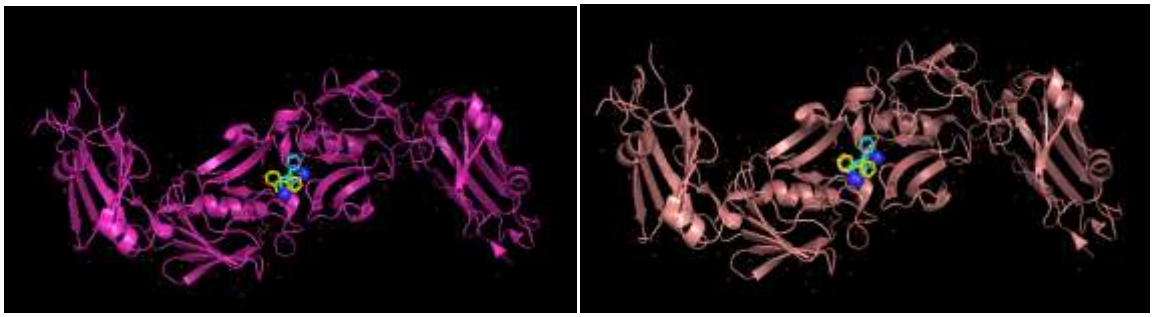
Docking

The designed molecules were docked against the selected target L-transpeptidases. The best and stable docked pose was selected based on the docking score and the basis of multiple interactions. On comparison, it was found that, interaction of amino acids Serine 533/296/609 Histidine 605/336/352 and Isoleucine 521 was most common amino acid residues for all the synthesised compounds as well as standard drugs INH and Pyrazinamide. Binding score for the synthesized compounds;
COMPOUND 01:-5.64,
COMPOUND 02:-6.27,
COMPOUND 03:-6.74,
COMPOUND 04:-5.88.
Standard drugs INH -6.8869 and Pyrazinamide -5.9686Kcal/mol.

DOCKING REPORTS



COMPOUND O1COMPOUND O2



COMPOUND 03 COMPOUND 04

ANALYTICAL & SPECTROSCOPICAL CHARACTERISTICS

The selected compounds were synthesized and purified by chromatographic techniques. The melting points of the synthesized compounds were determined by one end open capillary tube method. The temperature at which the compound starts losing its crystallinity and changes from solid to liquid form was found recorded. COMPOUND 01: 128 ^{o}C; COMPOUND 02: 134 ^{o}C; COMPOUND 03: 115 ^{o}C ; COMPOUND 04: 139 ^{o}C.All the synthesized Products obtained with a yield of about 80-85%. The structures of the final compounds were confirmed on the basis of spectral studies. All the newly synthesized compounds were characterized by IR, NMR spectroscopy.

COMPOUND 01
N[(Z)phenylmethylidene]aniline,
$C_{13}H_{11}N$ was obtained as a Light Brown color Solid;
FT-IR (KBr) cm^{-1}-2922 cm^{-1} (C-H), 2725 cm^{-1}(C=N), 1562 cm^{-1} (C=C), 1181 cm^{-1}(C-N)
1H-NMR- δ:6.9-7.8ppm (m, 7H Ar-H); 8.3ppm (s, 1H N-CH); 3.85ppm (s,3H N-H); MS m/z (M+): 280

COMPOUND 02
2-methoxy-4-[(Z)-(phenylimino)methyl]phenol,
$C_{14}H_{13}NO_2$ was obtained as a Light Yellow color Solid;
FT-IR (KBr) cm^{-1}-2935 cm^{-1} (C-H), 2836 cm^{-1} (C=N), 1744 cm^{-1}(C=C), 1161 cm^{-1} (C-N), 1080 cm^{-1} (C-O), 2960 cm^{-1} (OH).

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1H-NMR- (CDCI3) δ:6.9-7.8ppm (m, 8H C-H);8.4ppm (s, 1H N-CH);3.8ppm (s,3H N-H); MS m/z (M+): 245.

COMPOUND 03

2-[(Z)-(phenylimino) methyl]phenol,

$C_{13}H_{11}NO$ was obtained as a Brown color Solid

FT-IR (KBr) cm^{-1}- 2925 cm^{-1} (C-H), 2855 cm^{-1}(C=N), 1590 cm-1(C=C), 1276 cm^{-1} (C-N), 2657 cm^{-1}(OH)

1H-NMR δ:7-8ppm (m, 8H C-H);8.3ppm (s, 1H N-CH);3.10ppm (s,3H N-H); MS m/z (M+): 290

COMPOUND 04

N-[(1Z)-3-phenylpropylidene]aniline,

$C_{15}H_{15}N$ was obtained as a of white solid;

FT-IR (KBr) cm^{-1}- 2923 cm^{-1} (C-H), 2855 cm^{-1} (C=N), 1642 cm^{-1} (C=C), 1335 cm^{-1}(C-N).

1H-NMR :δ:6.9-7.8ppm (m, 7H Ar-H); 8.3ppm (s, 1H N-CH); 3.85ppm (s,3H N-H); MS m/z (M+): 280

Biological activity:

The in-vitro anti-tubercular activity of compounds were assessed against M. tuberculosis H37RV using Micro plate Alamar Blue assay (MABA).

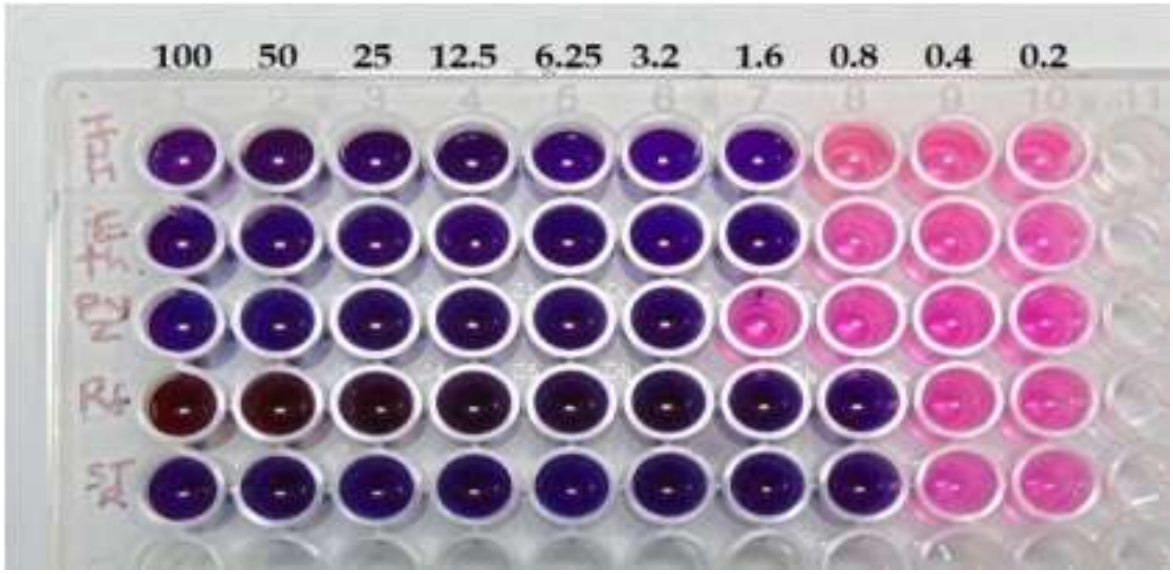
COMPOUND 01: 6.25 µg/ml.

COMPOUND 02: 6.25 µg/ml.

COMPOUND 03: 25 µg/ml.

COMPOUND 04: 25 µg/ml.

STANDARD DRUG PHOTOGRAGH



PHOTOGRAPH OF MABA METHOD

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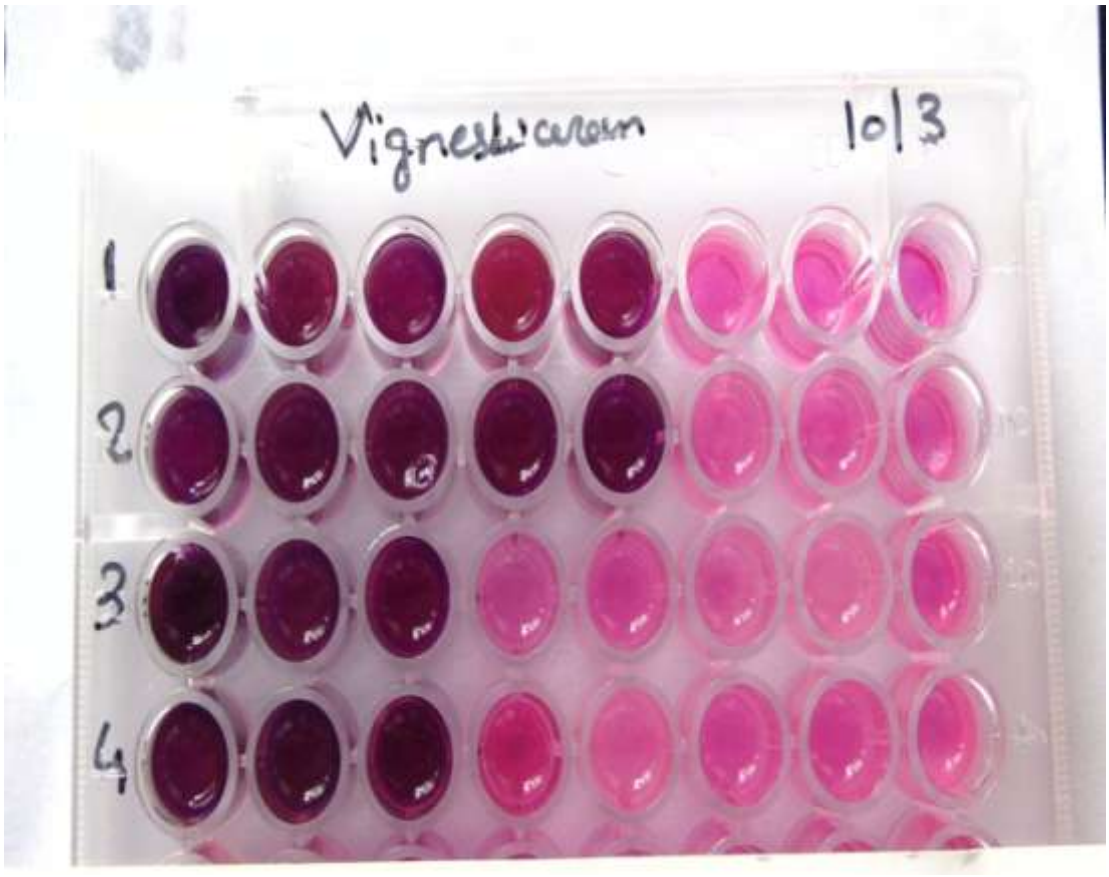

In-vitro anti-tubercular activity reports

All the synthesized compounds showed good and moderate activity against mycobacterium tuberculosis.

Results:

Sl. No.	Sample	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
01	01	S	S	S	S	S	R	R	R
02	02	S	S	S	S	S	R	R	R
03	03	S	S	S	R	R	R	R	R
04	04	S	S	S	R	R	R	R	R

S-Sensitive
R-Resistant

The compounds 01 & compounds 02 sensitive at 6.25 µg/ml, Compounds03 and
Compounds 04 Sensitive at 25 µg/ml.

IV. CONCLUSION

Our work concludes that our synthesized molecules are effective in inhibiting the target enzyme L, D-Transpeptidase-2 (3VAE), which is important forthe cell wall synthesis of Mycobacterium tuberculosis.

The synthesized compounds were active at 100 - 6.25µg/ml, which were compared to the

known anti-TB drugs: Pyrazinamide - 3.125µg/ml, Ciprofloxacin - 3.125µg/ml and Streptomycin - 6.25µg/ml.

Among all the compounds, Compound 01 and Compound 02 showed potent activity against Mycobacterium tuberculosis and Compound 03 and Compound 04 showed significant activity against Mycobacterium tuberculosis . Based on the anti-tuberculosis activity, docking and molecular design studies, the synthesized molecule can be considered as promising lead molecules for the development of new anti-tuberculosis medicines.

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