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Synthesis and Evaluation of Heterocyclic Derivative as Anti-Tubercular Agent

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Abstract: ***Tuberculosis is considered a worldwide health problem mainly due to co-infection with HIV and proliferation of multi-drug resistant strains. Sparking recent interest in finding new antitubercular agents with different chemical scaffolds and mechanisms of action. Sparking recent interest in finding new antitubercular agents with different chemical scaffolds and mechanisms of action. Mycobacterium tuberculosis shikimate kinase (MtSK), an enzyme present in the shikimate pathway in bacteria, is essential for the survival of the tubercle bacillus, representing an ideal target for therapeutic intervention given its absence in mammals. The last step in this pathway is performed by chorismate synthase (CS), which catalyzes the conversion of 5-enolpyruvylshikimate-3-phosphate (EPSP) to chorismate. In summary, we report new drug scaffolds targeting the essential protein MtSK that can be more selective antitubercular drugs.***

Keywords: Mycobacterium tuberculosis, Shikimate kinase, Chorismate synthase, Synthesis antitubercular drug, FT-IR, 13C NMR, 1H NMR, Mass Spectroscopy, In-silico studies, Biological testing, MABA Method

1. Introduction

1.1 Background

Tuberculosis remains one of the major global health threats leading to morbidity and mortality. One in three persons across the world representing 2-3 billion individuals are known to be infected with *Mycobacterium Tuberculosis*, of which 5-15% are likely to develop active TB disease during their lifetime. [1] There has been no much development in the first-line/classes of drugs for TB due to which totally drug resistant (TDR) strains deadpan to the current line of treatment have emerged. [2] Recently, USFDA granted accelerated approval to Johnson and Johnson's drug "Bedaquiline" to treat resistant TB. [3]Therefore, there is an imperative call for developing faster acting and effective new anti-tubercular agents, ideally belonging to new structural classes, to better fight against TB, including MDR-TB and XDR-TB, to cut down the duration of current treatment, to improve patient compliance and to provide effective treatment of dormant tuberculosis infection. [2]
Current anti-tubercular drugs chiefly target cellular processes involved in bacterial growth and are either bacteriostatic or bactericidal. These include cell wall synthesis inhibitors, nucleic acid synthesis inhibitors, protein synthesis inhibitors, and energy inhibitors. Newer drugs with novel targets are being exploited to cope up with the problems of multidrug tolerance and dormant TB populations. [5]

Recent development in mycobacterial molecular genetics tools have assisted the identification and validation of new drug targets essential for tubercular bacilli not only *in vitro* but also for its survival and persistence *in vivo*. [6]

2. Shikimic Acid Pathway

Shikimic acid pathway, also known as the chorismate biosynthesis pathway, is a seven step enzymatic reaction for conversion of two metabolites, phosphoenolpyruvate (PEP) of the glycolysis pathway and erythrose-4-phosphate of the non-oxidative branch of the pentose phosphate pathway, into chorismate. [7]It is a potential and favourable target for drug design. It is vital for survival of microorganisms and is exclusive to microorganisms. This pathway is not present in mammals which permit detection of targets that can probably decrease toxicity of drug candidates. The fact that shikimate pathway is essential for *M. tuberculosis* even in the existence of exogenous supplements as p-aminobenzoate, p-hydroxybenzoate and aromatic amino acids emphasize its importance as a drug target. Therefore, inhibition of critical enzymes involved in this pathway seems to be an attractive target for development of new anti-infective agents. [2]

One of the most potential enzymes of the shikimic acid pathway is Shikimate kinase (SK), which is involved in the fifth step of the pathway. It is responsible for catalyzing the ATP dependent phosphorylation of shikimate to form shikimate-3-phosphate. In *M. tuberculosis*, the *aroK gene* codes for *M. tuberculosis* Shikimate kinase (MtSk) which catalyzes the SK reaction. The disruption of *arok*gene has been shown to be essential for the viability of *M. tuberculosis* which makes it an attractive target for design of new molecules. [8]

The crystal structure of MtSK-MgADP-shikimate provides essential information for the design of SK inhibitors that target both the shikimate and ATP binding pockets or exceptionally the shikimate-binding site. [9] The shikimate binding site is characterized by a hydrophobic surface together with a number of hydrophilic charged residues which project into the cavity. The binding of shikimate to its cavity presents essential residues that build possible interactions of ligand to its protein. [2]

Literature survey shows that structure-based virtual screening protocols have been used to predict MtSk inhibitors. Docking simulations have recognized that potential inhibitors had a structural relationship to a triazole or a tetrazole hetero-aromatic system which may offer a candidate lead for the discovery of MtSk inhibitors. [10] The presence of the sulphur moiety as an electron rich centre is able to improve lipophilicity and modulate electron density of the triazole ring, as well as its interaction with hydrogen bond donors of the organism. [11] It was reported that the best scoring compounds contained a mercapto group and a triazole or tetrazole ring in the scaffold. [8]

Triazoles are an important group of heterocyclic compounds which are biologically active and of extensive significance in medicinal chemistry. It is known to exhibit anti-tubercular activity along with other biological activities like antimicrobial, CNS depressant, anti-HIV, cytotoxicity, anti-inflammatory, analgesic, anticonvulsant and many other activities and are capable of crossing the blood-brain barrier. [12] Hence it was decided to synthesize heterocyclic deivatives without mercapto group or sulphur atom to elucidate its importance in antitubercular activity using aminoguanidine bicarbonate.

3. Experimental Work

3.1 Synthesis of the Heterocyclic Molecules

The purity of the starting materials used in the reaction was confirmed by melting point and thin layer chromatography (TLC). The purity and structures of the synthesized compounds were confirmed by melting point, thin layer chromatography, infrared spectroscopy, nuclear magnetic resonance spectroscopy and mass spectroscopy where applicable. The melting points of the compounds synthesized were uncorrected and recorded by open glass capillary method on "**DBK Prog. Melting Point Apparatus**" and complied with the reported melting points wherever applicable. Analytical thin layer chromatography (TLC) was carried out on pre-coated TLC plates (Silica gel GF254).1H NMR spectra were recorded on "**FT-NMR 400 MHz Analyzer**" at SAIF NMR RESEARCH CENTRE, INDIAN INSTITUTE OF SCIENCE, BANGALORE. IR spectra were recorded using "**Bruker Optik GmbH, ALPHA-T**" at Dr. L. H. Hiranandai college of pharmacy, Ulhasnagar. Mass spectra of a few compounds were

recorded on "**Shimadzu LC-MS 8040 System"** at Powai, Mumbai.
All the chemicals, reagents and solvents used in this study were acquired from Molychem, Sigma Aldrich.

3.1.1. Synthesis of new molecule

Figure No.3.1.1: Scheme for synthesis cyclized derivatives

Where R is,

Table 3.1.1: Aldehyde substitution for synthesis of substituted aminguanidine derivatives

Compound	R
TVD2	

3.1.2. Step 1-Synthesis of Schiff base:

General Reaction:

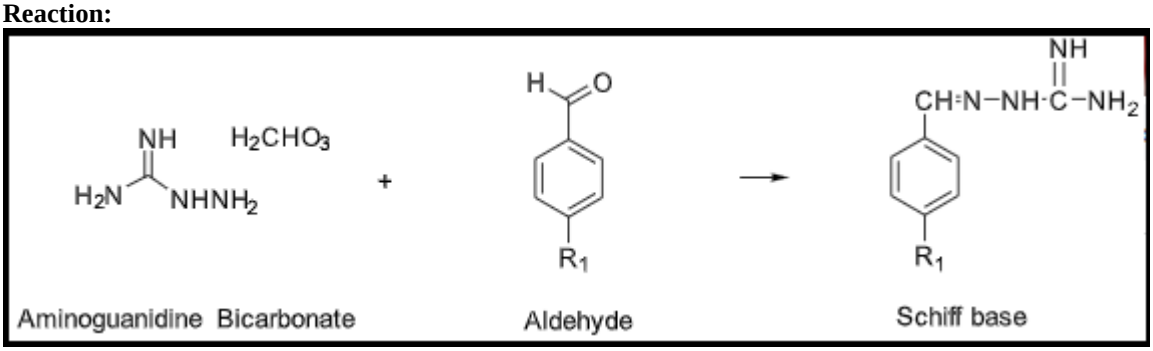

Figure 3.1.2.1: Scheme for synthesis Schiff bases.

General Procedure:
A mixture of aminoguanidine bicarbonate (1 mole) and substituted aldehyde (1 mole) in ethanol was heated under microwave at Power level 7 (420 W) for 10 min and 5 cycles and progress of reaction was monitored by TLC. After completion of reaction, the solution was cooled and added into crush ice with continuous stirring. Then dilute HCl was added till colour changed. Then sodium bicarbonate solution was added to neutralization and complete precipitation. The product thus obtained was filtered under vacuum, washed with cold water, dried and recrystallized from ethanol. All the derivatives (TVD2) were synthesized in the similar manner and characterized by spectral analysis.

TVD2: Synthesis of 2-[(4-Methylphenyl) methylideneamino] guanidine.

Molecular formula: $C_9H_{12}N_4$
Molecular weight: 176.1 g/m
TLC:
Stationary Phase: Silica Gel GF254
Mobile Phase: Ethyl Acetate: Benzene: Methanol (7: 3: 1)
Chromatogram: Single spot with Rf value = 0.56
Detection: Under short UV lamp in UV chamber.
Yield (%): 70.60 %

Melting Point (M. P.): 178-182°C

3.1.3. Step 2-Cyclization of Schiff Base:

General Reaction:

Figure 3.1.2.2: Scheme for synthesis Cyclized derivative from Schiff base

General Procedure:

To a solution of Schiff base in 10-20 ml of ethanol catalytic amounts of $FeCl_3$ wasadded. The contents were stirred at 60^0C on magnetic stirrer until the completion of reaction. The completion of reaction mixture was checked by thin layer chromatography. The product formed was filtered, washed with cold water, dried and recrystallized from ethanol. All the derivatives were synthesized in the similar manner and characterized by spectral analysis.

TVD2: 5-(4-Methylphenyl)-4H-1, 2, 4-triazol-3-amine

Molecular formula: $C_{10}H_{15}N_5$

Molecular weight: 205.26 g/m

TLC:

Stationary Phase: Silica Gel GF254

Mobile Phase: Ethyl Acetate: Benzene: Methanol (1: 1: 2)

Chromatogram: Single spot with Rf value = 0.46

Detection: Under short UV lamp in UV chamber.

Yield (%): 68.26 %

M. P.: 178-182°C

FT-IR Spectra of compoundTVD2:

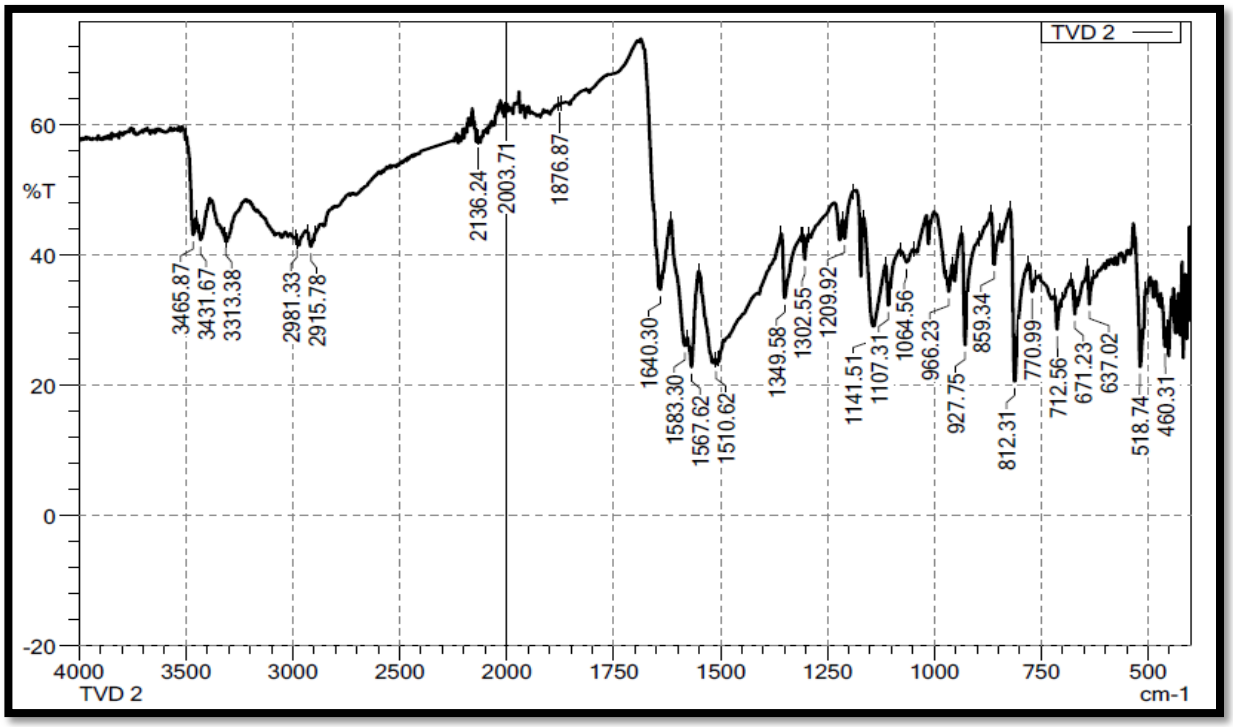

Figure 3.1.3.1: IR Spectra of compound TVD2

Table 3.1.3.2: IR values of compound TVD2.

Peak value (cm-1)	Groups
3465.87	- NH_2 stretching vibration
3313.38	- NH stretching vibration
2981.33	Aromatic CH stretching vibration
1640.30	- C=N stretching vibration
1583.30	- CH stretching vibration
2915.78	Aromatic –CH_3 stretching vibration

13C NMR of compound TVD2

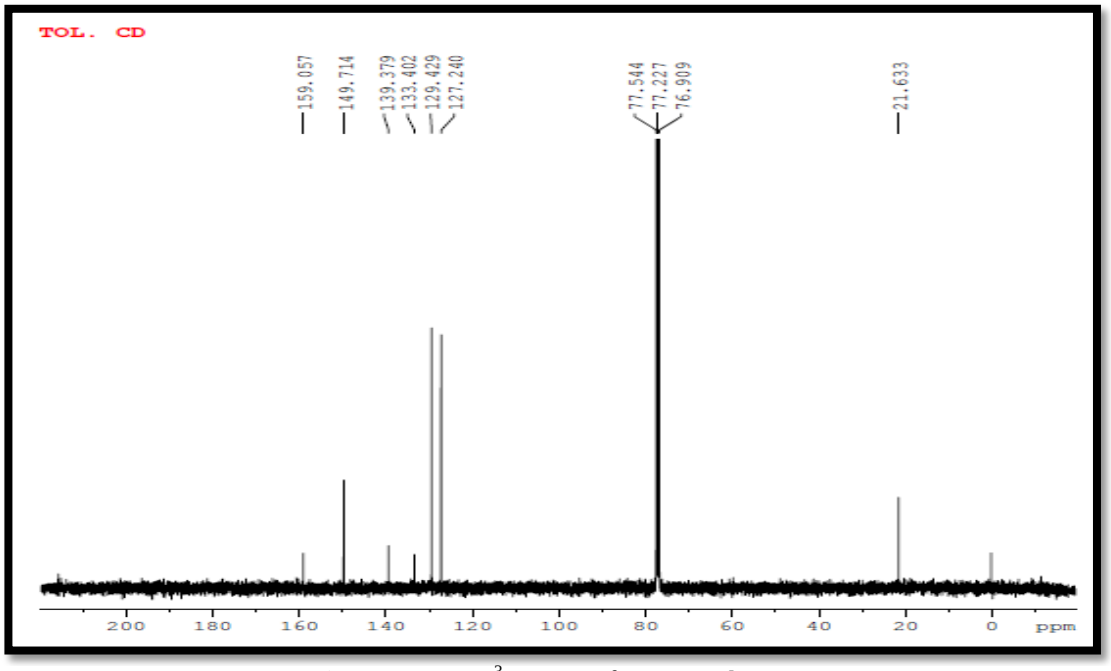

Figure 3.1.3.2: 1^3C NMR of compound TVD2

Table 3.1.3.3.1^3C NMR values of compound TVD2

Assignment	Carbon Type	Delta Value (ppm)
C1	>C-C	149.71
C2	>C-N	159.05
C3	C_6H_4 Aromatic Carbon	76.90
C4	C_6H_4 Aromatic Carbon	129.42
C5	C_6H_4 Aromatic Carbon	133.40
C6	C_6H_4 Aromatic Carbon	127.24
C7	C_6H_4 Aromatic Carbon	77.22
C8	C_6H_4 Aromatic Carbon	139.31
C9	- CH_3	21.63

1H NMR of compound TVD2

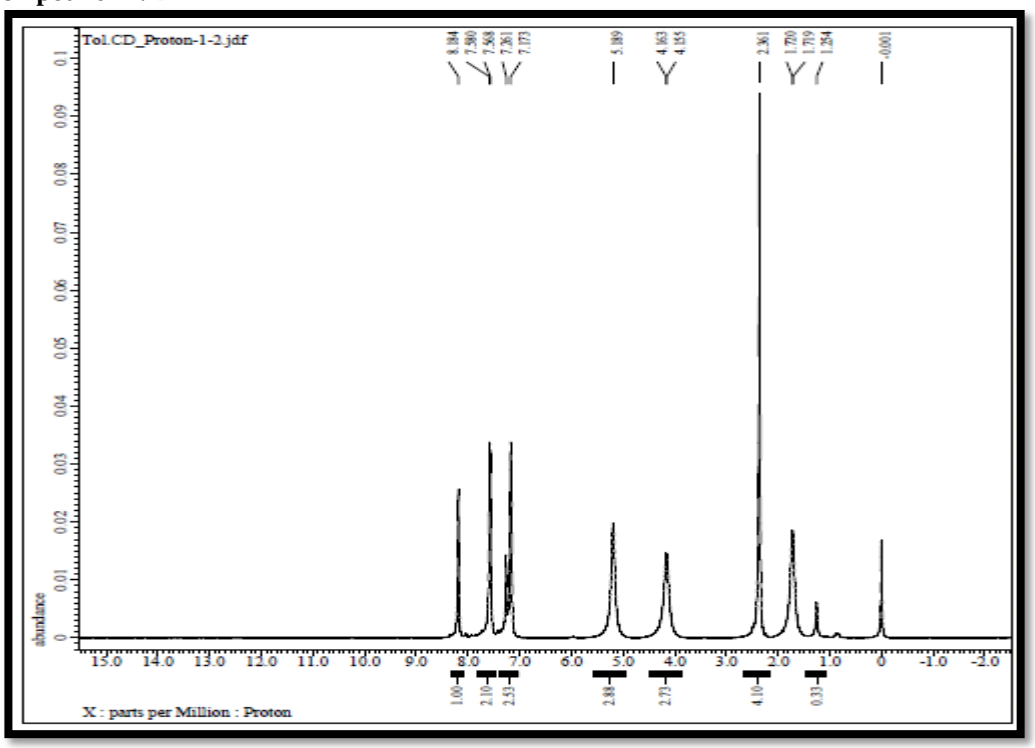

Figure No.3.1.3.3.1H NMR of compound TVD2

Table 3.1.3.4.1: H NMR values of compound TVD2

Group	Delta Value (ppm)	Number of protons	Multiplicity
- Ar-CH_3	2.461	3H	Multiplet
- NH_2	3.300	2H	Singlet
- Ar-H	8.078	1H	Doublet
- Ar-H	7.240	1H	Doublet
- NH	5.955	1H	Singlet

Mass Spectroscopy of compound TVD2:

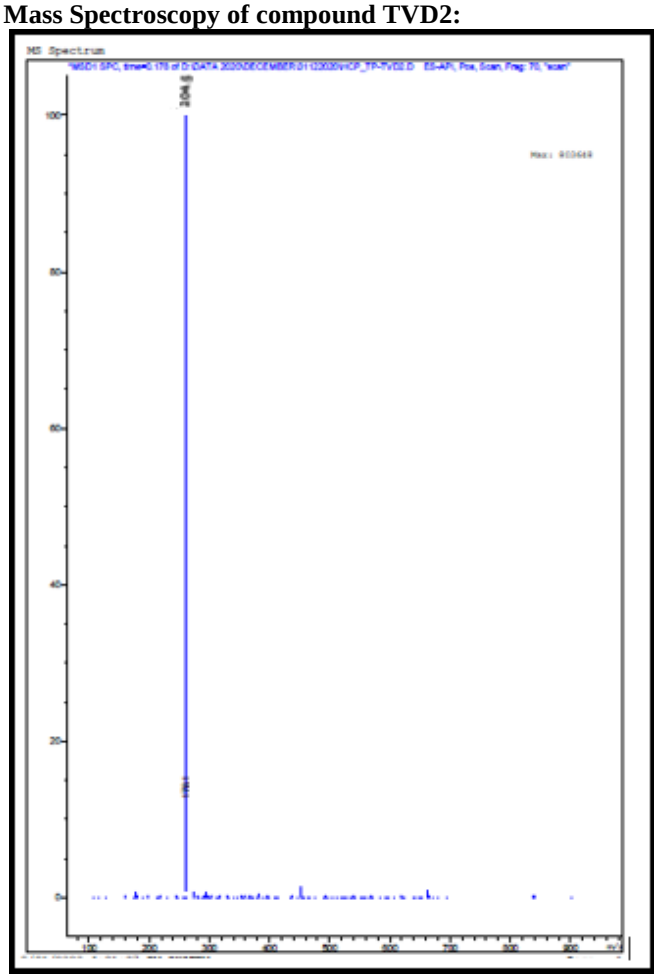

Figure 3.1.3.4: Mass of compound TVD2

Table 3.1.3.5: Mass values of compound TVD2

Compound	m/z Value
TVD2	204.3

3.2 In-Silico Studies

3.2.1 Drug Likeness Properties

- Chemical structures and SMILES notations of the Schiff bases and 1, 2, 4-triazol-3-amine derivatives were generated using ACD labs Chemsketch version 12.0. SMILES notations of the derivatives are read using an online server database, Molinspiration software (www.molinspiration. com, accessed on 6th April, 2021) to calculate various molecular properties.
- Calculation of molecular properties is important for satisfying Lipinski's rule of five, which is crucial for rational drug design.
- Molecular properties such as partition coefficient (Log P), topological polar surface area (TPSA), hydrogen bond donors and acceptors, rotatable bonds, number of atoms, molecular weight, and violations of Lipinski's rule of five were calculated to evaluate the drug likeness of the synthesized derivatives. The drug likeness properties of Schiff bases and 1, 2, 4-triazol-3-amine derivatives were compared with the standard drug INH and RIF and are illustrated in the **table no.1.**

3.2.2 Prediction of Admet Properties

- *In-silico* prediction of ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties is very important parameter for lead identification and optimization. admetSAR which is a free online server was used to predict ADMET properties. It enabled us to get data connected with known ADMET properties for different entities.
- The ADMET properties of Schiff bases and 1, 2, 4-triazol-3-amine derivatives were calculated using admetSAR online database (http: //lmmd. ecust. edu. cn/admetsar2, accessed on 6th May, 2019) and were compared with standard drug INH and RIF and are illustrated in the **table no.2.**

3.3.3. Prediction of Bioactivity

- The bioactivity scores of Schiff bases and 1, 2, 4-triazol-3-amine derivatives have been estimated by calculating the activity score of glycoprotein coupled receptor (GPCR) ligand, protease inhibitor, ion channel modulator, kinase inhibitor, nuclear receptor ligand, enzyme inhibitor.
- All these parameters were obtained using an online server database Molinspiration software (www.molinspiration. com, accessed on 6th April, 2021) and calculated bioactivity scores of all Schiff bases and 1, 2, 4-triazol-3-amine derivatives were compared with standard drug INH and RIF and are illustrated in the **table no.3.**

3.3 Biological Testing of the Series of Compounds

The series of compounds synthesized were tested for their antitubercular activity against *MTSK*H37Rv using MABA (MABA) method.

Procedure

1) The antimycobacterial activity of compounds were assessed against MTB using MABA.
2) This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method.
3) 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.
4) The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate.
5) The final drug concentrations tested were 100 to 0.2 µg/ml.
6) Plates were covered and sealed with parafilm and incubated at 37ºC for five days.
7) After this time, 25µl of freshly prepared 1: 1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs.

8) A blue colour in the well was interpreted as no bacterial growth, and pink colour was scored as growth.
9) The MIC was defined as lowest drug concentration which prevented the colour change from blue to pink.

4. Result and Discussion

Bacterial resistance to current antimicrobials and treatment failure have made it necessary for discovery of new molecules and a more focused, target-specific approach for the development of new therapeutics. Literature survey shows that structure-based virtual screening protocols have been used to predict Mycobacterium tuberculosis shikimate kinase (MtSK) inhibitors. Hence, in an attempt to synthesize and evaluate novel compounds active against TB, here, in this project, it was planned to synthesize and evaluation of heterocyclic derivatives as potential antitubercular agent.

This chapter includes detailed review of the results studies of heterocyclic derivatives, discussion on synthesis and characterization of molecules, *in-silico* studies which includes ADMET properties, drug-likeness properties and bioactivity score prediction, results of *in vitro* testing against *M. tuberculosis* H37RV.

4.1 Synthesis of Molecules

A series of molecules were synthesized by initially reacting substituted aromatic aldehydes with aminoguanidine bicarbonate to form Schiff base as an intermediates. In step 2, Schiff base which was further cyclized to form of 1, 2, 4-triazol-3 – amine derivatives in presence of FeCl3. A general method for the synthesis of molecules and outline of the work carried out is summarized in **Figure No.4.1**

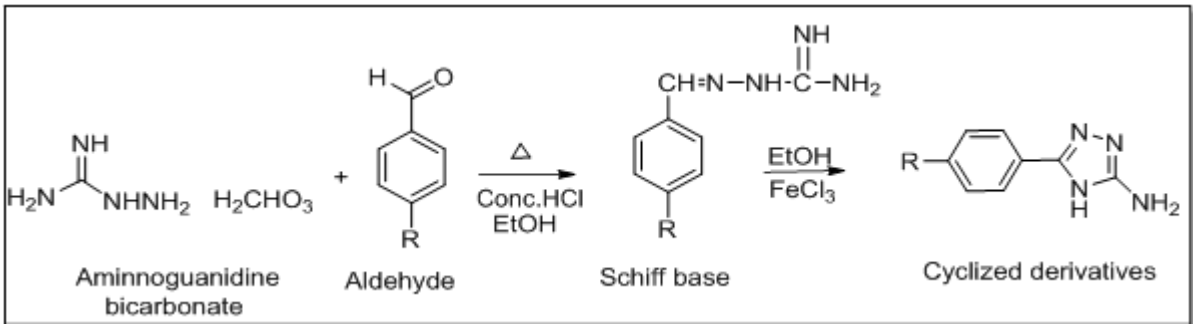

Figure 4.1: Scheme for synthesis cyclized derivatives

4.2 Characterisation of the synthesized compounds:

The synthesized compounds were purified by recrystallization and characterized spectroscopically using IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopy.

IR spectra of the compounds show NH stretching vibration of primary amine in the range of 3400-3350 cm-1. NH stretching vibration of secondary amine near 3200-3100 cm-1. C=N stretching vibration is observed in 1570-1450 cm-1 region which disappeared in Schiff base. C-N stretching vibration of 1, 2, 4-triazol-3-amine is in the range of 1650-1550 cm-1 which was not seen in Schiff bases. Aromatic C=C stretching vibration is observed in 1150-1100 cm-1 region. Aromatic C-H stretching vibration is observed in 3300-3200 cm-1.

1H NMR spectra of Schiff bases show singlet of-NH group of Aminoguanidine bicarbonate in the range of δ 11.5-11.1 and singlet for secondary amine is observed in the range of δ 9.5-9.0, aromatic protons show multiplet in the range of singlet for CH group of Schiff base is observed in the range of Final 1, 2, 4-triazol-3-amine derivatives show singlet of-NH group is in the range of δ 5-6, NH2 group is observed in the range of δ 3-5. Ar-CH group peaks are observed in range of δ 6.5-8.0. Ar-CH_3 group is observed in the range of δ 2.4-2.7 of 1, 2, 4-triazol-3-amine derivatives from Schiff bases.

13C NMR spectra of synthesized compounds are as follows. Chemical shift values of all the other aromatic carbons were observed at expected regions. The numbering of the carbons and their corresponding values are shown in the following section.

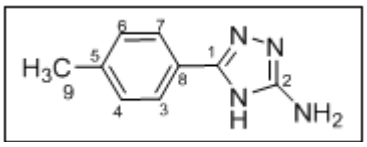

Figure 4.2.1: Structure of compound TVD2

Table 4.2.1: 13C NMR values of compounds TVD 2

Assignment	Carbon Type	13C NMR δ value (in ppm) of compound TVD2
C1	>C-C	149.71
C2	>C-N	159.05
C3, C7	C_6H_4 Aromatic Carbon	76.90, 77.22
C4, C6	C_6H_4 Aromatic Carbon	129.42, 127.24
C5	C_6H_4 Aromatic Carbon	133.40
C8	C_6H_4 Aromatic Carbon	139.31
C9	- CH_3	21.63

Mass spectrometry of the synthesized compound was recorded and was found (M+1) as molecular ion peak. Thus from the available spectral data of compounds (See Experimental Section), it can be concluded that the compounds synthesized are substituted 1, 2, 4-triazol-3-amine derivatives according to the predicted structure. Characterization of molecules confirms the structure of molecules and hence the mechanism of reaction of substituted 1, 2, 4-triazol-3-amine derivatives as shown in **Figure No.4.2.2.**

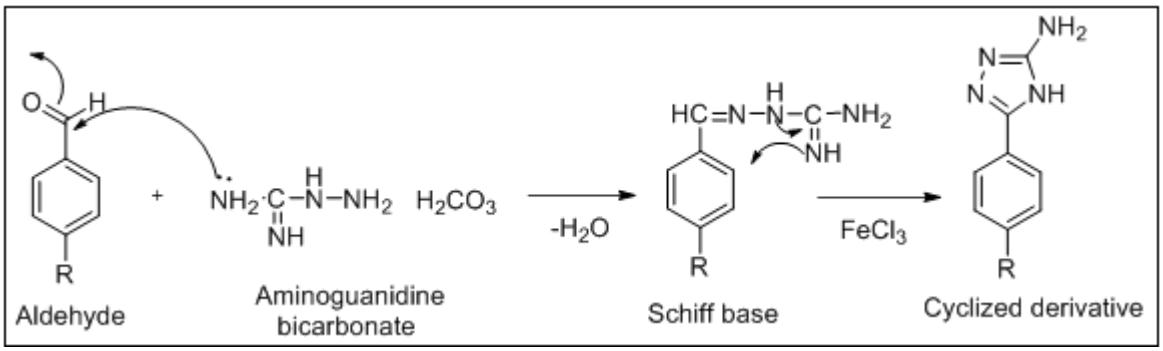

Figure 4.2.2: Mechanism for the synthesis 1, 2, 4-triazol-3 – amine derivatives

In the first step, the free aminoguanidine bicarbonate acts as a nucleophile and attacks the carbonyl group of aldehyde. Loss of water molecule results in the formation of Schiff base.

In the next step, the free amino acid of Schiff base acts as a nucleophile and attacks the carbonyl group of aldehyde. Loss of water molecule results in the formation of cyclization of Schiff bases take place in presence of FeCl3 resulting in formation of 1, 2, 4-triazol-3-amine derivatives.

4.3 In-Silico Studies:

4.3.1 Drug Likeness Properties

The drug likeness properties of the synthesized compounds are compared with standard drug on the grounds of their molecular weight, number of atoms, hydrogen bond acceptors, hydrogen bond donors, total polar surface area and number of rotating bonds, log P and N violation parameters in **Table No.4.3.1.**

Table 4.3.1: Drug likeness score of the synthesized derivatives.

Molecules (Compound Code)	Mol. wt. (Dal)	Total no. of Atom	nON	TPSA	nOHNH	No. of rotating bonds	Log P	N Violation	Volume
ISONIAZID	137.14	10	4	68.01	3	1	- 0.97	0	122.56
RIFAMPICIN	822.95	59	16	220.16	6	5	2.62	3	755.91
TVD2	174.21	13	4	67.60	3	1	1.76	0	159.97

On the basis of results obtained, all the compounds displayed drug-like characteristics based on Lipinski's rule of 5 that states if the compound, has certain pharmacological or biological activity, to make it an orally active drug in humans.

- The molecular weights of all Schiff bases and 1, 2, 4-triazol-3-amine derivatives were found to be less than 500 Daltons, and thus these molecules are predicted to be easily transported, diffused and absorbed as compared to large molecules.
- Number of hydrogen bond acceptors (nON) (Oxygen and Nitrogen atoms) is not observed more than 10.
- Number of hydrogen bond donors (nOHNH) (nitrogen or oxygen atoms with one or more H atoms) was found to be not more than 5.
- The calculated log P values of all derivatives were below 5 which is an indication for good water solubility.
- The topological polar surface area (TPSA) is calculated from number of oxygen and nitrogen atoms and by hydrogen atoms attached to them. Thus, the TPSA mimics the hydrogen bonding characteristic for a compound. TPSA was found to be not more than 140 Å for all derivatives indicating good intestinal absorption.
- A less than 10 No. of rotating bonds were observed.
- Zero N violations were observed for all derivatives.

Hence all molecules obey Lipinski's rule of five and therefore these molecules are anticipated to be easily transported, diffused and absorbed and are likely to be orally bioavailable.

4.3.2 Prediction of ADMET Properties

The biological permeability of synthesized compounds are compared with standard drug on the basis of blood brain barrier (BBB), human intestinal absorption (HIA), P-glycoprotein substrate, acute oral toxicity, carcinogenicity, rat acute toxicity and AMES toxicity in **Table No.4.3.2.**

Table 4.3.2: Predicted biological permeability of synthesized derivatives

Molecules	HIA	BBB	P-glycoprotein Substrate /inhibition	AMES Toxicity	Carcinogenicity	Acute Oral Toxicity	LD50 in Rats
ISONIAZID	1.000	0.9961	Non-substrate/ Non-inhibitor	Toxic	Carcinogenic	0.8032	1.167
RIFAMPICIN	0.8597	0.9738	Substrate/ inhibitor	Toxic	Non-Carcinogenic	0.7763	3.21
TVD2	1.0000	0.9141	Non-substrate/ Non-inhibitor	Non-Toxic	Non-Carcinogenic	0.7445	2.1796

- ADMET properties, as derived from admetSAR server, reveal tha molecules had better Human Intestinal Absorption (HIA) score than the control molecules, Greater HIA indicates that the compound could be better absorbed from the intestinal tract upon oral administration.
- The penetration through the Blood-Brain Barrier (BBB) for molecule showed good results.

- The prediction of the efflux by P-glycoprotein (P-gp), molecule came out to be non-substrate and non-inhibitor of P-gp similar to control molecule.
- The carcinogenic profile also revealed that all the ligands were non-carcinogenic similar to the control molecule RIF. However, control molecule INH was found to be carcinogenic.
- Molecule displayed lower acute oral toxicity than the control.
- Important information obtained was the computed LD50 dose in a rat model. Comparing the LD50 doses, a compound with a lower dose is more lethal than the compound having higher LD50. From our study, we found that moleculehad higher LD50, compared to the control.

4.3.3. Prediction of Bioactivity Score:

The bioactivity scores of the synthesized derivatives are compared with standard drug on the basis of GPCR ligand, ion channel modulator, nuclear receptor ligand, kinase inhibitor, protease inhibitor, enzyme inhibitor in **Table No.4.3.3.**

Table 5.3.3: Bioactivity score of the synthesized compounds

Molecules	GPCR Ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor	Ligand protease inhibitor	Enzyme inhibitor
ISONIAZID	- 1.39	- 1.45	- 1.05	- 2.33	- 1.23	- 0.66
RIFAMPICIN	- 2.10	- 3.27	- 3.04	- 2.89	- 1.61	- 2.42
TVD2	- 0.84	- 0.46	- 0.67	- 1.85	- 1.22	- 0.53

- A molecule having bioactivity score more than 0.00 is most likely to exhibit considerable biological activities, while values −0.50 to 0.00 are expected to be moderately active and if score is less than-0.50, it is presumed to be inactive.
- The results reveal that the physiological actions of Schiff bases and 1, 2, 4-triazol-3-amine derivatives involve mechanism inhibiting some enzymes which validates our design principle of inhibiting Shikimate kinase.
- The bioactivity score of compounds is an implicative of moderate interaction with the drug target.
- The compounds showed promising bioactivity scores. The compounds showed better bioactivity scores on comparison with standards taken for the study.

4.4 Biological Testing of the Series of Compounds:

The synthesized compounds in the present study were tested for their antitubercular activity against *M. tuberculosis* H37Rv by microplate alamar blue assay (MABA) method.

Table 4.4: MIC values of synthesized and standard compounds

Compound	MIC values (µg/ml)
INH	6.25
RIF	1.6
TVD2	25

- The results of *in vitro* antitubercular activity show that the synthesized 1, 2, 4-triazol-3-amine series is effective against *M. tuberculosis* at12.5 µg/ml Isoniazid.

Figure 4.4: (a) Anti-TB activity of synthesized compounds using Alamar Blue Dye

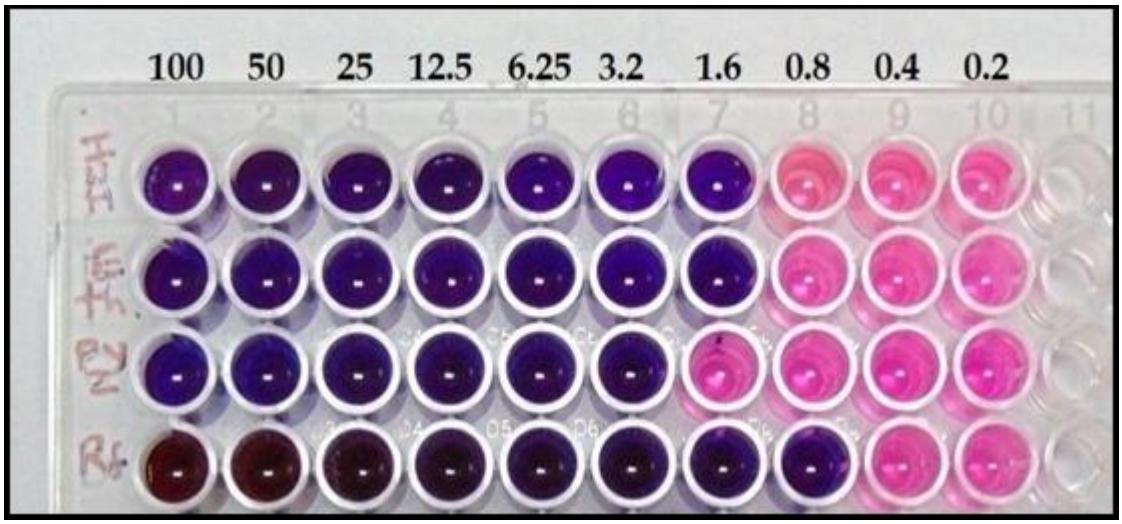

Figure 4.4: (b) Anti-TB activity of standard compounds using Alamar Blue Dye (Isoniazid, Ethambutol, Pyrazinamide, Rifampicin)

5. Conclusion

The results of *in vitro* antitubercular activity show that the synthesized 1, 2, 4-triazol-3-amine series is effective against *M. tuberculosis* at 12.5 µg/ml Isoniazid. Hence it can be concluded that synthesized 1, 2, 4-triazol-3-amine series is are not very active as compared to standard drugs and previously synthesized molecules in the laboratory with S in the ring or SH group on the ring. It implies that this is important for the Shikimate kinase inhibition activity. Hence future work in the laboratory would focus on modifying these molecules to improve their antitubercular activity.

References

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