# SYNTHESIS, DOCKING AND ANTITUBERCULER ACTIVITY OF TRIAZOLE-THIADIAZOLE HYBRIDE

[1]Trupti Chitre*, [2]Shubhangi Kumbhar, [3]Rasika Mulik, [4]Sadeecha Wani, [5]Rutuja Pawar,[6] Sheetal Parse, [7]Pooja Wagh , [8]Sheetal Patil and [9]Kalyani Asgaonkar

Associate Professor[1, 8, 9,] Student [2, 3, 4,5,6,7]

Department of Pharmaceutical Chemistry,

All India Shri Shivaji Memorial Society's College of Pharmacy, Kennedy Road, Near R.T.O., Pune-411001, M.S., India.

***Abstract:*** Aim of the present work was to synthesize some triazole and thiadiazole derivatives and evaluate them for their Anti-tubercular activity. Six triazole-thiadiazole hybrid derivatives were synthesized, subjected to docking studies and evaluated for their anti-tubercular activity using XTT Reduction Menadione Assay Protocol. The compounds , **5E** viz.,3-(3-nitrophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6(5H)-thione and **5F**, 3-(2,4-dichlorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6(5H)-thione have shown better activity as compared to other derivatives. The 5E has shown better docking score as compared to the standard used. Hybrids of triazole and thiadiazole thus can be further explored.

***Index Terms*:** Antitubercular, Triazole- Thiadiazole Hybrid, DNA gyrase, XTT Assay, Molecular Docking

## I. INTRODUCTION:

Tuberculosis (TB) is a leading cause of death worldwide from a single infectious agent called, *Mycobacterium tuberculosis* (MTB). Predominantly it infects lungs (pulmonary TB) but can also infect any other part of body (extra-pulmonary TB); if left untreated it destroys the body tissue by chronic inflammation and may culminate in death. About one third of the world's population is infected with MTB that causes TB. On an average 5–10% of these carriers become sick or infectious at some time during their life. [1-2]

Literature survey has revealed that derivatives of 1, 2, 4- triazole and 1,3,4- thiadiazole possesses broad spectrum of activities either alone or in hybrid scaffold. [3-26] In view of the promising anti-tubercular [27-41] activity of thiadiazole and 1,2,4-triazole derivatives we have designed 1,3,4-thiadiazole-1,2,4-triazole hybrids by incorporation of these two molecular entities in to a single framework. Further docking studies were performed on MTB DNA gyrase (PDB code: 2XCT) and studies of the minimum energy docked poses of these compounds revealed that they could fit into the binding pocket of DNA gyrase. [42]

## II. MATERIAL AND METHODS

### 2.1 Synthetic study

All chemicals and solvents were purchased from Sigma Aldrich and Merck, respectively. Melting points (mp) were determined by a Veego VMP-D apparatus and are uncorrected. Infrared (IR) spectra were recorded using KBr on a Varian-160 FTIR spectrometer using Diffuse Reflectance Attachment. $^1$H NMR spectra were measured on Jeol JNM ECX-400 P 0and Bruker Advance II-400 spectrometers in $CDCl_3$/ DMSO with TMS as internal standard. Mass Spectra were measured on a Jeol JMS-700 or Thermo Scientific Q-Exactive, Accela 1250 pump. Analytical thin-layer chromatography (TLC) was carried out on Merck's precoated silica-gel plates 60 $F_{254}$ and spots were visualized by irradiation with UV light (254 nm) .

#### 2.1.1. Step 1: Synthesis of Substituted benzoic esters from Substituted Benzoic acids (2A-2F):

Carboxylic acid (0.1 mole) was dissolved in 30 ml of dried ethanol or methanol in a dry RBF. To this, 0.1 mol of conc. $H_2SO_4$ was added drop wise with mechanical stirrer. The reaction mixture was refluxed for 7-8 hrs at 50-60$^0$c. Mixture was then allowed to cool and pH was neutralised by 10% Sodium Bicarbonate ($NaHCO_3$) solution to obtain the product. Reaction was monitored using TLC with mobile phase: n-Hexane: Ethyl Acetate (8:2) [43]

#### 2.1.2. Step 2: Synthesis of Substituted benzohydrazide from Substituted benzoester(3A-3F):

To a solution of ester (1 mmol, 1 equiv.), 99% hydrazine hydrate (3 mmol, 3.0 equiv.) was added drop-wise. The reaction mixture was refluxed for 5 hrs at 50$^0$c; after completion of the reaction, a solid product was formed, and the excess solvent was removed under reduced pressure. Reaction was monitored using TLC with mobile phase: Methanol: Chloroform (1:10) [44]

#### 2.1.3. Step 3: Synthesis of 4-amino-5-Substituted-4H-1,2,4-triazole-3-thiol from Substituted benzohydrazide(4A-4F):

To the solution of Potassium hydroxide (1.5 mmol, 1.5 equiv.) and absolute ethanol (25ml); substituted hydrazides (1 mmol, 1 equiv.), and carbon disulphide (1.5 mmol, 1.5 equiv.) was added drop-wise and mixture was refluxed for about 10 hrs. After completion of the reaction, the solvent was evaporated under reduced pressure to obtain the intermediate. To the crude product 99% Hydrazine Hydrate (25

ml) was added and was further refluxed for 10 hrs. The reaction mixture was then acidified with 10% HCl solution to obtain the final product. Reaction was monitored using TLC with mobile phase: n-Hexane: Ethyl Acetate (8:2) [44]

**2.1.4. Step 5: - Synthesis of 3-(Substituted)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6(5H)-thione from 4-amino-5-Substituted-4H-1,2,4-triazole-3-thiol(5A-5F):**

A mixture of 4-amino-5-Sub-4H-1,2,4-triazole-3-thiol (0.96 g,0.005 mol) and carbon disulphide (1 ml) in pyridine (25 ml) was refluxed for 8 hrs, then left to cool and poured on ice-cold water. The solid obtained was filtered, washed with water, dried and recrystallized from ethanol to give yellow plates identical with the product obtained. Reaction was monitored using TLC with mobile phase: n-Hexane: Ethyl Acetate (8:2) [45]

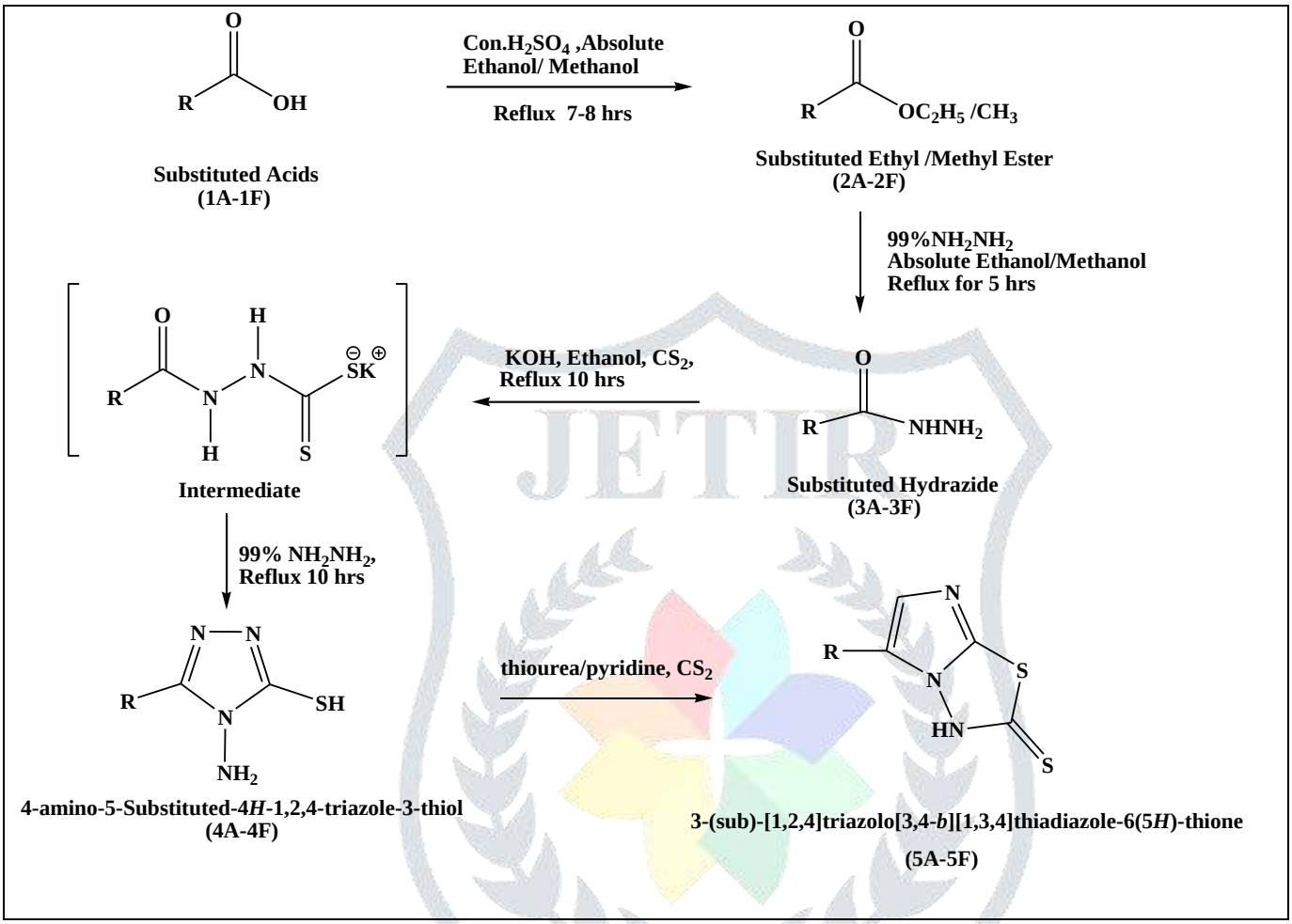


Fig 1: Synthetic route for 3-(sub)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole-6(5H)-thione

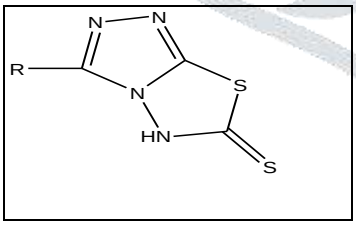

Fig 2: 3-(Substituted)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole-6(5H)-thione derivatives

Table 1: 3-(Substituted)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole-6(5H)-thione derivatives (5A-E).

| Sr No. | Compound Code | -R |
|---|---|---|
| 1 | 5A | 4-$NO_2$-$C_6H_5$ |
| 2 | 5B | 2,4di-$NO_2$-$C_6H_5$ |
| 3 | 5C | 3-Cl-$C_6H_5$ |
| 4 | 5D | 4-OH-$C_6H_5$ |
| 5 | 5E | 4-$NO_2$-$C_6H_5$ |
| 6 | 5F | 2,4-di-Cl-$C_6H_5$ |

**2.2 Docking:-**

The Glide (Schrodinger 2015) software was used to dock the synthesized molecules in the binding pocket of the DNA gyrase enzyme complexed with Sparfloxacin. PDB code was retrieved from the RCSB Protein Data Bank (PDB code: 2XCT). Receptor crystal was prepared by removing water molecules, metal ions and cofactors using the 'protein preparation wizard' in Maestro wizard 8.5.To ensure that active sites are not missed grids were generated by Receptor Grid Generation panel. The prepared receptor and the ligands were optimised to obtain least energy conformer. The ligands were docked within the active site of DNA gyrase using the 'Extra precision' Glide algorithm. Final scoring of docked ligand is carried out on the energy-minimized poses. The G-score was assessed along with the interactions. The docking of the synthesized compound was carried out along with the standard compound Sparfloxacin. [46-51]

**2.3. Antitubercular Activity:**

Antitubercular screening was performed for all the compounds using MTB, *H37Ra* and XRMA was determined by using the XTT Reduction Menadione Assay protocol. [52] Compound solutions were be prepared in 100% dimethyl sulfoxide (DMSO). Inoculated culture was be added into each well of 96 well plate except, blank. Test compounds were added to each well except blank and control. For dormant stage MTB, XTT assay was performed after 12 days. The compounds which showed more than 90% inhibition at 30µg/ml concentration in dormant assay for MTB *H37Ra* were further evaluated for dose response. Further their IC50 and MIC were also determined by using Origin software.

## III. RESULTS AND DISCUSSION:

**3.1. Characterization data of Synthesized compounds:**

3-(Substituted)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole-6(5H)-thione derivatives were prepared from variously substituted carboxylic acids and hydrazides according to the method described in the literature. (Table 1)

**3.1.1. 3-(4-nitrophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole-6(5H)-thione (5A):**

Colour: Pale yellow; yield: 17.91; mp: 214$^0$C; IR (KBr): 2331,2240,1500,1502 cm$^{-1}$ $^1$H NMR (500 MHz, Chloroform) δ 7.38 (d, *J* = 7.5 Hz, 1H), 7.14 (d, *J* = 7.5 Hz, 1H), ESI-MS m/z: 221[M]$^+$; HRMS calcd. for $C_9H_5N_5O_2S_2$: 278.99[M]$^+$.

**3.1.2. 3-(2,4-nitrophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole-6(5H)-thione (5B):**

Colour: Brownish Yellow; yield: 16.54; mp:190-199$^0$C; IR (KBr): 2331,2240,1500,1502 cm$^{-1}$ $^1$H NMR (500 MHz, Chloroform), δ 8.52 (d, *J* = 1.4 Hz, 1H), 8.04 (dd, *J* = 1.4 Hz, 1H), 7.82 (d, J = 7.5Hz, 1H), ESI-MS m/z: 253[M]$^+$; HRMS calcd. for $C_9H_4N_6O_4S_2$: 323.97 [M]$^+$.

**3.1.3. 3-(3-chlorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6(5H)-thione (5C):**

Colour: Pale yellow; yield: 14.36; mp:163-168$^0$C; IR (KBr): 2331,2240,1500, 610, cm$^{-1}$ $^1$H NMR (500 MHz, Chloroform), δ 7.83 (t, *J* = 4.7 Hz, 1H), 7.64 - 7.52 (m,1H), 7.47 – 7.35 (m, 2H), ESI-MS m/z: 209[M]$^+$;HRMS calcd. For $C9H_5ClN_4S_2$ :267.96 [M]$^+$.

**3.1.4. 3-(4-hydroxyphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6(5H)-thione (5D):**

Colour: Yellow; yield: 84.32; mp: 223-225$^0$C; IR (KBr): 2331,2240,1500,3600 cm$^{-1}$ $^1$H NMR (500 MHz, Chloroform), δ 7.49 (d, *J* = 7.5 Hz, 2H), 6.90 (d, *J* = 7.5 Hz, 2H), 4.00 (s, 1H). ESI-MS m/z: 192 [M]$^+$;HRMS calcd. for $C_9H_6N_4OS_2$: 304.03 [M]$^+$.

**3.1.5. 3-(3-nitrophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6(5H)-thione (5E):**

Colour: Brownish yellow ; yield: 09.98; mp: 153-154$^0$C ;IR (kBr): 2331,2240,1500,1300 cm$^{-1}$ $^1$H NMR (500 MHz, Chloroform), δ 8.36 (t, *J* = 1.4 Hz, 1H), 8.00 (ddt, *J* = 13.9, 7.5, 1.3Hz, 2H), 7.64 (t, *J* = 7.4 Hz, 1H), ESI-MS m/z: 221 [M]$^+$;HRMS calcd. For $C_9H_5N_5O_2S_2$: 278.99 [M]$^+$.

**3.1.6. 3-(2,4-dichlorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6(5H)-thione (5F):**

Colour: Pale yellow ; yield: 13.65; mp: 168-170$^0$C ;IR (kBr): 2331,2240,1500,610 cm$^{-1}$ $^1$H NMR (500 MHz, Chloroform), δ 7.57 (d, *J* = 7.5 Hz, 1H), 7.52 (d, *J* = 1.4 Hz, 1H), 7.35 (dd, *J* = 7.5, 1.4Hz, 1H), ESI-MS m/z: 243 $[M]^+$;HRMS calcd. For $C_9H_4Cl_2N_4S_2$: 301.93 $[M]^+$.

**3.2 Docking:**

The molecular docking studies were carried out of 3-(Substituted)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6(5H)-thione derivatives on DNA gyrase enzyme. The molecular basis of hydrogen bond interactions with the active binding pocket were analyzed. The Molecules were ranked on the basis on their G-Score (Table 2). Molecule 5E has shown best binding as compared to other molecules. The docking pose of 5E shows one hydrogen bond. Most of the molecules have shown better and favourable hydrophobic interactions of triazole and thiadiazole ring within the active site the receptor. G-score were found in the range of -5.66 to -8.93 kcal/mole. Molecule 5E showed good G-score. Rest molecules show comparable G-score with the standard drug.

Table 2: Molecular docking study– Glide score, per-residue interactions of ligands with the active site of DNA gyrase.

| Sr No | Molecule Name | Molecular Formula | X1 | X2 | G score | Hydrogen Bond | Good VDW | Bad VDW | Ugly VDW |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 5A | $C_9H_6N_5O_2S_2$ | $NO_2$ | - | -7.00 | 1 | 198 | 2 | 0 |
| 2 | 5B | $C_9H_6N_6O_4S_2$ | $NO_2$ | $NO_2$ | -7.65 | 1 | 213 | 2 | 0 |
| 3 | 5C | $C_9H_5ClN_4S_2$ | Cl | - | -6.03 | 1 | 242 | 4 | 0 |
| 4 | 5D | $C_9H_6N_4OS_2$ | OH | - | -5.66 | 1 | 203 | 2 | 0 |
| **5** | 5E | $C_9H_6N_5O_2S_2$ | $NO_2$ | - | -8.93 | 1 | 250 | 2 | 0 |
| 6 | 5F | $C_9H_4Cl_2N_4S_2$ | Cl | Cl | -6.91 | 1 | 213 | 4 | 0 |
| Standard Drug | Sparfloxacin | | | | -7.93 | 0 | 210 | 3 | 0 |

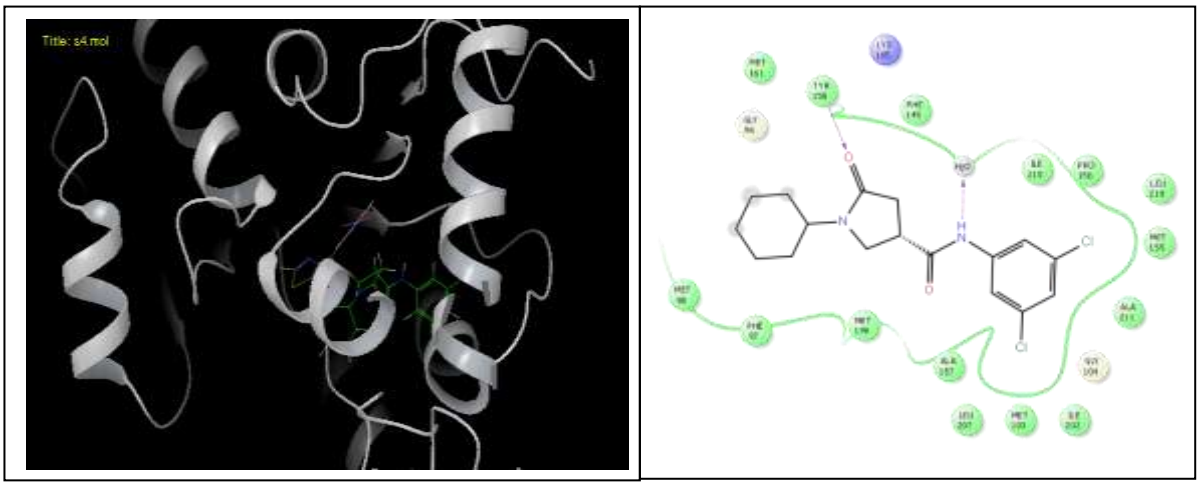

Fig 3**:** Docking pose of Docking Pose of 3-(3-nitrophenyl)-[1,2,4]triazolo[3,4 b][1,3,4]thiadiazole-6(5H)-thione (5E) in the active site of DNA gyrase.

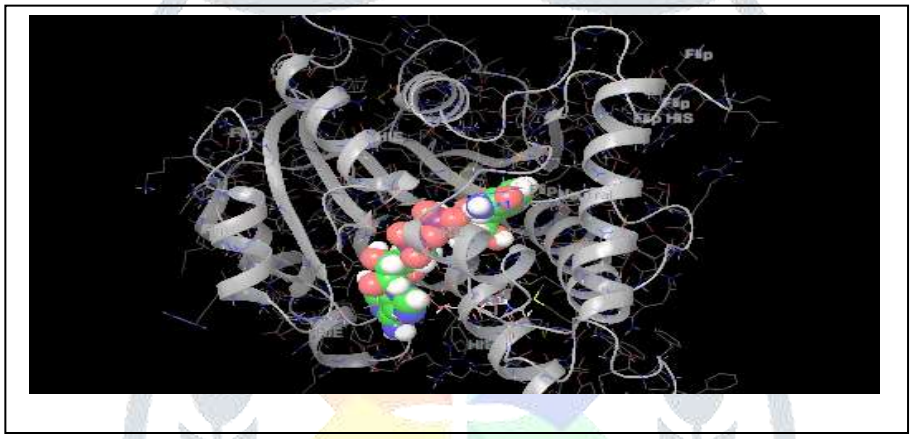

Fig 4 : Docking pose of Sparfloxacin in the active site of DNA gyrase

### 3.3. Biological Screening:

Following table represents the results of the Antitubercular Activity of Synthesised Compounds.

Table 3: Antitubercular Activity of Synthesised Compound**s.**

| Sample Code | MIC µg/ml* | IC50 µg/ml |
|---|---|---|
| 5A | >30 | >30 |
| 5B | >30 | >30 |
| 5C | 38.06 | 8.38 |
| 5D | 86.34 | 22.41 |
| 5E | 86.38 | 22.17 |
| 5F | 17.36 | 4.69 |

***** Rif (0.03)

## IV. CONCLUSION:

In the present work six derivatives of 3-(sub)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole-6(5H)-thione were synthesized, docked in the active cavity of DNA gyrase (2XCT) . Docking studies revealed some crucial interactions with that of standard. The 5E has shown better docking score as compared to the standard used. From the results of XTT Reduction Menadione Assay Protocol, it can be noted that, the compounds **5E** ,3-(3-nitrophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6(5H)-thione and **5F**, 3-(2,4-dichlorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6(5H)-thione have shown better activity amongst all the other derivatives. Hybrids of triazole and thiadiazole thus can be further explored for Antitubercular activity.

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**Conflict of Interest:** Authors declare no conflict of interest.

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