# SYNTHESIS, IN-SILICO AND IN-VITRO SCREENING OF NOVEL PYRROLYL THIAZOLIDINONE DERIVATIVES AS POTENT ANTI-TUBERCULAR AGENTS

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## Abstract

A series of novel Pyrrolyl thiazolidinone derivatives were synthesized by the reaction between Pyrrolyl hydrazide in presence of substituted aldehyde, ethanol to obtain Pyrrolyl Schiff Base; Further Pyrrolyl Schiff Base in presence of thioglycolic acid and Benzene refluxed for 12hr to yield Pyrrolyl thiazolidinone derivatives(4a-c). Through FT-IR, $^{1}$H NMR ,$^{13}$C NMR and Mass Spectroscopy the synthesized compound were successfully confirmed, Pyrrolyl derivatives weas thoroughly analyzed for their in-vitro antitubercular activity against Mycobacterium tuberculosis using $H_{37}$Rv Strains by using Microplate Almar Blue Assay (MABA) method, Isoniazid and Rifampicin used as reference standard drugs. Among all the synthesized compound 4b shows good activity when compared to standard drugs.

In-Silico, Molecular Docking Studies were carried out on newly synthesized compound Pyrrolyl thiazolidinone Derivatives(4a-c) using PyRx and Discovery studio software which shows the vital interaction and binding affinity.

**Keywords**: Pyrrole, IR, NMR, Molecular Docking, MABA Method.

## Introduction

**Tuberculosis** is a chronic infectious disease caused by Mycobacterium tuberculosis it is the worlds second common cause of death from infectious disease after AIDS [(1)].TB is a systemic disease with typical Pulmonary manifestation, which is transmitted from person to person by airborne bacteria[(2)]

Mycobacterium africanum, Mycobacterium bovis, Mycobacterium microtic, Mycobacterium canetti, Mycobacterium tuberculosis are five closely related Mycobacteria that produces the pandamic illness[(3)].Mycobacterium tuberculosis has been the major causative agent of tuberculosis, a disease that affects the lungs, referred as pulmonary TB and some other part as well, referred as extrapulmonary TB[(3,4)]Factors that increase the risk of developing active TB include undernutrition, alcohol consumption, smoking, diabetics. TB which resist to both isoniazid and rifampicin,the most powerful anti-TB drugs is called as MDR-TB 2[(5)]

First line and Second line drugs; The first line drugs that include Isoniazid, Rifimpacin, Ethambutol and streptomycin. The first line drugs combines with greatest level of efficacy with acceptable degree of toxicity and also responsible for the adverse reaction.Scond line drugs include Kanamycin, Cyclosterin, Ethionamide and Quinoline,Second line drugs shows Potentially nephrotoxicity[6]According to the WHO; In 2022, around 2 billion people were infected with TB,In 2018, 1.6 million TB-related deaths and In 2017,10 million new cases of TB were observed.[3,7,8]

It has become crucial for TB control to create a quick diagnostic test that can differentiate between active TB and LTBI or active TB and non-active TB, as well as detect M. tb infection.[9]

Tuberculosis is repeatedly diagnose using the GeneXpert assay, Sputum smear microscope and chest radiography. However, the culture method is considered as gold standard for detecting the causative agent of tuberculosis, Mycobacterium tuberculosis, but it is a time-consuming diagnosis with substantial contamination risks.[10]

Pyrrole is a five membered heterocyclic ring system has a fundamental role in living world. Pyrrole is unstable towards mineral acids, and is protonated in the second position. The resulting Pyrrylium cation polymerizes very readily to give high molecular mass pyrrole resins because of its highp-electron density,the characteristic reactions of Pyrrole are electrophilic substitution.[11]

Pyrrole derivatives have been found to possess a wide spectrum of activities, of which anti-TB is one of the prominent one.[12]Pyrrole derivatives are found in varieties of biological context as part of co-factor and natural product. Common naturally produced molecules containing pyrrole includes Vit.B12,bile pigments like bilirubin and biliverdin,porphyrins of heme, chlorophyll, chlorins, bacteriochlorins and porphyrinogens. One of the first synthesis of pyrrole containing molecule was that of haemin synthesized by E. Fischer in 1929.[13]

# Chemistry

**Apparatus**: Round bottom flask, Condenser, Magnetic stirrer, Measuring cylinder, Beaker, Boiling chips, Petri dish, Glass Rod

**Chemicals Required**: Ethyl-4-aminobenzoate,2,5-dimethoxy tetrahydrofuran, Glacial acetic acid, Hydrazine Hydrate , Ethanol , substituted aldehydes, thioglycolic acid, benzene ,sodium bicarbonate, 2,3-Dimethoxybenzaldehyde, vanillin, anisaldehyde. Ethyl acetate : Chloroform in the ratio 7 : 3 & Ethyl acetate : petroleum ether in the ratio 6 : 4 for TLC

**Software's**- Chemdraw Professional 16.0, Chem 3D 16.0, PyRx, Discovery studio

## Method:

- **Step-1 General procedure for synthesis of ethyl 4-(1H-pyrrol-1-yl) benzoate(I):** Compound (I) was synthesized by refluxing a mixture of ethyl 4-aminobenzoate (0.05 mol) in glacial acetic acid (7.5ml) and 2,5-dimethoxy tetrahydrofuran (0.05 mol) at 150-160$^0$ C for 45 min. Then the reaction mixture was poured onto crushed ice further it was neutralized with saturated solution of sodium bicarbonate, the separated solid was filtered and recrystallized with ethanol.

- **Step-2 Procedure for the synthesis of 4-(1H-pyrrol-1-yl) benzohydrazide (II):**
  Compound (II) was synthesized by refluxing a mixture of ethyl-4-(1H-pyrrol-1-yl) benzoate (I) (0.014 mol) with hydrazine hydrate (9.3ml) in absolute ethanol (9.3ml) for 3hr then reaction mixture was cooled. The obtained crystalline mass was filtered and recrystalized with ethanol.

- **Step-3 General synthesis of Schiff base derivatives of pyrrole (III):**
  Compound (III)was then synthesized by dissolving aromatic aldehyde(10mol) was dissolved in ethanol and was treated with a solution of benzoic acid hydrazide(10mol) or phenolic acid hydrazide(10mmol) in ethanol (25ml), followed by three drops of glacial acetic acid. The reaction mixture was then cooled down to ambient temperature and the resulting precipitate was filtered off by gravity filtration, dried and recrystalized from ethanol to give products.

- **Step-4 Synthesis of Pyrrolyl Thiozolidinone Derivative (IV):**
  To a solution of Schiff base, To compound (III) add 0.9 ml of thioghycolic acid and 15 ml of benzene, the mixture was refluxed on the water bath for 8-12hrs then cooled. The Upper organic layer was washed with sodium hydrogen carbonate solution $NaHCO_3$ and then with water, the Benzene was distilled off then the compound (VI) i.e Pyrrolyl thiazolidinone derivative was synthesized.

# Biological Activity

To the newly synthesized compounds with the MIC value was determined against *M. tuberculosis* strains H37 using Microplate Alamar Blue Assay (MABA) using isoniazid as the standard drug .The 96 wells plate received 100 µl of Middlebrook 7H9 broth and serial dilution of compounds were made directly on the plate with drug concentration of 0.2,0.4,0.8,1.6,3.125,6.25,12.5,25,50 and 100 µg/ml. Plate were covered and sealed with parafilm and incubated at 370 for 5days.Then, 25 µl of freshly prepared 1:1 mixture of almar blue reagent and 10% Tween 80 was added in the well was interpreted as no bacterial growth and pink colour in the was scored as growth .The MIC was defined as the lowest drug concentration ,which prevented colour change from blue to pink .Compounds 3c,3g and 3j showed significant antitubercular activity.Table-3 reveals antitubercular activity (MIC) data for all the synthesized compounds

# In-Silico Studies

The synthesized compounds were evaluated using molecular docking study software such as chemdraw Prossessional, chem3D 16.0, PyRx and Discovery studio. Based on the literature review (Decaprenylphosphoryl-beta-D-ribose oxidase of organism *Mycobacterium tuberculosis* H37Rv strain)6G83 strains was selected and downloaded from RCSB-Protein Data Bank (PDB). By using chemdraw and chem3D the ligand was prepared. Furthermore by using PyRx software the docking of protein and synthesized ligand molecule was performed, where the binding affinity of each synthesized compound was found and then the docked structure of protein-ligand complex was obtained. Visualization of Docked structure was done using Discovery Studio

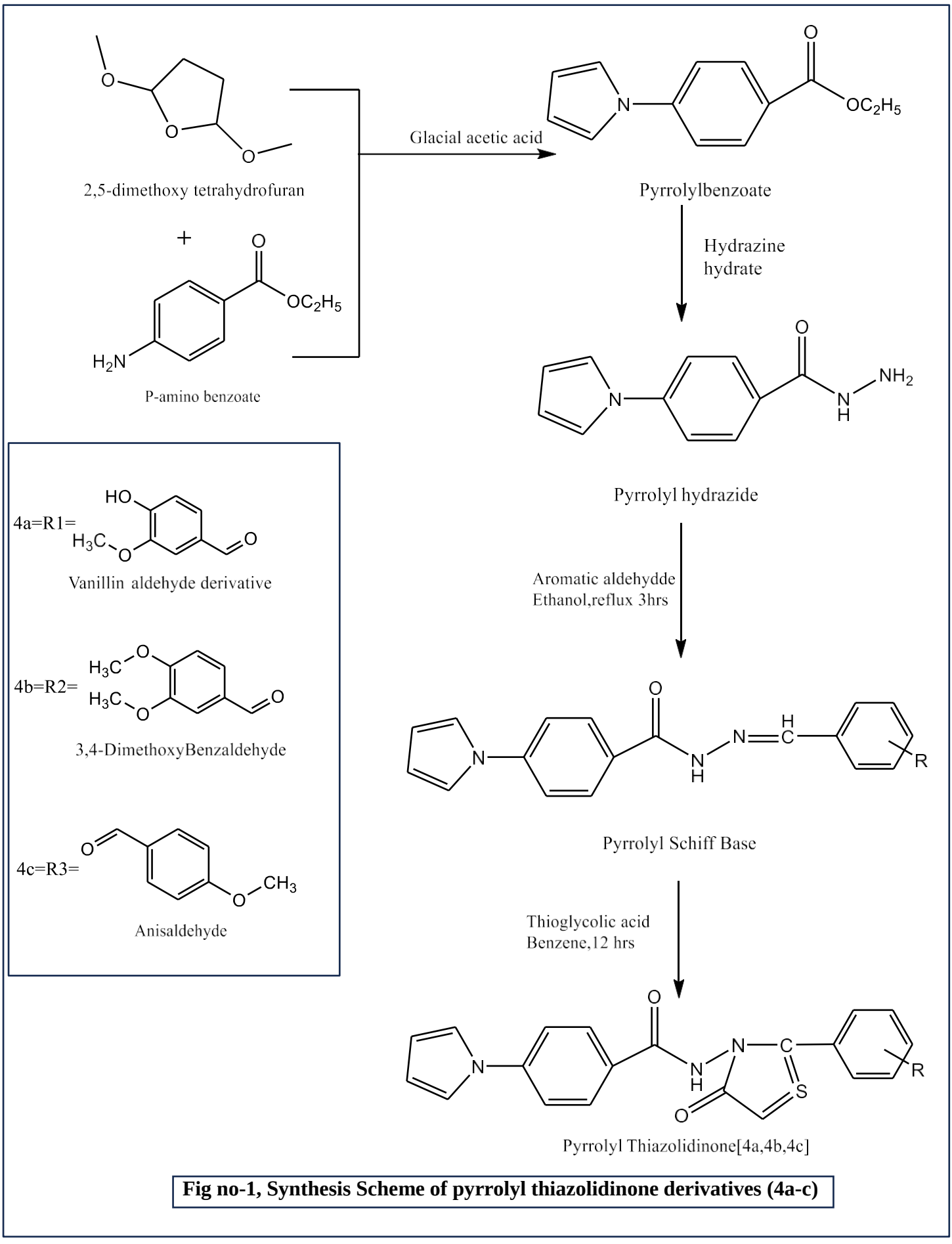
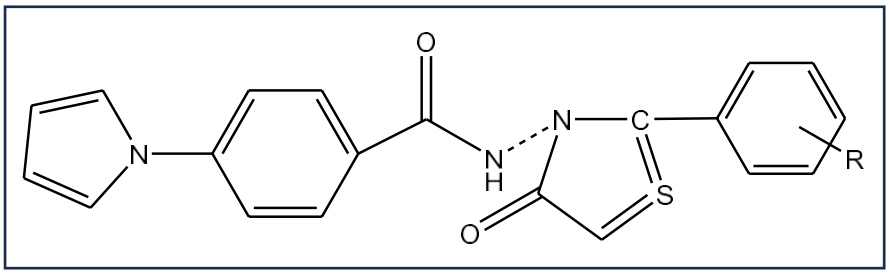



**Fig no-1, Synthesis Scheme of pyrrolyl thiazolidinone derivatives (4a-c)**

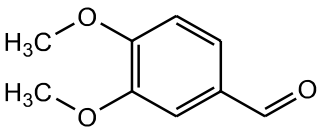
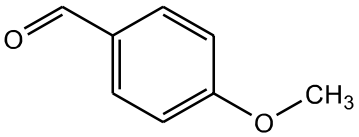
# Results and discussion

**Compound III (Pyrrolyl Schiff base derivatives) was synthesized and melting point is was found to be $215^0$c-$220^0$c**




**Compound IV (Pyrrolyl thiazolidinone derivatives) was synthesized and melting point is as follows;**

**Table no-1(Physicochemical data of compound III)**

| comp | R | Melting Point | Molecular Formula | Molecular weight | RF Value |
|---|---|---|---|---|---|
| 4a |  vanillin | $180^0$C | $C_{21}H_{17}N_3O_4S$ | 407 | 0.772 |
| 4b |  3,4-dimethoxy benzaldehyde | $187^0$C | $C_{22}H_{19}N_3O_4S$ | 421 | 0.80 |
| 4c |  Anisaldehyde | $184^0$C | $C_{21}H_{17}N_3O_3S$ | 391 | 0.82 |

Mobile phase: For compound 4a,4c. Ethylacetate: Chloroform (7:3); for 4b- Ethylacetate : n-petroleum ether (6:4) and Detecting agent: Iodine Chamber.

**The Spectral Data of The Newly Synthesized Pyrrole Derivatives(4a-c)**

- **FT-IR spectrum of 4a**:3205-90(-NH stretching);2918.74(-CH stretching);1685.81(-C=O stretching);1512.94(-C=C stretching);1278.64(-C-N stretching)

  **$^{1}$H NMR (DMSO) 500MHz (δppm)(4a):** 3.7-3.8(s 3H,-CH);4.1(d 1H,Ar-CH);6.0(s 1H,Ar-CH);6.35-6.38(s 3H,Pyrrole);6.8(s 1H,Pyrrole);6.9(d 2H,Pyrrole);7.0-7.8(d 5H,Ar-CH);8.4(s 1H,OH)

- **FT-IR spectrum of 4b:**3251.62(-NH stretching);2920.17(-CH stretching);1641.52(-C=O stretching);1507.23(-C=C stretching);1327.22(-CN stretching)

  **$^{1}$H NMR(CDCl3) 500MHz (δ ppm) 4b:** 2.0-2.3(m 6H, CH);3.9(d 1H,CH);6.3(s1H,Pyrrole);7.1(s 2H,Ar-CH)7.2(d 2H,Pyrrole);7.3(t 2H,Ar-CH);7.5(d 2H,Ar-CH);7.6(d 2H,Ar-CH);9.9(s 1H,NH)

  **$^{13}$C NMR($CDCl_3$) 500MHz (δ ppm) 4b:** 150.1($C_{24}$ of terminal benzene),149.5($C_{24}$ of terminal benzene),143.7($C_6$ of bridged benzene),128.9-129.2($C_9$ of bridged benzene),121.4($C_1$,$C_2$ of Pyrrole),118.9($C_{26}$ of terminal benzene),111.1($C_{23}$ of terminal benzene),110.4($C_2$,$C_3$ of Pyrrole),56.05($C_{30}$, $C_{28}$ of aliphatic compounds)

  **Mass Spectral Data:** Molecular Weight of the Compound 4b is 446 m/z

- **FT-IR spectrum of 4c**: 3217.33(-NH stretching);2918.74(-CH stretching);1717.24(-C=O stretching);1510.09(-C=C stretching);1327.22(-CN stretching)

  **$^{1}$H NMR(CDCl3) 500MHz (δ ppm)4c:** 0.8(s 2H,CH);1.2(s 8H,CH 3.7(d 2H,CH);6.3(s 1H,Pyrrole);7.0(s 1H,);7.2-7.4(d 2H,Ar-CH);7.7(d 1H,Ar-CH)

**Table No:6; Screening of Anti-tubercular activity by MABA method for newly synthesized compounds**

| Compounds | MIC µg/mL |
|---|---|
| 4a | 125µg/mL |
| 4b | 62.5µg/mL |
| 4c | 125µg/mL |
| **Rifampicin** | **0.98µg/mL** |
| **Isoniazid** | **1.95µg/mL** |

**Table No :5; Binding Affinity of Docked Ligand**

| Compound | Binding Affinity | Mode | RMSD/Lb | RMSD/Ub |
|---|---|---|---|---|
| 4a | -5.4 | 0 | 0.0 | 0.0 |
| 4b | -6.2 | 0 | 0.0 | 0.0 |
| 4c | -5.1 | 0 | 0.0 | 0.0 |

## Anti-tubercular activity result of compound (4a-c)

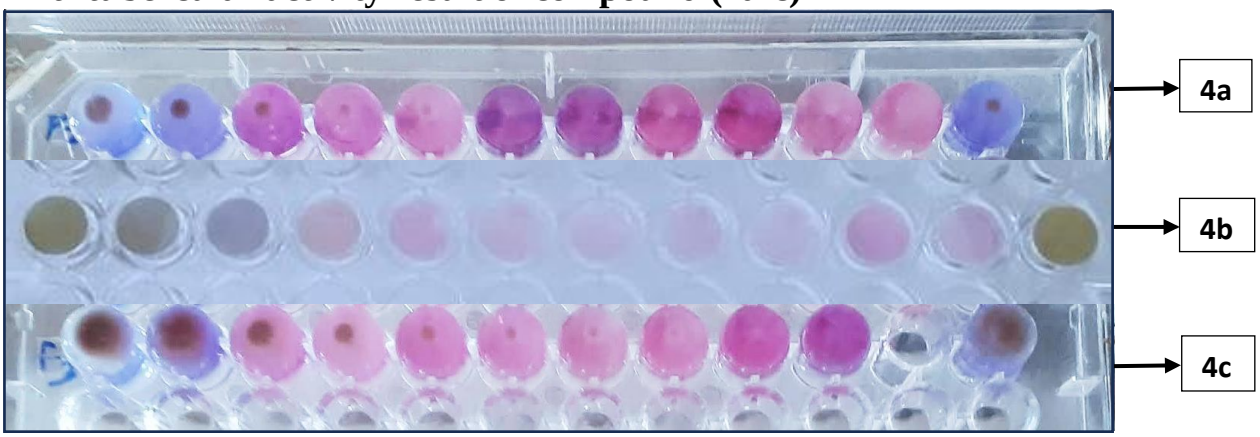


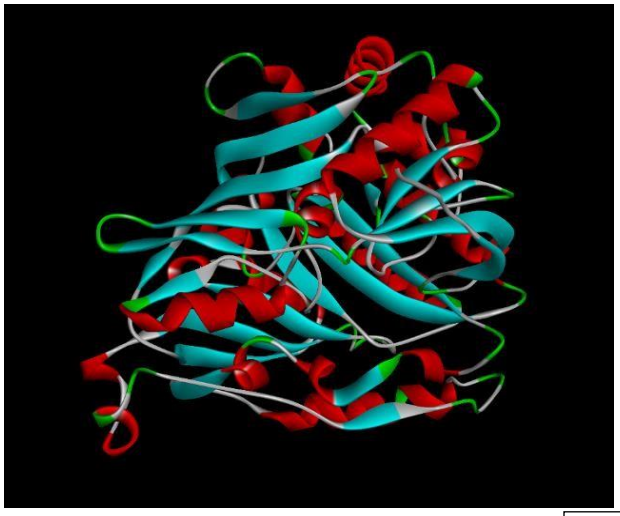

**Fig no-02**
**Compared with Standard [Rifampicin and Isoniazid] [fig;4]**

**Fig no-03**

Newly Synthesized compounds(4a-c) were tested for antitubercular activity using MABA method against H37Rv strain. The compound 4b shows good anti-tubercular activity with MIC value of 62.5µg/mL are shown in the Table no-6

## Molecular docking study for newly synthesized derivatives(4a-c)

By doing literature study, the crystal structure of protein Decaprenylphosphoryl-beta -D- ribose oxidase)6G83[14] was downloaded from Protein Data Base [PDB]



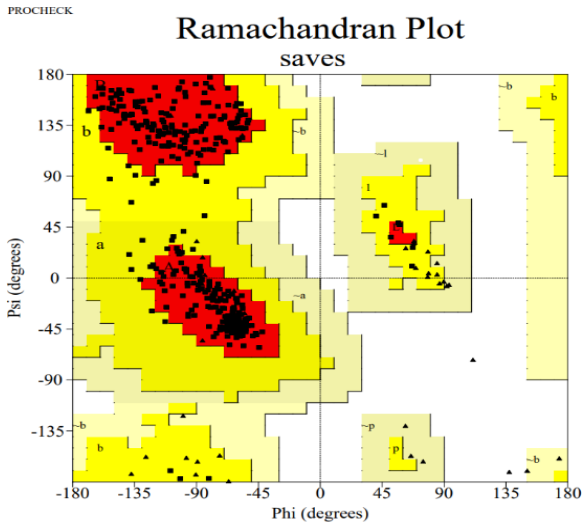
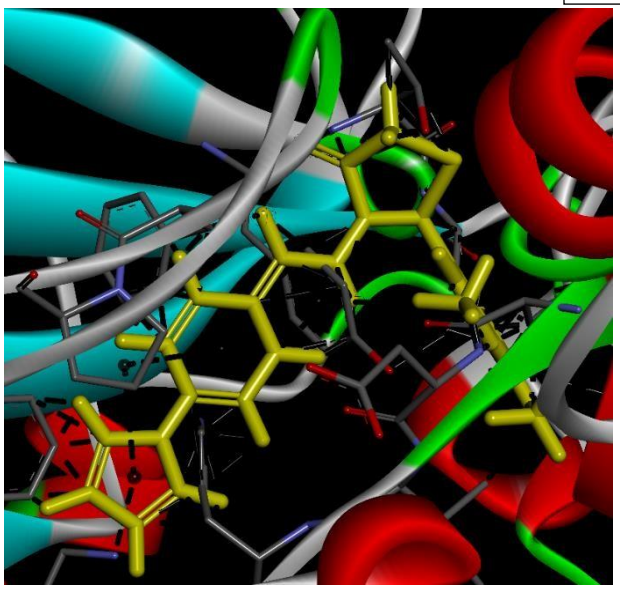



**Fig no- 04 & 05**



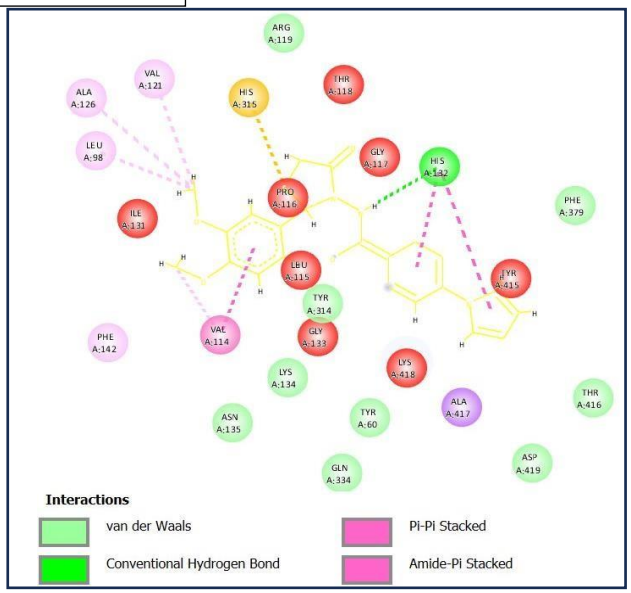
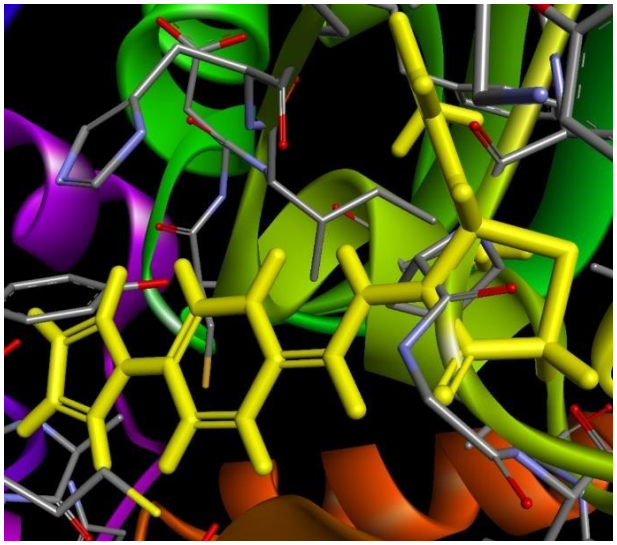



**Fig no-06 (3D& 2D) structure of compound 4a**



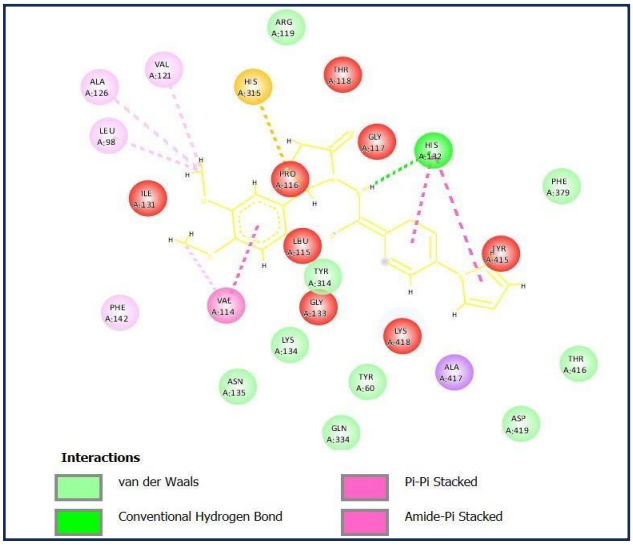



**Fig no-07 (3D& 2D) structure of compound 4b**

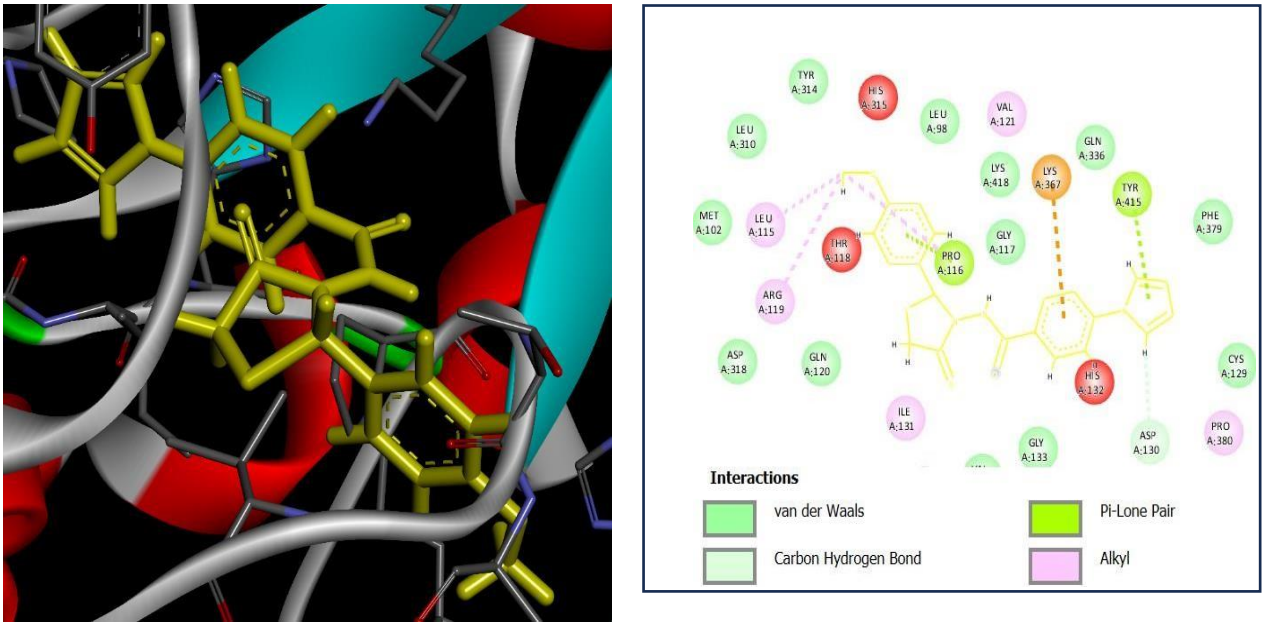



**Fig no-08 (3D& 2D) structure of compound 4c**

**Boilled EGG model**

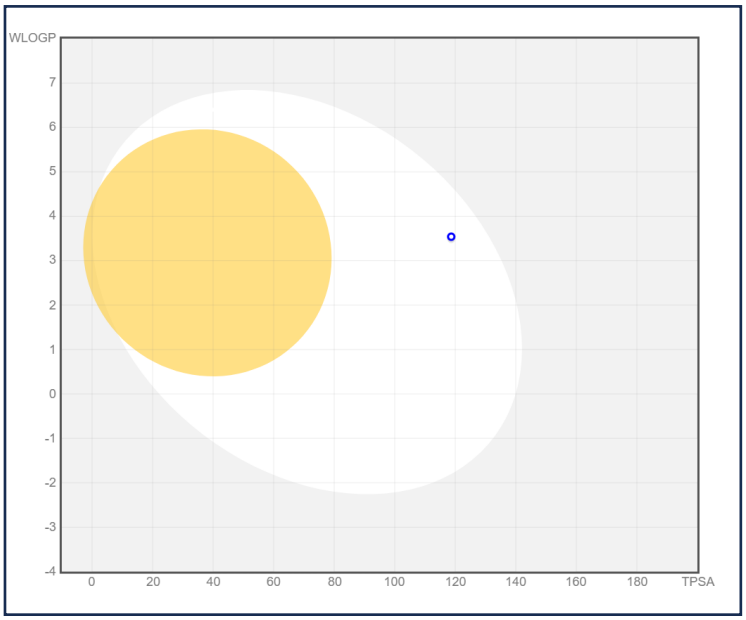



**Fig no-09 (4a)**

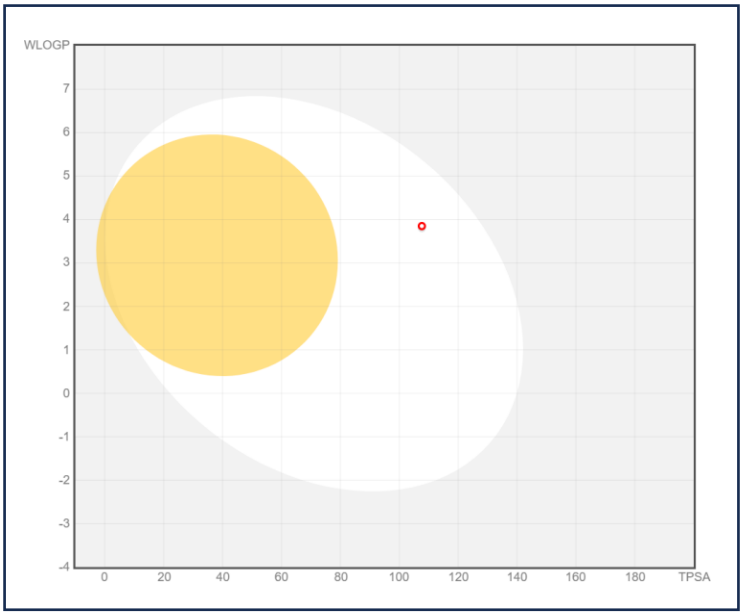



**Fig no-10(4b)**

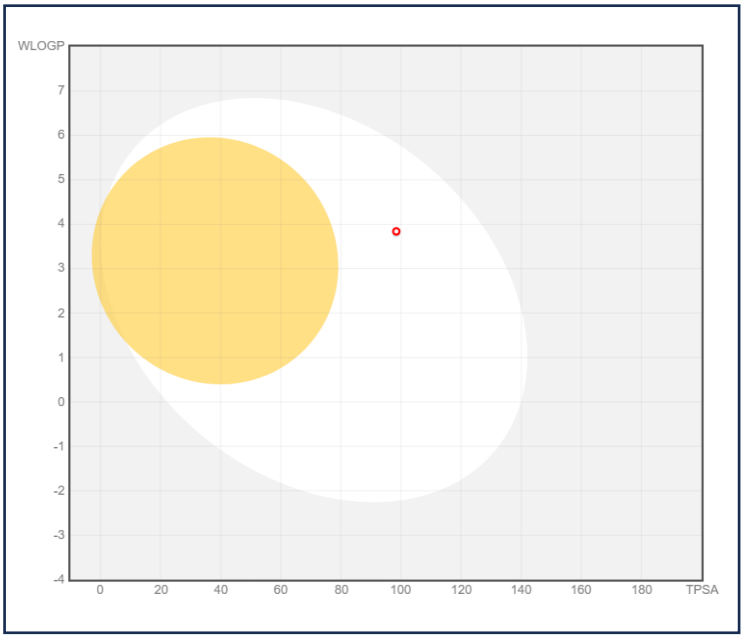



**Fig no-11(4c)**

In this research work, by the reaction between ethyl-4-aminobanzoate and 2,5-dimethoxy tetrahydrofuran in dried acetic acid the compound(I)was synthesized, The synthesized compound (1) was then reacted with hydrazine hydrate in absolute ethanol to yield the compound (2), the compound (2) in the presence of aromatic aldehyde and ethanol which was refluxed for 3hr yields the compound (3), Further the compound (3) was refluxed in the presence of thioglycolic acid and benzene for 12hrs to yield the compound(4a-c).

The Physicochemical data of newly synthesized Pyrrolyl thiazolidinone derivatives(4a-c) given in table no-01where the purity of the synthesized compound was confirmed and then the synthesized compound 4a-c subjected for spectral characterization data such as FT-IR,$^{1}$H NMR,$^{13}$C NMR and mass spectroscopy.

Antitubercular activity of the synthesized compound was carried out using *Mycobacterium tuberculosis* $H_{37}$Rv strain using the Microplate Alamar Blue Assay (MABA) method. The compound 4b exhibit good antitubercular activity with MIC value of 62.5µg/ml, And the compound 4a and 4c shows moderate activity with the MIC value of 125µg/ml when compared with the standard drugs isoniazid and rifampicin.

Further, In-silico studies were carried out using software Discovery studio and PyRx, which provides the data about the binding affinity for the docked ligand for the compound (4a-d) in which the compound 4b shows the good binding affinity of value of -6.2 against the target protein Decaprenylphosphoryl-beta-D-ribose oxidase(6G83), were as the compound 4a, 4c and 4d shows the value of-5.4, -5.1 and -5.8.

The boiled egg model was also studied and observed to know the lipophilicity and hydrophilicity of the synthesized compounds, from which we got to know that all the synthesized compounds were hydrophylic in nature.

## Conclusion

A novel series of Pyrrolyl thiazolidinone derivatives were synthesized by the reaction between Pyrrolyl Shiff Base in the Presence thioglycolic acid and benzene which was refluxed for 12hr to obtain Pyrrolyl thiazolidinone derivative (4a-c).The synthesized compound were characterized by the spectroscopic techniques such as FT-IR,$^{1}$H NMR,$^{13}$C NMR and mass spectra. The synthesized compound further screened for the antitubercular activity against *Mycobacterium tuberculosis* $H_{37}$Rv strain using the Microplate Alamar Blue Assay (MABA) method by using standard drug as rifampicin and isoniazid. The compound 4b shows good anti-tubercular activity with MIC value of 62.5µg/ml and the compound 4a and 4c shows the moderate activity with MIC value of 125µg/ml. Further, In-silico studies were carried out using software PyRx and Discovery studio, which provides the data about the binding affinity for the docked ligand, for the compound 4a-d in which the compound 4b shows the good binding affinity of value of -6.2 against the target molecule of Decaprenylphosphoryl-beta-D-ribose oxidase(6G83), were as the compound 4a, 4c and 4d shows the value of-5.4,-5.1 and -5.8. Through boiled egg model we can also conclude that all compounds are hydrophylic in nature.Hence, we conclude that this study will serve as a valuable guide for the further, design and synthesis of more effective pyrrole derivatives for the treatment of tuberculosis.

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