

DESIGN, SYNTHESIS AND ANTITUBERCULAR SCREENING OF CERTAIN NOVEL THIADIAZOLYL PYRROLIDINE CARBOXAMIDES AS ENOYL ACP REDUCTASE INHIBITORS

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ABSTRACT

A series of 5-oxo-1-phenyl-N-(5-substituted phenyl-1,3,4-thiadiazol-2-yl)pyrrolidine-3-carboxamides (3a-e) were synthesized by reacting 5-substituted -phenyl-1,3,4-thiadiazol-2-amine(2a-e) with 5-oxo-1-phenylpyrrolidine-3-carboxylic acid(1).The structures of the novel derivatives were confirmed by UV, IR, ¹HNMR and mass spectral data .The novel compounds were designed as *Mycobacterium tuberculosis* enoyl acyl carrier protein reductase (InhA) inhibitors based on docking studies and oral bioavailability scores based on Lipinski's rule evaluation. The synthesized leads were screened for antitubercular activity.

Keywords: Thiadiazolyl, Pyrrolidine, Enoyl

INTRODUCTION

Tuberculosis is one among the most dreadful infections affecting around two million casualties worldwide¹. The tedious duration of therapy and resistance developed by the microorganism has made the recurrence of the disease especially as MDR-TB and XDR-TB². Also for the past thirty years, no new TB drug has been discovered. The search for new entities has been focused now on the design of molecules acting as enzyme inhibitors. The target enzyme should play a vital role in any phase of the life cycle of the pathogen and should be absent in the host.

Enoyl acyl carrier protein reductase is a FAS II enzyme involved in the bacterial fatty acid biosynthetic pathway in the mycobacterium and other bacteria³. This enzyme is involved in fatty acid elongation in the cell wall synthesis. The prime TB drug isoniazid is reported to be a potent enoyl ACP reductase inhibitor but requires initial activation by Kat G, a catalase peroxidase enzyme⁴.

This led to the search for new antitubercular agents which can act as direct enoyl ACP reductase inhibitors without undergoing Kat G activation. Triclosan analogues⁵, pyrazole derivatives⁶, indole-5 amides⁷, pyrrolidine carboxamides⁸ etc are reported as direct enoyl ACP reductase inhibitors. Thiadiazoles are also reported to show a variety of biological activities like antitubercular⁹, antibacterial¹⁰, antifungal¹¹activities etc.

Based on this, an attempt was made to design a series of thiadiazolyl pyrrolidine carboxamides on the basis of drug design tools. Literature review and virtual screening¹² data has been utilized to

select the target enzyme and ligands. Lead optimization was done by screening for lipinski's rule¹³. The optimized ligands were docked with the enzyme. The leads designed were synthesized and characterized using UV, IR, ¹HNMR and mass spectral data and screened for antitubercular activity screening.

MATERIALS AND METHODS

The docking software used was autodock4.2 and the lead optimization was done through mol inspiration server. All the chemicals were procured from Sigma Aldrich, Hi-media and Merck. UV spectra were recorded on a JASCO V-530 UV/VIS spectrophotometer. IR spectra were done on a Shimadzu FTIR 8400S. PMR spectra were recorded on a Bruker300mhz NMR spectrometer. Mass spectra was obtained on a Shimadzu LCMS-2010 EV .Completion of the reaction was monitored by thin layer chromatography on perforated sheets of silica gel-G using iodine vapour for detection. The synthetic pathway is enumerated in the scheme and the physical data is given in Table 3.

Docking study

Selection of the target and lead

The target, enoyl acyl protein reductase(InhA) and the leads were selected based on the virtual screening from zinc database using iGEMDOCK v.2¹⁴.A small molecule library of 10,000 compounds were docked into the enzyme and the potential leads were discovered. Pyrrolidine carboxamide was selected as the potential lead. The structure of potential lead and the snap shots are depicted in Fig 1.

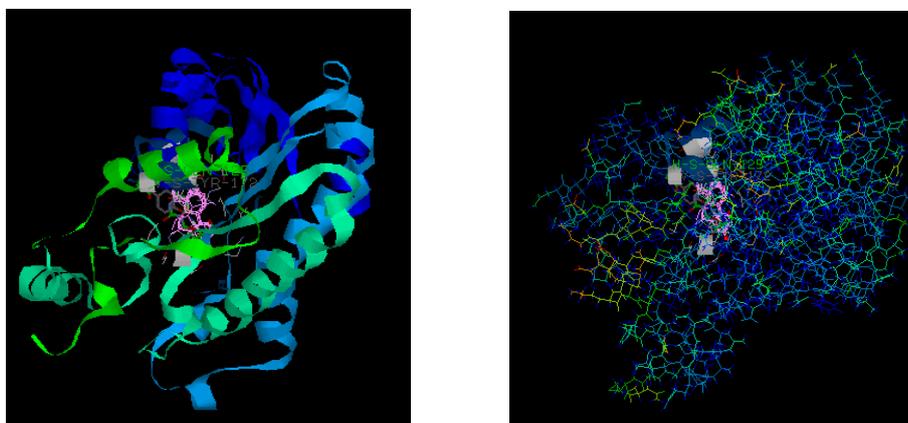


Fig. 1: Snapshots of ligands bound to enzyme InhA in iGEMDOCK v.

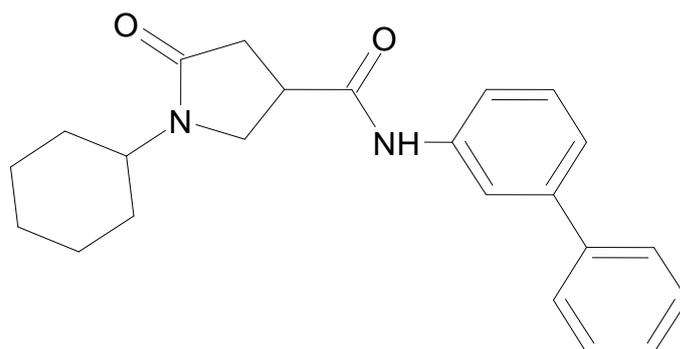


Table 1: Lipinski parameters

Sl no	Comp code	Log p	Molecular weight	No. of H donors	No. of H acceptors	No. of violations
01	3a	2.389	364.43	1	6	0
02	3b	3.517	398.87	1	6	0
03	3c	3.287	378.45	1	6	0
04	3d	2.895	394.45	1	7	0
05	3e	2.798	409.42	1	9	0

Lead optimization

Lead optimization was done through insilico Lipinski filter. Molinspiration server¹⁵ was used for this purpose. The structure drawn in the JME editor was subjected to calculate the druglikeness score through calculate the properties module. The datas are given in the table 1.

Molecular docking

The docking studies for all the derivatives (3a-e) were performed in the Autodock 4.2 version. The enzyme (pdb accession code: 2H71)¹⁶

was refined by removing water molecules and polar hydrogens and kollmann charges were added. Energy minimized ligands in pdb were subjected to calculation of Gasteiger-Huckel charges. Grid box for docking simulations were constructed with 60 points (with 0.375 Å spacing) in x, y, and z direction to be centered in the active site using Autogrid utility of the Autodock programme. The enzyme ligand complex was subjected to 2.5 million evaluations. The binding energies are compared with the dock score of the standard ligand, pyrrolidine carboxamide. The binding energy of pyrrolidine carboxamide is -8.23 kcal/mol. The snap shots are given in figure 1 and the binding energies are listed in Table 2.

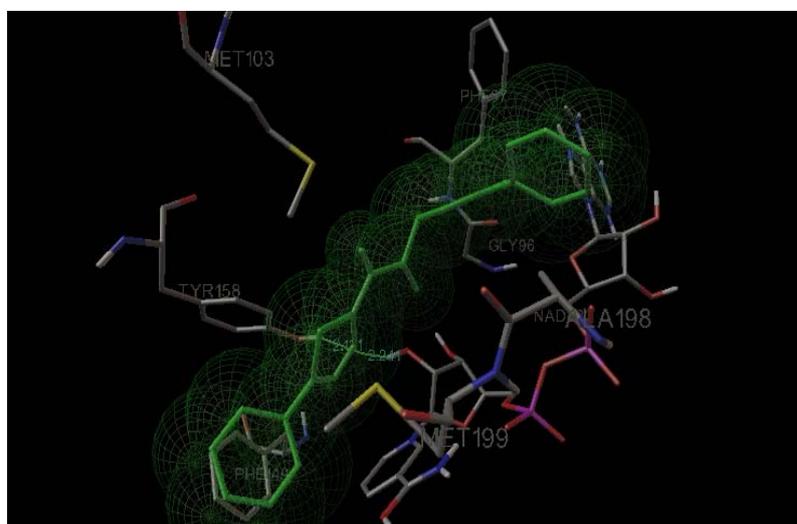
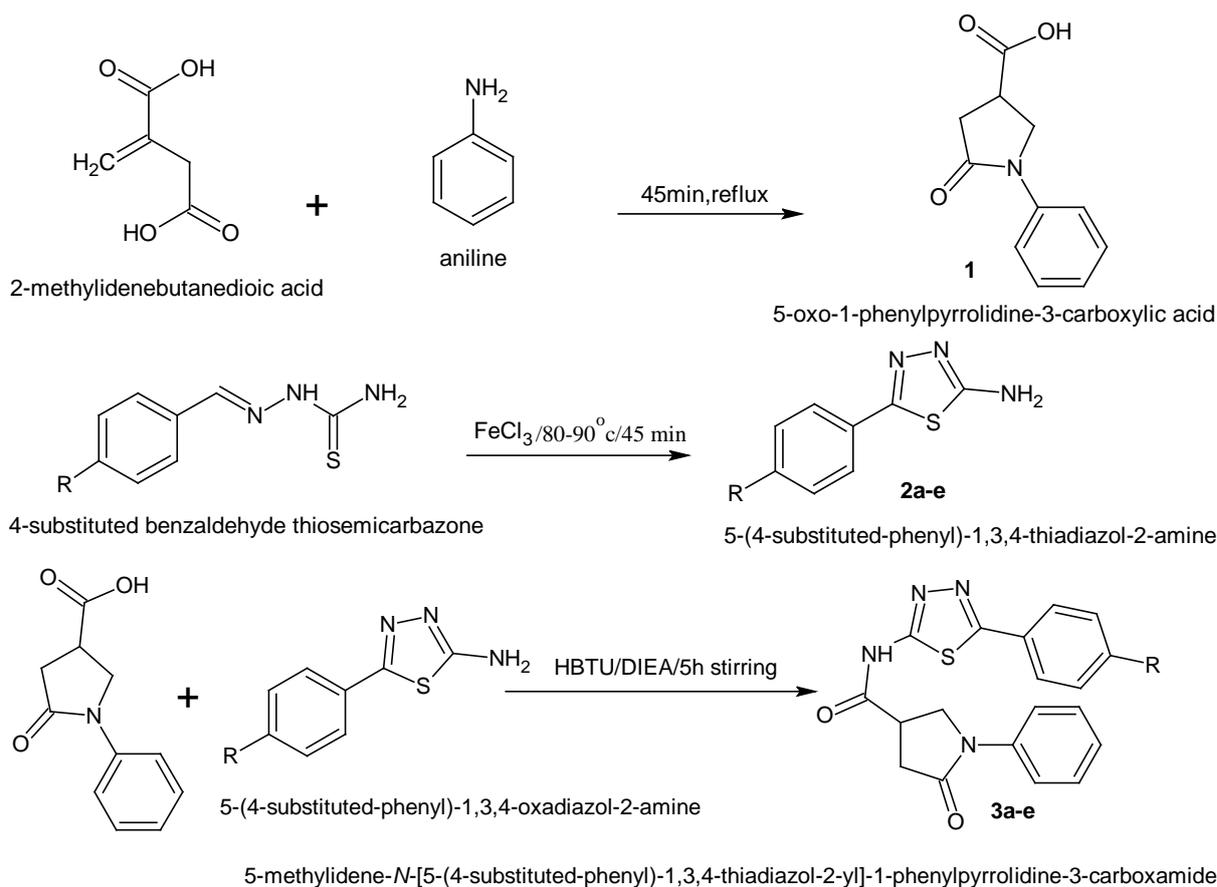


Fig. 2: Snap shot for the binding of 3a with InhA (a) ligand coloured in green (b) oxygen of ligand forms hydrogen bonds with TYR 158 and NAD

Table 2: Binding energies of docked structures

Sl no	Comp code	Binding Energy(Δkcal/mol)
01	3a	-9.14
02	3b	-8.65
03	3c	-7.35
04	3d	-9.36
05	3e	-9.75



SCHEME

Table 3: Physical data of the synthesized compounds

Sl no	Comp code	Molecular formulae	Melting point(°c)	Yield (%)
01	3a	C ₁₉ H ₁₆ N ₄ O ₂ S	249.3	54
02	3b	C ₁₉ H ₁₅ ClN ₄ O ₂ S	278.8	56
03	3c	C ₂₀ H ₁₈ N ₄ O ₂ S	251.9	66
04	3d	C ₂₀ H ₁₈ N ₄ O ₃ S	258.7	54
05	3e	C ₁₉ H ₁₅ N ₅ O ₄ S	239.2	65

TLC solvent: acetone/benzene: 4:6

EXPERIMENTAL

Synthesis of 5-oxo-1-phenylpyrrolidine-3-carboxylic acid (1)¹⁷

A mixture of itaconic acid (13.1gm, 0.1mol), aniline (9.2ml, 0.1mol) and water (200ml) was refluxed for one hour or until the odour of amine becomes faint after which the reaction mixture was chilled for one hour. The product filtered was washed with cold water and dissolved in dilute sodium hydroxide. The clear solution was treated with charcoal and filtered hot and acidified with dilute hydrochloric acid. The product filtered was recrystallised from water. m.p.: 189°C (lit¹²: 189-190°C)

Synthesis of 2-amino-5-(4-substituted) phenyl -aryl-1, 3, 4-thiadiazole (2a-e)¹⁸

Aromatic aldehydes (0.2mol) and thiosemicarbazide (0.2mol) was dissolved respectively in warm alcohol (300ml) and warm water (300ml) and mixed slowly with stirring. The aryl thiosemicarbazones obtained on cooling was filtered and recrystallised from 75% ethanol. Aryl thiosemicarbazone (0.05mol) already synthesized was suspended in 300ml of warm water. (0.15mol) of FeCl₃ in 300ml water was added with stirring. The

contents were heated for 45 minutes at 80-90°C. The solution was filtered in hot condition and (0.11mol) citric acid and (0.05mol) sodium citrate were added. The resulting solution was divided into four parts and to each part aq. NH₃ is added to neutralize the solution. The amine separated was filtered, dried and recrystallised from 50% alcohol.

Synthesis of 5-oxo-1-phenyl-N-[5-[4-substituted]-phenyl-1, 3, 4-thiadiazol-2-yl] pyrrolidine-3-carboxamides (3a-e)⁸

0.00189 mol of aryl 1, 3, 4-thiadiazol-2-amine (2a) and 0.0019 mol of 5-oxo-1-phenylpyrrolidine-3-carboxylic acid (1) were dissolved in 58ml of dry DMF.

HBTU (0.87gm, 0.0023mol) and DIEA (0.93ml, 0.0053 mol) were stirred for five hours at 23°C. The reaction was arrested by adding double the volume NaCl solution. The resulting mixture was extracted with ethyl acetate (3x50ml). The combined ethyl acetate layer was washed with 1N HCl then with saturated sodium bicarbonate followed by brine. The organic layer was filtered through anhydrous sodium sulphate. The residue left out after the evaporation of ethyl acetate is recrystallised from dichloromethane-methanol mixture.

Table 4: Antitubercular activity data

Sl no	Comp code	MIC($\mu\text{g/ml}$)
01	3a	25
02	3b	50
03	3c	50
04	3d	25
05	3e	25

Compound 3a: UV: λ_{max} 296 nm, IR (KBr): 3161.43 (amide NH), 3061.13 (aromatic CH stretch), 1701.27 (amide C=O), 1599.04 (thiadiazole C=N), 667.39 (thiazodiazole C-S) ^1H NMR(DMSO): δ 7.030-7.890 (m,10H, Ar-H),4.026-4.168 (dd,2H,-CH₂),3.381 (S,1H,-CH),2.729-2.956 (dd,2H, -CH₂); Mass (m/z): 366(M+2)

Compound 3b: UV: λ_{max} 301 nm, IR (KBr): 3161.43 (amide NH), 2902.96 (aromatic CH stretch), 1699.34 (amide C=O), 1597.11 (thiadiazole C=N), 734.90 (C-Cl), 667.39 (thiazodiazole C-S); ^1H NMR(DMSO): δ 7.128-7.981 (m,9H, Ar-H),4.036-4.174 (dd,2H,-CH₂),3.366 (S,1H,-CH),2.763-2.962 (dd,2H, -CH₂); Mass (m/z): 399(M⁺)

Compound 3c: UV: λ_{max} 295 nm, IR (KBr): 3153.72 (amide NH), 1703.20 (amide C=O), 1599.04 (thiadiazole C=N), 671.25 (thiazodiazole C-S); ^1H NMR(DMSO): δ 7.131-7.843 (m,9H, Ar-H),4.031-4.178 (dd,2H,-CH₂),3.633 (S,1H,-CH),2.733-2.931 (dd,2H, -CH₂),2.338 (s,3H, CH₃); Mass (m/z): 379(M+1)

Compound 3d: UV: λ_{max} 307 nm, IR (KBr): 3155.65 (amide NH), 3057.27 (aromatic CH stretch), 1697.41 (amide C=O), 1604.83 (thiadiazole C=N), 651.96 (thiazodiazole C-S); ^1H NMR(DMSO): δ 7.069-7.893 (m,9H, Ar-H),4.026-4.168 (dd,2H,-CH₂), 3.825 (S,3H,-OCH₃),3.670 (S,1H,-CH),2.756-2.923 (dd,2H, -CH₂); Mass (m/z): 395(M+1)

Compound 3e: UV: λ_{max} 347 nm, IR (KBr): 3155 (amide NH), 3080 (aromatic CH stretch), 1701.27 (amide C=O), 1597.04 (thiadiazole C=N), 1518 (aromatic N=O), 667.39 (thiazodiazole C-S); ^1H NMR(DMSO): δ 7.132-7.755 (m,9H, Ar-H),4.051-4.185 (dd,2H,-CH₂),3.508 (S,1H,-CH),2.756-2.923 (dd,2H, -CH₂); Mass(m/z): 410(M+1)

Antitubercular screening

The antimycobacterial activity of compounds were assessed against *M. tuberculosis* using microplate Alamar Blue assay (MABA) ¹⁹. The 96 well plates received 100 μl of the Middlebrook 7H9 broth containing *Mycobacterium tuberculosis* and serial dilution of compounds were made directly on plate. The final drug concentrations tested were 0.01 to 20.0 $\mu\text{l/ml}$. Plates were covered and sealed with parafilm and incubated at 37°C for seven days. After this time, 25 μl 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. The MIC data is given in table 4.

RESULTS AND DISCUSSION

The optimization of the lead obeyed the Lipinski's rule of five and showed good druglikeness score predicting the leads to show good oral bioavailability. The dock results showed that the oxygen of the pyrrolidine carboxamide formed hydrogen bond with Tyr 158 and NAD cofactor which was essential for a lead to act as enoyl ACP reductase inhibitor. The synthesized compounds were characterized by UV, IR, ^1H NMR and mass spectral data. The spectral datas confirms the successful formation of the newly synthesized compounds. All the compounds were obtained in good yields. The anti tubercular screening data showed that all the pyrrolidine carboxamides showed good anti tubercular activity. Compounds 3a, 3d and 3e showed excellent activity which is in correlation with the dock scores.

CONCLUSION

The present study has given an insight into the development of new direct *Mtb* Enoyl ACP reductase (InhA) inhibitors. The virtual screening technique helped in stream lining promising InhA inhibitors from a vast library of compounds thus highlighting the potential of the lead moiety, pyrrolidine carboxamide as *Mtb* InhA inhibitor. Various insilco tools like Lipinski filter and molecular docking has been utilized to conclude the relevance in synthesizing the leads. The synthesized derivatives showed good anti tubercular activity confirming that they were promising candidates as *Mtb* InhA inhibitor. Thus the insilco design has been helpful in synthesizing only promising molecules enabling the minimization of time spend for searching leads.

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