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SYNTHESIS, DOCKING AND BIOLOGICAL EVALUATION OF 1,4– DIHYDROPYRIDINE DERIVATIVES

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ABSTRACT
In the present study, a novel series of 1,4-dihydropyridine derivatives were synthesized and docking study was performed to rationalize the possible interactions between the synthesized compounds and active site.1,4-dihydropyridine derivatives were designed as Enoyl-acyl carrier protein reductase inhibitors. All compounds were screened for antimycobacterial activity against M.tuberculosis H37Rv using Microplate Alamar Blue Assay. Pyrazinamide (PZA) and Streptomycin were employed as the reference antimycobacterial agents. Among the series S1 found to be most potent while S2, S3, S4, S5, S6 were found to be less potent than S1.
Keywords: Antimycobacterial; Docking, MABA, Enoyl-acyl carrier protein reductase

1. INTRODUCTION

Tuberculosis (TB) is a pandemic disease and its causative agent Mycobacterium *tuberculosis* is one of the most prolific infectious agents affecting humans. The 196 countries reporting to WHO in 2008 notified 5.6 million new and relapse cases in 2007, of which 2.6 million (46%) were new smear-positive cases[I]. Furthermore, treatment of tuberculosis with human immuno deficiency virus infected patients (HIV) is difficult and results as the leading cause of death among HIV positive patients worldwide. Another factor which contributes to more number of deaths is the emergence of multiple drug resistance (MDR) [II-V] and totally drug-resistant tuberculosis (TDR-TB) [VI-VII]. Enoyl-acyl carrier protein reductase (ENR) is a key enzyme of the type II fatty acid synthesis (FAS) system. ENR is an attractive target for narrow spectrum antibacterial drug discovery because of its essential role in metabolism and its sequence conservation across many bacterial species. In addition, the bacterial ENR sequence and structural organization are distinctly different from those of mammalian fatty acid biosynthesis enzymes [VIII]. So ENR inhibitors can be designed for the development of new and potent antitubercular drugs. Several 1, 4-dihydropyridine derivatives have shown good inhibitory activity against ENR[IX]. In this work, some 1,4-dihydropyridine derivatives were synthesized, docked and screened for antimycobacterial activity. The newly synthesized heterocycle exhibited promising antimycobacterial activity. Till now, no new drug has been introduced since the discovery of Rifampin in spite of major advances that have been made in the drug discovery

process.Hence,there is an overwhelming need to develop novel antimycobacterial agents.Furthermore,solubility plays an important role for the development of drug in tuberculosis. Thus our aim was further refined to synthesize 1, 4-dihydropyridine derivatives and evaluate them for antimycobacterial activity.Herein we report the synthesis, docking and in vitro antimycobacterial activity of a series of 1, 4-dihydropyridine derivatives. The docking study was performed to rationalize the possible interactions between the synthesized compounds and the active site.

2. EXPERIMENTAL

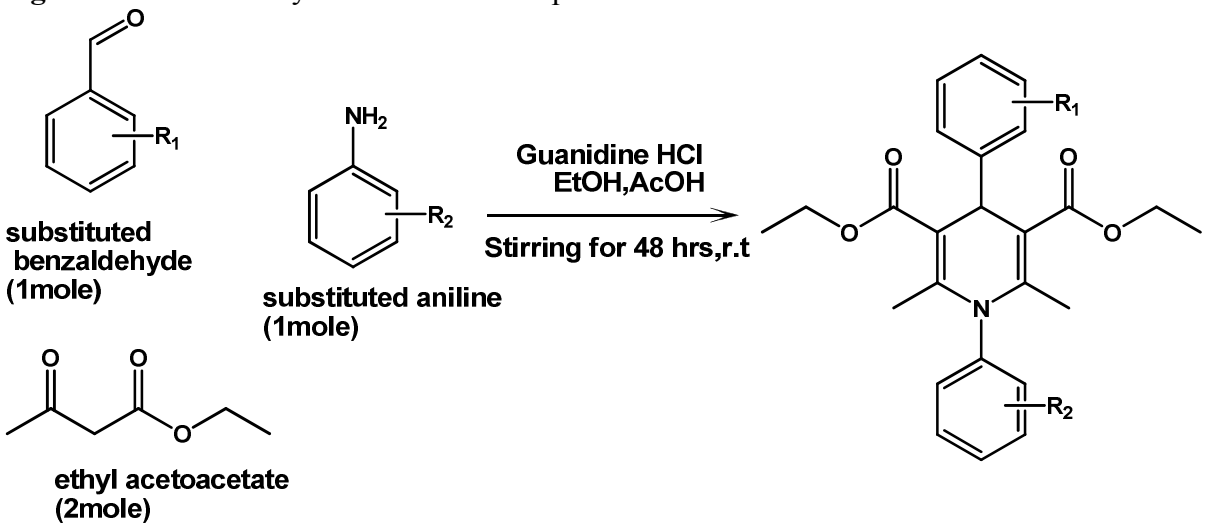
2.1. Material and Apparatus

All the reactions were carried out with dry, freshly distilled solvents under anhydrous conditions, unless otherwise noted. Melting points were determined by VeegoVMP-D Digital melting point apparatus and are uncorrected. FTIR spectra of the powdered compounds were recorded using KBr on a Varian-160 FTIR spectrometer using Diffuse Reflectance Attachment and are reported in cm^{-1} and ^{1}H NMR spectra were recorded on a Varian Mercury YH300 (300 MHz FT NMR) spectrophotometer using TMS as an internal reference (Chemical shift represented in ppm). LCMS were recorded on "2010EV LCMS Shimadzu" instrument by direct injection method. Purity of the compounds was checked on 'Silica Gel G' coated on thin layer chromatographic plate procured from Merck, eluent was the mixture of different polar and non-polar solvents in varying proportions and detection was done either by observing in UV light or exposure to iodine vapours as required. The synthetic route used for the title compounds is outlined in Scheme 1.

General Procedure:

A mixture of substituted aniline (1 mol), ethyl acetoacetate (2 mol), appropriate aldehyde **(**1 mol) acetic acid (1mole) and guanidine hydrochloride (0.015 mol), in catalytic amounts were taken in 10-15 ml absolute ethanol. The reaction mixture was stirred at room temperature for 48 hrs. The reaction was monitored by TLC (*n*-hexane: ethyl acetate, 60:40), upon completion the product was filtered and recrystallized with ethanol [XII].

Figure 1.Scheme for synthesis of title compound



2.2. Modelling Studies

2.2.1. Molecular Docking Protocol

The molecular docking tool, GLIDE (Schrodinger Inc.,USA) was used for ligand docking studies in to the enzyme ENR binding pocket.The crystal structures of ENR were obtained from protein data bank.(PDB Code: 2AQK).The protein structure was prepared for docking using 'protein preparation wizard'in Maestro wizard 8.5.The protein preparation uses the OPLS force field[XIII] for this purpose. Group grids were defined by centering them on the ligand in the crystal structure using the default box size. Ligprep 2.2 module utilized to produce the low energy conformer of ligands using MMFF94 force field [XIV].The lower energy conformations of the ligands were selected and were docked into the grid generated from protein structures using standard precision (SP) docking mode [XV].

Docking and Scoring Functions

The docked complexes of the designed compounds along with the ligand receptor poses have been shown in the Figure 2.The final evaluation is done with glide score (docking score) and single best pose is generated as the output for particular ligand.

G score=a***** vdw +b *****coul+Lipo+H bond +Metal+BuryP+Rot B+Site

Where, vdW: Vander Waal energy; Coul: Coulomb energy; Lipo: Lipolipophilic contact term; H Bond: hydrogen-bonding term; Metal: metal binding term; BuryP: penalty for buried polar groups; RotB: penalty for freezing rotatable bonds; Site: polar interactions at the active site; and the Coefficients of vdW and Coul are: a = 0.065, b = 0.130.

ADME Prediction

The ADME properties were calculated using Qikprop tool of Schrodinger software. It predicts both physicochemically significant descriptors and pharmacokinetically relevant properties. It also evaluates the acceptability of analogues based on Lipinski's rule of 5[XV, XVI] which is essential to ensure drug like pharmacokinetic profile while using rational drug design. All the analogues were neutralized before being used by Qikprop.

2.2.3. Antimycobacterial Activity

All the newly synthesized 1,4-dihydropyridine derivatives were assayed in vitro for antitubercular activity against M.tuberculosis H37Rv using Microplate Alamar Blue Assay (MABA). Pyrazinamide (PZA) and Streptomycin were employed as the reference antimycobacterial agents.

Microplate Alamar Blue assay (MABA): [XVII-XX]

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 Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate. The final drug concentrations tested were 0.01to 20.0 µg/ml.Plates were covered and sealed with parafilm and incubated at 37ºC for five days. After this time, 25µl of freshly prepared 1:1mixture of Almar 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.

3. RESULTS AND DISCUSION

Physical Characterization: Physical constant, R_f value was determined for all synthesized compounds. (Table1)

Table 1.Physicochemical properties of 1,4–dihydropyridine derivatives

Compound Code	R_1	R_2	mp(^oC) uncorrected	Molecular weight	R_f^*
S1	-Cl	3-Cl,4-F	134-136	491.11	0.68
S2	-H	3-Cl,4-F	125-129	457.92	0.56
S3	-OH	2,6 dichloro	130-134	490.38	0.72
S4	-H	2,6 dichloro	112-116	474.38	0.63
S5	-Cl	4-Cl	110-114	474.38	0.52
S6	-Cl	4-OH	118-122	455.15	0.67

Representative spectral data of product:

Compound S1: FTIR (KBr, cm^{-1}): 3062.41 (Aromatic C-H Stretch); 1253.5(C-O-C Stretch); 1389.21(C=C Stretch); 1650.77(C=O Stretch); 821.529 C-Cl Stretch); 1176.36(C-N Stretch); 794.52(C-F Stretch)^{1}H NMR ($CDCl_3$)δ(ppm): 1.29 (t, 3H-CH_3 of ethoxy); 4.20(q, 2H-CH_2 of ethoxy); 7.17(dd, 2H-ph); 7.37(dd,2H-ph);2.26(s,6H-CH_3); 7.33(d,2H-ph);7.16(d, 2H-ph); 6.04(dd, 2H-ph). MS (m/z%) 492.30(M^+).

Compound S2: FTIR (KBr, cm^{-1}): 3062.41 (Aromatic C-H Stretch); 1253.5(C-O-C Stretch); 1326.79 (C=C Stretch); 1650.77(C=O Stretch); 821.27(C-Cl Stretch); 1176.36(C-N Stretch); 796.52(C-F Stretch)

Compound S3: FTIR (KBr, cm^{-1}): 2981.41 (Aromatic C-H Stretch); 1253.5(C-O-C Stretch); 1326.79(C=C Stretch); 1650.77(C=O Stretch); 821.27(C-Cl Stretch); 1176.36(C-N Stretch); 3637.09(OH Stretch)

Compound S4: FTIR (KBr, cm^{-1}): 3062.41(Aromatic C-H Stretch); 1253.5(C-O-C Stretch); 1407.78(C=C Stretch); 1650.77(C=O Stretch); 759.81(C-Cl Stretch); 1141.65(C-N Stretch)

Compound S5: FTIR (KBr, cm^{-1}): 3151.15 (Aromatic C-H Stretch); 1253.5(C-O-C Stretch); 1407.78(C=C Stretch); 1646.50(C=O Stretch); 813.81(C-Cl Stretch); 1176.36(C-N Stretch)

Compound S6: FTIR (KBr, cm^{-1}): 3151.15 (Aromatic C-H Stretch); 1253.5(C-O-C Stretch); 1407.78(C=C Stretch); 1646.50(C=OStretch); 813.81(C-Clstretch); 1176.36 (C-N stretch); 3621.66(OH stretch)

Molecular Docking

The designed compounds were found to display good binding affinity to the receptor. G-score, H-Bond Interaction and Contacts .The more negative value of G-score indicates that the compound is more potent and good binding affinity (Table 2).G score of compound S1 was found to be -7.99 and G score of rest of the designed compounds were found be comparable with G-score of standard Isoniazid (G score:-6.61) indicated that designed compounds have good binding affinity for binding to inhA.The best poses obtained by docking results are reported in Fig. 2, where main interaction between ligands and receptors can be observed. Standard Isoniazid shows interaction with Lysine 165 amino acids by non covalent hydrogen bond. All designed compounds adopt a very similar conformation binding pocket, showing similar non-

covalent hydrogen binding with Lysine 165.It is well established and accepted fact that number of good Vander Waals interactions decides the binding affinity for any ligand with receptor enzyme protein and bad, ugly contacts indicate steric clashes after docking which should be less for good activity. Therefore we have analyzed the binding modes and abilities, considering the number of good, bad and ugly Vander Waals (vdW) interactions of the standard and designed compounds with active binding site.ADME Properties were analyzed using Qikprop and pharmaceutically relevant properties of 1,4 –dihydropyridine derivatives, which found to be significant are reported(Table 3) and are important for predicting the drug-like properties of molecules. These properties were:

1) Molecular weight (Mol_MW) (130 - 500)
2) Octanol/water partition coefficient (Log Po/w) (–2.0 – 6.5)
3) CNS Predicted central nervous system activity –2 (inactive), +2 (active)
4) Brain/blood partition coefficient (QPlogBB) (–3.0 – 1.2)
5) Percent human oral absorption (>80% is high, <25% is poor)

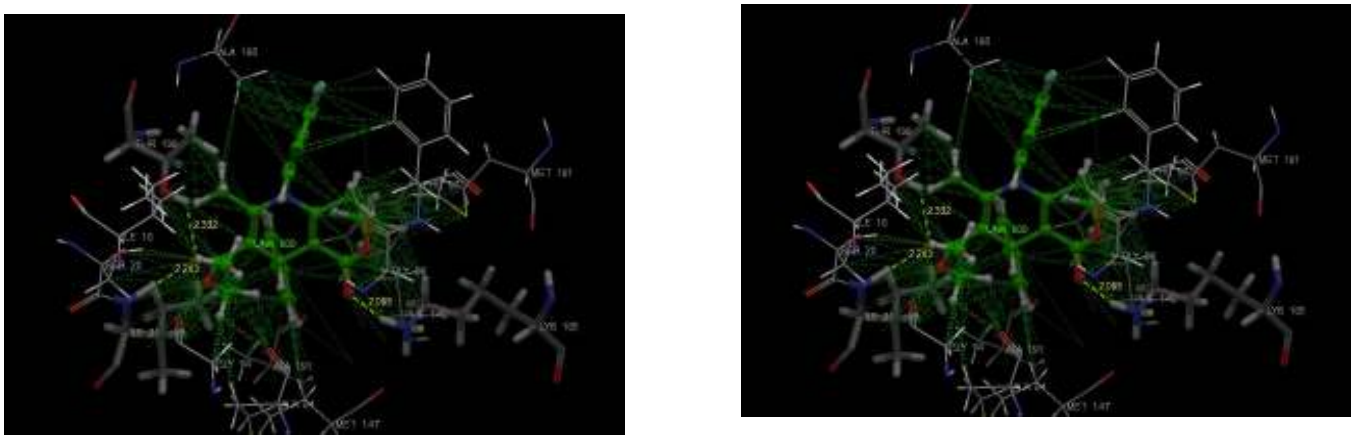
Antimycobacterial activity

Amongst the compound tested S1had shown good antimycobacterial activity against M.tuberculosis.S2,S3, S4 ,S5 and S6 were found less potent than S1(Table 4).The obtained result reveals that electron withdrawing group amend the lipophilicity of the test compounds, which in turn alters permeability across the bacterial cell membrane. Further, results shows that the presences of electron withdrawing groups at 2nd and 3rd position of N-1substituted benzene derivatives have shown good antimycobacterial activity.Antimycobacterial activity for synthesized compounds was expressed as the minimum inhibitory concentration (MIC) in µg/ml.The synthesized compounds were evaluated for antitubercular activity. Compounds were assayed for their antimycobacterial activity against *M. tuberculosis* $H_{37}Rv$. Antimycobacterial activity was carried out at 100, 50, 25 12.5,6.25,3.125,1.6 and 0.8µg/ml. (Table 4) For comparison, pyrazinamide and streptomycin was employed as the reference antitubercular agent. However remarkable activity was found for S1 compound which is comparable to Pyrazinamide while compound S2, S3 and S4 had shown activity comparable to Streptomycin. This is well supported by the docking studies performed, as more the G score of the test compounds better the activity and binding ability of molecule into the active site.

4. CONCLUSION

In present work a series of 1,4–dihydropyridine derivatives were synthesized and characterized. Molecular docking studies were performed to identify the possible interaction of ligand with receptor. And evaluated for their antimycobacterial activity. Most compounds exhibited significant antimycobacterial activity. However remarkable activity was found for S1compound which is comparable to Pyrazinamide while compound S2, S3 and S4 have shown activity comparable to Streptomycin. The obtained results reveal that electron withdrawing group at 2^{nd} and 3^{rd} position of N-1substituted benzene may have a considerable impact on the antitubercular activity of the synthesized compounds. As the docking score also supports this fact. Larger the G score better the binding affinity of test molecules and is reflected in antimycobacterial activity indicating a direct correlation between observed activity and G score. So, these factors collectively indicate the importance, simplicity and wide applicability of designed series as antimycobacterial agents.

Fig 2:(a) Docking interaction of Standard Isoniazid (b) Docking interaction of S1 Compound



(a) **(b)**

Table 2. Results of molecular docking studies using standard precision mode of Glide.

Sr. No	Title	G-score	H-Bond	Good VDW	Bad VDW	Ugly VDW
1.	S1	-7.99886	3	294	2	0
2.	Isoniazid	-6.61094	3	131	2	0
3.	S2	-6.43461	1	236	0	0
4.	S3	-6.25977	2	344	1	0
5.	S4	-6.20502	2	280	1	0
6.	S5	-5.97653	2	255	2	0
7.	S6	-4.71124	2	255	2	0

Table 3.Prediction of ADME properties of designed derivatives using qikprop.

Sr.no.	Title	Mol M.W.	logP o/w	logBB	% Human Oral Absorption	CNS
1.	S1	492.373	5.523	-0.319	100	-1
2.	S2	457.928	5.124	-0.419	100	1
3.	S3	474.383	4.001	-2.500	100	0
4.	S4	474.383	4.431	-0.393	100	0
5.	S5	490.382	5.666	-0.773	95.051	-1
6.	S6	455.937	5.022	-1.158	90.078	-1
7.	INH	137.141	-0.663	-0.778	66.959	-1

All designed compounds have shown the ADME properties in acceptable range.

Table 4.Antimycobacterial activity assay

Sr.no.	Compound code	MIC in µg/ ml (H37Rv)
1.	S1	3.125
2.	S2	6.25
3.	S3	6.25
4.	S4	6.25
5.	S5	12.5
6.	S6	12.5
7.	Pyrazinamide	3.125
8.	Streptomycin	6.25

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