Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2015, 7(2):153-159
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Synthesis, docking and biological evaluation of pyrrole-2-carbohydrazide derivatives

Shantkumar M. Biradar, Ganesh D. Mote*, Kalyani D. Asgaonkar and Trupti S. Chitre

Department of Medicinal Chemistry, Arvind Gavali College of Pharmacy, Jaitapur, Satara, Maharashtra, India

---

ABSTRACT

In the present study, a novel series of Pyrrole -2- carbohydrazide derivatives were synthesized and docking study was performed to rationalize the possible interactions between the synthesized compounds and active site. Pyrrole -2- carbohydrazide 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 GS4 found to be most potent .

Keywords: Antimycobacterial; Docking, MABA, Enoyl-acyl carrier protein reductase

---

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 [1]. 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) [2-5] and totally drug-resistant tuberculosis (TDR-TB) [6-7]. 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 [8]. So ENR inhibitors can be designed for the development of new and potent antitubercular drugs. Several Benzylidine pyrrole-2-carbohydrazide derivatives have shown good inhibitory activity against ENR [9-11]. In this work, some Benzylidine Pyrrole -2- carbohydrazide 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. Thus our aim was further refined to synthesize Benzylidine pyrrole-2- carbohydrazide and evaluate them for antimycobacterial activity.Herein we report the synthesis, docking and in vitro antimycobacterial activity of a series of Benzylidine pyrrole-2-carbohydrazide. The docking study was performed to rationalize the possible interactions between the synthesized compounds and the active site.

MATERIALS AND METHODS

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 ^1H 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). GCMS were recorded on "2010QP GCMS 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.

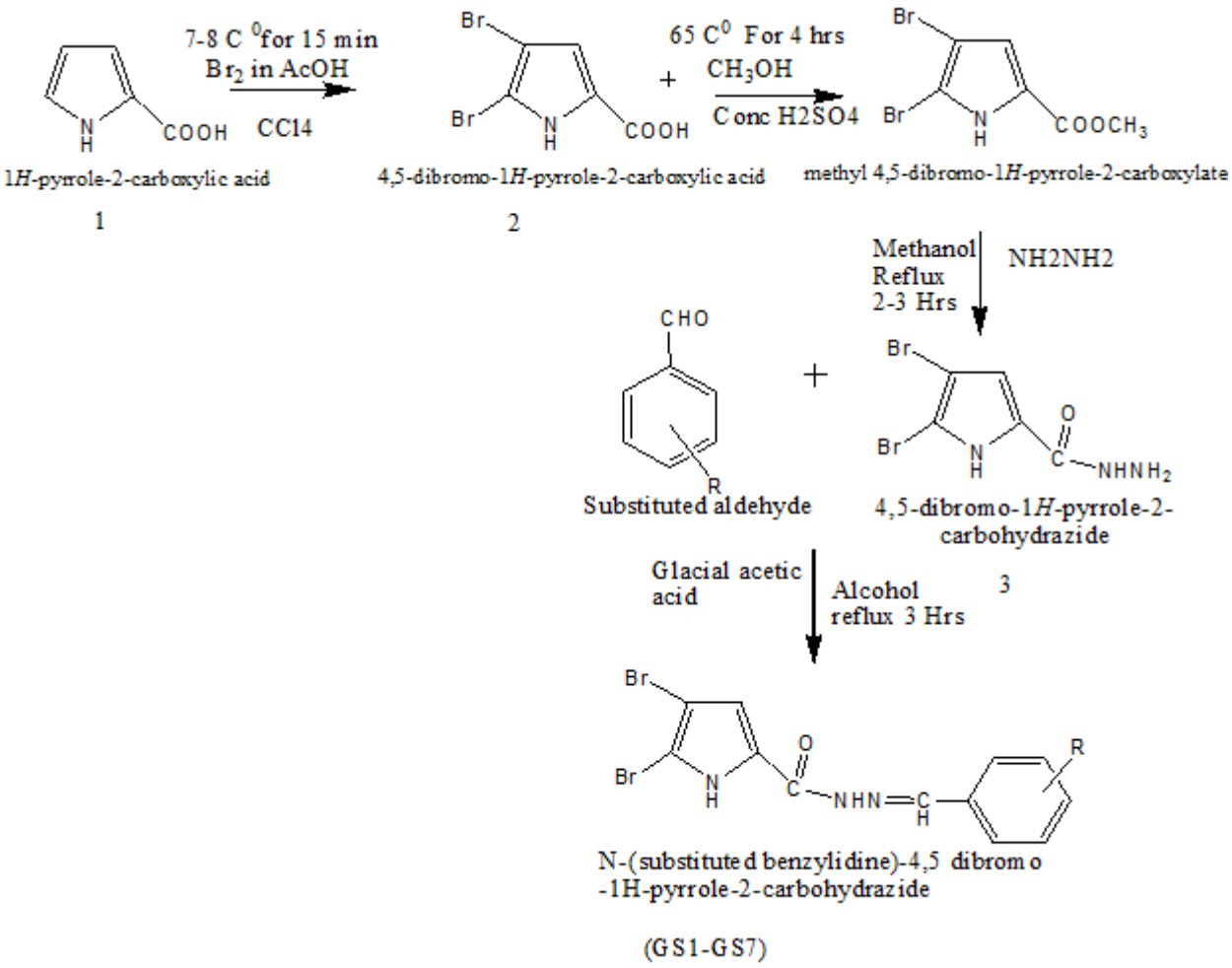


Figure 1.Scheme for synthesis of title compound

General Procedure:

Step-1: Bromination of pyrrole

A solution of 16.7 gm(0.083mol) of pyrrole-2- carboxylic acid in 100 ml acetic acid and 10ml of CCl_4 was stirred and cooled to slush. A solution of 26.6(0.166mol) in 50 ml of acetic acid added dropwise with continued cooling. Product which precipitated from the reaction mixture was crystalised from Propanol .

Step-2:Esterification of pyrrole

Mixture of 30gm (0.246mol) of 4,5 dibromo pyrrole -2-carboxylic acid and 80gm(10ml,2.5mol) of absolute methanol and 5gm(2.7ml) of conc. H_2SO_4 were placed 500 ml RBF.Excess of alcohol was distilled off by heating on water bath

The residue was poured into about 250 ml of water in seperatory funnel.Lower layer of pyrrole methyl carboxylate is extracted in CCl_4 and excess of acid is removed by adding of $NAHCO_3$ untill no further evaluation of CO_2 occurs. Filter the pyrrole methyl carboxylate and product collected was colourless solid

Step-3: Formation of acid hydrazide
1ml of hydrazine hydrate was placed in test tube fitted with reflux condenser .Then 1gm pyrrole methyl carboxylate was added dropwise and mixture was heated under reflux for 15 min.

Absolute ethanol was added through condenser to produce clear solution and refluxed to for 2-3 hours, crystals of acid hydrazide filtered and recrystalised from ethanol

Step-4:formation substituted benzylidine dibromo -1H pyrrole-2-carboxylic acid

Equimolar quantities of carbohydrazide and substituted aldehyde were refluxed in alcohol for 3 hrs in presence of few drops of glacial acetic acid.

The solvent was evaporated and product was poured on cold water ,filtered and dried.The crude solid was recrystalised in appropriate solvent system to give the product

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 enol ACP CO reductase binding pocket.The crystal structures of enoyl ACP CO reductase were obtained from protein data bank.(PDB Code: 2IDZ).The protein structure was prepared for docking using 'protein preparation wizard'in Maestro wizard 9.0.The protein preparation uses the OPLS force field[13] 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[14].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 [15].

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.

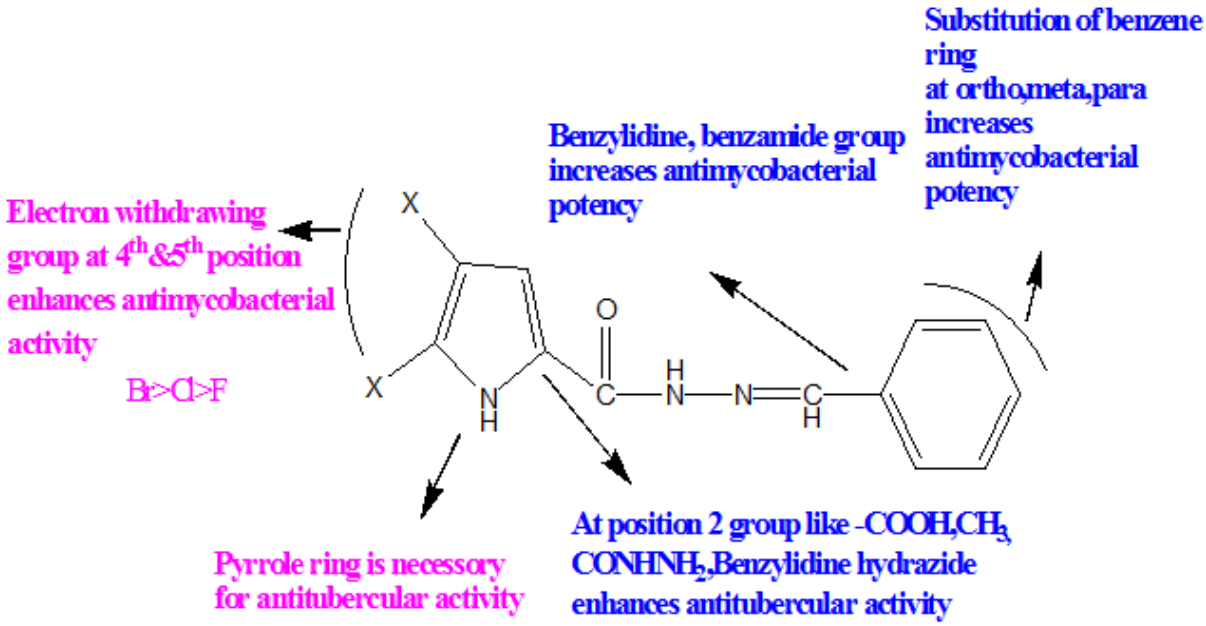
ADME Prediction
The ADME properties were calculated using Qikprop tool of Schrodinger software. It predicts both physicochemically significant descriptors and pharmacokinetically relevant properties. It also evaluates the acceptability of analogues based on Lipinski's rule of 5[15, 16] which is essential to ensure drug like pharmacokinetic profile while using rational drug design. All the analogues were neutralized before being used by Qikprop.

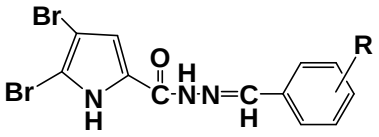
2.2.3. Antimycobacterial Activity
All the newly synthesized benzylidine Pyrrole carbohydrazide der were assayed in vitro for antitubercular activity against *M.tuberculosis* H37Rv using Microplate Alamar Blue Assay (MABA). Isoniazide was employed as the reference antimycobacterial agents.

Microplate Alamar Blue assay (MABA): [17-20]
Sterile deionized 200ul 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.

RESULTS AND DISCUSION

Physical Characterization: Physical constant, Rf value was determined for all synthesized compounds.
Pharmacophore optimized by SAR study





R=3-OC2H5,4-OH(GS1)
=3,4 OH(GS2)
=2,5 DIMETHOXY(GS3)
=4-DIMETHYLAMINE(GS4)
=3-Cl(GS5)
=2-NO_2(GS6)
=3-NO_2(GS7)

Representative Physiochemical and spectral data of product:
Compound GS1: 3-ethoxy-4-hydroxy benzylidine-4,5 dibromo -1H-pyrrole-2-carbohydrazide: Yield: 70%;m.p 178-182 ^{0}C; Rf = 0.7 (EtOAc/n-hexane7:3);FTIR (KBr, cm^{-1}): 3451.54 (pyrrole N-H Stretch); 3274.54.5(C-OH Stretch); 3085.55(aromatic C-H Stretch); 1855.19(C=O Stretch1635.34(N-H aromatic bend); 1376.93(C-O-H bend); 1187.94(C-N Stretch),1041.37(C-O-Cstretch);524(C-Br stretch)^{1}H NMR (400MHz, DMSO) δ(ppm): 7.0(s,1H,CHpyrrole);5.0(s,1H,NHpyrrole);8.0(s,1H,NH);8.1(s,1H,CH);
7.0(d,1H,CH);6.7(d,1H,CH);5.0(m,1H,OH);1.33(d,5H,C_2H_5)

Compound GS2: 3,4-hydroxy benzylidine-4,5 dibromo -1H-pyrrole-2-carbohydrazide: Yield:80%; m.p 220-222 ^{0}C ; Rf = 0.67 (EtOAc/n-hexane7:3);FTIR (KBr, cm^{-1}): 3463.53 (pyrrole N-H Stretch); 3262.97(C-O-H Stretch); 3070.12 (aromatic C-H Stretch); 1774.19(C=O amide Stretch); 1608.34(N-H aromatic bend); 1361.15(C-OH bend); 1060.66 (C=Nstretch) 644.248 (C-BrStretch); ^{1}HNMR (400MHz,DMSO) δ(ppm): 7.0(s,1H,CHpyrrole); 5.0(s,1H,NHpyrrole); 8.0(s,1H,NH); 8.1(s,1H,CH); 7.0(d,1H,CH); 6.6(d,1H,CH); 5.0(m,1H,OH); 5.0(m,1H,OH); 6.9(s,1H,CH); MS(m/z) 402.90(M^+).

Compound GS3: 2,5 dimethoxy benzylidine-4,5 dibromo -1H-pyrrole-2-carbohydrazide; Yield:90%; m.p 202-204 ^{0}C ; Rf = 0.72 (EtOAc/n-hexane7:3);FTIR (KBr, cm^{-1}): 3209.93 (pyrrole N-H Stretch); 3036.37(aromatic-H Stretch); 1858.08(C=O amide Stretch);1647.88(N-H bend); 1269.9(C-N Stretch); 1176.36(C-N Stretch); 1041.37(C-O-C Stretch);521.65(C-Br stretch) ^{1}H NMR (DMSO) δ(ppm): 7.0(s,1H,CH pyrrole); 5.0(s,1H,NH pyrrole); 8.0(s,1H,NH); 8.1(s,1H,CH); 7.0(q,1H,CH); 3.73(d,3H,OCH_3) 6.7(d,1H,CH); 6.7(d,1H,CH); 3.73(s,3H,OCH_3); MS (m/z) 430.095(M^+).

Compound GS4: 4-dimethylamino benzylidine-4,5 dibromo -1H-pyrrole-2-carbohydrazide; Yield:75%; m.p 179-181 ^{0}C ; Rf = 0.63 (EtOAc/n-hexane7:3);FTIR (KBr, cm^{-1}): 32331.15 (pyrrole N-H Stretch); 3031.55(aromatic-H

stretch);1880.86 (C=O amide Stretch);1294(C-N-C stretch) 1060.66(C=N benzylidine Stretch); 1600.63(N-H aromatic bend); 691.355(C-Br Stretch) ^{1}H NMR (DMSO)δ(ppm): 7.0(s,1H,CH pyrrole);5.0(s,1H,NH pyrrole);8.0(s,1H,NH);8.1(s,1H,CH);7.4(d,1H,CH);6.6(s,1H,CH)2.85(s,3H,CH_3); 2.85(s,3H,CH_3); 6.6(d,1H,CH); 7.4(s,1H,CH); MS (m/z) 402.042(M^+).

Compound GS5: 3-chloro benzylidine-4,5 dibromo -1H-pyrrole-2-carbohydrazide; Yield:65%; m.p 181-183 ^{0}C ; Rf = 0.52 (EtOAc/n-hexane7:3);FTIR (KBr, cm^{-1}): 3202.18 (pyrrole N-H Stretch); 3061.44(aromatic C-H stretch);1769.37 (C=O amide Stretch);1294(C-N-C stretch) 1060.66(C=N benzylidine Stretch); 1669.52(N-H aromatic bend); 691.355(C-Br Stretch) ^{1}H NMR (DMSO)δ(ppm): 7.0(s,1H,CH pyrrole);5.0(s,1H,NH pyrrole);8.0(s,1H,NH);8.1(s,1H,CH); 7.5(d,1H,CH);7.2(d,1H,CH);7.3(s,1H,CH);7.6(s,1H,CH);MS (m/z) 430.095(M^+).

Compound GS6: 2-nitro benzylidine-4,5 dibromo -1H-pyrrole-2-carbohydrazide; Yield:85%; m.p 194-196 ^{0}C ; Rf = 0.67 (EtOAc/n-hexane7:3);FTIR (KBr, cm^{-1}):3523.31(pyrrole N-H stretch)2340.19(C-N-O stretch) 3021.91 (Aromatic C-H Stretch); 1817.58(C=O amide stretch)1650.77(N-H bend); 1075.12 (C-N stretch); 682.677(C-Br stretch) ^{1}H NMR (DMSO)δ(ppm): 7.0(s,1H,CH pyrrole);5.0(s,1H,NH pyrrole);8.0(s,1H,NH);8.1(s,1H,CH); 7.9(d,1H,CH);7.7(d,1H,CH);7.6(d,1H,CH);MS (m/z) 413.111(M^+).

Compound GS7: 3-nitro benzylidine-4,5 dibromo -1H-pyrrole-2-carbohydrazide; Yield:60%; m.p 196-198 ^{0}C ; Rf = 0.66 (EtOAc/n-hexane7:3);FTIR (KBr, cm^{-1}): 3580.08 (pyrrole N-H Stretch); 3220.54 (Aromatic C-H Stretch); 2352.73(C-N-O stretch)1897.61(C=O amide stretch); 1079.94 (C-N stretch); 536.114(C-Br stretch) MS (m/z%)^{1}H NMR (DMSO)δ(ppm): 7.0(s,1H,CH pyrrole);5.0(s,1H,NH pyrrole);8.0(s,1H,NH);8.1(s,1H,CH); 8.0(d,1H,CH);7.6(d,1H,CH);8.2(s,1H,CH);8.6(s,1H,CH)MS(m/z) 413.111(M^+).

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 -7.1381 and G score of rest of the designed compounds were found be comparable with G-score of standard Isoniazid (G score:-7.1005) 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 Benzylidine pyrrole-2- carbohydrazide 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

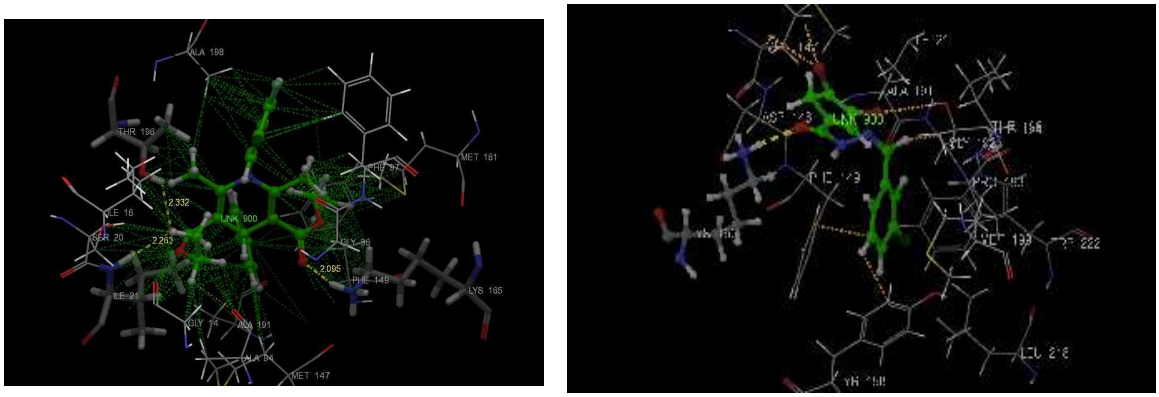
Amongst the compound tested GS4 had shown good antimycobacterial activity against M.tuberculosis.GS1,GS2,GS3, GS5 ,GS6 and S7 were found less potent than GS4(Table 4).The obtained result reveals that electron withdrawing group amend the lipophilicity of the test compounds, which in turn alters permeability across the bacterial cell membrane. Further, results shows that the presences of electron withdrawing groups at 2nd and 3rd position of benzene derivatives have shown good antimycobacterial activity.Also substitution at para position to benzene by electron releasing group i.e dimethyl amine shows better 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, isoniazid was employed as the reference antitubercular agent. However remarkable activity was found for GS4 compound which is comparable to isoniazid while compound GS2, GS3 and GS5,GS6,GS7 had shown good response to MABA assay. 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.

CONCLUSION

In present work a series of Benzylidine pyrrole-2- carbohydrazide 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 GS4 compound which is comparable to Isoniazid while compound GS2, GS3 and GS5,GS6,GS7 had shown positive response to the MABA assay. The obtained results shows that the presences of electron withdrawing groups at 2nd and 3rd position of benzene derivatives have shown good antimycobacterial activity.Also substitution at para position to benzene by electron releasing group i.e dimethyl amine shows better antimycobacterial activity. 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 GS4 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.	GS1	-6.01571	1	245	13	0
2.	GS2	-5.88602	1	217	6	0
3.	GS3	-5.33502	1	231	4	0
4.	GS4	-7.138179	1	277	6	0
5.	GS5	-5.68179	1	217	6	0
6.	GS6	-5.12194	2	173	4	0
7.	GS7	-5.73224	1	260	13	0
8	INH	-7.10055	3	161	1	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.	GS1	430	5.523	-0.319	100	0
2.	GS2	402	5.124	-0.419	85.66	-2
3.	GS3	430.095	4.001	-2.500	100	0
4.	GS4	402.042	4.431	-0.393	100	0
5.	GS5	430.095	5.666	-0.773	100	1
6.	GS6	413.111	5.022	-1.158	100	0
7.	GS7	413.111	-0.663	-0.778	100	1
8	INH				100	

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

Table 4.Antimycobacterial activity assay

Sr.no.	Compound code	MIC in μM
1.	GS1	>50.00
2.	GS2	50.00
3.	GS3	>50.00
4.	GS4	12.50
5.	GS5	50.00
6.	GS6	50.00
7.	GS7	50.00

Acknowledgments
We are grateful to Dr. Ashwini. R. Madgulkar, Principal, AISSMS College of Pharmacy, Pune, for providing us infrastructure for carrying out this work. We would also like to thank Dr.Sanjiv Yadav ,junior scientist of CSIR-CDRI Luknow us for antimycibacterial assay.

REFERENCES

[1] WHO.Tuberculosis.November **2010**.
[2] Dye,C.;Williams B.;Espinal M.; Raviglion M.; *Science*, (**2002)**295,2042-2046.
[3] Morris S.;Bai G.; Suffys P.;Portilo L.;Fairchok M.; Rouse D. *Infectious Diseases*, (**1995**)171, 954-960.
[4] Telzak E.; Sepkowitz K.; Alpert P.; Mannheimer S.; Mederd F.; ElSad W.; Blum S.; Gagliardi A.; Alomon N.;Turett G. *The New England Journal of Medicineogy* (**1995**) , 333,907-912.
[5] Basso L.; Blanchard J.; *Advances in Experimental Medicine and Biology* (**1998**),456, 115-144.
[6] http://www.nature.com/news/totally-drug-resistant-tb-emerges-in-india accessed on date: 09-04-2012.
[7] Velayati A.;Masjedi M.;Farnia P.;Tabarsi P.;Ghanavi J.; ZiaZarifi A.; Hoffner S.*Chest (***2009**),136, 420-425.
[8] Khisimuzi M.; Melvin S.Current *Opinion in Pharmacology* (**2006**),6,459–467.
[9] Delaine, T.; Bernardes-Génisson, V.; Quémard, A.; Constant, P.; Meunier, B.;Bernadou, J. *Eur. J. Med. Chem.* (**2010**), 45, 4554-4560
[10] Khoshnevizadeh M.; Edraki N.;Javidnia K.;Alborzi A.;Pourabbas B.;Mardenah J.; Miri R. *Bioorg .Med. Chem.*(**2009**),17,1579-1587
[11] Broussy S.; Bernardes Génisson V.; Quémard A.; Meunier B.; Bernadou *J. J.Org. Chem*. (**2005**),70,10502-10508
[12] Denis M. Bailey ,Robert E. Johnson,U Joseph Salvador;*journal of medicinal chemistry* 16(11) 1299-1304
[13] Vogels textbook of practical organic chemistry;B.S Furnis,A.J Hannaford,P.W.G Smith,A.R Tatchell;5th edition ,pg no-1077
[14] Vogels textbook of practical organic chemistry;B.S Furnis,A.J Hannaford,P.W.G Smith,A.R Tatchell;5th edition ,pg no-1269-1270
[15] B.P Malikarjuna et al *European journal of medicinal chemistry*(**2009**),44,4739-4744
[16] Jorgensen W.;Maxwell D.;Tirado R.*Journal of American Chemical Society* (**1996**),118,11225-11236.
[17] Hayes M.; Stein M.; Weiser J.*The Journal of Physical Chemistry*(**2004**),108, 3572-3580.
[18] Friesner R.; Murphy R.; Repasky M.; Frye L.; Greenwood J.; Halgren T.; Sans chagrin P.; Mainz D. *Journal of Medicinal Chemistry* (**2006**), 49, 6177
[19] Elmer W.; Stephen D.;William M.; Paul C.;Washing C. Text book of Diagnostic Microbiology 5 Lippincott Pub, J. B. Lippincott Co., Philadelphia, (**2002**) p. 125.
[20] Collins L.; Franzblau S.; *Antimicrob. Agent. Chemother*(**1997**), 41, 1004-1009.
[21] Srivastava T.;Gaikwad A.; Haq W.;Sinha S.; Katti S. *ARKIVOC* (**2005**), 2, 120-130.
[22] Lourenço C.; Desouza V.; Pinheiro A.; Ferreira M.; Gonçalves R.; Nogueira T.; Peralta M. *ARKIVOC*,(**2007**),15,181-191.
[23] Morgan J.; Haritakul R.; Keller P.*Bioorganic Medicinal Chemistry Letters* (**2003**),13, 1755-1757.