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SYNTHESIS AND SCREENING OF METHYL 4-(SUB) PHENYL-1H-PYRROLE-3-CARBOXYLATESAS POTENTIAL ANTI-TUBERCULAR AGENTS

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ABSTRACT

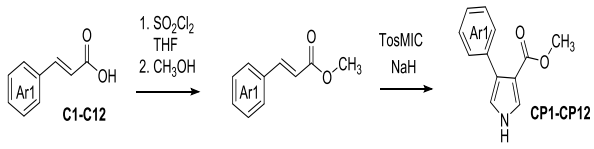
Cinnamic acids are versatile molecules to synthesize potential antimicrobial agents. Here, we studied van Leusen reaction on cinnamic acid methyl esters to obtain corresponding methyl 4-(sub) phenyl-1H-pyrrole-3-carboxylates in appreciable yield. The synthesized compounds are screened for antimicrobial and anti-tubercular activities. Two compounds methyl 4-(3,4,5-trimethoxyphenyl)-1H-pyrrole-3-carboxylate (**CP11**) and methyl 4-(1H-indol-3-yl)-1H-pyrrole-3-carboxylate (**CP12**)have shown high potency (MIC 3.12µg/mL) against *Mycobacterium tuberculosis* H37Rv. The compounds did not show antifungal activity against tested fungi at 100µg/mL and did not inhibit the tested bacteria at concentrations > 50 µg/mL indicating selective toxicity against Mtb. Further, the drug-inhibitor combined study revealed that the compounds CP11 and CP12 showed synergistic activity with rifampicin, ciprofloxacin and streptomycin but not with isoniazid. The docking simulations were conducted on *Beta-Ketoacyl-Acyl Carrier Protein Synthase III (1hzp), MabA (1uzn) ,Transferase (5ld8), Oxidoreductase (5mtp)*. The docking score showed correlation with the results from in vitro anti TB activity and also selective inhibitory tendency of the synthesized compounds towards MabA. The correlation between minimum inhibition versus docking scores gives that MabA protein could be the most probable site of action for the selected ligand.

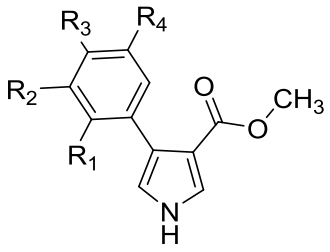
INTRODUCTION

Mycobacterium tuberculosis (Mtb), an airbor ne pathogen, is responsible for tuberculosis (TB)[1].A number of factors, such as poor or inconsistent dosage, delayed diagnosis, prolonged therapy, and assessment of drug-resistant strains generating multidrug-resistant (MDR-TB) and extensively drug-resistant (XDR-TB),have aggravated the complex situations of TB[2].Mycobacterial cell wall production provides desirable protein targets for the development of targeted anti-TB drugs. Isoniazid and ethambutol are two important first-line anti-TB medications which prevents the formation of cell wall[3]. Inorder to produce fatty acids of Mtb cell wall; condensation, keto reduction, dehydration, and enoyl reduction cycles are to be done repeatedly. These processes are catalysed by four enzymes: β-ketoacyl synthase (KAS), β-ketoacylreductase (KR), b-hydroxyacyldehydrase (DE), and enoylreductase (ER)[4]. All of these enzymes

were found to be essential for Mtb and thus identified as potential targets for anti TB drug development**.** The pyrrole component is used in a wide range of therapeutically effective chemicals, such as fungicides, antibiotics, anti-inflammatory medications[5], cholesterol lowering medications[6],antitumor agents[7] and many more.Several types of natural and synthetic compounds containing two aryl groups on adjacent positions in five-membered heterocyclic derivatives shows a diverse of biological and chemical properties and biomedical attributes[8]. Based on numerous applications, the heterocyclic pyrrole may serve as a privileged scaffold for the elimination of Mtb by targeting selected enzymes[9]. As a result, considerable efforts have been put to create useful procedures for the synthesis of pyrrole units that incorporates essential structural diversity. The reaction of tosylmethylisocyanide (TOSMIC) with a Michael acceptor to produce a 3,4-disubstituted pyrrole is one such technique[10].Substituted 1H-pyrrole-3-carboxylates have been found to have calcium channel activating activity[11] and anti-microbial property[12]. Compounds having pyrrole along with other pharmacophores have been found to have different pharmacological activities[13]. Compounds like BM212 and its analogues with a pyrrole nucleus have been found to exhibit potent anti -tubercular activity[14],[15]. Our interest in the potential anti-tubercular properties of methyl 4-(sub) phenyl-1H-pyrrole-3-carboxylates was prompted by these reports about the anti-tubercular activity of pyrrole analogues.

SCHEME OF WORK





code	R_1	R_2	R_3	R_4
CP1	H	H	H	H
CP2	H	H	CH_3	H
CP3	H	H	OCH_3	H
CP4	H	H	F	H
CP5	H	H	Cl	H
CP6	H	H	$N(CH_3)_2$	H
CP7	H	F	F	H
CP8	H	OCH_3	OCH_3	H
CP9	OCH_3	H	OCH_3	H
CP10	Cl	H	Cl	H
CP11	H	OCH_3	OCH_3	OCH_3
CP12	3-indolyl			

2. EXPERIMENTAL WORK:

2.1Chemistry

The starting materials used in our synthesis are known and are obtained in bulk by using published protocols. The physicochemical and spectral data of the synthesized compounds are compared with published data to confirm their structure. In brief, cinnamic acids are obtained by using Knoevenagel condensation reaction between respective aryl aldehyde and malonic acid.

2.2. Synthesis of methyl, 4-aryl 1H-pyrrole 3-carboxylates (CP1-CP12)

To a solution of cinnamic acid (5 mmol) in THF (10 mL), thionyl chloride is added and stirred at room temperature for 30 mins. Then 5mL of methanol was added and the reaction mixture was refluxed till the methyl ester formation is completed (~ 3hrs) as indicated by t.l.c. Then, the reaction mixture is distilled to remove solvent and after usual workup, the product is obtained as viscous liquid. This is used for the next reaction without further purification. Appropriate methylcinnamate (5 mmol) and p-toluenesulfonyl methyl isocyanide (TosMIC, 5 mmol) are dissolved in 25 mL of diethyl ether: DMSO (2:1). This solution is added dropwise to a stirred suspension of sodium hydride (1.2 equivalent) in 10 mL diethyl ether in ice cold condition. Once the addition is over, the temperature of the reaction mixture is allowed to slowly reach room temperature (~ 15 mins) with continued stirring. Then the reaction was quenched by adding cold water and the mixture is extracted with diethyl ether (10mL X 3). The combined organic extracts are dried over anhydrous $MgSO_4$ and evaporated to obtain the crude compound. This is further

purified by column chromatography to obtain the target compounds CP1-CP12.

2.2.1 Methyl 4-phenyl-1H-pyrrole-3-carboxylate (CP1)

Yield:78%. **Mp:**181-182^{o}C. **^{1}H NMR (DMSO-d6, 400MHz): δ=** 3.78 (s, 3H), 6.88 (s, 1H), 7.43-7.26 (m, 5H), 7.62 (s, 1H), 11.22 (br s, 1H). **^{13}C NMR (DMSO-d6, 100MHz): δ=** 50.8, 116.5, 121.3, 125.3, 127.5, 128.1, 129.5, 130.1, 135.2, 168.2. **IR (KBr)** ν_{max}1629(C=Caromatic),1695(C=O),2932, 3095 (C-H Str), 3110 (NH). **MS** m/z 202 (MH^+).Mol Formula $C_{12}H_{11}NO_2$; calcd C, 71.63; H, 5.51; N 6.96; found C, 71.61; H 5.51, N 6.95.

2.2.2 Methyl 4-(4-hydroxyphenyl)-1H-pyrrole-3-carboxylate (CP2)

Yield:72%, **Mp:** 155-157 ^{o}C.**^{1}H NMR (DMSO-d6, 400MHz): δ=** 2.33 (s, 3H), 3.72 (s, 3H), 6.95 (s, 1H), 7.09 (d, *J* = 7.8 Hz, 2H), 7.45 (d, *J* = 7.8 Hz, 2H), 7.55 (s, 1H), 11.68 (br s, 1H). **^{13}C NMR (DMSO-d6, 100MHz): δ=** 21.2., 50.5, 115.2, 118.8, 126.8, 127.4, 129.1, 130.2, 134.4, 135.5, 168.7. **IR** (KBr) ν_{max}1645 (C=Caromatic),1682(C=O),2895, 3026 (C-H Str), 3112 (NH). **MS** m/z 216 (MH^+). Mol Formula $C_{13}H_{13}NO_2$; calcd C, 72.54; H, 6.09; N, 6.51; found C, 72.53; H, 6.07; N, 6.50.

2.2.3 Methyl 4-(4-methoxyphenyl)-1H-pyrrole-3-carboxylate (CP3)

Yield: 26%, **Mp:** 154-155 ^{o}C.**^{1}H NMR (DMSO-d6, 400MHz): δ=** 3.69 (s, 3H), 3.68 (s, 3H), 6.86 (m, 3H), 7.35 (d, J = 7.8Hz, 2H), 7.45 (s, 1H), 11.47 (br s, 1H). **^{13}C NMR (DMSO-d6, 100MHz): δ=** 55.5, 60.2, 116.7, 118.2, 123.7, 130.1, 130.8, 132.6, 135.0, 162.9, 169.8. **IR** (KBr) ν_{max}1648(C=Caromatic),1680(C=O), 2924, 3052 (C-H str), 3115 (NH). **MS** m/z 232 (MH^+). Mol Formula$C_{13}H_{13}NO_3$; calcd C, 67.52; H, 5.67; N, 6.06; found C, 67.50; H, 5.65; N, 6.06.

2.2.4 Methyl 4-(4-fluorophenyl)-1H-pyrrole-3-carboxylate (CP4)

Yield: 84%, **Mp:** 191-192 ^{o}C.**^{1}H NMR (DMSO-d6, 400MHz): δ=** 3.64 (s, 3H), 6.94 (s, 1H), 7.13 (m, 2H), 7.45-7.50 (m, 3H), 11.56 (br s, 1H). **^{13}C NMR (DMSO-d6, 100MHz): δ=** 51.8, 114.3, 116.5, 128.2, 130.8, 131.6,132.7, 135.4, 161.4, 166.1. **IR** (KBr) ν_{max}1636 (C=Caromatic),1658(C=O), 2894, 3086 (C-H str), 3112 (NH)**MS** m/z 220 (MH^+). Mol Formula$C_{12}H_{10}FNO_2$; calcd C, 65.75; H, 4.60; N, 6.39; found C, 65.75; H, 4.61; N, 6.37.

2.2.5 Methyl 4-(4-chlorophenyl)-1H-pyrrole-3-carboxylate (CP5)

Yield: 58%, **Mp:** 171-173 ^{o}C.**^{1}H NMR (DMSO-d6, 400MHz): δ=** 3.78 (s, 3H), 7.03 (s, 1H), 7.36 (d, *J* = 6.6 Hz, 2H), 7.55 (d, J = 6.6Hz), 7.62 (s, 1H), 11.72 (br s, 1H). **^{13}C NMR (DMSO-d6, 100MHz): δ=** 55.7, 116.7, 124.6, 129.0, 131.3, 132.7, 135.6, 135.7, 139.1, 169.6. **IR** (KBr) ν_{max}1635(C=Caromatic),1698(C=O),2853, 3656(C-H Str), 3118 (NH). **MS** m/z 236 (MH^+), 238 (MH^++2). Mol Formula$C_{12}H_{10}ClNO_2$; calcd C, 61.16; H, 4.28; N, 5.94; found H, 61.15; H, 4.26; N, 5.92.

2.2.6 Methyl 4-(4-(dimethylamino)phenyl)-1H-pyrrole-3-carboxylate (CP6)

Yield: 56%, **Mp:** 168-169 ^{o}C.**^{1}H NMR (DMSO-d6, 400MHz): δ=** 2.81 (s, 6H), 3.75 (s, 3H), 6.79 (d, J = 7.3Hz, 2H), 7.01 (s, 1H), 7.33 (d, J = 7.3Hz, 2H), 7.65 (s, 1H), 11.28 (br s, 1H). **^{13}C NMR (DMSO-d6, 100MHz): δ=** 41.5, 52.3, 112.8, 115.2, 124.1, 126.9, 129.5, 131.2, 136.1, 148.7, 166.9. **IR** (KBr) ν_{max}1639(C=Caromatic),1690(C=O),2937, 3095 (C-H Str), 3112 (NH). **MS** m/z 245 (MH^+), Mol Formula$C_{14}H_{16}N_2O_2$; calcd C, 68.83; H, 6.60; N, 11.47; found C, 68.82; H, 6.60; N, 11.46.

2.2.7 Methyl 4-(3,4-difluorophenyl)-1H-pyrrole-3-carboxylate (CP7)

Yield: 92%, **Mp:** 183-184 ^{o}C.**^{1}H NMR (DMSO-d6, 400MHz): δ=** 3.72 (s, 3H), 7.01 (s, 1H), 7.28-7.25 (m, 3H), 7.55 (s, 1H), 11.15 (br s, 1H). **^{13}C NMR (DMSO-d6, 100MHz): δ=** 52.1, 112.5, 115.5, 119.2, 123.9, 125.4, 128.7, 134.2, 135.5, 143.9, 150.6, 167.1. **IR** (KBr) ν_{max}1628 (C=Caromatic),1669(C=O), 2915, 3081 (C-H str), 3115 (NH). **MS** m/z 237 (MH^+), Mol Formula$C_{12}H_9F_2NO_2$; calcd C, 60.76; H, 3.82; N, 5.90; found C, 60.75; H, 3.82; N, 5.90.

2.2.8 Methyl 4-(3,4-dimethoxyphenyl)-1H-pyrrole-3-carboxylate (CP8)

Yield: 42%, **Mp:** 161-163 ^{o}C.**^{1}H NMR (DMSO-d6, 400MHz): δ=** 3.64 (s, 3H), 3.76 (s, 6H),, 6.89 – 6.92 (m, 2H), 6.98 (d, J =8Hz, 1H), 7.09 (s, 1H), 7.46 (m, 1H), 11.49 (br s,

1H). **^{13}C NMR (DMSO-d6, 100MHz): δ=** 50.8, 56.01, 111.2, 112.0, 113.7, 119.1, 121.2, 125.5, 126.2, 128.2, 147.7, 148.3, 165.1. **IR** (KBr) ν_{max}1642(C=Caromatic),1685(C=O),2890, 3026(C-H Str), 3112 (NH). **MS** m/z 262 (MH^+), Mol Formula$C_{14}H_{15}NO_4$; calcd C, 64.36; H, 5.79; N, 5.36; Found C, 64.35; H, 5.76; N, 5.37.

2.2.9 Methyl 4-(2,4-dimethoxyphenyl)-1H-pyrrole-3-carboxylate (CP9)

Yield: 45%, **Mp:** 175-176 ^oC.**^{1}H NMR (DMSO-d6, 400MHz): δ=** 3.68 (s, 3H), 3.81 (s, 3H), 3.92 (s, 3H), 6.67 (m, 2H), 7.01 (s, 1H), 7.28 (m, 1H), 7.62 (s, 1H), 11.8 (br s, 1H). **^{13}C NMR (DMSO-d6, 100MHz): δ=** 51.8, 55.9, 56.7, 102.4, 110.4, 115.2, 118.7, 123.4, 127.1, 131.6, 133.8, 157.4, 158.5, 167.7. **IR** (KBr) ν_{max}1638(C=Caromatic),1686(C=O),2937, 3095 (C-H Str), 3110 (NH). **MS** m/z 262 (MH^+), Mol Formula$C_{14}H_{15}NO_4$; calcd C, 64.36; H, 5.79; N, 5.36; found C, 64.36; H, 5.78; N, 5.36.

2.2.10 Methyl 4-(2,4-dichlorophenyl)-1H-pyrrole-3-carboxylate (CP10)

Yield: 87%, **Mp:** 200-201 ^oC.**^{1}H NMR (DMSO-d6, 400MHz): δ=** 3.57 (s, 3H), 6.88 (s, 1H), 7.33 (d, J = 12Hz, 1H), 7.36 (dd, J = 4, 12Hz, 1H), 7.49 (s, 1H), 7.59 (d, J = 4Hz, 1H), 11.63 (br s, 1H).**^{13}C NMR (DMSO-d6, 100MHz): δ=** 50.9, 113.8, 120.2, 121.0, 125.1, 126.9, 128.6, 132.1, 133.6, 134.3, 134.9, 164.5. **IR** (KBr) ν_{max}1632 (C=C aromatic), 1661(C=O), 2923, 3056 (C-H Str), 3115 (NH). **MS** m/z 270 (MH^+), 272 (MH^++2) Mol Formula$C_{12}H_9Cl_2NO_2$; calcd C, 53.36; H, 3.36; N, 5.19; found C, 53.36; H, 3.35; N, 5.18.

2.2.11 Methyl 4-(3,4,5-trimethoxyphenyl)-1H-pyrrole-3-carboxylate (CP11)

Yield: 35%, **Mp:** 187-188 ^oC.**^{1}H NMR (DMSO-d6, 400MHz): δ=** 3.66 (s, 3H), 3.67 (s, 3H), 3.76 (s, 6H), 6.77 (s, 2H), 7.01 (s, 1H), 7.48 (s, 1H), 11.55 (br s, 1H). **^{13}C NMR (DMSO-d6, 100MHz): δ=** 50.9, 56.4, 60.4, 106.9, 112.1, 119.6, 125.5, 126.5, 131.0, 136.5, 152.5, 165.1. **IR** (KBr) ν_{max}1637(C=C aromatic), 1684 (C=O), 2925, 3092 (C-H Str), 3110 (NH). **MS** m/z 292 (MH^+), Mol Formula$C_{15}H_{17}NO_5$; calcd C, 61.85; H, 5.88; N, 4.81; found C, 61.87; H, 5.89; N, 4.81.

2.2.12 Methyl 4-(1H-indol-3-yl)-1H-pyrrole-3-carboxylate (CP12)

Yield: 74%, **Mp:** 191-192 ^oC.**^{1}H NMR (DMSO-d6, 400MHz): δ=** 3.77 (s, 3H), 6.81-6.92 (m, 2H), 7.12 (m, 1H), 7.35-7.40 (m, 2H), 7.64 (m, 2H), 11.82 (br s, 1H). **^{13}C NMR (DMSO-d6, 100MHz): δ=** 52.5, 109.2, 110.2, 112.8, 121.2, 121.5, 122.6, 127.5, 128.2, 132.1, 137.6, 167.5. **IR** (KBr) ν_{max}1632(C=Caromatic),1680(C=O), 2925, 3015 (C-H Str), 3112 (NH). **MS** m/z 241 (MH^+), Mol Formula$C_{14}H_{12}N_2O_2$; calcd C, 69.99; H, 5.03; N, 11.66; found C, 69.99; H, 5.02; N, 11.65.

2.3. BIOLOGICAL ACTIVITY

2.3.1 Anti-bacterial activity:

To determine minimum inhibitory concentrations (MICs), all synthesized twelve compounds were tested (Minimum inhibitory concentration). The nutritional broth dilution technique was used, as recommended by the Clinical and Laboratory Standards Institute (formerly known as National Committee for Clinical Laboratory Standards, 2000).

2.3.2 Anti-Fungal activity:

Antifungal activity was determined by using the agar well diffusion method

2.3.3 Anti-tubercular activity:

Lowenstein Jensen (LJ) medium growth was suspended in sterile Middlebrook 7H9 broth supplemented with 0.2% glycerol and 10% OADC (oleate-albumin-dextrosecatalase) enrichment, with a 1:20 dilution used as the MABA inoculum.

2.4. Molecular Modelling and Docking Simulations

The PDB structures of the target proteins, *Beta-Ketoacyl-Acyl Carrier Protein Synthase III (1hzp), MabA (1uzn) ,Transferase (5ld8), Oxidoreductase (5mtp)*were obtained from www.rcsb.org (Table 1). The co-crystallized ligand was extracted from the protein complex and saved separately. Protein structures were chosen on the basis of resolution (2 Angstroms) and availability of X-ray crystallographic structures.

Ligand and protein preparation

Chemdraw19.0 was used to draw the ligands as well as optimise ligand shape and perform MM2 energy minimization on the 3D

structures. Proteins were prepared using Molegro Virtual Docker. Water molecules and other co crystallised ligands were removed and missing amino acid residues were added. Detection of binding cavities was done to identify the ligand binding site. The prepared protein (with and without the co-crystallised ligand) was stored as a PDB file using this software. The prepared proteins were opened using Auto Dock and kollman charges were added and the protein was saved in the pdbqt format. The standard ligand was extracted from the protein structure, and a pdbqt file was created using Autodock tools.

Grid box preparation and docking

Docking experiments were performed with prepared ligands and the experimental control inhibitors against *Beta-Ketoacyl-Acyl Carrier Protein Synthase III (1hzp), MabA(1uzn) ,Transferase (5ld8), Oxidoreductase (5mtp)*proteins. Grid box parameters (Table 2) were set by using Autodock Tools (ADT), a free graphic user interface of MGL software packages (version 1.5.7) (Morris et al. 2009). The molecular docking program AutoDockVina (version 1.2.0) (Trott and Olson 2010) was employed to perform the docking experiment. Grid preparation was done using standard protocol. Docking experiments were run using the software AU DOCKER LE[16]. The obtained results were visualized using PyMol software[17].LIGPLOT interactions were plotted by using LigPlot + software (http://www.ebi.ac.uk/thornton-srv/software/LIGPLOT/).Statistical analysis of the docking results versus the obtained MIC values was done to identify the most probable target protein for the selected ligands using linear regression analysis. The results of the docking experiments are discussed in the following section.

TABLE 1: **Critical interactions observed between the co crystallised ligand and amino acids**

Protein	***Co crystallised ligand***	***Active pocket site amino acid***	***Dock score***
Beta-Ketoacyl-Acyl Carrier Protein Synthase III (1hzp)	Lauric acid	ASN-274	-5.5
MabA(1uzn)	NADP NICOTINAMIDE-ADENINE-DINUCLEOTIDE PHOSPHATE	ASN-24,ARG-25,ILE-27,ARG-47,ASP-61,VAL-62,ASN-88,GLY-90	-8.9
Transferase (5ld8)	~{N}-(1-methylindazol-6-yl)butane-1-sulfonamide	GLU-120, GLU-199	-6.8
Oxidoreductase (5mtp)	2-(2-methylphenoxy)-5-[(4-phenyl-1H-1,2,3-triazol-1-yl)methyl]phenol	GLY-192, GLN-214	-7.5

3. RESULTS AND DISCUSSION

3.1. Chemistry

We have created a total of 12 different compounds. Spectral analysis, including NMR, IR, and mass investigations, was used to confirm the identity of the synthesised substances.

3.2. Biology

3.2.1. *In Vitro* Anti tubercular Activity

The MABA technique was used to conduct an in-vitro anti-tubercular screening on the produced compounds (Table 5). The antitubercular action of the aryl pyrrole carboxylates was examined at various concentrations, including 100, 50, 25, 12.5, 6.25, 3.12, 1.6, and 0.8 g/mL. To ensure perfect solubility, DMSO was used in the preparation of the samples. The studied compounds with the highest potency were CP11 and CP12, with MICs of 3.2 g/mL[3].

3.2.2.*In Vitro*Antifungal and Antibacterial

The synthesized compounds did not show antifungal activity against tested fungi at 100µg/mL and did not inhibit the tested bacteria at concentrations > 50 µg/mL[3]

3.3.Docking results: The retrieved proteins docked with the ligands CP1 to CP12. Among the tested compounds CP7, CP12 and CP5

gives the best dock scores in all the 4 selected proteins.The binding energy obtained for CP12 with MabA were -7.0 kcal/mol. The binding free energy of CP12 with MabA was comparable with the control inhibitor isonicotinic-acyl-NADH(−8.9 kcal/mol).The residues **GLY-139, PRO-183, ILE-186, THR-191**involved in hydrogen bond interactions with the ligand CP12.The residues **TYR-158, LYS-165, ALA-191, GLY-192** involved in hydrogen bond interactions with the ligand CP12,those amino acids key amino acids in the active pocket of InhA protein.The residues **GLY-152** involved in hydrogen bond interactions with the ligand CP12 those amino acids key aminoacids in the active pocket of Beta-Ketoacyl-Acyl Carrier Protein Synthase III protein.The residue **GLY -117** involved in hydrogen bond interactions with the ligand CP12 with KasA protein. The amino acid residues which are involved in polar interaction may lead to inhibition of enzyme activity. This may affect mycolic acid biosynthetic pathway.

TABLE 2: Gridbox box parameters for selected proteins

Protein	***Centre***			***size***		
	x	**y**	**z**	**x**	**y**	**z**
Beta-Ketoacyl-Acyl Carrier Protein Synthase III (1hzp)	-2.917	46.000	19.333	40	28	18
MabA(1uzn)	6.139	20.194	14.306	22	34	34
Transferase (5ld8)	9.972	27.722	-7.028	24	26	32
Oxidoreductase (5mtp)	13.500	-5.583	54.444	26	42	20

Table 5: Anti-tubercular, Antifungal and Antimicrobial activity of synthesized compounds (CP1 to CP12)

CODE	**Mtb**	**Antifungal**			**Antibacterial**			
		AN	**AF**	**CA**	**SA**	**EF**	**EC**	**PA**
CP1	50	>100	>100	>100	100	100	>100	>100
CP2	50	>100	>100	>100	100	100	>100	>100
CP3	50	>100	>100	>100	>100	>100	>100	>100
CP4	25	>100	>100	>100	50	100	50	100
CP5	50	>100	>100	>100	50	100	50	50
CP6	25	>100	>100	>100	50	100	50	50
CP7	6.25	>100	>100	>100	50	100	50	50
CP8	12.5	>100	>100	>100	50	100	100	50
CP9	12.5	>100	>100	>100	50	50	100	50
CP10	6.25	>100	>100	>100	50	50	50	50
CP11	3.12	>100	>100	>100	100	100	50	100
CP12	3.12	>100	>100	>100	25	50	50	50

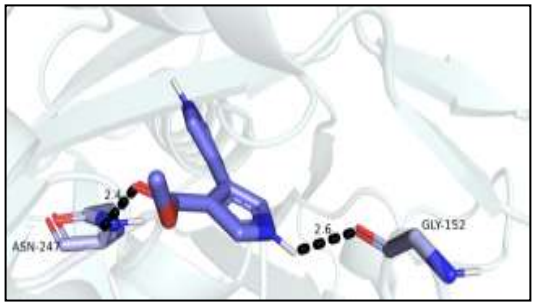
TABLE 6: Docking Scores of Synthesized Compounds

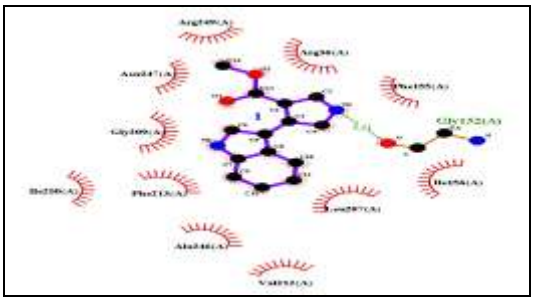
Ligands	***1hzp***	***1uzn***	***5ld8***	***5mtp***	***MIC***
CP1	-6.8	-6.8	-6.9	-7.0	50
CP2	-6.7	-6.5	-7.2	-7.1	50
CP3	-6.9	-6.6	-6.6	-7.4	50
CP4	-7.0	-6.5	-7.5	-7.4	25
CP5	-7.0	-6.6	-7.5	-7.4	50
CP6	-6.6	-6.5	-6.6	-7.4	25
CP7	-7.4	-6.7	-7.5	-7.5	6.25
CP8	-6.1	-6.5	-6.2	-7.3	12.5
CP9	-6.4	-6.5	-6.1	-7.3	12.5
CP10	-6.6	-6.3	-6.5	-7.3	6.25
CP11	-6.1	-6.7	-6.3	-7.6	3.12
CP12	-7.0	-7.0	-7.5	-8.1	3.12

Table 7: Interactions

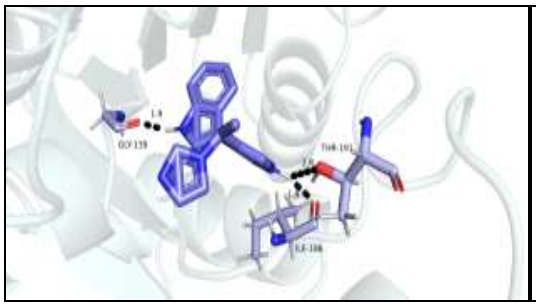
Ligand	*Protein name*	*Predicted binding energy*	*Interaction with amino acid residues*
CP7	Keto acyl	-7.4	Asn-247
	MabA	-6.7	Ile-27, Gly-28, Gly-90
	Transferase	-7.5	GLY-200
	Oxidoreductase	-7.5	ASP-148, TYR-158
CP12	Keto acyl	-7.0	GLY-152
	MabA	-7.5	GLY-139,PRO-183,ILE-186,THR-191
	Transferase	-7.5	GLY-117
	Oxidoreductase	-8.1	TYR-158, LYS-165, ALA-191, GLY-192

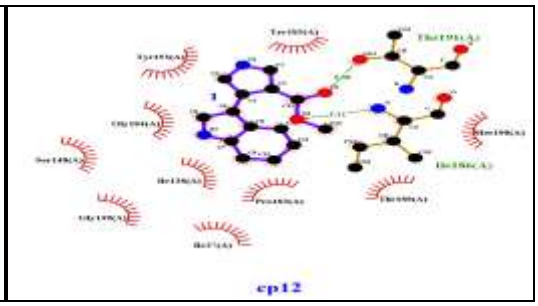
Beta-Ketoacyl-Acyl Carrier protein synthase III (1hzp)
CP12



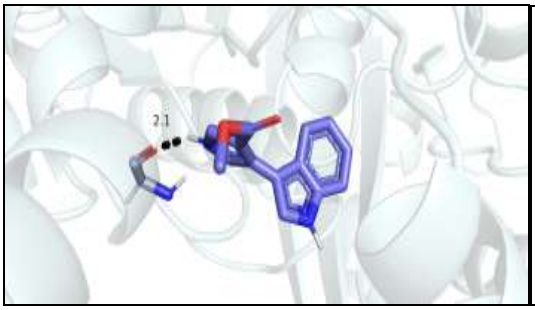


MabA(1UZN)
CP12





KasA(5LD8)
CP12



InhA (5MTP)
CP12

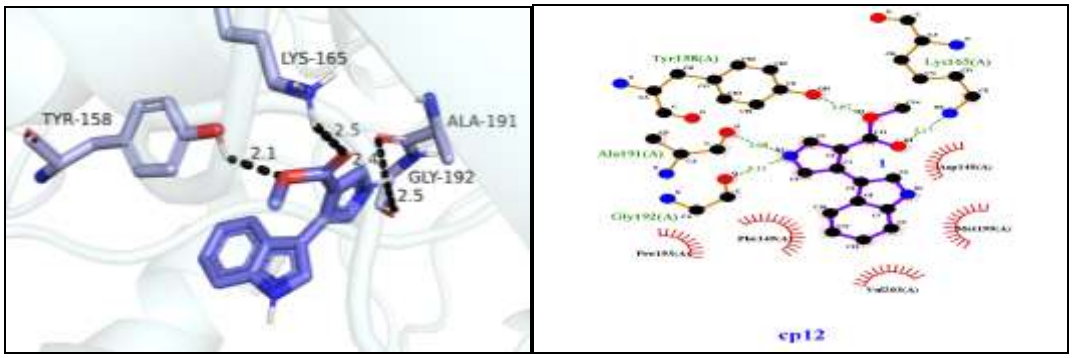


Fig 1: Binding interactions of the top-ranking molecule **CP12**at the active site of Beta-Ketoacyl-Acyl Carrier protein synthase III (1hzp**),**MabA,KasA(5LD8) andInhA (5MTP) protein along with their 3D interactions. The protein is represented as pale cyan ribbon and interacting residues of the protein as light blue colour ball and stick model. The ligands are represented in slate blue colour stick model.

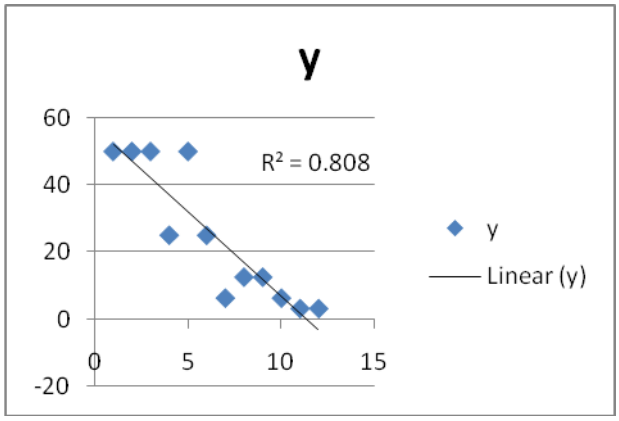


Fig 2: Correlation plot between MIC (µg/ml) and Docking Scores (Kcal/mol)

CONCLUSION:

The synthesized compounds were screened for anti-tubercular activity against M. tuberculosis H37Rv using MABA assay. Further, the most potent compounds were subjected to axenic assay and MTT cell survival assays against Mtb H37Ra (MTCC 300). Here, the Two compoundsmethyl 4-(3,4,5-trimethoxyphenyl)-1H-pyrrole-3-carboxylate (**CP11**) and methyl 4-(1H-indol-3-yl)-1H-pyrrole-3-carboxylate (**CP12**)have shown high potency (MIC 3.12µg/mL) against *M. tuberculosis* H37Rv.Here the synthesized compounds doesn't show any antibacterial activity against using serial dilution technique. The test compounds CP12 showed maximum potency with 25µg/ml against *S.aureus* out of the remaining tested compounds.Hence, the synthesized compounds don't show any activity against *A.niger*, *A flavus*, *C. albicans*.The liner regression analysis showed that the MABA protein could be the most probable site of action for the selected ligand based on r^2 value (0.80). The results of the *in silico* studies need to be further validated by *in vitro* ligand binding studies.The results of these compounds antitubercular activity are supported by docking analysis.The experimental data (MIC) and docking score of CP compounds against MabA have a correlation of 0.80, indicating thatMabA's docking simulation parameters are effective in reproducing the experimental orientation of produced compounds.

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