Synthesis and Evaluation of Novel Heterocycles as Anti-Tubercular Agents

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ABSTRACT

Tuberculosis, an infectious disease, has been reported to cause 1.5 million death in 2020 and the possibility is more among people with HIV. The continuous global spread of tuberculosis is mostly due to multi-drug-resistant tuberculosis. Drug resistance generated against mutant *Mycobacterium tuberculosis* strains poses a problem for drug development in identifying new molecules or compounds to treat the evolved resistant strains. Mimicking the vital mycobacterial cell wall component fatty acid, mycolic acids the present study aims to design and synthesize a series of Pyrazole derivatives through molecular hybridization approach which are proposed to block mycobacterial cell wall biosynthetic pathway (FAS II) by inhibiting *InhA* enzyme. Hence, in this study, we have focussed on the synthesis of pyrazole, oxo-pyrimidine derivatives from chalcone derivatives synthesised through cyclo-condensation with hydrazine hydrate and urea, respectively and evaluated for their anti-tubercular activity against Mycobacterium tuberculosis H37Rv strain by Microplate Alamar Blue assay (MABA) method. Among the synthesised pyrazole derivatives, **compound 4** with bromo phenyl and tolyl substitution showed excellent anti-tubercular activity when compared with the oxo-pyrimidine derivatives with MIC 3.12µg/ml.

Key words: anti-tubercular, pyrazole, oxo-pyrimidine derivatives, chalcones

INTRODUCTION

Tuberculosis caused by Mycobacterium tuberculosis is the thirteenth leading cause of mortality globally and due to the fact that Tuberculosis is carried from person to person through the air and that endemic TB areas have incredibly high transmission rates, this has gained widespread public attention. Transmission of TB happens remarkably similarly to other Tuberculosis, usually known as TB is an ancient illness,

and the skeletal remains of people under study revealed that TB has been a problem for mankind for thousands of years. One of the health-related goals included in the United Nations' Sustainable Development Goals is to put an end to the tuberculosis epidemic by the year 2030. According to 2021, Global Tuberculosis report by WHO, TB caused about 1.5 million deaths in the year 2020. In addition, the pervasiveness of the co-infection of HIV/TB, extensively drug resistant and multi drug resistance increase the challenges of treatment which causes the widespread of the disease and also increases the mortality.

First line and second line anti-tubercular drugs on prolonged usage are proved to be toxic. Hence few compounds like Pretomanid containing (imidazo oxazine nucleus) in combination with linezolid and bedaquiline for the treatment of multi-drug resistant tuberculosis was granted approval by the Food and Drug Administration of the United States in August 2019, as an outcome of its successful performance in a phase III clinical study. But these drugs are recommended only for multi drug resistant tuberculosis and are associated with adverse effects, hence the need the novel antitubercular agents with better activity and the less toxicity profile is required to put an end for TB.

Literatures shows that various heterocyclic compounds that are part of the natural metabolites like flavonoids, alkaloids and steroids having wide range of bioactivities can be considered in the treatment of TB. Of the heterocyclic compounds, which are essential in the treatment of wide range of microbial infections, N-heterocycles such as indoles, triazoles, thiazoles, and pyrazoles played a significant role in the development of anti-tubercular agents. Several literature proves the effects of N-heterocycles as anti-tubercular agents, in which Pyrazole, a five membered heterocyclic compound with two nitrogen atoms attached adjacent to each other was found to have a broad range of the biological activities like antimicrobial, antiviral, anticancer, anti-inflammatory, ACE inhibitory, anticonvulsant and neuroprotective etc., Drugs such as Razaxaban- Xa inhibitor, Lonazolac- NSAID, Penthiopyrad-fungicide, and Celecoxib-selective COX-2 inhibitor were found to have pyrazole pharmacophore.

Several researches are carried out as a result of the wide range of biological activities combined with the successful usage of pyrazole-containing medications in clinics. Secondly, the dihydro-derivative of pyrazole (pyrazoline) is another important aza-heterocycle which has gained attention by the chemist because of the stability of

the ring. Chemists have experimented a variety of structural variations of pyrazolines leading to the development of newer anti-inflammatory, analgesic, antibacterial, anticancer, antidepressant, and anticancer agents. The azole functionality inside the ring pyrazole enhances lipophilicity, allowing the medication to passively diffuse through the Mtb cell wall, according to structure activity relationship analysis. Therefore, there exist a great interest in the development of novel pyrazole and pyrazoline derivatives to act as anti- tubercular agents and this work represents the synthesis using substituted aldehydes and acetophenone derivatives.

MATERIALS AND METHODS

A sequences of Pyrazole derivatives were designed, optimized and subjected to molecular docking to determine the binding energy and admet SAR and Osiris which helps to identify the risks during clinical development before synthesis.

Molecular Docking

A series of Pyrazole hybrid derivatives were drawn using Chem draw and they were saved in mol format. 3D structures of the compounds were generated using Avogadro and optimization was done to generate possible tautomeric states and low energy conformers. Energy minimization of the compounds were done by MMFF94 as the force field. The InhA protein (4TRN.pbd) was downloaded in PDB format from RSCB-Protein Data Bank. Protein's three-dimensional crystal structure was generated by eliminating water molecules, refining bond orders and addition of hydrogens. Using the Molegro molecular viewer, protein minimization was carried out. Auto dock tools was used to generate the grid box at the active site of the receptor. The generated low energy poses were docked using Auto dock software into the active site of *InhA* protein (4TRN.pdb). Using Lamarckian evolutionary method, the binding score was noted and interactions of the docked compounds were displayed using Discovery Studio.

ADMET Profile

To compute *in-silico* pharmacokinetic properties, the software program admetSAR was utilized, which facilitated in calculating the solubility, BBB and logP values. OSIRIS properties were used to predict the organ toxicities and toxicological outcomes of experimental drugs. Carcinogenicity, mutagenicity, reproductive effects, drug-likeness, and irritants were assessed and recorded in this study, to evaluate the toxicity risk.

ROUTE OF SYNTHESIS

General Procedure for the synthesis of Substituted Pyrazole and oxo-Pyrimidine derivatives:

Step 1: Equimolar quantities of substituted aldehyde derivatives (0.013M) and substituted acetophenone derivatives (0.013M) was added and dissolved in 65 ml of ethanol in a round bottom flask and stirred using magnetic stirrer. 60 percent aqueous sodium hydroxide solution (40 ml) were introduced to the reaction mixture dropwise which is mixed thoroughly at room temperature. The reaction is monitored by TLC using (n-hexane: ethyl acetate 3:2) after 2 hours. On completion of the reaction, the liquid was neutralized with 2M HCl and allowed to cool in an ice bath, to yield a yellow precipitate. Then it is filtered using vacuum filtration technique, dried and recrystallized from ethanol resulting in chalcone derivative.

Step 2: Hydrazine hydrate (0.01 M) or Urea (0.005 M), with substituted Chalcone derivatives (0.01 M), and sodium acetate (0.01 M) or Acetic acid (30 ml) were dissolved in 12 mL ethanol and refluxed for 6-8 hours at 120^0C. The reaction progress was monitored with TLC. The reaction mixture was concentrated under reduced pressure and poured into ice water. The resulting precipitate was filtered, washed and dried with water and recrystallized using appropriate solvents.

RESULTS AND DISCUSSION:

Synthesis:

The designed compounds which showed better binding energy on docking were synthesized by two steps.

Step 1: Various chalcones derivatives were synthesised using Claisen–Schmidt condensation. Here, an aromatic aldehyde interacts with an aryl methyl ketones or methyl ketone in the presence of a base to form an alpha, beta-unsaturated ketone (enone). Substituted acetophenones at room temperature with suitable aldehydes dissolved in sodium hydroxide solution on continuous stirring for 2-3 hours resulted in chalcone derivatives. The completion of the reaction was monitored by TLC using n-hexane and ethyl acetate (3:2). Figure 1 represents the synthetic scheme of chalcone derivatives.

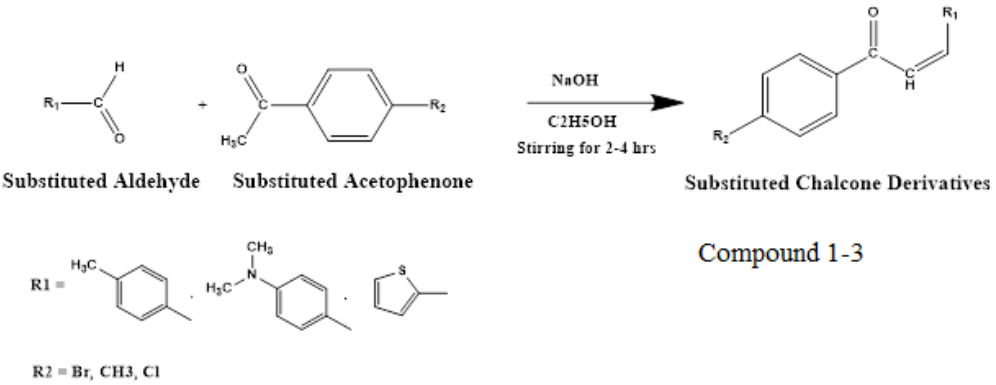


Figure 1: Synthesis of substituted chalcone derivatives from substituted aldehyde and substituted Acetophenone

Step 2: Various heterocyclic derivatives such as pyrazole, oxo-pyrimidine were synthesized from chalcone derivatives through cyclo-condensation with hydrazine hydrate and urea, respectively. The completion of the reaction was monitored by TLC using n-hexane and ethyl acetate (3:2). The obtained precipitate was recrystallized using ethanol. Figure 2 represents the scheme for the synthesis of pyrazole and oxo-pyrimidine derivatives.

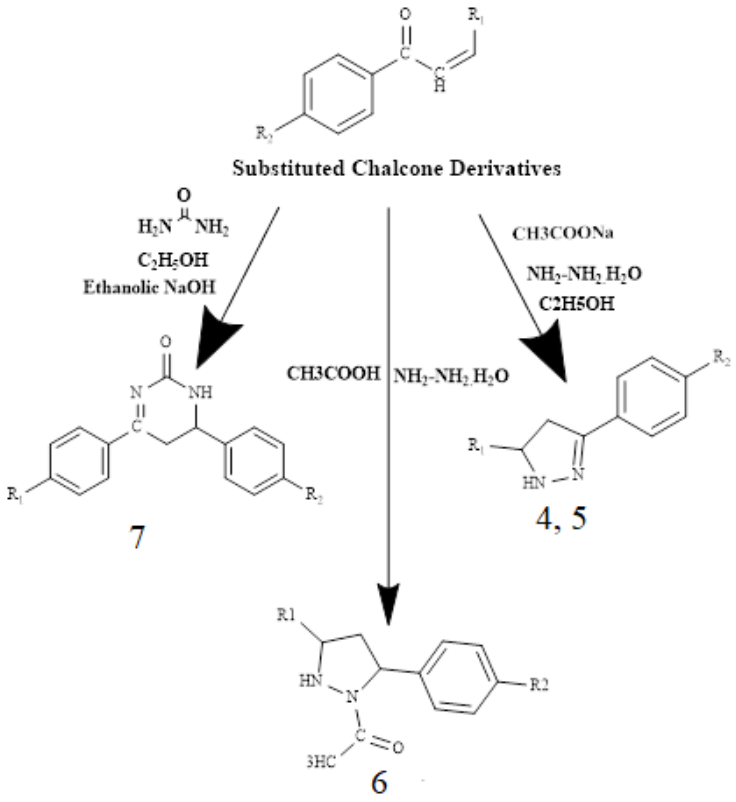
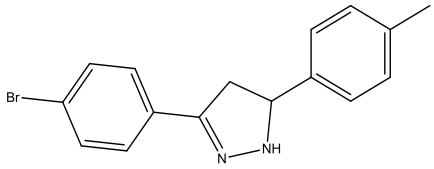


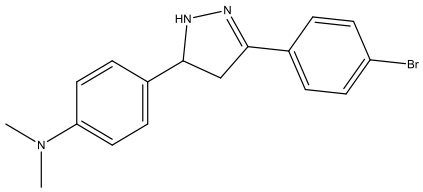
Figure 2: Synthesis of substituted Pyrazole and oxo-Pyrimidine derivatives from substituted chalcone derivatives

Compound 4: 3-(4-bromophenyl)-5-(p-tolyl)-4,5-dihydro-1H-pyrazole



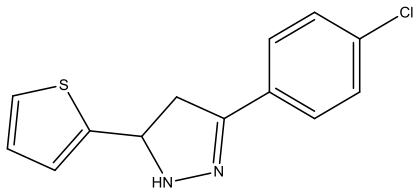
Light yellow color solid; yield 61%; Melting point (m.p) = 219^{0}C; R_f (n-hexane: Ethyl acetate 3:2): 0.58; FT-IR (KBr, cm^{-1}) ν: 3400 (N-H amide), 2919.31 (C-H aromatic), 1070 (-C-N-), 1680.99 (-C=N-), 1484 (C=C Aromatic Alkene), 523 (C-Br). MS (ESI) Calculated m/z for $C_{16}H_{15}N_2Br$ (315.21), obtained m/z 315 (M^+).

Compound 5: 4-(3-(4-bromophenyl)-4,5-dihydro-1H-pyrazol-5-yl)-N, N-dimethyl aniline



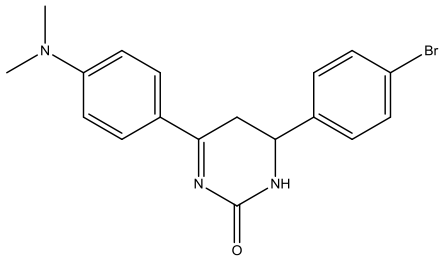
Yellow color solid; yield 61%; Melting point (m.p) = 215^{0}C; R_f (n-hexane: Ethyl acetate 3:2): 0.61; FT-IR (KBr, cm^{-1}) ν: 3350 (N-H amide), 2845 (C-H aromatic), 1163 (-C-N-), 1651.09 (-C=N-), 1675.21 (C=C Aromatic Alkene), 592.16 (C-Br). MS (ESI) Calculated m/z for $C_{17}H_{18}N_3Br$ (344.25), obtained m/z 343 (M^++1)

Compound 6 : 3-(4-chlorophenyl)-5-(thiophen-2-yl)-4,5- dihydro-1H-pyrazole



Light brown color solid; yield 55%; Melting point (m.p) = 213 ^{0}C; R_f (n-hexane: Ethyl acetate 3:2): 0.63; FT-IR (KBr, cm^{-1}) ν: 3475 (N-H amide), 2925 (-C-H-), 1088.84 (-C-N-), 1650 (-C=N-), 1589.37 (-C=O- amide) 1488.11 (-C=C-Aromatic Alkene), 700.17 (-C-Cl). MS (ESI) Calculated m/z for $C_{15}H_{15}N_2ClOS$ (306.81), obtained m/z 305 (M^++1).

Compound 7: 6-(4-bromophenyl)-4-(4-(dimethyl amino) phenyl)-5,6-dihydropyrimidin-2(1H)-one

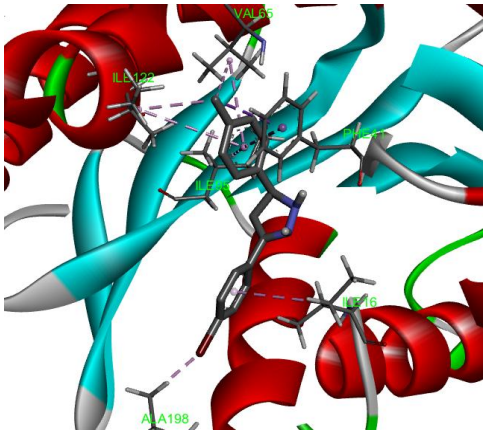


Yellow color solid; yield 67%; Melting point (m.p) = 220 ^{0}C; R_f (n-hexane: Ethyl acetate 3:2): 0.61; FT-IR (KBr, cm^{-1}) ν: 3355 (N-H amide), 2914.49 (C-H aromatic), 1227.71 (-C-N-), 1680.99 (-C=N-), 1565.26 (C=C Aromatic Alkene), 515.97 (C-Br), 1510 (-C=O), 3078.44 (=C-H). MS (ESI) Calculated m/z for $C_{18}H_{18}N_3Br$ O (372.26), obtained m/z 372.65 (M^+).

***IN SILICO* STUDIES:**

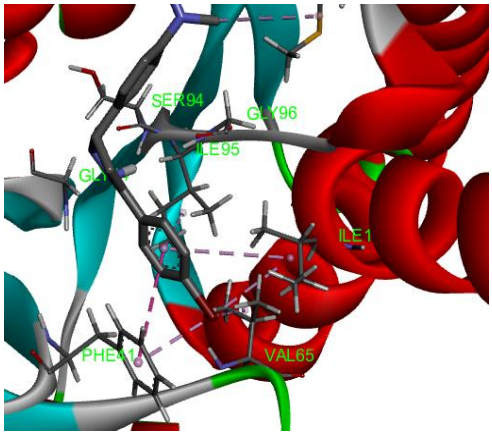
A total of 6 Pyrazole and oxo pyrimidines hybrids were designed and binding modes were analysed through molecular docking approach. The 3D low energy conformers were generated through Discovery studio 2021 and flexible docking was done in the catalytic pocket of *InhA* protein (4TRN.pdb). Based on autodocking score, binding energy, visual analysis of binding modes and their interaction profile four compounds were selected. The molecular docking values for the selected four compounds were represented in **Table 1,** the docking score and binding energy was observed in the range of -7.69 to -8.72 Kcal/mol against standard isoniazid -5.18. The 3D docked poses of the Compounds **4** to **7** were illustrated in Table 1. The compounds **4 to 7** interacted with catalytic residues by forming hydrogen bonding and hydrophobic interactions. The residues involved in the binding are Ser20, Ile21, Thr39, Phe41, Ser94, Gly96 and Lys65. The high scored compound **6 (-8.72 Kcal/mol)** formed hydrogen bonding and π-π stacking interactions with the residues mainly SER 20, PHE 41, SER 94 and GLY 96.

Table 1: 3D and 2D Docked poses of Compounds 4, 5, 6 and 7



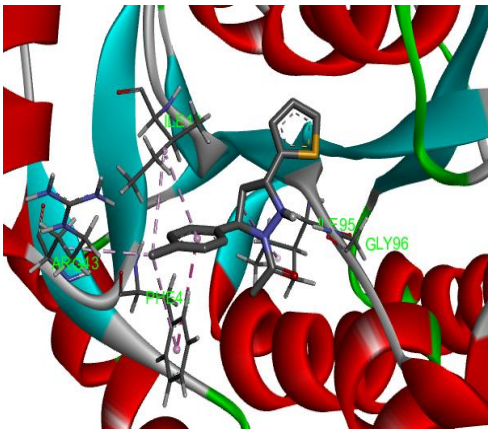
Compound 4

Binding energy -7.9



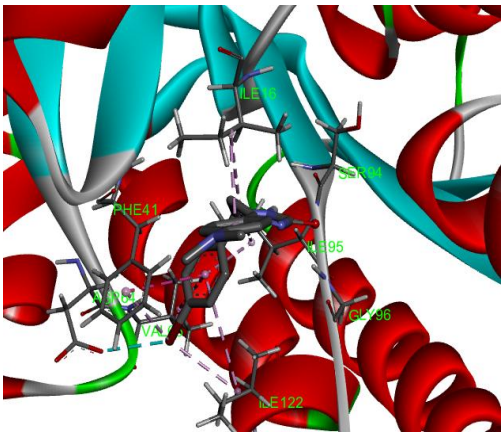
Compound 5

Binding energy -7.76



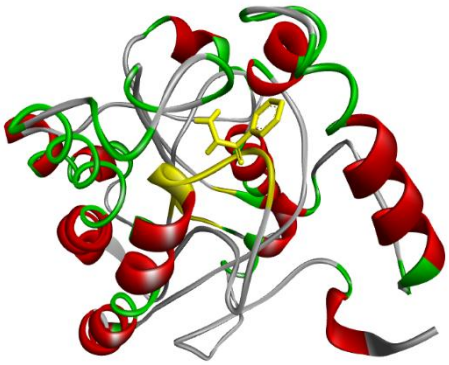
Compound 6

Binding energy -8.72



Compound 7

Binding energy -7.69



Standard (Isoniazid)

Binding energy -5.18

ADMET STUDIES:

The calculated ADMET properties by Swiss ADME and OSIRIS property explorer were concluded in Table 2 and this indicated that all the properties are within recommended limits. The lipophilicity (log P) of synthesized compounds iis a key physicochemical property which is closely correlated with its biological activity and was observed in the range of 2.77 to 3.07. All compounds were non toxic expecting for compound 5 and 7 which were found to toxic towards carcinogenicity.

Table 2: ADMET properties of compound 4 to 7 using Swiss ADME and OSIRIS explorer Property

Compound	Solubility	BBB	LogP	Mutagenicity	Carcinogenicity	Irritant	Reproductive effects	Drug-likeness
4	-4.89	Yes	3.05	G	G	G	G	0.26
5	-4.81	Yes	2.91	G	R	G	G	0.63
6	-3.85	Yes	3.07	G	G	G	G	7.01
7	-4.50	Yes	2.77	G	R	G	G	0.49

BBB: Blood brain barrier; logP: Lipophilicity, R-Toxic and G- Non toxic

LIPINSKI'S RULE OF FIVE

The physiochemical properties of the designed compounds were noted and summarized by using *Molinspiration* online webserver.

Table 3: Physicochemical properties of compounds 4 to 7

Compound	Molecular Weight	TPSA	Rotatable Bonds	Hydrogen Donors	Hydrogen Bond Acceptor	Violation
4	315.21	24.39	2	2	1	0
5	344.26	27.63	3	3	1	0
6	306.82	32.34	2	3	1	0
7	372.27	44.70	3	4	1	0

TPSA-Total Polar surface area

***IN VITRO* ANTI-TUBERCULAR ACTIVITY**

***Mycobacterium tuberculosis* growth inhibition assay (MABA Method)**

MABA assay protocol was used to determine the minimum inhibitory concentration (MIC) of all the synthesized compounds. The synthesized compounds were screened against *M. tuberculosis* H37Rv using serial dilution technique in Middlebrook 7H9 broth medium. Isoniazid (INH) was used as the standard drug. Standard Strain used: ***Mycobacteria tuberculosis*** (Vaccine strain, H37 RV strain): ATCC No- 27294. The MIC of the synthesized compounds are presented in Table 4

Table 4: Anti-tubercular activity of synthesized compounds

COMPOUND	MIC (µg/mL)
4	3.12
5	12.5
6	6.25
7	25
ISONIAZID (STD)	1.6
PYRAZINAMIE (STD)	3.125

The results of antitubercular activity for the four compounds indicated that the compound 4 with bromo phenyl and tolyl substitution at the pyrazole nucleus showed least concentration of about 3.12 µg/mL when compared with the standard Pyrazinamide which showed minimum inhibitory concentration at 3.12 µg/mL. Compound 5 with bromo phenyl and dimethyl aniline substitution at the pyrazole nucleus showed lesser anti-tubercular activity among the pyrazole derivatives. Compound 7 with bromophenyl and dimethyl amino phenyl substitution on the pyrimidine nucleus showed the least anti-tubercular activity.

CONCLUSION

In conclusion, the four pyrazole and oxo-pyrimidine derivatives synthesized from substituted chalcones were found to exhibit anti-tubercular activity. ADMET studies prove that all the compounds obeyed Lipinski's rule of five and they were nontoxic towards hepatotoxicity and mutagenicity. These compounds when tested for their ability to inhibit the growth of M. tuberculosis, it was found that the pyrazole derivatives with bromo phenyl and tolyl substitution showed excellent anti-tubercular activity with MIC 3.12µg/ml, while the oxo-pyrimidine derivatives showed the least anti-tubercular activity when compared with the standard Isoniazid and Pyrazinamide. Further, compound 4 can be modified to obtain an excellent anti-tubercular agent.

Declaration of Competing Interest

The authors claim no conflict of interest

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