Design and Synthesis of Some Novel Anti-tubercular Agents

AN EXECUTIVE SUMMARY OF THE THESIS

SUBMITTED TO

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BY

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Introduction

TB is the major death-causing disease all around the world especially, in the developing countries. TB was the leading cause of mortality from a single infectious agent, until the coronavirus pandemic in 2019. It is a granulomatous, contagious airborne disease proved to be fatal if not treated. TB is caused by *Mycobacterium* species and generally affects the lungs, but it can also affect other body parts as well[1]. Earlier TB was successfully controlled by a set of drugs namely, isoniazid (**2**), rifampicin (**3**), ethambutol (**4**) and pyrazinamide (**5**). These drugs are the first line agents used primarily to cure the disease. During the later years, other drugs such as fluorouinolones, ofloxacin, cycloserine (**7**) etc were introduced to aid the first-line agents.[2] These are also known as second line drugs. There are more than two dozen drugs available to treat TB. Still, TB remains one of the major concerns to the healthcare professionals, as it is associated with development of resistance, patient's incompliance, serious side effects and long duration of treatment[3].

Development of resistance against available drugs have been the major concern for quite some time now. When the patient is non-responsive to the first line agents like, isoniazid (**2**) and rifampicin (**3**), it known as Multidrug resistance (MDR).[4] If the resistance has been developed against first line drugs and one of the second line drugs, then it's called extensive drug resistance (XDR). If the patient is non-responsive to any treatment given, then its a case of severe total drug resistance (TDR). Resistance may be caused by mutation in one or more chromosomal genes in *Mtb*.[5] There is lot of research going on the field of TB, yet only three drugs namely bedaquiline (**17**), delamanid (**19**) and pretomanid (**18**) have been approved lately by USFDA for the treatment of resistant TB. But, unfortunately bedaquilline resistant strains of *Mtb* have also been reported lately.[6]

There is an urgent need to address the deadliest communicable disease, Tuberculosis. Presently, there are over two dozen anti-tubercular agents present in the market to tackle the disease. Yet, it is still spreading and proving the available anti-TB drugs inefficient. The main reason for failure of the existing drugs is attributed to the development of resistance by *Mycobacterium Tuberculosis*, patient incompliance and long durations of treatment.

The Mycobacterium cell wall is the first point of contact between the host and *Mtb*. The cell wall has always been an effective target for developing anti-TB agents. Many available drugs such as isoniazid, cycloserine, ethambutol etc. inhibit cell wall synthesis. There are various enzymes involved in the biosynthesis of cell wall such as InhA, Kas A, DprE1, MmpL3

etc. which regulate the cell permeability. Thus, targeting these enzymes could be an effective approach for design and development of newer anti-TB agents.

DprE1 is the most exploited target at present for development of newer anti-TB agents. DprE1 enzyme is involved in the synthesis of decaprenylphosphoryl-β-*D*-arabinofuranose (DPA) which is the only source of arabinan for synthesis of arabinogalactan layer of the cell wall. At present there are only four DprE1 inhibitors present in the clinical trials, namely BTZ043, PBTZ169, TBA-7371 and OPC-167832. Thus, it was planned to target DprE1 for designing and development of novel anti-TB agents.

The brief research methodology and the key findings

The overall work is divided into two sub-categories since two different approaches were used to carry out the research work. These approaches are stated as below:

1. **Design and development of novel anti-TB agents based on the existing DprE1 inhibitors**

A vast literature is available on the enzyme DprE1 and its inhibitors. The available literature indicates towards the diversity of chemical scaffolds as DprE1 inhibitors. The various scaffolds that exhibit DprE1 inhibitory activity are azaindoles (**24**), benzothiazinones (**25-26**, **35**), benzothiazoles (**36**), pyrazolopyridine (**42**) etc. So, it was thought logical to develop a common pharmacophoric features using reported DprE1 inhibitors. The so developed common pharmacophoric features could be used for design of newer DprE1 inhibitors.

2. Design and development of novel anti-TB agents based on hybrid approach

From the literature, it has been found that chalcones (**43**, **48**, **50**, **52** and **56**) are biologically active moieties having vast range of biological activity including anti-TB activity[7–10]. Some of the reports also indicated that chalcones exhibit binding to the DprE1 enzyme via hydrogen bonding, π-π interactions and van der Waals interactions[11,12]. Further, chalcones and related compounds are not explored to the extent as anti-tubercular agents. Thus, it was thought logical to explore chalcone scaffold (**Figure 1**).

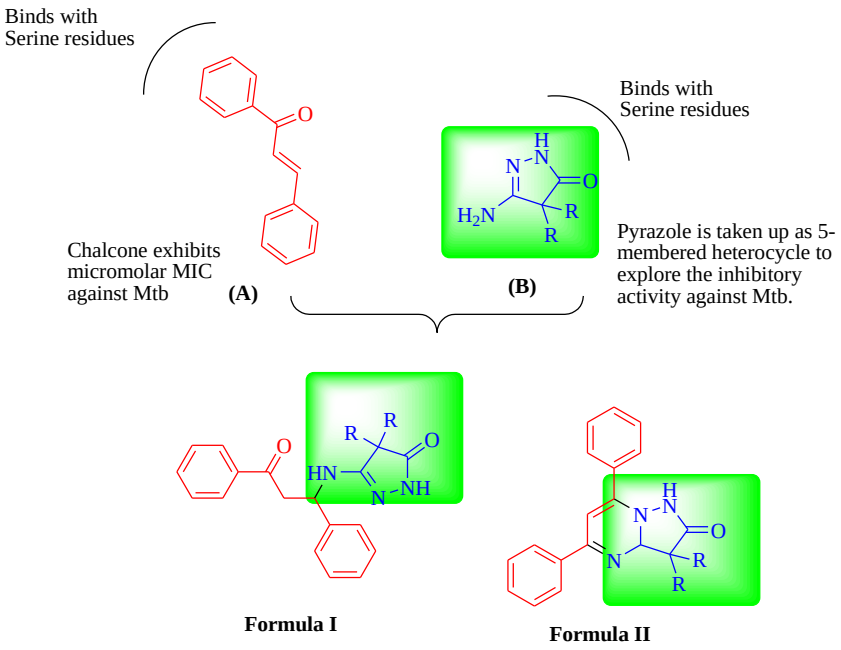


Figure 1: Designing of pyrazole fused dihydrochalcone derivatives (**I, II**) using hybrid approach

Thus, the work has been subcategorized based on the approach used for design and development of compounds.

SECTION I

Based on the structure of reported DprE1 inhibitors, a pharmacophore model was generated having common structural features. The generated pharmacophore model was used as a filter in the virtual screening of Asinex database to search for newer scaffold as DprE1 inhibitor. Other filters such as molecular docking and rule of five was also applied to get a hit molecule. This hit molecule was further optimized using molecular docking and ADMET predictions to get a lead compound (**Figure 4**)

Pharmacophore model

The present work aimed to identify the minimium structual features required for DprE1 inhibition. To serve the aim, a definite pharmacophore model was generated using reported DprE1 inhibitors. Active and inactive threshold pIC_{50} were fixed to 6.9 and 5.0 respectively and applied to the dataset of 140 compounds, giving 41 active, 15 inactive and 84 moderately active compounds. For generation of pharmacophore model, all the 41 active compounds were used. Pharmacophoric sites were created to give six types of structural features such as aromatic ring (R), negative (N), positive (P), hydrophobic (H), H-bond donor (D), and acceptor (A). The

software was restricted to find a minimum of 3 and maximum of 5 sites. Based on the active structures of the dataset, several 3-point and 4-point hypothesis were generated. AHRR, AAR, AAH, ARR, HRR and AHR were found to be most probable hypothesis. All the generated hypothesis were evaluated. The survival score ranged from 2.714 to 3.310 (**Table 1**).

Table 1: Data of the top ranked pharmacophore models

ID	Survival score	Survival inactive	Post hoc score	Site	Vector	Selectivity	Match	Energy
AHRR.28	2.990	1.641	2.991	0.63	0.897	1.359	22	2.254
AHRR.29	2.714	1.782	2.714	0.51	0.850	1.362	22	1.185
AAR.4	3.310	2.432	3.310	0.76	0.901	0.848	21	0.467

The highest ranked survival score for 3-point hypothesis was obtained for AAR.4 i.e. 3.310 whereas 4-point hypothesis AHRR.28 and AHRR.29 were scored as 2.990 and 2.714 respectively as shown in **Table 1**. The best model (AHRR.28) containing one hydrogen bond acceptor, one hydrophobic group and two aromatic rings is shown in **Figure 2**.

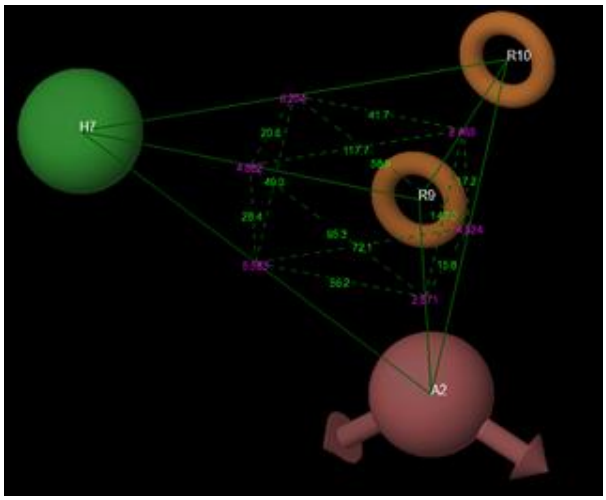


Figure 2: Pharmacophore model AHRR.28. Orange coloured torus represents aromatic rings (R9 and R10), green coloured sphere represents hydrophobic group (H7), and light magenta coloured vector represents hydrogen bond acceptor group (A2). Values in purple colour represent distances and in green colour represent the angles between the pharmacophoric features

The angles and distances of AHRR.28 among the pharmacophore are given in **Table 2** and **3**. The top three ranked pharmacophore models AHRR.28, AAR.4 and AHRR.29 were then used to align the molecules to build atom-based 3D-QSAR analysis. The best model was denoted by various statistical measures. The accuracy of the generated QSAR model was verified by the statistical data such as R^2 and Q^2.

Table 2: Angles between the different pharmacophoric features of AHRR.28 model

Site 1	Site 2	Site 3	Angle
H7	A2	R9	56.2
H7	A2	R10	72.1
R9	A2	R10	15.8
A2	H7	R9	28.4
A2	H7	R10	49.0
R9	H7	R10	20.6
A2	R9	H7	95.3
A2	R9	R10	147.0
H7	R9	R10	117.7
A2	R10	H7	58.9
A2	R10	R9	17.2
H7	R10	R9	41.7

Table 3: Distance between the different pharmacophoric features of AHRR.28 model

Site 1	Site 2	Distance (Å)
A2	H7	5.583
A2	R9	2.671
A2	R10	4.924
H7	R9	4.662
H7	R10	6.204
R9	R10	2.465

Virtual screening

Virtual screening has been proved to be a competent technique for the identification of hits using already available database in public/private domain. Various filters were applied to the Asinex database consisting of 2,12,526 compounds, so as to get initial hits as DprE1 inhibitors. The virtual screening results are illustrated in **Figure 3.**

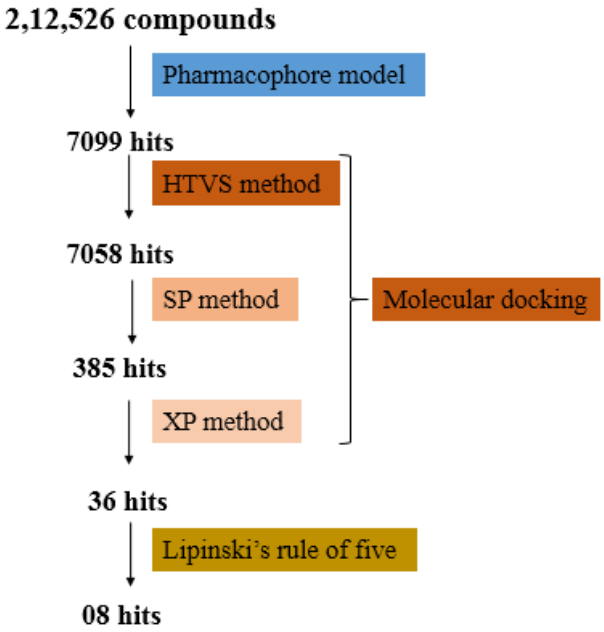
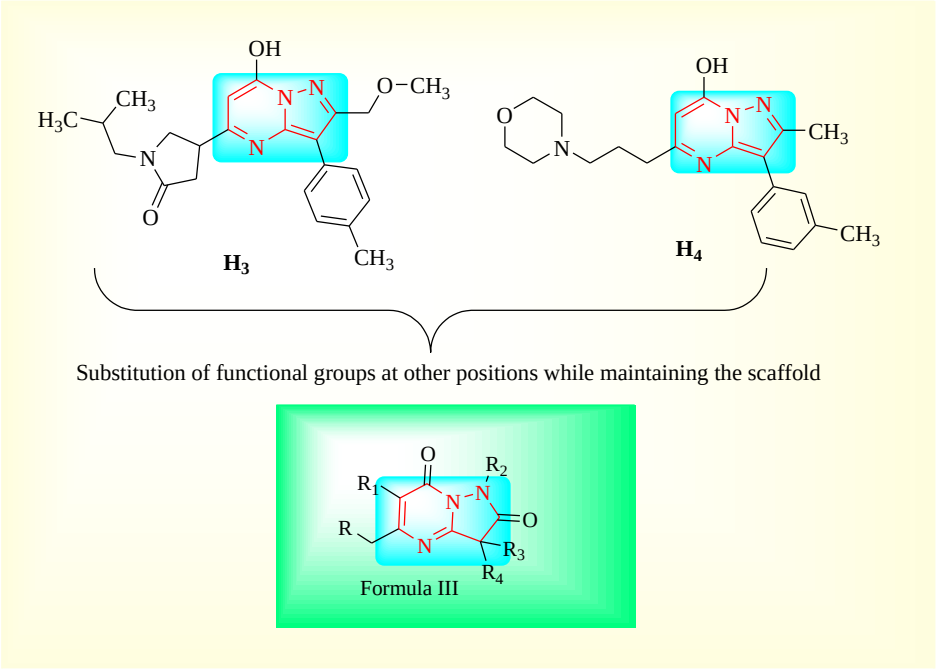


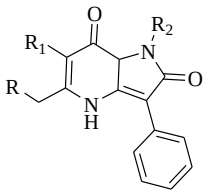
Figure 3: Flow chart of virtual screening



Wherein, R= -Cl, -NH-CH_2-C_6H_5, -NH-CH_2-4-C_6H_4-Cl, -NH-CH_2-4-C_6H_4-F, -NH-CH_2-4-C_6H_4-CH_3, -NH-CH_2-4-C_6H_4-OCH_3, -NH-CH_2-3,4-C_6H_3-*di*Cl, -NH-C_6H_5, -NH-2-C_6H_4-Cl, -NH-4-C_6H_4-F, -NH-4-C_6H_4-CH_3, -NH-4-C_6H_4-OCH_3, pyridin-4-ylamino, pyrrolidin-1-yl, piperazin-1-yl, morpholino, 4-methylpiperazin-1-yl
R_1=Cl/H; R_2= Br/H; R_3= -C_6H, -CH-C_6H_5, R_4= H, -CH-C_6H_5

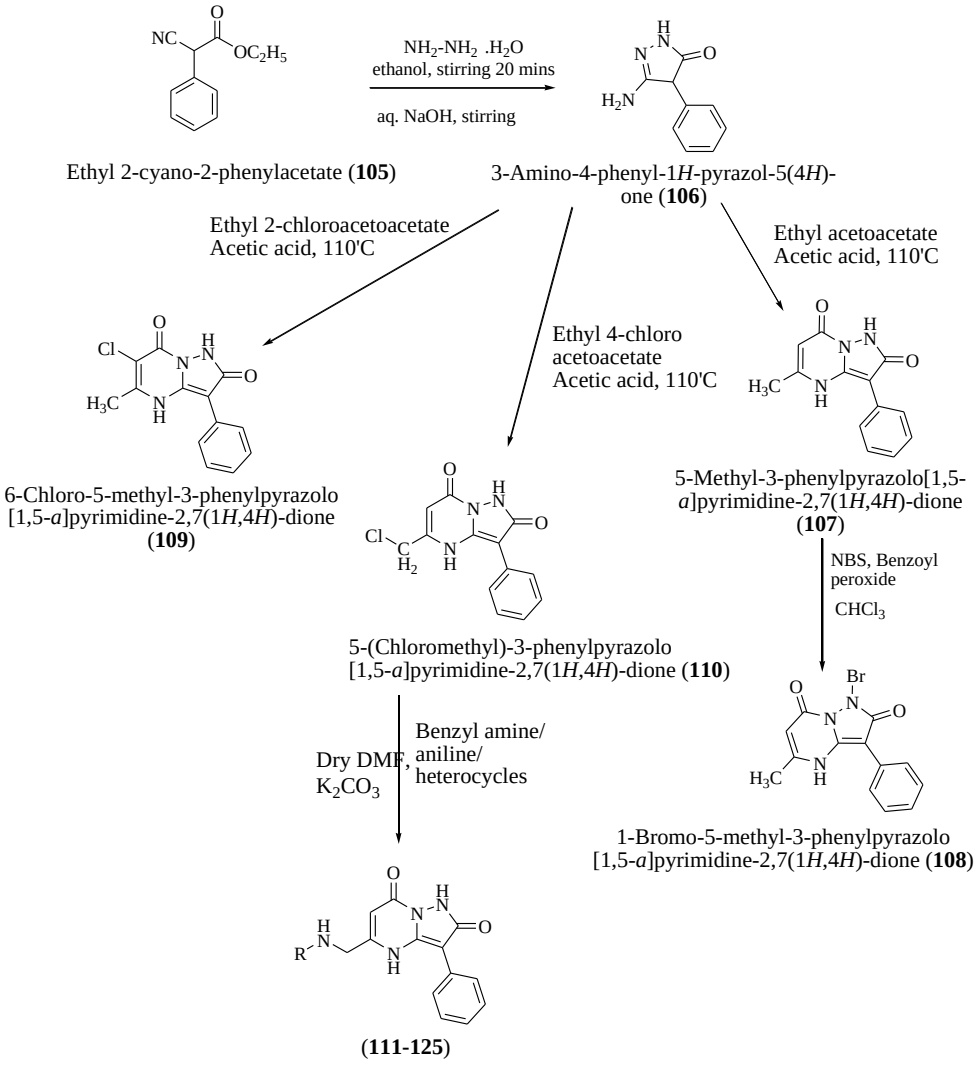
Figure 4: Designing of pyrazolo[1,5-*a*]pyrimidine-2,7(1*H*,4*H*)-dione compounds based on the hits obtained by virtual screening

For the synthesis of the aimed compounds (**Formula IIIa**) the following steps have been utilized as explained in **Scheme 1**.



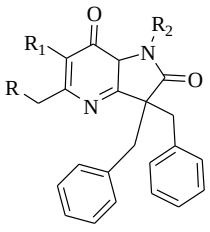
Formula IIIa

3-Amino-4-phenyl-*1H*-pyrazol-5(*4H*)-one (**106**) was synthesized by reacting commercially available ethyl-2-cyano-2-phenylacetate (**105**) with hydrazine hydrate 99%. The synthesized compound was further reacted with various ethyl acetoacetates in presence of glacial acetic acid to yield substituted 3-phenylpyrazolo[1,5-*a*]pyrimidine-2,7(1*H*,4*H*)-dione (**107, 109-110**) by substitution and subsequent cyclization. It was aimed to synthesize three different substituted 3-phenylpyrazolo[1,5-*a*]pyrimidine-2,7(1*H*,4*H*)-dione (**107, 109-110**). The first class of derivative (**107, 109-110**) was reported as such. The second category of 3-phenylpyrazolo[1,5-*a*]pyrimidine-2,7(1*H*,4*H*)-dione (**107**) was brominated with *N*-bromosuccinimide to give 1-bromo5-methyl-3-phenylpyrazolo[1,5-*a*]pyrimidine-2,7(1*H*,4*H*)-dione (**108**). The third category of compounds was synthesized by substitution reaction of the compound (**110**) with various amines such as benzyl amine, anilines and heterocyclic amine.

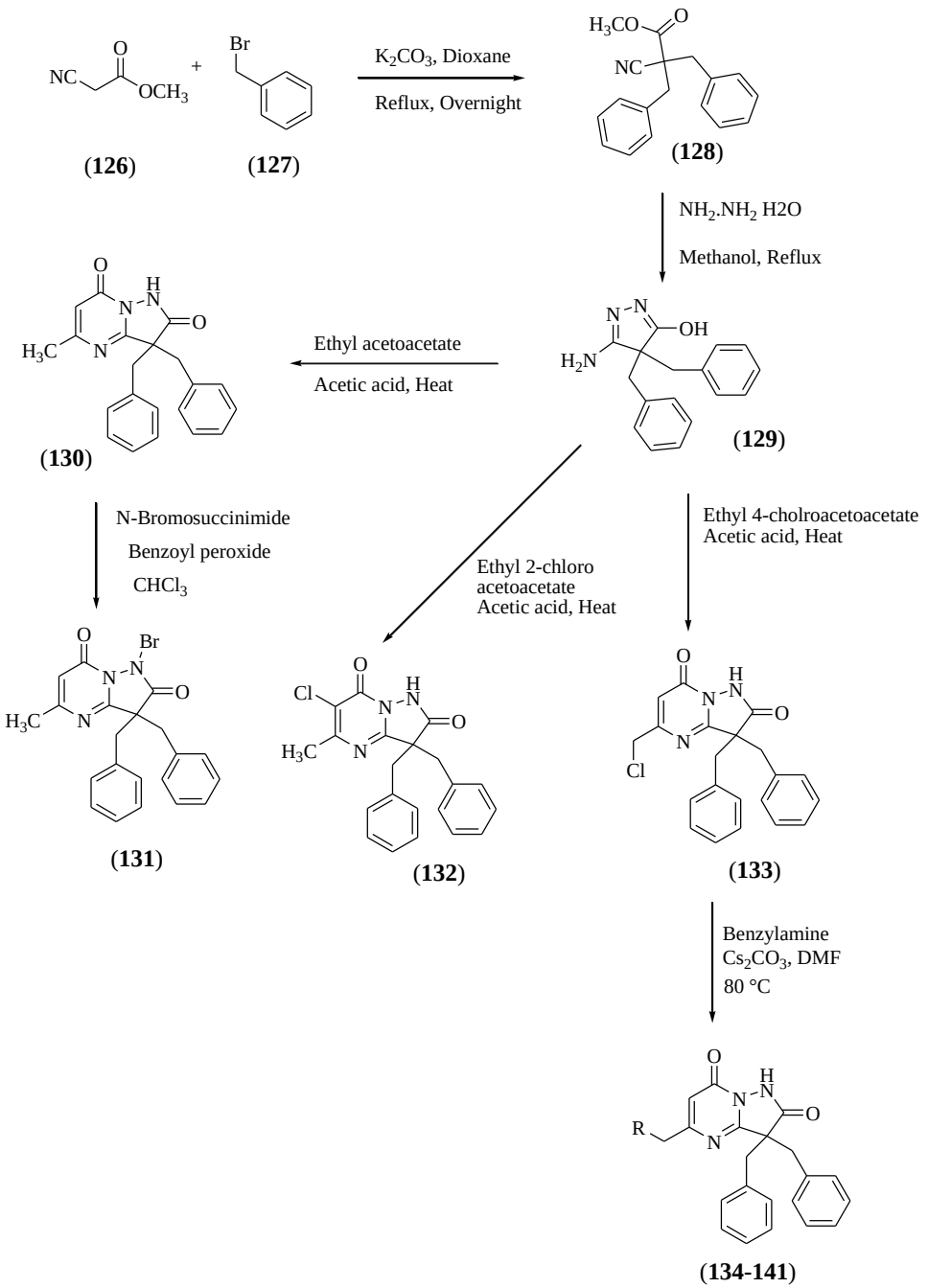


Scheme 1: Synthesis of compounds of 3-phenylpyrazolo[1,5-*a*]pyrimidine-2,7(1*H*,4*H*)-dione (**Formula IIIa**) based compounds

The designed compounds having **formula III-b** were synthesized by implementing **scheme 2**.

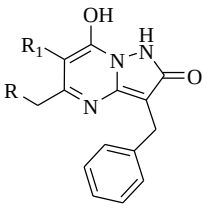


Formula III-b

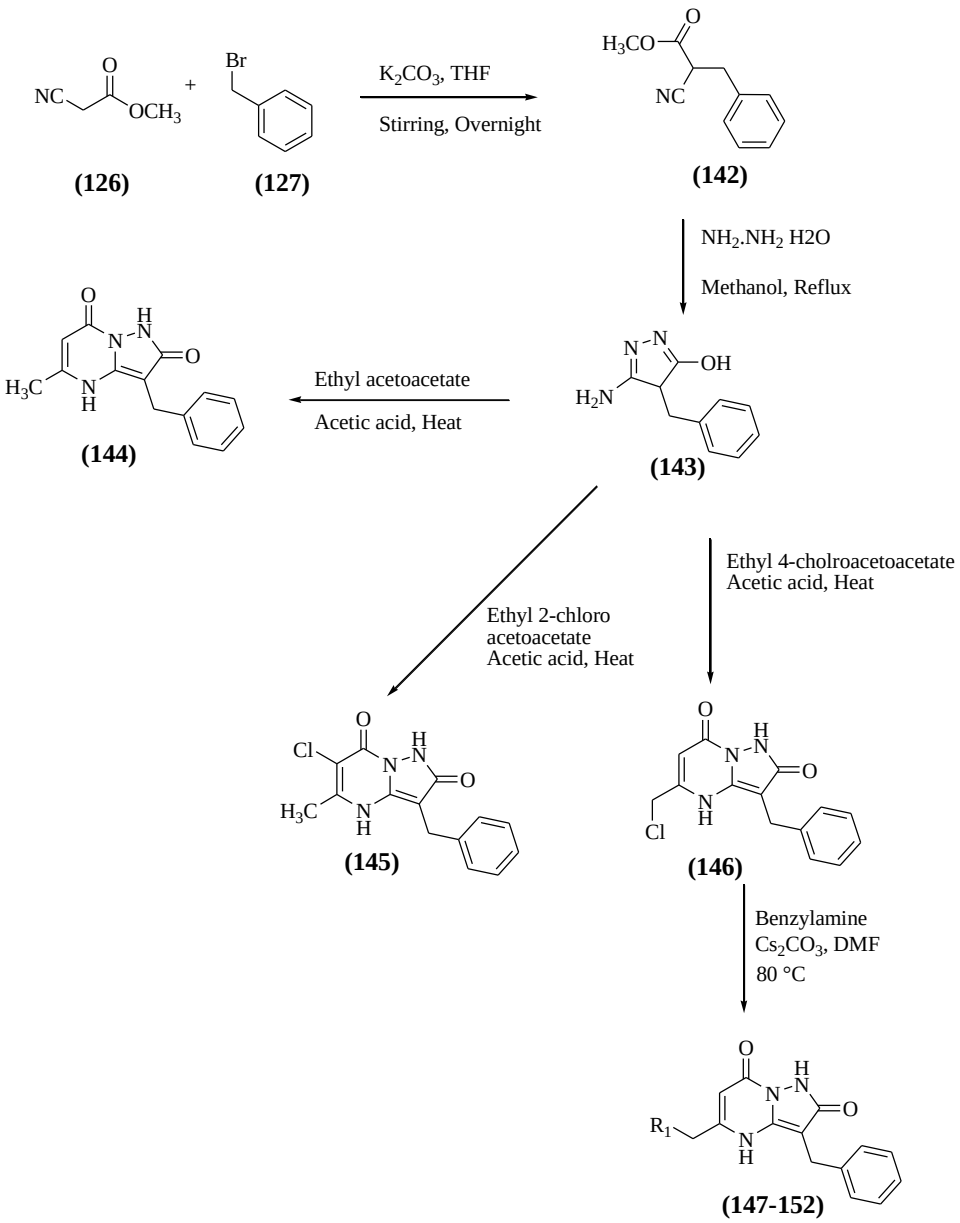


Scheme 2: Synthesis of disubstituted pyrazolo-pyrimidine derivatives **(Formula IIIb)**

The designed compounds having **formula III-c** were synthesized by implementing **scheme 3**.



Formula III-c



Scheme 3: Synthesis of 3-benzyl-7-hydroxy-5-methylpyrazolo[1,5-*a*]pyrimidin-2(1*H*)-one derivatives

Biological screening of synthesized compounds as anti-TB agents using MABA assay

The prepared compounds were evaluated for their percentage inhibition against *Mycobacterium bovis* (BCG). Results are shown in **Table 4** and **Figure 5**. Overall, seven compounds (**109, 118, 130, 132, 144, 145 and 146**) out of 38 compounds exhibited inhibition of the bacteria. It was found that compound **118** and **132** was exhibiting 90.87% inhibition, comparable to that of standard drugs. Compounds **130, 144, 145,** and **109** having chlorine in the 6th and 5th position respectively are showing inhibition, thus it can be agreed that presence of chlorine atom is favorable for inhibitory activity.

Table 4: Anti-tubercular activity of the synthesized compounds

Comp ID	% Inhibition	Comp ID	% Inhibition	Comp ID	% Inhibition
108	NI	**122**	NI	**140**	NI
109	**55.1**	**123**	NI	**141**	NI
110	**3**	**124**	NI	**144**	**55.1**
111	NI	**125**	NI	**145**	**60.21**
112	NI	**130**	**80.1**	**146**	**42**
113	NI	**131**	NI	**147**	NI
114	NI	**132**	**90.87**	**148**	NI
115	NI	**133**	NI	**149**	NI
116	NI	**134**	NI	**150**	NI
117	NI	**135**	NI	**151**	NI
118	**90.87**	**136**	NI	**152**	NI
119	NI	**137**	NI	**Isoniazid**	**93.46**
120	NI	**138**	NI	**Rifampicin**	**96.09**
121	NI	**139**	NI	**Blank**	**0.06**

NI: No inhibition at 10µg/mL.

Inspired from the results of the primary screening, compound (**118**) was taken up for further studies and it was found that compound (**118**) was exhibiting an MIC value of ≤1.5µg/ml.

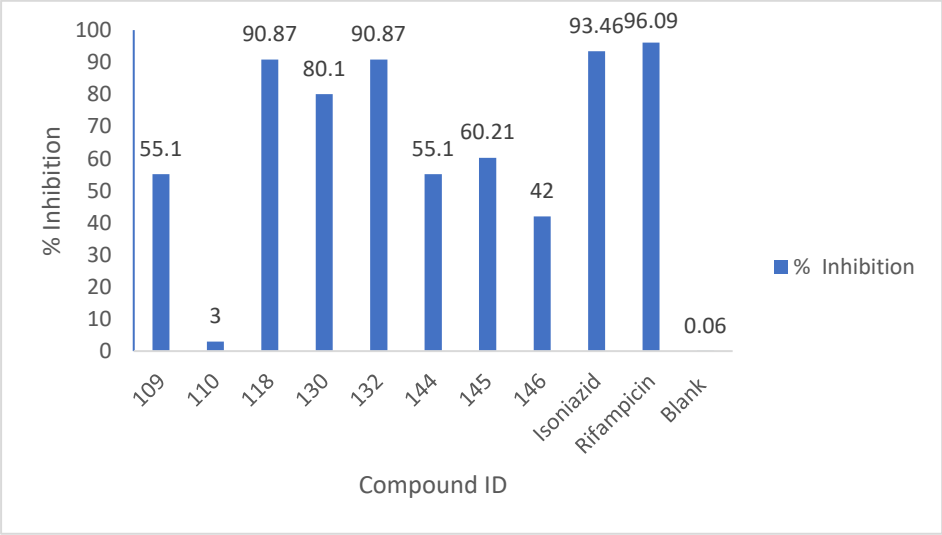


Figure 5: Percentage inhibition of *Mycobacterium bovis* (BCG) by the synthesized compounds using MABA assay.

Cell viability assay

As compound (**118**) was found to be significantly active, its safety aspect was assessed by performing cell viability assay using human lung adenocarcinoma cell line A549. This cell viability assay was based on resazurin dye. Results indicated that the compound (**118**) does not show any significant reduction in cell viability at 1.5 µg/ml and 3.125 µg/ml at any point of time. However significant reduction in cell viability was observed at the concentration of 6.25 µg/ml after 6th day of treatment. The results are demonstrated in **Figure 6**.

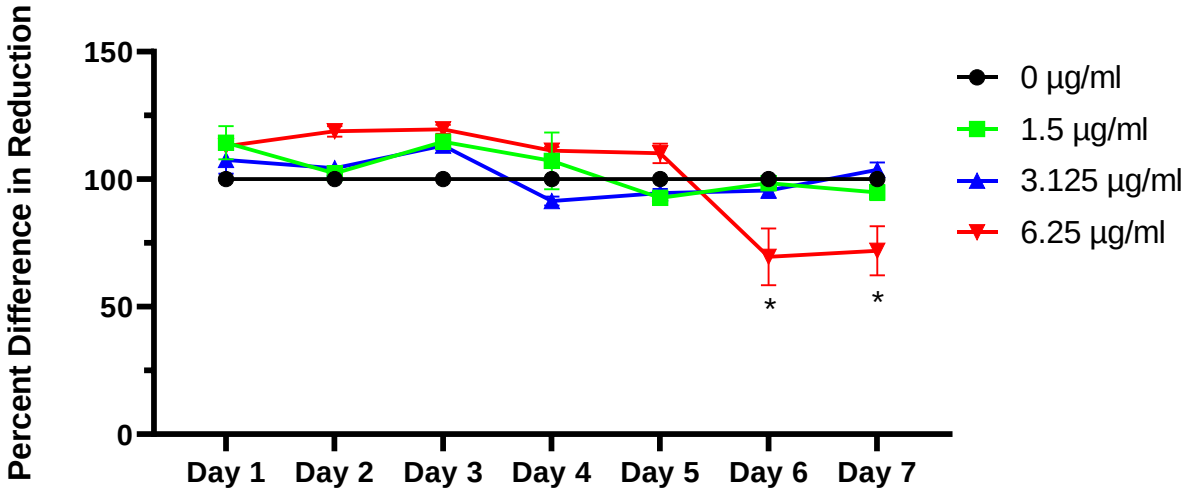


Figure 6: Impact of compound (**118**) concentrations -1.5µg/ml, 3.125µg/ml and 6.25 µg/ml on cell viability in A549 cell line. Percent difference in reduction of Resazurin in A549 cells upon treatment with compound at 1.5µg/ml, 3.125µg/ml and 6.25 µg/ml final concentration. The graph represents percent reduction in resazurin compared to untreated positive control at each day. Each concentration was evaluated in triplicates and Error bars depict the standard error of the mean. T-test was used to calculate statistical significance. *$p \leq 0.05$, n=3

Molecular docking, ADMET studies and simulation studies of the synthesized compounds

Computational studies such as molecular docking, ADME predictions, molecular simulations provide an insight to the behaviour of the molecule with the enzyme within the body. These studies also help in deducing the mode of the action as well as figuring out the pharmacokinetics of the compounds. The results were found to be within the range.

Molecular simulations studies of compound (118)

The molecular simulation studies of compound (**118)** show the stability of complex in a time period of 1ns. For the simulation studies different parameters of molecular dynamics has been taken into consideration which includes the RMSD analysis, RMSF analysis and radius of gyration (R_g). From the RMSD analysis it was observed that the complex attains stability

for a very short span of time and the RMSD keep on fluctuating during the given time period window. The deviation occurs in between RMSD 3.0- 4.2 nm for a time period 0.84ns. In last 0.2ns the deviation of complex occurs in between 2.0-3.0 nm.

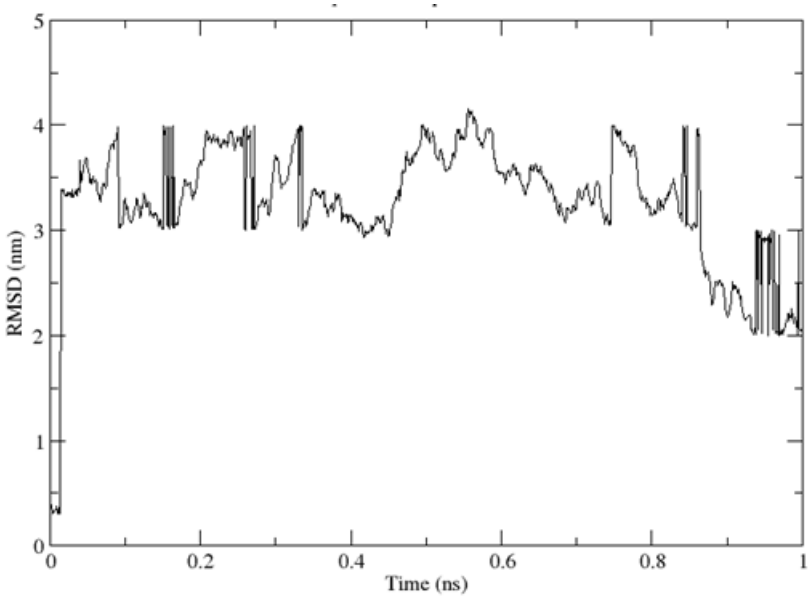


Figure 7: RMSD of complex (**118)**

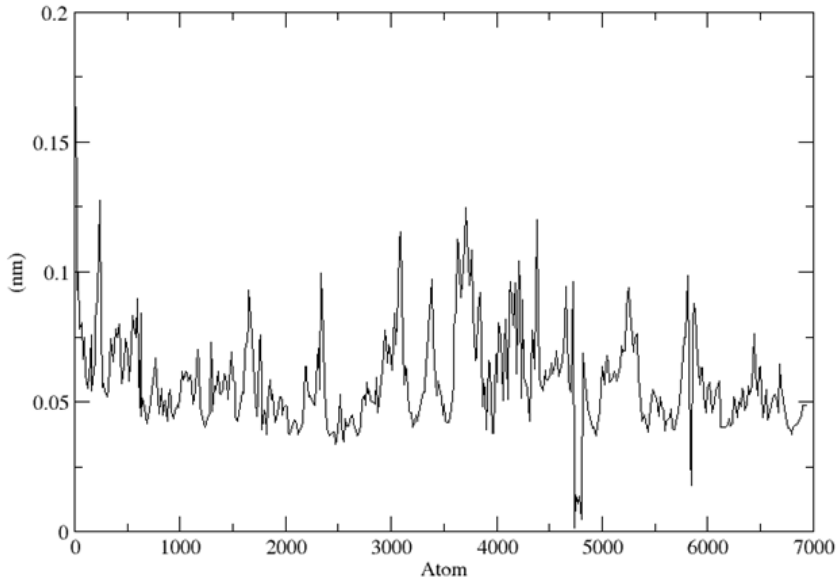


Figure 8: RMS of complex (**118)**

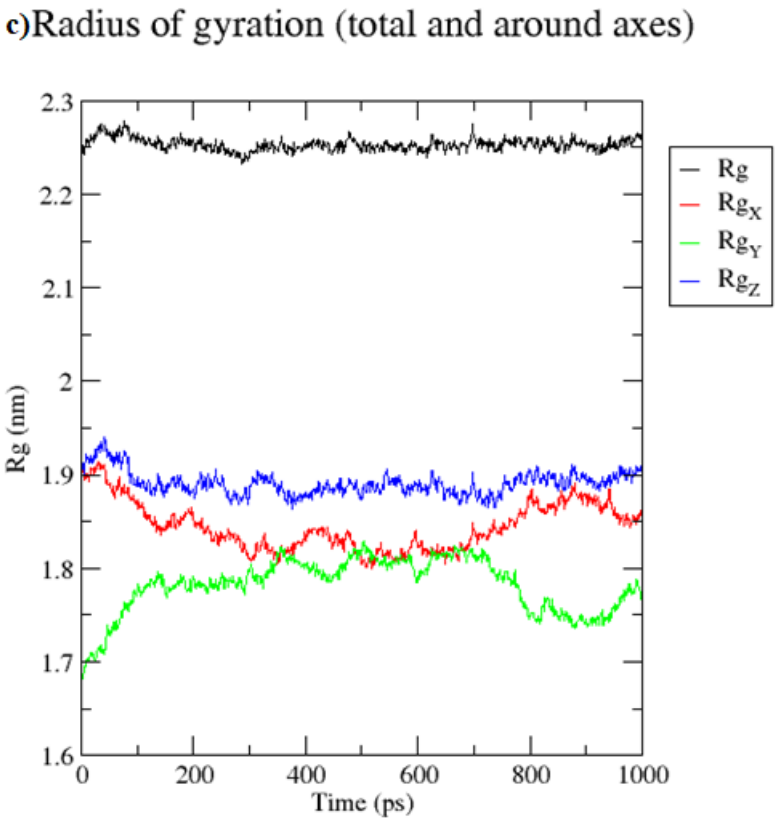


Figure 9: Radius of gyration of complex (**118)**

Additionally, the RMSF studies revealed that the atoms in the complex shows steep fluctuations in the given time period with a low stability profile. The atoms show stability within the plot but only for short intervals. The Root mean square fluctuations observed in between 0.038-0.132 nm. The radius of gyration studies determined for this complex indicates towards the instability and shows no significant deviations between 100-1000ps and the R_g remains in the range 0.25-0.27nm (**Figures 7-9**).

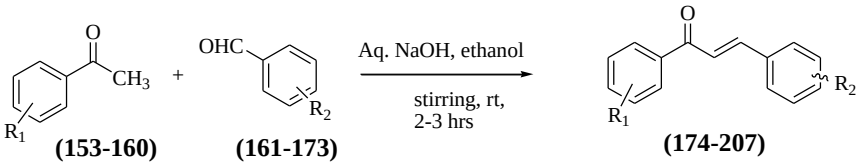
SECTION II

The compounds designed by implementing the hybrid approach of fusing pyrazolone and chalcones are described here.

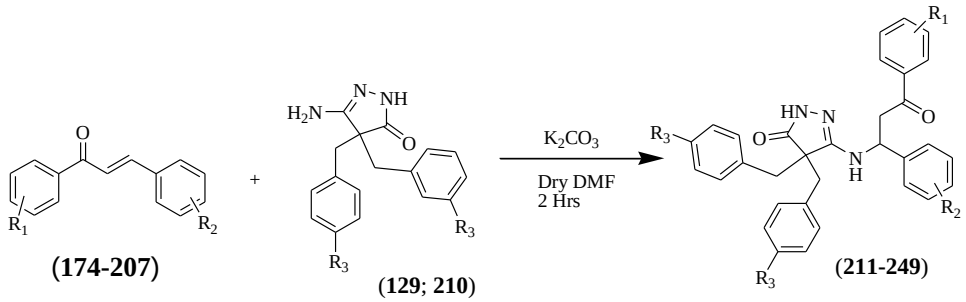
Synthesis of 3-(3-oxo-1,3-diphenylpropylamino)-4,4-dibenzyl-1*H*-pyrazol-5(4*H*)-one derivatives (211-249)

To prepare substituted chalcone derivatives (**174-207**), commercially available substituted benzaldehydes were reacted with substituted acetophenones in presence of a strong base (**Scheme 4**). These substituted chalcones (**174-207**) were fused with 3-amino-4,4-dibenzyl-1*H*-pyrazol-5(4*H*)-one (**129**) in presence of weak base such as anhydrous potassium carbonate in DMF to yield the desired compounds (**211-249**). The reaction proceeds via

Michael-aza addition of NH_2 of the pyrazolone on the double bond of the chalcone to offer final compounds (**211-249**) (**Scheme 5**).



Scheme 4: Synthesis of chalcone derivatives

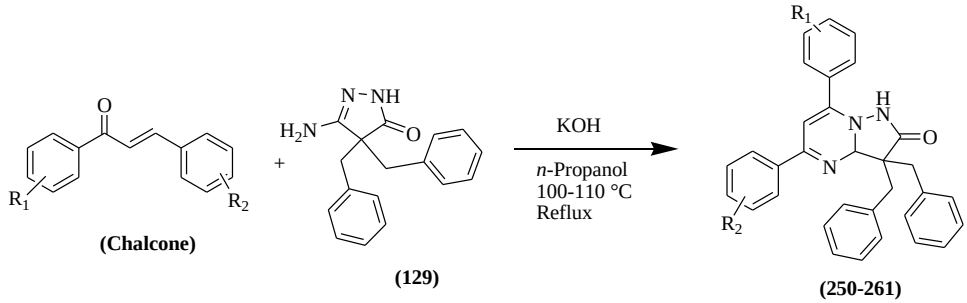


Scheme 5: Synthesis of final compounds **(211-249)**

4.7.2 Synthesis of 3,3-dibenzyl-3,3*a*-dihydro-5,7-diphenylpyrazolo[1,5-*a*]pyrimidin-2(1*H*)-one derivatives (250-261)

3-Amino-4,4-dibenzyl-1*H*-pyrazol-5(4*H*)-one (**129**) was synthesized by using a method discussed in **Scheme 2**. Substituted/unsubstituted chalcones were synthesized by reacting different benzaldehyde with various acetophenones in presence of KOH and aqueous ethanol as discussed in section S**cheme 4**.

The desired final compounds (**250-261**) were obtained by condensing substituted chalcones (**CH1-34**) with 3-amino-4,4-dibenzyl-1*H*-pyrazol-5(4*H*)-one (**129**) in presence of potassium hydroxide in *n*-propanol. The reaction proceeds via Michael-aza addition of -NH_2 on the double bond of chalcone followed by cyclization reaction to give pyrazolo[1,5-*a*]pyrimidin2(1*H*)-one scaffold (**Scheme 6**).



Scheme 6: Synthesis of final compounds (**250-261**)

Biological screening of synthesized compounds as anti-TB agents by MABA assay

The prepared compounds were evaluated for their percentage inhibition against *Mycobacterium bovis* (BCG). Overall, four compounds (**213, 214, 216** and **220**) out of 51 compounds exhibited inhibition of the bacteria. It was found that compound **213** and **220** was exhibiting 91% and 93% inhibition, comparable to that of standard drugs. Compounds **214** and **220** having fluorine and bromine respectively in their structure, are showing inhibition, thus it can be agreed that presence of halogen atom is favorable for inhibitory activity. The results are shown in **Table 5** and **Figure 10**. Compound (**216**) exhibited MIC value of 1.5 µg/mL.

Table 5: Anti-tubercular activity of the synthesized compounds (**211-39** and **250-261**)

Comp ID	% Inhibition	Comp ID	% Inhibition	Comp ID	% Inhibition
211	NI	**228**	NI	**245**	NI
212	NI	**229**	NI	**246**	NI
213	91	**230**	NI	**247**	NI
214	79	**231**	NI	**248**	NI
215	NI	**232**	NI	**249**	NI
216	89	**233**	NI	**250**	NI
217	NI	**234**	NI	**251**	NI
218	NI	**235**	NI	**252**	NI
219	NI	**236**	NI	**253**	NI
220	93	**237**	NI	**254**	NI
221	NI	**238**	NI	**255**	NI
222	NI	**239**	NI	**256**	NI
223	NI	**240**	NI	**257**	NI
224	NI	**241**	NI	**258**	NI
225	NI	**242**	NI	**259**	NI
226	NI	**243**	NI	**260**	NI
227	NI	**244**	NI	**261**	NI
Isoniazid	93.46	**Rifampicin**	96.09	**Blank**	0.06

NI: No inhibition at 10 µg/mL; isoniazid: 0.04 µg/mL; rifampicin: 0.08 µg/mL

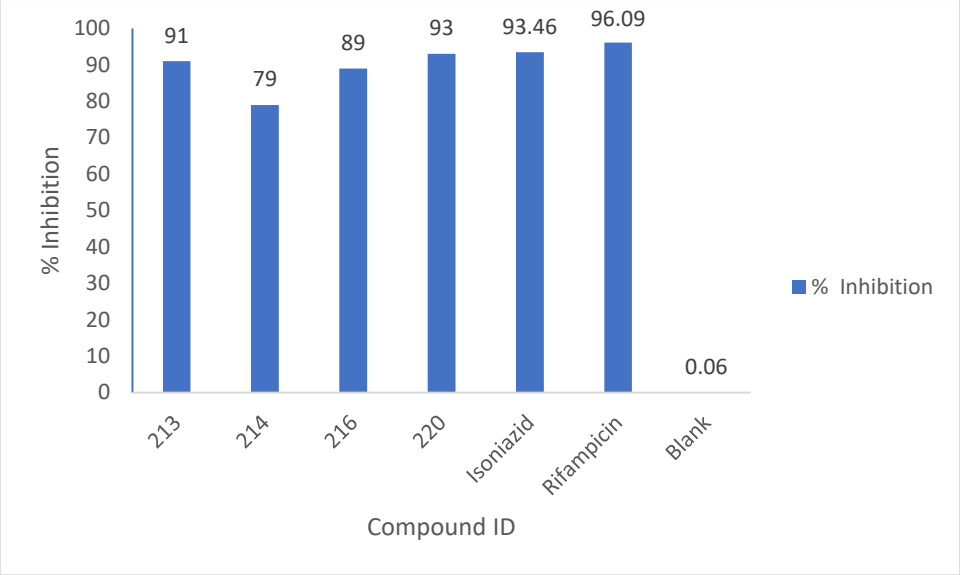
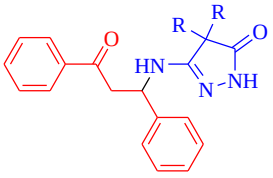


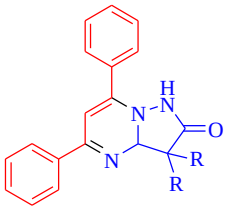
Figure 10: Percentage inhibition of *Mycobacterium bovis* (BCG) by the synthesized compounds using MABA assay.

Conclusions and the recommendations/ suggestions

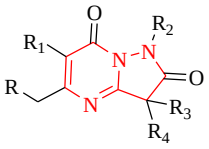
A thorough literature survey encouraged us to perform pharmacophore modelling to search for common structural features present in the reported DprE1 inhibitors. As a result, a pharmacophore model having AHRR (Hydrogen bond acceptor, Hydrophobic group and aromatic rings) having four feature was developed. With an aim to identify some non-reported scaffold as DprE1 inhibitors, Virtual screening was performed on Asinex database. Results of virtual screening provided eight leads out of which two were pyrazolopyrimidines. These pyrazolopyrimidine hits were optimized to get a lead molecule (**Formula III**).



Formula I



FormulaII



Formula III

Hybrid approach has been one of the widely used techniques to develop new drugs. Thus, with an aim to explore some of the reported but not explored scaffolds such as pyrazole and chalcones were fused together via hybrid approach to get some novel compounds (**Formula I** and **Formula II**).

Current work discloses design, synthesis, in silico studies and biological study of novel anti-TB agents. Synthesis of the compounds was done by adopting five general synthetic schemes.

Compounds with **Formula III** were synthesized using **Scheme 1-3**, while hybrid compounds with **Formula I** and **Formula II** were synthesized by using **Scheme 5** and **Scheme 6**.

The synthesized compounds were evaluated for their anti-TB activity by using MABA assay. It was noteworthy, that some compounds of were exhibiting excellent MIC value of 1.2 µg/mL.

Overall, pyrazolopyrimidine derivative (**109**, **118**, **130**, **132**, **144** and **145**) showed noticeable inhibition of *Mycobacterium Bovis* (BCG). Wherein compound (**118 and 132**) demonstrated MIC value of 1.2 µg/mL. Four hybrid compounds (**213**, **214**, **216** and **220**) exhibited good inhibition of *Mycobacterium Bovis* (BCG). Wherein. Wherein compound (**216**) demonstrated MIC value of 1.2 µg/mL.

Results of docking studies indicated that anti-TB activity of the synthesized compounds could be a result of binding to the enzyme DprE1. Most of the compounds showed binding affinity similar to the standard compounds while some of the derivatives demonstrated better binding affinity than the standard compound. This was further supported by molecular dynamics simulations studies, that resulted in the stable complex formation of the compound (**118**) and the enzyme. ADMET predictions of the synthesized compounds indicated towards the drug-likeness of the compounds, with a few exceptions. Genotoxicity predictions by Nexus Derrek indicated that a majority of compounds are non-mutagenic.

Bibliography and webliography

(1) *WHO, World Tuberculosis Report, 2022*.

(2) Chikhale, R. V.; Barmade, M. A.; Murumkar, P. R.; Yadav, M. R. Overview of the Development of DprE1 Inhibitors for Combating the Menace of Tuberculosis. *J. Med. Chem.* **2018**, *61* (19), 8563–8593. https://doi.org/10.1021/acs.jmedchem.8b00281.

(3) Sharma, A.; De Rosa, M.; Singla, N.; Singh, G.; Barnwal, R. P.; Pandey, A. Tuberculosis: An Overview of the Immunogenic Response, Disease Progression, and Medicinal Chemistry Efforts in the Last Decade toward the Development of Potential Drugs for Extensively Drug-Resistant Tuberculosis Strains. *J. Med. Chem.* **2021**, *64* (8), 4359–4395. https://doi.org/10.1021/acs.jmedchem.0c01833.

(4) Riccardi, G.; Pasca, M. R. Trends in Discovery of New Drugs for Tuberculosis Therapy. *J. Antibiot. (Tokyo).* **2014**, *67* (9), 655–659. https://doi.org/10.1038/ja.2014.109.

(5) Singh, V.; Chibale, K. Strategies to Combat Multi-Drug Resistance in Tuberculosis. *Acc. Chem. Res.* **2021**, *54* (10), 2361–2376. https://doi.org/10.1021/acs.accounts.0c00878.

(6) Ghajavand, H.; Kamakoli, M. K.; Khanipour, S.; Dizaji, S. P.; Masoumi, M.; Jamnani, F. R.; Fateh, A.; Siadat, S. D.; Vaziria, F. High Prevalence of Bedaquiline Resistance in Treatment-Naive Tuberculosis Patients and Verapamil Effectiveness. *Antimicrob. Agents Chemother.* **2019**, *63* (3), 1–13. https://doi.org/10.1128/AAC.02530-18.

(7) Zhang, M.; Prior, A. M.; Maddox, M. M.; Shen, W. J.; Hevener, K. E.; Bruhn, D. F.; Lee, R. B.; Singh, A. P.; Reinicke, J.; Simmons, C. J.; Hurdle, J. G.; Lee, R. E.; Sun, D. Pharmacophore Modeling, Synthesis, and Antibacterial Evaluation of Chalcones and Derivatives. *ACS Omega* **2018**, *3* (12), 18343–18360. https://doi.org/10.1021/acsomega.8b03174.

(8) Marrapu, V. K.; Chaturvedi, V.; Singh, S.; Singh, S.; Sinha, S.; Bhandari, K. Novel Aryloxy Azolyl Chalcones with Potent Activity against Mycobacterium Tuberculosis H37Rv. *Eur. J. Med. Chem.* **2011**, *46* (9), 4302–4310. https://doi.org/10.1016/j.ejmech.2011.06.037.

(9) Ramesh, D.; Joji, A.; Vijayakumar, B. G.; Sethumadhavan, A.; Mani, M.; Kannan, T. Indole Chalcones: Design, Synthesis, in Vitro and in Silico Evaluation against Mycobacterium Tuberculosis. *Eur. J. Med. Chem.* **2020**, *198*, 112358. https://doi.org/10.1016/j.ejmech.2020.112358.

(10) Muradás, T. C.; Abbadi, B. L.; Villela, A. D.; Macchi, F. S.; Bergo, P. F.; De Freitas, T. F.; Sperotto, N. D. M.; Timmers, L. F. S. M.; De Souza, O. N.; Picada, J. N.; Fachini, J.; Da Silva, J. B.; De Albuquerque, N. C. P.; Habenschus, M. D.; Carrão, D. B.; Rocha, B. A.; Junior, F. B.; De Oliveira, A. R. M.; Mascarello, A.; Neuenfeldf, P.; Nunes, R. J.; Morbidoni, H. R.; Campos, M. M.; Basso, L. A.; Rodrigues-Junior, V. S. Pre-Clinical Evaluation of Quinoxaline-Derived Chalcones in Tuberculosis. *PLoS One* **2018**, *13* (8), 1–13. https://doi.org/10.1371/journal.pone.0202568.

(11) Yalcin, G.; Burmaoglu, S.; Yildiz, I.; Algul, O. Molecular Docking Studies on Fluoro-Substituted Chalcones as Potential DprE1 Enzyme Inhibitors. *J. Mol. Struct.* **2018**, *1164*, 50–56. https://doi.org/10.1016/j.molstruc.2018.02.087.

(12) Kumar, G.; Siva Krishna, V.; Sriram, D.; Jachak, S. M. Pyrazole–Coumarin and Pyrazole–Quinoline Chalcones as Potential Antitubercular Agents. *Arch. Pharm. (Weinheim).* **2020**, *353* (8). https://doi.org/10.1002/ardp.202000077.