3. Aim and objectives

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 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:

3.1 Design and development of novel anti-TB agents based on the existing DprE1 inhibitors

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

3.1 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 (**Figure 3.1**). 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.

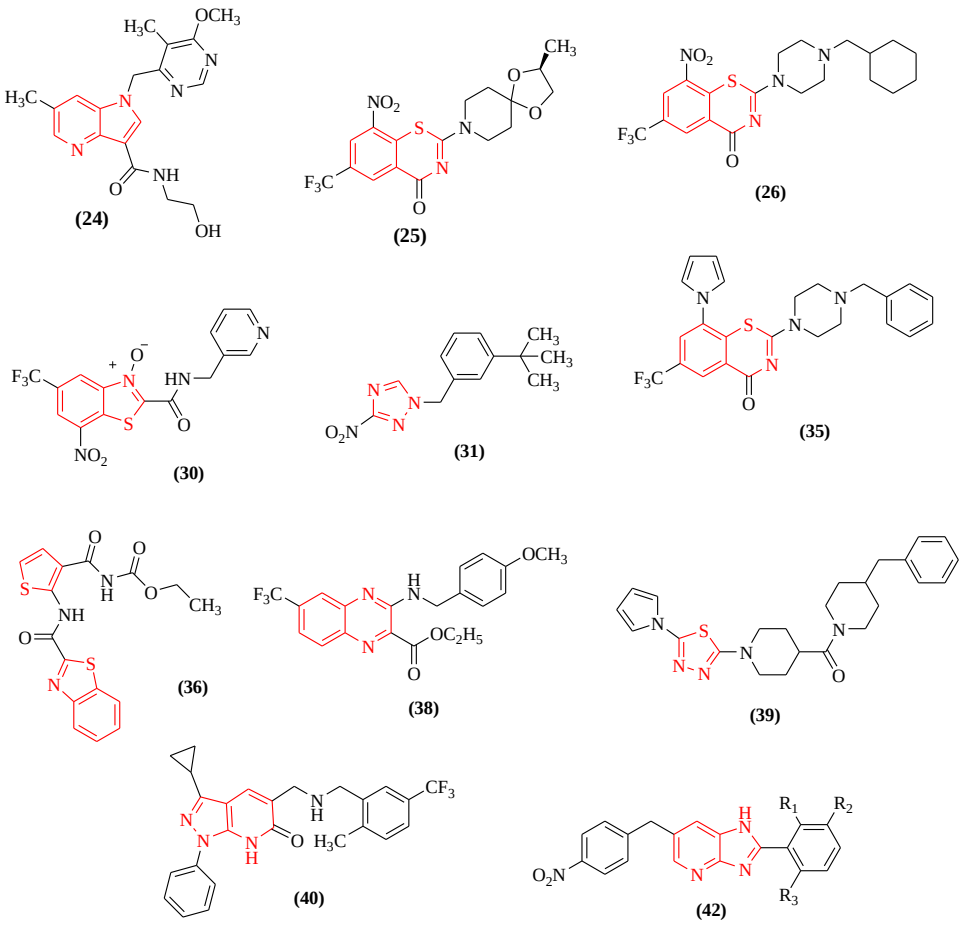

Figure 3.1: Some of the reported DprE1 inhibitors (for development of pharmacophore model)

Although there is diverse range of scaffolds reported with DprE1 inhibitory properties. Still, not a single compound could reach to the market. Thus, there is a need to explore new moieties which could demonstrate DprE1 inhibitory properties. This purpose can be resolved by virtual screening as there is strong chance to get some unexplored scaffolds with DprE1 inhibitory activity. The hits obtained by virtual screening can be optimized by using various techniques such as docking, ADME predictions etc. The resulting compounds so obtained could be synthesized and evaluated for their anti-tubercular activity.

Therefore, the aims and objectives of the present study is:

1. To develop a pharmacophore model by using reported DprE1 inhibitors to identify the essential structural features require to exert the DprE1 inhibition.

2. To perform the virtual screening in quest of novel scaffolds by applying filters like pharmacophore model, molecular docking, Lipinski rule of five etc.
3. To optimize the hits through various techniques such as molecular docking, ADMET predictions etc.
4. To design, synthesize and characterize the compounds.
5. To carry out biological screening of the synthesized compounds for anti-TB activity.
6. To carry out molecular simulations' studies of the active compounds.

3.2 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[1–4]. 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[5,6]. Further, chalcones and related compounds are not explored to the extent as anti-tubercular agents. Thus, it was thought logical to explore chalcone scaffold.

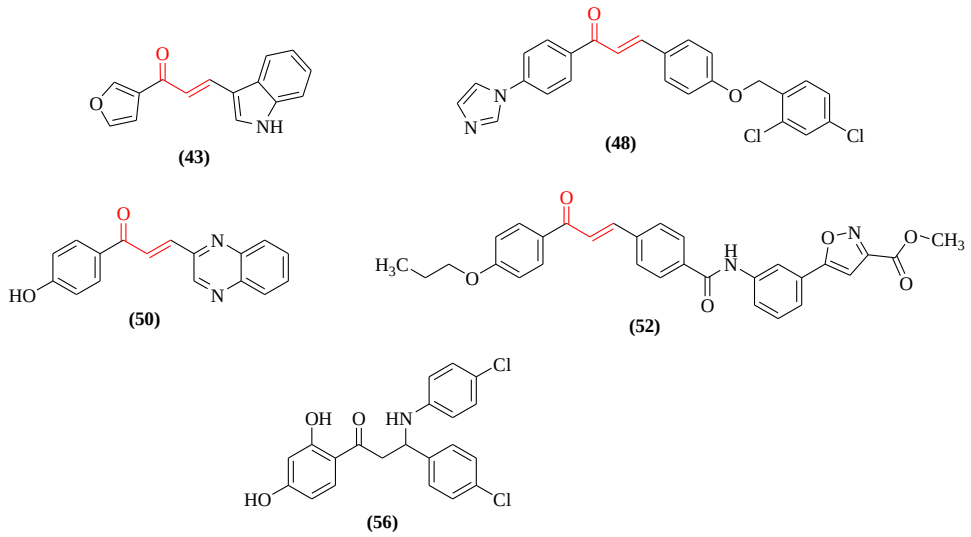

Figure 3.2: Some of the reported Chalcone derivatives as anti-TB agents

It was also found that the five membered heterocycles especially *N*-containing heterocycles such as triazole, thiadiazole etc exhibit good DprE1 inhibitory activity. From the literature[7–16], it was found that heterocycles such as pyrazole, triazole, thiadiazole, hydantoin exhibit significant DprE1 inhibitory and anti-TB activity in the micromolar range.

During the literature review, it was observed that most of the five membered heterocycles like triazole and thiadiazole were explored for anti-TB activity and DprE1

inhibitory activity. Moieties like pyrazole have been reported to exerted anti-TB activity and DprE1 inhibitory activity, but it hasn't been explored thoroughly. So, it was thought logical to explore pyrazole moiety for anti-TB activity.

Thus, we thought to combine two active scaffolds i.e., chalcone (**A**) and pyrazole (**B**) to form a new hybrid molecule. For designing the hybrid molecule, two approaches were used. Firstly, pyrazole was fused with the chalcones via Michael aza-addition to obtain **Formula I (Figure 3.3)**. Secondly, pyrazole was fused with chalcones via Michael aza-addition-cyclization reaction to obtain **Formula II** having pyrazolopyrimidine moiety. The designing of the **Formula II** is also favoured by the fact that most of the reported DprE1 inhibitors are bicyclic heterocycles containing one more nitrogen in the ring such as azaindoles (**24**), imidazopyridine (**40**), pyrazolopyridones (**42**) etc. The designing of the hybrid molecules is shown in **Figure 3.3**.

Further, molecular docking was carried out for the designed **Formula** (**I** and **II**). It was found that the binding affinity of the designed compounds was found to be in the range of -9.2 to -11.6 Kcal/mol, similar or higher than the binding affinity of the standard ligand -9.5 Kcal/mol. The ADMET properties were also predicted, for the designed compounds and the results indicated towards the optimum physicochemical properties of the designed compounds.

The designed compounds were found to show some important interactions within the active site of the ligand. The most of the designed compounds were found to interact with CYS 387, a prominent amino acid residue for the inhibition of DprE1. Other interactions observed were, interactions with Serine, Valine, Glycine, Threonine etc.

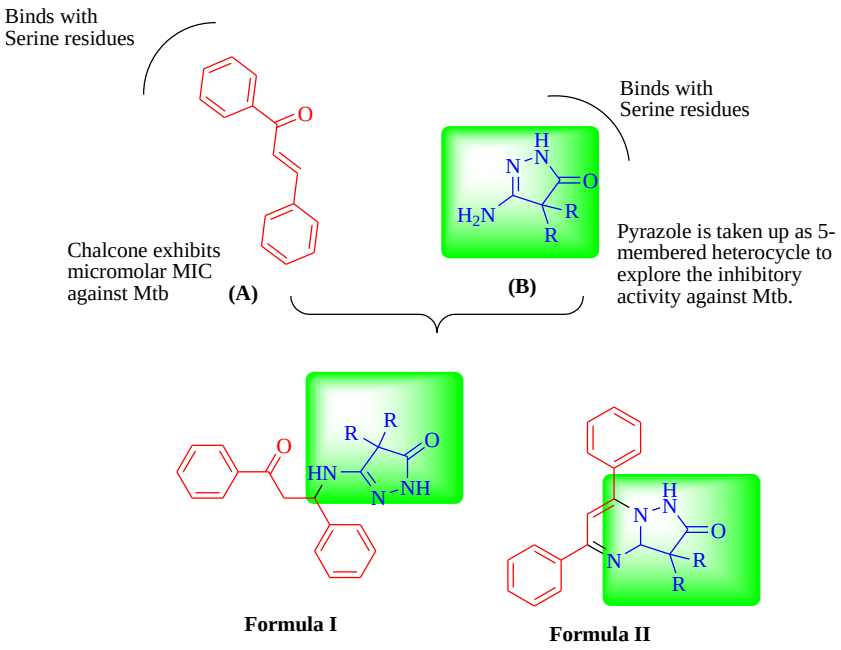

Figure 3.3: Designing of pyrazole fused dihydrochalcone derivatives (**I, II**) using hybrid approach

Thus, the broad aims and objectives of the research is:

1. To design the hybrid compounds having **Formula I** and **II** based on dihydrochalcone and establish the synthetic scheme.
2. To synthesize and carry out characterization of the designed compounds.
3. To carry out molecular docking to examine the binding of the compounds with DprE1enzyme.
4. To carry out biological screening of the synthesized compounds for anti-tubercular activity.

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