OSDD Approach to TB Drug Discovery

In an effort towards discovery and development of novel drugs against *Mtb,* OSDD is pursuing multi pronged strategy that encompasses

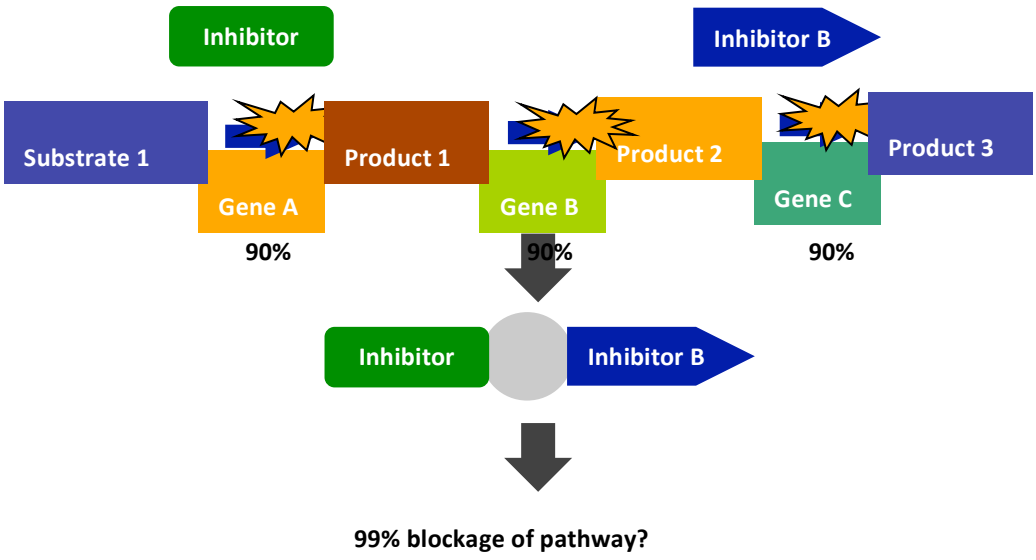
- Target Based
- Whole Cell Based
- Structure Based

Target Based Strategy

Conventionally, target based drug discovery is aimed at achieving maximum inhibition of a single 'essential' drug target with potential to kill the bacteria. However, in the field of anti-infectives in contrast to the 'whole cell based' strategy 'target based discovery' has been less successful. This suggests that an alternative approach needs to be pursued in target based discovery. One of the approaches is to pursue 'multi target' inhibition where in multiple targets of a pathway/s, are inhibited by a single compound. In this strategy,

- multiple but related (structurally) targets are sought to be inhibited by the same compound
- The inhibition of the individual targets could be 99% inhibition or less but combined with inhibition of multiple targets may lead to 99%
- inhibition of the pathway.

The approach has the potential to achieve a higher efficiency by overcoming redundancy and achieving maximal pathway inhibition.



Schematic Representation of Targeting Multiple Targets with Single compound

Multi target approach of inhibiting FAALs and FACLs as anti TB drugs (CSIR-IGIB & Jubilant Chemsys) (more details on sysborg- link)

FAALs were identified as a novel class of FadD enzymes ((Trivedi et al ;Nature;2004). FAAL and FACL take part in critical step of acyladenylate formation and are the key nodal points in lipid metabolism. FAAL are a group of closely related enzyme with high sequence similarity of 70-80%. The homology with FACL is 20-30%. They both have similar substrate specificity providing the rationale for simultaneous targeting of both the enzymes, which could result in greater efficacy than targeting single enzyme. The aim is to design small molecule inhibitors that could simultaneously target not only FAALs but also FACLs. Given that acyl adenylate is a reaction intermediate of FACL proteins and is a product of FAAL proteins, designing non-hydrolysable analogues of acyl adenylate could act as a multi-pronged approach to disrupt several pathways. This approach has been validated using, the substrate analogue for acyladenylate i.e. Acylsulphamoyl adenosine, which was found to inhibit at micromolar IC50 and showed visible effect in cell morphology. The treated cell showed lack of lipid coat and irregular shape of cell, establishing the mode of action of such inhibition. The crystal structure of FACL 3 with LAMS inhibitor has been obtained, which facilitates structured based drug design.
The current target of inhibition, are two proteins FACL6, FACL19 and FAAL28, FAAL32 and FAAL13. Optimization of LAMS inhibitor to improve the potency, SAR and drug like properties in collaboration with Jubilant is in progress. About 200 compounds have been designed, synthesized and tested so far. Non-radioactive assays are being developed for throughput screening for identification of novel scaffolds.

Targeting GlmU of Mtb for anti TB (National Institute of Immunology/IIT-Kanpur, BITS-Hyderabad), more details on sysborg- link
GlmU (N-acetylglucosamine-1-phosphate uridyltransferase), a bifunctional enzyme, produces UDP-GlcNAc, an essential precursor for the biosynthesis of peptidoglycan and lipopolysaccharide has been shown to be essential for survival. This was established through transposon mutagenesis experiments, which showed that the *M. tuberculosis glmU* to be an essential gene. Modeling of the M. tuberculosis GlmU, purification of M. tuberculosis GlmU and standardization of uridyltranferase and acetyltransferase assays has been carried out. Additionally computer based docking of compound libraries followed by de novo design of inhibitors has yielded a few scaffolds that are being investigated. A high throughput assay (96 well format) for GlmU acetyltransferase and uridyltransferase assay has been standardized. Medium through put screening of the OSDD compound library has identified few hits that need to further validated and optimized. A structure based drug design approach will be followed. The essentiality of GlmU in TB is being confirmed by gene knock down strategy.

DapA and DapB (CSIR-IGIB and Anthem Biosciences). More details on sysborg- link.

DapA (DHDPS, dihydrodipicolinate synthase) and DapB (DHDPR dihydrodipicolinate reductase) enzymes are involved in synthesis of DAP (diaminopimelic acid), which is an intermediate of the (S)-lysine biosynthetic pathway and also a constituent of the cell wall. Absence of DAP results in cell lysis. DapA and DapB play a crucial role in cell wall synthesis, leading to the synthesis of D- lysine, the biosynthetic pathway of which is absent in mammals making it is 'selective drug target' for *Mtb*. X crystal structure of DHDPS (dihydrodipicolinate synthase) and coupled enzyme assays have been reported, suggesting the feasibility of the target for drug discovery.

This project utilizes the in silico docking expertise at CSIR-IMTECH and CSIR-IGIB. Preliminary enzymatic assay was set up at CSIR-IGIB, which was further optimized at a CRO to develop a robust and characterized assay and optimized for throughput screening. Primary screening of CSIR-IICT library for DapA/B is ongoing and about 3000 compounds have been screened so far. In addition, the co-crystal structure of DapA of Mtb with KPA has been solved by scientists at IGIB in collaboration with IISc, which will aid the design of inhibitors

Mur Pathway (Acharya Narendrev Dev College, ANDC) more details on sysborg- link

From the systems levels analysis of Mtb metabolome many of the mur pathway enzymes involved in cell wall biosynthesis were predicted as drug targets. A one pot enzyme assay to assay for all the Mur pathway enzymes is being set up.

Sigma Factors (Indian Institute of Science): more details on sysborg- link

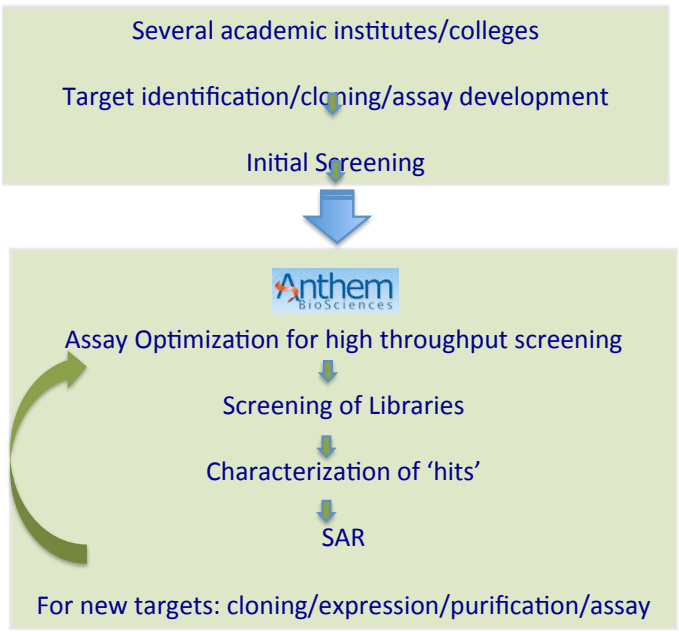
Multiple sigma factors (Sig B, C, E, F, H,M) have been predicted to be 'critical' targets by 'interactome' analysis of Mtb (PLoS One. 2012;7(7):e39808). While targeting single sigma factors may affect Mtb survival, inhibition may be incomplete as it can be seen from interactome studies that multiple sigma factors are critical and need to be targeted. Hence, a strategy to inhibit 'multiple targets' by a single compound needs to be employed. A hypothesis to inhibit multiple sigma factors by a common peptide/compound that disrupts the RNA polymerase-sigma factor interaction surface as a means of targeting *Mtb* is being tested experimentally.

Methionine Amino Peptidase MAP (CSIR-IICT & CSIR-NIIST) more details on sysborg- link

MAPs are involved in removal of the initiator methionine from newly synthesized peptides. Hit identification studies random screening followed by structure based design are ongoing for identifying inhibitors targeting MAP A and B with emphasis on MAP B which has been shown to be essential by anti

sense based targeting in *Mtb*. In parallel gene knock down strategy is being pursued to validate the essentiality of MAP B in *Mtb*. Crystal structure of MAP B is available and studies are ongoing to decipher crystal structure of MAP A of *Mtb*.

Target Based Screening



Target	PI/Institution
FAAL/FACL	R.Ghokale/IGIB
DapA/B	Ramchandran/IGIB
GlmU	Vinay Nandicoori/NII
NAD Ligase	Ravishankar/CDRI
MAP	Anthony/IICT Nampoothri/NIIST
Sigma Factors	IISc/OSDD unit
Ribosome Biogenesis	Balaji/IIT-K
Mur Pathway	U.Bajpai/ANDC
Rv0079-DATIN	Niyaz/HU
Mym operon	S.Sharma/MH
Rv1258c efflux pump	Inshad/IIIM
MMPL3	S. Reddy/NCL

Screening		Hit to Lead	Pre Clinical	Clinical
Whole Cell based	**Target based**			
• 30,000 compound library (CSIR-IIIM) • CSIR Diverse Compound library • OSDDchem library • Natural Product Library • Phage Therapy	• GlmU • DapA/DapB • Sigma Factors • Mur Pathway • NAD Ligase • MAP • Ribosome & translation inhibitors	• CDRI-830 • LAMS • IIIM hits	Candidates under evaluation • Amlodipine • Thioamide Boosters • Verapamil	• Pa 824 • Pa-M-Z combination • Two other Candidates under evaluation