WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 5, 514-522. Review Article ISSN 2277– 7105

TARGET ENZYMES IN MYCOBACTERIUM TUBERCULOSIS

Mrunmayee Toraskar* **and Pravin khude**

Department of Pharmaceutical Chemistry, Bharati Vidyapeeth's College of Pharmacy, Sector 8, C.B.D, Belapur, Navi Mumbai-400614, Maharashtra, India.

Article Received on
08 March 2016,
Revised on 29 March 2016,
Accepted on 20 April 2016
DOI: 10.20959/wjpr20165-6145

***Corresponding Author**
Mrunmayee Toraskar
Department of Pharmaceutical Chemistry, Bharati Vidyapeeth's College of Pharmacy, Sector 8, C.B.D, Belapur, Navi Mumbai-400614, Maharashtra, India.

ABSTRACT

When antibiotic loses its ability to effectively control or kill bacterial growth, it leads to antibiotics resistance. Bacteria acquire resistance in various possible ways; by genetic mutation and by acquiring resistance from another bacterium with existing resistant genes etc. Recently, almost 80% strains of Escherichia coli have become resistant to ampicillin, which has been used to treat E.coli infection since 1960's. Same is for the Staphylococcus aureus strains causing infection which have become resistant to methicillin. Mycobacterium tuberculosis (M.tb) which has become multi-drug resistant in 1990's, is the most dangerous among the drug resistant bacteria. For this reason, searching for new target enzyme is the current objective. Catalyzing enzyme malate synthase and isocitratelyase helps escape two CO_2 steps in kerb cycle, taking part in glyoxylate shunt leading to formation of malate and acetyl CoA as a net product. β-Carbonic anhydrases (β-CAs) belonging to family of Carbonic anhydrase, present in bacteria and plant kingdom plays a important role through interconversion between carbon dioxide and bicarbonate. β-CAs expressed in M. Tb which acts as target enzyme for anti-tb agents, may help in design of new anti-tb agents. Another enzyme target is β-hydroxy acyl dehydratase (HadAB) which is the catalyzing enzyme in FAS-II reaction for fatty acid synthesis. Conserved gene HadA, HadB and HadC, in their combined form of HadAB and/or HadBC heterodimer is involved in third step reaction of FAS-II. Thorough understanding of structural information of enzyme target can help synthesize new anti-bacterial drugs which may inhibit growth of bacteria and their activity.

KEYWORD: Malate synthase, Isocitratlyase, β-carbonic anhydrase, and β-hydroxy acyl dehydratase.

INTRODUCTION

Indeed, it was a huge success in human health life expectance till 1990's. After that some bacteria, among them M.tb has become parallel to human success through developing resistance against known first line drugs which had proved to be useful to kill and inhibit the bacterial growth. Streptomycin resistant M.tb has put forth the question and to search for new emerging target enzymes to stab bacteria at their ground state.

Here our review is focusing on four enzymes

1. Malate synthase
2. Isocitratelyase
3. β-carbonic anhydrase
4. β-hydroxy acyl dehydratase.

Malate synthase and isocitratelyase emerged as target enzymes involved in glyoxylate shunt present in dormant M.tb.[1]In latent state Mycobacterium tuberculosis undergoes inactive state, due to insufficient nutrient environment it leads to activation of glyoxylate shunt, there in the starting two initial step are same as that of kreb cycle skipping two carbon steps in kreb cycle, resulting in netproduct malate and acetyl CoA, with the help from two enzyme involved in glyoxylate shunt, Malate sythase(MS) and Isocitratelyase(ICL) (Fig1).[2] β-CAs a metalloenzymes, belonging to family of Carbonic anhydrase known to be present in bacteria and plant, plays very important role in bacteria for physiological processes by converting carbon dioxide to bicarbonate. β-CAs protein structure is dimer, tetramer or octamers, containing the zinc metal in active site, which is essential for the catalysis. Sulphonamide class of compound and few other which interact through zinc metal for inhibition of catalytic processes which are required for physiological role in bacteria.[4,3] β-hydroxy acyl dehydratase(HadAB) enzyme involved in catalytic reaction in FAS-II, in combined form i.e. heterodimer, HadAB.[5,6] It is the target enzyme in drug resistant M.tb. These all above mentioned enzymes may act as effective targets for development of newer anti-TB agents with detail studies.

1. MALATE SYNTHASE

In glyoxylate shunt, the two steps from kreb cycle which involves CO_2generating steps are skipped. And it uses two carbon molecules like acetate, fatty acid and ethanol as source. Malate synthase enzyme plays pivotal role in glyoxylate shunt producing malate which is virulence factor for pathogenesis.[7,8] Active site of Malate synthase is composed of residues

as Glu-427, Asp-455 which binds with $Mg^{2+}$and residue Arg-338 which forms hydrogen bonds with glyoxylate. Acetyl CoA shows interaction residues Try-126, Pro-538, Val-18 and Pro-536. Since the MS are not present in mammals, they make promising target. They exists in two isoforms MSA and MSG. By utilizing this structural data, inhibitors can be designed to inhibit the activity of MS.

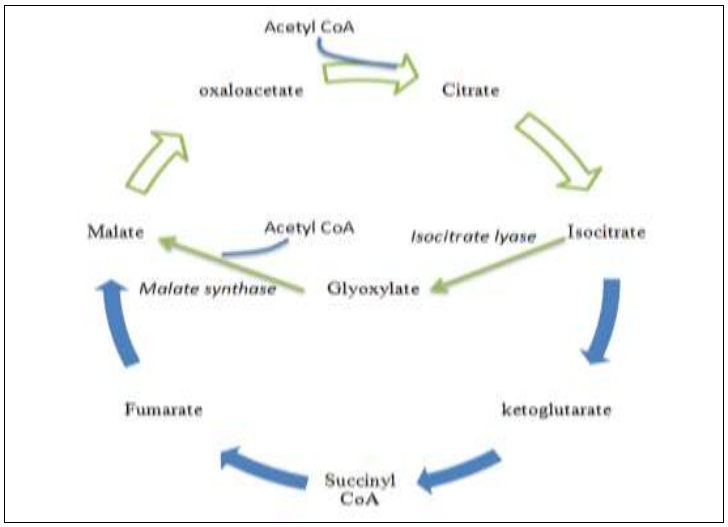

Fig1: Glyoxylate shunt cycle

2. ISOCITRATE LYASE

It started from the 1950's when Hans L. Kornberg and co-workers elucidated the presence of glyoxylate cycle in micro-organisms. From the strong investigation it becomes clear that the glyoxylate cycle bypasses the decarboxylation steps of the citric acid cycle. Glyoxylate shunt functions as anaplerotic in TCA cycle, that means glyoxylate cycle generates extra molecule succinate which fuels up TCA cycle and allows other intermediates to be withdrawn and be used for anabolism. Activation of glyoxylate cycle in Mycobacterium tuberculosis occurs due to low nutrient environment. These two key enzymes of cycle isocitratelyase and malate synthase which work sequentially resulting in net product malate and acetyl CoA required for gluconeogenesis.

Isocitratelyase one of the specific enzyme in glyoxylate cycle catalyses the conversion of isocitrate to succinate and glyoxylate. Expression of ICL is upregulated during infection of macrophages by Mycobacterium tuberculosis and disruption of ICL gene inhibits the persistence of M.tb in macrophages and in mice.[9] Besides the presence of ICL1 in the glyoxylate cycle, some M.tb strains express a second enzyme ICL2 with same activity. ICL2 is observed to come into action when the intracellular isocitrate levels increase above a

certain threshold. Inhibition of this enzymes leads to further inhibition of gluconeogenesis required by the bacteria.[10]

3. MALATE SYNTHASE AND ISOCITRATELYASE INHIBITORS

As described above the function of malate synthase and isocitratelyase enzymes in the glyoxylate shunt. The need for inhibitors for inhibition of this enzyme present in pathway is required, since the glyoxylate cycle enzymes are not present in mammals. In current scenario, there is new invention for malate synthase inhibitors patented by Freundlich et. al. Substituted diketo acid, comprising of structure (Fig.2), Where R^1 is a phenyl, a naphthyl, a thienyl, bithiophenyl, imidazolyl, benzothienyl, furanyl, benzofuranyl, pyrimidine and R^2 is H, OH, NH_2or a pharmacologically acceptable salt.[11]

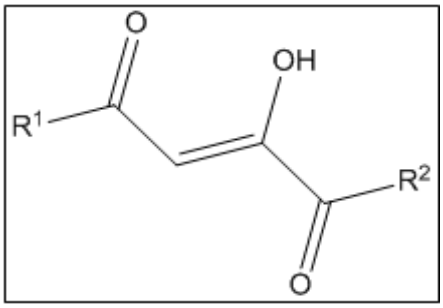

Fig. 2

Anti-tuberculosis drug targeting ICL, salicylanilide, benzanilide, 3-nitro propionamide and pthalazinyl derivative have also been shown.[12] An reviewed synthetic compound pthalazinyl derivatives, phthalazin-4-ylacetamide, 5-Nitro-2,6-dioxohexahydro-4-pyrimidinecarboxamides and 3-Nitropropionamide derivative, shown to have IC_{50}0.1μM, respectively.[13]

4. BETA CARBONIC ANHYDRASE

The carbonic anhydrases (CA) are zinc metalloenzymes that catalyze the simple interconversion of CO_2 and HCO_3^-($CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$). A reaction fundamental to many biological processes such as photosynthesis, respiration, renal tubular acidification, and bone resorption. These metalloenzymes evolved independently into five distinct groups α-CA, β-CA, δ-CA, γ-CA, and ζ-CA.[14,15,16,17]β-class of carbonic anhydrase are widely spread among domains of life, present in chloroplast of both plant and algae as well as enzyme from diverse species of Archaea and Bacteria.[18,19,20] β-CAs are oligomers, generally formed of 2-6 monomers of molecular weight of 25-30kDa.[21] These enzyme from

M. tuberculosis is encoded by gene Rv1284, Rv3588c and Rv3273 gene belonging to β-class.[22] The mentioned CAs reaction mechanism depends on the presence of zinc-coordinated hydroxide as the reactive species. Indeed, the zinc co-ordination with protein ligand in the domain of α-CA, γ-CA, and δ-CA is consistent by at least one water molecule in the active site structure. On contrary, β-CA shows a considerable difference of the zinc ligation within the class. The β-CA have notably different zinc ligation comprising one histidine and two cysteine with fourth co-ordination position occupied by either a water molecule or an aspartyl side chain.[19,20,21,22,23]

4.1. Overall structure

The two β-CA enzymes from M. Tuberculosis after cloning and crystallizing with X-ray crystallography technique are Rv1284 and Rv3588creported at the resolution of 1.75-2.00 Å. There is no enzymatic activity found for the Rv1284 gene, whereas for the gene Rv3588c activity was detected, because the gene Rv1284 active site is small and occupied with solvent. Rv1284 crystal structure contains two dimers which form the part of tetramer via crystallographic two-fold axis. Crystal structure ofRv3588c contains single subunit that forms dimer by the interaction around a crystallographic two-fold axis. In Rv1284, all of the metal ligands are contributed by one subunit of the dimer. Residues Cys35, His88, Cys91 and a water molecule coordinated with the metal ion (Fig2A from reference[24]). A thiocyanate molecule is also positioned in the active site of Rv3588c, though it is not co-ordinate to the metal ion. In Rv3588c, the active site residue Asp53 displaces the water molecule, and coordinated directly to the zinc ion, so breaking a potential salt link to Arg55(Fig2B, C from reference[24]).[24]

4.2 β-Carbonic anhydrase Inhibitors

Some clinically significant used drugs, such as acetazolamide (AAZ), methazolamide (MZA), ethoxyzolamide (EZA), Topiramate (TPM), benzolamide (BZA), celecoxib (CLX) have shown β-CAs inhibition. There are comparable results available in micro molar range for the series of simpler sulphonamide and sulfamate compounds synthesized.[21]Other than sulfonamide group, investigation on series of carboxylic acids for their interactions with three β-CAs strains from M. tuberculosis i.e. mtCA1, mtCA2 and mtCA3 have also done.[25]

5. β-HYDROXYACYL-ACP DEHYDRATASE

Mycobacterium tuberculosis shows tolerance against various drugs by developing a lipid rich outer layer in its cell wall. Lipid rich layer plays major role in pathogenesis and responsible

for the bacillus persistency within the host cell.[26] The layer composed of long chain fatty acid derivative of which one of the prevalent components is mycolic acid (MA). MA are crucial not only for the cell wall architecture but also contribute to the drug resistance by forming a selectively permeable envelope. There are two distinct fatty acid synthesis (FAS) pathways that come in sequence, FAS-I and FAS-II. FAS-I is mammalian cytoplasmic like single enzyme, multi domain, that has the ability to extend fatty acid up to C_{20}-C_{26}starting from acetyl-CoA.

The bacterial or plant FAS-II is a disintegrated system in enzymes where it further elongates the FAS-I products to full length fatty acids required for MA synthesis. In M. tuberculosis, the process is initiated by the condensation of acyl-CoA and malonyl ACP, a reaction catalysed by M.tbfabH, a β-keto acyl-ACP synthase. The newly form β-keto acyl-ACP is first reduced by a β-keto acyl-ACP reductase (MabA) to a β-hydroxy acyl-ACP intermidate which is then dehydrated by β-hydroxyacyl-ACP-dehydratase to form an enoyl-ACP intermidate. This is further reduced by an enoyl-ACP-reductase (InhA) to yield an ACP-bound acyl chain that is two carbons long. Gene encoding FAS-II enzymes are essential for mycobacterium survival, conditional depletion, or inactivation of these enzymes leads to bacterial lysis.[25,26] This makes FAS-II enzymes an attractive target for drug development. Among seven M. tuberculosis proteins contained in a double hot-dog fold. Of these, two genes, Rv3538 and Rv0636, were proposed to be essential for the mycobacterial growth. The bioinformatic analysis and drug inhibition studies together suggested that the putative protein encoded by Rv0636 was likely the FAS-II dehydratase.[7]

5.1 β-hydroxy acyl dehydratase inhibitors

Flavonoids act as growth inhibitor in E. coli and P. Falciparum, that have shown exact action as inhibition of enzymes in fatty acid biosynthesis. Butein, isoliqirtigenin, 2,2',4-trihydroxychalcone, Quercetin, and fisetin, these five flavonoids were initially tested for in vivo inhibitory activity on M. Bovis BCG, which is recognized as close surrogate of M.tb. MIC_{99}calculated values indicated anti-mycobacterial potency for each flavonoids.[27]

From bioinformatics approach the product of Rv0636 as a candidate has generated, that might represent the unknown β-hydroxy ACP dehydratase component of M.tbFAS-II. Since, the overexpression of Rv0636 in M.bovis conferred resistance to growth inhibition of butein and four other significantly relevant drugs and relieved inhibition of fatty acid and mycolic acid biosynthesis in vivo. Futhermore, after overexpression of Rv0636 in M.smegmatis FAS-II

was less sensitive of these inhibitors in invitro. Here M.bovis is close surrogate for M.tb inhibition studies reviled that Rv0636 is the strong candidate for β-Had ACP dehydratase enzyme of M.tb FAS-II. [28]

Clinically used anti-TB drugs like thiacetazone (TCA) and isoxyl (ISO) as well as flavonoids inhibit the enzyme activities of the M.tb HadAB complex. The first crystal structure of the M.tb HadAB complex bound with flavonoids inhibitors, depicted inhibition, which has revel in the publication(PDB codes: 4RLT, 4PLV, 4RLW).[29]

6. CONCLUSION

Though drugs of diverse chemical structures acting through different mechanisms are available for the treatment of M. tuberculosis, there are still many problems associated with the currently available compounds. Therefore, medicinal chemists worldwide are designing, synthesizing and evaluating a variety of novel molecules for inhibiting the bacterium's growth, with a special emphasis on identifying new drug targets. This review presents an overview of the few enzyme targets of Mycobacterium tuberculosis which may be the promising target for the development of drugs for TB therapy.

7. REFERENCES

1. Smith CV, Sharma V, Sacchettini JC. TB drug discovery: addressing issues of persistence and resistance. Tuberculosis, 2004; 84(1): 45-55.
2. Dunn MF, Ramirez-Trujillo JA, Hernandez-Lucas I. Major roles of isocitrate lyase and malate synthase in bacterial and fungal pathogenesis. Microbiology, 2009; 155(10): 3166-75.
3. Nishimoria I, Minakuchia T, Marescab A, Cartab F, Scozzafava A, T Supuran C. The β-carbonic anhydrases from Mycobacterium tuberculosis as drug targets. Current pharmaceutical design, 2010; 16(29): 3300-9.
4. Supuran CT, Scozzafava A. Carbonic anhydrases as targets for medicinal chemistry. Bioorganic & medicinal chemistry, 2007; 15(13): 4336-50.
5. Heath RJ, Rock CO. Roles of the FabA and FabZ β-hydroxyacyl-acyl carrier protein dehydratases in Escherichia coli fatty acid biosynthesis. Journal of Biological Chemistry, 1996; 271(44): 27795-801.
6. Slama N, Leiba J, Eynard N, Daffe M, Kremer L, Quémard A, Molle V. Negative regulation by Ser/Thr phosphorylation of HadAB and HadBC dehydratases from

Mycobacterium tuberculosis type II fatty acid synthase system. Biochemical and biophysical research communications, 2011; 412(3): 401-6.

7. Lohman JR, Olson AC, Remington SJ. Atomic resolution structures of Escherichia coli and Bacillus anthracis malate synthase A: Comparison with isoform G and implications for structure-based drug discovery. Protein Science, 2008; 17(11): 1935-45.
8. McKinney JD, zu Bentrup KH, Munoz-Elias EJ, Miczak A, Chen B, Chan WT, Swenson D, Sacchettini JC, Jacobs WR, Russell DG. Persistence of Mycobacterium tuberculosis in macrophages and mice requires the glyoxylate shunt enzyme isocitrate lyase. Nature, 2000; 406(6797): 735-8.
9. Shukla H, Kumar V, Singh AK, Singh N, Kashif M, Siddiqi MI, Krishnan MY, Akhtar MS. Insight into the structural flexibility and function of Mycobacterium tuberculosis isocitrate lyase. Biochimie, 2015; 110: 73-80.
10. Beeckmans S. Glyoxylate Cycle. Physiology, 2009; 159-179.
11. Freundlich J, Sacchettini, J. Inhibitors of mycobacterium tuberculosis malate synthase, methods of making and uses thereof. US Patent, US, 2014; 8,664,255 B2
12. Sharma R, Das O, Damle SG, Sharma AK. Isocitrate lyase: a potential target for anti-tubercular drugs. Recent patents on inflammation & allergy drug discovery, 2013; 7(2): 114-23.
13. Lee Y, Wahab H, Choong Y. Potential Inhibitors for Isocitrate Lyase of Mycobacterium tuberculosis and Non-M. Tuberculosis: A Summary. BioMed Research International, 2015; 2015: 1-20.
14. Hewett-Emmett D, Tashian RE. Functional diversity, conservation, and convergence in the evolution of the α-, β-, and γ-carbonic anhydrase gene families. Molecular phylogenetics and evolution, 1996; 5(1): 50-77.
15. Lane TW, Morel FM. Regulation of carbonic anhydrase expression by zinc, cobalt, and carbon dioxide in the marine diatom Thalassiosira weissflogii. Plant Physiology, 2000; 123(1): 345-52.
16. Supuran CT. Carbonic anhydrase inhibitors and activators for novel therapeutic applications. Future medicinal chemistry, 2011; 3(9): 1165-80.
17. Lane TW, Morel FM. A biological function for cadmium in marine diatoms. Proceedings of the National Academy of Sciences, 2000; 97(9): 4627-31.
18. Smith K. Prokaryotic carbonic anhydrases. FEMS Microbiology Review, 2000; 24(4): 335-366.

19. Smith KS, Ferry JG. A plant-type (β-class) carbonic anhydrase in the thermophilic methanoarchaeon Methanobacterium thermoautotrophicum. Journal of bacteriology, 1999; 181(20): 6247-53.
20. Covarrubias AS, Bergfors T, Jones TA, Hogbom M. Structural mechanics of the pH-dependent activity of β-carbonic anhydrase from Mycobacterium tuberculosis. Journal of Biological Chemistry, 2006; 281(8): 4993-9.
21. Supuran CT. Carbonic anhydrases as drug targets-an overview. Current topics in medicinal chemistry, 2007; 7(9): 825-33.
22. Nishimori I, Minakuchi T, Vullo D, Scozzafava A, Innocenti A, Supuran CT. Carbonic anhydrase inhibitors. Cloning, characterization, and inhibition studies of a new β-carbonic anhydrase from Mycobacterium tuberculosis. Journal of medicinal chemistry, 2009; 52(9): 3116-20.
23. Supuran CT, Scozzafava A. Carbonic anhydrase inhibitors and their therapeutic potential. Expert Opinion on Therapeutic Patents, 2000; 10(5): 575-600.
24. Covarrubias AS, Larsson A M, Hogbom M, Lindberg J, Bergfors T, Bjorkelid C, Mowbray SL, Unge T, Jones TA. Structure and function of carbonic anhydrases from Mycobacterium tuberculosis. Journal of Biological Chemistry, 2005; 280(19): 18782-9.
25. Maresca A, Vullo D, Scozzafava A, Manole G, Supuran CT. Inhibition of the β-class carbonic anhydrases from Mycobacterium tuberculosis with carboxylic acids. Journal of enzyme inhibition and medicinal chemistry, 2013; 28(2): 392-6.
26. Biswas R, Dutta A, Dutta D, Hazra D, Banerjee D, Basak A et al. Crystal structure of dehydratase component HadAB complex of mycobacterial FAS-II pathway. Biochemical and Biophysical Research Communications, 2015; 458(2): 369-374.
27. Gurvitz A, Hiltunen J, Kastaniotis A. Heterologous Expression of Mycobacterial Proteins in Saccharomyces cerevisiae Reveals Two Physiologically Functional 3-Hydroxyacyl-Thioester Dehydratases, HtdX and HtdY, in Addition to HadABC and HtdZ. Journal of Bacteriology, 2009; 191(8): 2683-2690.
28. Brown A, Papaemmanouil A, Bhowruth V, Bhatt A, Dover L, Besra G. Flavonoid inhibitors as novel antimycobacterial agents targeting Rv0636, a putative dehydratase enzyme involved in Mycobacterium tuberculosis fatty acid synthase II, Microbiology, 2007; 153(10): 3314-3322.
29. Doddareddy M, Pattubala A, Hibbs D. Structure Based Design of Mtb Had AB Inhibitors useful in the Treatment of Tuberculosis. International Journal of Applied Sciences& Engineering, 2015; 1(1): 59-64.