Ind. J. Pharm. Edu. Res., 2024; 58(1s):s58-s66.
https://www.ijper.org

Review Article

Molecular Insights into Salicylate Synthase: From Medicinal Chemistry Perspective

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ABSTRACT
Tuberculosis is a pathogenic infection that has created trepidation around the globe due to its repercussion on the health of patients. The iron transport mechanism in mycobacterium plays a pivotal role in bacterial survival; whilst this can be considered an emerging drug target. Salicylate synthase is a member of the MST family and this enzyme is distrait in mammals. It is the first enzyme for the synthesis of mycobactin (siderophores) which is a determinant for iron transport. The present all-embracing review provides overall coverage of the biological and structural outlook of the enzyme salicylate synthase and its inhibitors reported in recent years. Furthermore, this review sheds limelight on the development of novel and more potent salicylate synthase inhibitors.

Keywords: *Mycobacterium tuberculosis*, Anti-tubercular drug targets, Siderophores, Mycobactin, Salicylate synthase (MbtI).

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Received: 25-01-2023;
Revised: 09-02-2023;
Accepted: 03-11-2023.

INTRODUCTION

Tuberculosis is a pathogenic disease infecting people from the ancient period and the origin of this pathogen was also found in ancient mummies however the causative organism was discovered by Robert Koch in 1882.[1,2] *Mycobacterium tuberculosis* (Mtb) affiliated to the class of prokaryotic bacteria that contain cell wall made up of peptidoglycan, arabinogalactan, outer membrane and mycolyl-arabinogalactan-peptidoglycan complex that supports the upper myco-membrane, this enables bacteria to survive severe environments more easily. However, determining the bactericidal activity of chemotherapeutic agents remains difficult because the bacterium does not appear to enter the active phase once it has entered the target organ.[3] Mtb exists in the host in different forms, as a free mycobacterium or inside the macrophage.[4] According to the World Tuberculosis Report 2021, there were 5.8 million diagnosed cases worldwide in 2020. However, it is assumed that this reduction in cases is due to COVID-19's impact on access to diagnosis and treatment for tuberculosis, which led to an escalation in the estimated number of deaths from the disease to 1.3 million.[5] *Mycobacterium tuberculosis* is still infecting a huge population every year and affects millions more, with an enormous impact on families and communities around the globe.

Because of the increase in drug resistance, it is believed that treatment for tuberculosis is becoming ineffective. The preferred treatment of tuberculosis is Directly Observed Therapy (DOT) which have an alarming adverse effects and most importantly patient incompliance. The mycobacterium tends to develop resistance against drugs including isoniazid, rifampicin, ethambutol, streptomycin, etc. DOT's treatment has severe adverse effects like hepatitis, exanthema, dyspepsia and arthralgia that may lead to mortality in patients.[6] Due to patient incompliance, there was the emergence of new mycobacterial strains that are Multi-Drug Resistant (MDR), Extensively Drug-Resistant (XDR) strains and Totally Drug Resistant (TDR).[7] About 0.150359 million diagnosed cases of drug-resistant TB underwent treatment, while 2.8 million patients received TB preventative care in 2020.[5] Now new anti-tubercular drugs were discovered (Figure 1) namely Bedaquiline, Delamanide and Pretomanid in the years 2012, 2014 and 2019 respectively.

DOI: 10.5530/ijper.58.1s.5

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These drugs are relatively safe despite having horrifying adverse effects.[8] Bedaquiline inhibits ATP synthase in the mitochondria and resistance to this drug may result from mutations in the trans membrane oligomeric C subunit (*atpE*) and transcriptional receptor (Rv0678) genes, which code for the MmpS5-MmpL5 efflux pump.[9] The mechanism of delamanid involves inhibition of the biosynthesis of methoxymycolic acid and ketomycolic acid, the resistance to this drug may be caused by mutation in the five genes *fbiC, fbiB, fbiA, fgd1, ddn* related with F_{420} biosynthetic pathway or prodrug activation. In case of pretomanid, it targets cell wall biosynthesis and cause respiratory poisoning by means of nitric oxide release. It is also reported that it targets pentose phosphate pathway which apparently causes accumulation of lethal methylglyoxal.[10,11] To combat the above adverse events, there is a need for the development of new anti-tubercular agents that portray minimal adverse effects with maximal potency against *M. tuberculosis*.

Various drug targets have been studied extensively for the development of new and unique anti-tubercular agents, the study of crystallographic structures and some of these novel targets were reported by Mori *et al*., namely Isocitrate lyase (PDB-1F61,6XPP), Methylmalonyl-CoA Mutase (MCM) (PDB-6OXC,6OXD), Fumarate Hydratase (PDB-5F91), Enoyl-Acyl Carrier Protein Reductase (InhA) (PDB-6R9W,6XQ9), Dihydrofolate Reductase (DHFR) (PDB-6DDP,6DE5), Alanine Racemase (Alr) (PDB-6SCZ), L-Aspartate-α-Decarboxylase (PanD) (PDB-6OYY), Decaprenylphosphoryl-β-D-ribose-2'-oxidase (DprE1) (PDB-6HEZ), Cytochromes CYP124 and CYP121 (PDB-6T0K,6T0L,6RQ0), Ser/Thr Protein Kinase B (PknB) (PDB-5U94), Adenosine Kinase (AdoK) (PDB-6C67,6C9N,6C9Q,6C9V), Thymidylate Kinase (TMPK) (PDB-1W2G,5NQ5,1N5K,6YT1), tRNA (guanine37-N^1)-methyltransferase (TrmD) (PDB-6JOF,5ZHN,5ZHL), Nicotinic Acid Mononucleotide (NaMN) Adenylyltransferase (NadD) (PDB-6BUV), Enhanced Intracellular Survival (Eis) Transferase (PDB-6VUZ,6B3T,6P3U), 2-Succinyl-5-enolpyruvyl-6-hydroxy-3-cyclohexadiene-1-carboxylate Synthase (MenD) (PDB-6O0N), Tryptophan Synthase (TrpAB) (PDB-6USA), Salicylate Synthase (MbtI) (PDB-6ZA4,6ZA5), Malate Synthase G (GlcB) (PDB-3SB0), β-ketoacyl-AcpM Synthase (KasA) (PDB-5W2P,6P9K,6P9L,6P9M), Dethiobiotin Synthase (DTBS) (PDB-6CVE,7L1J) and etc.[12] This review aims to disclose exclusively enzymology, crystal structure, and inhibitor development of two important molecular target salicylate synthase with the emphasis on medicinal chemistry perspective.

Biology of salicylate synthase

Salicylate synthase (MbtI) of *mycobacterium tuberculosis* belongs to the family of chorismate-utilizing enzyme (MST family), which is responsible for siderophore synthesis for the engulfment of the free or bounded iron across the cell which is a crucial nutrient for the survival and growth of *Mycobacterium tuberculosis*.[13] *Mycobacterium tuberculosis* in the host cell, can acquire extracellular or intracellular iron, extracellularly iron is bound to various transport proteins and intercellular iron is present in erythrocytes.[14,15] Mtb resides in the host in two forms namely in the dormant stage inside the macrophage and as a free mycobacterium. Mtb procures iron from the extracellular matrix by invading the transporter proteins (lactoferrin, transferrin and ferritin) and in the circulation by the lysis of erythrocytes encountering the heme.[16-18] The Mtb inhabiting the macrophage faces iron deficiency even though the concentration of iron is high intracellularly in the macrophage.[19] To satisfy the need for iron in macrophages inhabiting *mycobacterium tuberculosis* siderophores plays a starring role in the iron diffusion across macrophagic plasma membrane, an alternative source for iron is the destruction of red blood cells creating hemoglobin as a vital source.[20]

Siderophores are iron-chelating molecules released by bacteria to aid in the transport and uptake of iron.[21,22] These are the iron scroungers that extract iron from lactoferrin, transferrin and ferritin. To engulf iron in the bacterium it forms chelates with ferric ions like hexadentate and octadentate complex.[23] Siderophores are categorized into four types depending on the ferric ion binding motif that are carboxylates, catecholate, hydroxamate and phenolate, some of the examples of siderophores includes mycobactin and exochelin.[24-26]

Mtb biosynthesize two mycobactins namely carboxymycobactin and mycobactin constituting of hydroxamate and phenolate functionality[27] both mycobactin allocates indistinguishable core comprising of oxazolidine ring and five amino acids derived from salicylate. The mycobactin are lipophilic molecules that anchors to the cell wall and plasma membrane because of their extended aliphatic tail. On the contrary, carboxymycobactin is lipophobic due to its short tail that ends with carboxylate functionality.[28,29] Exochelin is the non-virulent siderophores produced by *Mycobacterium smegmatis* and *Mycobacterium neoaurum* that are hydrophilic in nature which indicates the ongoing tuberculosis infection.[30,31]

Biosynthesis of mycobactin

Numerous biosynthetic pathways are associated in the biosynthesis of mycobactin. In the biosynthesis of mycobactin two genes are involved, *mbt-1 and mbt-2*.[32,33] The *mbt-1* constitutes of ten genes *mbtA* to *mbtJ* namely salicyl AMP ligase (*mbtA),* non-ribosomal peptide synthetase (NRPS; *mbtB, mbtE and mbtF)*, polyketide synthase (PKS; *mbtC and mbtD),* the role of MbtJ and MbtH are still unknown, these enzymes are accountable for the synthesis of the core structure of mycobactin.[34] The *mbt*2 consists of four gene *mbtK* to *mbtN* that are determinants for the synthesis of the lipophilic aliphatic side chain. After the genesis of acyl side chain by MbtL, MbtM, MbtN, this side chain transfers to

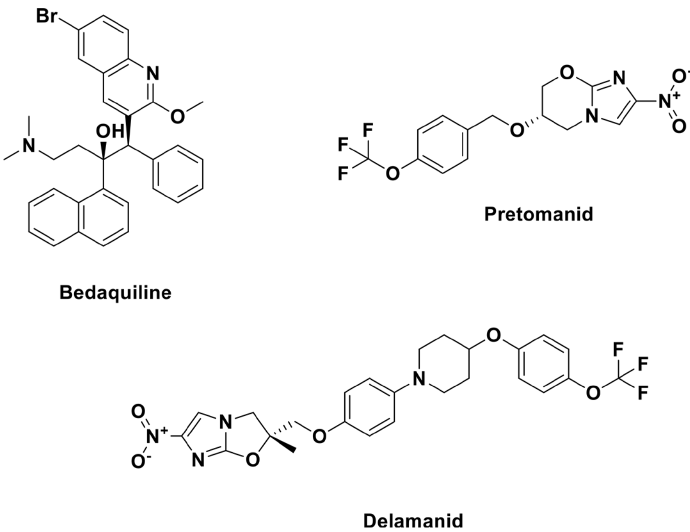


Figure 1: Newly marketed antitubercular drugs.

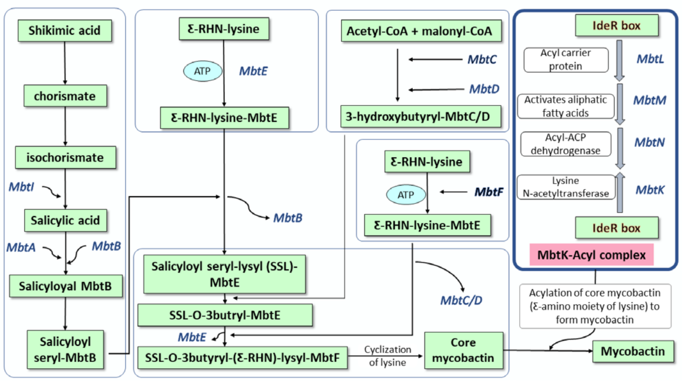


Figure 2: Biosynthetic pathways for mycobactin.

core mycobactin with the help of complex of Mbtk with fatty acyl which results in the biosynthesis of functional mycobactin Figure 2.

The pathway for the biosynthesis of mycobactin involves two key intermediates chorismate and isochorismate and are converted to salicylate which is a key biomolecule in the biosynthesis of mycobactin. Chorismate is a secondary metabolite that is biosynthesized by the shikimic acid pathway which is further transformed to isochorismate in presence of MbtI. MbtI a primary enzyme is involved in the synthesis of mycobactin and catalyzes two steps in the synthesis of salicylic acid i.e., chorismate is converted to isochorismate that is a reversible reaction and chorismate or isochorismate to salicylate which is an irreversible reaction.[35] The activation of salicylic acid is dependent on MbtA and MbtB and further transfer of salicylate to thiolate domain via an acyl adenyl intermediate. MbtB is a fragment of megasynthatase comprising three NRPS's and two polyketides synthase that aggregate the core structure.[36,37]

The alternative pathway for the synthesis of mycobactin is initiated from hydroxylation of L-lysine to N^6 hydroxyl-l-lysine which is catalyzed by MbtG and MbtH leads to the exact folding of MbtB, MbtE and MbtF.[38] The synthesis of mycobactin is synchronized by an iron-dependent regulator (IdeR), IdeR represses gene is associated with the synthesis of mycobactin and genes responsible for the import and export of mycobactin.[39,40] The assembly of the aliphatic acyl side chain occurred in presence of MbtL, MbtM and MbtN and its linkage is catalyzed by MbtK. The above process clearly focuses on the assembly of mycobactin.

Mtb siderophores export and import

As discussed, two siderophores mycobactin and carboxymycobactin are implicated in the relocation of iron across the plasma membrane by iron reuptake mechanism as portrayed in Figure 3. Mycobactins are attached to the cell membrane in the cell wall region via various transporter proteins.[41] Transport of mycobactins across the membrane are facilitated by the enzyme mycobacterial membrane protein large (MmpL i.e., MmpL4 and MmpL5) together with their corresponding small membrane-associated proteins (MmpS i.e. MmpS4 and MmpS5).[42] The small proteins MmpS4 and MmpS5 are responsible in the Fe^{3+} ion accession similarly, the large proteins MmpL4/5 and MmpS4/5 are responsible for mycobactin export whereas not for mycobactin import.[43] Once the siderophores are exported out of the cell membrane they are recycled although the non-chelated siderophores uptake mechanism which is still unknown.

Sequence similarity[44]

Crystal structure of MbtI and it's signaling pathway

The sequence similarity search of the enzyme was studied and similarity is mentioned in Figure 4. The crystallographic structure of MbtI was determined from X-Ray crystallographic method.[44-46] Salicylate synthase is a bilobed protein comprising of antiparallel beta sheets and helixes. The beta-sheets consist of two parts: subdomain 1 (10 strands) and subdomain 2 (11 strands). The helix surrounds the beta sheets and the central part of the crystal structure symbolizes the lipophilic cavity. The cofactor magnesium ion which is pivotal in the formation of salicylate

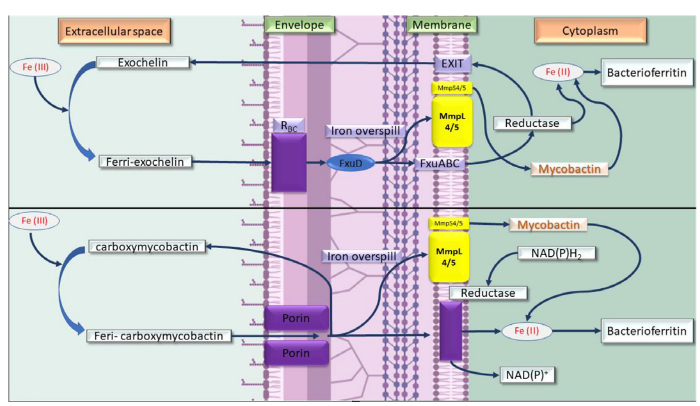


Figure 3: Iron transport system.

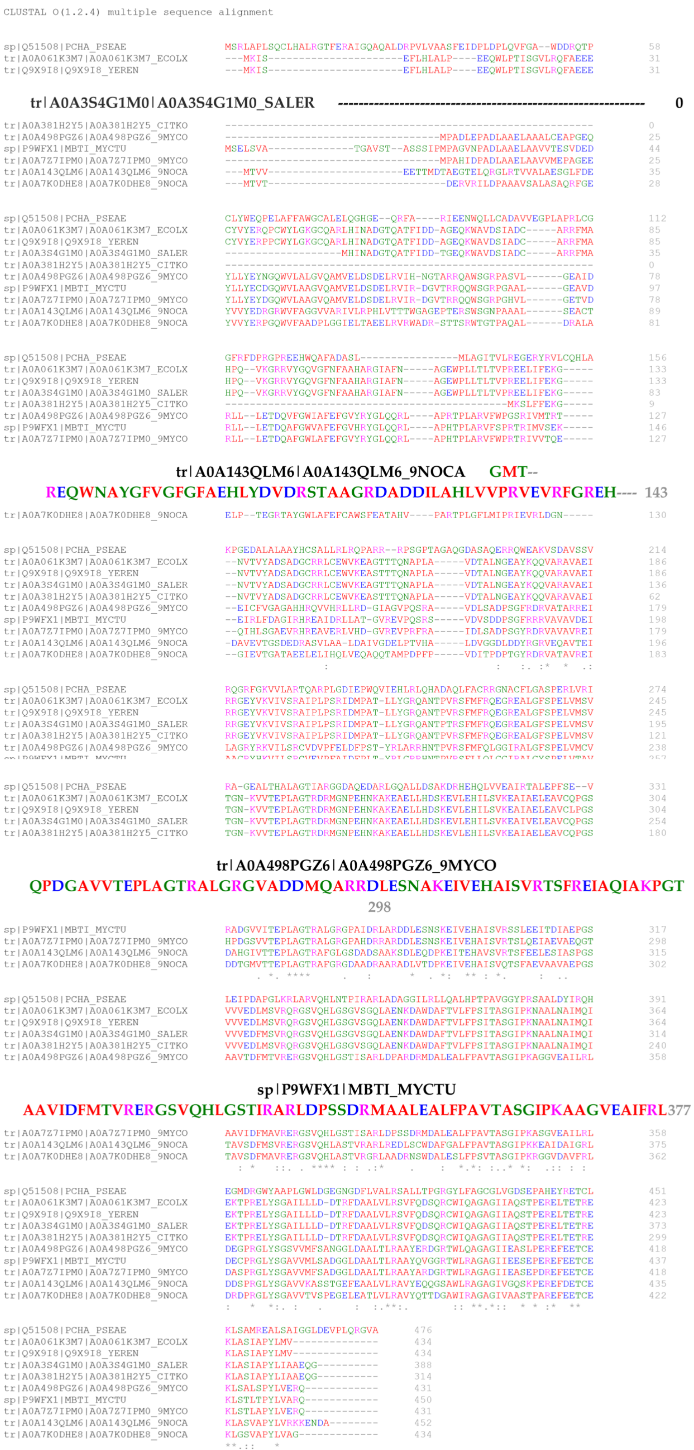


Figure 4: Sequence similarity assessment.

from chorismate is placed in the active site of MbtI which is embodied in the Figure 5.[47,48] Manos-Turvey *et al.*, revealed the open and closed conformations of the MbtI. The elastic residues array from Leu268 - Lys293 and Met324 - Gly336 for both open and closed chain are common in both the conformation of the enzyme.[46] These elastic residues in the open conformer are leaned upward (colored magenta) while in the closed conformer the

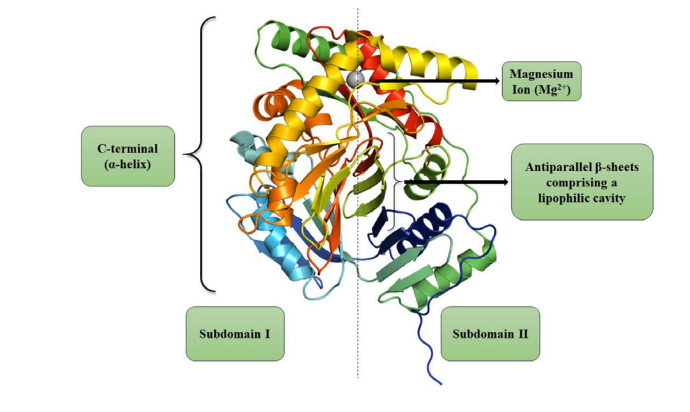


Figure 5: Crystal structure of MbtI revealed by structural analysis.

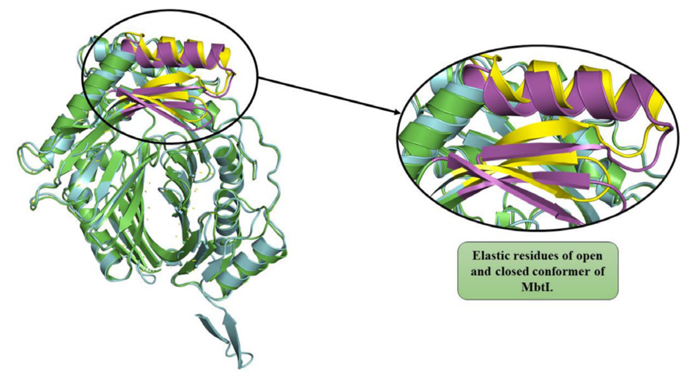


Figure 6: Elastic residues of open and close conformer of MbtI (PDB: 3LOG, 2G5F).

residues bend towards the active site (colored yellow) as depicted in the Figure 6.

Biologically salicylate synthase (MbtI) has isochorismate synthase (i.e., chorismate isomerase) and isochorismate-pyruvate lyase activity. The isochorismate synthase and isochorismate-pyruvate lyase activities are pH dependent. At pH 7, MbtI shows isochorismate synthase functional activity but at pH 7.5 the isochorismate-pyruvate lyase activity escalates and the principal product formed is salicylate and if the pH is 8 then MbtI transform isochorismate as a substrate to salicylate. The salicylate synthase and isochorismate activity of MbtI depends on magnesium ion. The acidic residues of two amino acids Glu297 and Glu434 forms interaction with the Mg^{2+} ion and the carboxylic acid end of the chorismate is involved in the interaction with the cofactor Mg^{2+} ion. Lys205 and Glu252 are responsible for the isomerization of chorismate to form isochorismate. Pyruvate elimination activity of MbtI shows that there are no residues of the active site that are in direct contact with the ligand and hence, the elimination of the pyruvate group may have alternative way.

Tactics for development of MbtI inhibitors

MbtI inhibitors hinder the initial step for synthesizing siderophores by averting the mycobactin as shown in Figure 7.

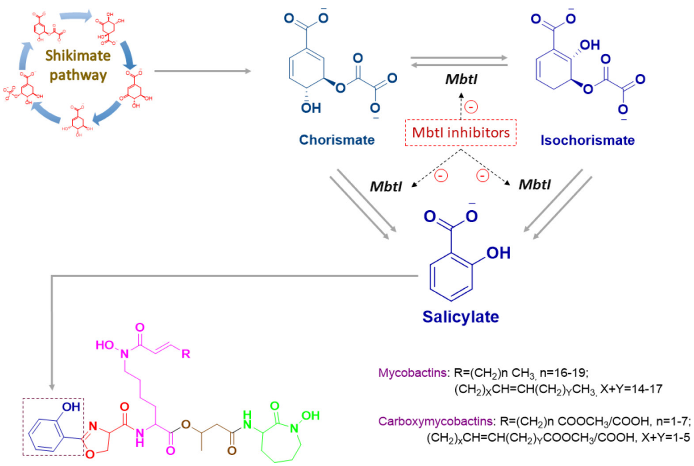


Figure 7: Possible strategies for the development of MbtI inhibitors.

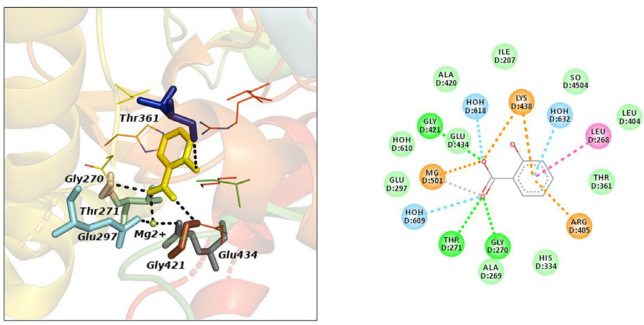


Figure 8: Complex of salicylate acid with Mg^{2+} ion (PDB: 6ZA5).

The information related to the docking of the molecules, selectivity and inhibitors mechanism of action were studied with an aid of X-ray crystallographic structures of salicylate synthase ligand complexes.[12] The binding of the salicylic acid in the active pocket of the MbtI initiates the synthesis of mycobactin which has a pivotal role for the growth as well as survival of mycobacterium tuberculosis. The salicylic acid fitted in the active pocket interacted with the critical amino acids namely Gly270, Thr271, Glu297, Thr361, Gly421 and Glu434 established by hydrogen bond interactions. The amino acids Glu297 and Glu434 interacted via salt bridge with the cofactor Mg^{2+} ion,[48] as depicted in the Figure 8.

MbtI inhibitors are classified in two main types based on their chemical modifications, namely Transition State Inhibitors (TSI) and Non-Transition State Inhibitors (NTSI). The TSI's and NTSI's bind within the active site specifically the hydrophobic region by interacting with the amino acids in the C helix and the interactions via magnesium salt bridge is dependent as well as independent based on the chemical structure of the ligands. The (4R,5S,6R)-4-amino-5-(2-carboxyethyl)-6-hydroxycyclohex-1-ene-1-carboxylic acid is paradigm of TSI interacted with Lys205, Gly270, Gly421, Arg430 and Glu431 in the active site as portrayed in the Figure 9. The 5-(3-cyanophenyl) furan-2-carboxylic acid is an example of NTSI interacted with

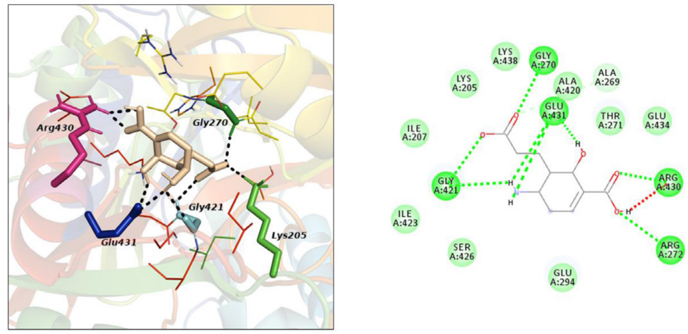


Figure 9: Complex of transition state inhibitor with MbtI.

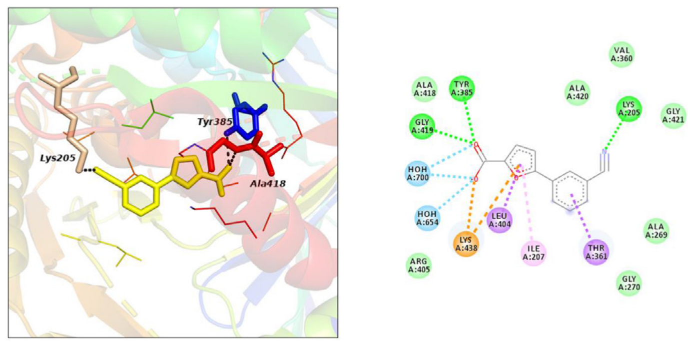


Figure 10: Complex of non-transition state inhibitor with MbtI (PDB: 6ZA4).

Lys205, tyr 385 and Ala418 in the active pocket shown in the Figure 10. the above data provides the information about the structural requirements for development of MbtI inhibitors.[47,48]

The recent development of MbtI inhibitors

Manos-Turvey *et al.*, documented the inhibitory studies of MbtI inhibitors. The enzyme inhibitory assayed revealed that compound 1 inhibited MbtI with Ki value of 11 μM. Molecular modelling studies stated that 1, 2 and 3 interacted with both isomers E and Z were engaged in the active region of MbtI by developing ionic interaction of carboxylate functional group with Mg^{+2} ion, the hydroxyl functional group interacted via hydrogen bond with Lys438 and the bulkier groups occupied the side chain hydrophobic cavity.[49]

Chi *et al.*, explained the binding pattern of inhibitors in the elastic active cavity of MbtI. A correlation amid inhibitor potency and binding mode of the antagonist was established. The molecular modelling studies stated that lipophilic substituents like phenyl functionality attached with the enolpyruvyl side chain flicked protein backbone by 180° and studies also stated that flexibility in the side chain of the MbtI protein played the role in escalating the potency of designed inhibitors. Flexibility in the ligand was observed at the ether linkage which was also crucial for the MbtI inhibition.[50]

The phenylacralate-derived inhibitors were given by Manos-Turvey as a powerful salicylate synthase inhibitor.

Compound 4 was found to be the most active among series of molecules with MIC_{50} value of 25 µM. All the inhibitors have showed hydrophilic character due to which it portrayed minimal anti-tubercular activity. Due to the minimal lipophilic character of the designed molecules the work was further extended.[51]

Ferrer *et al*., worked on the perspicacity of various mechanisms of salicylate synthase with an aid of molecular modelling. Here the authors stated the role of magnesium ion for the conversion of chorismate to salicylate in the presence of isochorismate synthase and isochorismate puruvate lyase. According to the QM/MM and MD simulation, the existence of Mg^{2+} in the enzyme's active site dislocated the Lys293 7.89 Å more than the absence of Mg^{2+} 1.90 Å, further the negatively charged Glu297 migrated inside the active site with 2.17 Å. Due to the Mg^{+2} ion water molecule migrated towards the active site from the solvent region with the distance of 2.2 Å with its corresponding pKa values of Lys293 10.72 without Mg^{+2} and 12.28 with Mg^{+2} ion and similarly the pKa values for Glu297 5.66 without Mg^{+2} and 6.55 with Mg^{+2} ion. The QM/MM MD simulation studies clearly stated that the presence of magnesium ion is responsible for the catalytic pursuit of isochorismate synthase and isochorismate pyruvate lyase.[52]

New MbtI inhibitors consisting heterocyclic scaffolds benzoisothiozolones, diaryl sulphones and benzimidazol-2-thiones were identified through high throughput screening. The benzisothiazoles derivatives were reported as irreversible inhibitors which interacted with four system residues of the MbtI. The benzimidazole-2-thione derivatives (IC_{50} = 9.2 to >200 µM) were identified as the best derivatives amongst the other classes which were stated as reversible non-competitive inhibitors that escalated the potency of inhibition and hence considered as novel scaffold against novel MbtI.[53]

Liu and Aldrich divulged optically active molecules as MbtI inhibitors as an anti-tubercular agent. Authors synthesized the optically active ammonium salt of compound 5 with two stereocenters. 5 inhibited MbtI with 10% dependence at 100 µM. The results of biological activity were confirmed by molecular modelling studies which detailed that 5 fitted well in the active pocket by interacting in the hydrophobic region. Ligand and structure-based approach for recognition of new potent MbtI inhibitors were reported. The pharmacophore modelling studies reported ten hypotheses which were found to be prototypical with a max fit value of 5. Based on ligand-based pharmacophores model six structure-based pharmacophore model were developed that constituted hydrogen bond acceptor feature and nitrogen-hydrogen bond donor features that interacted with Lys438 and Lys205 respectively. The validation of the generated pharmacophores was analyzed based on goodness of hit score of 0.89 (ligand based) and 0.97 (structure based). Seventy-three hits were identified from zinc database by applying filters like ADMET properties, pharmacophore-based screening. Molecular docking studies unveiled that seventeen hits were chosen based on binding mode analysis and all the inhibitors occupied the active site of MbtI. Molecular dynamics stimulation affirmed that the inhibitors represented stability in active cavity of the protein by a minimal confirmational change in the surrounding amino acids.[54]

Zang and co-workers published chorismate utilizing enzymes transition state inhibitors synthesis. The replacement of hydroxyl functionality with amino functionality yielded compound 6 which was disqualified due to its non MbtI inhibitory activity at 100 µM that was found to be very low.[55]

Pini and their collaborator reported chromone derivatives as Mtb salicylate synthase inhibitors directed by computational modelling studies. Enzyme inhibitory assay exemplified that compound 7 inhibited salicylate synthase with IC_{50} value of 55.8 µM and corresponding residual activity of 23% discretely. Molecular docking experiments divulged that 7 occupied profoundly the active site of the salicylate synthase establishing hydrogen bond between carbonyl functionality of chromone with Lys205 and hydroxy group with Gly270. The carboxylic acid group interacted Mg ion and Glu421. Further the molecular dynamics simulation of 7 was performed for 100 ns which stated that 7 portrayed the stability in the active site throughout the simulation and the interaction that were observed in the docking studies were retained in the simulation.[56]

5 substituted furan-2-carboxylic acids as salicylate synthase inhibitors were reported by Chiarelli and group. The designing of the molecules was undertaken with an aid of virtual screening and pharmacophore modelling. From the molecular modelling studies five compound were identified. Compound 8 inhibited MbtI with IC_{50} value of 7.6 µM and depicted encouraging anti-tubercular activity with MIC^{99} of 156 µM.[57]

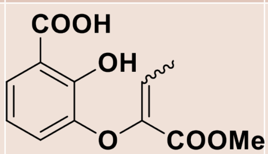
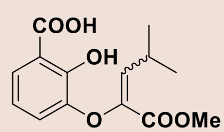
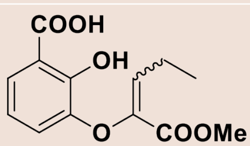
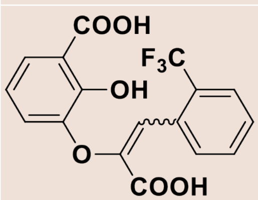
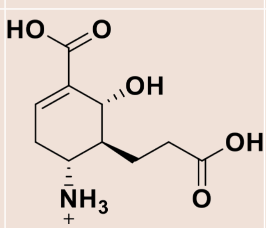
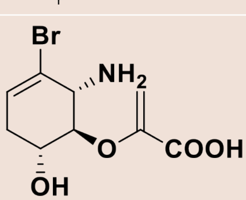
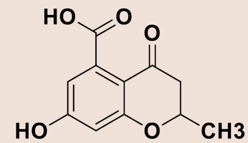
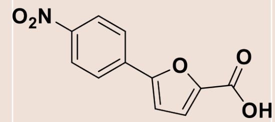
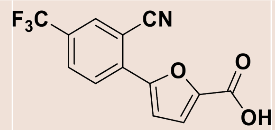
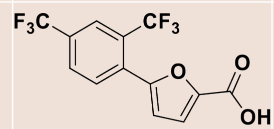
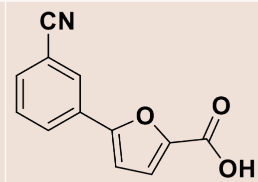
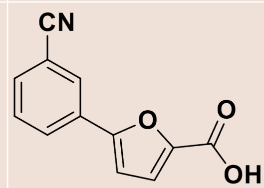
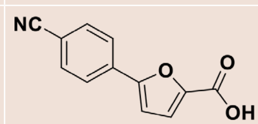
Chiarelli *et al*., reported SAR of the 5-Phenylfuran-2-carboxylic acid analogous as salicylate synthase inhibitors as anti-tubercular agents. Enzyme inhibitory assay stated that compounds 9 and 10 inhibited salicylate synthase with its corresponding IC_{50} value of 18.5 and 13.1 µM from which 10 portrayed potency and selectivity. Compound 10 was screened for its antiproliferative activity against MRC-5 (human foetal lung fibroblast) with its inhibition potential of IC_{50} value >100 µM. Molecular docking studies said that 10 resides in the active region of MbtI establishing hydrophobic interactions with Ile207, Pro251, Thr361 and Leu404 and the carboxylic functionality formed salt bridge with Mg^{2+} Cofactor in the active site. The MD simulation studies reveled 10 did not maintain stability in the active area of the macromolecule.[58]

Mori *et al*., outlined the role of magnesium in the salicylate synthase for discovery of novel drugs against *mycobacterium tuberculosis*. Compound 11 selectively and potentially subdued the MbtI with its corresponding IC_{50} value of 6.3 µM and residual activity of 3.1%. Findings of the molecular docking studies stated

11 accommodated in the catalytic region of MbtI inception of hydrogen bond interactions with Lys205, Tyr385, Gly490, Arg405 and Lys438 also manifested ionic interaction with Mg^{2+} ion. Further, the MD stimulation revealed that the binding mode of 11 was Mg^{2+} independent. The overall studies stated that the binding of inhibitors to MbtI are independent of Mg^{2+} ion.

Mori and Stelitano reported five membered heterocyclic carboxylic acids as antimycobacterial agents targeting salicylate synthase. 5-substituted five membered heterocyclic motifs namely: thiophene, 1,3-thaizole, 1,3-oxazole, 1*H*-imidazole, 1,3,4-oxadiazole and 1,4 substituted 1,2,3-triazole carboxylic acids were synthesized. Compounds 12 and 13 inhibited MbtI with IC_{50} values of 6.3 and 7.6 µM respectively. Structures of all the inhibitors are quoted in Table 1.

Table 1: MbtI inhibitors.

Comp. No.	Comp. No. in original paper	Chemical Structure	MbtI IC_{50}/Ki (µM)	Year of report
1	39		11	2010
2	40		-	2010
3	41		-	2010
4	24		-	2012
5	4		-	2015
6	1		-	2017
7	1		55.8	2018
8	1a		7.6	2018
9	1g		18.5	2019
10	1h		13.1	2019
11	10		6.3	2020
12	I		6.3	2021
13	II		7.6	2021

Mutations in salicylate synthase

Mutation is a natural phenomenon in which the amino acid sequence of a protein is altered, leads to a change in the enzymatic activity.[59] Mutations in MbtI are artificially induced by His-tagged method divulged by Zwahlen *et al.,* The amino acids K205A, L268A, T271A, H334M and R405A displayed mutant activity in the absence or presence of Mg^{2+} ion in MbtI mutants created by Quick Change mutagenesis and for the Glu252Gly there was no change observed in the activity. Sequence comparison stated that the MbtI was 31% cognate and 45% similar of *S. marcescens* with chorismate-binding (TrpE) subunit of enzyme as compared with wild-type MbtI.

CONCLUSION

For the identification and blooming of novel anti-tubercular agents, salicylate synthase is a contentious target. The primary role of salicylate synthase is spotted in first step for synthesis of siderophore namely carboxymycobactin and mycobactin, these molecules are responsible for iron import and export across the cell which is responsible for cell survival therefore it makes target more crucial for discovery of small molecules against salicylate which can later be modified into potential anti-tubercular molecules. Thus, for the current therapy for treatment of tuberculosis is trivial and has its own drawback so this target salicylate synthase may be considered for the development novel antitubercular agents. Structure based drug discovery strategies were employed for the discovery and development of numerous salicylate synthase inhibitors. Topical review highlights the pharmacological, biochemical and molecular modelling that tackles and delineate salicylate synthase inhibitors till date. Furthermore, it focuses on advancements of potential TS and NTS inhibitors against this target.

ACKNOWLEDGEMENT

Dr. Ravindra Kulkarni as the corresponding author expresses heartfelt gratitude to the Principal, Poona College of Pharmacy, Pune for his indispensable encouragement and also thank Ritesh, Mehavi and Harshali. Also, all the authors obliged to late Dr. Subhash Bodhankar for his continuous encouragement and insightful comments.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

Mtb: *Mycobacterium tuberculosis*; **MDR:** Multi-drug resistant; **XDR:** Extensively drug-resistant; **TDR:** Totally drug resistant; **MCM:** Methylmalonyl-CoA Mutase; **TrpAB:** Tryptophan Synthase; **MbtI:** Salicylate Synthase.

SUMMARY

Tuberculosis is a pathogenic infection that has created trepidation around the globe due to its repercussion on the health of patients. Numerous enzyme targets have been identified for the progression of tuberculosis from which salicylate synthases is explored in this review. Varieties of inhibitors have been developed against MbtI but there is no drug in market against this target so it remains as a target in limelight for discovery of novel anti-tubercular agents/ drugs. This comprehensive report provides the medicinal chemistry aspects of the MbtI and its inhibitors developed in the time scale of a decade.

REFERENCES

1. Zink AR, Grabner W, Nerlich AG. Molecular identification of human tuberculosis in recent and historic bone tissue samples: The role of molecular techniques for the study of historic tuberculosis. Am J Phys Anthropol. 2005;126(1):32-47. doi: 10.1002/ajpa.10409, PMID 15386245.
2. Daniel TM. The history of tuberculosis. Respir Med. 2006;100(11):1862-70. doi: 10.1016/j.rmed.2006.08.006, PMID 16949809.
3. Chiaradia L, Lefebvre C, Parra J, Marcoux J, Burlet-Schiltz O, Etienne G, *et al*. Dissecting the mycobacterial cell envelope and defining the composition of the native mycomembrane. Sci Rep. 2017;7(1):12807. doi: 10.1038/s41598-017-12718-4, PMID 28993692.
4. Gengenbacher M, Kaufmann SH. Mycobacterium tuberculosis: success through dormancy. FEMS Microbiol Rev. 2012;36(3):514-32. doi: 10.1111/j.1574-6976.2012.00331.x, PMID 22320122.
5. World Health Organization. Global tuberculosis report. 2021;2021.
6. Suárez I, Fünger SM, Kröger S, Rademacher J, Fätkenheuer G, Rybniker J. The diagnosis and treatment of tuberculosis. Dtsch Arztebl Int. 2019;116(43):729-35. doi: 10.3238/arztebl.2019.0729, PMID 31755407.
7. Migliori GB, Tiberi S, Zumla A, Petersen E, Chakaya JM, Wejse C, *et al*. MDR/XDR-TB management of patients and contacts: Challenges facing the new decade. The 2020 clinical update by the Global Tuberculosis Network. Int J Infect Dis. 2020;92S:S15-25. doi: 10.1016/j.ijid.2020.01.042. PMID 32032752.
8. Shyam M, Shilkar D, Verma H, Dev A, Sinha BN, Brucoli F, *et al*. The mycobactin biosynthesis pathway: A prospective therapeutic target in the battle against tuberculosis. J Med Chem. 2021;64(1):71-100. doi: 10.1021/acs.jmedchem.0c01176, PMID 33372516.
9. Ismail N, Ismail NA, Omar SV, Peters RPH. *In vitro* study of stepwise acquisition of rv0678 and atpE mutations conferring bedaquiline resistance. Antimicrob Agents Chemother. 2019;63(8):e00292-19. doi: 10.1128/AAC.00292-19, PMID 31138569.
10. Rifat D, Li SY, Ioerger T, Shah K, Lanoix JP, Lee J, *et al*. Mutations in fbiD (Rv2983) as a novel determinant of resistance to pretomanid and delamanid in Mycobacterium tuberculosis. Antimicrob Agents Chemother. 2020;65(1):e01948-20. doi: 10.1128/AAC.01948-20, PMID 33077652.
11. Yang JS, Kim KJ, Choi H, Lee SH. Delamanid, bedaquiline, and linezolid minimum inhibitory concentration distributions and resistance-related gene mutations in multidrug-resistant and extensively drug-resistant tuberculosis in Korea. Ann Lab Med. 2018;38(6):563-8. doi: 10.3343/alm.2018.38.6.563, PMID 30027700.
12. Mori M, Villa S, Ciceri S, Colombo D, Ferraboschi P, Meneghetti F. An outline of the latest crystallographic studies on inhibitor-enzyme complexes for the design and development of new therapeutics against tuberculosis. Molecules. 2021;26(23):7082. doi: 10.3390/molecules26237082, PMID 34885662.
13. Doherty CP, host. Pathogen interactions: the role of iron. J Nutr. 2007;137(5):1341-4. doi: 10.1093/jn/137.5.1341, PMID 17449603.
14. Theil EC. The ferritin family of iron storage proteins. Adv Enzymol Relat Areas Mol Biol. 1990;63:421-49. doi: 10.1002/9780470123096.ch7, PMID 2407067.
15. Wally J, Buchanan SK. A structural comparison of human serum transferrin and human lactoferrin. Biometals. 2007 Jun;20(3-4):249-62. doi: 10.1007/s10534-006-9062-7, PMID 17216400.
16. Ratledge C, Dover LG. Iron metabolism in pathogenic bacteria. Annu Rev Microbiol. 2000;54:881-941. doi: 10.1146/annurev.micro.54.1.881, PMID 11018148.
17. Quadri LE. Assembly of aryl-capped siderophores by modular peptide synthetases and polyketide synthases. Mol Microbiol. 2000;37(1):1-12. doi: 10.1046/j.1365-2958.2000.01941.x, PMID 10931301.
18. Miethke M, Marahiel MA. Siderophore-based iron acquisition and pathogen control. Microbiol Mol Biol Rev. 2007;71(3):413-51. doi: 10.1128/MMBR.00012-07, PMID 17804665.
19. Ratledge C. Iron, Mycobacteria and tuberculosis. Tuberculosis (Edinb). 2004;84(1-2):110-30. doi: 10.1016/j.tube.2003.08.012, PMID 14670352.
20. Wren BW, Stabler RA, Das SS, Butcher PD, Mangan JA, Clarke JD, *et al*. Characterization of a haemolysin from *Mycobacterium tuberculosis* with homology to a virulence factor of *Serpulina hyodysenteriae*. Microbiology (Reading). 1998;144(5):1205-11. doi: 10.1099/00221287-144-5-1205, PMID 9611795.
21. Snow GA. Mycobactins: iron-chelating growth factors from mycobacteria. Bacteriol Rev. 1970;34(2):99-125. doi: 10.1128/br.34.2.99-125.1970, PMID 4918634.
22. De Voss JJ, Rutter K, Schroeder BG, Barry CE 3rd. Iron acquisition and metabolism by mycobacteria. J Bacteriol. 1999;181(15):4443-51. doi: 10.1128/JB.181.15.4443-4451.1999, PMID 10419938.
23. Ratledge C, Patel PV, Mundy J. Iron transport in *Mycobacterium smegmatis*: the location of mycobactin by electron microscopy. J Gen Microbiol. 1982;128(7):1559-65. doi: 10.1099/00221287-128-7-1559, PMID 6181183.
24. Adilakshmi T, Ayling PD, Ratledge C. Mutational analysis of a role for salicylic acid in iron metabolism of *Mycobacterium smegmatis*. J Bacteriol. 2000;182(2):264-71. doi: 10.1128/JB.182.2.264-271.2000, PMID 10629169.
25. Quadri LE. Biosynthesis of mycobacterial lipids by polyketide synthases and beyond. Crit Rev Biochem Mol Biol. 2014;49(3):179-211. doi: 10.3109/10409238.2014.896859, PMID 24625105.
26. Ratledge C, Ewing M. The occurrence of carboxymycobactin, the siderophore of pathogenic mycobacteria, as a second extracellular siderophore in *Mycobacterium smegmatis*. Microbiology (Reading). 1996;142(8):2207-12. doi: 10.1099/13500872-142-8-2207, PMID 8800816.
27. Dragset MS, Poce G, Alfonso S, Padilla-Benavides T, Ioerger TR, Kaneko T, *et al*. A novel antimycobacterial compound acts as an intracellular iron chelator. Antimicrob Agents Chemother. 2015;59(4):2256-64. doi: 10.1128/AAC.05114-14, PMID 25645825.
28. Gobin J, Moore CH, Reeve JR Jr, Wong DK, Gibson BW, Horwitz MA. Iron acquisition by *Mycobacterium tuberculosis*: Isolation and characterization of a family of

iron-binding exochelins. Proc Natl Acad Sci U S A. 1995;92(11):5189-93. doi: 10.1073/pnas.92.11.5189, PMID 7761471.

29. Wong DK, Gobin J, Horwitz MA, Gibson BW. Characterization of exochelins of *Mycobacterium avium*: Evidence for saturated and unsaturated and for acid and ester forms. J Bacteriol. 1996;178(21):6394-8. doi: 10.1128/jb.178.21.6394-6398.1996, PMID 8892850.
30. Sharman GJ, Williams DH, Ewing DF, Ratledge C. Determination of the structure of exochelin MN, the extracellular siderophore from *Mycobacterium neoaurum*. Chem Biol. 1995;2(8):553-61. doi: 10.1016/1074-5521(95)90189-2, PMID 9383459.
31. Sharman GJ, Williams DH, Ewing DF, Ratledge C. Isolation, purification and structure of exochelin MS, the extracellular siderophore from *Mycobacterium smegmatis*. Biochem J. 1995;305(1):187-96. doi: 10.1042/bj3050187, PMID 7826328.
32. Krithika R, Marathe U, Saxena P, Ansari MZ, Mohanty D, Gokhale RS. A genetic locus required for iron acquisition in *Mycobacterium tuberculosis*. Proc Natl Acad Sci U S A. 2006;103(7):2069-74. doi: 10.1073/pnas.0507924103, PMID 16461464.
33. Quadri LE, Sello J, Keating TA, Weinreb PH, Walsh CT. Identification of a *Mycobacterium tuberculosis* gene cluster encoding the biosynthetic enzymes for assembly of the virulence-conferring siderophore mycobactin. Chem Biol. 1998;5(11):631-45. doi: 10.1016/s1074-5521(98)90291-5, PMID 9831524.
34. Chavadi SS, Stirrett KL, Edupuganti UR, Vergnolle O, Sadhanandan G, Marchiano E, *et al*. Mutational and phylogenetic analyses of the mycobacterial mbt gene cluster. J Bacteriol. 2011;193(21):5905-13. doi: 10.1128/JB.05811-11, PMID 21873494.
35. Mori M, Stelitano G, Chiarelli LR, Cazzaniga G, Gelain A, Barlocco D, *et al*. Synthesis, characterization, and biological evaluation of new derivatives targeting MbtI as antitubercular agents. Pharmaceuticals (Basel). 2021;14(2):155. doi: 10.3390/ph14020155, PMID 33668554.
36. Sritharan M. Iron homeostasis in *Mycobacterium tuberculosis*: Mechanistic insights into siderophore-mediated iron uptake. J Bacteriol. 2016;198(18):2399-409. doi: 10.1128/JB.00359-16, PMID 27402628.
37. McM ahon MD, Rush JS, Thomas MG. Analyses of MbtB, MbtE, and MbtF suggest revisions to the mycobactin biosynthesis pathway in *Mycobacterium tuberculosis*. J Bacteriol. 2012;194(11):2809-18. doi: 10.1128/JB.00088-12, PMID 22447909.
38. Patel K, Butala S, Khan T, Suvarna V, Sherje A, Dravyakar B. Mycobacterial siderophore: A review on chemistry and biology of siderophore and its potential as a target for tuberculosis. Eur J Med Chem. 2018;157:783-90. doi: 10.1016/j.ejmech.2018.08.030, PMID 30142615.
39. Schmitt MP, Predich M, Doukhan L, Smith I, Holmes RK. Characterization of an iron-dependent regulatory protein (IdeR) of *Mycobacterium tuberculosis* as a functional homolog of the diphtheria toxin repressor (DtxR) from Corynebacterium diphtheriae. Infect Immun. 1995;63(11):4284-9. doi: 10.1128/iai.63.11.4284-4289.1995, PMID 7591059.
40. Gold B, Rodriguez GM, Marras SA, Pentecost M, Smith I. The *Mycobacterium tuberculosis* IdeR is a dual functional regulator that controls transcription of genes involved in iron acquisition, iron storage and survival in macrophages. Mol Microbiol. 2001;42(3):851-65. doi: 10.1046/j.1365-2958.2001.02684.x, PMID 11722747.
41. Greenwood KT, Luke RK. Enzymatic hydrolysis of enterochelin and its iron complex in *Escherichia coli* K-12. Properties of enterochelin esterase. Biochim Biophys Acta. 1978;525(1):209-18. doi: 10.1016/0005-2744(78)90216-4, PMID 150859.
42. Wells RM, Jones CM, Xi Z, Speer A, Danilchanka O, Doornbos KS, *et al*. Discovery of a siderophore export system essential for virulence of *Mycobacterium tuberculosis*. PLOS Pathog. 2013;9(1):e1003120. doi: 10.1371/journal.ppat.1003120, PMID 23431276.
43. Domenech P, Reed MB, Barry CE 3rd. Contribution of the *Mycobacterium tuberculosis* MmpL protein family to virulence and drug resistance. Infect Immun. 2005;73(6):3492-501. doi: 10.1128/IAI.73.6.3492-3501.2005, PMID 15908378.
44. UniProt n.d. [cited Aug 25, 2022] Available from: https://www.uniprot.org/.
45. Harrison AJ, Yu M, Gårdenborg T, Middleditch M, Ramsay RJ, Baker EN, *et al*. The structure of MbtI from *Mycobacterium tuberculosis*, the first enzyme in the biosynthesis of the siderophore mycobactin, reveals it to be a salicylate synthase. J Bacteriol. 2006;188(17):6081-91. doi: 10.1128/JB.00338-06, PMID 16923875.
46. Manos-Turvey A, Bulloch EM, Rutledge PJ, Baker EN, Lott JS, Payne RJ. Inhibition studies of *Mycobacterium tuberculosis* salicylate synthase (MbtI). ChemMedChem. 2010;5(7):1067-79. doi: 10.1002/cmdc.201000137, PMID 20512795.
47. Zwahlen J, Kolappan S, Zhou R, Kisker C, Tonge PJ. Structure and mechanism of MbtI, the salicylate synthase from *Mycobacterium tuberculosis*. Biochemistry. 2007;46(4):954-64. doi: 10.1021/bi060852x, PMID 17240979.
48. Mori M, Stelitano G, Gelain A, Pini E, Chiarelli LR, Sammartino JC, *et al*. Shedding X-ray light on the role of magnesium in the activity of *Mycobacterium tuberculosis* salicylate synthase (MbtI) for drug design. J Med Chem. 2020;63(13):7066-80. doi: 10.1021/acs.jmedchem.0c00373, PMID 32530281.
49. Liu Z, Liu F, Aldrich CC. Stereocontrolled synthesis of a potential transition-state inhibitor of the salicylate synthase MbtI from *Mycobacterium tuberculosis*. J Org Chem. 2015;80(13):6545-52. doi: 10.1021/acs.joc.5b00455, PMID 26035083.
50. Chi G, Manos-Turvey A, O'Connor PD, Johnston JM, Evans GL, Baker EN, *et al*. Implications of binding mode and active site flexibility for inhibitor potency against the salicylate synthase from *Mycobacterium tuberculosis*. Biochemistry. 2012;51(24):4868-79. doi: 10.1021/bi3002067, PMID 22607697.
51. Manos-Turvey A, Cergol KM, Salam NK, Bulloch EM, Chi G, Pang A, *et al*. Synthesis and evaluation of *M. tuberculosis* salicylate synthase (MbtI) inhibitors designed to probe plasticity in the active site. Org Biomol Chem. 2012;10(46):9223-36. doi: 10.1039/c2ob26736e, PMID 23108268.
52. Ferrer S, Martí S, Moliner V, Tuñón I, Bertrán J. Understanding the different activities of highly promiscuous MbtI by computational methods. Phys Chem Chem Phys. 2012;14(10):3482-9. doi: 10.1039/c2cp23149b, PMID 22307014.
53. Vasan M, Neres J, Williams J, Wilson DJ, Teitelbaum AM, Remmel RP, *et al*. Inhibitors of the salicylate synthase (MbtI) from *Mycobacterium tuberculosis* discovered by high-throughput screening. ChemMedChem. 2010;5(12):2079-87. doi: 10.1002/cmdc.201000275, PMID 21053346.
54. Maganti L, Grandhi P, Ghoshal N. Integration of ligand and structure-based approaches for identification of novel MbtI inhibitors in Mycobacterium tuberculosis and molecular dynamics simulation studies. J Mol Graph Model. 2016;70:14-22. doi: 10.1016/j.jmgm.2016.09.004, PMID 27639087.
55. Zhang XK, Liu F, Fiers WD, Sun WM, Guo J, Liu Z, *et al*. Synthesis of transition-state inhibitors of chorismate utilizing enzymes from bromobenzene cis-1,2-dihydrodiol. J Org Chem. 2017;82(7):3432-40. doi: 10.1021/acs.joc.6b02801, PMID 28282140.
56. Pini E, Poli G, Tuccinardi T, Chiarelli LR, Mori M, Gelain A, *et al*. New chromane-based derivatives as inhibitors of *Mycobacterium tuberculosis* salicylate synthase (MbtI): preliminary biological evaluation and molecular modeling studies. Molecules. 2018;23(7):1506. doi: 10.3390/molecules23071506, PMID 29933627.
57. Chiarelli LR, Mori M, Barlocco D, Beretta G, Gelain A, Pini E, *et al*. Discovery and development of novel salicylate synthase (MbtI) furanic inhibitors as antitubercular agents. Eur J Med Chem. 2018;155:754-63. doi: 10.1016/j.ejmech.2018.06.033, PMID 29940465.
58. Chiarelli LR, Mori M, Beretta G, Gelain A, Pini E, Sammartino JC, *et al*. New insight into structure-activity of furan-based salicylate synthase (MbtI) inhibitors as potential antitubercular agents. J Enzyme Inhib Med Chem. 2019;34(1):823-8. doi: 10.1080/14756366.2019.1589462, PMID 30889995.
59. Walhekar V, Bagul C, Kumar D, Muthal A, Achaiah G, Kulkarni R. Topical advances in PIM kinases and their inhibitors: Medicinal chemistry perspectives. Biochim Biophys Acta Rev Cancer. 2022;1877(3):188725. doi: 10.1016/j.bbcan.2022.188725, PMID 35367531.

Cite this article: Bire N, Walhekar V, Makane O, Yelchatwar P, Muthal A, Bodhankar S, *et al*. Molecular Insights into Salicylate Synthase: From Medicinal Chemistry Perspective. Indian J of Pharmaceutical Education and Research. 2024;58(1s):s58-s66.