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Review Article

Deciphering Molecular Insights into Isocitrate Lyase and their Inhibitors: A Detailed Display through Medicinal Chemistry Window

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ABSTRACT

Aim: Isocitrate lyase is the ancient glyoxylate cycle enzyme that catalyzes isocitrate cleavage into succinate and glyoxylate. The glyoxylate cycle is a key metabolic pathway as indicated by significant data on many host pathogens, which confirm the fundamental existence, in particular, causing life-threatening diseases, including tuberculosis. **Objectives:** Mycobacterium tuberculosis Isocitrate lyase and other organisms, which express ICL, have attracted the attention of molecular biologists and medicinal chemists due to their indispensable role in normal cellular metabolic pathways and even resistance. Numerous studies that blocked genes expressing ICL and gene knockout in experimental animals indicated retarded growth. The origins of these inhibitor studies approaches are categorized into mycobacterial and non-mycobacterial Isocitrate lyases. **Conclusion:** Nonetheless, these inhibitors can be either synthetic or natural, while in this study, the authors emphasize catalytic enzyme information of Isocitrate lyase.

Keywords: Isocitrate Lyases, Mycobacterium tuberculosis, Glyoxylate cycle, Krebs cycle.

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INTRODUCTION

Isocitrate Lyase (ICL) is an enzyme that catalyzes glyoxylate cycle cleavage from isocitrate to glyoxylate. This is also referred to as isocitrate, isocyritase, isocitratase, glyoxylate-lyase threo-DS-isocitrate and glyoxylate-lyase isocitrate.[1] Campbell *et al*., have first identified the catalytic isocitrate lyase in Pseudomonas aeruginosa as a reversible cleavage of DS-isocitrate.[2]

As degradation of oxygen and nutrients in Tricarboxylic Acid (TCA) and Krebs cycle occurs, an alternative mechanism for gluconeogenesis exists which is known as the glyoxylate cycle. The glyoxylate cycle has been identified by Kornberg and Madsen as a "modified tricarboxylic acid cycle," which involves dehydrogenase, citrate synthase and aconitases. ICL is not only important for human cellular biochemical cycle but also essential for seed germination particularly in higher plants, microbial pathogenicity and sustainability.[3]

According to enzyme biochemistry, ICL an enzyme that causes the threo-DS-isocytic acid of an aldol to be separated into succinate and glyoxylic acid. So, one substratum, i.e. isocitrate and two intermediates; succinate and glyoxylate can be said for this enzyme. ICL is also first glyoxylate enzyme in metabolism that is keys to carbohydrate synthesis. The glyoxylate cycle helps to overcome several TCA cycle beta-oxidation steps.

Role of ICL

Isocitrate lyase is an oxoacid lyase that possesses the ability to cleave carbon-carbon bonds. Glyoxylate cycle is a metabolic pathway, which assists in the synthesis of carbohydrates. There are a few similarities amid Krebs cycle and glyoxylate shunt as depicted in Figure 1. The glyoxylate shunt is a shortcut for gluconeogenesis, as an excess of oxaloacetate is used to produce glucose. It bypasses two carbon dioxide generating steps that also allow the bacteria to sustain. The conversion of isocitrate to succinate and glyoxylate is catalyzed by isocitrate lyase; this is then followed by addition of acetyl Co-A to glyoxylate which

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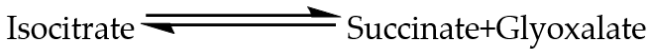
then synthesizes malate in the presence of the enzyme Malate Synthase (MS). Malate synthase and isocitrate lyase are the two most important biocatalysts of glyoxylate shunt.

The resemblance of the glyoxylate cycle to the TCA cycle is seen in the initial phase of the cycle as acetyl CoA is the only substrate present in both the respective cycles. Although the source of acetyl CoA is dissimilar for both the cycles, the point at which TCA and glyoxylate can be differentiated is when acetyl CoA is transformed to isocitrate.



Malate synthase catalyzes the glyoxylate-acetyl CoA condensation reaction. It reacts to a thioester by the carbonyl glyoxylate and methyl acetyl CoA group. This is then hydrolyzed to generate L-malate and HSCoA. The glyoxylate cycle's net role is to generate C4 compounds from C2, i.e., two acetyl CoA's in each cycle. In addition to the energy output, the glyoxylate process is a sequence of reactions involving the conversion of acetates into succinates. For bacteria and fungi, they thrive on this acetate because it is the main carbon source within the macrophages under aggressive conditions where there is no glucose. Therefore, skipping the two beta-oxidation steps to preserve carbon for sufficient supply of TCA intermediates is healthy and acceptable for the biosynthesis of carbohydrates in a few micro-organisms.[4]

Presence of ICL in Nature

A simplified form of TCA is a glyoxylate loop that requires two enzymes, i.e. Malate synthase and isocitrate lyase and later is widely spread across plants, bacteria, fungi, protozoans and algae. The existence of some microorganisms on compounds that are metabolically similar to butyrate contributes to the role of the process of glyoxylate.[5] Acetate conversion to acetyl-CoA is catalyzed in presence of acetyl-coenzyme A. The glyoxylated loop synthesis of acetyl-CoA helps to synthesize malate and succinate. Many microorganisms that cause severe human illness require enzymes including MS and ICL, like Mycobacteriaceae responsible for tuberculosis and leprosy diseases, Pseudomonadaceae responsible for diseases from hospital; Trypanosomatidae containing the five species of Leishmania, an insidious human disease; Saccharomycetaceae a sophisticated family of yeasts and fungi, which cause many fungal diseases. For certain cases including protozoa and mycobacteria, a functioning glyoxylate cycle allow us to regulate pathogens because it is believed that this mechanism does not operate for humans. ICL-directed inducers such as nitropropionate and itaconate help to regulate the development of many glyoxylated microorganisms with a cycle.[6] Recently, it has been documented that this pathway is chief to microbial rancor for fungal and bacterial domains microorganisms.[7] Here is a comprehensive description of glyoxylate pathway regulation in different microorganisms (Table 1).

Mycobacterium tuberculosis

A gram-negative bacterium that has been afflicting human beings for thousands of years. This is an intracellular pathogen that is naturally resistant to most antibiotics. The precise factors that intercede with this tolerance are not well established. Nandakumar and colleagues used metabolomic profiling to classify a specific set of metabolic alterations associated with three commonly used and widely marketed anti-tubercular drugs, isoniazid, rifampicin and streptomycin activities. Surprisingly, given the cellular approach, all three drugs cause MTB's ICLs activation. In an extensive review, possible mechanistic attributes for antibiotic tolerance (*in vitro*) MTB were described.[8] The deficient strain of ICL was tested in a MTB mouse model and was one of the most strongly attenuating MTB mutants. TB catalyzes only two common biochemical reactions; both utilize the same active site and play similar roles in the metabolism of fatty acids.[9] Further functions have been identified for ICL in nutrient hunger and hypoxia acclimatization, which are not related to metabolism of fatty acid but are similar to conditions that MTB is thought to have in the host. In another study, a pathway closely linked to the glyoxylate cycle was discovered and identified as being involved in fatty acid metabolism, called the citramalate cycle. Compared to the wild type strains of MTB in the above-mentioned three anti-tubercular drugs, the weakness of the strain with ICL deficiency was more demonstrated. The resistance was chemically saved when MTB was co-incubated along with an antioxidant. Such data indicate that attenuating effects of MTB's ICL deficiency could lead to multiple physiological functions being deprived. Such findings helped to define the role of MTB's ICLs as a key component in antioxidant defense mechanism.[10]

ICL Crystal Structure

To reconnoiter the mechanism of isocitrate lyase inhibition, it is significant to understand its function in conjunction with the microorganism. These crystal structures enable us to understand the active position within the molecule and the important amino acids that bind with the ligand (inhibitor), which play a decisive role in the inhibition of enzymes. The last decade of twentieth century has revealed the insights of amino acid sequence and crystal structures of ICL from numerous organisms. Among all the studied, ICL from E. coli was highly established, which showed four highly conserved regions 177 to 201, 231 to 241, 310 to 324 and 348 to 356. K193, 194, H184 and 197 were two amino acids conserved in the first conserved region. Site directed mutagenic studies showed that K194 and S319 were found to be functional conserved amino acids whereas C195 was extremely important for catalytic activity.

Crystal structure of MTB ICL

Target processes for traditional antibiotics are typically based on bacterial cell wall growth and division, chromosome replication and cell wall biosynthesis. Nonetheless, in combination with the

current anti-TB therapy isocitrate lyase inhibitors can help to accelerate the eradication of this infection. Sharma and colleagues carried out an exhaustive analysis of MTB ICL three-dimensional structure. In a combination of 3-Bromopyruvate and 3-Nitropropionate, which are the basic prototype inhibitors, tuberculosis isocitrate lyase recognizes the properties of this enzyme.[11]

Binding sites of 3-Bromopyruvate and 3-Nitropropionate

Ternary complex of ICL with glyoxylate and 3-Nitropropionate, the non-reactive succinate, is useful for clarifying the mechanical properties. Determination of structure of mutant ICL in which a nuclear active site C191 has been converted into an ICL to achieve nitropropionate and glyoxylate binding stability. ICL active site was located at the ends of the C-terminal of the β-beaches from among α/β containers. Apparent density for glyoxylate, 3-Nitropropionate and Mg^{2+} was observed in non-crystallographic symmetry and different maps were contoured. Glyoxylate has been successfully bound by interaction and hydrogen association with the residues of the protein S91 OG, G92 N, W93 N and R228 NH2 to the active site Mg^{2+} ion (Figure 2). They were unable to make a distinction between the 3-Nitrpropionate carboxylate and nitro groups. Succinate molecule that formed unique hydrogen bonds between one group of carboxylates and the N313 ND1, E295 OE2, R228 NH1 and G192 N residual side chains. In comparison, Hydrogen bonds were formed in the 2nd Carboxylate with T347 OG, N313 ND2, S315 OG, S317 OG and H193 ND1 (Figure 2). The succinate C2 and C3 methylene carbons filled the protein surface with W93, T347 and L348 residues. The orientation formed was 3.9 Å from C2 succinate to glyoxylate aldehyde carbon and 3.2 Å from the S191 hydroxyl group of the mutant C191S.

3-Bromopyruvate triumphantly inhibits ICL by dehalogenation of inhibitor that backs to the formation of lone covalent adduct with the active site nucleophile C191. The second carboxylate of succinate had previously been found to be composed of pyruvyl and hydrogen bonded to remaining side chains of H193 ND1, Asn313 ND2, S315 OG, S317 OG, T347 OG1 and one water molecule. Residue C191 in the mutant C191S is adopted as an almost identical conformation with Ser191, suggesting no further rearrangement of the amino acid residues in the active site for the pyruvyl movement. Within the aforementioned complex, the

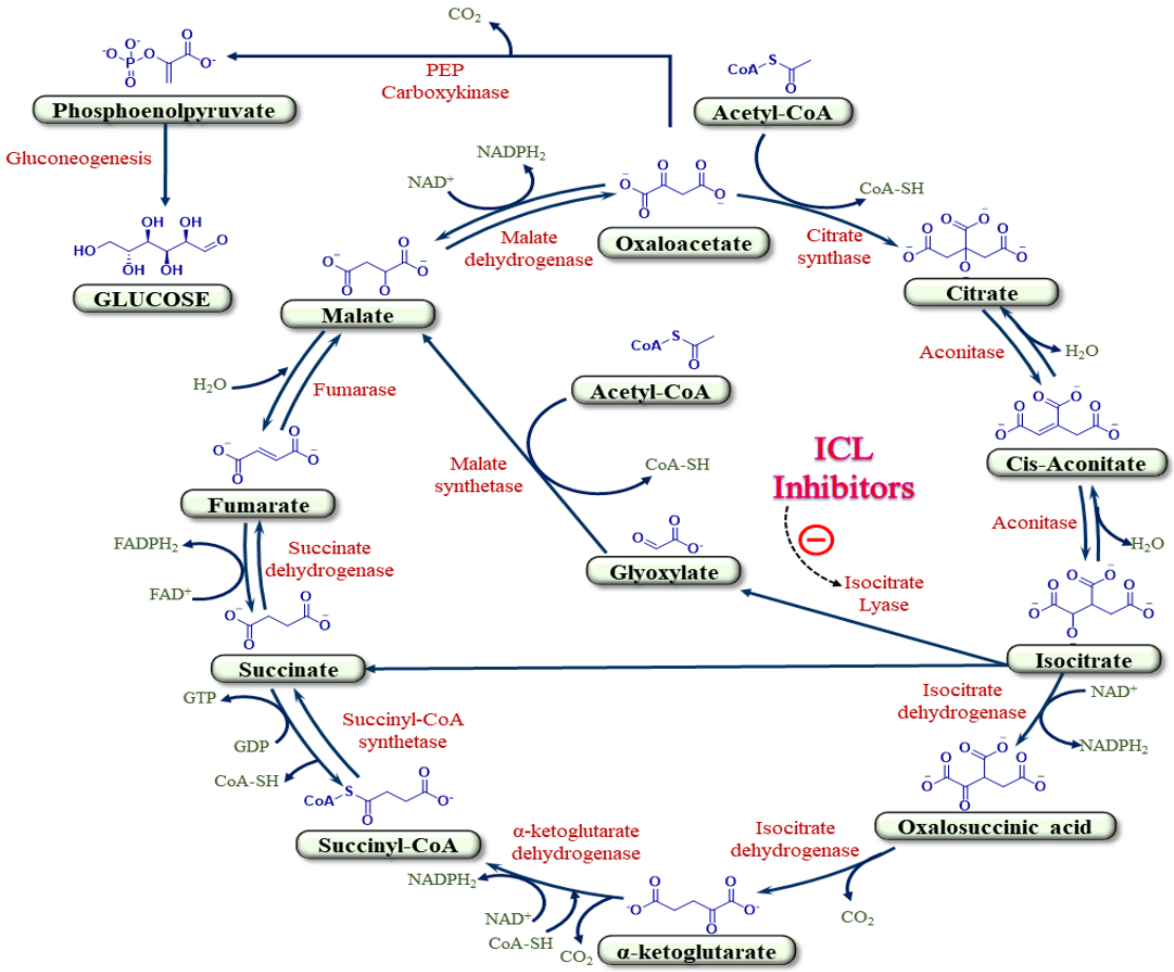


Figure 1: Isocitrate lyase's role in the glyoxylate shunt.

Table 1: List of microorganisms in which isocitrate lyase is detected. It also contains the essentiality of ICL towards the respective microorganism.

Sl. No.	Name of Microorganism	Host	Disease caused	Essentiality of ICL enzyme for pathogenicity
1.	*Candida albicans*	Humans	Candidiasis	Essential for pathogenicity.
2.	*Leptosphaeria maculans*	Crucifer plants	Blackleg of crucifer plants.	Essential for pathogenicity.
3.	*Magnaporthe grisea*	Agriculturally important crops, rice, barley, rye, wheat.	Generation of appressorium.	Essential for pathogenicity.
4.	*Stagonospora nodorum*	Wheat and other agriculturally important crops	Leaf and glume blotch disease.	Essential for pathogenicity.
5.	*Colletotrichum lagenariu*	Agriculturally important plants like mango, cucumber, watermelon, etc.,	Anthracnose	Only required at the initial stage of infection.
6.	*Paracoccidioides brasiliens*	Humans	Paracoccidioidomycosi	Essential for pathogenesis.
7.	*Talaromyces marneffe*	Humans	Systemic mycosis	Essential for pathogenesis.
8.	*Salmonella enteric*	Humans and mice	Typhoid fever	Essential for perseverance during chronic infection, but not for acute lethal infection in mice.
9.	*Rhodococcus equi*	Foals, cats, dogs and pigs.	Pyogranulomatous pneumoni.	Essential for pathogenesis.
10.	*Rhodococcus fascian*	Monocots and dicots.	Leafy gall disease.	Essential for pathogenesis.
11.	*Pseudomonas aeruginosa*	Humans	Cystic fibrosis.	Essential for pathogenesis.
12.	*Bradyrhizobium japonicum*	Soybean	Nitrogen fixing and legume-root nodulation.	Essential for pathogenesis.
13.	*Sinorhizobium melilot*	Soil	Nitrogen fixing symbiosis.	Not essential for the Rhizobium-legume symbiosis.
14.	*Mycobacterium tuberculosis*	Humans	Tuberculosis	Essential for pathogenicity.
15.	*Dianthus caryophyllus*	Petals of senescent plants	Petal senescence	Essential for plant cycle.
16.	*Musa cavendishii*	Irradiated banana	Delay ripening	Essential to delay ripening in irradiated banana.
17.	*Aspergillus fumigates*	Humans	Chronic pulmonary infection, or allergic disease in immuno competent host.	Not essential for pathogenesis.
18.	*Cryptococcus neoformans*	Humans and animals	Cryptococcal meningitis.	Not essential for pathogenesis.

binding site for glyoxylate has been identified with the solvent molecules that also coordinate the Mg^{2+} ion (Figure 3).[11]

Conformational changes induced by inhibitors

When the indigenous structure and the ICL inhibitor bound forms were juxtaposed, a huge conformational difference was defined, particularly in the two areas which control the entry into the active site. The first region consisted of a complex site loop comprising the residue of 185-196 ICL signature sequences; the second field comprising the residues 411-428 (last 18 residues). While the apoenzyme was found in open conformation, the C91 in the active site loop was noticed to be comparatively distant from other catalytic residues and highly accessible to solvents. In the 'open' conformation, the active site loop was suggested to be versatile as residues H193 and L194 were identified in the asymmetric unit for their low electron density. As the inhibitor binds, mobility in the loop takes place by 10-15 Å, thus making the structure in a "closed" conformation. The access of the closed conformation bound by the inhibitor to the catalytic site is blocked by the loop of active site amino acids 185-196

in contrast to the open conformation of the free enzyme. This closure facilitated the passage of the neighboring subunits' 411-428 residues. The last 11 residues were found in a disordered state, while the apparent electron density in the inhibited enzyme crystal was defined for all excepting the C-terminus residue 428. The C-finish locks the loop of the active site into the catalytic position as the resulting orientation is right above the active site loop. This two-step conformational rearrangement is attributed to Mg^{2+} conformational drift by 2.5 Å bonded in a strongly electronegative depression in the apoenzyme formed by D108, D153, K155 and K182. This Mg^{2+} activation occurs because of succinate binding, allowing Lys189 of active site loop to create electrostatic interactions confined in the region while allowing the active site loop to close on bound substrates.

The findings above have been outlined in the following points. Where either nitropropionate occupied the binding site or the pyruvyl movement, the loop closure occurs only and suggests that succinate in addition to glyoxylate is necessary to induce loop motion residence. In both complexes, the interactions that are responsible for closure can be reduced to the typical carboxylate banding in the pocket of the residue H193, N313, S315, S317 and T34, since glyoxylate is often used as crystals of 3-Bromopyruvate. Once both residues are binding, they suffer repercussions, except for the T347 residue, which is located in both bonded and borderless states. The residues of N313, S315 and S317 at the end of the β14 strand of C-terminals shift from 1 to 2 Å when it is binding, while H193 is labelled with a versatile active site circuit. The active site C191 was found to be deprotonated by 193.

MTB ICL as the catalytic basis for bacterial mode inhibition was reviewed by Park and coworkers, which states the coalition of ligand with binding structural forms of ICL. Sharma and coworkers concluded that ICL-focused drugs function in combination with currently available medicines and significantly reduce the chemotherapy duration. It also shows that successful drug screens against intracellular pathogens are very much required to assess the differences between *in vitro* and *in vivo* microbial metabolism. This increased the attractive potential for ICL inhibitors to be effective not just for treating *M. Tuberculosis*, but also other recurrent infections.[11]

Structure of ICL2

Leung *et al.*, reported ligand free ICL2 crystal structure derived from Mtb CDC1551 developed at resolution of 1.8 Å. The crystal structure is tetramer of A4 type with 766 residues in each monomer resulting in ~200 Å extended structure. The flexible linker (residues 591-602) connects N-terminal (residues 1-590) and C-terminal (residues 603-766) domains. In similar fashion to ICL1, N-terminal domain is constituted with α/β core and conserved catalytic motif 213KKCGH217 and an extra helical substructure (helices α10-α16; amino acid residues 278-427) which is absent in ICL1. A peculiar C-terminal was found, and such type of structure is uncommon in other bacterial strains. A barrel-like structure is formed from C terminal ends of two monomers.[12]

Crystal structure of Magnaporthe oryzae and *Fusarium graminearum* ICL

Worldwide large annual economic losses are caused by the two most damaging pathogens *Magnaporthe oryzae* and *Fusarium graminearum*. The former is a pathogen of corn, while the latter causes Fusarium headband in wheat, barley and corn. Research by Wang and colleagues found that ICL was deficient in *M. oryzae* mutants show reduced virulence when compared to their corresponding wild type strains, which suggest that ICL is the focus of antifungal agents' production. The position of the cysteine

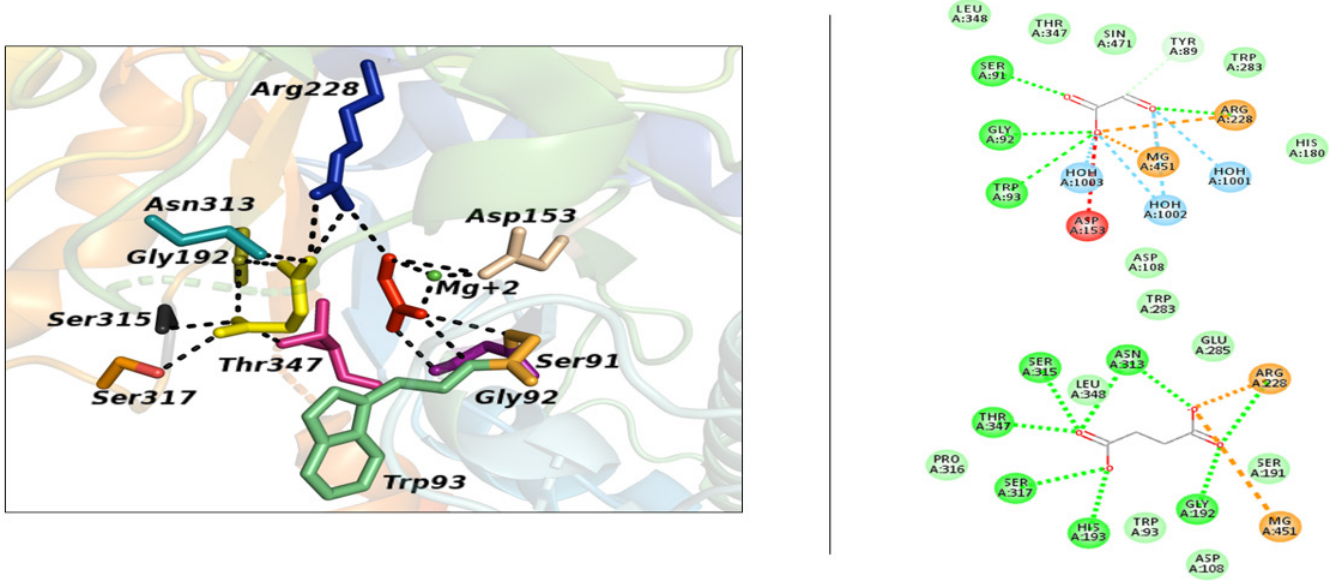


Figure 2: 2a and 2b) 3D and 2D interactions of glyoxylate and succinate in inhibition of *M. tuberculosis* ICL by 3-nitropropionate (PDB: 1F8I).

in the catalytic loop of M. oryzae is seen in the last section. The ligand-free structure of *Magnaporthe oryzae* ICL (MoICL) was identified as well and the ICL structure of *Fusarium graminearum* (FgICL) complexed with Mg^{2+} and malonate was involved with Mg^{2+}, glyoxylate and glycerol. Also known for catalytical loop and C-terminal region, ligand-induced conformation changes were unveiled. Detailed information on structural and functional analyses of fungal ICLs has been reported in an activity study using various mutant ICLs.[13]

MoICL-Mg^{2+}-glyoxylate-glycerol-complex active site and ligand-induced conformational changes

In the break of four crystallographically distinct subunits, the binding site for a ligand free metal ion was allowable. In subsequent structural determinations of both quaternary complex (MoICL-Mg^{2+}-glyoxylate-glycerol) and ternary complex (FgICL-Mn^{2+}-malonate) validation tests were performed for the presence of a metal ion at a propound site. The location of metal binding site was found at around 13 Å profound from the barrel boundary. Mg^{2+} was probably the metal ion in complex structure of MoICL as the crystallization was done with 2 mM $MgCl_2$.[13]

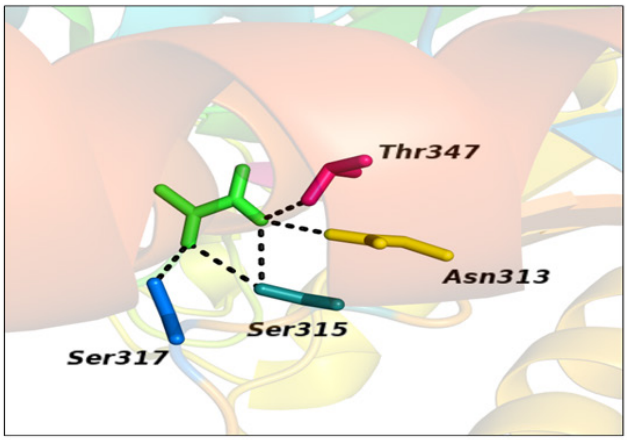
The isocitrate substrate buffer was used to soak MoICL crystal to define the binding locations at the proposed active position for ICL (Mg^{2+} and glyoxylate) products and the chemical glycerol used for cryoprotection. Because of its univocal electron density, the above quaternary complicated information for metal coordination has shown the binding mode of glycerol, glyoxylate and catalytic loop stabilization (the area between 4th β4 and its α8-containing catalytic residue C215). In comparison to the stable structure of the quaternary complex, in ligand-free form, the catalytic loop region was strongly disordered.[13]

Locking process of metal and ligands

The movement of glyoxylate was directly coordinated with metal ion in 4 subunits, while glycerol binding was observed in three subunits only. In the 2.5 Å lengths of potential ligands, the metal ion coordinating shell supported an octahedral structure. In interior, residue D177 and the one water molecule have been used for the axial ligands which are closely connected to the barrel folding opening along with two well defined water molecules supporting equatorial ligands and two carbonyl oxygen atoms of glyoxylate. The axial water molecule, which coordinated with metal, was situated adjacent to the second ligand and glycerol binding site by 2.6 Å. Nevertheless, glyoxylate molecule was located on the equatorial plane. Near the catalytic loop at the active site entrance was identified the glycerol binding site. Specific interactions on the active site via the residues of the ICLs family appeared to regulate the binding of glyoxylate and glycerol. Y104, S106, H204 and R252 (Figure 4) were in a gap of 3.5 Å of glycerol connected within 3.5 Å of the C215, R252, E405, N433, S435 and T467 residues of the side chain. Various contacts were made by central atoms in the newly described catalytic loop with glycerol.[13]

The catalytic loop stabilization

One of the remarkable structural characteristics in the quaternary complex containing K213 to A219 was catalytic loop stabilization. As seen earlier, the catalytic loop was extremely disordered in a ligand-free shape, creating empty space in the active site completely for solvent. However, the binding between glyoxylate and glycerol stabilized the loop. In the absence of glycerol in one subunit the catalytic loop was still disordered, it was thus concluded that the binding site of the glycerol was critical for catalytic loop stabilization. Following these observations, extensive interactions between glycerol and catalytic loop residues were observed. No apparent differences were found in active site residues after ligand



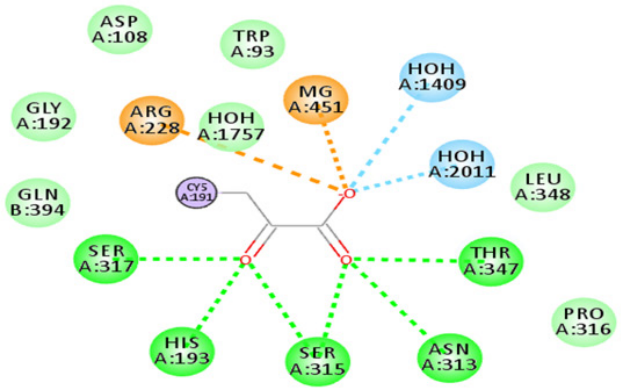


Figure 3: 3a and 3b) 3D and 2D interactions of pyruvate in inhibition of *M. tuberculosis* ICL by 3-bromopyruvate (PDB: 1F8M).

binding, except for the above-mentioned interactions. For the 513 Cα atoms, the RMSD was calculated between the ligand-free form and the quaternary complex.[13]

Malonate binding and conformational changes of the FgICL- Mn^{+2}-malonate complex

Only in unit A was the Mn^{+2} binding and malonate found, which had the catalytic loop stabilized. The catalytic loop, which is in subunit B, was degraded and metal ion and malonate were both absent in the active site. The eventually organized region was the C-terminal area. The regulated C-terminal region which is in subunit B was component of active site present in subunit A, contributing to the complete closure of the active site of subunit A, which includes Mn^{+2} ion and malonate. The later found region of the C-terminal consisted of the V526-F545 residue of subunit B, which was almost identical to that of the forging a previous C-terminal. Newly established interactions, including catalytic loops, were observed between residues on the edge of the active site entrance and this C-terminal region. The metal ion binding site was similar to the quaternary complex (MoICL-Mg^{2+}-glyoxylate-glycerol). The equatorial positions of the two water molecules were not glyoxylated, which resulted in an octahedral arrangement coordinated with all the water molecules except for the axial ligand and D177. The correlation was found between the malonate and glyoxylate binding sites. It was associated not only with residues of the seventh β7 and its linking helix but also with residues of the catalytic loop. Bonding with S106, C215, H217, N432, S434, S436 and T466 may be rendered by malonate as shown in Figure 5.[13]

ICL inhibitors

For all the species which contain this enzyme, ICL for MTB receives the greatest attention as it is connected with the most serious infection in over 30% of the world population.[14] As we know, MTB survives with the collusion of various metabolic pathways at both stages, i.e. active and dormant. Identifying a rife drug target that takes advantage of various metabolic paths is difficult for both phases. Therefore, MTB's drug target analysis is critical for increasing stage.[15] MTB ICL is considered one of the most stubborn and difficult organisms/enzymes because its growth is sluggish, and the risk of infection is increased. To research the live MTB strain, training and bio-safety level three facilities are required. Many scientists have therefore proposed several strategies to inhibit ICL. The source of these inhibitor studies strategies is divided into tubercular and Non-tubercular ICL. These inhibitors can never be less synthetic or human.[16]

Synthetic MTB ICL Inhibitors

In addition to the acquisition of synthetic compounds, the usual strategy advice is given for obtaining analog or derivatives of the existing potential inhibitor, regardless of MTB or non-MTB ICL. This helps both to find new inhibitors and to enhance the potential of existing inhibitors.

Sriram and coworkers synthesized and evaluated different 3-Nitropropionamides for log and starvation process crops *Mycobacterium tuberculosis, Mycobacterium smegmatis* and isocitrate lyasis *in vitro* activities. The 1 (1-Cyclopropyl-7-[3,5-dimethyl-4-(3-nitropropanoyl) piperazin-1-yl]-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid) was the most potent compound with a MIC value of 0.16 and a MIC value of 0.04 μM/mL against log and starvation culture of MTB, respectively. Other amides showed an increase in MIC values of 287 μM/mL precisely and a value above 257.5 μM/mL against dormant bacilli. Methyl and methoxy electron donating groups were introduced to the aniline ring slightly better than before, while electron with drawing groups were approximately doubled. As expected, fluoroquinolone derivatives

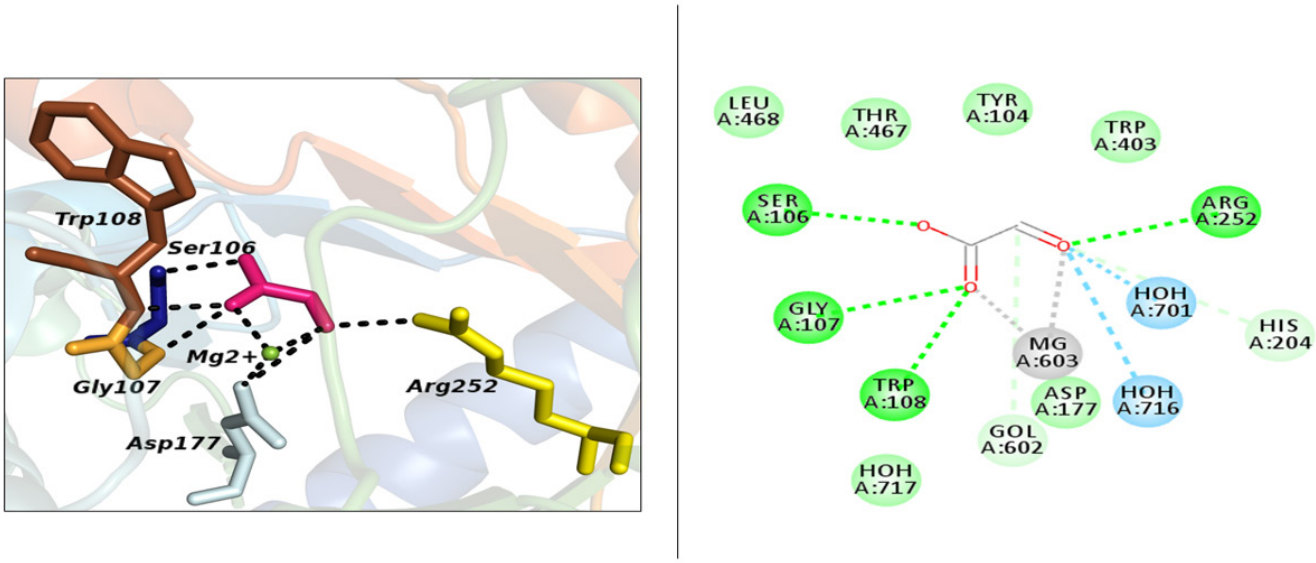


Figure 4: 4a and 4b) 3D and 2D interactions of glyoxylate in inhibition of *M. oryzae* ICL (MoICL) (PDB: 5E9G).

Table 2: Potency of Itaconic Anhydride, XHD-1 and XHD-2 against MTB ICL Activity.

Sl. No.	Name of the inhibitor	Concentration mg/mL	% Inhibition (mg/mL)
1.	Itaconic anhydride	0.03	90
2.	XHD-1	0.05	52
3.	XHD-2	0.05	75

have shown excellent activity. However, the open-chain secondary amides manifested weak activity. Four additional compounds with a concentration of 62.5 mg/mL in Vero cells were tested for their cytotoxicity; there was no toxicity in most compounds. A fluoroquinolone was non-toxic in this concentration and selectivity index of 3185 for the starved mycobacterial culture. Of the ten compounds screened, three have successfully inhibited ICL with IC_{50} values less than 1 µM with fluoroquinolone movement. Compounds with N-(4-halogen)phenyl or 4-piperazin-1-yl moieties, in comparison with standard 3-nitropropionic acid which gave the IC_{50} value of 116.0 µM, have showed better activity (36.80 µM).[17]

Ji and coworkers performed a HTS of 124 Mannich bases, one of which 2, was identified with consequential inhibitory activity against Mycobacterium tuberculosis ICL at 0.05 mg/mL concentration. The IC_{50} of this compound (2, 4-[1-(4-chlorophenyl)-5-(6-methoxynaphthalen-2-yl)-3-oxo pentylamino] benzoic acid) was assayed as 53.5 µM. With the help of simulated 3D structure of 2, it was reported that this compound might exert by wedging in the isocitrate lyase active site.[18]

Another HTS screening of 5,000 compounds performed by Zhang and colleagues where compounds with a novel potential scaffold that was chosen to reach isocitrate lyase with a clement inhibitory effect. The plum compound 3 was with an IC_{50} value of 134.4 µM and a MIC of 0.5 µg/mL. It was therefore concluded that isocitrate lyase inhibition was just a puny side-effect of this compound. *In vitro*, studies of 3 not only inhibited all sensitive strains of 0.54 µg/mL, but also most resistant isolates of less than 2.16 µg/mL. Existing cross-resistance to new TB drugs was shown to be less susceptible to XDR-TB (MIC99 4.33 µg/mL). The cytotoxicity assessment of 3 was performed using two cell lines with IC_{50} results of 60.26 µg and an estimated selectivity range of between 7.5-120. This compound will influence species that can actively reproduce together with a bacteriostatic effect. It was shown that the dosage given to MDR-TB contaminated mice was not responsible for efficacy.[19]

Sriram *et al.*, tested 2-[(4-Bromo-2-fluorobenzyl]-4-oxo-2,4-dihydro-1-phthalazinyl]acetic acid hydrazones *in vivo* and *in vitro* substituted benzaldehyde, benzophenone and acetophenone for the enzyme isocitrate lyase of mycobacterial strains. 2-[3-(4-bromo-2-fluorobenzyl)-4-oxo-3,4-dihydrophthalazine-1-yl]-N'-(4-nitrobenzylide) aceto hydrazide 4, was substituted with 4-NO_2, showed lower MIC against MTB (0.18 and less than 0.09 µg/mL log-phase and strain MDR-TB respectively). This obstructed the growth of six atypical MTB of up to 12.25 which include M. *segmentis, M. microti., M. vaccae., M. phlei., M. fortuitum* and *M. kansasii.* This compound was identified as nontoxic to Vero cells at IC_{50} of 122.5 µM. Many derivatives have affected MTB's latent community in the comparatively monumental range of 2.88 to 8.91 µM. This 25 mg/kg compound (R=4-NO_2) partially protects the lungs and spleen tissues from murine tuberculosis infection. During the SAR study, there has been an improvement in the activity by electron moieties (nitro, halogens) on the ring of benzaldehyde, while electron-donating groups have decreased it. Benzaldehydes have been shown to be superior compared to benzophenones and acetophenones, while acetopherones have been less than both groups. At 10 µM seven compounds decreased mycobacterial isocitrate lyase enzymatic activity. The most powerful inhibitors with the rate of 61.6% were the 3-Nitro derivatives (R=3-NO_2), while the other derivatives displayed a reduction in ability of R=2-CF_3; 4-NO_2; 4-Br; 4-F with a comparatively inhibitory level of approximately 45-55%.[20]

New 2-[3-(4-Bromo- 2-fluorobenzyl)-4-oxo-3, 4-dihydro-1-phthalazinyl] acetic acid amides were screened by Sriram *et al.*, in both *in vitro* and *in vivo* log and starvation-phase against eight mycobacterial species of tuberculosis ICL. The 5 was one of the many studied compounds that inhibited all tested mycobacterial species including MDR-TB strain with MIC values in 0.08-5.05 µg/mL. Amides from substituted anilines disclosed a decreased ICL inhibitory activity when compared to piperazines.[21]

A study conducted by Sriram and colleagues examined the production, development and isocitrate lyase inhibitory activity of several 5-Nitro-2-furoic acid hydrazones with benzaldehyde, furan-2-carbaldehyde and acetophenone. Among the synthesized compounds, 6 with activity of 2.65 and 10.64 µg/mL against log and starved-phase cultures of mycobacterium respectively were found to be the most active compound. In Vero cells, cytotoxicity was acceptable, whereas the MIC of other hydrazides ranged to 48.22 µg/mL. The activity has been improved as substitutes for benzaldehyde, furanyl and acetophenone based hydrazones by electron-withdrawing groups. The activity has been depleted by substitutes like hydroxyl and methoxy groups. N'-[1-(4-bromophenyl), ethylidene]-5-nitrofuran-2-carbohydrazide was noticed to be the most potent compound. The compound 6 has been shown to have an outstanding ICL inhibitory activity

at 10 µM; the remaining hydrazones displayed inhibition levels in the range of 19.8 to 73.1%. Benzaldehyde derivatives were less favorable than acetophenone derivatives. Compared to halogen(s), methyl, or hydrogen, the presence of the nitro group improved activity. The molecular docking studies further confirmed the high interactions of compounds to the active site of isocitrate lyases.[22]

Kratky *et al.*, reviewed new lead structure modified on three sites 7; R1=halogenated aryl, mercaptomethoxy group; R2=H, NH_2; R3=substituted phenyl, 1H-pyrrol-1-yl and reported for mycobacterial isocitrate lyase inhibition. One derivative which manifested the greatest rate of isocitrate lyase inhibitory activity with IC_{50} of 1.2 µM (R1=mercaptomethoxy, R2=NH_2, R3=3-Amino-5-chlorophenyl). It caused a dwindling inhibition of CYP450 and glutathione diminution. With promising findings led the molecule for clinical trials, however recently no supporting report has been published.[23]

A series of 5-Nitro-2,6-dioxohexahydropyrimidine -4-carboxamides was reported against both wild and resistant MTB and M. smegmatis. At the concentration value of 10 µM, some of the compounds inhibited 20-45% isocitrate lyase. The most potent *in vitro* antimycobacterial agent with the value of MIC 0.17 µg/mL against multiplying and MDR-TB strains was evaluated by be 1-Cyclopropyl-6-fluoro-8-methoxy-7-[3-methyl-4-(5-nitro-2,6-dioxo hexahydro pyrimidine-4-carbonyl) piperazin-1-yl]-4-oxo-1,4-dihydro quinoline-3-carboxylic acid 8, it had IC_{50} greater than 111.5 µM on Vero cells. A significantly weaker efficiency was shown by the simpler compounds possessing lone substituted aromatic ring, as an amid part of the molecules when compared to fluoroquinolone derivatives. The compound with, 8 was the most active compound with inhibitory concentration of 2.17 and 1.11 µg/mL against sensitive and resistant MTB bacteria, respectively. Although at more significant concentrations than 3.96 µM with fluoroquinolones preeminence, dormant mycobacteria were inhibited too. The compound 7-[3,5-dimethyl-4-(5-nitro2,6-dioxohexah ydropyrimidine -4-carbonyl)piperazin-1-yl]-1-ethyl-6,8-di fluoro-4-oxo-1,4-dihydro quinoline-3-carboxylic acid provided the highest activity and was evaluated to cause the most substantial isocitrate lyase inhibition at 10 µM (45.7%) from five experimental compounds. When compared to the cyclopropyl group, ethyl group was described to be boon for the substitution of 1,4-dihydroquinoline nitrogen. Similarly, there was an increase in the inhibition rates when another fluorine group was introduced at position 8 of 1, 4-Dihydroquinoline as compared to hydrogen and methoxy moiety.

Sriram *et al.*, synthesized and evaluated a series of 5-substituted-1-(arylmethyl/alkylmethyl)-1H-indole,2,3-dione-3-(N-hydroxy/methoxy-thiosemi carbazone) compounds for antimycobacterial and anti-HIV activities against log and starved-phase organisms. 2-(1-{[4- (4-Chlorophenyl) piperazin-1-yl] methyl}-5-methyl-2-oxo indolin -3-ylidene)-N-methoxyhydrazinecarbothioamide 9 showed exemplary inhibition of replicating HIV virus. At the same time, in antimycobacterial screening, it effectively inhibited both log- and starved-phase cultures with MIC of 3.30 and 12.11 µg/mL respectively.[29]

Shingnapurkar *et al.*, performed an experiment which concluded that four compounds (R=H, OH; X and Y=CH, N; R=H, OH; X and Y=CH, N, 10 and 11) their supposedly, copper complexes were capable inefficiently inhibiting the growth of M. tuberculosis H37Rv. Perhaps due to the retained inherent activity of INH and Cu^{2+}, their derivatives were superior among all the compounds. The study also concluded that hydrazones of pyruvate could conceivably affect the isocitrate lyase biochemistry, which was attested by docking of Schiff bases into the active site of ICL for scrutinizing their interactions. The structural similarities with known inhibitors and the facts that pyruvate hydrazides afford chelate formation with Mg^{2+}, which is a desideratum for enzymatic function were responsible for the mechanism of

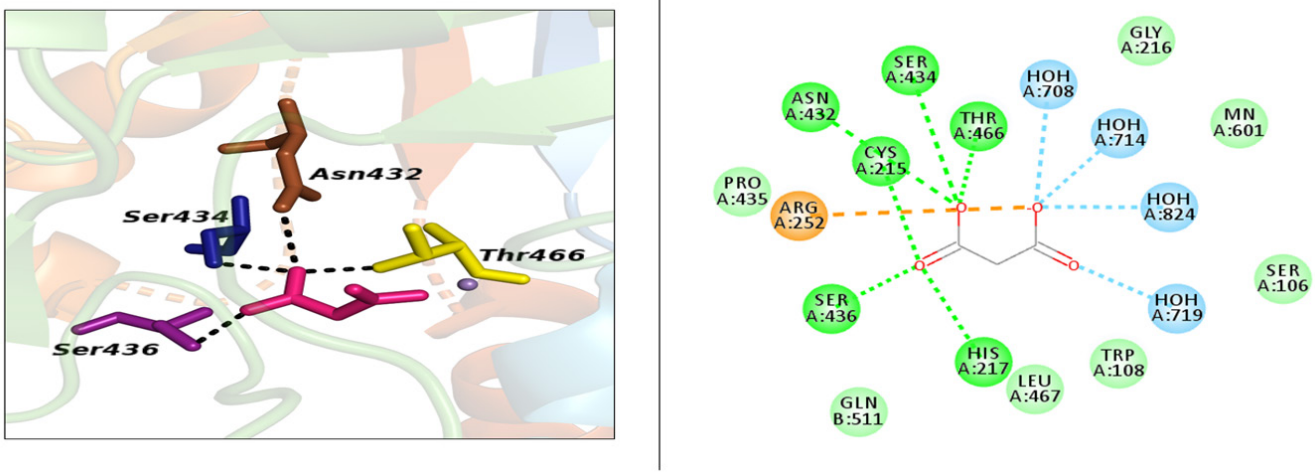


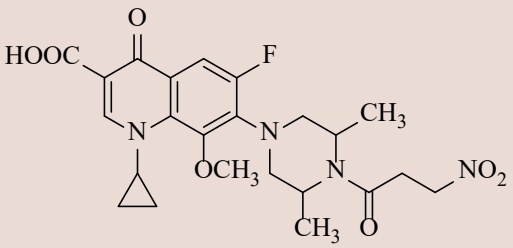
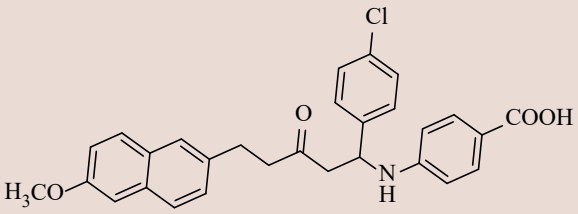
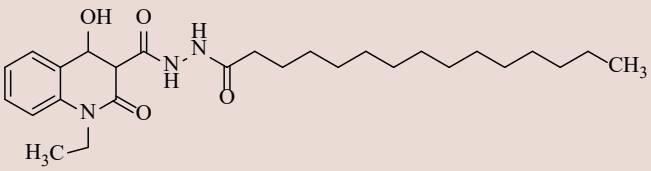
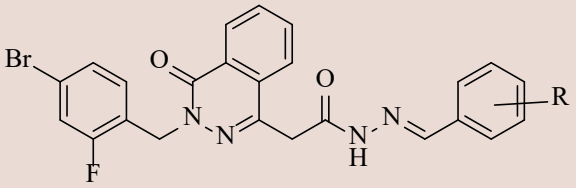
Figure 5: 5a and 5b) 3D and 2D interactions of malonate in inhibition of *F. graminearum* ICL (FgICL) (PDB: 5E9H).

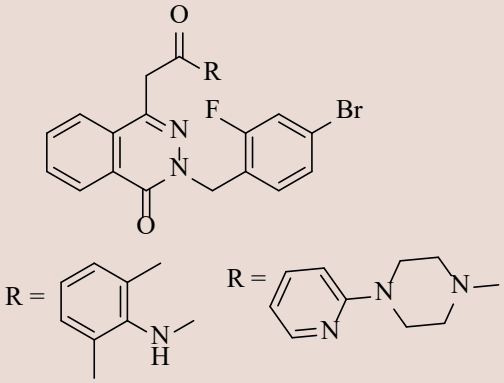
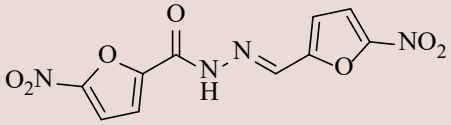
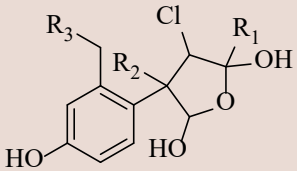
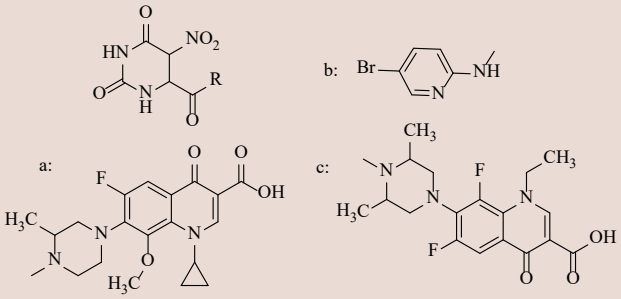
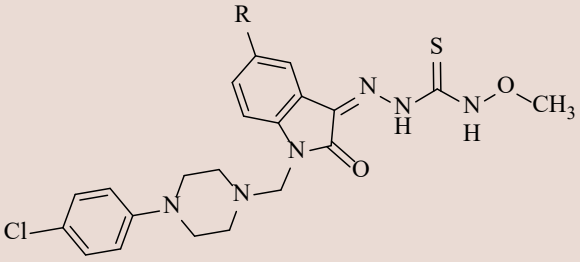
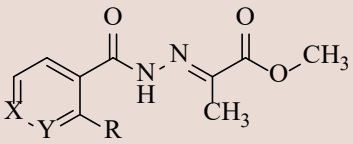
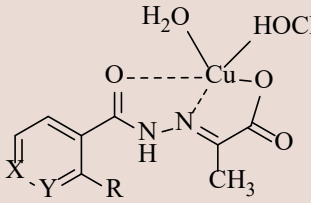
inhibition. The postulated assumptions of ICL inhibition were only based on computationally driven docking study, while no additional activity or any activity against mycobacteria have been reported.[30]

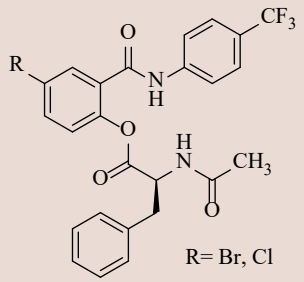
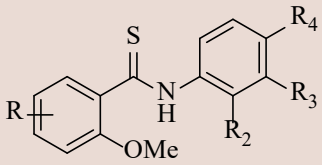
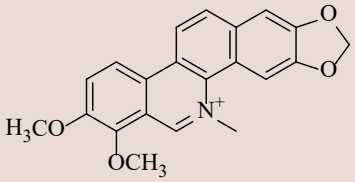
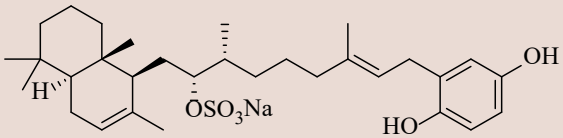
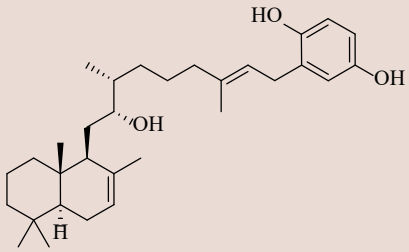
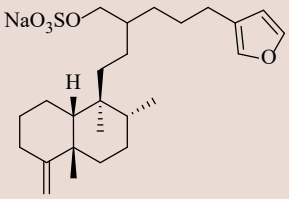
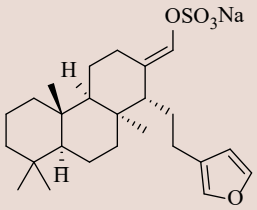
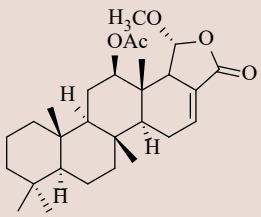
Vinšová group revealed outcomes of three studies on the isocitrate lyase inhibition and the antimycobacterial properties of diverse substituted benzanilides and thiobenzanilides. Initially, 2-hydroxy-N-phenylbenzamides (salicylanilides) and their esters were probed. The activity of parent salicylanilides was improved by its esters, which showed the MIC values within 0.25-2 μg/mL range, against drug-sensitive MTB. Smallest MIC values were shown by (S)-4-Bromo/chloro-2-[4- (trifluoromethyl) phenyl carbamoyl]phenyl 2-acetamido-3-pheny lpropanoate (12; R=Br or Cl). In general, potent *in vitro* antimycobacterial activity and least cytotoxicity was observed from salicylanilide esters with N-acetyl-L-phenylalanine followed by pyrazinoates, benzoates and benzenesulfonates. With the superiority of (S)-4-bromo-2-[4- (trifluoromethyl) phenylcarbamoyl] phenyl 2-acetamido-3- phenylpropanoate (R=Br), eight of the nineteen derivatives inhibited MTB isocitrate lyase at 10 μM within a range of 10-22%, while the strongest inhibition was provided by 5-Chloro-2- hydroxy-N-[4-(trifluoromethyl) phenyl] benzamide and its pyrazinoate at 100 μM (59%). When compared to benzoates and benzenesulfonates, salicylanilide esters with lower lipophilicity provided more influential inhibition.[24]

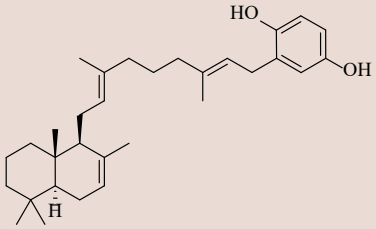
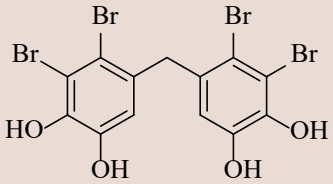
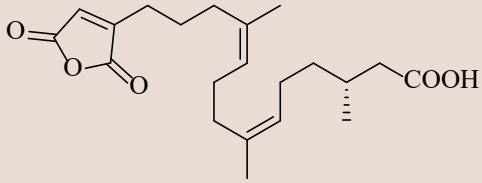
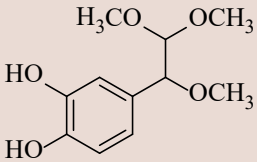
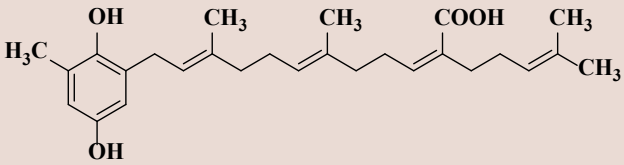
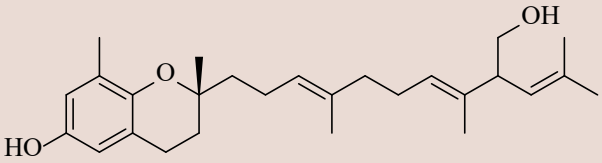
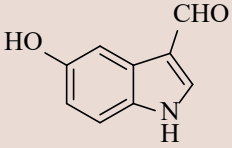
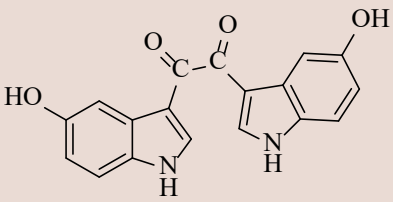
One of the reports includes the study performed by Kozic and coworkers in which the study investigated forty-two N-(2-Hydroxyphenyl)-2-methoxybenzamides and their thioxo analogues for their antimycobacterial activity and the capability for isocitrate lyase inhibition. Derivatives of 4-chloro were less active than 5-Chloro-2-methoxybenzoic acid derivatives while on aniline substitution when compared to chlorine and bromine, derivatives of CF_3 and 4,5-Dichloro were more effective. Ambivalent effects on the activities of the most potent benzanilides were brought by thionation. The thioamides were found to be improved inhibitors of ICL in comparison to amides and their two derivatives (13; R1=4- or 5-Cl, R2=OH, R3=H, R4=CF_3) which exhibited an activity commensurable to 3-NP at 10 μM with 21 and 23% inhibitory activity. However, no inhibition was provided by most of the benzanilides and some thiobenzanilides. Analogous benzoxazoles were noticed inactive, indicating that C=O(S) group is requisite for the inhibition.

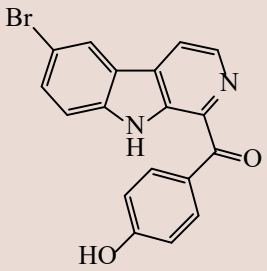
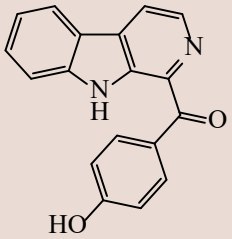
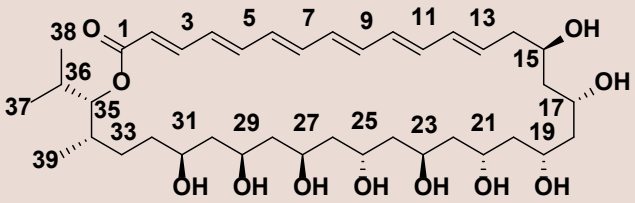
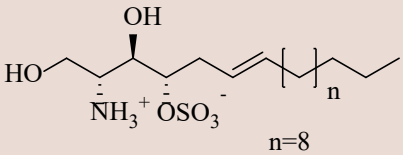
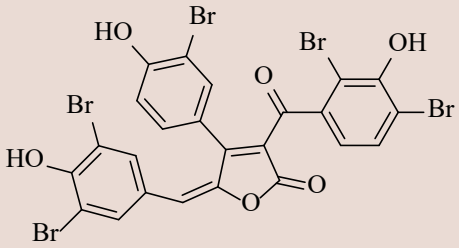
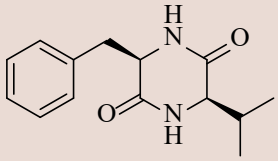
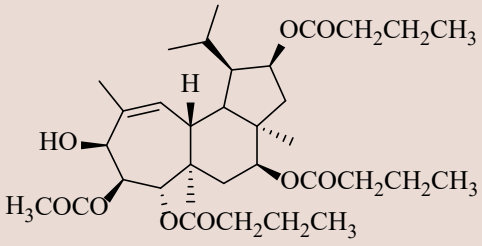
Table 3: ICL inhibitors.

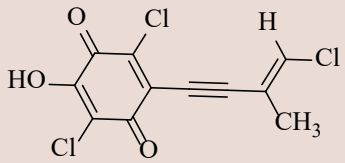
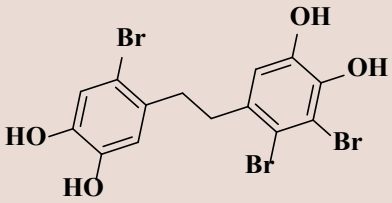
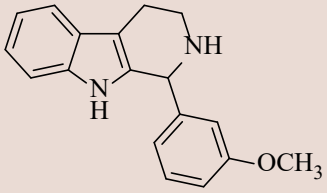
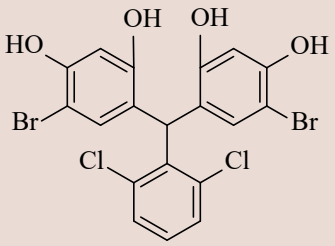
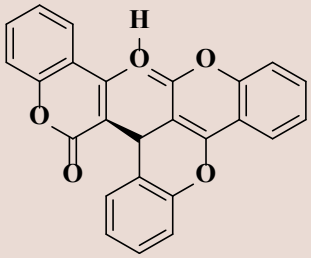
Comp. No.	Comp. No. in original paper	Chemical Structure	Mbtl IC_{50} / Ki / MIC (μM)	Year of report
1	22		0.16	2011
2	Ydcm67		53.5	2011
3	I2906		134.4	2010
4	7a-7r		100.8-129.3	2009

5	5j,5w		>126.4, < 116.52	2010
6	4o		2.65	2010
7	-		1.2	2012
8	-		0.17, 2.17, 3.96	2012
9	-.		3.30	2012
10	-		-	2012
11	-		-	2012

12	2a,2c		0.25-2	2012
13	-		126	2012
14	-		-	2006
15	1		12.6	2007
16	3		15	2007
17	2		67.4	2007
18	8		5	2011
19	2		40.8	2011

20	1		31.3	2008
21	2		2.0	2007
22	13		-	2008
23	3		14.6-200	2008
24	18		50-95	2008
25	-		118.4	2008
26	6		247	2009
27	7		89	2009

28	1		50.2	2012
29	7		48.2	2012
30	1	 84	10.8	2012
31	1	 n=8	2	2012
32	1		7.62	2013
33	Cyclo(L-Phe-L-Val)		27	2019
34	4		-	2008

35	-		5.2	1990
36	-		0.28-1.02	2010
37	9j		14.77	2010
38	-		9.6	2010
39	2_045593399		62.5	2020

Categories of synthetic derivatives such as Mannich bases, peptide inhibitors, DNAzymes and pyruvate-isoniazid analog with their copper complex drifted the attention of medicinal chemists for ICL inhibitory effects. Li and coworkers introduced the notion of silencing DNAzymes mediated ICL gene. In this study, it has shown that numerous designed DNAzymes possess the capability to specifically slice ICL mRNA hence, causing interruption of expression of ICL in macrophage. However, no effect was seen of DNAzymes towards *in vitro* tubercular bacteria growth when combined with isoniazid. Out of 124 Mannich bases screened, Ydcm67 was stated as one of the superior inhibitors, but no *in vivo* data disclosed. The inhibition rate for peptide inhibitors was found to be 38.82-47.92%. Yin and coworkers concluded that due to the small size of these peptide inhibitors, it might anticipate drug delivery issue. However, Liu and coworkers reported a few optimizations for peptide inhibitors. Initially, the report revealed that a phage peptide library was screened to obtain 29 potential inhibitors against MTB ICL and then molecular docking simulation was executed to vindicate the hit. They accomplished synthesizing 12 peptides out of twenty-nine which were efficiently docked into ICL active site, the highest IC_{50} amongst these was reported to be 126 μM in bioassay. The analog of copper complex of pyruvate isoniazid, resulted in 6-92% ICL inhibition; however, it still needs supplemental investigation for the exact mechanism of inhibition.

MTB ICL Inhibitors of Natural Compounds

Bai and colleagues reported the first natural compound from HTS screening against MTB ICL. Traditional Chinese medicines (TCMs) are considered to have strong folklore-based efficacy and are inexpensive and many TCMs are used to treat tuberculosis. Instigating a simple high-performance HTS screening of ICL inhibitors allowed TCMs bioactive extracts to investigate novel anti-tuberculosis agents and to study active components. The study was aimed to screen 465 Chinese traditional medicines against MTB ICL. Zings and Illicium verum, both of which have

a 47.7 µg/mL and 18.2 µg/mL inhibitory effects, were recorded as IC_{50} values. Research to assess the strength of itaconic anhydride (standard medicine) together with 465 extracts with an ultimate concentration of 0.05 mg/mL against ICL activity was performed. The inhibition above 50% was affected by blazoning. Of these, Illicium verum (XHD-1) and Zingiber officinale (XHD-2) extracts have been found to inhibit ICL (Table 2).[25]

A natural quaternary benzo phenanthridine alkaloid called chelerythrine 14 is a Chelidonium majus root extract with an anti-mycobacterial activity potential. The possible mechanism of action of chelerythrin against MyCBT, with the aid of commercial oligonucleotide microarrays, has been revealed by Liang and coworkers to examine genome-wide transcriptional alterations that have been triggered by treatment, including submicromolar concentrations of chelerythrin. The report also stated that chelerythrine was able to inhibit isocitrate lyase by 5.0-fold in gene ICL (Rv0467).[26]

Non-MTB ICL Natural Inhibitors

Natural compounds possess healing potential and are illustrious in many parts of the world. In the sojourn to explore natural products such as ICL inhibitors, marine sponges and algae have given exceptional results.

Candida albicans and Magnaporthe grisea

Natural sesterpene ICL inhibitors including halisulfates were isolated from M. grisea and halisulfate1 15 demonstrated ICL inhibitory activity IC_{50} of 12.6 µM. Halisulfate1 also inhibited in concentration dependent on the formation of appressoria by M. grisea. A study of appressoria formation under the influence of M. grisea revealed that halisulfate1 inhibited appressoria formation and conidia germination significantly which as comparable to Δicl mutant. Hydrolyzed product of halisulfate1, hydrohalisulfate 16 and halisulfate 17 have also been evaluated for *M. grisea* ICL inhibitory activity. Hydrohalisulfate retained ICL inhibitory activity of halisulfate1 and even found seven-fold potent activity when compared with bromopropionic acid which is a standard ICL inhibitor. Some more sesterpenes were reported from the extracts of sponge *Coscinoderma* sp. Isolated compounds were screened for isocitrate lyase, Na^+/K^+ ATPases inhibitory and cytotoxicities. One of the suvanine salts 18 found to show *C. albicans* derived isocitrate lyase inhibitory activity IC_{50} value of 5 µM and cytotoxicity against K562 cell line LC_{50} of 16 µM.[27] Hyatell sp., a type of sponge produced different scalarane sesterpenes along some reported secondary metabolites. Some of the scalarane sesterpenes consist of oxidized furan scaffold and lactam ring. All the isolated compounds were evaluated for antibacterial, isocitrate lyase inhibitory and cytotoxicity activities. One such oxidized furan ring containing scalarane sesterpene 19 inhibited ICL with IC_{50} of 40.8 µM and LC_{50} of 38.4 µM against K562 cell lines.[28] Sesterterpene sulfates were found from the extracts of Dysidea sp a tropical sponge and reported compounds were subjected for biological evaluation. One of the sesterterpene sulfate bearing furan ring was found to possess C. albicans ICL inhibitory potency with IC_{50} of 31.3 µM. Also, these sesterterpene sulfates were reported with antibacterial activity against a wide range of organisms 20.[29] Bromophenols have been known to own wide range of biological activities, six bromophenol secondary metabolites were identified from methanolic extracts of red algae *Odonihalia corymbifera* belong to rhodomelaceae family. One of such bromophenol derivative 21 showed M. grisea ICL inhibitory activity of 2.0 µM which subsequently correlated with effective hindered oppressoria formation.[30]

Profuse number of new sesterpenoids compounds were isolated from sponge *sarcotragus* sp. The numerous isolated compounds have demonstrated moderate gram positive and gram-negative antibacterial activities however devoid of antifungal activity. One compound 22 also showed moderate ICL inhibitory activity of *C. albicans*.[31] Five new dihydrostyrenes were isolated from Jaspis species and reported with wide range of biological activities such as cytotoxicity, induction of metamorphosis, angiogenic inhibitory activity. These dihydrostyrenes 23 also displayed ICL inhibitory potency of *C. albicans* and *S. aureus* with IC_{50} in the range of 14.6-200 µg/mL.[32] Meroterpenoids and meroditerpenoids from natural sources are found to be moderate isocitrate lyases inhibitors. Structurally novel eleven meroditerpenoids along with some compounds whose structures are reported have been extracted from brown alga *Sargassum siliquastrum*. Some of the nohocols and isonahocols exhibited antioxidants exhibited significant antioxidants in DPPH model of radical scavenging activity. Also, some of nahocols and sargahydroquinoic acids were moderated ICL inhibitors of C. albicans with IC_{50} in the range of 50-95 µg/mL 24.[33] Chromene class meroterpenoids were revealed from *Sargassum silguastrum* a tan alga and screened for *C. albicans* ICL and Na^+/K^+ ATPases from porcine cerebral cortex. One of the isolated compounds 25 unveiled isocitrate lyases inhibitory activity of 118.4 µM and the same compound also showed moderate antibacterial activity with MIC in the range of 12.5-25 µg/mL against tested organisms except *E. coli*.[34]

Hyrtios sp., a type of marine sponge reported to produce secondary metabolite alkaloids bearing carboline and indole scaffolds which exhibited moderate isocitrate lyases inhibitory activity of C. albicans. Two compounds among the reported 5-Hydroxyindole-3-carbaldehyde 26 and hyrtiosin B 27 has ICL inhibitory activity IC_{50} of 247.0 and 89.0 µM respectively.[35] This study extended the interest of exploring alkaloids as isocitrate lyase inhibitors to Synoicum sp. Six new β-carboline eudistomins alkaloids have been characterized and subjected for synthetic modifications. All the natural and synthetic β-carboline alkaloids were screened for cytotoxic, sortase A, isocitrate lyase, antimicrobial and Na^+/K^+ ATPase inhibitory activities. One natural 28 and synthetic 29 derivatives exhibited ICL inhibitory activity of 50.2 and 48.2 µM.[36]

Marine actinomycete Streptomyces sp., produced in laboratory culture thirty six membered polyenol lactones Bahamaolides A and B. Bahamaolide A 30 displayed moderate isocitrate lyase of *C. albicans* inhibitory activity of IC_{50} of 10.8 µM and moderate antifungal activity was also determined at 12.5 µg/mL.[37] Extracts of *Spirastrella abata* a sponge yielded three sphingosine 4-sulfates and one lysophosphatidylglycerol which were subjected for cytotoxic, isocitrate lyase and Na^+/K^+ ATPase inhibitory activities. One of the sphingosine 4-sulfates 31 displayed ICL IC_{50} of 2 µM.[38] Dark red ascidian Synoicum sp., expressed four tris-aromatic furanones and two bis-aromatic diesters. A triphenyl furanone 32 inhibited ICL with IC_{50} of 7.62 µM.[39]

Culture of marine-derived *Streptomyces puniceus* Act1085 produced five different diketopiperazines that were structurally cyclic dipeptides. One of the cyclic dipeptide, Cyclo(L-Phe-L-Val) 33 was found to be *C. albicans* derived isocitrate lyase inhibitor with IC_{50} of 27 µM which was mediated through disruption of gene transcription of ICL.[40] Sponge Phoorbas sp., reported to form new ten polyoxygenated diterpenes and six gagunin secondary metabolized in cultures and monitored their cytotoxicity and isocitrate lyase inhibitory activities. Structurally characterized 34 compounds demonstrated satisfactory cytotoxic activity against K-562 and weak ICL inhibitory activities.[41] Broth cultures of a basidiomycete, Mycena sp., ceded new chlorinated benzoquinone derivative Mycenon 35. Lineweaver-Burk plot of ICL inhibitory studies derived from *Ricinus communis, Acinetobacter calcoaceticus* and *Neurospora crassa* interpreted IC_{50} of 5.2 µM, 11 µM and 7.4 µM respectively. Mycenon also exhibited encouraging antibacterial and antifungal activities.[42]

Synthetic non-MTB ICL inhibitors

Non-tubercular ICL helped in discovering the most established inhibitors (synthetic compounds) for ICL that are itaconate, 3- Nitropropionate and 3-Bromopyruvate with *Ki* value of 120, 120 and 3 µM, respectively. Itaconate and 3-Nitropropionate are analogues of succinate while 3-Nitropropionate is the analog of glyoxylate. As these inhibitors are toxic and possess the capability to inhibit some cardinal metabolism enzymes at *in vivo* level, hence, these inhibitors are not being developed into drugs. Itaconate was found to affect the growth of rats while causing hypertonicity towards cat blood pressure. The 3-Nitropropionate caused neurotoxicity, whereas 3-Bromopyruvate seemed to be an energy blocker. Hence, these compounds are used only as control in ICL inhibitory experiments. To check the inhibitory potential of many synthetic compounds against Candida albicans ICL, like derivatives of hydroquinone, bromophenols 36, indole-containing natural compounds 37 and dimer of brominated resorcinol 38. Their IC_{50} values were reported as 0.28-1.02 µM, 14.77 µM, 21 µM and 9.6 µM respectively. da Silva *et al.*, performed molecular dynamic and virtual screening studies consisting of large database of compounds. Numerous hits containing carboxamide, β-carboline and lactone scaffold showed encouraging activities. The compound 39 was found to be exemplary compound with 100% Isocitrate lyase inhibitory activity at 62.5 µg/mL against dimorphic fungus *Paracoccidioides brasiliensis* and MIC value of 15.6 µg/mL.[43] Structures of all the inhibitors are quoted in Table 3.

CONCLUSION

Since 2000, when the crystal structure of the MTB ICL was published, momentum of drug discovery has gained so that it can be better understood. Detailed research on the metabolic routes of different microorganisms, in particular fungal and bacterial infection, for public health, crop production and animal welfare are functional in many respects. The life cycle of the glyoxylate cycle appears to be an effective metabolic pathway as substantial evidence in many host pathogens supports its critical features. Along with other pathogens, ICL's fundamental work has also demonstrated its promise as a drug target for tuberculosis's most tenacious disease. It functions as a latent TB infection flair drug target.

Contradictory evidence has shown that ICL remains healthy and tireless for MTB. The method of study can usually be divided into two methods for discovering more possible ICL inhibitors: biological experiments and *in silico* approaches. The biological test is typically conducted as a full cell assay or as an enzyme test for high-performance screening. To further minimize failure costs during lead detection, virtual screening or ensemble porting may be integrated in the silicon method rather than biological testing into the existing screening strategy. Potential inhibitors are likely to be hit better because, unlike traditional virtual screening, the degree of freedom was etched by ensemble docking during the molecular docking process. In computer-aided drug design, ensemble docking is rather an *in silico* approach developed and still not applied in ICL studies. The logical design of drugs can also be seen as a complementary strategy, as both lead detection and lead optimization are accomplished. A combination of both logical drug design and modern HTS (4D docking) can be much better than either one.

The 'popular' unassuming inhibitors including itaconic acid and anhydride, 3-bromopyruvates, oxalates and 3-Nitropropionates, cannot be utilized due to their unexpected biotoxicity with no added special effects. The analysis reveals various promising compounds with inhibition of lyase isocitrate, low toxicity and good bioavailability. Some promising classes include phthalazine-4-ylacetamide, certain fluoroquinolones, furan and tetrahydrofuran. A derivative 1-Cyclopropyl 7-[3, 5-dimethyl-4-(3-nitropropanoyl]-6-fluoro-8-methoxy-4-oxy-1, 4-dihydro quinoline-3-carboxylic acid was found to have the highest activity (IC_{50} 0.10±0.01 µM) with a molecule motivation of 3-nitropropionamide. The result with an IC_{50} of 1.2 µM was positive and another standardized plumage called 'Jainin' was tetrahydrofuran-2,5-diol, 4-Amino-4-(3-amino-5-chloroben zyl)-4-hydroxyphenyl), 3-Chloro-2-(mercaptomethoxy). All

data possibly indicate zero direct inter relationship between inhibition of isocitrate lyase and anti-mycobacterial activity *in vitro*. The molecules that enjoy both high activities against aggressively growing mycobacteria and isocitrate lyase inhibition will potentially have great advantages to combat mycobacterial spread. Nonetheless, analogously high IC_{50} values up to the micro-molar were observed in some of the molecules mentioned, with the recorded isocitrate lyase inhibition (e.g., I2906, benzanilides and thiobenzanilides). Such compounds cannot simply inhibit only mycobacterial isocitrate but also interfere with other cell objectives. Thus, it is safe to say that the suppression of lyase isocitrate activity is only an 'added value' to a complex operation against active mycobacteria, especially where the inhibition levels are fragile. The information gained about the function of this pathway, pathogenesis in various pathogens and promising structures of inhibitory lead molecules is therefore significant as they give enormous opportunities to create unique and more effective ICL inhibitors could be employed to contain several fungal and bacterial diseases. This review may provide insides of the research conducted by various medicinal chemists of the following unexplored targets for development of novel antitubercular agents.

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CONFLICT OF INTEREST

All the authors claimed there was no conflict of financial interest in the preparation of the article.

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