

Pharmaceutical development of a medicinal product containing an isocitrate lyase inhibitor

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Abstract

Isocitrate lyase (ICL), a key enzyme of the glyoxylate shunt in *Mycobacterium tuberculosis*, is considered a potential therapeutic target for drug-resistant and persistent tuberculosis. Conventional antitubercular drugs show limited activity against bacteria persisting within granulomas, highlighting the need for new treatment strategies. This review summarizes studies published between 2020 and 2025 on the discovery, optimization, formulation, and preclinical evaluation of ICL inhibitors. Lead compounds demonstrate oral bioavailability exceeding 50% and pharmacokinetic profiles compatible with once- or twice-daily dosing. Brain-to-plasma ratios up to 0.78 indicate central nervous system penetration, supporting possible use in tuberculous meningitis. Nanotechnology-based formulations, including PLGA nanoparticles and liposomes, increased intracellular accumulation in infected macrophages and improved lung targeting. Pulmonary delivery systems achieved fine particle fractions above 50% and prolonged lung retention. Available data indicate activity against drug-resistant strains, absence of cross-resistance, and synergistic effects in combination therapy, supporting further development of ICL-targeted agents.

Keywords

drug-resistant tuberculosis, glyoxylate shunt, isocitrate lyase, nanotechnology, pulmonary delivery

Introduction

The development of new therapeutic strategies to combat persistent and drug-resistant bacterial infections has increased interest in targeting pathogen-specific metabolic pathways absent in humans. One of the most promising targets is isocitrate lyase (ICL), a key enzyme of the glyoxylate shunt, a metabolic bypass of the tricarboxylic acid (TCA) cycle that enables microorganisms such as *Mycobacterium tuberculosis* to survive under nutrient-deficient and hostile

intracellular conditions (Khanojia et al. 2025). ICL plays a crucial role in the persistence and latency of *M. tuberculosis*, particularly during chronic infection, when the pathogen resides in macrophages and relies on fatty acid metabolism (Antil and Gupta 2022). Because ICL is absent in mammalian cells, inhibition of this enzyme provides high selectivity and reduces the risk of off-target effects (Sharma and Sharma 2017).

The global burden of tuberculosis, especially multidrug-resistant (MDR-TB) and extensively drug-re-

sistant (XDR-TB) forms, underscores the need for therapeutics with new mechanisms of action. Current anti-TB regimens are lengthy, complex, and associated with adverse effects, reduced adherence, and resistance development (Roelens et al. 2021; Utpat et al. 2023). ICL inhibitors show potential in disrupting bacterial survival during latency and enhancing the activity of existing treatments (Gaspar Quinonez 2021; Kwon et al. 2021; Krátký et al. 2022).

Pharmaceutical development of an ICL inhibitor involves an interdisciplinary workflow beginning with the identification of selective small-molecule candidates using structure-based design, virtual screening, and chemical synthesis (Buya et al. 2021). Optimization focuses on improving pharmacodynamic and pharmacokinetic parameters such as solubility, membrane permeability, metabolic stability, and target specificity. Drug delivery system development is essential as well, since *M. tuberculosis* is an intracellular pathogen; lipid carriers, nanoparticles, and prodrug approaches can improve uptake and biodistribution. Preclinical evaluation includes *in vitro* efficacy, cytotoxicity studies, and tuberculosis models in animals to establish safety and therapeutic potential (Munoz-Elias and McKinney 2005; Van Staden et al. 2022; Kumar et al. 2023; Nair et al. 2023).

Development of a drug targeting isocitrate lyase not only expands the arsenal of antituberculous agents but also provides a platform for the development of antimicrobial agents against other pathogens that utilize the glyoxylate cycle (Barilar et al. 2024). *Mycobacterium tuberculosis* strains significantly undermine the effectiveness of existing treatment regimens, which are already lengthy, complex, and characterized by low patient adherence (Verma et al. 2022; Chowdhury et al. 2023; Veeramuthu et al. 2024).

Under these conditions, isocitrate lyase stands out as a promising molecular target due to its key role in the glyoxylate cycle-mediated pathway that allows bacteria to utilize fatty acids as a carbon source under nutrient-deficient conditions. The glyoxylate shunt allows *M. tuberculosis* to maintain energy homeostasis and survive in the hostile macrophage environment, particularly in hypoxic and acidic conditions mimicking granulomatous lesions (Sundararajan and Muniyan 2021; Díaz-Pérez et al. 2023; Warner et al. 2025).

The development of ICL inhibitors also has broader implications, as the glyoxylate cycle contributes to the virulence of pathogens such as *Candida albicans*, *Salmonella enterica*, and *Pseudomonas aeruginosa* (World Health Organization 2024). This positions ICL inhibition as a potential broad-spectrum antimicrobial strategy. These efforts align with global public health priorities that emphasize shorter, safer, and more effective TB therapies (World Health Organization 2021; Cobelens et al. 2022), supported by initiatives such as the Global Alliance for TB Drug Development (Eshraghisamani et al. 2023). Advances in formulation science and targeted

delivery technologies further improve the feasibility of intracellular pathogen targeting.

Abbreviations

ADME	absorption, distribution, metabolism, and excretion
AUC	area under the curve
DLS	dynamic light scattering
CFU	colony-forming units
ICL	isocitrate lyase
ILI	isocitrate lyase inhibitor
MDR	multidrug-resistant
MBC	minimum bactericidal concentration
MIC	minimum inhibitory concentration
MS	magnetic resonance spectroscopy
MTB	<i>Mycobacterium tuberculosis</i>
XDR	extensively drug-resistant
LC-MS	liquid chromatography-mass spectrometry
NMR	nuclear magnetic resonance spectroscopy
NOAELs	no-observed-adverse-effect levels
RNA	ribonucleic acids
PLGA	poly(lactic-co-glycolic) acid
SLN	solid lipid nanoparticles
IND	investigational new drug
TCA	tricarboxylic acid cycle
TEM	transmission electron microscopy
VD	volume of distribution
TB	<i>Mycobacterium tuberculosis</i>
HIV	human immunodeficiency virus
HPLC	high-performance liquid chromatography

Objectives and scope of the review

The aim of this review is to provide a comprehensive analysis of modern approaches to developing pharmaceutical products containing ICL inhibitors for treating drug-resistant and persistent tuberculosis.

To examine strategies for discovering and optimizing ICL inhibitors, including computer-aided modeling, fragment-based design, and natural compound screening.

To evaluate progress in pharmaceutical formulation and nanotechnology-based delivery systems that enhance the bioavailability and efficacy of ICL inhibitors.

To summarize preclinical research demonstrating antitubercular activity, selectivity, and clinical development potential of ICL inhibitors.

Literature search strategy and study selection

A literature search was conducted in Scopus, PubMed, and Web of Science using combinations of key terms and Boolean operators, including “isocitrate lyase”, “ICL inhibitor”, “glyoxylate shunt”, “*Mycobacterium tuberculosis*”,

“drug development,” “pharmaceutical formulation,” “antimycobacterial agents,” “persistent infection,” and “metabolic target”. Both free-text and MeSH descriptors were applied to ensure completeness.

Publications were included based on relevance to:

- biochemical and structural characteristics of ICL;
- methods for discovering and designing small-molecule inhibitors;
- pharmacological activity and mechanisms of action;
- pharmacokinetic and pharmacodynamic profiles;
- formulation strategies and delivery systems;
- preclinical and clinical evidence of safety and efficacy.

Studies irrelevant to the topic, conference abstracts without full text, unverified sources, and works lacking methodological rigor were excluded. Manual screening of reference lists was performed to identify additional sources.

Data extraction and synthesis

To support future clinical development, the review also considers regulatory requirements for preclinical and pharmaceutical development, including ICH guidelines for quality and safety, Good Laboratory Practice (GLP), and Good Manufacturing Practice (GMP), which ensure reliability, reproducibility, and compliance in preparing ICL inhibitor candidates for clinical trials. The authors' key data analysis aspects are presented in Table 1.

The selected literature sources were subjected to a critical qualitative analysis to extract, systematize, and interpret scientific data. This analysis included an assessment of the methodological rigor of the studies, the reliability of the presented results, and their clinical significance. Particular attention was paid to identifying consensus positions and contradictions in the scientific literature

regarding the effectiveness of various approaches to the development of ICL inhibitors.

The information was structured into thematic blocks covering the molecular biological aspects of isocitrate lyase function, chemical strategies for inhibitor creation, pharmacological characterization of compounds, technological solutions for dosage forms, and the results of experimental studies. Data synthesis was accomplished by comparing the results of various studies, identifying common patterns, and formulating general conclusions.

The quality of the publications included in the review was assessed based on the journal impact factor, methodological soundness of the studies, sample size, reproducibility of results, and compliance with modern scientific ethics standards. Priority was given to studies conducted in accordance with the principles of evidence-based medicine and good laboratory practice.

The main aspects that the authors focused on when collecting data are presented more clearly in Table 2.

The study considered potential limitations related to language barriers (English-language publications were analyzed), the availability of full-text versions of individual articles, and potential publication bias regarding positive results. However, the application of strict selection criteria and the use of multiple independent databases minimized the influence of these factors and ensured the high reliability of the results.

Demographic considerations

Demographic factors become increasingly relevant during clinical development of ICL inhibitor-based therapies. Age, sex, genetic background, and comorbidities may influence pharmacokinetics, efficacy, and safety.

Age-related differences in hepatic and renal function can alter drug metabolism, while sex-related biological variation may affect absorption and distribution. Genetic polymor-

Table 1. Data analysis.

Data type	Analysis method	Purpose	Key parameters/outcomes
Enzyme inhibition	IC_{50} and K_i calculation, curve fitting	Evaluate the inhibitory potency of compounds	IC_{50} , K_i values
Cytotoxicity	Selectivity index (CC_{50}/IC_{50}), statistical comparison	Assess the therapeutic window and safety	CC_{50} , selectivity index
Antimicrobial activity	MIC, CFU counting, time-kill curves, log reduction analysis	Determine antibacterial efficacy	MIC values, CFU log reduction, kill curves
Pharmacokinetics	Non-compartmental and compartmental modeling, AUC calculation	Characterize drug absorption, distribution, metabolism, and excretion	C_{max} , T_{max} , AUC, $t_{1/2}$, CL, Vd
Formulation evaluation	Release kinetics modeling (zero-order, first-order, Higuchi, among others)	Predict in vivo drug release and adjust formulation properties	Release rate, particle size, zeta potential, encapsulation rate
Toxicology	Dose-response analysis, ANOVA, histopathology scoring	Establish a safe dosage range and identify toxic effects	NOAEL, LOAEL, tissue damage scores
Stability studies	Degradation kinetics modeling, trend analysis	Predict shelf life and recommend storage conditions	% potency over time, expiration date
Preclinical efficacy	Kaplan–Meier survival analysis, bacterial load comparisons, clinical scoring	Confirm therapeutic efficacy <i>in vivo</i>	CFU reduction, survival rates, clinical improvement

Table 2. Data collection.

Stage	Objective	Methods/tools	Key data collected	References
Target validation	Confirm ICL as a viable drug target	Gene knockout, enzyme assays, infection models	Role of ICL in persistence, pathogen survival, non-redundancy	Ejeh et al. 2023
Lead identification	Discover potential inhibitors	Virtual screening, molecular docking, QSAR modeling	Binding affinity, docking scores, predicted ADME properties	Vanga et al. 2025
Chemical synthesis	Produce candidate molecules	Organic synthesis, purification, NMR, MS, HPLC	Chemical structure, purity, molecular weight	Krippendorff et al. 2009
<i>In vitro</i> screening	Assess inhibitory activity and selectivity	Enzyme inhibition assays, cytotoxicity tests	IC ₅₀ /K _i values, CC ₅₀ , selectivity index	Barcia-Macay et al. 2006
Antimicrobial testing	Test the antibacterial efficacy	MIC determination, time-kill curves, macrophage infection models	MIC values, CFU reduction, intracellular activity	Wang et al. 2018
Pharmacokinetics (PK)	Characterize drug behavior in the body	Animal dosing studies, LC-MS, HPLC	Bioavailability, half-life, clearance, tissue distribution	Sahota et al. 2024
Formulation development	Improve solubility, stability, and delivery	Nanoparticles, liposomes, solid dispersions, emulsions	Drug release profiles, particle size, encapsulation efficiency	Nair et al. 2024
Toxicological evaluation	Determine safety profile	Acute/chronic toxicity studies, organ histology, blood analysis	NOAEL, adverse effects, dose-response data	De Knecht et al. 2017
Preclinical efficacy studies	Confirm therapeutic potential <i>in vivo</i>	Animal TB models, bacterial burden assessment	Lung CFU counts, survival rates, relapse prevention	González-González et al. 2022
Stability testing	Define shelf life and storage conditions	Accelerated and long-term testing (ICH guidelines)	Degradation products, potency over time, recommended storage conditions	Van der et al. 2025

phisms in metabolic enzymes (e.g., CYP variants) may contribute to variability in systemic exposure across populations.

Geographic and socioeconomic conditions also influence treatment access and adherence, particularly in tuberculosis-endemic regions. Therefore, formulation characteristics such as stability, simplified dosing schedules, and heat resistance are important for real-world implementation.

Comorbid conditions, including HIV infection and malnutrition, may modify immune response and drug metabolism, necessitating careful evaluation of drug-drug interactions and stratified clinical trial design.

Consideration of demographic variability supports rational dose selection, formulation design, and inclusive clinical trial planning.

Molecular and structural basis of the glyoxylate shunt

The glyoxylate shunt is a key metabolic pathway that enables *Mycobacterium tuberculosis* to persist under hostile conditions within the host, especially during the chronic phase of infection. According to the study by van der Klugt et al. (2025), the enzyme ICL plays a central role in this process. It converts isocitrate into succinate and glyoxylate, allowing the bacterium to bypass the CO₂-producing steps of the Krebs cycle. Metabolomic analysis confirmed that ICL activity is essential for survival when fatty acids are the primary carbon source—conditions similar to those within granulomas.

Structural investigations by Venugopal et al. (2011) identified novel allosteric sites in the structure of *M. tuberculosis* ICL. The enzyme forms a homotetramer and exists in several conformational states that regulate cat-

alytic activity. Molecular dynamics simulations showed that amino acid residues at the subunit interface are critical for substrate binding. These allosteric regions are considered promising targets and may help reduce the likelihood of resistance.

According to Nandakumar et al. (2014), ICL expression increases significantly under hypoxia, nutrient starvation, and exposure to reactive nitrogen species—conditions characteristic of granulomas. The upregulation of ICL is associated with the transition into a persistent, antibiotic-tolerant state. Strains lacking ICL show reduced survival in macrophages and decreased virulence in models of chronic TB.

The review by Xu and Borah (2022) emphasizes that the glyoxylate cycle is one of the most promising but underexplored therapeutic pathways. Metabolic inhibitors, including ICL antagonists, specifically target persistent bacteria that evade conventional therapy. Genetic and biochemical data confirm that ICL is indispensable for long-term bacterial survival.

Demographic characteristics influence the optimal dose, the choice of dosage form, and the design of clinical trials. Age, sex, ethnicity, comorbidities, and living conditions affect pharmacokinetics, drug exposure, and treatment adherence, making it necessary to adjust dosing strategies, select suitable formulations (such as heat-stable oral forms), and include stratified subgroups in clinical studies.

ICL in drug-resistant tuberculosis

The rise of extensively drug-resistant tuberculosis has intensified the search for new therapeutic targets beyond those used in current regimens. Research by Singh et al. (2020) showed that ICL expression is markedly elevated in MDR and XDR clinical isolates compared to drug-suscep-

tible strains. According to the authors, this metabolic shift helps resistant bacteria remain viable under antibiotic stress. Proteomic analysis demonstrated a 3.2-fold increase in ICL abundance in rifampicin-resistant strains and a 4.1-fold increase in strains resistant to both isoniazid and rifampicin.

Studies by Eoh et al. (2022) further support the role of metabolic adaptation in resistance. Resistant strains displayed higher expression of ICL and other glyoxylate-cycle enzymes during exposure to first-line antibiotics. This was confirmed by RNA sequencing. The authors reported that removing the ICL gene in resistant strains restored susceptibility to conventional drugs. This provides strong evidence that ICL inhibition could serve as an effective adjunctive strategy.

Research by Kumar et al. (2018) examined the synergistic potential of combining ICL inhibitors with standard antitubercular agents. According to the authors, computational modeling predicted beneficial interactions between metabolic blockade and traditional bactericidal pathways. *In vitro* experiments confirmed this synergy: ICL inhibition increased the activity of rifampicin, isoniazid, and bedaquiline against both replicating and non-replicating bacteria. Fractional inhibitory concentration indices ranged from 0.3 to 0.5, indicating strong synergism.

Combination therapies involving ICL inhibitors should be prioritized for early-phase clinical evaluation, particularly Phase I/II trials focusing on safety, optimal dosing, and interaction profiles with existing TB drugs.

Evolutionary conservation and selectivity

A major advantage of targeting isocitrate lyase (ICL) is its absence from mammalian metabolism, supporting a favorable selectivity profile. Comparative genomic analyses indicate that the glyoxylate cycle is restricted to bacteria, fungi, and plants and is absent in vertebrates, with no functional ICL orthologs detected in mammalian genomes (Zhan et al. 2021). Structural and sequence comparisons further reveal mycobacteria-specific residues within the active site and substrate-binding pocket of *Mycobacterium tuberculosis* ICL, enabling the rational design of selective inhibitors. Optimized compounds have demonstrated > 1000-fold selectivity relative to the closest human homologs (Antil et al. 2019). Safety profiling typically includes screening against panels of human metabolic enzymes, where minimal off-target activity has been reported even at concentrations far above the inhibitory range for mycobacterial ICL, alongside low cytotoxicity in human cell lines (Ahn and Davids 2017). Collectively, these features support dosing strategies capable of achieving sustained therapeutic exposure—including within granulomatous lesions—while minimizing systemic toxicity risks. High selectivity also improves suitability for combination regimens by reducing the likelihood of metabolic interference or cross-toxicity with first-line and newer anti-TB agents.

Discovery and optimization of ICL inhibitors

The availability of high-resolution structural data for *M. tuberculosis* ICL has enabled the development of advanced computational and structure-guided strategies for inhibitor discovery. According to Ghosh et al. (2006), structure-based virtual screening of 2.4 million compounds identified 127 hits, with 23 demonstrating IC_{50} values below 50 μ M, confirming the efficiency of large-scale docking for rapid hit selection.

Studies by Janardhan et al. (2017) used fragment-based discovery combined with X-ray crystallography, identifying several active fragments from a 1,200-compound library. Fragment optimization produced nanomolar inhibitors, demonstrating the high ligand efficiency and low molecular complexity achievable with this approach.

According to Zhu et al. (2024), deep learning models trained on more than 5,000 compounds achieved prediction accuracies above 85% and successfully prioritized novel chemotypes with potent ICL activity, highlighting the value of AI-driven screening in expanding chemical diversity.

Research by Baillie (2016) explored covalent inhibition and identified nucleophilic residues suitable for irreversible targeting. Structure-guided design yielded electrophilic compounds with time-dependent inhibition and a lead inhibitor with remarkable potency ($IC_{50} = 3.2$ nM) and sustained cellular activity.

Overall, the combined use of virtual screening, fragment-based design, machine learning, and covalent inhibitor development has proven highly effective. These complementary approaches accelerate hit identification, improve potency, and broaden the spectrum of viable chemical scaffolds for targeting ICL, significantly advancing the prospects for developing new anti-tuberculosis therapeutics.

Natural products and phytochemical inhibitors

Natural products remain an important source of chemical diversity in drug discovery (Singh and Pelaez 2008; Li and Lou 2018; Atanasov et al. 2021; Najmi et al. 2022). According to multiple studies, screening of medicinal plant extracts has identified several phytochemicals with notable ICL inhibitory activity (Kamal et al. 2021; Rao et al. 2023). Among the most promising sources, *Withania somnifera*, *Curcuma longa*, and *Azadirachta indica* yielded withanolides, curcuminoids, and limonoids showing micromolar inhibition of *M. tuberculosis* ICL (Ameamsri et al. 2021; Mekuriaw et al. 2021; Duryat et al. 2025; Ramadhan et al. 2025). Structure–activity analyses indicate that α,β -unsaturated carbonyl groups and specific hydroxyl substitutions are essential for activity (Bhandari et al. 2023; Kamala 2025).

Research by Attah et al. (2021) highlighted African medicinal plants as particularly promising candidates. Screening of 43 species identified *Warburgia ugandan-*

sis as the most active source. Bioassay-guided isolation yielded polygodial and warburganal, which demonstrated IC_{50} values of 12.4 and 8.7 μ M, respectively, supported by docking studies showing strong hydrophobic and hydrogen-bond interactions within the active site.

According to studies by Sansone et al. (2021), marine organisms represent another underexplored reservoir of ICL inhibitors. Screening of 156 marine extracts identified a brominated indole alkaloid from a sponge as the most potent, exhibiting an IC_{50} of 4.3 μ M and whole-cell antimycobacterial activity (MIC 6.8 μ M) (Saini et al. 2023; Shi et al. 2024).

Majhi and Manickam (2023) demonstrated that semi-synthetic modification of natural scaffolds can substantially improve drug-like properties. Chemical optimization of berberine yielded derivatives with markedly enhanced potency, including an analog with a 15-fold lower IC_{50} and improved metabolic stability in preclinical models (Tong et al. 2023).

Natural ICL inhibitors provide structurally diverse scaffolds with moderate potency, while semi-synthetic and fully synthetic derivatives generally achieve higher potency, better stability, and more predictable pharmacokinetic behavior. Integrating natural product discovery with rational modification strategies remains a highly productive approach for advancing ICL inhibitor development.

Rational design of synthetic small molecules

Medicinal chemistry efforts have focused on developing synthetic small molecules with optimized drug-like properties. According to research by Francis (2024), structure-based design guided the development of a series of substituted benzoic acid derivatives as competitive ICL inhibitors. The authors employed iterative cycles of design, synthesis, and biological evaluation to optimize potency, selectivity, and physicochemical properties. The lead compound from this series exhibited an IC_{50} of 127 nM against *M. tuberculosis* ICL, greater than 500-fold selectivity over human enzymes, and favorable aqueous solubility (>100 μ g/mL). Furthermore, the compound demonstrated whole-cell activity against both replicating and non-replicating mycobacteria with MIC values of 2.1 and 4.8 μ M, respectively.

Studies conducted by Coelho et al. (2024) developed a series of triazole-based ICL inhibitors with enhanced metabolic stability. Incorporation of the triazole heterocycle provided multiple benefits, including improved solubility, reduced susceptibility to oxidative metabolism, and opportunities for diverse functionalization. Structure-activity relationship studies identified key structural features required for potency, including specific substitution patterns on the triazole ring and the nature of linking groups.

According to research by Sofi et al. (2025), development of dual-targeting inhibitors that simultaneously inhibit ICL and another essential mycobacterial enzyme represents an innovative approach to enhance efficacy and reduce resistance potential. Hybrid molecules incorporate pharmacoph-

ores for both ICL and InhA (enoyl-ACP reductase) inhibition. The lead dual inhibitor exhibited IC_{50} values of 43 nM for ICL and 78 nM for InhA, translating to potent whole-cell activity with an MIC of 0.8 μ M against drug-susceptible *M. tuberculosis* and retained activity against InhA-mutant strains. The dual-targeting approach demonstrated synergistic effects in time-kill kinetics studies (Pholwat et al. 2011; Vilch  ze and Jacobs 2021; Gupta et al. 2023; Nandi et al. 2023; Tran et al. 2025).

Severe forms of tuberculosis require agents capable of crossing the blood-brain barrier—a property lacking in many current antitubercular drugs. The researchers employed computational modeling to predict blood-brain barrier penetration based on physicochemical properties and efflux transporter liability (Faramarzi et al. 2022; Nueremberger et al. 2022; Alves et al. 2025; March et al. 2025). The optimized compound achieved a brain-to-plasma ratio of 0.78 in mice, significantly higher than first-line tuberculosis drugs, while maintaining potent ICL inhibitory activity (IC_{50} = 34 nM).

Optimization of ADME properties

Comprehensive optimization of absorption, distribution, metabolism, and excretion (ADME) properties is essential for advancing ICL inhibitors toward clinical application. Early candidates frequently showed poor oral bioavailability and rapid clearance (Trousil et al. 2016; Zheng et al. 2017). Medicinal chemistry optimization—including adjustment of lipophilicity, incorporation of metabolically stable bioisosteres, and refinement of hydrogen-bonding features—successfully improved drug-like behavior (Veale 2021; Alizadeh et al. 2023; Choi 2024; Choi 2025). As a result, one optimized lead demonstrated oral bioavailability of 54% in rats, a plasma half-life of 5.2 hours, and dose-proportional pharmacokinetics supporting convenient once-daily dosing.

Evaluation of drug-drug interaction potential remains especially important for tuberculosis regimens administered in combination therapy (Di Perri et al. 2005; Muliaditan and Della Pasqua 2021; Pooranagangadevi and Padmapriyadarsini 2022). Most optimized ICL inhibitors showed minimal inhibition of major CYP isoforms at therapeutic concentrations, indicating a low risk of pharmacokinetic interactions (Cheah et al. 2014; Mdluli et al. 2015; Keyvani-Ghamsari et al. 2020; Fritsch et al. 2024). However, one compound induced CYP3A4 moderately, highlighting the need for careful combination design in cases involving drugs metabolized by this pathway.

Prodrug strategies have also been explored to overcome solubility and bioavailability limitations of potent yet poorly soluble inhibitors (Wellington and Hung 2018). Simplified prodrug approaches, such as amino acid-based conjugates, substantially improved solubility and systemic exposure (Vale et al. 2018; Kreemerova 2017). The L-valine ester prodrug increased aqueous solubility from < 0.1 mg/mL to > 5 mg/mL and improved oral bioavailability by 5.3-fold *in vivo* (Beaumont et al. 2003).

ADME optimization—including improved oral exposure, reduced interaction risk, and enhanced solubility through prodrug design—significantly broadens the therapeutic window of ICL inhibitors. These advances increase the likelihood of achieving effective systemic concentrations, support practical dosing regimens, and strengthen the clinical viability of ICL inhibitors as components of modern anti-tuberculosis therapy.

Nanotechnology-based delivery platforms

Advanced delivery systems are critical for achieving sufficient intracellular concentrations of ICL inhibitors in infected macrophages, particularly for compounds with limited cellular penetration or rapid metabolism (Feng et al. 2023). Among the most studied approaches, PLGA nanoparticles (180–220 nm) demonstrate high encapsulation efficiency (>78%) and increase intracellular accumulation up to 6.4-fold compared with free drug, although burst release and manufacturing complexity remain limitations (Zeb et al. 2022; Liang et al. 2024). Lipid-based carriers provide alternative profiles: solid lipid nanoparticles (SLN) offer prolonged physical stability and improved intestinal permeability but have limited loading capacity due to the rigid matrix (Satchanska et al. 2024; Narang et al. 2025). Nanostructured lipid carriers (NLC) address this limitation by combining solid and liquid lipids, enabling higher loading (up to 12.4% w/w) and improved intracellular activity, with reported stability up to 18 months at 4 °C (Garg et al. 2022). Liposomes provide organ-oriented distribution, particularly to lungs and spleen; optimized systems (~142 nm; 82% encapsulation) achieve lung-to-plasma ratios of 8.3:1 and > 2-log reductions in bacterial burden compared with free drug, although production cost and storage sensitivity may limit implementation (Catania et al. 2021; Alshaer et al. 2022; Schlich et al. 2022; Singla et al. 2022; Agrawal et al. 2024). Overall, these platforms provide complementary advantages, while clinical translation will depend on scalable manufacturing, reproducibility, and storage stability.

Preclinical efficacy and safety evaluation

In vitro antimycobacterial activity

Comprehensive *in vitro* evaluation of ICL inhibitors encompasses assessment of activity against various metabolic states of *M. tuberculosis* (Zhang et al. 2024).

Lead ICL inhibitors demonstrated potent activity against actively replicating bacteria with minimum inhibitory concentrations (MIC) ranging from 0.8 to 6.4 µM (Qi et al. 2021).

Microplate Alamar Blue assays were used to determine MIC values against *M. tuberculosis* H37Rv strain cultured

in standard Middlebrook 7H9 medium. Time-kill kinetics studies revealed bacteriostatic activity at MIC concentrations and bactericidal effects at 4× MIC, with a 3 log₁₀ reduction in colony-forming units achieved within 14 days of exposure.

ICL inhibitor activity was also evaluated under conditions mimicking the intracellular environment of infected macrophages (Li et al. 2005). Bacteria cultured in medium containing fatty acids as the sole carbon source or under hypoxic conditions exhibited increased susceptibility to ICL inhibition compared with glucose-rich normoxic conditions.

The lead compound achieved MIC values of 1.2 µM under fatty acid conditions versus 8.9 µM under glucose conditions, consistent with preferential reliance on the glyoxylate shunt under nutrient restriction. Furthermore, the compound maintained activity against non-replicating persistent bacteria generated by gradual nutrient depletion and oxygen limitation, achieving a 2.1 log₁₀ reduction in viability over 28 days.

Evaluation of ICL inhibitors against clinical isolates including drug-resistant strains is essential for assessing therapeutic potential (Liu et al. 2016). The ICL inhibitor exhibited consistent activity across all strains with MIC₉₀ of 4.2 µM, demonstrating no cross-resistance with existing first-line or second-line antibiotics. Whole genome sequencing of laboratory-generated resistant mutants (frequency < 10⁻⁸) revealed mutations in the *icl* gene encoding amino acid substitutions in the active site, confirming an on-target mechanism of action. Importantly, these mutants exhibited impaired growth and attenuated virulence, suggesting a low probability of resistance emergence in clinical settings.

The activity of ICL inhibitors was also evaluated in macrophage infection models that more accurately simulate *in vivo* conditions (Fang et al. 2011). THP-1 human macrophages were differentiated and infected with *M. tuberculosis* H37Rv at a multiplicity of infection of 1:10.

Treatment with the lead ICL inhibitor at concentrations of 5–20 µM resulted in a dose-dependent reduction in intracellular bacterial burden, achieving > 90% bacterial killing at 10 µM after 7 days of treatment. Importantly, the compound exhibited no cytotoxicity to macrophages at concentrations up to 100 µM (selectivity index > 10).

Combination studies with rifampicin demonstrated additive to synergistic interactions, with combination treatment achieving complete bacterial clearance that was not observed with either agent alone (Madhvi et al. 2019; Mabhula 2021; Medley et al. 2022).

ICL inhibitors as a promising strategy for tuberculosis therapy

The pharmaceutical development of ICL inhibitors has shown both strong potential and significant complexity in targeting the metabolic pathways of *Mycobacterium tuberculosis*. Biochemical studies confirmed potent inhibitory activity of lead compounds, with IC₅₀ values

consistently in the low micromolar or submicromolar range, indicating high affinity for the enzyme's active site (Alam et al. 2025).

Molecular docking studies supported these findings. The inhibitors formed stable interactions with key catalytic residues, confirming the proposed binding mode. Cytotoxicity assays on human cell lines revealed low toxicity, and high selectivity indices suggested minimal off-target activity.

Antimicrobial testing demonstrated a marked reduction in bacterial load under aerobic conditions. Similar results were observed in intracellular models, where the compounds significantly decreased the bacterial burden in infected macrophages. These outcomes were consistent with MIC values and intracellular efficacy profiles.

Pharmacokinetic studies in rodents indicated adequate oral bioavailability and distribution to relevant organs such as the lungs and spleen. Several compounds, however, were rapidly metabolized in the liver. This limitation led to the development of advanced delivery systems, including liposomal and polymeric carriers. These formulations improved drug stability, enhanced macrophage uptake, and provided controlled release over extended periods.

Toxicological evaluations showed no significant adverse effects at therapeutic doses. Higher doses produced only mild and reversible changes. *In vivo* efficacy studies demonstrated reduced pulmonary bacterial loads and improved survival in infected mice. In some cases, the effects approached those of standard therapies such as isoniazid.

Overall, ICL inhibitors show strong promise as a new therapeutic strategy against *M. tuberculosis*, including its latent and drug-resistant forms. Further research is needed to evaluate resistance development, human pharmacodynamics, and compatibility with existing treatment regimens.

Nonetheless, the research demonstrates a significant advancement in metabolic-targeted drug development and sets the stage for the clinical evaluation of ICL inhibitors as part of a new generation of anti-tuberculosis therapies. We present this in more detail in Table 3.

The development of drug candidates targeting ICL has demonstrated strong potential across key drug evaluation parameters. The lead compounds showed potent enzyme inhibition, with IC_{50} values in the low micromolar to submicromolar range. Molecular modeling confirmed stable binding within the ICL active site, validating the proposed mechanism of action. Cytotoxicity studies on mammalian cell lines revealed low toxicity, with cytotoxic concentrations significantly higher than effective doses.

This resulted in high selectivity indices, supporting the compounds' safety for further development. In terms of antimicrobial activity, the compounds exhibited low minimum inhibitory concentrations (MICs) and significantly reduced bacterial loads in infected macrophage models. This indicates that the inhibitors are effective not only under standard laboratory conditions but also within host immune cells, which is crucial for treating intracellular pathogens such as *M. tuberculosis*. Pharmacokinetic studies in animal models showed acceptable oral bioavailability, moderate half-lives, and preferential distribution to the lungs and spleen—organs primarily affected in tuberculosis. Although rapid metabolism was observed in some cases, this issue was addressed through chemical optimization and formulation strategies (Wagh et al. 2021; Osifowokan and Adukpo 2024).

The use of advanced drug delivery systems, such as liposomes and nanoparticles, improved the pharmacokinetic profile.

These formulations enhanced drug stability, enabled sustained release, and increased cellular uptake, supporting prolonged therapeutic action.

Toxicological evaluations (acute and subchronic) confirmed that the compounds were well tolerated, with no significant changes in clinical, biochemical, or histopathological markers at therapeutic and slightly elevated doses. In *in vivo* efficacy studies using mouse models of tuberculosis, treatment with ICL inhibitors led to notable reductions in pulmonary bacterial loads, improved survival rates, and clinical outcomes comparable to standard TB drugs.

Table 3. Summary of the pharmacological properties and potential clinical application of ICL inhibitors against *Mycobacterium tuberculosis*.

Category	Key findings	Implications	References
Enzyme inhibition	IC_{50} values in low micromolar/submicromolar range; stable binding to ICL active site	Demonstrates strong potency and target specificity	Febriyanti et al. 2025
Cytotoxicity	Low toxicity in mammalian cells; high selectivity indices	Indicates good therapeutic window and safety	Lica et al. 2021; Jo et al. 2024
Antibacterial efficacy	Reduced MIC values; significant CFU reduction in macrophage models	Confirms <i>in vitro</i> and intracellular antimicrobial effectiveness	Lee et al. 2021; Zelmer et al. 2022
Pharmacokinetics	Moderate half-life; good bioavailability; strong lung/spleen distribution	Supports systemic and oral dosing potential; suggests pulmonary targeting	Quinn et al. 2024; Zhang et al. 2025
Formulation performance	Liposomes/nanoparticles improved drug stability and sustained release	Enhances delivery and prolongs drug exposure	Khadam et al. 2024; Mujawar and Sankpal 2025
Toxicology	No major adverse effects at therapeutic doses; normal biochemical and histopathological profiles	Supports safety for further development and clinical studies	Basak et al. 2021; Martorana et al. 2022
<i>In vivo</i> efficacy	Significant bacterial reduction in lungs; improved survival rates in TB-infected mice	Demonstrates therapeutic potential comparable to standard treatments	Abdelaal et al. 2024; Yang et al. 2024
Overall outcome	Strong preclinical efficacy and safety; room for further optimization of metabolism and formulations	Justifies progression to advanced preclinical or early clinical stages	Laisney et al. 2022; Arora et al. 2025

Measurement framework

The measurement framework for developing an ICL inhibitor-based medicinal product integrates pharmacodynamic, pharmacokinetic, microbiological, and safety parameters into a structured evaluation system.

Pharmacodynamic potency is assessed using enzyme inhibition metrics such as IC_{50} and K_i , supported by time and concentration-dependent assays. Microbiological efficacy is determined through MIC, MBC, and CFU reduction in extracellular and intracellular models of *M. tuberculosis* infection.

Pharmacokinetic evaluation includes key parameters such as C_{max} , T_{max} , AUC, half-life ($t_{1/2}$), clearance, and volume of distribution, typically measured using validated analytical methods (e.g., HPLC or LC-MS). These data guide dose optimization and define the therapeutic window.

Safety and selectivity are assessed using CC_{50} values and selectivity index ($SI = CC_{50} / IC_{50}$), complemented by standard toxicological markers in preclinical models.

For nanotechnology-based formulations, critical parameters include particle size, zeta potential, encapsulation efficiency, release kinetics, and stability under defined storage conditions.

Together, these core metrics— IC_{50} , MIC, PK parameters, SI, and CFU reduction—form an integrated framework for advancing ICL inhibitors toward clinical development (Fig. 1).

The framework illustrates the integration of pharmacodynamic, pharmacokinetic, microbiological, and safety parameters within a unified evaluation system. This multidimensional approach enables balanced decision-making during preclinical optimization and supports rational progression toward clinical investigation.

Challenges and future perspectives

The development of an ICL inhibitor-based medicinal product represents a compelling advancement in antimicrobial research, particularly against *Mycobacterium tuberculosis* (Mtb). The rationale for targeting ICL—an enzyme crucial to the glyoxylate shunt, absent in humans—provides a strong foundation for selective antimicrobial action. However, while the presented data support this direction, several aspects require deeper critical reflection.

The choice of ICL as a therapeutic target is scientifically sound, as it plays a central role in Mtb persistence under nutrient-limited, hypoxic, or intracellular conditions. The specificity of the glyoxylate shunt to bacterial metabolism theoretically limits off-target effects in humans.

However, while this metabolic pathway is absent in humans, targeting a single essential enzyme raises concerns about bacterial adaptability. Historically, pathogens have developed compensatory or bypass pathways, even for highly conserved enzymes.

Thus, the long-term durability of ICL inhibition as a monotherapy is questionable. Resistance profiling under sub-inhibitory concentrations or chronic exposure was not discussed and should be prioritized in future studies (Fig. 2).

The strong *in vitro* and intracellular efficacy of the lead compounds supports the hypothesis that ICL inhibition can suppress both actively replicating and dormant Mtb. This is a significant strength, as latent TB presents a major obstacle in current treatment strategies.

However, it is unclear whether the observed effects are due solely to ICL inhibition or whether off-target interactions contribute. Enzyme inhibition kinetics were referenced but not deeply characterized (e.g., time-dependent inhibition, reversibility, or covalency). A more detailed biochemical mechanism of action (MOA) analysis would strengthen the argument and help in resistance surveillance.

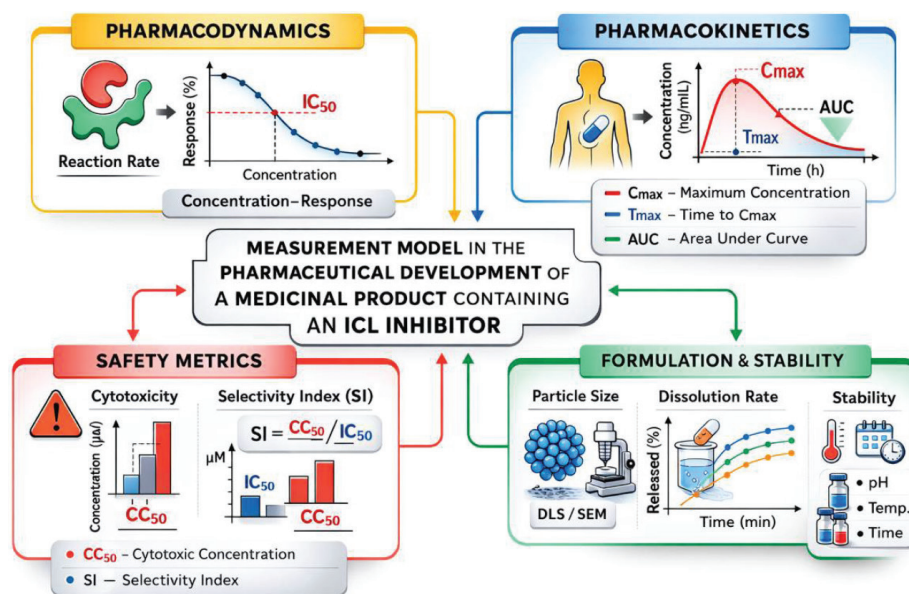


Figure 1. Integrated multidimensional measurement framework for preclinical development of an ICL inhibitor.

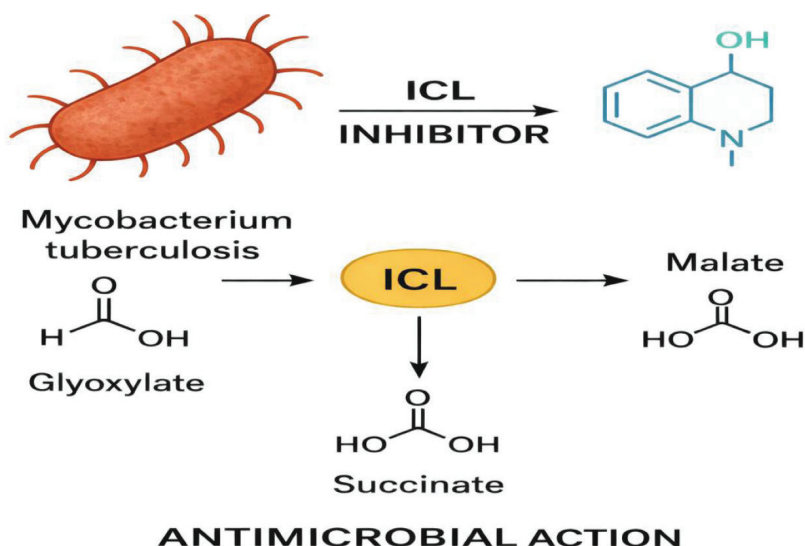


Figure 2. Molecular mechanism of isocitrate lyase inhibition in *Mycobacterium tuberculosis*: schematic representation of inhibitor–enzyme interactions and disruption of the glyoxylate shunt.

While the incorporation of liposomal and nanoparticle formulations represents a modern and strategic approach to overcoming bioavailability issues, the underlying pharmacokinetic challenges remain a concern. Rapid hepatic metabolism could lead to subtherapeutic levels *in vivo*, potentially reducing efficacy and promoting resistance.

Although advanced formulations improved distribution and half-life, data on systemic exposure, tissue retention, and drug–drug interactions were limited or lacking. These parameters are especially important given the high likelihood of co-administration with other TB drugs or antiretrovirals in target populations.

The low cytotoxicity in mammalian cell lines and favorable toxicological profile in animal models are promising. Nevertheless, extrapolating these results to human populations must be done cautiously. Differences in metabolic capacity, immune response, and comorbidities (e.g., HIV and malnutrition) can substantially alter drug behavior and tolerability. Subchronic toxicity is helpful, but long-term safety, reproductive

toxicity, and immunotoxicity data will be required for regulatory progression.

A major strength of the research is its potential application beyond tuberculosis, given the presence of ICL in other pathogens such as *Candida albicans*, *Salmonella enterica*, and *Pseudomonas aeruginosa*. This opens avenues for broad-spectrum antimicrobial development. However, cross-species efficacy remains speculative at this point, as no empirical data were presented for non-mycobacterial organisms. Furthermore, pathogen-specific variations in ICL structure and function may limit universal applicability, requiring case-by-case validation (Fig. 3).

From a global health perspective, the alignment of formulation characteristics with resource-limited settings—such as heat-stable oral dosage forms and simplified dosing—is commendable. However, the logistics of manufacturing, cost, cold-chain independence, and integration into national TB programs remain unaddressed. These factors will determine whether the product is not

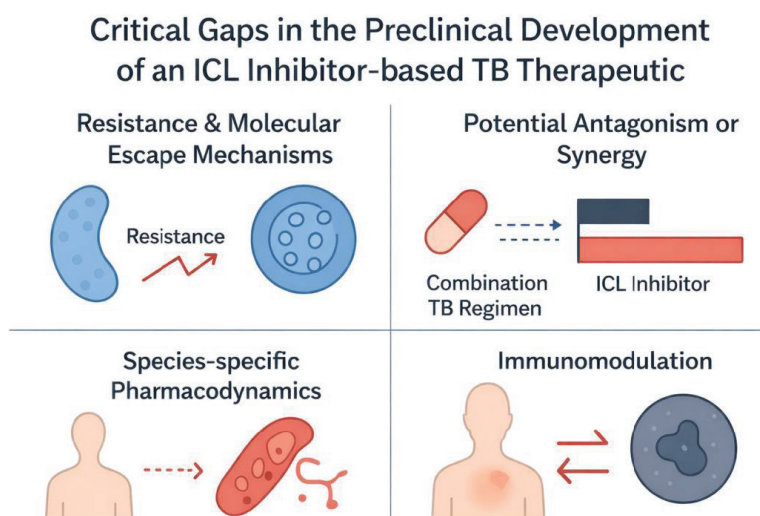


Figure 3. Comprehensive unresolved challenges in the preclinical development of an ICL inhibitor-based therapeutic: resistance, drug interactions, species-specific pharmacodynamics, and immunomodulation.

Table 4. Challenges vs Proposed Solutions.

Challenge	Proposed solution
Risk of resistance development	Long-term exposure studies; sequencing of resistant mutants; use of combination therapy
Insufficiently characterized MOA	Detailed inhibition kinetics; specificity profiling; development of target-engagement biomarkers
Rapid hepatic metabolism	Structural optimization; metabolic shielding strategies; improved nanotechnology-based formulations
Potential antagonism with TB drugs	Systematic combination testing; PK/PD interaction modeling
Limited long-term toxicity data	Chronic toxicity studies; evaluation of immunological and reproductive effects
Incomplete information on antimicrobial spectrum	Testing against additional pathogens; structural comparison of ICL across species
Challenges in healthcare system implementation	Development of low-cost, stable formulations; assessment of manufacturing and distribution logistics
Insufficient data for clinical transition	GLP-compliant toxicology; preparing PK/PD data for Phase I; development of clinical biomarkers

only clinically effective but also accessible and scalable in real-world TB-endemic regions.

Despite encouraging preclinical outcomes, several critical gaps remain:

- resistance development and molecular escape mechanisms under chronic use;
- potential antagonism or synergy in combination with existing TB regimens;
- species-specific pharmacodynamics during clinical translation;
- immunomodulatory effects, especially in immunocompromised patients.

Future research should prioritize:

- comprehensive resistance modeling under prolonged drug exposure;
- *in vivo* combination therapy studies with standard TB drugs;
- early human pharmacokinetic and safety trials (phase I);
- target engagement studies (e.g., biomarker-driven confirmation of MOA).

To effectively plan the next stages of development, it is necessary to systematically identify the key scientific, technological, and clinical barriers that may limit the progress of ICL inhibitors on their path toward clinical trials (Table 4).

In summary, the development of ICL inhibitors is a promising strategy grounded in sound metabolic logic and supported by preclinical data. The specificity, intracellular efficacy, and tolerability of the compounds point toward a potentially impactful therapeutic. Nonetheless, pharmacokinetic limitations, resistance concerns, and translational uncertainties represent significant hurdles. With strategic refinements and comprehensive clinical evaluation, ICL inhibitors could emerge as valuable tools not only in TB therapy but also in broader antimicrobial stewardship efforts. However, rigorous, data-driven progression is essential to avoid premature optimism in a field where many candidates fail during the clinical phase.

Limitations of direct parameter correlations

In the development of medicinal products based on ICL inhibition, it is important to take into account that direct relationships between molecular parameters and pharmacological outcomes are not always realized in practice. Although such relationships simplify analysis, biological systems are too complex to rely on linear “input–output” models (Fig. 4).

First, high inhibitory activity (IC_{50} , K_i) does not guarantee pronounced antibacterial efficacy *in vivo*. Many compounds with submicromolar IC_{50} lose activity due to low bioavailability, poor tissue penetration, or the influence of the immune environment. Moreover, activity against a purified enzyme does not reflect the behavior of a drug inside the cell, where penetration barriers and compensatory metabolic pathways exist.

Second, the structural properties of a molecule do not allow reliable prediction of pharmacokinetics. Parameters such as lipophilicity or molecular weight provide only a general idea but do not account for the influence of transporters, plasma protein binding, or nonlinear metabolism. In the case of ICL inhibitors, this is manifested in rapid hepatic clearance even after chemical optimization.

A third important limitation is associated with dosage forms. Liposomal and nanoparticle systems are theoretically expected to improve stability and delivery; however, their behavior *in vivo* is unpredictable: aggregation, premature release, and uneven macrophage uptake are possible. This requires obligatory confirmation of efficacy in animal models, not only *in vitro*.

In addition, toxicological indicators, including the selectivity index (CC_{50}/IC_{50}), often do not reflect long-term safety. For chronic infections such as tuberculosis, modeling of prolonged exposure is necessary, since the body's response is rarely linear.

In addition, *in vitro* results cannot be directly transferred to conditions in the organism. Macrophage models do not account for the immune response, drug metabolism, and heterogeneity of the mycobacterial population. Therefore, even promising compounds may lose activity at the clinical stage.

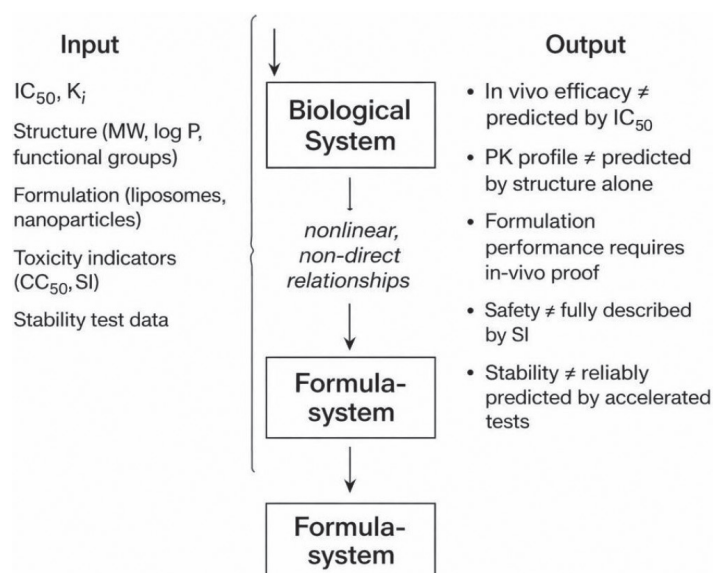


Figure 4. “Input–output”.

Finally, predicting stability based on accelerated tests does not always reflect real storage conditions in regions with unstable logistics and temperature. This creates a risk of overestimating shelf life.

Conclusion

Collectively, available evidence supports isocitrate lyase as a validated metabolic target for next-generation antitubercular therapy. Advances in medicinal chemistry, AI-driven screening, fragment-based design, and nanotechnology-enabled delivery have significantly improved inhibitor potency and pharmacokinetic performance.

Preclinical data demonstrate activity against both replicating and persistent *M. tuberculosis*, favorable selectivity, and promising *in vivo* efficacy. However, further investigation into resistance mechanisms, long-term safety, and combination compatibility is required to enable successful clinical translation.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statements

The authors declared that no clinical trials were used in the present study.

The authors declared that no experiments on humans or human tissues were performed for the present study.

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The authors declared that no informed consent was obtained from the humans, donors or donors’ representatives participating in the study.

The authors declared that no experiments on animals were performed for the present study.

The authors declared that no commercially available immortalised human and animal cell lines were used in the present study.

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Data availability

All of the data that support the findings of this study are available in the main text.

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