Targeting *Mtb* isocitrate lyase for the treatment of tuberculosis

R. P. Bhusal,[1] K. Patel,[1] B. X.C. Kwai,[1] G. Bashiri,[2] J. Sperry,[1] I.K.H. Leung[1] and J. Reynisson[3*]

1. *School of Chemical Sciences, The University of Auckland, Private Bag 92019, Victoria Street West, Auckland 1142, New Zealand*
2. *School of Biological Sciences, The University of Auckland, Private Bag 92019, Victoria Street West, Auckland 1142, New Zealand*
3. *School of Pharmacy & Bioengineering, Keele University, Newcastle-under-Lyme, Staffordshire, ST5 5GB, United Kingdom*

* *Email: j.reynisson@keele.ac.uk*

Introduction

The ability of *Mycobacterium tuberculosis* (*Mtb*) to preferentially utilise lipids as its carbon source is a metabolic feature that enables chronic infection. Isocitrate lyase (ICL) isoforms 1 and 2 are key enzymes in this, through their roles in the glyoxylate and methylcitrate cycles (Figure 1). Both enzymes are essential for *in vivo* growth and virulence, but most studies have focused on the roles and structure of ICL1, leaving our understanding of ICL2 hampered by the lack of structural, functional and mechanistic insight.

The aims of this project are to:

- Develop assays to quantify the potency of ICL inhibitors
- Develop novel inhibitors of *Mtb* ICL
- Establish the role of the ICL2 isoform and its potential druggability

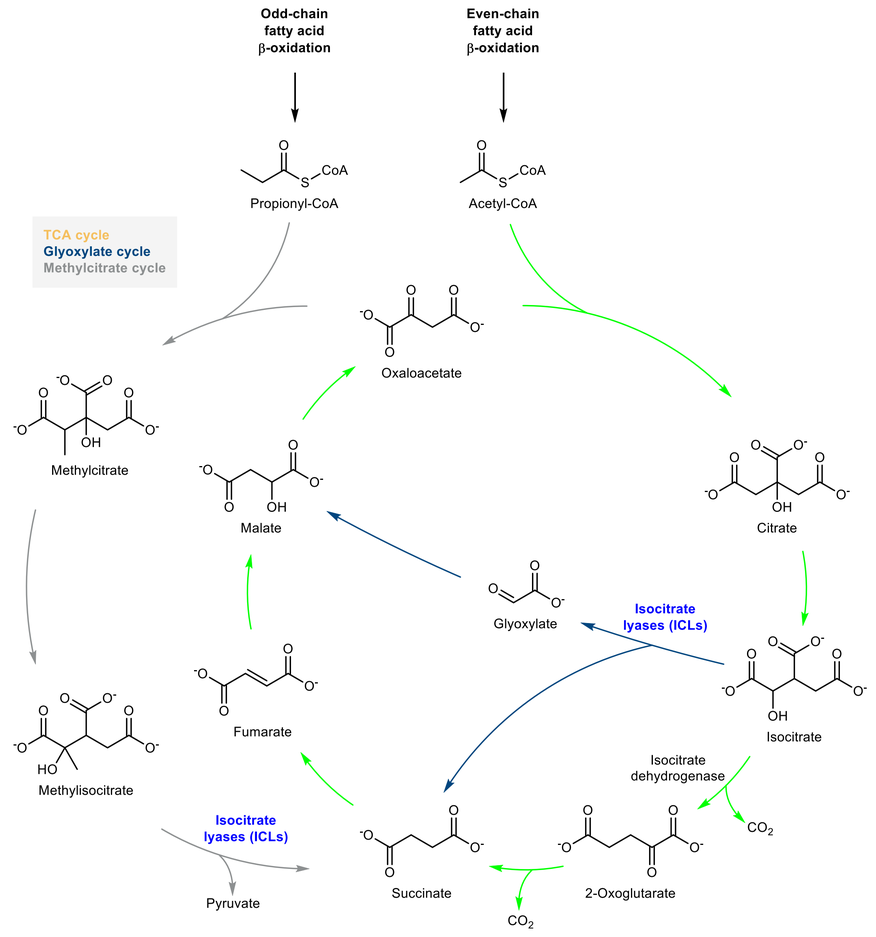

Figure 1 *ICL catalyses the conversion of isocitrate to glyoxylate and succinate in the glyoxylate cycle, and the conversion of methylisocitrate to pyruvate and succinate in the methylcitrate cycle.*

NMR-based kinetics and inhibition assay

^{1}H nuclear magnetic resonance (NMR) spectroscopy is an established technique for the study of enzyme kinetics. It enables direct monitoring of reaction kinetics in real time. Accurate and quantitative information can be obtained by following changes in the peak area of the resonances associated with the substrate and/or reaction products (Figure 2).

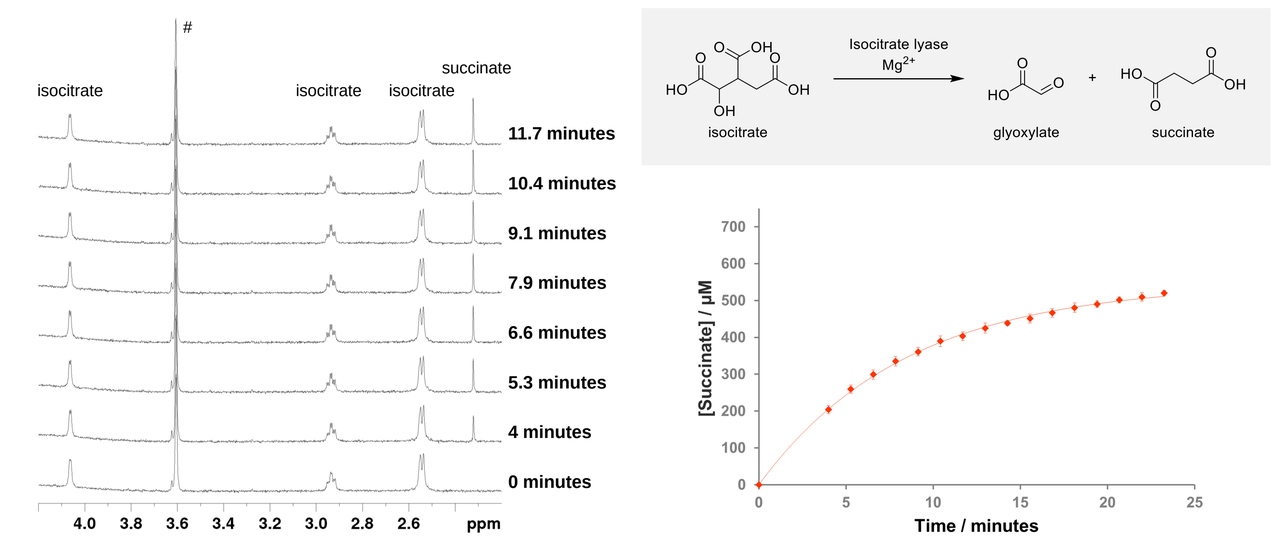

Figure 2 *^{1}H NMR spectroscopy to monitor ICL1-catalysed turnover of isocitrate into succinate. The hashtag (#) indicates Tris/Tris-D11 peak.*

Thermal shift assay

Thermal shift assay is a widely-used method to study protein–ligand interactions. The principle of a thermal shift assay is based on the premise that ligand binding can stabilise or destabilise protein to thermal denaturing, and therefore lead to a shift in the protein's melting temperature (Figure 3).

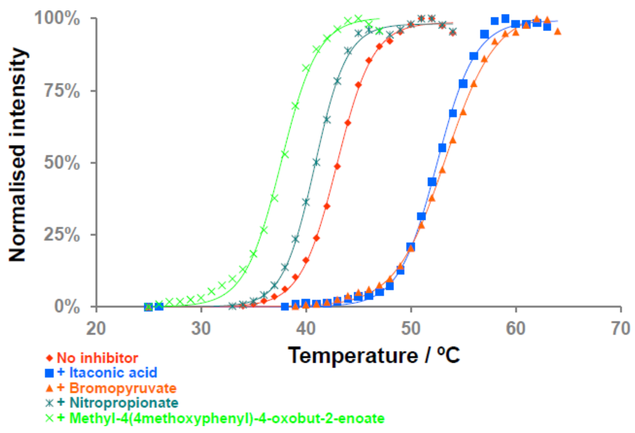

Figure 3 *Protein melt curve for ICL1 inhibitors.*

Virtual screening to identify new ICL inhibitors

Virtual high-throughput screening is a cost-effective and efficient strategy to identify inhibitors of a target protein.
Using the crystal structure of ICL1 (PDB ID: 1F8I), a screen was performed with the Inter-BioScreen Ltd natural product collection. 9050 compounds were screened and fourscoring functions, GoldScore, ChemScore, Piecewise Linear Potential and Astex Statistical Potential, were used. 41 compounds were selected for experimental testing, and two were found to inhibit ICL1 (Figure 4).

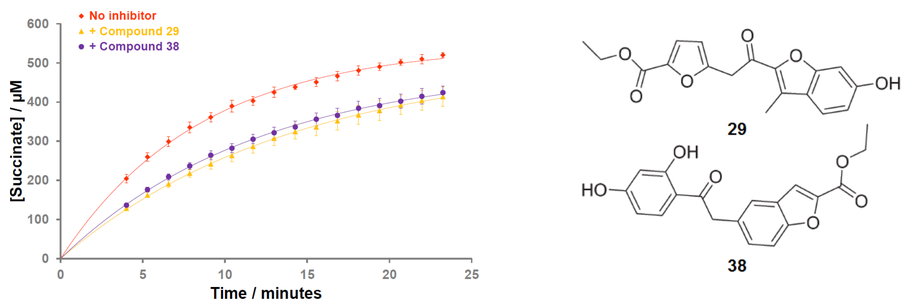

Figure 4 *Two new inhibitors of ICL1 were identified by virtual screening and confirmed by the ^{1}H NMR and thermal shift assays.*

Structure of ICL2 reveals an unusual C-terminal domain

Mtb ICL2 crystallised as a tetramer, with four subunits forming an elongated structure. Each subunit comprises two distinct domains connected by a flexible linker (Figure 5). The C-terminal domain of *Mtb* ICL2 is unique to this isoform, with no sequence homology to known proteins. Structural searches revealed similarities to members of the Gcn5-related N-acetyltransferase (GNAT) superfamily.

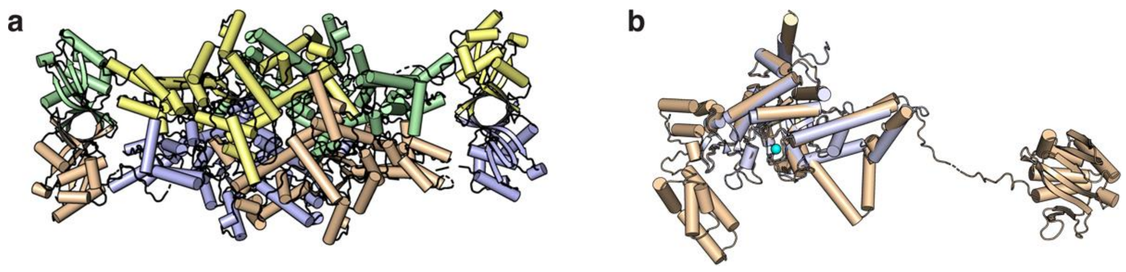

Figure 5 *(a) The four subunits of Mtb ICL2 (shown in different colours) form an elongated structure; (b) Each ICL2 subunit comprises two distinct domain. The N-terminal domain (wheat) structurally resembles ICL1 (light blue). The C-terminal domain, which is specific to ICL2, is separated from the N-terminal domain by a long and flexible linker.*

References

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