

The fall of the mycobacterial cell wall: interrogating peptidoglycan synthesis for novel anti-TB agents

Cheng-Yu Chiang and Nicholas P. West

School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane, Queensland, Australia

ABSTRACT

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* has been a threat to human health for thousands of years and still leads to millions of deaths each year. TB is a disease that is refractory to treatment, partially due to its capacity for in-host persistence. The cell wall of mycobacteria, rich in mycolic acid, is broadly associated with bacterial persistence together with antimicrobial and immunological resistance. Enzymes for the biosynthesis of bacterial peptidoglycan, an essential component of the cell wall, have been addressed and considered as appealing drug targets in pathogens. Significant effort has been dedicated to finding inhibitors that hinder peptidoglycan biosynthesis, many with demonstrated enzymatic inhibition *in vitro* being published. One family of critical biosynthetic enzymes are the Mur enzymes, with many enzyme specific inhibitors having been reported. However, a lesser developed strategy which may have positive clinical implications is to take advantage of the common structural and catalytic characteristics among Mur enzymes and to allow simultaneous, multiple Mur inhibition, and avert the development of drug resistance. *M. tuberculosis* relies on these essential Mur enzymes, with the best-known subset being Mur ligases, but also utilizes unique functions of atypical transpeptidases resulting in peptidoglycan peptide cross-linking beneficial to the bacteria's capacity for chronic persistence in humans. A systematic review is now needed, with an emphasis on *M. tuberculosis*. The urgent development of novel anti-TB agents to counter rapidly developing drug resistance requires a revisit of the literature, past successes and failures, in an attempt to reveal liabilities in critical cellular functions and drive innovation.

Subjects Biochemistry, Microbiology, Infectious Diseases, Pharmacology

Keywords Peptidoglycan, Tuberculosis, Inhibitor, Mur ligase, Transpeptidase, Mycobacteria, Cell wall

INTRODUCTION

Tuberculosis (TB), a highly contagious disease, is caused by the bacteria *Mycobacterium tuberculosis* and remains a global health concern today. According to the WHO, 10.6 million people fell ill with TB in 2022 of which 1.4 million people lost their lives ([World Health Organization, 2023](#)). Treatment regimens are protracted and costly, even for drug susceptible cases. The persistence of *M. tuberculosis*, resulting in latent TB infection, dictates a long treatment regimen, leading to unwanted side effects, reduced patient compliance and drug resistance. Front-line treatment includes a biphasic strategy with four drugs (isoniazid, rifampicin, ethambutol and pyrazinamide) administered for an

Submitted 10 June 2024
Accepted 4 October 2024
Published 14 November 2024

Corresponding author
Nicholas P. West, n.west@uq.edu.au

Academic editor
Nagendran Tharmalingam

Additional Information and
Declarations can be found on
page 23

DOI 10.7717/peerj.18404

© Copyright
2024 Chiang and West

Distributed under
Creative Commons CC-BY 4.0

OPEN ACCESS

initial 2-month intensive phase, and a subsequent 4-month continuation phase comprising isoniazid, rifampicin. Drug resistance in TB is primarily due to spontaneous chromosomal mutations of *M. tuberculosis* and subsequent selection (Migliori et al., 2012). The treatment success rate of multidrug-resistant (MDR) TB and extensively drug-resistant (XDR) TB, was reported as just 60% and 57% respectively in 2022 for the American region and it is projected to be lower globally (World Health Organization, 2023). According to 2022 guidelines an improved 6-month treatment regime composed of bedaquiline, pretomanid, linezolid and moxifloxacin or a 9-month all-oral treatment regime is recommended for MDR-TB patients. However, the evidence of efficacy for these modern regimes is limited and longer treatment is often required (World Health Organization, 2022). In cases which prove to be refractory to treatment, surgical resectioning of diseased lung remains an option (World Health Organization, 2022; World Health Organization, 2023). The cost of MDR-TB treatment is impossible for most of the patients in developing countries, ranging from 1,500 USD to 8,000 USD, and up to 200,000 USD in developed countries (Akalu et al., 2023; Katrak, Wang & Barry, 2023). These intensify the challenge of managing TB and novel therapeutic agents for TB are urgently needed.

The persistence of *M. tuberculosis* is in part due to the highly specialised mycobacterial cell wall. The mycobacterial cell wall complex is primarily composed with three components; mycolic acid, arabinogalactan and peptidoglycan (mAGP) (Jarlier & Nikaido, 1994). Mycolic acid, a very long chain fatty acid (60–90 carbons), forms the outermost layer of mAGP and acts as the major permeation barrier (Brennan & Nikaido, 1995; Jarlier & Nikaido, 1994). Arabinogalactan contributes to structural rigidity, permeation selectivity, and provide the anchor sites for mycolic acids and peptidoglycan (Alderwick et al., 2015). Peptidoglycan, onto which the arabinogalactan layer is covalently bound in mycobacteria, is present in all bacteria and serves as the essential component in maintaining cellular morphology and resistance to osmotic pressure, while maintaining flexibility for cell expansion and division (Cabeen & Jacobs-Wagner, 2005). It is well-acknowledged that the mAGP complex largely attributes the intrinsic resistance of mycobacteria (Jarlier & Nikaido, 1994; Mdluli et al., 1998; Nikaido & Jarlier, 1991).

The importance of peptidoglycan, and its absence from mammalian cells, makes it an attractive target for antibiotic development. The backbone of peptidoglycan consists of alternating N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc) linked via $\beta(1 \rightarrow 4)$ glycosidic bond. The pentapeptide side chain comprises L-alanine (L-Ala), D-glutamic acid (D-Glu), meso-diaminopimelic acid (m-DAP), and dimeric D-alanine (D-Ala-D-Ala), anchored on the lactyl group of MurNAc is responsible for the formation of a crosslinking bridge with the adjacent glycan strand. Inter-peptide crosslinking predominantly occurs between the carboxyl group of D-Ala at position four of one peptide and mDAP in the third position on the adjacent pentapeptide stem (3 \rightarrow 4 linkage), establishing the rigid and mesh-like structure of peptidoglycan (Fig. 1) (Raghavendra, Patil & Mukherjee, 2018). In mycobacteria, a unique peptide linkage pattern can exist during non-replicative persistence or under environmental stress where linkage occurs between diaminopimelic acid of adjacent chains (Lavollay et al., 2008; Wayne & Sohaskey, 2001).

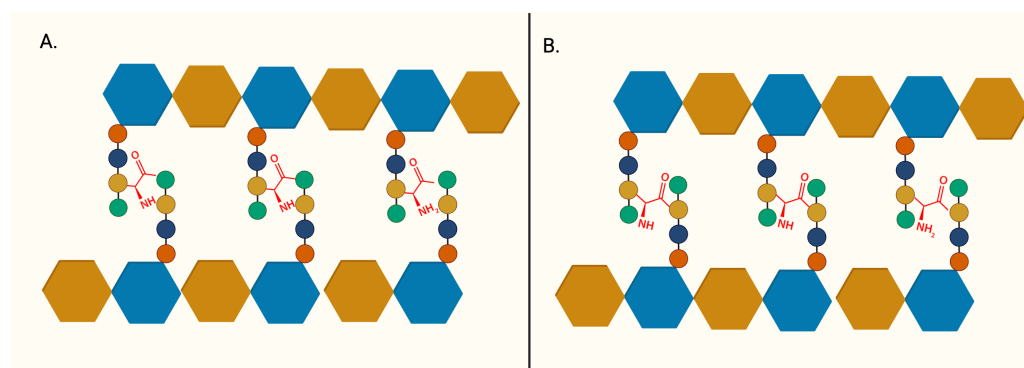


Figure 1 The structure of peptidoglycan crosslinking. (A) Typical 4-3 interpeptide crosslinking bridge of bacterial peptidoglycan. (B) Atypical 3-3 interpeptide crosslinking bridge found in mycobacteria, particularly during persistent or non-replicating state. The blue hexagon represents UDP-MurNAc and the brown hexagon represents GlcNAc. L-Alanine (red circle), D-glutamic acid (yellow circle), D-alanine (green circle), and m-diaminopimelic acid (green circle) are represented in the peptide crosslinking bridge.

Full-size DOI: [10.7717/peerj.18404/fig-1](https://doi.org/10.7717/peerj.18404/fig-1)

These unique features of mycobacterial peptidoglycan provide insights which might be exploited for clinical use in the future. This review summarises the published inhibitors for essential enzymes in peptidoglycan biosynthesis, including efforts to interrogate the atypical transpeptidases of *M. tuberculosis*, which can potentially act as the starting point of novel anti-tubercular therapeutic agents. We hope this review serves as a guide and a catalyst to prime beneficial strategies and future collaborative investigations between the broad, multi-disciplinary TB research community.

SURVEY METHODOLOGY

The literature in scope with the topic of this review was assessed with PubMed, Google Scholar, and Web of Science using key terms: “Mur ligases”, “Mur C ligase”, “Mur D ligase”, “Mur E ligase”, “Mur F ligase”, “Mur X”, “Mur G”, “Mur J”, “peptidoglycan biosynthesis”, “bacterial cell wall biosynthesis” in combination with “inhibitors”, “*Mycobacterium tuberculosis*”, “drug development”, “drug screening”, “antitubercular”, “antibacterial”, “structure–activity relationships”, “multitarget inhibitor”, “antibiotics”, “capuramycin”, “ β -lactamase”, “ β -lactam”, “carbapenem” with “AND” and “OR” operators in search query to ensure comprehensive and unbiased literature search was conducted. The literature identified was evaluated by their pertinence to the topic and all related articles are thoroughly reviewed. The enzyme inhibition activity, antimicrobial activity and reference of compounds mentioned in the text are listed in [Table 1](#).

Synthesis of peptidoglycan

Peptidoglycan synthesis has been thoroughly studied in many organisms since its discovery in 1952 ([Park, 1952](#)). Nevertheless, the precise peptidoglycan synthesis pathway in *M. tuberculosis* remains ambiguous. The generally accepted assumption is that mycobacterial peptidoglycan is synthesised similarly to that of *Escherichia coli* due to the

Table 1 List of identified compounds with the corresponding target, IC₅₀, MIC and reference.

ID	Target	IC ₅₀	MIC	Reference
1	MurC	<i>E. coli</i> : 42 nM	Not determined	Reck et al. (2001)
2	MurC	<i>E. coli</i> : 2.3 μM	Not determined	Ehmann et al. (2004)
3	MurC	Not specified 12–24 μM	<i>E. coli</i> : >65 μM <i>S. aureus</i> : 31–93 μM	Sim et al. (2002)
4	MurC	<i>E. coli</i> : 30.2 μM <i>K. pneumoniae</i> : 26.4 μM <i>P. mirabilis</i> : 41.4 μM	No antimicrobial activity	Zawadzke et al. (2008)
5	MurC	<i>E. coli</i> : 12 nM <i>P. aeruginosa</i> : <10 nM	<i>E. coli</i> ΔTO1C: 3.13 μM <i>P. aeruginosa</i> -546: 1.56 μM	Hameed et al. (2014) Humnabadkar et al. (2014)
6	MurD	<i>E. coli</i> : 0.7–9.0 μM	Not determined	Horton et al. (2003)
7	MurD	<i>E. coli</i> : 10 μM	No antimicrobial activity	Tomašić et al. (2011)
8	MurD	<i>E. coli</i> : 34 μM	<i>S. aureus</i> : 128 μg/mL <i>E. faecalis</i> : 128 μg/mL	Zidar et al. (2011)
9	MurD	<i>S. aureus</i> : 22.33 μM	<i>S. aureus</i> : 2 μg/mL <i>K. pneumoniae</i> : 2 μg/mL <i>E. coli</i> : 16 μg/mL	Jupudi, Azam & Wadhwani (2019)
10	MurD	<i>S. aureus</i> : 13.37 μM	<i>S. aureus</i> : 32 μg/mL <i>K. pneumoniae</i> : 4 μg/mL <i>E. coli</i> : 16 μg/mL	Jupudi, Azam & Wadhwani (2019)
11	MurD	<i>E. coli</i> : 85 μM	Not determined	Humljan et al. (2008)
12	MurD	<i>E. coli</i> : 8.4 μM	No antimicrobial activity	Sosić et al. (2011)
13	MurE	<i>M. tuberculosis</i> : 57 μM	<i>M. tuberculosis</i> : 4 μg/mL <i>M. bovis</i> BCG: 5 μg/mL	Guzman et al. (2010)
14	MurE	<i>M. tuberculosis</i> : 36 μM	<i>M. tuberculosis</i> : 10 μg/mL <i>M. smegmatis</i> : 10 μg/mL <i>S. aureus</i> : 0.5 μg/mL	Guzman et al. (2011)
15	MurF	<i>S. pneumoniae</i> : 8 μM	Not determined	Gu et al. (2004)
16	MurF	<i>S. pneumoniae</i> : 1 μM	Not determined	
17	MurF	<i>S. pneumoniae</i> : 22 nM	Not determined	Stamper et al. (2006)
18	MurF	<i>S. pneumoniae</i> : 0.42 μM <i>E. coli</i> : 81 μM <i>S. aureus</i> : 91 μM	<i>S. pneumoniae</i> : 16–32 μg/mL	Hrast et al. (2013)
19	MurF	<i>E. coli</i> : 26 μM	LPS-defective <i>E. coli</i> : 8–16 μg/mL <i>S. aureus</i> : 8–16 μg/mL <i>E. faecalis</i> : 8–16 μg/mL <i>E. faecium</i> : 8–16 μg/mL	Baum et al. (2007)
20	MurF	<i>E. coli</i> : 24 μM	<i>E. coli</i> : 8 μg/mL <i>S. aureus</i> : >32 μg/mL	Baum et al. (2009)
21	MurF	<i>E. coli</i> : 29 μM	<i>E. coli</i> : 8 μg/mL <i>S. aureus</i> : 32 μg/mL	Baum et al. (2009)
22	MurD MurE MurF	<i>E. coli</i> MurD: 2 μM <i>S. aureus</i> MurE: 6 μM <i>E. coli</i> MurF: 2 μM	<i>E. coli</i> : >128 μg/mL <i>P. aeruginosa</i> : 128 μg/mL <i>E. faecalis</i> : >128 μg/mL <i>S. aureus</i> : >128 μg/mL	Tomašić et al. (2010)

(continued on next page)

Table 1 (continued)

ID	Target	IC ₅₀	MIC	Reference
23	MurD MurE	<i>E. coli</i> MurD: 690 µM <i>E. coli</i> MurE: 89 µM	<i>E. faecalis</i> : 128 µg/mL <i>S. aureus</i> : No activity <i>H. influenzae</i> : No activity	<i>Perdih et al. (2014)</i>
24	MurC MurD MurE MurF	<i>E. coli</i> MurC: 94 µM <i>E. coli</i> MurD: 92 µM <i>E. coli</i> MurE: 32 µM <i>E. coli</i> MurF: 12 µM	<i>S. aureus</i> : 256 µg/mL <i>E. faecalis</i> : >256 µg/mL <i>H. influenzae</i> : 256 µg/mL	<i>Perdih et al. (2015)</i>
25	MurC MurD MurE MurF	<i>E. coli</i> MurC: 41 µM <i>E. coli</i> MurD: 60 µM <i>E. coli</i> MurE: 93 µM <i>E. coli</i> MurF: 89 µM	Not determined	<i>Perdih et al. (2014)</i>
26	MurD MurE	<i>E. coli</i> MurD: 8.2 µM <i>S. aureus</i> MurD: 6.4 µM <i>E. coli</i> MurE: 180 µM <i>S. aureus</i> MurE: 17 µM	<i>S. aureus</i> : 8 µg/mL <i>E. faecalis</i> : 64 µg/mL <i>E. coli</i> : >128 µg/mL	<i>Tomašić et al. (2012a)</i> ; <i>Tomašić et al. (2012b)</i>
27	MurX	550 nM	<i>M. smegmatis</i> : 6.25 µg/mL <i>M. avium</i> : <0.063 µg/mL <i>M. intracellulare</i> : <0.063 µg/mL	<i>Hotoda et al. (2003a)</i> ; <i>Hotoda et al. (2003b)</i>
28	MurX	<i>M. tuberculosis</i> : 41 nM	<i>M. tuberculosis</i> : 0.037 µM	<i>Tran et al. (2017)</i>
29	Transpeptidase	Not determined	<i>M. tuberculosis</i> : 0.1 µg/mL <i>M. abscessus</i> : 0.4 µg/mL	<i>Gupta et al. (2021)</i>

high level of homology between the enzymes involved (*Glauner, Höltje & Schwarz, 1988*). The biosynthesis of peptidoglycan can be spatially separated into three stages; cytoplasmic stage, which is responsible for the biosynthesis of peptidoglycan precursors, membrane-associated stage, which involves attachment of precursors to the inner membrane, and ultimately extracellular stage, where the polymerisation and crosslinking occurs (*Raghavendra, Patil & Mukherjee, 2018*).

The cytoplasmic stage of peptidoglycan synthesis begins with the conversion of fructose-6-phosphate to Uridine-diphosphate-GlcNAc (UDP-GlcNAc), requiring the activity of three enzymes, GlmS, GlmM, and GlmU (*Van Heijenoort, 2001*). GlmS, an aminotransferase, is responsible for the conversion of fructose-6-phosphate into glucosamine-6-phosphate (*Durand et al., 2008*). Subsequent conversion to glucosamine-1-phosphate is catalysed by GlmM, a mutase (*Li et al., 2012*). The acetylation and uridylation of glucosamine-1-phosphate is conducted by the bifunctional enzyme, GlmU, which completes the synthesis of UDP-GlcNAc (*Zhang et al., 2009*). The conversion of UDP-GlcNAc to UDP-MurNAc subsequently takes place due to the activities of the enolpyruvyl transferase and the enolpyruvyl reductase, MurA and MurB respectively. MurA transfers the enolpyruvyl moiety to UDP-GlcNAc, followed by the reduction catalysed by MurB, which converts the enolpyruvyl moiety to D-lacoyl, yielding UDP-MurNAc (*Benson et al., 1993*; *Jankute et al., 2015*; *Xu et al., 2014*). Thereafter, the assembly of the pentapeptide side chain is facilitated by sequential activities of functionally similar ATP-dependent ligases, MurC, MurD, MurE, and MurF (*Barreteau et al., 2008*) in parallel with hydrolysis of ATP (*Fig. 2*), resulting in the addition of L-Ala, D-Glu, mDAP and D-Ala-D-Ala, respectively,

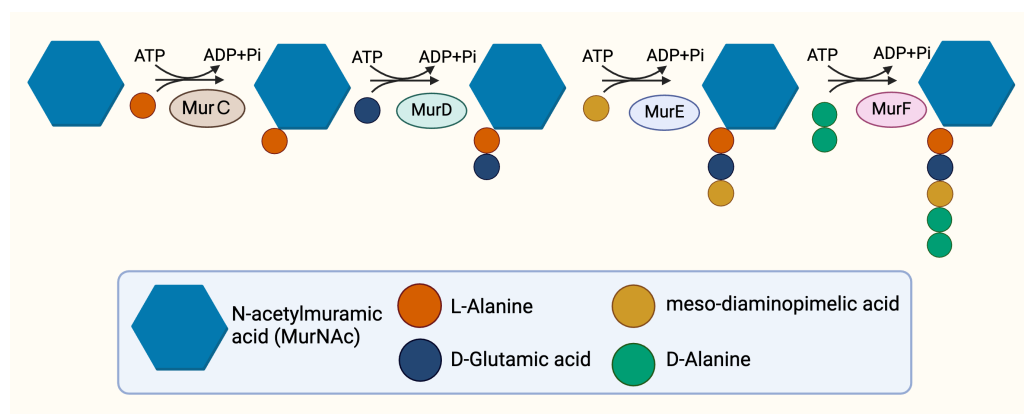


Figure 2 The cytoplasmic synthesis of Park's nucleotide.

Full-size [DOI: 10.7717/peerj.18404/fig-2](https://doi.org/10.7717/peerj.18404/fig-2)

and ultimately resulting in the generation of Park's nucleotide (UDP-MurNAc-L-ala-D-Glu-mDAP-D-Ala-D-Ala) (Munshi et al., 2013).

Following the generation of Park's nucleotide (UDP-MurNAc-pentapeptide), the association with cytoplasmic membrane is facilitated by MurX/MraY, creating a membrane-bound peptidoglycan precursor (Lipid I) (Ben et al., 2013). Next, GlcNAc is attached to Lipid I by MurG, resulting in Lipid II, the monomeric units of peptidoglycan (Jha et al., 2012). It is generally believed that Lipid II is flipped to the periplasm by a "flippase" called MurJ, but the detailed mechanism remains unexplored (Sanduo et al., 2018; Sham et al., 2014). Thereafter, polymerisation and crosslinking of the monomeric units occur in the periplasm in the last stage of peptidoglycan synthesis, which involves transglycosylation and transpeptidation of the monomeric units. Bifunctional enzymes, PonA1 and PonA2 are associated with polymerisation and crosslinking of monomeric units. The glycosyltransferase domain of PonA1 and PonA2 achieve polymerisation by ligating the GlcNAc moiety with muranyl moiety while the transpeptidase domain contributes to the formation of crosslinking between mDAP and D-Ala (3 → 4 linkage) of the adjacent pentapeptide side chain, yielding mature peptidoglycan (Fig. 3) (Hett, Chao & Rubin, 2010; Sauvage et al., 2008). Ultimately, the biosynthesis of peptidoglycan is completed after anchoring to arabinogalactan by Lcp1 phosphotransferase (Harrison et al., 2016).

Mur ligase inhibitors for combating TB

Cell wall peptidoglycan biosynthesis has been heavily investigated and exploited as an antibiotic target since the discovery of penicillin. However, both β -lactam and glycopeptides are ineffective against *M. tuberculosis* due to robust β -lactamases and the intrinsic resistance imparted by the *M. tuberculosis* cell wall (Chambers et al., 1995; Li, Zhang & Nikaido, 2004; Nikaido, 2001). Frontline TB drugs established the viability of targeting cell wall in TB treatment such as isoniazid targets mycolic acid synthesis, ethambutol targets arabinogalactan synthesis, and D-cycloserine targets D-Ala:D-Ala ligase (Prosser & De Carvalho, 2013; Timmins & Deretic, 2006; Zhu et al., 2018). However, peptidoglycan

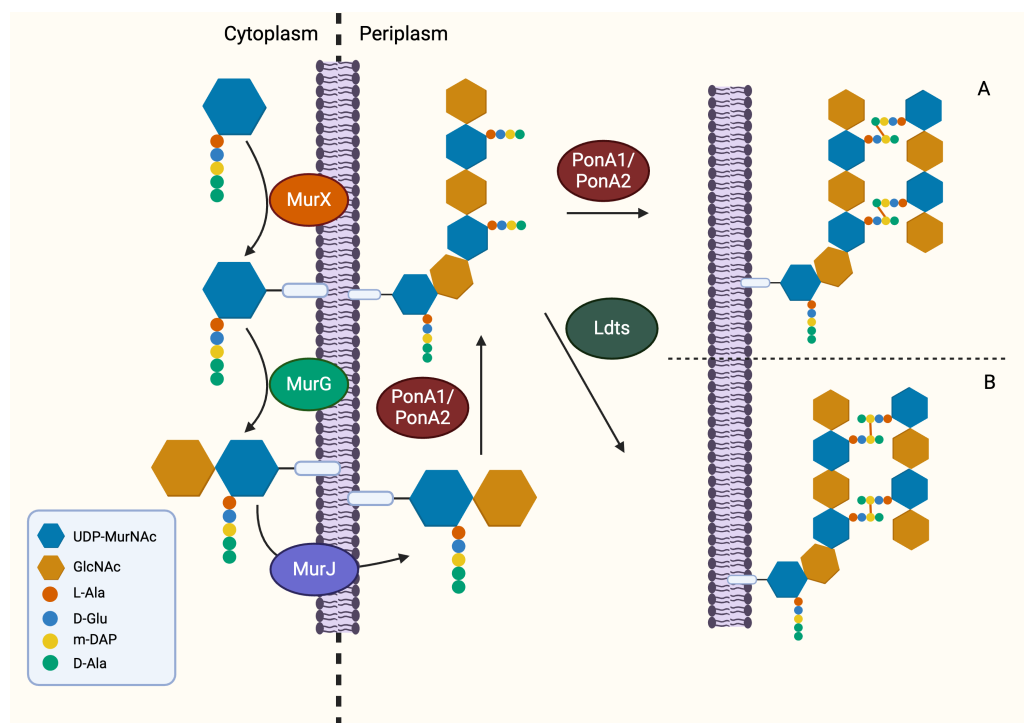


Figure 3 The membrane-associated stage and the extracellular stage of peptidoglycan synthesis. Two distinctive linkage can be facilitated by different enzymes in transpeptidation, the ultimate step of peptidoglycan synthesis. (A) The transpeptidation domain of PonA1/PonA2 facilitate the formation of 3-4 linkage and (B) Ldt_{MT1-5} facilitate formation of 3-3 linkage which is commonly seen in dormant or non-replicating Mtb.

Full-size [DOI: 10.7717/peerj.18404/fig-3](https://doi.org/10.7717/peerj.18404/fig-3)

inhibitors approved for TB therapy are limited. According to 2022 guidelines, β -lactam-based antibiotic, imipenem-cilastatin or meropenem, can be used in the treatment of MDR or XDR-TB cases. However, due to the lack of clinical evidence, they are conditionally recommended and only administered when frontline and second-line medication cannot be used (*World Health Organization, 2022*). Recent clinical trials on meropenem also demonstrated minimal improvement in treatment outcomes (*De Jager et al., 2022*). The D, D-transpeptidases responsible for the formation of typical 3 \rightarrow 4 crosslinking bonds are the essential target for β -lactam antibiotics (*Ghuysen, 1991*). Previous research indicated that an exceptionally high percentage (80%) of atypical 3 \rightarrow 3 linkage are formed by L, D-transpeptidases in dormant *M. tuberculosis* during non-replicating persistence (*Fig. 1*) (*Lavollay et al., 2008*). Thus, sole inhibition of D, D-transpeptidases by β -lactam antibiotics is insufficient in clearing a mycobacterial infection (*Gupta et al., 2010*). Recently, carbapenems which target both D, D-transpeptidase and L, D-transpeptidase have been used in treating MDR-TB and XDR-TB (*Kumar et al., 2019; Van Rijn et al., 2019*).

There is currently great interest in developing novel anti-tubercular drugs targeting the early stages of peptidoglycan synthesis with Mur ligases being of particular interest. There are good reasons for the interest here, including; the Mur ligases are present in all clinically important bacterial pathogens but absent in mammals, providing potential

board-spectrum inhibition and selectivity ([Kouidmi, Levesque & Paradis-Bleau, 2014](#)), and; the conserved structure of binding sites among Mur ligases can be exploited by designing inhibitors with potential multitarget inhibition which target multiple Mur ligases. The probability of resistance against multitarget inhibitors reduces exponentially because at least two advantageous mutations are required in the same generation to develop resistance. Hence, multitarget inhibitors can be clinically beneficial in difficult-to-treat bacteria such as *M. tuberculosis* ([Silver, 2007](#)).

The only published protein structure of Mur ligases from *M. tuberculosis* remains MurE ([Basavannacharya et al., 2010](#)); nonetheless, the X-ray crystallography of Mur ligases from different species has been solved, aiding rational drug design. Homology models of Mur ligases for *M. tuberculosis* were constructed based on the amino acid sequence similarity with these corresponding homologues. The crystal structure of MurC from *Haemophilus influenzae* was utilised as a template for homology modelling of *M. tuberculosis* MurC, while *Escherichia coli* was exploited for MurD modelling and *Streptococcus pneumoniae* was exploited for MurF modelling ([Kotnik et al., 2007](#); [Mol et al., 2003](#); [Moraes et al., 2015](#); [Yan et al., 2000](#)). Since good structural homology is observed, it is expected that inhibitors of Mur ligases of other species might inhibit mycobacterial Mur ligases as well. Herein, we review the inhibitors of Mur ligases from various bacterial species which might serve as a good starting point in developing mycobacterial Mur ligase inhibitors.

Inhibitors of MurC

MurC facilitates the initial step of the pentapeptide side chain assembly and is the preliminary step in the cytoplasmic stage of peptidoglycan synthesis. Enormous effort has been devoted to the discovery and development of molecules which interfere with this catalytic process ([Fig. 4](#)). Many published MurC inhibitors were transition state analogues such as phosphinate and Banzofuran acyl-sulfonamide.

Initially, a series of phosphinate compounds were synthesised as transition state analogue inhibitors for MurC. The phosphinate inhibitors demonstrated significant potency against MurC with the most potent compound exhibiting an IC_{50} of 42 nM ([Fig. 4](#), compound 1) ([Marmor et al., 2001](#); [Reck et al., 2001](#)). Due to the structural similarity of Mur ligases, phosphinates were found to be capable of inhibiting MurD-F, which will be discussed in the following sections.

Benzofuran acyl-sulfonamides was identified as an inhibitor of MurC with an IC_{50} value of 2.3 μ M ([Fig. 4](#), compound 2). However, it displayed significant protein binding liabilities which led to notable reduction in MurC inhibition ([Ehmann et al., 2004](#)).

Besides transition state analogues, phosphate or diphosphate mimetics are commonly seen in Mur ligase inhibitors ([Maitra et al., 2019](#)). These compounds generally incorporate a heterocyclic moiety such as rhodanine or thiazodindione. Benzylidene rhodanines ([Fig. 4](#), compound 3) were identified as MurC inhibitors, with many exhibiting low micro-molar inhibition ($IC_{50} = 12\text{--}24 \mu$ M). The whole-cell inhibitory activity of benzylidene rhodanine was also evaluated, with negligible antibacterial activity displayed for *E. coli* ($MIC > 65 \mu$ M) and modest activity observed in methicillin-resistant *Streptococcus aureus* ($MIC = 31\text{--}93 \mu$ M) ([Sim et al., 2002](#)). Two ATP-competitive inhibitors ([Fig. 4](#),

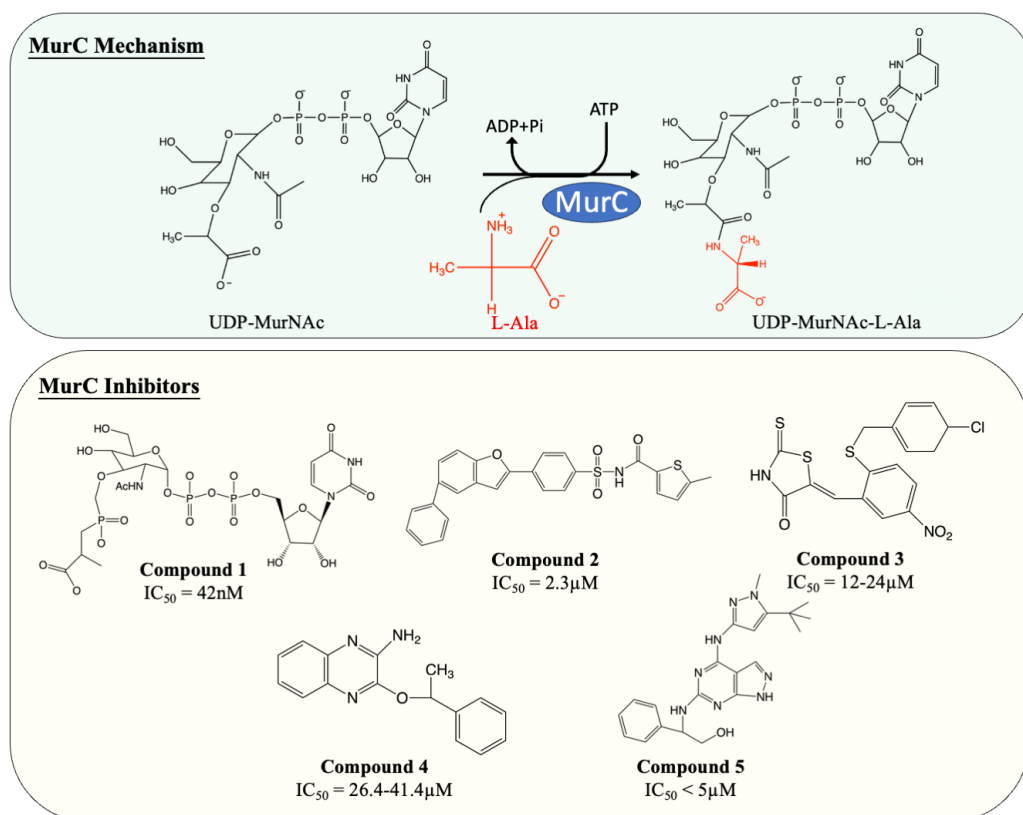


Figure 4 The catalytic mechanism of MurC with inhibitors and corresponding IC₅₀ value.

Full-size [DOI: 10.7717/peerj.18404/fig-4](https://doi.org/10.7717/peerj.18404/fig-4)

compound 4 and 5) were discovered *via* high-throughput screening (HTS). The potency of compound 4 (a quinoxaline) was evaluated against MurC isolated from six microorganisms (*E. coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Haemophilus influenzae*, *Acinetobacter baylyi*, *Pseudomonas aeruginosa*). Interestingly it only inhibit MurC isolated from Enterobacteriaceae (IC₅₀*E. coli* = 30.2 μM, IC₅₀*K. pneumoniae* = 26.4 μM, IC₅₀*P. mirabilis* = 41.4 μM) but failed to inhibit MurC from other bacterial families. This suggests that there are distinct structural features in Enterobacterial MurC which are required for the inhibition by the quinoxaline (Zawadzke *et al.*, 2008). Nevertheless, compound 4 was not antibacterial even with highly drug-susceptible, efflux-defective *E. coli* strain (*E. coli* ΔTO1C) (Zawadzke *et al.*, 2008).

Pyrazolopyrimidine (compound 5) inhibited MurC isolated from *E. coli* and *P. aeruginosa* at nanomolar levels (IC₅₀ of 12 nM and <10 nM respectively). In contrast to quinoxaline, pyrazolopyrimidines displayed antibacterial activity in both TO1C-mutated *E. coli* and efflux-deficient *P. aeruginosa* (MIC_{*E. coli* ΔTO1C} = 3.13 μM, MIC_{*P. aeruginosa* 546} = 1.56 μM) (Hameed *et al.*, 2014; Humnabadkar *et al.*, 2014). Nevertheless, no antibacterial activity was observed in wild-type *E. coli* and *P. aeruginosa*, suggesting the low permeability and high efflux rate restrain the development of pyrazolopyrimidine as an antibacterial

agent and was not further tested on *M. tuberculosis* (Hameed et al., 2014; Humnabadkar et al., 2014).

Apart from chemical entities, short peptide ligands were also examined as MurC inhibitors. Two peptide MurC inhibitors (N-Asp-His-Arg-Asn-Pro-Asn-Tyr-Ser-Trp-Leu-Lys-Ser-COOH and N-Cys-Gln-Asp-Thr-Pro-Tyr-Arg-Asn-Cys-COOH) was discovered *via* phage display, but IC₅₀ values of 1.5 mM and 0.9 mM respectively were disappointing (El Zoeiby et al., 2003). Feglymycins of *Streptomyces*, a 13-mer peptide antibiotic was also revealed with exceptional capability to inhibit both MurA and MurC of *E. coli* (IC₅₀ = 3.4 μM and 0.3 μM, respectively) and *S. aureus* (IC₅₀ = 3.5 μM and 1.0 μM, respectively) (Hänchen et al., 2013). Additionally, feglymycin demonstrated antibacterial activity on three drug-resistant *S. aureus* strains with MICs between 0.25 μM to 1 μM, highlighting the potential of MurC as a novel pathway in combating rapid-emerging resistance and warranting further consideration (Hänchen et al., 2013).

Inhibitors of MurD

MurD is responsible for the subsequent ligation of D-Glu onto L-Ala, which results in the formation of UDP-MurNAc-L-Ala-D-Glu. It is known that MurD possesses high stereospecificity toward D-Glu. The binding sites for D-Glu are conserved across almost all bacteria, which made MurD an ideal target for antibiotic development. A significant amount of effort has been dedicated to finding MurD inhibitors (Fig. 5). In 2003, a macrocyclic MurD inhibitor (Fig. 5, compound 6) was discovered *via* a structure-based rational design. The synthesised macrocyclic inhibitors demonstrated inhibition against MurD at the low micromolar range (IC₅₀ range between 0.7–9.0 μM) (Horton et al., 2003). However, no further progress was documented in the development of this molecule which might be due to the lack of any reported antibacterial activity.

More recently, virtual screening has been heavily deployed due to time and cost efficiency in identifying inhibitory scaffolds, which can then be subsequently optimised to improve potency and antibacterial activity. A lead compound containing D-Glu moiety was discovered as a MurD inhibitor *via* virtual screening, exploiting the high specificity towards D-Glu. Preliminary structural optimisation of the D-Glu-based scaffold showed that a compound containing a 5-benzylidenerhodanine can inhibit MurD in micromolar concentration (IC₅₀ = 174–206 μM) (Tomašić et al., 2009). Further optimisation was conducted and led to the discovery of 5-benzylidenerhodanine and 5-Benzylidenethiazolidine-2,4-dione, inhibitors with improved potency (IC₅₀ = 85 μM and 45 μM, respectively) (Zidar et al., 2010). The binding mode of 5-Benzylidenerhodanine was evaluated through X-ray crystallography and revealed the compound binds to the active site of MurD, suggesting the compounds are the ideal starting point of developing a MurD-targeting antibiotic (Zidar et al., 2010). In another study, the lead compound (Fig. 6, compound 7) was developed by combining 2-thioxothiazolidin-one-ring, a uracil mimicry that binds to MurD, and glutamic acid and demonstrated good potency against MurD with IC₅₀ of 10 μM (Tomašić et al., 2011). Further exploration of the structure–activity relationship (SAR) on the scaffold demonstrated three novel 5-benzylidenethiazolidin-4-one inhibitors that can inhibit MurD with IC₅₀ less than 50 μM (Zidar et al., 2011).

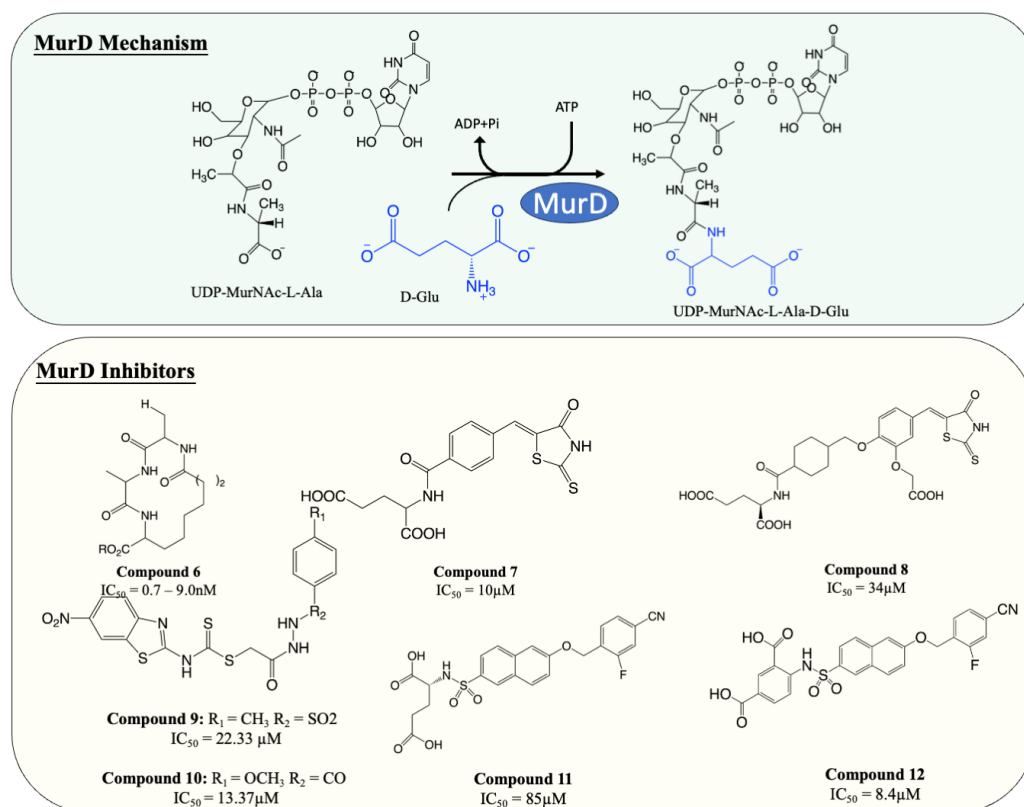


Figure 5 The catalytic mechanism of MurD with inhibitors and corresponding IC_{50} value.

Full-size [DOI: 10.7717/peerj.18404/fig-5](https://doi.org/10.7717/peerj.18404/fig-5)

Unfortunately, these compounds lacked antibacterial activity, with only one compound (Fig. 6, compound 8) displaying poor antibacterial activity against Gram-positive *S. aureus* and *E. faecalis* with MIC of 128 $\mu\text{g/mL}$ (Zidar *et al.*, 2011).

In 2019, a virtual screening based on *S. aureus* MurD structure followed by docking energy and molecular dynamics (MD) simulation also successfully identified another scaffold. The following *in vitro* data indicated that the identified hit displayed significant inhibitory activity ($IC_{50} = 7 \mu\text{M}$) and mild antibacterial activity (MIC = 128 $\mu\text{g/mL}$) against *S. aureus* and *Bacillus subtilis*, suggesting it was a promising candidate for development (Azam & Jupudi, 2019). Subsequent structural modifications were performed on the scaffold to improve the affinity towards MurD. The modified compounds demonstrated inhibitory activity with IC_{50} ranged from 13.37–79.27 μM . Among the modified molecules, the benzothiazol-2-ylcarbamidithioate substitutes, compound 9 and 10 (Fig. 5), possess the greatest inhibitory property against *S. aureus* with IC_{50} of 22.33 and 13.37 μM respectively. Furthermore, unlike most of the Mur ligases inhibitors, compound 13 and 14 exhibited significant bactericidal activity against multiple microorganisms (MIC_{*S. aureus*} = 2 and 32 $\mu\text{g/mL}$, respectively. MIC_{*K. pneumoniae*} = 2 and 4 $\mu\text{g/mL}$, respectively. MIC_{*E. coli*} = 16 and 16 $\mu\text{g/mL}$, respectively) compared to the current standard treatment ciprofloxacin and gentamicin (MIC_{*S. aureus*} = 2 and 8 $\mu\text{g/mL}$, respectively) (Jupudi, Azam & Wadhwani,

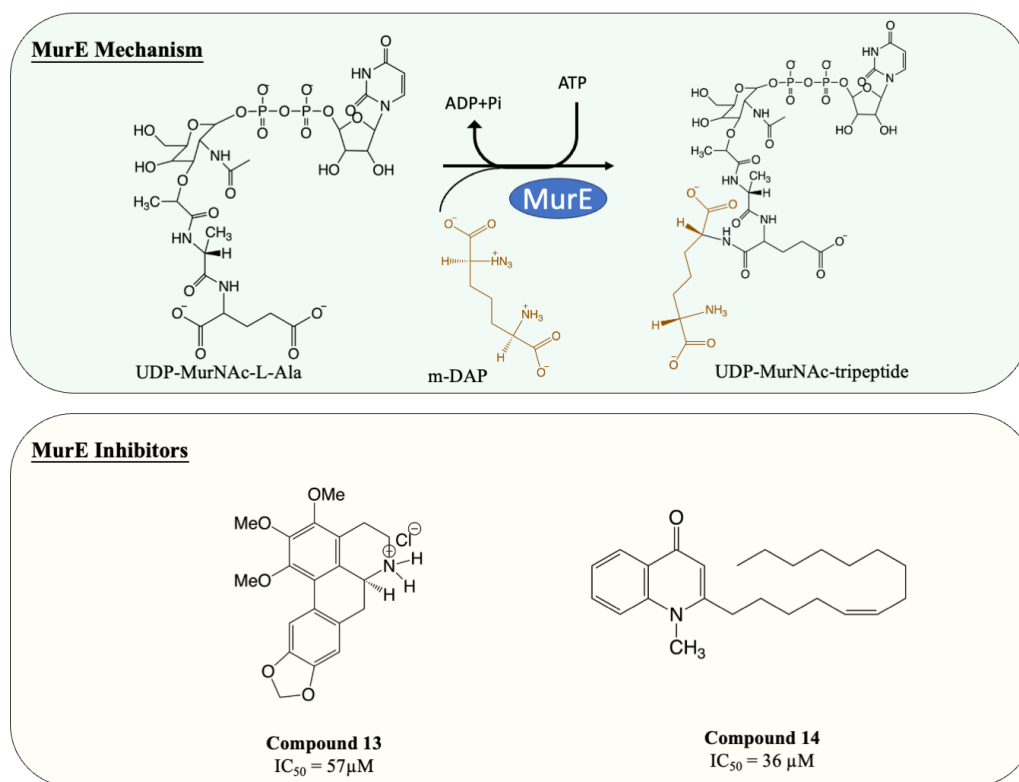


Figure 6 The catalytic mechanism of MurE with inhibitors and corresponding IC_{50} value.

[Full-size DOI: 10.7717/peerj.18404/fig-6](https://doi.org/10.7717/peerj.18404/fig-6)

2019). The promising antibacterial activity in a broad spectrum of bacteria warrants further investigation and requires validation on *M. tuberculosis*.

Another compound, naphthalene-N-sulfonyl-d-Glu, was designed to mimic the tetrahedral transition state of MurD. The inhibitors exhibited the competitive inhibition mode to D-Glu with the IC_{50} value of $280 \mu\text{M}$. The structure of inhibitor-MurD complex also demonstrated that the inhibitor binds to the enzyme active site *via* hydrogen bond and lipophilic interaction formed between D-Glu moiety and D-Glu-binding pocket (Kotnik *et al.*, 2007). The subsequent structural optimisation fully elucidated that the D-Glu moiety and sulphonamide bond are essential for MurD inhibition as D-Glu provides specificity and sulphonamide bond provides geometric similarity with the tetrahedral intermediate (Obreza & Gobec, 2004). The structural optimisation demonstrated that introducing arylalkyloxy substituent on position 6 of the naphthalene further improved the inhibition, with compound 11 (Fig. 5) being the most potent compound in this study with IC_{50} at $85 \mu\text{M}$ (Humljan *et al.*, 2008). The superior potency was backed by a free binding energy calculation, indicating the incorporation of 4-cyanophenyl of compound 11 contributes to the most potent binding compared to other analogues (Perdih, Bren & Solmajer, 2009).

The naphthalene-N-sulfonyl-D-Glu inhibitors were further developed into second-generation sulphonamide inhibitors by rigidising the D-Glu moiety. The resulting compound 12 (Fig. 5) exhibited a further 10-fold improvement in inhibition activity with

the IC₅₀ of 8.4 μM compared to the parent compound 11 (Sosič *et al.*, 2011). The recent computational analysis of second-generation sulphonamide inhibitors also elucidated that the rigid D-Glu moiety allows them to be more favourable in binding with the calculated binding free energy of −3.4 kcal/mol, compared to unrigidised inhibitor, with the binding value of −2.6 kcal/mol and further attention is warranted in this space (Perdih, Wolber & Solmajer, 2013). Unfortunately, these compounds were found to be ineffective in inhibiting bacterial growth, which might be attributed to poor penetration into bacterial cell walls. Despite the lack of antibacterial activity on the species tested, the enzyme potency and specificity demonstrated suggests an opportunity for further development as anti-TB agents (Sosič *et al.*, 2011).

Inhibitors of MurE

MurE facilitates the attachment of m-DAP onto D-Glu moiety of UDP-MurNAc-dipeptide, yielding UDP-MurNAc-tripeptide. Interestingly, despite there being fewer MurE inhibitors published, many of the MurE inhibitors demonstrated inhibition of the *M. tuberculosis* MurE, as well as antitubercular activity. Previous studies have shown that analogues of m-DAP and tetrahedral transition state (phosphinates) both possess potency against MurE (Auger *et al.*, 1996; Strancar *et al.*, 2007). Another scaffold, 3-methoxynordomesticine, which was isolated from the Colombian plant *Ocotea macrophylla*, was identified with anti-tubercular properties. The most potent compound (Fig. 6, Compound 13) exhibited MurE-inhibiting activity IC₅₀ of 57 μM and anti-tubercular activity with MIC <5 μg/mL against *M. tuberculosis* H37Rv. Further structural optimisation of 3-methoxynordomesticine is suggested to reduce cytotoxicity while improving affinity for MurE (Guzman *et al.*, 2010). Similar to 3-methoxynordomesticine, (S)-Leucosine isolated from the Colombian Lauraceae tree *Rhodostemonodaphne crenaticupula* was also found to hinder the growth of *M. tuberculosis*. An explorative SAR study was conducted focused on the common tetrahydroisoquinoline scaffold between 3-methoxynordomesticine and (S)-Leucosine. After three rounds of optimisations, three tetrahydroisoquinolines were able to inhibit *M. tuberculosis* MurE with IC₅₀ < 111 μM, with antimycobacterial activity displayed (MIC = 20–60 μg/mL), conferring tetrahydroisoquinolines as MurE-targeting anti-tubercular agents (Guzman *et al.*, 2015).

An alternative scaffold identified, N-methyl-2-alkenyl-4-quinolone, was isolated from *Euodia rutaecarpa*, a Chinese medical plant and display antimycobacterial activity with MIC of 1 μg/mL against *Mycobacterium smegmatis* (Wube *et al.*, 2011). A subsequent study elucidated the antimycobacterial property of N-methyl-2-alkenyl-4-quinolones (Fig. 6, compound 14) by demonstrating inhibition against *M. tuberculosis* MurE (IC₅₀ = 36–72 μM). Docking simulation revealed that quinolones interact to the pocket near N-terminus uracil recognition site of MurE with a competitive inhibition pattern against UDP-MurNAc-dipeptide substrate observed (Guzman *et al.*, 2011).

Inhibitors of MurF

MurF is responsible for the ligation of D-ala-D-ala dipeptide during cytoplasmic peptidoglycan synthesis, which is also the ultimate step in Mur ligases cascade. Significant

numbers of MurF inhibitors have been investigated. Two MurF-inhibiting leads (Fig. 7, compound 15 & 16) ($IC_{50} = 8 \mu M$ and $1 \mu M$, respectively) were reported *via* affinity selection screening by Abbott lab, followed by several rounds of structure-based optimization (Gu *et al.*, 2004). Guided by the crystal structures reported, the optimisation successfully improved the potency by 40-fold, improving the IC_{50} from $1 \mu M$ to 22 nM (Fig. 7, compound 17), albeit the improved compound still lacked bactericidal activity (Gu *et al.*, 2004; Stamper *et al.*, 2006). The mechanism of action of compound 17 was further elucidated by the following co-crystallisation study, which shows that compound 17 occupies the uridine-ribose binding pocket on the N-terminal domain and induces conformational closure (Longenecker *et al.*, 2005). Even though the compound was never adapted commercially due to the absence of antibacterial activity, this study established a profound insight into the interaction between the compound and MurF. More recently, two SAR studies designed and synthesised a series of cyanothiophene MurF inhibitors by introducing structural modifications on compound 15, followed by potency examination on MurF from three species (*S. pneumoniae*, *E. coli* and *S. aureus*) and MIC determination (Hrast *et al.*, 2014; Hrast *et al.*, 2013). Ultimately, the modified compounds (Fig. 7, compound 18) successfully enhanced the inhibition activity to nanomolar level towards MurF of *S. pneumoniae*, yet only poor inhibition was demonstrated in *E. coli* and *S. aureus* ($IC_{50} < 100 \mu M$) (Hrast *et al.*, 2013). Co-crystallisation demonstrated that the newly developed cyanothiophene-based inhibitors interact with MurF in a similar fashion to the parent compound which induced interdomain closure, and several modified compounds display moderate bactericidal activity against *S. pneumoniae* and *E. coli* (MIC = 16–32 $\mu g/mL$) (Gu *et al.*, 2004; Hrast *et al.*, 2014; Hrast *et al.*, 2013). Moreover, multiple computational approaches were exploited in validating and optimising the compound-enzyme interaction of compound 15 and compound 16 as well as design new MurF inhibitors based on the scaffold, which now require further *in vivo* examination (Azam & Jupudi, 2017; Khedkar, Malde & Coutinho, 2007; Taha *et al.*, 2008).

A series of MurF-inhibiting compounds, 8-hydroxyquinoline, was also determined with significant potency ($IC_{50} = 0.33\text{--}25 \mu M$) and moderate bactericidal activity (MIC = 8–32 $\mu g/mL$) (Baum *et al.*, 2007). However, the subsequent assays showed that the antibacterial activity of 8-hydroxyquinoline was predominantly contributed by its well-known chelating property (Fraser & Creanor, 1975; Rohde *et al.*, 1977). To circumvent this issue, another compound, 4-phenylpiperidine (Fig. 7, compound 19), discovered *via* 8-hydroxyquinoline-based pharmacophore modelling, showed bactericidal activity *via* MurF inhibition ($IC_{50} = 26 \mu M$) in LPS-modified *E. coli* (MIC = 8 $\mu g/mL$) but absent in wild type *E. coli* (MIC > 32 $\mu g/mL$) due to poor cell penetration (Baum *et al.*, 2007). The pharmacophore model also led to the discovery of two diarylquinolines (Fig. 7, compound 20 and 21) which displayed MurF-inhibiting properties with IC_{50} of 24 and 29 μM , respectively. Moreover, compound 20 and 21 demonstrated antibacterial activity in both Gram-positive and Gram-negative species (MIC = 2–8 $\mu g/mL$) with accumulated MurF reactants and decreased MurF products, suggesting that the antibacterial activity of diarylquinolines was induced by MurF inhibition (Baum *et al.*, 2009). This finding

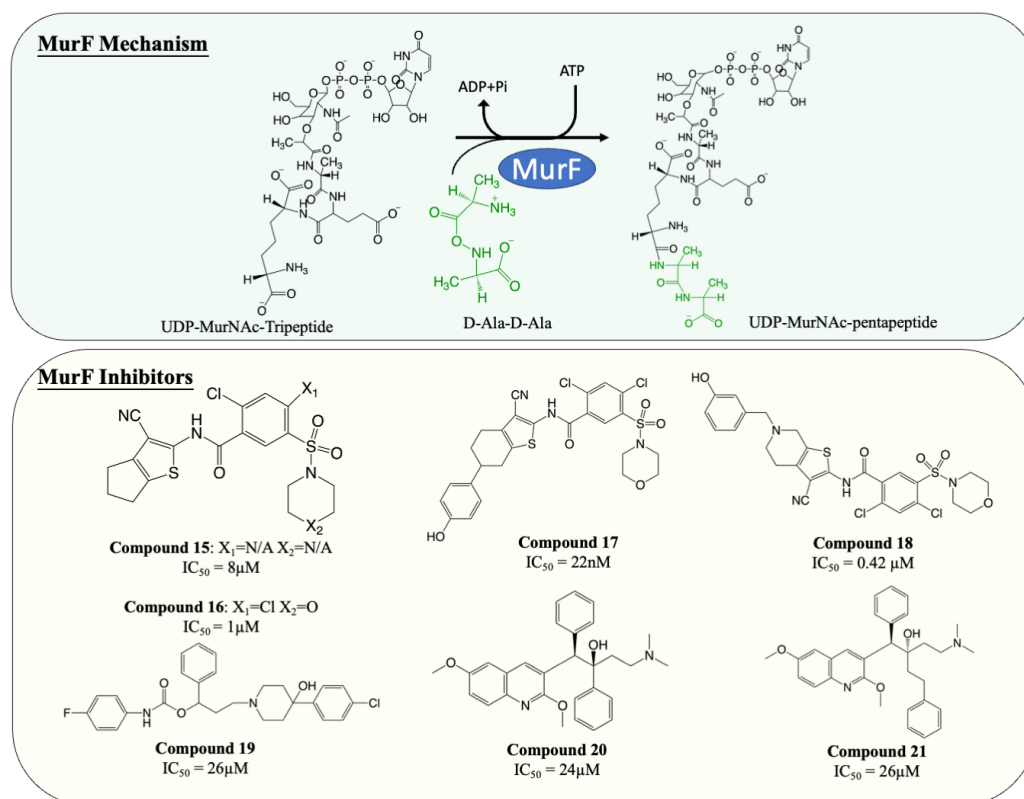


Figure 7 The catalytic mechanism of MurF with inhibitors and corresponding IC_{50} value.

[Full-size DOI: 10.7717/peerj.18404/fig-7](https://doi.org/10.7717/peerj.18404/fig-7)

warranted the development of a MurF inhibitor into novel anti-TB chemotherapy, with diarylquinolines showing promising results in a broad spectrum of bacteria.

Multitarget inhibitors

It stands to reason that multitarget inhibitors may represent a therapeutic benefit over single-target inhibitors in certain conditions (Silver, 2007). The threshold for potency may decrease with the synergistic inhibition of multiple pathway enzymes, reducing the costs associated with treatment and the risk of off target effects. Further, the development of resistance is less likely to occur since it would require simultaneous mutations on two or more targeted genes in a single generation (Škedelj et al., 2011). Mur ligases are ideal targets for designing multitarget inhibitors due to the similar structures and catalytic mechanisms among Mur ligases family (Bouhss et al., 1999). The highly conserved residues in ATP-binding sites and UDP-substrate-binding sites of Mur also underline the viability of designing compounds that interact and potentially inhibit multiple ligases simultaneously (El Zoeiby et al., 2003). The published multitarget Mur inhibitors can be generally categorised into three main types: (A) competitive ATP inhibition, (B) Inhibitor mimicking UDP-MurNAc moiety of UDP substrate, and (C) compounds which mimic the common tetrahedral intermediate of Mur ligases (Tomašić et al., 2010).

Targeting ATP-binding site

ATP-binding pocket of Mur ligases, which was identified as a highly conserved region among the Mur ligases family, were investigated as candidate sites for multitarget inhibitors (*Bouhss et al., 1999; Bouhss et al., 1997*). Different strategies were deployed to identify competitive inhibitors that target ATP-binding on Mur ligases (*Šink et al., 2008*). Structure-based virtual screenings were exploited which led to the discovery of several compounds that exhibited modest potency against MurC and MurD by competitive inhibition. Those compounds act as the starting points for the design of multiple Mur ligase inhibitors targeting the ATP-binding site (*Tomašić et al., 2012a; Tomašić et al., 2012b*). Additionally, N-Acylhydrazones was identified as a multitarget Mur inhibitor *via* compound library screening. During optimisation, the potency against MurC and MurD was observed on N-acylhydrazone bearing 2,3,4-trihydroxyphenyl group, with IC₅₀ of 123 μM and 230 μM respectively. Results also showed that the inhibitor predominantly occupies the ATP-binding site with partial binding to UDP-binding pocket, leads to the simultaneous inhibition of MurC and MurD (*Šink et al., 2008*).

UDP-substrate binding

UDP-substrate-binding pockets are another highly conserved region in Mur ligases since the UDP-moiety is present throughout the entire Mur ligase cascade. The 5-benzylidenethiazolidin-4-ones analogue (*Fig. 8*, compound 22) which incorporated rhodanine as a diphosphate surrogate and the 2,3,4-hydroxyphenoyl group as hydrogen-bond-forming moiety was designed to bind within the ATP- or UDP-binding site. The design of the compound was successful, with the resulting compound potency against MurD-F in the low micromolar range (IC₅₀ = 2–6 μM). Kinetic analysis revealed that the inhibition of 5-benzylidenethiazolidin-4-ones was contributed solely by the interaction with UDP-binding pocket. Unfortunately, 5-benzylidenethiazolidin-4-ones was devoid of antibacterial activity (*Tomašić et al., 2010*). Surprisingly, the analogues of UDP-MurNAc which were expected to induce multiple Mur ligases inhibition only demonstrated potency against MurE of *M. tuberculosis*, indicating further optimisation is required for the scaffold (*Hervin et al., 2020*).

Tetrahedral intermediate mimics

A frequently exploited strategy in design of MurD inhibitors is mimicking MurD tetrahedral intermediate, which is commonly achieved by phosphinate or N-sulfonyl derivatives (*Kotnik et al., 2007; Obreza & Gobec, 2004*). The discovery of the peptide-sulfonamides bearing D-Glu group which inhibit both MurD and MurE have shown that product-like MurD inhibitors can serve as a substrate-like inhibitor for MurE with noteworthy potency displayed in both enzymes (*Humljan et al., 2006*). Moreover, it was observed by structural comparison that the MurD residues responsible for binding of D-Glu also present in MurE active site (*Gordon et al., 2001*). These results accentuate the possibility of designing dual (MurD/MurE) inhibitor *via* MurD tetrahedral intermediate mimicry.

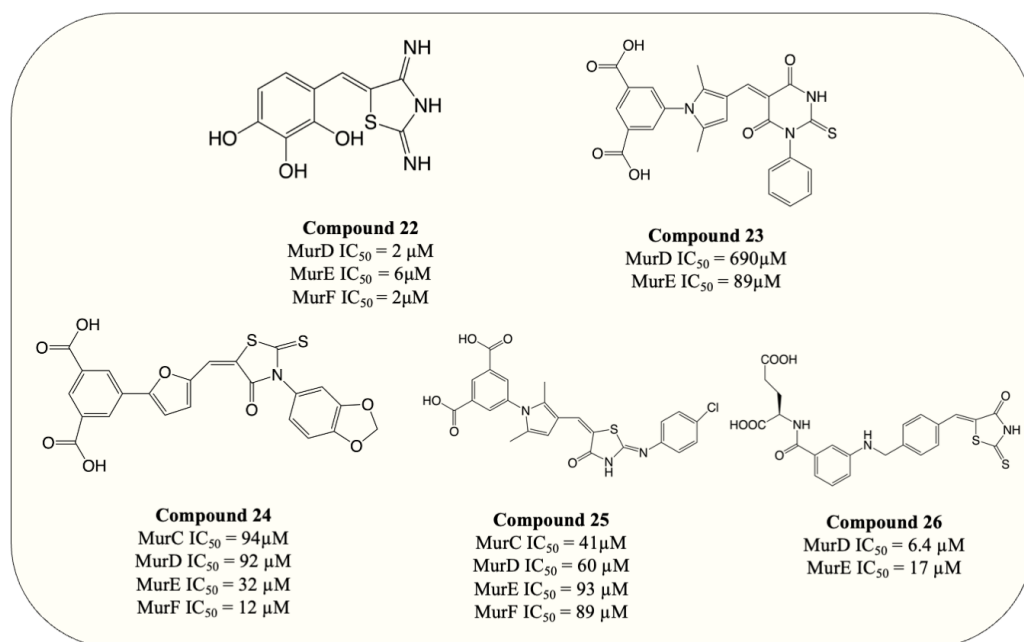


Figure 8 Multi-target inhibitors of Mur ligases.

Full-size DOI: [10.7717/peerj.18404/fig-8](https://doi.org/10.7717/peerj.18404/fig-8)

Arresting the catalytic activity

To search for D-Glu-based dual inhibitors, a pharmacophore-based virtual screening followed by potency assessment revealed that benzene 1,3-dicarboxylate moiety serves as a rigid replacement for D-Glu and led to greater potency against both MurD and MurE (Perdih, Bren & Solmajer, 2009). Subsequently, those discovered leads, benzene-1,3 dicarboxylate bearing 2,5-dimethylpyrrole (Fig. 8, compound 23) and benzene-1,3-dicarboxylate bearing furane moiety (Fig. 8, compound 24) were selected for further optimisation. The optimisation on compound 23 demonstrated that incorporation of the rhodanine moiety (compound 25) resulted in enhanced potency and expansion of the inhibition to all four Mur ligases (MurC-F) (Perdih et al., 2014). Also the optimisation of compound 24 showed that it exhibited inhibitory property in all four Mur ligases (MurC-F) in low micromolar level (Perdih et al., 2015). Interestingly, these two similar scaffolds displayed different inhibitory mode against MurD substrates. Benzene-1,3 dicarboxylate 2,5-dimethylpyrrole (Fig. 8, compound 23) exhibited competitive inhibition to D-Glu while partial uncompetitive and partial non-competitive inhibition to ATP and UMA respectively. Alternatively, furan-based benzene 1,3-dicarboxylic acid (Fig. 8, compound 24) display apparent competitive inhibition to ATP (Perdih et al., 2014; Perdih et al., 2015). Hence it appears that Benzene-1,3 dicarboxylate 2,5-dimethylpyrrole fall into UDP-substrate mimicry inhibitor category while furan-based benzene 1,3-dicarboxylic acid act as an ATP-competitive inhibitor. Unfortunately, antibacterial property was devoid in all benzene 1,3-dicarboxylic acid analogues, with only one furan-based compound showing modest activity against *S. aureus* (MIC = 32 μ g/mL) (Perdih et al., 2015). Similarly, another D-Glu based multitarget inhibitor was developed *via* modification of previously

identified MurD inhibitor. The resulting compound (Fig. 8, compound 26) exhibited dual inhibition against both MurD and MurE ($IC_{50} = 6.4$ and $17 \mu M$, respectively) and bactericidal activity against *S. aureus* ($MIC = 8 \mu g/mL$) (Tomašić et al., 2012a; Tomašić et al., 2012b). The following research validates the formation of inhibitor-MurE complex, which is mainly contributed by hydrogen bond formed with central domain and docking of D-Glu and thiazolidine-4-one ring towards UDP-MurNAc-tripeptide-binding pocket and uracil-binding pocket respectively (Azam & Jupudi, 2019). To summarise, despite the lack of antibacterial activity in species tested, the development of multitarget chemotherapy is of great interest for species like *M. tuberculosis* where slow growth characteristics may represent entirely different outcomes.

Inhibitors of MurX

Following the sequential cytoplasmic synthesis of UDP-MurNAc-pentapeptide, the next stage of peptidoglycan synthesis, known as membrane-associated stage, facilitates the translocation of this peptidoglycan precursor.

The UDP-MurNAc-pentapeptide is transported to the periplasm *via* three crucial enzymes MurX, MurG and MurJ. Likewise, the essentiality and specificity of these enzymes make them the ideal target for antibiotics. Hence, besides exploiting Mur ligases due to their structural resemblance, work has also focussed on targeting essential enzymes involved in the membrane-associated stage of peptidoglycan synthesis. Capuramycin, a nucleoside natural inhibitor, was identified as inhibitory against MurX/MraY (MurX in *M. tuberculosis*, MraY in other bacteria), which anchors completed UDP-MurNAc-pentapeptide to lipid carrier, forming lipid I (Hotoda et al., 2003b; Yamaguchi et al., 1986). Since then, extensive SAR studies have been conducted to enhance the potency of capuramycin analogues. Among thousands of compounds tested, SQ-641 (Fig. 9, compound 27) exhibited the most promising anti-mycobacterial activity with $MIC < 0.063 \mu g/mL$ despite the high IC_{50} observed ($550 ng/mL$) (Hotoda et al., 2003a; Hotoda et al., 2003b). On the other hand, the parental compound, capuramycin, displayed significantly low IC_{50} ($IC_{50} = 10 ng/mL$) but higher MIC ($MIC = 8 \mu g/mL$). It is hypothesised that the increased anti-mycobacterial activity of SQ-641 could be attributed to higher lipophilicity, which also leads to low solubility and significantly impairs intracellular and *in vivo* activity (Bogatcheva et al., 2011). Several formulations were developed and examined to overcome this deficiency including α -tocopheryl polyethylene glycol 1,000 succinate formulation, which was tested on the murine model and demonstrated better *in vivo* activity (Nikonenko Boris et al., 2009). However, the low drug loading of the formulation makes it impractical for human use. Other formulations such as phospholipid-based nanoemulsion and nanocarrier drug cocktail also enhance the *in vivo* activity of SQ-641 and warrant further search (D'Addio et al., 2015; Nikonenko et al., 2014).

Since the discovery of capuramycin, researchers have been focusing on synthesising analogues and conducting SAR without understanding the enzyme structure. The structure of mycobacterial MurX has not yet been solved, but the crystal structure of the homologue, MraY from *Aquifex aeolicus* (MraY_{AA}) has been resolved and exploited for rational inhibitor design based on protein-inhibitor interaction (Ben et al., 2013). The interaction between

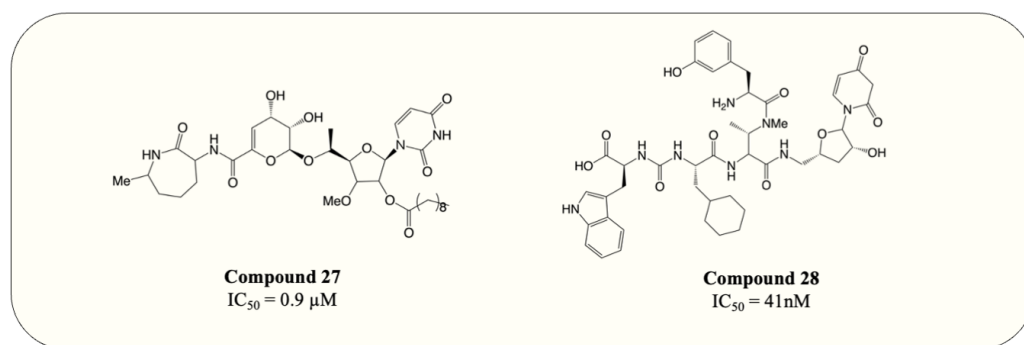


Figure 9 MurX inhibitors with the corresponding IC₅₀ value.

Full-size [DOI: 10.7717/peerj.18404/fig-9](https://doi.org/10.7717/peerj.18404/fig-9)

MraY_{AA} and a collection of nucleoside natural product inhibitors was investigated. The studies reveal that nucleoside inhibitors induce similar conformational changes of MraY upon binding and expose cryptic binding sites not present in the apoenzyme (Chung *et al.*, 2016). A uridine-binding pocket is formed in MraY to accommodate the common uridine moiety in all nucleosides upon binding. The uracil ring of uridine moiety is involved in an extensive hydrogen bonding network with loop C and loop D (Chung *et al.*, 2016). The importance of uracil is also evident in SAR studies where major modification of uracil resulted in the loss of inhibition (Heib *et al.*, 2019; Wiegmann, Koppermann & Ducho, 2018). Besides the common uridine-binding pocket, the uridine-adjacent pocket which varies in different inhibitors plays a significant role in inhibitor affinity and selectivity. For capuramycin, the uridine-adjacent pocket interacts with a hydroxyl group in 3,4-dihydroxy-3,4-dihydro-2H-pyran moiety by hydrogen bonding, which leads to a 10-fold difference in inhibition compared to analogues without the hydroxyl group (Mashalidis *et al.*, 2019; Muramatsu, Ishii & Inukai, 2003). Furthermore, the unique caprolactam moiety of capuramycin occupies another hydrophobic binding pocket inaccessible to other nucleosides, contributing to its activity (Mashalidis *et al.*, 2019). Interestingly, capuramycin does not form hydrogen bonding with loop E as other nucleoside inhibitors, which provides the opportunity for further optimisation (Mashalidis *et al.*, 2019).

Another natural product, sansanmycin, was found to be active against *M. tuberculosis* with MIC = 9.5 μM (Li *et al.*, 2011). Inspired by the favourable antimycobacterial effects, analogue library synthesis and screening were conducted to further enhance the anti-TB activity. The study successfully generated potent compounds (Fig. 9, compound 28) that demonstrated antimycobacterial activity at the nanomolar concentration (MIC = 37 nM). A MurX-MurG coupled assay displayed the addition of sansanmycin halting the formation of lipid II (IC₅₀ = 41 nM), suggesting the likely mechanism of action for sansanmycin (Tran *et al.*, 2017). Another SAR study utilised the crystal structure of MraY_{AA} to predict the mycobacterial MurX structure model and aim to enhance the potency of sansanmycin with the structure-based analogue design. The 18 compounds generated exhibited significant *in vitro* anti-mycobacterial activity (MIC = 5.9–192.3 nM) as well as activity against intracellular *M. tuberculosis* (MIC = 0.5–12.5 μM) (Tran *et al.*, 2021). It is

also worth noting that the compound with the highest *in vitro* potency only has modest intracellular activity, possibly attributed to its lower membrane permeability. Subsequently, the analogues are screened for inhibitory activity against *M. tuberculosis* MurX. Intriguingly, it is discovered that compounds bearing amide linkage are significantly more potent than those with ester linkage despite similar *in vitro* and intracellular anti-mycobacterial activity ($IC_{50} = 20\text{--}60\text{ nM}$ vs $IC_{50} = 614\text{--}2,617\text{ nM}$) ([Tran et al., 2021](#)). It is suggested that the ester-bearing compounds act as prodrugs and the ester moiety is hydrolysed during *in vitro* assay, while this hypothesis still requires further examination.

Inhibitors of MurG & MurJ

In contrast to other members in the Mur enzyme family, of which extensive research has been conducted, there is limited information on MurG and MurJ, which are responsible for the membrane-associated stage of peptidoglycan synthesis. MurG, as a glycosyltransferase, which attaches the GlcNAc moiety of UDP-GlcNAc to MurNAc in lipid I, yielding lipid-linked β -(1,4) disaccharide peptidoglycan precursor, known as lipid II ([Mohammadi et al., 2007](#)). The cytoplasmic facing lipid II is then flipped to the periplasm by lipid II flippase, MurJ, and undergoes polymerisation and cross-linking ([Kuk, Mashalidis & Lee, 2017](#)). Despite several HTS studies for MurG inhibitors being completed in the early 2000s, they mainly focused on *E. coli* MurG, with the mode of action for the identified inhibitors remaining unknown ([Helm et al., 2003](#); [Ramachandran et al., 2006](#)). A recent study utilised a computational drug discovery pipeline including homology modelling, pharmacophore generation, and virtual compounds screening to investigate potential *M. tuberculosis* MurG inhibitors. This pipeline successfully identified 10 potential lead compounds for *M. tuberculosis* MurG while further *in vitro* validation is not provided ([Saxena et al., 2018](#)). For MurJ, the current literature notably lacks published examples to date. Despite there being antibiotics that interact with lipid II, such as vancomycin and ramoplanin, it is demonstrated that these two antibiotics do not bind directly to MurJ ([Bolla et al., 2018](#)). In summary, despite the importance of MurG and MurJ in peptidoglycan synthesis, the inhibition of MurG and MurJ remain largely unexplored and therefore holds significant potential for future research in the development of antibacterial agents and combatting existing issues of drug resistance.

Polymerisation and crosslinking

After the translocation of lipid II, polymerisation and crosslinking occur to form the rigid peptidoglycan structure. This process is facilitated by bifunctional enzymes PonA1 and PonA2 which compose the transglycosylase and transpeptidase activities. In fact, it is well established that this enzymatic process can be an ideal drug target as β -lactams such as penicillin restricts this process to achieve antibacterial activity. Unfortunately, β -lactam antibiotics have limited clinical significance in the current *M. tuberculosis* treatment regime mainly credited to the innate β -lactamase (BlaC) encoded in mycobacteria ([Flores et al., 2005](#)). It has been demonstrated that β -lactams co-delivered with β -lactamase inhibitors increases activity compared to β -lactam alone ([Li et al., 2018](#)). Furthermore, a high level of the atypical transpeptidation (3 \rightarrow 3 linkage) which occurs in mycobacteria facilitated by

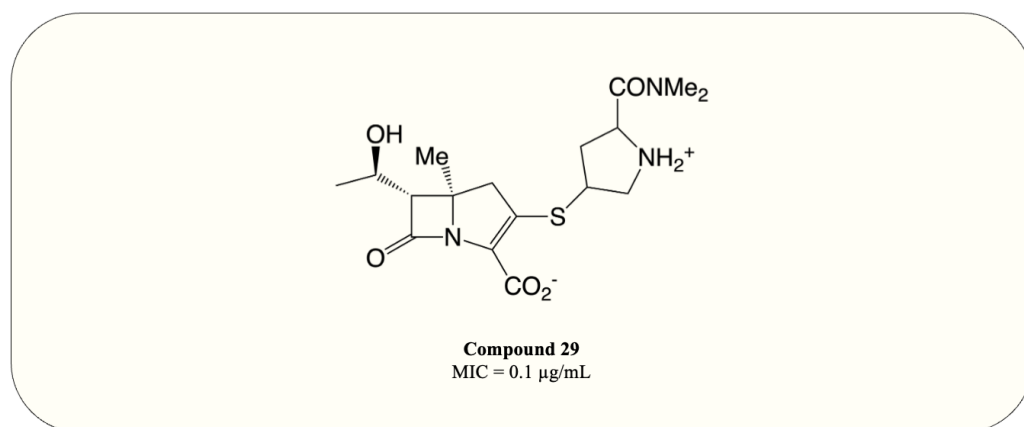


Figure 10 The optimised carbapenem with MIC value evaluated for *M. tuberculosis*.

Full-size [DOI: 10.7717/peerj.18404/fig-10](https://doi.org/10.7717/peerj.18404/fig-10)

unique L,D-transpeptidases (Ldts), also contributes to resistance against β -lactams through (Gupta *et al.*, 2010).

Recently, carbapenem was discovered and became increasingly important in combatting drug-resistant bacteria. As a broad spectrum antibiotic, carbapenem demonstrates the ability to inhibit *M. tuberculosis* PBPs (PonA1/PonA2). Meropenem, an FDA-approved carbapenem, combined with clavulanate, a BlaC inhibitor, achieves a low MIC in MDR-TB and non-replicating TB (Hugonnet *et al.*, 2009). Intriguingly, despite possessing the β -lactam ring, evidence shows that carbapenem is a poor substrate for BlaC with a slow hydrolysis rate which can minimize the impact made by BlaC (Sun, Fan & Zhong, 2021). Moreover, beyond the capability of inhibiting PBPs and BlaC, carbapenems also exhibit potency against Ldts which facilitates the unique $3 \rightarrow 3$ crosslinking during the non-replicative stage (Cordillot *et al.*, 2013; Dub  e *et al.*, 2012). Thus, carbapenem impedes mycobacterial peptidoglycan synthesis at multiple steps, making it an ideal scaffold for further optimisation. In fact, imipenem is now included in MDR-TB and NTM treatment regimes improved clinical outcomes observed (Chavan *et al.*, 2020).

A carbapenem optimisation study conducted in 2021 demonstrated that methyl substitution on C5 α greatly improves the potency against *M. tuberculosis* (MIC = 0.1 $\mu\text{g/mL}$) even in the absence of β -lactamase inhibitor (Fig. 10, compound 29). The optimised compound fully acylates Ldt_{MT2}, proving the mode of action (Gupta *et al.*, 2021). This result elucidates the potential of carbapenems as antimycobacterial agents and warrants further investigation.

Amongst all β -lactams, carbapenems possess the most potent activity for inhibiting Ldts (De Munnik *et al.*, 2020). Compounds active against Ldt_{MT2} and BlaC were identified as hit compounds with pIC₅₀ ranging 5.55–7.99. Several new classes of Ldt inhibitors including α -chloro ketones, maleimides, acrylamides, fumaryl amides, an ebsulfur analogue, isatins, and nitriles have now been reported. The most active compounds are those that covalently interacting with Cys354 of Ldt, which resides in the active site and is catalytically essential (De Munnik *et al.*, 2023). To summarise, despite *M. tuberculosis*

possessing potent β -lactamase and unique 3 \rightarrow 3 linkage, the recent development of carbapenem and identification of other novel analogues exhibits promising developments towards pan-crosslinking inhibition of peptidoglycan, providing hope in the fight against drug-resistant TB.

CONCLUSIONS

Peptidoglycan is an essential component of bacterial cell walls, and hence bacterial viability, with enzymes dedicated to its biosynthesis being therefore critical. The exclusiveness and essentiality of PG biosynthetic enzymes make them a very attractive targets for antibiotics development. Furthermore, the structural and functional relationships amongst several of these enzymes may offer the opportunity for simultaneous inhibition, leading to improved bactericidal potency and potentially circumventing the rapid development of drug resistance ([Silver, 2007](#); [Silver & Bostian, 1993](#)). These properties of essential targets identify the pathway as an ideal candidate towards the development of effective and safe anti-Tb drugs.

An unresolved question relates to the observations that many potent inhibitors of enzyme function do not possess antibacterial effects. These compounds are hampered by their capacity for cell wall penetration, and/or efficient efflux systems in many bacterial pathogens, including *M. tuberculosis* ([Dinesh, Sharma & Balganes, 2013](#); [Hameed et al., 2014](#); [Humnabadkar et al., 2014](#); [Silver, 2006](#)). The bacteriostatic or bactericidal effect of these inhibitors is significantly improved in some bacteria when pre-exposed to cell envelop permeabilizers, demonstrating cell entry bottleneck ([Baum et al., 2007](#); [Hameed et al., 2014](#)). Future endeavours could focus on improved drug delivery strategies. Cell entry can be achieved by chemical optimisation of the molecule promoting passive diffusion or conjugation with a moiety that promotes active uptake. A “pro-drug” strategy is widely used to with pharmacologically active molecules to mask hydrophilic groups and increase the permeability of the compounds. The pharmacological inactive groups are then removed by enzymatic or chemical transformation in the bacterial cytoplasm ([Rautio et al., 2008](#)).

M. tuberculosis represents a great many challenges for the development of novel chemotherapies directed to its elimination. The enormous volume of research conducted to date on the peptidoglycan synthesis pathway, and its inhibition, when taken together highlights opportunities for continued efforts and novel strategies for the development of effective inhibitors. Concepts of multi-target inhibitors, pan-transpeptidase inhibitors, and compound pro-drugging for improved permeability are future tasks which are reasonable, and highly desirable, goals for researchers in the field.

ACKNOWLEDGEMENTS

We thank Dr. Emily Strong for reviewing the manuscript.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

Cheng-Yu Chiang is a recipient of a University of Queensland Graduate School Scholarship for Higher Degree Research. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures

The following grant information was disclosed by the authors:

University of Queensland Graduate School Scholarship for Higher Degree Research.

Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Cheng-Yu Chiang conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Nicholas P. West conceived and designed the experiments, authored or reviewed drafts of the article, and approved the final draft.

Data Availability

The following information was supplied regarding data availability:

This is a literature review.

REFERENCES

- Akalu TY, Clements ACA, Wolde HF, Alene KA. 2023.** Economic burden of multidrug-resistant tuberculosis on patients and households: a global systematic review and meta-analysis. *Scientific Reports* **13**:22361 DOI [10.1038/s41598-023-47094-9](https://doi.org/10.1038/s41598-023-47094-9).
- Alderwick LJ, Harrison J, Lloyd GS, Birch HL. 2015.** The mycobacterial cell wall—peptidoglycan and arabinogalactan. *Cold Spring Harbor Perspectives in Medicine* **5**:a021113-a021113 DOI [10.1101/cshperspect.a021113](https://doi.org/10.1101/cshperspect.a021113).
- Auger G, Van Heijenoort J, Vederas JC, Blanot D. 1996.** Effect of analogues of diaminopimelic acid on the meso-diaminopimelate-adding enzyme from *Escherichia coli*. *FEBS Letters* **391**:171–174 DOI [10.1016/0014-5793\(96\)00619-9](https://doi.org/10.1016/0014-5793(96)00619-9).
- Azam MA, Jupudi S. 2017.** Insight into the structural requirements of thiophene-3-carbonitriles-based MurF inhibitors by 3D-QSAR, molecular docking and molecular dynamics study. *Journal of Receptors and Signal Transduction* **37**:522–534 DOI [10.1080/10799893.2017.1360354](https://doi.org/10.1080/10799893.2017.1360354).
- Azam MA, Jupudi S. 2019.** Structure-based virtual screening to identify inhibitors against *Staphylococcus aureus* MurD enzyme. *Structural Chemistry* **30**:2123–2133 DOI [10.1007/s11224-019-01330-z](https://doi.org/10.1007/s11224-019-01330-z).

- Barreteau H, Kovač A, Boniface A, Sova M, Gobec S, Blanot D. 2008. Cytoplasmic steps of peptidoglycan biosynthesis. *FEMS Microbiology Reviews* 32:168–207 DOI 10.1111/j.1574-6976.2008.00104.x.
- Basavannacharya C, Moody PR, Munshi T, Cronin N, Keep NH, Bhakta S. 2010. Essential residues for the enzyme activity of ATP-dependent MurE ligase from *Mycobacterium tuberculosis*. *Protein Cell* 1:1011–1022 DOI 10.1007/s13238-010-0132-9.
- Baum EZ, Crespo-Carbone SM, Foleno BD, Simon LD, Guillemont J, Macielag M, Bush K. 2009. MurF inhibitors with antibacterial activity: effect on mucopeptide levels. *Antimicrobial Agents and Chemotherapy* 53:3240 DOI 10.1128/AAC.00166-09.
- Baum EZ, Crespo-Carbone SM, Klinger A, Foleno BD, Turchi I, Macielag M, Bush K. 2007. A MurF inhibitor that disrupts cell wall biosynthesis in *Escherichia coli*. *Antimicrobial Agents and Chemotherapy* 51:4420 DOI 10.1128/AAC.00845-07.
- Ben CC, Jinshi Z, Robert AG, Do-Yeon K, Ziqiang G, Jiyong H, Pei Z, Seok-Yong L. 2013. Crystal structure of MraY, an essential membrane enzyme for bacterial cell wall synthesis. *Science* 341:1012–1016 DOI 10.1126/science.1236501.
- Benson TE, Marquardt JL, Marquardt AC, Etzkorn FA, Walsh CT. 1993. Overexpression, purification, and mechanistic study of UDP-N-acetylenolpyruvylglucosamine reductase. *Biochemistry* 32:2024–2030 DOI 10.1021/bi00059a019.
- Bogatcheva E, Dubuisson T, Protopopova M, Einck L, Nacy CA, Reddy VM. 2011. Chemical modification of capuramycins to enhance antibacterial activity. *Journal of Antimicrobial Chemotherapy* 66:578–587 DOI 10.1093/jac/dkq495.
- Bolla JR, Sauer JB, Wu D, Mehmood S, Allison TM, Robinson CV. 2018. Direct observation of the influence of cardiolipin and antibiotics on lipid II binding to MurJ. *Nature Chemistry* 10:363–371 DOI 10.1038/nchem.2919.
- Bouhss A, Dementin S, Parquet C, Mengin-Lecreulx D, Bertrand JA, Le Beller D, Dideberg O, Van Heijenoort J, Blanot D. 1999. Role of the ortholog and paralog amino acid invariants in the active site of the UDP-MurNAc-L-alanine:D-glutamate ligase (MurD). *Biochemistry* 38:12240–12247 DOI 10.1021/bi990517r.
- Bouhss A, Mengin-Lecreulx D, Blanot D, Van Heijenoort J, Parquet C. 1997. Invariant amino acids in the mur peptide synthetases of bacterial peptidoglycan synthesis and their modification by site-directed mutagenesis in the UDP-MurNAc:L-alanine ligase from *Escherichia coli*. *Biochemistry* 36:11556–11563 DOI 10.1021/bi970797f.
- Brennan PJ, Nikaido H. 1995. The envelope of mycobacteria. *Annual Review of Biochemistry* 64:29–63 DOI 10.1146/annurev.bi.64.070195.000333.
- Cabeen MT, Jacobs-Wagner C. 2005. Bacterial cell shape. *Nature Reviews Microbiology* 3:601–610 DOI 10.1038/nrmicro1205.
- Chambers HF, Moreau D, Yajko D, Miick C, Wagner C, Hackbarth C, Kocagöz S, Rosenberg E, Hadley WK, Nikaido H. 1995. Can penicillins and other beta-lactam antibiotics be used to treat tuberculosis? *Antimicrobial Agents and Chemotherapy* 39:2620 DOI 10.1128/AAC.39.12.2620.
- Chavan VV, Dalal A, Nagaraja S, Thekkur P, Mansoor H, Meneguim A, Paryani R, Singh P, Kalon S, Das M, Ferlazzo G, Isaakidis P. 2020. Ambulatory management of pre- and extensively drug resistant tuberculosis patients with imipenem delivered

- through port-a-cath: a mixed methods study on treatment outcomes and challenges. *PLOS ONE* 15:e0234651 DOI 10.1371/journal.pone.0234651.
- Chung BC, Mashalidis EH, Tanino T, Kim M, Matsuda A, Hong J, Ichikawa S, Lee S-Y. 2016.** Structural insights into inhibition of lipid I production in bacterial cell wall synthesis. *Nature* 533:557–560 DOI 10.1038/nature17636.
- Cordillot M, Dubée V, Triboulet S, Dubost L, Marie A, Hugonnet J-E, Arthur M, Mainardi J-L. 2013.** In vitro cross-linking of *Mycobacterium tuberculosis* peptidoglycan by Ldt-transpeptidases and inactivation of these enzymes by carbapenems. *Antimicrobial Agents and Chemotherapy* 57:5940–5945 DOI 10.1128/aac.01663-13.
- D’Addio SM, Reddy VM, Liu Y, Sinko PJ, Einck L, Prud’homme RK. 2015.** Antitubercular nanocarrier combination therapy: formulation strategies and in vitro efficacy for rifampicin and SQ641. *Molecular Pharmaceutics* 12:1554–1563 DOI 10.1021/mp5008663.
- De Jager V, Gupte N, Nunes S, Barnes GL, Van Wijk RC, Mostert J, Dorman SE, Abulfathi AA, Upton CM, Faraj A, Nuermberger EL, Lamichhane G, Svensson EM, Simonsson USH, Diacon AH, Dooley KE. 2022.** Early bactericidal activity of meropenem plus clavulanate (with or without rifampin) for tuberculosis: the comrade randomized, phase 2A clinical trial. *American Journal of Respiratory and Critical Care Medicine* 205:1228–1235 DOI 10.1164/rccm.202108-1976OC.
- De Munnik M, Lang PA, De Dios Anton F, Cacho M, Bates RH, Brem J, Rodríguez Miquel B, Schofield CJ. 2023.** High-throughput screen with the L, d-transpeptidase Ldt(Mt2) of *Mycobacterium tuberculosis* reveals novel classes of covalently reacting inhibitors. *Chemical Science* 14:7262–7278 DOI 10.1039/d2sc06858c.
- De Munnik M, Lohans CT, Langley GW, Bon C, Brem J, Schofield CJ. 2020.** A fluorescence-based assay for screening β -lactams targeting the *Mycobacterium tuberculosis* transpeptidase Ldt(Mt2). *ChemBioChem* 21:368–372 DOI 10.1002/cbic.201900379.
- Dinesh N, Sharma S, Balganes M. 2013.** Involvement of efflux pumps in the resistance to peptidoglycan synthesis inhibitors in *Mycobacterium tuberculosis*. *Antimicrobial Agents and Chemotherapy* 57:1941 DOI 10.1128/AAC.01957-12.
- Dubée V, Triboulet S, Mainardi J-L, Ethève-Quelquejeu M, Gutmann L, Marie A, Dubost L, Hugonnet J-E, Arthur M. 2012.** Inactivation of *Mycobacterium tuberculosis* transpeptidase Ldt by carbapenems and cephalosporins. *Antimicrobial Agents and Chemotherapy* 56:4189–4195 DOI 10.1128/aac.00665-12.
- Durand P, Golinelli-Pimpaneau B, Moulleron S, Badet B, Badet-Denisot M-A. 2008.** Highlights of glucosamine-6P synthase catalysis. *Archives of Biochemistry and Biophysics* 474:302–317 DOI 10.1016/j.abb.2008.01.026.
- Ehmann DE, Demeritt JE, Hull KG, Fisher SL. 2004.** Biochemical characterization of an inhibitor of *Escherichia coli* UDP-N-acetylmuramyl-L-alanine ligase. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics* 1698:167–174 DOI 10.1016/j.bbapap.2003.11.006.

- El Zoeiby A, Sanschagrin F, Darveau A, Brisson J-R, Levesque RC. 2003. Identification of novel inhibitors of *Pseudomonas aeruginosa* MurC enzyme derived from phage-displayed peptide libraries. *Journal of Antimicrobial Chemotherapy* 51:531–543 DOI 10.1093/jac/dkg010.
- Flores AR, Parsons LM, Pavelka , Martin S. 2005. Genetic analysis of the β -lactamases of *Mycobacterium tuberculosis* and *Mycobacterium smegmatis* and susceptibility to β -lactam antibiotics. *Microbiology* 151:521–532 DOI 10.1099/mic.0.27629-0.
- Fraser RS, Creanor J. 1975. The mechanism of inhibition of ribonucleic acid synthesis by 8-hydroxyquinoline and the antibiotic lomofungin. *The Biochemical Journal* 147:401–410 DOI 10.1042/bj1470401.
- Ghuysen J-M. 1991. Serine β -lactamases and penicillin-binding proteins. *Annual Review of Microbiology* 45:37–67 DOI 10.1146/annurev.mi.45.100191.000345.
- Glauner B, Höltje JV, Schwarz U. 1988. The composition of the murein of *Escherichia coli*. *Journal of Biological Chemistry* 263:10088–10095 DOI 10.1016/S0021-9258(19)81481-3.
- Gordon E, Flouret B, Chantalat L, Van Heijenoort J, Mengin-Lecreulx D, Dideberg O. 2001. Crystal structure of UDP-N-acetylmuramoyl-l-alanyl-d-glutamate:meso-Diaminopimelate Ligase from *Escherichia coli*. *Journal of Biological Chemistry* 276:10999–11006 DOI 10.1074/jbc.M009835200.
- Gu YG, Florjancic AS, Clark RF, Zhang T, Cooper CS, Anderson DD, Lerner CG, McCall JO, Cai Y, Black-Schaefer CL, Stamper GF, Hajduk PJ, Beutel BA. 2004. Structure–activity relationships of novel potent MurF inhibitors. *Bioorganic & Medicinal Chemistry Letters* 14:267–270 DOI 10.1016/j.bmcl.2003.09.073.
- Gupta R, Al-Kharji N, Alqurafi MA, Nguyen TQ, Chai W, Quan P, Malhotra R, Simcox BS, Mortimer P, Brammer Basta LA, Rohde KH, Buynak JD. 2021. Atypically modified carbapenem antibiotics display improved antimycobacterial activity in the absence of β -lactamase inhibitors. *ACS Infectious Diseases* 7:2425–2436 DOI 10.1021/acsinfecdis.1c00185.
- Gupta R, Lavollay M, Mainardi JL, Arthur M, Bishai WR, Lamichhane G. 2010. The *Mycobacterium tuberculosis* protein LdtMt2 is a nonclassical transpeptidase required for virulence and resistance to amoxicillin. *Nature Medicine* 16:466–469 DOI 10.1038/nm.2120.
- Guzman JD, Gupta A, Evangelopoulos D, Basavannacharya C, Pabon LC, Plazas EA, Muñoz DR, Delgado WA, Cuca LE, Ribon W, Gibbons S, Bhakta S. 2010. Anti-tubercular screening of natural products from Colombian plants: 3-methoxynordomesticine, an inhibitor of MurE ligase of *Mycobacterium tuberculosis*. *Journal of Antimicrobial Chemotherapy* 65:2101–2107 DOI 10.1093/jac/dkq313.
- Guzman JD, Pesnot T, Barrera DA, Davies HM, McMahon E, Evangelopoulos D, Mortazavi PN, Munshi T, Maitra A, Lamming ED, Angell R, Gershater MC, Redmond JM, Needham D, Ward JM, Cuca LE, Hailes HC, Bhakta S. 2015. Tetrahydroisoquinolines affect the whole-cell phenotype of *Mycobacterium tuberculosis* by

- inhibiting the ATP-dependent MurE ligase. *Journal of Antimicrobial Chemotherapy* 70:1691–1703 DOI 10.1093/jac/dkv010.
- Guzman JD, Wube A, Evangelopoulos D, Gupta A, Hüfner A, Basavannacharya C, Rahman MM, Thomaschitz C, Bauer R, McHugh TD, Nobeli I, Prieto JM, Gibbons S, Bucar F, Bhakta S. 2011. Interaction of N-methyl-2-alkenyl-4-quinolones with ATP-dependent MurE ligase of *Mycobacterium tuberculosis*: antibacterial activity, molecular docking and inhibition kinetics. *Journal of Antimicrobial Chemotherapy* 66:1766–1772 DOI 10.1093/jac/dkr203.
- Hameed PS, Manjrekar P, Chinnappattu M, Humnabadkar V, Shanbhag G, Kedari C, Mudugal NV, Ambady A, De Jonge BLM, Sadler C, Paul B, Sriram S, Kaur P, Guptha S, Raichurkar A, Fleming P, Eyermann CJ, McKinney DC, Samban-damurthy VK, Panda M, Ravishankar S. 2014. Pyrazolopyrimidines establish MurC as a vulnerable target in *Pseudomonas aeruginosa* and *Escherichia coli*. *ACS Chemical Biology* 9:2274–2282 DOI 10.1021/cb500360c.
- Hänchen A, Rausch S, Landmann B, Toti L, Nusser A, Süßmuth RD. 2013. Alanine scan of the peptide antibiotic feglymycin: assessment of amino acid side chains contributing to antimicrobial activity. *ChemBioChem* 14:625–632 DOI 10.1002/cbic.201300032.
- Harrison J, Lloyd G, Joe M, Lowary TL, Reynolds E, Walters-Morgan H, Bhatt A, Lovering A, Besra GS, Alderwick LJ. 2016. Lcp1 is a phosphotransferase responsible for ligating arabinogalactan to peptidoglycan in *Mycobacterium tuberculosis*. *mBio* 7:e00972-00916 DOI 10.1128/mBio.00972-16.
- Heib A, Niro G, Weck SC, Koppermann S, Ducho C. 2019. Muraymycin nucleoside antibiotics: structure-activity relationship for variations in the nucleoside unit. *Molecules* 25:22 DOI 10.3390/molecules25010022.
- Helm JS, Hu Y, Chen L, Gross B, Walker S. 2003. Identification of active-site inhibitors of MurG using a generalizable, high-throughput glycosyltransferase screen. *Journal of the American Chemical Society* 125:11168–11169 DOI 10.1021/ja036494s.
- Hervin V, Arora R, Rani J, Ramchandran S, Bajpai U, Agrofoglio LA, Roy V. 2020. Design and synthesis of various 5'-Deoxy-5'-(4-substituted-1, 2 3-triazol-1-yl)-uridine analogues as inhibitors of *Mycobacterium tuberculosis* Mur Ligases. *Molecules* 25:4953 DOI 10.3390/molecules25214953.
- Hett EC, Chao MC, Rubin EJ. 2010. Interaction and modulation of two antagonistic cell wall enzymes of mycobacteria. *PLOS Pathogens* 6:e1001020 DOI 10.1371/journal.ppat.1001020.
- Horton JR, Bostock JM, Chopra I, Hesse L, Phillips SEV, Adams DJ, Johnson AP, Fishwick CWG. 2003. Macrocyclic inhibitors of the bacterial cell wall biosynthesis enzyme mur D. *Bioorganic & Medicinal Chemistry Letters* 13:1557–1560 DOI 10.1016/S0960-894X(03)00176-8.
- Hotoda H, Daigo M, Furukawa M, Murayama K, Hasegawa CA, Kaneko M, Muramatsu Y, Ishii MM, Miyakoshi S-I, Takatsu T, Inukai M, Kakuta M, Abe

- T, Fukuoka T, Utsui Y, Ohya S. 2003a. Synthesis and antimycobacterial activity of capuramycin analogues. Part 2: acylated derivatives of capuramycin-related compounds. *Bioorganic & Medicinal Chemistry Letters* 13:2833–2836 DOI 10.1016/S0960-894X(03)00597-3.
- Hotoda H, Furukawa M, Daigo M, Murayama K, Kaneko M, Muramatsu Y, Ishii MM, S-i Miyakoshi, Takatsu T, Inukai M, Kakuta M, Abe T, Harasaki T, Fukuoka T, Utsui Y, Ohya S. 2003b. Synthesis and antimycobacterial activity of capuramycin analogues. Part 1: substitution of the azepan-2-one moiety of capuramycin. *Bioorganic & Medicinal Chemistry Letters* 13:2829–2832 DOI 10.1016/S0960-894X(03)00596-1.
- Hrast M, Anderluh M, Knez D, Randall CP, Barreateau H, O'Neill AJ, Blanot D, Gobec S. 2014. Design, synthesis and evaluation of second generation MurF inhibitors based on a cyanothiophene scaffold. *European Journal of Medicinal Chemistry* 73:83–96 DOI 10.1016/j.ejmech.2013.11.031.
- Hrast M, Turk S, Sosič I, Knez D, Randall CP, Barreateau H, Contreras-Martel C, Dessen A, O'Neill AJ, Mengin-Lecreulx D, Blanot D, Gobec S. 2013. Structure–activity relationships of new cyanothiophene inhibitors of the essential peptidoglycan biosynthesis enzyme MurF. *European Journal of Medicinal Chemistry* 66:32–45 DOI 10.1016/j.ejmech.2013.05.013.
- Hugonnet J-E, Tremblay LW, Boshoff HI, Barry CE, Blanchard JS. 2009. Meropenem-clavulanate is effective against extensively drug-resistant *Mycobacterium tuberculosis*. *Science* 323:1215–1218 DOI 10.1126/science.1167498.
- Humljan J, Kotnik M, Boniface A, Šolmajer T, Urleb U, Blanot D, Gobec S. 2006. A new approach towards peptidosulfonamides: synthesis of potential inhibitors of bacterial peptidoglycan biosynthesis enzymes MurD and MurE. *Tetrahedron* 62:10980–10988 DOI 10.1016/j.tet.2006.08.030.
- Humljan J, Kotnik M, Contreras-Martel C, Blanot D, Urleb U, Dessen A, Šolmajer T, Gobec S. 2008. Novel naphthalene-n-sulfonyl-d-glutamic acid derivatives as inhibitors of MurD, a key peptidoglycan biosynthesis enzyme. *Journal of Medicinal Chemistry* 51:7486–7494 DOI 10.1021/jm800762u.
- Humnabadkar V, Prabhakar KR, Narayan A, Sharma S, Gupta S, Manjrekar P, Chinnaoattu M, Ramachandran V, Hameed SP, Ravishankar S, Chatterji M. 2014. UDP-N-acetylmuramic acid L-alanine ligase (MurC) inhibition in a tolC mutant *Escherichia coli* strain leads to cell death. *Antimicrobial Agents and Chemotherapy* 58:6165–6171 DOI 10.1128/AAC.02890-14.
- Jankute M, Cox JAG, Harrison J, Besra GS. 2015. Assembly of the mycobacterial cell wall. *Annual Review of Microbiology* 69:405–423 DOI 10.1146/annurev-micro-091014-104121.
- Jarlier V, Nikaido H. 1994. Mycobacterial cell wall: structure and role in natural resistance to antibiotics. *FEMS Microbiology Letters* 123:11–18 DOI 10.1111/j.1574-6968.1994.tb07194.x.
- Jha RK, Katagihallimath N, Hota SK, Das KS, De Sousa SM. 2012. An assay for exogenous sources of purified MurG, enabled by the complementation of *Escherichia coli*

- murG(Ts) by the *Mycobacterium tuberculosis* homologue. *FEMS Microbiology Letters* **326**:161–167 DOI [10.1111/j.1574-6968.2011.02446.x](https://doi.org/10.1111/j.1574-6968.2011.02446.x).
- Jupudi S, Azam MA, Wadhvani A. 2019. Synthesis, molecular docking, binding free energy calculation and molecular dynamics simulation studies of benzothiazol-2-ylcarbomodithioates as *Staphylococcus aureus* MurD inhibitors. *Journal of Receptors and Signal Transduction* **39**:283–293 DOI [10.1080/10799893.2019.1663538](https://doi.org/10.1080/10799893.2019.1663538).
- Katrak S, Wang R, Barry P. 2023. Costs of treating multidrug-resistant TB in California in 2022. *The International Journal of Tuberculosis and Lung Disease* **27**:864–866 DOI [10.5588/ijtld.23.0150](https://doi.org/10.5588/ijtld.23.0150).
- Khedkar SA, Malde AK, Coutinho EC. 2007. Design of inhibitors of the MurF enzyme of *Streptococcus pneumoniae* using docking, 3D-QSAR, and de novo design. *Journal of Chemical Information and Modeling* **47**:1839–1846 DOI [10.1021/ci600568u](https://doi.org/10.1021/ci600568u).
- Kotnik M, Humljan J, Contreras-Martel C, Oblak M, Kristan K, Hervé M, Blanot D, Urleb U, Gobec S, Dessen A, Solmajer T. 2007. Structural and functional characterization of enantiomeric glutamic acid derivatives as potential transition state analogue inhibitors of MurD ligase. *Journal of Molecular Biology* **370**:107–115 DOI [10.1016/j.jmb.2007.04.048](https://doi.org/10.1016/j.jmb.2007.04.048).
- Kouidmi I, Levesque RC, Paradis-Bleau C. 2014. The biology of Mur ligases as an antibacterial target. *Molecular Microbiology* **94**:242–253 DOI [10.1111/mmi.12758](https://doi.org/10.1111/mmi.12758).
- Kuk ACY, Mashalidis EH, Lee S-Y. 2017. Crystal structure of the MOP flippase MurJ in an inward-facing conformation. *Nature Structural & Molecular Biology* **24**:171–176 DOI [10.1038/nsmb.3346](https://doi.org/10.1038/nsmb.3346).
- Kumar P, Singh UB, Lamichhane G, Story-Roller E. 2019. Repurposing of carbapenems for the treatment of drug-resistant tuberculosis. In: Hasnain SE, Ehtesham NZ, Grover S, eds. *Mycobacterium tuberculosis: molecular infection biology, pathogenesis, diagnostics and new interventions*. Singapore: Springer Singapore, 497–514.
- Lavollay M, Arthur M, Fourgeaud M, Dubost L, Marie A, Veziris N, Blanot D, Gutmann L, Mainardi J-L. 2008. The peptidoglycan of stationary-phase *Mycobacterium tuberculosis* predominantly contains cross-links generated by L, D-Ttanspeptidation. *The Journal of Bacteriology* **190**:4360 DOI [10.1128/JB.00239-08](https://doi.org/10.1128/JB.00239-08).
- Li S, Kang J, Yu W, Zhou Y, Zhang W, Xin Y, Ma Y. 2012. Identification of *M. tuberculosis* Rv3441c and *M. smegmatis* MSMEG_1556 and essentiality of *M. smegmatis* MSMEG_1556. *PLOS ONE* **7**:e42769–e42769 DOI [10.1371/journal.pone.0042769](https://doi.org/10.1371/journal.pone.0042769).
- Li F, Wan L, Xiao T, Liu H, Jiang Y, Zhao X, Wang R, Wan K. 2018. In vitro activity of β -lactams in combination with β -lactamase inhibitors against *Mycobacterium tuberculosis* clinical isolates. *BioMed Research International* **2018**:3579832 DOI [10.1155/2018/3579832](https://doi.org/10.1155/2018/3579832).
- Li Y-B, Xie Y-Y, Du N-N, Lu Y, Xu H-Z, Wang B, Yu Y, Liu Y-X, Song D-Q, Chen R-X. 2011. Synthesis and in vitro antitubercular evaluation of novel sansan-mycin derivatives. *Bioorganic & Medicinal Chemistry Letters* **21**:6804–6807 DOI [10.1016/j.bmcl.2011.09.031](https://doi.org/10.1016/j.bmcl.2011.09.031).

- Li X-Z, Zhang L, Nikaido H. 2004. Efflux pump-mediated intrinsic drug resistance in *Mycobacterium smegmatis*. *Antimicrobial Agents and Chemotherapy* 48:2415 DOI 10.1128/AAC.48.7.2415-2423.2004.
- Longenecker KL, Stamper GF, Hajduk PJ, Fry EH, Jakob CG, Harlan JE, Edalji R, Bartley DM, Walter KA, Solomon LR, Holzman TF, Gu YG, Lerner CG, Beutel BA, Stoll VS. 2005. Structure of MurF from *Streptococcus pneumoniae* co-crystallized with a small molecule inhibitor exhibits interdomain closure. *Protein Science* 14:3039–3047 DOI 10.1110/ps.051604805.
- Maitra A, Munshi T, Healy J, Martin LT, Vollmer W, Keep NH, Bhakta S. 2019. Cell wall peptidoglycan in *Mycobacterium tuberculosis*: An Achilles' heel for the TB-causing pathogen. *FEMS Microbiology Reviews* 43:548–575 DOI 10.1093/femsre/fuz016.
- Marmor S, Petersen CP, Reck F, Yang W, Gao N, Fisher SL. 2001. Biochemical characterization of a phosphinate inhibitor of *Escherichia coli* MurC. *Biochemistry* 40:12207–12214 DOI 10.1021/bi015567m.
- Mashalidis EH, Kaeser B, Terasawa Y, Katsuyama A, Kwon D-Y, Lee K, Hong J, Ichikawa S, Lee S-Y. 2019. Chemical logic of MraY inhibition by antibacterial nucleoside natural products. *Nature Communications* 10:2917 DOI 10.1038/s41467-019-10957-9.
- Mdluli K, Swanson J, Fischer E, Lee RE, Barry 3rd CE. 1998. Mechanisms involved in the intrinsic isoniazid resistance of *Mycobacterium avium*. *Molecular Microbiology* 27:1223–1233 DOI 10.1046/j.1365-2958.1998.00774.x.
- Migliori GB, Dara M, De Colombani P, Kluge H, Raviglione MC. 2012. Multidrug-resistant tuberculosis in Eastern Europe: still on the increase? *European Respiratory Journal* 39:1290–1291 DOI 10.1183/09031936.00214411.
- Mohammadi T, Karczmarek A, Crouvoisier M, Bouhss A, Mengin-Lecreulx D, Den Blaauwen T. 2007. The essential peptidoglycan glycosyltransferase MurG forms a complex with proteins involved in lateral envelope growth as well as with proteins involved in cell division in *Escherichia coli*. *Molecular Microbiology* 65:1106–1121 DOI 10.1111/j.1365-2958.2007.05851.x.
- Mol CD, Brooun A, Dougan DR, Hilgers MT, Tari LW, Wijnands RA, Knuth MW, McRee DE, Swanson RV. 2003. Crystal structures of active fully assembled substrate- and product-bound complexes of UDP-N-Acetylmuramic acid:l-alanine ligase (MurC) from *Haemophilus influenzae*. *Journal of Bacteriology* 185:4152 DOI 10.1128/JB.185.14.4152-4162.2003.
- Moraes GL, Gomes GC, Monteiro De Sousa PR, Alves CN, Govender T, Kruger HG, Maguire GEM, Lamichhane G, Lameira J. 2015. Structural and functional features of enzymes of *Mycobacterium tuberculosis* peptidoglycan biosynthesis as targets for drug development. *Tuberculosis* 95:95–111 DOI 10.1016/j.tube.2015.01.006.
- Munshi T, Gupta A, Evangelopoulos D, Guzman JD, Gibbons S, Keep NH, Bhakta S. 2013. Characterisation of ATP-dependent Mur ligases involved in the biogenesis of cell wall peptidoglycan in *Mycobacterium tuberculosis*. *PLOS ONE* 8:e60143 DOI 10.1371/journal.pone.0060143.

- Muramatsu Y, Ishii MM, Inukai M. 2003.** Studies on novel bacterial translocase inhibitors, A-500359s: II. Biological activities of A-500359 A, C, D and G. *The Journal of Antibiotics* **56**:253–258 DOI [10.7164/antibiotics.56.253](https://doi.org/10.7164/antibiotics.56.253).
- Nikaido H. 2001.** Preventing drug access to targets: cell surface permeability barriers and active efflux in bacteria. *Seminars in Cell & Developmental Biology* **12**:215–223 DOI [10.1006/scdb.2000.0247](https://doi.org/10.1006/scdb.2000.0247).
- Nikaido H, Jarlier V. 1991.** Permeability of the mycobacterial cell wall. *Research in Microbiology* **142**:437–443 DOI [10.1016/0923-2508\(91\)90117-s](https://doi.org/10.1016/0923-2508(91)90117-s).
- Nikonenko B, Reddy Venkata M, Bogatcheva E, Protopopova M, Einck L, Nacy Carol A. 2014.** Therapeutic efficacy of SQ641-NE against *Mycobacterium tuberculosis*. *Antimicrobial Agents and Chemotherapy* **58**:587–589 DOI [10.1128/aac.01254-13](https://doi.org/10.1128/aac.01254-13).
- Nikonenko Boris V, Reddy Venkata M, Protopopova M, Bogatcheva E, Einck L, Nacy Carol A. 2009.** Activity of SQ641, a Capuramycin analog, in a murine model of tuberculosis. *Antimicrobial Agents and Chemotherapy* **53**:3138–3139 DOI [10.1128/aac.00366-09](https://doi.org/10.1128/aac.00366-09).
- Obreza A, Gobec S. 2004.** Recent advances in design, synthesis and biological activity of aminoalkylsulfonates and sulfonamidopeptides. *Current Medicinal Chemistry* **11**:3263–3278 DOI [10.2174/0929867043363659](https://doi.org/10.2174/0929867043363659).
- Park JT. 1952.** Uridine-5'-pyrophosphate derivatives, II. Isolation from *Staphylococcus aureus*. *Journal of Biological Chemistry* **194**:877–884 DOI [10.1016/S0021-9258\(18\)55843-9](https://doi.org/10.1016/S0021-9258(18)55843-9).
- Perdih A, Bren U, Solmajer T. 2009.** Binding free energy calculations of N-sulphonyl-glutamic acid inhibitors of MurD ligase. *Journal of Molecular Modeling* **15**:983–996 DOI [10.1007/s00894-009-0455-8](https://doi.org/10.1007/s00894-009-0455-8).
- Perdih A, Hrast M, Barreteau H, Gobec S, Wolber G, Solmajer T. 2014.** Benzene-1, 3-dicarboxylic acid 2 5-dimethylpyrrole derivatives as multiple inhibitors of bacterial Mur ligases (MurC–MurF). *Bioorganic & Medicinal Chemistry* **22**:4124–4134 DOI [10.1016/j.bmc.2014.05.058](https://doi.org/10.1016/j.bmc.2014.05.058).
- Perdih A, Hrast M, Pureber K, Barreteau H, Grdadolnik SG, Kocjan D, Gobec S, Solmajer T, Wolber G. 2015.** Furan-based benzene mono- and dicarboxylic acid derivatives as multiple inhibitors of the bacterial Mur ligases (MurC–MurF): experimental and computational characterization. *Journal of Computer-Aided Molecular Design* **29**:541–560 DOI [10.1007/s10822-015-9843-6](https://doi.org/10.1007/s10822-015-9843-6).
- Perdih A, Wolber G, Solmajer T. 2013.** Molecular dynamics simulation and linear interaction energy study of D-Glu-based inhibitors of the MurD ligase. *Journal of Computer-Aided Molecular Design* **27**:723–738 DOI [10.1007/s10822-013-9673-3](https://doi.org/10.1007/s10822-013-9673-3).
- Prosser GA, De Carvalho LPS. 2013.** Kinetic mechanism and inhibition of *Mycobacterium tuberculosis* d-alanine:d-alanine ligase by the antibiotic d-cycloserine. *The FEBS Journal* **280**:1150–1166 DOI [10.1111/febs.12108](https://doi.org/10.1111/febs.12108).
- Raghavendra T, Patil S, Mukherjee R. 2018.** Peptidoglycan in Mycobacteria: chemistry, biology and intervention. *Glycoconjugate Journal* **35**:421–432 DOI [10.1007/s10719-018-9842-7](https://doi.org/10.1007/s10719-018-9842-7).

- Ramachandran V, Chandrakala B, Kumar Vidya P, Usha V, Solapure Suresh M, De Sousa Sunita M. 2006. Screen for inhibitors of the coupled transglycosylase-transpeptidase of peptidoglycan biosynthesis in *Escherichia coli*. *Antimicrobial Agents and Chemotherapy* 50:1425–1432 DOI 10.1128/aac.50.4.1425-1432.2006.
- Rautio J, Kumpulainen H, Heimbach T, Oliyai R, Oh D, Järvinen T, Savolainen J. 2008. Prodrugs: design and clinical applications. *Nature Reviews Drug Discovery* 7:255–270 DOI 10.1038/nrd2468.
- Reck F, Marmor S, Fisher S, Wuonola MA. 2001. Inhibitors of the bacterial cell wall biosynthesis enzyme MurC. *Bioorganic & Medicinal Chemistry Letters* 11:1451–1454 DOI 10.1016/S0960-894X(01)00251-7.
- Rohde W, Cordell B, Webster R, Levinson W. 1977. Inhibition of amino acyl tRNA synthetase activity by copper complexes of two metal binding ligands N-Methyl isatin beta-thiosemicarbazone and 8-hydroxyquinoline. *Biochimica et Biophysica Acta (BBA) - Nucleic Acids and Protein Synthesis* 477:102–111 DOI 10.1016/0005-2787(77)90226-X.
- Sanduo Z, Lok-To S, Frederick AR, Kelly PB, William PR, John JM, Debora SM, Thomas GB, Andrew CK. 2018. Structure and mutagenic analysis of the lipid II flippase MurJ from *Escherichia coli*. *Proceedings of the National Academy of Sciences of the United States of America* 115:6709–6714 DOI 10.1073/pnas.1802192115.
- Sauvage E, Kerff F, Terrak M, Ayala JA, Charlier P. 2008. The penicillin-binding proteins: structure and role in peptidoglycan biosynthesis. *FEMS Microbiol Reviews* 32:234–258 DOI 10.1111/j.1574-6976.2008.00105.x.
- Saxena S, Abdullah M, Sriram D, Guruprasad L. 2018. Discovery of novel inhibitors of *Mycobacterium tuberculosis* MurG: homology modelling, structure based pharmacophore, molecular docking, and molecular dynamics simulations. *Journal of Biomolecular Structure and Dynamics* 36:3184–3198 DOI 10.1080/07391102.2017.1384398.
- Sham LT, Butler EK, Lebar MD, Kahne D, Bernhardt TG, Ruiz N. 2014. MurJ is the flippase of lipid-linked precursors for peptidoglycan biogenesis. *Science* 345:220–222 DOI 10.1126/science.1254522.
- Šink R, Kovač A, Tomašić T, Rupnik V, Boniface A, Bostock J, Chopra I, Blanot D, Mašič LP, Gobec S, Zega A. 2008. Synthesis and biological evaluation of n-acylhydrazones as inhibitors of MurC and MurD ligases. *ChemMedChem* 3:1362–1370 DOI 10.1002/cmdc.200800087.
- Škedelj V, Tomašić T, Mašič LP, Zega A. 2011. ATP-binding site of bacterial enzymes as a target for antibacterial drug design. *Journal of Medicinal Chemistry* 54:915–929 DOI 10.1021/jm101121s.
- Silver LL. 2006. Does the cell wall of bacteria remain a viable source of targets for novel antibiotics? *Biochemical Pharmacology* 71:996–1005 DOI 10.1016/j.bcp.2005.10.029.
- Silver LL. 2007. Multi-targeting by monotherapeutic antibacterials. *Nature Reviews Drug Discovery* 6:41–55 DOI 10.1038/nrd2202.

- Silver LL, Bostian KA. 1993. Discovery and development of new antibiotics: the problem of antibiotic resistance. *Antimicrobial Agents and Chemotherapy* 37:377–383 DOI 10.1128/aac.37.3.377.
- Sim MM, Ng SB, Buss AD, Crasta SC, Goh KL, Lee SK. 2002. Benzylidene rhodanines as novel inhibitors of UDP-N-acetylmuramate/l-alanine ligase. *Bioorganic & Medicinal Chemistry Letters* 12:697–699 DOI 10.1016/S0960-894X(01)00832-0.
- Sosič I, Barreateau H, Simčič M, Šink R, Cesar J, Zega A, Grdadolnik SG, Contreras-Martel C, Dessen A, Amoroso A, Joris B, Blanot D, Gobec S. 2011. Second-generation sulfonamide inhibitors of d-glutamic acid-adding enzyme: activity optimisation with conformationally rigid analogues of d-glutamic acid. *European Journal of Medicinal Chemistry* 46:2880–2894 DOI 10.1016/j.ejmech.2011.04.011.
- Stamper GF, Longenecker KL, Fry EH, Jakob CG, Florjancic AS, Gu Y-G, Anderson DD, Cooper CS, Zhang T, Clark RF, Cia Y, Black-Schaefer CL, McCall J Owen, Lerner CG, Hajduk PJ, Beutel BA, Stoll VS. 2006. Structure-based optimization of MurF inhibitors. *Chemical Biology & Drug Design* 67:58–65 DOI 10.1111/j.1747-0285.2005.00317.x.
- Strancar K, Boniface A, Blanot D, Gobec S. 2007. Phosphinate inhibitors of UDP-N-acetylmuramoyl-L-alanyl-D-glutamate: L-lysine ligase (MurE). *Archiv der Pharmazie* 340:127–134 DOI 10.1002/ardp.200600191.
- Sun L, Fan H, Zhong S. 2021. Evaluating the covalent binding of carbapenems on BlaC using noncovalent interactions. *Journal of Molecular Modeling* 27:161 DOI 10.1007/s00894-021-04760-x.
- Taha MO, Atallah N, Al-Bakri AG, Paradis-Bleau C, Zalloum H, Younis KS, Levesque RC. 2008. Discovery of new MurF inhibitors via pharmacophore modeling and QSAR analysis followed by in-silico screening. *Bioorganic & Medicinal Chemistry* 16:1218–1235 DOI 10.1016/j.bmc.2007.10.076.
- Timmins GS, Deretic V. 2006. Mechanisms of action of isoniazid. *Molecular Microbiology* 62:1220–1227 DOI 10.1111/j.1365-2958.2006.05467.x.
- Tomašić T, Kovač A, Klebe G, Blanot D, Gobec S, Kikelj D, Mašič LP. 2012b. Virtual screening for potential inhibitors of bacterial MurC and MurD ligases. *Journal of Molecular Modeling* 18:1063–1072 DOI 10.1007/s00894-011-1139-8.
- Tomašić T, Kovač A, Simčič M, Blanot D, Grdadolnik SG, Gobec S, Kikelj D, Peterlin Mašič L. 2011. Novel 2-thioxothiazolidin-4-one inhibitors of bacterial MurD ligase targeting d-Glu- and diphosphate-binding sites. *European Journal of Medicinal Chemistry* 46:3964–3975 DOI 10.1016/j.ejmech.2011.05.070.
- Tomašić T, Šink R, Zidar N, Fic A, Contreras-Martel C, Dessen AA, Patin D, Blanot D, Müller-Premru M, Gobec S, Zega A, Kikelj D, Mašič LP. 2012a. Dual inhibitor of MurD and MurE ligases from *Escherichia coli* and *Staphylococcus aureus*. *ACS Medicinal Chemistry Letters* 3:626–630 DOI 10.1021/ml300047h.
- Tomašić T, Zidar N, Kovač A, Turk S, Simčič M, Blanot D, Müller-Premru M, Filipič M, Grdadolnik SG, Zega A, Anderluh M, Gobec S, Kikelj D, Peterlin Mašič L. 2010. 5-Benzylidenethiazolidin-4-ones as multitarget inhibitors of bacterial mur ligases. *ChemMedChem* 5:286–295 DOI 10.1002/cmdc.200900449.

- Tomašić T, Zidar N, Rupnik V, Kovač A, Blanot D, Gobec S, Kikelj D, Mašič LP. 2009. Synthesis and biological evaluation of new glutamic acid-based inhibitors of MurD ligase. *Bioorganic & Medicinal Chemistry Letters* 19:153–157 DOI 10.1016/j.bmcl.2008.10.129.
- Tran W, Kusay AS, Hawkins PME, Cheung C-Y, Nagalingam G, Pujari V, Ford DJ, Stoye A, Ochoa JL, Audette RE, Hortle E, Oehlers SH, Charman SA, Linington RG, Rubin EJ, Dowson CG, Roper DI, Crick DC, Balle T, Cook GM, Britton WJ, Payne RJ. 2021. Synthetic sansanmycin analogues as potent *Mycobacterium tuberculosis* translocase I inhibitors. *Journal of Medicinal Chemistry* 64:17326–17345 DOI 10.1021/acs.jmedchem.1c01407.
- Tran AT, Watson EE, Pujari V, Conroy T, Dowman LJ, Giltrap AM, Pang A, Wong WR, Linington RG, Mahapatra S, Saunders J, Charman SA, West NP, Bugg TDH, Tod J, Dowson CG, Roper DI, Crick DC, Britton WJ, Payne RJ. 2017. Sansanmycin natural product analogues as potent and selective anti-mycobacterials that inhibit lipid I biosynthesis. *Nature Communications* 8:14414–14414 DOI 10.1038/ncomms14414.
- Van Heijenoort J. 2001. Formation of the glycan chains in the synthesis of bacterial peptidoglycan. *Glycobiology* 11:25R–36R DOI 10.1093/glycob/11.3.25R.
- Van Rijn SP, Zuur MA, Anthony R, Wilffert B, van Altena R, Akkerman OW, de Lange WCM, van der Werf TS, Kosterink JGW, Alffenaar JC. 2019. Evaluation of carbapenems for treatment of multi- and extensively drug-resistant *Mycobacterium tuberculosis*. *Antimicrobial Agents and Chemotherapy* 63:e01489-18 DOI 10.1128/aac.01489-18.
- Wayne LG, Sohaskey CD. 2001. Nonreplicating persistence of *Mycobacterium tuberculosis*. *Annual Review of Microbiology* 55:139–163 DOI 10.1146/annurev.micro.55.1.139.
- Wiegmann D, Koppermann S, Ducho C. 2018. Aminoribosylated analogues of muraymycin nucleoside antibiotics. *Molecules* 23:3085 DOI 10.3390/molecules23123085.
- World Health Organization. 2022. WHO consolidated guidelines on tuberculosis module 4: treatment drug-resistant tuberculosis treatment. Geneva: WHO. Available at <https://www.who.int/publications/i/item/9789240063129>.
- World Health Organization. 2023. Global tuberculosis report 2023. Geneva: WHO. Available at <https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2023>.
- Wube AA, Hüfner A, Thomaschitz C, Blunder M, Kollroser M, Bauer R, Bucar F. 2011. Design, synthesis and antimycobacterial activities of 1-methyl-2-alkenyl-4(1H)-quinolones. *Bioorganic & Medicinal Chemistry* 19:567–579 DOI 10.1016/j.bmc.2010.10.060.
- Xu L, Wu D, Liu L, Zheng Q, Song Y, Ye L, Sha S, Kang J, Xin Y, Ma Y. 2014. Characterization of mycobacterial UDP-N-acetylglucosamine enolpyruvyle transferase (MurA). *Research in Microbiology* 165:91–101 DOI 10.1016/j.resmic.2014.01.004.
- Yamaguchi H, Sato S, Yoshida S, Takada K, Itoh M, Seto H, Otake N. 1986. Capuramycin, a new nucleoside antibiotic: taxonomy, fermentation, isolation and characterization. *The Journal of Antibiotics* 39:1047–1053 DOI 10.7164/antibiotics.39.1047.

- Yan Y, Munshi S, Leiting B, Anderson MS, Chrzas J, Chen Z. 2000.** Crystal structure of *Escherichia coli* UDPMurNAc-tripeptide d-alanyl-d-alanine-adding enzyme (MurF) at 2.3 Å resolution. Edited by I, Wilson. *Journal of Molecular Biology* **304**:435–445 DOI [10.1006/jmbi.2000.4215](https://doi.org/10.1006/jmbi.2000.4215).
- Zawadzke LE, Norcia M, Desbonnet CR, Wang H, Freeman-Cook K, Dougherty TJ. 2008.** Identification of an inhibitor of the MurC enzyme, which catalyzes an essential step in the peptidoglycan precursor synthesis pathway. *Assay and Drug Development Technologies* **6**:95–103 DOI [10.1089/adt.2007.114](https://doi.org/10.1089/adt.2007.114).
- Zhang Z, Bulloch EM, Bunker RD, Baker EN, Squire CJ. 2009.** Structure and function of GlmU from *Mycobacterium tuberculosis*. *Acta Crystallographica Section D Biological Crystallography* **65**:275–283 DOI [10.1107/s0907444909001036](https://doi.org/10.1107/s0907444909001036).
- Zhu C, Liu Y, Hu L, Yang M, He ZG. 2018.** Molecular mechanism of the synergistic activity of ethambutol and isoniazid against *Mycobacterium tuberculosis*. *Journal of Biological Chemistry* **293**:16741–16750 DOI [10.1074/jbc.RA118.002693](https://doi.org/10.1074/jbc.RA118.002693).
- Zidar N, Tomašić T, Šink R, Kovač A, Patin D, Blanot D, Contreras-Martel C, Dessen A, Premru MM, Zega A, Gobec S, Mašič LP, Kikelj D. 2011.** New 5-benzylidenethiazolidin-4-one inhibitors of bacterial MurD ligase: design, synthesis, crystal structures, and biological evaluation. *European Journal of Medicinal Chemistry* **46**:5512–5523 DOI [10.1016/j.ejmech.2011.09.017](https://doi.org/10.1016/j.ejmech.2011.09.017).
- Zidar N, Tomašić T, Šink R, Rupnik V, Kovač A, Turk S, Patin D, Blanot D, Contreras-Martel C, Dessen A, Premru MM, Müller Zega A, Gobec S, Mašič LP, Peterlin, Kikelj D. 2010.** Discovery of Novel 5-benzylidenethiazolidine-2,4-dione inhibitors of MurD ligase. *Journal of Medicinal Chemistry* **53**:6584–6594 DOI [10.1021/jm100285g](https://doi.org/10.1021/jm100285g).