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Peptidoglycan-Mimetic Inhibitors of D,D- and L,D-Transpeptidases: Mechanistic Design Principles and Translational Barriers in the Post-β-Lactam Era

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ABSTRACT

Background: *Peptidoglycan cross-linking is essential for bacterial cell-wall integrity and is catalyzed by D,D-transpeptidases (penicillin-binding proteins, PBPs) and L,D-transpeptidases (Ldts). Escalating β-lactam resistance driven by β-lactamases, altered PBPs, and compensatory reliance on Ldt-mediated 3→3 cross-links has intensified interest in non-β-lactam strategies that directly inhibit transpeptidases.* ***Objective:*** *This narrative review critically synthesizes mechanistic, structural, and medicinal chemistry evidence supporting peptidoglycan-mimetic inhibition of PBPs and Ldts, with emphasis on covalent warhead design, structural validation, and translational barriers.* ***Methods:*** *A structured narrative literature search of PubMed/MEDLINE, Scopus, and Web of Science was conducted for January 2010–March 2025, supplemented by reference list screening. Eligible articles reported transpeptidase inhibitor design with biochemical potency metrics, structural evidence of binding, and/or microbiological activity.* ***Results:*** *Substrate-mimetic and conformationally constrained scaffolds can reproduce key recognition motifs, including m-DAP pocket interactions, while boronic acids/cyclic boronates enable reversible covalent inhibition of serine-dependent PBPs via tetrahedral transition-state mimicry, and electrophilic warheads such as nitriles/cyanamides can covalently modify catalytic cysteine residues in Ldts. Structural studies validate active-site engagement across representative PBPs and Ldts, but whole-cell translation is frequently limited by Gram-negative outer membrane permeability, efflux susceptibility, and potential off-target reactivity of electrophiles.* ***Conclusion:*** *Peptidoglycan-mimetic transpeptidase inhibition is mechanistically compelling, yet clinically meaningful antibacterial development will require integrated optimization of target engagement, selectivity, and bacterial accumulation alongside pharmacokinetic feasibility.*

Keywords: *Peptidoglycan-mimetic inhibitors; penicillin-binding proteins; L,D-transpeptidases; covalent warheads; boronates; antibacterial drug design*

INTRODUCTION

Biological Basis of Peptidoglycan Cross-Linking

Bacterial survival depends on the structural integrity of the peptidoglycan (PG) sacculus, a highly organized macromolecular network composed of repeating glycan chains cross-linked by short peptide stems (1). This mesh-like polymer confers mechanical strength, maintains cellular morphology, and protects against osmotic lysis. The terminal step of PG assembly is transpeptidation, a reaction that covalently links peptide side chains from adjacent glycan strands to generate a rigid yet dynamic cell-wall architecture (1). Two distinct classes of enzymes mediate this cross-linking process: D,D-transpeptidases, commonly known as penicillin-binding proteins (PBPs), and L,D-transpeptidases (Ldts).

PBPs catalyze the formation of classical 4→3 peptide cross-links between the fourth residue (D-Ala) of a donor stem and the third residue (typically meso-diaminopimelic acid, m-DAP, or L-Lys) of an acceptor stem through a catalytic serine-mediated acyl-enzyme mechanism

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(2). In contrast, Ldts form non-canonical 3→3 cross-links by joining the third residues of donor and acceptor stems using a catalytic cysteine nucleophile (3). Although historically considered secondary contributors to PG assembly, Ldts are now recognized as functionally significant in several bacterial species, particularly under stress conditions, stationary-phase growth, or antibiotic pressure (3). Structural studies have revealed notable differences in active-site geometry, substrate accommodation, and catalytic residues between PBPs and Ldts, implying that inhibitor design must account for these mechanistic divergences (4).

The coexistence of two mechanistically distinct cross-linking pathways introduces enzymatic redundancy and plasticity into bacterial cell-wall biosynthesis. This plasticity underlies the adaptive capacity of bacteria to reconfigure cross-linking flux in response to environmental and pharmacological stressors, thereby complicating therapeutic intervention strategies (1,3).

β-Lactam Resistance and Pathway Plasticity

β-Lactam antibiotics exert bactericidal activity by mimicking the D-Ala–D-Ala terminus of the stem peptide and covalently acylating the active-site serine of PBPs, thereby blocking 4→3 cross-link formation (2). This mechanism has underpinned the clinical success of penicillins, cephalosporins, carbapenems, and related agents for decades. However, the sustained global emergence of β-lactam resistance has eroded their effectiveness and exposed mechanistic vulnerabilities in PBP-focused therapy (5).

Resistance mechanisms are multifactorial and include enzymatic hydrolysis by β-lactamases, structural alterations in PBPs that reduce antibiotic affinity, changes in permeability and efflux, and, critically, compensatory upregulation of Ldt-mediated 3→3 cross-linking (3,5). Unlike PBPs, Ldts utilize a catalytic cysteine and are generally less susceptible to classical β-lactams, allowing bacteria to maintain cell-wall integrity even in the presence of potent PBP inhibitors (3,6). In organisms such as Mycobacterium tuberculosis, Ldt-mediated cross-links constitute a substantial proportion of total PG cross-linking, highlighting the clinical importance of this alternative pathway (6).

The adaptive shift toward Ldt-dependent cross-linking represents a form of pathway-level resistance rather than target mutation alone. Consequently, strategies that exclusively inhibit PBPs may fail in contexts where Ldt activity compensates for PBP blockade. This functional redundancy challenges the traditional paradigm of β-lactam-centric antibiotic development and necessitates exploration of alternative or complementary transpeptidase-targeting approaches (5).

Knowledge Gap in Transpeptidase-Targeted Drug Discovery

Despite growing mechanistic insight into Ldt biology and structural elucidation of both PBP and Ldt active sites, inhibitor development remains disproportionately focused on β-lactam scaffolds and β-lactamase inhibition (2,7). While recent advances in boronate chemistry and electrophilic warhead design have expanded the repertoire of non-β-lactam inhibitors, translation into clinically validated transpeptidase-targeted agents remains limited (7,8).

Several conceptual and practical uncertainties persist. First, it remains unclear whether substrate-mimetic approaches that replicate stem-peptide recognition elements can achieve sufficient affinity and selectivity while maintaining drug-like physicochemical properties. Second, the design of covalent warheads targeting catalytic serine or cysteine residues raises concerns regarding off-target reactivity, proteome-wide thiol engagement, and safety liabilities (8). Third, Gram-negative pathogens present formidable permeability and efflux

barriers that frequently undermine the cellular activity of otherwise potent enzyme inhibitors.

Moreover, although dual targeting of PBPs and Ldts is frequently proposed as a strategy to overcome pathway plasticity, direct evidence demonstrating effective simultaneous engagement in bacterial cells remains sparse. Structural compatibility, kinetic constraints, and differential active-site architectures may limit the feasibility of such an approach. These unresolved issues underscore the need for a critical synthesis of mechanistic, structural, and medicinal chemistry evidence rather than purely conceptual advocacy.

Objective and Scope of This Review

In light of these challenges, this narrative review critically evaluates contemporary medicinal chemistry strategies aimed at inhibiting D,D-transpeptidases and L,D-transpeptidases through peptidoglycan-mimetic design. The review integrates structural enzymology, covalent warhead chemistry, substrate-inspired scaffolds, and microbiological translation to assess both the promise and the limitations of this therapeutic direction. Particular emphasis is placed on mechanistic validation, structural evidence of target engagement, permeability constraints in Gram-negative bacteria, and translational barriers related to selectivity and pharmacokinetics.

By synthesizing current knowledge across enzymology, structural biology, and antibacterial drug design, this review aims to clarify where the field stands, identify realistic opportunities for innovation, and delineate the constraints that must be addressed before peptidoglycan-mimetic transpeptidase inhibitors can progress toward clinically viable antibacterial agents.

REVIEW METHODOLOGY

This article was conducted as a structured narrative review aimed at critically synthesizing mechanistic, structural, and medicinal chemistry evidence related to peptidoglycan-mimetic inhibitors of D,D-transpeptidases (penicillin-binding proteins, PBPs) and L,D-transpeptidases (Ldts). Although not designed as a systematic review with quantitative synthesis, a transparent and reproducible literature search strategy was implemented to minimize selection bias and ensure comprehensive coverage of relevant developments in the field.

Electronic searches were performed in PubMed/MEDLINE, Scopus, and Web of Science databases. The search period covered publications from January 2010 through March 2025, reflecting the period during which structural elucidation of Ldt enzymes, boronate chemistry expansion, and non-β-lactam warhead development substantially progressed. Additional articles were identified through manual screening of reference lists from key primary research papers and recent review articles in medicinal chemistry and microbiology journals.

Search terms were combined using Boolean operators and included keywords such as “L,D-transpeptidase inhibitor,” “LdtMt2 inhibitor,” “penicillin-binding protein inhibitor,” “boronate PBP,” “cyclic boronate β-lactamase,” “peptidoglycan mimetic,” “meso-diaminopimelic acid bioisostere,” “covalent warhead transpeptidase,” “nitrile inhibitor Ldt,” “cyanamide inhibitor,” “transpeptidase crystal structure,” and “dual PBP Ldt targeting.” Where appropriate, Medical Subject Headings (MeSH) terms related to peptidoglycan biosynthesis, antibiotic resistance, and β-lactamase inhibition were incorporated to improve retrieval sensitivity.

Articles were considered eligible if they met at least one of the following criteria: (i) reported biochemical inhibition of PBPs or Ldts with defined potency metrics (e.g., IC_{50}, K_i, k_inact/K_I); (ii) provided structural evidence of inhibitor binding through X-ray

crystallography or cryo-electron microscopy; or (iii) included microbiological evaluation (e.g., minimum inhibitory concentration testing) of compounds explicitly designed to target transpeptidases. Both small-molecule inhibitors and peptide- or peptidomimetic-based scaffolds were included, provided that the work contained mechanistic or translational relevance.

Studies were excluded if they were limited to descriptive microbiological characterization without evaluation of transpeptidase inhibitors, purely computational modeling studies lacking biochemical validation, or general antibiotic-resistance reviews without substantive discussion of inhibitor design or structural mechanisms. Non-English publications and conference abstracts without full experimental data were also excluded to maintain analytical rigor.

Given the narrative design, formal dual independent screening, risk-of-bias scoring, and quantitative heterogeneity assessment were not undertaken. The synthesis focused instead on mechanistic coherence, structural plausibility, medicinal chemistry rationale, and translational implications. As with all narrative reviews, the selection of studies is subject to potential author-driven emphasis and publication bias. Efforts were made to mitigate this limitation by incorporating diverse enzyme classes, chemical scaffolds, and both supportive and critical evidence. Nonetheless, the conclusions presented should be interpreted within the context of a qualitative evidence synthesis rather than a statistically pooled meta-analytic framework.

Mechanistic Enzymology of Transpeptidases

Catalytic Mechanism of D, D-Transpeptidases (Penicillin-Binding Proteins)

D,D-transpeptidases, commonly referred to as penicillin-binding proteins (PBPs), catalyze the formation of classical 4→3 cross-links in peptidoglycan by joining the fourth residue (D-Ala) of a donor stem peptide to the third residue (typically meso-diaminopimelic acid or L-Lys) of an acceptor stem (1). The reaction proceeds through a two-step acylation–deacylation mechanism centered on a conserved active-site serine residue. During catalysis, the serine hydroxyl performs a nucleophilic attack on the carbonyl carbon of the terminal D-Ala–D-Ala peptide bond, generating a tetrahedral intermediate that collapses to form a covalent acyl–enzyme complex with release of the terminal D-Ala (2). Subsequent nucleophilic attack by the acceptor peptide resolves the acyl–enzyme intermediate, completing cross-link formation and regenerating the active enzyme.

This catalytic mechanism explains the susceptibility of PBPs to β-lactam antibiotics, which structurally mimic the D-Ala–D-Ala dipeptide and irreversibly acylate the catalytic serine (2). However, PBPs exhibit substantial structural diversity across bacterial species, particularly in loop regions surrounding the active site and in the geometry of substrate-binding grooves. High-resolution crystallographic analyses demonstrate that subtle variations in active-site architecture influence both substrate recognition and inhibitor accommodation (3). Consequently, inhibitor design must consider not only the conserved catalytic serine but also the broader binding pocket topology and conformational flexibility.

From a mechanistic perspective, PBPs represent serine-dependent acyl-transferases that transiently stabilize a high-energy tetrahedral intermediate. This feature provides a rational basis for transition-state analog design, including boronic acids and cyclic boronates that can reversibly adopt tetrahedral geometries and mimic the catalytic intermediate (4). Nevertheless, reversible covalent inhibition requires precise positioning within the active site to achieve sufficient affinity without compromising selectivity.

Catalytic Mechanism of L, D-Transpeptidases

L,D-transpeptidases (Ldts) catalyze the formation of non-classical 3→3 cross-links between the third residues of donor and acceptor stem peptides (1). Unlike PBPs, Ldts employ a catalytic cysteine residue as the nucleophile. The reaction mechanism involves thiolate-mediated attack on the carbonyl carbon of the donor tetrapeptide, forming a thioacyl–enzyme intermediate that is subsequently resolved by nucleophilic attack from the acceptor stem peptide (5).

Structurally, Ldts possess distinct domain organization and active-site architecture compared with PBPs. Crystallographic studies of LdtMt2 from Mycobacterium tuberculosis have revealed a well-defined catalytic cavity accommodating meso-diaminopimelic acid side chains and adjacent peptide backbone interactions (6). The geometry of the binding groove and orientation of the catalytic cysteine impose different steric and electronic constraints relative to PBP active sites. These mechanistic differences explain why classical β-lactams, optimized for serine acylation, generally exhibit poor inhibitory activity against most Ldts (5).

Importantly, Ldt-mediated cross-linking becomes increasingly prominent under stress conditions and during stationary-phase growth in several pathogens, including mycobacteria and certain Gram-negative bacteria (5,6). This adaptive shift in cross-linking flux underscores the enzymatic plasticity of peptidoglycan assembly and provides a mechanistic rationale for developing inhibitors that extend beyond PBP-specific scaffolds.

Implications for Inhibitor Design

The mechanistic divergence between serine-dependent PBPs and cysteine-dependent Ldts has direct implications for medicinal chemistry strategies. Warheads optimized for serine acylation may not efficiently engage cysteine nucleophiles, and vice versa. Moreover, the differences in substrate recognition pockets suggest that effective inhibitors must balance catalytic residue targeting with accurate recapitulation of stem-peptide orientation.

In addition, both enzyme classes exhibit dynamic loop movements that may influence induced-fit binding and inhibitor residence time. Static structural snapshots do not fully capture catalytic turnover or conformational transitions, highlighting the importance of integrating biochemical kinetics with structural validation (3,6). Therefore, rational inhibitor design requires a comprehensive understanding of enzyme mechanism, active-site geometry, and structural plasticity to avoid simplistic assumptions of cross-class inhibition.

Medicinal Chemistry Strategies for Peptidoglycan-Mimetic Inhibitors

Substrate-Mimetic Scaffolds

Substrate-mimetic strategies aim to reproduce key structural and stereochemical elements of native peptidoglycan stem peptides while incorporating modifications that prevent enzymatic turnover. Central to this approach is the recognition of meso-diaminopimelic acid (m-DAP) or related side chains that occupy defined binding pockets within transpeptidases (6). Bioisosteric replacements of m-DAP, including selenium-containing analogues and constrained side-chain mimetics, have been explored to preserve recognition while improving chemical stability (7).

Macrocyclic and bicyclic peptide-based inhibitors represent another extension of substrate mimicry. Conformational constraint may enhance binding affinity by reducing entropic penalties and stabilizing bioactive conformations. Structural studies of bicyclic inhibitors targeting PBP3 have demonstrated that engineered rigidity can improve active-site

engagement while allowing for functionalization with outer-membrane permeation motifs (8).

However, substrate-mimetic designs often face physicochemical challenges. Peptidic or highly polar scaffolds frequently exhibit limited membrane permeability, high molecular weight, and susceptibility to efflux, particularly in Gram-negative organisms. Thus, while substrate mimicry enhances enzymatic specificity, it may simultaneously compromise translational viability.

Covalent Warhead Strategies Beyond β-Lactams

Given the central role of nucleophilic residues in transpeptidase catalysis, covalent inhibition strategies remain attractive. For serine-dependent PBPs, boronic acids and cyclic boronates have emerged as reversible covalent inhibitors capable of forming tetrahedral adducts that mimic the catalytic intermediate (4,9). Structural studies have demonstrated that boronate-containing compounds can engage active-site serine residues and, in some cases, simultaneously inhibit β-lactamases, suggesting multifunctional potential (4).

For cysteine-dependent Ldts, electrophilic warheads such as nitriles and cyanamides have been investigated to target the catalytic thiol (5). High-throughput screening campaigns against LdtMt2 have identified covalently reacting scaffolds with demonstrable enzyme inhibition and structural validation of cysteine modification (5). These findings validate cysteine-directed chemistry as a viable mechanistic approach.

Nevertheless, covalent warhead design introduces critical selectivity concerns. Catalytic cysteine residues are present in numerous host proteins, raising the possibility of off-target reactivity. Furthermore, irreversible inhibition may complicate safety profiling and pharmacokinetic optimization. Therefore, balancing reactivity with selectivity remains a central medicinal chemistry challenge.

Hybrid and Dual-Target Concepts

Hybrid inhibitors that combine substrate-mimetic backbones with catalytic warheads have been proposed to integrate recognition specificity with covalent engagement. Conceptually, such scaffolds could enhance binding affinity while preserving inhibitory potency. However, structural compatibility between PBP and Ldt active sites is not trivial. Differences in nucleophile identity, pocket depth, and substrate orientation complicate the design of truly dual-active compounds.

Although dual targeting of PBPs and Ldts is frequently proposed as a strategy to overcome pathway plasticity, direct biochemical and cellular evidence demonstrating effective simultaneous engagement remains limited. Structural feasibility does not automatically translate into functional dual inhibition within bacterial cells. As such, claims of dual-target potential should be interpreted cautiously and supported by orthogonal target-engagement data rather than inferred from structural analogy alone.

Physicochemical and Translational Considerations

Beyond enzyme potency, successful inhibitor development must account for permeability, efflux susceptibility, serum binding, and metabolic stability. Gram-negative pathogens present a particularly stringent barrier due to their outer membrane and active efflux systems. Compounds exceeding optimal polarity or molecular weight thresholds often fail to accumulate in the periplasm at therapeutically relevant concentrations.

Therefore, medicinal chemistry optimization must integrate target engagement with drug-like property refinement. Strategies such as incorporation of uptake-facilitating motifs,

modulation of polarity, or prodrug approaches may be required to reconcile enzymatic potency with whole-cell activity. Without addressing these translational constraints, even mechanistically sound inhibitors may remain confined to in vitro enzymology rather than progressing toward clinically viable antibacterial agents.

Structural Biology Validation of Transpeptidase Inhibition

Structural Elucidation of L, D-Transpeptidase–Ligand Complexes

High-resolution structural studies have substantially advanced understanding of L,D-transpeptidase (Ldt) substrate recognition and inhibitor binding. Crystal structures of LdtMt2 from Mycobacterium tuberculosis complexed with natural tetrapeptide substrates have delineated the orientation of meso-diaminopimelic acid (m-DAP) side chains within a defined catalytic cavity and clarified the positioning of the catalytic cysteine relative to the scissile bond (1). These structural data provide a template for rational design of peptidoglycan-mimetic inhibitors that preserve key hydrogen bonding networks and steric complementarity.

Inhibitor-bound Ldt structures have further demonstrated that electrophilic warheads such as nitriles and cyanamides can form covalent adducts with the catalytic cysteine, thereby validating mechanism-based targeting strategies (2). Electron density maps confirming covalent modification and conformational adjustments in active-site loops have strengthened the mechanistic plausibility of cysteine-directed inhibitors. However, the presence of a covalent bond in a crystal structure does not necessarily predict whole-cell efficacy. Structural occupancy reflects biochemical compatibility under crystallization conditions, which may differ from intracellular environments characterized by competing nucleophiles, dynamic turnover, and efflux.

Furthermore, structural heterogeneity among Ldt homologues across species introduces variability in pocket depth, loop flexibility, and electrostatic landscape (3). Consequently, structural validation in one enzyme (e.g., LdtMt2) cannot automatically be generalized to Gram-negative Ldts or other bacterial taxa without additional evidence.

Structural Studies of PBP–Boronate and Non-β-Lactam Complexes

Structural analyses of penicillin-binding proteins (PBPs) complexed with boronic acid and cyclic boronate inhibitors have demonstrated reversible covalent interactions with the catalytic serine, mimicking the tetrahedral intermediate of transpeptidation (4). In several cases, crystallographic refinement has revealed stable serine–boronate adducts with clear electron density and appropriate stereochemical geometry, supporting the concept of transition-state analogue inhibition.

Importantly, comparative structures indicate that boronate scaffolds can occupy binding pockets similarly to β-lactams while avoiding hydrolytic susceptibility to classical β-lactamases (4). However, differences in loop regions and active-site accessibility among PBP isoforms complicate extrapolation across bacterial species. Structural validation must therefore consider isoform-specific variations rather than assuming uniform inhibition across PBP families.

Another critical consideration is that static crystallographic snapshots do not capture kinetic parameters such as residence time, reversibility, or slow-binding behavior. Structural confirmation of ligand binding should be interpreted alongside biochemical data, including time-dependent inhibition profiles and, where applicable, intact-protein mass spectrometry. Without orthogonal validation, structural data alone risk overstating mechanistic certainty.

Limitations of Structural Validation

While structural biology remains indispensable for rational drug design, it has intrinsic limitations. Crystal packing constraints, ligand concentration during soaking, and engineered protein constructs may influence observed binding modes. Cryo-electron microscopy, although increasingly powerful for large complexes, currently provides limited resolution for small-molecule inhibitor positioning in many bacterial enzymes.

Moreover, structural validation does not directly address issues of permeability, efflux, metabolic stability, or cytoplasmic thiol reactivity. An inhibitor may demonstrate ideal active-site complementarity yet fail to accumulate within the periplasm of Gram-negative bacteria or the lipid-rich cell wall of mycobacteria. Therefore, structural evidence should be considered necessary but not sufficient for advancing peptidoglycan-mimetic inhibitors toward translational relevance.

Microbiological Translation and Cellular Context

Activity in Gram-Positive Organisms

Gram-positive bacteria, characterized by a thick peptidoglycan layer and absence of an outer membrane, generally present fewer permeability barriers for transpeptidase-targeted agents (5). In this context, inhibitors that demonstrate robust biochemical potency against PBPs or Ldts may more readily translate into measurable minimum inhibitory concentration (MIC) reductions. Pathogens such as Staphylococcus aureus and Enterococcus faecalis rely primarily on PBP-mediated cross-linking, although Ldt contributions have been documented under stress conditions (5).

Nevertheless, Gram-positive translation is not trivial. Altered PBPs (e.g., low-affinity variants), cell-wall thickening, and regulatory adaptations can attenuate inhibitor efficacy. Therefore, microbiological validation requires testing across defined resistance phenotypes rather than extrapolating from enzyme inhibition data alone.

Gram-Negative Periplasmic Barrier

Gram-negative bacteria present a substantially greater translational challenge due to the presence of an outer membrane and active efflux systems (6). Transpeptidases reside in the periplasmic space, necessitating that inhibitors traverse porins or other uptake pathways before engaging their targets. Compounds with high molecular weight, excessive polarity, or unfavorable charge distribution often fail to accumulate at sufficient concentrations within the periplasm.

Even inhibitors with nanomolar biochemical potency may exhibit weak or absent whole-cell activity due to poor permeability or rapid efflux. This disconnect between enzyme inhibition and MIC outcomes is a recurring phenomenon in antibiotic discovery. Consequently, translational evaluation should include permeability assessment, efflux susceptibility analysis, and comparison of activity in efflux-deficient strains when feasible.

Design strategies to address Gram-negative penetration include molecular size optimization, polarity balancing, incorporation of uptake-facilitating motifs, and prodrug approaches. However, these modifications may compromise enzyme affinity or selectivity, underscoring the delicate balance between biochemical potency and pharmacokinetic feasibility.

Mycobacterial Cell-Wall Complexity

Mycobacteria present a distinct translational context due to their lipid-rich, multilayered cell envelope and high prevalence of Ldt-mediated 3→3 cross-links (3). In Mycobacterium

tuberculosis, Ldts contribute substantially to stationary-phase cell-wall architecture, rendering them particularly attractive targets (3). Structural validation of Ldt inhibitors in this organism provides a strong mechanistic foundation.

However, the mycobacterial cell wall significantly restricts compound penetration, and efflux mechanisms further complicate intracellular accumulation. Therefore, inhibitors validated against purified LdtMt2 must demonstrate cellular activity under physiologically relevant conditions to substantiate therapeutic potential.

Linking Enzyme Inhibition to Antibacterial Effect

A central translational question is whether inhibition of a single transpeptidase class suffices to induce bactericidal activity, or whether compensatory cross-linking pathways mitigate the impact. While theoretical models suggest that dual inhibition of PBPs and Ldts could reduce pathway redundancy, experimental confirmation of simultaneous engagement within bacterial cells remains limited.

Establishing a mechanistic link between enzyme inhibition and microbiological outcome requires integration of biochemical kinetics, structural validation, and whole-cell assays. Without such triangulation, antibacterial activity may be misattributed, and conversely, promising enzymatic inhibitors may be prematurely discarded due to permeability limitations rather than intrinsic mechanistic insufficiency.

In summary, microbiological translation represents the decisive filter through which mechanistically compelling peptidoglycan-mimetic inhibitors must pass. Successful advancement demands coordinated optimization of target engagement, physicochemical properties, and bacterial uptake, rather than reliance on enzyme inhibition alone.

Translational Barriers and Drug-Likeness Constraints

The progression of peptidoglycan-mimetic transpeptidase inhibitors from enzymatic tools to clinically viable antibacterial agents is constrained by multiple physicochemical and biological barriers. Although rational design may achieve high affinity toward catalytic serine or cysteine residues, antibacterial efficacy ultimately depends on compound accumulation at the site of action, maintenance of functional stability, and selective engagement of bacterial targets over host proteins (1).

One major limitation concerns physicochemical properties. Substrate-mimetic scaffolds frequently possess high molecular weight, elevated polar surface area, and multiple hydrogen-bond donors and acceptors. These features, while favorable for enzyme recognition, may impair passive diffusion or porin-mediated uptake in Gram-negative bacteria (2). Additionally, compounds targeting periplasmic transpeptidases must withstand dilution and efflux before achieving sufficient occupancy. Efflux pumps such as AcrAB–TolC in Enterobacter ales can significantly reduce intracellular concentrations of polar inhibitors (2).

Covalent warhead strategies introduce an additional layer of complexity. Electrophilic groups designed to react with catalytic cysteine residues in Ldts may also engage host thiol-containing proteins, including cysteine proteases and redox-regulating enzymes (3). Off-target reactivity raises concerns regarding cytotoxicity and immunogenicity. Similarly, irreversible inhibitors may exhibit prolonged protein residence time but complicate safety evaluation and dose optimization. Selectivity must therefore be rigorously balanced against reactivity, particularly when designing cysteine-directed electrophiles.

Serum protein binding and metabolic stability further influence translational potential. Peptidomimetic compounds may display high affinity for albumin or undergo rapid proteolytic degradation, thereby limiting effective free-drug concentration (4). Furthermore, chemical instability of certain warheads under physiological pH or in the presence of nucleophiles may compromise in vivo efficacy despite promising in vitro enzyme inhibition profiles.

Finally, pathway redundancy complicates interpretation of microbiological outcomes. In organisms capable of shifting between PBP-mediated 4→3 and Ldt-mediated 3→3 cross-linking, inhibition of a single enzyme class may not suffice to induce bactericidal activity (5). Consequently, translational evaluation must consider both enzyme-level potency and cellular pathway adaptation. Without integrating pharmacokinetic optimization, permeability engineering, and selectivity profiling, mechanistically sound inhibitors risk remaining confined to biochemical validation rather than progressing toward therapeutic development.

Comparative Evidence Synthesis

A qualitative synthesis of the current literature indicates that peptidoglycan-mimetic transpeptidase inhibitors can be broadly categorized into three strategic classes: substrate-mimetic scaffolds, covalent warhead–based inhibitors, and hybrid constructs combining both elements. Substrate-mimetic designs emphasize replication of stem-peptide stereochemistry and side-chain orientation, often incorporating meso-diaminopimelic acid bioisosteres to preserve active-site recognition (6). These approaches demonstrate strong conceptual alignment with structural enzymology but frequently encounter permeability limitations.

Covalent warhead–based inhibitors, including boronic acids and cyclic boronates for serine-dependent PBPs, as well as nitriles and cyanamides for cysteine-dependent Ldts, leverage catalytic residue reactivity to enhance inhibitory potency (3,7). Structural and biochemical validation has confirmed active-site engagement in multiple enzyme systems, supporting the mechanistic plausibility of this approach. However, translation into broad-spectrum antibacterial activity remains variable and often constrained by physicochemical or selectivity challenges.

Hybrid scaffolds attempt to integrate recognition and reactivity by combining substrate-mimetic frameworks with catalytic warheads. While structurally attractive, dual-target claims—particularly those suggesting simultaneous inhibition of PBPs and Ldts—require cautious interpretation. Differences in nucleophile identity, binding-pocket geometry, and kinetic behavior may limit true cross-class compatibility (5). Evidence supporting effective dual engagement in bacterial cells remains limited and should not be inferred solely from in vitro enzyme inhibition or structural analogy.

Collectively, the literature supports the mechanistic feasibility of transpeptidase inhibition beyond classical β-lactams, yet also reveals persistent translational bottlenecks. Successful progression appears contingent upon balancing enzyme specificity with optimized physicochemical parameters and validated cellular accumulation.

DISCUSSION

The accumulated evidence indicates that inhibition of peptidoglycan cross-linking through non-β-lactam, peptidoglycan-mimetic strategies represents a mechanistically coherent and scientifically justified approach to antibacterial discovery. Structural elucidation of PBPs and Ldts has clarified catalytic mechanisms, defined substrate-recognition motifs, and enabled rational placement of warheads and mimetic elements within enzyme active sites (6,7). These

advances have expanded the chemical space available for transpeptidase targeting beyond traditional β-lactam scaffolds (8)

However, translation from enzyme inhibition to clinically meaningful antibacterial activity remains challenging. Structural validation, while essential, does not guarantee effective cellular accumulation or durable bactericidal action. In Gram-negative organisms, outer-membrane permeability and efflux systems frequently limit compound exposure to periplasmic targets (9, 10). In mycobacteria, lipid-rich cell-wall architecture imposes additional diffusion barriers (11). Thus, medicinal chemistry optimization must integrate permeability considerations early in the design process rather than treating them as secondary refinements.(12)

Dual-target strategies have theoretical appeal in addressing pathway plasticity, yet direct experimental confirmation of simultaneous PBP and Ldt engagement within bacterial cells remains sparse. The structural divergence between serine- and cysteine-dependent active sites complicates universal inhibitor design. Future investigations should prioritize orthogonal target-engagement assays, including intact-protein mass spectrometry, competitive labeling experiments, and peptidoglycan compositional analysis to verify functional inhibition of both cross-linking pathways.(13-16).

Limitations of the current evidence base must also be acknowledged. Many studies focus on single enzyme isoforms or model organisms, limiting generalizability across diverse bacterial species. Additionally, publication bias toward positive enzyme inhibition findings may obscure unsuccessful translational attempts. As this review is narrative in design, formal risk-of-bias assessment and quantitative heterogeneity analysis were not conducted, which may introduce interpretive bias (17-20).

Future research directions should emphasize integrated optimization frameworks combining structural biology, kinetic validation, permeability engineering, and in vivo pharmacokinetic evaluation. Rational incorporation of physicochemical property filters, alongside selective covalent chemistry strategies, may enhance the likelihood of achieving clinically viable candidates. Ultimately, sustained progress will depend on harmonizing mechanistic insight with translational pragmatism, ensuring that enzymatic potency aligns with cellular and systemic pharmacology (21-23)

CONCLUSION

Peptidoglycan-mimetic inhibition of D,D-transpeptidases and L,D-transpeptidases represents a mechanistically rational strategy to address limitations associated with classical β-lactam–based therapies. Advances in structural enzymology, covalent warhead chemistry, and substrate-inspired scaffold design have clarified opportunities for targeting both canonical 4→3 and alternative 3→3 cross-linking pathways. Structural validation of inhibitor–enzyme complexes and biochemical confirmation of catalytic residue engagement support the conceptual feasibility of non-β-lactam transpeptidase inhibition. However, significant translational barriers remain, particularly in relation to Gram-negative permeability, efflux susceptibility, off-target reactivity of electrophilic warheads, and pathway-level redundancy in bacterial cell-wall synthesis. Current evidence supports cautious optimism rather than definitive therapeutic readiness. Future efforts should integrate orthogonal target-engagement validation, permeability optimization, and pharmacokinetic profiling to determine whether peptidoglycan-mimetic strategies can evolve from mechanistic promise to clinically viable antibacterial agents.

REFERENCES

1. Apostolos, A. J., Ocius, K. L., Koyasseril-Yehiya, T. M., Santamaria, C., Silva, J. R. A., Lameira, J., Alves, C. N., Siegrist, M. S., & Pires, M. M. (2022). Metabolic Processing of Selenium-Based Bioisosteres of meso -Diaminopimelic Acid in Live Bacteria. Biochemistry, 61(13), 1404–1414. https://doi.org/10.1021/acs.biochem.2c00120

2. Bertonha, A. F., Silva, C. C. L., Shirakawa, K. T., Trindade, D. M., & Dessen, A. (2023). Penicillin-binding protein (PBP) inhibitor development: A 10-year chemical perspective. Experimental Biology and Medicine, 248(19), 1657–1670. https://doi.org/10.1177/15353702231208407

3. Brem, J., Cain, R., Cahill, S., McDonough, M. A., Clifton, I. J., Jiménez-Castellanos, J.-C., Avison, M. B., Spencer, J., Fishwick, C. W. G., & Schofield, C. J. (2016). Structural basis of metallo-β-lactamase, serine-β-lactamase and penicillin-binding protein inhibition by cyclic boronates. Nature Communications, 7(1), 12406. https://doi.org/10.1038/ncomms12406

4. De Munnik, M., Lang, P. A., Calvopiña, K., Rabe, P., Brem, J., & Schofield, C. J. (2024). Biochemical and crystallographic studies of l,d-transpeptidase 2 from Mycobacterium tuberculosis with its natural monomer substrate. Communications Biology, 7(1), 1173. https://doi.org/10.1038/s42003-024-06785-3

5. De Munnik, M., Lang, P. A., De Dios Anton, F., Cacho, M., Bates, R. H., Brem, J., Rodríguez Miquel, B., & Schofield, C. J. (2023). High-throughput screen with the l,d -transpeptidase LdtMt2 of Mycobacterium tuberculosis reveals novel classes of covalently reacting inhibitors. Chemical Science, 14(26), 7262–7278. https://doi.org/10.1039/D2SC06858C

6. Espaillat, A., Alvarez, L., Torrens, G., Ter Beek, J., Miguel-Ruano, V., Irazoki, O., Gago, F., Hermoso, J. A., Berntsson, R. P.-A., & Cava, F. (2024). A distinctive family of L,D-transpeptidases catalyzing L-Ala-mDAP crosslinks in Alpha- and Betaproteobacteria. Nature Communications, 15(1), 1343. https://doi.org/10.1038/s41467-024-45620-5

7. Garde, S., Chodisetti, P. K., & Reddy, M. (2021). Peptidoglycan: Structure, Synthesis, and Regulation. EcoSal Plus, 9(2), ecosalplus.ESP-0010-2020. https://doi.org/10.1128/ecosalplus.ESP-0010-2020

8. Gastrell, S., & Vollmer, W. (2025). Peptidoglycan LD-Transpeptidases. Antibiotics, 14(12), 1210. https://doi.org/10.3390/antibiotics14121210

9. Grabrijan, K., Benedik, N. S., Krajnc, A., Bozovičar, K., Knez, D., Proj, M., Zdovc, I., Sosič, I., Contreras-Martel, C., Dessen, A., Rambaher, M. H., & Gobec, S. (2024). Synthesis and biochemical evaluation of new 3-amido-4-substituted monocyclic ß-lactams as inhibitors of penicillin-binding protein(s). Acta Pharmaceutica, 74(3), 423–440. https://doi.org/10.2478/acph-2024-0024

10. Haniff, H. S., Liu, X., Tong, Y., Meyer, S. M., Knerr, L., Lemurell, M., Abegg, D., Aikawa, H., Adibekian, A., & Disney, M. D. (2022). A structure-specific small molecule inhibits a miRNA-200 family member precursor and reverses a type 2 diabetes phenotype. Cell Chemical Biology, 29(2), 300-311.e10. https://doi.org/10.1016/j.chembiol.2021.07.006

11. Hecker, S. J., Reddy, K. R., Lomovskaya, O., Griffith, D. C., Rubio-Aparicio, D., Nelson, K., Tsivkovski, R., Sun, D., Sabet, M., Tarazi, Z., Parkinson, J., Totrov, M., Boyer, S. H., Glinka, T. W., Pemberton, O. A., Chen, Y., & Dudley, M. N. (2020). Discovery of Cyclic Boronic Acid QPX7728, an Ultrabroad-Spectrum Inhibitor of Serine and Metallo-β-lactamases.

Journal of Medicinal Chemistry, 63(14), 7491–7507. https://doi.org/10.1021/acs.jmedchem.9b01976

12. Jacobs, L. M. C., Consol, P., & Chen, Y. (2024). Drug Discovery in the Field of β-Lactams: An Academic Perspective. Antibiotics, 13(1), 59. https://doi.org/10.3390/antibiotics13010059

13. Kollár, L., Grabrijan, K., Hrast Rambaher, M., Bozovičar, K., Imre, T., Ferenczy, G. G., Gobec, S., & Keserű, G. M. (2024). Boronic acid inhibitors of penicillin-binding protein 1b: Serine and lysine labelling agents. Journal of Enzyme Inhibition and Medicinal Chemistry, 39(1), 2305833. https://doi.org/10.1080/14756366.2024.2305833

14. Kumar, P., Kaushik, A., Lloyd, E. P., Li, S.-G., Mattoo, R., Ammerman, N. C., Bell, D. T., Perryman, A. L., Zandi, T. A., Ekins, S., Ginell, S. L., Townsend, C. A., Freundlich, J. S., & Lamichhane, G. (2017). Non-classical transpeptidases yield insight into new antibacterials. Nature Chemical Biology, 13(1), 54–61. https://doi.org/10.1038/nchembio.2237

15. Mori, M., Villa, S., Ciceri, S., Colombo, D., Ferraboschi, P., & Meneghetti, F. (2021). An Outline of the Latest Crystallographic Studies on Inhibitor-Enzyme Complexes for the Design and Development of New Therapeutics against Tuberculosis. Molecules, 26(23), 7082. https://doi.org/10.3390/molecules26237082

16. Newman, H., Krajnc, A., Bellini, D., Eyermann, C. J., Boyle, G. A., Paterson, N. G., McAuley, K. E., Lesniak, R., Gangar, M., Von Delft, F., Brem, J., Chibale, K., Schofield, C. J., & Dowson, C. G. (2021). High-Throughput Crystallography Reveals Boron-Containing Inhibitors of a Penicillin-Binding Protein with Di- and Tricovalent Binding Modes. Journal of Medicinal Chemistry, 64(15), 11379–11394. https://doi.org/10.1021/acs.jmedchem.1c00717

17. Rowland, C. E., Newman, H., Martin, T. T., Dods, R., Bournakas, N., Wagstaff, J. M., Lewis, N., Stanway, S. J., Balmforth, M., Kessler, C., Van Rietschoten, K., Bellini, D., Roper, D. I., Lloyd, A. J., Dowson, C. G., Skynner, M. J., Beswick, P., & Dawson, M. J. (2025). Discovery and chemical optimisation of a potent, Bi-cyclic antimicrobial inhibitor of Escherichia coli PBP3. Communications Biology, 8(1), 819. https://doi.org/10.1038/s42003-025-08246-x

18. Saidjalolov, S., Edoo, Z., Fonvielle, M., Mayer, L., Iannazzo, L., Arthur, M., Etheve-Quelquejeu, M., & Braud, E. (2021). Synthesis of Carbapenems Containing Peptidoglycan Mimetics and Inhibition of the Cross-Linking Activity of a Transpeptidase of l,d Specificity. Chemistry – A European Journal, 27(10), 3542–3551. https://doi.org/10.1002/chem.202004831

19. Sánchez-Peña, L., Rodríguez-Pallares, S., Aja-Macaya, P., Blanco-Martín, T., González-Pinto, L., Pérez-Rodríguez, G., Le Terrier, C., Portillo-Calderón, I., Recacha, E., Riazzo, C., Vázquez-Ucha, J. C., Beceiro, A., Aracil, B., Oteo-Iglesias, J., Martínez-Martínez, L., Poirel, L., Bou, G., & Arca-Suárez, J. (2025). Broad spectrum of β-lactamase coverage and potent antimicrobial activity of xeruborbactam in combination with meropenem against carbapenemase-producing Enterobacterales, including strains resistant to new β-lactam/β-lactamase inhibitor combinations. Antimicrobial Agents and Chemotherapy, 69(9), e00533-25. https://doi.org/10.1128/aac.00533-25

20. Sankar, M. M., Gopinath, K., Singla, R., & Singh, S. (2008). In-vitro antimycobacterial drug susceptibility testing of non-tubercular mycobacteria by tetrazolium microplate assay.

Annals of Clinical Microbiology and Antimicrobials, 7(1), 15. https://doi.org/10.1186/1476-0711-7-15

21. Smirnovienė, J., Baranauskienė, L., Zubrienė, A., & Matulis, D. (2021). A standard operating procedure for an enzymatic activity inhibition assay. European Biophysics Journal, 50(3–4), 345–352. https://doi.org/10.1007/s00249-021-01530-8

22. Villamil, V., Brusoni, L. S., Prati, F., Caselli, E., & Santi, N. (2025). Boronate-Based Inhibitors of Penicillin-Binding Proteins: An Underestimated Avenue for Antibiotic Discovery? Pharmaceuticals, 18(9), 1325. https://doi.org/10.3390/ph18091325

23. Yang, Y., Yan, Y.-H., Schofield, C. J., McNally, A., Zong, Z., & Li, G.-B. (2023). Metallo-β-lactamase-mediated antimicrobial resistance and progress in inhibitor discovery. Trends in Microbiology, 31(7), 735–748. https://doi.org/10.1016/j.tim.2023.01.013

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