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MINI-REVIEW ARTICLE

The Potential of Mur Enzymes as Targets for Antimicrobial Drug Discovery

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Abstract: The extensive development in the strains of resistant bacteria is a potential hazard to public health worldwide. This necessitates the development of newer agents with the antibacterial property having new mechanisms of action. Mur enzymes catalyze the steps related to the biosynthesis of peptidoglycan, which constitutes a major part of the cell wall in bacteria. Peptidoglycan increases the stiffness of the cell wall, helping it to survive in unfavorable conditions. Therefore, the inhibition of Mur enzymes may lead to novel antibacterial agents that may help in controlling or overcoming bacterial resistance. Mur enzymes are classified into MurA, MurB, MurC, MurD, MurE, and MurF. Until-date, multiple inhibitors are reported for each class of the Mur enzymes. In this review, we have summarized the development of Mur enzyme inhibitors as antibacterial agents in the last few decades.

Keywords: Antibacterial agents, inhibitor, ligase, mur enzymes, peptidoglycan, antimicrobial.

1. INTRODUCTION

An antimicrobial agent is a substance that eradicates or hinders microbial growth, including bacteria, viruses, fungi, *etc.* and is, therefore, used to eradicate microbial infections in humans, animals, and plants. The quick evolution of multi-drug-resistant strains of a variety of microbes after the use of antimicrobials in humans and others leading to antimicrobial resistance (AMR) is amongst the leading global public health concerns. Considering the harmful effects of antimicrobial resistance on human health [1, 2], there is an immediate requirement for the advancement of new therapeutics with antimicrobial properties having novel mechanisms of action, which are directed toward the novel target (s).

In this review, we are focusing on the development of antibacterial agents in the last few decades against enzymes implicated in the bacterial peptidoglycan biosynthesis pathway. The synthesis of peptidoglycan may be one of the rich sources of valid druggable targets for the invention of effective antibacterial therapeutics [3, 4]. Peptidoglycan, also known as murein, is known to be a part of the cell wall in bacteria [5, 6]. It is ubiquitous among bacteria and located outside the cytoplasmic membrane of the bacteria [7]. The various Mur enzymes are the biocatalyst for the synthesis of peptidoglycan which is required for bacterial survival because it contributes to the rigidity of the cell wall and helps the bacteria to survive in unfavourable environments [8, 9]. Over the past several decades, a lot of research has been focused on the synthesis of bacterial peptidoglycan for the identification of novel antibacterial therapeutics. Peptidoglycan biosynthesis involves two main steps, the first of which is catalyzed by Mur enzymes that occurs in the cytoplasm [10, 11]. Mur enzymes can be classified into MurA (UDP-N-acetylglucosamine enolpyruvyl transferase), MurB (UDP-N-acetylenolpyruvoylglucosamine reductase), MurC (UDP-N-acetylmuramate--L-alanine ligase), MurD (UDP-N-acetyl muramoyl-L-alanine--D-glutamate ligase), MurE (UDP-N-acetylmuramoyl-L-alanyl-D-glutamate--2,6-diaminopimelate ligase), and MurF (UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase) [12]. During the recycling process of peptidoglycan in selected Gram-negative bacteria, there is another enzyme, Mpl, which links tripeptide l-Ala-γ-d-Glu-*meso*-A_2pm to UDP-MurNAc [13, 14]. As every enzyme has its inhibitor, there are different inhibitors for each class of Mur enzymes [15].

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2. MUR ENZYMES: A PROMISING TARGET FOR THE DEVELOPMENT OF ANTIBACTERIAL AGENTS

Enzymes serve as catalysts to accelerate chemical reactions by lowering the activation energy of the chemical reaction. These enzymes belong to six main groups, namely oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases [16, 17]. The chemical agents capable of abolishing or inhibiting the catalytic role of such enzymes are known as enzyme inhibitors which can be reversible or irreversible depending upon the mechanism of inhibition [18]. An antimicrobial agent is a substance that plays an important function in killing or minimizing the growth of microorganisms. The discovery of penicillin was further proceeded by the development of different classes of antibacterial agents, such as macrolides, sulphonamides, streptomycin, *etc.* However, the overuse of antimicrobial agents is associated with a critical problem of multidrug resistance, which implies resistance towards the standard antibacterial therapy.

Additionally, the emergence of widespread multidrug-resistant organisms will limit the therapy option with only a few antimicrobial agents. In the process of discovering antibacterial drugs, researchers also need to focus on the concept of targeting multiple targets for developing promising antibacterial drugs. It is also advisable to explore new molecules obtained from natural, marine, or microbial sources [19-21].

Peptidoglycan biosynthetic processes are significant targets for antibacterial therapy. Drugs interfering with normal biosynthesis and peptidoglycan formation serve as powerful antibacterial agents [22]. Peptidoglycan creates a mesh-like network and maintains the rigidity and structure of the cell wall of bacteria. In addition, peptidoglycan protects the bacterial cell by preventing its lysis. The bacterial cell wall acts as a hypotonic medium in the absence of a peptidoglycan layer, eventually leading to cell rupture. Hence, the peptidoglycan layer is a vital component of bacteria contributing to its survival as it gives mechanical strength to withstand higher osmotic pressure inside and preserves the shape of the cell [23, 24].

As peptidoglycans are important for bacterial survival, their biosynthetic pathway represents a crucial aim for the advancement of new agents against bacteria with good specificity because peptidoglycan is present in bacterial cells and not in mammalian cells [25, 26]. Mur enzymes catalyze the cytoplasmic steps in the peptidoglycan precursors' biosynthesis. Therefore, Mur enzymes are promising targets for the advancement of antibacterial agents [27-29].

3. BIOSYNTHESIS OF PEPTIDOGLYCAN

Mur enzymes are mandatory for the formation of bacterial cell wall peptidoglycan. The cell wall of bacteria is made up of N-acetylmuramyl-peptides (NAM) and N-acetylglucosamine (NAG) [30]. These two units are arranged alternately and linked together by peptide chains, which protect the bacterial cell wall from being destroyed by osmotic pressure. Besides, these macromolecules are essential to give shape to the bacterial cell wall and enable the anchoring of other components, such as proteins and polysaccharides [31, 32]. Biosynthesis of the peptidoglycan cell wall can be considered a complex process as it involves different types of membrane and cytoplasmic and extracellular steps (Fig. **1**).

In the first stage, the formation of monomer subunits takes place, *i.e.*, UDP-MurNAc-pentapeptide from UDP-GlcNAc using GlmS synthase, GlmM synthase, and GlmU synthase. Different enzymes play different roles in the synthesis of peptide moieties, such as MurC, MurD, MurE, and MurF, which contribute to the addition of different types of components, such as D-glutamic acid, L-alanine, meso-diaminopimelic acid (A2pm) or L-lysine and D-alanyl-D-alanine to UDP-MurNAc [31, 33, 34]. The formation of peptide subunits commences with the addition of L-alanine to UDP-MurNAc along with the addition of D-glutamic acid and dipeptide D-alanine, following the same with the help of various Mur synthases (MurC, MurD, MurF) followed by the formation of UDP-MurNAc-pentapeptide [35].

The membrane step involves the formation of a lipid intermediate, which starts with the transference of phospho--MurNAc-pentapeptide from the cytoplasmic step to the membrane site acceptor, so-called undecaprenyl phosphate (C55-P) [31]. The catalysis of this reaction is done by MraY (translocase), resulting in the synthesis of undecaprenyl-pyrophosphoryl-MurNAc-pentapeptide (Lipid I). The reaction then continues with the delivery of the GlcNAc molecule UDP-GlcNAc to lipid I [36, 37]. This reaction is catalyzed by MurG and leads to the formation of undecaprenyl-pyrophosphoryl-MurNAc-(pentapeptide)-GlcNAc (Lipid II). Briefly, in the cytoplasmic membrane, bactoprenol, which is a lipid carrier, is involved in the transport of peptidoglycan precursors through the cell membrane. Bactoprenol then pounces on the UDP-MurNAc penta, giving rise to a lipid, PP-MurNac penta. UDP-GlcNAc is then transferred to MurNAc, leading to the formation of a disaccharide as well as a precursor to peptidoglycan, lipid-PP-MurNAc penta-GlcNAc. After the molecule is carried through the membrane, it is added to the growing glycan chain, though the exact mechanism of the entry of the precursor into the membrane is not clear. The subsequent reaction is called transglycosylation, where the lipid-PP from the glycan chain is moved by the hydroxyl group of the GlcNAc attached to the MurNAc in the glycan. This displacement is caused by the enzyme transglycosylase. Peptidoglycan cross-linking is thus formed by this complex path. Here it is important to mention that the transmembrane lipid transporter proteins, also known as flippase, which are situated in the membrane and belong to ABC transporter or P4-type ATPase families, primarily contribute to the motion of phospholipid molecules between the two leaflets that make the cell membrane (transverse diffusion, also called "flip-flop" transition). A vital step in this pathway is the export of Lipid II, the lipid-linked cell wall monomer, by its transporter MurJ. The mechanism of the transbilayer movement of Lipid II moderated by MurJ is still unclear. It is assumed that attaching the Lipid II to those residues during the transport causes a conformational change in

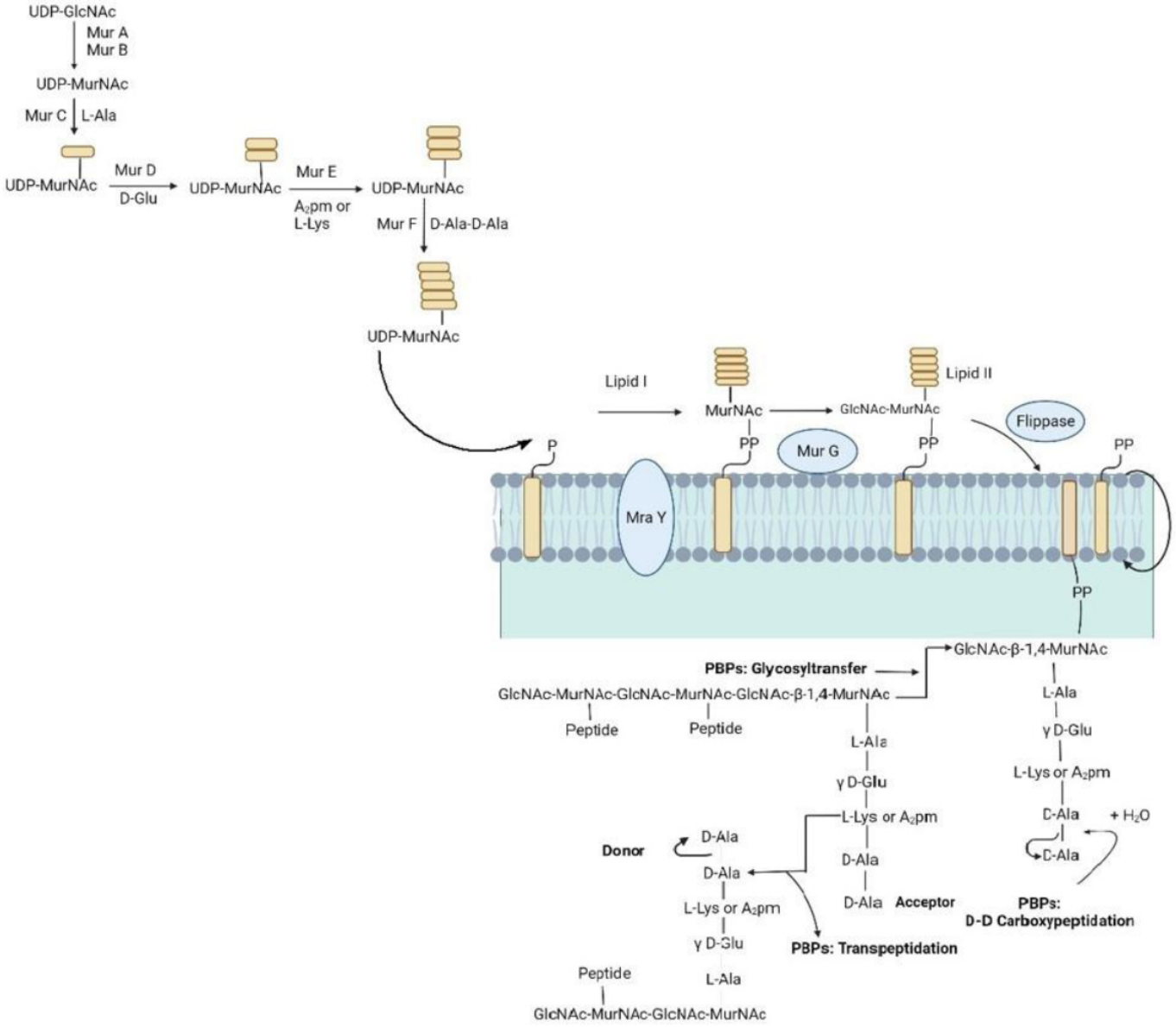

Fig. (1). Peptidoglycan biosynthesis of the cell wall of bacteria. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

MurJ, which is needed to progress through the Lipid II transport cycle. UDP-N-acetyl muramic acid (UDP-MurNAc) is formed from UDP-N-acetylglucosamine (UDP-GlcNAc). This process is catalyzed by both MurA and MurB enzymes.

4. INTERRELATION OF FUNCTIONAL AND STRUCTURAL ENVIRONMENT BETWEEN DIFFERENT MUR LIGASES

A group of Mur ligase enzymes composed of MurA, MurB, MurC, MurD, MurE, and MurF is known as Mur ligases. Mur ligases serve as the targets for antibacterial drugs as they are considered the family of enzymes that ligate the cell wall of bacteria. During the building of bacterial cell walls from peptidoglycan, these Mur ligases exhibit an important function in the addition of a short polypeptide to UDP-D-acetylmuramic acid. For example, MurC causes the addition of L-alanine, MurD causes the addition of D-glutamate, MurE adds meso-diaminopimelate or L-lysine, while MurF adds D-alanyl-D-alanine to peptidoglycans. All these Mur ligases are similar to each other concerning their topology. MurC and MurF enzymes have a three-domain structure which includes the N-terminal domain, central domain, and C-terminal domain, all of which contribute to the attachment of UDP-MurNAc, ATPase, as well as GTPase that contributes to the attachment to the incoming amino acid [38-41].

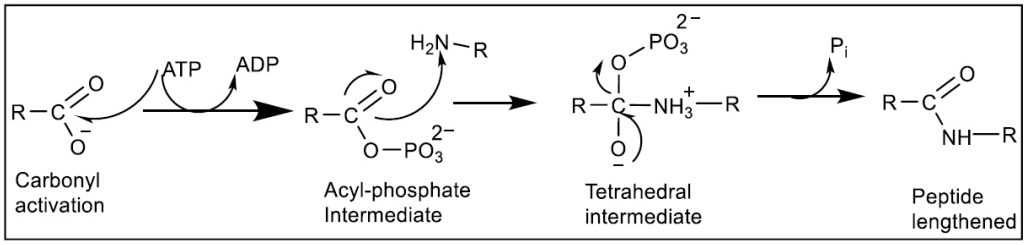

Fig. (2). The mechanism involved in the formation of the peptide bonds by the catalytic action of Mur ligases.

The ATP binding sites of the four different Mur ligases, MurC, MurD, MurE, and MurF, are 10-20% identical in terms of the primary sequence, and the enzymes have a very similar ATP binding site with common ATP-binding consensus sequence GXXGKT/S [42]. Furthermore, the structures of these Mur ligases possess four homologous regions, including nucleotide fold, hydrophobic region (Glu and His amino acids located in between the two acidic residues in the middle of the proteins), a dyad composed of a set of concentrated acidic residues, and the fourth region includes a hydrophobic patch. These regions seem critical for the Mur ligase activity [43, 44]. The ligation of amino acids is catalyzed by Mur ligases, resulting in the formation of a phosphate bond to form a peptide chain from UDP-MurNAc [42]. The process starts with ATP binding, followed by the nucleotide substrate and then dipeptide. ATP consists of several charged groups where an SN2 reaction is involved. During the reaction, the phosphate group is displaced with the amino group. Fig. (**2**) depicts the catalytic mechanism of Mur ligases.

5. INSIGHT INTO THE THREE-DIMENSIONAL STRUCTURES OF MUR ENZYMES

Through the analysis of multiple X-ray crystal structures of Mur enzymes, it has been shown that these ligases possess three similar domains, including the N-terminal domain (NTD), central domain (CD), and C-terminal domain (CTD) [12]. The first domain is bound to the nucleotide substrate. In *E. coli* Mur ligases, the α helices are present in varying numbers, such as three α-helices in MurF, four α-helices in MurC and MurD while, two α-helices and five parallel β-sheets in MurE. The nucleotide substrate also possesses UDP moiety, which is bound with MurC and MurD enzymes in opposite orientations for the accommodation of a longer substrate. However, for interaction with MurE and MurF, the pyrophosphate moiety of UDP is extended out towards the N-terminal domain. MurE and MurF enzymes bind to UDP in such a way that the loop extending from the third domain surrounds the uridine group and is connected with four hydrogen atoms in the diphosphate moiety [45]. They show basic N-terminal alpha and beta fold after recognizing the lengthening nucleotide of four helices in MurC and MurD while two helices and five parallel β-sheets in MurE. Additionally, MurE consists of a specific N-terminal binding pocket in which the enzyme has a preference towards γ rather than α carboxyl group of the UDP-MurNAc-peptide for peptide bond formation [46].

For the central domain, MurC consists of seven parallel β-sheets, whereas there are six parallel β-sheets in the case of MurD, MurE, and MurF. Besides, there are varying numbers of alpha-helices surrounding the beta-sheets, which are seven for MurD and MurE, four for MurC and eight for MurF. This second domain also plays a role in the binding of ATP because it consists of the binding fold of mononucleotide. Besides, the two important residues, glutamic acid and histidine, are also present for controlling magnesium ions. The stabilization of the second magnesium binding site in MurD, MurE, and MurF is then achieved by the carbamylated lysine residue and glutamic acid residue present in MurC [38].

For the third domain, there is also the presence of both α-helices and β-sheets in which five α-helices surround the five parallel and one antiparallel β-sheets. Rossmann dinucleotide binding fold is also present in this domain for amino acid substrate binding. For example, in MurC, the presence of histidine residue plays a key role in creating a hydrophobic pocket which results in complementary binding with the short side chain of L-Ala. In addition, the purpose of Mur ligase having the C-terminal is to cover the ATP binding site as well as to help in the orientation of the amino acid by inserting a loop into the active site. The interaction between amino acid and the phosphate group of ATP causes the formation of a complete form of the arginine residue. Following this, the nucleophilic attack is observed when there is a binding of ADP, which results in the shifting of position in the arginine residue [45, 46, 38].

6. MURA (UDP-N-ACETYLGLUCOSAMINE ENOLPYRUVYL TRANSFERASE)

MurA enzyme is responsible for the catalytic activity in the first step. In this step, enolpyruvate is transferred from phosphoenolpyruvate to the third position of UDP-GlcNAc, leading to the formation of a UDP-GlcNAc enolpyruvate [8, 47, 48]. This enzyme functions as transferase, which acts on the second carbon of phosphoenolpyruvate (PEP) to break the CO bond. MurA is composed of 418 amino acid residues and has two globular domains linked by a double-stranded linker [49, 50]. Each domain is composed of six helices and three four-stranded β-sheets [51]. The catalytic site of the MurA enzyme is located in a deep pocket between the two globular domains [52].

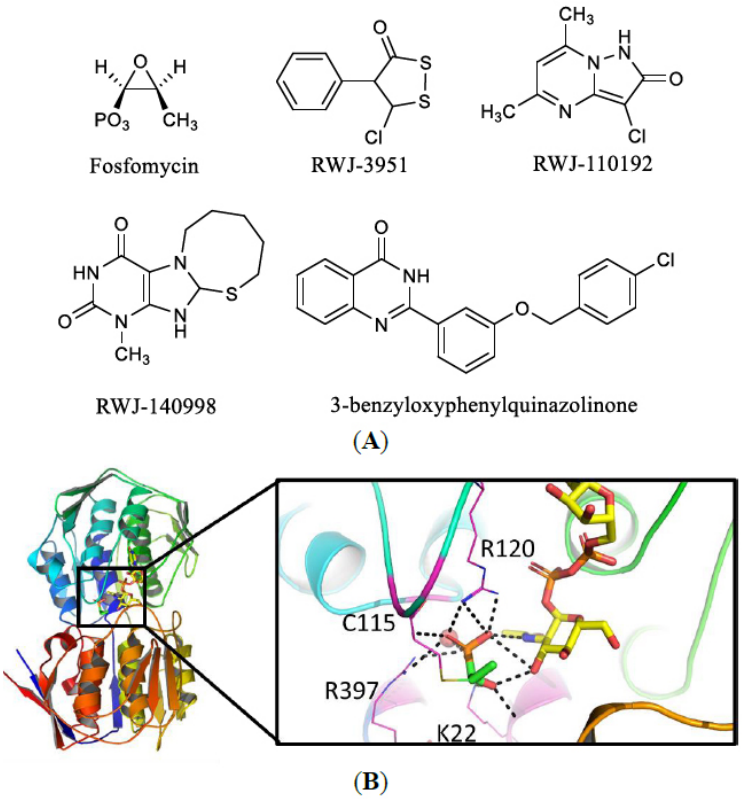

Fig. (3). (**A**) Chemical structures of fosfomycin, cyclic disulfide RWJ-3981, pyrazolopyrimidines RWJ-110192, purine RWJ-140998 derivative, and 3-benzyloxyphenylquinazolinone, (**B**) Depiction of the protein-ligand interaction of Fosfomycin with MurA (PDB-ID: 1UAE). Protein is represented in cartoons, ligands/cofactors are represented in sticks, and amino acids are represented in lines. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

This first stage is vital for the subsequent steps of peptidoglycan biosynthesis. Therefore, MurA enzyme inhibitors can act as excellent antibacterial agents [53, 54]. Examples of MurA enzyme inhibitors include fosfomycin, cyclic disulfide RWJ-3981, the purine analog RWJ-140998, and the pyrazolopyrimidine RWJ-110192. By screening a chemical library, compounds RWJ-3981, RWJ-110192, and RWJ-140998 were identified as inhibitors of the *E. coli* MurA enzyme by the researchers. RWJ-140998 is a purine and contains the 2, 4-dioxopyrimidine ring, which is also found in the uracil portion of the UNAG substrate. For RWJ-3981, the centroid of the sulfur-containing ring was predicted to bind 7.1 Å from the sulfur of the catalytic Cys115. The carbonyl oxygen of the inhibitor may form strong hydrogen bonds with Arg120 and Arg397. The sulfur atoms can make hydrophobic contacts with the side chain carbon atoms of Met90 and Arg91. For RWJ-110192, the centroid of the rings was predicted to bind farther from Cys115 than for RWJ-3981. Fig. (**3**) depicts the chemical structures of selected MurA inhibitors and the protein-ligand interaction of Fosfomycin with MurA (PDB-ID: 1UAE).

Fosfomycin is a product of natural origin with a broad spectrum of antibacterial activity. Despite its name (ending in -omycin), Fosfomycin is not a macrolide but a member of a novel class of phosphonic antibiotics. Fosfomycin (originally known as phosphonomycin) was discovered in a joint effort of Merck and Co. and Spain's Company Espanola de Penicilina y Antibiotic (CEPA). It was first isolated by screening broth cultures of Streptomyces *fradiae* isolated from soil samples for the ability to cause the formation of spheroplasts by growing bacteria. The discovery was described in a series of papers published in 1969. CEPA began producing fosfomycin on an industrial scale in 1971 at its Aranjuez facility. This enzyme catalyzes the committed step in peptidoglycan biosynthesis, namely the ligation of phosphoenolpyruvate (PEP) to the 3'-hydroxyl group of UDP-N-acetylglucosamine. This pyruvate moiety provides the linker that bridges the glycan and peptide portion of peptidoglycan. Fosfomycin is a PEP analog that inhibits MurA by alkylating an active site cysteine residue (Cys 115 in the *Escherichia coli* enzyme). Fosfomycin enters the bacterial cell through the glycerophosphate transporter [55, 56].

Fosfomycin is used clinically as an antibacterial agent successfully [57] and is also useful in treating urinary tract infections caused by bacteria in women and is also used to treat infections outside the urinary tract [58]. It enters actively into the bacteria *via* the L-α-glycerophosphate and the glucose-6-phosphate uptake systems [59] and acts as an equivalent of phosphoenol pyruvate, which binds covalently to the active site having cysteine residue. The impairment in fos-

fomycin uptake or overproduction of the low-affinity transferase enzyme leads to resistance in the chromosomally encoded fosfomycin-resistant bacterial strains [60-62]. In some bacteria, namely *Serratia marcescens*, *Klebsiella pneumoniae*, *Enterobacter cloacae,* and *Staphylococcus epidermidis*, the resistance is due to the enzymatic transformation of the fosfomycin [63-66]. The *Mycobacterium tuberculosis* (Mtb) is resistant to fosfomycin because of the existence of an aspartate residue in place of the cysteine residue at the active site of the MurA enzyme [67]. Cyclic disulphide, pyrazolopyrimidine, and purine analogues (Fig. **3**) are another three classical inhibitors of *Escherichia coli* MurA [68]. Their chemical properties and mechanisms of action are different from that of fosfomycin. Cyclic disulphide and purine derivatives are irreversible inhibitors, while pyrazolopyrimidine derivatives are reversible inhibitors. These compounds are reported to inhibit *Staphylococcus aureus*, *Enterococcus faecalis*, and *Enterococcus faecium* [69, 70]. The synthesis and evaluation of a series of quinazolinone-based compounds against *E. coli* MurA revealed 3-benzyloxyphenylquinazolinone with promising inhibitory activity against MurA (IC_{50} = 8 μM). Derivatives of diarylmethane, a substituted imidazole, and sesquiterpene lactones are reported to inhibit MurA with IC_{50} values ranging between 0.2-0.9 μM. It is worth mentioning that the half maximal inhibitory concentration (IC_{50}) is a measure of the potency of a substance in inhibiting a specific biological or biochemical function. IC_{50} is a quantitative measure that indicates how much of a particular inhibitory substance (*e.g.*, drug) is needed to inhibit *in vitro* a given biological process or biological component by 50% [1]. The biological component could be an enzyme, cell, cell receptor, or microorganism. IC_{50} values are typically expressed as molar concentration.

According to the phylogenetic analysis, there are two different categories of MurA transferases. The first category of transferases exists throughout all bacteria except gram-negative *Mycoplasma* species. The second category occurs as a copy of a duplicate gene only in the low gram-positive bacteria. Both the categories are structurally and functionally very much alike, and one enzyme can act as an alternate for the other [71]. The purine analogues, namely acyclic disulphide and pyrazolopyrimidine derivatives, are the novel inhibitors of the *E. coli* MurA enzymes. In acyclic disulphide, the amino acid cysteine contains a thiol group and readily forms disulfides, linking two cysteine units together. This simple linkage has important consequences for protein shape as this covalent interaction restricts the conformations. Classical theory suggests that disulfide bonds stabilize proteins by reducing the entropy of the denatured state. More recent theories have attempted to expand this idea, suggesting that in addition to configurational entropic effects, enthalpic and native-state effects occur and cannot be neglected. Whereas the heterocyclic fusion of pyrimidine and pyrazole ring resulted in the formation of pyrazolopyrimidines. The amino group on pyrazolopyrimidine allowed modifications of the molecules through binding to sugars or amino acids. The IC_{50} of those compounds was found to be lesser compared to fosfomycin when preincubated with MurA. There was a reduction in the IC_{50} value at least five times, owing to the presence of UDP-N-acetylglucosamine during preincubation, indicating that these compounds might interact with the enzyme in a particular manner that is increased by UDP-N-acetylglucosamine such as fosfomycin. There was probably no similarity in the structure of the three compounds to fosfomycin. The compounds showed antibacterial activity but not specifically by MurA inhibition, as inhibition of DNA, RNA, and protein synthesis was also seen. In comparison to fosfomycin, the minimum inhibitory concentrations (MICs) of these compounds were similar to those of the test strains [61]. The compounds were proposed to attach firmly with MurA but not covalently. They appeared to attach non--covalently to the MurA enzyme at or near the site of binding of fosfomycin.

7. MURB (UDP-N-ACETYLENOLPYRUVOYLGLUCOSAMINE REDUCTASE)

MurB enzyme is responsible for the catalytic activity in the second step of the synthesis of peptidoglycan. This step comprises the conversion of UDP-N-acetylglucosamine (UDP-GlcNAc) into UDP-N-acetyl muramic acid (UDP-MurNAc). The enolpyruvate moiety is reduced to D-lactate in the second stage to produce UDP-N-acetyl muramic acid (UDP-MurNAc). MurB enzyme catalyzes this process as a reductase [72, 73].

The MurB reductase plays a catalytic role in the two steps of the reaction. Firstly, flavin adenine dinucleotide (FAD) is reduced by two electrons from NADPH to form a firmly bound flavin, followed by the movement of the identical electron at the third carbon of the enol ether, which eventually leads to the reduction of the vinyl bond on the second carbon by a quenching process. The active site, Ser-229, is important for the carbanion or enol intermediate quenching as it is of proton origin [74]. The X-ray crystal structures of the *E. coli* MurB in a substrate (UDP-N-acetylglucosamine enolpyruvate)-free (PDB-ID: 1MBT), substrate-bound forms (PDB-IDs: 2MBR, 1MBB, 1UXY), and inhibitor-bound form (PDB-ID: 2Q85) are deposited in the protein data bank [75-79]. MurB has three domains where domain 1 is comprised of 3-67 residues and composed of six β-strands and α-helix. Domain 2 is comprised of 68-201 residues and has nine β-strands and two α-helices, while domain 3 has 219-326 residues and is composed of six β-strands and three α-helices [77, 80]. Fig. (**4**) gives a schematic view of the substrate-bound *E. coli* MurB crystal structure and depicts the chemical structure of the MurB inhibitor.

Given that MurB enzymes are necessary for the bacteria to stay alive, this enzyme is a suitable target for antibacterial drugs for the treatment of infections since the homologue in eukaryotic cells is absent. MurB inhibitors are targeted to be antibacterial agents. They are effective in inhibiting *S. aureus*, *S. pneumoniae,* and *E. coli* [81]. Examples of MurB enzyme inhibitors include 4-thiazolidinone analogues and naphthyl tetronic acid. Fig. (**4**) depicts the MurB fold and binding mode of naphthyl tetronic acid as a MurB inhibitor (PDB-ID: 2Q85).

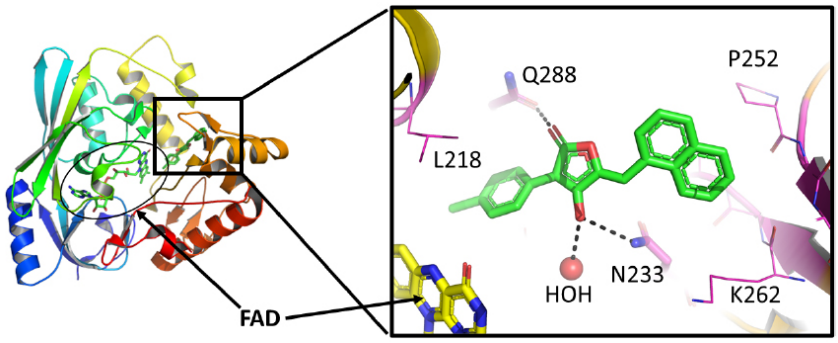

Fig. (4). A schematic view of the substrate-bound *E. coli* MurB crystal structure and depiction of the binding mode of naphthyl tetronic acid as MurB inhibitor (PDB-ID: 2Q85). Protein is represented in cartoons, ligands/cofactors are represented in sticks, and amino acids are represented in lines. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

A novel class of 2,3,5-Trisubstituted-4-thiazolidinone derivatives is reported as MurB enzyme inhibitors [82]. Structure-activity relationship studies from the general structure of 4-thiazolidinone show that R1 (*e.g.*, n-butyl), R2 (*e.g.*, t-butyl-m-phenoxy benzaldehyde), and R3 (*e.g.*, hydrazide) side chains at 2nd, 3rd and 5th positions, respectively, are essential for inhibitory activity. The inhibitory activity is high when the R1 at the 2nd position has an n-butyl group, and the R2 at the 3rd position has aromatic rings, especially t-butyl-m-phenoxy benzaldehyde. t-butyl-m-phenoxy benzaldehyde is a bulky group that can fill the big hydrophobic pockets in the MurB enzyme. Besides, the inhibitory action of MurB enzyme inhibitors disappears when the R2 position is substituted with a simple phenyl group. The R3 side chain at the 3rd position does not affect MurB enzyme inhibition. This is evident from the fact that the substitution of the R3 position with methyl or hydrazide moiety results in an effect that is not significant [83]. Further, naphthyl tetronic acid is another class of MurB inhibitor; an X-ray crystallographic structure of it complexed with MurB enzyme (PDB-ID: 2Q85) is deposited in the protein data bank. Although the complete detail of this molecule is yet to be published, based on X-ray crystallographic data of MurB and using the UDP--sugar substrate as a guide, surrogates of the diphosphate moiety were developed. The main objective of the template was to imitate the main interactions of the diphosphate with the enzyme and to align the resultant side chains in such a manner that they would occupy space similar to the glucosamine and uridine moieties of the substrate. In the above study, the microbiological activity for the 4-thiazolidinone compounds was not specified apparently due to a deficiency of such activity. The four Mur ligases, MurC, MurD, MurE, and MurF, catalyze the addition of a short polypeptide to UDP-N-acetylmuramic acid. Frankia strains of cluster-2 and cluster-3 contain two copies of murC, while the strains of cluster-1 and cluster-4 contain only one. Phylogenetically, the protein encoded by the murC gene shared only by cluster-2 and cluster-3, termed MurC1, groups with MurC proteins of other Actinobacteria. The protein encoded by the murC gene found in all Frankia strains, MurC2, shows a higher similarity to the MurC proteins of plants than of Actinobacteria. MurC2 could have been either acquired *via* horizontal gene transfer or gene duplication and convergent evolution, while murC1 was subsequently lost in the cluster-1 and cluster-4 strains. In the nodules induced by the cluster-2 strains, the expression levels of murC2 were significantly higher than those of murC1. Thus, there is clear sequence divergence between both types of Frankia MurC, and Frankia murC1 is in the process of being replaced by murC2, indicating selection in favor of murC2. Nevertheless, protein modelling showed no major structural differences between the MurCs from any phylogenetic group examined [84].

8. MURC (UDP-N-ACETYLMURAMATE--L-ALANINE LIGASE)

MurC enzyme is highly essential for peptidoglycan biosynthesis. They are mainly responsible for the addition of the L-alanine (Ala) residue onto the nucleotide precursor UDP-MurNAc. The major role of the MurC enzyme is to catalyze ATP-dependent ligation of Ala and UDP-N-acetylmuramic acid (UNAM), thus forming UNAM-Ala as the end product. To form this end product, the MurC enzyme acts as a non-ribosomal peptide ligase and forms an amide bond between Ala and UNAM with the help of ATP [85, 86]. The crystal structure of *E. coli* MurC is determined at 2.6 Å resolution in the apo-form (PDB-ID: 2F00) [87, 88]. Similar to other Mur enzymes, this enzyme has a modular multi-domain structure (Fig **5**). Since the MurC enzyme is vital in the formation of pentapeptide, the inhibition of the same will alter the formation of the peptidoglycan wall of bacteria. There are different types of inhibitors for MurC enzymes which include pyrazolopyrimidine derivatives, phosphonate derivatives, and benzylidene rhodamine derivatives. Fig. (**5**) shows the chemical structures of MurC inhibitors. Studies about structure-activity relationships have revealed that the tert-butyl and methyl-substituted pyrazole moiety should be retained in pyrazolopyrimidine derivatives [89, 90]. However, different side chains are inserted into the pyrimidine ring, which causes the minimum inhibitory concentration to be varied. It was seen that the activity is optimized with the side chain attachment, as shown in Fig. (**5**). Concerning phosphonate derivatives, it was found that the

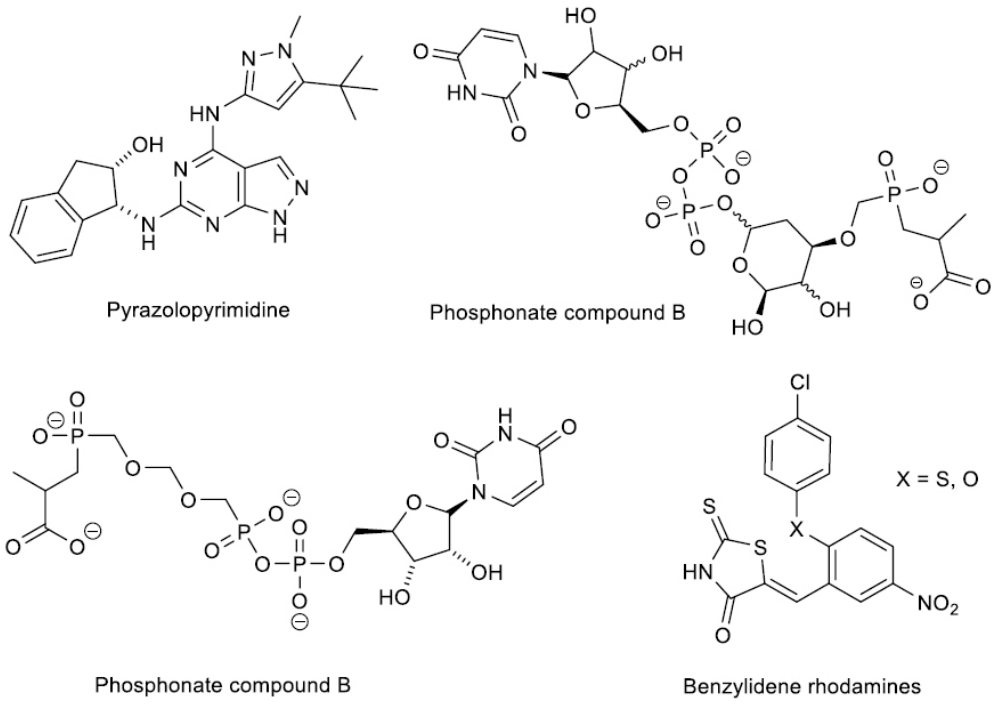

Fig. (5). Chemical structures of various classes of MurC inhibitors.

acyl phosphate moiety is responsible for the inhibitory action of the MurC enzyme. Phosphonate compound A, as shown in Fig. (**5**), can inhibit the MurC enzyme as it may bind tightly with it. The same applies to phosphonate compound B.

Furthermore, the phosphonate compound A is deficient in the methyl group in the lactic acid moiety; however, in phosphonate compound B, the hexose moiety is attached to the 1,3-propanediol group. The reason they are specifically potent against MurC is due to the formation of ether linkage in the alpha-position of the phosphonate group in compounds A and B, which makes them bind tightly. It can be concluded that compound A has the highest inhibitory activity as compared with others. This may be due to the presence of des-methyl muramic acid moiety attached to a 1,3-propanediol group of compound A [91]. The compound exhibited mixed-type inhibition about all three enzyme substrates indicating that it forms dead-end complexes with multiple enzyme states. Isothermal titration calorimetry (ITC) was applied to validate these findings. Analogues of Ala were tested as inhibitors of the *E. coli* MurC and were found to be competitive against Ala. Inhibition by other Ala analogues was also shown with the *E. coli* enzyme but without specification of the type of inhibition.

In benzylidene rhodamine derivatives, the inhibitory activity of different compounds was evaluated against the MurC enzyme. It was found that only a few compounds had inhibitory potency. Compounds with X as O or S, as depicted in Fig. (**5**), showed similar activity as benzylidene rhodamine, which is essential for inhibitory action.

9. MURD (UDP-N-ACETYLMURAMOYL-L-ALANINE--D-GLUTAMATE LIGASE)

MurD ligase has a similar role as the MurC enzyme. MurD is majorly involved in the addition of D-glutamic acid to UDP-MurNAc-L-Ala in the presence of ATP with the formation of acyl-phosphate and tetrahedral intermediates [92]. MurD enzyme allows the addition of second amino acid (D-glutamic acid) on the peptide stem [88, 93]. There is a large number of X-ray crystal structures of apo- and substrate-bound MurD deposited in the protein data bank. Fig. (**6**) depicts cartoon views of selected MurD crystal structures. The X-ray crystal structure of *E. coli* MurD in the presence of its UDP-sugar substrate revealed it to be composed of three globular domains (Fig. **6**) [94]. Domain 1 is responsible for the linking of the UDP moiety of the UDP sugar substrate. This domain includes 1-93 residues and contains a five parallel β-sheet encircled by four α-helices [95]. Domain 2 includes 94-298 residues and contains a central six parallel β-sheet enclosed by seven α-helices, while domain 3 includes 299-437 residues and consists of a six β-sheet (five parallel and one antiparallel strand enclosed by five α-helices [96, 41]. Furthermore, the MurD enzyme contains the N-terminal, which is accountable for the binding of UDP--MurNAc-1-Ala, the central part in charge of the ATP binding, and the C-terminal area accountable for the D-glutamic acid binding.

The *E. coli* MurD is normally present in the cytoplasm, which causes the failure of most of the MurD inhibitors to function as they show poor penetration across the cytoplasmic membrane of bacteria. There are several types of MurD

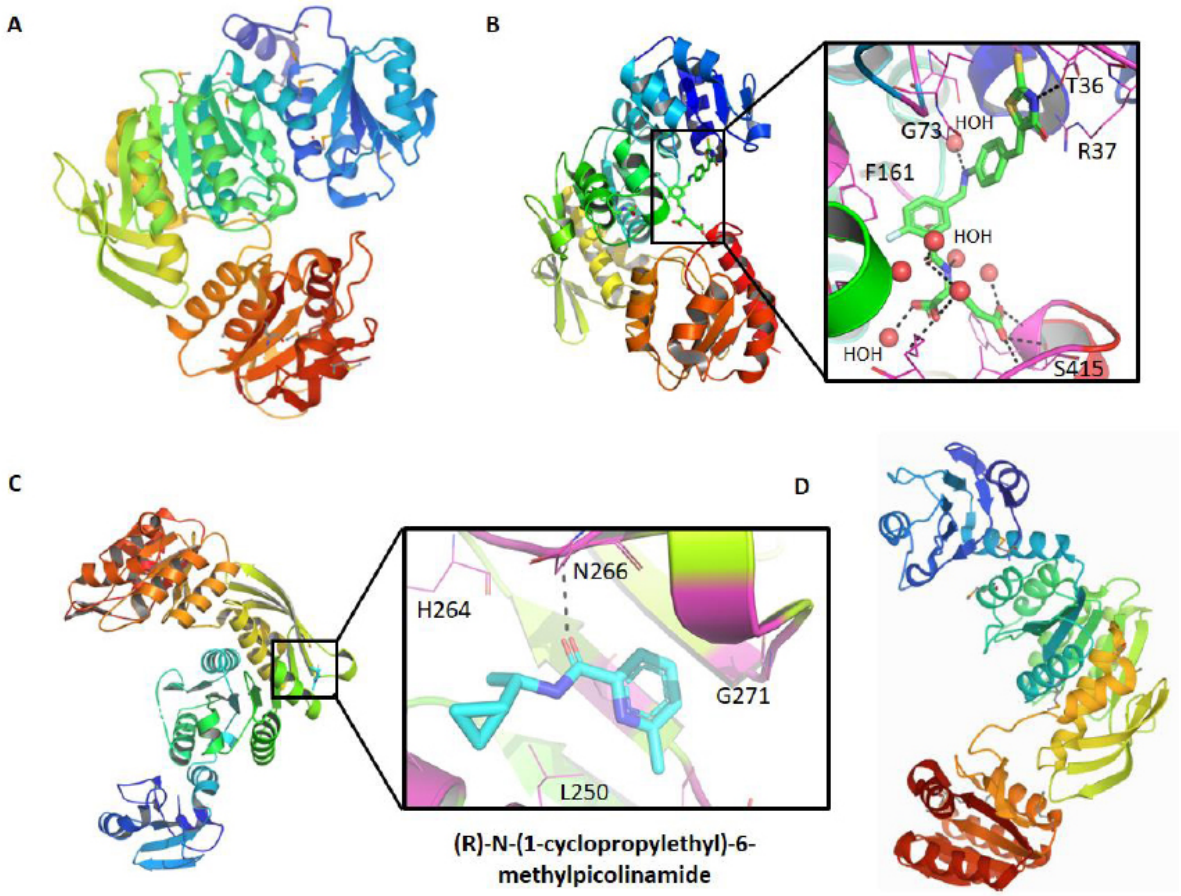

Fig. (6). A schematic depiction of cartoon view of (**A**) *E. coli* MurC (PDB-ID: 1F00), (**B**) *E. coli* MurD (PDB-ID: 2Y68), (**C**) *E. coli* MurE (PDB-ID: 7B61), and (**D**) *E. coli* MurF (PDB-ID: 1GG4) enzymes. Protein is represented in cartoon, ligands/cofactors are represented in sticks, and amino acids are represented in lines. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

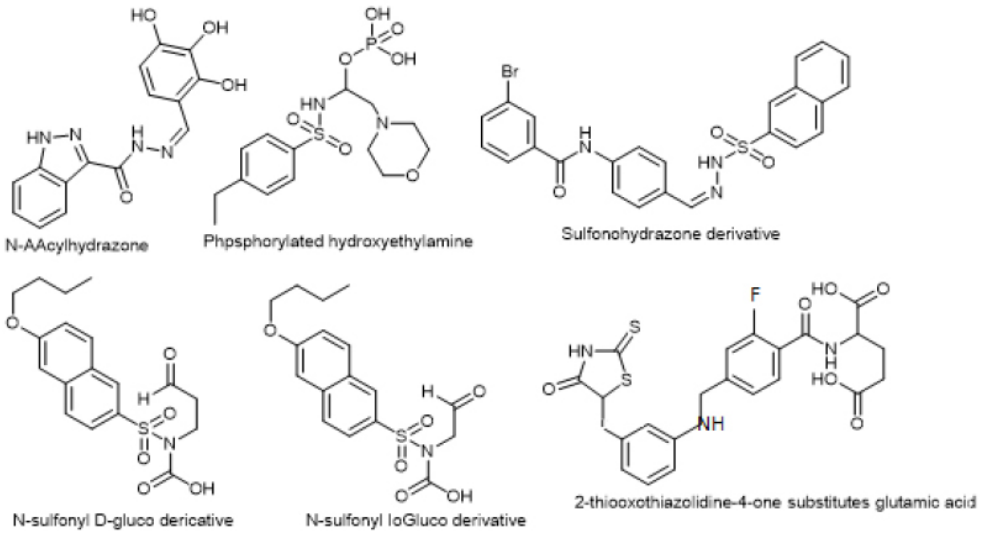

Fig. (7). Chemical structures of various classes of MurD inhibitors.

inhibitors reported in the literature [97]. Fig. (**7**) depicts the chemical structures of a few MurD inhibitors. Firstly, phosphonate derivatives possess MurD inhibitory effects. Different substitutions on phosphonate derivatives can produce different inhibitory effects. The first one includes carbamate and amide substituted phosphonate derivatives with high potency to inhibit MurD. This is because it can be substituted with trans-cinnamoyl, 3-hydroxy substituted trans-cinnamoyl, and 3,4- methylenedioxyphenyl ring in the structure. The compounds substituted with methylene and D-alanyl groups are less potent than the above compounds.

Furthermore, compounds that contain the nitrobenzene sulfonyl group are also potent inhibitors. On the other hand, sulphonamide-substituted phosphonate derivatives are the other domain substitution that contributes to forming MurD inhibitors. Compounds that consist of *o*-nitrobenzene sulfonyl and m-nitrobenzene sulfonyl substituents have the higher inhibitory potency in this series. However, this sulphonamide substitution is less effective than carbamate and amide substitution [98].

Besides these, the second type of derivatives includes 2-oxoindolinylidene and 2-thiooxothiazolidine-4-one derivatives. Compounds with pyridine ring structure can bind with the central domain spaces, while the presence of 2-oxoindolinylidene enables attachment to uracil binding sites with the greatest area. This allows these inhibitors to have higher chances of interaction with protein molecules, as depicted using the X-ray crystal structure of MurD bound with 2-thiooxothiazolidine-4-one derivative in Fig. (**6**) (PDB-ID: 2Y68) [99].

The third type of derivatives includes naphthalene-N-sulfonyl-D-glutamic acid derivatives. Agents with 2-naphthalene substituted N-sulfonyl-D-glutamic acid and aryl alkyloxy moiety that is substituted at position-6 and position-7 can increase the inhibitory activities. Substitution of 6-(aryl) allyloxy naphthalene side chains gives lipophilic properties to the derivatives, while naphthalene rings provide hydrophobic interactions [100]. Other derivatives include macrocyclic inhibitors, which act as novel compounds that inhibit the formation of peptidoglycan through MurD inhibition [101]. Polycyclic derivatives are also one of the potent inhibitory compounds for the MurD enzyme. Studies have shown that 9H-xanthine substituted polycyclic compounds will have greater inhibitory effects than other substituted polycyclic derivatives. This type of substitution produces the most potent inhibitory action in *E. coli* [102]. The most potentially designed MurD inhibitor possesses phosphinic acid having a tetrahedral geometry at the dipeptide center, which could be enzymatically phosphorylated, leading to a close counterpart of the normal reaction intermediate. It also withholds the charged UDP moiety probably vital for binding. Based on the belief that the structure of N-acetylmuramic acid could be vital for the potency of the amide-forming enzyme inhibitors, another research group carried out the synthesis of a new generation of inhibitors involving this characteristic. The potency was enhanced by more than three orders of magnitude as compared to Tanner's inhibitor due to the inclusion of muramic acid and the control of the stereochemical configuration of the L-amino phosphinate. This was evident from an IC_{50} value of <1 nM in comparison to an IC_{50} value of 680 nM for Tanner's inhibitor. Recently, a series of N-(5-phthalimidopentanoyl)-, N-[2-(2-ethoxy)acetyl]- and N-(7-oxooctanoyl)-phosphono- and phosphinoalanine derivatives were synthesized and investigated for inhibition of MurD. Concerning substrate analogues, the effect of different analogues of D-glutamic acid on the *E. coli* enzyme was evaluated, among which some showed weak inhibition. Additionally, N-acetylmuramic acid derivatives were also synthesized and evaluated as promising inhibitors of MurD; however, no such potential inhibitors were found [103].

10. MURE (UDP-N-ACETYLMURAMOYL-L-ALANYL-D-GLUTAMATE--2,6-DIAMINOPIMELATE LIGASE)

UDP-MurNAc-tripeptide ligase (MurE) is one of the enzymes from the class of ATP- dependent ligase family. MurE is considered the only Mur ligase which demonstrates substrate specificity for various bacterial species. Generally, gram-positive bacteria contain an L-lysine residue, and gram-negative bacteria possess meso-diaminopimelic acid (meso-A_2pm) group at the third position of the peptidoglycan peptide moiety. It is worth mentioning that most rod-shaped gram-positive bacteria also contain diaminopimelic acid (DAP) residue at this position, including bacilli and mycobacteria. Peptidoglycan is the principal component of the cell wall of bacteria which chemically consists of disaccharide and pentapeptide-stem. The disaccharide, made up of N-acetyl-glucosamine and N-acetyl-muramic acid, is preserved in all eubacteria. However, the pentapeptide-stem and bridge structure change from species to species. During synthesis, MurE is responsible for controlling the addition of lysine or diaminopimelate moiety into the stem peptides of peptidoglycan structure in a specific manner [104]. There is a large number of X-ray crystal structures of apo- and substrate-bound MurD deposited in the protein data bank. The X-ray crystal structure of the *E. coli* MurE complexed with its product, UDP-N-acetylmuramyl-tripeptide, revealed three globular domains, out of which two domains bear a topology equivalent to that of MurD (Fig. **6**). Domain 1 includes 1-88 residues and contains five β-sheets encircled by two α-helices, while domain 2 includes 90-338 residues and contains a central six parallel β-sheet encircled by seven α-helices. Domain 3 includes 340-497 residues and consists of six β-sheets enclosed by five α-helices [105].

MurE shows a vital function in adding the third residue of the peptidoglycan peptide moiety. The stereochemistry of this third position is vital to regulate the extent of antibiotic resistance as well as the cell characteristics and morphology. Hence, MurE ligase catalyzes the addition of L-lysine or meso-diaminopimelic acid to form UDP-N- acetylmuramoyl-L-Ala-D-Glu-L-Lys/A2pm, and this will indirectly make the enzyme act as a probable aim for the emergence of antibacterial agents [106, 107]. Besides, if MurE successfully carries the correct complementary amino acid substrate, it will be able to maintain the peptidoglycan integrity because

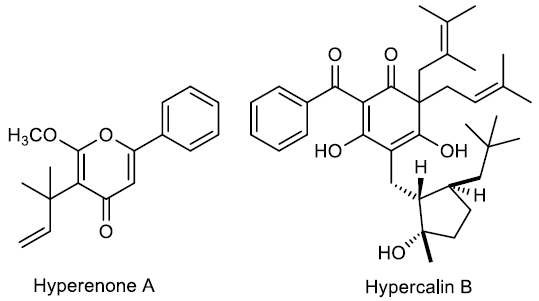

Fig. (8). MurE inhibitors from *Hypercium acmosepalum.*

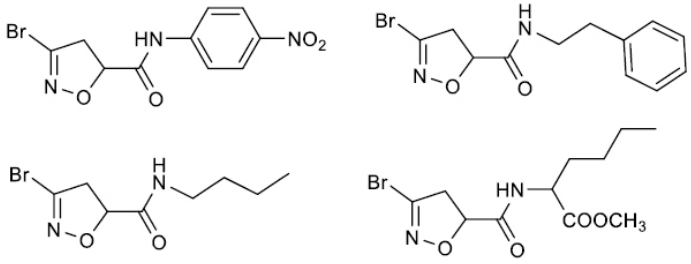

Fig. (9). Isoxazole derivatives as MurE inhibitors.

it will enable the third residue of the peptidoglycan peptide moiety to involve in the cross-linkages of the glycan strand leading to peptidoglycan integrity. However, if it is not complimentary, it will lead to alterations in morphology and even cause the lysis of bacterial cells [108]. There are several inhibitors of MurE ligases. Fig. (**8**) depicts the chemical structures of a few MurE inhibitors from *Hypercium acmosepalum*.

For example, an antibacterial isolated from *Hypercium acmosepalum* was found to hinder ATP-dependent MurE ligase of *Mycobacterium tuberculosis*. Both hyperenone A and hypercalin B were isolated from the aerial parts of *Hypercium acmosepalum* using hexane and chloroform extracts derived from the same. Both these agents are capable of exhibiting antibacterial activity against multidrug-resistant strains of *Staphylococcus aureus*. However, only hyperenone A show growth inhibitory activity against *Mycobacterium tuberculosis* and *Mycobacterium bovis*. When both agents were tested on *E. coli*, they did not show any inhibitory action. However, when tested on ATP-dependent MurE ligase of *Mycobacterium tuberculosis*, hyperenone A showed inhibitory action, while no effect was observed on the enzyme activity with hypercalin B [109]. Fig. (**9**) depicts the isoxazole class of MurE enzyme inhibitors. For example, 3-bromo-4, 5-dihydroisoxazole derivatives, showed inhibitory action without cytotoxicity.

The designing of the first phosphinate inhibitor of MurE with structural features was done based on the MurD inhibitor reported earlier. This compound hindered the reaction catalyzed by MurE with an IC_{50} value of 1.1 μM. Many diaminopimelic acid analogues were also evaluated as substrates or competitive inhibitors of MurE [110].

11. MURF (UDP-N-ACETYLMURAMOYL-TRIPEPTIDE--D-ALANYL-D-ALANINE LIGASE)

D-alanyl-D-alanine, which is one of the four amide bond-forming enzymes (MurF) from *Streptococcus pneumoniae*, plays a key role in bacterial survival because it is involved in catalyzing the last cytoplasmic step of peptidoglycan biosynthesis [109]. It catalyzes the ATP-dependent formation of UDP-MurNAc-pentapeptide, being the most important part of the cell wall of bacteria [111, 112]. The cell wall of *Staphylococcus aureus* consists of a peptidoglycan structure that is highly cross-linked, where most of the pentaglycine branches of muropeptide units are combined with the amino group of lysine and nearly all monomeric and acceptor muropeptides bear a carboxyl-terminal D-alanyl-D-alanine residue. Following this, D-alanyl-D-alanine catalyses the formation of dipeptide followed by attachment of the dipeptide to UDP-N-acetylmuramic acid-tripeptide by MurF, thus forming UDP-linked MurNAc-pentapeptide which is the building block of peptidoglycan. This dipeptide plays an important role as its peptide bond boosts the cross-linking reaction in the periplasmic space where there is the absence of ATP, leading to the assemblage of the peptidoglycan [113]. Besides, MurF also utilizes D-amino acids as dipeptide substrates and receives a wider range of various substrates in comparison to all other types of Mur ligases. In addition, MurF also shows the capability to incorporate non-canonical forms of D-amino acids into the peptidoglycan, such as D-methionine. Due to environmental stresses, such

as the entry into the stationary phase, the non-canonical D-amino acids are usually produced, thus resulting in the remodelling of the peptidoglycan composition and structure. Various MurF ligase inhibitors have been found. The crystal structure of the *E. coli* MurF enzyme revealed that it consists of three domains (PDB-ID: 1GG4) (Fig. **6**) [114, 115]. Fig. (**10**) depicts the chemical structures of MurF inhibitors. The first reported MurF enzyme inhibitor was 4-phenylpiperidine. It interferes with cell wall biosynthesis and shows antibacterial activity. It shows its inhibitory action with a minimum inhibitory concentration of 8-16 μg/ml against *E. coli* and even includes cell lysis. Additionally, from the MurF binding assay, it was noticed that a series of 8- hydroxyquinolines caused the inhibition of the *E. coli* enzyme by binding to it. This proved that the antibacterial activity is also exhibited by pharmacophoric modelling of 8-hydroxyquinolines which otherwise have restricted antibacterial activity. However, by using permeability enhancers, such as polymyxin B nonapeptide, there is a possibility of having a reduction in the MIC values. In the present study novel, lead compounds were identified by the researchers, which could inhibit the biological activity of the MurF protein. It plays a major role in the last step of the peptidoglycan biosynthesis pathway. The predicted three-dimensional structure of the MurF protein was evaluated. Molecular dynamics simulation studies were performed over 100 ns to assess the stability and flexibility of the predicted protein. The result of high throughput virtual screening rendered the top three compounds Zinc-12134489, May bridge-11911, and Specs-10474 with high docking scores -6.3, -6.1, -5.9 kcal/-mol and binding energies of -46.2, -35.4, -47.7 kcal/mol, respectively. The compounds were predicted to establish strong hydrogen bond interactions with Thr28, Asp29, Lys43, Asn46, Phe47, Asp133, and Lys148 residues. Density functional theory described the higher reactivity of the screened compounds. Low binding gap values of the top three hits, ranging from -0.04 eV to -0.06 eV, defines higher chemical reactivity. Molecular dynamics simulation studies were performed for three protein-ligand complexes that showed acceptable RMSD and RMSF ranges. Predicted ADME properties of the top lead compound were in the acceptable range. Thus, the identified three inhibitors were predicted to show significant inhibitory activities against MurF that could be exploited to overcome the filarial infections after experimental validation [116]. The Gram-negative oral pathogen *Tannerella forsythia* strictly depends on the external supply of the essential bacterial cell wall sugar N-acetylmuramic acid (MurNAc) for survival because of the lack of the common MurNAc biosynthesis enzymes MurA/MurB. The bacterium thrives in a polymicrobial biofilm consortium. Thus, it is plausible that it procures MurNAc from MurNAc-containing peptidoglycan (PGN) fragments (muropeptides) released from cohabiting bacteria during natural PGN turnover or cell death. There is indirect evidence that in *T. forsythia*, an AmpG-like permease (Tanf_08365) is involved in cytoplasmic muropeptide uptake. In E. coli, AmpG is specific for the import of N-acetylglucosamine (GlcNAc)-anhydroMurNAc(-peptides), which are common PGN turnover products, with the disaccharide portion as a minimal requirement. Currently, it is unclear which natural, complex MurNAc sources *T. forsythia* can utilize and which role AmpG plays therein [117].

The diarylquinolines, DQ1 and DQ2, represented in Fig. (**10**), have the same structure as the compound, but there is only one difference which is at the beta position to the quinoline ring in DQ2. These two compounds have very limited inhibitory action due to their low permeability into the cells. Hence, both of them will not show inhibitory action against the wild type of *E. coli* and gram-negative bacteria. DQ2 can act on gram-positive bacteria because it is more flexible compared to DQ1, which has a phenethyl group. However, the problem related to low permeability can be resolved by adding permeability enhancers, such as polymyxin B nonapeptide. Cyanothiophene derivatives as MurF ligase inhibitors from *Streptococcus pneumoniae* and *E. coli* were explored (Fig. **10**). Through methodical structural modifications of the parent compounds, they found a micromolar inhibitor of MurF from *S. pneumoniae* (IC_{50} = 0.3 μM), *E. coli* ((IC_{50} = 55 μM), and *S. aureus* (IC_{50} = 120 μM) [118]. Phosphinate inhibitors of MurF known so far are aminoalkylphosphinate compounds synthesized as transition-state analogues. They operate as reversible competitive inhibitors of the *E. coli* MurF enzyme, with Ki values in the range of 200 to 700 mM. No antibacterial activity was exhibited by these compounds on standard strains. The non-hydrolyzable ATP analogue AMP-PCP was evaluated on the MurF-catalysed reaction and revealed to be an effective inhibitor of ATP hydrolysis and was competitive with ATP.

One of the main aspects to be considered in the design of future Mur synthetase inhibitors is the conserved binding patterns and the common kinetic mechanism amongst MurC, MurD, MurE, and MurF. An inhibitor will possibly attach to and hinder more than one enzyme that identifies homologous binding motifs. This would result in much more effective inhibition of the pathway. Additionally, the frequency of the rise of resistance to such an inhibitor would be much lesser as mutations causing resistance would require to take place simultaneously in more than one target gene.

12. DEVELOPMENT OF MULTI-TARGETS-BASED INHIBITORS

Multi-target inhibitors are a better approach to inhibiting Mur enzymes effectively. The blocking of nucleotide substrate binding is mainly observed in MurC, MurD or MurE, and MurF [119, 120]. This is because all these Mur ligases share common characteristics concerning UDP-binding mode. By blocking either one of the groups of enzymes, binding effects will be interrupted. An example of a compound that can perform this action is 5-benzylidene thiazolidine-4-one. It is capable of inhibiting MurD, MurE, and MurF. The second target of action can be the ATP binding site. This is because most of the Mur ligases with ATP-binding domains have similar structural confirmations and sequences, and the mechanism is not closely related to the human enzymes utilizing ATP [121]. The specialty of the ATP binding site of bacteria is that the Mur ligases consist of

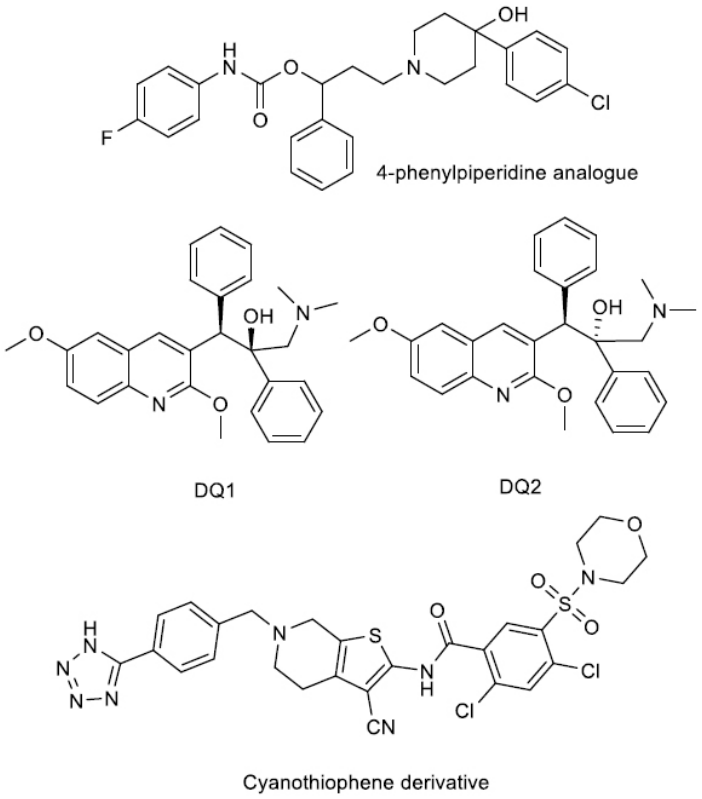

Fig. (10). 4-phenylpiperidine, diarylquinoline, and cyanothiophene derivatives as MurF inhibitors.

P-loop confirmation, which is different from the ATP-binding loop in the human system [9, 122]. Compounds, such as N-acylhydrazone can act as potent inhibitors of MurC and MurD as both are likely to aim for the ATP binding site. Since the amino acid substrate is needed for the catalytic reaction of bacteria, the inhibition of this function can be done by mimicking the structure of the amino acid substrate. Phosphonate derivative is the first analogue of Mur enzyme inhibitors, and the compound consists of a dipeptide analogue connected to uridine diphosphate, a spacer being hydrophobic [123, 124]. These derivatives are reported to inhibit both MurC and MurD ligases. Sulphonamide derivatives like naphthalene-N-sulfonyl-D-glutamic acid have been designed as the MurD enzyme inhibitors [118, 125]. This is because of the presence of 1,3-dicarboxylic acid in sulphonamide derivatives which can inhibit MurC and MurD. Another mechanism of multi-target inhibitors utilizes the conformational change of Mur ligase towards substrate binding. These features allow the researchers to develop compounds that can be capped inside the inactive open state, thus, interfering with the binding of the substrate on the active site [126]. Fig. (**11**) depicts the chemical structures of selected inhibitors of multiple Mur enzymes. The screening of a series of ATP-competitive kinase inhibitors on *E. coli* Mur ligases was conducted, and five scaffolds were found that inhibit at least two of these ligases (MurC, D, and F) [11]. The furan-containing aza-stilbene derivative (Fig. **11**) showed a competitive inhibition of MurD activity towards D-glutamic acid, and NMR studies revealed that it binds to the binding site of D-glutamic acid, independent of the enzyme closure caused by ATP [24]. Meanwhile, in the same series, a thiazoline-containing aza-stilbene derivative (IC_{50} MurC: 82, MurD: 85, MurE: 150, and MurF: 71 μM) was also found to exhibit comparable inhibition of multiple Mur ligases (MurC-MurF) and exhibited moderate antibacterial activity (Fig. **11**). An attempt was done to replace the diphosphate group of UDP-Mur NAc with a 1,2,3-triazolo spacer where, out of several compounds synthesized and tested, one *N*-acetylglucosamine analogue emerged as the best inhibitor against the *Mycobacterium tuberculosis* MurA-MurF enzymes reconstruction pathway with a 56% inhibition at 100 μM [17]. Further, the optimization of benzene-1,3-dicarboxylic acid to 2,5-dimethylpyrrole derivatives was done and found to exhibit dual MurD/MurE inhibition properties, which were found in the virtual screening campaign. Compounds were analyzed against *E. coli* MurC-MurF enzymes in biochemical inhibition assays where the compounds containing benzene-1,3-dicarboxylic acid 2,5-dimethylpyrrole linked with five-membered rhodanine moiety exhibited inhibition of multiple MurC-MurF ligases in the micromolar range [127] (Fig. **11**). In another study, furan-based benzene-1,3-dicarboxylic acid derivatives were reported to act as multiple MurC-MurF ligase inhibitors [128]. Table **1** represents the various Mur enzyme inhibitors that have been studied so far [30].

Table 1. Various mur enzyme inhibitors.

S. No.	Class of Inhibitor	Enzyme Targeted
1	Benzothioxalone derivatives	Mur A
2	Cyclic disulfide, pyrazolopyrimidine and purine analogues	
3	Sulfonyloxy anthranilic acid derivatives	
4	Imidazole derivatives	
5	Aminotetralone derivatives	
6	Peptide derivatives	
7	Sesquiterpene lactones derivatives	
8	Thimerosal, thiram and ebselen	
9	Tulipalines, tuliposides and their derivatives	
10	Terreic acid	
11	Avenaciolides	
12	4-thiazolidinones derivatives	Mur B
13	3,5-dioxopyrazolidines derivatives	
14	5-hydroxy-1H-pyrazole-3 (2H) -one derivatives	
15	Imidazolinones derivatives	
16	Pyrazolopyrimidines derivatives	Mur C
17	Phosphinates derivatives	
18	Benzylidene rhodamine derivatives	
19	Phosphinate derivatives	Mur D
	Carbamate and amide substituted phosphinates derivative	
	Sulfonamide substituted phosphinates derivatives	
20	2-oxoindolinylidene derivatives	
21	Naphthalene-N-sulfonyl-D-glutamic acid derivative	
22	Macrocyclic inhibitors	
23	N-sulfonyl-glutamic acid derivatives	
24	Coumarin derivatives	
25	Polycyclic derivatives	
26	Inhibitors from *Hypericum acmosepalum* (hyperenone A and hypercalin B)	Mur E
27	Quinolones derivatives	
28	Aporphine alkaloid derivatives	
29	Isoxazole derivatives	
30	Thiazolylaminopyrimidine derivatives	Mur F
31	4-phenylpiperidine derivative	
32	Diarylquinone derivatives	
33	Miscellaneous inhibitors	
	Inhibitors from *S. pneumoniae*	
	(-) -epigallocatechin gallate	
Multitarget Inhibitors		
34	Naphthyl tetronic acid derivatives	Mur A-Mur E
35	Furan-based benzene mono- and dicarboxylic acid derivatives	Mur C-Mur F
36	N-acylhydrazones derivatives	Mur C and Mur D
37	5-benzylidenethiazolidin-4-ones derivatives	Mur D, Mur E and Mur F
38	Feglymycin	Mur A and Mur C

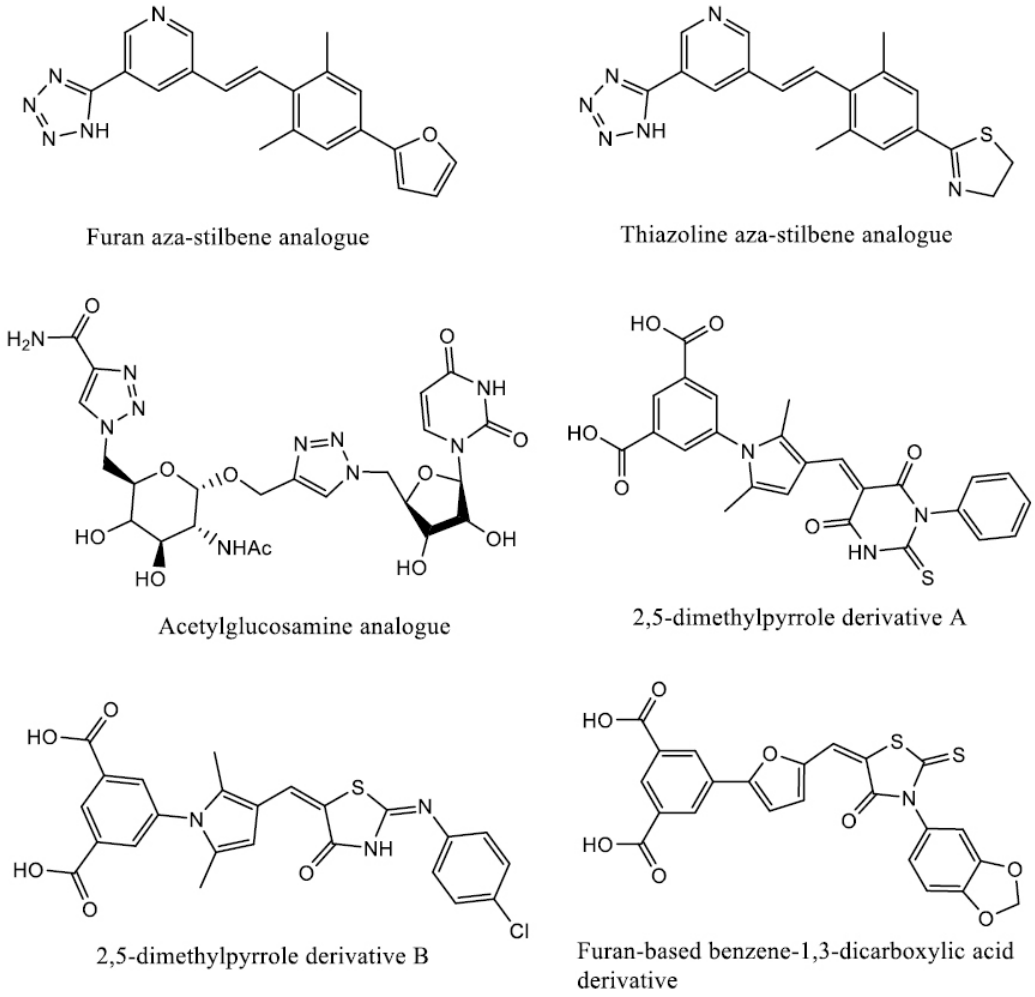

Fig. (11). Chemical structures of various compounds known to inhibit multiple Mur ligases.

13. ANTIBIOTIC DEVELOPMENT OF INHIBITORS WITHIN BACTERIAL CYTOPLASM: A NEW ARENA THROUGH PERIPHERAL GLYCOSYLTRANSFERASE MURG GENE

The development of novel antibiotics targeting bacterial cell wall synthesis is a relevant approach to designing novel inhibitors. The biosynthesis of peptidoglycan in the cell wall of bacteria is a complicated process that involves enzymatic reactions occurring in the cytoplasm for the formation of nucleotide precursors. The synthesis of lipid-linked intermediates takes place through polymerization reactions on the inside and outside of the cytoplasmic membrane. The cytoplasmic steps of the biosynthesis of peptidoglycan can be categorized into four types of reactions that result in the synthesis of UDP-N-acetylglucosamine from fructose 6-phosphate, UDP-N-acetylmuramic acid from UDP-N-acetylglucosamine, UDP-N-acetylmuramyl-pentapeptide from UDP-N-acetylmuramic acid and D-glutamic acid and dipeptide D-alanyl-D-alanine concerned different Mur genes (Fig. **1**). Both processes have reactions whose catalysis is done within the cytoplasm. Initially, the formation of UDP--MurNAc (UM) by MurA and MurB gene and attachment of amino acids by MurC onto UM, D, E, F to generate UM- di, tri and pentapeptide take place (Fig. **1**). The possible existence of different cytoplasmic complex molecules by Mur enzymes is well established [92]. Mur genes exist in a single operon, and their pairs are generally joined to give rise to a single polypeptide. Mur enzymes could exist as a complex whose build-up makes the accessibility of small molecules to their active sites limited. Bacterial morphogenesis is a method that is associated closely with the biosynthesis of peptidoglycan. Lipid II, which is the basic building block of peptidoglycan, is formed through the action of Mur enzymes A-F in the cytoplasm of bacteria (Fig. **1**). As Lipid II is vital for both the elongation of the cell wall and division, these enzymes are mandatory for the working of both the elongasome and the divisome. The divisome and elongasome are bacterial protein complexes that are responsible for peptidoglycan synthesis during cell division and elongation, respectively. The divisome is a membrane protein complex with proteins on both sides of the cytoplasmic membrane. In

gram-negative cells, it is located in the inner membrane. The divisome is nearly ubiquitous in bacteria, although its composition may vary between species. The elongasome is a modified version of the divisome without the membrane-constricting FtsZ-ring and its associated machinery. The elongasome is present only in non-spherical bacteria and directs lateral insertion of PG along the long axis of the cell, thus allowing cylindrical growth (as opposed to spherical growth, as in *cocci*).

Moreover, MurE and MurF interact with a peripheral glycosyltransferase gene which is a molded discrete oligomer. The oligomeric assembly of MurG may allow it to play a bonafide scaffolding role for a latent Mur complex, thus leading to the effective transportation of peptidoglycan-building blocks toward the inner membrane leaflet. This prompts the fact that MurG can serve as a scaffold for Mur enzyme, helping restrict the diffusion of peptidoglycan intermediate within the cytoplasm and regulating them towards the membrane's inner side [13]. The structural nature of the existence of such complex molecules is still not studied properly to date. MurG is a necessary enzyme interacting and/or colocalizing with other Mur genes, that is, MurD, E, and F. It is a peripheral membrane protein interacting with cytoplasmic membrane phospholipids. It is observed that MurG acts as a dimer; however, the structural determinants and functional importance of this arrangement have not been stated to date. The glycosyltransferase gene also links a GlcNAc moiety to Lipid I, leading to the formation of Lipid II, whose translocation is further done by flippases towards the periplasmic space. As there is a lack of research on the structure and role of this arrangement, it provides immense scope for developing novel inhibitors as antimicrobials targeting the peripheral glycosyltransferase MurG gene.

CONCLUSION

Multidrug resistance is undoubtedly a global public health concern and mandates an urgent need to develop novel antibacterial therapy targeting novel targets in bacteria. The system for the biosynthesis of peptidoglycan is one of the rich sources of valid druggable targets for the discovery and development of effective antibacterial therapeutics. Mur enzymes play imperative roles in the biosynthesis of bacterial peptidoglycan. It is one of the major targets for developing antibacterial agents. The inhibition of Mur enzymes can lead to the destruction of bacterial peptidoglycan, thus, inhibiting the bacteria. Hence, Mur enzyme inhibitors are good antibacterial agents and can be called antibiotics. Generally, Mur enzyme inhibitors can act on the six major families of Mur enzymes, viz. MurA, MurB, MurC, MurD, MurE, and MurF. These Mur enzymes have different functions in the formation of bacterial peptidoglycan. Their inhibitors act differently to inhibit each enzyme specifically. The antibacterial drug development as multiple Mur enzyme inhibitors is essential as the problem of bacterial resistance is very common at present. Thus, more antibacterial agents targeting novel pathways are needed to treat a variety of resistant bacterial infections effectively.

AUTHORS' CONTRIBUTION

Yadu Nandan Dey and Dharmendra Kumar generated the concept, searched the literature, and drafted the manuscript. Nandan Sarkar and Kuldeep Kumar Roy contributed significantly to the preparation of the manuscript and the literature review. Dheeraj Bisht, Deepak Kumar, Bitasta Mandal, and Mogana R contributed to the literature review and correction of the manuscript. All authors of this manuscript have read and approved the manuscript for submission.

LIST OF ABBREVIATIONS

AMR = Antimicrobial Resistance

CD = Central Domain

CEPA = Company Espanola de Penicilina y Antibiotic

CTD = C-terminal Domain

FAD = Flavin Adenine Dinucleotide

MICs = Minimum Inhibitory Concentrations

NAG = N-acetylglucosamine

NTD = N-terminal Domain

PEP = Phosphoenolpyruvate

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

All the authors of this manuscript declare that there is no conflict of interest in any financial or non-financial means.

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