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Mur Ligase Inhibitors as Anti-bacterials: A Comprehensive Review

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Abstract: Exploring a new target for antibacterial drug discovery has gained much attention because of the emergence of Multidrug Resistance (MDR) strains of bacteria. To overcome this problem the development of novel antibacterial was considered as highest priority task and was one of the biggest challenge since multiple factors were involved. The bacterial peptidoglycan biosynthetic pathway has been well documented in the last few years and has been found to be imperative source for the development of novel antibacterial agents with high target specificity as they are essential for bacterial survival and have no homologs in humans. We have therefore reviewed the process of peptidoglycan biosynthesis which involves various steps like formation of UDP-N-acetylglucosamine (GlcNAc), UDP-N-acetylmuramic acid (MurNAc) and lipid intermediates (Lipid I and Lipid II) which are controlled by various enzymes like GlmS, GlmM, GlmU enzyme, followed by Mur Ligases (MurA-MurF) and finally by MraY and MurG respectively. These four amide ligases MurC-MurF can be used as the source for the development of novel multi-target antibacterial agents as they shared and conserved amino acid regions, catalytic mechanisms and structural features. This review begins with the need for novel antibacterial agents and challenges in their development even after the development of bacterial genomic studies. An overview of the peptidoglycan monomer formation, as a source of disparity in this process is presented, followed by detailed discussion of structural and functional aspects of all Mur enzymes and different chemical classes of their inhibitors along with their SAR studies and inhibitory potential. This review finally emphasizes on different patents and novel Mur inhibitors in the development phase.

Keywords: Peptidoglycan biosynthesis, Mur inhibitors, Antibacterial agents, Chemical classes, SAR, patents.

1. INTRODUCTION

Any compound which is generally used to inhibit or kill bacterial populations because of particular bacterial infection is called as an antibacterial agent. Depending on the intended purpose they either inhibit the growth (bacteriostatic) or kill (bactericidal) respectively [1, 2]. The literature review indicates that such antibacterial drugs are reported from natural, marine, semisynthetic or synthetic origin.

Commonly, different terms like Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), and Minimum Antibiotic Concentration (MAC) are used to determine the efficacy of antibacterial drugs [3]. Effectiveness of antibacterial agent depends on the actual concentration of the drug reaching the site of infection, but it may vary according to the intactness of the host defence mechanism. Toxicity and selectivity are the two essential properties possessed by good antibacterial agents. Antibacterial agent must be highly selective for its target and must have a low degree of toxicity to avoid any sort of tissue damage. For this, the target must be metabolic pathways or enzymes present only in the bacteria and totally absent in the mammalian cells [4].

1.1. Antibacterial Drug Discovery Over Time Scale

The use of antimicrobial agents to treat different types of microbial infections was a milestone in the development of modern medicines. Due to extensive use of present molecules and low rate of new molecule development the problem of antimicrobial resistance is increasing day-by-day [5, 6].

Antibacterial drug discovery starts with first miracle molecule penicillin, which was discovered in 1928, but it was used during the Second World War in the 1940s. It indicates a stringent condition by regulatory agencies in the drug approval process. Later on, different classes of antibacterial agents like sulfonamides, streptomycin, and tetracyclines, macrolides, glycopeptides, cephalosporins were developed and marketed in the late 1960s. Cephalosporin's a class of antibacterial agent becomes the first choice of prescriber upto a large extent hence second and third generation of cephalosporin was marketed upto mid-1980s, but later on cases of resistance to cephalosporin's were also reported [7, 8].

Because of several reasons in the early 1980s discovery of new drug was reduced to drastic levels [9, 10]. Very few organizations were engaged in antibacterial agents and their continuous effort led to the discovery of a new class of Oxazolidinones in 1999-2001. Merging or takeover of small companies by top ones led to decrease in output of novel antibacterial agents (Fig. 1).

1.2. Need to Study Antibacterial Agents

One of the greatest achievements in the field of medicine during the 20th century was use of antimicrobial agents to treat microbial infections, but this success did not remain steady for a long period of time as several factors were responsible. This may lead to new problems of Multidrug Resistance (MDR) strain of bacteria, which was resistant to routine standard antibacterial therapy [11]. Multidrug Resistance (MDR) strains of bacteria are gaining focus nowadays due to exposure of patient to severe infections and if not treated properly becomes fatal [12,13]. This led to extensive use of available antibacterial agents, which may again worsen the present conditions; so the demand for novel antibacterial agents was increasing day-by-day [14].

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Fig. (1). Discovery void in antibacterial drug discovery.

1.3. Discovery Void

In the 1970-1990s, many big pharmaceutical firms stopped antibacterial drug discovery due to different reasons which result in discovery of very few molecules from the class of antibacterial agents [15]. Due to this discovery void (Fig. 1) it was not possible to fulfil the increasing demand of novel antibacterial agents from health professionals to treat microbial infections of the patient. Since then, problem of bacterial resistance has become more prominent which demands novel antibacterial agents [16, 17].

1.4. Challenges in Antibacterial Drug Discovery

There is big difference in the demand and supply of novel antibacterial agents with newer mechanism of action to avoid problem of antimicrobial resistance due to multistep processes of discovery and development of antibacterial drugs (Fig. 2). Development of novel antibacterial agents depends on several factors like unreliable results against infection with present drugs, varying antibiotic consumption patterns, evidence of secondary infections, stringent condition for approval by different regulatory agencies, less productivity of industry, multiple steps in clinical trials, control of regulatory agencies over drug pricing, merging of pharmaceutical companies [05, 18].

Two main important approaches can be adopted in the process of new drug development, of which first approach involves development of novel molecules useful for the society and for which grant from different government agencies were provided. Secondly, it involves development of molecule required in actual market and for which, the initiative of a pharmaceutical company is important as it plays an important role in the capital investments [19-22].

A decision made by pharmaceutical companies about the investments in novel antibacterial drug discovery program was taken on the basis of extensive study of several factors like need of new molecules in the market, consumer potential, i.e. actual patient population, advantages and limitations of novel agents over present molecules, presence of competitor brands if any, calculation of the

amount invested and time period required for its return [22]. Due to all these factors numbers of novel antibacterial drug discovery programs currently running were limited and out of which very few of them reached the final step and achieved their desired goals [23].

It can be observed from different studies that success rate of antibacterial molecules in clinical development is much higher as compared to other class of drugs [24, 25]. Even bacterial genomics are studied extensively, but molecules developed by this approach are limited and the main reason behind this was selection of target spectrum, which involves selection of target families which had same properties but were easy to differentiate [5].

1.5. Need to Exploit Different Targets for Better Antibacterial Drugs

The process of development of new molecules of any class involves some basic approach like chemical modification of existing analogues, newer molecules with novel mechanism of action, new molecules by using recent techniques like High throughput screening (HTS), or molecules from natural [26-29], marine [30-32] and microbial source.

2. BIOSYNTHESIS OF PEPTIDOGLYCAN LAYER

Peptidoglycan (PG) is the vital component of bacterial cell wall, hence also called as “biochemical machinery” or less commonly as “murein”. It forms mesh like network and has the function of conservation of rigidity and shape of bacterial cells. Besides this, it also protects cell from bursting due to turgor pressure [33, 34]. In the absence of peptidoglycan layer, the cells will act as a hypotonic medium which lead to swelling and bursting of cells, from this it can be concluded that the presence of peptidoglycan layer is essential for bacterial survival as it plays a role in cell rigidity, maintenance and cell division [35].

Present in the periplasm is affected by changes in ionic strength, pH value, osmolality on the cell's environment. To overcome this problem cell must have rigid and robust peptidoglycan to grow under diverse situations [36].

Fig. (2). Schematic pathway for discovery and development of antibacterial drug.

Peptidoglycan is found to be one of the best targets for antibacterial drug discovery as it is absent in mammalian cell and present only in bacterial cell. Short strands of glycan are joined together to form a continuous mesh like structure of peptidoglycan *i.e.* “Sacculus”. Thickness of sacculus layer varies depending on types of bacteria, in case of Gram negative species, thickness of PG layer is 3-6 nm and for Gram positive species, thickness of Peptidoglycan layer is 10-20 nm as it is multi layered [34].

Amino acid like phenylalanine, leucine and isoleucine are not the components of the normal peptidoglycan assembly, hence their presence indicates improper removal of amino acids, which directly result in errors in the report of a peptidoglycan structure [33].

Peptidoglycan or murein is an essential component of bacterial cell wall made up of short glycan chains of alternating N-acetyl muramoyl-peptides (MurNAc) and N-acetyl glucosamine (GlcNAc) which are cross linked with each other [37, 38]. A variation in cell's turgor pressure is responsible for expansion & shrinkage of peptidoglycan layer. It is intriguing fact that peptidoglycan layer with its crucial, steady role is constantly modified [39].

Peptidoglycan layer has a high degree of cross linking and hence must be hydrolyzed by specific enzymes in order to study the structural behaviour [40]. Presence of peptidoglycan layer is essential criterion for taxonomic classification because Gram positive bacteria shows great variation in the structure of peptidoglycan layer, but in case of the Gram negative bacteria structure of peptidoglycan layer are uniform and does not show any variations [41].

Several problems arise due to the presence of the peptidoglycan layer like inhibition of entry of hydrophilic compounds. The remedy for this is to use carrier mediated transport, which is the normal mechanism of cell for uptake of solutes from outside to inner parts of the cell. Efflux may take place for such hydrophilic compounds which enter into the cell [42, 43]. Bactoprenol, a lipophilic mole-

cule plays decisive role in passage of precursor molecules from cytoplasm to exterior of cell wall membrane to increase chain length of growing peptidoglycan [44, 45].

2.1. Historical Perspectives

Study of peptidoglycan structure and intermediates involved in biosynthesis is well documented in the field of antibacterial drug discovery. Here we are trying to cover the brief history of peptidoglycan layer study, and antibacterial drug discovery.

It begins with the discovery of penicillin by Alexander Fleming which act as a specific inhibitor of cell wall synthesis, with advancement in technique of structural detailing of Peptidoglycan like its monomer assembly, different peptidoglycan precursors like Transpeptidase (TPs) & Glycosyltransferase (GTase) along with their inhibitors. Discovery of biosynthetic pathway of peptidoglycan ends with a determination of lipid intermediates *i.e.* Lipid I and Lipid II upto mid-1960 [46-48].

Later on, efforts were made in the direction of *in vitro* peptidoglycan synthesis and satisfactory results were obtained up to some extent [49]. Advancement in the analytical technique results in the detection of other intermediates and different mechanisms due to use of more sophisticated techniques like radiolabelling and other chromatographic methods [33].

2.2. Pathway for Biosynthesis of Bacterial Peptidoglycan

Biosynthesis of bacterial peptidoglycan are studied almost in different species and detail information about biosynthetic pathway is available. There are two stages involved in biosynthesis of bacterial peptidoglycan. First stage consists of the formation of monomer subunit due to activity of enzymes present in the cytoplasm or at cytoplasmic membrane. Disaccharide – (peptide) – pyrophosphate undecaprenol is the lipid intermediate formed at the end of the first

Fig. (3). Peptidoglycan biosynthesis pathway.

stage. Second stage start with translocation of lipid intermediates outside the cytoplasmic membrane which then result into polymerization reaction due to the presence of two different enzymes, i.e. Glycosyltransferase (GTase) which perform polymerization of glycan strand & Transpeptidase (TPase) which perform formation of peptide cross linking (see Fig.3) [50-55].

At least in case of *Escherichia coli* all genes involved in peptidoglycan formation were identified and isolated, cloned and sequenced successfully. Due to availability of large amount pure enzymes, advancement in the study was possible. Owing to high specificity at each step and their presence in bacteria only, Peptidoglycan synthesis inhibitors are considered as a good candidate for anti-bacterial drug discovery [50].

2.3. Assembly of Monomer Unit

Formation of monomer units is carried out in a sequential manner, starting from the fructose-6-phosphate to final lipid intermediate via UDP-N-acetylglucosamine. This pathway has two essential features. Firstly, it involves the high degree of specificity reflected in every step which contributes to the final structure of peptidoglycan. Secondly, transfer of monomer units from cytoplasm to outer polymerization site through hydrophobic membranes. Pathway of peptidoglycan biosynthesis was established after detailed study of all the precursors and enzymes involved in it (see Fig.4) and in-vitro assay was developed for enzymes involved in each step of peptidoglycan biosynthesis [51-57].

2.4. Steps Involved in Assembly of Monomer Unit

Formation of peptidoglycan monomer units is a multistep process and involves four important steps as follows [50]:

2.4.1. Step I: Formation of UDP-N-acetylglucosamine

Variation in precursor is due to the type of bacteria because in gram positive bacteria teichoic acid is utilized as precursor and in case of Gram negative bacteria UDP-N-acetylglucosamine (UDP-GlcNAc), along with other precursors [66]. Formation of UDP-GlcNAc from fructose-6-phosphate involves four consecutive steps

catalyzed by three enzymes i.e. GlmS synthase, GlmM synthase, GlmU synthase [50]

2.4.1.1. GlmS Synthase

GlmS gene present in *E. coli* is found to be helpful in conversion of D-fructose-6-phosphate into Glucosamine-6-phosphate, which is catalyzed by L-glutamine [65]. Homogeneity of GlmS transferase is reported in *E. coli* and *Thermus thermophilus* after purification [58, 59]. It is the enzyme having two domains which differ from each other in structure and function. The first domain C-terminal performs the isomerization of fructose-6-phosphate to Glucosamine-6-phosphate and for this conversion ammonia gets utilized. The second domain N-terminal carries the conversion of glutamine to glutamate and ammonia. Both the domains are crystallized and its 3D structures are studied using high resolution [60-62].

2.4.1.2. Phosphoglucosamine Mutase (GlmM)

The second step involves formation of Glucosamine-6-phosphate and glucosamine-1-phosphate and it takes place in place of phosphoglucosamine mutase. It is characterized in *E. coli* as glmM gene [62] and ureC gene in *H. pylori* [63], as femD or FemR315 gene in *S. aureus* [64] and as ORF540 in *P. aeruginosa* [65]. This enzyme is active only in its phosphorylated form catalysis by an enzyme is according to ping-pong bi-bi mechanism. Ser-102 is clearly identified as phosphorylation site of enzyme [66].

2.4.1.3. GlmU Synthase

GlmU synthase is the two domain enzyme like GlmS synthase and involved in the last step of UDP-GlcNAc formation i.e. acetylation and uridylation respectively. Helical arm and three GlmU molecules assemble to each other to form trimeric arrangements. The C-domain catalyses acetylation of Glucosamine-1-phosphate into N-acetylglucosamine-1-phosphate and now this N-acetylglucosamine-1-phosphate is converted into UDP-GlcNAc by uridylation carried out by N-terminal domain. Both these two domains are attached to each other with long α -Acetylation is followed by uridylation but the steps are not interdependent, Acetyl transferase activity

Fig. (4). Enzymes involved in assembly of the peptidoglycan monomer unit.

is lost on treating it with thiol containing reagents but uridyl transferase activity remains unaffected [67, 68].

2.5. Step II: Formation of UDP-N-acetyl Muramic Acid

Formation of UDP-N-acetylglucosamine is followed by formation of UDP-N-acetyl muramic acid which is actually a two-step process. In the first step formation of UDP-Glc-Nac enolpyruvate takes place by transferase when enolpyruvate from phosphoenolpyruvate (PEP) is transferred to position 3 of GlcNAc residue. Second step ends with the formation of UDP-Mur-NAc by the action of reductase, which causes reduction of enolpyruvate moiety to D-lactoyl [50].

2.5.1. MurA Transferase

Mechanism of action of MurA is complex as it is followed by inducing fit mechanism and involves different changes in the conformation of the enzymes. MurA act on the C-2 position of phosphoenolpyruvate (PEP) which finally result into the cleavage of C-O bond. MurA has two domain structures with 418 amino acid residues. C-terminal portion of the sequence residues is associated with domain 1 while N-terminal portion of the sequence residues is associated with domain 2. Both the domains consist of six helices and 4 stranded β -sheets. Fosfomycin a natural broad spectrum antibiotic shows inhibition to MurA by showing covalent adduct with the active sites of the enzyme. Resistance to the action of Fosfomycin is because of change in the active site of enzymes due to mutational changes [69].

2.5.2. MurB Reductase

The MurB perform its mechanism in the two steps. The first step involves formation of tightly bound Flavin when reduction of FAD takes place by two electrons given by NADPH. The second step involves the transfer of same electron at C-3 position of Enol ether and it finally result in reduction of a vinyl bond by the process of quenching at C-2 position [70]. Active site Ser-229 acts as a source of proton and hence responsible for quenching of the carbanion or the enol intermediates [71].

2.6. Step III: Formation of UDP-MurNAc-peptides

Formation of monomer units involves stepwise addition of different components like L-alanine, D-glutamic acid, diaminopimelic acid (diamino acid) and a dipeptide D-alanine-X (generally X is D-

alanine) to UDP-MurNAc. This conversion involves highly specific enzymes called as Mur synthetase or Mur ligase. The Detail study of all the Mur synthatases (MurC, MurD, MurE, and Mur F) are performed in case of *E. coli* and their identification, cloning, characterization and overproduction are studied in detail. They are present in mra cluster of *E. coli* chromosome at 2 min consist gene for both the important functions, i.e. cell division and peptidoglycan synthesis [72,73]. Besides all the essential Mur synthatases members, other non-essential member is reported like Mpl which has a function to catalyze the addition of tripeptide to UDP-MurNAc in the process of peptidoglycan synthesis [74, 75]. Mur ligase belong to the class of ADP-peptide forming synthase, but the presence of other enzyme like, Cyanophlein synthetase & folylpoly- γ -L-glutamate synthatase (FPGS) belonging to this class were also reported in different studies [76, 77].

Synthesis of peptide subunit involves the sequential addition of different components in four steps. It starts with addition of L-alanine to UDP-MurNAc followed by addition of D-Glutamic acid, diamino acid (diaminopimelic acid) & dipeptide D-alanine-X in presence of MurC, MurD and MurF respectively and end with the formation of UDP-MurNAc-pentapeptide. Details of Mur synthatase are discussed in much depth in the later parts of the present article.

2.7. Step IV: Formation of Lipid Intermediates

Formation of lipid intermediates (Lipid I &II) is a two step process. In the first step MraY catalyses the transfer of phospho-MurNAc-pentapeptide group of UDP-MurNAc-pentapeptide to undecaprenyl phosphate to yield MurNAc-(pentapeptide)-pyrophosphoryl undecaprenol (lipid I). The second step involves the addition of N-acetylglucosamine subunits to this in presence of MraG and leads to formation of GlcNAc-MurNAc-(pentapeptide)-pyrophosphoryl undecaprenol (lipid II) [50].

2.8. Significance of Supportive Pathways

Formation of the peptidoglycan monomer unit is a multistep process and utilizes different substrates like D-Glutamic acid, diamino acid (diaminopimelic acid) & dipeptide D-alanine-X at different levels which are produced from different supportive pathways. Out of all the substrates formation pathways of diaminopimelic acid are reported in different studies [78, 79].

2.9. Sources of Disparity in Structure of Peptidoglycan Monomer Unit

Structural modification in the peptidoglycan monomer unit can take place at different stages of synthesis; before, during or after monomer unit formation. Mur synthetase are known for its substrate specificity, but variation in monomer unit may arise because of changes in amino acid sequence of peptide subunits. Bacteria related factors like mutation, alteration in the growth condition, and exposure to antibiotic treatment also has importance in the study of changes in monomer unit as they alter different parameters of monomer unit like chain length of glycan, degree of cross linking etc. [80].

2.10. Significance of Peptidoglycan Layer

Synthesis of peptidoglycan layer involves several steps which are catalyzed by different Mur ligases like MurC, MurD, MurE and MurF (see Fig. 5). The presence of this enzyme is important from the survival aspect of bacteria and they are absent in all eukaryotic cells, hence they can be utilized as excellent targets for antibacterial drug discovery [81, 82].

Pentapeptide or stem peptide moiety which is attached to the C-3 position of MurNAc is has variation in composition in of different bacteria. In case of Gram-positive bacteria it is made up of L-alanine- γ -D-glutamate-L-lysine-D-alanine-D-alanine while in case of Gram-negative bacteria L-alanine- γ -D-glutamate-diaminopimelate (meso-DAP)-D-alanine-D-alanine [83, 84].

Structure of Lipid II intermediate is complex type & it has two ends, i.e. one non reducing (acceptor) and other growing (donor) end. Formation of the peptidoglycan layer from Lipid II involves two steps: In first step non reducing end of Lipid II is attached to the growing terminal of glycan chain in the presence of glycosyl transferase (GTase) of penicillin binding proteins (PBPs). In second

step pre-existing glycopeptide chain is cross linked with this residue and all this takes place in presence of Transpeptidase (TPase) [83, 84].

2.11. Peptidoglycan Transglycosylation

Peptidoglycan transglycosylation takes place at outer side of cytoplasmic membrane due to Glycosyltransferase (GTase) which perform functions of polymerization of glycan strands. It provides valuable information to understand mechanisms of Lipid II polymerization [85, 86].

2.12. Peptidoglycan Transpeptidation

Transpeptidation occurs as an essential step in peptidoglycan layer formation due to presence of Transpeptidase (TPase) and it performs the function of cross linking of glycan strands [83].

2.13. Peptidoglycan Modification:

The mammalian immune system has complex nature and due to this it can identify several structural features of murein even if the though bacteria can cause a different type of infections. The basic reason behind this type of infection is a structural modification of peptidoglycan which is carried out by bacteria due to modification in glycan strand [87]. For the purpose of protection against lysozyme mediated cleavage, bacteria modify their C-6 position of MurNAc by o-acetylation [88].

2.14. Regulation of Peptidoglycan Biosynthesis

The outer membranes of Gram-negative bacteria consist of specialized lipoproteins LpoA and LpoB & are required for proper functioning of penicillin binding proteins (PBPs) [89, 90]. Recently the role of lipoproteins in activation of peptidoglycan synthetases has been revealed by using fluorescence microscopy techniques [91].

Fig. (5). Different structural features of Mur Ligases enzymes.

3. MUR LIGASES

Mur ligases are the family of enzymes which ligases the bacterial cell walls. They have an important role in the biosynthesis of peptidoglycan hence, their structure, function and dynamic nature is reported in literature. There are six enzymes belonging to this family and they are MurA, MurB, MurC, MurD, MurE and MurF respectively (see Fig. 5). All the four Mur enzymes (MurC-MurF) consist of three domain structures, namely N-terminal, central domain, C-terminal domain which are responsible for binding of UDP-MurNAc, ATPases and GTPases, binding of the incoming amino acid residues respectively [92].

3.1. MurA as Vital Target for Novel Antibacterial Agents

Globally problems of antibiotic resistance are at its alarming stage and to resolve these problems two approaches can be adopted. The first being, safer use of existing antibacterial agents, but this approach is not found to be much permissible hence second approach involve discovery of novel antibacterial agents. An ideal characteristic of any antibacterial drug is that it should have a target which is generally absent in mammals. In case of bacterial peptidoglycan synthesis different steps are involved which are catalyzed by different Mur enzymes, hence inhibitors targeting single or multiple enzymes during Peptidoglycan synthesis can be adopted as a good tool to design novel antibacterial agents.

The Gram negative bacteria consist of low Guanine to Cytosine content and only a copy of murA gene, while Gram positive bacteria consist of two genes (murA & murZ) which are coded for MurA and MurB respectively. It indicates that the presence of both these genes is therefore important for the survival of Gram positive bacteria.

3.2. Important Functional Aspects of MurA

Purification and characterization of more from different bacteria are reported in literature, but detail study of different aspects was carried out using *E. coli* and it is mentioned (see Table 1).

Table 1. Purification and characterization of MurA of different bacteria.

Sr. No.	Purification and characterization of MurA of different bacteria	Reference
1.	<i>E. cloacae</i>	[93]
2.	<i>E. coli</i>	[94-96]
3.	<i>M. tuberculosis</i>	[97]
4.	<i>S. pneumonia</i>	[98]
5.	<i>P. aeruginosa</i>	[112]

Bacterial Peptidoglycan synthesis is a highly complex process and involves a series of steps, but it starts with formation of enolpyruvyl UDP-N-acetylglucosamine (EP-UDP-GlcNAc) by transfer of enolpyruvate from phosphoenolpyruvate (PEP) to the 3'-hydroxyl group of UDP-N-acetylglucosamine (UDP-GlcNAc) and this step is catalyzed by MurA. Apart from fosfomycin all other agents failed to reach the clinical use as antibacterial agents and the main problem associated with most of them was lack of target selectivity [99].

The detail study of MurA enzyme highlights the presence of five amino acid residues and their conservancy in various bacterial species is given (see Table 2).

Table 2. Amino acid residues of MurA enzyme.

Sr. No.	Amino acid residues	Role/ Function Performed	Reference
1.	Lys22 residue	Covalent bond formation with PEP and Fosfomycin	[100,101]
2.	Cys115	Product release and final deprotonation of C-3 atom	[102]
3.	Asp305	Product release and final deprotonation of C-3 atom	[102]
4.	Asp369	Interaction between different antibiotics and MurA enzyme	[103]
5.	Leu370	Interaction between different antibiotics and MurA enzyme	[103]

S. aureus being Gram positive bacteria have both MurA and MurZ enzymes due to presence of murA and murZ genes (murA2 or murAB gene) respectively [94]. A recent study indicates that inhibition of murA gene results into high reduction of peptidoglycan content (upto 26%) as compared to inhibition of murZ gene (only up to 03%). The conclusion drawn from this study was as MurA was more essential cellular enzyme as compared to MurZ [104].

Gram positive and Gram negative bacteria shows similar homology when compared by using amino acid sequence but it was a surprising fact that the crystal structures of Gram negative bacteria were studied as much depth as compared to Gram positive bacterial [104].

3.3. Structural Features of Mur Enzymes

The literature survey indicates that different Mur enzymes are obtained from different bacteria and they show many structural similarities [105, 106]:

- They consist of two domain topologies which have active sites present at their interphase.
- Proportion of α -helices and β -sheets were similar in both the domains.
- Presence of Cys 115 residue at active sites plays a crucial role in functional aspects of the enzyme.

3.4. Inhibitors of MurA

MurA is the enzyme from the Mur ligase family which is involved in the step of conversion of UDP-GlcNAc into UDP-GlcNAc-enolpyruvate in presence of phosphoenol pyruvate (PEP). This step was important as other steps in peptidoglycan biosynthesis are dependent on it. The inhibitors of MurA ligases are as follow:

3.4.1. Benzothioxalone Derivatives

New inhibitors of MurA belonging to series of benzothioxalone derivatives **1-9** have been identified by using HTS approach Fig. (6). Most of them are inhibiting *E. coli* MurA while some are inhibiting MurA and MurZ of *S. aureus* with IC₅₀ values of 0.25 to 18.54 μ M & 1.0 – 51.46 μ M respectively [99].

3.4.2. Cyclic Disulfide, Pyrazolopyrimidine, And Purine Analogues

Baum *et al.* reported three novel inhibitors of *E. coli* MurA by HTS technique to the chemical library & these were a cyclic disul

Fig. (6). Benzothioxalone derivatives.

phide RWJ-3981 (10), a pyrazolopyrimidine RWJ-110192 (11), and a purine RWJ-140998 (12) (see Fig. 7),

Fig. (7). Cyclic disulfide, pyrazolopyrimidines and purine derivatives.

They differ from fosfomycin chemically and also by mechanism of action, out of these three compounds 10-12 show irreversible patterns of inhibition while Compound 11 show reversible patterns of inhibition. All these three compounds are found to be more effective than Fosfomycin as they show 50% minimum inhibitory concentration.

On testing for antibacterial activity the compound shows good result against *S. aureus*, *E. faecalis*, and *E. faecium* which are good, but indicate the nonspecific mechanism of action of bacterial inhibition hence further efforts on the research of these compounds were restricted [107].

3.4.3. Sulfonyloxy Anthranilic Acid Derivatives

Two derivatives from this class T6361 (13) & T6362 (14) are reported by High throughput screening (HTS) as an inhibitor of *E. cloacae* MurA enzyme, these acts by competitive inhibition of UDP-N-acetylmuramoyl-L-alanyl-D-glutamate (UMAG) (see Fig. 8). T6361 (13) differs from Fosfomycin as it shows time dependent inhibition and hence found to act by reversible mode of action.

T6361 (13) causes inhibition between open to close transitions and it does not inhibit active site which is desirable for every inhibitor. Due to this novel mechanism of inhibition, these inhibitors represent a novel class of MurA inhibitors [105, 108].

3.4.4. Imidazole Derivatives

Two new compounds Cpd01 (15) and Cpd2 (16), derivatives of diarylmethane and substituted imidazole respectively, were discovered during whole cell peptidoglycan synthesis assay and found to show successful inhibition of peptidoglycan synthesis (see Fig. 9). Out of these two, only compound 15 were found to be effective (IC_{50} values of $6\mu\text{M}$). Antibacterial and time dependent inhibition was shown by compound 15 but for which it required pre incubation with UNAG. The only problem associated with compound 15 was a higher concentration ($>32.3\mu\text{g/ml}$) required to show antibacterial action, this problem is solved by structural modification of the compound 15 [109].

3.4.5. Aminotetralone Derivatives

Two novel MurA inhibitor compounds 17 & 18 were identified from this class when high throughput screening (HTS) of the compounds was performed by multinational companies (see Fig. 10). These compounds show *E. coli* MurA inhibition with IC_{50} values of 3.1 and $8.5\mu\text{M}$ respectively; in addition, they also inhibit both MurA and MurZ from *S. aureus* (IC_{50} values of 12–23 μM). The inhibitory action shown by them was found to be irreversible.

Fig. (8). Sulfonyloxy-anthranilic acid derivatives.

Fig. (9). Imidazole derivatives.

SAR studies show that α -amino ketone moiety was essential for their inhibitory action. Compounds **17** & **18** were not promiscuous inhibitors as they involved in inactivation of two mammalian enzymes, *i.e.* malate dehydrogenase and chymotrypsin [110, 111].

3.4.6. Peptide Derivatives

In order to identify peptide inhibitors of *P. aeruginosa* two peptide libraries consisting of 109 C-7-C and 12-mer peptides are

Fig. (10). Aminotetralones derivatives.

successfully screened. Four rounds of scanning were performed to screen libraries. The only one peptide showing MurA inhibitory action at a concentration of IC_{50} , 200 μ M was PEP 1354 peptide. It showed time dependent and competitive action. It is the first and only reported peptide inhibitor [112].

Fig. (11). Sesquiterpene lactones derivatives.

3.4.7. Sesquiterpene lactones derivatives

Cnicin (19) and **Cynaropicrin (20)** are the two potent natural inhibitors of MurA belonging to class sesquiterpene lactones (SLs) (see Fig. 11). They show inhibitory action against *E. coli* MurA (IC_{50} =16.7 and 19.5 μ M) and *P. aeruginosa* MurA (IC_{50} =10.3 and 12.1 μ M, respectively). From the SAR studies it can be concluded that the ester side chain of **Cnicin (19)** and **Cynaropicrin (20)** is essential for them to show MurA inhibitory action. Selectivity of these agents belonging to the class was not proven hence more experiments must be performed in that direction [113].

3.4.8. Miscellaneous Inhibitors

3.4.8.1. Thimerosal, Thiram, and Ebselen

It was reported in literature that three compounds, **Thimerosal (21)**, **Thiram (22)**, and **Ebselen (23)** showed inhibitory action against *H. influenza* MurA (IC₅₀ values, 0.1 to 0.7 μ M) during screening of a chemical library. Structurally they differ from fosfomycin as Thimerosal is an ethyl (2-mercaptobenzoato-S) mercury sodium salt, Thiram is a 1-(dimethylthiocarbamoyl-disulfanyl)-N,N-dimethyl- methanethioamide and Ebselen consist of benzoisoselesazolone moiety (see Fig. 12).

Fig. (12). Thimerosal, Thiram and Ebselen.

They differ from fosfomycin not only structurally but also in their mode of action as they binds to active site of MurA in open conformation and fosfomycin binds to the active site of MurA in closed conformation [107].

3.4.8.2. Tulipalines, Tuliposides, and their Derivatives

These are the compounds belonging to the class of natural MurA inhibitors and present in the anther of plants in high concentration. Ubukata & co-workers synthesized (+) -6-Tuliposide B (24). Different other compounds of tulip are reported like; **Tulipaline A (25)** & **Tulipaline B (26)**, and their corresponding glycosides **1-Tuliposide A (27)** & **1-Tuliposide B (28)** were tested for anti MurA property (see Fig. 13).

SAR study indicates that 3', 4'-dihydroxy-2'-methylenebutanoate (DHMB) moiety is responsible for MurA inhibitory action of the compounds of this class. **Cnicin (19)** a potent natural inhibitor of MurA belonging to the class of sesquiterpene lactones (SLs) also consist DHMB moiety hence acts in the similar way as that of tuliposides and its derivatives [114, 115].

3.4.8.3. Terreic Acid

Terreic acid (29) is a fungal metabolite obtained from *Aspergillus terreus* and is reported to show inhibitory action against both *E. cloacae* MurA and *E. coli* MurA. **Terreic acid (29)** shows many similarities like fosfomycin as both consist epoxide ring in their structure, presence of UNAG, covalent binding to thiol group of Cys115 near the active site of MurA. Even after many similarities with fosfomycin they show 50 times less potency for MurA inhibition (see Fig. 14).

Terreic acid (29) has certain features different from fosfomycin as it binds to open conformation of the enzyme while the latter is bound to the closed conformation of the enzyme. Target specificity of **Terreic acid (29)** is questionable as it shows its antibacterial action beside than MurA inhibition [116].

Fig. (13). Tulipalines, tuliposides and their derivatives.

Fig. (14). Terreic acid.

3.4.8.4. Avenaciolides

Recently four avenaciolide derivatives **30-33** were isolated from *Neosartorya fischeri*, out of which three had significant antimicrobial activity against MRSA (see Fig. 15). They show their action by inhibition of peptidoglycan biosynthesis. From SAR Studies it can be concluded that α , β -unsaturated carbonyl group is responsible for their antibacterial activity. They inhibit MRSA and fosfomycin resistant bacteria, hence useful to solve problems of antibiotic resistance [117].

3.5. Inhibitors of MurB

As discussed previously, the MurB enzymes catalyze the synthesis of UDP-Nacetylmuramic acid (UDP-MurNAc) from enolpyruvyl- UDP-N-acetylglucosamine (EP-UNAG). MurB is considered to be one of the enzymes essential for bacterial survival [118]. MurB is an attractive target for antibacterial drug discovery due to the absence of a homologue in eukaryotic cells. X-ray structural analysis of MurB from *S. aureus* [119], *S. pneumoniae* [120], *E. coli* [121-124], have been reported in literature and it is used in designing of different classes of MurB inhibitors [125].

There are different inhibitors of MurB ligases reported like:

Fig. (15). Avenaciolide derivatives.

3.5.1. 4-thiazolidinones Derivatives

2, 3, 5-Trisubstituted-4-thiazolidinones derivatives (34) act as inhibitors of MurB enzyme because overlay of the thiazolidinone core of the diphosphate region is of EP-UNAG substrate is confirmed by X-ray crystallographic Studies [126] (see Fig. 16).

Fig. (16). 4-Thiazolidinones derivatives.

SAR study of generalized 4-thiazolidinone structure indicates that three side chains R₁, R₂ & R₃ are important in the structure of thiazolidinones. Any change in these side chains affects the extent of MurB enzyme inhibition. Most active compounds from this series have been obtained when R₁ chain is substituted with n-butyl group. R₂ Position shows better suitability for aromatic substituents, but when the simple phenyl group is presents at this position no MurB inhibitory action was found. If a bulky side chain like t-butyl-m- phenoxy benzaldehyde is replaced at R₂ position, high degree of MurB enzyme inhibition can be achieved as it is supposed to fill large hydrophobic pockets present in the enzyme. R₃ side chain has very little influence because when it is replaced with methyl or hydrazide moiety it shows lack of activity and from this, generalized conclusion can be made that R₃ side chain plays minor role in the binding of MurB enzyme. Stereochemistry of R₁ was studied by applying chiral amino acids, but the same was not studied for R₂ [126].

3.5.2. 3, 5-Dioxypyrazolidines Derivatives

In a recent study, four compounds from the class of 3, 5-dioxypyrazolidines **35-38** were identified as inhibitors of MurB enzyme by studying their inhibitory activities on *S.aureus* MurB and *E.coli* MurB. These inhibitors show good activity against antibiotic resistant strains of bacteria as they bind to active sites of MurB which are adjacent to FAD cofactor [127].

Fig. (17). General structure of 3, 5-dioxypyrazolidines derivatives.

The inhibitory activities of these compounds **35-38** against soluble peptidoglycan biosynthetic enzymes are summarized (see Fig. 17 & Table 3) [127].

Table 3. 3, 5-Dioxypyrazolidines inhibitors of MurB.

Compound	R ₁	<i>E. coli</i> MurB IC ₅₀ (μM)	<i>S. aureus</i> MurB IC ₅₀ (μM)
35		4.1	4.3
36		5.3	10.3
37		6.8	5.1
38	-	35.0	24.5
Fosfomycin	-	>300	>300

All compounds present in the above table are showing multi target inhibition of different enzymes of Mur family like MurA, MurB and MurC. This multi target inhibition is achieved because Inhibitors of MurB fit into the active sites of MurA, MurB & MurC. From the above table it can be concluded that compounds from the class of 3, 5-dioxypyrazolidines show MurB inhibition, with good antibacterial action and minimize peptidoglycan synthesis [127].

3.5.3. 5-hydroxy-1H-pyrazol-3 (2H) -one Derivative

It is reported in literature that 5-hydroxy-1H-pyrazol-3 (2H) -one act as inhibitor of *Escherichia coli* UDPN- acetyl enolpyruvyl glucosamine reductase (MurB) (see Fig. 18). MurB inhibition potency can be enhanced by increasing lipophilicity of C-4 substitute of 5-hydroxy-1H-pyrazol-3(2H)-one inhibitor. Most of the 5-hydroxy- 1H-pyrazol-3 (2H) -ones inhibit *E.coli* MurB and PG biosynthesis and also show antibacterial activity against Gram positive bacteria (see Table 4) [128].

5-hydroxy-1H-pyrazol-3(2H)-one derivative

Fig. (18). General structure of 5-hydroxy-1H-pyrazol-3(2H)-one derivative.

Table 4. 5-Hydroxy-1H-pyrazol-3 (2H) -one inhibitors of MurB.

Compound	R ₁	R ₂	MurB IC ₅₀ (μm)
39	Ph	(4-Cl) Ph	54
40	Ph	(4-Br) Ph	42
41	Ph	Ph	57
42	(3-Cl) Ph	(4-Cl) Ph	8.6
43	(3-Cl) Ph	(4-CF ₃) Ph	8.7
44	(3,4-Cl) Ph	(4-CH ₃) Ph	4.8
45	(3,4-Cl) Ph	(3,4-Cl) Ph	4.8

3.5.4. Imidazolinones Derivative

From the SAR study of this class it can be concluded that lipophilic substituent at N-1 is essential for good MurB inhibition, which provides whole cell antibacterial activity to them (see Table 5). Compound **51** and **52** have these structural features hence show good MurB Inhibition. Compounds **46** & **47** have less potency as they lack lipophilic group at N-1 position (see Fig. 19) [129].

3.6. Inhibitors of MurC

MurC is cytoplasmic enzyme required in peptidoglycan biosynthesis of phase I reactions and it catalyze ATP-dependent ligation of L-alanine (Ala) and UDP-*N*-acetylmuramic acid (UNAM) to form UDP-*N*-acetylmuramyl-L-alanine (UNAM-Ala). The enzyme is a non-ribosomal peptide ligase, which utilizes ATP to form an amide bond between L-alanine and UNAM. MurC is involved in the synthesis of pentapeptide precursor hence inhibition of MurC cause problems in peptidoglycan biosynthesis [130, 131].

Besides this method, evaluation of kinetic mechanism of *E. coli* uridine diphosphate-*N*-acetylmuramate (UNAM): alanine ligase or MurC gene products are reported by using initial velocity method which involve double reciprocal plot of activity verses substrate concentration [132]. Data collected by initial velocity method is precious as it focuses into the effect on kinetic mechanism of enzyme by changing the substrate concentrations [133, 134]. Specific inhibition patterns of product are also useful in determination of substrate addition and product release rates [135, 136].

There are many inhibitors of MurC ligases reported like

3.6.1. Pyrazolopyrimidines Derivatives

SAR study of different molecules indicates that the *t*-butyl, methyl substituted pyrazole was kept as it is and change in side chain was carried out (see Fig. 20). A few key molecules from this

Imidazolinones derivatives

Fig. (19). General structure of imidazolinone derivatives.

Table 5. Imidazolinones inhibitors of MurB.

Compound	R	R ₁	MurB IC ₅₀ (μm)	MIC (μg/mL)
46	H	H	>118	-
47	CH ₃	CH ₂ CO ₂ H	>101	-
48	H	CH ₃	>115	-
49	H	Allyl	40	4
50	H	Benzyl	60	2
51	H	3,4-dichlorobenzyl	15	4
52	H	2-chlorophenethyl	25	4

series are mentioned (see Table 6). The difference in the IC₅₀ values of all the members of this class is due to the presence of different side chains. Compound **58** shows best activity against MurC, but it is not found to be active against wild type of bacteria [133].

3.6.2. Phosphinates Derivatives

Phosphinate inhibitors are reported (see Table 7) for Mur ligase, as these ligases involve acyl phosphate intermediates in their actions. Compound **65** & **66** are capable to show MurC inhibitory action because of their tight binding with MurC (see Fig. 21). Compound **65** lacks methyl group of lactic acid moiety and in case of compound **66**, hexose group is substituted by 1, 3- propanediol group. Synthesis & biological evaluation of these compounds is reported from their synthetic precursors. An ether linkage in α -position of the phosphinate moiety of compounds **65** & **66** made them potent inhibitors of MurC as compared to other members of same series because formation of this ether linkage was considered as the rate determining factor.

Compounds **65-68** were tested for inhibition of MurC from *E. coli* and it was concluded that compound **65** was most potent with IC₅₀ values of 49 nM. Three folds decrease in activity of compound **65** and observed when des-methyl muramic acid moiety is substituted with flexible 1, 3-propanediol moiety. From SAR study it was clear that the presence of UDP moiety is essential for members of this series to show potent inhibition, compound **67** & **68** are inactive just because of absence of UDP moiety [137].

Fig. (20). General structure of pyrazolopyrimidine derivatives.

Table 6. Pyrazolopyrimidines inhibitors of MurC.

Compounds	R	Pae MurC IC ₅₀ (μ m)	Eco MurC IC ₅₀ (μ m)
53		0.011	0.101
54		0.013	0.188
55		0.001	<0.005
56		0.008	0.011
57		0.007	0.060
58		<0.0005	0.002

59		0.008	0.040
60		0.009	Nd
61		0.004	0.021
62		<0.0005	0.004
63		0.0012	0.015
64		0.008	0.057

3.6.3. Benzylidene Rhodamine Derivatives

Systemic search and discovery of benzylidene rhodamine series as an effective MurC inhibitor by high throughput screening was reported [138]. From this series about 40 compounds were synthesized and tested for their MurC inhibitory potency, out of which only 5 compounds **69-73** were found to show MurC inhibitory action. Compound **69** (removal of a CH₂ group) & **70** (replacement of S with O) showed comparable activity as that of benzylidene rhodamine 1. Compound **71** & **72** showed low potency as compared to benzylidene rhodamine 1 as they consist of different benzylidene substitutes at their C-5 position. If N-3 position of benzylidene rhodamine 1, 3 and 6 is substituted with methyl, ethyl, allyl, aryl, methyl acetyl group it results into the generation of inactive compounds. From this it can be concluded that free NH group may be essential to bind with active site of MurC and hence all N-substituted products are found to be inactive. Substitution at C-5 position does not affect the potency of compound to large extent (see Fig. 22).

Benzylidene rhodamine **69** & **70** were tested for their whole cell inhibitory action and after evaluation it was found that rhodamine **69** & **71** shows optimum inhibition against Gram-positive methicillin resistant, *Staphylococcus aureus* (MRSA). Benzylidene rhodamines are easy to synthesize as their building blocks are available commercially hence found to be an attractive route towards MurC inhibitor discovery [138].

Fig. (21). General structure of phosphinate derivatives.

Table 7. Phosphinates inhibitors of MurC.

Compounds	MIC (IC ₅₀) μm
65	49
66	60
67	Inactive
68	>100

Fig. (22). Benzylidene rhodamine derivatives.

3.7. Inhibitors of Mur D

Mainly four enzymes from class of Mur ligases (MurC, D, E & F) are involved in peptide stem of bacterial peptidoglycan biosynthesis. Out of these four Mur ligases MurD cause ATP-dependent addition of d-glutamic acid to UDPMurNac- l-Ala, a reaction which involves acyl-phosphate and tetrahedral intermediates [139].

E. coli MurD is the ligase, which are studied for three dimensional (3D) structures and after that 3D structure of MurD from other sources and other Mur ligases were determined successfully [140-143].

MurD shows substrate specificity as it causes the addition of second amino acid in the form of d-glutamic acid on the peptide stem of peptidoglycan biosynthesis [144, 145]. Mature peptidoglycan may show minor variations in the amino acid due to the pres-

ence of other Mur ligases [146, 147]. After the study of addition of different amino acids or their derivatives it can be concluded that: (i) α -amino acids should be always present in d configuration. (ii) Presence of the anionic group at C-4 position. (iii) Optimum distance between two acid groups must be maintained [148].

Several nutraceuticals were evaluated and reported for their docking scores. Diosgenin was found to be the best of all the tested Nutraceuticals with the highest activity. Hence it will play a key role in the experimental design and development of mycobacterium tuberculosis MurD [149].

MurD show few compounds like dl-homocysteic acid, d-erythro-3- and -4-methylglutamic acid, and two cyclic analogs of Glu (five- or six-membered ring) as substrate for it, apart from d-Glu [145]. MurD differs from MurC as the former is showing synthesis of adenosine tetraphosphate intermediate while later is not showing such intermediates [146]. The 3D structure of *E. coli* MurD shows presence of three domains. The N-terminal domain is responsible for the binding of UDP-MurNAc-l-Ala, the central domain binds ATP and the C-terminal domain binds d-glutamic acid. Beside this the 3D structure of MurD from *Streptococcus agalactiae* [147] and *Thermotoga maritima* [148] were also reported and the domain arrangement are found to be somewhat similar to *E. coli* MurD.

MurD was present in both open and closed conformation & it depends on C-terminal. If C-terminal domain is rotated towards the centre and then it led to formation of the closed conformation & if C-terminal domain is rotated away from the centre and then it led to formation of the open conformation [150, 151]. *E. coli* MurD was generally expressed in cytoplasm, it had molecular weight of 49.3KDa and a single chain monomer of 486 amino acids length was also observed [152]. Most of the MurD inhibitor synthesized fails to show potency against bacteria as they show poor penetration of bacterial cytoplasm, efflux or non-selective binding to other proteins [153].

The use of computational study approach involves techniques like quantum mechanical/molecular mechanical (QM/MM) molecular approach. It was utilized for MurD ligase from *E. coli* for evaluation of the possible reaction pathways which finally leads to tetrahedral intermediate formation. Comparison between kinetic mechanism and available structural data was carried out by applying the replica path method (RPATh) to generate reaction pathways between initial starting structures and corresponding tetrahedral reaction intermediates. Data generated by this study is found to be helpful for designing of novel MurD ligase inhibitors [154].

There are many inhibitors of MurD ligases reported like

3.7.1. Phosphinate Derivatives

3.7.1.1. Carbamate & Amide Substituted Phosphinates Derivative

From the class of Carbamate & amide substituted phosphinates inhibitors (see Table 8), all the compounds **75-77** are found to be more potent than compound **74** as they have trans-cinnamoyl, 3-hydroxy substituted trans-cinnamoyl and 3, 4-methylenedioxy ring substituted trans-cinnamoyl moiety respectively in their structures. Compound **78** & **79** have methylene group & D-alanyl substitution respectively hence were found to be less potent inhibitors of MurD enzyme. Compounds **80** & **81** show presence of nitrobenzyl sulfonyl moiety in their structures and compound **81** is found to be more potent inhibitor than compound **80**.

3.7.1.2. Sulfonamide Substituted Phosphinates Derivatives

Presences of alanine and glutamate residues are essential for activity of phosphinate inhibitors. Phosphinate inhibitors **74-91** were synthesized and tested for their inhibitory action on MurD of *E. coli*. Results of the study were expressed in terms of residual activity (RA) of the enzyme in presence of 1mM inhibitor (see Fig. 23).

From the class of sulfonamide substituted phosphinates inhibitors compound **82-91** (see Table 9). The two most active compounds from this series are compound **89** & **90**, which consists of o-nitrobenzylsulfonyl and m-nitrobenzyl sulfonyl substituents, respectively (see Fig. 24). All the members from this series were found to be less potent in MurD inhibition than compound **76** [155].

3.7.2. 2-oxoindolinyldene Derivatives

N-(5-(5-nitro-2-oxo-1, 2-dihydro-3H-indol-3-ylidene) 4-oxo-2-thioxo-1, 3-thiazolidin-3-yl) nicotinamide (compound **92**) show potent inhibition of MurD enzymes of *E. coli* and belong to novel class of 2-oxoindolinyldene derivative (see Fig. 25). The credit of discovery of 2-oxoindolinyldene derivative goes to the database at Lek Pharmaceuticals. Out of the three domains of MurD an enzyme compound **92** shows interactions only with N-terminal and central domain but shows no interaction with C-terminal.

Compound **92** differs from other class of MurD inhibitors as pyridine ring present in its structure occupy a place in the central binding domain. The 2-oxoindolinyldene ring present in their structures occupy a larger volume in the Uracil binding pocket as compared to other MurD inhibitors hence compound **92** show a high degree of interactions with the protein residues [156].

3.7.3. Naphthalene-N-sulfonyl-D-glutamic Acid Derivative

Study on Naphthalene-N-sulfonyl-D-glutamic acid derivatives as MurD inhibitors are reported in literature [157]. All the compounds from this series [93-107] show their IC_{50} values ranging between 80 and 600 μ M (see Fig. 26). From SAR study it can be concluded that agent having 2-naphthalene substituted N-sulfonyl-D-glutamic acid and aryl alkyloxy substituents at the 6- and 7-position show the potent inhibitory action as compared to the other members of same series. 6-(aryl) alkyloxy naphthalene side chain is responsible for binding affinity and lipophilicity of the compounds. Naphthalene ring present in inhibitor was responsible for hydrophobic interaction shown by inhibitory activity. IC_{50} values (see Table 10) indicate the inhibitory potential of different members of this class [158].

3.7.4. Macrocyclic Inhibitors

A series of novel macrocyclic compounds [108-115] inhibiting peptidoglycan biosynthesis by inhibiting MurD enzyme are synthesized and reported in the literature by using different techniques of computer based molecular designing. IC_{50} values (see Table 11) indicate the inhibitory potential of different members of this class [159].

3.7.5. N-sulfonyl-glutamic Acid Derivatives

Synthesis and study of inhibitory action of N-sulphonyl-glutamic acid derivative as MurD Inhibitors is reported in literature (see Fig. 28) [160]. The study is based on no-substitution of compounds and its effect on inhibitory potency, i.e. IC_{50} values (see Table 12). Compound **117** is found to be much more potent than its L-stereoisomer compound **116** due to the contribution of the lipophilic tail in the structure. Compound **119** (IC_{50} =105 μ m) becomes most active from this series due to presence of 4-cyano phenyl group in the lipophilic moiety. Compound **121** & **122** are showing moderate MurD inhibitory action. In case of compound **124**, γ -carboxylic group is omitted and due to this, its IC_{50} value also changed around >2000 μ M which is much more than the other members of same series [160].

3.7.6. Coumarin Derivatives

Three derivatives [128-130] are reported from this class of inhibitors and their potency is expressed in terms of residual activity (RA) at a concentration of 100 μ m of the compounds. Residual activity (RA %) values (see Table 13) indicate the inhibitory potential of different members of this class (see Fig. 29) [161].

Fig. (23). General structure of carbamate & amide substituted phosphinate derivatives.

Table 8. Carbamate & amide substituted phosphinates inhibitors of MurD.

Compound	R ₁	Residual Activity (RA %)
74		41±2
75		28±2
76		23±2
77		17±1
78		36±1
79		44±1
80		19±3
81		8±1

Fig. (24). General structure of sulfonamide substituted phosphinate derivatives.**Table 9.** Sulfonamide substituted phosphinates inhibitors of MurD.

Compound	R ₂	RA%
82		54 ± 5
83		52 ± 2
84		55 ± 1
85		40 ± 1
86		49 ± 2
87		50 ± 2
88		37 ± 2
89		30 ± 5
90		22 ± 2
91		472

Fig. (25). 2-oxoindolinyldene derivatives.

Fig. (26). General structure of Naphthalene-N-sulfonyl-D-glutamic acid derivatives.

Table 10. Naphthalene-N-sulfonyl-D-glutamic acid inhibitors of MurD.

Compound	R	IC ₅₀ (μm)
93		590
94		305
95		280
96		170
97		176
98		192
99		630
100		>1000
101		400
102		239
103		132
104		105

(Table 10) Contd....

Compound	R	IC ₅₀ (μm)
105		100
106		85
107		122

Fig. (27). General structure of Macrocyclic inhibitors.

Table 11. Macrocyclic inhibitors of MurD.

Compound	n	Chirality at (*)	X	IC ₅₀ (μm)
108	2	S	H	5.1
109	2	R	H	1.5
110	3	S	H	3.2
111	3	R	H	1.5
112	2	S	OH	2.3
113	2	R	OH	0.7
114	2	S	O ^t Bu	9.0
115	2	R	O ^t Bu	1.9

Fig. (28). General structure of N-sulfonyl-glutamic acid derivatives.

3.7.7. Polycyclic Derivatives

During a structure-based virtual screening, compound **131** of class 9H-xanthene is found which is capable of showing *E. coli* MurD enzyme Inhibition (IC₅₀=10μm). It is found that the compound is among one of the most potent MurD inhibitor (see Fig. **30**) [162].

3.8. Inhibitors of Mur E

Stereochemistry of third position of amino acid in pentapeptide structure is important and responsible to control degree of antibiotic resistance and cell morphology. MurE is the enzyme from the class of Mur ligases and control addition of lysing or diaminopimelate moiety into stem peptides of peptidoglycan structure.

There are many inhibitors of MurE ligases reported like

3.8.1. Inhibitors from *Hypericum Acnosepalum*

Recently, two compounds from the *Hypericum* genus, **Hyperenone A (132)** and **Hypercalin B (133)** are obtained from hexane and chloroform extracts of the different aerial parts of plant *Hypericum acnosepalum*. Both the structures have been studied for NMR and Mass spectroscopy (see Fig. **31**).

Hyperenone A (132) and **Hypercalin B (133)** are capable of showing antibacterial activity against MRSA with MIC values of 2–128 mg/L and 0.5–128 mg/L, respectively. Both agents did not show any action against *E. coli* and have been found to be non-toxic to mammalian macrophage cells. Both have been tested for MurE inhibitory activity against MurE ligase of *M. tuberculosis* and from the study it has been concluded that MurE selectively inhibited by **Hyperenone A (132)** and remains unaffected by **Hypercalin B (133)** [163].

Fig. (29). General structure of coumarin derivatives.

3.8.2. Quinolones Derivatives

Some of the members of the novel N-methyl-2-alkenyl-4-quinolones series are capable to inhibit MurE ligase of *M. tuberculosis* and MRSA. The five compounds **134-138** with IC₅₀ value ranging between 40-200 mM, taken into consideration. Members of

Table 12. N-sulfonyl-glutamic acid inhibitors of MurD.

Compound	R ₁	R ₂	X	Substitution	IC ₅₀ (μm)
116		H	C,C	2,6	710
117		H	C,C	2,6	280
118		H	C,C	2,6	170
119		H	C,C	2,6	105
120		H	C,C	2,6	810
121		H	C,C	2,6	590
122		H	C,C	2,6	305
123		H	C,C	2,6	239
124		H	C,C	2,6	>2000
125		H	C,C	1,5	>1000
126		CH ₃	N,N	2,6	-
127		H	C,C	2,6	-

this series do not show any inhibition against *E. coli* and *P. aeruginosa* (see Fig. 32). Change in the inhibitory potential of all these quinolones is due to different modification carried on alkenyl side chain of structure. Compound **135** & **137** are found to be most potent inhibitors from this series as they show MIC values of 10 μm each [164].

Table 13. Coumarin inhibitors of MurD.

Compound	R ₁	RA%
128		107
129		132
130		111

3.8.3. Aporphine Alkaloid Derivatives

Aporphine alkaloid derivatives are found to inhibit *M. tuberculosis* MurE ligase enzyme at low concentration. Total seven compounds **139-145** reported from these series and compound **139** (3-methoxynordomesticine) show highest activities with low MIC values (see Fig. 33).

Fig. (30). Polycyclic derivatives.

Fig. (31). Inhibitors from Hypericum acmosepalum.

Fig. (32). Quinolones derivatives.

From the study of MurE inhibition pattern of all the compounds it can be concluded that compounds **139-141** had IC_{50} values less than 100 mM. Compound **143** & **144** are moderate inhibitors of MurE Enzyme and compound **142** & **144** has been found to be inactive. Compound **145** has MurE inhibition potency but biggest problem associated with it is of its low selectivity [165].

3.8.4. Isoxazole Derivatives

3-bromo-4, 5-dihydroisoxazole derivatives **146-149** are the special class of MurE enzyme inhibitors of peptidoglycan biosynthesis without any cytotoxicity [166, 167]. Inhibition modes of these derivatives are not clear as it does not clear about how they inhibit the enzyme MurE (see Fig. 34). The C-3 position of the ring is important and any change in the ring substituents causes alteration in the MurE inhibitory potency of the compounds. These compounds are not showing inhibitory action of MurE enzyme, unless they correctly position in the catalytic pockets. From this it can be

concluded that MurE inhibitors with a high degree of selectivity for targets can be developed from the class of inhibitors.

Besides MurE, these derivatives also inhibit several enzymes in the human body, involved in different metabolic pathways like aldehyde-CoA/alcohol dehydrogenase (alcohol metabolism) and pyrroline-5-carboxylate decarboxylase (glutamate and proline metabolism) which is not desirable [168].

3.9. Inhibitors of MurF

D-Ala-D-Ala is an important component for the formation of UDP-N-acetylmuramic acid pentapeptide which is considered as a vital component in the peptidoglycan biosynthesis. The catalytic conversion is brought about in presence of MurF enzyme. Hence MurF provide potential targets for drug design as its inhibition cause inhibition of important steps in the peptidoglycan biosynthesis process [169].

It is reported that highly active form of Glutathione S-transferase (GST) -MurF enzyme fusion of highly active form is obtained by affinity chromatography in single step reaction for study of the kinetic mechanism of MurF in *E. coli* [170]. Glutathione S-transferase (GST)-MurF enzymes separate from each other with the help of a polyglycine spacer. PK-LDH coupled spectrophotometric assay method is used for monitoring of activity of purified fusion protein [171]. However, variations in experimental conditions resulted in conclusion that high concentration of salt causes inhibition of the substrate. Now such conditions are used to study steady state kinetics. Understanding of the kinetic mechanism of such reaction helps in designing framework for novel inhibitors [170,171].

There are many inhibitors of MurF ligases reported like:

3.9.1. Thiazolylaminopyrimidine Derivatives

Baum *et al.* reports three compounds from the class thiazolylaminopyrimidine (see Fig. 35). Compounds **150-152** have IC_{50} values of 7.5, 2.5 & 2.5 μ M. From these values it can be concluded that compounds **151** & **152** were three times more potent than compound **150** [169].

3.9.2. 4-phenylpiperidine Derivative

4-phenylpiperidine (4-PP) show MurF enzyme inhibition with a MIC value of 8-16 μ g/ml and was obtained by pharmacophoric modelling of 8-hydroxyquinolines which itself has limited antibacterial activity. Mechanism of action of **4-phenylpiperidine (153)** is not clearly understood, but was thought to act by interfering with the biosynthesis of cell wall (see Fig. 36). Reduction in the MIC values of **4-phenylpiperidine (153)** can be achieved by using permeability enhancer like polymyxin B nonapeptide (PMBN) [172].

3.9.3. Diarylquinone Derivatives

Two compounds of this class, i.e. compound **154** & **155** had MIC values of 8-16 μ g/ml. Compound **155** has the same structure as the compound **154** except it differs at β - position to quinolone ring (see Fig. 37). The compound **154** & **155** has limited permeability into cells, which minimize the inhibition potential; hence they were not showing inhibitory action against wild type of *E. coli* and Gram-negative bacteria. The issue of this limited permeability would be resolved by the addition of permeability enhancer like polymyxin B nonapeptide (PMBN). Compound **155** acted on gram-positive bacteria like, *S. aureus* MurF and this extra flexibility than compound **154** was due to presence of a phenethyl group in compound **154** [173].

3.9.4. Miscellaneous Inhibitors

Compounds **156** & **157** were the inhibitors of MurF ligase reported from Abbott which were obtained from *S. pneumoniae* with IC_{50} values of 8 μ M & 1 μ M (see Fig. 38) [174, 175].

From SAR study of compound **157** it can be concluded that compound consists of three different rings attached to each other

Fig. (33). Aporphine alkaloid derivatives.

with short linkers. The 3-cyano- 4, 5, 6, 7-tetrahydrobenzo[b] thiophene moiety is present in the structure of compound **157**. Two parts of compound **157** i.e. dichloro-substituted phenyl part and thiophene ring part are attached to each other with amide bond. The phenyl moiety is attached to the morpholine ring by sulfonamide bond. Amide nitrogen plays important role in H-bond formation while oxygen in the morpholine moiety forms weak H-bonds [176].

All the three moieties present here, i.e. Cyano group at the C-3 position of thiophene, a NH amide linker, and the chlorine ortho to the amide linkage are essential for MurF inhibition of compounds **156** & **157**. From this it can be concluded that there is the presence of both cyano group and NH amide linker as they were participating in the main process of the Hydrogen bonding interaction with Mur F ligase enzyme. The presence of chlorine atom at ortho position to amide linker is important as it provides proper orientation to interaction between the amide linker and the phenyl ring [177].

M. O. Taha *et al.* reported a new series of 39 inhibitors of MurF enzyme developed from techniques like QSAR analysis, Pharmacophoric modelling and in-silico screening. Compound **158** is found to be the most potent inhibitor of MurF enzyme as show IC₅₀ value of 0.022 μ M [178].

Stamper *et al.* reported series of MurF inhibitors from which the most potent member was compound **159**, which show IC₅₀ values of 0.022 μ M. From this value it can be concluded that compound **159** has much more potency than other members of the same class.

From the SAR study it can be concluded that structure of compound **159** has three parts, i.e. a central ring, 3-cyanothiophene ring and polar sulfonamide. The central and 3-cyanothiophene ring are attached to each other with small amide linker. The presence of two chlorine atoms on ortho and para position of the central ring helps in its higher potency. 3-cyanothiophene moiety attached to the saturated ring further showed some attachments. Polar sulphonamide group is attached with morpholine ring, and oxygen atom of this ring provides additional chances of interaction between enzyme and the compound [179].

(-) -epigallocatechin gallate (**160**) shows inhibitory action against MurF enzyme with IC₅₀ values of 9 μ M. It was discovered during natural product screening of compounds inhibiting peptidoglycan biosynthesis in bacteria. The presence of many hydroxyl groups in the structure of (-) -epigallocatechin gallate (**160**) has contributed for its MurF inhibition potential [180].

4. MULTITARGET INHIBITORS OF MUR ENZYMES

Biosynthesis of peptidoglycan involves several stages hence it is a complex process involving three different classes of enzyme like cytoplasmic soluble enzymes (MurA-F), membrane bound enzymes (MraY & MraG) and lastly enzymes which are acting externally to cytoplasmic membrane (transglycosylases & transpeptidases). Study of all these is important as they control one or many steps in the process of peptidoglycan biosynthesis [181]. Design of

Fig. (34). Isoxazole derivatives. **Fig. (35).** Thiazolylaminopyrimidine derivatives. **Fig. (36).** 4-phenylpiperidine derivative.

Fig. (37). Diarylquinone derivatives.

Fig. (38). Miscellaneous inhibitors of Mur F.

inhibitors of multiple Mur enzymes is important as it works according to multi-target hypothesis and it minimizes the incidence of drug resistance [182].

Different Multitarget inhibitors of Mur Enzyme are reported like

4.1. Naphthyl Tetronic Acid Derivatives

Mansour *et al.* 2007 has prepared & evaluated 11 derivatives from class of naphthyl tetronic acid for their Mur inhibitory potential against multiple Mur enzymes. All derivatives of this class were prepared in the process which involve three steps and for this 3-

bromo-4-methoxy-5H-furan- 2-one and suitably substituted aldehydes were used as precursors.

From the SAR study it can be concluded that C3 α & C5 β substitution is favourable. Broad spectrum activity for the Mur enzymes inhibition which is highly desirable can be obtained by replacement of naphthyl group with p-chlorophenyl substitution. Further changes at C5 α methylidene moiety leads into generation of new compounds; has the potential to inhibit set of all nine enzymes used to assess Mur inhibition potential of these agents.

It can be thus concluded that compounds **161** & **162** show highest Mur inhibition potential with lowest MIC values (see Fig. 39) [183]. Interaction between the three portions of the compound, the furanone core, the naphthyl moiety, and chlorophenyl side chain are determined at a resolution of 2.5 K. Compounds **161** & **162** show Mur A-Mur E inhibitory action [184].

Fig. (39). Naphthyl tetronic acid derivatives.

4.2. Furan-based Benzene Mono- and Dicarboxylic Acid Derivatives

Inhibition of MurC-F by furan based benzene mono and dicarboxylic acid derivatives were also reported. From the series of these compounds, only compound **163** & **164** (see Fig. 40) show significant MurC-F inhibition activity with lowest IC₅₀ values (see Table 14) [185].

Fig. (40). General structure of Furan-based benzene-1,3-dicarboxylates derivatives.

By using structure based virtual screening approach, the novel class of glutamic acid derivatives, *i.e.* benzene 1, 3-dicarboxylic acid derivatives were reported when MurD crystal structure has been co-crystallized with N-sulfonyl glutamic acid inhibitors. From this series compound **165** & **166** are found to possess dual MurD &

Table 14. Furan based benzene-1,3-dicarboxylates inhibitors of Mur ligase enzyme.

Compound	R ₁	MurC (IC ₅₀)	MurD (IC ₅₀)	MurE (IC ₅₀)	MurF (IC ₅₀)
163		94 μM	92 μM	32 μM	12 μM
164		46 μM	95 μM	272 μM	19 μM

MurE inhibitory activity (see Fig. 41). Compound **165** consists of furan linker which joins rhodamine moiety to the benzene 1, 3-dicarboxylate and hence dual inhibitor of MurD (IC₅₀=270 μm) & MurE (IC₅₀=32 μm). Compound **166** also shows dual inhibition MurD (IC₅₀=690 μm) & MurE (IC₅₀=89 μm) and preferably less favorable than compound **165** as it consist 2-thioxodihydropyrimidine-4, 6-dione moiety [186].

Fig. (41). benzene-1,3-dicarboxylates derivatives.

A novel series of benzene-1, 3-dicarboxylic acid 2, 5-dimethylpyrrole derivatives has been reported showing compound **167** with highest activity against multiple bacterial mur ligases (MurC-MurF). It also shows inhibition against MurC, MurD, MurE and

MurF at IC_{50} values of 41 μ m, 60 μ m, 93 μ m, 89 μ m respectively. As per SAR study it has been found that substituted benzene moiety must be present at R_1 position to show inhibitory activity against multiple bacterial mur ligases (MurC-MurF). This multiple bacterial mur ligases (MurC-MurF) activity persist due to coupling of five membered rhodamine moiety with benzene-1, 3-dicarboxylic acid 2, 5-dimethylpyrrole ring [187].

4.3. N-acylhydrazones Derivatives

Synthesis and inhibitory potential of N-acylhydrazone compound series is reported in the literature which involve a series of compound inhibiting MurC and MurD with IC_{50} values in the range of 100-300 μ m [188]. Compound **168** has been the most potent inhibitor from the 37 members reported from this class as it possess 2, 3, 4-trihydroxyphenyl group at R_2 position and had IC_{50} values of 123 μ m and 230 μ m for MurC and MurD respectively (see Fig. 42).

Fig. (42). N-Acylhydrazones derivatives.

4.4. Thiazolidinone Derivatives

4.4.1. 4-thiazolidinone Derivatives

Synthesis, characterization and evaluation of six new 4-thiazolidinone derivatives against gram positive and gram negative bacterial strain is reported [189]. All the compounds show MIC values in the range of 100-400 μ g/ml. Compounds **169** & **170** with lowest MIC values were most potent members of this series (see Fig. 43) [189].

Fig. (43). 4-thiazolidinone derivatives.

4.4.2. 5-benzylidenethiazolidin-4-ones Derivatives

Several compounds belonging to the class of hydroxy-substituted 5-benzylidenethiazolidin-4-ones also were synthesized and evaluated for their Mur ligases inhibitory potential [190]. Compound **171** is found to be the most potent member of this series as it inhibits MurD, MurE and MurF with IC_{50} values of 2 mm, 2 mm and 6 mm respectively, thus showing multiple target inhibition of the Mur ligases enzyme. Apart from antibacterial activity other studies, like NMR, genotoxicity and mutagenicity were carried out on the compound **171**. It has been found to be insoluble in toluene. In order to reduce exocyclic bond hydroxyl groups, compound **171** has been acetylated by using acetic anhydride (see Fig. 44). From

the SAR studies, it would be concluded that trihydroxyphenyl substituted Rhodamine moieties present in compound **171** were essential for activity. Substitution of this molecule by any other moiety causes reduction in the inhibitory potency for MurD.

Fig. (44). 5-benzylidenethiazolidin-4-ones derivatives.

4.5. 5-amido-1-(2, 4-dinitrophenyl)-1H-4-pyrazolecarbonitriles Derivatives

Members of class 5-amido-1-(2, 4-dinitrophenyl)-1H-4-pyrazolecarbonitriles were known to show antibacterial activity against some of the gram positive strain of bacteria. Their synthesis and SAR studies are reported in literature [191]. Compounds **172** & **173** (see Fig. 45) of this class show good antibacterial property which has been due to large polarizable, and electron rich substituted benzamide moieties present in their structures.

Fig. (45). 5-amido-1-(2, 4-dinitrophenyl)-1H-4-pyrazolecarbonitriles derivatives.

4.6. 5-Arylidene-thiazolidine-2, 4-dione Derivatives

Beside antibacterial activities thiazolidines show other activities. Synthesis & evaluation of new derivatives are reported from this class with substitution at position 5 of thiazolidine nucleus. All the members of this series were shown antimicrobial activity with MIC values ranging from 2-16 μ g/mL. Compound substituted with arylidene group at position 5 showed remarkable antibacterial action. Compound **174**, (see Fig. 46) where R is 3-methoxy or 4-hydroxy group has been found to be the best compound from all the members as it has least MIC values [192].

Fig. (46). 5-Arylidene-thiazolidine-2,4-dione derivatives.

4.7. Phosphorylated hydroxyethylamines Derivatives

Phosphorylated hydroxyethylamines were known for their Mur ligases inhibition (MurC to MurF). They show IC_{50} values in the micro molar range which make them ideal inhibitors of Mur ligases. The best inhibitors, 1-(4-methoxyphenylsulfonamido)-3-morpholinopropan-2-yl dihydrogen phosphate (**175**) from this series show presence of methoxy group at para position of the benzene ring (see Fig. 47). From SAR study it has been concluded that the phosphate group attached to the hydroxyl moiety of the hydroxyethylamine core is required by compounds of this series to show good antibacterial activity [193].

Fig. (47). Phosphorylated hydroxyethylamines derivatives.

4.8. Miscellaneous

4.8.1. Feglymycin

Feglymycin (176) isolation and synthesis was reported in literature [194]. **Feglymycin (176)** affects only on the initial steps in the biosynthesis of peptidoglycan and it does not have any influence on later on steps (see Fig. 48). **Feglymycin (176)**, a 13-mer peptide and its structure consist of several non proteinogenic amino acids. Even though mechanism of **Feglymycin (176)** is not clear, it can be concluded that it has been the first natural inhibitor of MurC enzyme. Besides MurC it could also inhibit MurA to some extent, but it tells that MurC is the main target for a **Feglymycin (176)** which has 10 times more sensitive for MurC than MurA [195].

Fig. (48). Feglymycin.

4.9. Patents On Mur Inhibitors

Details of patents, their description and references for different Mur inhibitors are discussed in this section (see Table 15). Inhi-

tors of Mur A-1 polypeptides are essential as they are used in anti-bacterial therapy. Procedure for the production of MurA-1 polypeptides and detection method for any other polypeptides having structural homology with MurA-1 present in the hosts has been already reported. Mur A-1 polypeptides Inhibitors can be used to treat diseases of bacterial origin as they have the ability to inhibit bacterial cell wall formation [196].

Most of the inhibitors act on crosslinking steps in peptidoglycan biosynthesis but rather than this inhibition of the different steps involved in the stem peptides are desirable. Process of isolation of purified MurA protein from *S. pneumoniae* also has been reported in literature. Such MurA protein can be utilized for identification of novel MurA inhibitors. By detail study of structure of MurA protein, novel inhibitors of MurA were developed by structure based drug design approach [197]. Methods for production of MurA polypeptide has been already reported and successfully utilized in screening methods for antibacterial compound drug design [198].

El-Sherbeini *et al.* has reported isolation, purification, nucleic acid sequence and methods for detection of inhibitors of MurC protein from *P. aeruginosa*. Amount of MurC in peptidoglycan biosynthesis can be modified by the application of special compound i.e. modulator. The modulator has the ability to affect vital processes like transcription, translation, post translational folding of proteins. Mostly antisense strands were used as modulators for this approach [199].

Hoskins *et al.* reported a method for MurD protein production and detection kit for identification of inhibitors of MurD enzyme of *S. pneumoniae*. For this they have made use of vectors which causes transformation in the host cells. Now in such transformed host cells MurD enzyme has been expressed which is isolated in its pure form and used for detection of inhibitors of the same enzyme. This MurD protein can be prepared by recombinant method which is tedious as it involves several steps [200]. In another patents Hoskins *et al.* has also reported about MurD genes and compounds encoded by this gene from *S. pneumoniae* [201].

Fig. (49). Enramycin B.

El-Sherbeini *et al.* has reported isolation, purification, nucleic acid sequence and methods for detection of inhibitors of MurD protein from *S. aureus*, *S. pyogenes* & *P. aeruginosa*. He also mentioned about modulators that can be used for MurD expression and assay procedures for MurD inhibitors [202-204].

Table 15. Patents on Mur Inhibitors.

Sr. No.	Patent Number	Description	References
1.	US 6,228,627 B1	Procedure for production of MurA-1 polypeptides and method for identification of its structural homologues	[196]
2.	USO05691161A	Method for isolation of purified MurA protein from <i>S. pneumoniae</i>	[197]
3.	US 6,346,396 B1	MurA polypeptide production process & its use in screening assay of antibacterial compounds	[198]
4.	US 7,022,829 B1	Isolation, purification, nucleic acid sequence and methods for detection of inhibitors of MurC protein from <i>P. aeruginosa</i> and role of modulator in detection of novel inhibitors.	[199]
5.	US 5,681,694	Method for MurD protein production and detection kit for identification of inhibitors of MurD enzyme of <i>S. pneumoniae</i>	[200]
6.	US 5,834,270	MurD genes and compound encoded by this gene from <i>S. pneumoniae</i>	[201]
7.	US 6,534,284 B1 US 6,746,858 B1 US 6,890,910 B1	Isolation, purification, nucleic acid sequence and methods for detection of inhibitors of MurD protein from <i>S. aureus</i> , <i>S. pyogenes</i> & <i>P. aeruginosa</i>	[202-204]

4.10. Mur Inhibitors in Clinical Trials

4.10.1. Enramycin B

Enramycin B (Enduracidin B) is the molecule of synthetic origin and act as MurG inhibitor which belong to class of Lipodepsipeptide. It inhibits peptidoglycan biosynthesis and act against multidrug resistance gram positive bacteria. This compound is still in development stage (see Fig. 49) [205].

CONCLUSION

Peptidoglycan biosynthesis pathway has become attractive target since last decades for antibacterial drug discovery. Among the different enzymes involved in peptidoglycan biosynthesis pathway, Mur ligase family has gained much attention of researchers because of absence of human analogue. Clear understanding of the structures of all Mur enzymes has motivated the researchers to develop new inhibitors which result into number of chemical classes of Mur inhibitors. Some findings of structural similarities of Mur enzymes encouraged development of new multiple target inhibitors of Mur enzymes. Some molecules in clinical trials would encourage our hope for some new antibacterial agents in market in coming years.

LIST OF ABBREVIATIONS

Alr	= Alanine racemase (EC 5.1.1.1)
Ddl	= D-Ala- D-Ala ligase (EC 6.3.2.4)
DHMB	= 3', 4'-Dihydroxy-2'-methylenebutanoate
EP-UNAG	= Enolpyruvyl- UDP- <i>N</i> -acetylglucosamine
FAD	= Flavin adenine dinucleotide
FPGS	= Folylpolyl- γ -L-glutamate synthatases
GTase	= Glycosyltransferase (EC 2.4.1.129)
GlmS	= L-glutamine:D-fructose-6-phosphate amidotransferase (EC 2.6.1.16)
GlmM	= Phosphoglucosamine mutase (EC 5.4.2.10)
GlmU	= Bifunctional enzyme, which includes <i>N</i> -acetylglucosamine-1-phosphate uridyltransferase (EC 2.7.7.23) and glucosamine-1-phosphate <i>N</i> -acetyl-transferase (EC 2.3.1.157)
Lipid I	= MurNAc-(pentapeptide)-pyrophosphoryl undecaprenol

Lipid II	= GlcNAc-MurNAc-(pentapeptide)- pyrophosphoryl undecaprenol
Mpl	= Murein peptide ligase
MRSA	= Methicillin resistant <i>S. aureus</i>
MraY	= UDP- <i>N</i> -acetylmuramyl-pentapeptide phototransferase (EC 2.7.8.13)
MurA	= UDP- <i>N</i> -acetylglucosamine enolpyruvyl transferase (EC 2.5.1.7)
MurB	= UDP- <i>N</i> -acetylenolpyruvylglucosamine reductase (EC 1.1.1.158)
MurC	= UDP- <i>N</i> -acetylmuramoyl-L-alanine synthetase (EC 6.3.2.8)
MurD	= UDP- <i>N</i> -acetylmuramoyl-L-alanyl-D-glutamate synthetase (EC 6.3.2.9)
MurE	= UDP- <i>N</i> -acetylmuramyl-tripeptide synthetase (EC 6.3.2.13)
MurF	= UDP- <i>N</i> -acetylmuramyl-pentapeptide synthetase (EC 6.3.2.10)
MurG	= UDP- <i>N</i> -glucosamine- <i>N</i> -acetylmuramyl- (pentapeptide) pyrophosphoryl-undecaprenol <i>N</i> -acetyl glucosamine transferase (EC 2.4.1.227)
MurI	= Glutamate racemase (EC 5.1.1.3)
NADPH	= Nicotinamide adenine dinucleotide phosphate
PBP	= Penicillin binding proteins
PEP	= Phosphoenolpyruvate
PG	= Peptidoglycan
PLP	= Pyridoxal 5'-phosphate
PMBN	= Polymyxin B nonapeptide
PMB	= <i>p</i> -Methoxybenzyl protecting group
4-PP	= 4-Phenylpiperidine
SLs	= Sesquiterpene lactones
TPs	= Transpeptidase
UDP-MurNAc-	= UDP- <i>N</i> -acetylmuramyl-pentapeptidepentapeptide
UDP-GlcNAc	= UDP- <i>N</i> -acetylglucosamine

UDP-MurNAc	=	UDP- <i>N</i> -acetylmuramic acid
UMAG	=	UDP- <i>N</i> -acetylmuramoyl-L-alanyl-D-glutamate
UNAG	=	UDP- <i>N</i> -acetylglucosamine

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

- Rachakonda S, Cartee L. Challenges in antimicrobial drug discovery and the potential of nucleoside antibiotics. *Curr Med Chem* 2004; 11(6): 775-93.
- Chambers HF, Sande MN. Goodman and Gilman's The Pharmacological Basis of Therapeutics 1996.
- Schentag JJ. Correlation of pharmacokinetic parameters to efficacy of antibiotics: relationships between serum concentrations, MIC values, and bacterial eradication in patients with gram-negative pneumonia. *Scand J Infect Dis Suppl* 1990; 74: 218-34.
- Sefton AM. Mechanisms of antimicrobial resistance: their clinical relevance in the new millennium. *Drugs* 2002; 62(4): 557-66.
- Projan SJ, Shlaes DM. Antibacterial drug discovery: is it all downhill from here? *Clin Microbiol Infect* 2004; 10(Suppl. 4): 18-22.
- Powers JH. Antimicrobial drug development--the past, the present, and the future. *Clin Microbiol Infect* 2004; 10(Suppl. 4): 23-31.
- National nosocomial infections surveillance (NNIS) report, data summary from October 1986-April 1996, issued May 1996. A report from the National Nosocomial Infections Surveillance (NNIS) system. *Am J Infect Control* 1996; 24(5): 380-8.
- Dancer SJ. The problem with cephalosporins. *J Antimicrob Chemother* 2001; 48(4): 463-78.
- Shlaes D, Levy S, Archer G. Antimicrobial resistance: new directions. *ASM American Society for Microbiology News* 1991; 57: 455-8.
- Lederberg J, Shope RE, Oaks SC Jr, Eds. Emerging Infections: Microbial Threats to Health in the United States 1992.
- Kesselheim AS, Outtersen K. Improving antibiotic markets for long-term sustainability. *Yale J Health Policy Law Ethics* 2011; 11(1): 101-67.
- Carmeli Y, Eliopoulos G, Mozaffari E, Samore M. Health and economic outcomes of vancomycin-resistant enterococci. *Arch Intern Med* 2002; 162(19): 2223-8.
- Sunenshine RH, Wright MO, Maragakis LL, et al. Multidrug-resistant *Acinetobacter* infection mortality rate and length of hospitalization. *Emerg Infect Dis* 2007; 13(1): 97-103.
- Capitano B, Leshem OA, Nightingale CH, Nicolau DP. Cost effect of managing methicillin-resistant *Staphylococcus aureus* in a long-term care facility. *J Am Geriatr Soc* 2003; 51(1): 10-6.
- Theuretzbacher U. Future antibiotics scenarios: is the tide starting to turn? *Int J Antimicrob Agents* 2009; 34(1): 15-20.
- Theuretzbacher U. Resistance drives antibacterial drug development. *Curr Opin Pharmacol* 2011; 11(5): 433-8.
- Piddock LJ. The crisis of no new antibiotics--what is the way forward? *Lancet Infect Dis* 2012; 12(3): 249-53.
- Spellberg B, Powers JH, Brass EP, Miller LG, Edwards JE Jr. Trends in antimicrobial drug development: implications for the future. *Clin Infect Dis* 2004; 38(9): 1279-86.
- DiMasi JA, Hansen RW, Grabowski HG, Lasagna L. Research and development costs for new drugs by therapeutic category. A study of the US pharmaceutical industry. *Pharmacoeconomics* 1995; 7(2): 152-69.
- Mehl B, Santell J. Projecting future drug expenditures--2001. *Am J Health Syst Pharm* 2001; 58(2): 125-33.
- Falagas ME, Fragoulis KN, Karydis I. A comparative study on the cost of new antibiotics and drugs of other therapeutic categories. *PLoS One* 2006; 1: e11.
- DiMasi JA, Hansen RW, Grabowski HG. The price of innovation: new estimates of drug development costs. *J Health Econ* 2003; 22(2): 151-85.
- Peck CC. Drug development: improving the process. *Food Drug Law J* 1997; 52(2): 163-7.
- Keim KL, Calcagno J, Leventer SM, Harris HW. Unlocking the value of failed CNS trials. *Curr Drug Disc* 2003; 3: 29-33.
- Dimasi JA. Risks in new drug development: approval success rates for investigational drugs. *Clin Pharmacol Ther* 2001; 69(5): 297-307.
- Singh SB, Barrett JF. Empirical antibacterial drug discovery--foundation in natural products. *Biochem Pharmacol* 2006; 71(7): 1006-15.
- Butler MS. The role of natural product chemistry in drug discovery. *J Nat Prod* 2004; 67(12): 2141-53.
- Newman DJ, Cragg GM, Snader KM. Natural products as sources of new drugs over the period 1981-2002. *J Nat Prod* 2003; 66(7): 1022-37.
- Clardy J, Fischbach MA, Walsh CT. New antibiotics from bacterial natural products. *Nat Biotechnol* 2006; 24(12): 1541-50.
- Penesyan A, Kjelleberg S, Egan S. Development of novel drugs from marine surface associated microorganisms. *Mar Drugs* 2010; 8(3): 438-59.
- Haefner B. Drugs from the deep: marine natural products as drug candidates. *Drug Discov Today* 2003; 8(12): 536-44.
- Blunt JW, Copp BR, Hu WP, Munro MH, Northcote PT, Prinsep MR. Marine natural products. *Nat Prod Rep* 2009; 26(2): 170-244.
- Schumann P. 5- Peptidoglycan Structure. *Methods Microbiol* 2011; 38: 101-29.
- Egan AJ, Biboy J, van't Veer I, Breukink E, Vollmer W. Activities and regulation of peptidoglycan synthases. *Philos Trans R Soc Lond B Biol Sci* 2015; 370(1679): 20150031.
- van Heijenoort J. Recent advances in the formation of the bacterial peptidoglycan monomer unit. *Nat Prod Rep* 2001; 18(5): 503-19.
- Pazos M, Peters K, Vollmer W. Robust peptidoglycan growth by dynamic and variable multi-protein complexes. *Curr Opin Microbiol* 2017; 36: 55-61.
- Bouhss A, Trunkfield AE, Bugg TD, Mengin-Lecreux D. The biosynthesis of peptidoglycan lipid-linked intermediates. *FEMS Microbiol Rev* 2008; 32(2): 208-33.
- Rogers HJ, Perkins HR, Ward JB. Microbial cell walls and membranes 1980.
- Goodell EW, Schwarz U. Release of cell wall peptides into culture medium by exponentially growing *Escherichia coli*. *J Bacteriol* 1985; 162(1): 391-7.
- Young KD. Bacterial shape. *Mol Microbiol* 2003; 49(3): 571-80.
- Schleifer KH, Kandler O. Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* 1972; 36(4): 407-77.
- Chopra I. Molecular mechanisms involved in the transport of antibiotics into bacteria. *Parasitology* 1988; 96(Suppl.): S25-44.
- Hogan D, Kolter R. Why are bacteria refractory to antimicrobials? *Curr Opin Microbiol* 2002; 5(5): 472-7.
- Meeske AJ, Riley EP, Robins WP, et al. SEDS proteins are a widespread family of bacterial cell wall polymerases. *Nature* 2016; 537(7622): 634-8.
- Cho H, Wivagg CN, Kapoor M, et al. Bacterial cell wall biogenesis is mediated by SEDS and PBP polymerase families functioning semi-autonomously. *Nat Microbiol* 2016; 1: 16172.
- Fleming A. Classics in infectious diseases: on the antibacterial action of cultures of a penicillium, with special reference to their use in the isolation of *B. influenzae* by Alexander Fleming, Reprinted from the British Journal of Experimental Pathology 10:226-236, 1929. *Rev Infect Dis* 1980; 2(1): 129-39.
- Salton MR, Horne RW. Studies of the bacterial cell wall. II. Methods of preparation and some properties of cell walls. *Biochim Biophys Acta* 1951; 7(2): 177-97.
- Anderson JS, Matsushashi M, Haskin MA, Strominger JL. Biosynthesis of the peptidoglycan of bacterial cell walls. II. Phospholipid carriers in the reaction sequence. *J Biol Chem* 1967; 242(13): 3180-90.
- Izaki K, Matsushashi M, Strominger JL. Glycopeptide transpeptidase and D-alanine carboxypeptidase: penicillin-sensitive enzymatic reactions. *Proc Natl Acad Sci USA* 1966; 55(3): 656-63.
- van Heijenoort J. Recent advances in the formation of the bacterial peptidoglycan monomer unit. *Nat Prod Rep* 2001; 18(5): 503-19.
- Tiyanont K, Doan T, Lazarus MB, Fang X, Rudner DZ, Walker S. Imaging peptidoglycan biosynthesis in *Bacillus subtilis* with fluorescent antibiotics. *Proc Natl Acad Sci USA* 2006; 103(29): 11033-8.

- [52] Ward JB. Biosynthesis of peptidoglycan: points of attack by wall inhibitors. *Pharmacol Ther* 1984; 25(3): 327-69.
- [53] Bugg TD, Walsh CT. Intracellular steps of bacterial cell wall peptidoglycan biosynthesis: enzymology, antibiotics, and antibiotic resistance. *Nat Prod Rep* 1992; 9(3): 199-215.
- [54] Ghuysen JM, Hakenbeck R. Cell-wall-associated proteins in Gram-positive bacteria. *Bacterial cell wall* 1994; 27: 217.
- [55] Mengin-Lecreulx D, van Heijenoort J. Characterization of the essential gene *glmM* encoding phosphoglucosamine mutase in *Escherichia coli*. *J Biol Chem* 1996; 271(1): 32-9.
- [56] Walsh CT. Enzymes in the D-alanine branch of bacterial cell wall peptidoglycan assembly. *J Biol Chem* 1989; 264(5): 2393-6.
- [57] Wu HC, Wu TC. Isolation and characterization of a glucosamine-requiring mutant of *Escherichia coli* K-12 defective in glucosamine-6-phosphate synthetase. *J Bacteriol* 1971; 105(2): 455-66.
- [58] Badet B, Vermoote P, Haumont PY, Lederer F, LeGoffic F. Glucosamine synthetase from *Escherichia coli*: purification, properties, and glutamine-utilizing site location. *Biochemistry* 1987; 26(7): 1940-8.
- [59] Badet-Denisot MA, Fernandez-Herrero LA, Berenguer J, Ooi T, Badet B. Characterization of L-glutamine:D-fructose-6-phosphate amidotransferase from an extreme thermophile *Thermus thermophilus* HB8. *Arch Biochem Biophys* 1997; 337(1): 129-36.
- [60] Isupov MN, Obmolova G, Butterworth S, *et al*. Substrate binding is required for assembly of the active conformation of the catalytic site in Ntn amidotransferases: evidence from the 1.8 Å crystal structure of the glutaminase domain of glucosamine 6-phosphate synthase. *Structure* 1996; 4(7): 801-10.
- [61] Teplyakov A, Obmolova G, Badet-Denisot MA, Badet B, Polikarpov I. Involvement of the C terminus in intramolecular nitrogen channeling in glucosamine 6-phosphate synthase: evidence from a 1.6 Å crystal structure of the isomerase domain. *Structure* 1998; 6(8): 1047-55.
- [62] Jolly L, Ferrari P, Blanot D, Van Heijenoort J, Fassy F, Mengin-Lecreulx D. Reaction mechanism of phosphoglucosamine mutase from *Escherichia coli*. *Eur J Biochem* 1999; 262(1): 202-10.
- [63] De Reuse H, Labigne A, Mengin-Lecreulx D. The *Helicobacter pylori* *ureC* gene codes for a phosphoglucosamine mutase. *J Bacteriol* 1997; 179(11): 3488-93.
- [64] Jolly L, Wu S, van Heijenoort J, de Lencastre H, Mengin-Lecreulx D, Tomasz A. The *femR315* gene from *Staphylococcus aureus*, the interruption of which results in reduced methicillin resistance, encodes a phosphoglucosamine mutase. *J Bacteriol* 1997; 179(17): 5321-5.
- [65] Tavares IM, Jolly L, Pompeo F, *et al*. Identification of the *Pseudomonas aeruginosa* *glmM* gene, encoding phosphoglucosamine mutase. *J Bacteriol* 2000; 182(16): 4453-7.
- [66] Jolly L, Ferrari P, Blanot D, Van Heijenoort J, Fassy F, Mengin-Lecreulx D. Reaction mechanism of phosphoglucosamine mutase from *Escherichia coli*. *Eur J Biochem* 1999; 262(1): 202-10.
- [67] Mengin-Lecreulx D, van Heijenoort J. Copurification of glucosamine-1-phosphate acetyltransferase and N-acetylglucosamine-1-phosphate uridylyltransferase activities of *Escherichia coli*: characterization of the *glmU* gene product as a bifunctional enzyme catalyzing two subsequent steps in the pathway for UDP-N-acetylglucosamine synthesis. *J Bacteriol* 1994; 176(18): 5788-95.
- [68] Gehring AM, Lees WJ, Mindiola DJ, Walsh CT, Brown ED. Acetyltransferase precedes uridylyltransferase in the formation of UDP-N-acetylglucosamine in separable active sites of the bifunctional *GlmU* protein of *Escherichia coli*. *Biochemistry* 1996; 35(2): 579-85.
- [69] Moraes GL, Gomes GC, Monteiro de Sousa PR, *et al*. Structural and functional features of enzymes of *Mycobacterium tuberculosis* peptidoglycan biosynthesis as targets for drug development. *Tuberculosis (Edinb)* 2015; 95(2): 95-111.
- [70] Benson TE, Marquardt JL, Marquardt AC, Etzkorn FA, Walsh CT. Overexpression, purification, and mechanistic study of UDP-N-acetylenolpyruvylglucosamine reductase. *Biochemistry* 1993; 32(8): 2024-30.
- [71] Benson TE, Walsh CT, Massey V. Kinetic characterization of wild-type and S229A mutant *MurB*: evidence for the role of Ser 229 as a general acid. *Biochemistry* 1997; 36(4): 796-805.
- [72] Hara H, Yasuda S, Horiuchi K, Park JT. A promoter for the first nine genes of the *Escherichia coli* *mra* cluster of cell division and cell envelope biosynthesis genes, including *ftsI* and *ftsW*. *J Bacteriol* 1997; 179(18): 5802-11.
- [73] Mengin-Lecreulx D, Ayala J, Bouhss A, van Heijenoort J, Parquet C, Hara H. Contribution of the *Pmra* promoter to expression of genes in the *Escherichia coli* *mra* cluster of cell envelope biosynthesis and cell division genes. *J Bacteriol* 1998; 180(17): 4406-12.
- [74] Mengin-Lecreulx D, van Heijenoort J, Park JT. Identification of the *mpl* gene encoding UDP-N-acetylmuramate: L-alanyl-gamma-D-glutamyl-meso-diaminopimelate ligase in *Escherichia coli* and its role in recycling of cell wall peptidoglycan. *J Bacteriol* 1996; 178(18): 5347-52.
- [75] Park JT, Raychaudhuri D, Li H, Normark S, Mengin-Lecreulx D. MppA, a periplasmic binding protein essential for import of the bacterial cell wall peptide L-alanyl-gamma-D-glutamyl-meso-diaminopimelate. *J Bacteriol* 1998; 180(5): 1215-23.
- [76] Eveland SS, Pompliano DL, Anderson MS. Conditionally lethal *Escherichia coli* murein mutants contain point defects that map to regions conserved among murein and folyl poly-gamma-glutamate ligases: identification of a ligase superfamily. *Biochemistry* 1997; 36(20): 6223-9.
- [77] Bouhss A, Mengin-Lecreulx D, Blanot D, van Heijenoort J, Parquet C. Invariant amino acids in the *Mur* peptide synthetases of bacterial peptidoglycan synthesis and their modification by site-directed mutagenesis in the UDP-MurNAc:L-alanine ligase from *Escherichia coli*. *Biochemistry* 1997; 36(39): 11556-63.
- [78] Born TL, Blanchard JS. Structure/function studies on enzymes in the diaminopimelate pathway of bacterial cell wall biosynthesis. *Curr Opin Chem Biol* 1999; 3(5): 607-13.
- [79] Cox RJ, Sutherland A, Vederas JC. Bacterial diaminopimelate metabolism as a target for antibiotic design. *Bioorg Med Chem* 2000; 8(5): 843-71.
- [80] Schleifer KH, Hammes WP, Kandler O. Effect of endogenous and exogenous factors on the primary structures of bacterial peptidoglycan. *Adv Microb Physiol* 1976; 13: 245-92.
- [81] Lugtenberg EJ, De Haas-Menger L, Ruyters WH. Murein synthesis and identification of cell wall precursors of temperature-sensitive lysis mutants of *Escherichia coli*. *J Bacteriol* 1972; 109(1): 326-35.
- [82] Clarke AJ, Dupont C. O-acetylated peptidoglycan: its occurrence, pathobiological significance, and biosynthesis. *Can J Microbiol* 1992; 38(2): 85-91.
- [83] Sobhanifar S, King DT, Strynadka NC. Fortifying the wall: synthesis, regulation and degradation of bacterial peptidoglycan. *Curr Opin Struct Biol* 2013; 23(5): 695-703.
- [84] Lovering AL, Safadi SS, Strynadka NC. Structural perspective of peptidoglycan biosynthesis and assembly. *Annu Rev Biochem* 2012; 81: 451-78.
- [85] Lovering AL, de Castro LH, Lim D, Strynadka NC. Structural insight into the transglycosylation step of bacterial cell-wall biosynthesis. *Science* 2007; 315(5817): 1402-5.
- [86] Huang CY, Shih HW, Lin LY, *et al*. Crystal structure of *Staphylococcus aureus* transglycosylase in complex with a lipid II analog and elucidation of peptidoglycan synthesis mechanism. *Proc Natl Acad Sci USA* 2012; 109(17): 6496-501.
- [87] Sukhithasri V, Nisha N, Biswas L, Anil Kumar V, Biswas R. Innate immune recognition of microbial cell wall components and microbial strategies to evade such recognitions. *Microbiol Res* 2013; 168(7): 396-406.
- [88] Hadi T, Pfeffer JM, Clarke AJ, Tanner ME. Water-soluble substrates of the peptidoglycan-modifying enzyme O-acetylpeptidoglycan esterase (Ape1) from *Neisseria gonorrhoeae*. *J Org Chem* 2011; 76(4): 1118-25.
- [89] Paradis-Bleau C, Markovski M, Uehara T, *et al*. Lipoprotein cofactors located in the outer membrane activate bacterial cell wall polymerases. *Cell* 2010; 143(7): 1110-20.
- [90] Vollmer J, Schiefer A, Schneider T, *et al*. Requirement of lipid II biosynthesis for cell division in cell wall-less *Wolbachia*, endobacteria of arthropods and filarial nematodes. *Int J Med Microbiol* 2013; 303(3): 140-9.
- [91] Turner RD, Hurd AF, Cadby A, Hobbs JK, Foster SJ. Cell wall elongation mode in Gram-negative bacteria is determined by peptidoglycan architecture. *Nat Commun* 2013; 4: 1496.
- [92] Smith CA. Structure, function and dynamics in the *mur* family of bacterial cell wall ligases. *J Mol Biol* 2006; 362(4): 640-55.
- [93] Molina-López J, Sanschagrin F, Levesque RC. A peptide inhibitor of *MurA* UDP-N-acetylglucosamine enolpyruvyl transferase: the first committed step in peptidoglycan biosynthesis. *Peptides* 2006; 27(12): 3115-21.

- [94] Mendgen T, Scholz T, Klein CD. Structure-activity relationships of tulipalines, tuliposides, and related compounds as inhibitors of MurA. *Bioorg Med Chem Lett* 2010; 20(19): 5757-62.
- [95] Shigetomi K, Shoji K, Mitsuhashi S, Ubukata M. The antibacterial properties of 6-tuliposide B. Synthesis of 6-tuliposide B analogues and structure-activity relationship. *Phytochemistry* 2010; 71(2-3): 312-24.
- [96] Sheehan JC, Lawson WB, Gaul RJ. The structure of terreic acid. *J Am Chem Soc* 1958; 80: 5536-8.
- [97] Chang CM, Chern J, Chen MY, et al. Avenaciolides: potential MurA-targeted inhibitors against peptidoglycan biosynthesis in methicillin-resistant *Staphylococcus aureus* (MRSA). *J Am Chem Soc* 2015; 137(1): 267-75.
- [98] Eschenburg S, Kabsch W, Healy ML, Schönbrunn E. A new view of the mechanisms of UDP-N-acetylglucosamine enolpyruvyl transferase (MurA) and 5-enolpyruvylshikimate-3-phosphate synthase (AroA) derived from X-ray structures of their tetrahedral reaction intermediate states. *J Biol Chem* 2003; 278(49): 49215-22.
- [99] Brown ED, Vivas EI, Walsh CT, Kolter R. MurA (MurZ), the enzyme that catalyzes the first committed step in peptidoglycan biosynthesis, is essential in *Escherichia coli*. *J Bacteriol* 1995; 177(14): 4194-7.
- [100] Crick DC, Mahapatra S, Brennan PJ. Biosynthesis of the arabinogalactan-peptidoglycan complex of *Mycobacterium tuberculosis*. *Glycobiology* 2001; 11(9): 107R-18R.
- [101] Dhalla AM, Yanchunas J Jr, Ho HT, Falk PJ, Villafranca JJ, Robertson JG. Steady-state kinetic mechanism of *Escherichia coli* UDP-N-acetylenolpyruvylglucosamine reductase. *Biochemistry* 1995; 34(16): 5390-402.
- [102] Tao K, Makino K, Yonei S, Nakata A, Shinagawa H. Purification and characterization of the *Escherichia coli* OxyR protein, the positive regulator for a hydrogen peroxide-inducible regulon. *J Biochem* 1991; 109(2): 262-6.
- [103] Perichon B, Tankovic J, Courvalin P. Characterization of a mutation in the parE gene that confers fluoroquinolone resistance in *Streptococcus pneumoniae*. *Antimicrob Agents Chemother* 1997; 41(5): 1166-7.
- [104] Shahab M, Verma M, Pathak M, Mitra K, Misra-Bhattacharya S. Cloning, expression and characterization of UDP-N-acetylglucosamine enolpyruvyl transferase (MurA) from *Wolbachia* endosymbiont of human lymphatic filarial parasite *Brugia malayi*. *PLoS One* 2014; 9(6): e99884.
- [105] Oberdorfer G, Binter A, Ginj C, Macheroux P, Gruber K. Structural and functional characterization of NikO, an enolpyruvyl transferase essential in nikkomycin biosynthesis. *J Biol Chem* 2012; 287(37): 31427-36.
- [106] Geha RM, Chen K, Wouters J, Ooms F, Shih JC. Analysis of conserved active site residues in monoamine oxidase A and B and their three-dimensional molecular modeling. *J Biol Chem* 2002; 277(19): 17209-16.
- [107] Jin BS, Han SG, Lee WK, et al. Inhibitory mechanism of novel inhibitors of UDP-N-acetylglucosamine enolpyruvyl transferase from *Haemophilus influenzae*. *J Microbiol Biotechnol* 2009; 19(12): 1582-9.
- [108] Baum EZ, Montenegro DA, Licata L, et al. Identification and characterization of new inhibitors of the *Escherichia coli* MurA enzyme. *Antimicrob Agents Chemother* 2001; 45(11): 3182-8.
- [109] Miller K, Dunsmore CJ, Leeds JA, et al. Benzothioxalone derivatives as novel inhibitors of UDP-N-acetylglucosamine enolpyruvyl transferases (MurA and MurZ). *J Antimicrob Chemother* 2010; 65(12): 2566-73.
- [110] Schönbrunn E, Eschenburg S, Krekel F, Luger K, Amrhein N. Role of the loop containing residue 115 in the induced-fit mechanism of the bacterial cell wall biosynthetic enzyme MurA. *Biochemistry* 2000; 39(9): 2164-73.
- [111] El Zoeiby A, Sanschagrin F, Levesque RC. Structure and function of the Mur enzymes: development of novel inhibitors. *Mol Microbiol* 2003; 47(1): 1-12.
- [112] Du W, Brown JR, Sylvester DR, et al. Two active forms of UDP-N-acetylglucosamine enolpyruvyl transferase in gram-positive bacteria. *J Bacteriol* 2000; 182(15): 4146-52.
- [113] Eschenburg S, Priestman MA, Abdul-Latif FA, Delachaux C, Fassy F, Schönbrunn E. A novel inhibitor that suspends the induced fit mechanism of UDP-N-acetylglucosamine enolpyruvyl transferase (MurA). *J Biol Chem* 2005; 280(14): 14070-5.
- [114] Barbosa MD, Yang G, Fang J, Kurilla MG, Pompliano DL. Development of a whole-cell assay for peptidoglycan biosynthesis inhibitors. *Antimicrob Agents Chemother* 2002; 46(4): 943-6.
- [115] Dunsmore CJ, Miller K, Blake KL, et al. 2-Aminotetralones: novel inhibitors of MurA and MurZ. *Bioorg Med Chem Lett* 2008; 18(5): 1730-4.
- [116] Ramaswamy S, Musser JM. Molecular genetic basis of antimicrobial agent resistance in *Mycobacterium tuberculosis*: 1998 update. *Tuber Lung Dis* 1998; 79(1): 3-29.
- [117] Bachelier A, Mayer R, Klein CD. Sesquiterpene lactones are potent and irreversible inhibitors of the antibacterial target enzyme MurA. *Bioorg Med Chem Lett* 2006; 16(21): 5605-9.
- [118] Mengin-Lecreulx D, Flouret B, van Heijenoort J. Cytoplasmic steps of peptidoglycan synthesis in *Escherichia coli*. *J Bacteriol* 1982; 151(3): 1109-17.
- [119] Benson TE, Harris MS, Choi GH, et al. A structural variation for MurB: X-ray crystal structure of *Staphylococcus aureus* UDP-N-acetylenolpyruvylglucosamine reductase (MurB). *Biochemistry* 2001; 40(8): 2340-50.
- [120] Sylvester DR, Alvarez E, Patel A, Ratnam K, Kallender H, Wallis NG. Identification and characterization of UDP-N-acetylenolpyruvylglucosamine reductase (MurB) from the Gram-positive pathogen *Streptococcus pneumoniae*. *Biochem J* 2001; 355(Pt 2): 431-5.
- [121] Benson TE, Walsh CT, Hogle JM. X-ray crystal structures of the S229A mutant and wild-type MurB in the presence of the substrate enolpyruvyl-UDP-N-acetylglucosamine at 1.8-Å resolution. *Biochemistry* 1997; 36(4): 806-11.
- [122] Sylvester DR, Alvarez E, Patel A, Ratnam K, Kallender H, Wallis NG. Identification and characterization of UDP-N-acetylenolpyruvylglucosamine reductase (MurB) from the Gram-positive pathogen *Streptococcus pneumoniae*. *Biochem J* 2001; 355(Pt 2): 431-5.
- [123] Dhalla AM, Yanchunas J Jr, Ho HT, Falk PJ, Villafranca JJ, Robertson JG. Steady-state kinetic mechanism of *Escherichia coli* UDP-N-acetylenolpyruvylglucosamine reductase. *Biochemistry* 1995; 34(16): 5390-402.
- [124] Sarver RW, Rogers JM, Epps DE. Determination of ligand-MurB interactions by isothermal denaturation: application as a secondary assay to complement high throughput screening. *J Biomol Screen* 2002; 7(1): 21-8.
- [125] Katz AH, Caufield CE. Structure-based design approaches to cell wall biosynthesis inhibitors. *Curr Pharm Des* 2003; 9(11): 857-66.
- [126] Andres CJ, Bronson JJ, D'Andrea SV, et al. 4-Thiazolidinones: novel inhibitors of the bacterial enzyme MurB. *Bioorg Med Chem Lett* 2000; 10(8): 715-7.
- [127] Yang Y, Severin A, Chopra R, et al. 3,5-dioxypyrazolidines, novel inhibitors of UDP-N-acetylenolpyruvylglucosamine reductase (MurB) with activity against gram-positive bacteria. *Antimicrob Agents Chemother* 2006; 50(2): 556-64.
- [128] Gilbert AM, Failli A, Shumsky J, et al. Pyrazolidine-3,5-diones and 5-hydroxy-1H-pyrazol-3(2H)-ones, inhibitors of UDP-N-acetylenolpyruvyl glucosamine reductase. *J Med Chem* 2006; 49(20): 6027-36.
- [129] Bronson JJ, DenBleyker KL, Falk PJ, et al. Discovery of the first antibacterial small molecule inhibitors of MurB. *Bioorg Med Chem Lett* 2003; 13(5): 873-5.
- [130] Marmor S, Petersen CP, Reck F, Yang W, Gao N, Fisher SL. Biochemical characterization of a phosphinate inhibitor of *Escherichia coli* MurC. *Biochemistry* 2001; 40(40): 12207-14.
- [131] Falk PJ, Ervin KM, Volk KS, Ho HT. Biochemical evidence for the formation of a covalent acyl-phosphate linkage between UDP-N-acetylmuramate and ATP in the *Escherichia coli* UDP-N-acetylmuramate:L-alanine ligase-catalyzed reaction. *Biochemistry* 1996; 35(5): 1417-22.
- [132] Emanuele JJ Jr, Jin H, Yanchunas J Jr, Villafranca JJ. Evaluation of the kinetic mechanism of *Escherichia coli* uridine diphosphate-N-acetylmuramate:L-alanine ligase. *Biochemistry* 1997; 36(23): 7264-71.
- [133] Segel IH. Enzyme kinetics 1975.
- [134] Viola RE, Cleland WW. Initial velocity analysis for terreactant mechanisms. *Methods Enzymol* 1982; 87: 353-66.
- [135] Investigations of Rates and Mechanisms of Reaction Bernasconi, CF Cleland WW, Bernasconi CF. *Techniques of Chemistry* 1986; 791-871.

- [136] Hameed P S, Manjrekar P, Chinnapattu M, *et al.* Pyrazolopyrimidines establish MurC as a vulnerable target in *Pseudomonas aeruginosa* and *Escherichia coli*. *ACS Chem Biol* 2014; 9(10): 2274-82.
- [137] Beveridge TJ. Ultrastructure, chemistry, and function of the bacterial wall. *Int Rev Cytol* 1981; 72: 229-317.
- [138] Sim MM, Ng SB, Buss AD, Crasta SC, Goh KL, Lee SK. Benzylidene rhodanines as novel inhibitors of UDP-N-acetylmuramate/L-alanine ligase. *Bioorg Med Chem Lett* 2002; 12(4): 697-9.
- [139] Šink R, Barreateau H, Patin D, Mengin-Lecreulx D, Gobec S, Blanot D. MurD enzymes: some recent developments. *Biomol Concepts* 2013; 4(6): 539-56.
- [140] Vollmer W, Blanot D, de Pedro MA. Peptidoglycan structure and architecture. *FEMS Microbiol Rev* 2008; 32(2): 149-67.
- [141] Schleifer KH, Kandler O. Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* 1972; 36(4): 407-77.
- [142] Münch D, Roemer T, Lee SH, Engeser M, Sahl HG, Schneider T. Identification and in vitro analysis of the GatD/MurT enzyme-complex catalyzing lipid II amidation in *Staphylococcus aureus*. *PLoS Pathog* 2012; 8(1): e1002509.
- [143] Schleifer KH, Plapp R, Kandler O. Identification of threo-3-hydroxyglutamic acid in the cell wall of *Microbacterium lacticum*. *Biochem Biophys Res Commun* 1967; 28(4): 566-70.
- [144] Pratiel-Sosa F, Acher F, Trigalo F, Blanot D, Azerad R, van Heijenoort J. Effect of various analogues of D-glutamic acid on the D-glutamate-adding enzyme from *Escherichia coli*. *FEMS Microbiol Lett* 1994; 115(2-3): 223-8.
- [145] Kotnik M, Humljan J, Contreras-Martel C, *et al.* Structural and functional characterization of enantiomeric glutamic acid derivatives as potential transition state analogue inhibitors of MurD ligase. *J Mol Biol* 2007; 370(1): 107-15.
- [146] Bouhss A, Dementin S, van Heijenoort J, Parquet C, Blanot D. Formation of adenosine 5'-tetraphosphate from the acyl phosphate intermediate: a difference between the MurC and MurD synthetases of *Escherichia coli*. *FEBS Lett* 1999; 453(1-2): 15-9.
- [147] Wheeler R, Chevalier G, Eberl G, Gomperts Boneca I. The biology of bacterial peptidoglycans and their impact on host immunity and physiology. *Cell Microbiol* 2014; 16(7): 1014-23.
- [148] Favini-Stabile S, Contreras-Martel C, Thielens N, Dessen A. MreB and MurG as scaffolds for the cytoplasmic steps of peptidoglycan biosynthesis. *Environ Microbiol* 2013; 15(12): 3218-28.
- [149] Teimouri M, Kamrani H. In-Silico Testing Of Nutraceutical Against The MurD Enzymes From *Mycobacterium Tuberculosis*. 2016; 5(11)
- [150] Bertrand JA, Fanchon E, Martin L, *et al.* "Open" structures of MurD: domain movements and structural similarities with folypolyglutamate synthetase. *J Mol Biol* 2000; 301(5): 1257-66.
- [151] Perdihi A, Kotnik M, Hodoscek M, Solmajer T. Targeted molecular dynamics simulation studies of binding and conformational changes in *E. coli* MurD. *Proteins* 2007; 68(1): 243-54.
- [152] Moraes GL, Gomes GC, Monteiro de Sousa PR, *et al.* Structural and functional features of enzymes of *Mycobacterium tuberculosis* peptidoglycan biosynthesis as targets for drug development. *Tuberculosis (Edinb)* 2015; 95(2): 95-111.
- [153] Zidar N, Tomasić T, Šink R, *et al.* Discovery of novel 5-benzylidenerhodanine and 5-benzylidenethiazolidine-2,4-dione inhibitors of MurD ligase. *J Med Chem* 2010; 53(18): 6584-94.
- [154] Perdihi A, Hodoscek M, Solmajer T. MurD ligase from *E. coli*: Tetrahedral intermediate formation study by hybrid quantum mechanical/molecular mechanical replica path method. *Proteins* 2009; 74(3): 744-59.
- [155] Štrancar K, Blanot D, Gobec S. Design, synthesis and structure-activity relationships of new phosphinate inhibitors of MurD. *Bioorg Med Chem Lett* 2006; 16(2): 343-8.
- [156] Simčič M, Pureber K, Kristan K, Urleb U, Kocjan D, Grdadolnik SG. A novel 2-oxoindolinylidene inhibitor of bacterial MurD ligase: Enzyme kinetics, protein-inhibitor binding by NMR and a molecular dynamics study. *Eur J Med Chem* 2014; 83: 92-101.
- [157] Humljan J, Kotnik M, Contreras-Martel C, *et al.* Novel naphthalene-N-sulfonyl-D-glutamic acid derivatives as inhibitors of MurD, a key peptidoglycan biosynthesis enzyme. *J Med Chem* 2008; 51(23): 7486-94.
- [158] Simcic M, Hodošek M, Humljan J, *et al.* NMR and molecular dynamics study of the binding mode of naphthalene-N-sulfonyl-D-glutamic acid derivatives: novel MurD ligase inhibitors. *J Med Chem* 2009; 52(9): 2899-908.
- [159] Horton JR, Bostock JM, Chopra I, *et al.* Macrocyclic inhibitors of the bacterial cell wall biosynthesis enzyme MurD. *Bioorg Med Chem Lett* 2003; 13(9): 1557-60.
- [160] Perdihi A, Bren U, Solmajer T. Binding free energy calculations of N-sulphonyl-glutamic acid inhibitors of MurD ligase. *J Mol Model* 2009; 15(8): 983-96.
- [161] Perdihi A, Hrst M, Barreateau H, Gobec S, Wolber G, Solmajer T. Inhibitor design strategy based on an enzyme structural flexibility: a case of bacterial MurD ligase. *J Chem Inf Model* 2014; 54(5): 1451-66.
- [162] Li Z, Francisco GD, Hu W, *et al.* 2-Phenyl-5,6-dihydro-2H-thieno[3,2-c]pyrazol-3-ol derivatives as new inhibitors of bacterial cell wall biosynthesis. *Bioorg Med Chem Lett* 2003; 13(15): 2591-4.
- [163] Osman K, Evangelopoulos D, Basavannacharya C, *et al.* An anti-bacterial from *Hypericum acmosepalum* inhibits ATP-dependent MurE ligase from *Mycobacterium tuberculosis*. *Int J Antimicrob Agents* 2012; 39(2): 124-9.
- [164] Guzman JD, Wube A, Evangelopoulos D, *et al.* Interaction of N-methyl-2-alkenyl-4-quinolones with ATP-dependent MurE ligase of *Mycobacterium tuberculosis*: antibacterial activity, molecular docking and inhibition kinetics. *J Antimicrob Chemother* 2011; 66(8): 1766-72.
- [165] Guzman JD, Gupta A, Evangelopoulos D, *et al.* Anti-tubercular screening of natural products from Colombian plants: 3-methoxynordomesticine, an inhibitor of MurE ligase of *Mycobacterium tuberculosis*. *J Antimicrob Chemother* 2010; 65(10): 2101-7.
- [166] Pinto A, Tamborini L, Cullia G, Conti P, De Micheli C. Inspired by Nature: The 3-Halo-4,5-dihydroisoxazole Moiety as a Novel Molecular Warhead for the Design of Covalent Inhibitors. *ChemMedChem* 2016; 11(1): 10-4.
- [167] Jöst C, Nitsche C, Scholz T, Roux L, Klein CD. Promiscuity and selectivity in covalent enzyme inhibition: a systematic study of electrophilic fragments. *J Med Chem* 2014; 57(18): 7590-9.
- [168] Orth R, Böttcher T, Sieber SA. The biological targets of acivicin inspired 3-chloro- and 3-bromodihydroisoxazole scaffolds. *Chem Commun (Camb)* 2010; 46(44): 8475-7.
- [169] Gu YG, Florjancic AS, Clark RF, *et al.* Structure-activity relationships of novel potent MurF inhibitors. *Bioorg Med Chem Lett* 2004; 14(1): 267-70.
- [170] Anderson MS, Eveland SS, Onishi HR, Pompliano DL. Kinetic mechanism of the *Escherichia coli* UDPMurNAc-tripeptide D-alanyl-D-alanine-adding enzyme: use of a glutathione S-transferase fusion. *Biochemistry* 1996; 35(50): 16264-9.
- [171] Duncan K, van Heijenoort J, Walsh CT. Purification and characterization of the D-alanyl-D-alanine-adding enzyme from *Escherichia coli*. *Biochemistry* 1990; 29(9): 2379-86.
- [172] Taha MO, Atallah N, Al-Bakri AG, *et al.* Discovery of new MurF inhibitors via pharmacophore modeling and QSAR analysis followed by in-silico screening. *Bioorg Med Chem* 2008; 16(3): 1218-35.
- [173] Stamper GF, Longenecker KL, Fry EH, *et al.* Structure-based optimization of MurF inhibitors. *Chem Biol Drug Des* 2006; 67(1): 58-65.
- [174] Comess KM, Schurdak ME, Voorbach MJ, *et al.* An ultraefficient affinity-based high-throughput screening process: application to bacterial cell wall biosynthesis enzyme MurF. *J Biomol Screen* 2006; 11(7): 743-54.
- [175] Monaghan RL, Barrett JF. Antibacterial drug discovery--then, now and the genomics future. *Biochem Pharmacol* 2006; 71(7): 901-9.
- [176] Bebrone C, Lassaux P, Vercheval L, *et al.* Current challenges in antimicrobial chemotherapy: focus on β -lactamase inhibition. *Drugs* 2010; 70(6): 651-79.
- [177] Longenecker KL, Stamper GF, Hajduk PJ, *et al.* Structure of MurF from *Streptococcus pneumoniae* co-crystallized with a small molecule inhibitor exhibits interdomain closure. *Protein Sci* 2005; 14(12): 3039-47.
- [178] Agarwal AK, Fishwick CW. Structure-based design of anti-infectives. *Ann N Y Acad Sci* 2010; 1213: 20-45.
- [179] Turk S, Hrst M, Sosić I, *et al.* Biochemical characterization of MurF from *Streptococcus pneumoniae* and the identification of a new MurF inhibitor through ligand-based virtual screening. *Acta Chim Slov* 2013; 60(2): 294-9.
- [180] Murakami R, Muramatsu Y, Minami E, *et al.* A novel assay of bacterial peptidoglycan synthesis for natural product screening. *J Antibiot* 2009; 62(3): 153-8.

- [181] Walsh C. Antibiotics: Actions, Origins, Resistance (American Society for Microbiology Press, Washington, DC).
- [182] Silver LL. Multi-targeting by monotherapeutic antibacterials. *Nat Rev Drug Discov* 2007; 6(1): 41-55.
- [183] Perdih A, Hrast M, Pureber K, *et al.* Furan-based benzene mono- and dicarboxylic acid derivatives as multiple inhibitors of the bacterial Mur ligases (MurC-MurF): experimental and computational characterization. *J Comput Aided Mol Des* 2015; 29(6): 541-60.
- [184] Šink R, Kovač A, Tomasić T, *et al.* Synthesis and biological evaluation of N-acylhydrazones as inhibitors of MurC and MurD ligases. *ChemMedChem* 2008; 3(9): 1362-70.
- [185] Rausch S, Hänchen A, Denisiuk A, Löhken M, Schneider T, Süßmuth RD. Feglymycin is an inhibitor of the enzymes MurA and MurC of the peptidoglycan biosynthesis pathway. *ChemBioChem* 2011; 12(8): 1171-3.
- [186] Perdih A, Kovač A, Wolber G, Blanot D, Gobec S, Solmajer T. Discovery of novel benzene 1,3-dicarboxylic acid inhibitors of bacterial MurD and MurE ligases by structure-based virtual screening approach. *Bioorg Med Chem Lett* 2009; 19(10): 2668-73.
- [187] Perdih A, Hrast M, Barreateau H, Gobec S, Wolber G, Solmajer T. Benzene-1,3-dicarboxylic acid 2,5-dimethylpyrrole derivatives as multiple inhibitors of bacterial Mur ligases (MurC-MurF). *Bioorg Med Chem* 2014; 22(15): 4124-34.
- [188] Dettner F, Hänchen A, Schols D, Toti L, Nusser A, Süßmuth RD. Total synthesis of the antiviral peptide antibiotic feglymycin. *Angew Chem Int Ed Engl* 2009; 48(10): 1856-61.
- [189] Gupta A, Singh R, Sonar PK, Saraf SK. Novel 4-Thiazolidinone Derivatives as Anti-infective Agents: Synthesis, Characterization and Antimicrobial Evaluation.
- [190] da Silva IM, da Silva Filho J, Santiago PB, *et al.* Synthesis and antimicrobial activities of 5-Arylidene-thiazolidine-2,4-dione derivatives. *BioMed Res Int* 2014; 2014: 316082.
- [191] Zampini IC, Cuello S, Alberto MR, *et al.* Antimicrobial activity of selected plant species from "the Argentine Puna" against sensitive and multi-resistant bacteria. *J Ethnopharmacol* 2009; 124(3): 499-505.
- [192] Tomasić T, Zidar N, Kovač A, *et al.* 5-Benzylidenethiazolidin-4-ones as multitarget inhibitors of bacterial Mur ligases. *ChemMedChem* 2010; 5(2): 286-95.
- [193] Sadeghian H, Sadeghian A, Pordel M, *et al.* Design, synthesis, and structure-activity relationship study of 5-amido-1-(2, 4-dinitrophenyl)-1H-4-pyrazolecarbonitrils as DD-carboxypeptidase/penicillin-binding protein inhibitors with Gram-positive antibacterial activity. *Med Chem Res* 2010; 19: 103-19.
- [194] Mansour TS, Caufield CE, Rasmussen B, *et al.* Naphthyl tetronic acids as multi-target inhibitors of bacterial peptidoglycan biosynthesis. *ChemMedChem* 2007; 2(10): 1414-7.
- [195] Vértessy L, Aretz W, Knauf M, Markus A, Vogel M, Wink J. Feglymycin, a novel inhibitor of the replication of the human immunodeficiency virus. Fermentation, isolation and structure elucidation. *J Antibiot* 1999; 52(4): 374-82.
- [196] Wallis NG. Inventor; Smithkline Beecham Corporation, assignee. Mur A-1 from *Streptococcus pneumoniae*. United States patent US 6,228,627. 2001 May.
- [197] Skatrud PL, Peery RB. Inventors; Eli Lilly, assignee. Nucleic acid compounds. United States patent US 5,691,161. 1997 Nov.
- [198] Huang J, Jiang X, Payne D, Van Horn S, Wallis NG. Inventors. MurA. United States patent US 6,346,396. 2002 Feb.
- [199] El-Sherbeini M, Azzolina B. Inventors; Merck & Co., Inc., assignee. MurC gene and enzyme of *Pseudomonas aeruginosa*. United States patent US 7,022,829. 2006 Apr.
- [200] Hoskins JA, Peery RB, Skatrud PL, Wu CY. Inventors; Eli Lilly, assignee. MurD protein method and kit for identification of inhibitors. United States patent US 5,681,694. 1997 Oct.
- [201] Hoskins JA, Peery RB, Skatrud PL, Wu CY. Inventors; Eli Lilly, assignee. Biosynthetic gene murD from *streptococcus pneumoniae*. United States patent US 5,834,270. 1998 Nov.
- [202] El-Sherbeini M, Wong KK, Geissler WM. Inventors; Merck & Co., Inc., assignee. Isolated polynucleotide selected from polynucleotide encoding polypeptide having specified amino acid sequence, and polynucleotide fully complementary to that polynucleotide. United States patent US 6,534,284. 2003 Mar.
- [203] El-Sherbeini M, Wong KK. Inventors; Merck & Co. Inc., assignee. MurD protein and gene of *Streptococcus pyogenes*. United States patent US 6,746,858. 2004 Jun.
- [204] El-Sherbeini M, Azzolina B. Inventors; Merck & Co., Inc., assignee. MurD protein and gene of *Pseudomonas aeruginosa*. United States patent US 6,890,910. 2005 May 10.
- [205] <http://www.abcam.com/enramycin-b-enduracidin-b-ab143613.html>