A REVIEW ON NOVEL TARGETS WITH THEIR BIOLOGIAL PATHWAYS FOR ANTI-MICROBIAL ACTIVITY

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ABSTRACT

Antimicrobial agents have played a significant role in saving human lives and it is an advanced modern medicine for over 70 years. Nevertheless, the emergence of drug resistance shows a serious threat to the effectiveness of the drug treatments. This antimicrobial resistance (AMR) will occur when microorganism develop resistance to antimicrobial agents, leaving them ineffective and unresponsive. This growing phenomenon possess a threat to global public health, as the treatment and management of resistance infection become increasingly challenging. So, highlighting the urgent need for new and improved antimicrobial drugs with novel targets to mitigate cross resistance. This review explores the innovative strategies for discovering novel antimicrobial agents which can combat AMR, by focusing on several promising bacterial target for the drug development. Example, for its potential target includes biomolecules which involved in cell wall biosynthesis, cell membrane function, isoprenoid biosynthesis, folic acid synthesis, protein biosynthesis, fatty acid biosynthesis.

KEYWORDS: *Antimicrobial resistance, potential targets, drug development, biological pathways.*

1. Introduction

Antimicrobials are the agents which designed to destroy or inhibit the growth of microorganisms, including bacteria, viruses, fungi, parasites etc. These agents are categorized based on their mechanism of action and effects, such as bacteriostatic or bactericide. Thus, antimicrobials play a crucial role to treat and prevent infections in a range of conditions, endocarditis, gingivitis, prophylactic or suppressive therapy as per surgical antimicrobials, prophylaxis in immunocompromised patients with HIV/AIDS, traumatic injuries, neutropenia etc. [1]

Over 70 years, antimicrobial agents have played a key role in treating infections caused by pathogenic microbes and significantly saving lives and shaping modern medicines. However, their widespread use of these agents has led to evolutionary pressure on microbes, resulting in the emergence of drug-resistant strains. This resistance has decreased the effectiveness of these agents, and this led to their withdrawal from common use also. Drug resistance has become a major challenge in 20^{th} century, with remarkable examples including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococci* (VER), which have appeared due to the overuse and misuse of antibiotics. This growing resistance causes significantly human suffering and obstacle to effective infection control. [2]

The ongoing issue of antimicrobial resistance (AMR) underscores the urgent need for new and improved antimicrobial agents because resistance leads to ineffective standard treatments, leading to prolonged infections and increased spread. Resistant organisms can also cause treatment failures, increased healthcare costs, and potentially lead to a return to a pre-antibiotic period or era. [3] One key mechanism of AMR involves alterations in the molecular targets of antimicrobials. In response, researchers have developed a new drug by modifying existing molecules so that to enhance their efficacy and safety. [1]

However, the problem is that the pathogens are evolving or often adapt quickly, and new drugs couldn't be developed fast. This situation demands developing a new resistance mechanism and continuing to avoid problems. To combat AMR, several strategies are being explored. These include developing inhibitor to resistant enzymes, which can be used alongside antibiotics to maintain their effectiveness. [4] The literature survey focuses on identifying novel targets for antibiotics, such as cell wall biosynthesis, amino acid synthesis, fatty acid biosynthesis, folic acid synthesis. Rationally choosing targets based on these differences is pivotal to developing effective treatments. [4-5] Despite progress in identifying new antimicrobial targets and developing innovative treatments, the threat of AMR remains significant. Continued research into various targets such as cell wall biosynthesis and amino acid synthesis, is critical to finding effective solutions. [5] This review aims to explore novel antimicrobial targets and alternative approaches to antimicrobial agent development, emphasizing the need for ongoing innovation to combat against AMR.

2. Inhibiting pathogenicity through anti-virulence factors

Bacteria use various tools, known as virulence factors, to harm their host. These virulence factors help bacteria stick to and invade the host, initiate an infection, and evade the immune system. The key virulence factors include:

1. Adherence Factors: Help bacteria stick to host tissues.
2. Invasion Factors: Aid in penetrating of host cells.

3. Bacterial capsules protect the bacteria, while their toxins damage host tissues.

4. Siderophores: Gather iron, which bacteria need to grow.

To fight bacterial infections, one approach is to target and inhibit these virulence factors instead of killing bacteria directly. This will lessen the chance of bacteria in developing resistance. Strategies include blocking the signals, bacteria use to coordinate attacks, disrupting toxins, preventing adhesion to the host cells, and targeting specific genes involved in the infection process. [2]

2.1 Anti-toxins

Toxins are harmful substance produced by bacteria that can trigger immune response in the host. These toxins act on the host cell and stimulate the production of specific antibodies known as antitoxins. Monoclonal antibodies (mAbs) can be created target and neutralize bacterial toxins or surface structures, these anti-toxins mAbs help reduce bacterial virulence by neutralizing exotoxins (Directly blocking the harmful effects of toxins), Promoting phagocytosi**s** (Helping immune cells engulf and destroy bacteria), Enhancing complement activity (using the immune system's complement proteins to kill bacteria), Independent killing (killing bacteria without relying on the immune system). [6]

2.1.1 Toxin neutralization

Toxin neutralization is a key mechanism by which monoclonal antibodies (mAbs) combat bacterial toxins. These mAbs work by binding to toxins, forming complexes that are cleared from the body by the reticuloendothelial systems. The effectiveness of these mAbs depends on how well they bind to their target toxins. [7]

Examples of approved monoclonal antibodies (mAbs) were:

a) **Raxibacumab:** It is the first biologic product that has an anti-protective antigen (APA), approved management of anthrax infection with antimicrobial agents and preventing the toxin's components from entering host cells.
b) **Obiltoxaximab:** It is also another APA mAbs that was approved to protect against anthrax toxin by inhibiting PA binding to cellular receptors on host cells. [8]
c) **Bezlotoxumab:** It is a human IgG1 was approved to reduce recurrence of *Clostridium difficile* infection (CDI) who are receiving antimicrobial for CDI and are at high risk for CDI recurrence. In host cell, it binds and inhibits toxin B. Hence, it prevents toxin B-mediated inactivation of Rho GTPase and downstream signalling pathways in cells. Bezlotoxumab is mainly used for the prevention of recurrent *Clostridioides difficile* infection (CDI) and not for its treatment

In addition to these agents, many monoclonal antibodies are currently in clinical development.

2.1.2 Immunoconjugates

This strategy involves using antibodies to deliver antibacterial agents directly to microbes inside cells. The antibodies are designed to bind specifically to bacteria, leading them into phagolysosomes, where the attached antibacterial agents are released to kill the bacteria. This method is particularly effective against drug resistant strains, like vancomycin-resistant *S. aureus*. [9]

The approach has several advantages:

a) **Improved Pharmacokinetics**: Antibody-drug conjugates generally have longer half-lives, meaning they stay in the body longer and continue to work effectively.
b) **Reduced Toxicity**: By targeting the drug specifically to the bacteria, less of the drug is exposed to the rest of the body, minimizing side effects.
c) Reviving Failed Drugs: Agents that didn't work well in previous trials due to poor pharmacokinetics or high toxicity might be effective when delivered directly to the target bacteria.

An example of this is the use of Bismuth -213 linked to an antibody targeting *Pneumococcal* capsular polysaccharide, which has shown effective in killing bacteria in the lab. Presently, there is a clinical trial (DSTA-4637S) assessing an antibody linked to a rifamycin analogue, which targets on surface proteins of *S. aureus* to convey the drug particularly to and kill the bacteria inside the cells. In summary, immunoconjugates are a promising approach that combines targeted delivery with potent antibacterial agents, offering improved effectiveness and reduced side effects in the treatment of bacterial infections. [9]

2.1.3 Immunomodulatory monoclonal antibodies (mAbs)

It can enhance the body's ability to clear bacterial infections by boosting the immune system. One example is the of anti-PD-1 mAbs in treating tuberculosis (TB). PD-1 is a protein on T cells that can inhibit their activity, and its ligands decrease the effectiveness of T cells in fighting TB.

In TB patients, standard treatments can reduce PD-1 and its ligands, but this still may not fully restore T cell's ability to produce cytokines and respond to TB antigens. Essentially, these antibodies help reinvigorate the immune system, improving its capacity to fight the infection.

2.2 Biofilm formation inhibitor

Biofilm formation by bacteria is a significant problem in infections, as biofilms protect bacteria and increase their resistant to treatments. Biofilm can be up to 1000 times more resistant to drugs free-floating bacteria, making infections harder to treat and increasing risk of in-vivo toxicity.

To tackle this, researchers are exploring various strategies:

a) **Cyclic dimeric guanosine monophosphate (C-di-GMP) Modification**: The c-di-GMP is a signalling molecule that promotes biofilm formation. By modifying or reducing c-di-GMP levels, it is not possible to decrease biofilm formation. For example, nitric oxide (NO) can stimulate enzymes that break down c-di-GMP, leading to the dispersal of biofilm and a shift to a more vulnerable plantonic state.
b) **Quorum sensing inhibition**: Quorum sensing is a communication method that bacteria use to coordinate biofilm formation. Inhibiting this process can disrupt biofilm development.
c) **Anti-microbial peptides**: Synthetic peptides, such as those derived from natural sources (e.g. human cathelidin LL-37) and antimicrobial peptide 1018, can inhibit biofilm formation. These peptides work by binding to, and degrading key signalling molecules involved in biofilm maintenance.

Overall, these approaches aim to prevent or disrupt biofilm formation, making bacterial infections more susceptible to treatment.

2.3 Teichoic Acid

Teichoic acids are crucial components of the bacterial cell wall, particularly in gram-positive bacteria. They play several important roles, including helping bacteria survive in hostile environments, resisting antibiotics, aiding in surface colonization, maintaining cell shape, regulating ion balance, and avoiding host immune defences. Because humans lack teichoic acids, they present an attractive target for new antibiotics.

Inhibiting teichoic acid synthesis can make bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA), more susceptible to antibiotics like beta-lactams. Research has focused on disrupting this process, particularly targeting the D-alanylation pathway, which modifies the cell wall and affects its charge. Several agents have been identified for their ability to inhibit teichoic acid synthesis, targocil (targets the biosynthesis of teichoic acids), tarocins (Also inhibit teichoic acid production), tunicamycin (A broad-spectrum antibiotic that interferes with the synthesis of various cell wall components, including teichoic acid). These inhibitors can potentially resensitize resistant bacteria to existing antibiotics and are a promising area of research for combating antibiotic-resistant infection. [10] The teichoic acid inhibitor structures are given in fig. 1 and 2.

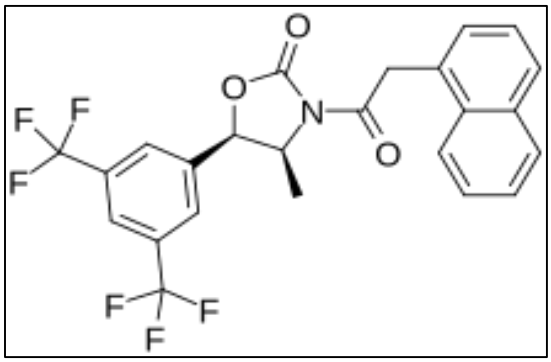

Fig.1: Inhibitor structure of tarocin A

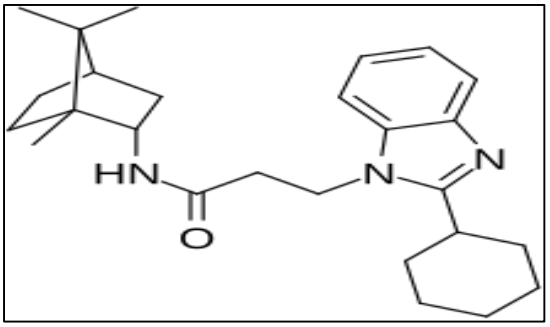

Fig.2: Inhibitor structure of tarocin B

2.4 Quorum sensor synthesis inhibitors

Autoinducers are signaling chemicals that is produced by bacteria to aid in population estimation and communication. Bacteria basically multiply to increase their population when the autoinducer level is too low. The pathogenicity grows, biofilms form, and their synthesis is auto induced when a threshold number is achieved.

Quorum sensing (QS) is therefore a possible target for novel antimicrobial drugs that inhibit QS activation in vivo, hence lowering pathogenicity. Blocking the effects of QS compounds does not apply selective pressure for emergency resistance because QS does not directly contribute to the development of bacteria. Quorum quenching, another name for QS inhibition, can be accomplished by using antagonistic chemicals to block receptors, preventing the formation of autoinducers, and using hydrolytic enzymes to break down the autoinducers. [11,12]

2.4.1. Acyl homoserine lactone synthesis inhibitors

In most bacteria QS system is done by producing of signal substance called acyl homoserine lactones (AHL). By inhibiting AHL synthase QS system can be obstructed. Some substrate analogues, including butyryl S-adenosylmethionine (butyryl-SAM), acyl-carrier-protein (holo-ACP), sinefungin and L/D-S-adenosylhomocysteine, can block AHL production in vitro. But the mechanism of action is not yet understood clearly. [13]

3. Novel targets and their pathways available for antimicrobial activity

There are various targets and as well as enzymes involved in antimicrobial activity. The various targets with their biological pathways are given in fig.3.

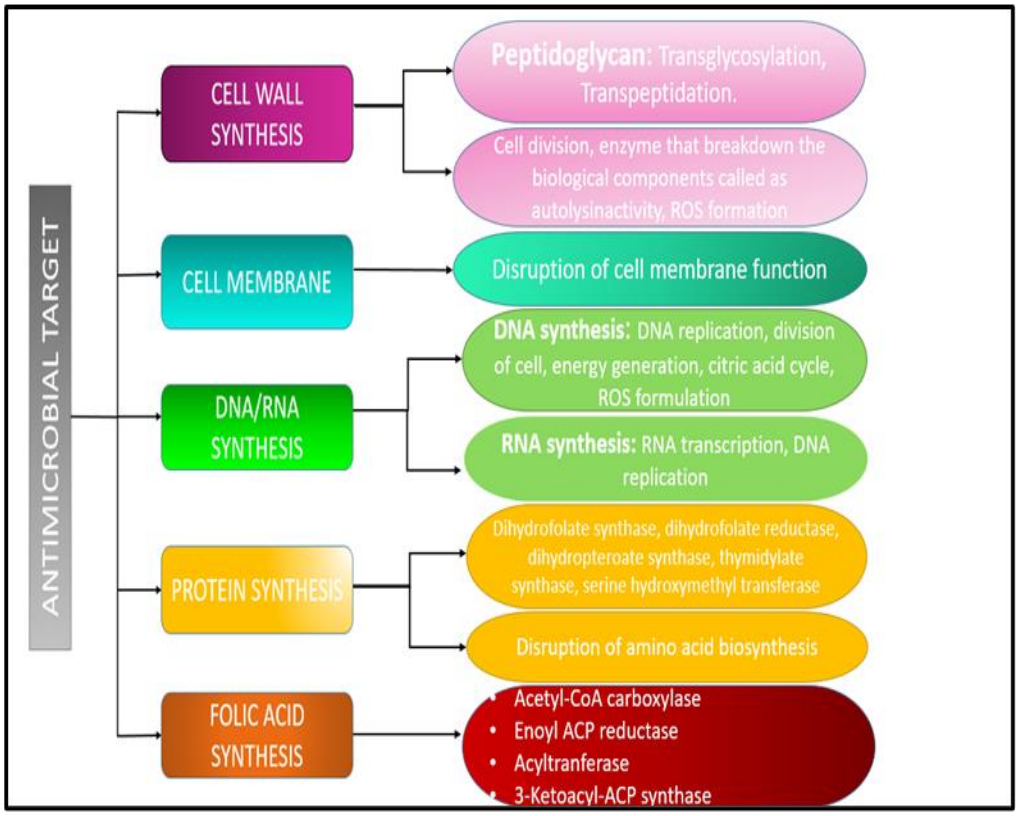

Fig. 3: Various primary antimicrobial target with their biological pathways

3.1 Cell wall biosynthesis

Peptidoglycan (PG) is a vital component of bacterial cell walls, it consists of alternating sugars (N-acetylglucosamine, GlcNAc, N-acetylmuramic acid and MurNAc) linked to a pentapeptide chain. Its synthesis begins in the cytoplasm, where UDP-GlcNAc is created from fructose-6-phosphate by Glutamine-fructose-6-phosphate amidotransferase (Glm) enzymes.

At that time, UDP-N-acetylmuramyl-pentapeptide (UDP-Mpp) is produce from UDP-GlcNAc by Mur enzyme (MurA through MurF). The process continuous when the enzyme MraY transfer the MurNAc-pentapeptide from UDP-Mpp to a lipid carrier called undecaprenyl phosphate (C55-P), forming lipid I, resulting in lipid II. This lipid II is then moved across the membrane to the outer side by a flippase, where it integrates into the growing peptidoglycan layer. This layer is necessary for supporting cell wall structure and integrity. Targeting these enzymes offers a novel approach for developing new antibiotics. [14]

3.1.1 Undecaprenyl phosphate (C55-P) and lipid II

Undecaprenyl phosphate (C55-P) is a notable molecule which is crucial for the construction of bacterial cell wall. 55-carbon long chain isoprene lipid is necessary. It is also known as bactoprenol which is involved in the formation of peptidoglycan, teichoic acids, lipopolysaccharides, entero antigen and capsule polysaccharides. The enzyme UppS catalyzes the synthesis of undecaprenyl pyrophosphate from precursors, which is then dephosphorylated by UppP to form undecaprenyl phosphate. The MraY and MurG enzymes catalyze the straight transfers of the MurNAc-pentapeptide (M-pep) and GlcNAc from the peptidoglycan nucleotide precursors onto the C55-P lipid, generating the lipid I and lipid II intermediates, consecutively. The lipid II is then shifted to the outer side of the cytoplasmic membrane, where the polymerization reactions catalyzed by the penicillin-binding proteins (PBPs) and the shape, elongation, division and sporulation family of proteins occur. Hence, the carrier lipid is released in C55-PP form and should be dephosphorylated before being reused for de novo peptidoglycan (or other cell wall components) synthesis. [15] Colicin M (ColM) is assigned into the periplasmic membrane, where it mainly targets lipid II. The various antimicrobial agents including glycopeptides such as vancomycin, nisin, ramoplanin and mannopeptimycins inhibits the lipid II enzyme. It cleaves the phosphoester bond that connects the C55 lipid to the pyrophosphoryldisaccharide-peptide, effectively degrading lipid II. By doing so, ColM disrupts the normal cycle of peptidoglycan synthesis, inhibiting bacterial cell wall assembly. ColM acts as a potent antibacterial agent by interfering within the critical steps in peptidoglycan synthesis pathway, ultimately compromising bacterial unity and feasibility. [16] The inhibitors structures for the enzymes C55-P are given in the fig.4.

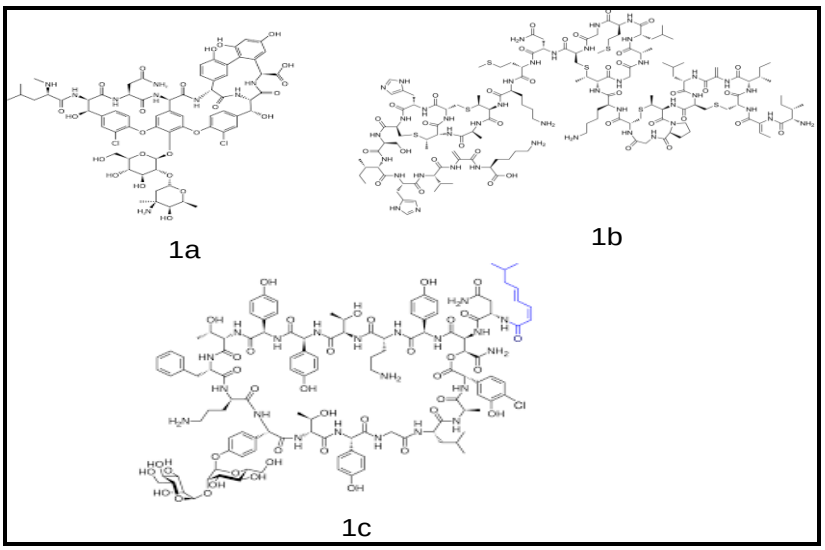

Fig. 4: Structures of Inhibitors for C55-P target; 1a - vancomycin, 1b - nisin, 1c - Ramoplanin

3.1.2 UDP-N-Acetylmuramate (Mur) enzymes

The Mur enzymes, also known as UDP-N-acetylmuramate. Ligases are essential components in bacterial cell wall biosynthesis, specifically in the formation of peptidoglycan, a critical structural component that maintains bacterial cell shape and integrity and it is a target for many antibiotics due to its role in bacterial cell wall synthesis. Mur enzymes catalyse the ligation of UDP-N-acetylmuramic acid (UDP-MurNAc) with L-alanine or D-alanine, which is the first step in the formation of the peptide stem that connects adjacent peptidoglycan strands. This process involves several enzymatic steps and is tightly regulated to ensure proper cell wall formation. This process is fundamental for maintaining the structural integrity and shape of bacterial cells, protecting them from osmotic stress and environmental challenges.

There are various types of Mur enzyme such as Mur A, Mur B, Mur C, Mur, D, Mur E, Mur F etc. MurA is known as UDP-GlcNAc enolpyruvyl transferase catalyzes the transfer of an enol pyruvate moiety from phosphoenol pyruvate (PEP) to Uridine diphosphate N-acetylglucosamine (UDP-GlcNAc) with phosphate. Its mechanism of action is an addition–elimination one: Asp305 (numbering of MurA from *Escherichia coli*) and Cys115 of MurA catalyze the anti-addition of PEP on UDP-GlcNAc to generate the matching tetrahedral intermediate, after which UDP-GlcNAc enolpyruvate is enabled by syn-elimination with the help of nicotinamide adenine dinucleotide phosphate (NADPH). [17]

MurB is also known as UDP-GlcNAc enolpyruvate reductase, which catalyzes the stereoselective reduction of UDP-GlcNAc enolpyruvate to UDP-MurNAc. The presence of FAD is an important element which is located on the active sites of this enzyme. The creation of the FAD-MurB is the initial stage in the mechanism. In addition, with the development of these the action of the NADPH-MurB complex is caused by the reduction of FAD by NADPH. Transferring the H4 (pro-S) from NADPH to the FAD intermediates FADH2-MurB. [18]

MurC-F ligases catalyze the sequential synthesis of tri-, tetra-, and penta-peptides by MurC, MurD, MurE, and MurF, which results in the creation of the lipid I precursor (UDP MurNAc-pentapeptide,) from UDP-MurNAc. The protein sequence characterisation for MurC-F enzyme has been carried out on various gram-positive and gram-negative bacterial strains. Although there are variances throughout species, they all have the same structural topology, that is, a protein having an active site that is conserved that is split into three distinct domains. [19,20]. The inhibitors structure of MurA-G enzyme is given in fig.5, 6, and 7. Fig.8 shows the pathways and the enzymes with their inhibitors involve in Mur target.

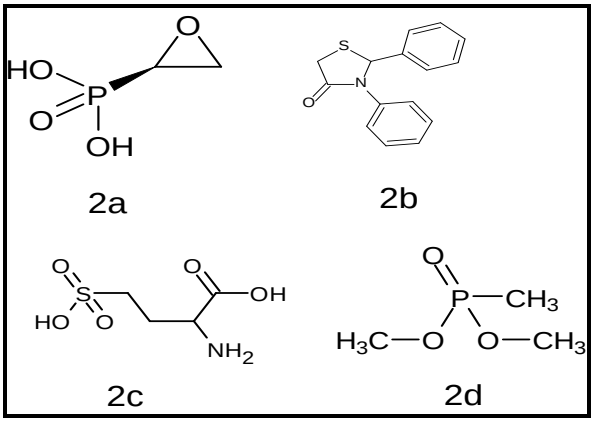

Fig. 5: Inhibitors Structures of MurA to MurE; 2a - Fosfomycin, 2b – 4-Thiazolidinone, 2c – D Homocysteic acid, 2d - Phosphinate

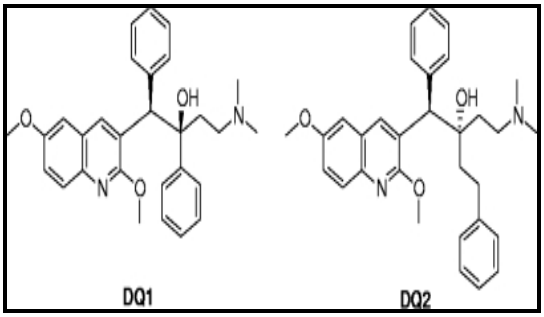

Fig.6: Inhibitors structures of MurF

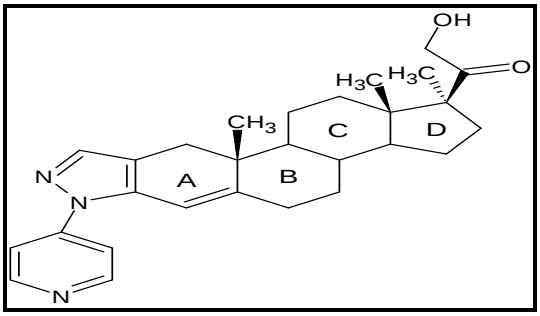

Fig.7: Inhibitors structures of MurG - Murgocil

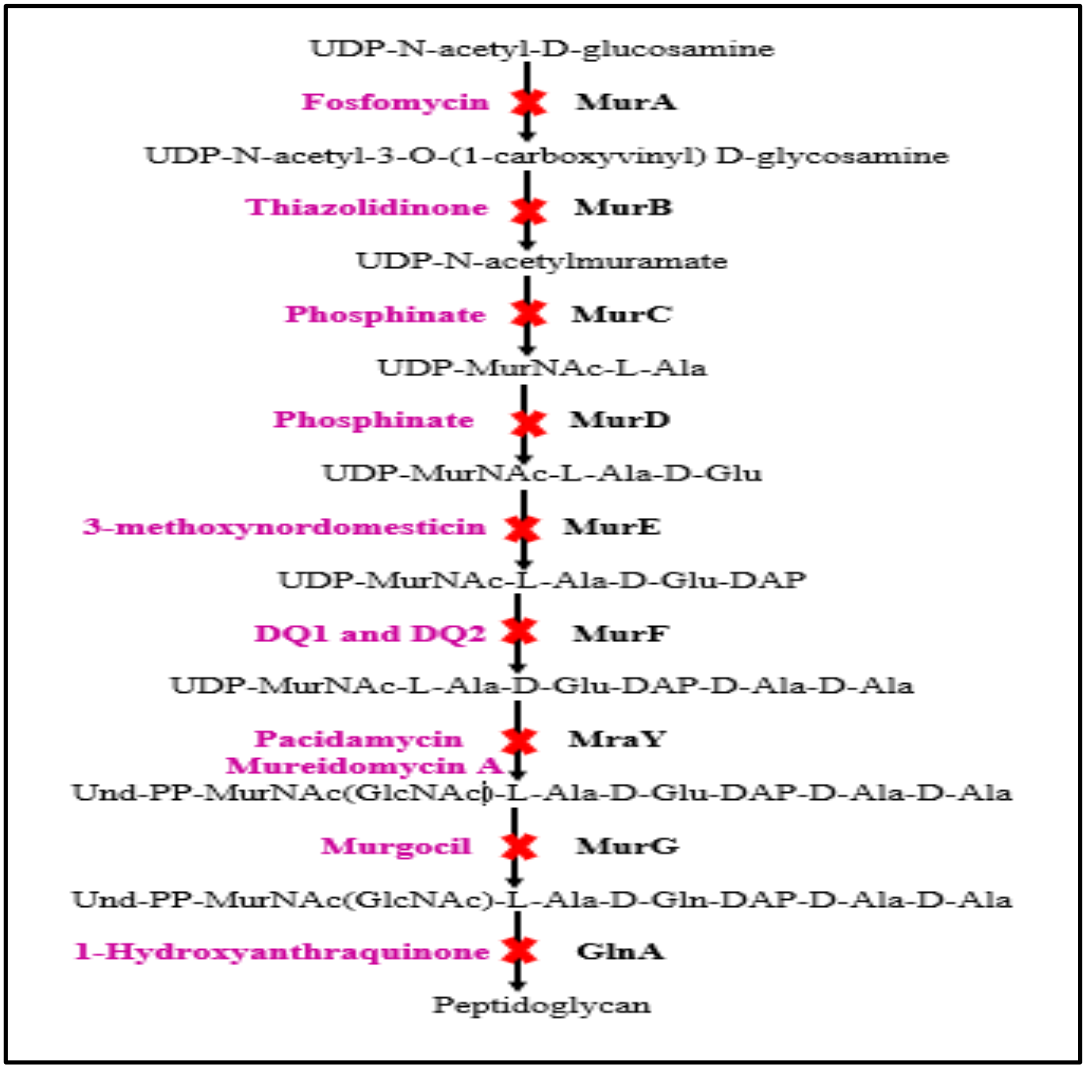

Fig.8: Pathway of Mur enzymes with their inhibitors

3.1.3 Glutamine A (GlnA)

Glutamine (Gln) is a major molecule in nitrogen metabolism and thus, it acts as the nitrogen donor for several cellular nitrogen-containing molecules. It has been seen that most of the pathways that drive nutrition and cellular metabolism, especially those involved with Gln metabolism, play significant roles in enhancing the severity of various bacterial pathogens. Glutamate and ammonia are transformed to Gln by the glnA encoded enzyme (glutamine synthetase, GS) in an ATP-driven reaction in most cells. As a major enzyme in nitrogen assimilation, GS plays an important role in *Salmonella enterica* growth and it is said to control the important traits in the bacteria including the mobility-related and virulence-related traits due to the existence of Gln. Also, glnA is one of the virulence factors that promotes the pathogenicity of *Streptococcus* , its severe growth attenuation in mouse models has been attributed to the deletion of glnA gene. The molecule is an essential amino acid and shows an important role in many bacterial processes, including the cell wall and as key protein residues. GS can direct the Gln metabolism that shows biofilm formation. Earlier, CNS was considered non-pathogenic with little to no virulence factors. But recently the discoveries and increased isolation rates by several investigators have shown that *S. xylosus* is highly pathogenic and can cause a large-scale infection under favourable conditions. The toxicity of *S. xylosus* and its biofilm formation is still unclear under the role of GS. Currently, GS is recognised as an effective target in drugs for curing human diseases induced by the pathogenic bacteria such as *Mycobacterium tuberculosis*. [21] Fig.8 shows the pathways and the enzymes with their inhibitors involve in Mur target. The inhibitor structures of GlnA enzymes are given in fig.9.

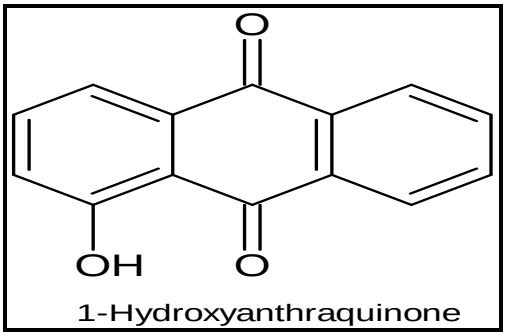

Fig.9: Inhibitor structure of GlnA

3.1.4 Phospho-MurNac-pentapeptide translocase (MraY)

Phosphate-MurNac-pentapeptide transpotentator or MraY plays a role in the bacterial cell wall synthesis pathway and is an enzyme that is part of the polyprenyl-phosphate N-acetylhexosamine 1-phosphate-transferase (PNPT) family. MraY is a highly interesting target for the discovery of novel antibiotic drugs because it is necessary for the survival of bacteria but absent in eukaryotes.

During peptidoglycan biosynthesis, the enzyme Phospho-N-acetylmuramoyl-pentapeptide-transferase (MraY) is responsible for the formation of lipid I and it catalyze the transfer of the phosphor MurNAcpentapeptide group to the undecaprenyl phosphate. The fact that it is the target of five classes of naturally occurring nucleoside inhibitors that have been isolated from Streptomyces species that exhibit strong antibacterial activity—liposidomycins/ caprazamycins, capuramycins, mureidomycins, muraymycins, and tunicamycin which makes it a promising candidate for the development of antibiotics. [22]

The lipid lipoligacy is catalyzed by phosphate-MurNAc-pentapeptide transpotentator (MraY), an integral membrane enzyme that is necessary for the first membrane-associated and initiated phase in peptidoglycan synthesis. With the help of the Mg^{2+} cofactor, it converts phospho-MurNAc-pentapeptides from the hydrophilic substrate uridine diphosphate-MurNAc-pentapeptide (UM5A) into undecaprenyl phosphate (C55-P), resulting in the production of uridine monophosphate (UMP) and undecylene-P–P-MurNAc-pentapeptide (lipid intermediate I). [23] The inhibitors structure of MraY enzymes is given in fig.10.

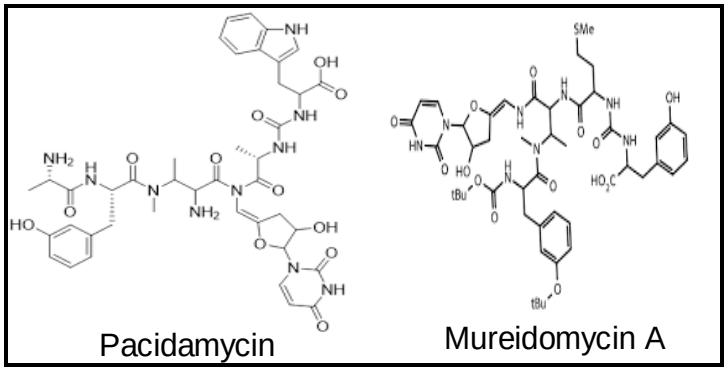

Fig. 10: Inhibitors for MraY targets

3.2 Fatty acid biosynthesis

Fatty acid biosynthesis (Fab) is important to produce phospholipids. Fatty acid biosynthesis in bacteria takes place by a various kind of fatty acid synthase II (FASII), while in mammals it is accomplished by a single multifunctional type I synthase. Because of this distinguishing feature, type II fatty acid synthase might act as an ideal target for the development of a novel antimicrobial agent. Almost all the FAS II enzymes are required for bacterial survival and, in general, form good targets for the recognition of antimicrobial agents. Discovering substances that inhibit acetyl-CoA carboxylase is a viable target site because fatty acid production starts with the carboxylation of acetyl-CoA by acetyl-CoA carboxylase, which yields malonyl-CoA. [13] The inhibitor structure of FAS enzymes is given in fig.12.There are many natural products which inhibit the growth of bacteria by blocking acetyl-CoA such as andrimid and moiramide B. [24] The elongation step will begin with the carboxylation of acetyl-CoA by acetyl-CoA carboxylase to produce malonyl-CoA, in fatty acid cycle. Because of these features, FabH has been targeted for drug identification, and this phase is targeted by many natural compounds. [25] Two natural antifungal drugs called cerulenin and thiolactomycin target FabB/FabF, an essential inhibitor of type II fatty acid production. The generation of certain lipid-containing components, such as cell membranes, depends on fatty acid synthesis (FAS) in bacteria.

Mammalian fatty acid synthesis is controlled by a single multifunctional enzyme-acyl carrier protein (ACP) complex known as type I, whereas bacterial fatty acid synthesis is carried out by a group of separate enzymes known as type II. [26] The enzymatic pathway for Fatty acid synthesis is given in fig.11. The inhibitors structures for FAS target are given in fig.13.

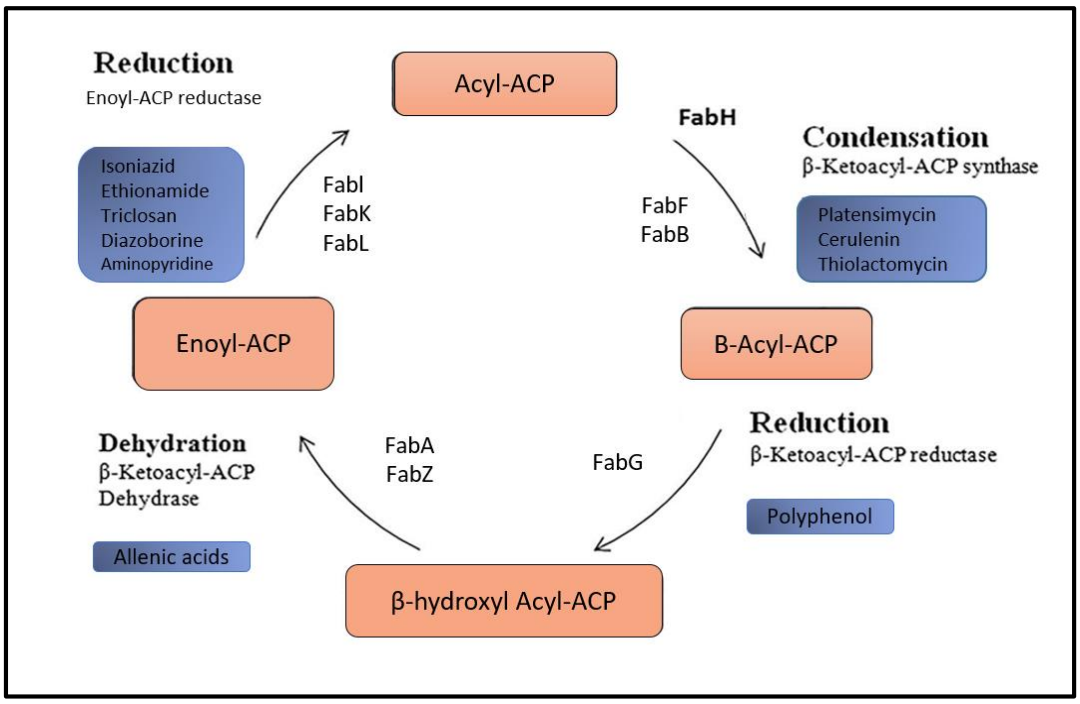

Fig.11: Enzymatic pathway for Fatty acid synthesis

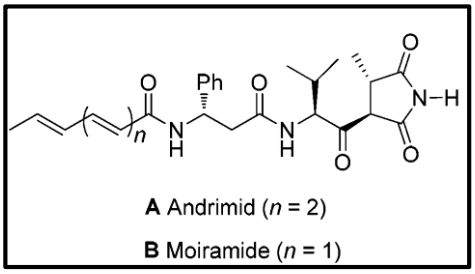

Fig.12: Inhibitors structure of FAS enzyme

3.2.1 Enoyl-[acyl-carrier-protein] (ACP) reductase (FabI)

The last stage in each elongation cycle was finalized by the bacterial target FabI, Enoyl [acyl carrier protein] reductase (InhA), a crucial enzyme involved in type-II fatty acid biosynthesis. It has enough NADH-dependent activity to enable a β-oxidation reversal to function effectively. After being cloned and purified, *Bacillus subtitis* FabI (bsfabl) showed characteristics like those of *E. coli* FabI, such as a preference for NADH as a cofactor over NADPH. For the synthesis of butyrate and longer-chain carboxylic acids, overexpression of the bsFabl gene was just as successful as the *E. coli* FabI mutant. [27]

3.2.2 β-Ketoacyl-Actl Carrier Protein Synthase lll (FabH)

In bacterial fatty acid synthesis (FAS), FabH plays a key role in the initiation of fatty acid chain elongation cycle. It is also essential for the survival of bacteria and for the maintenance of cell membrane processes. [28]

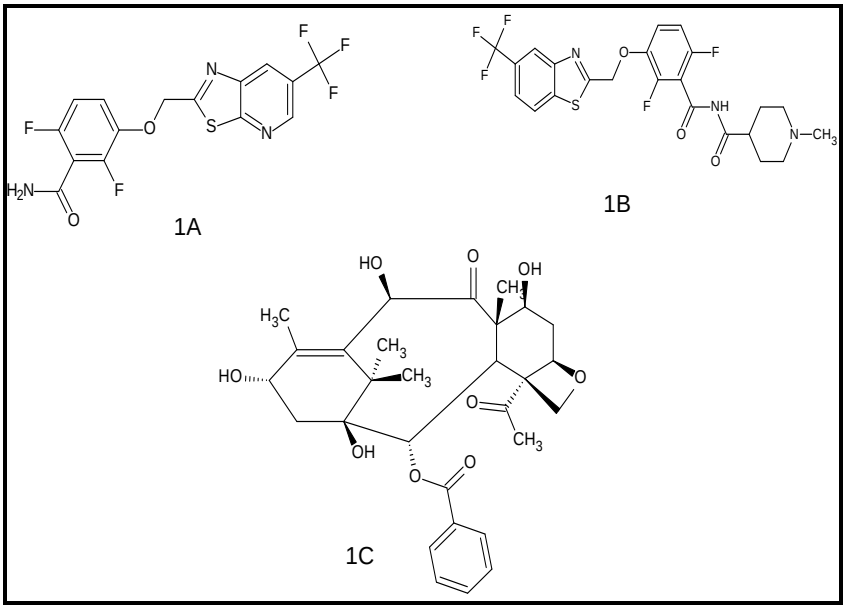

Fig.13: Inhibitors for FAS target 1A- TXA707, 1B- TXA709, 1C- Paclitaxel (Taxol)

3.3 Cell membrane target

The most potential target in which the bacteria need to survive is the cell membrane. The nomination of membrane-active agents to combat against bacteria will be affected by the dissimilarity in lipid composition and membrane structure between bacteria and eukaryotic organisms. [29] The examples of negatively charged phospholipids are lipoteichoic acids, cardiolipin, phosphatidylglycerol, and lipopolysaccharides that has been announced to be produced by bacteria. The negatively charged phospholipids like cardiolipin and phosphatidylglycerol can be found on the surface of cell membranes while anionic lipids are restricted to the monolayer that exists inside the cell in eukaryotic cytoplasm membranes. [30] Several antimicrobial medications can end the synthesis of lipopolysaccharide at various stages and because of this process, the bacteria's cell membranes become more permeable, making this protective layer futile and making them more at risk to destruction. On the top of that, by preventing solutes from escaping through the outer membrane, antimicrobial agents will result in death. Thus, blocking the production of bacterial cell membranes presents a potential approach to developing medications that target Gram-negative bacteria exclusively. [31] Phospholipids including phosphatidylglycerol, cardiolipin, and phosphatidylethanolamine may be targets for an antimicrobial agent. Another contrast is that eukaryotic membranes have exposed lipids on the outside, while phosphatidylethanolamine and anionic lipids are primarily restricted to the cytoplasmic surface. This change will help in the development of novel antibacterial drugs that target the lipids of bacteria. [32]

3.3.1. Cardiolipin

When cationic antimicrobial peptides are present, cardiolipin a unique phospholipid will separate into a domain. Daptomycin acts by directly interacting, in a calcium-dependent manner, with the cell membrane's division septum. Cardiolipin synthase mutations have been observed in *Enterococcus faecalis*, which is resistant to daptomycin. Daptomycin becomes redirected and confine in different membrane regions because of mutations that makes no longer connect with the septum. [33]

3.3.2. Menaquinone (vitamin K 2) biosynthesis inhibitor

Menaquinones (MK) are vital essential components of the cell membrane and are involved in the synthesis of cytochromes, endospores, pathogenicity, and the electron transport chain in the majority of anaerobic, mycobacteria and gram-positive bacteria. Instead of utilizing MK for respiration, mammals use ubiquinone. Since humans are unable to synthesis MK, they must obtain it from their diet or intestinal microbiota. Therefore, the MK biosynthesis pathway could be a target for the creation of new antimicrobial drugs to treat illnesses brought on by MK-using microbes. The chorismate pathway produces MK, and inhibitors of biosynthetic enzymes have been found. Analogs of the enzyme's substrates or cofactors make up the majority of inhibitors. Respective enzymes, including MenF, MenD, MenH, MenC, MenE, MenB, MenA, and MenG, are involved in the conventional pathway. [34] There are several known inhibitors for these enzymes. Vancomycin is less effective than lysocin E, a naturally occurring chemical that works by binding directly to menaquinone inside the membrane. [35] Ro 48-071 inhibits the MenA enzyme in Mycobacteria. [36]

3.3.3. WalK/WalR

The WalK/WalR two component system (TCS) is an essential for bacterial growth and cell envelope remodelling, it is hugely conserved signalling pathway in gram positive bacteria including some main pathogen especially in *Clostridiodes defficile*, an important pathogen which are responsible for numerous hospitalization and deaths. This system is important and essential for bacterial growth, cell wall metabolism for maintaining the integrity of the peptidoglycan (PG) sacculus, which surrounds bacterial cells shield them from internal pressure. [37]

In term of its structure, WalK feature a large extracellular domain lie close (nestled) between a transmembrane segment. [38] This configuration allows the WalK to sense environmental segment effectively while WalR has conserve receiver and effector domain, which characteristic of the OmpR family of response regulators. These domains are crucial for its role in regulating transcription. [39] Functionally, in some histidine kinases, like WalK acts as a bifunctional kinase and phosphatase in *Bacillus anthraces* while the WalR helps to response the regulator that manages gene expression. So, this system can regulate genes involves in all envelope biogenesis particularly in PG hydrolase. While phosphatase activity for WalK in *Bacillus subtilis* hasn’t been experimentally confirmed, it is suggested by computational analysis. [40]

Recent research shows that fragments of peptidoglycan generated during remodelling can attach to WalK’s extracellular domain influencing its activity and consequently the phosphorylation state of WalR. When WalR is phosphorylated, it can be activated or repress a variety of genes, many of which are crucial for proper cell envelope formulation and function, especially these coding for PG hydrolases. This understanding of the WalRK TCS not only sheds light on bacterial potential as a target for new antibiotics, given its crucial role in cell wall metabolism and response to environmental signals, making it a key player in the survival of gram-positive bacteria. Its conservation and conservancy across species highlight its significance, and ongoing investigation into its mechanisms and functions may pave the way for new antimicrobial therapies. [41] The inhibitors structures of WalK/WalR enzymes are given in fig.14.

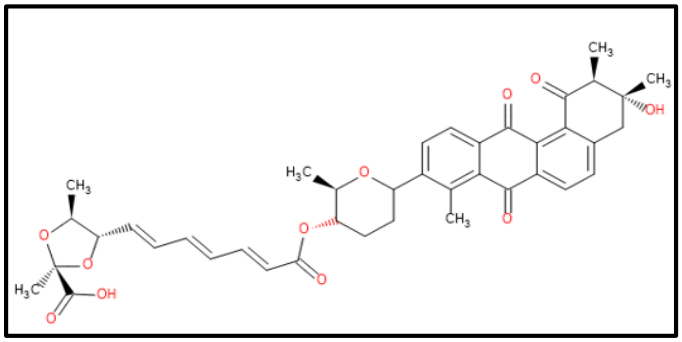

Fig.14: inhibitor structure of Waldiomycin

3.4 Cell division target

At the core of bacteria cell division is the guanosine triphosphate (GTPase) Filamenting temperature-sensitive mutant Z (FtsZ), which is crucial for forming a ring-link structure known as the z-ring at the midpoint of the cell. [42] This Z-ring acts as a scaffold for recruiting other essential proteins, such as FtsA and ZapA, B, C, and D, which are necessary for the subsequent steps in cell division. Since these protein-protein interactions, particularly between FtsZ and FtsA, are essential for bacterial survival, targeting them offers a promising approach for developing new antimicrobial agents. [43,44]

Inhibiting the cell division process by focusing on FtsZ, the central mediator of cell division, has emerged as a potential strategy for new antibiotics. These agents either disrupt the normal function and dynamics of FtsZ or trigger a protease to degrade FtsZ, leading to bacterial cell death. Examples of such bactericidal agents include PC190723 and Viriditoxin, which specifically target and inhibit FtsZ to prevent cell division. [45]

3.4.1. Filamenting temperature-sensitive mutant Z (FtsZ)

"Filamenting temperature-sensitive mutant Z" FtsZ is a protein encoded by the ftsZ gene that assembles into a ring at the future site of bacterial cell division which is also known as the Z ring. [46] This FtsZ protein is essential for bacterial cell division and an ideal target for novel anti-bacterial drugs, which involved in cell division forming single-stranded filaments by guanosine 5-triphosphate (GTP) dependent polymerization and assemble into the contractile ring (Z ring) and form in the inside of the cytoplasmic membrane where it marks the future site of the septum of dividing bacterial cell. FtsZ is a prokaryotic homologue of the eukaryotic protein tubulin. [47]

In most of the bacteria, archaea, chloroplasts and some mitochondria, FtsZ is establish where it is very crucial for cell division. FtsZ assembles the cytoskeletal scaffold of the Z ring that, along with additional proteins, constricts to divide the cell in two and FtsZ is essential for the germ and is widely conserved throughout bacteria. It exhibits all the necessary features of a new target for antibiotic compounds. FtsZ is the first protein to confine at the division site and recruits other proteins involved in bacterial cell division. Besides serving as a scaffold for the other cell division proteins, FtsZ itself may exert cytokinetic forces that guide to cell division. [48]

3.5 Lipopolysaccharide biosynthesis

Liposaccharide (LPS) are the complex molecules, and it is made up of the outer leaflet of the outer membrane of most gram-negative bacteria is referred to as an endotoxin. It is consisting of a hydrophilic polysaccharide chain and hydrophobic component is referred to as lipid A. [49] For the bioactivity of endotoxin lipid A is responsible, and immune cells will acknowledge it as a pathogen associated molecule. [50] In *Escherichia coli*, many of the enzymes and gene coding are essential for the biosynthesis of lipopolysaccharides have been recognize. LPS structure can vary from one bacterium to another, and current discovery have disclosed that additional enzymes and gene products that can change the basic structure of LPS in some bacteria. LPS molecules are liable for the survival of bacteria, particularly in pathogens. [51] Some LPS variants can be the source for human diseases such as septic shock. The biosynthesis of LPS has been diligently studied to develop strategies to control gram-negative pathogens and to cure septic shock. While LPS is distributes on the surface of bacterial cells. Its synthesis is starts with the transport of polysaccharides and it involves in structural modification of lipopolysaccharide, virulence of bacteria. [52]

3.5.1 UDP-(3-O-acyl)-N-acetyl glucosamine deacetylase (LpxC)

The LpxC [UDP-(3-O-acyl)-N-acetyl glucosamine deacetylase] enzyme is widely conserved over Gram-negative microbes, and it is main for synthesizing lipid A enzyme. The key protein in the biosynthesis of lipopolysaccharides, which are the major component of the external membrane. Gram-negative microbes, such as Escherichia coli, have sequentially modified leading to constrained treatment choices. This is due to their lipopolysaccharide (LPS) external film components, which serve as endotoxins and ensures the protection of Gram-negative microscopic organisms from anti-microbial. [52] The LpxC protein induces the committed step of LPS biosynthesis, making LpxC an optimistic target for antibacterial agents. [53]

The Kinetic analysis aims that LpxC works by a general acid-base mechanism, with glutamate being the general base. Numerous potent LpxC inhibitors have been identified, and most of them accommodate a hydroxamate group that targets the catalytic zinc ion. They recently searched compound CHIR-090 as a powerful antibacterial agent that inhibits the growth of *E. coli* and *P. aeruginosa*, with a potency equal to that of the FDA-approved antibiotic ciprofloxacin. [54]

When developing new antibiotics to combat gram-negative bacteria, the bacterial enzyme LpxC, which catalyzes the initial committed step of lipid A biosynthesis, is a promising target. The Zn2+-dependent non-metal structure, catalytic reaction mechanism, and control have been well studied. Since the enzyme is highly conserved in Gram-negative bacteria and has no sequence homology with any mammalian protein, it can be used to create gram-negative selective antibacterial treatments with few off-target effects. It is required for the growth and viability of gram-negative bacteria. The substrate analog TU-514, the aryloxazoline L-161, the sulfonamide, the N-aroyl-L-threonine derivative CHIR-090, the sulfone-containing pyridone LpxC-3, and the uridine-based inhibitor are just a few examples of the small-molecule LpxC inhibitors that have been developed and exhibit a variety of inhibitory and antibacterial properties.

A Zn2+-binding hydroxamate moiety coupled to a structural feature that addresses the hydrophobic tunnel or the UDP binding site is shared by many of these compounds. [55,56] The inhibitors structures of LpxC enzymes are given in fig.15.

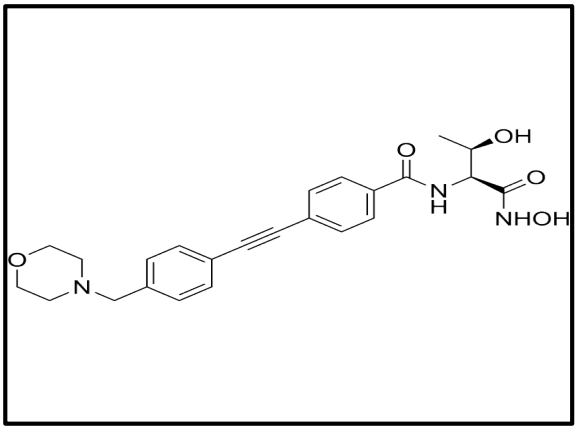

Fig.15: Inhibitor structure of LpxC (CHIR-090)

4. Conclusion

Due to increase in anti-microbial resistance and unavailability of potential anti-microbial to stop these microorganisms is becoming a major challenge in future. Combating this resistance requires new and improved anti-microbial agents. However, microbes often adapt quickly, creating new resistance mechanism. A set of potential targets have been exploding which are found to be effective in the antimicrobial activity. Several novel targets like cell wall synthesis, cell membrane synthesis, fatty acid biosynthesis inhibitor and cell division target have been utilised to check anti-microbial resistance action. These targets have shown a promising effect. The continuous research for antimicrobial resistance action and the increased resistance of microorganisms have posed a major drawback for the health care society. Inspite of the various new antimicrobial agents found by the researchers, it still presents a great challenge to overcome this anti-microbial resistance action. The review here focuses to explore various new antimicrobial targets and development which is found to combat anti-microbial resistance action and create new innovations.

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