Development of Antitubercular Agents: Potential for β-lactum Based Compounds

Mohammad Asif
Department of Pharmacy, GRD(PG)IMT, Dehradun, (Uttarakhand), 248009, India
E-mail: aasif321@gmail.com

Abstract: Development of antitubercular agents is the need to improve tuberculosis control. In recent years there is an enhanced activity in the development of new drugs for TB. Some compounds are presently in clinical development, while others are being investigated preclinically in an attempt to explore new molecules for the target based treatment of TB. Simultaneously some new targets are being identified and validated for their practical usefulness. Structures based on β-lactum could have considerable potential in the development of target based anti-TB agents. This review provides an overview of the drugs that are being clinically used and the compounds that are in advanced stages of clinical studies.

Keywords: β-lactum, Tuberculosis, Drug development, Chemotherapy.

Introduction: Mycobacterium tuberculosis (M. tuberculosis), which causes tuberculosis (TB), is a slow growing bacterium and was evolved more than ten thousand years ago from a soil bacterium [1-3]. Tuberculosis is a respiratory transmitted disease affecting nearly 32% of the world’s population, more than any other infectious disease. Among the infected individuals, approximately eight million develop active TB and almost two million of these die from this disease per year. Of new TB cases, 95% occur in developing countries every year and approximately one million young women per year are victimized with this disease in the developing world [4-7]. The occurrence of this disease is linked to dense population, poor nutrition, and poor sanitation. Tuberculosis, an airborne communicable disease caused by transmission of aerosolized droplets of M. tuberculosis [8-10]. The primary source of infection is viable tubercular bacilli, expelled in the environment by a patient with active TB. Mycobacterium is a genus of bacteria, which grows slowly under aerobic conditions and is distinguished by acid-fast staining. They are Grampositive, non-motile, rod-shaped, obligate aerobic bacteria that belong to the order actinomycetales, family Mycobacteriaceae [11,12]. Several species, including M. tuberculosis, M. bovis, M. africanum, M. microti, M. avium, and M. leprae, are intracellular pathogens of higher vertebrates (12). The cell wall of Mycobacterium species in its full structural and functional integrity is essential for its growth and survival in the infected host. M. tuberculosis possesses a cell wall dominated by covalently linked mycolic acids, arabinogalactan and peptidoglycan (AGP), the mycolic acids of which are complimented by glycolipids such as α,α-trehalose monomycolate (TMM) [13,14]. This mycolic acid based permeability barrier shields the organism from environmental stress and contributes to disease persistence and the refractoriness of M. tuberculosis to many antibiotics. One of the most prominent macromolecular entities of mycobacterial cell wall is arabinan, a common constituent of both arabinogalactan (AG) and lipoarabinomannan (LAM) [15-18]. In the chemical setting of mycolylarabinogalactanpeptidoglycan complex, AG forms an integral part of the cell wall proper, whereas LAM, based on a phosphatidylinositol anchor, apparently exists in a state of flux. LAM is an essential part of the cell wall core, anchored in the cell membrane and transversing the cell wall as well as appearing as an excretery product. LAM has been implicated as a key surface molecule in host-pathogen interactions. The biosynthetic pathways leading to the formation of the key mycobacterial cell wall components AG and mycolic acids are the target for the rational design of new antitubercular agents. The complete genome sequence of the best-characterized strain of M. tuberculosis H37Rv has been determined [19,20]. Moreover, the TB-structural genomics consortium has undertaken an extensive study to determine and analyze the structures of over 400 proteins from M. tuberculosis including 40 novel folds and 200 new families of protein structures. The database of linked structural and functional information generated will have lasting impact in understanding the M. tuberculosis pathogenesis and for structure based drug design. Further, recent strategies that target various pathways related virulence, including inhibition toxin function, toxin delivery, regulation of virulence expression and bacterial adhesion could provide a number of new targets for novel anti-TB drugs [21,22].

Beta-Lactam Antibiotics: The structure of the cell membrane of bacteria is unique and does not have any mammalian analogs. The cell membrane protects bacteria cells from lysis, which can occur as a result of different osmotic pressures between the cytoplasm and the surrounding medium. The main component of bacterial cell membranes is a mixed polymer known as murein or peptidoglycan. Peptidoglycan is a long polysaccharide chain that is cross-linked with short peptides. Polysaccharide chains
are made up of two varying aminosugars-\(N\)-acetylmuramic acid and \(N\)-acetylglucosamine. For example, Staphylococcus aureus golden staphylococci, a tetrapeptide made of L-alanine, D-glutamic acid, L-lysine, and D-alanine, is joined to every one of the \(N\)-acetylmuramic acid units, forming side chains of glycan chains. Many of these tetrapeptides are cross-linked with one another either directly or with short peptide chains. In S. aureus, L-lysine of one of the tetrapeptides is bound by a pentaglycine chain to D-alanine of the other. This kind of structure gives it a certain rigidity to bacterial membranes. The peptidoglycan layer of Gram-negative bacteria is thinner than that of Gram-positives, and it has fewer cross-transversal links. The synthesis of peptidoglycan of bacterial cell membranes can be divided into three stages based on where the reaction takes place. The first stage occurs in the cytoplasm, which results in the synthesis of precursor units-uridindiphospho-\(N\)-acetylmuramyl pentapeptide. Such an antibiotic, for example, cycloserine, the drug most frequently used to treat TB, blocks synthesis of cell membranes at this stage by competitive inhibition of the stage of introducing alanine into a pentapeptide [23-26]. Diffusion of beta-lactam antibiotics across this membrane is only possible through transmembrane channels made of proteins called porins. It has been shown that beta-lactam antibiotics diffuse through porine channels, and the ease of this process varies depending on their size, charge, and hydrophilic properties. Accordingly, the idea of the possible mechanism of resistance for Gram-negative bacteria being the inability of beta-lactam antibiotics to get desired penicillin binding proteins (PBPs) is also unlikely. The second mechanism of resistance to beta-lactam antibiotics can appear as a change in target PBP, which is expressed in a reduction in the affinity to beta-lactam molecules. Finally, the most important mechanism of resistance to beta-lactam antibiotics is the production of beta-lactamase by the bacteria. Beta-lactamases break the C–N bond in the beta-lactam ring of antibiotics. Since its existence is absolutely necessary for reacting with PBP, a break in the beta-lactam ring leads to a loss of antibacterial activity. There are many beta-lactamases and they can be classified differently: by type of substrate, replacement of genes chromosomes or plasmids), and place of production. Some of these enzymes directly hydrolyze penicillins penicillinases), others hydrolyze cephalosporins cephalosporinases), and others extend to a broad spectrum of substrates. Some bacteria have the ability to induce synthesis of beta-lactamase. Synthesis of beta-lactamase, which in a normal condition is suppressed, is induced in the presence of some beta-lactam antibiotics. Thus, beta-lactam antibiotics can inhibit the process of synthesis of bacterial cell membranes in different ways, thus causing them to die quickly [27-30].

The term “antibiotic” was given by Selman Waksman in 1942 to substances produced by microorganisms that inhibit the growth of other microorganisms [31]. However, today the term antibiotic is used for substances or antimicrobial agents from natural or synthetic molecule, that kill or inhibit the growth of microbes by specific interactions with bacterial targets, without harming the eukaryotic host harboring the infecting bacteria [32,33]. An antimicrobial agent must have the potency (enter into the bacterial cell) and access (able to reach the target) in order to exert its antimicrobial action [34]. The major classes of antibiotics inhibit or kill the bacteria mainly by targeting a) cell-wall biosynthesis, b) protein synthesis, c) DNA replication and repair, d) disruption of bacterial membrane, and e) folic acid synthesis [33, 35] (Table 1).

Antibiotic resistance and mechanisms: Antibiotic resistance can be defined as “the ability of a microorganism to resist the antibiotic pressure and survive” [33,37], in contrast to the susceptible bacteria which will be eliminated. The effect of an antibiotic can either be bacteriostatic or bactericidal based upon the antibiotic target and concentration. Bacterial susceptibility to a particular antibiotic can be defined from both a microbial and a clinical point of view. From a bacterial point of view, a susceptible bacterium belongs to a sub-microbial and a clinical point of view. From a bacterial point of view, a susceptible bacteria can be divided into susceptible, intermediate susceptible, or resistant to antibiotics [38]. Mechanisms of resistance are found within bacteria either intrinsically or they may be acquired. The intrinsic resistance refers to existence of resistance genes as part of the genome encoding mechanisms intrinsically found in the population of the bacteria (genus or species) [32]. For instance, Gram-negative bacteria are intrinsically resistant to glycopeptides and macrolides due to their impermeable outer membrane [39]. Further, due to the lack of a cell-wall, Mycoplasma intrinsically shows resistance to \(\beta\)-lactams and other cell-wall biosynthesis targeting antibiotics [39]. In contrast, the acquired resistance mechanisms are attained by bacteria through mutations or mechanisms of horizontal gene transfer such as transformation, conjugation, and transduction [40, 41]. For instance, many \(\beta\)-lactamase genes are acquired by bacteria through mobile genetic elements such as plasmids [42-44], transposons [45], and insertion sequence common region (ISCR) elements [46]. Plasmids can replicate independently within bacteria and also...
transfer between bacterial cells and species, spreading resistance [33, 43]. Further, the rapid generation time of bacteria assist them to evolve quickly and hence become resistant to antibiotics with in a short period of time [40]. Generally bacteria exhibits biochemical resistance by three different mechanisms (Figure 1); a) by reducing their permeability into the cell and/or by active efflux mechanism [47,48], b) by structurally altering the antibiotic targets [49], c) by enzymatic modification or inactivation of the antibiotic before reaching the targets [4, 50]. Bacteria can combine these mechanisms to exhibit resistance towards antibiotics [51].

### Table 1. Classification of antimicrobials based on their target site [36].

<table>
<thead>
<tr>
<th>Target site</th>
<th>Target</th>
<th>Antibiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibition of cell wall synthesis</td>
<td>Penicillin binding proteins, D-alanyl-D-alanine, Muropeptide transport</td>
<td>Penicillins, Cephalosporins, Carbapenems, Monobactams, Daptomycin, Glycopeptides</td>
</tr>
<tr>
<td>Inhibition of protein synthesis</td>
<td>30s and 50s subunits of the ribosome</td>
<td>Tetracyclines, Chloramphenicol, Macrolides, Aminoglycosides, Linco-samides, Oxazolidinones, Streptogramins</td>
</tr>
<tr>
<td>Interference of nucleic acid synthesis</td>
<td>DNA gyrase, DNA structure integrity, RNA polymerase</td>
<td>Quinolones, Nitroimidazoles, Rifampicin</td>
</tr>
<tr>
<td>Disruption of bacterial membrane</td>
<td>Phospholipid structure</td>
<td>Polymixins</td>
</tr>
<tr>
<td>Inhibition of folic acid pathway</td>
<td>Dihydrofolate reductase, Dihydropteroate synthetase</td>
<td>Sulphonamides, Trimethoprim</td>
</tr>
</tbody>
</table>

**Figure 1**: Representation of bacterial resistance mechanisms [35].

A brief overview of the different mechanisms will be given below with the main focus on resistance to β-lactams.

**Reduced permeability and active efflux**: In Gram-negative bacteria, the outer membrane contains protein channels, formed by porin proteins important for nutrient transportation into the cell [52]. In order to prevent the entry of antibiotics, bacteria reduce the access of antibiotics mainly by changing the outer membrane (in Gram-negative bacteria) and cell wall (in Gram-positive bacteria). Gram-negative pathogens like *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* show resistance to antibiotics like β-lactams by altering the porins or by loss of porins [53]. As an example, the combination of deletion of outer membrane porins with the production of plasmid-mediated AmpC β-lactamas in *K. pneumoniae* can confer resistance to imipenem [54]. Another strategy is expelling the antibiotics out of the bacterial cell by active efflux through membrane bound efflux pumps [48]. *P. aeruginosa* harbour several efflux pumps like MexAB-OprM, MexCD-OprJ, and MexXY-OprM with various spectre of substrate profiles that includes different groups of antibiotics including β-lactams [55].

**Target alteration**: Bacteria can alter the targets of antibiotics structurally reducing the affinity for antibiotics. For instance, modification of penicillin binding proteins (PBPs) which are the main targets for β-lactams reduces the affinity for β-lactams [56]. The most known example is methicillin resistant *Staphylococcus aureus* (MRSA) which is achieved by the acquisition of an altered PBP (PBP2a or
PBP2’) by the mecA gene [57]. Also in Gram-negative bacteria such as A. baumannii [58] and P. aeruginosa [59] altered PBPs have been implicated in resistance towards β-lactams.

**Enzymatic inactivation or modification:** Most of the antibiotics are characterized by ester or amide bonds, which are hydrolytically susceptible, targeted by certain bacterial enzymes, and render them inactive [60]. β-lactamases are the major resistance mechanisms in this respect (discussed later). Modification of the antibiotic molecule is a major resistance mechanism in Gram-negatives to aminoglycosides conferred by aminoglycoside modifying enzymes [61].

**β-Lactam antibiotics:** The first antibiotic was accidentally discovered by Sir Alexander Fleming in 1928 from a mould culture of *Penicillium notatum*, which was able to kill *Staphylococci* [64]. The active substance from *Penicillium* was named “penicillin” [62]. Howard Florey and Ernst Boris Chain were able to produce penicillin in large quantities [63], which was first used to treat bacterial infections during the Second World War saving many lives and penicillin became known as the “magic bullet”. The discovery of penicillin revolutionized treatment of infectious diseases and became a milestone for the modern medicine. Fleming, Florey and Chain were awarded the Nobel Prize of Medicine and Physiology in 1945 for the discovery and application of penicillin. Later in 1949 the structure of penicillin was solved by x-ray crystallography and the β-lactam ring of penicillin was identified as the key functional property [64]. Since the discovery of penicillin, β-lactams have been our most important antibiotic group (>65% worldwide market) for the past 70 years and are used to treat infections caused by both Gram-negative and Grampositive bacteria [67, 65]. Based on the structure and discovery, β-lactams can be classified into four major groups; penicillins, cephalosporins, carbapenems, and monobactams (Figure 2).

The β-lactams are either natural or semisynthetic molecules, characterized by a basic nucleus of a four-membered lactam ring containing three carbon atoms and one nitrogen atom. In further development, the β-lactam ring is fused with a five or six membered ring in a bicyclic ring structure to enhance biological activity, β-lactamase stability, and reduce toxicity [47, 66]. The β-lactam ring is fused with a five-membered thiazolidine ring for penicillins, and to a six-membered dihydrotiazine ring for cephalosporins. Carbapenems have an additional ring that is similar to that of penicillins but is unsaturated and the sulphur atom is replaced by a carbon atom. In contrast, monobactams have no fused structures to the β-lactam ring. Further, different β-lactams belonging to same group are distinguishable by their side chain groups (such as R1, R2 and possibly R3) [66]. The four different classes of β-lactam antibiotics are described below in brief.

**Penicillins:** Penicillin was discovered in 1928 by Alexander Fleming, who noticed that one of his experimental cultures of staphylococcus was contaminated with mold, which caused the bacteria to lyse. Since mold belonged to the family *Penicillium*, he named the antibacterial substance penicillin. A crude substance made up of a few low-molecular substances, which were penicillins F,G,K,O,V, X). Penicillin G benzylpenicillin), the most active of these, was suggested for clinical trials in 1941. Drugs of the penicillin group are effective for infections caused by Gram-positive bacteria streptococcus, pneumococcus, and others), spirochaetae, and other pathogenic microorganisms. Drugs of this group are ineffective with respect to viruses, mycobacteria TB, fungi, and the majority of Gram-negative microorganisms. The production of penicillin was an extremely important milestone in the development of microbiology, chemistry, and medicine, signifying the creation of the powerful antibiotic industry and formation of modern biotechnology. There have been attempts to chemically synthesize penicillins; however, no practical methods have been found. An extremely important progress in the development of penicillins took place in 1959, when the penicillin nucleus, 6-aminopenicillanic acid 6-APA), was removed from the side chain and isolated from a culture of *Penicillium chrysogenum*. Subsequent acylation of 6-APA by various acid derivatives led to the formation of a large number of semisynthetic penicillins. Penicillin benzylpenicillin, penicillin G) is made in huge amounts (tens of thousands of tons) by the microbiological industry. Penicillin can be made by many types of *Penicillium* fungi, and also by a few types of *Aspergillus* fungi. In industrial conditions, culture fluids are made that contain more than 30 mg/mL of penicillin. About two-thirds of the produced penicillin is used for making 6-APA [35-40]. Despite the possibility of pure chemical deacylation, the most prospective way of making 6-APA is an enzymatic method of hydrolyzing benzylpenicillin molecules using immobilized penicillinamidase, an enzyme isolated from practically all penicillin-producing fungi. It should be noted that 6-APA itself is practically devoid of antibiotic properties. However, by acylating it with various acid derivatives, more than 30,000 semisynthetic penicillins have been made, of which less than 30 are currently used in medicine. Variations of the acyl regions of the side chain in penicillin molecules produces significant changes in the properties of resulting compounds. It was discovered that the side chain of the acyl region of the molecule determines the antimicrobial spectrum, sensitivity to β-lactams, and the unique pharmacokinetic features of a
specific penicillin. The unique feature of a few semisynthetic penicillins meticillin, oxacillin, cloxacillin is their efficacy with respect to a culture of microorganisms staphylococci (group 1; cephaloglycin and group 2; ceftrizine), miscellaneous derivatives such as ceftibuten (group 3), oral cephalosporin prodrugs such as cefuroxime (group 4), aryloxyimino derivative nonesterified compounds such as cefixime (group 5), and carbacephems such as loracarbef (group 6) [69]. The cephamycins are structurally similar to cephalosporins, but the cephalosporin nucleus is fused with a 7-alpha-methoxyl group. The additional group gives high level resistance to class A β-lactamaes [47]. Cephamycins are produced from actinomycetes. The first semisynthetic cephamycins was cefoxitin.

Carbapenems: The first carbapenem discovered was thienamycin from the culture filtrate of Streptomyces cattleya [70-72]. Thienamycin was unstable at pH >8, and thus was not suitable for clinical use [71]. Carbapenems can be divided into either natural origin such as thienamycin, or synthetic origin such as imipenem. Imipenem (N-formimidoyl thienamycin is a chemically stable compound compared to thienamycin, and was the first carbapenem approved for clinical use [73, 74]. The basic structure of carbapenem contains a four member β-lactam ring fused to a five member thiazolidinic secondary ring through the nitrogen and adjacent tetrahedral carbon atom [45]. The side chains fused to the core structure influence the antimicrobial activity [45, 46]. Carbapenems are the most potent class of β-lactams, and exhibits high activity against Gram-positive, Gram-negative, and anaerobic bacteria [75, 77]. Imipenem has a non-substituted group at position 1 to the basic thienamycin nucleus. Meropenem is structurally different with a methyl group. Other carbapenems approved for clinical use includes ertapenem and doripenem [75, 78].

Monobactams: SQ-26180 was the first monocyclic β-lactam derived naturally from Chromobacterium violaceum [79]. Later, this compound was successfully developed by in 1985 by demethoxylation at the C3 position and substitution with a 2-amino-5-thiazolyl oxime moiety into aztreonam [80]. Aztreonam is the only monobactam in clinical use and show high activity against Enterobacteriaceae and good efficacy against P. aeruginosa [81]. An overview of the antibacterial spectrum of β-lactams is presented in Table 2.

Mechanism of action of β-lactams: The β-lactams exert their bactericidal activity primarily on the cell-wall biosynthesis in bacteria. In the 1960s, the structure of the bacterial cell wall and the mechanism of its biosynthesis were described [83, 84]. The cell wall is a protective barrier for the bacterium in order to maintain the rigidity and to resist the internal osmotic pressure, and participates in cell division [85]. The bacterial cell wall is mainly composed of peptidoglycan; a complex polymer consisting of linear
Figure 2: The core structures of β-lactams.
Table 2. Classes of β-lactams and the antibacterial spectrum [82].

<table>
<thead>
<tr>
<th>β-lactam</th>
<th>Chemical class</th>
<th>Examples</th>
<th>Spectrum of activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillins</td>
<td>Penicillins</td>
<td>Penicillin-G, Penicillin-M</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Semi-synthetic β-</td>
<td>Amoxicillin, Ampicillin</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>lactams</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>1st generation</td>
<td>Cephalothin, Cefazolin</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>2nd generation</td>
<td>Cefoxitin, Cefuroxime</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>3rd generation</td>
<td>Ceftazidime, Cefotaxime</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>4th generation</td>
<td>Cefepime, Cefpirome</td>
<td>+</td>
</tr>
<tr>
<td>Penems</td>
<td>Carbapenems</td>
<td>Imipenem, Meropenem</td>
<td>+</td>
</tr>
<tr>
<td>Monobactams</td>
<td>Monobactams</td>
<td>Aztreonam</td>
<td>+</td>
</tr>
</tbody>
</table>

+: good activity; ±: reduced activity.

glycans interlinked by peptide chains and sugars, responsible for shape and integrity of the cell wall [83, 86]. The glycans chains are composed of alternating units of N-acetylmuramic acid (MurNAc or NAM) and N-acetylglucosamine (GlcNAc or NAG), which are linked to sugars by β-1-4 glycosidic bonds [86]. The neighbouring glycan subunits are interlinked either by direct linkage between peptide subunits of one chain with other peptide chains or by a short (5 amino acid long) peptide bridge between two peptides to form a rigid network [86]. The cell wall biosynthesis is performed by a series of membrane located transpeptidase enzymes, penicillin binding proteins (PBPs), due to their ability to bind penicillin molecules [56, 87, 88]. PBPs involved in peptidoglycan synthesis include activities such as glycosyltransferase, transpeptidase, and carboxypeptidase activities and are responsible for the cross-linking between the peptidoglycan subunits [49]. Many variants of PBPs are described (PBP1, PBP2, PBP2A, PBP2B, PBP3-PBP6), and categorized as low and high molecular weight PBPs [89]. In general, β-lactams target the cell wall biosynthesis by binding and inhibiting the PBP. The β-lactam nucleus mimics the terminal D-alanyl-D-alanine residue of the peptide and interfere with the serine hydroxyl group of PBPs inhibiting the transpeptidation reaction [90].

β-lactamases: The effectiveness of β-lactams relies upon their accessibility to their targets (PBP) and ability to inhibit them. The most common mechanism of resistance to β-lactams in bacteria is the production of hydrolytic enzymes, termed β-lactamases, which inactivate the β-lactams by disrupting the amide bond of their β-lactam ring [47, 85]. In Gram-positive bacteria, β-lactamases are either bound to the cytoplasmic membrane or excreted into the extracellular space, whereas in Gram-negative bacteria they are located in the periplasmic space [34]. β-lactamases are structurally related to PBPs and it is suggested that they might have evolved from the β-lactam binding enzymes of the cell wall biosynthesis [87]. The first β-lactamase was reported in Escherichia coli in 1940 [91], before the clinical release of penicillin. Since then β-lactamases have been reported in Gram-positive, Gram-negative bacteria and mycobacteria [92, 93]. So far more than 1000 β-lactamases have been reported [32, 94]. These enzymes are either chromosomally encoded or the genes are located on mobile genetic elements such as plasmids or transposons [95]. Consequently, bacteria are able to acquire β-lactamase genes and become resistant to β-lactams.

Classification of β-lactamases: β-lactamases show great diversity and different schemes have been proposed to classify them based on functional and biochemical properties [40, 96], as well as amino acid sequence similarities [97]. In the Ambler molecular classification scheme β-lactamases are classified into four different molecular classes, class A, B, C, and D based on amino acid sequence criteria (Table 3) [97]. The Ambler molecular classification can be grouped structurally into two super families; serine β-lactamases (class A, B, and D) and metallo-β-lactamases (class B). Although, serine β-lactamases and metallo-β-lactamases hydrolyze the β-lactams, the catalytic mechanism is notably different between them. The serine β-lactamases have a serine residue for the catalytic activity, while metallo-β-lactamases have catalytic Zn²⁺ ions important for the catalytic activity. The Bush and Jacoby classification scheme of β-lactamases is based on substrate/inhibitor specificity (Table 3) [98], and has recently been updated in order to accommodate newly discovered β-lactamases [99].
Table 3. Functional, molecular classification, and properties of β-lactamases [98,100].

<table>
<thead>
<tr>
<th>Bush-Jacoby Group</th>
<th>Molecular Class</th>
<th>Preferred substrates</th>
<th>Inhibition</th>
<th>Representative enzymes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C</td>
<td>Penicillins, Cephamycins, Cephalosporins, Aztreonam</td>
<td>-</td>
<td>MIR-1, CMY-2, FOX-1, P99</td>
</tr>
<tr>
<td>1e</td>
<td>C</td>
<td>Penicillins, Cephamycins, E-S cephalosporins, Aztreonam</td>
<td>-</td>
<td>GC1, CMY-37</td>
</tr>
<tr>
<td>2a</td>
<td>A</td>
<td>Penicillins</td>
<td>+</td>
<td>PCI1 and other staphylococcal Penicillinases</td>
</tr>
<tr>
<td>2b</td>
<td>A</td>
<td>Penicillins, early cephalosporins</td>
<td>+</td>
<td>TEM-1, TEM-2, SHV-1, TLE-1</td>
</tr>
<tr>
<td>2be</td>
<td>A</td>
<td>Penicillins, Monobactams, E-S cephalosporins</td>
<td>+</td>
<td>TEM-10, TEM-26, SHV-2 to SHV-6, CTX-M-15, CTX-M-44, PER-1, SFO-1, VEB-1, ESBLs</td>
</tr>
<tr>
<td>2br</td>
<td>A</td>
<td>Penicillins, early cephalosporins</td>
<td>±</td>
<td>TEM-30, TME-76, TEM-103, SHV-10, SHV-26</td>
</tr>
<tr>
<td>2ber</td>
<td>A</td>
<td>Penicillins, monobactams, E-S cephalosporins</td>
<td>+</td>
<td>TEM-50, TEM-68, TEM-89</td>
</tr>
<tr>
<td>2c</td>
<td>A</td>
<td>Carbenicillins</td>
<td>+</td>
<td>PSE-1, CARB-3</td>
</tr>
<tr>
<td>2d</td>
<td>D</td>
<td>Cloxacin or Oxacillin</td>
<td>±</td>
<td>OXA-1, OXA-10, PSE-2</td>
</tr>
<tr>
<td>2de</td>
<td>D</td>
<td>Penicillins, E-S cephalosporins</td>
<td>±</td>
<td>OXA-11, OXA-15</td>
</tr>
<tr>
<td>2df</td>
<td>D</td>
<td>Carbapenems, Cloxacin</td>
<td>±</td>
<td>OXA-23, OXA-48</td>
</tr>
<tr>
<td>2e</td>
<td>A</td>
<td>Cephalosporins</td>
<td>+</td>
<td>CepA</td>
</tr>
<tr>
<td>2f</td>
<td>A</td>
<td>Penicillins, Cephamycins, Cephalosporins, Carbapenems</td>
<td>+</td>
<td>IMI-1, KPC-2, KPC-3, NMC-A, SME-1, GES-2</td>
</tr>
<tr>
<td>3a</td>
<td>B</td>
<td>Penicillins, Cephamycins, Carbapenems, Cephalosporins</td>
<td>-</td>
<td>IMPs, VIMs, NDMs, GIa-1, Bcll, CcrA, L1, AIM-1, FEZ-1</td>
</tr>
<tr>
<td>3b</td>
<td>B</td>
<td>Carbapenems</td>
<td>-</td>
<td>CphA, Shh-1</td>
</tr>
</tbody>
</table>

CA: Clavulanic acid; EDTA: Ethylenediaminetetraacetic acid; E-S: expanded spectrum; +: positive; -: negative; ±: partially inhibited.

Since the discovery of 6-amino penicillanic acid (penicillin) in 1929 [101], β-lactams have been one of the most successfully used classes of antibiotics. They are irreversible inhibitors of peptidoglycan-cross-linking enzymes, D,D-transpeptidases and D,D-carboxypeptidases [102]. β-lactams are rarely used in chemotherapy of TB, however, because of the limited permeability of the mycobacterial cell envelope, expression of inactivating enzymes (β-lactamases), and involvement of β-lactam-insensitive targets in peptidoglycan transpeptidation. The pharmacophore of the β-lactams is a highly reactive four-membered azetidinone ring which is generally fused to a five- or six-membered ring. There is an absolute requirement for the β-lactam ring and a carboxylic acid on the fused ring (or an electron withdrawing moiety such as the sulfonyl as in monobactams). An amide a to the β-lactam ring is preferred. Conformationally, this core resembles the acyl-D-alanyl-D-alanine moiety of the natural substrate. The serine nucleophile in the enzyme active site attacks the electrophilic carbonyl of the β-lactam amide leading to ring opening and irreversible acylation of the enzyme [103-105]. The β-lactams are commonly used in combination with β-lactamase inhibitors such as clavulanic acid (CA), sulbactam, and tazobactam which themselves are β-lactams. A variety of β-lactams have been tested for in vitro efficacy against \textit{M. tuberculosis} (Table 2). However, despite activity of some β-lactams against \textit{M. tuberculosis} in vitro, especially in the presence of β-lactamase inhibitors, none have to date shown good efficacy in vivo. Amoxicillin/CA was found to be ineffective in mice [106].
and imipenem showed only a 16-fold reduction in bacterial burden in lungs of infected mice over 4 weeks of treatment. Furthermore, only a modest decrease in viable numbers was seen in sputum of patients receiving amoxicillin/CA or ampicillin/sulbactam monotherapy [107]. The poor in vivo efficacy may be due to the intracellular environment of *M. tuberculosis*, making it difficult for drugs to penetrate the phagosomal compartment. Additionally, the bacterial physiology in vivo may be different making it less responsive to β-lactam therapy, although the recent demonstration of activity of meropenem/CA against non-replicating persistent *M. tuberculosis* has raised the possibility of use of this carbapenem against TB [103, 108]. CP-5484 (a carbapenem with activity against MRSA) [108,109] is currently in preclinical development for use against tuberculosis. Additionally, the meropenem/CA combination has shown potent activity against strains of *M. tuberculosis* [103-105] and is currently being investigated for possible clinical use.

### Table 4. Basic structures of clinically relevant β-lactams and their pharmacologic properties

<table>
<thead>
<tr>
<th>β-lactam class</th>
<th>Spectrum of activity</th>
<th>Inactivation</th>
<th>Adverse effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penams</td>
<td>G-positives</td>
<td>Classical β-lactamases, Carbapenemases</td>
<td>Diarrhea, hypersensitivity, anaphylaxis, pseudomembranous colitis, yeast infections</td>
</tr>
<tr>
<td>Cephems</td>
<td>G-negatives</td>
<td>Extended spectrum β-lactamases, carbapenemases, Cephalosporinases</td>
<td>Diarrhea, hypersensitivity, pseudomembranous colitis, yeast infections</td>
</tr>
<tr>
<td>Carbapenems</td>
<td>G-positives, G-negatives, anaerobes</td>
<td>Renal dehydropeptidase, carbapenemases</td>
<td>Diarrhea, anaphylaxis, pseudomembranous colitis, nephrotoxicity, neurotoxicity</td>
</tr>
<tr>
<td>Monobactams</td>
<td>Selective G-negatives</td>
<td>Extended spectrum β- lactamases, carbapenemases</td>
<td>Diarrhea, pseudomembranous colitis, yeast infections</td>
</tr>
</tbody>
</table>

### Table 5 Biological activity of select β-lactams against *M. tuberculosis* H37Rv in the presence or absence of clavulanic acid [103-104]

<table>
<thead>
<tr>
<th>Compound</th>
<th>MIC (mM)</th>
<th>Compound</th>
<th>MIC (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-CA</td>
<td>+CA</td>
<td>-CA</td>
</tr>
<tr>
<td>Amoxicillin (21a)</td>
<td>&gt;250</td>
<td>0.5-3</td>
<td>Meropenem (21e)</td>
</tr>
<tr>
<td>Ampicillin (21b)</td>
<td>&gt;25</td>
<td>13</td>
<td>Imipenem (21f)</td>
</tr>
<tr>
<td>Ceftriaxone (21c)</td>
<td>&gt;230</td>
<td>7-28</td>
<td>Aztreonam (21g)</td>
</tr>
<tr>
<td>Cephalothin (21d)</td>
<td>&gt;40</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>

**Beta-lactamase inhibitors**

**Clavulanic acid and sulbactam:** An addition of beta-lactamase inhibitors, such as clavulanic acid and sulbactam to penicillins or to aminopenicillins of a broad spectrum of action significantly expands their antimicrobial spectrum. Clavulanic acid is isolated from *Streptomyces clavuligerus*, and sulbactam, a sulfone of penicillanic acid, is synthesized from 6-APA. Both compounds have extremely weak antibacterial properties and act by forming irreversible complexes with beta-lactamase, which inactivates the enzyme, and as a result the beta-lactam antibiotic has time to destroy the microorganism. Currently, a number of combined drugs containing various combinations of beta-lactamase antibiotics and inhibitors are used [106-110].

All cephalosporins, and thus this compound cannot be formally numbered with cephalosporins, cephamicins, or penicillins; however, in terms of pharmacological action, it is related to all three of the antibiotics listed above, and it is classified as a third-generation cephalosporin. Moxalactam is resistant to the action of beta-lactamase, penicillinase, and cephalosporinase, which are produced by Gram-negative and Gram-positive bacteria. Many strains of a number of microorganisms that possess multiple resistance to other antibiotics—semisynthetic penicillins, cephalosporins, and aminoglycosides—are sensitive to moxalactam. This drug is used for infections of the respiratory organs, urinary tract, abdominal cavity, as well as for gynecological infections, infections of the bones, joints, skin, soft tissues, and for gonorrhea. Synonyms of this
drug are latamoxef, festamoxin, moxacef, moxam, and many others [115].

**Discussion:** Tuberculosis is one of the most devastating bacterial diseases, with increasing rates of morbidity and mortality, despite the presence of effective chemotherapy and Bacillus-Calmette-Guerin (BCG) vaccine. The success of *M. tuberculosis* lies in its ability to spread by aerosol droplets, evade the host immune system and to persist in pulmonary granulomas. The advancement in the field of molecular and cellular microbiology and the availability of transcriptome and proteome data of *M. tuberculosis* have aided in understanding the pathogenesis of this organism for developing more effective drugs. The current strategy of drug design is to identify gene products, which are essential for survival and virulence. To date, several gene products of mycobacteria, ranging from proteins involved in cell wall synthesis to energy generation and from entry into host to persistence, have been shown to be essential for the survival or virulence of *M. tuberculosis*. These proteins and their associated pathways are considered as promising drug targets against *M. tuberculosis* and several of these have been patented protected. Herein, we enlist drug targets against *M. tuberculosis* for which patents have been filed and issued during the last ten years. The significance of these drug targets in the development of drug is also discussed [116-124]. This review presents a comprehensive account of the pivotal information for drug discovery and drug design to all researchers involved in tuberculosis research.

**Conclusion:** In conclusion, we can confirm that in general quinolones are particularly adapt to be used as antitubercular agents. Finally, the selectivity and the consistent ability to reduce the onset of cross resistance of triazolquinolones, probably due to a different mechanism of action toward quinolones, lead them to be good candidates for further development.

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