LINEZOLID AND ITS DERIVATIVES: THE PROMISING THERAPEUTIC CHALLENGE TO MULTIDRUG-RESISTANT PATHOGENS

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Abstract

Linezolid is the main drug representative of the oxazolidinones, widely used in the clinical practice to treat severe Gram-positive infections for some decades. The uniquely particular mechanism of action of Linezolid, with a block of ribosomal assembling before the initiation of bacterial protein synthesis has been studied in various bacteria and linked mainly to mutations in the ribosomal 50S subunit. Over the years, a large amount of clinical and pharmacokinetic data have been accumulated, relating to linezolid use in different patient groups (obesity, enteral feeding, renal failure, neonates, and paediatrics) and in different clinical conditions (sepsis syndrome, skin and soft tissue infection, diabetic foot infection, pneumonia, bone and joint infection, infection of the central nervous system, eye infection, and neutropenic sepsis).

In 2001 Linezolid resistance started emerging in *Staphylococcus aureus* and *Enterococcus faecium* clinical isolates and once again the attention of researchers has been caught looking for new antimicrobials with improved efficacy and reduced toxicity, therefore, subsequent studies have been designed to modify the oxazolidinone structure in order to improve safety profile, to extend spectrum of antibacterial activity and to obtain reliable activity against strains resistant to Linezolid.

A better understanding of the molecular mechanisms of Linezolid's derivatives should contribute to the development of new tools to predict therapeutic failures in high-risk patients. Meanwhile, pharmacological strategies such as the use of Linezolid or its derivatives and also combination regimens may serve as valuable approaches to increase and/or preserve Linezolid's activity against multidrug-resistant pathogens.

Keywords: *Linezolid, mode of action, bacterial resistance, clinical data, Linezolid's derivatives.*

Introduction

The findings in antibiotic therapy over the past 100 years have had a major impact on human health with the discovery of potent and effective agents able to treat many infections previously associated with a high incidence of mortality. Most of these drugs were derived from natural products and were discovered by broad screening of bacterial cultures, looking for agents that cause inhibition of bacterial growth. Although these agents were complex molecules difficult to synthesize, they were effective compounds in the treatment of infections caused by Gram-positive and Gram-negative bacteria [1,2].

Despite the success of these drugs, the resistance to several antibiotic classes began to emerge in Gram-positive bacteria during the 1990s and, increasingly, different bacterial species (i.e. *Staphylococcus, Streptococcus, Enterococcus* and *Pseudomonas* spp.) began to develop resistance to known antibiotics, renewing, thus, the need and the interest to discover new antibacterials [3,4].

Oxazolidinones are a class of synthetic antibiotics discovered in the 1980s with a good activity against Gram-positive bacteria, as well as several anaerobes and *Mycobacterium tuberculosis* [5]. These drugs are also active against Methicillin- or Vancomycin-Resistant Staphylococci (MRS or VRS), and Vancomycin- or Penicillin-Resistant Enterococci (VRE or PRE). During the years, multi-resistant Gram-positive cocci were spreading to the community and the spectrum of available antimicrobial compounds for an effective control of these relevant infections was significantly compromised in selection and clinical efficacy by the emerging and spread of methicillin-resistant and more recently glycopeptide-resistant Gram-positive microbial strains. The first oxazolidinone derivative Linezolid, together with the recently licensed quinupristin–dalfopristin, daptomycin, and tigecycline, followed by glycopeptides, fluoroquinolones and other experimental compounds, represent an effective response to the great majority of these concerns, for an innovative mechanisms of action, for a maintained or enhanced activity against multi-resistant pathogens, for an effective pharmacokinetic/pharmacodynamic properties, for a frequent possibility of synergistic activity with other compounds effective against Gram-positive pathogens, and a diffuse potential for a safe and easy administration [6,7].

1. Oxazolidinones: Mode of Action and Spectrum of Activity

Oxazolidinones inhibit bacterial protein synthesis by binding to a site on the bacterial 23S ribosomal RNA of the 50S ribosomal subunit, thus preventing the 70S ribosomal unit formation and the initiation phase of translation [8].This is a unique mechanism, because other protein synthesis inhibitors interfere with polypeptide extension. Many studies have elucidated the mechanism of action of this antimicrobial drug, identifying the A site of peptidyltransferase center (PTC) as the exact binding location of oxazolidinones, that interfere, thus, with the binding of the aminoacyl moiety of the incoming aminoacyl-tRNA [9].

Moreover, an interesting study examined *in vitro* activity of Tedizolid phosphate against Linezolid-resistant isolates and found that the 23S rRNA-peptidyl-transferase portion could be an additional interaction site of Tedizolid phosphate with ribosome, indicating as augmented antibacterial activity of this drug [10]. Additional competition binding experiments with chloramphenicol and lincomycin suggested that the oxazolidinones did not affect the peptide elongation or translation steps of protein synthesis [9]. Marks et al. [11], in a recent paper, showed that chloramphenicol and Linezolid stall ribosomes in specific mRNA locations by *in vitro* experiments. Bacterial cells treatment with high concentrations of these antibiotics leads to preferential arrest of translation in defined sites, resulting in redistribution of the ribosomes on mRNA. Antibiotic-mediated inhibition of protein synthesis is most efficient when the nascent peptide in the ribosome carries an alanine residue and, to a lesser extent, serine or threonine in its penultimate position. In contrast, the inhibitory action of the drugs is counteracted by glycine either at the nascent-chain C terminus or at the incoming aminoacyl-tRNA. The context-specific action of chloramphenicol explains the operation of the mechanism of inducible resistance that relies on programmed drug-induced translation arrest [11]. Crosslinking experiments in living bacteria with radiolabeled, photoactive oxazolidinone derivatives, as well as NMR and molecular modeling results,

further defined the site of action in the bacterial ribosome [8,12,13].

All clinically important Gram-positive bacteria are sensitive to Linezolid, such as *Enterococcus faecium* and *Enterococcus faecalis* (including vancomycin-resistant enterococci), *Staphylococcus (S.) aureus* (including methicillin-resistant *Staphylococcus aureus*, MRSA), *Streptococcus agalactiae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus viridans*, *Listeria monocytogenes*, and *Corynebacterium* species, that are very susceptible with minimum inhibitory concentrations below 0.5 mg/L. Linezolid is also highly effective *in vitro* against several mycobacteria. Linezolid is considered bacteriostatic against most of the organisms but it has some bactericidal activity against streptococci. Although Linezolid has a bacteriostatic effect *in vitro*, some authors have observed that it acts as a bactericidal antibiotic *in vivo*, by inhibiting the production of toxins by staphylococci and streptococci. On the contrary, Linezolid has no clinically significant effect on most of the Gram-negative bacteria; e.g. *Pseudomonas* and the Enterobacteriaceae are not susceptible. *In vitro*, it is very active against *Pasteurella multocida*, *Fusobacterium*, *Moraxella catarrhalis*, *Legionella*, *Bordetella*, and *Elizabethkingia meningoseptica*, and moderately active against *Haemophilus influenzae*, with a minimum inhibitory concentration of 8 mg/L for 90% of strains [14,15,16].

2. Oxazolidinones: Mechanism of Bacterial Resistance

Linezolid is the main drug representative of the oxazolidinones, introduced in 2000 in the clinical practice to treat severe Gram-positive infections, including MRSA and VRE. The spontaneous frequency of resistance to oxazolidinone antimicrobials is relatively low when compared to other antimicrobial agents. This is in large part due to the fact that most of the bacteria possess multiple copies of rRNA, necessitating mutations in multiple copies of the 23S rRNA central loop of domain V gene target to increase Minimal Inhibitory Concentration (MIC) [14]. Moreover, the uniquely particular mechanism of action of Linezolid, with a block of ribosomal assembling before the initiation of bacterial protein synthesis, makes very improbable the emerging of cross resistance with other antimicrobial compounds. Single episodes of

Linezolid resistance have until now been anecdotally reported, especially after long-term and low dosage courses, although it appears to be extremely rare among Staphylococci, while linezolid-resistant Enterococci have been occasionally reported in intensive care units, reports for both are now increasingly common [6].

The molecular mechanism of resistance to oxazolidinones, such as Linezolid, has been studied in various bacteria and linked mainly to mutations in the ribosomal 50S subunit. Gram-positive bacteria usually develop resistance to Linezolid as the result of a point mutation, known as G2576T, in which a guanine base is replaced with thymine in the domain V region of 23S rRNA genes [17,18]. This is the most common mechanism of resistance in staphylococci, and the only one known to date in isolates of *E. faecium*. Other mechanisms have been identified in *Streptococcus pneumoniae* (including mutations in an RNA methyltransferase that methylates G2445 of the 23S rRNA and mutations causing increased expression of ABC transporter genes) [19] and in *Staphylococcus epidermidis* [20,21].

Acquisition of the *cfr* gene resulted in resistance to Linezolid, as well as other antimicrobials such as phenicols, lincosamides, pleuromutilins, streptogramin A and 16-member-ring macrolide antibiotics [16]. Mutations in genes encoding the ribosomal proteins L3 and L4 associated with the PTC have also been associated with Linezolid resistance, while the Tedizolid proved effective against these mutations (although the MICs are increased) even when they are combined with the *cfr* gene [22].

A study by Locke et al. [23] showed that Tedizolid also required multiple mutations in the 23S rRNA in order to occur the initial stepwise MIC increases for MRSA ATCC 33591, in contrast to Linezolid that involved single mutations, identified in both strains resulting in two- to four-fold changes in MIC with five to eight serial passages. The same study also reported that spontaneous mutations, conferring reduced susceptibility to Tedizolid, are less frequent than those conferring reduced susceptibility to Linezolid [23]. Another study by Shaw et al. [24] reported the activity of Tedizolid against Linezolid-resistant strains demonstrating that Tedizolid maintained MIC values between 0.5 and 1 mg/l against MRSA strains that possess the *cfr* gene. This

is an important finding due to the possibility that Tedizolid will achieve sufficient tissue concentrations to treat these Linezolid-resistant infections. Researchers trying to explain Tedizolid's activity versus Linezolid-resistant MRSA stated that optimization of the C- and D-rings allowed interaction with more highly conserved regions of the PTC binding site [24].

In the 2015 the STAR Program developed the analysis of 6884 Gram-positive clinical isolate collected from multiple US and Europe site as part Surveillance Tedizolid Activity and Resistance Program. The data collected showed a MIC of 0.25 ug/ml against *Staphylococcus* spp. and only for 13 strains showed a MIC≥1ug/ml. The resistance mechanism confirm the presence of the *cfr* methyltransferase gene, mutations on gene encoding 23S rRNA and mutations in the gene encoding ribosomal proteins L3 and L4 [25].

As reported by Mendes et al. [16] the scenario of the mechanism of resistance is more complex and consist of a greater number of mutations in ribosomal proteins in conjunction with modifications in 23S rRNA and/or presence of *cfr* gene. Moreover, the Coagulase-negative isolates (CoNS) can serve as a reservoir for *cfr*. *S. aureus* fortunately does not seem to behave like *S. epidermidis* and other species of CoNS with regard to resistance mechanisms. Among Linezolid non-susceptible or resistant clinical isolates, *S. aureus* remains rare in the original surveillance programs and most isolates demonstrate a single Linezolid resistance mechanism [16].

3. Linezolid: Clinical Data

Linezolid was widely used in the treatment of Gram-positive infections for more than a decade; it is the only antibiotic active against the most multiply-resistant Gram-positive bacteria by an oral preparation with 100% bioavailability and an extensive volume of distribution.

Over the years, a large amount of clinical and pharmacokinetic data have been accumulated, relating to linezolid use in different patient groups (obesity, enteral feeding, renal failure, neonates, and paediatrics) and in different clinical conditions (sepsis syndrome, skin and soft tissue infection, diabetic foot infection, pneumonia, bone and joint infection, infection of the central nervous system, eye infection, and neutropenic sepsis) [26].

The indications for linezolid use approved by the U.S. Food and Drug Administration (FDA) are the treatment of vancomycin-resistant *Enterococcus faecium* infections, with or without bacterial invasion of the bloodstream; nosocomial pneumonia (hospital-acquired) and community-acquired pneumonia caused by *S. aureus* or *S. pneumoniae*; complicated skin and skin structure infections (cSSSI) caused by susceptible bacteria, including diabetic foot infection, unless complicated by osteomyelitis (infection of the bone and bone marrow); and uncomplicated skin and soft tissue infections caused by *S. pyogenes* or *S. aureus* [16].

Linezolid appears to be as safe and effective for use in children and newborns as it is in adults [27].

A large meta-analysis of randomized controlled trials found linezolid to be more effective than glycopeptide antibiotics (such as vancomycin and teicoplanin) and beta-lactam antibiotics in the treatment of skin and soft tissue infections (SSTIs) caused by Gram-positive bacteria [28] and smaller studies appear to confirm its superiority over teicoplanin in the treatment of all serious Gram-positive infections [29].

As far as complicated skin and soft tissue infections are concerned, in 2005 a randomized study was conducted on 1200 hospitalized patients with confirmed or suspected methicillin-resistant staphylococcal infection and with common disorders, such as severe cellulitis, cutaneous abscess and surgical wound infection [30]. The comparison between Linezolid and vancomycin proved a evident overall superiority of Linezolid with a greater cure rate and the involved pathogens were methicillin-resistant Staphylococci in 42% of cases, followed by methicillin-sensitive Staphylococci and coagulase-negative Staphylococci[30].

Moreover, if consider methicillin-resistant staphylococcal infections only, a greater percentage of success rate was obtained with linezolid over vancomycin (88.6% vs 66.9% respectively), paralleling the better bacteriological success rate of linezolid over vancomycin [30].

In the treatment of diabetic foot infections, linezolid appears to be cheaper and more effective than vancomycin. In a 2004 open-label study, it was as

effective as ampicillin/sulbactam and Amoxicillin/clavulanic acid, and far superior in patients with foot ulcers and no osteomyelitis, but with significantly higher rates of adverse effects. [31]. A 2008 meta-analysis of 18 randomized controlled trials, however, found that linezolid treatment failed as often as other antibiotics, regardless of whether patients had osteomyelitis [32-34].

Moreover, several studies increased and proved recognition of the clinical potential of linezolid, leading to the inclusion of linezolid in the treatment guidelines of the American Thoracic Society and those of the Infectious Disease Society of America (IDSA), as an initial choice for patients with a suspected nosocomial pneumonia caused by methicillin-resistant Staphylococci revealed a greater survival rate and clinical cure with linezolid compared with vancomycin [33-36].

In another study [37] which compared the efficacy of linezolid and teicoplanin in 430 patients with differently located, ascertained or presumed Gram-positive infections, linezolid proved as effective as teicoplanin in patients suffering from pneumonia (96% vs 93%), but infections complicated by bacteremia had a greater response rate when linezolid was administered (88.5% vs 56.7%; p<0.001) [37].

The use of Linezolid in other relevant infectious processes is increasing day by day. The excellent tissue penetration of linezolid makes this oxazolidinone drug extremely promising for the approach to difficult-to-treat endocarditis with or without bacteremia, central nervous system infections, and bone and joint infections, caused by resistant Gram-positive cocci [6]. In the past, it was thought that the deep infections, such as osteomyelitis and infective endocarditis, should be treated with bactericidal antibiotics, and not bacteriostatic ones. Nevertheless, preclinical studies were conducted to assess the efficacy of linezolid for these infections, and the drug has been used successfully to treat them in clinical practice. Linezolid appears to be a reasonable therapeutic option for infective endocarditis caused by multi-resistant Gram-positive bacteria, despite a lack of high-quality evidence to support this use [38]. Results in the treatment of enterococcal endocarditis have varied, with some cases treated successfully and others not responding to therapy [39,40]. Low- to medium-quality evidence is also mounting for its use in bone and joint infections, including chronic osteomyelitis, although adverse effects are a significant concern when long-term use is necessary [41,42].

Moreover, a very interesting activity of linezolid has been demonstrated both in vitro and in vivo against susceptible and especially multi-drug resistant *Mycobacterium tuberculosis*, and a synergistic activity may be exploited with a broad spectrum of fluoroquinolones, although the clinical significance of those associations needs to be clinically confirmed by controlled clinical trials [43,44].

Linezolid has been studied as an alternative to vancomycin in the treatment of febrile neutropenia in cancer patients when Gram-positive infection is suspected [45]. It is also one of few antibiotics that diffuse into the vitreous humor, and may therefore be effective in treating endophthalmitis (inflammation of the inner linings and cavities of the eye) caused by susceptible bacteria. Again, there is little evidence for its use in this setting, as infectious endophthalmitis is treated widely and effectively with vancomycin injected directly into the eye [6].

4. Side Effects

Similar to other antimicrobial agents, the most common adverse events during therapy with oxazolidinones affect the gastrointestinal tract and the Central Nervous System. Typically, effects are rather unspecific and symptom such as nausea, vomiting, and headache usually are mild and readily reversible. Also, cases of diarrhea as a result of alteration of intestinal microflora are well-known side effects of any therapy. In addition, linezolid and other oxazolidinones reveal some characteristic types of toxicity such as hemato-toxicity and neurotoxicity, which is relevant and have to be considered as part of the benefit-risk evaluation if a therapy with these drugs is initiated [46-48].

Linezolid is generally well tolerated, it is a relatively safe antibiotic when given for short periods. It can be used in people of all ages and in people with liver disease or poor kidney function. Common side effects with short-term use include headache, diarrhea, rash, and nausea. Serious side effects may include serotonin syndrome, bone marrow suppression, and high blood lactate levels, particularly when used for more than two weeks. If

used for longer periods it may cause nerve damage, including optic nerve damage, which may be irreversible [49].

Several rare but important toxicities have emerged, including an irreversible peripheral neuropathy, a partially reversible optic neuropathy and severe lactic acidosis. Both the peripheral and optic neuropathies appear to occur after prolonged therapy (median 4 months and 10 months, respectively), whereas lactic acidosis has been reported after as little as 1 week of therapy.

Monitoring hematology is recommended during linezolid administration, as prolonged use of linezolid has been associated with myelotoxicity and mitochondrial toxicity [50,51]. Mitochondrial toxicity has been mainly linked with many of the common adverse reactions. Use of linezolid, especially by patients with co-morbidities, such as sepsis, thiamine deficiency, and cirrhosis, increases plasma lactate concentrations. Linezolid can also induce hepatic aminotransferases and has been associated with cholestasis [50,51].

Linezolid has been associated with myelosuppression, including anemia, leukopenia, pancytopenia, and thrombocytopenia. It is recommended that complete blood counts be monitored weekly in patients who take linezolid, especially those who take it for more than 2 weeks (124[R]) [49]. The mechanism of the anemia has been described and is thought to be inhibition of mitochondrial respiration. It can be managed relatively easily with transfusions. The thrombocytopenia is progressive and may require drug withdrawal; a mechanism for this effect has not been described. A bone marrow biopsy in a patient who developed thrombocytopenia 7 days after starting to take linezolid showed adequate numbers of normal-looking megakaryocytes. This finding alone argues against marrow suppression and supports an immune-mediated mechanism of platelet destruction (125[A]) [52].

Peripheral neuropathy associated with linezolid is typically reported as paresthesia and numbness of the distal extremities, with the lower extremities being more commonly affected than the upper extremities, usually in association with allodynia [53]. Even with peripheral neuropathy, the onset of symptoms occurs during prolonged treatment (range, 3-12 months). The mechanisms responsible for the peripheral neuropathy remains unclear. Lee et al. [54] have suggested that linezolid-associated TON may be the result of mitochondrial toxicity, analogous to TON associated with metabolic conditions (e.g., nutritional deficiency, Leber's hereditary optical neuropathy) or with other antimicrobials (e.g., chloramphenicol, ethambutol), in which impaired mitochondrial protein synthesis leads to axonal death.

5. Linezolid's Derivatives

Since the discovery of Linezolid and its subsequent approval, there have been several research reports evidencing its effectiveness and broad spectrum of activity against Gram-positive bacteria, particularly, against serious MRSA and VRE infections. Over the years, structure-activity relationships (SAR) studies have highlighted the structural features important for the biological activity described in Figure 1A [55]. It has been defined the importance of N-aryl group, the requirement of the *S*-configuration at C-5 position of oxazolidinone ring for antibacterial activity, the positive influence on antibacterial activity together with a favorable safety profile concerning the presence of an electron-donating amino substituent on the aromatic ring and the strength of the antibacterial activity and efficacy due to the additional presence of fluorine atoms on the phenyl ring.

In 2001 Linezolid resistance started emerging in *S. aureus* and *E. fecium* clinical isolates and once again the attention of researchers has been caught looking for new antimicrobials with improved efficacy and reduced toxicity [56,57]. Therefore, subsequent studies have been designed to modify the oxazolidinone structure in order to improve safety profile, to extend spectrum of antibacterial activity and to obtain reliable activity against strains resistant to linezolid. Among the second-generation oxazolidinones, four linezolid analogues have been identified as promising antibacterial candidates and actually under active investigation in clinical trials. Tedizolid, marketed as tedizolid phosphate prodrug (Figure 1B), was approved by the Food and Drug Administration (FDA) in June 2014 for the treatment of MRSA skin infections. Two key structural features respect to linezolid are present in its scaffold: the presence of C-5 hydroxymethyl group in place of the acetamide group and an additional hetero-aromatic D-ring. Taken together, these structural aspects lead

to about 16 times higher activity against linezolid-resistant sthaphylococci than linezolid [58]. Radezolid (Figure 1C) is a completely synthetic oxazolidinone and it exhibits respect to tedizolid a phenyl C-ring in place of the pyridine C-ring, a spacer between C- and D-rings and the acetamide moiety at the 5-position of the oxazolidinone ring-A as target linezolid [59]. Its antibacterial activity has been evaluated 2-8-fold more potent than linezolid against several Gram positive bacteria, including some linezolid resistant strains [60]. Phase II clinical trials in community-acquired pneumonia and uncomplicated skin infections have been performed and completed, but there is no evidence to progress the phase III studies.

Structurally closed to linezolid is Sutezolid (Figure 1D), that differs only by the replacement of morpholine with thiomorpholine ring-C. Recently, it has been recognized as a potential anti-tubercular compound for the treatment of drug-susceptible TB (DS-TB) and MDR-TB. It is presently undergoing phase II a clinical studies demonstrating that it is active in humans [61]. AZD5847 (Figure 1E) is another oxazolidinone developed by AstraZeneca and actually stopped in phase II clinical trials to assess safety and efficacy [62]. Werngren et al. in 2014 reported the *in vitro* antibacterial activity of AZD5847 against 146 geographically different clinical isolates of *Mycobacterium tuberculosis* displaying its equal efficiency against drug-sensitive strains, multidrug-resistant strains and extensively drug resistant strains [63].

On the ground of the promising results with Ranbezolid (Figure 1F) [64], an oxazolidinone analogue with an additional 5-nitrofuran heteroaromatic ring, Khalaj et al. [65] reported a series of linezolid analogues bearing a nitroaryl-1,3,4-thiadiazole moiety as additional group linked to C-ring. Among the synthesized compounds, the c stood out as the most potent of the series displaying high activity against Gram-positive bacteria at non-cytotoxic concentrations.

Promising results for the antimicrobial activity against MRSA and linezolid-resistant strains were reported by Guo et al. [66] with a series of new tricyclic fused oxazolidinones. In initial studies they found compound a (Figure 2) as potential candidate displaying an activity against linezolid-resistant strains from 8- to 16-fold higher than linezolid and an excellent pharmacokinetic profile, but with the drawback of the poor solubility as a limitation to intravenous formulation.

In the second report, then, the aim of improving the solubility, maintaining the antimicrobial activity and a good PK profile, was reached by incorporating polar groups, destroying the molecular planarity and using pro-drugs. The SAR studies led to the identification of the compound b (Figure 2) which showed excellent solubility, a good pharmacokinetic profile with high in vivo activity for treating MRSA and MSSA infected mice [67,68].

Moreover, Verdino et al. [69] have reported, as a part of their ongoing studies on finding new promising antibacterial agents, the synthesis of a new series of linezolid analogs with a C-5 side chain modification with regard to the target linezolid. They replaced the acetamide group in the side-chain linked to the oxazolidinone C-5 position with urea and thiourea moieties (Figure 3). The planning of these derivatives was based on a computational study which highlighted the potential replacement of the acetoamide methyl group in the side chain with larger groups allowing a more favorable fitting into the binding pocket of ribosomes carrying mutations. The antibacterial studies revealed that the analogue f was very promising and highly active against methicillin- susceptible and methicillin-resistant *S. aureus* strains.

Afterwards, Parisi et al. [70] showed that the incorporation of Linezolid analogues in suitable delivery systems could be used as a strategy to enhance the antimicrobial activity.

Córdova-Guerrero et al. [71] synthesized ten novel 3-oxazolidin-2-one analogues devoid of rings B and C and evaluated the *in vivo* antibacterial activity against several MRSA clinical strains. Among the series, compound a was shown to be a potential antimicrobial agent with low toxicity against the tested strains (Figure 4).

Recently, Wang et al. [72] have disclosed a library of oxazolidinone analogues with different aromatic rings at the piperazine N-4 position (Figure 5). They investigated *in vitro* and intracellular activity of these oxazolidinones against *Mycobacterium tuberculosis* and clinical isolates of *Staphylococcus aureus*. The SAR studies demonstrated the positive influence of the piperazine on the biological activity and the negative effect of introducing a polar or

bulky group into the N-heteroaryl moiety. Among the synthesized compounds, a showed higher antimicrobial activity than linezolid against both the standard and clinical *S. aureus* strains. As to the screening for in vitro anti-TB activity, compounds a and b were the most active and their activity against clinical pathogens was similar to that of linezolid.

Another interesting second-generation oxazolidinone is MRX-I from MicuRx Pharmaceuticals Inc.. (Figure 6) The key structural features of the molecule is dihydropyridoneheterocycle replacing the morpholine C-ring in linezolid and the presence of three fluorine atoms in the phenyl B-ring [73]. MRX-I showed potent activity against Gram-positive pathogens including MRSA, penicilli-resistant *Streptococcus pneumoniae* (PRSP) and VRE. In addition, its high activity and oral bioavailability were coupled with reduced myelosuppression, hematological toxicity and monoamine oxidase inhibition (MAO). Phase II clinical trials evaluating the efficacy, safety and tolerability versus linezolid in the treatment of patients with ABSSSIs are ongoing in China and US.

Yang et al. have reported a series of new teraryloxazolidinone compounds characterized by the presence of a pyrazoyl ring as C-ring substituted at the 4-position with an aromatic N-heterocycle (Figure 7) [74]. The SAR studies showed that, among all the synthesized compounds, 13f was a promising drug candidate exhibiting high antibacterial activity in vitro with low toxicity on L02, HEK 293, THP-1 and K562 cells. Interestingly, its phosphate derivative had high solubility in water and displayed good *in vivo* efficacy in MRSA systemic infection mice models together with a notable PK profile compared to linezolid [74].

Later, the same group reported a series of 13 analogues with different substituents at the C-5 position of oxazolidinone ring with the aim to examine the influence on the toxicity profile [75] (Figure 8). Most of these compounds did not exhibit any cytotoxicity towards HEK293A and L02 cells at concentration lower than 400 µg/mL, in particular analogues 14a-f showed high antibacterial activity with MIC values around 2-4 µg/mL. However, the potential antibacterial candidate was e which showed an appropriate balance between antibacterial activity and safety profile with interesting *in vivo* results.

Recent structural modifications of the linezolid scaffold are related to the replacement of morpholine ring with its bioisoestere 2-oxa-6-azaspiro[3.3] heptane (Figure 9) and the introduction of different aromatic or aliphatic groups at C-5 oxazolidinone position [76]. The SAR studies identified various compounds with promising antibacterial and antitubercular activity and reasonable tolerance limits for further studies as oral drug candidates. Among them, compound 15 showed antibacterial activity on *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* strains similar to linezolid and compound 16 was characterized by an antitubercular profile similar to linezolid.

Conclusions

Linezolid is an agent with remarkable properties; it is the only antibiotic active against multi-resistant Gram-positive bacteria with an excellent oral bioavailability and effective penetration at therapeutic concentrations to almost every organ in the body, so it is a suitable agent for a wide range of infections caused by susceptible bacteria.

Treatment of severe multidrug-resistant pathogenic infections is an important clinical challenge for clinicians worldwide. As one of the few compounds with in vitro bactericidal activity, Linezolid has risen as an interesting alternative in the management of these infections. A better understanding of the molecular mechanisms of Linezolid's derivatives should contribute to the development of new tools to predict therapeutic failures in high-risk patients. Meanwhile, pharmacological strategies such as the use of Linezolid or its derivatives and also combination regimens may serve as valuable approaches to increase and/or preserve Linezolid's activity against multidrug-resistant pathogens.

The future therapeutic research promises the development of novel compounds aimed at intervening favorably against the unavoidable increase of drug resistance frequency and levels against the present reference compounds. This interest in finding new and more efficient agents effective against the emerging multidrug-resistant pathogens with a better toxicity profile is also proved by the high number of Linezolid's derivatives studied so far. The structure-activity relationship

studies represent a promising tool in the development of new drug candidates with desirable features such as ability to reach target site, potency against a range of bacteria with even more than one target within bacteria and low toxicity profiles.

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Figure 1. Structure of: A)Linezolid; B) Tedizolid; C) Radezolid; D) Sutezolid; E) AZD5847; F) Ranbezolid.
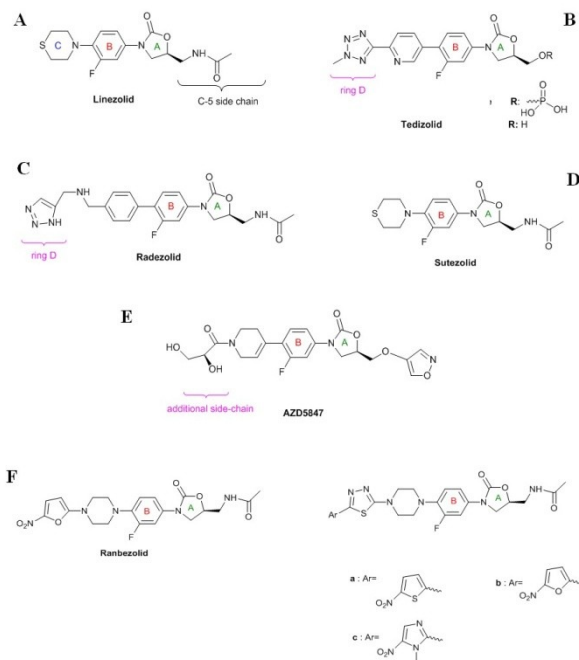
Figure 2. Tricyclic fused oxazolidinone derivatives.
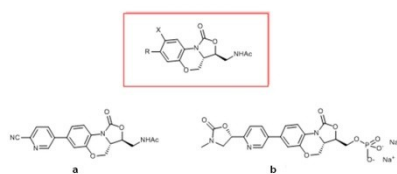
Figure 3. Linezolid analogues with C-5 side chain modifications.
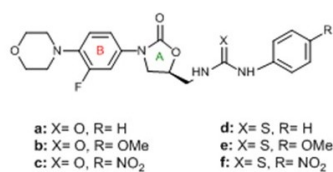
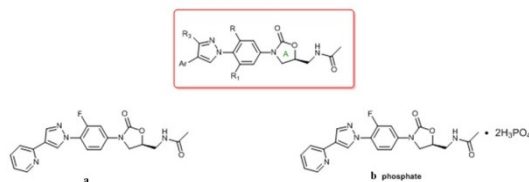**Figure 4.** New 3-oxazolidin-2-one analogues devoid of rings B and C.
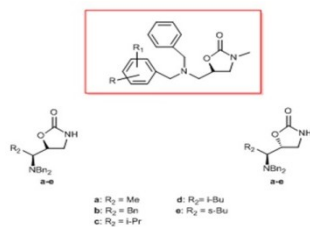
Figure 5. Oxazolidinone analogues bearing various aromatic rings at the piperazine N-4 position.
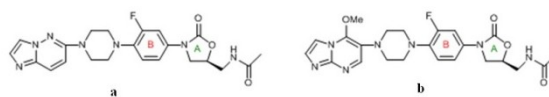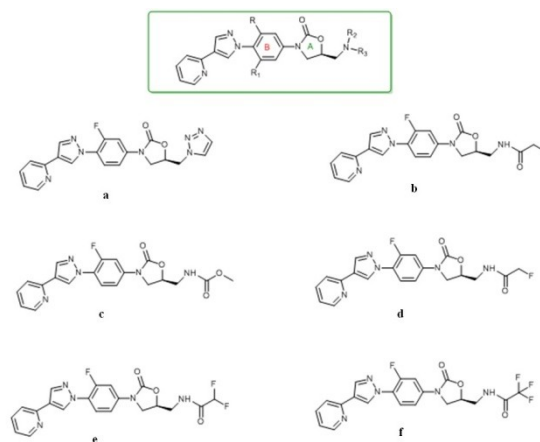

Figure 6. MRX-I structure and biological activity.

Figure 7. Structures of new teraryloxazolidinone derivatives.

Figure 8. Structures of teraryloxazolidinone derivatives bearing different substituents at the C-5 position of oxazolidinone ring.

Figure 9. Novel azaspiro analogs of Linezolid.