International Journal of Pharmaceutics 461 (2014) 163–170

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical nanotechnology

Enhanced cellular uptake by "pharmaceutically oriented devices" of new simplified analogs of Linezolid with antimicrobial activity

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ARTICLE INFO

Article history:
Received 4 October 2013
Received in revised form
19 November 2013
Accepted 23 November 2013
Available online 1 December 2013

Keywords:
Drug carriers
Enhanced cellular uptake
Oxazolidinones
Antimicrobial activity
Simplified analogs of Linezolid

ABSTRACT

The aim of the present study was to enhance cellular uptake of simplified analogs of Linezolid by their incorporation into suitable delivery devices in order to improve the antimicrobial activity of these novel synthesized oxazolidin-2-one derivatives.

The oxazolidin-2-one derivatives were synthesized by developing a rather simple one-pot reaction starting from oxiranylmethanol and several primary amines. Three delivery devices were prepared by following different synthetic approaches, such as single-step free radical grafting, precipitation polymerization and nano-emulsion. Finally, the antimicrobial activity of the novel synthesized compounds, without any vehicle and after their incorporation into the delivery devices, was evaluated against *Escherichia coli* and *Saccharomyces cerevisiae* by performing time-kill analyses.

The synthesized oxazolidinones exhibited modest antimicrobial activity against *E. coli* and *S. cerevisiae* (MIC 16 μg/mL). A good activity was, instead, highlighted after their incorporation into the prepared delivery devices (lecithin-based nano-emulsion, poly(N-vinyl-pyrrolidone)-methacrylic acid grafted copolymer and spherical polymeric nanoparticles) (MIC ≤ 4 μg/mL). The incorporation into suitable vehicles, indeed, reduced by 4 times the normal MICs of the newly synthesized oxazolilidin-2-ones and represents an effective strategy to overcome cellular penetration constraints.

1. Introduction

The oxazolidinones, a new class of synthetic clinically important antimicrobial agents, are active against the sensitive and resistant Gram +ve organisms.

Initial studies designed to elucidate the oxazolidinones mechanism of action evidenced that these drugs inhibit the formation of the initiation complex in bacterial translation systems by preventing the formation of the N-formylmethionyl-tRNA ribosome-mRNA ternary complex (Swaney et al., 1998). The identification of the drug-binding site at the ribosome, as well as the mechanism of action of these antimicrobial agents, is not fully understood. However, recent works have highlighted the interaction of oxazolidinones (e.g. Linezolid, Fig. 1.) with the 50S A-site pocket at the peptidyltransferase center (PTC) of the ribosome, which overlaps the aminoacyl moiety of an A-site bound tRNA, inhibiting the protein synthesis process in whole cells of Gram +ve bacteria (Wilson et al., 2008). On the other hand, the inhibition of protein synthesis of Gram −ve bacteria, such as *Escherichia coli* (*E. coli*), has been observed only at higher doses (Muller and Schimz, 1999).

Keeping in mind the aforementioned interesting antimicrobial activities of oxazolidinones and that the infections caused by Gram −ve bacteria are emerging nowadays (Michalska et al., 2013), the preparation of some new oxazolidin-2-ones potentially active against this kind of microorganisms and investigate their formulation and cellular uptake could be of relevant interest.

Since the main target sites for antibacterial agents are located within the cell, at the cytoplasmic membrane level or within the cytoplasm, the wall structure of Gram −ve bacteria offers a complex barrier system to these compounds (Denyer and Maillard, 2002). The phenomenon of cellular impermeability, indeed, is due to the presence of an outer envelope represented by a bilayer structure

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http://dx.doi.org/10.1016/j.ijpharm.2013.11.048

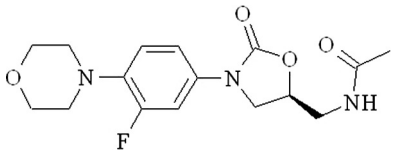


Fig. 1. Chemical structure of Linezolid.

consisting of lipopolysaccharides and phospholipids. Thus, antimicrobial agents have to cross this outer membrane in order to get their principal target site.

Limited cellular penetration could reduce the effectiveness of many antimicrobial treatments and, in view of this fact, the present study was focused on the exploration of antimicrobial activity of the synthesized therapeutic agents alone and after their incorporation into "pharmaceutically oriented devices".

In order to modulate the cellular uptake of these compounds, three different strategies were adopted. The novel synthesized oxazolidin-2-ones were, indeed, incorporated into drug delivery carriers of different nature and structure, such as lecithin-based nano-emulsion, poly(N-vinyl-pyrrolidone)-methacrylic acid grafted copolymer (PVP-MAA) and spherical nanoparticles (SNs).

The antimicrobial activity of the compounds, without any vehicle and after their incorporation into the delivery devices, was evaluated against *E. coli* and *Saccharomyces cerevisiae* (*S. cerevisiae*) by performing time-kill analyses according to the method of the Clinical and Laboratory Standards Institute (Clinical and Laboratory Standards Institute, 1999).

The choice of these kinds of vectors, characterized by such a different construction, allows to hypothesize several possible mechanisms involved in the enhancement of cellular uptake and, consequently, in the improvement of the antimicrobial activity.

2. Materials and methods

2.1. Materials and instrumentation

Commercial reagents were purchased from Aldrich, Acros Organics and Alfa Aesar and were used without additional purification.

Melting points were determined on a Gallenkamp melting point apparatus.

The IR spectra were recorded on a Fourier Transform Infrared Spectrometer FT/IR-4200 for KBr pellets.

GC/MS analyses were performed using a 17AA-V3 230 VLV spectrometer or a 6890N Network GC System (Agilent Technologies Inc., Palo Alto, CA, USA) equipped with an HP-5MS (30 m × 0.25 mm, PhMesiloxane 5%) capillary column. The mass detector was operated in the ionization chemical mode (CI-CH_4).

ESI-MS was performed using a spectrometer LC–MS Waters alliance 2695 (ESI+).

^{1}H NMR (300 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker 300 spectrometer. Chemical shifts are expressed in parts per million downfield from tetramethylsilane as an internal standard.

Thin layer chromatography (TLC) was performed on silica gel 60F-264 (Merck).

The scanning electron microscopy (SEM) photographs were obtained with a Jeol JSMT 300 A; the surface of the samples was made conductive by the deposition of a gold layer on the samples in a vacuum chamber. Approximate range in particle size was determined employing an image processing and analysis system, a Leica DMRB equipped with a LEICA Wild 3D stereomicroscope.

Dynamic light scattering (DLS) analysis was performed using a 90 Plus Particle Size Analyzer (Brookhaven Instruments Corporation, New York USA) at 25.0 ± 0.1 °C by measuring the autocorrelation function at 90°. The laser was operating at 658 nm.

2.2. Synthesis of simplified analogs of Linezolid

2.2.1. Preparation of (RS)-3-alkyl (or aryl)-5-hydroxymethyloxazolidin-2-ones (**5a,b**) (general method)

To a stirred solution of the appropriate primary amines **3a,b** (8.62 mmol) in MeOH (3 mL), (RS)-oxiranylmethanol **2** (7.84 mmol) was slowly added. The reaction mixture was stirred further at room temperature for 12 h to give intermediates **4a,b**. Diethylcarbonate (9.09 mmol) and anhydrous MeONa (0.74 mmol) were then added and the resulting mixture was heated under reflux for 12 h. After cooling to room temperature, the reaction mixture was evaporated under reduced pressure to afford an oil residue. This oil was taken up with EtOAc and the organic layer washed with water. Then it was separated, dried (Na_2SO_4) and concentrated under reduced pressure to give a solid or oil product, which was purified using silica gel column chromatography.

2.2.2. (RS)-3-Allyl-5-hydroxymethyl-oxazolidin-2-one (**5a**)

White solid, using $CHCl_3$:MeOH (9:1) as eluent, yield 45%; mp 82 °C. IR spectrum, ν, cm^{-1}: 3413, 2924, 1733, 1645, 1263, 765. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 3.18 (1H, br.s, OH); 3.33–3.61 (4H, m, CH_2NCH_2); 3.77–3.86 (2H, m, CH_2OH); 4.51–4.59 (1H, m, $CHCH_2OH$); 5.14–5.26 (2H, m, $CH_2CH{=}CH_2$); 5.63–5.78 (1H, m, $CH_2CH{=}CH_2$). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 154.69; 133.85; 119.54; 76.03; 67.38; 59.61; 42.60. Mass spectrum (EI, 70 eV), m/z (I_{rel}, %): 157 [M]$^+$ (100), 126 (74), 98 (89), 82 (98), 68 (88), 56 (44). Found, %: C 53.46; H 7.01; N 8.88. $C_7H_{11}NO_3$. Calculated, %: C 53.49; H 7.05; N 8.91.

2.2.3. (RS)-5-Hydroxymethyl-3-phenethyl-oxazolidin-2-one (**5b**)

White solid, using EtOAc:hexane (4:1) as eluent, yield 50%; mp 93 °C. IR spectrum, ν, cm^{-1}: 3444, 2927, 1735, 1603, 752. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (J, Hz): 2.78–2.86 (2H, t, CH_2Ph); 3.22–3.53 (6H, m, CH_2NCH_2, $CHOH$, OH); 3.69–3.77 (1H, dd, J = 3.30; J = 12.60, $CHOH$); 4.41–4.50 (1H, m, $CHCH_2OH$); 7.11–7.26 (5H, m, Ar). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 158.16, 138.25, 128.66, 128.59, 126.58, 73.72, 62.64, 46.09, 45.35, 33.72. Mass spectrum (EI, 70 eV), m/z (I_{rel}, %): 221 [M]$^+$ (36), 130 (100), 104 (55), 77 (19), 56 (18). Found, %: C 65.18; H 6.79; N 6.29. $C_{12}H_{15}NO_3$. Calculated, %: C 65.14; H 6.83; N 6.33.

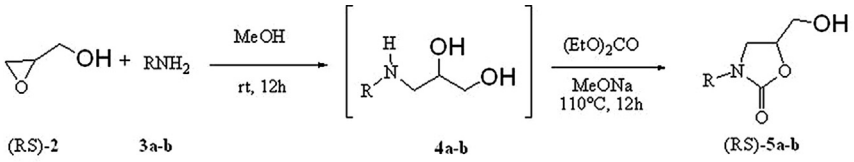
2.3. Synthesis of "pharmaceutically oriented delivery devices"

2.3.1. Poly(N-vinyl-pyrrolidone)-methacrylic acid (PVP-MAA) grafted copolymer

Single-step grafting of methacrylic acid (MAA) onto poly(N-vinyl-pyrrolidone) (PVP, average M.W. 360,000, Sigma–Aldrich) was carried out by employing hydrogen peroxide/ascorbic acid as biocompatible and water soluble redox pair (Parisi et al., 2013).

In a 25 mL glass tube, 4.0 g of PVP were dissolved in 15 mL of distilled water, then 1 mL of distilled MAA and 0.5 mL of H_2O_2 1.0 M containing 0.025 g of ascorbic acid were added. The mixture was maintained under stirring at 25 °C for 3 h under atmospheric air. The resulting PVP-MAA grafted copolymer was purified by successive washing steps with distilled water, recovered by filtration and, finally, dried overnight in a vacuum oven set at 40 °C.

An amount of the synthesized PVP-MAA copolymer (128 mg) was immersed in 7.7 mL of a phosphate buffer solution (pH 7.4). Then, 0.3 mL of a solution in DMSO containing 32 mg of the antimicrobial agent were added and the obtained mixture was sonicated for 30 min.



Scheme 1. Synthesis of oxazolidinones **5a** and **5b**.

2.3.2. Spherical nanoparticles (SNs)

Spherical polymeric nanoparticles were prepared by precipitation polymerization employing methacrylic acid and ethylene glycol dimethacrylate (EGDMA) as functional monomer and cross-linking agent, respectively (Puoci et al., 2004).

In a 100 mL round bottom flask, distilled MAA (8 mmol) were dissolved in a mixture of acetonitrile (20 mL) and methanol (20 mL), then EGDMA (10 mmol) and 2,2-azoisobutyronitrile (AIBN, 50 mg) were added. The polymerization mixture was degassed in a sonicating water bath, purged with nitrogen for 10 min cooling with an ice bath. The flask was then gently agitated (40 rpm) in an oil bath. The temperature was increased from room temperature to 60 °C within 2 h, and then kept at 60 °C for 24 h. At the end of the reaction, the resulting nanoparticles were filtered, washed with 100 mL of ethanol, 100 mL of acetone and 100 mL of diethyl ether and, successively, dried overnight under vacuum at 40 °C.

An amount of the resulting SNs (128 mg) was immersed in 3 mL of a solution in ethanol containing 32 mg of the antimicrobial agent and soaked for 3 days at room temperature. Then the solvent was removed under reduced pressure.

2.3.3. Nano-emulsion based on soybean lecithin

Nano-emulsion containing the antimicrobial agent was prepared by the following procedure.

The emulsifier and the emulsifier adjuvant, such as soybean lecithin (1.2%, w/w) and tween 80 (1.2%, w/w) respectively, were added to soybean oil as the oil phase (10%, w/w). Lecithin was stirred with heating to 80 °C until it was completely dissolved in the oil phase and the obtained mixture was cooled to room temperature. Then, 0.3 mL of a solution in DMSO containing the antimicrobial agent (0.24%, w/w) was added and mixed homogeneously. The mixture was added dropwise into a 2.25% glycerol solution preheated at 40 °C, and then homogenized for 10 min at 24,000 rpm using a homogenizer. After the completion of the emulsification, the pH was adjusted to neutrality with NaOH (Kim and Lee, 1999).

2.4. Biological analyses

2.4.1. Yeast and bacterial strains

Yeast strains Y10000, wild-type, was provided by the EUROFAN resource center EUROSCARF (Frankfurt, Germany). The cells were grown in rich medium containing 2% Bactopeptone and 1% yeast extract (YP), supplemented with fermentable 2% glucose carbon sources (Iacopetta et al., 2010). The final pH was adjusted to 4.8.

The *E. coli* strains ATCC 8739 was provided by REMEL. The cells were grown in LB medium containing 10 g/L Tryptone, 5 g/L yeast extract and 0.5 g/L NaCl (Iacopetta et al., 2010; Santoro et al., 2011). The final pH was adjusted to 7.4.

Minimum inhibitory concentrations (MICs) were determined by the agar dilution method as described by the Clinical and Laboratory Standards Institute (CLSI) (Clinical and Laboratory Standards Institute, 2012).

2.4.2. Time-kill studies

Time-kill analyses were performed according to the method of the CLSI (Clinical and Laboratory Standards Institute, 1999).

Test organisms incubated in appropriate medium over night at 30 °C (yeast) or 35 °C (bacteria) were diluted with fresh medium to ca. 104 CFU/mL and the diluted cultures were pre-incubated for 2 h. Each drug, solubilized in dimethyl sulfoxide (DMSO) was added to the cultures at concentrations of 1× MIC. Samples (0.1 mL) of the cultures were removed at 0, 2, 4, 6 and 24 h of incubation and serial 10-fold dilutions were prepared in saline. The number of viable cells was determined as described in the literature (Jung et al., 2012).

3. Results and discussion

3.1. Chemistry

Different methods for the synthesis of oxazolidin-2-one compounds were reported in the literature (Lamanna et al., 2004; Osa et al., 2005; Robles-Machín et al., 2006; Ella-Menyea and Wang, 2007; Adibpour et al., 2010; Lancelot et al., 1993, 1996; Saturnino et al., 2000, 2004).

In the present study, we report a rather simple one-pot synthesis of two compounds (**5a,b**) starting from racemic (RS)-oxiranylmethanol (**2**) and different primary amines (**3a,b**) (Scheme 1). Thus, compound **2** was allowed to react with different primary amines (**3a,b**) in methanol, to give the corresponding 3-substituted-amino-1,2-propanediols intermediates (**4a,b**). The latter were then treated with equimolar amounts of diethylcarbonate in the presence of catalytic amounts of sodium methoxide to give N-substituted-5-hydroxymethyl-oxazolidin-2-ones (**5a,b**) in 45–87% yield (Table 1).

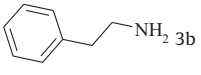
3.2. Synthesis of "pharmaceutically oriented delivery devices"

3.2.1. PVP-MAA grafted copolymer

Methacrylic acid was grafted onto a preformed polymeric backbone, such as poly(N-vinyl-pyrrolidone), by a free radical procedure involving a one-pot reaction at room temperature. Hydrogen peroxide/ascorbic acid redox pair was employed as water-soluble and biocompatible initiator system allowing to perform the grafting process in aqueous media. In view of this fact, the use of organic solvents and the generation of any kind of toxic reaction byproducts are avoided.

The mechanism of redox pair reaction (Fig. 2.) consists of the oxidation of ascorbic acid (AA) by hydrogen peroxide with the formation of hydroxyl and ascorbate radicals (Curcio et al., 2009). Hydroxyl radical represents the most reactive radical among the reactive oxygen species and its reaction with PVP chain generates macroradicals centered in three possible positions according to the lability of the hydrogen atoms present in the polymeric

Table 1
Synthesized oxazolidinones.

Cpd	RNH_2	Rdt %
5a	 3a	45
5b	 3b	50

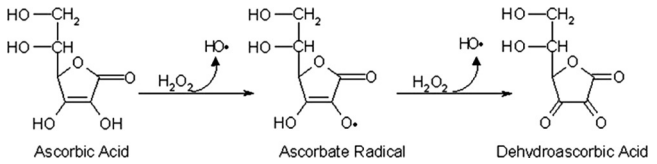


Fig. 2. Mechanism of AA/H_2O_2 redox pair reaction.

structure. Literature (Barros et al., 2006), indeed, reported that C—H bonds of PVP α-positioned to a heteroatom or a carbonyl are lower in energy, mainly due to the stabilization of the radical product. Generated macroradicals react with MAA monomer which is graft-copolymerized onto the PVP preformed polymeric chain.

Poly(N-vinyl-pyrrolidone) is a polymeric material characterized by chemical stability, low toxicity, biocompatibility with living tissues, general bioinertness and water solubility due to the presence of the polar lactam group which increases its hydrophilicity (Liu et al., 2013). On the other hand, the non-polar methylene groups make this material lipophilic. All these features make PVP widely employed as biomaterial and excipient in various fields, such as pharmaceutical, cosmetic, biomedical and food.

The grafting of hydrophilic molecules onto the surface of a material could improve its biocompatibility regulating protein adsorption and cell adhesion. In this study, the conjugation of PVP with methacrylic acid aimed to increase the hydrophilicity of the copolymer, providing pH-sensitivity to the system, and the choice of MAA was dictated to the broad applications of methacrylate polymers in biotechnology and biomedicine (Pérez et al., 2006).

PVP-MAA and the respective control polymer (PVP) were characterized by Fourier Transform IR spectrophotometry. By comparing the two IR spectra (Fig. 3), the PVP-MAA spectrum (trace B) showed the appearance of a new signal at 1717 cm^{-1} ascribable to the C=O stretching vibration of the carboxylic groups of methacrylic acid.

3.2.2. Spherical nanoparticles (SNs)

In the last years, polymeric nanoparticles have received considerable attention due to their potential applications as drug delivery systems.

Precipitation polymerization represents a simple and efficient method to obtain uniform polymeric particles in the absence of any stabilizer. The size and size distribution of particles are involved in size-dependent cellular uptake (Zhang et al., 2009) and this polymerization technique allows to have a better control on size and shape.

The polymerization system consists of only monomer, cross-linker, initiator and solvent as components (Li and Stover, 1993) and, in the first stage of the process, monomers form oligomer radicals. Then, the formed oligomers crosslink and the obtained crosslinked nuclei aggregate into larger particles leading to the formation of the final polymer beads.

In a precipitation polymerization process, the adopted concentrations of monomer, cross-linking agent and initiator affect the size of synthesized particles. The diameter of polymeric spheres, indeed, increases with increasing monomer or initiator concentration (Puoci et al., 2004); on the other hand, the particles size decreases as the cross-linker percentage increases (Shim et al., 2004).

In the present study, spherical nanoparticles (SNs) were prepared by precipitation polymerization employing MAA, EGDMA and AIBN as functional monomer, cross-linking agent and initiator, respectively. For this purpose, in the prepolymerization feed the employed MAA/EGDMA molar ratio was equal to 8:10 (Cirillo et al., 2009). This ratio represents, indeed, the optimal value to obtain nanospheres with the desired characteristics such as spherical shape and nanometer size.

Spherical geometry and the practical monodispersion of the prepared nanospheres were confirmed by scanning electron micrographs (Fig. 4).

3.2.3. Nano-emulsion based on soybean lecithin

An emulsion is a mixture of two immiscible liquids, such as water and oil, with one dispersed in the other (Leal-Calderon et al., 2007). The preparation of emulsions requires the use of surfactants in the aim to decrease the interfacial tension and maintain the stability of the formulation.

Nano-emulsions represent promising drug delivery vehicles due to their small size, biocompatibility, relative stability and ability to protect drugs from hydrolysis and enzymatic degradation under physiological conditions (Jafari et al., 2007).

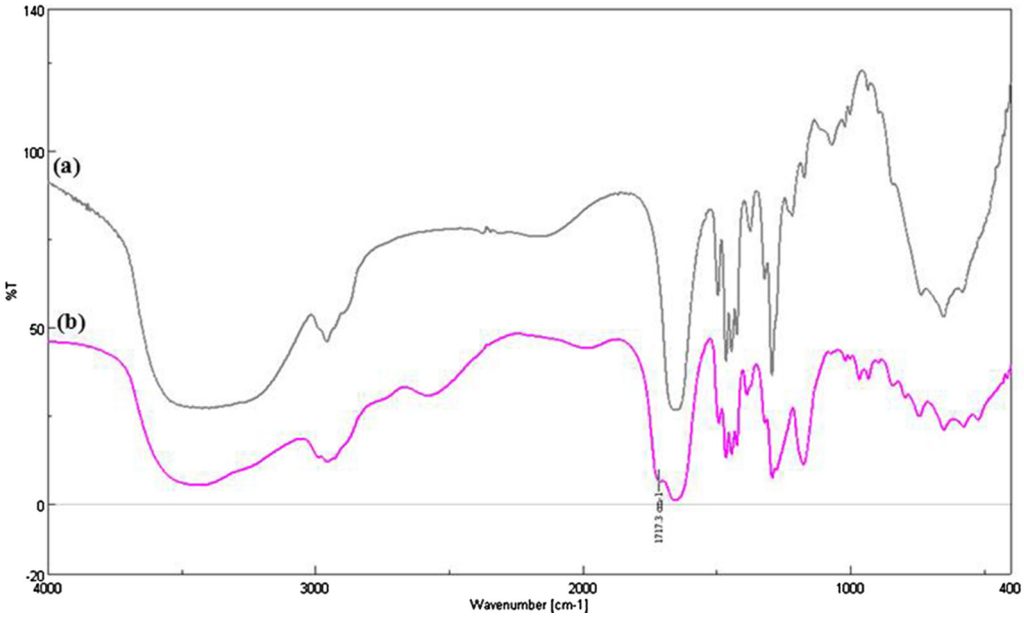


Fig. 3. FT-IR spectra of PVP (a) and PVP-MAA (b).

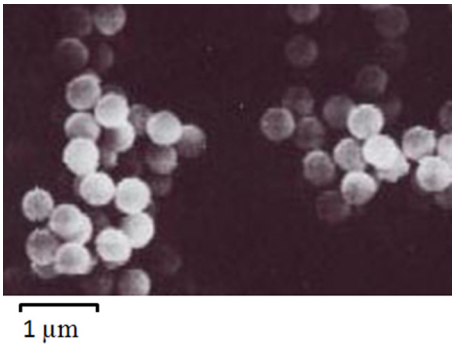


Fig. 4. Scanning electron micrograph of SNs.

In this report, nano-emulsions containing the antimicrobial agents were prepared using soybean lecithin and tween 80 as emulsifier and emulsifier adjuvant, respectively. The droplet size distribution was measured by dynamic light scattering (DLS) and it was found to be 259.6 nm (effective diameter) with a PDI of 0.268 (Fig. 5).

3.3. Time-kill curves

The oxazolidinones represent a novel chemical class of synthetic antimicrobial agents active against a large spectrum of sensitive and resistant Gram +ve organisms due to a unique mechanism of protein synthesis inhibition involving the binding at the P site of the ribosomal 50S subunit (Bozdogan and Appelbaum, 2004). However, protein synthesis of Gram −ve bacteria was affected only at higher doses by these molecules.

In this study, new oxazolidin-2-ones potentially active against Gram −ve bacteria were synthesized by following a simple one-step reaction and their formulation and cellular uptake were investigated.

Two different kinds of microorganisms, such as *E. coli* and *S. cerevisiae*, were chosen to evaluate the antimicrobial activity by performing time-kill analyses. The first one was chosen because it is a common type of bacteria and represents one of the most

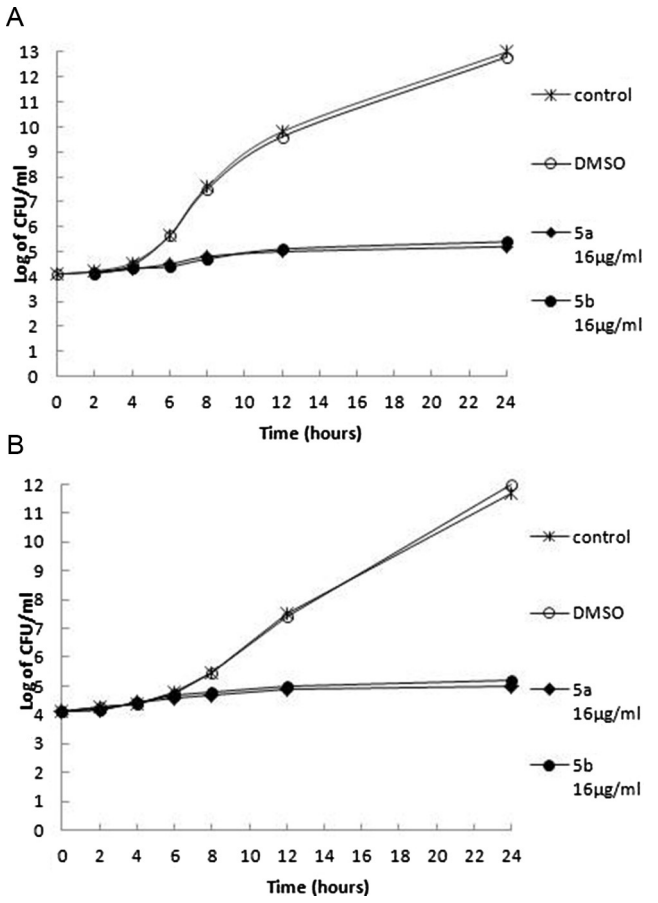


Fig. 6. Time-kill curve profiles of **5a** and **5b** against *E. coli* (panel A) and *S. cerevisiae* (panel B).

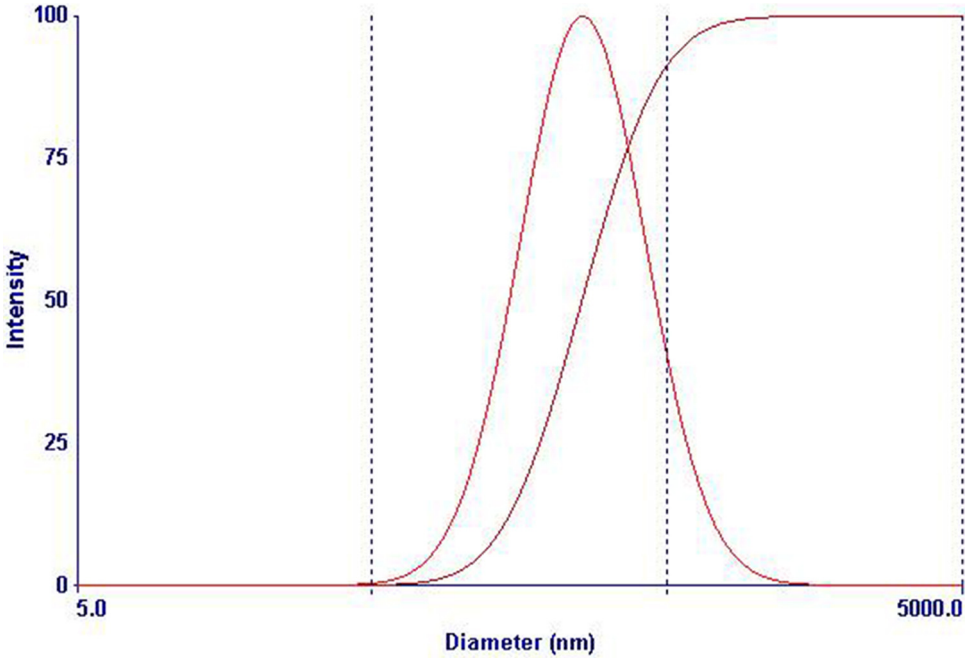


Fig. 5. Droplet size distributions of lecithin-based nano-emulsion determined by dynamic light scattering.

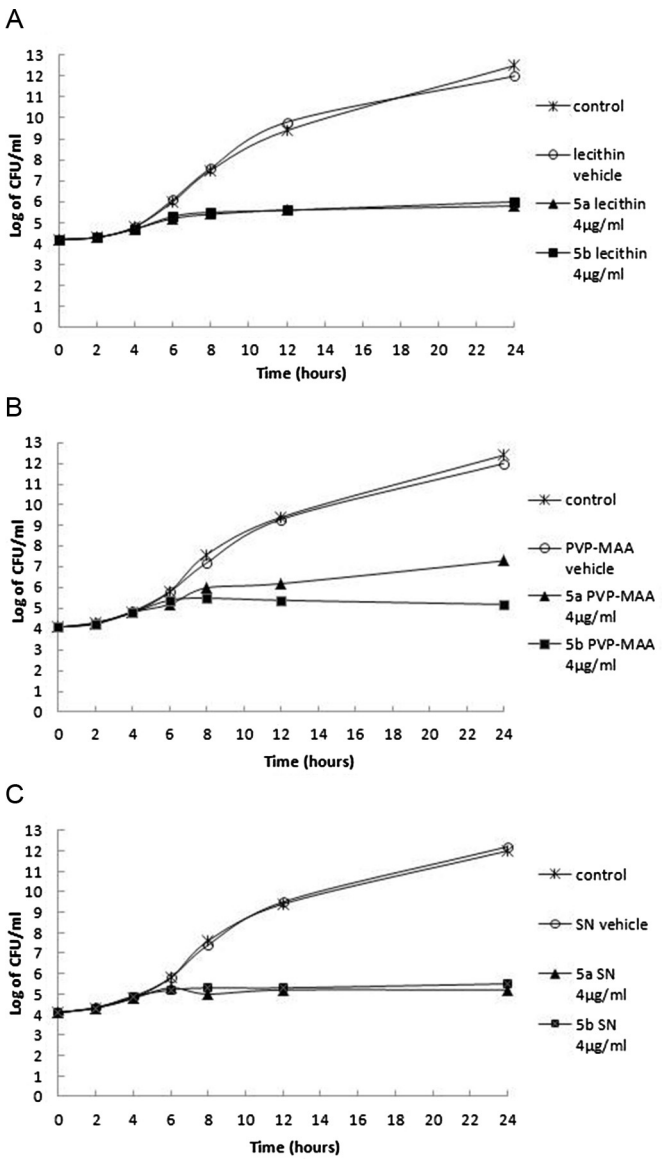


Fig. 7. Time-kill curve profiles of **5a** and **5b** against *E. coli*, after incorporation into lecithin-based nano-emulsion (panel A), PVP-MAA (panel B) and SNs (panel C).

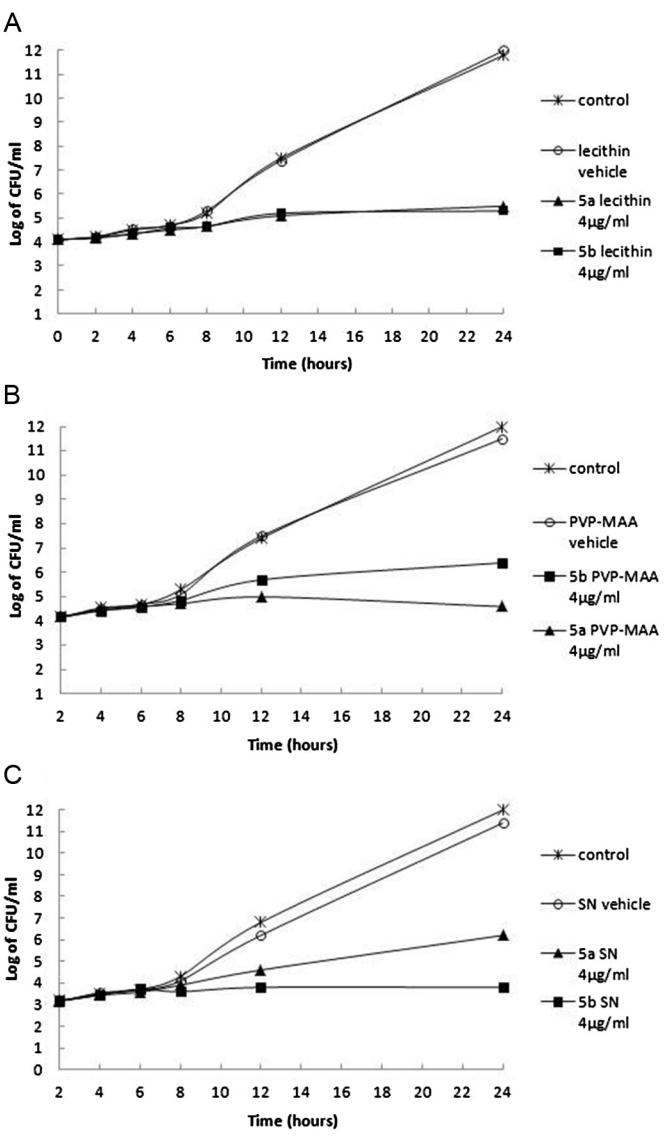


Fig. 8. Time-kill curve profiles of **5a** and **5b** against *S. cerevisiae*, after incorporation into lecithin-based nano-emulsion (panel A), PVP-MAA (panel B) and SNs (panel C).

frequent causes of many widespread bacterial infections; *S. cerevisiae* was selected due to the construction of its cell wall which is typical of other relevant and dangerous Saccharomycetes such as *Saccharomyces albicans*.

The synthesized oxazolidin-2-ones (**5a** and **5b**), without any vehicle, showed modest antimicrobial activity against *E. coli* (Fig. 6, panel A) and *S. cerevisiae* (Fig. 6, panel B) (MIC 16 μg/mL) in comparison with the results obtained using the microorganisms alone.

The problem could be probably due to the cellular impermeability of these microorganisms to the antimicrobial agents. Poor cellular uptake, indeed, could be responsible for the disappointing activity against bacteria that the synthesized compounds exhibited.

In order to overcome this drawback, **5a** and **5b** molecules were incorporated into three different carriers prepared according to reported literature: nano-emulsion based on soybean lecithin (Kim and Lee, 1999), PVP-MAA grafted copolymer (Parisi et al., 2013) and spherical polymeric nanoparticles (Puoci et al., 2004).

We supposed that the chosen vehicles have the ability to improve the cellular uptake of the synthesized oxazolidin-2-ones derivatives by several possible mechanisms.

PVP-MAA copolymer is characterized by a poly(N-vinyl-pyrrolidone) backbone with polymethacrylic acid grafted side chains attached to it. PVP is an interesting material from a biological point of view due to the presence of the lactam group in the pyrrolidone moiety which makes its structural feature similar to those of proteins (Liu et al., 2013). The performance of the prepared grafted copolymer as drug device depends on its lipophilicity/hydrophilicity ratio. Since membrane proteins also have hydrophilic and hydrophobic domains, these structural features may influence protein interactions with the copolymer.

Nano-emulsions containing the antimicrobial agents were, instead, prepared using soybean lecithin and tween 80 as emulsifier and emulsifier adjuvant, respectively. The results obtained employing this formulation could be attributed to its cellular permeability due to the presence of lecithin which is a complex mixture containing phospholipids, the main components of the biological membranes. Furthermore, the use of delivery systems at the nanoscale may potentially increases the passive cellular absorption mechanisms allowing the antimicrobial agent to act from the inner side on the cytoplasmic membrane.

Finally, spherical nanoparticles (SNs) were prepared by precipitation polymerization employing MAA and EGDMA as

functional monomer and cross-linking agent, respectively. Polymeric nanoparticles, indeed, are attracting significant research interest due to their unique properties and potential applications in different fields such as drug delivery and therapy. Their application in biomedical field involves the interaction with cells, which is influenced by the small size and the possibility to overcome cellular barriers by different endocytic pathways including phagocytosis (the uptake of large particles) (Sahay et al., 2010). Based on these considerations, the enhanced antimicrobial effect of novel synthesized Linezolid analogs observed employing SNs as carrier could be ascribable to the endocytic uptake of particulate systems which provides improved access for drugs.

The time-kill curve profiles of compounds **5a** and **5b,** after their incorporation into the carriers, confirmed our supposition showing significant activity against *E. coli* and *S. cerevisiae* (Fig. 7 and Fig. 8, respectively).

The obtained results, indeed, evidenced the efficiency of all the tested carriers in improving the antimicrobial activity and could be attributed to the different nature of the prepared delivery devices.

Significant differences were observed, between the log10 CFU per milliliter counts at 24 h for all killing curves versus those for the growth controls and to those of vehicle alone (Fig. 7), with nanoemulsion (Fig. 7, panel A) and SNs formulations (Fig. 7, panel C). Although PVP-MAA vehicle, **5b** loaded, showed a similar behavior compared to other formulations, it proved to be less efficient for **5a** (Fig. 7, panel B). Therefore, the results of time-kill analyses showed that **5b** (with every vehicle) and **5a** (only with lecithin or SNs vehicles) has antibacterial activity against *E. coli* that begins to be evident after six hours from the addition of formulations and it remains for 24 h.

The time-kill effect of **5a** and **5b** against *S. cerevisiae* was similar to that against *E. coli*, only when lecithin was used as vehicle (Fig. 8, panel A). Conversely, PVP-MAA and SNs vehicles have been found to be less efficient for **5b** and **5a**, respectively (Fig. 8, panel B and C).

4. Conclusion

In the present study, we reported the synthesis of new oxazolidin-2-ones (**5a** and **5b**), as simplified analogs of Linezolid, and their incorporation into suitable "pharmaceutically oriented devices" in the aim to enhance their cellular uptake and improve the antimicrobial activity.

The synthesized derivatives showed modest antimicrobial activity against *E. coli* and *S. cerevisiae* (MIC 16 μg/mL). As hypothesized, a good activity was instead highlighted after their incorporation into the prepared delivery devices such as lecithin-based nano-emulsion, PVP-MAA grafted copolymer and SNs (MIC $\leq$ 4 μg/mL).

The obtained results clearly indicated that the incorporation into suitable drug carriers could be an effective and very promising strategy to enhance the cellular uptake of the synthesized therapeutic agents and, consequently, improve their antimicrobial effect. The presence of vehicles, indeed, reduced by 4 times the normal MIC values of the newly synthesized compounds improving their antimicrobial activity against two different kinds of microorganisms, Gram −ve bacteria and yeasts, at the same time.

Conflict of interest

The authors report no declarations of interest.

Acknowledgments

We wish to thank Italian Minister of University and Research for financial support of this work.

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