

ANTIMICROBIAL ACTIVITIES OF AMPHIPHILIC DERIVATIVES OF α -AMINO ACIDS

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ABSTRACT

Antimicrobial products are very important to the health of the society. The increasing resistance of some bacteria to existing antimicrobials require searching of new active substances with antimicrobial activities. This review is devoted to the newly synthesised amphiphilic derivatives of proteinogenic α -amino acids and study of their antimicrobial properties. For this purpose, classification of the basis of both - head charge and type of the amino acid is presented with focus on the cationic representatives as possessing well-expressed antimicrobial activities. Data about representatives of amphiphilic α -amino acid derivatives with proven antimicrobial activity has been summarised, presented in tables and compared. Results obtained up to now show that arginine derivatives are the most promising, but also gemini derivatives of lysine and arginine amphiphiles have higher antimicrobial activity than monomeric ones. Even though these compounds have lower toxicity than conventional surfactants, they are still more toxic than accepted for drug formulations, so to be used in terms of antibiotics. Characteristics of the newly designed α -amino acid amphiphilic derivatives should be improved before their antimicrobial potential becomes practically applicable.

Keywords: α -amino acid derivatives, antimicrobial activity, amphiphiles, bacterial strains, microorganisms, cationic amphiphiles, polar head group, hydrophobic chain.

INTRODUCTION

Nowadays the world is facing serious difficulties with the antimicrobial resistance of a large number of pathogenic microorganisms. Many conventional antibiotics are considered less effective or ineffective against some infectious diseases caused by some bacteria and/or fungi. A vivid example of this issue is the resistance showed by some strains of *Escherichia coli* and *Klebsiella pneumoniae*, which can cause urinary infections, against ciprofloxacin. Their resistance to this antibiotic has varied throughout the years from 8.4 % to 92.9 % for *Escherichia coli* and from 4.1 % to 79.4 % for *Klebsiella pneumoniae* in different countries [1]. For this reason, many researchers are focused on finding molecules that have the capacity to fight against the antibiotic resistance of microorganisms, which, at the

same time, are harmless to the macroorganisms, non-toxic, biodegradable and affordable. Components that could fit in this description as promising antimicrobial are the surface-active amphiphilic derivatives of α -amino acids, especially the cationic representatives [2, 3].

Amphiphilic molecules in general consist of a polar hydrophilic group and non-polar hydrophobic moiety, called *head* and *tail*, respectively. As their name (*amphis* - “both”, “double” and *philia* - expresses affinity) and common structure indicate, these molecules possess dual affinity which means that they have both hydrophilic and lipophilic properties. The amphiphiles are classified according to different criteria [4]. Natural representatives of amphiphiles, produced by different microorganisms, are also known as biosurfactants. A subclass of the biosurfactants is the class of the so called lipopeptides that are types of molecules which contain

one or more alpha-amino acid residues in their polar groups, connected to a lipid moiety – the hydrophobic tail. Generally, biosurfactants are produced by bacteria of the *Bacillus* species, although data about other origins are also available [5]. On the basis of the moieties in the hydrophylic head and hydrophobic tail they are divided into subclasses, the main of which are surfactin, fengycin and iturin. Some of their representatives are proven to have antimicrobial and antitumor activity [6 - 8]. Isolation of biosurfactants is a laborious and an expensive process, which alternative is synthetic preparation of amino acid containing amphiphiles. Experimental studies of such synthetic amphiphiles confirm that a large amount of these molecules are surface active agents. Important characteristics of these molecules are: simple structure, biodegradability, biocompatibility and low toxicity [8, 9]. The availability of alpha-amino acids in terms of structure, especially the proteinogenic ones, makes them even more close to the natural surfactants, i.e. biosurfactants [5, 8].

In this review we aim to summarize the information found in the literature about the antimicrobial activities of synthetically obtained amphiphilic alpha-amino acid derivatives, as a possible alternative to the conventional antibiotics.

RESULTS AND DISCUSSION

Amphiphilic derivatives of amino acids

Classification

As it was mentioned in the introduction part, the amphiphilic molecules containing amino acids can be categorized according to different criteria.

According to the type of the head group and its charge, they are divided into *cationic*, *anionic*, *amphoteric* and *non-ionic* amphiphilic derivatives [5, 8, 9].

The *cationic derivatives* have a positive charged head group. The alpha-amino acids frequently included in the synthesis of cationic amphiphiles are Arg, Lys and His. Some examples of such molecules are cocoyl arginine ethyl ester, used as an additive to disinfectants and antimicrobial agents [10] and N-acyl lysine methyl esters, proven to display antimicrobial properties [11].

In the *anionic amphiphiles* the head carries negative charge. Derivatives of Gly in the form of sarcosinates are wide used as anionic surfactants in sunscreen products, mouthwash products and shampoos

because of their mild nature [8, 12]. Although, anionic surfactants are produced mainly as derivatives of Asp and Glu because of the additional carboxylic group in their side chain. Other commercial anionic surfactants used in cosmetics and as dispersing agents are sodium N-cocoyl-L-glutamate and potassium N-cocoyl glycinate known under the trade names Amisoft CS-22 and Amilite GCK-12, respectively [13, 14].

The *amphoteric derivatives*, also known as zwitterionic, contain both acidic and basic sites and thus change charge by varying pH of the medium. In acidic medium they behave as cationic molecules, while in alkaline solution they behave as anionic molecules [8]. Best examples are lauroyl lysine (LL) and alkoxy (2-hydroxypropyl) arginine, used mainly in skin creams, hair conditioners and lubricants [15].

Non-ionic amphiphiles possess head group with no formal charge [8]. The most used alpha-amino acids in this type of molecules are Lys, Val and Phe. Al-Sabagh et al. have worked in the field by synthesizing eight derivatives of L-Phe and L-Leu, which have good foaming and detergency properties [8, 16].

According to the molecular structure (number of heads and tails), the amphiphilic molecules are divided into several classes, some of which are presented in Fig.1. The simplest structure, called *monomeric amphiphil* concern single-chain molecules that contain one head group connected to one tail moiety (Fig. 1(A)). *Bolaamphiphile molecules* (Fig. 1(B)), called also bipolar amphiphilic molecules are built by two polar heads connected each other by a hydrophobic chain with certain length (spacer) [4, 17]. *Dimeric amphiphiles* known also as gemini surfactants (Fig. 1(C)) are characterized by two polar groups, linked with a hydrophobic spacer and two more hydrophobic tails attached to the both heads. *Double-chained amphiphiles* have one polar head and two hydrophobic tails (Fig.1 (D)). Obviously, more varieties of amphiphilic structures exist, like triple chain molecules, etc., but the most often synthesized and studied are presented in the Fig. 1 [4, 6, 7].

As it is well known, each amino acid possesses at least two functional groups in its molecule, including both a carboxylic group and an amino group. That is why it can be a building element of an amphiphilic structure through reacting with a long hydrophobic residue in any of the depicted in Fig. 2 ways. The attachment the hydrophobic chain to the alpha-amino acid can be at the

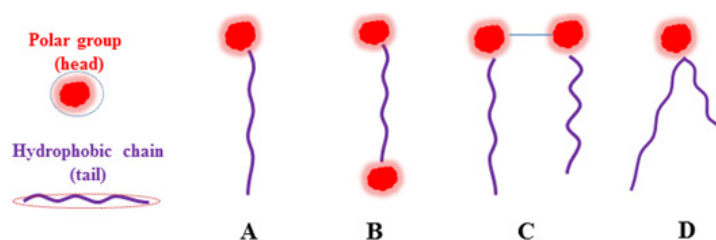


Fig. 1. Amphiphile classification according to the number of polar heads and tails: A) Monomeric, B) Bolaform, C) Gemini (dimeric) surfactant, D) Double-chained amphiphile.

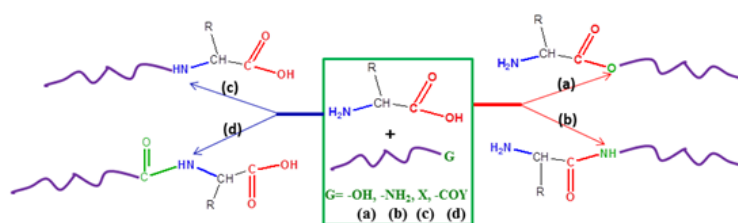


Fig. 2. General scheme for the preparation of alpha-amino acid amphiphilic derivatives: (a) esterification of the carboxylic group, (b) amidation of the carboxylic group, (c) alkylation of the aminogroup, (d) amidation of the aminogroup.

side of the amino group or at the side of the carboxylic group, as it is shown in Fig. 2, but it can be achieved also through reacting in the side chain of the amino acid. This leads to the formation of acyl, alkyl, amide and ester linkage [18, 19]. The process can be clearly synthesis or enzymatic, or even mixed - partially chemical and partially enzymatic reactions.

Attachment of the hydrophobic tail to the amino acid molecule is presented in Fig. 2. It can be realized through esterification (a) or amidation (b) of the carboxylic group (C-side), as well as through alkylation (c) or amidation (d) of the aminogroup (N-side).

Supramolecular structures of the amphiphiles in solutions

Amphiphilic molecules usually adsorb at interfaces, like air/water and oil/water such decreasing the interfacial tension. They order orienting the hydrophilic part to water and hydrophobic part to the air (or the oil) phase, forming a monolayer. The minimum amount of surfactant required to reduce the maximum surface tension of water is known as critical micelle concentration (CMC) or critical aggregation concentration (CAC).

At his concentration supramolecular complexes like micelles or other aggregates are formed [20 - 22]. These supramolecular complexes can have various shapes and sizes depending on different factors, but mainly on the molecule composition, which includes the hydrophobic part of the substance - its structure and length, as well as the nature of the polar head. Examples of such complexes are Langmuir monolayers, micelles, vesicles, liposomes, etc. [4]. The micelles could be in the shape of a sphere, bilayer or cylindrical [20]. The most common shapes are displayed on Fig. 3.

When the solution contains non-polar organic solvents, inverse micelles are being formed. In this case, the hydrophilic groups gather in the center forming the core of the micelles, and the hydrophobic tails come out of the core, directed to the surrounding solvent [21]. Micelles form when the concentration of the substance in the solution is higher than the critical micellar concentration and the temperature of the media is higher the temperature of the formation of the micelles, which is known as Krafft temperature or Krafft point for ionic surfactants [8]. CMC is one of the main characteristic properties of surfactants, alongside with the surface

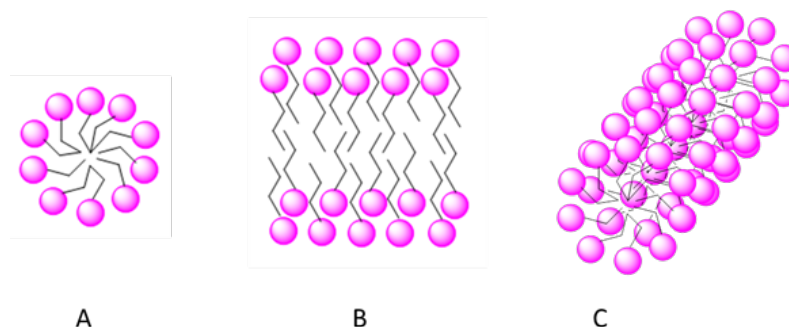


Fig. 3. Common shapes of micelles: A) a sphere, B) a bilayer, C) a cylindrical shape.

tension. After they reach the CMC, the surface tension remains stable even if we change the concentration of the amphiphilic molecules [22].

The amino acid amphiphilic derivatives have lower CMC than the conventional surfactants. The monomeric ones with long hydrophobic chain have lower CMC than these with shorter chain, which becomes even lower when the amino acid residues have free carboxylic, amide or hydroxyl group due to the formation of hydrogen bonds between the heads [18, 23]. The gemini amphiphilic molecules (Fig.1(C)) have lower CMC than their monomeric analogues and are better at lowering the surface tension due to the formation of hydrocarbon film at the air-water interface [18]. The surface tension of the amphiphilic amino acid derivatives is increasing with the length of the hydrophobic chain in the molecule and is closely connected to the number of the chains [8, 24]. Generally, the gemini surfactants exhibit lower surface tension than the monomeric ones [18]. Also, they form larger micelles than their monomeric analogs [18, 25].

Antimicrobial activities of amphiphilic alpha-amino acid derivatives

As it was mentioned earlier, there are many natural amphiphilic molecules that possess antimicrobial activity against various infections [26]. Those that to have this property are the

Cationic alpha-amino acid amphiphiles are considered to resemble the most the structure of natural lipopeptides and therefore they may share the same mechanism of action [2]. Actually, compounds found to manifest antimicrobial activities are these, containing amino acids with cationic side chains. Moreover, there

is evidences that if an amphiphilic derivative possesses a free primary amine group it easily accumulates in Gram-negative bacterial strains [27, 28].

Amphiphilic alpha-amino acid derivatives with antimicrobial activity have been synthesized and reported by various researchers in the field. Most of the newly synthesized molecules are arginine-based [29 - 32] and lysine-based [2, 11, 33], although some researchers synthesize and characterize antimicrobial amphiphiles with other amino acids, for example alanine-based [34]. The antimicrobial effects of these derivatives are closely related to a few principles, which will be discussed below. Apart from derivatives that contain only one amino acid, there is also data for dipeptide-based amphiphilic molecules that have antimicrobial properties [35]. Anionic and non-ionic amphiphilic alpha-amino acid derivatives usually do not display antimicrobial activity.

Principles and mechanism of the antimicrobial activity of the cationic alpha-amino acid derivatives

Antimicrobial activity of cationic derivatives depends also on the structure and cell characteristics of Gram-positive and Gram-negative bacterial strains. They have the ability to interact with negatively charged molecules in the bacterial membrane, such as lipoteichoic acids for Gram-positives and lipopolysaccharides for Gram-negatives [2, 36]. These molecules are considered relatively less effective against fungi due to the low amount of negatively charged molecules in their cell membrane [2, 37].

The mechanism of antimicrobial activity of cationic amphiphilic molecules includes two main steps -

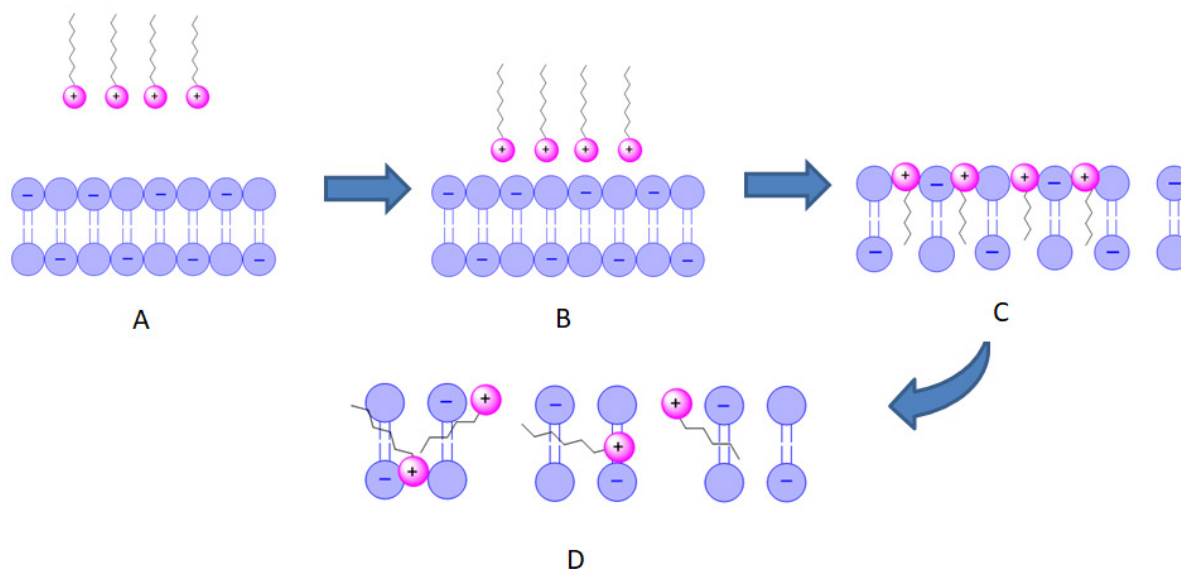


Fig. 4. Scheme of the mechanism of antimicrobial activity of cation amphiphilic alpha-amino acid derivatives: A - initial state, B - stage of electrostatic interactions, C - entering in the cell membrane and altering the bilayer, D - disruption of the lipid bilayer.

attachment of the molecules to the cell membrane favored by electrostatic interactions with the counterions in it and modification of the lipid bilayer of the membrane to facilitate the transport of intracellular content outside of the cell through the cell membrane [2, 29]. A schematic description of the whole antimicrobial mechanism is presented in Fig. 4. In the first step major role play the positively charged heads of the amphiphiles by interacting with the negatively charged molecules in the cell membrane (Fig. 4(B)), while in the second step the hydrophobic tails alter the bilayer of the membrane (Fig. 4(C)). For better performance in the second step, an optimum proportion between the hydrophobic and the polar moieties of the amphiphile are presented. All these steps eventually lead to the death of the cell (Fig. 4 (D)) as a result of the leakage of its inner cellular content [38].

According to the mechanism described above, these molecules are more active towards Gram-positive bacterial strains because of the higher negative charge in their membranes compared to the other bacterial cell membranes [29]. However, it was found that in case the amphiphilic molecules contain a free primary amine group, they are more active against Gram-negative bacteria [39]. Huan Li et al. reported synthesis of amphiphilic derivatives with free amine group of the amino acids that are bonded through a peptide (amide)

bond to an aliphatic amine with hydrocarbon chain varying in length. They tested the newly synthesized peptidomimetics against Gram-positive strains of *Staphylococcus aureus* and *Bacillus subtilis* and the Gram-negative strains of *Escherichia coli* and *Salmonella enterica*, and discovered that the molecules are more active against the Gram-negative strains [39]. This finding could be explained by the presence of primary amines. Also, their major advantage against most antibiotics is that they lead to the disruption of the cell membrane unlike antibiotics, which mostly affect the biosynthetic pathways in the cell or different parts of the cell itself [2, 18].

Characteristics of the antimicrobial activity of the amphiphilic alpha-amino acid derivatives

The antimicrobial activity of the cationic amphiphiles highly depends on their structure, size of the molecules, self-assembly effects and their hydrophilic-lipophilic balance [2, 18, 40].

The counter anion of the cationic molecule is very important for the activity of the cationic molecules. The counter anion should be soluble and easy to remove once it reaches the target cell [41, 42]. Another important feature is the cationic head group architecture, which is represented by its type and number of the amino

acid residues and also by its pK_a values. For example, the cationic charge of amino acid-based amphiphiles depends on the pH values of the media and thus under certain conditions such a molecule could act as a cationic amphiphile and possesses antimicrobial activity, but when the conditions are changed, it could lose its activity. These type amphiphiles are considered pH sensitive as the cationic charge density is proportional to the pK_a values of their head groups. Under physiological conditions, molecules with pK_a values higher than 9 have shown better antimicrobial activity compared to molecules with pK_a values below 7, especially against Gram-negative microorganisms [2, 40]. In addition, increasing the number of the head groups in the amphiphilic molecules leads to increasing the cationic charge density and thus the antibacterial activity rises [43, 44].

The hydrophobicity effects play important role in the antimicrobial activity of the cationic amphiphiles, especially in the second stage of the mechanism where the hydrophobic chains cause lysis to the cell membrane and eventually the death of the cell itself [45]. The length of the hydrocarbon tail shares non-linear dependence with the antimicrobial activity, elongation of the tail leads to increasing in the activity up to a certain length, at further elongation a decrease of the activity is being noticed. This phenomenon is known as the *cut-off effect* [46]. Molecules with chain lengths of 12-14 carbon atoms are considered to possess optimal antimicrobial activity because there is a stable ratio between the amounts of free volume beneath the hydrophobic residue and the rate of the interactions between the chain and the cellular lipids [40, 46, 47]. The same could valid for all kinds of cationic amphiphilic molecules that mimic the antimicrobial peptides [48]. The number of chains also influences the extent of the antimicrobial effects and there is evidence that cationic gemini amphiphilic molecules display better antimicrobial activity than their monomeric counterparts [2, 40, 49]. Moreover, the length of the spacer is also important for the activity because gemini alpha-amino acid derivatives with longer spacer tend to be more flexible and can connect easily to the negatively charged molecules in the cell [50]. Using amino acids with aromatic group in their side chains increases the hydrophobicity of the molecule, but if amino acids that have hydroxyl groups in the side chains are included, such as tyrosine, the hydrophobicity decreases alongside with the antimicrobial activity [40,

49]. To determine which moiety has stronger effect on the antimicrobial activity, Ghosh et al. designed three series of amphiphilic molecules with aromatic and alkyl chains connected to a lysine residue [51]. Their results showed that compared to the short alkyl chain, the aromatic ring had a stronger effect on the antimicrobial activity, but as the length of the alkyl chain increased, a greater impact on the antimicrobial activity of the amphiphilic molecules was observed [40, 51].

The self-assembly effect and the aggregation properties of the amphiphilic amino acid derivatives also play a key role for their antimicrobial properties. It can be influenced by the CMC/CAC values and also the sizes and forms of the aggregates. When the minimal inhibitory concentration (MIC) of the molecules is lower than their CAC, the antimicrobial activity is only dependent on the electrostatic-hydrophobic balance of the amphiphile. Also, the dependence between CAC and the antimicrobial activity is again non-linear and characterized with a *cut-off effect* [40, 52]. On the other hand, if the MIC value is above the CAC, then the aggregation properties have influence on the antimicrobial effects by promoting the activity of the amphiphilic amino acid derivatives. The aggregates are more potent antimicrobials than the lone molecules due to the rise of the density of the negative charge and their local concentration in the cell. The supermolecular complexes that form are usually of different shapes and sizes, but those molecules that form smaller aggregates are more likely to enter the microbial cell unhindered [40]. Qi et al. have investigated different amphiphiles with lysine residues to determine the influence of the aggregates formed and the spacer length on the antimicrobial potency of the molecules [53]. The results indicate that when the molecules form long fiber aggregates, the CMC increases, which leads to less active antimicrobial amphiphiles, while aggregates in the forms of short rods and spheres with small sizes can enter easily in the cell membrane and disrupt it at lower MIC values [40, 53].

Representatives of amphiphilic alpha-amino acid derivatives with proven antimicrobial activity

Many researchers have synthesized and characterized a huge number of amphiphilic alpha-amino acid derivatives over the years and have studied their antimicrobial activities. Some research works are concentrated on derivatives of only one alpha-amino

acid, while others investigate series of derivatives and compare the final results. This part of the review is focused mainly on the information available in the literature for compounds that are already synthesized and proven to have antimicrobial properties.

Arginine derivatives

The most popular and well-known antimicrobial amphiphilic molecules derived from alpha-amino acids are the arginine-based amphiphilic molecules.

The presence of free quinidine group, which is an essential part of arginine, facilitates the transport of the amphiphilic molecules to liposomal and cell membrane by interacting easily with the negatively charged molecules there [54]. It is reported that the presence of arginine in the derivatives is responsible for increasing the antimicrobial effects in histone-derived antimicrobial peptides [55]. Some of these derivatives and the bacterial strains, against which they are proven to have antibacterial activities, are listed in Table 1.

Table 1. Some arginine derivatives that possess antibacterial activity.

Arginine-based amphiphilic derivatives	Antimicrobial activity against bacterial strains:	References:
N ^ω -Octanoyl-L-Arginine ethyl ester	<i>Staphylococcus aureus</i> ATCC 6538	[31]
N ^ω -Nonanoyl-L-Arginine ethyl ester	Gram-positive: <i>Staphylococcus aureus</i> ATCC 6538, <i>Listeria monocytogenes</i> ATCC 751; Gram-negative: <i>Escherichia coli</i> ATCC 8739, <i>Salmonella</i> spp.;	[31]
N ^ω -Myristoyl-L-Arginine ethyl ester	<i>Staphylococcus aureus</i> ATCC 6538, <i>Escherichia coli</i> ATCC 8739	[31]
N ^α ,N ^ω -bis(N ^α -acylarginine)α,ω-alkylendiamides (spacer: 2-10 carbon atoms)	Gram-positive: <i>Bacillus cereus</i> var. <i>mycoides</i> ATCC 11778, <i>Bacillus subtilis</i> ATCC 6633, <i>Staphylococcus aureus</i> ATCC 25178, <i>Staphylococcus epidermidis</i> ATCC 155-1; Gram-negative: <i>Alcaligenes faecalis</i> ATCC 8750, <i>Bordetella bronchiseptica</i> ATCC 4617, <i>Citrobacter freundii</i> ATCC 11606, <i>Enterobacter aerogenes</i> ATCC 10938;	[56]
Arginine O-alkyl ester dihydrochloride (alkyl chain: 8, 10 and 12 carbon atoms)	Gram-positive: <i>Staphylococcus aureus</i> , <i>Staphylococcus epidermidis</i> , <i>Streptococcus faecalis</i> ; Gram-negative: <i>Escherichia coli</i> , <i>Klebsiella pneumoniae</i> , <i>Citrobacter freundii</i> , <i>Serratia marcescens</i> ;	[2]
Bis(N ^α -caproyl-L-arginine)-1,3-propanediamine dihydrochloride	<i>Staphylococcus aureus</i> ATCC 9144, <i>Escherichia coli</i> ATCC 10356	[29]
Arginyl alkyl amide dihydrochloride salts (alkyl chain: 12 and 14 carbon atoms)	Gram-positive: <i>Bacillus cereus</i> var. <i>mycoides</i> ATCC 1178, <i>Bacillus subtilis</i> ATCC 6633, <i>Staphylococcus aureus</i> ATCC 25178, <i>Staphylococcus epidermis</i> ATCC 155-1; Gram-negative: <i>Bordetella bronchiseptica</i> ATCC 4617, <i>Citrobacter freundii</i> ATCC 1160, <i>Enterobacter aerogenes</i> ATCC 10938, <i>Salmonella typhimurium</i> ATCC 14028	[30]

According to several comparative studies and reviews, some of the most active types of antimicrobial agents of the arginine derivatives are the N^α-acyl arginine alkyl esters, especially their hydrochlorides [2, 31].

Lysine derivatives

The lysine-based amphiphilic derivatives are also ones of the most researched amphiphilic derivatives of alpha-amino acids. They possess two amino groups - one in the main chain and one at the end of the side chain. This fact supports the expectations of scientist that the lysine derivatives would display antimicrobial activity, even against the prone to resistance bacterial strains because of the free primary amino groups [28]. Many different derivatives could be synthesized. However the majority of synthesized components are single chain and N^α- or N^ε-acyl lysine derivatives [2]. Some of lysine-amino acid derivatives and the bacterial strains that they affect are listed in Table 2.

Several research results and comparative studies indicate that the lysine amphiphilic derivatives are less active than their arginine alternatives [2, 11, 55]. Other important fact is that both lysine and arginine gemini amphiphilic derivatives have higher antimicrobial activity compared to their monomeric analogues [2].

Other amino acid derivatives

Apart from arginine and lysine derivatives, there are other amphiphilic compounds derived from other α-amino acids, either neutral or anionic side chain, reported to have antimicrobial activity. The common thing in all of them is the free amino group which ensures their antimicrobial properties [39, 52]. Some of these compounds and the bacterial species that these molecules are active against are listed in Table 3.

There are also dipeptide amphiphilic derivatives that have antimicrobial activities on the basis of proline, phenylalanine and tryptophan [35]. There is also evidence of amphiphilic molecules that have antifungal activities. For example, gemini bis-quaternary surfactants, derivatives of bis-alanine esters, that are active against *Candida albicans*, were synthesized by Luczynski et al. [34].

Application and perspectives of antimicrobial amphiphilic alpha-amino acids derivatives

As we considered earlier, the amphiphilic alpha-amino acid derivatives that possess antimicrobial activity are these that have cationic charge in aqueous solutions. Although there are many newly synthesized derivatives with different charge, some of which are

Table 2. Some lysine derivatives that possess antibacterial activity.

Lysine-based amphiphilic derivatives	Antimicrobial activity against bacterial strains:	References:
Chloride salts of N ^α -Layroyl lysine methyl/ethyl esters	Gram-positive: <i>Staphylococcus aureus</i> , <i>Staphylococcus epidermidis</i> , <i>Bacillus. Subtilis</i> Gram-negative: <i>Escherichia coli</i> , <i>Klebsiella pneumoniae</i> , <i>Pseudomonas aeruginosa</i>	[2]
N ^ε -acyl lysine methyl esters (alkyl chain: 12, 14 and 16 carbon atoms)	Gram-positive: <i>Bacillus cereus</i> var. <i>mycoides</i> , <i>Enterococcus hirae</i> , <i>Micrococcus luteus</i> , <i>Staphylococcus aureus</i> : Gram-negative: <i>Escherichia coli</i> (apart from the palmitoyl derivative), <i>Klebsiella pneumoniae</i> (only for the lauroyl derivative)	[11]
N,N'-Bisbromoacetyl-L-lysine ethyl esters (acetyl chain: 8 and 10 carbon atoms)	<i>Staphylococcus aureus</i> and <i>Escherichia coli</i>	[33]
Chloride salt of lysine O-lauroyl ester	<i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> and <i>Escherichia coli</i>	[57]

Table 3. Other alpha-amino acid derivatives that possess antimicrobial activity.

Amphiphilic alpha-amino acid derivatives	Antimicrobial activity against bacterial strains:	References:
L-Phenylalanine alkyl esters (alkyl chain: up to 20 carbon atoms)	Gram-positive: <i>Staphylococcus aureus</i> ATCC, 29213, <i>Staphylococcus aureus</i> ATCC 25923, <i>Staphylococcus epidermis</i> ATCC 12228 Gram-negative: <i>Escherichia coli</i> ATCC 22922, <i>Pseudomonas aeruginosa</i> ATCC 27853, <i>Salmonella typhimurium</i> ATCC 14028, <i>Klebsiella pneumonia</i> ATCC 13883;	[52]
L-Tyrosine alkyl esters (alkyl chain: 8, 10, 12 and 14 carbon atoms)	Gram-positive: <i>Staphylococcus aureus</i> ATCC, 29213, <i>Staphylococcus aureus</i> ATCC 25923, <i>Staphylococcus epidermis</i> ATCC 12228; Gram-negative: <i>Escherichia coli</i> ATCC 22922, <i>Pseudomonas aeruginosa</i> ATCC 27853, <i>Salmonella typhimurium</i> ATCC 14028, <i>Klebsiella pneumonia</i> ATCC 13883;	[52]
N^α-alkyl-L-Phenylalanine (alkyl chain: up to 16 carbon atoms)	Gram-positive: <i>Staphylococcus aureus</i> , <i>Bacillus subtilis</i> , Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA); Gram-negative: <i>Escherichia coli</i> , <i>Salmonella enterica</i> ;	[39]
N^α-lauroyl-L-Leucine/Valine/Isoleucine	Gram-positive: <i>Staphylococcus aureus</i> , <i>Bacillus subtilis</i> , Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA); Gram-negative: <i>Escherichia coli</i> , <i>Salmonella enterica</i> ;	[39]

synthesized and characterized by our group, none of them are reported to have antimicrobial properties [58, 59]. However, some of these molecules can develop excellent nanosystems that are appropriate for drug delivery. In this way they can participate in the fight against drug resistant pathogens [18, 60]. Castillo et al. discovered that some anionic amino acid derivatives can enhance the antimicrobial effectiveness of different preservatives and thus supporting their action [12]. The cationic alpha-amino acid amphiphilic molecules are considered as good alternative to conventional antibiotics. Several research works are concentrated in comparison studies of such molecules and common antibiotics. The results show that some derivatives are nearly equally potent as some antibiotics. This is visible

of the results from the study performed by Castillo et al., who compared the newly synthesized bis(Na-caproyl-L-arginine)-1,3-propanediamine dihydrochloride and chlorhexidine dihydrochloride and observed nearly similar results [29]. They even show better activity than some conventional cationic surfactants, which also have antimicrobial properties. Pérez et al. compared their newly synthesized lysine derivatives to the conventional surfactant hexadecyl trimethyl ammonium bromide and registered lower activity of the latter than of their molecules [11].

Apart from good antimicrobial activity, the alpha-amino acid derivatives must contain low cytotoxicity and good biodegradability in order to become a part of the antimicrobial drugs. Even though they are less toxic

than the conventional surfactants due to the presence of alpha-amino acids [18], many derivatives have cytotoxicity, which makes them potential antitumor agents, but hinders their application as antimicrobial drugs since they are considered as harmful not only to the bacterial cell, but also to the mammalian cell [43, 61]. Molecules that have antibacterial activity along with low toxicity profile are long chain cationic arginine derivatives [2, 18, 19]. Researchers are in the process of developing strategies in order to minimize the toxicity of the cationic amino-acid derivatives. For example, Lozano et al. developed catanionic vesicles of cationic arginine-glycerol-based compounds with changed positive to negative charge with the aim of decreasing their cytotoxicity [62]. Still, there is a lot of work to do until the antimicrobial potential of alpha-amino acid amphiphilic derivatives could be practically applicable [2].

CONCLUSIONS

The amphiphilic alpha-amino acid derivatives are molecules that enter deeper and deeper into our everyday life. They are surface active, biodegradable and easy to synthesize. Their cationic derivatives are reported to possess antimicrobial properties, especially the arginine-containing molecules, which are most common. These molecules are considered as good alternatives to conventional antibiotics. However, most of them are toxic not only for the microorganisms, but also for the macroorganisms. For this reason they have not yet found their place in the fight against drug resistant bacteria and their application is scarce. New designs and techniques should be invented in order to convert their antimicrobial potential into actual antimicrobial product.

Acknowledgements

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