Peptoids As Source of Compounds Eliciting Antibacterial Activity

Isabel Masip[£], Enrique Pérez-Payá[#] and Angel Messeguer[£,*]

[£]*Department of Biological Organic Chemistry, I.I.Q.A.B. (C.S.I.C.). J. Girona, 18. E-08034 Barcelona, Spain*

[#]*Fundación Valenciana de Investigaciones Biomédicas, CSIC. E-46010 València, Spain*

Abstract: *N*-Alkylglycine oligomers (peptoids) constitute a family of non-natural peptidomimetics attractive for the early drug discovery process because of their physicochemical features, easy of adaptation to combinatorial chemistry approaches and their proteolytic stability. Consequently, peptoid libraries have found application for discovering hits against a wide diversity of pharmaceutical targets, among which different examples of antibacterials are found. In the present work, research efforts addressed towards the identification of peptoids as antibacterial agents are discussed.

Keywords: *N*-Alkylglycine trimers, peptoids, combinatorial chemistry, chemical libraries, antibacterial activity, bacterial resistance.

INTRODUCTION

Bacterial resistance to the available drugs is recognized as a major problem in infectious diseases. Since their discovery, antibiotics have proved to be effective to control bacterial infections. Unfortunately, nowadays some pathogens exhibit resistance to many drugs that were initially effective. In fact, more than 70% of hospital-acquired infections in the US show resistance to at least one of the main antibacterial agents commonly used [1,2].

The selection of the most appropriate antibacterial agents for tackling a given infectious pathology demands the previous knowledge of the mechanisms of resistance that could emerge as consequence of that treatment. Marketed antibiotics act mainly against the bacterial cell wall or blocking crucial biosynthesis pathways (DNA gyrase, RNA polymerase or protein synthesis). In this sense, bacteria have developed defences by mutation or by acquiring new genes from other bacteria. These defences may cause the alteration in the permeability of the cell wall or the modification of the enzymatic processes intervening in the biosynthesis of the cell wall. In addition, they may induce the inactivation of the drug or stimulate the action of the efflux pumps to throw the antibacterial compound out.

Although a more appropriate use of current antimicrobial agents would result in a reduction of the resistance phenomenon and therefore in an extension of the effective life of these drugs, the development of new antibacterial agents appears to be the most attractive challenge to fight against the emergence of resistance [3]. In this context, one simple possibility is to think in the analoguing strategy; however, it would be highly probable that the mode of action of the molecules generated by this route will be the same as that operating with the precursors. Consequently, the development of a rapid reaction of bacterial resistance will be also highly expected. This fact probably accounts for the limited number of compounds approved for antibacterial activity in recent years. Thus, seeking for new antibacterial agents with a novel mechanism of action emerges as the best approach to overcome bacterial resistance. In this context, the recent progress on bacterial genomics and proteomics may permit the identification and validation of new protein targets essential for the bacteria survival. From this knowledge it will be more feasible the development of new chemical entities, peptides and non-peptides, which could act efficiently over these targets. In fact, several pharmaceutical companies are working actively along this line.

*Address correspondence to this author at the Department of Biological Organic Chemistry, I.I.Q.A.B. (C.S.I.C.). J. Girona, 18. E-08034 Barcelona, Spain; Tel: 34-93-4006121; Fax: 34-93-2045904; E-mail: ampqob@iiqab.csic.es

1. ANTIBACTERIAL PEPTIDES

Although it is known that antibacterial peptides are important components of the innate defences of living species, it has been in the recent years when their importance as antibacterials has been recognized [4,5]. Many of these peptides show a potent *in vitro* activity against microorganisms resistant to conventional antibiotics at concentrations ranging from 0.25 to 4 μg/mL [4]. Among the advantages offered by these peptides are their ability to kill target cells rapidly, the unusual broad activity spectra showed against some of the more serious antibiotic resistant pathogens in clinics and the relative difficulty in selecting resistant mutants *in vitro*.

The antibacterial peptides discovered so far -over 500- [6] exhibit great structural diversity, although they have also common structural patterns. In general, active compounds both from endogenous source or obtained synthetically are composed of hydrophobic and cationic amino acids (particularly Lys and Arg), which are organised in the molecule as an amphipathic structure. More than 50% of these peptides are arranged as linear α-helices, (*cf.* magainins [7], cepropins [8] and cathelicidins [9]). A second group is that formed by a relatively rigid antiparallel β-sheet stabilised by intramolecular disulphide bonds. This is the case of α- and β-defensins [10,11] and protegrins [12] Finally, a third group is comprised by peptides lacking Cys, but that are rich in Pro, Trp or His residues, such as PR39 [13] indolicidin [14] and histatin, respectively. Peptides composed of all D-amino acids, in place of the respective L-

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amino acids, typically retain full antibiotic potency while exhibiting enhanced resistance to enzymatic proteolysis [15]. Short linear or cyclic amphiphilic peptides that contain both L- and D-amino acids can be also generated with various degrees of selectivity and antibacterial potency [16]. Recently, protease resistant antibacterial peptides composed of β-amino acids have been also reported [17]. The main site of action of most antimicrobial peptides [16] is the cytoplasmic membrane. The cationic antimicrobial peptide interacts with the negatively charged phospholipids of the bacterial membrane leading to the displacement of these lipids, alteration and depolarization of the membrane structure and further collapse into vesicles and micelles, or in the creation of pores that allow the entry of the peptide and solutes into the cell and efflux of cellular contents [18,19].

The growing problem of the bacterial resistance to conventional antibiotics has driven the pharmaceutical companies to investigate compounds with novel modes of action. This is the case of antibacterial cationic peptides, where their mode of action against the bacterial membrane makes difficult the selection of resistant organisms, since it would involve extensive modifications and reorganisation of the lipid membrane. Actually, the studies carried out on these cationic peptides confirm that the emergence of resistance is less probable than from conventional antibiotics, and for this reason, the development of antimicrobial peptides as human therapeutic agents is an area of intense research, with some molecules in advanced stage evaluation, such as MSI-78 (Magainin Pharmaceuticals), IB-367 (Intrabiotics) or human lactoferricin (AM Pharma).

2. PEPTIDOMIMETICS

Despite the great potential as antibacterial agents, peptides present known drawbacks relative to their drug-likeness character. In general, peptide drugs exhibit poor oral bioavailability, a short half-life, rapid biodegradation by proteases, and multiple actions and fluctuations in their pharmacokinetic profile. Consequently, the development of peptidomimetics capable of maintaining the biological activity elicited by antibacterial peptides, but counteracting their pharmacological limitations, has been a challenge for medicinal chemistry in recent years.

The use of peptoids has been one of the most representative approach to meet this goal [20,21]. Peptoids are structurally characterised by the formal displacement of the side chain from the chiral α-carbon of the peptide to the nitrogen atom, resulting in an *N*-substituted oligoglycine. The main advantages of peptoids are: higher metabolic stability, since proteases cannot cleave easily this tertiary amide bond [22,23], and high flexibility due to conformational mobility originated by both *cis* and *trans* amide bond rotamers, which expands the exploration of the conformational space [20,21]. Moreover, peptoids do not show spatial restrictions because of the absence of chirality at the α-carbon and from the lack of intramolecular hydrogen bonding interactions between amide N-H and carbonyl groups characteristic of peptides and inducers of secondary structure. Nevertheless, peptoids can adopt stable helical structures in the presence of α-chiral side chains [24,25].

The broad molecular diversity achievable together with the inherent conformational flexibility of these molecules makes peptoids suitable to interact with a broad variety of biological targets. Thus, it has been reported the discovery of a Tat/TAR RNA inhibitor that suppresses HIV-1 replication [26,27] and the combinatorial approaches that led to the discovery of cationic peptoid reagents for gene delivery [28] and for the identification of antagonists of the $TRPV_1$ receptor channel in analgesia [29].

From the synthetic point of view, peptoids are readily prepared through the solid-phase "submonomer" synthetic methodology reported by the group of Chiron [30] This methodology uses primary amines as source for the different amino side-chain substituents, which provides a wide chemical diversity and also facilitates the application of combinatorial techniques for the easy production of peptoids libraries. Moreover, the ease of preparation of peptoids has made possible the recent development of a photolithographic procedure for the synthesis of peptoid arrays for protein ligand discovery [31].

2.1 Peptoids with Antibacterial Activity

A) Analogues of Antibacterial Peptides

Patch *et al.* have reported the synthesis of active peptoid analogues of magainin, a linear cationic, amphipathic helical peptide [32]. These peptoids are composed of different combinations of 12-17mer cationic hydrophilic (*N*Lys), α-chiral aliphatic hydrophilic ((*S*)-*N*-(*sec*butyl)glycine) and α-chiral aromatic lipophilic moieties ((*S*)-*N*-(1-phenethyl)glycine), to stabilise α-helical conformations and preserve the amphipathic features of the molecule. Compounds shown in Fig. **1** exhibit potent and selective

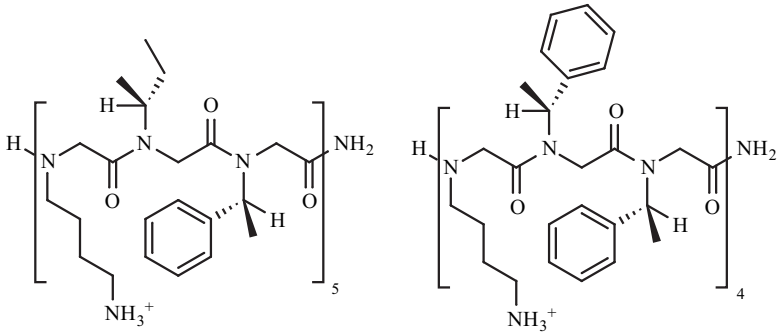


Fig. (1). Peptoid mimics of magainin-2 amide.

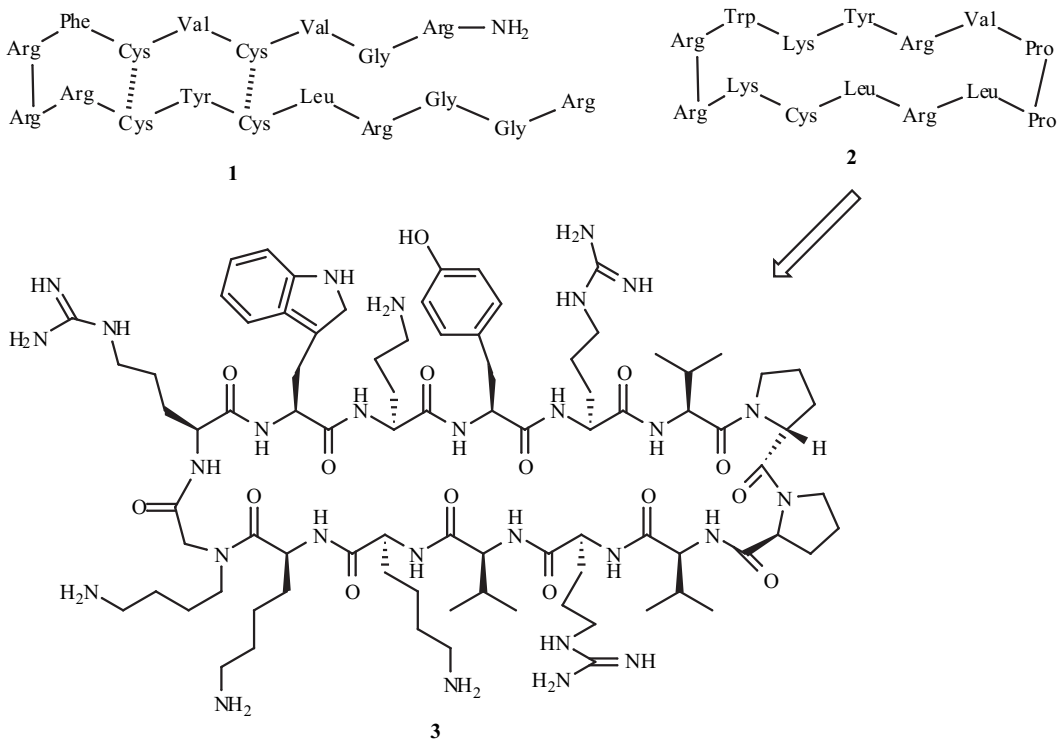


Fig. (2). Structure of a hybrid peptide-peptoid showing β-hairpin conformation and eliciting potent antibacterial activity [33].

antibacterial activity against both Gram-positive (*Bacillus subtilis*) and Gram-negative (*Escherichia coli*) bacteria with MIC values in the low micromolar range, and no haemolytic activity against human erythrocytes.

Along the same line, Shankaramma *et al.* prepared a family of peptoids that mimic another antibacterial cationic peptide, protegrin-I (**1**, Fig. **2**), and conserve its β-hairpin amphiphilic structure [33]. In **1**, the β-hairpin structure is constrained by two disulfide bridges (Fig. **2**). The first generation of peptidomimetics described by this group was obtained by fixing the desired conformation through the insertion of selected aminoacids of a macrocyclic peptidomimetic composed of a 12-mer-peptide loop to a hairpin stabilising template of D-Pro-L-Pro or xanthene, as shown in **2** (Fig. **2**) [34]. Activity against Gram-positive and Gram-negative bacteria was observed for this compound; noticeably, it showed also lower haemolytic activity on red blood cells in comparison with protegrin-I. At this point, a second generation of peptidomimetics was envisaged to improve proteolytic stability. The strategy involved the insertion of a peptoid unit in a position where the original NH bond was not intervening in cross-strand H-bonding of the regular β-hairpin. The resulting hybrid structure **3** (Fig. **2**) exhibited antimicrobial activities against Gram-positive and Gram-negative bacteria (MIC~8-30 μg/mL in *Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853 and *Staphylococcus aureus* ATCC29213). These activities were in the same range to that of protegrin-I (MIC 4 μg/mL against the three bacterial strains), but with improved selectivity for human erythrocytes (less than 1% haemolytic activity at 100 μg/mL).

Studies performed by Oh *et al.* showed that replacement of amide bonds of antimicrobial peptides by introduction of *N*-substituted glycine moieties at the *N*-terminal residues decreases α-helicity while retaining antimicrobial activity due to the increase of hydrophobic interactions [35]. This effect was studied by the incorporation of peptoid monomers in a membrane active decapeptide (KSL) against *Candida albicans*. Compounds showed in Fig. **3** have MIC values between 6 and 17 μg/mL against a panel of Gram-positive and Gram-negative bacteria.

$$RNHCH_2CONRCH_2CO\text{-}WFKVKFKk\text{-}CONH_2$$

MP4 $R = CH_2CH_2CH_2N(CH_3)_2$

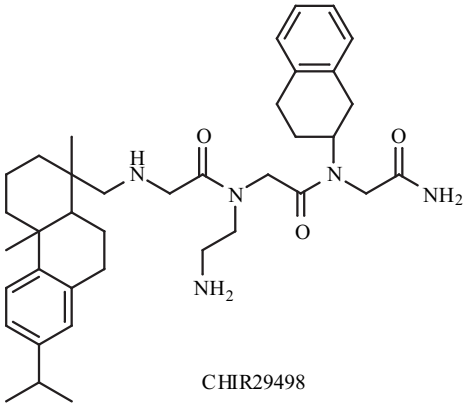
MP5 $R = CH_2CH_2NH_2$

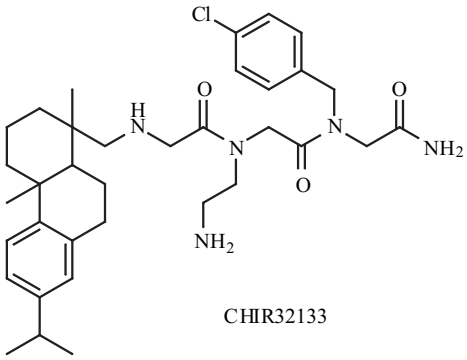
Fig. (3). Structure of peptidomimetics reported by Oh *et al.* [35].

B) Combinatorial Libraries of Peptoids

In recent years, combinatorial chemistry has found a vast application in the early stages of drug discovery. Chemical libraries have efficiently speeded up the exploration of the chemical diversity for the identification of hits and eventually lead compounds. As mentioned above, the modular scaffold of *N*-alkylglycine oligomers made them amenable for combinatorial strategies. In this context, the screening of two peptoid libraries has also permitted the identification of new antibacterial compounds.

The first peptoid library described with antibacterial activity was reported by a group from Chiron Corporation [36,37]. This library, optimised from a previous one, was constituted by 845 trimers of *N*-alkylglycines distributed into 65 pools and constructed using the split and pool methodology. The screening of this library against different Gram-positive and Gram-negative strains revealed that a combination of hydrophobicity and basic sites, especially in the middle position, was necessary to elicit high antibacterial activity (Fig. **4**). Complementary assays carried

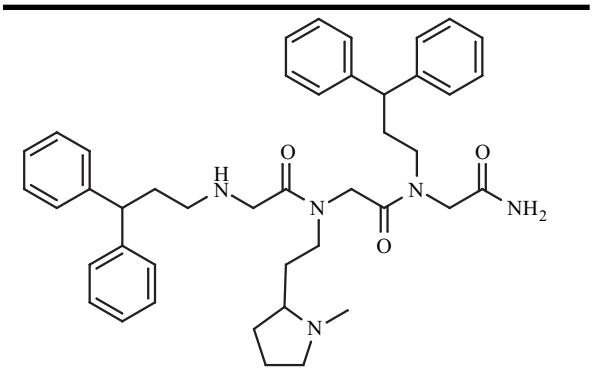




Strain	MIC (μg/mL)	
	CHIR29498	CHIR32133
E. coli ATCC 25922	20	40
P. aeruginosa ATCC 27853	20	40
S. aureus ATCC 25923	20	10
E. faecalis ATCC 29212	10	20

Fig. (4). Structures of the most active antibacterial peptoids identified from the screening of the library reported by the Chiron group [36].

out with active peptoids derived form this library showed that their mechanism of action did not interfere with protein synthesis. On the other hand, the structural features of these active peptoids could make possible their access to the targets situated in the bacterial membrane, similarly to the active peptides mentioned above, although this assumption remains to be confirmed. Finally, it is worth of mention that the identified active peptoids are smaller than the smallest antibacterial peptides, either natural (indolicidin, 13-mer) [38] or synthetic (6-mer) [39].



Strain	**MIC (μg/mL)**
E. coli ATCC 25922	62
P. aeruginosa ATCC 27853	31
S. aureus ATCC 25923	31
E. faecalis ATCC 29212	31

Fig. (5). Antibacterial peptoid identified from the screening of the library reported by M. Humet *et al*. and further one-step structural optimisation [39].

Recently, another combinatorial library, containing more than 10.000 *N*-alkylglycine trimers and constructed under the positional scanning format, has been reported. The screening of this library for compounds exhibiting antibacterial activity permitted the identification of several peptoids with moderate activity against a panel of Gram-positive and Gram-negative bacteria [40]. Although the defined peptoids deduced from the library deconvolution showed modest selectivity ratios, the most promising compound served as a hit, and its antibacterial activity was improved one order of magnitude by one point diversity substitution (Fig. **5**). Once again, the occurrence of a basic site in the internal position, in this case a tertiary amino moiety, was required to elicit activity. Activities in the low micromolar were identified both in Gram-positive and Gram-negative bacteria, being the compound showed in Fig. **5**, the most promising hit.

In conclusion, the screening of libraries of peptoids constructed either as split and mix or positional scanning formats has permitted the identification of *N*-alkylglycine trimers exhibiting activities potent and selective enough in front of bacterial strains of interest as pharmaceutical targets. Although it can be accepted that for different pharmacological and pharmaceutical reasons it is hardly conceivable to imagine peptoids as an end-point drug molecule, their structural simplicity makes these peptidomimetics susceptible of broad structural manipulation and therefore of drug-like property optimisation. Thus, in front of those strategies seeking for increasing complexity on the hit rate, which gives less room for optimisation of the drug-like profile, the use of peptoid libraries establishes a valuable link between modern drug discovery technologies and the currently revisited historical lead approach [41,42]. On the other hand, peptoids are molecules presenting a great conformational mobility; in

this sense, each *N*-alkylglycine oligomer can be envisaged as a small dynamic combinatorial library [43]. This conformational mobility can also generate selectivity problems due to interactions with targets other than that desired. When this is the case, freezing of the active conformation by means of synthesizing the appropriate conformationally restricted analogues could lead to an improvement of the activity and selectivity of these compounds. Active research along this approach is in progress in our laboratories.

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