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1,4-Dihydropyridine Cationic Peptidomimetics with Antibacterial Activity

Inbal Lapidot[1,2] · Amnon Albeck[1] · Gary Gellerman[2] · Shimon Shatzmiller[2] · Flavio Grynszpan[2]

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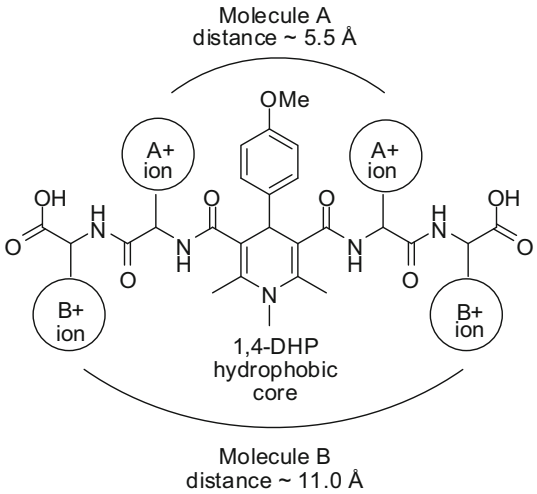
Abstract We synthesized new broad spectrum antibacterial cationic peptidomimetics centered on a hydrophobic 1,4-dihydropyridine (1,4-DHP) scaffold. The synthesis involves the preparation of the scaffold in a three step Hantzsch reaction followed by simultaneous coupling of the 1,4-DHP scaffold to two dipeptides bearing cationic side chains. The synthesized peptidomimetics were found to have no measurable toxic hemolytic effect against mammalian red blood cells. The compounds were found to have antibacterial activity against Gram-(−) and Gram-(+) bacteria with MICs in the range of 35–100 μg/mL. These cationic antimicrobial peptidomimetics will lead to more effective antibacterial drug candidates based on a synthetically accessible scaffold.

Graphical Abstract



Keywords Peptidomimetics · 1, 4-dihydropyridine scaffold · Broad spectrum anti-bacterial · Anti-microbial · Cationic peptides · Drug design

Abbreviations

1,4-DHP	1,4-dihydropyridine
BDZP	Benzodiazepine
HOBt	*N*-hydroxyl benzotriazole
MIC	Minimal inhibitory concentration
RBC	Red blood cells

Introduction

Bacterial antibiotic resistance is a major health threat. It is therefore important to explore new molecular architectures with broad antibacterial activity that are particularly

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✉ Shimon Shatzmiller
shimon120@gmail.com

✉ Flavio Grynszpan
flaviog@ariel.ac.il

[1] Department of Chemistry, Bar-Ilan University, Ramat Gan, Israel

[2] Department of Biological Chemistry, Ariel University, Ariel, Israel

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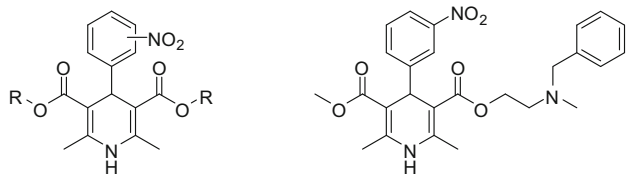
effective against multi-resistant Gram-positive bacteria (Hancock 2001; Niu et al. 2012).

Antibacterial agents can be classified in three major groups according to their mechanism of action: (a) cell wall synthesis and disruption, (b) protein synthesis, and (c) DNA synthesis. One of the most promising approaches in the development of antibacterial agents of broad action is the application of a model based on cationic oligopeptides capable of disrupting the bacterial cell wall. Cationic peptides can be found in all forms of life as natural defense against pathogens (Brown and Hancock 2006; Jenssen et al. 2006). Inspection of naturally-occurring antibacterial peptides shows a general pattern of short amino acid sequences including two cationic amino acids, like lysine or arginine, bracketing a sequence of two to three hydrophobic amino acids (Faccone et al. 2014; Toke 2005; Uggerhoj et al. 2015). Many naturally-occurring antibacterial peptides acquire a well-defined three-dimensional structure only upon binding to a membrane such as a bacterial cell wall, when the side chains of the cationic amino acids interact with negatively charged phosphodiester groups in the membrane (Zaiou 2007). For cationic peptides having an α-helix secondary structure, the minimal separation distance between two cationic residues located on the same polar face is 5.4 Å corresponding to one turn of the helix. The second step in this molecular ladder represents a vertical distance of 10.8 Å corresponding to two turns of the helix.

One of the main disadvantages of peptide drugs is their potential metabolic instability while being quickly cleared from the body. A peptidomimetic approach designed to improve the metabolic stability and retain the desired antibacterial activity seems very attractive (Marr et al. 2006). We reasoned that a 1,4-dihydropyridine (1,4-DHP) scaffold could be used to mimic the hydrophobic peptide gap between two cationic residues. The 1,4-DHP structure is a well-known pharmacophore considered to be a "privileged structure" that, when appropriately substituted, can cause different biological responses. 1,4-DHPs were first reported by Fleckenstein (1983) for the treatment of coronary diseases (Neal et al. 2000). Specifically, 4-Aryl-1,4-DHPs of the nifedipine type (**1**, **2**, and **3** in Fig. 1) were found to bind and stabilize the inactive conformation of calcium channel pump proteins thereby acting as calcium channel blockers useful for the treatment of cardiovascular diseases (Janis et al. 1987). More than 20 years have passed after the introduction of the prescription drug nifedipine (**1**, aka: Adalat, Procardia, Afeditab, Nifediac, etc. Fig. 1) and many 1,4-DHP analogs have been synthesized since then. Several second-generation commercial products have also appeared on the market (e.g., **2, 3**, Fig. 1, Bossert and Vater 1989).



1; nifedipine; R=Me,2-NO 2
2; nitrendipine; R=Et,3-NO 2

3; nicadipine

Fig. 1 4-Aryl-1,4-dihydropyridines

In the past, 1,4-DHPs were subjected to Mannich reaction yielding 2-alkyl-1-(1′-dihydropyridinyl methyl) benzimidazoles having significant antibacterial activity (Mane et al. 1995). Since then, focus has also been given to the ability of 1,4-DHP derivatives to revert multi-drug resistance (Carosati et al. 2012).

The nature of the hydrophobic linker is not the main factor determining the antibacterial activity of cationic peptides. Therefore, it is tempting and intriguing to examine the possibility of using 1,4-DHP, an accessible scaffold with advantageous pharmacological properties, in the preparation of cationic peptidomimetics with a potentially broad bactericide spectrum. We concentrated our efforts on the implementation of the 1,4-DHP scaffold as a bridge between two cationic Lys residues at different distances, mimicking one or two turns of the α-helix. Since in some cases antibacterials can cause hemolytic anemia, we decided to test the minimal microbial inhibitory concentration (MIC) of the synthesized peptidomimetic compounds as well as the ability to induce lysis of human red blood cells (RBC), as a measure of toxicity.

The synthesized cationic peptidomimetcs include a central hydrophobic 1,4-DHP moiety flanked at both sides by units of the same dipeptide in a C_{term}-to-*N*-[1,4-DHP]-*N*-to-C_{term} fashion. The selected dipeptides are based on Lys-Leu-OMe and Pro-Lys-OMe, which are commercially available. In the Lys-Leu-OH derivative, the two cationic Lys residues can be directly connected to the 1,4-DHP scaffold. The resulting molecule can adopt a conformation that aligns the two cation bearing side chains separated by a distance of 5.5–6.0 Å, as estimated from a 3D minimized model using the MM2 program as implemented by Chem3D (ChemOffice Ultra 10.0, CambrigeSoft). A distance of about 11 Å can be achieved in a similar way, in the Pro-Lys-OH derivative (Fig. 2).

The main features of our design are the use of two cationic Lys residues, separated by a vertical distance which resembles one or two α-helix full turns, committed to the disruption of the bacterial membrane, and the hydrophobic 1,4-DHP core with its outstanding pharmacokinetic features bridging between the two cations.

The synthesis of the 1,4-DHP moiety is based on a general strategy which relies on the Hantzsch reaction

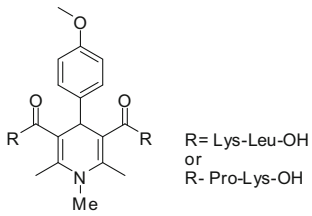


Fig. 2 Design of the 1,4-DHP based cationic peptidomimetics with potential antibacterial activity

(Fig. 3) (Hantzsch 1881). This multicomponent reaction facilitates the preparation of dihydropyridine derivatives in good yields by condensation of an aldehyde bearing the R^3 group and two β-ketoester equivalents in the presence of ammonia. The resulting dihydropyridine (**4**) can then be *N*-methylated. Hydrolysis of the esters at positions 3 and 5 yields a diacid (**6**) ready for coupling to the dipeptide of choice (Fig. 3).

N-hydroxybenzotriazole (HOBt) (Chan and Cox 2007) was used to simultaneously activate the two acid groups in molecule **6**, and to reduce the racemization of the dipeptides while building the two amide bonds. After coupling and deprotection, the terminal esters were hydrolyzed, affording the corresponding free acids **8** and **10** (Fig. 4).

In order to evaluate the toxicity of the synthesized 1,4-DHP peptidomimetic compounds toward mammalian cells, the ability to induce lysis in human erythrocytes (hRBC) was measured. Neither of compounds **8** and **10** displayed any significant in vitro hemolytic activity (observed lysis <3 %) at concentrations up to 1000 μg/mL.

The antibacterial activity of **8** and **10** was tested using the dilution method to determine their minimum inhibitory concentrations (MICs) (Andrews 2001) on *E. coli* as a representative of Gram-(−) bacteria, and on *S. aureus* as a representative of Gram-(+) bacteria.

The synthesis and evaluation of antibacterial activity of a series of benzodiazepine derivatives decorated with cationic Lys residues was accomplished in a parallel effort (Zats et al. 2015). Two benzodiazepine derivatives, compounds **11** and **12** (Table 1), were used as positive (substantial antibacterial activity) and negative (no substantial antibacterial activity) controls, respectively (Zats et al. 2015).

The synthesis of the benzodiazepine core was achieved in six steps. Full details of the synthesis and antibacterial activities of the benzodiazepine peptidomimetics were disclosed elsewhere (Zats et al. 2015). The preparation of these benzodiazepine peptidomimetic compounds can be divided in of two segments. The first segment deals with the six-step synthesis of the benzodiazepine scaffold bearing two functional groups amenable for the attachment of amino acids (Cepanec et al. 2006; Kazmierski 1999; Selnick et al. 1997). The second segment involves the solid phase peptide synthesis (SPPS) of the desired benzodiazepine containing peptides.

It was found that 1,4-DHP derivatives **8** and **10** have substantial activity against both Gram-(−) and Gram-(+) bacteria, and are therefore considered to be broad spectrum antibacterial agents. Compound **10** is significantly more active than compound **8**, suggesting that the separation between the two cationic residues mimicking two full α-helix turns is preferable (Table 1). This distance is reminiscent of that present in compound **11**. It is currently believed that the antibacterial activity displayed by **8**, **10** and **11** is caused by the presence of the two cationic groups following the known mechanism of membrane disruption (Glukhov et al. 2008).

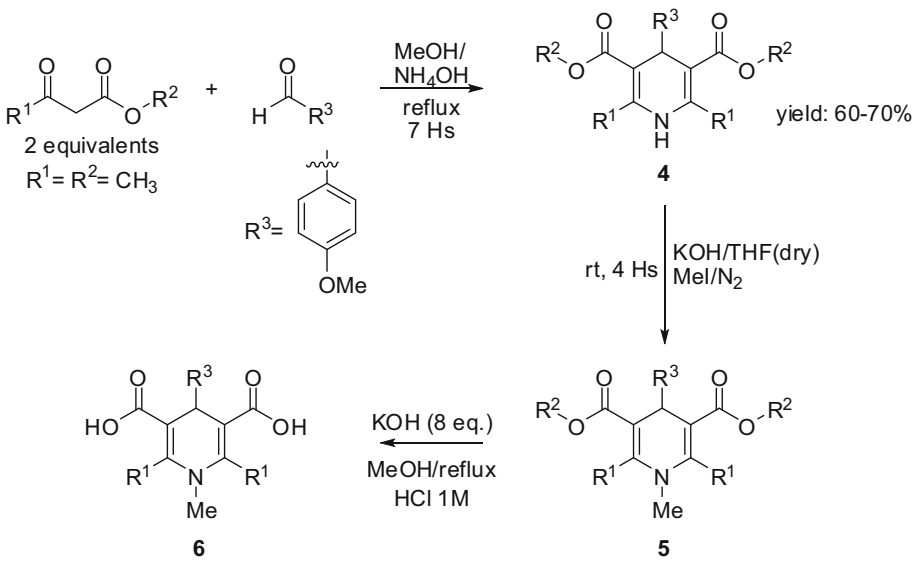


Fig. 3 Synthesis of the 1,4-DHP hydrophobic core

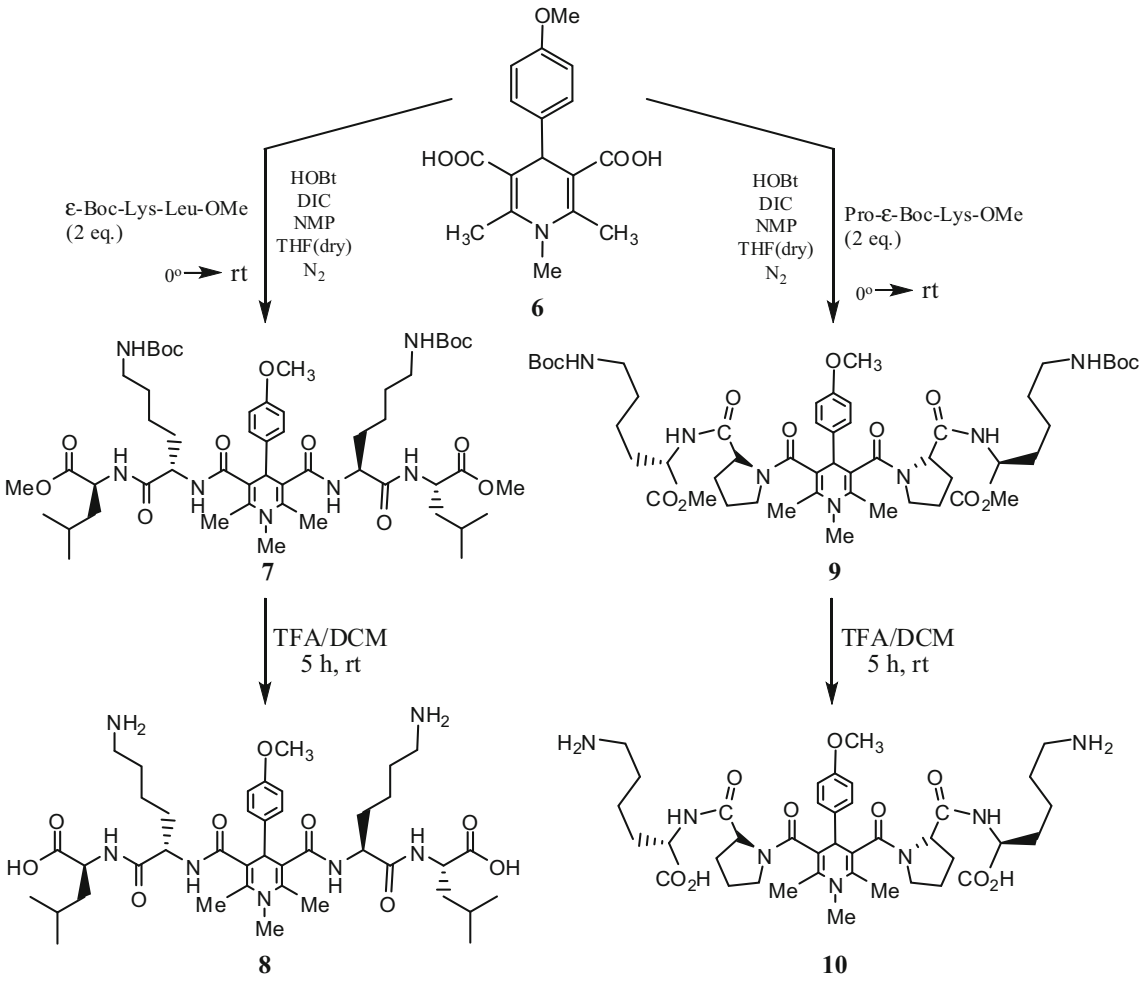
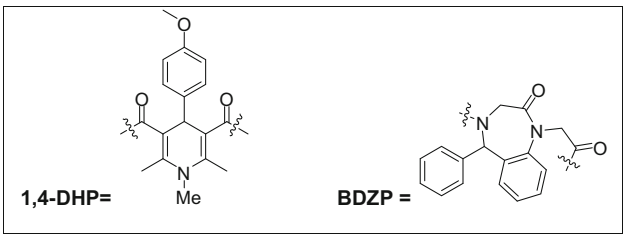


Fig. 4 Synthesis of peptidomimetic 1,4-DHP derivatives **8** and **10**

Table 1 Antimicrobial activity against Gram-negative and Gram-positive bacteria

Compound	Peptidomimetic sequence	MIC (μg/mL)	
		E. coli	*S. aureus*
8	Leu-Lys-**1,4-DHP**-Lys-Leu	95	100
10	Lys -Pro-**1,4-DHP**-Pro- Lys	36	45
11	Lys-**BDZP**-Cys-Lys-NH_2	25	12.5
12	H-**BDZP**-Ile-Lys-NH_2	>200	>200



1,4-DHP 1,4-dihydropyridine scaffold, *BDZP* benzodiazepine scaffold

In summary, new broad spectrum antibacterial cationic peptidomimetics based on a 1,4-DHP moiety were prepared and tested. Further optimization of such 1,4-DHP based cationic peptidomimetics will probably lead to more effective antibacterial drug candidates.

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