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Design of antimicrobial compounds based on peptide structures

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Abstract—New antimicrobial compounds are of major importance because of the growing problem of bacterial resistance and antimicrobial peptides have been gaining a lot of interest. Their mechanism of action, however, is often obscure. Here a set of non-peptidic compounds with antimicrobial activity are presented that have been designed based on criteria derived from three-dimensional structures of antimicrobial peptides. Even though only a small set of compounds has been designed, the activity immediately matches that of the original peptides, supporting the proposed criteria for activity, i.e. not the peptidic nature of antimicrobial peptides is responsible for their activity but rather the proper arrangement of the relevant functional groups.

Bacterial resistance to existing drugs is a constantly growing problem that, combined with a decline in the development of new antibiotics, presents a significant threat to human health.[1–3] The identification of new antimicrobial agents is therefore of considerable importance. In recent years, antibacterial and antifungal peptides have gained a lot of interest, due to their potential use as a new generation of therapeutic agents.[4,5] These peptides exhibit activity against a broad spectrum of microbes, albeit with fairly low activity. However, resistance against them has rarely been reported, even though they are evolutionary ancient weapons of higher animals.[4] Unfortunately, their mechanism of action is still not clear and its elucidation would form a sound basis for the further development of pharmaceutical compounds.

In two recent papers we have determined the structure of the antimicrobial peptide *cyclo*-(Arg-Arg-Trp-Trp-Arg-Phe) and several analogues using solution NMR spectroscopy and have described their potential interactions with a biological membrane using extensive molecular dynamic simulations.[6–8] In a membrane mimicking environment, the peptide exhibits an amphipathic structure that differs considerably from the one found in aqueous solution. The hydrophobic part is formed by the aromatic side chains while the hydrophilic part is made up of the peptide backbone. The aromatic side chains protrude into the lipid chains of the membrane, the guanidine groups form contacts to charged lipid head groups, and the backbone faces the outside of the membrane. When tryptophan is replaced by tyrosine or arginine is replaced by lysine the structure of the peptide does not change, the backbone forms similar β-turns positioning the amino acid side chains in similar directions. The activity, however, is changed considerably.[9] On the other hand, scrambling of the original sequence in a way that the three aromatic side chains are next to each other (cyclo(RRWWFR) and cyclo(RRWFWR)) changes the backbone structure but does not affect the activity of the peptides [to be published]. From those findings we conclude that the peptide backbone merely presents the scaffold for the orientation of the side chains of the amino acids and that the antimicrobial activity requires a sufficient number of indole rings and guanidinium groups. It should then be possible to design an antimicrobial compound with similar activity as the original peptide by using a simpler scaffold that is capable of positioning the amino acids in a proper manner.

To test this hypothesis, we have chosen to synthesize a small set of compounds based on trimesic acid as the scaffold with indole rings and guanidinium groups attached (Scheme 1). The structural rationale behind the design of the compounds is shown in Figure 1 where the structure of the peptide bound to detergent micelles as determined by NMR is overlaid with a simple model of the new compound. As can be seen the trimesic acid is capable of positioning the relevant functional groups in a way comparable to that in the peptide. To assess the

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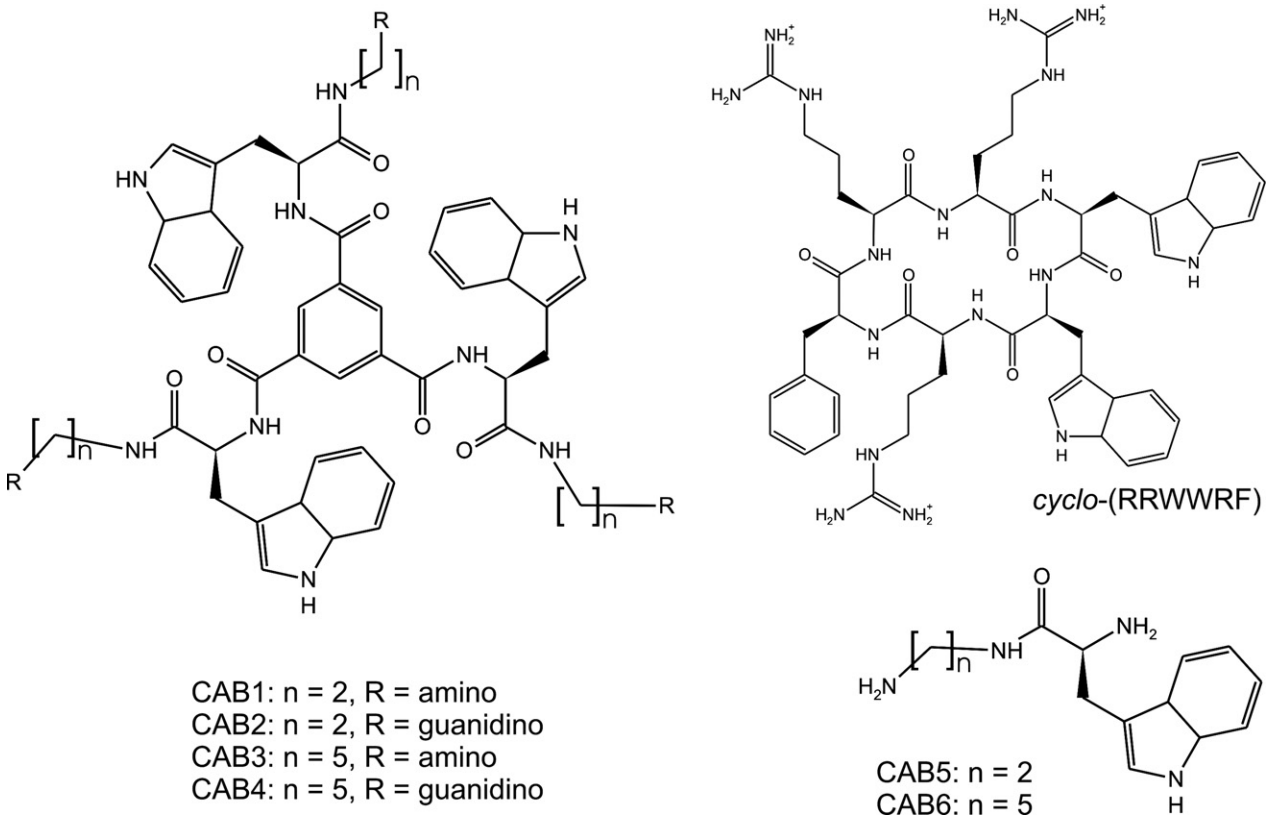


Scheme 1. Structure of the non-peptidic analogs of antimicrobial peptides together with the structure of cyclo(RRWWRF) and the 'side chain' compounds CAB5 and CAB6.

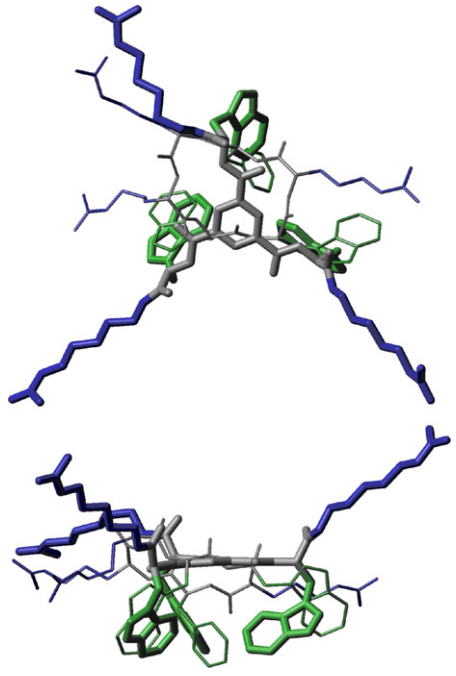
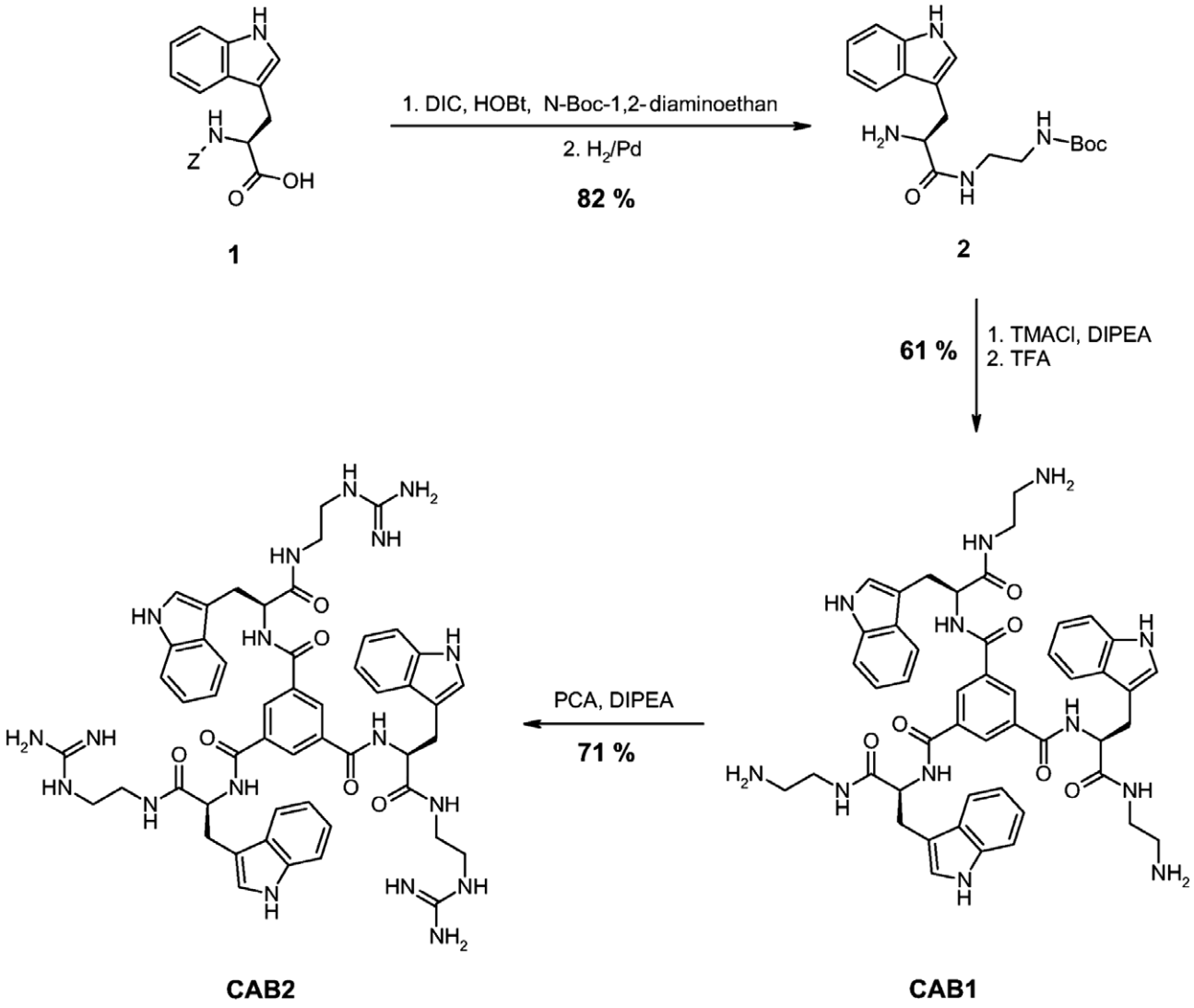


Figure 1. Comparison of a model of CAB4 (thick) and the NMR structure of cyclo(RRWWRF) (thin)[6]. The model was created by orienting the 'side chains' in the same way as in the peptide followed by an energy minimization. This shows that the indoles (green) can adopt a similar orientation as the aromatic side chains of the peptide. Likewise, the guanidine moieties can be oriented in the same way as the arginine side chains. A structure determination of CAB4 bound to the micelle has so far been precluded by the symmetry of the compound.

importance of the guanidinium groups, we synthesized and investigated analoges carrying amino instead of guanidinium groups. To allow for variation in the positioning of the aromatic rings and the charged groups relative to each other, two different chain lengths were used for the attachment of the charged groups. The synthesis of the four compounds is exemplified in Scheme 2 by the synthesis of CAB1 and CAB2 containing ethane linkers. CAB3 and CAB4 contained pentane chains and were synthesized in a similar way.

The biological activity of the new compounds was tested on two bacterial strains, *Escherichia coli* and *Bacillus subtilis*, as representatives of gramnegative and grampositive bacteria, respectively. CAB4 possesses three guanidinium moieties attached to pentane linkers. It turned out to be the most potent compound, having minimal inhibitory concentrations in the low micromolar range against both *E. coli* and *B. subtilis* (Table 1). This activity is similar to the one of the *cyclo*-(Arg-Arg-Trp-Trp-Arg-Phe) peptide. The compound CAB3 is the corresponding triamine. It is less effective in inhibiting bacterial growth and is therefore comparable to the peptide *cyclo*-(Lys-Lys-Trp-Trp-Lys-Phe).

In order to check whether fragments of these components display any antimicrobial activity, CAB5, CAB6, and trimesic acid were also tested (Table 1). They were completely inactive demonstrating that the linkage of three tryptophans with guanidines or amines is essential for the activity.



Scheme 2. Synthesis of CAB1 and CAB2. TMACl, trimesic acid chloride. PCA, pyrazole carboxamidine.[11]

Table 1. Antimicrobial activities and erythrocyte lysis of the developed compounds

	R	*n*	MIC *E. coli* (μM)	MIC *B. subtilis* (μM)	Erythrocyte lysis at 100 μM (%)
CAB1	Amino	2	>125	31.3	1.2
CAB2	Guanidino	2	>125	3.9	5.1
CAB3	Amino	5	62.5	31.3	0.4
CAB4	Guanidino	5	15.6	2.0	22.6
cyclo(RRWWRF)[a]	–	–	6.3	3.1	24
cyclo(KKWWKF)[a]	–	–	25	25	10
CAB5	–	–	>250	>250	<1
CAB6	–	–	>250	>250	<1
Trimesic acid	–	–	>250	>250	<1

[a] Reproduced with permission from.[8] CAB5, (*S*)-2-amino-*N*-(2-aminoethyl)-3-(1*H*-indol-3-yl)propanamide; CAB6, (*S*)-2-amino-*N*-(5-aminopentyl)-3-(1*H*-indol-3-yl)-propanamide.

A different behavior was seen for CAB1 and CAB2 which have their amino and guanindino moieties coupled to an ethyl linker. Like CAB3 and CAB4 they were active against *B. subtilis*. However, they were inactive against *E. coli*. This indicates that the distance between the charged groups and the aromatic rings has an impact on the activity but given the small set of compounds tested it is not possible to explain the observed selectivity. Expanding the set of compounds to overcome these limitations is in progress. Additionally, the symmetry of the compounds has so far precluded the determination of their micelle-bound structures. It was, however, possible to obtain information on the orientation of the peptides using methods described previously.[6] The compounds orient parallel to the micelle surface with the aromatic rings pointing into the micelle and the

guanidinium groups on the surface. Together with the size of the compounds this is a clear indication that no channel formation is taking place and that the compounds rather act according to the carpet model.[10]

In conclusion, we have been able to design non-peptidic compounds with antimicrobial activity based on principles derived from the structure of antimicrobial peptides determined using solution NMR-spectroscopy. Even though the design was simple, the activities of the new compounds immediately match those of the original peptides.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.01.075.

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