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Application of the Suzuki–Miyaura cross-coupling to increase antimicrobial potency generates promising novel antibacterials

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Abstract—Antimicrobial peptides have been recognized as a novel class of antibiotics, and several candidates are currently in clinical trials. In this work, a tripeptide derivative containing 4-iodo phenylalanine has been derivatized through the Suzuki–Miyaura cross-coupling. This has enabled the rapid and efficient synthesis of an array of tripeptide derivatives encompassing novel biaryl moieties. The peptide derivatives show high activity against Gram-positive bacteria.

A prolonged and uncritical use of antibiotics throughout the western world has led to widespread bacterial resistance to most commercially available antibiotics. This disturbing development, combined with the fact that only a few new classes of antibacterial agents have been introduced into the clinic during the last decades, has created an urgent need for the development of novel antibiotics. In this respect, cationic antimicrobial peptides are a promising class of future antibacterial agents.[1–4]

Cationic antimicrobial peptides (CAPs) are less likely to induce resistance in bacteria, as has been shown by several research groups.[5–8] This reduced incidence of resistance development most probably stems from the unique mechanism of action of antimicrobial peptides. Although no exact mode of action has been established, and considerable debate is still going on, most antimicrobial peptides are thought to act on or at least to involve an action on bacterial membranes.[3,9,10] Several models have been put forward to explain the interactions between CAPs and bacterial membranes[11] but no consensus has been reached to date. A common mechanism of action of all CAPs discovered is also highly unlikely as a wide range of biological activities have been found for different classes of CAPs.

There are several challenges regarding the development of CAPs into future drugs. Our group has for a number of years focused on developing extremely small CAPs in order to overcome these obstacles. In our research, we have identified a pharmacophore of short CAPs,[12] exemplified by the dipeptide ester derivative H_2N-Arg-Trp-OBn shown in Figure 1 and we are currently undertaking further studies to produce clinical candidates.

Toward this end, we have found, as earlier demonstrated for longer CAPs,[13–15] that introduction of bulky non-natural amino acids can dramatically increase the antimicrobial activity of dipeptide derivatives and lead to highly active derivatives of the general formula Xxx-Arg-Y (see Fig. 2).[16]

In addition, some Arg-Tbt-Arg-Y tripeptide (see Fig. 2 for Tbt structure) derivatives were found to be highly active. Arg-Tbt-Arg-NHBn in particular displayed a

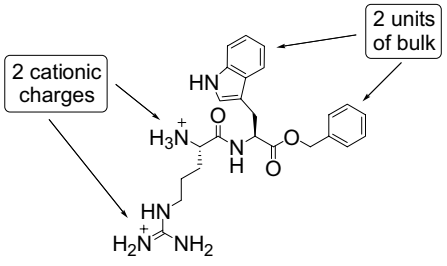

Figure 1. Pharmacophore for short CAPs containing natural residues.

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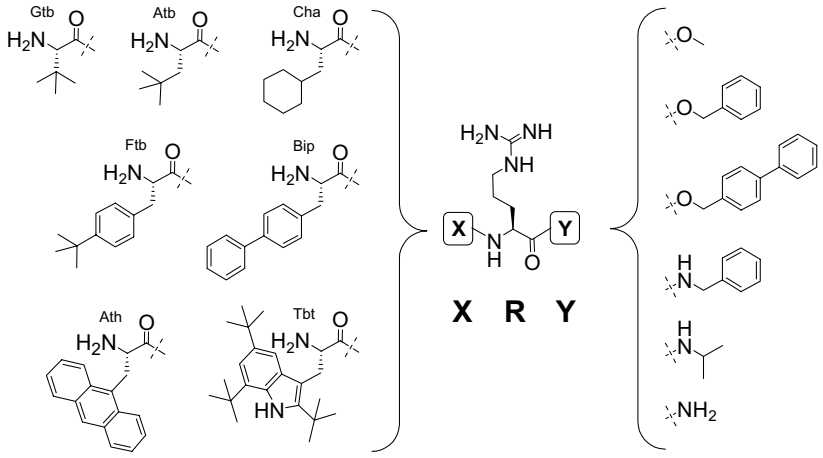


Figure 2. Antibacterial dipeptides with the general formula X-Arg-Y.

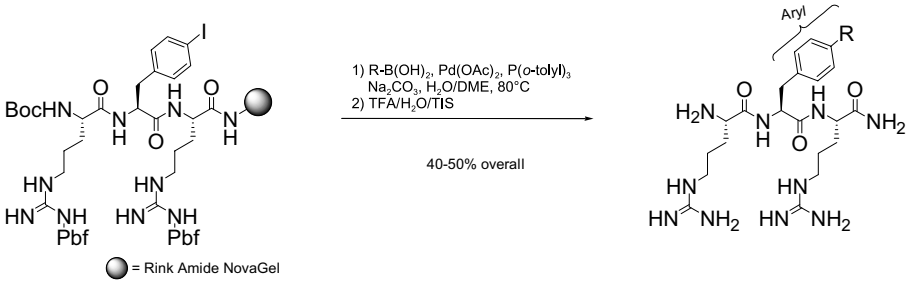
broad range of antimicrobial activity against *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus* as well as *Methicillin-resistant S. aureus* (MRSA) and *Methicillin-resistant S. epidermidis* (MRSE) and Glycopeptide intermediate-resistant *S. aureus* (GISA).[17]

In the current study, we have prepared a tripeptide derivative containing the unnatural amino acid 4-iodo phenylalanine with the intention of preparing a library of novel tripeptides through Suzuki–Miyaura[18] cross-coupling reactions between the 4-iodo phenylalanine tripeptide scaffold and a variety of boronic acids.

The Suzuki–Miyaura cross-coupling reactions are one of the most important methods for C–C bond formation in organic synthesis (for recent reviews, see [19,20]), the reaction tolerates a wide variety of functional groups, and a number of different solvents and catalysts can be used to effectuate the coupling reaction. We decided to adopt a strategy in which a library of antimicrobial compounds could be made from a common resin-bound tripeptide scaffold. For this purpose Boc-Arg(Pbf)-Phe(4-I)-Arg(Pbf)-Rink Amide[21] (see Scheme 1) was prepared on a NovaGel resin. We chose to use Arg as the cationic moiety as this residue has previously been demonstrated to improve antibacterial activity compared to peptides containing Lys.[22] However, it should be noted that Arg may confer higher toxicity toward human cells than Lys.[23] The use of a Rink Amide linker ensured *C*-terminal amides, thereby giving the peptides a total charge of +3. In contrast to earlier studies, no further modifications to the *C*-terminal were made. This was done in order to exclude any additional contribution to the antimicrobial effect from the *C*-terminal group. Hence, the data set is designed to show only the effect of the different aryl moieties (Scheme 1 and Fig. 3) on the biological activity of the different Arg-X-Arg-NH_2 cross-coupling products.

The scaffold peptide was prepared using Fmoc-based peptide chemistry on a Rink Amide NovaGel resin using a manual peptide synthesis vessel (Scheme 1) under inert atmosphere. HBTU, HOBt, and DIPEA mediated couplings were performed in DMF and removal of the *N*-terminal Fmoc-protecting group was performed using 20% piperidine in DMF.[24] The peptide synthesis proceeded uneventfully producing the fully protected resin-bound tripeptide in 98% yield (by weight) after extensive drying.

Treatment of a small aliquot of the peptide-resin with cleavage cocktail afforded after purification H-Arg-Phe(4-I)-Arg-NH_2, peptide **1**. Several catalysts were tested for the Suzuki–Miyaura cross-coupling reaction on the resin bound aryl iodide.[25] A combination of 0.2 equiv $Pd(OAc)_2$ and 0.4 equiv P(*o*-tolyl)$_3$ with an excess of Na_2CO_3 in a water/DME mixture ensured complete conversion of the starting material at 80 °C.[26]



Scheme 1. Suzuki–Miyaura coupling on tripeptide scaffold.

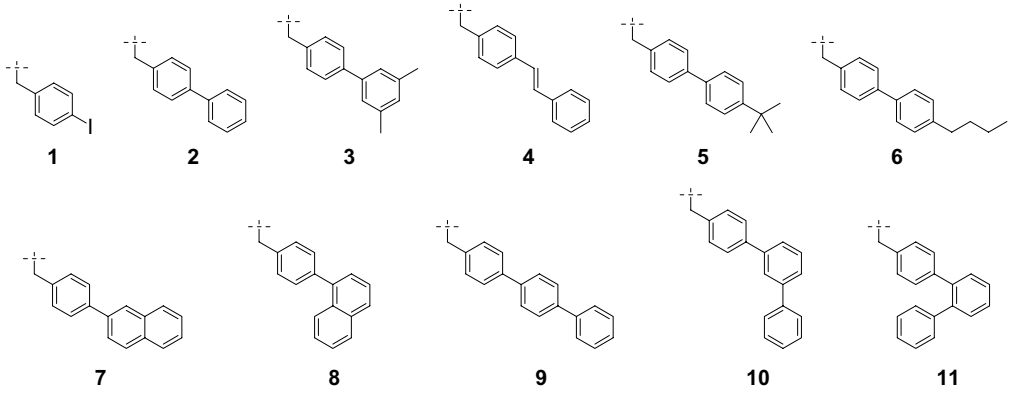


Figure 3. Structure of the aryl side chain of X in Arg-X-Arg-NH_2.

The catalyst, excess of base, and boronic acid were removed from the resin in several consecutive washing steps, after which the resin was treated with a cleavage cocktail (Scheme 1) to afford the crude cross-coupling product as an oil after evaporation. The crude product was dissolved in a water–acetonitrile mixture and any insoluble material was removed by filtration. RP-HPLC purification afforded pure tripeptide derivatives **3**–**11** in 40–50% overall yield after lyophilization. H-Arg-Bip-Arg-NH_2, peptide **2**, was also prepared as a control using standard solid-phase methods.[24]

All peptides were tested for their ability to inhibit growth of *Streptococcus pyogenes*, *S. aureus*, *MRSA*, and *MRSE*. The results are expressed as the minimal inhibitory concentration (MIC) and are summarized in Table 1.

The scaffold tripeptide, peptide **1**, did not show any antibacterial activity up to 160 μM (100 μg/mL) against *Strept. pyogenes*, *S. aureus* or MRSA and a minimal inhibitory concentration (MIC) of 116 μM against MRSE. H-Arg-Bip-Arg-NH_2, peptide **2**, was also found to be inactive up to 180 μM (100 μg/mL), except for MRSE where a MIC of 59 μM was found. The lack of antimicrobial activity of peptides **1** and **2** is not surprising, as they do not fulfill the criteria for the pharmacophore of short CAPs.[12]

Table 1. Antibacterial activity (MIC in μM)

Compound	*Streptococcus pyogenes*	*Stapyhlococcus aureus*	*MRSA*	*MRSE*
1	na[a]	na	na	na
2	na	na	na	na
3	na[b]	na	na	9.0
4	na	na	na	14
5	25	11	9.7	4.1
6	16	9.0	7.0	4.1
7	na	25	25	5.0
8	na	na	29	8.3
9	20	9.1	8.3	4.8
10	na	16	11	4.8
11	na	28	24	6.4

[a] Peptides **1** and **2** were tested at concentrations up to 100 μg/ml (na, no activity within concentration range tested).
[b] Peptides **3**–**11** were tested at concentrations of 20 μg/ml or lower.

All the derivatives **3**–**11** which were prepared by the Suzuki–Miyaura cross-coupling reaction showed a significant increase in antimicrobial activity compared to the control peptides **1** and **2**. Peptides **3** through **11** were only tested at peptide concentrations below 20 μg/mL. In general, *Strept. pyogenes* was found to be the least susceptible bacterium in the test panel, while all peptides were most active against MRSE. In previous studies MRSE has also been found to be particularly susceptible to short antimicrobial peptides.[12] MRSA was also found to be more susceptible to the tripeptide derivatives than the non-resistant *S. aureus*.

Interestingly, for peptides with isomeric side chains higher antimicrobial activity was always observed for those peptides with a biaryl moiety with a more elongated shape. This is evident for *t*-butyl and *n*-butyl derivatives **5** and **6** where the presence of the more elongated *n*-alkyl chain leads to higher activity. A similar structure–activity relationship was observed for the 1-naphthyl and 2-naphthyl derivatives **7** and **8** and also for the terphenyl derivatives **9**–**11**. A corresponding shape dependence of the antimicrobial activity has previously been found for 15-residue bovine lactoferricin derivatives. Peptides containing 2-naphthylalanine were more active than those containing 1-naphthylalanine.[15]

The antibacterial effect of peptides **3**–**11** is governed by the presence of a certain combination of cationic and bulky structural motifs, which is in agreement with the pharmacophore.[12] In the present study, only the side chain of the bulky and lipophilic amino acid differs among **3**–**11**, and thus hydrophobicity of the peptides becomes the most significant property responsible for the observed variation in antibacterial activity. Hence, the overall hydrophobicity was evaluated by measuring the retention time (t_R) of each individual peptide using RP-HPLC.[24,27] A clear correlation between retention on the C_{18} column (i.e., affinity for the hydrophobic column surface) and antibacterial activity was found (Fig. 4) for MRSA. No antibacterial activity within the concentration range tested was found

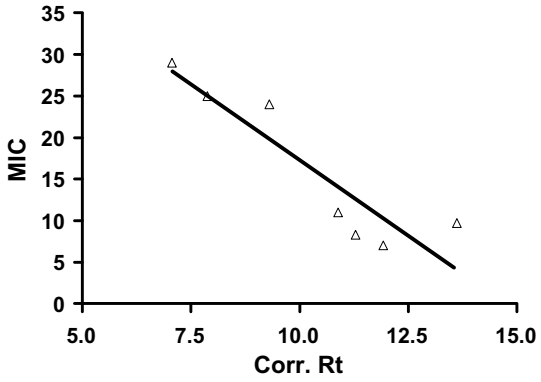


Figure 4. Correlation between corrected t_R and antibacterial activity.

for peptides eluting faster than about 7 min (corrected for column void volume).[24] Peptide **6**, the most hydrophobic in the series, was also found to be the most active derivative overall, however only marginally more active than peptides **5** and **9**. Peptides **6**, **7** and **9** were all found to elute at higher acetonitrile concentrations, that is, display an overall higher hydrophobicity, than their isomeric counterparts (**5**, **8** and **10** and **11**, respectively). Thus, peptides containing a more elongated side chain were found to be more hydrophobic which in turn correlated with a higher antibacterial activity. Recently, the introduction of fluorine atoms or trifluoromethyl groups into antimicrobial peptides has resulted in increased hydrophobicity and thus higher antibiotic potency of short CAPs.[28]

The peptides were also tested for hemolysis of human erythrocytes.[24] Most of the peptides were found to be non-hemolytic within the concentration range tested (up to 500 μg/mL). Peptide **6** showed a 40% hemolysis at 500 μg/mL, while peptide **9** was the most hemolytic in the series with an EC_{50} value of 420 μg/mL against human erythrocytes. The low hemolytic property in combination with the high antimicrobial activity of the peptides adds to the probability of developing such peptide derivatives into clinical candidates for the future development of a drug effective against multidrug resistant pathogens.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.12.049.

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