Ph.D. Theses

# Synthesis and Antimicrobial Activity Investigation of Peptides Derived from NCR169 and NCR147

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**2023**

## 1. Introduction

A multifaceted problem triggered by the development of antimicrobial resistance (AMR) has become a significant danger to global health, and WHO listed the ESKAPE (*Enterococcus faecalis, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Escherichia coli*) group as the top priority pathogens bacteria. The excessive use of traditional antibiotics was the main factor behind the emergence of AMR. As a result, scientists are now focusing on exploring alternative approaches to fighting antimicrobial resistance. Taking inspiration from nature, antimicrobial peptides (AMPs) are attracting increasing interest due to their unique advantages over conventional antibiotics, making them promising agents for clinical applications. AMPs possess certain distinctive structural features that differentiate them from conventional antibiotics. Typically, these peptides consist of fewer than 100 amino acids and tend to have a higher proportion of positively charged and hydrophobic residues. Apart from their structural dissimilarities, AMPs frequently directly interact with the bacterial cell membrane instead of targeting intracellular processes. One benefit of AMPs is their ability to target different biological sites compared to traditional antibiotics. Furthermore, a notable characteristic of numerous AMPs is their diverse range of mechanisms that collectively contribute to their overall antimicrobial effectiveness. Since many AMPs interact with lipid components in the bacterial cell membrane, they often exhibit wide-ranging antimicrobial activity.

AMPs can be classified based on their sources, structural features, the abundance of certain amino acids, like glycine, cysteine, and leucine-rich peptides, and biological activities. In nature, AMPs can be found ubiquitously in living organisms. As for their structures, AMPs are divided into 4 classes: extended peptide chain forming α-helices; peptide chain in β-sheet structure; extended linear peptide chain; and hybrid peptide chain in a loop structure formed by a single internal disulfide bond. Additionally, based on their biological activities, AMPs can be categorized into 4 broad groups: antibacterial, antifungal, anticancer, and antiviral AMPs.

One subset of AMPs is known as nodule-cysteine-rich peptides (NCR). NCR peptides are produced through the symbiotic relationship between nitrogen-fixing legumes and rhizobia within nodule cells, leading to rhizobia differentiation. Researchers have identified approximately 700 NCR peptides from a well-studied legume model called *Medicago truncatula*, which forms symbiotic associations with *Sinorhizobium meliloti*. Some studies have indicated that certain members of the NCR group, such as NCR247 and NCR335, exhibit antimicrobial properties like defensins, primarily due to the presence of conserved cysteine residues that could form disulfide bridges.

## 2. Objectives

Two main areas are covered and presented in this thesis: the synthesis of peptides and the antimicrobial activity evaluation of the synthesized peptides. The main objective of this study was to synthesize AMPs derived from NCR169 and NCR147 with enhanced antimicrobial activity. Determining the active part from

each parent peptide's sequence is important for developing the antimicrobial profile of each peptide. Another key factor addressed in this study is the role of certain amino acids in the antimicrobial profiles of peptides.

In the first part of this research, a full sequence of NCR169 was synthesized along with its derivatives, including the smaller fragments, followed by a biology assay for antimicrobial activity. We aimed to determine the active core fragment of NCR169 against pathogen microorganisms. We then utilized our findings from the first step. We synthesized several derivatives of the active NCR169's fragment, with some modifications to the initial sequence, to investigate the role of certain amino acids in the stability of the peptide and how they could affect the activity. The best sequence would then be the parent compound in the next phase. Following the result of our previous stage, we synthesized analogs with the modification of amino acid at a specific position with a modified amino acid that contains D- and L- forms and an unnatural side chain. This approach investigated the effect of different isoforms and side chain types on antimicrobial activity.

In the second part of this project, we synthesized the full sequence of NCR147 and its derivatives, including its shorter fragments, followed by a biology assay for antimicrobial activity. We aimed to determine the active core fragment of NCR147 against some pathogen bacteria. The active fragment of this step was then modified utilizing fluorinated tryptophan to enhance the antimicrobial activity.

## 3. Experimental Methods

Solid-phase peptide synthesis (SPPS) protocol was applied to generate the desired peptides. This work was conducted in the peptide laboratory Department of Medical Chemistry, University of Szeged. All amino acids utilized in the synthesis process were protected with the fluorenylmethyloxycarbonyl (Fmoc) protecting group. The resin used for this work was TentaGel S RAM, with diisopropylcarbodiimide (DIC) as an activator and Oxyma as a base activator. The cleaving cocktail to detach the ultimate peptide from the resin at the last phase of the synthesis was a mixture of trifluoroacetic acid (TFA)/water (95:5), plus dithiothreitol (DTT) 3% (*w/v*) and triisopropylsilane (TIS) 3% (*w/v*). For analysis and purification purposes, we conducted it through analytical high-performance liquid chromatography (HPLC) and electrospray ionization mass spectroscopy (ESI-MS).

The antimicrobial activity was evaluated at the Biological Research Center, Szeged, Hungary. We conducted a minimum bactericidal concentration (MBC) assay using potassium-phosphate buffer (PBB) to determine the antibacterial activity of the synthesized peptides. The bacterial strain used for this assay were *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 8739, ATCC 35218, and ATCC 25922), *Salmonella enterica* (ATCC 13076), *Klebsiella pneumoniae* (NCTC 13440), *Acinetobacter baumannii* (ATCC 17978) and the Gram-positive strains were *Enterococcus faecalis* (ATCC 29212), *Listeria monocytogenes* (ATCC 19111), and *Staphylococcus aureus* (HNCMO112011 and ATCC 25923). In the last part of this work, we utilized 8 Gram-negative bacteria

for antibacterial assay. Those bacteria are *Pseudomonas syringae* pv. tomato, *Pseudomonas syringae* pv. tabaci, *Pseudomonas gladioli*, *Xanthomonas campestris*, *Xanthomonas malvaceae*, *Erwinia chrysanthemi*, *Erwinia carotovora*, and *Agrobacterium tumefaciens*. In addition, we carried out a hemolysis assay for some active NCR169 derivatives against human red blood cells.

We performed an antifungal assay to explore the antifungal activity of some active NCR169 peptides. The parameter used for this assay was the minimal inhibitory concentration (MIC) against several fungi strains. Those strains were *Candida albicans* (ATCC 10231, SC 5314, and SZMC 1458), *Candida auris* (0381), *Candida glabrata* (CBS 138), *Candida parapsilosis* (CBS 604), and *Candida tropicalis* (CBS 94). Furthermore, we investigated the biofilm formation of *C. albicans* and *C. tropicalis* strains, following the XTT reduction assay protocol, and we analyzed their morphological features using bright-field microscopy and scanning electron microscopy. We have also assessed the viability of human keratinocytes (HaCaT) towards our active NCR169 derivatives.

## 4. Prospective Novel Findings

**1. We established that the C-terminal of the NCR169 peptide was the core region responsible for the NCR169 antimicrobial activity.**

1.1. The Alphafold structural prediction of NCR169 showed that this peptide contained different properties in the elongated N-terminal, and the C-terminal preserved a short antiparallel β-sheet (Figure 1). This result was aligned with the publication from the Isozumi group. However, the structural prediction of NCR169C$_{17-38}$ displayed the absence of the β-sheet and was replaced by an alpha-helical structure (Figure 1).**[1]**

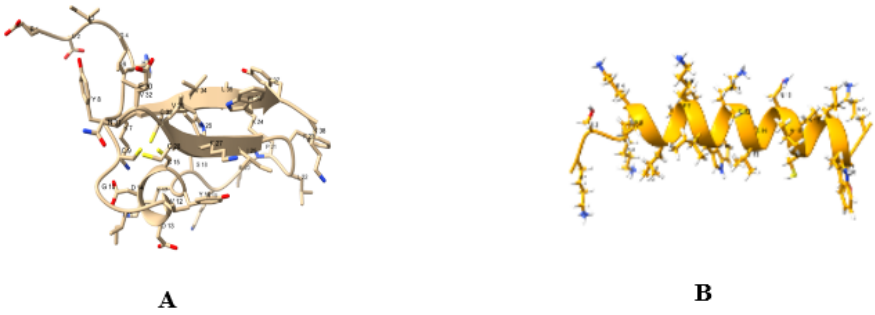


**Figure 1**. Structural Prediction of (**A**) NCR169 and (**B**) NCR169C$_{17-38}$

1.2 Among a whole sequence of NCR169, 6 shorter fragments, an oxidized form, and an elongated form with a StrepII short fragment consisting of 8 amino acids (WSHPQFEK) attached to the C-terminal peptides that we have synthesized, the NCR169C$_{17-38}$ was able to terminate most of the tested bacteria with MBC of 3.1 μM (Table 1). This peptide was more potent than the NCR169 full sequence and the other derivatives. Furthermore, the antibacterial profile of NCR169C$_{17-38}$ was better than carbenicillin and levofloxacin as controls. **[1]**

**Table 1.** Minimal bactericidal concentrations (MBC; in μM) of the studied peptides on different pathogens after 3 h of treatment in PPB. Gram-negative *E. c., Escherichia coli* (ATCC 8739)*; S. e., Salmonella enterica* (ATCC 13076)*; K. p., Klebsiella pneumoniae* (NCTC 13440)*; A. b., Acinetobacter baumannii* (ATCC 17978)*; P. a., Pseudomonas aeruginosa* (ATCC 27853). Gram-Positive: *E. f., Enterococcus faecalis* (ATCC 29212)*; L. m., Listeria monocytogenes* (ATCC 19111)*; S. a., Staphylococcus aureus* (HNCMO112011).

| **Peptides** | **Gram-Negative** | | | | | **Gram-Positive** | | |
|---|---|---|---|---|---|---|---|---|
| | ***E. c.*** | ***S. e.*** | ***K. p.*** | ***A. b.*** | ***P. a.*** | ***E. f.*** | ***L. m.*** | ***S. a.*** |
| NCR169 | 3.1* | 3.1* | 12.5* | 3.1 | 12.5* | - | 12.5* | 6.3* |
| $NCR169C_{17-38}$ | 1.6 | 3.1 | 3.1 | 3.1 | 3.1 | 6.3 | 3.1 | 3.1 |
| Carbenicillin | 1280 | 640 | >10240 | 5120 | 10240 | 5120 | 80 | 640 |
| Levofloxacin | 5.0 | 1.3 | 320 | 20 | 1.3 | 160 | 320 | 2.5 |

**The best MBC value was measured, but there were more than two dilution step differences in replicate experiments.*

## 2. We determined that the presence of tryptophans in the $NCR169C_{17-38}$ sequence was essential for the peptide's antimicrobial profile, and the double substitution of cysteines with serines could boost the antimicrobial activity.

2.1. We proved, through the structural prediction by the AlphaFold program, that the modifications on the $NCR169C_{17-38}$ sequence, specifically on the cysteine and tryptophan residues with serine and alanine, did not alter the secondary structure of the native peptide significantly (Figure 2). **[1]**

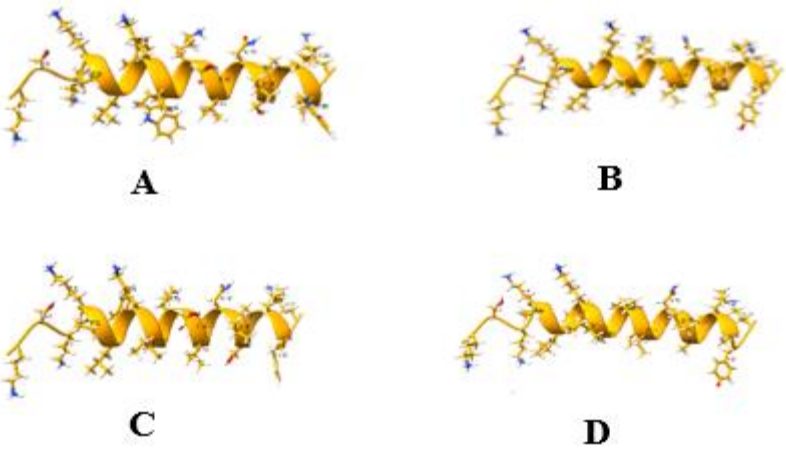


**Figure 2**. Structural Predictions of (**A**). NCR169C$_{17-38}$ C$_{12,17}$/S; **(B)**. NCR169C$_{17-38}$ W$_{10,20}$/A; (**C**). NCR169C$_{17-38}$ W$_{10,20}$/A, C$_{12,17}$/S; **(D)**. NCR169C$_{17-38}$ W$_{10,20}$C$_{12,17}$/A

2.2. The antibacterial in vitro studies of our NCR169C$_{17-38}$ derivatives proved that preserving the tryptophans at their original positions and substituting two cysteines with serines (NCR169C$_{17-38}$C$_{12,17}$/S) would improve the MBC value of the peptide (Table 2). The comparison of the structural predictions of these peptides with their antibacterial activity established that the backbone of the peptide's sequence was not the key factor to its biological activity. Still, certain amino acids' side chains might play a vital role in the activity **[1].**

**hTable 2**. Minimal bactericidal concentrations (MBC, in μM) of peptides against Gram-negative: *E. c., Escherichia coli* (ATCC 8739)*; S. e., Salmonella enterica* (ATCC 13076)*; K. p., Klebsiella pneumoniae* (NCTC 13440)*; A. b., Acinetobacter baumannii* (ATCC 17978)*; P. a., Pseudomonas aeruginosa* (ATCC 27853)*, and* Gram-Positive: *E. f., Enterococcus faecalis* (ATCC 29212)*; L. m., Listeria monocytogenes* (ATCC 19111)*; S. a., Staphylococcus aureus* (HNCMO112011).

| **Peptides** | **Gram-Negative** | | | | | **Gram-Positive** | | |
|---|---|---|---|---|---|---|---|---|
| | ***E. c.*** | ***S. e.*** | ***K. p.*** | ***A. b.*** | ***P. a.*** | ***E. f.*** | ***L. m.*** | ***S. a.*** |
| NCR169$C_{17–38}$ | 1.6 | 3.1 | 3.1 | 3.1 | 3.1 | 6.3 | 3.1 | 3.1 |
| NCR169$C_{17–38}C_{12,17}$/S | 1.6 | 1.6 | 3.1 | 1.6 | 3.1 | 3.1 | 3.1 | 1.6 |
| NCR169$C_{17–38}W_{10,20}$/A | 3.1 | - | 12.5 | 3.1 | 3.1 | - | 25 | - |
| NCR169$C_{17–38}W_{10,20}$/A, $C_{12,17}$/S | 6.3 | 6.3 | 25 | 12.5 | 3.1 | 25 | 3.1 | 6.3 |
| NCR169$C_{17–38}W_{10,20}C_{12,17}$/A | 6.3 | 6.3 | 25 | 12.5 | 3.1 | - | 6.3 | 6.3 |

*-: inactive up to 25 μM*

## 3. We discovered that the NCR169$C_{17-38}C_{12,17}$/S derivatives containing fluorinated tryptophans are the most potent antibacterial peptides in the NCR169$C_{17-38}$ series.

3.1. We generated a series of NCR169$C_{17-38}C_{12,17}$/S through the modification on tryptophans at the 10$^{th}$ and 20$^{th}$ positions with 5 commercial tryptophan derivatives in racemic forms, except for 5-fluoro tryptophan, which was in D- and L- enantiomeric forms. We successfully synthesized and purified 16 new NCR169$C_{17-38}C_{12,17}$/S derivatives. **[1]**

3.2. Our in vitro studies revealed that substitution of tryptophan at the 10th position with 6-fluro tryptophan enhanced the antibacterial activity against all tested bacteria, with MBC varied between 0.8 to 1.6 μM for NCR169$C_{17\text{-}38}C_{12,17}$/S-10$W^{6\text{-}F}$I. NCR169$C_{17\text{-}38}C_{12,17}$/S-10$^{W6\text{-}F}$II also displays similar antibacterial profiles (Table 4). However, this peptide could only terminate *Salmonella enterica* with an MBC of 6.3 μM. Peptides containing 5-fluoro tryptophan also presented good MBC activity between 1.6 to 6.3 μM towards the pathogen bacteria. **[1]**

**Table 3**. Minimal bactericidal concentrations (MBC; in μM) of the modified peptides on different pathogens after 3 h of treatment in phosphate buffer (PPB). *E. f., Enterococcus faecalis* (ATCC 29212)*; S. a., Staphylococcus aureus* (HNCMO112011)*; K. p., Klebsiella pneumoniae* (NCTC 13440)*; A. b., Acinetobacter baumannii* (ATCC 17978)*; P. a., Pseudomonas aeruginosa* (ATCC 27853)*; E. c., Escherichia coli* (ATCC 8739)*; L. m., Listeria monocytogenes* (ATCC 19111); *S. e., Salmonella enterica* (ATCC 13076). The two most active peptides are in bold.

| **Peptides** | **Gram-Negative** | | | | | **Gram-Positive** | | |
|---|---|---|---|---|---|---|---|---|
| | ***E. c.*** | ***S. e.*** | ***K. p.*** | ***A. b.*** | ***P. a.*** | ***E. f.*** | ***L. m.*** | ***S. a.*** |
| NCR169$C_{17\text{–}38}C_{12,17}$/S-10$W^{5\text{-}F\text{-}L}$ | 1.6 | 1.6 | 3.1 | 1.6 | 1.6 | 1.6 | 1.6 | 1.6 |
| NCR169$C_{17\text{–}38}C_{12,17}$/S-10$W^{5\text{-}F\text{-}D}$ | 1.6 | 3.1 | 3.1 | 1.6 | 3.1 | 1.6 | 6.3 | 3.1 |
| NCR169$C_{17\text{-}38}C_{12,17}$/S-20$W^{5\text{-}F\text{-}L}$ | 3.1 | 3.1 | 3.1 | 3.1 | 6.3 | 3.1 | 3.1 | 1.6 |
| NCR169$C_{17\text{-}38}C_{12,17}$/S-10$W^{6\text{-}F}$ I | 0.8 | 1.6 | 1.6 | 1.6 | 0.8 | 1.6 | 1.6 | 0.8 |
| NCR169$C_{17\text{-}38}C_{12,17}$/S-10$W^{6\text{-}F}$ II | 0.8 | 6.3 | 1.6 | 0.8 | 1.6 | 0.8 | 1.6 | 0.8 |

3.3. We proved through hemolysis assay that NCR169$C_{17\text{-}38}$, along with NCR169$C_{17\text{-}38}C_{12,17}$/S and its two most active derivatives (NCR169$C_{17\text{-}}$

$_{38}C_{12,17}$/S-10W$^{6\text{-}F}$I and NCR169C$_{17\text{-}38}$C$_{12,17}$/S-10W$^{6\text{-}F}$II) did not trigger hemolysis in human red blood cells. It indicates that these peptides may not threaten human cells when interacting with bacterial membranes (Figure 3). **[1]**

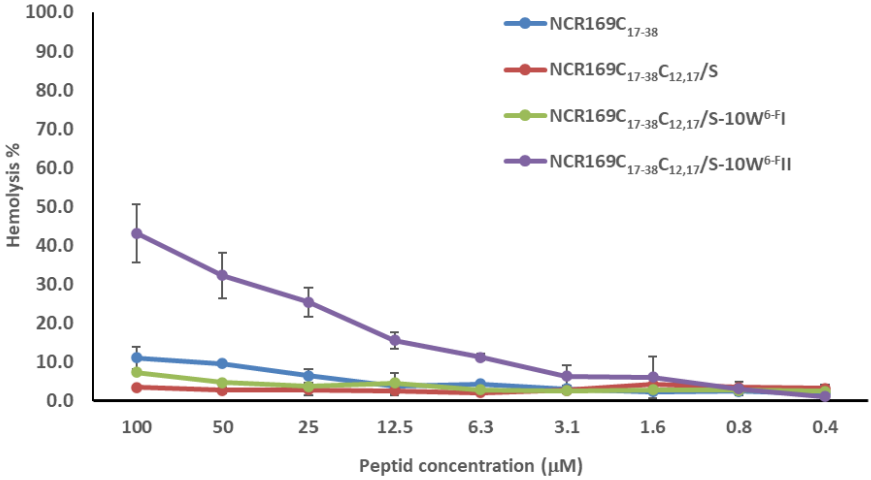


**Figure 3**. Hemolysis Activity of NCR169C$_{17\text{-}38}$ and its Most Active Derivatives

**4. We confirmed that the sequence close to the NCR147 C-terminal NCR147 possessed relatively better activity than its parent compound and other NCR147 shorter fragments.**

4.1. We revealed that the NCR147 has a short antiparallel β-sheet (T22 to V25 and R30 to W35) at the C-terminal and various properties, including a β strand (D8 to D10) at the elongated N-terminal (Figure 4). The β-sheet structure was also present in NCR147$_{13\text{-}36}$ and NCR147$_{20\text{-}36}$.

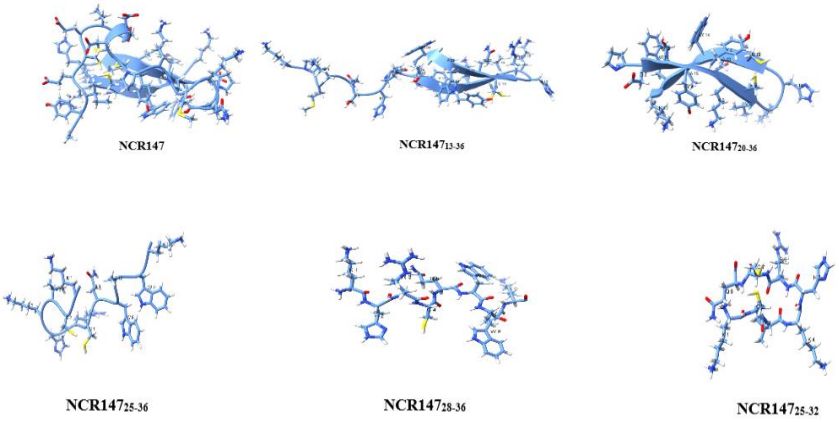


**Figure 4.** Structural Predictions of NCR147 and its shorter fragments

4.2. In our initial investigation, we made a significant finding that the NCR147 variant with a -COOH group at the C-terminal displayed activity against *Escherichia coli* (MBC: 25 µM)*, Pseudomonas aeruginosa* (MBC: 12.5 µM)*, Listeria monocytogenes* (MBC: 12.5 µM), and *Acinetobacter baumannii* (MBC: 3.125 µM) **[3]**. Subsequently, we synthesized the NCR147 amidated peptide alongside 5 shorter fragments and 3 NCR147$_{25-36}$ derivatives, wherein tryptophan was substituted with alanine.

4.3. The antibacterial assay of the amidated NCR147 series unveiled that NCR147 has low antibacterial activity, with MBC around 50 µM (Table 5). The NCR147$_{25-36}$ has slightly better antibacterial activity (MBC between 25 to 50 µM), whereas the NCR147C$_{20-36}$ could terminate *Listeria monocytogenes* with MBC of 6.25, *Acinetobacter baumannii* and *Enterococcus faecalis* with MBC of 12.5 µM. However, the later peptide

was weak against *Salmonella enterica*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* (MBC around 100 μM).

**Table 4**. Minimal bactericidal concentrations (MBC; in μM) of the studied peptides on different pathogens after 3 h of treatment in PPB (20 mM). Gram-negative: *E. c., Escherichia coli* (ATCC 8739)*; S. e., Salmonella enterica* (ATCC 13076)*; K. p., Klebsiella pneumoniae* (NCTC 13440)*; A. b., Acinetobacter baumannii* (ATCC 17978)*; P. a., Pseudomonas aeruginosa* (ATCC 27853). Gram-Positive: *E. f., Enterococcus faecalis* (ATCC 29212)*; L. m., Listeria monocytogenes* (ATCC 19111)*; S. a., Staphylococcus aureus* (HNCMO112011).

| **Peptides** | **Gram-Negative** | | | | | **Gram-Positive** | | |
|---|---|---|---|---|---|---|---|---|
| | ***E. c.*** | ***S. e.*** | ***K. p.*** | ***A. b.*** | ***P. a.*** | ***E. f.*** | ***L. m.*** | ***S. a.*** |
| NCR147 | 50 | 50 | 50< | 50 | 50 | 50< | 25 | 50< |
| $NCR147_{13-36}$ | 12.5 | 50 | 100< | 12.5 | 6.25 | 50 | 12.5 | 50 |
| $NCR147C_{20-36}$ | 50 | 100< | 100< | 12.5 | 25 | 12.5 | 6.25 | 100< |
| $NCR147_{25-32}$ | 50< | 50< | 50< | 50< | 50< | 50< | 50< | 50< |
| $NCR147_{25-36}$ | 25 | 50 | 50< | 25 | 25 | 50< | 50< | 50 |
| $NCR147_{28-36}$ | 6.25 | 25 | 100< | 6.25 | 12.5 | 100< | 100< | 25 |

## 5. We verified that incorporating fluorinated tryptophans into the $NCR147_{25-36}$ sequence resulted in a considerable improvement in antibacterial effectiveness.

5.1. We synthesized some derivatives of $NCR147_{25-36}$ by accommodating 5-fluoro-L- tryptophan at 9 and 11 residues in the sequence. The utilization of this tryptophan derivative into the sequence was intended to investigate the effect of fluorinated tryptophan on the antibacterial activity of $NCR147_{25-36}$.

5.2. The results of the antibacterial assay using the ESKAPE group plus *Salmonella enterica* and *Listeria monocytogenes* flaunted the $NCR147_{25-36}W_{9,11}/W^{5\text{-}F\text{-}L}$ as the most promising antibacterial peptide among the NCR147 derivatives (Table 5). This peptide could terminate half of the tested pathogens with an MBC of 3.125 μM. Single substitution of fluorinated tryptophan in the 9th or 11th residue also developed the antibacterial activity of the peptide, but not as good as the double substitution peptide. Nonetheless, these $NCR147_{25-36}$ analogs displayed weak activity against *Klebsiella pneumoniae* and *Enterococcus faecalis*, with MBC around 50 μM.

**Table 5.** Minimal bactericidal concentrations (MBC; in μM) of the $NCR147_{25-36}$ derivatives on different pathogens after 3 h of treatment in PPB (20 mM). Gram-negative: *E. c., Escherichia coli* (ATCC 8739)*; S. e., Salmonella enterica* (ATCC 13076)*; K. p., Klebsiella pneumoniae* (NCTC 13440)*; A. b., Acinetobacter baumannii* (ATCC 17978)*; P. a., Pseudomonas aeruginosa* (ATCC 27853). Gram-Positive: *E. f., Enterococcus faecalis* (ATCC 29212)*; L. m., Listeria monocytogenes* (ATCC 19111)*; S. a., Staphylococcus aureus* (HNCMO112011).

| **Peptides** | **Gram-Negative** | | | | | **Gram-Positive** | | |
|---|---|---|---|---|---|---|---|---|
| | ***E. c.*** | ***S. e.*** | ***K. p.*** | ***A. b.*** | ***P. a.*** | ***E. f.*** | ***L. m.*** | ***S. a.*** |
| $NCR147_{25-36}W_9/W^{5\text{-}F\text{-}L}$ | 12.5 | 12.5 | 50< | 25 | 12.5 | 50< | 50 | 12.5 |
| $NCR147_{25-36}W_{11}/W^{5\text{-}F\text{-}L}$ | 6.25 | 6.25 | 50< | 6.25 | 6.25 | 50< | 12.5 | 6.25 |
| $NCR147_{25-36}W_{9,11}/W^{5\text{-}F\text{-}L}$ | 3.125 | 3.125 | 50< | 3.125 | 6.25 | 50< | 3.125 | 3.125 |

5.3. We further investigated the antibacterial profile of NCR147 peptide with its shorter fragments and the derivatives of $NCR147_{25-36}$ against 8 Gram-

negative bacteria that caused several plant diseases. The results demonstrated similar trends, in which the full sequence of NCR147 has low MBC values, whereas the shorter fragments like $NCR147_{13-36}$ and $NCR_{25-36}$ were active against half of the tested bacteria. Surprisingly, $NCR147_{25-36}W_{11}/W^{5\text{-}F\text{-}L}$ displayed better activity against *Pseudomonas syringae* pv. tabaci (MBC: 1.6 μM), *Pseudomonas syringae* pv. tomato (MBC: 3.125 μM), and MBC of 6.25 μM against *Erwinia carotovora* and *Xanthomonas malvaceae*. The $NCR147_{25-36}W_{9,11}/W^{5\text{-}F\text{-}L}$ exhibited comparable activity, albeit slightly less potent, against *Erwinia carotovora* (MBC: 12.5 μM).

**Table 6.** Minimal bactericidal concentrations (MBC; in μM) of the NCR147 and its derivatives on different pathogens after 3 hr of treatment in PPB (20 μM). *P. s. tom., Pseudomonas syringae* pv. tomato*; X.c., Xanthomonas campestris; E. ch., Erwinia chrysanthemi; A. t., Agrobacterium tumefaciens; E. ca., Erwinia carotovora; P. g., Pseudomonas gladioli; X. m., Xanthomonas malvaceae; P. s. tab., Pseudomonas syringae* pv. tabaci.

| **Peptides** | ***P. s. tom.*** | ***X. c.*** | ***E. ch.*** | ***A. t.*** | ***E. ca.*** | ***P. g.*** | ***X. m.*** |
|---|---|---|---|---|---|---|---|
| NCR147 | 50< | 50< | 50< | 50< | 50< | 50< | 25 |
| $NCR147_{13-36}$ | 12.5 | 50< | 50< | 50< | 12.5 | 50< | 12.5 |
| $NCR147_{25-36}$ | 12.5 | 50< | 50< | 50< | 12.5 | 50< | 25 |
| $NCR147_{28-36}$ | 6.25 | 50< | 50< | 50< | - | 50< | 6.25 |
| $NCR147_{25-32}$ | 50< | 50< | 50< | 50< | 50< | 50< | 50< |
| $NCR147_{25-36}W_9/W^{5\text{-}F\text{-}L}$ | 12.5 | 50< | 50< | 50< | 12.5 | 50< | 12.5 |
| $NCR147_{25-36}W_{11}/W^{5\text{-}F\text{-}L}$ | 3.125 | 50< | 50< | 50< | 6.25 | 50< | 6.25 |
| $NCR147_{25-36}W_{9,11}/W^{5\text{-}F\text{-}L}$ | 3.125 | 50< | 50< | 50< | 12.5 | 50< | - |

## 5. Potential Applications

This Ph.D. thesis primarily aimed to synthesize peptides derived from NCR169 and NCR147 peptides and investigate their antimicrobial activity. We expected that the results of our study could be beneficial for further developing AMPs as therapeutic agents. Our research findings are still in the early stages of the drug development process. Nonetheless, these may be good recommendations for preclinical research to assess their safety, efficacy, and pharmacological properties through in vitro and in vivo assays. Besides applications for medicine, our findings could be applied to agricultural management since some of our peptides are active against plant pathogenic bacteria.

## 6. Scientific Publications

Identification number in the Hungarian Collection of Scientific Publications (MTMT):

10084078

Publications Related to Dissertation:

1. **Dian H.O. Howan**, Sándor Jenei, János Szolomajer, Gabriella Endre, Éva Kondorosi, Gábor K. Tóth,
   Enhanced Antibacterial Activity of Substituted Derivatives of NCR169C Peptide
   International Journal of Molecular Sciences, 24 (2023) 1-13
   $IF_{2022} = 5.57$
2. Bettina Szerencsés, Attila Gácser, Gabriella Endre, Ildikó Domonkos, Hilda Tiricz, Csaba Vágvölgyi, János Szolomajer, **Dian H.O. Howan**, Gábor K. Tóth, Ilona Pfeiffer, Éva Kondorosi
   Symbiotic NCR Peptide Fragments Affect the Viability, Morphology and Biofilm Formation of *Candida* Species
   International Journal of Molecular Sciences, 22 (2021), 1-20
   $IF_{2021} = 6.03$
3. Rui M. Lima, Balaji B. Rathod, Hilda Tiricz, **Dian H.O. Howan**, Mohamad A. Al Bouni, Sándor Jenei, Edit Timár, Gabriella Endre, Gábor K. Tóth, Éva Kondorosi
   Legume Plant Peptides as Sources of Novel Antimicrobial Molecules Against Human Pathogens
   Frontiers in Molecular Biosciences, 9 (2022), 870460
   $IF_{2022} = 5.0$

**ΣIF = 16.6**

Publication Unrelated to Dissertation

1. Attila Bajtai, István Ilisz, **Dian H.O. Howan**, Gábor K. Tóth, Gerhard K.E. Scriba, Wolfgang Lindner, Antal Péter

Enantioselective Resolution of Biologically Active Dipeptide Analogs by High-Performance Liquid Chromatography Applying *Cinchona* Alkaloid-Based Ion-Exchange Chiral Stationary Phases
Journal of Chromatography A, 1611 (2020), 460574
$IF_{2020} = 4.76$

**Total IF = 21.36**

Conference Participation Related to Dissertation

1. **Dian H.O. Howan**, Sándor Jenei, Mohamad Anas Al Bouni, Éva Kondorosi, Gábor K. Tóth
   Synthesis and Investigations of the Antimicrobial NCR169 Analogs
   Peptidkémiai Munkabizottság Tudományos Ülése, 11-13 October 2021, Hungary (oral presentation)
2. János Szolomajer, Bettina Szerencsés, **Dian H.O. Howan**, Ilona Pfeiffer, Éva Kondorosi, Gábor K. Tóth
   Növényi Eredetű Peptidek Antifungális Hatása
   Peptidkémiai Munkabizottság Tudományos Ülése, 11-13 October 2021, Hungary (oral presentation)