Synthesis, biological evaluation and membranotropic properties of quinoline-antimicrobial peptide conjugates as antibacterial drugs

Pierre Laumaillé1*, Alexandra Dassonville-Klimpt1, Sophie Da Nascimento1, Catherine Mullié1, François Peltier1,2, Claire Andréjak1,3, Sandrine Castelain1,2, Sandrine Morandat4, Karim El Kirat4, Pascal Sonnet1

1 AGIR, EA 4294, UFR of Pharmacy, Jules Verne University of Picardie, 80037 Amiens, France; 2 Department of Bacteriology, Amiens University Hospital, 80054 Amiens, France 3 Respiratory and Intensive Care Unit, Amiens University Hospital, 80054 Amiens, France 4 Laboratory of Biomechanics and Bioengineering, UMR CNRS 7338, Compiègne University of Technology (UTC), 60205 Compiègne, France

* Corresponding author: pierre.laumaille@etu.u-picardie.fr
Synthesis, biological evaluation and membranotropic properties of quinoline-antimicrobial peptide conjugates as antibacterial drugs

Antibiotics
Antimicrobial peptides
Drug resistant bacteria/mycobacteria

| Name  | MIC (µM) | HC50 (µM) |
|-------|----------|-----------|
|       | S. aureus | E. faecalis | E. coli | P. aeruginosa |       |
| WK    | 45.7     | ND        | 45.7    | 45.7          | ND    |
| Q-WK  | 1.2      | 0.6       | 2.4     | 2.4           | 0.9   |
| C5    | 40.6     | 40.6      | 40.6    | >324          | 350   |

Membranotropic effect on S. aureus model

biological study
physico-chemical study

5th International Electronic Conference on Medicinal Chemistry
1-30 November 2019

sponsors: MDPI, pharmaceuticals
Abstract:

Tuberculosis and nosocomial infections are among the most frequent cause of death in the world. Mycobacteria such as *Mycobacterium tuberculosis* and ESKAPE bacteria are pathogens particularly implicated in these infectious diseases. The lack of antibiotics with novel mode of action associated with the spread of drug resistant bacteria make the fight against these infections particularly challenging.

Using antimicrobial peptides (AMPs) to restore or to broaden antibacterial activity of antibiotics is an interesting strategy to fight resistant strains. For example, the conjugation between chloramphenicol and ubiquicidine gives a conjugate with increased activity against *Escherichia coli* and reduced toxicity against neutrophils compared to chloramphenicol alone.

During previous work on the development of new anti-infective drugs, we identified a series of quinolines active against Gram-positive bacteria such as *Staphylococcus aureus* and *Enterococcus faecalis*. Concerning Gram-negative bacteria, some of them were active on *E. coli* but not against *Pseudomonas aeruginosa*. In order to broaden the antibacterial spectrum of this heterocycle core, we synthesized quinoline-based conjugates with short AMP sequences. Their antibacterial activities against a panel of bacteria and mycobacteria will be discussed. Membranotropic properties study through tensiometry measures on bacterial mimetic membrane models was carried out to elucidate their mechanism of action.

References:
1. (a) WHO, Global tuberculosis report 2017; (b) Khan, H. A., Baig, F. K. & Mehboob. Nosocomial infections: Epidemiology, prevention, control and surveillance, *Asian Pac. J. Trop. Biomed.* 2017, 7, 478–482.
2. (a) Arnusch et al. Enhanced Membrane Pore Formation through High-Affinity Targeted Antimicrobial Peptides. *PLoS ONE* 2012 7:e39768; (b) Chen et al. Bacteria-Targeting Conjugates Based on Antimicrobial Peptide for Bacteria Diagnosis and Therapy. *Mol. Pharm.* 2015, 12, 2505.
3. Jonet, A.; Dassonville-Klimpt, A.; Sonnet, P.; Mullié, C. Side chain length is more important than stereochemistry in the antibacterial activity of enantiomerically pure 4-aminoalcohol quinoline derivatives. *J. Antibiot. (Tokyo)* 2013, 66, 683–686.
4. Laumaillé, P.; Dassonville-Klimpt, A.; Peltier, F.; Mullié, C.; Andrájak, C.; Da-Nascimento, S.; Castelain, S.; Sonnet, P.; Synthesis and study of new quinolineaminoethanols as anti-bacterial drugs, *Pharmaceuticals* 2019, 12(2), 91.
5. Strøm, M. B. et al. The Pharmacophore of Short Cationic Antibacterial Peptides, *2003*, 46, 3–6.

Keywords: Quinoline, AMP, AMP conjugates, antibacterial drugs, membranotropic properties
Introduction : Aims of the project

• **Tuberculosis** (caused by typical mycobacteria like *M. tuberculosis*) is one of the 10 first causes of death worldwide: 10 million of people infected and 1.7 million of people killed each year in 2017.

• **Atypical mycobacteria** (*M. avium, M. abcessus*) are responsible of a lot of infections, mainly pulmonary infections, between 0.5 and 2 cases for 100000 people a year.

• **Nosocomial infections** in hospitals: 1.4 million of people infected worldwide, 5-10 % of hospitalized people.

Problems of antibiotics resistance (*M. tuberculosis, S. aureus, P. aeruginosa*).

➔ There is an urgent need of designing new antimicrobial compounds to fight antibiotics resistance.
Introduction: Conjugation with AMPs

- Conjugation between antibiotics and antimicrobial peptides (AMPs) can increase and/or broaden antimicrobial properties of antibiotics. Many examples in the literature.

➢ **dpMtx**: activity against *M. tuberculosis* increased.

![Methotrexate](image1)

Methotrexate: IC\(_{50}\) > 10 µM against *M. tuberculosis* H37Ra

![dpMtx](image2)

dpMtx: IC\(_{50}\) 950 nM against *M. tuberculosis* H37Ra

Peirera et al, ACS, 2015

➢ **chloramphenicol-ubiquicidine\(_{29-41}\)**: activity against *E. coli* increased and toxicity against neutrophiles reduced.

![Chloramphenicol](image3)

Chloramphenicol: MIC = 6.2 µM on *E. coli*

0.24 \(\times\) 10\(^9\) neutrophiles/L of blood

![Chloramphenicol-ubiquicidine\(_{29-41}\)](image4)

chloramphenicol-ubiquicidine\(_{29-41}\): MIC = 3.8 µM on *E. coli*

0.98 \(\times\) 10\(^9\) neutrophiles/L of blood

Chen et al. Mol. Pharm. 2015 12, 2505
Introduction : Conjugation with AMPs (2)

Interest of the antibiotic-AMP conjugation in this project :

➔ To fight mycobacteria in latent phase (more resistant against antibiotics) and in rapide replication phase.

➔ To help antibiotics to translate through bacterial membrane (Gram negative bacteria and mycobacteria) and through macrophage membrane (mycobacteria).

Cell wall of Gram-negative bacteria

Cell wall of mycobacteria
Introduction: conjugates design (1)

- **AMPs** = short peptides (few tens of aminoacids (AA)) with high proportion of hydrophobic AAs and positively charged AAs. It is possible to functionalize the C-terminal extremity.

- Some aminoquinoline-methanols (**AQM**)s developed by the research team showed good antibacterial properties against Gram + bacteria.

\[
\begin{align*}
\text{R} &= \text{C}_6\text{H}_{13}, \text{MIC} = 9.8 \, \mu\text{M against } S. \text{ aureus} \text{ and } E. \text{ faecalis} \\
\text{R} &= \text{C}_7\text{H}_{15}, \text{MIC} = 2.4 \, \mu\text{M against } S. \text{ aureus} \text{ and } E. \text{ faecalis}
\end{align*}
\]

- **Objectives**: Synthesis of **AQM-AMPs** conjugates with antibacterial (Gram + et Gram -) and antimycobacterial (typical and atypical) properties.
Introduction : conjugates design (2)

Some peptide-X and linker-peptide-X were synthesized as reference.

**linker:**

1) \[\text{H}_2\text{N}-\text{CHR}-\text{COOH}\]

2) \[\text{H}_2\text{N}-\text{CHR}-\text{N}-\text{CHR}-\text{COOH}\]

**X:**

- \(-\text{NH}_2\)
- \(-\text{OBn}\)
- \(-\text{OH}\)

**Peptide:**

- \(-\text{RWRW}\)
- \(-\text{RWRWRW}\)
- \(-\text{RCyRCyRCy}\)
- \(-\text{MLLKKLLKKM}\)
- \(-\text{WKWLKKWIK}\)
Introduction: Summary

Membranotropic Study
- Tensiometry measures on membrane models

Biological evaluation
- MIC on S. aureus, E. faecalis, E. coli, P. aeruginosa, M. avium, M. abscessus, M. smegmatis

Chemical synthesis
Solid support

Secondary structure determination
- Circular dichroism
  - Not shown here

Cytotoxicity
- Hemolysis tests

5th International Electronic Conference on Medicinal Chemistry
1-30 November 2019
Results and discussion: retrosynthesis

Quinoline epoxide 5 is the precursor of all conjugates 9 and 10.
Results and discussion: peptidic synthesis

Solid phase synthesis with peptide synthesizer, Fmoc strategy, 3 different approaches depending of the desired C-term functionnalization.

**Strategy 1**
- RINK resin
- 1) Fmoc-AA\_i\_GP, HBTU, HOBt, DIEA
- 2) piperidine, NMP

\[
\text{Fmoc-NH} \rightarrow \text{PG\_i\_i=1,n-(AA\_i\_i=1,n)NH} \rightarrow H_2N-(AA\_i\_i=1,n)_{\text{NH}_2}
\]

12-100%

GABA linker is considered as an aminoacid on this scheme

PG = Protecting group (Boc, Pbf).

**Strategy 2**
- SASRIN resin
- 1) Fmoc-AA\_i\_GP, HBTU, HOBt, DIEA
- 2) piperidine, NMP

\[
\text{Fmoc-AA\_i=1,n-O} \rightarrow \text{PG\_i\_i=1,n-(AA\_i\_i=1,n)O} \rightarrow H_2N-(AA\_i\_i=1,n)_{\text{OH}}
\]

16-57%

**Strategy 3**
- SASRIN resin
- 1) Fmoc-AA\_i\_GP, HBTU, HOBt, DIEA
- 2) piperidine, NMP

\[
\text{Fmoc-AA\_i=1,n-O} \rightarrow \text{PG\_i\_i=1,n-(AA\_i\_i=1,n)O} \rightarrow H_2N-(AA\_i\_i=1,n)_{\text{OBn}}
\]

6-20%
Results and discussion: AQM-AMP conjugates synthesis

AQM-AMP conjugates are obtained by nucleophilic substitution between the AMP and the quinoline epoxide 5, then by resin cleavage. Concerning conjugates with diamine linker, few steps are necessary before the coupling. The conjugates are obtained with a yield between 1.7 and 29%.
Results and discussion: AMPs biological activity

Good activity (MIC < 25 µM) for GABA-RCyRCyRCy-NH₂, RWRW-OBn, RWRWRW-OBn et MLLKKLLKKM-OH.

All the compounds are inactive against *M. avium* and *M. abscessus* (MIC > 100 µg/mL).
Results and discussion: AQM-AMP conjugates biological activity

MIC < 10 µM for most compounds
AQM-AMPs more active than AMPs alone

For *M. avium* and *M. abscessus*, MIC > 64 µg/mL for all tested compounds.
Results and discussion

Membranotropic properties

Membrane mimetics models:
- E. coli
- S. aureus
- Cellule hépatique

Lipids alone:
- POPG
- POPC
- DOPE
- CL

Physico-chemical study carried out on membrane mimetics models (mix of lipids to simulate a cell membrane) and on the lipids alone. 3 models: E. coli, S. aureus and hepatic cell.
Results and discussion: choice of tested compounds

5 sequences with the most interesting activity against the 4 strains of bacteria, alone (N° 1-5) or conjugated with AQM (N° 6-10), with C5 (N°11) as a reference.

| N° | name   | core | peptide       | X     | MIC (µM) |      |      |      |      | HC₅₀ (µM) |
|----|--------|------|---------------|-------|----------|------|------|------|------|-----------|
|    |        |      | S. aureus CIP103.429 |      | E. faecalis CIP 103214 |      | E. coli DSM 1103 |      | P. aeruginosa DSM 1117 |      |
| 1  | RW4    | /    | RWRW           | NH₂   | >162     | >162 | >162 | >162 |       | ND        |
| 2  | RW6    | /    | RWRWRW         | NH₂   | 56       | ND** | >113 | >113 |       | ND        |
| 3  | RCy6   | /    | RCyRCyRCy      | NH₂   | 7.8      | ND   | 3.9  | 7.8  |       | ND        |
| 4  | MLK    | /    | MLLKKLLKKM     | OH    | 96       | ND   | >96  | 96   |       | >1150     |
| 5  | WK     | /    | WKWLKKWKIK     | OH    | 45.7     | ND   | 45.7 | 45.7 |       | ND        |
| 6  | Q-RW4  | Quinoline | RWRW           | NH₂   | 1.8      | 7.3  | 14.6 | 7.3  |       | 22.3*     |
| 7  | Q-RW6  | Quinoline | RWRWRW         | NH₂   | 2.8      | 2.8  | 5.6  | 2.8  |       | 8.8*      |
| 8  | Q-RCy6 | Quinoline | RCyRCyRCy      | NH₂   | 5.8      | ND   | 95.7 | 47.9 |       | 4.6*      |
| 9  | Q-MLK  | Quinoline | MLLKKLLKKM     | OH    | 4.9      | 2.4  | 9.8  | 9.8  |       | 17.1*     |
| 10 | Q-WK   | Quinoline | WKWLKKWKIK     | OH    | 1.2      | 0.6  | 2.4  | 2.4  |       | 0.9*      |
| 11 | C5     | Quinoline | /              | /     | 40.6     | 40.6 | 40.6 | >324 |       | 350       |

* Reading after 24h (1h for the other)
** Not determined
Results and discussion : principle of physico-chemical study

Determination of Maximal Insertion Pressure (MIP) :

Use of Wilhelmy plate (a tank with a monolayer of lipids at the interface, in which a piece connected to a tensiometer is immersed to measure surface pressure):

- Measure of surface pressure at the interface air/peptide solution.
- Measure of surface pressure at the interface air/water.
- Plot of this difference of surface pressure ($\Delta \pi$) for different initial pressure ($\pi_i$) of lipid.

Decreasing slope $\Rightarrow$ insertion into the lipid layer. Horizontal slope $\Rightarrow$ adsorption onto the lipid layer.

Extrapolation for $\pi_i=0$ gives the MIP.

$\text{MIP} = \text{pressure of lipid above which the compound can’t insert into the lipid layer any more.}$

If $\text{MIP} < \text{physiological pressure of membrane lipids (30-35 mN.m}^{-1})$
$\Rightarrow$ The compound can’t insert into a biological membrane.
Results and discussion: *E. coli* model

We can observe an adsorption onto the lipid monolayer. MLK et WK induce a stronger interaction (higher $\Delta \pi$). Conjugates AQM-AMPs interact more strongly than AMPs alone.
Results and discussion: inter-models comparison

Study on two new models (hepatic cell model and S. aureus model) of C5 (ref) and the more effective AQM-AMPs on E. coli model (Q-MLK et Q-WK).

\[
\begin{align*}
\Delta \pi (\text{mN.m}^{-1}) & \quad \pi_1 (\text{mN.m}^{-1}) \\
\text{Q-WK Comparison} & \\
\text{S. aureus} & \quad \text{E. coli} & \quad \text{Hepatic cell}
\end{align*}
\]

\[
\begin{align*}
\Delta \pi (\text{mN.m}^{-1}) & \quad \pi_1 (\text{mN.m}^{-1}) \\
\text{Q-MLK Comparison} & \\
\text{S. aureus} & \quad \text{E. Coli} & \quad \text{Hepatic cell}
\end{align*}
\]

| MIP S. aureus (mN.m\(^{-1}\)) | Q-WK | 42 |
|-------------------------------|------|----|
| Q-MLK                         | 50.6 |
| C5                            | 32   |

We can see an adsorption for hepatic cell model (horizontal slope) and E. coli model but an insertion for S. aureus model (decreasing slope) for the 3 compounds. Q-WK and Q-MLK could be able to insert into a cell (MIP > 35 mN.m\(^{-1}\)).
Results and discussion: investigation on lipids from the models

Comparaison Q-WK

MIP Q-WK (mN.m⁻¹)

|            | S. aureus | CL | PG |
|------------|-----------|----|----|
| MIP        | 42        | 53 | 36 |

Similarity of physico-chemical behavior (insertion) between CL and PG (lipids from S. aureus model). Better interaction with CL (MIP = 53 mN.m⁻¹).

Concerning PE (main lipid of E. coli model) and PC (main lipid of hepatic cell model), Δπ smaller than complete model→ Synergy or influence of minoritary lipid to explain the difference.
Results and discussion: focus on Q-WK

The AMP part (WK) induce an adsorption behavior (no MIP) and the AQM part (C5) induce an insertion behavior (MIP = 32 mN.m\(^{-1}\)) ➔ The conjugate Q-WK shows a stronger insertion behavior (MIP= 42 mN.m\(^{-1}\))

This trend is the same for CL but for PG, C5 does not insert into the lipid monolayer.

| MIP S. aureus (mN.m\(^{-1}\)) | Q-WK | WK | C5 |
|-------------------------------|------|-----|-----|
| Q-WK                          | 42   |     |     |
| WK                            |      |     |     |
| C5                            | 32   |     |     |

\[\Delta \pi \text{ (mN.m}^{-1}\)]

\[\pi_i \text{ (mN.m}^{-1}\)]
Conclusions

- 12 AQM-AMPs conjugates synthesized in 1-3 steps (from AMP and quinoline epoxide) with low yields (2-30 %).
  12 AMPs synthesized with various yields (6-100 %).

- AQM-AMPs conjugates are generally active against Gram-positive and Gram-negative bacteria, but not against mycobacteria (except *M. smegmatis* for some of them). They show hemolytic properties. AMPs alone and quinoline alone are less active than the AQM-AMP conjugates (and less hemolytic).

- WKWLKWIK sequence shows strong interaction on *S. aureus* model, with a global insertion behavior (quinoline => insertion and AMP => adsorption).

| nom    | MIC(µM) | HC₅₀ (µM) |
|--------|---------|-----------|
|        | *S. aureus* | *E. faecalis* | *E. coli* | *P. aeruginosa* |     |
|        | CIP103.429 | CIP 103214 | DSM 1103 | DSM 1117 |     |
| WK     | 45.7     | ND        | 45.7     | 45.7     | ND  |
| Q-WK   | 1.2      | 0.6       | 2.4      | 2.4      | 0.9 |
| C5     | 40.6     | 40.6      | 40.6     | >324     | 350 |

- Further physico-chemical studies are planned on a *M. tuberculosis* model and on liposome (to work with a bilayer model and not a monolayer model, which will allow to study other properties like translocation through a membrane).
Acknowledgments

I would like to thank all the AGIR team, especially François Peltier and Claire Andréjak for the realisation of the antimycobacterial tests, Sophie Da Nascimento for her help concerning peptide synthesis and purification, Catherine Mullié for having trained me for antibacterial tests, Sandrine Morandat and Karim El Kirat for having welcomed me in their lab and trained me for the physico-chemical study, Alexandra Dassonville-Klimpt and Pascal Sonnet for the supervision of my work, and my funders « Région Hauts-de-France »