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

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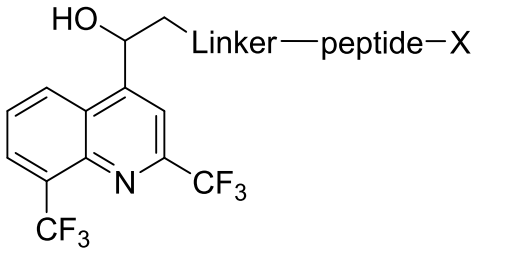
Synthesis, biological evaluation and membranotropic properties of quinoline-antimicrobial peptide conjugates as antibacterial drugs

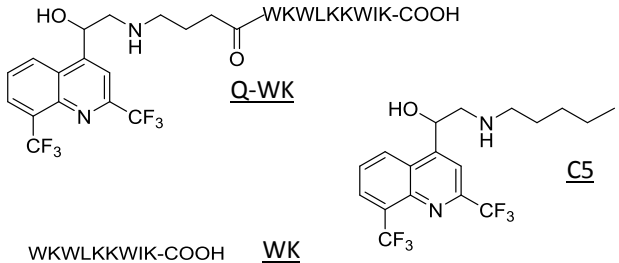
Antibiotics

Antimicrobial peptides

Drug resistant bacteria/mycobacteria



library of compounds

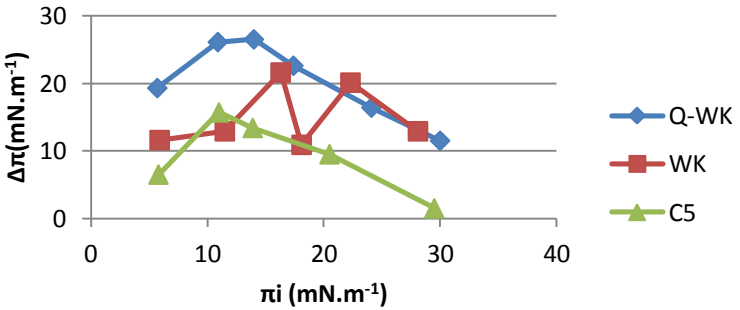


lead compounds

Name	MIC (μM)				HC_{50} (μM)
	***S. aureus* CIP103.429**	***E. faecalis* CIP 103214**	***E. coli* DSM 1103**	***P. aeruginosa* 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

biological study

Membranotropic effect on *S. aureus* model



physico-chemical study

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[1]. 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$_{29\text{-}41}$ gives a conjugate with increased activity against *Escherichia coli* and reduced toxicity against neutrophils compared to chloramphenicol alone [2].

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*[3,4]. In order to broaden the antibacterial spectrum of this heterocycle core, we synthesized quinoline-based conjugates with short AMP sequences[5]. 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.; Andréjak, 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.

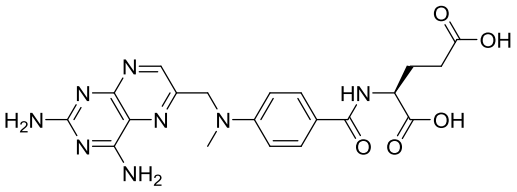
Problems of antibiotics resistance (*M. tuberculosis, S. aureus, P. aeruginosa*).

➔ There is an urgent need of designing new antimicrobial compounds to fight antibiotics resistance.

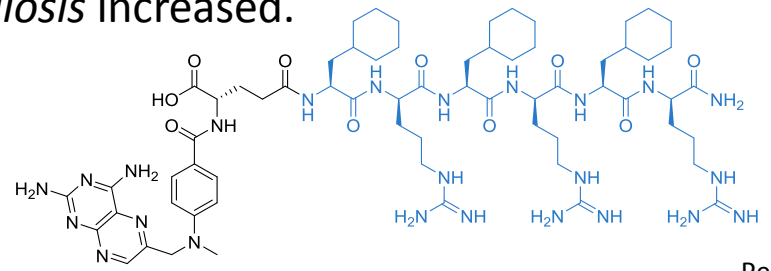
Introduction : Conjugation with AMPs

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

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



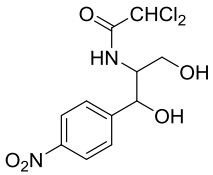
Methotrexate : IC_{50} > 10 µM against *M. tuberculosis H37Ra*



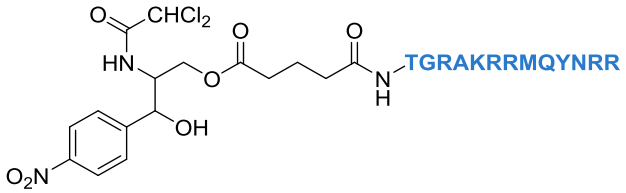
Peirera et *al*, ACS, **2015**

dpMtx : IC_{50} 950 nM against *M. tuberculosis H37Ra*

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



Chloramphenicol : MIC = 6.2 µM on *E. coli*
0,24.10^9 neutrophiles/L of blood



Chen *et al. Mol. Pharm.* **2015** 12, 2505

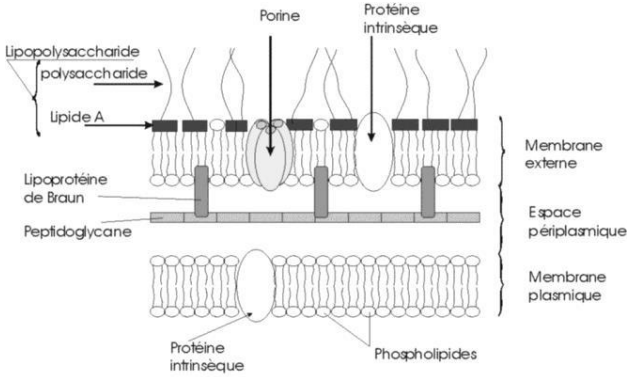
chloramphenicol-ubiquicidine$_{29-41}$: MIC = 3.8 µM on *E. coli*
0,98.10^9 neutrophiles/L of blood

Introduction : Conjugation with AMPs (2)

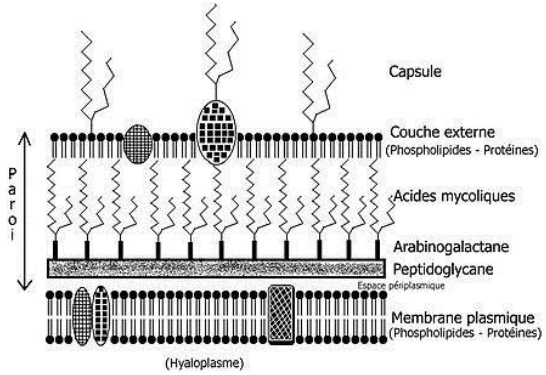
Interest of the antibiotic-AMP conjugation in this project :

➔ 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

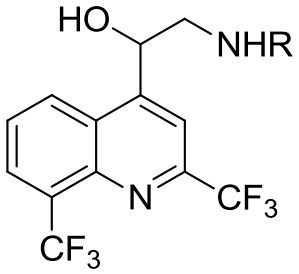


Cell wall of mycobacteria

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.

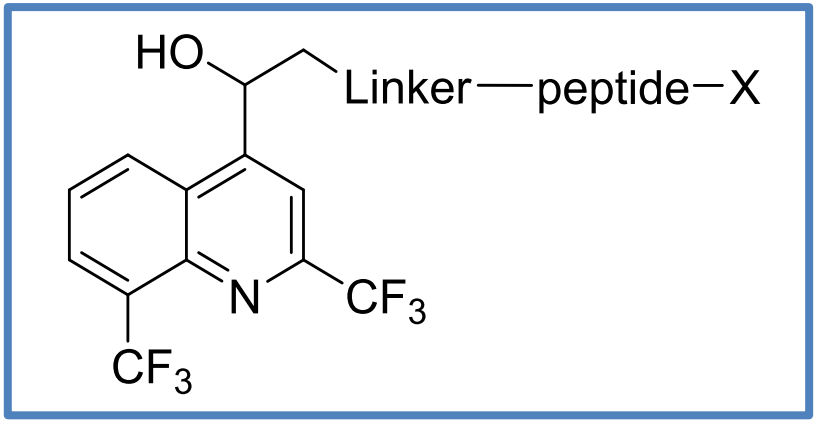
- Some aminoquinoline-methanols (**AQMs**) developped by the research team showed good antibacterial properties against Gram + bacteria.



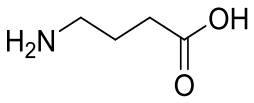
R = C_6H_{13} , MIC = 9.8 µM against *S. aureus* and *E. faecalis*
R = C_7H_{15} , MIC = 2.4 µM against *S. aureus* and *E. faecalis*

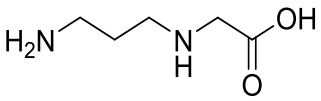
- **Objectives** : Synthesis of **AQM-AMPs** conjugates with antibacterial (Gram + et Gram -) and antimycobacterial (typical and atypical) properties.

Introduction : conjugates design (2)



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

linker : 1) 

2) 

X : - NH_2
- OBn
- OH

Peptide : -RWRW
- RWRWRW
- RCyRCyRCy
- MLLKKLLKKM
- WKWLKKWIK

Introduction : Summary

Membranotropic Study

Tensiometry measures on membrane models

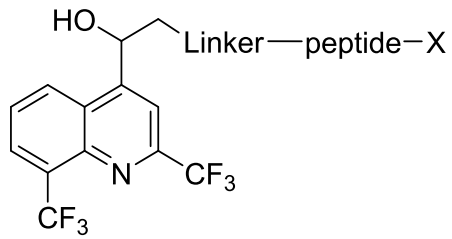
Secondary structure determination

Circular dichroisme

Not shown here

Chemical synthesis

Solid support



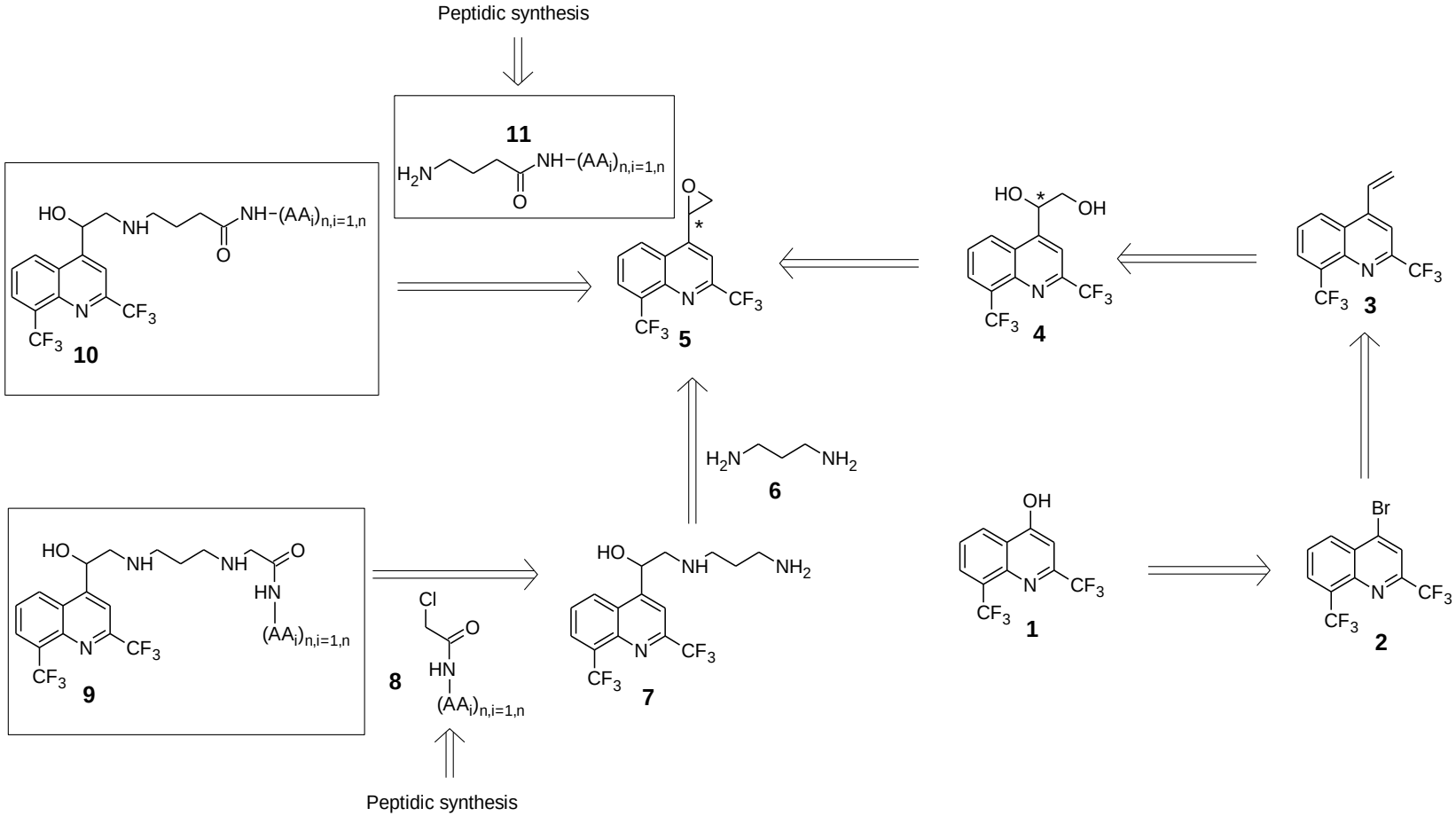
Biological evaluation

MIC on *S. aureus*, *E. faecalis*, *E. coli*, *P. aeruginosa*, *M. avium*, *M. abscessus*, *M. smegmatis*

Cytotoxicity

Hemolysis tests

Results and discussion : retrosynthesis

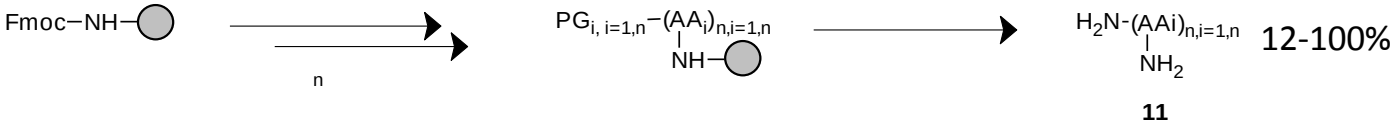


Quinoline epoxide **5** is the precursor of all conjugates **9** and **10**.

Results and discussion : peptidic synthesis

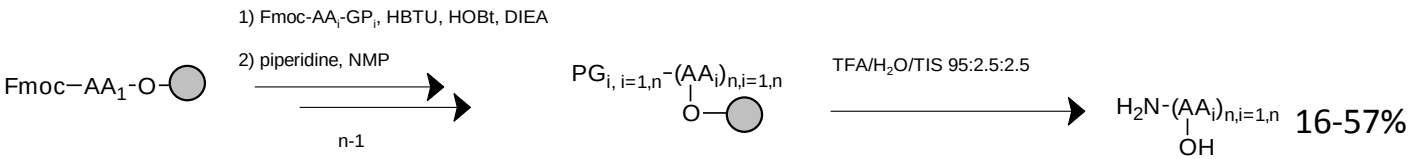
Solid phase synthesis with peptide synthesizer, Fmoc strategy,
3 different approaches depending of the desired *C*-term functionnalization.

Strategy 1 RINK resin

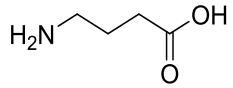


11 12-100%

Strategy 2 SASRIN resin

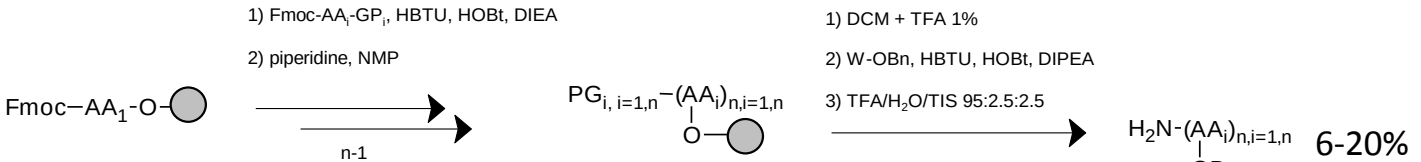


11 16-57%



GABA linker is considered as an aminoacid on this scheme
PG = Protecting group (Boc, Pbf).

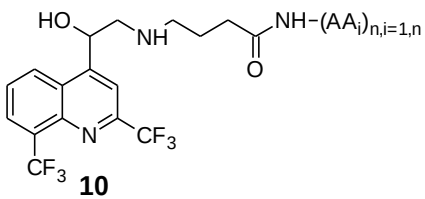
Strategy 3 SASRIN resin



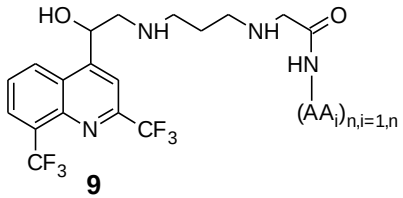
11 6-20%

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%.

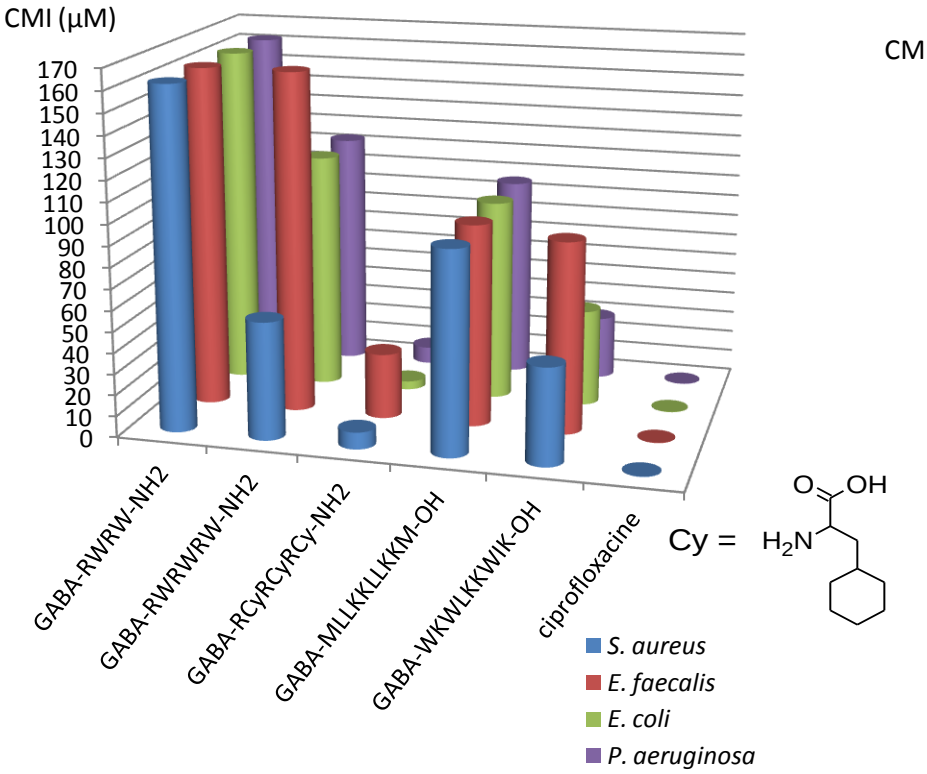


10



9

Results and discussion : AMPs biological activity



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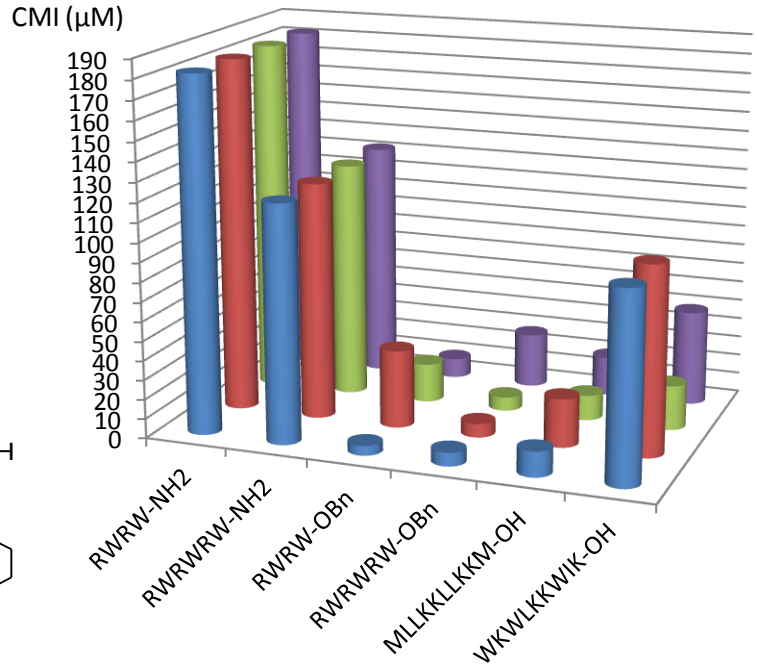
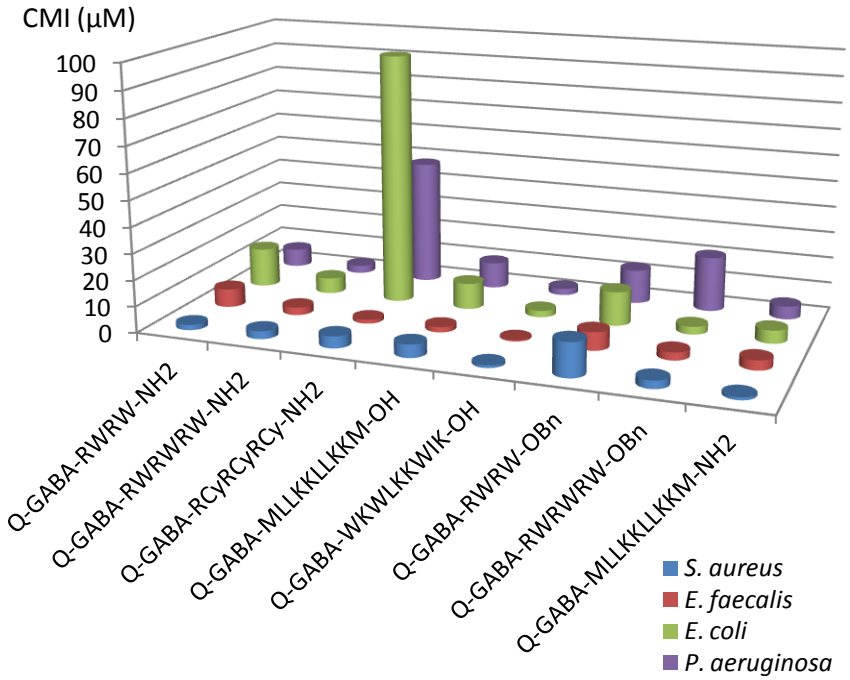
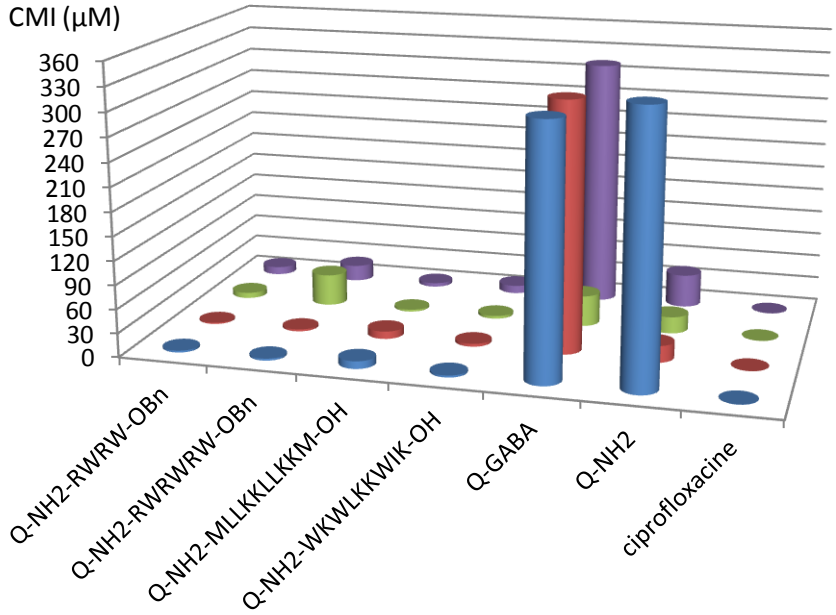


Good activity (MIC < 25 µM) for GABA-RCyRCyRCy-NH_2, RWRW-OBn, RWRWRW-OBn et MLLKKLLKKM-OH.

All the compounds are inactive against *M. avium* and *M. abcessus* (MIC > 100 µg/mL).

Results and discussion : AQM-AMP conjugates biological activity



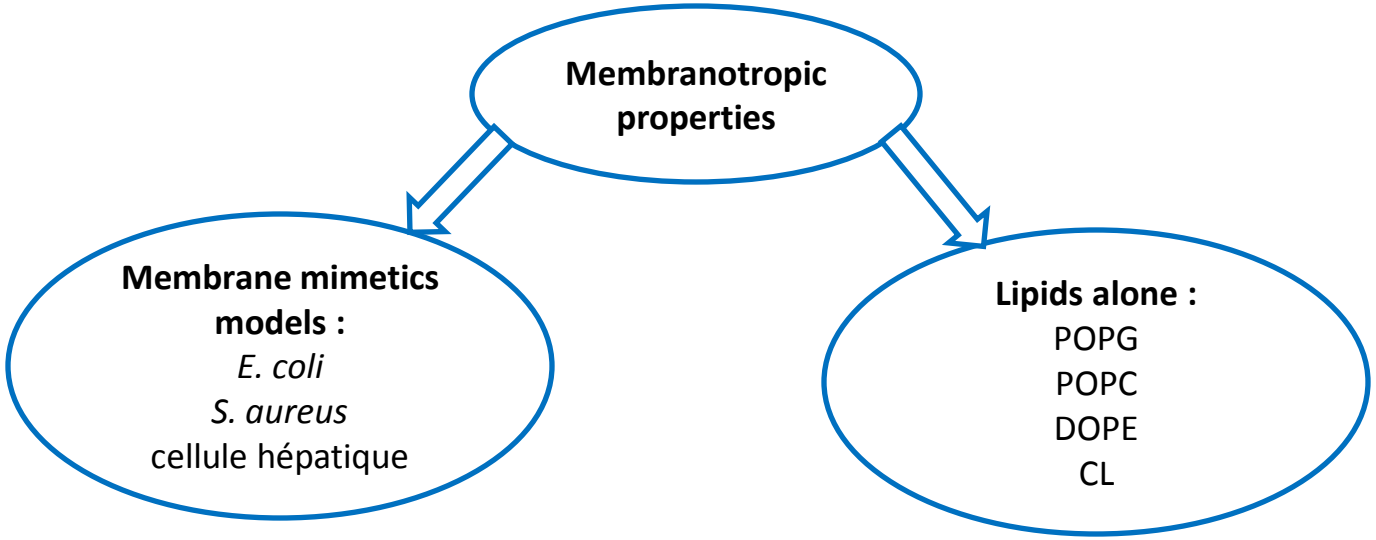


MIC< 10 µM for most compounds
AQM-AMPs more active than AMPs alone

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

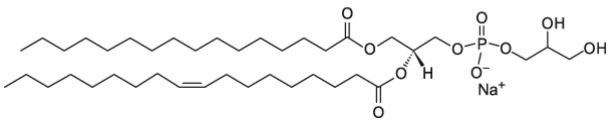
core	linker	sequence	*C*-term	MIC (µM)	
				M. smegmatis **ATCC 607** **MH medium**	***M. smegmatis*** **ATCC 607** **7H9 medium**
Quinoline	GABA	RWRW	NH_2	3.6	3.6
Quinoline	GABA	RWRWRW	NH_2	5.6	2.8
Quinoline	diamine	RWRWRW	Obn	10.3	>41

Results and discussion

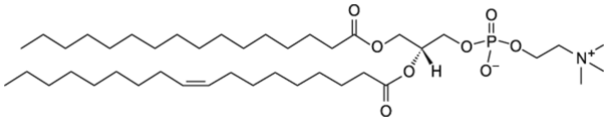


Physico-chemical study carried out on membrane mimetics models (mix of lipids to simulate a cell membrane) and on the lipids alone.
3 models : *E. coli*, *S. aureus* and hepatic cell.

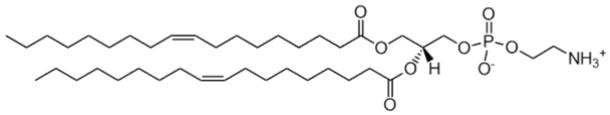
POPG



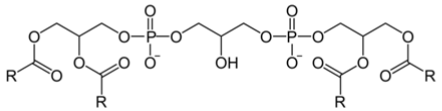
POPC



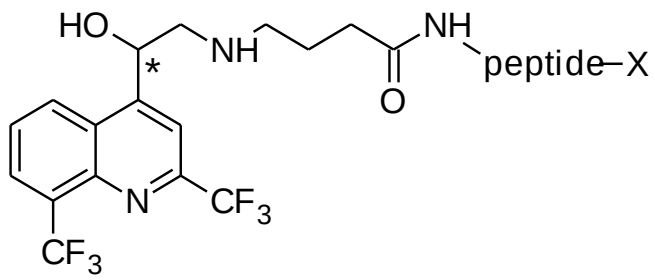
DOPE



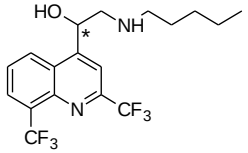
CL



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.

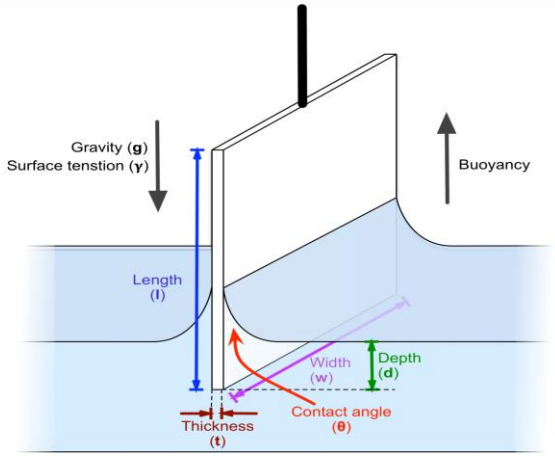


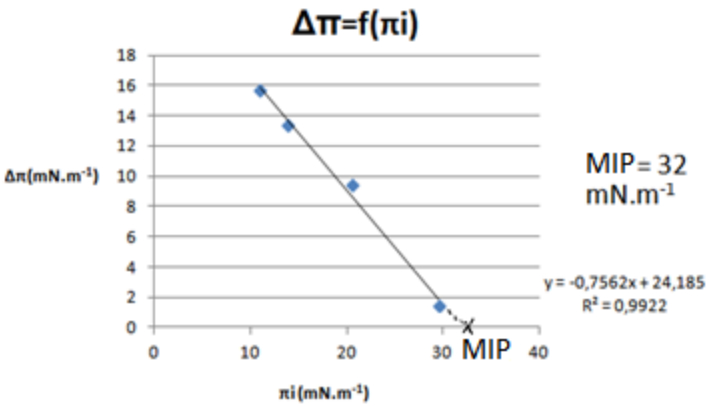
N°	name	core	peptide	X	MIC (µM) *S. aureus CIP103.429*	MIC (µM) *E. faecalis CIP 103214*	MIC (µM) *E. coli DSM 1103*	MIC (µM) *P. aeruginosa DSM 1117*	HC_{50} (µM)
1	RW4	/	RWRW	NH_2	>162	>162	>162	>162	ND
2	RW6	/	RWRWRW	NH_2	56	ND**	>113	>113	ND
3	RCy6	/	RCyRCyRCy	NH_2	7.8	ND	3.9	7.8	ND
4	MLK	/	MLLKKLLKKM	OH	96	ND	>96	96	>1150
5	WK	/	WKWLKKWIK	OH	45.7	ND	45.7	45.7	ND
6	Q-RW4	Quinoline	RWRW	NH_2	1.8	7.3	14.6	7.3	22.3*
7	Q-RW6	Quinoline	RWRWRW	NH_2	2.8	2.8	5.6	2.8	8.8*
8	Q-RCy6	Quinoline	RCyRCyRCy	NH_2	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	WKWLKKWIK	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 (π_i) of lipid.

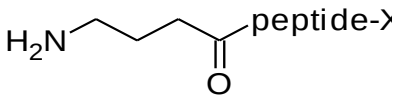
Decreasing slope➔ insertion into the lipid layer.
Horizontal slope➔ adsorption onto the lipid layer.

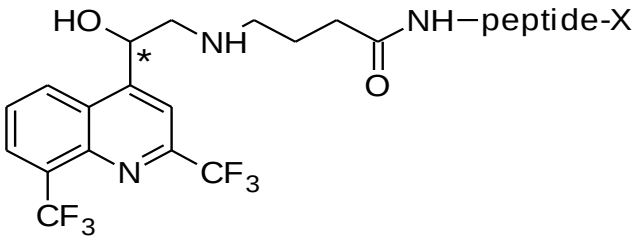
Extrapolation for π_i=0 gives the MIP.

MIP = pressure of lipid above which the compound can't insert into the lipid layer any more.

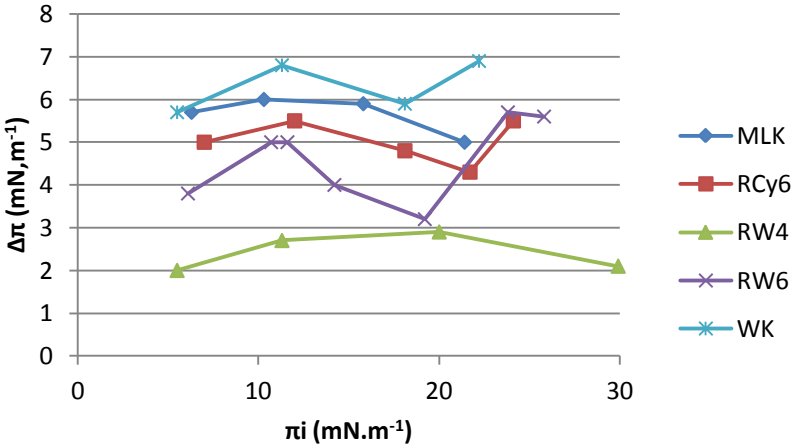
If MIP < physiological pressure of membrane lipids (30-35 $mN.m^{-1}$)
➔ The compound can't insert into a biological membrane.

Results and discussion : *E. coli* model

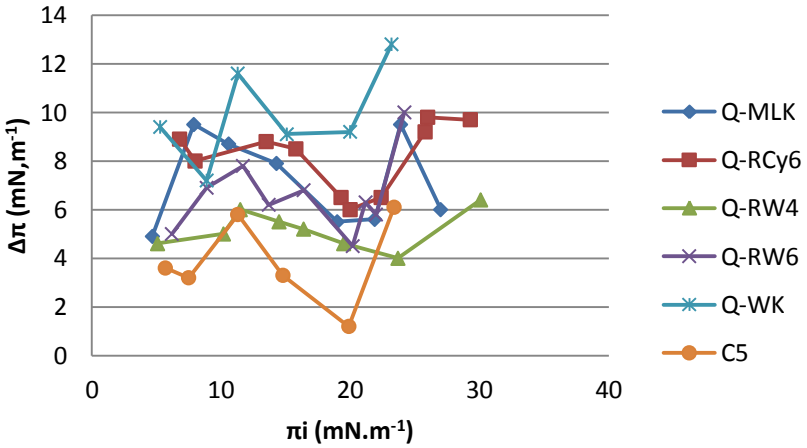




MIP on *E. coli*



MIP on *E. coli*

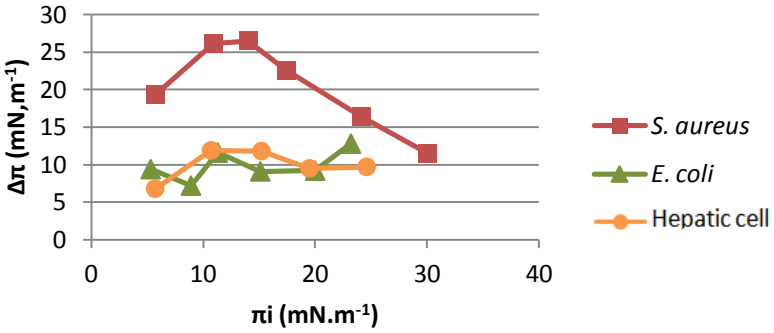


We can observe an adsorption onto the lipid monolayer.
MLK et WK induce a stronger interaction (higher Δπ).
Conjugates AQM-AMPs interact more strongly than AMPs alone.

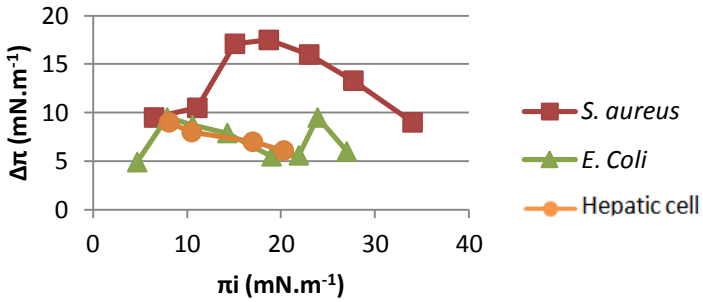
Results and discussion : inter-models comparison

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).

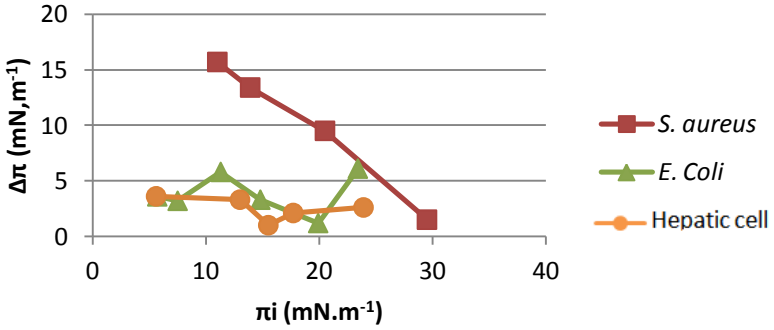
Q-WK Comparison



Q-MLK Comparison



C5 Comparison

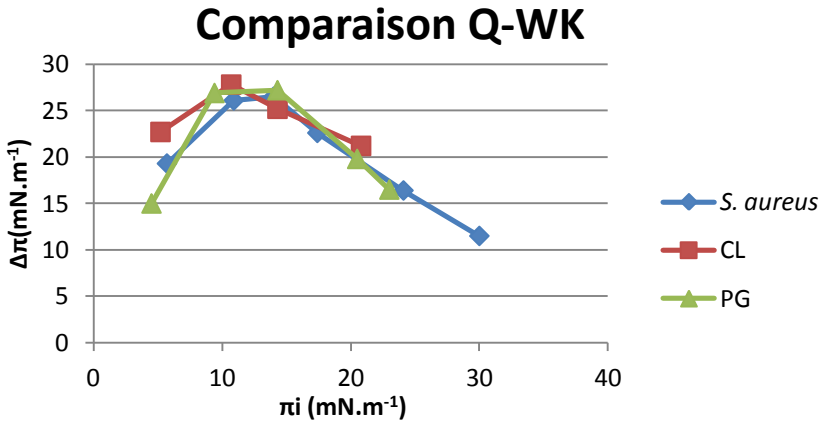


MIP *S. aureus* (mN.m^{-1})	
Q-WK	42
Q-MLK	50.6
C5	32

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.

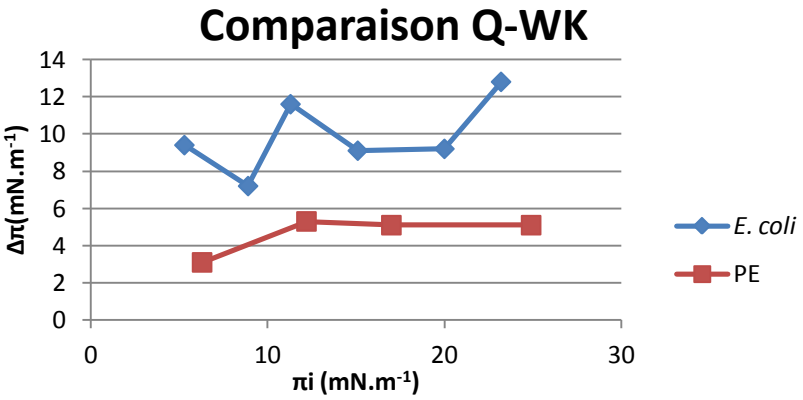
Q-WK and Q-MLK could be able to insert into a cell (MIP > 35 mN.m^{-1}).

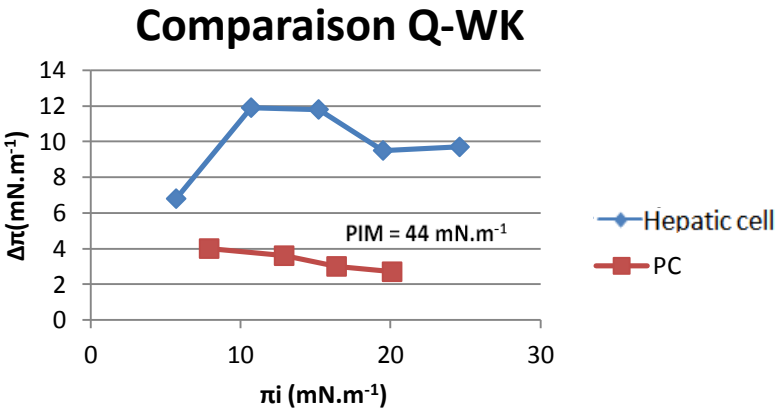
Results and discussion : investigation on lipids from the models



MIP Q-WK (mN.m^{-1})	
S. aureus	42
CL	53
PG	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}).

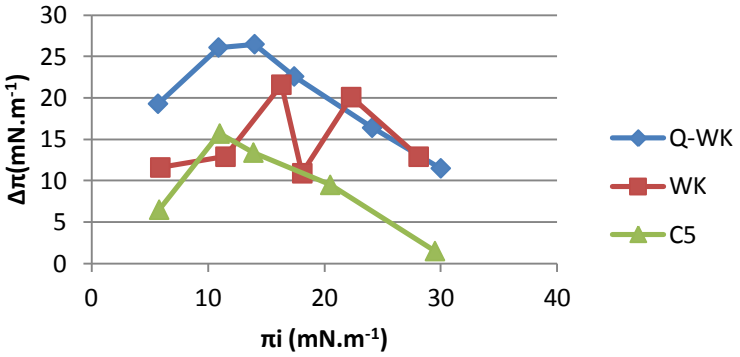




Concerning PE (main lipid of *E. coli* model) and PC (main lipid of hepatic cell model),
Δπ smaller than complete model➔ Synergy or influence of minoritary lipid to explain the difference.

Results and discussion : focus on Q-WK

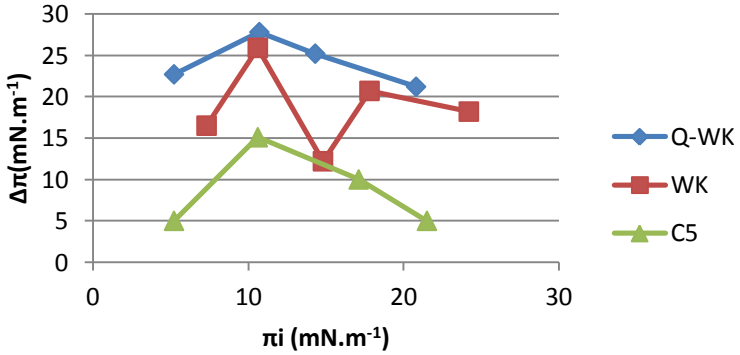
S. aureus comparison



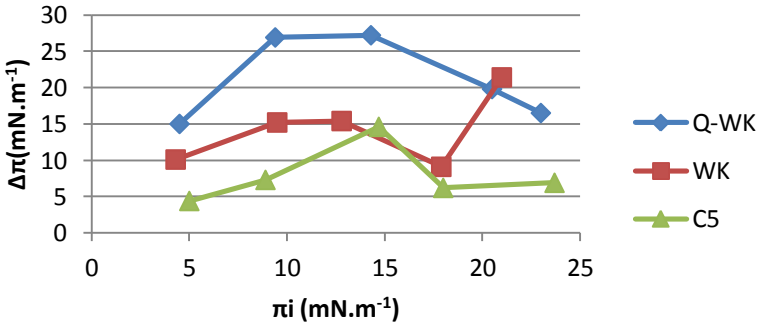
The AMP part (WK) induce an adsorption behavior (no MIP) and the AQM part (C5) induce an insertion behavior (MIP = 32 $mN.m^{-1}$)
➔ The conjugate Q-WK shows a stronger insertion behavior (MIP= 42 $mN.m^{-1}$).
This tend is the same for CL but for PG, C5 does not insert into the lipid monolayer.

MIP *S. aureus* ($mN.m^{-1}$)	
Q-WK	42
WK	/
C5	32

CL comparison



PG comparison



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)				HC_{50} (µM)
	***S. aureus* CIP103.429**	***E. faecalis* CIP 103214**	***E. coli* DSM 1103**	***P. aeruginosa* 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 »