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# From Hymenoptera venom to bioactive peptides

Yasmine Boughanmi; Kamel Mabrouk; Hamza Olleik; Chloé Mollet; Soioulata Aboudou; Harold de Pomyers; Didier Gigmes; Marc Maresca

1-Introduction

2- Strategy

3- Results

- Screening of venom libraries
- Identification of molecules
- Physicochemical and pharmacological characterizations
- SAR
- Mechanism of action

4- Conclusions & Perspectives

# 1- Introduction : Venoms as sources of bioactive molecules

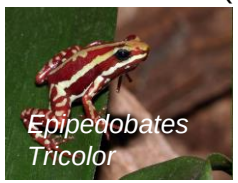
**131I-TM-601** - Chlorotoxin

**Anti-cancer**

**Captoten®** (*Pentapharm*)


- **Captopril** : a dipeptide mimetic derived from bradikinin-potentiating peptide
- Angiotensin converting enzyme inhibitor
- **Antihypertensive**

**ABT-594** (*Abbot*)




- **Epibatidine**: a small cyclic molecule
- Neuronal nicotinic acetylcholine receptor agonist
- **Neuropatic Pain**

**Defibrase®** (*Pentapharm*)


- **Botroxobin**: a 32 kDa peptide
- Fibrinogenolytic
- **acute cerebral infarction**

**Exenatide** (*Amylin Pharmaceuticals*)

- **Exendin-4** : a 39 aa peptide
- Glucagon like-peptide-1
- **Type-2 diabetes and related metabolic disorders**

**NPS 1506** (*NPS Pharmaceuticals*)


- **Delucemine** : a small cyclic molecule
- NMDA receptor antagonist
- **Neuroprotective agent/ Alzheimer**

**Integrilin®** (*Schering-Plough*)

- **Eptifibatide** : a cyclic heptapeptide derived from venom peptide
- Platelet glycoprotein IIb/IIIa receptor antagonist
- **Acute coronary syndrome**

**Prialt™** (*Elan*)


- **Ziconotide derived from the ω-conotoxin :** a 25 aa peptide
- Blocking the pre-synaptic N-type Calcium channels
- **Severe Chronic Pain**

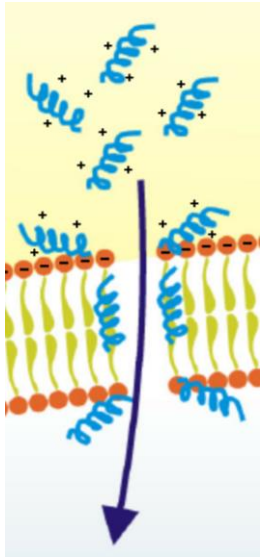
# 1- Introduction :Venoms and AMP

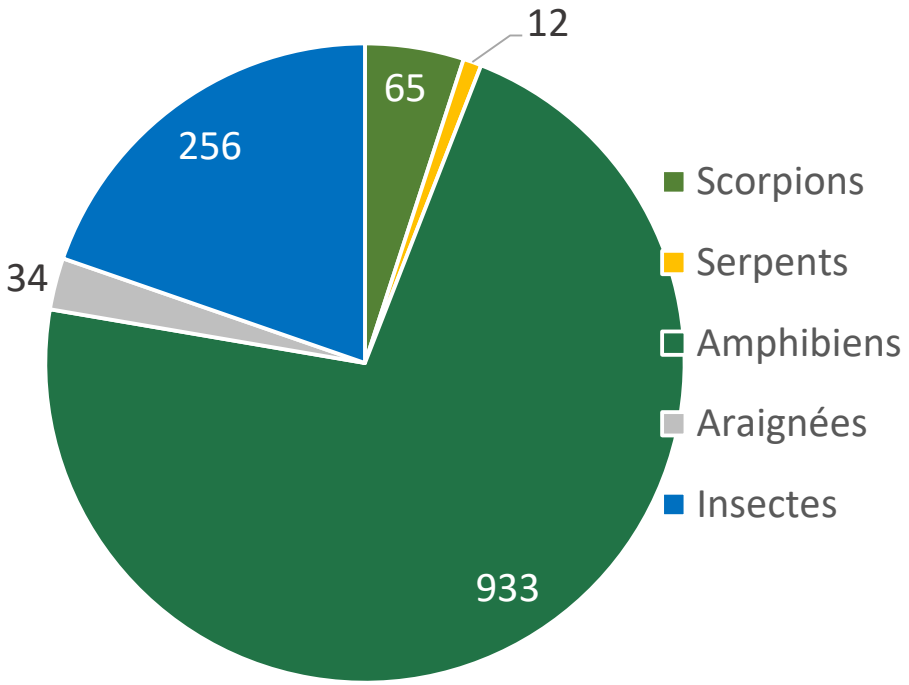
**1300 PAMs isolated from venoms**

**considerable effectiveness, large antibacterial spectrum**

**distinct (bacterial membrane) or multiple mechanism of action**

**Cationic / > 50% hydrophobic / amphiphilic**

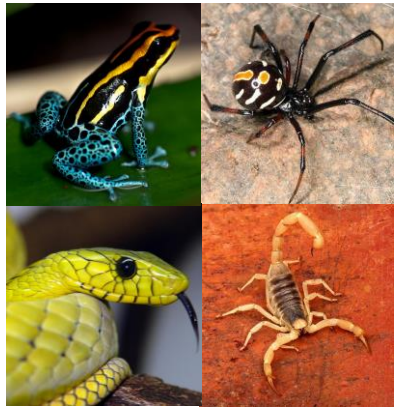




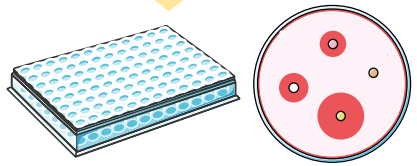

Number of AMPs isolated from the venoms of these animals, according to AMP database.

# **3-Results:** Screening

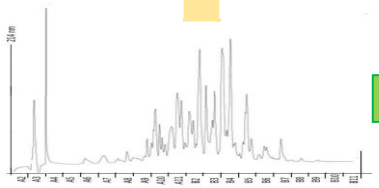
**IDENTIFY** **ISOLATE** **CHARACTERIZE**



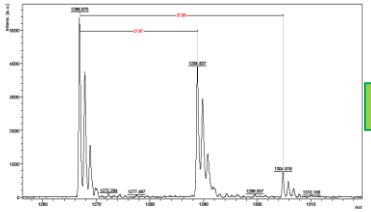
**Collection of venoms**



**Evaluation of the <u>antimicrobial activity</u>**

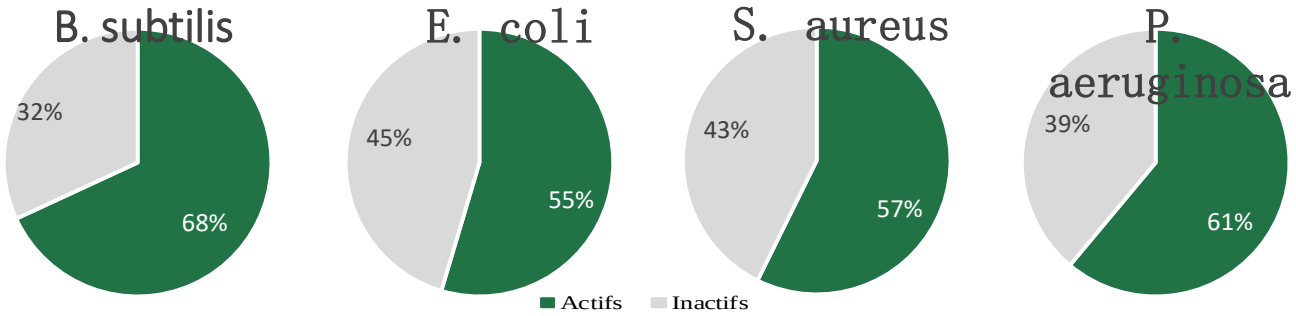


**<u>Purification</u> by RP-HPLC: venoms = 25 fractions / sub-fractions**



**<u>Characterization:</u> MALDI-Tof / Sequencing / MSMS**

**Synthesis of analogues** activity, toxicity, mechanism,...




60% have antibacterial activity

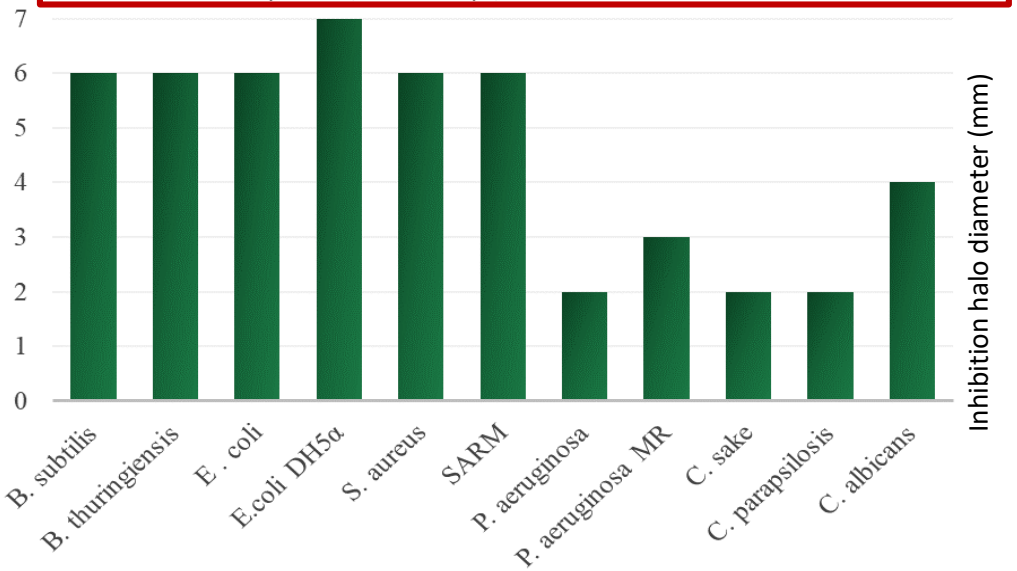
40% inhibit G+, G-, pathogens & non-pathogens

5% specific pathogens

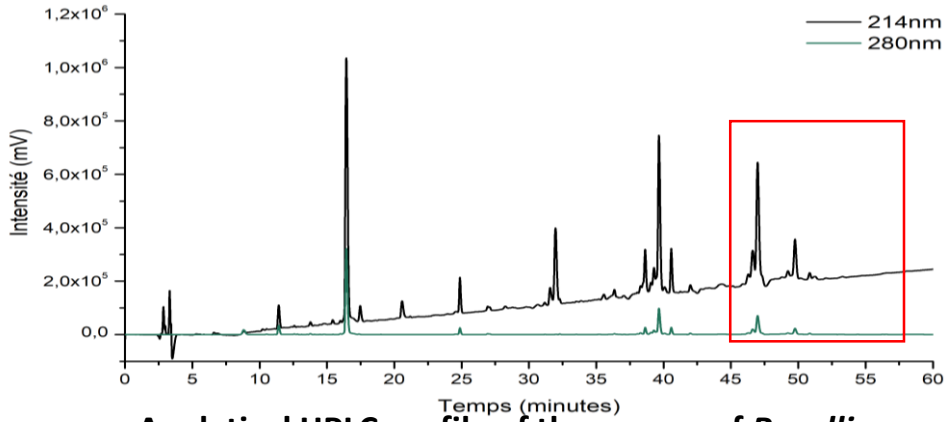
# 3-Results: Physico-chemical Characterization

**Broad spectrum of antimicrobial activity :**

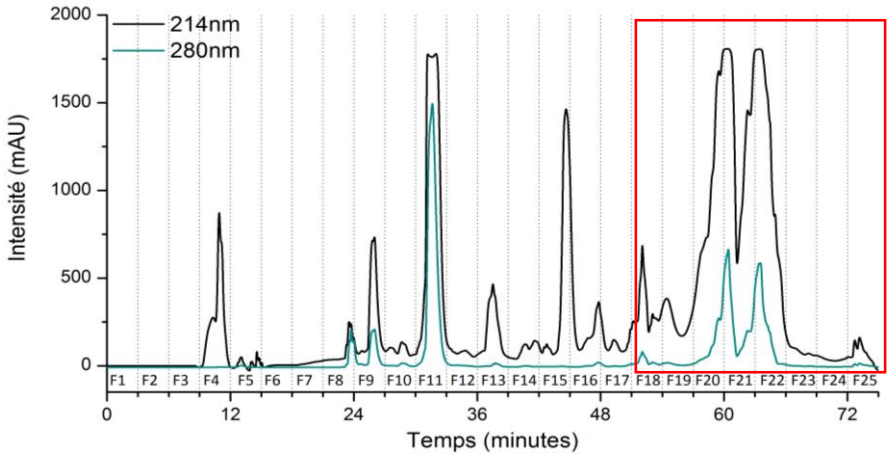
- multi-resistant bacteria (MRSA/P.aeruginosa)
- bacillus G+/ G- (B. thurigiensis/ E. coli)
- G+ coccus
- yeasts (Candida sp.)




… From the venom of *Polistes gallicus*

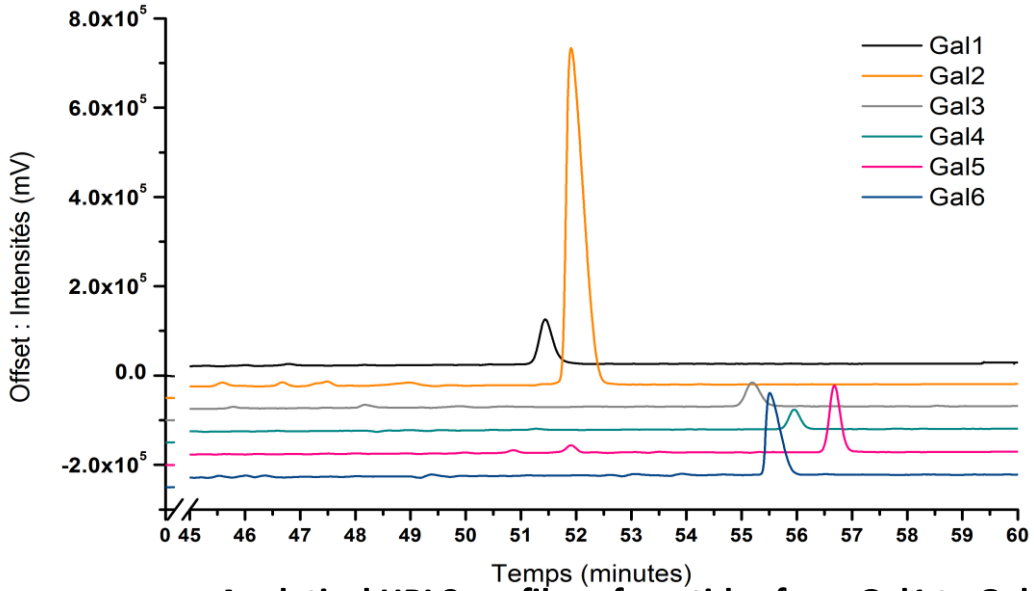



**Analytical HPLC profile of the venom of *P. gallicus***




**Preparative HPLC profile of 50 mg of *P. gallicus* venom**

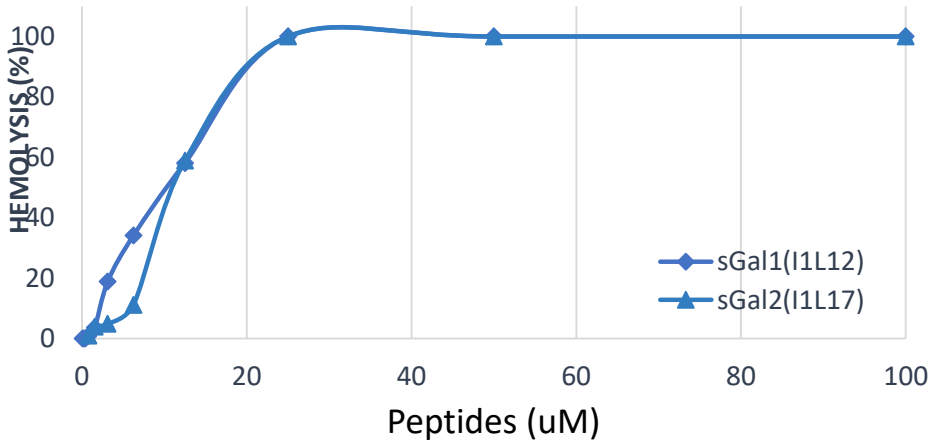
Separation into 25 fractions
19 Sub Fractions
10 sub-sub fractions




**Analytical HPLC profiles of peptides from Gal1 to Gal6**

# 3-Results: Peptide Synthesis and SAR

| Peptides | Sequences | MIC (µM) | | | | $MHC_{50}$ (µM) | Therapeutic index | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | P. aeruginosa | S. aureus | E. coli | B. subtilis | | P. aeruginosa | S. aureus | E. coli | B. subtilis |
| $_{S}Gal1(I_1L_{12})$ | ILSAILGLLKNL | 100 | 6,25-3,1 | 100-50 | 3,13-1,56 | 10 | 0,1 | 1,6-3,2 | 0,1-0,2 | 3,2-6,4 |
| $sGal1(I_1K_{10})$ | ILSAILGLLK | - | 50-25 | 100-50 | 25-12,5 | 135 | - | 2,7-5,4 | 1,35-2,7 | 5,4-10,8 |
| $_{S}Gal1(I_1L_9)$ | ILSAILGLL | - | 100 | - | 100 | | | | | |
| $_{S}Gal1(L_2K_{10})$ | LSAILGLLK | - | - | - | - | | | | | |
| $_{S}Gal1(L_2L_9)$ | LSAILGLL | - | - | - | - | | | | | |
| $_{S}Gal1(L_8L_{12})$ | LLKNL | - | - | - | - | ND | | | | |
| $_{S}Gal1(L_6L_{12})$ | LGLLKNL | - | - | - | - | | | | | |
| $_{S}Gal1(S_3L_{12})$ | SAILGLLKNL | - | - | - | - | | | | | |
| $_{S}Gal1(S_3L_9)$ | SAILGLL | - | - | - | - | | | | | |



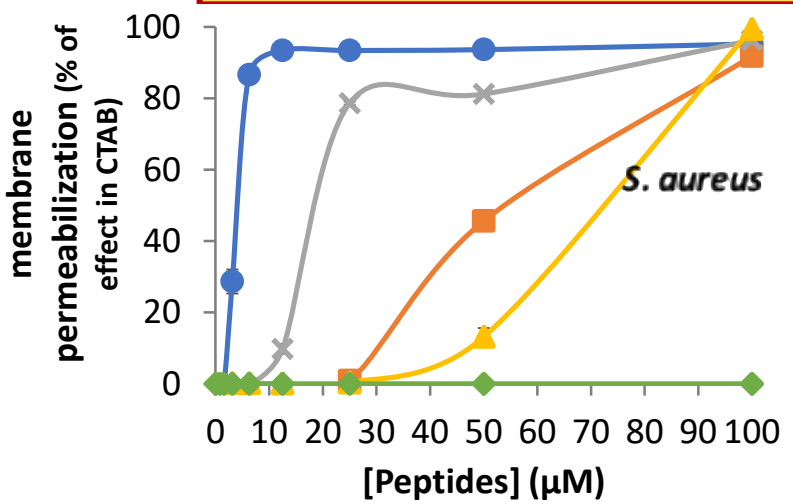
# 3-Results: Peptide Synthesis and SAR

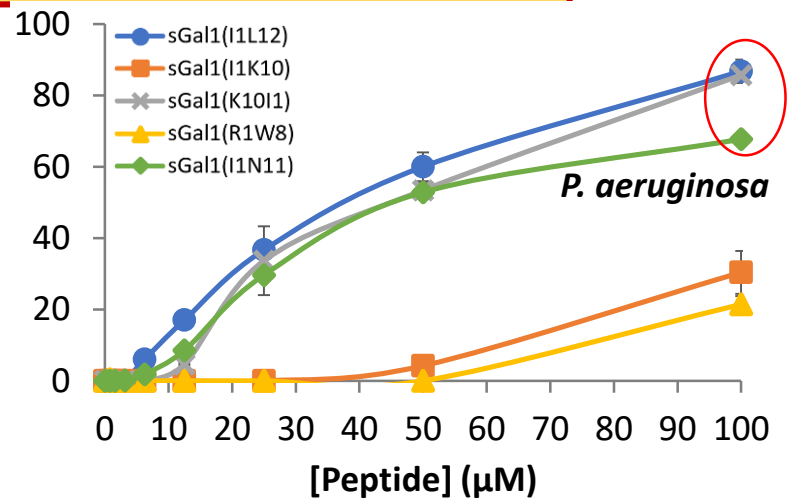
| | | MIC (µM) | | | | | Therapeutic Index | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Peptides | Sequences | P. aeruginosa | S. aureus | E. coli | B. subtilis | $CMH_{50}$ (µM) | P. aeruginosa | S. aureus | E. coli | B. subtilis |
| $_{S}Gal1(I_1L_{12})$ | ILSAILGLLKNL | 100 | 6,25-3,1 | 100-50 | 3,13-1,56 | 10 | 0,1 | 1,6-3,2 | 0,1-0,2 | 3,2-6,4 |
| $_{S}Gal1(L_{12}I_1)$ | LNKLLGLIASLI | 50 | 3,13-1,56 | 50-25 | 3,13-1,56 | 36 | 0,72 | 11,5-23 | 0,72-1,5 | 11,5-23 |
| $_{S}Gal1(_{D}I_1L_{12})$ | $_{D}$(ILSAILGLLKNL) | 100 | 6,25 | 100 | 3,13 | 25 | 0,25 | 4 | 0,25 | 8 |
| $_{S}Gal1(I_1K_{10})$ | ILSAILGLLK | - | 50-25 | 100-50 | 25-12,5 | 135 | - | 2,7-5,4 | 1,35-2,7 | 5,4-10,8 |
| $_{S}Gal1(K_{10}I_1)$ | KLLGLIASLI | - | 12,5-6,25 | 100-50 | 6,25-3,13 | 30 | - | 2,4-4,8 | 0,3-0,6 | 4,8-9,6 |
| $_{S}Gal1(K_1I_8)$ | KLLGLILI | - | 25 | -/50 | 12,5-6,25 | 20 | - | 0,8 | 0,4 | 1,6-3,2 |
| $_{S}Gal1(R_1W_8)$ | RLLGLWLW | - | 25-12,5 | - | 12,5 | 225 | - | 9-18 | - | 18 |
| $_{S}Gal1(I_1N_{11})$ | ILKAIKKLLKN | 6,25-3,13 | -/100 | 50-25 | 12,5-6,25 | >10000 | 1600-3200 | - | 200-400 | 800-1600 |

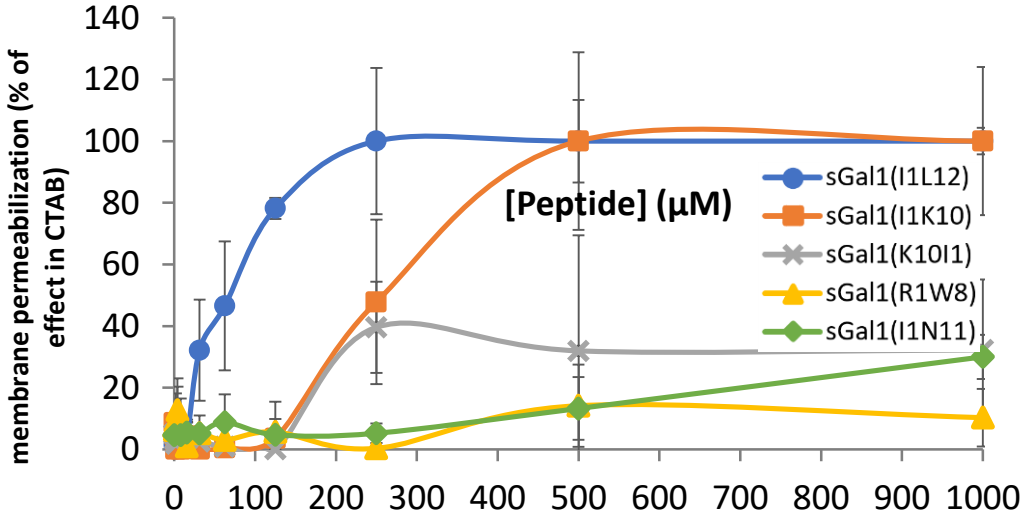
**$_{S}Gal1(I_1N_{11})$** : No hemotoxicity up to 10 mM!

# 3-Results: Pharmacological characterizations

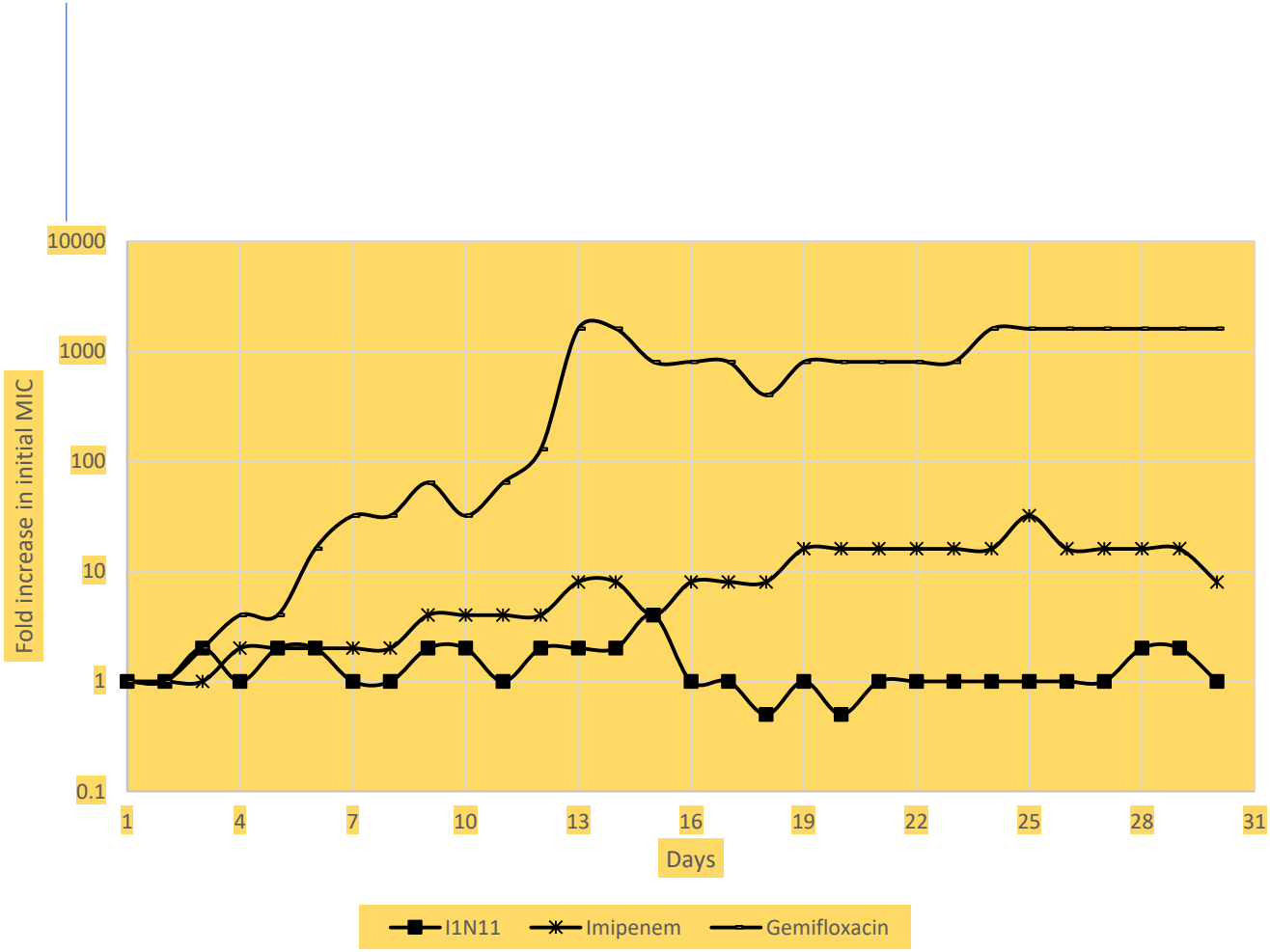
**Bacterial growth inhibition by membrane permeabilization**



# 3-Results: AntibioResistance and SAR




| Peptides | Primary Structure | MW (Da) | size | Net Charge |
|---|---|---|---|---|
| | | | | |
| gallicune-1 ($I_1N_{11}$) | ILKAIKKLLKN | 1281 | 11 | +5 |
| gallicune-1 (i1D-n11D) | iLKAIKKLLKn | 1281 | 11 | +5 |
| (gallicune-1) ($I_1N_{11}$) D | Ilkaikkllkn | 1281 | 11 | +5 |
| $I_1L_9$ | ILKAIKKLL | 1039 | 9 | +4 |
| $I_1K_7$ | ILKAIKK | 813 | 7 | +4 |
| $L_2L_9$ | LKAIKKLL | 926 | 8 | +4 |
| $L_2k_{10}$ | LKAIKKLLk | 1054 | 8 | +5 |
| $K_3N_{11}$ | KAIKKLLKN | 1055 | 9 | +5 |
| Ac $I_1N_{10}$ | Ac-IKAIKKLLKN | 1210 | 10 | +4 |
| $I_1K_{10}$ | ILKAIKKLLK | 1167 | 10 | +5 |
| $i_{1D}k_{10D}$ | iLKAIKKLLk | 1167 | 10 | +5 |
| ($i_1k_{10}$*)D | Ilkaikkllk | 1167 | 10 | +5 |

# 3-Results: Pharmacological characterizations

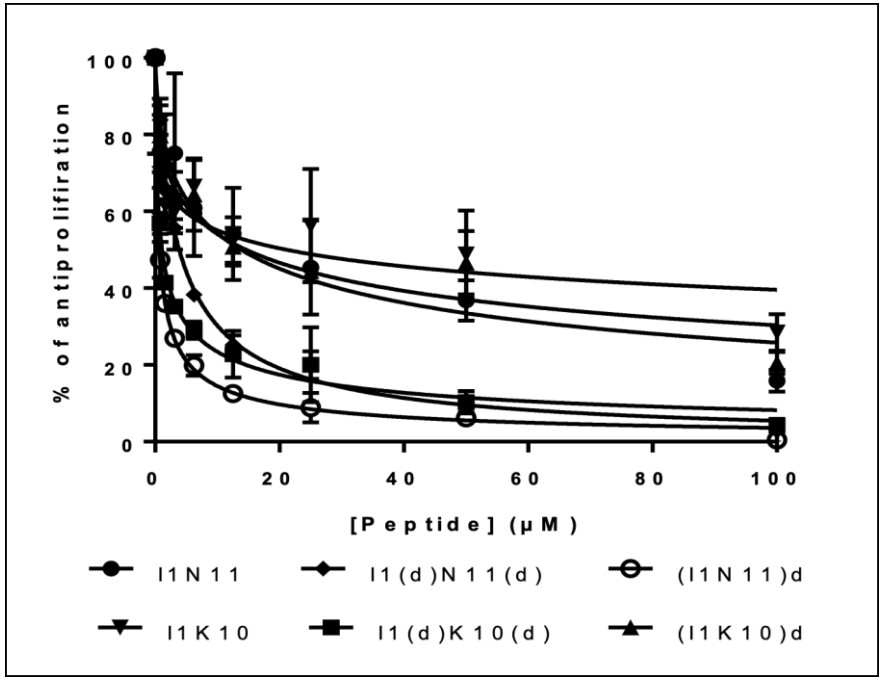
| MIC (µM) | | I1N11 | I1(d)N11(d) | (I1N11)d | I1K10 | I1(d)K10(d) | (I1K10)d |
|---|---|---|---|---|---|---|---|
| Gram+ | L. lactis | 1.5 | 1.5 | 1.5 | 3.125 | 0.78 | 1.5 |
| | B. subtilis (Nisin-resistant) | 12.5 | 6.25 | 6.25 | 12.5 | 3.12 | 12.5 |
| | A. gandavensis | 3.12 | 1.5 | 1.5 | 3.12 | 0.78 | 1.5 |
| Gram - | E. coli EHEC K88 | 100 | 25 | 50 | 100 | 6.25-12.5 | 50 |
| | P. aeruginosa | 6.25 | 1.5 | 1.5 | 3.125 | 0.78 | 3.125 |
| | P. aeruginosa FQR | 25 | 6.25-12.5 | 6.25-12.5 | 12.5-25 | 3.12-6.25 | 12.5-25 |
| | P. aeruginosa 01 | 25 | 6.25 | 6.25 | 12.5 | 3.125 | 12.5 |
| | S. flexneri | 50 | 12.5 | 12.5 | 25 | 3.12-6.25 | 12.5 |
| | C. farmeri | 12.5 | 6.25 | 6.25 | 25 | 3.12 | 12.5 |
| | C. rodentium | 50 | 25 | 12.5 | 100 | 12.5 | 50 |
| | S. enterica | 100 | 25 | 25 | 50 | 12.5 | 50 |
| | V. alginolyticus | 0.78 | 0.78 | 0.78 | 1.56 | 0.78 | 0.78-1.56 |
| | H. pylori | 100 | 25 | 50 | 50 | 12.5 | 50 |
| Mycobact | M. smegmatis | 25 | 25 | 25 | 50 | 12.5 | 6.25 |

# 3-Results: Pharmacological characterizations

| Cytottoxicity (C50) and MIC of P. aeruginosa | | | | | | |
|---|---|---|---|---|---|---|
| | I1N11 | I1(d)N11(d) | (I1N11)d | I1K10 | I1(d)K10(d) | (I1K10)d |
| **MIC PA** | 6.25 | 1.5 | 1.5 | 3.125 | 0.78 | 3.125 |
| **IMR90** | 892.5 | 362.1 | 653.1 | 368.4 | 782.7 | 364.8 |
| **HUVEC** | 825.9 | 435.5 | 869 | 781.8 | 814.3 | 682.8 |
| **BEAS** | 820.9 | 390.8 | 843.4 | 389.8 | 866.6 | 725.8 |

| Therapeutic Index (IC50 compared to P. aeruginosa atcc) | | | | | | |
|---|---|---|---|---|---|---|
| | I1N11 | I1(d)N11(d) | (I1N11)d | I1K10 | I1(d)K10(d) | (I1K10)d |
| **IMR90** | 142.8 | 241.4 | 435.4 | 117.888 | 1003.462 | 116.736 |
| **HUVEC** | 132.144 | 290.3333 | 579.3333 | 250.176 | 1043.974 | 218.496 |
| **BEAS** | 131.344 | 260.5333 | 562.2667 | 124.736 | 1111.026 | 232.256 |

MIA Paca




**IC50 1.08 uM (I1DK10D) to 23.02 uM I1K10**

# 3-Conclusions & Perspectives

**1. Screening**

Collection of venoms

Antimicrobial activity

Inhibition test: liquid & solid medium

**Active venoms**

- % against : G+ & G- /non-pathogenes & pathogenes?
- %? Pathogènes only

Identification of undescribed venoms

Bibliographic study

Identification of molécules

**PERSPECTIVES**

- Test against multi-resistant strains?
- Anti-biofilm effect?
- Resistance induction?
- Synergy test (PAMs, antibiotics)?
- Xenograft in mice

European patent Peptide
Lead in development
With potential applications
in several health fields

# Thank you

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Dr. Régine Lebrun

Plateforme Protéomique et Spectrométrie de Masse
Dr. Patrick Fourquet

Plateforme d'AAA
Jean-Pierre Andrieu

*Synthétiser un peptide reste un "art".*