The 9th International Electronic Conference on Medicinal Chemistry (ECMC 2023)
01–30 November 2023 | Online

New 2-heteroaryl-4-aminoquinolines to tackle *Pseudomonas aeruginosa* virulence

Chaired by Dr. Alfredo Berzal-Herranz and Prof. Dr. Maria Emília Sousa

pharmaceuticals MDPI

Marie Hanot [1,*], Elodie Lohou [1], François Peltier [1] and Pascal Sonnet [1]

[1] Laboratoire AGIR, UR 4294, Université de Picardie Jules Verne (UPJV), UFR de pharmacie, 1 rue des louvels, 80037 Amiens, France

* Corresponding author: marie.hanot@u-picardie.fr

Abstract

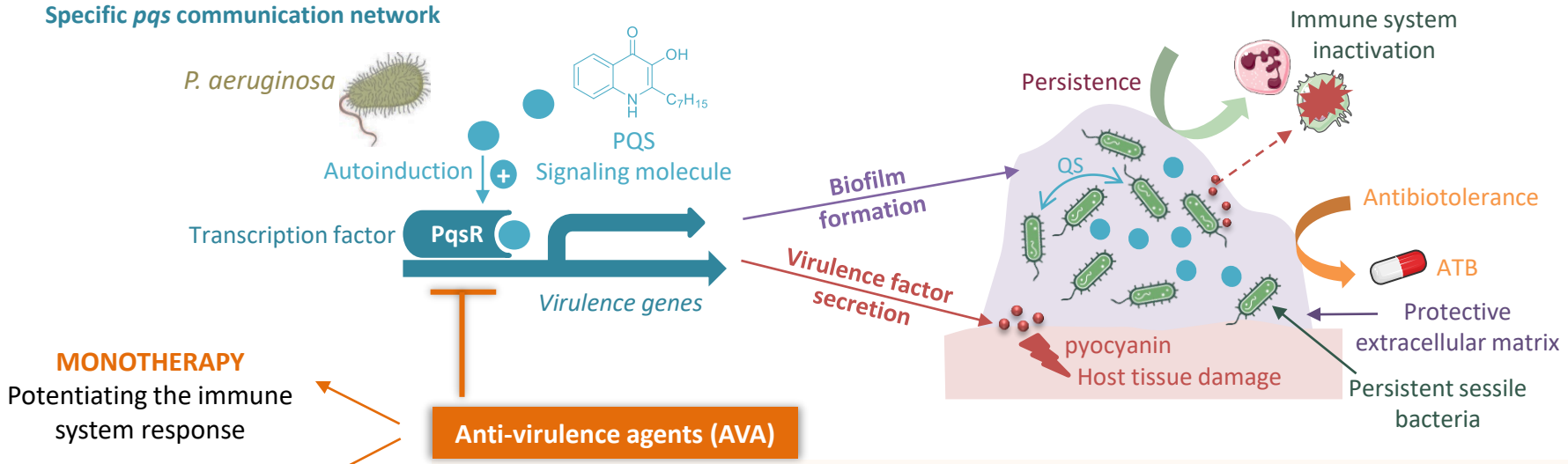
The multi-drug resistant pathogen *Pseudomonas aeruginosa* has been designated by the WHO as a high-priority for the development of new anti-infective treatments. Among Gram-negative bacteria, this species secretes a characteristic cytotoxic pigment called pyocyanin and is able to form biofilms that act as protective barriers against the immune system and antibiotics. Such pathogenicity is mainly regulated by the quorum sensing (QS) pathways that orchestrate the bacterial communication according to the population density. *P. aeruginosa* possesses a specific QS system : *pqs*. In this circuit, the transcription factor PqsR stimulates the expression of virulence-related genes *via* recognition of its auto-inducer PQS (*Pseudomonas* Quinolone Signal). This notably controls the secretion of pyocyanin and the establishment of biofilms. Therefore, the development of QS inhibitors as anti-virulence agents (AVA) able to tackle *P. aeruginosa* without affecting bacterial growth appears as a promising strategy to circumvent the selection pressure mediated by conventional antibiotherapy. Ultimately, they could restore the efficacy of antibiotics in dual therapy or potentiate the immune system response in monotherapy. In particular, the design of PqsR inhibitors seems like a sustainable approach to combat *P. aeruginosa* specifically. In the literature, benzamide-benzimidazole, indole-naphtalene and benzofurane-aminoquinoline hybrids have been reported as such quenchers. Meanwhile, our team discovered a hit 2-heteroaryl-4-quinolone that displays interesting anti-biofilm and anti-pyocyanin activities. By structural analogy with these bi-aromatic molecules, we have recently developed a new family of 2-heteroaryl-4-aminoquinolines with promising anti-virulence properties. The presentation describes the synthesis of our new AVA as well as their physicochemical and biological evaluation.

Keywords: Multi-resistant bacteria; *Pseudomonas aeruginosa*; Biofilm; Quorum Sensing; anti-virulence agents.

The 9th International Electronic Conference on Medicinal Chemistry
01–30 November 2023 | Online

New 2-heteroaryl-4-aminoquinolines to tackle *Pseudomonas aeruginosa* virulence

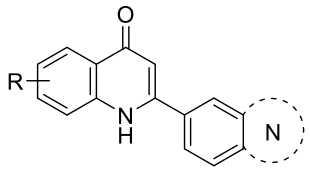
Graphical Abstract



Introduction

Design strategy of novel 2-heteroaryl-4-aminoquinolines as anti-virulence agents (AVA)

1st AVA family developed in AGIR lab
*Hit compound **IQO-1***



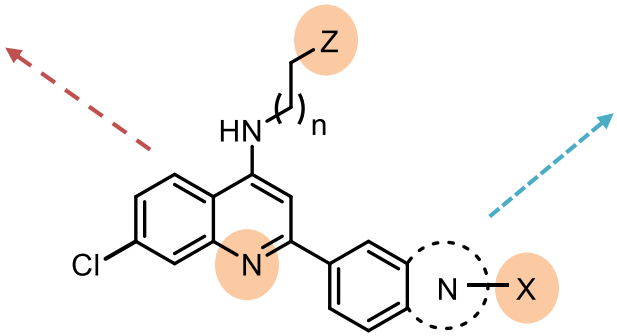
2-heteroaryl-4-quinolone
IQO-1
(R=7-Cl)

Validated biological prerequisites

- ✓ No bacteriostatic effect on *P. aeruginosa*
- ✓ Low to moderate cytotoxicity in a human HepG2 cell line: CC_{50} = 97% (**IQO-1**)

Anti-virulence efficacy in *P. aeruginosa* PAO1 strain

- ✓ Anti-biofilm activity: 34 and 32% inhibition at 25 and 50 µM, respectively
- ✓ Anti-pyocyanin activity: 35% inhibition at 100 µM

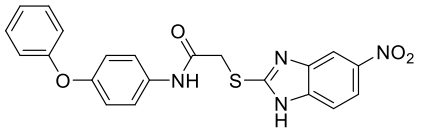


2-heteroaryl-4-aminoquinolines

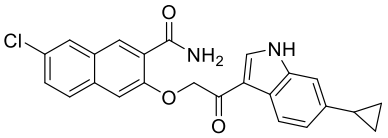
n = 1-7
Z = NH_2, NHR_1, NR_2R_3, …
X = H, $(CH_2)_{0-1}COOR_4$, …

Ionizable functions at physiological pH
→ Facilitated transport across the lipopolysaccharidic diderm barrier of Gram-negative bacteria *via* porins

Bi-aromatic PqsR inhibitors described in the literature



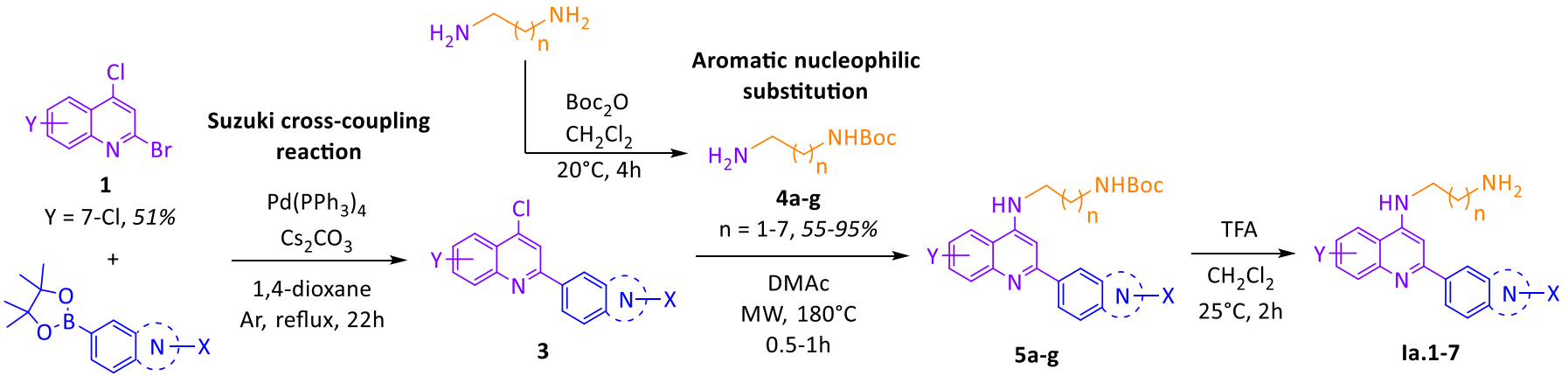
Benzamide-benzimidazole
M64 (IQS-1)
PqsR inhibition (IC_{50}): 0.32[a]/1.22[b] µM
Anti-biofilm activity [a]: 50% inhibition at 10 µM
Anti-pyocyanin activity (IC_{50}) [b]: 0.3 µM
Tested strains: [a]PAO1, [b]PA14
No cytotoxicity on lung epithelial cells (A549)



Indole-naphtalene
SPR-00305 (IQS-2)
Anti-PQS activity (IC_{50}): 0.05-0.25 µM
Anti-pyocyanin activity (IC_{50}): 0.05-0.25 µM
Tested strain: PA14
Eukaryotic cytotoxicity: not determined

Results & Discussion

Synthesis of 2-heteroaryl-4-aminoquinolines

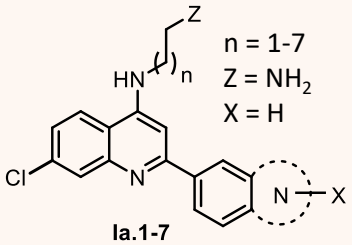


Key aminoquinoline hybrids				Final products		
X	**n**	**5**	**Yield (%)**	**Ia**	**Yield (%)**	**Purity* (%)**
H	1	**5a**	54	**Ia.1**	75	92
H	2	**5b**	45	**Ia.2**	96	99
H	3	**5c**	46	**Ia.3**	93	93
H	4	**5d**	31	**Ia.4**	81	91
H	5	**5e**	28	**Ia.5**	59	96
H	6	**5f**	68	**Ia.6**	88	93
H	7	**5g**	40	**Ia.7**	55	96

Results & Discussion

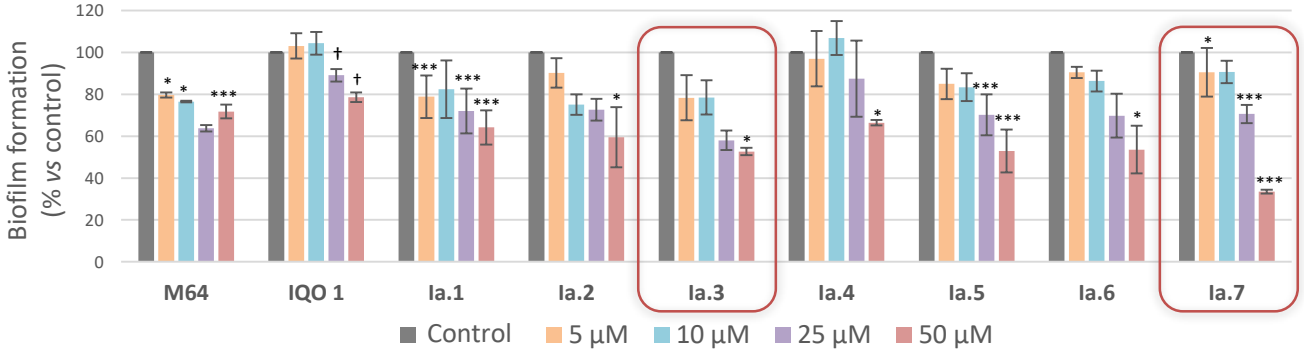
Biological prerequisite study

No effect on pseudomonal growth → **Validation of the biological prerequisite for the development of AVA**



Anti-virulence evaluation

Anti-biofilm activity (PAO1 strain) → Biofilm staining with crystal violet and quantification *via* UV/Vis spectrometry



Anti-biofilm activities at 50 µM

Ia.1-7 > **M64** & **IQO-1**

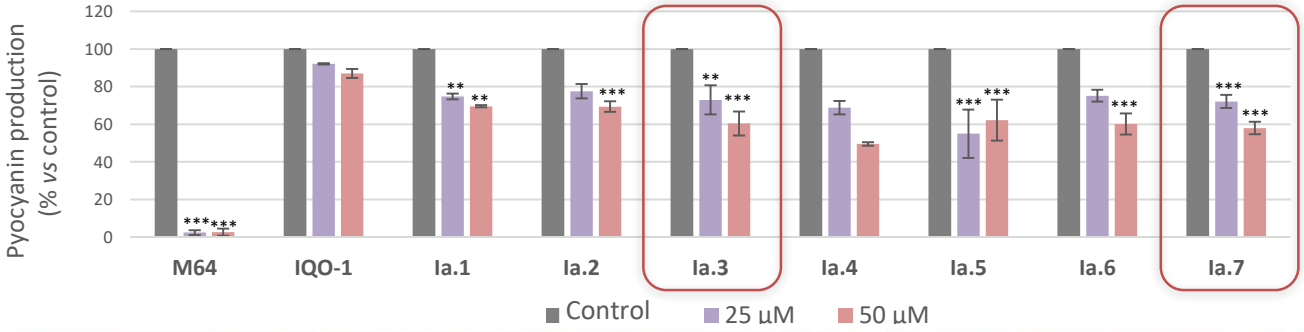
Anti-pyocyanin activities at 50 µM

Ia.1-7 << **M64**

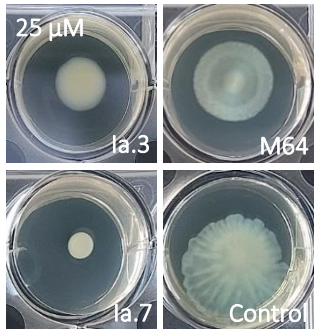
Ia.1-7 > **IQO-1**

Most potent compounds: Ia.3 & Ia.7

Anti-pyocyanin activity (PAO1 strain) → Pyocyanin extraction and quantification *via* UV/Vis spectrometry

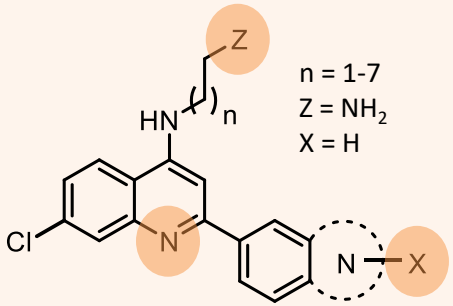


Anti-swarming activity (PAO1 strain)



Conclusion & Perspectives

Synthesis of seven new 2-heteroaryl-4-aminoquinolines



Ia.1-7

✓ 5 steps
Global yields : 5-21 %

✓ Ionizable functions at physiological pH (*in silico* and experimental results)
→ Facilitated penetration of the outer membrane *via* porins

Compounds Ia.3 & Ia.7 (n = 3 & 7)
✓ No bacteriostatic activity on *P. aeruginosa*
✓ Potent anti-virulence activity on the PAO1 strain
- Anti-biofilm activity : 47 & 67% inhibition at 50 µM
- Anti-pyocyanin activity : 40 & 42% inhibition at 50 µM
- Anti-swarming activity from 25 µM

	IQO-1	M64	Ia.3 (n=3)	Ia.7 (n=7)
Eukaryotic cytotoxicity on a human HepG2 cell line CC_{50} (µM)	97 ± 5	58 ± 7	8 ± 3	**39 ± 2**

Moderate cytotoxicity on human cells for hybrid **Ia.7** (*vs* **Ia.3**)
→ **New hit AVA**

→ Ongoing pharmacomodulations on the bi-aromatic scaffold to expand the efficacy screening

The 9th International Electronic Conference on Medicinal Chemistry (ECMC 2023)
01–30 November 2023 | Online

New 2-heteroaryl-4-aminoquinolines to tackle *Pseudomonas aeruginosa* virulence

Chaired by Dr. Alfredo Berzal-Herranz and Prof. Dr. Maria Emília Sousa

pharmaceuticals MDPI

Marie Hanot [1,*], Elodie Lohou [1], François Peltier [1] and Pascal Sonnet [1]

[1] Laboratoire AGIR, UR 4294, Université de Picardie Jules Verne (UPJV), UFR de pharmacie, 1 rue des louvels, 80037 Amiens, France

* Corresponding author: marie.hanot@u-picardie.fr