Development of new anti-virulence agents to tackle multi-resistant *Pseudomonas aeruginosa*

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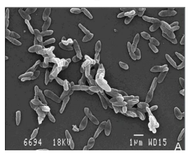
A new therapeutic anti-virulence strategy against *Pseudomonas aeruginosa*

P. aeruginosa



- Nosocomial opportunistic gram-negative bacillus responsible for lung, bloodstream, skin, ocular and urinary tract infections
- Major cause of chronic and lethal infections in cystic fibrosis patients [1]
- Part of the ESKAPEE group of multi-drug resistant bacteria designated by the WHO as a priority for the development of new treatments

Pathogenicity :

- Formation of persistent biofilms that act as protective barriers against the immune system and antibacterial agents
- Secretion of virulence factors like pyocyanin implicated in the host infection process [2]

→ The design of non-bactericidal agents able to quench virulence pathways appears as a promising approach. They could restore the efficacy of conventional antibiotics (ATBs) when used in combination therapy, without creating selection pressure issues (Fig. 1).

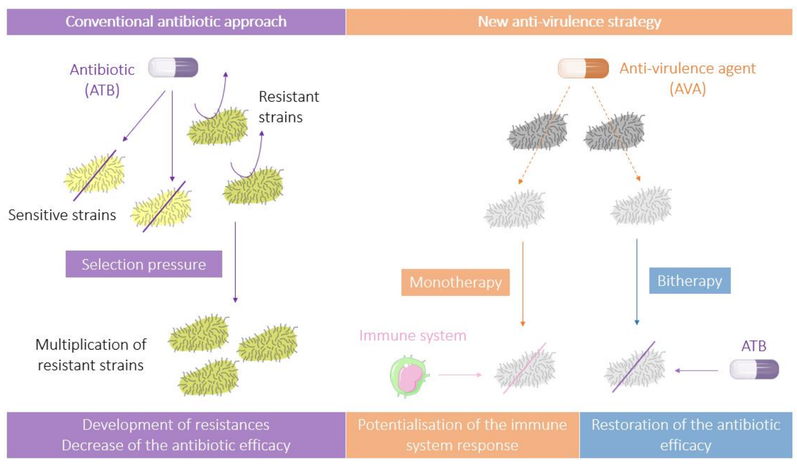


Figure 1: New anti-virulence strategy *vs* conventional antibiotic approach

A pool of targets for the development of anti-virulence agents (AVAs) is the Quorum Sensing : a bacterial communication network responsible for virulence pathway expression according to the population density. In *P. aeruginosa* specific quorum sensing system *pqs*, the transcription factor PqsR regulates the activation of virulence-related genes *via* recognition of its auto-inducer PQS (Pseudomonas Quinolone Signal). This circuit stimulates the secretion of virulence factors like pyocyanin as well as the establishment of biofilms. Therefore, the design of AVAs that inhibit PqsR appears to be a sustainable strategy to combat *P. aeruginosa* specifically (Fig. 2).[3,4]

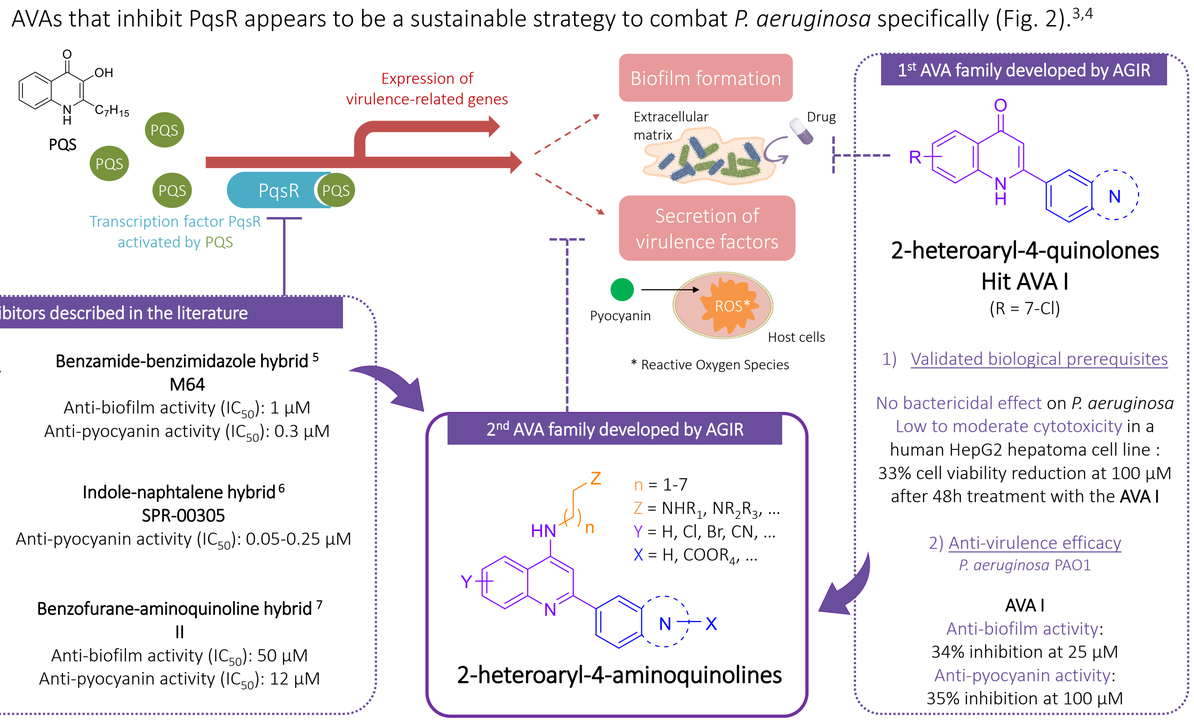


Figure 2: Development of 2-heteroaryl-4-aminoquinolines as new AVAs

Several bi-aromatic PqsR inhibitors have been described in the literature. Among them, the compounds **M64** and **SPR-00305** are currently in preclinical stage.[5,6] Moreover, our team designed a series of 2-heteroaryl-4-quinolones and discovered the **hit AVA I**. Another recently reported building block **II** possessing an aminoquinoline moiety displays interesting anti-virulence properties.[7] By structural analogy, we aim to develop a new family of 2-heteroaryl-4-aminoquinoline hybrids as new AVAs potentially inhibiting PqsR.

Synthesis of 2-heteroaryl-4-aminoquinolines

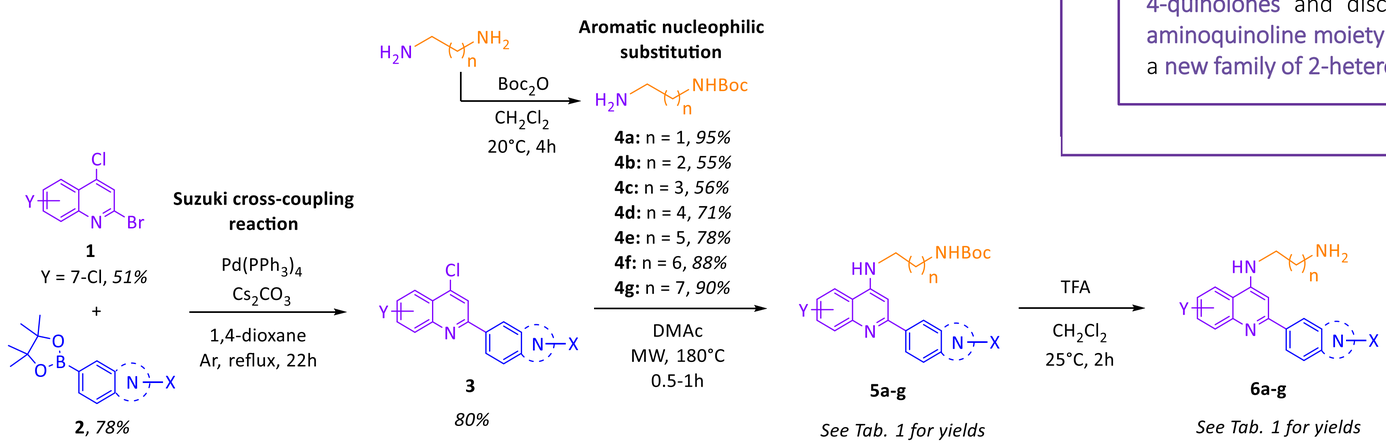


Figure 3: Synthesis of new 2-heteroaryl-4-aminoquinolines

The synthesis of the key 4-aminoquinoline hybrids **5** was achieved *via* two key steps. First, intermediates **3** were obtained by Suzuki cross-coupling reaction between 2-bromo-4-chloroquinoline precursors **1** and heteroarylboronic esters **2** that were prepared beforehand. Then, a microwave-enhanced aromatic nucleophilic substitution was performed on the 2-heteroaryl-4-chloroquinoline **3** with the *N*-Boc-protected diamines **4a-g** (Fig. 3). Finally, protecting group removal under acidic conditions provided the expected products **6a-g** with excellent yields (Tab. 1).

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

*HPLC analysis

Table 1: Key aminoquinoline hybrids and final products

Physicochemical and biological prerequisite study

In silico predicted physicochemical properties				
Compounds			*clogP*$_{o/w}$*	pKa**
Ciprofloxacin			0.28	6.2 (COOH) 8.6 (N_2, piperazine)
Hit AAV I			2.82	12.36 (OH, 4-quinolinol) 4.53 (N_1, 4-quinolinol)
	X	Y	n	
6a	H	7-Cl	1	2.58	7.1 (N, quinoline) 10.1 (NH_2)
6b	H	7-Cl	2	2.96	7.5 (N, quinoline) 10.2 (NH_2)
6c	H	7-Cl	3	3.32	7.6 (N, quinoline) 10.2 (NH_2)
6d	H	7-Cl	4	3.64	7.6 (N, quinoline) 10.2 (NH_2)
6e	H	7-Cl	5	4.03	7.6 (N, quinoline) 10.2 (NH_2)
6f	H	7-Cl	6	4.36	7.6 (N, quinoline) 10.2 (NH_2)
6g	H	7-Cl	7	4.67	7.6 (N, quinoline) 10.2 (NH_2)

Table 2: *clogP*$_{o/w}$ and pKa values for 2-heteroaryl-4-aminoquinolines calculated *via* *Qikprop and **Epik softwares.

Contrary to Gram-positive bacteria, Gram-negative bacteria possess a lipopolysaccharidic diderm barrier that diminishes the permeability to therapeutic molecules as well as their chances of reaching their target. This is why drugs have to fulfill several physicochemical prerequisites. Our 2-heteroaryl-4-aminoquinolines make no infraction to the Lipinski rules. *clogP*$_{o/w}$ values reveal a stronger lipophilicity of compounds **6e-g** than hybrids **6a-d** that could be in favor of a passive diffusion (Tab. 2). Anyway, the presence of ionizable functions at physiological pH should facilitate the cell entry *via* porins.

Hybrids **6a-c** were tested for their bactericidal activity. They showed no effect on bacterial growth that is a favorable result for AVAs (Tab. 3).

MIC (µg/mL)			
Compounds	*S. aureus* CIP 103.29	*P. aeruginosa* DSM 1117	*E. coli* DSM 1103
Ciprofloxacin	0.06	0.06	0.06
6a	≈ 256	≈ 256	≈ 256
6b	≈ 256	≈ 256	≈ 256
6c	≈ 256	≈ 256	≈ 256

Table 3: MIC of 2-heteroaryl-4-aminoquinolines 6a-c on three ESKAPEE bacterial strains.

Anti-virulence evaluation

The evaluation of anti-pyocyanin properties of the synthesized products was carried out on *P. aeruginosa* PAO1 strain measuring the pigment concentration by UV/Vis spectrometry. Compounds **6a, 6b** and **6c** revealed more efficient to inhibit pyocyanin production than the hit **AAV I** with a decrease of 30.5, 30.7 and 43.4% at 50 µM, respectively (Fig. 4).

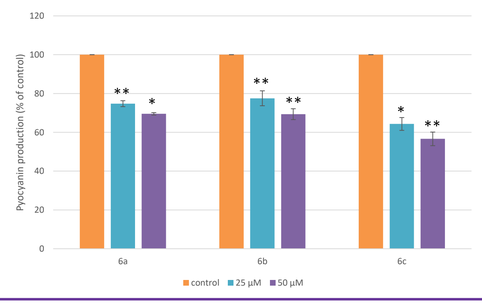


Figure 4: Evaluation of pyocyanin production on *P. aeruginosa* PAO1 strain.
Measurement of the pigment concentration by UV/Vis spectrometry after 48h growth in the presence or absence of new 2-heteroaryl-4-aminoquinolines at different concentrations. Bars represent the mean ± SD of at least three independent experiments performed in triplicate. *p < 0.05 and **p < 0.01 *vs* control (Mann-Whitney's test : p values < 0.05 were considered significant).

Conclusion and perspectives

Seven new 2-heteroaryl-4-aminoquinoline hybrids have been synthetized with global yields of 5 to 21%. Promising physicochemical and anti-virulence properties have been highlighted for non-bactericidal molecules **6a-c**. Further biological evaluations including anti-biofilm, anti-pyocyanin and cytotoxicity assays as well as extended pharmacomodulations on the bi-aromatic scaffold are ongoing to expand the efficacy screening.

References : 1. Malhotra, S. *et al. Clin. Microbiol. Rev.* **2019**, *32*, e00138-18. 2. Jurado-Martín, I. *et al. IJMS* **2021**, *22*, 3128. 3. Duplantier, M.; Lohou, E.; Sonnet, P. *Pharmaceuticals* 2021, *14*, 1262. 4. Rather, M.A. *et al. Microbiol. Res.* **2022**, *264*, 127173. 5. Starkey, M. *et al. PLoS Patholog.* **2014**, *10*, e1004321. 6. Zahler, R. WO **2016**/112088. 7. Aleksic, I. *et al. ACS Chem. Biol.* **2019**, *14*, 2800-2809.