Tackling multi-drug resistance in *Pseudomonas aeruginosa* thanks to a new promising anti-virulence strategy

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INTRODUCTION

The antibiotic resistance constitutes a critical public health issue. Among incriminated multi-drug resistant microorganisms, ***Pseudomonas aeruginosa* has been pointed out by the WHO as a priority threat**. This Gram-negative bacteria (GNB) is responsible for numerous nosocomial infections, usually lethal for patients suffering from cystic fibrosis. **Its ability to develop biofilms reinforces its pathogenicity and intrinsic drug resistance. Its virulence is orchestrated by the quorum sensing (QS) that refers to a sophisticated communication network** (Figure 1). QS molecular pathways rely on the release and perception of autoinducers (AIs). The extracellular concentration of these signaling molecules acts as a population density indicator. The biomass growth provokes an increased secretion of AIs inducing the expression of QS-associated genes *via* the activation of specific transcription factors. This stimulation ensures the biosynthesis of essential proteins for the synchronisation of bacteria colonies regarding the environmental medium and especially those implicated in the virulence pathways. Three interconnected QS systems regulate *P. aeruginosa* pathogenicity. Taking into account the widespread occurrence of *N*-acyl-homoserine lactone-mediated communication *las* and *rhl* circuits in GNB, the third species-specific *pqs* network appears as a pool of promising therapeutic targets for the development of inhibitors. The main AI of this circuit is the 2-heptyl-3-hydroxy-4(1*H*)-quinolone named *Pseudomonas* quinolone signal (PQS) that activates the PqsR transcriptional regulator.[1]

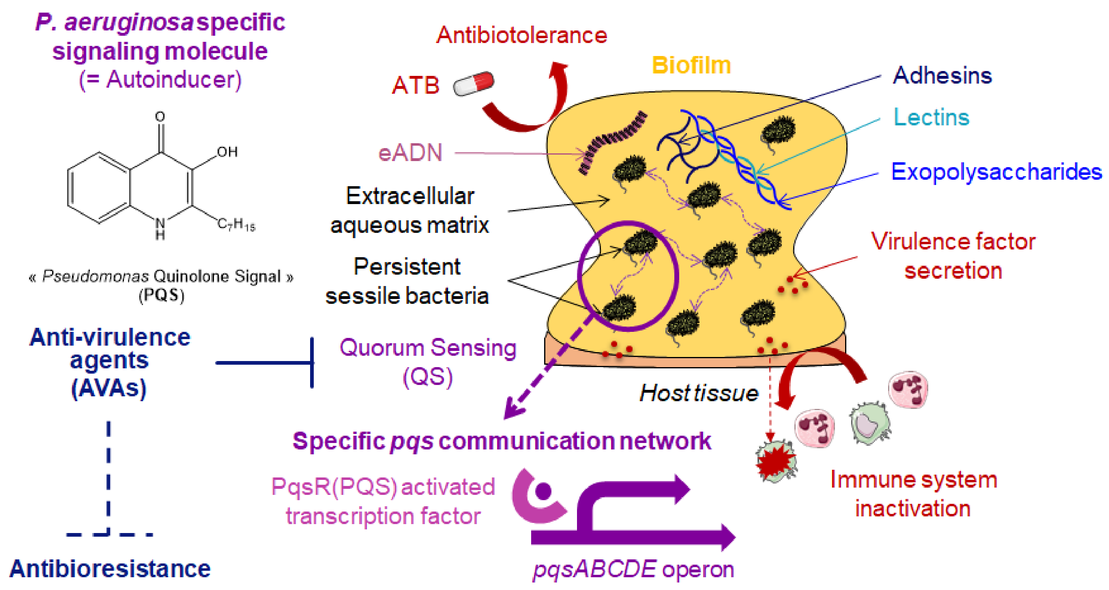


Figure 1. QS-orchestrated *P. aeruginosa* pathogenicity.

In the last decades, the interest of a quorum silencing pharmacological approach has emerged. Indeed, the selective pressure put on sensitive bacteria by conventional antimicrobial molecules causing their death promotes resistant strain survival. **Non-bactericidal anti-virulence agents (AVAs) could increase pathogen sensibility to the host immune system response in monotherapy. In combination therapy, they could restore the efficiency of current antibiotics (ATBs) by inhibiting the formation of the hermetic barrier provided by biofilms.**[1]

RESULTS AND DISCUSSION

Design, synthesis and *in silico* physicochemical study of new 2-heteroaryl-4-quinolones

A benzamide-benzimidazole hybrid appears as one of the most promising PqsR inhibitor in preclinical stage.[2] It revealed the best anti-virulence activity among all reported in the literature. Several alkylquinolone autoinducer analogs also demonstrated efficient anti-pyocyanin and anti-biofilm properties. With this in mind, our team has recently developed **a novel family of biaryl quinolone-based hybrids as AVAs** through a transdisciplinary research methodology (Figure 2).

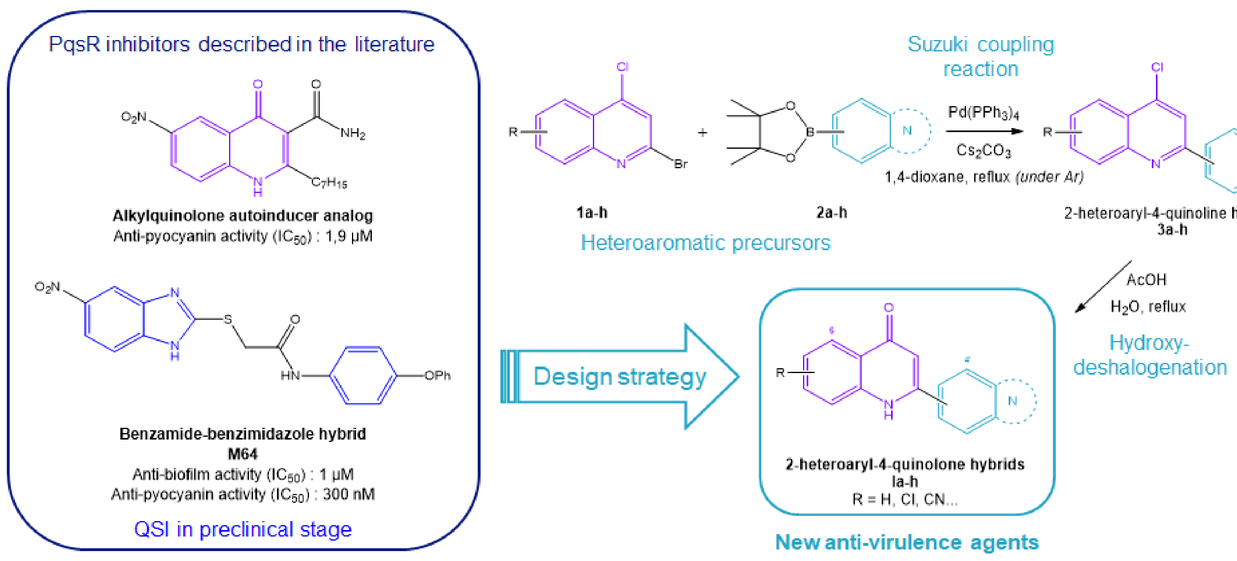


Figure 2. Design and synthesis of new biaryl quinolone-based hybrids as anti-virulence agents.

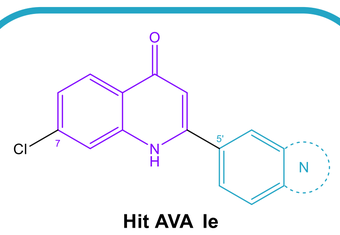
Heteroaromatic precursors				2-heteroaryl-4-quinoline hybrids 3a-h		2-heteroaryl-4-quinolone hybrids Ia-h		
2-bromo-4-chloroquinoline (R)		Pinacol heteroarylboronic (pinB) ester		Yields (%)		Yields (%)		$clogP_{o/w}$ (*QikProp, Schrödinger software*)
1a	H	**2a**	4'-pinB	**3a**	78	**Ia**	81	2.417
1c	7-Cl			**3b**	63	**Ib**	98	2.882
1a	H	**2b**	5'-pinB	**3c**	90	**Ic**	77	2.370
1b	6-CN			**3d**	83	**Id**	61	1.647
1c	7-Cl			**3e**	82	**Ie**	81	2.818
1a	H	**2c**	6'-pinB	**3f**	62	**If**	53	2.414
1b	6-CN			**3g**	32	**Ig**	quantitative	1.697
1c	7-Cl			**3h**	58	**Ih**	68	2.881

Moderate lipophilicity of new synthetized 2-heteroaryl-4-quinolones
⇔ Transport inside the bacterial cell by passive diffusion or endocytosis as for PQS ?

Biological evaluations

↳ Prerequisites

① No effect on *P. aeruginosa* bacterial growth
(DSM 1117 strain)
⇔ Expected result for AVAs
② Low to moderate cytotoxicity
in a human HepG2 hepatoma cell line after 48 h of treatment at 100 µM



Hit AVA Ie

Anti-biofilm activity
34% inhibition at 25 µM
Anti-pyocyanin activity
35% inhibition at 100 µM
(P. aeruginosa PAO1 strain)

↳ Anti-biofim activity

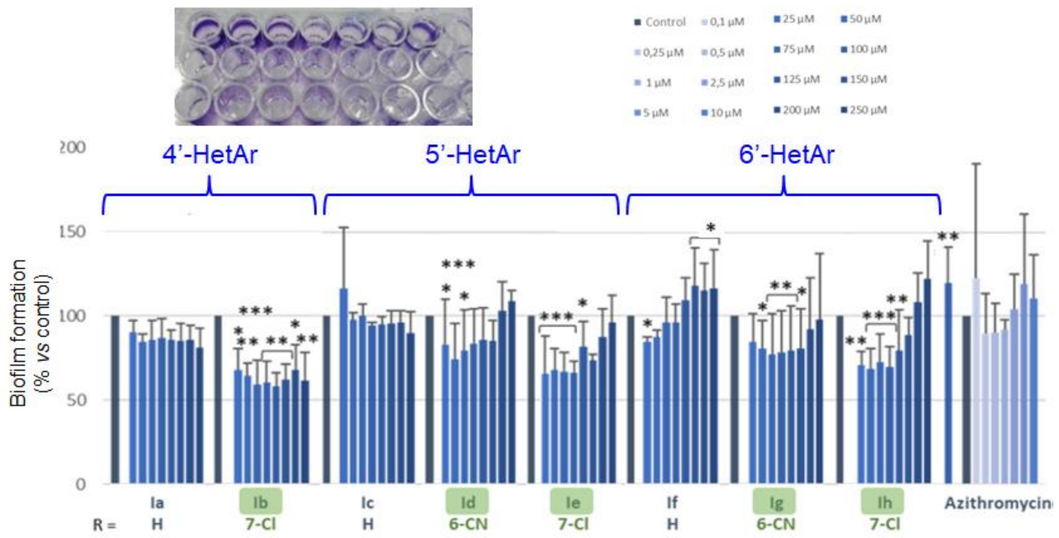


Figure 3. Evaluation of biofilm formation on *P. aeruginosa* PAO1 strain by quantitative analysis thanks to crystal purple dyeing, after 24h of growth in the presence or absence of new 2-heteroaryl-4-quinolones or azithromycin reported as an anti-biofilm reference (IC_{50} = 6.75 µM)[3] at different concentrations. Bars represent the mean ± SD of at least three independent experiments performed in triplicate. **p* < 0.05; ***p* < 0.01 and * *p* < 0.001 *vs* control (Mann-Whitney's test : *p* values < 0.05 were considered significant).**

↳ Anti-pyocyanin activity

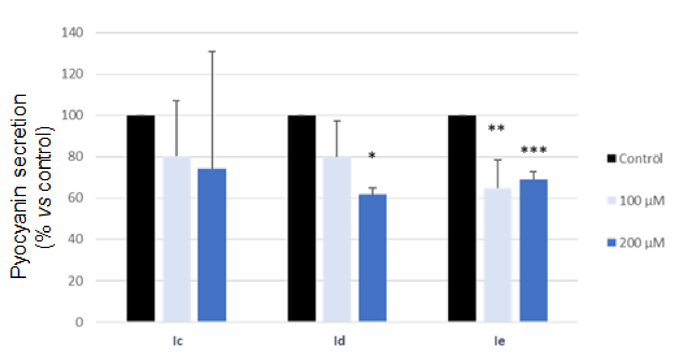


Figure 4. Evaluation of pyocyanin secretion on *P. aeruginosa* PAO1 strain by measurement of the specific pigment concentration by UV/Vis spectrometry, after 48h of growth in the presence or absence of new 2-heteroaryl-4-quinolones at different concentrations. Bars represent the mean ± SD of at least three independent experiments performed in triplicate. **p* < 0.05; ***p* < 0.01 and * *p* < 0.001 *vs* control (Mann-Whitney's test : *p* values < 0.05 were considered significant).**

CONCLUSION

Eight quinolone-based hybrids have been synthesized in 4-5 steps with global yields of 10 to 51%. **The 7-chloro derivative Ie exhibited promising anti-biofilm and anti-pyocyanin properties without affecting the bacterial growth**. Following the structure-activity and structure-property relationship studies, extended pharmacomodulations on the biaryl scaffold are currently under progress to expand the efficacy screening and improve the drugability of the hit AVA **Ie**, especially its capacity to infiltrate *P. aeruginosa* lipopolysaccharidic diderm barrier.

REFERENCES

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