ECA 2023
The 3rd International Electronic Conference on Antibiotics
Rise of Antibiotic Resistance: Mechanisms Involved and Solutions to Tackle it
01–15 December 2023 | Online

New 2-heteroaryl-4-aminoquinolines as *Pseudomonas aeruginosa* virulence quenchers

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A NEW THERAPEUTIC ANTI-VIRULENCE STRATEGY AGAINST MULTI-DRUG RESISTANT *P. AERUGINOSA*

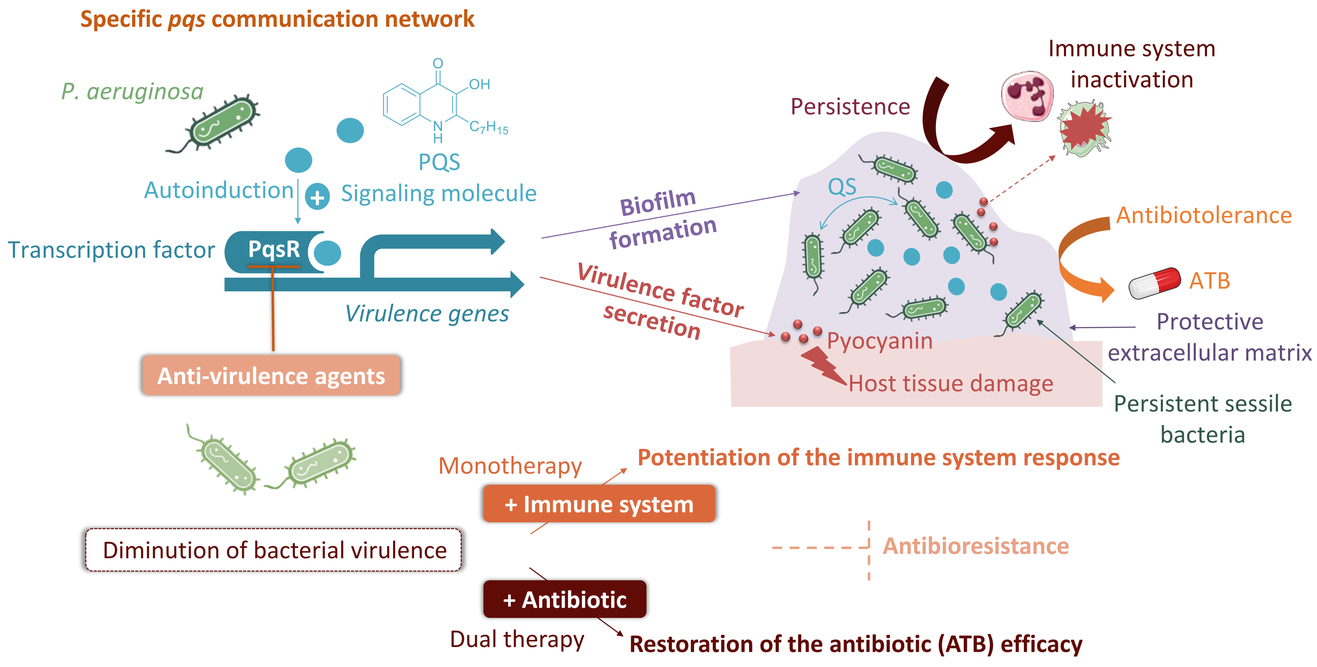


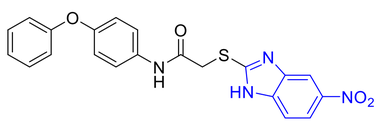
Figure 1: Inhibiting quorum sensing (QS) to quench pseudomonal virulence and antibioresistance

In the struggle against multi-drug resistant bacterial infections, the opportunistic pathogen *Pseudomonas aeruginosa* has been identified by the WHO as a priority for the development of new treatments.[1] This Gram-negative bacterium produces a characteristic cytotoxic pigment called pyocyanin and is able to form biofilms that act as protective barriers against the immune system and antibiotics (ATB). Its pathogenicity is coordinated by the quorum sensing (QS) that is the bacterial communication network responsible for pathogenicity expression according to the population density. In the *P. aeruginosa* specific QS 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 pyocyanin as well as the establishment of biofilms (Fig. 1).[2]

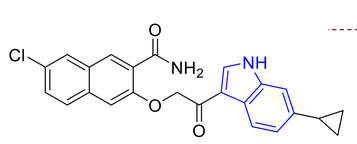
Therefore, the development of quorum quenchers that disrupt connections without affecting bacterial growth appears as a promising strategy to circumvent selection pressure issues over sensitive strains mediated by conventional antibiotherapy. These new anti-virulence agents (AVA) could restore the efficacy of antibiotics when used in dual therapy or potentiate the immune system response in monotherapy (Fig. 1). In particular, the design of PqsR inhibitors as AVA seems like a sustainable approach to combat *P. aeruginosa* specifically.[3]

Bi-aromatic molecules targeting PqsR have been reported in the literature.[4-5] Meanwhile, our team discovered a hit 2-heteroaryl-4-quinolone compound that displays interesting anti-biofilm and anti-pyocyanin activities. By structural analogy, we have recently developed a new family of 2-heteroaryl-4-aminoquinolines, as potential PqsR inhibitors with anti-virulence properties (Fig. 2).

Bi-aromatic PqsR inhibitors described in the literature

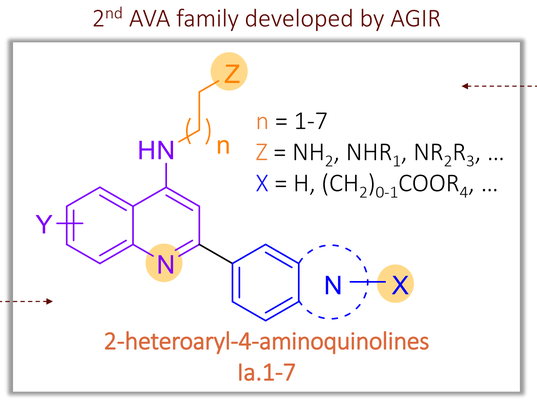


Benzamide-benzimidazole hybrid (M64) [4]
PqsR inhibition (IC_{50}): 0.32[a]/1.22[b] µM
Anti-biofilm activity[b]: 50% inhibition at 10 µM
Anti-pyocyanin activity (IC_{50})[b]: 0.3 µM
Tested strains: [a]PAO1, [b]PA14
No cytotoxicity on lung epithelial cells (A549)



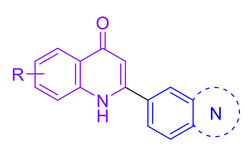
Indole-naphtalene hybrid (SPR-00305) [5]
Anti-PQS activity (IC_{50}): 0.05-0.25 µM
Anti-pyocyanin activity (IC_{50}): 0.05-0.25 µM
Tested strain: PA14
Eukaryotic cytotoxicity: not determined

2nd AVA family developed by AGIR



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

1st AVA family developed by AGIR



2-heteroaryl-4-quinolones
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} = 95 µM (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

Figure 2: Design strategy of new anti-virulence agents (AVA)

SYNTHESIS OF 2-HETEROARYL-4-AMINOQUINOLINES

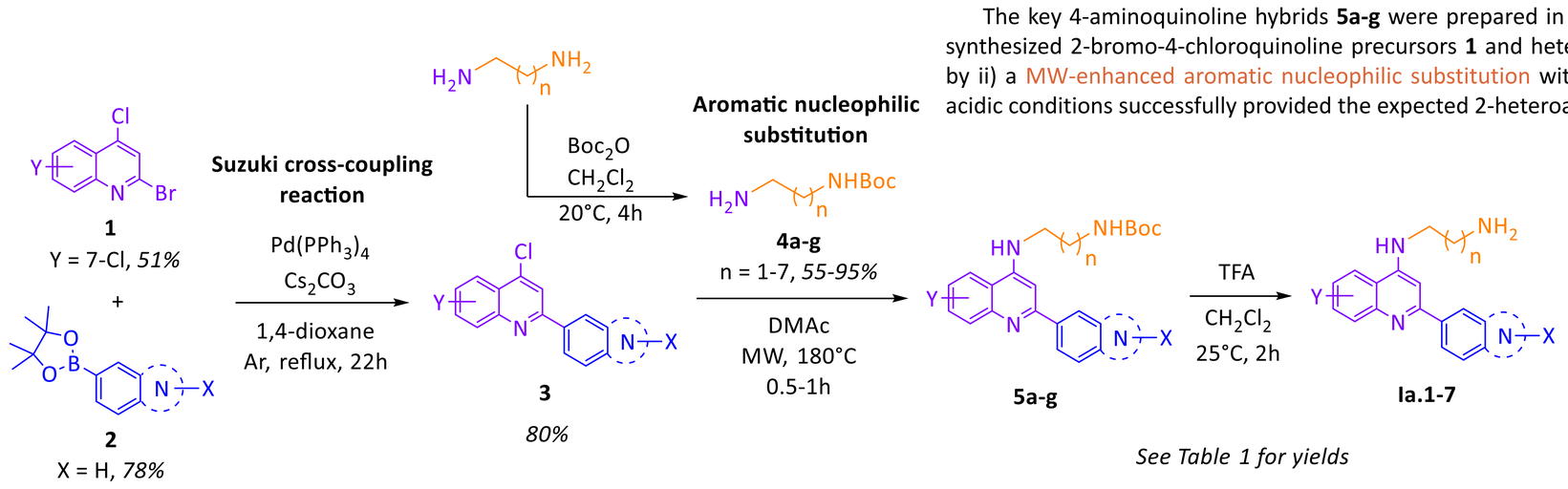


Figure 3: Synthesis of new 2-heteroaryl-4-aminoquinolines

The key 4-aminoquinoline hybrids **5a-g** were prepared in two main steps : i) a Suzuki cross-coupling reaction between the previously synthesized 2-bromo-4-chloroquinoline precursors **1** and heteroarylboronic esters **2** that afforded the bi-aromatic derivatives **3**, followed by ii) a MW-enhanced aromatic nucleophilic substitution with the *N*-boc protected diamines **4a-g**. The cleavage of protecting groups in acidic conditions successfully provided the expected 2-heteroaryl-4-aminoquinolines **Ia.1-7**.

Table 1: Yields and purity of final products synthesized from key bi-protected aminoquinoline hybrids

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

* HPLC analysis

PHYSICOCHEMICAL AND BIOLOGICAL PREREQUISITE STUDY

Table 2: *In silico* physicochemical properties of 2-heteroaryl-4-aminoquinolines and references, MIC determination on *P. aeruginosa* PAO1 strain and CC_{50} evaluation *via* a MTT assay on the human HepG2 cell line.

Compounds	X	Y	n	clog$P_{o/w}$*	pKa**	MIC in µg/mL (µM) (n = 3x3)	Eukaryotic cytotoxicity CC_{50} (µM) (n = 3)
Ciprofloxacin				0.28	6.2 (COOH) 8.6 (N_2, piperazine)	0.25	181
IQO-1				2.82	4.5 (N, 4-quinolinol) 12.4 (OH, 4-quinolinol)	>128 (434)	97 ± 5
M64				3.93	X	>128 (304)	58 ± 7
Ia.1	H	7-Cl	1	2.58	7.1 (N, quinoline) 10.1 (NH_2)	64 (190)	12 ± 2
Ia.2	H	7-Cl	2	2.96	7.5 (N, quinoline) 10.2 (NH_2)	64 (182)	13 ± 6
Ia.3	H	7-Cl	3	3.32	7.6 (N, quinoline) 10.2 (NH_2)	>128 (351)	8 ± 3
Ia.4	H	7-Cl	4	3.64	7.6 (N, quinoline) 10.2 (NH_2)	>128 (338)	27 ± 6
Ia.5	H	7-Cl	5	4.03	7.6 (N, quinoline) 10.2 (NH_2)	>128 (326)	20 ± 2
Ia.6	H	7-Cl	6	4.36	7.6 (N, quinoline) 10.2 (NH_2)	>128 (315)	27 ± 4
Ia.7	H	7-Cl	7	4.67	7.6 (N, quinoline) 10.2 (NH_2)	>128 (304)	39 ± 2

Calculated with * Qikprop and ** Epik softwares

Drugs have to fulfill several physicochemical prerequisites in order to cross the lipopolysaccharidic diderm barrier of Gram-negative bacteria. Compounds **Ia.1-7** make no infraction to the Lipinsky rule of five. The presence of ionizable functions at physiological pH should facilitate the intracellular entry *via* porins. Their lipophilicity determined *via* clog$P_{o/w}$ values could favor passive diffusion through the cytoplasmic membranes (Tab. 2). Furthermore, the 4-aminoquinoline hybrids revealed no effect on pseudomonal growth which is favorable for the development of AVA.

ANTI-VIRULENCE EVALUATION

Table 3: Anti-virulence activities of 2-heteroaryl-4-aminoquinolines and references at 50 µM.

	M64	IQO-1	6a	6b	6c	6d	6e	6f	6g
Anti-biofilm activity (% inhibition)	28	32	36	40	47	34	47	46	67
Anti-pyocyanin activity (% inhibition)	97	NS*	30	31	40	NS	38	40	42

* Not significant

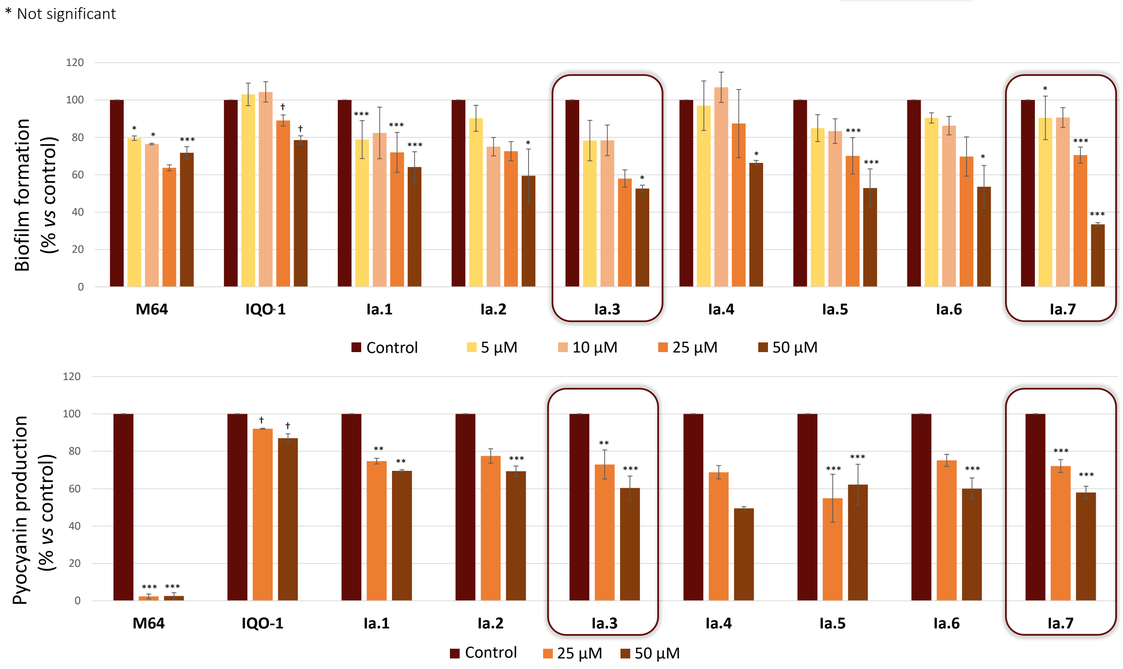


Figure 4: Evaluation of biofilm production on *P. aeruginosa* PAO1 strain
Quantitative analysis *via* crystal violet staining following 24h growth in presence or absence of tested products at different concentrations. Bars represent the mean ± SD of at least three independent experiments performed in triplicate. **p* < 0.05, ***p* < 0.01 and *** p < 0.005 *vs* control (Mann-Whitney's test : *p* values < 0.05 were considered significant). † Not significant for this assay.

Figure 5: 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 tested products 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 < 0.005 *vs* control (Mann-Whitney's test : *p* values < 0.05 were considered significant).

The newly synthesized 2-heteroaryl-4-aminoquinolines **Ia.1-7** displayed an interesting dose-dependent anti-virulence efficiency (Fig. 4 & Fig. 5). They appeared more potent to inhibit biofilm formation than the reference **M64** and showed better activities than the previous hit compound **IQO-1**. Particularly, **Ia.3** and **Ia.7** reduced biofilm formation by 47 and 67% at 50 µM, respectively. Both were also able to inhibit pyocyanin secretion by 40 and 42% at 50 µM, respectively (Tab. 3).

CONCLUSION AND PERSPECTIVES

Seven new 2-heteroaryl-4-aminoquinolines have been synthetized in five steps with global yields of 5 to 21%. Their physicochemical and biological druggability as AVA with no effect on bacterial growth has been highlighted as well as their promising anti-virulence properties. In particular, hybrids **Ia.3** and **Ia.7** displayed the most interesting anti-pyocyanin and anti-biofilm activities. Since the compound **Ia.7** appeared less cytotoxic towards human cells than **Ia.3** (CC_{50} = 39 *vs* 8 µM), it was evidenced as our new hit AVA. Extended pharmacomodulations on the bi-aromatic scaffold are now ongoing to expand the efficacy screening.

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