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Fumiquinazoline related alkaloids with antibacterial, anti-biofilm and efflux pump inhibition properties

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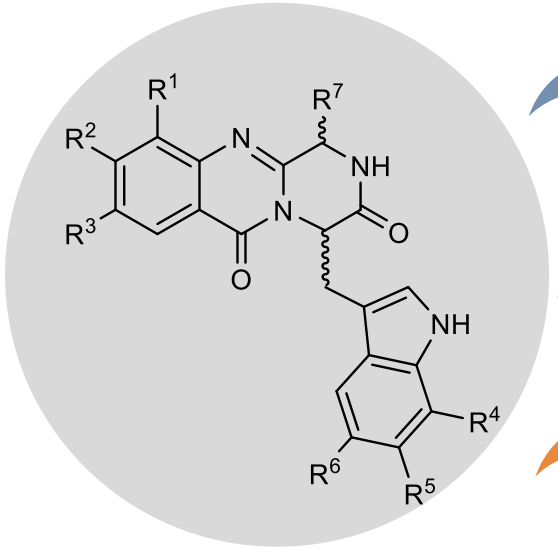
Mariana C. Almeida [1, 2], Nikoletta Szemerédi [3], Fernando Durães [1,2], Diana I. S. P. Resende [1, 2,*], Paulo M. da Costa [2,4], Madalena Pinto [1, 2], Gabriella Spengler [3], Emília Sousa [1, 2,*]

1 Laboratório de Química Orgânica e Farmacêutica, Departamento de Ciências Químicas, FFUP – Faculdade de Farmácia, Universidade do Porto, Porto, Portugal
2 CIIMAR- Centro Interdisciplinar de Investigação Marinha e Ambiental, Matosinhos, Portugal.
3 Department of Medical Microbiology, Albert Szent-Gyorgyi Health Center and Albert Szent-Gyorgyi Medical School, University of Szeged, Hungary.
4 ICBAS – Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal.
Corresponding author: dresende@ff.up.pt, esousa@ff.up.pt

ciimar Centro Interdisciplinar de Investigação Marinha e Ambiental
U.PORTO FACULDADE DE FARMÁCIA UNIVERSIDADE DO PORTO
LQOF Laboratório de Química Orgânica e Farmacêutica
ALBERT SZENT-GYÖRGYI MEDICAL SCHOOL UNIVERSITY OF SZEGED

Fumiquinazoline related alkaloids with antibacterial, anti-biofilm and efflux pump inhibition properties

Multi-step synthetic approach



Small library of fumiquinazoline related alkaloids

Antibacterial
4 active compounds

Anti-biofilm
4 active compounds

Efflux pump inhibition
3 active compounds

Abstract

With antimicrobial resistance reaching critical levels worldwide, the development of new compounds that are effective against resistant bacterial pathogens or that can potentiate the effect of known antibiotics is an urgent need. The formation of biofilms and the overexpression of efflux pumps are some of the most common causes of drug resistance. Previous work from our group has shown that alkaloids related to the fumiquinazolines have antibacterial potential. Herein we aimed to synthesize a small library of fumiquinazoline related alkaloids and to study their antibacterial and antibiofilm activities as well as their capacity to inhibit bacterial efflux pumps. To achieve these goals, two naturally occurring alkaloids, as well as several new derivatives, were synthesized through a multi-step synthetic pathway. The screening of their antibacterial activities was achieved by determination of the minimum inhibitory concentration of each compound against a panel of clinically relevant bacterial species. Several compounds exhibited promising activities against Gram-positive bacteria. Then, using the ethidium bromide accumulation assay it was possible to identify some compounds with capacity to inhibit efflux pumps. Some of the synthesized alkaloids also showed anti-biofilm potential, reinforcing the idea that fumiquinazoline related alkaloids can constitute a key strategy to fighting antimicrobial resistance.

Keywords: Antibacterial; Anti-biofilm; Efflux pump inhibition; Fumiquinazolines.

Introduction

Emergence of microorganisms resistant to the known antibiotics

Development of efficient antimicrobial agents is a priority

Common resistance mechanisms

Overexpression of efflux pumps

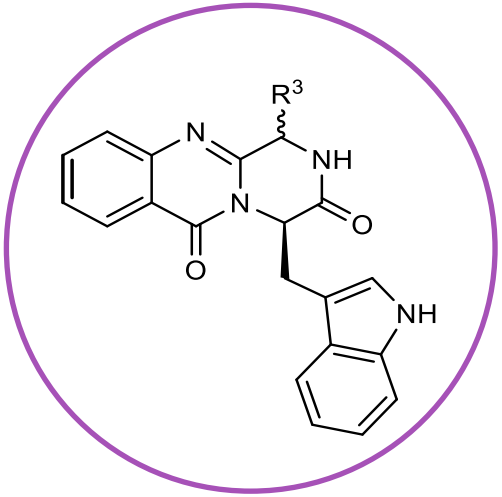
- Render the antibiotics ineffective by expelling them from the microorganism

Formation of biofilms

- Complex structures highly resistant to antimicrobial drugs

1. Nathan, C. *Nature Reviews Microbiology* **2020,** *18* (5), 259-260. 2. Durães F. *et al. Pharmaceuticals*, **2021**, *14*, 572.

Introduction



Fumiquinazoline and related alkaloids

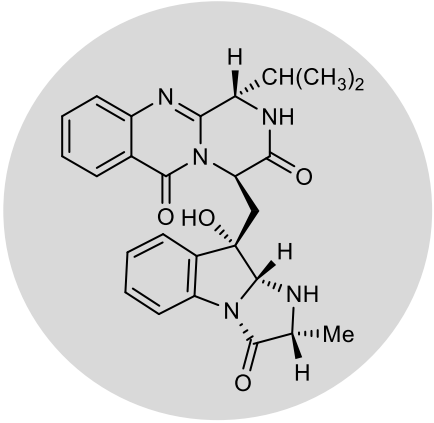
- **Specialized metabolites**

  Produced by both marine and terrestrial organisms

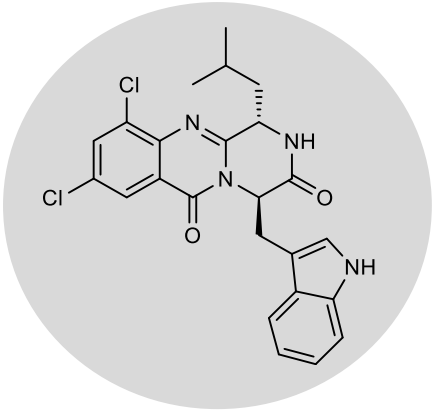
- **Privileged Structure**
- **Diverse bioactivities**

→ Relevant antimicrobial properties

Promising examples →



Neofiscalin A



Synthetic derivative

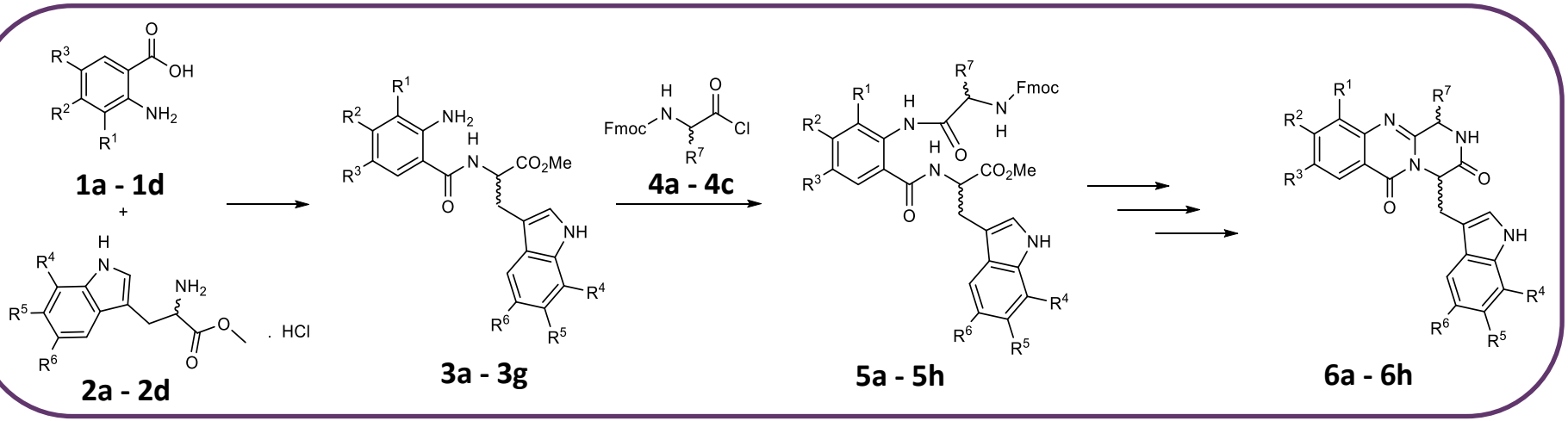
3. Resende D. I. S. P. *et al. Natural Product Reports,* **2019**, *36*, 7-34. 4. Bessa L. J. *et al. FEMS Microbiology Letters,* **2016,** *363*, fnw150.
5. Long, S. *et al. RSC Advances.* **10,** 31187-31204.

Aims

- ✓ Synthesis of new halogenated fumiquinazoline related alkaloids
- ✓ Structure elucidation of the synthesized compounds
- ✓ Evaluation of antibacterial activity
- ✓ Assessment of anti-biofilm and efflux pump inhibition capacity

Synthesis

Multi-step synthetic pathway



Molecular structure elucidation ..... NMR techniques

..... HRMS

Confirmed the expected structures

NMR = Nuclear Magnetic Resonance
HRMS = High Resolution Mass Spectrometry

6. Wang H. *et al. The Journal of Organic Chemistry*, **2000**, *65*, 1022-1030.

Biological activity screening

Antibacterial activity

MIC determination → **Broth microdilution method according to the CLSI guidelines**

Against a panel of clinically relevant bacterial strains

4 active compounds against Gram-positive bacteria

→ **MIC = 1.56 – 100 µM**

Hit compound 6h

→ MIC = 1.56 - 32 µM against *Staphylococcus aureus,* including MRSA

→ MIC = 64 µM against *Enterococcus faecalis*, including VRE

Cytotoxicity On the NIH/3T3 mouse fibroblast cell line →

Compounds **6a**-**6d** showed no cytotoxicity (IC_{50} >100 µM)

Compounds **6e**-**6h** showed IC_{50} = 47 – 58 µM

MIC = Minimum Inhibitory Concentration MRSA = Methicillin-Resistant *S. aureus* VRE = Vancomycin Resistant *E. faecalis*

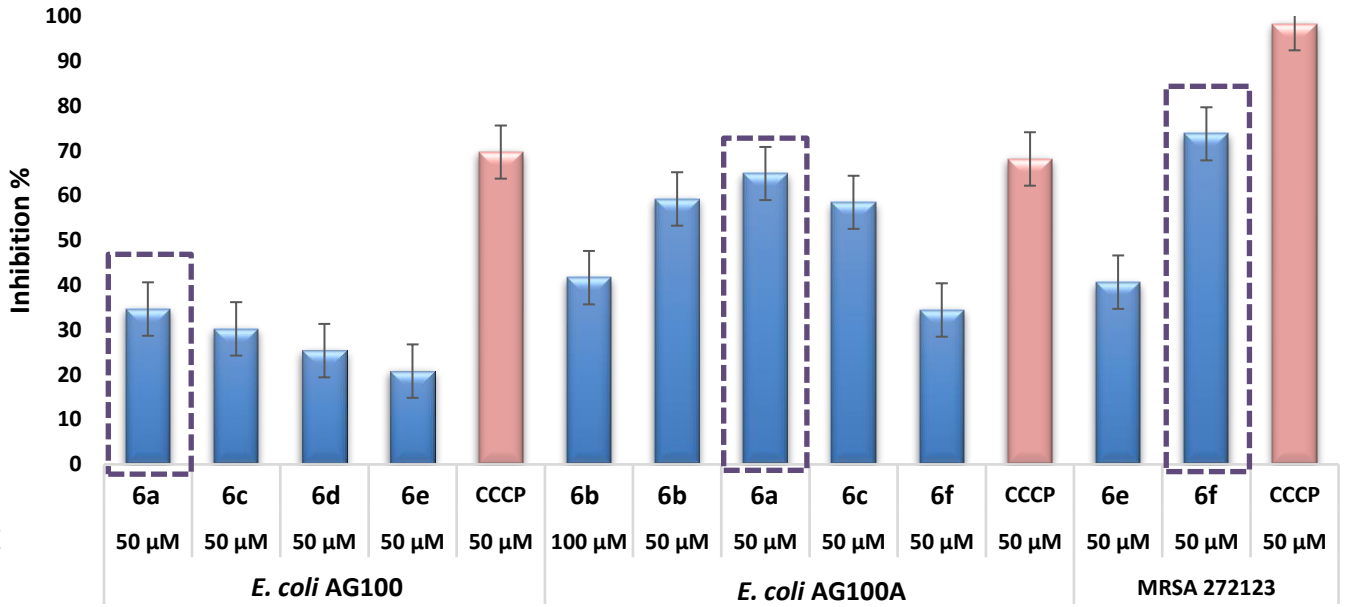
Biological activity screening

Anti-biofilm activity

Capacity to inhibit biofilm formation of *Escherichia coli* and MRSA

E. coli AG100 – expressing the AcrAB-TolC efflux pump

E. coli AG100A – AcrAB-TolC deleted mutant



Hit compound 6a ➡ 34.7% and 65% inhibition of *E. coli* (AG100 and AG100A) biofilm formation

Hit compound 6f ➡ 73.8% inhibition of MRSA biofilm formation

Positive control: CCCP = Carbonyl cyanide *m*-chlorophenyl hydrazone

Biological activity screening

Efflux pump inhibition

Ethidium bromide accumulation assay

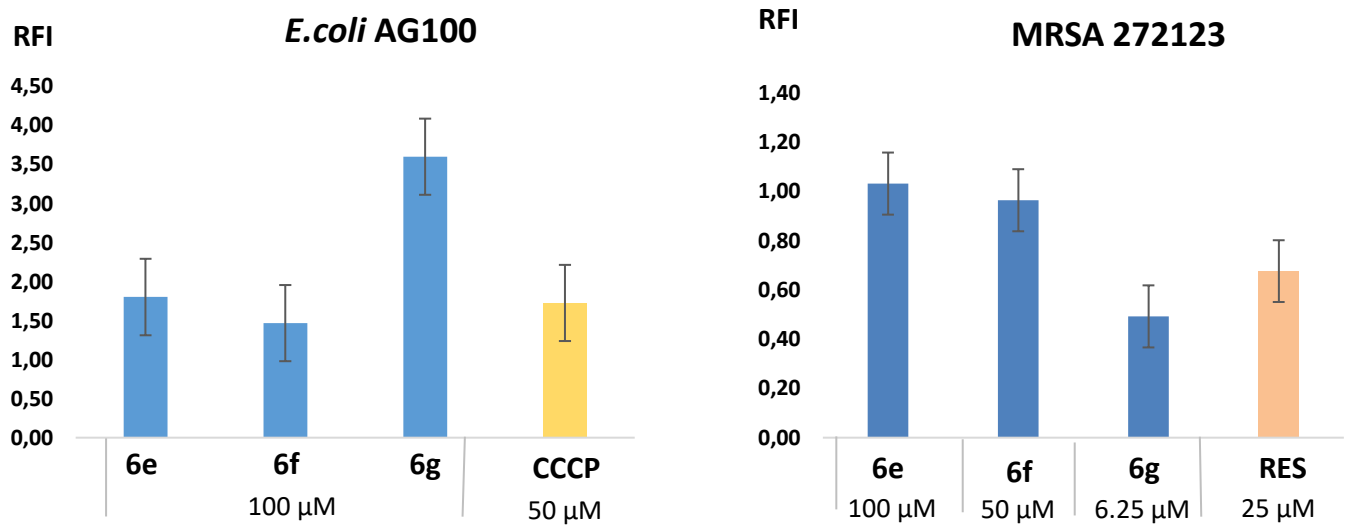
3 active compounds

Hit compound 6g

Active against *E. coli* AG100 at 50 µM and 100 µM and against *E. coli* AG100A at 100 µM

Active against MRSA at MIC/2 (6.25 µM)

Examples:



RFI = Relative Fluorescence Index

Positive controls: CCCP = Carbonyl cyanide *m*-chlorophenyl hydrazone ; RES = Reserpine

Conclusions

Obtained compounds

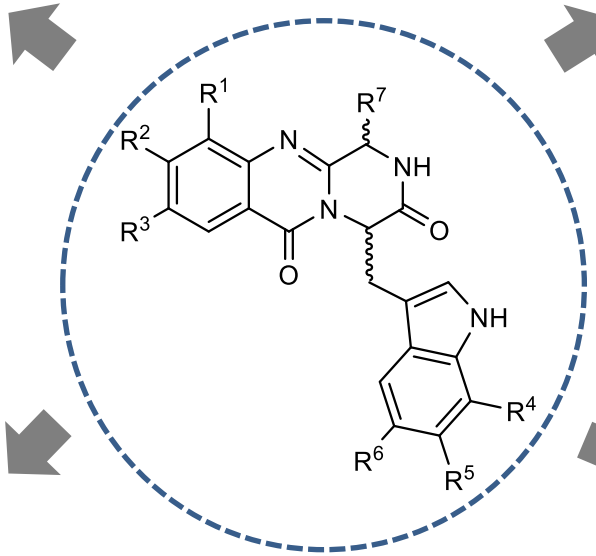
2 naturally-occurring compounds (**6a**-**6b**)

6 new alkaloids (**6c**-**6h**)

Anti-biofilm capacity

Hit compounds **6a** and **6f**

Inhibition of *E. coli* and MRSA biofilm formation



Antibacterial activity

Hit compound **6h**

Active against Gram-positive bacteria, including VRE and MRSA

Efflux pump inhibition

Hit compound **6g**

Inhibition of *E. coli* and MRSA efflux pumps

Acknowledgments

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