The 7th International Electronic Conference on Medicinal Chemistry (ECMC 2021)
01–30 NOVEMBER 2021 | ONLINE

Fumiquinazoline related alkaloids: Synthesis and evaluation of their antibacterial activities

Mariana C. Almeida[1,2], Diana I. S. P. Resende[1,2*], Paulo M. da Costa[2,3], Madalena Pinto[1,2], 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]ICBAS – Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal.

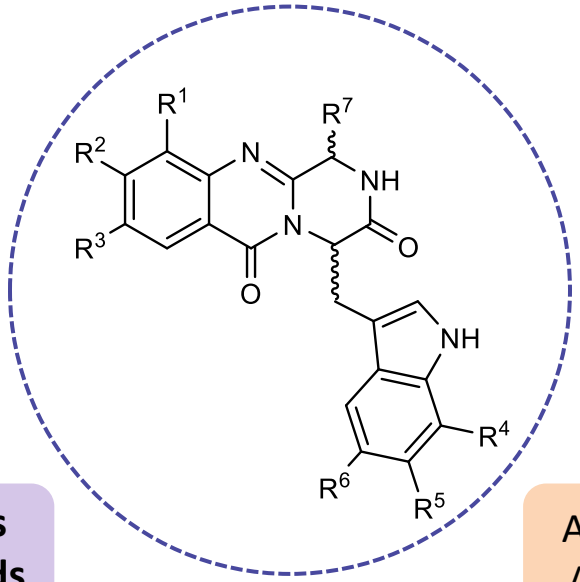
***** Corresponding author: dresende@ff.up.pt

Fumiquinazoline related alkaloids: Synthesis and evaluation of their antibacterial activities

Synthesis

Multi-step approach

6 new halogenated alkaloids
2 natural occurring compounds



Evaluation of antibacterial activity

5 active compounds against Gram-positive bacteria

Most promising compound:

Active against MRSA (MIC = 8 µg/mL)
Active against VRE (MIC = 32 µg/mL)

Abstract

Antimicrobial resistance has become a major threat to public health worldwide making the discover of new antimicrobial agents, with innovative chemistry and modes of action, a global priority. Indole alkaloids related to fumiquinazolines have shown several biological activities, including antimicrobial potential. Therefore, our project aims to synthesize new alkaloids related to the fumiquinazolines and to evaluate their antibacterial activities.

Herein, we present the synthesis of two naturally occurring compounds as well as of new fumiquinazoline related alkaloids through a multi-step synthetic pathway. Structure elucidation of the compounds was made by NMR and HRMS. To assess their antibacterial potential, the minimum inhibitory concentration (MIC) of each compound against a panel of four clinically relevant bacterial species (which includes both reference and multi-resistant strains) was determined. Additionally, a preliminary synergism study was made. The most promising alkaloid showed activity against methicillin resistant *Staphylococcus aureus* (MRSA) comparable to the natural product neofiscalin A (MIC = 8 µg/mL) as well as activity against vancomycin resistant *Enterococcus faecalis* (VRE) (MIC = 32 µg/mL).

Keywords – Antimicrobial; Fumiquinazolines; Medicinal Chemistry.

Introduction

↑ Antimicrobial resistance

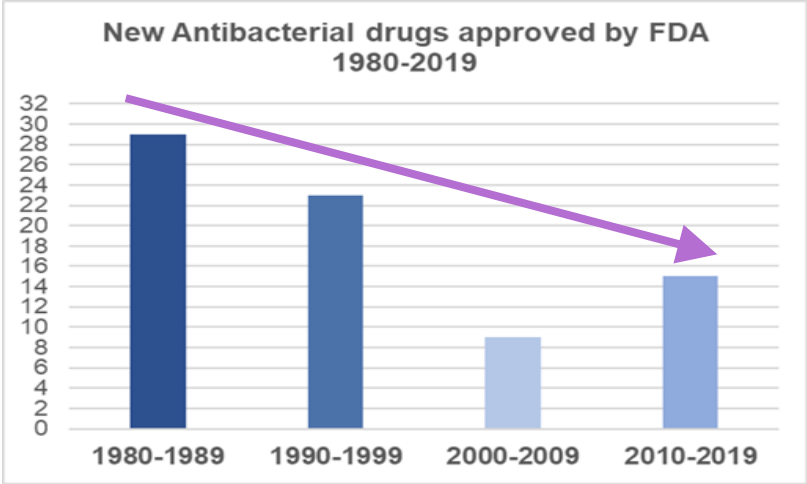
↓ Number of approved antimicrobial drugs

Most big pharmaceuticals have abandoned the research of antimicrobials

Why?

Economic reasons

Scientific challenges



Adapted from: Dheman, N. *et al Clinical Infectious Diseases,* **2020**.

FDA = Food and Drug Administration

Urgent need to find new and effective antimicrobial agents

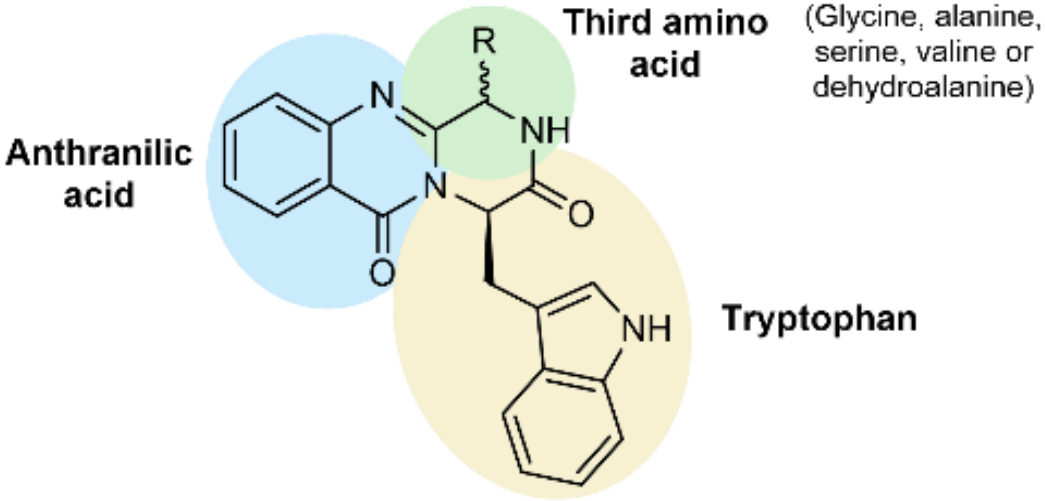
1. Durand, G. A.; Raoult, D.; Dubourg, G., *Int. J. Antimicrob. Agents* **53**, 371-382 (2019) ; 2. Renwick, M.; Mossialos, E., *Expert Opin. Drug Discov.* **13,** 889-892 (2018) ; 3. Silver, L. L., *Clin. Microbiol. Rev.* **24**, 71-109 (2011).

Introduction

More than three-quarters of the currently used antibiotics correspond to natural products or their derivatives

Mostly produced by fungi and bacteria

Fumiquinazolines and related alkaloids



Specialized metabolites

Produced by both marine and terrestrial organisms

Privileged Structure

Quinazolinone and indole moieties

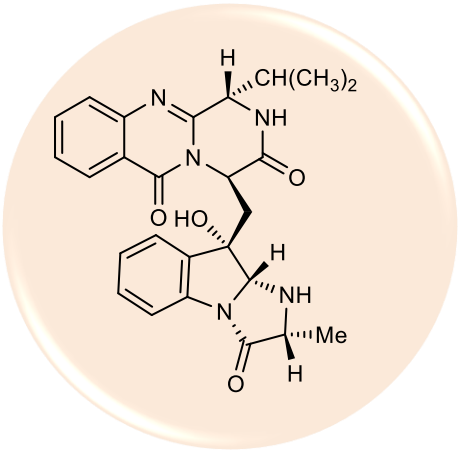
Diverse bioactivities

Relevant antimicrobial properties

1. Durand, G. A.; Raoult, D.; Dubourg, G., *Int. J. Antimicrob. Agents* **53**, 371-382 (2019) ; 4. Resende, D. I. S. P. *et al*. *Nat. Prod. Rep.* **36,** 7-34 (2019)

Introduction

Promising examples

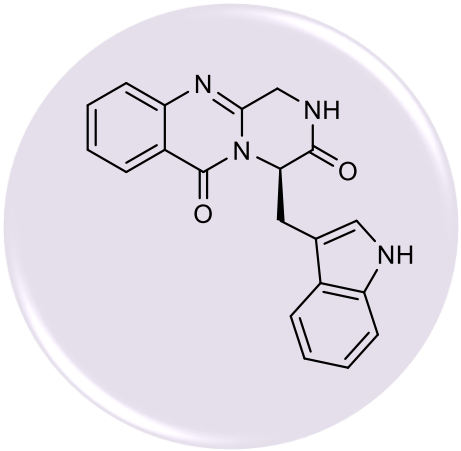


Neofiscalin A

Antibacterial

S. aureus and *E. faecalis*
(Including MRSA and VRE)

MIC = 8 µg/mL

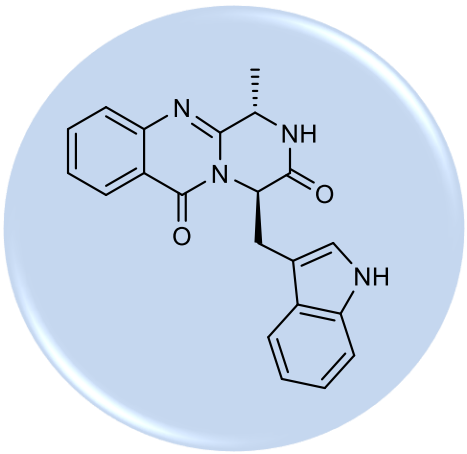


Glyantrypine

Antiviral

Active against H1N1

IC_{50}= 100-150 µM

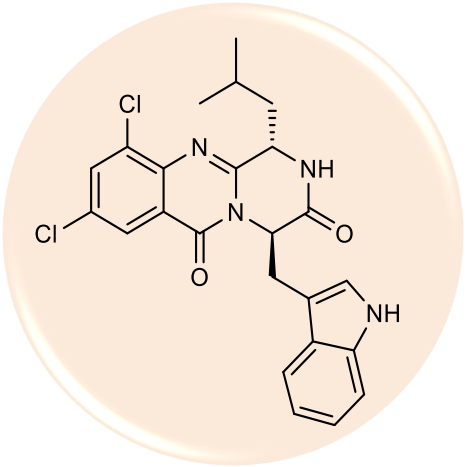


Fumiquinazoline F

Antifungal

Active against several
phytopathogenic fungi

MIC = 12.5-25 µg/mL



Synthetic derivative

Antibacterial

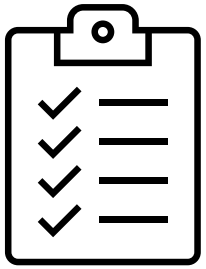
Active against *S. aureus*
(Including MRSA)

MIC = 8 µg/mL

MIC = Minimum inhibitory concentration

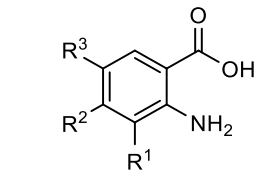
4. Resende, D. I. S. P. *et al. Nat. Prod. Rep.* **36,** 7-34 (2019) ; 5. Bessa, L. J. *et al. FEMS Microbiol. Lett.* **363,** (2016) ; 6. Long, S. *et al. RSC Adv.* **10,** 31187-31204 (2020).

Aims



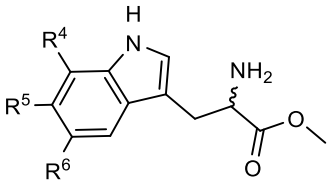
- Synthesis of new halogenated fumiquinazoline related alkaloids
- Structure elucidation of the synthesized compounds
- Evaluation of antibacterial activity of the synthesized compounds

Synthesis



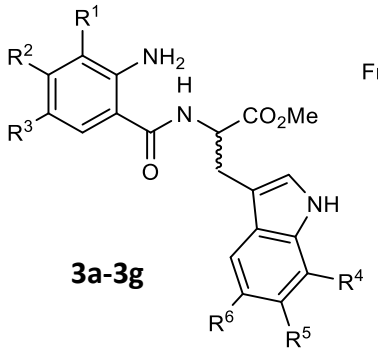
1a-1d

+

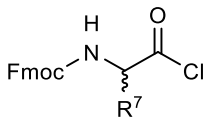


. HCl

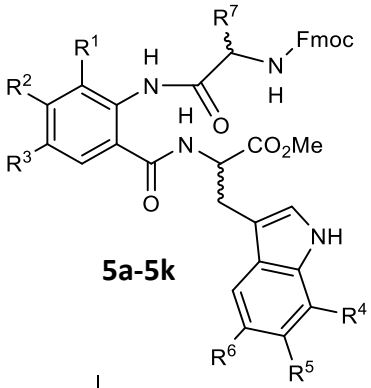
2a-2d



3a-3g



4a, 4b, 4c



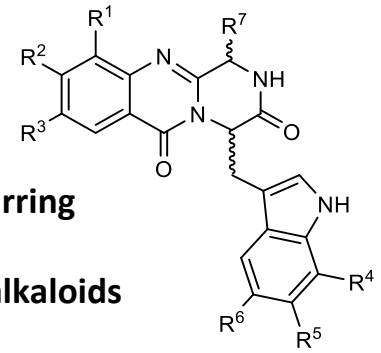
5a-5k

Multi-step approach

Linear dipeptide

Linear tripeptide

Cyclization



6a and 6b: Naturally occurring compounds

6c-6h: New halogenated alkaloids

7. Wang, H. & Ganesan, A. *J. Org. Chem.* **65,** 1022-1030 (2000) ; 8. He, F. & Snider, B. B., *J. Org. Chem.* **64**, 1397-1399 (1999)

Structure elucidation and purity assessment

Purity assessment by HPLC-DAD

- Peak purity above 94%
- Chromatographic purity above 93%

NMR techniques

High Resolution Mass Spectrometry

Confirmed the purposed structures

Melting points measurement

Optical rotation measurement

NMR = Nuclear magnetic resonance

HPLC-DAD = High performance liquid chromatography with diode array detection

Antibacterial activity evaluation

MIC determination

Broth microdilution method

4 reference bacterial strains

- ***E. coli*** ATCC 25922
- ***P. aeruginosa*** ATCC 27853
- ***S. aureus*** ATCC 29213
- ***E. faecalis*** ATCC 29212

3 clinically relevant strains

Methicillin-resistant *S. aureus* 66/1 (**MRSA**)
Vancomycin-resistant *E. faecalis* B3/101 (**VRE**)
Cefotaxime resistant strain *E. coli* SA/2 (**ESBL**)

5 active compounds against the Gram-positive bacterial species tested

6e-h and related alkaloid 7

Most promising compound

Halogenated alkaloid 6h

MIC = 32 µg/mL against VRE (*E. faecalis* B3/101)

MIC = 8 µg/mL against MRSA (*S. aureus* 66/1)

ESBL = Extended spectrum beta-lactamase

Antibacterial activity evaluation

Synergism Study

Agar disk diffusion method

Broth microdilution method

Investigation of possible synergism between the compounds and known antibiotics

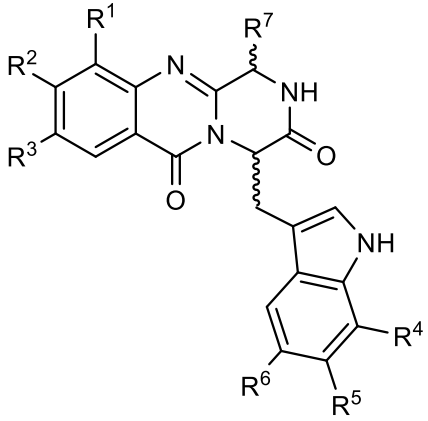
- Oxacillin against MRSA (*S. aureus* 66/1)
- Vancomycin against VRE (*E. faecalis* B3/101)
- Cefotaxime against ESBL (*E. coli* SA/2)

Potential synergism of 6g and 7 with vancomycin against *E. faecalis* B3/101

Able to reduce the MIC from 516 to 256 µg/mL when tested at 16 µg/mL

Conclusions

Synthetized compounds



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

6 new alkaloids (6c-6h)

Hit compound: 6h

- Comparable activity with natural product neofiscalin A against MRSA
- Active against *E. faecalis*, contrary to previously reported halogenated derivatives

Halogenated fumiquinazoline related alkaloids constitute a novel and relevant approach in the development of new antibacterial agents

Acknowledgments

This research was supported by national funds through FCT - Foundation for Science and Technology within the scope of UIDB/04423/2020, UIDP/04423/2020 (Group of Natural Products and Medicinal Chemistry CIIMAR), and under the project PTDC/SAU-PUB/28736/2017 (reference POCI-01-0145-FEDER-028736), co-financed by COMPETE 2020, Portugal 2020 and the European Union through the ERDF and by FCT through national funds, as well as CHIRALSINTESE_APSFCT_IINFACTS_2021 and R&D&I ATLANTIDA (reference NORTE-01-0145-FEDER-000040), supported by NORTE2020, through ERDF. Mariana C. Almeida acknowledges the BYT+ scholarship to CIIMAR.