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Indolylmethyl pyrazinoquinazoline alkaloids inspired by the sea: Total synthesis and evaluation of antimicrobial activities

Mariana da Costa Almeida

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Trabalho realizado sob orientação da Doutora Diana Resende e coorientação da Professora Doutora Madalena Pinto e do Professor Doutor Paulo Martins da Costa

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FACULDADE DE FARMÁCIA
UNIVERSIDADE DO PORTO

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Part of this work was/will be presented in the following communications:

Articles:

- Almeida, M. C.; Resende, D. I. S. P.; da Costa, P. M.; Pinto, M. M. M.; Sousa, E., "Tryptophan derived natural marine alkaloids and synthetic derivatives as promising antimicrobial agents." *European Journal of Medicinal Chemistry* **2021**, *209*, 112945. DOI: 10.1016/j.ejmech.2020.112945

Oral communications:

- Mariana Almeida; Diana Resende*; Paulo Costa; Madalena Pinto*; Emília Sousa, "Synthetic studies towards fumiquinazoline related alkaloids", Oral communication presented in SIFFUP, Simpósio de Investigação da Faculdade de Farmácia da Universidade do Porto, 2ª edição, Faculdade de Farmácia da Universidade do Porto, 24th November 2020.
- Mariana C. Almeida, Diana I. S. P. Resende*, Paulo M. Costa, Madalena Pinto*, Emília Sousa, "Fumiquinazoline related alkaloids: synthesis and preliminary antibacterial assessment", Oral communication presented in IJUP'21, Encontro de Investigação Jovem da Universidade do Porto, 14ª Edição, Online Meeting, 5-7th May 2021.
- Mariana C. Almeida, Diana I. S. P. Resende*, Paulo M. da Costa, Madalena Pinto*, Emília Sousa Marine inspired fumiquinazoline related alkaloids: synthesis and assessment of antibacterial potential, Flash talk presented in Blue Think Conference 2021, 23rd-24th September 2021 (Accepted).

Poster communications:

- Mariana Almeida; Diana Resende*; Paulo Costa; Madalena Pinto*; Emília Sousa, "Initial steps on the synthesis of new antimicrobial fumiquinazoline related alkaloids", Poster communication presented at 6th International Electronic Conference on Medicinal Chemistry, 6th November 2020.
- M. C. Almeida; D. I. S. P. Resende; P. Costa; M. Pinto; E. Sousa. "Towards the synthesis of antimicrobial fumiquinazoline related alkaloids". Poster communication presented in Concurso de Pósteres das Olimpíadas Universitárias da Bioquímica - Edição Especial COVID-19 (Ref. R9), 6th March 2021.

- Mariana C. Almeida, Diana I. S. P. Resende*, Paulo M. Costa, Madalena Pinto*, Emília Sousa “Synthesis of alkaloids related to the fumiquinazolines and preliminary evaluation of their antibacterial potential”, Poster communication presented in Concurso de Pósteres das VI Jornadas da Bioquímica, Online meeting, 7-9th May 2021.
- Mariana C. Almeida, Diana I. S. P. Resende*, Paulo M. Costa, Madalena Pinto*, Emília Sousa “Synthesis and preliminary antibacterial activity evaluation of fumiquinazoline related alkaloids”, Poster communication presented in 7th Portuguese Young Chemists Meeting, Online meeting, 19-21st May 2021.

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Abstract

Drug resistance is rising to alarming levels, constituting a major threat to public health worldwide. Therefore, the research and development of new and effective antimicrobial agents is extremely important in the current society. Fumiquinazolines and related alkaloids, which can be produced by both marine and terrestrial organisms, comprise a large variety of compounds with diverse biological activities, including relevant antimicrobial properties. For this reason, these alkaloids represent a very interesting family of compounds in medicinal chemistry.

In this work, it was possible to synthesize a small library of fumiquinazoline related alkaloids using a multi-step approach. The obtained compounds include the natural products glyantrypine (**12**) and fumiquinazoline F (**16**) as well as six new halogenated alkaloids (**43a-f**). The structural elucidation of all synthesized alkaloids was successfully attained by several complementary methods such as NMR spectroscopy (^{1}H-NMR, ^{13}C-NMR, HSQC, HBMC) and HRMS. Melting points and optical rotation were also measured and found to be according to the literature for the known compounds. Purity of all alkaloids was evaluated by HPLC-DAD. In addition, it was also possible to obtain and characterize by NMR several intermediates, namely seven linear dipeptides and four linear tripeptides.

The synthesized alkaloids and their intermediates as well as a related alkaloid obtained in a previous work (**DR127**), were screened for antibacterial activity against four representative bacterial species and against three relevant resistant clinical strains. Compounds **43c-f** and **DR127** showed relevant activities against the tested Gram-positive bacterial species (*Staphylococcus aureus* and *Enterococcus faecalis,* (MIC = 8 – 32 μg/mL). Compound **43f** emerged as a *hit* compound being active against both sensitive and methicillin resistant *S. aureus* (MIC = 16 and 8 μg/mL respectively) and against sensitive and vancomycin resistant *E. faecalis* (MIC = 32 μg/mL). A synergism study with several known antibiotics was also made and compounds **43c** and **DR127** showed a potential synergism with vancomycin towards *E. faecalis* B3/101 (MIC reduction from 512 μg/mL to 256 μg/mL) that should be further investigated.

Key words: Antimicrobial; Fumiquinazolines; Alkaloids; Medicinal Chemistry.

Resumo

A resistência antimicrobiana está a atingir níveis alarmantes, constituindo uma ameaça para a saúde pública global. Assim, a pesquisa e desenvolvimento de novos agentes antimicrobianos eficazes é extremamente importante na sociedade atual. As fumiquinazolinas e alcaloides relacionados, produzidos por organismos terrestres e marinhos, incluem uma grande variedade de compostos com diversas atividades biológicas de entre as quais a atividade antimicrobiana é uma das que mais se destaca. Por esta razão, estes alcaloides constituem uma família de compostos muito interessante em química medicinal.

Neste projeto, foi possível sintetizar uma pequena biblioteca de alcaloides relacionados com as fumiquinazolinas através de uma abordagem que inclui vários passos reacionais. Os compostos obtidos incluem os produtos naturais gliantripina (**12**) e fumiquinazolina F bem como seis novos alcaloides halogenados (**43a-f**). A respetiva elucidação estrutural foi feita com recurso a várias técnicas complementares como espetroscopia de ressonância magnética nuclear (^{1}H-NMR, ^{13}C-NMR, HSQC, HBMC) e espetrometria de massa de alta resolução. Os pontos de fusão e a rotação ótica dos alcaloides foram também avaliados e estão de acordo com a literatura para os compostos conhecidos. Para além disso, a pureza dos compostos foi avaliada com recurso a HPLC-DAD. Foi ainda possível sintetizar e caracterizar por RMN vários intermediários, nomeadamente sete dipéptidos e quatro tripéptidos lineares.

A atividade antibacteriana dos alcaloides sintetizados e dos seus intermediários bem como de um outro alcaloide obtido num trabalho anterior (**DR127**), foi avaliada contra quatro espécies bacterianas representativas e contra três estirpes clínicas resistentes. Os compostos **43c-f** e **DR127** mostraram atividade contra as espécies bacterianas Gram-positivo testadas (*Staphylococcus aureus* e *Enterococcus faecalis,* (MIC = 8 – 32 μg/mL). O composto **43f** surgiu como um composto *hit* sendo ativo contra *S. aureus* sensível e resistente à meticilina (MIC = 16 e 8 μg/mL, respetivamente) e contra *E. faecalis* sensível e resistente à vancomicina (MIC = 32 μg/mL). Foi ainda feito um estudo para avaliar possíveis sinergismos com diversos antibióticos conhecidos tendo sido observado um potencial sinergismo entre a vancomicina e os compostos **43c** e **DR127** (Redução da MIC de 512 μg/mL para 256 μg/mL) que teria de ser futuramente explorado de forma mais completa.

Palavras-chave : Antimicrobianos; Fumiquinazolinas; Alcaloides; Química Medicinal.