5th International Electronic Conference on Medicinal Chemistry

1-30 November 2019

chaired by Dr. Jean Jacques Vanden Eynde

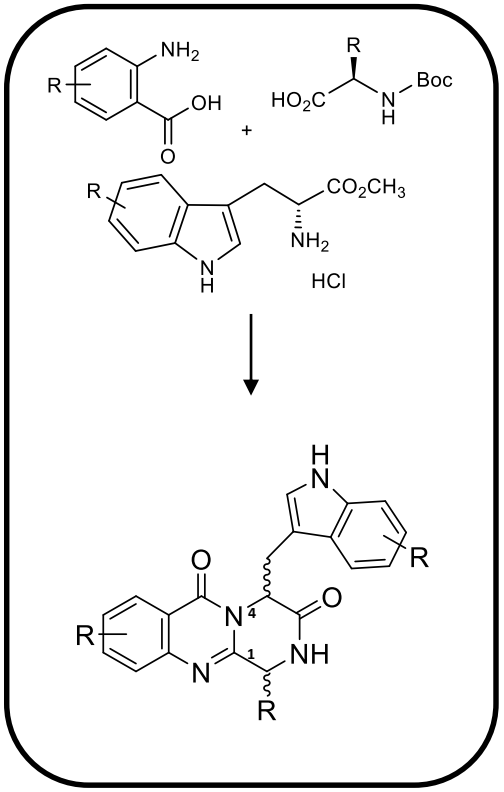
sponsored by pharmaceuticals

Synthetic Strategies Towards Bioactive Nature-Inspired Indole-Containing Alkaloids

Long S [1], Resende D [1,2], Pereira-Terra P [2,3], Freitas-Silva J [2,3], Silva A [4], Tiritan ME [1,2,5], Kijjoa A [2,3], Pinto E [2,6], Costa P [2,3], Pinto M [1,2], Sousa E[1,2,*]

[1] Laboratório de Química Orgânica e Farmacêutica, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal;
[2] Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), Terminal de Cruzeiros do Porto de Lexões, Av. General Norton de Matos s/n, 4450-208 Matosinhos, Portugal;
[3] ICBAS—Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal;
[4] Organic Chemistry, Natural Products and Food stuffs (QOPNA), Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal
[5] CESPU, Instituto de Investigação e Formação Avançada em Ciências e Tecnologias da Saúde (IINFACTS), Rua Central de Gandra, 1317, 4585-116 Gandra PRD, Portugal,
[6] Laboratório de Microbiologia, Departamento de Ciências Biológicas, Faculdade de Farmácia, Universidade do Porto, Portugal.
***** Corresponding author: esousa@ff.up.pt

Synthetic Strategies Towards Bioactive Nature-Inspired Indole-Containing Alkaloids



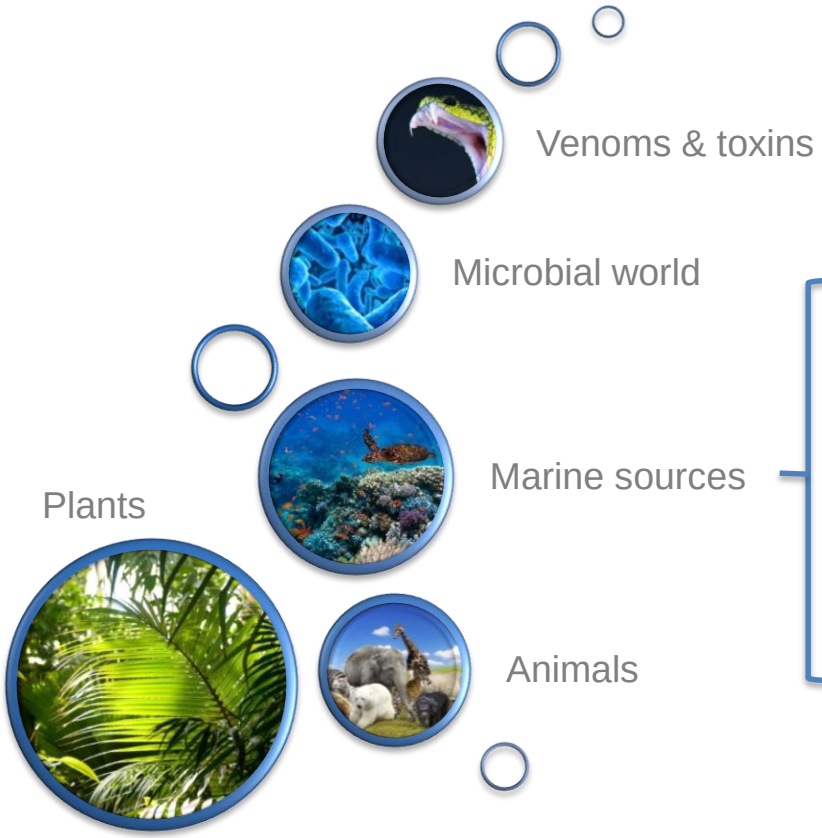
Synthesis	**- Method A** One-pot microwave-assisted approach **- Method B** Mazurkiewicz-Ganesan approach
Molecular Modifications	- C1 and C4 stereochemistry - C1 side chain and stereochemistry - Substitution at Ant moiety
Biological Activities	- Antibacterial - Antitumor - Neuroprotection

Abstract: Currently drug resistance is rising to dangerously high levels worldwide and threatening our ability to treat even common infectious diseases. Secondary metabolites, especially alkaloids containing an indole group and structurally related to fumiquinazolines, are of crucial importance in the area of drug discovery, having representatives such as fiscalin B that was reported as substance P antagonist and neofiscalin A, a potent antibacterial agent active in both reference and multidrug-resistant isolates. Herein, the synthesis of quinazolinone alkaloid derivatives containing an indole moiety is reported, using two different methodologies – a highly efficient three-component one-pot microwave-assisted and a multi-step Mazurkiewicz-Ganesan approach. With this approach, 38 derivatives were obtained in low to moderate yields and were further tested for their antitumor, neuroprotection, antibacterial, and antifungal activities. While 16 compounds exhibited weak to moderate tumor cell growth inhibitory activity, other four compounds showed potential for in vitro neuroprotection in Parkinson disease. It was also observed for some derivatives a good antibacterial activity against clinical *Staphylococcus aureus* resistant to methicillin (MRSA). Structure-activity relationship was established and four hit compounds containing the quinazolinone scaffold emerged as potential drug candidates.

Keywords: Alkaloids; fiscalins; resistance; enantioselective; bioactive.

Introduction

Sources of Natural Products



Nature

Important source of medicinal agents: more than half of the commercialized drugs are extracted from natural sources or produced by chemical synthesis using natural products as models or as starting material

Discovery of **new lead** compounds from **marine sources**

Important source of **pharmacologically active** metabolites

Introduction

Research Group

Interdisciplinary Centre of Marine and Environmental Research

Research Line: Marine Biotechnology

GROUP: MEDICINAL CHEMISTRY: DRUG DISCOVERY AND DRUG DESIGN

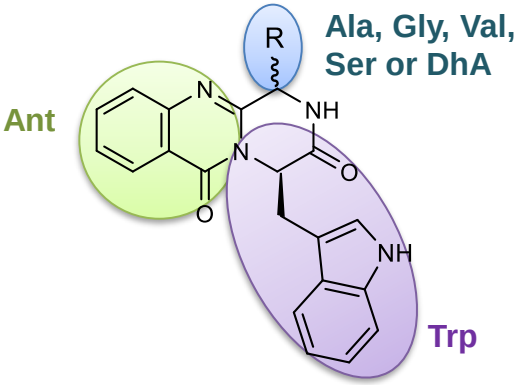
AIM: Search for new pharmacologically active compounds from natural or synthetic origin

Introduction

Fumiquinazolines - Structure and biosynthesis

Fumiquinazolines

- Secondary metabolites produced by fungi of marine or terrestrial sources
- Quinazolinones with a fused piperazine system linked to an indole moiety
- Biosynthesis: incorporation of the β-amino acid anthranilate (anthranilic acid, **Ant**), tryptophan (**Trp**), and an additional amino acid (**Ala, Gly, Val, Ser or DhA**)



Biosynthesis

Glycine-derived alkaloids

Gly

- Glyantrypines
- Cottoquinazolines
- Versiquinazolines.

Alanine-derived alkaloids

Ala

- Fumiquinazolines
- Ardeemins
- Aniquinazolines
- Fumigatosides

Valine-derived alkaloids

Val

- Fiscalins
- Cladoquinazolines
- Quinadolines
- Neosartoryadins

Introduction

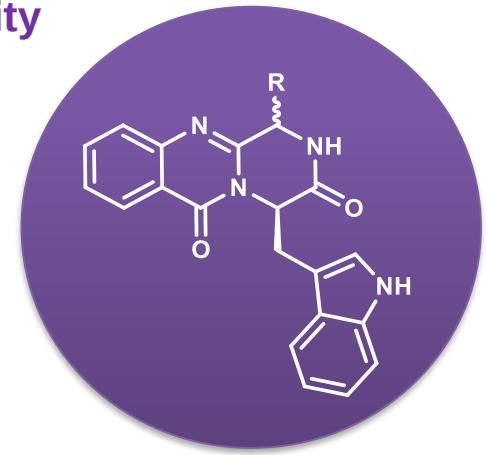
Fumiquinazolines - Isolation and bioactivity

Antitumor Activity

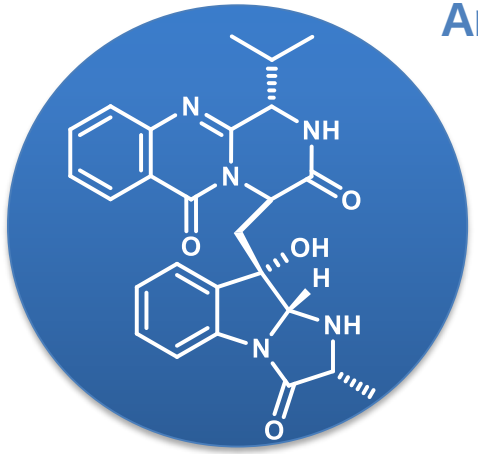
Mostly investigated on
Natural Products
Lacking SAR studies

Other activities

Substance P antagonists
Antinfluenza-H1N1



Glyantrypine, R = H
Fumiquinazoline F, R = Me (*S*)
Fumiquinazoline G, R = Me (*R*)
Fiscalin B, R = *i*-Pr (*S*)



Neofiscalin A
Marine sponge
Neosartorya siamensis
KUFA 0017

Antimicrobial

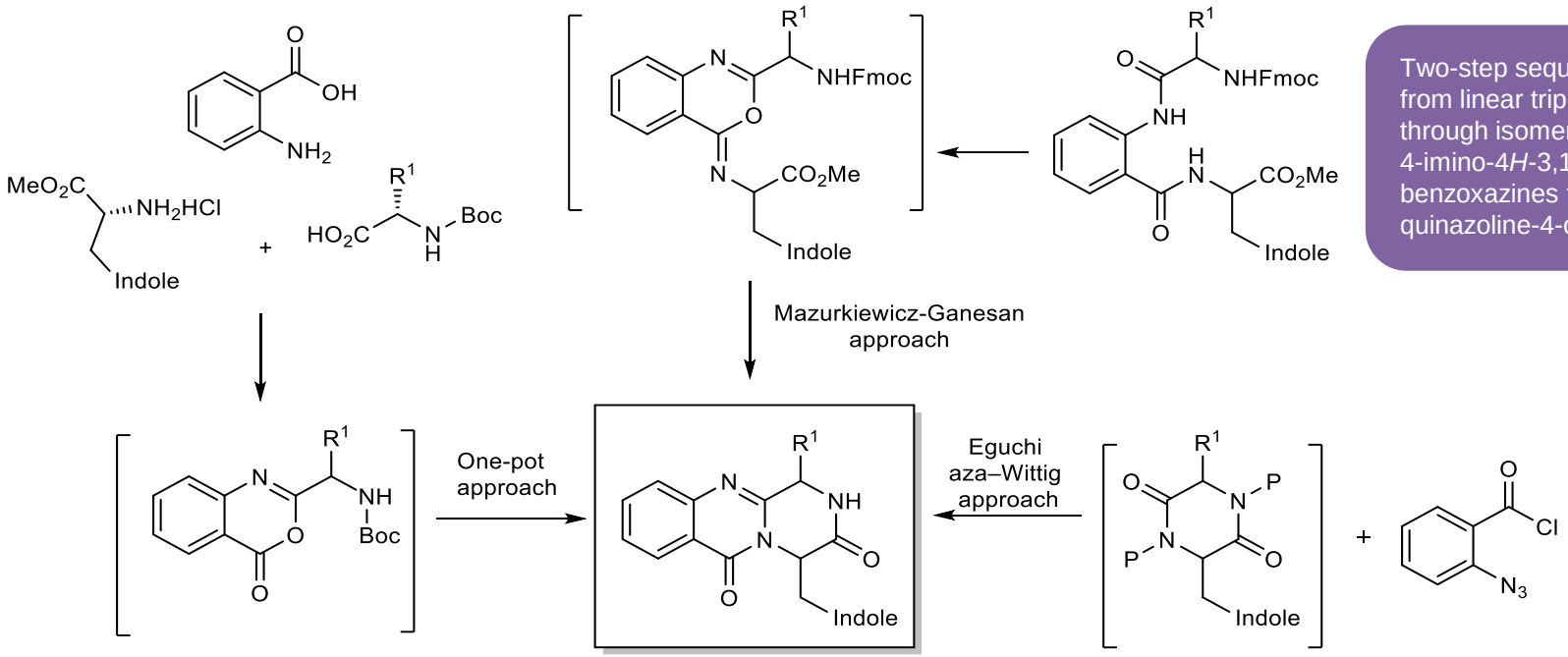
In MDR models
Stereochemistry dependent
Total synthesis not yet report

SAR = structure activity relationship
MDR = multidrug resistant

Resende, D. I. S. P.; Boonpothong, P.; Sousa, E.; Kijjoa, A.; Pinto, M. M. M., Chemistry of the fumiquinazolines and structurally related alkaloids. *Natural Product Reports* **2019,** *36*, 7-34

Introduction

General methodologies for the synthesis of pyrazino[2,1-*b*]quinazoline-3,6-diones



Two-step sequence from linear tripeptide through isomerization of 4-imino-4*H*-3,1-benzoxazines to the quinazoline-4-ones

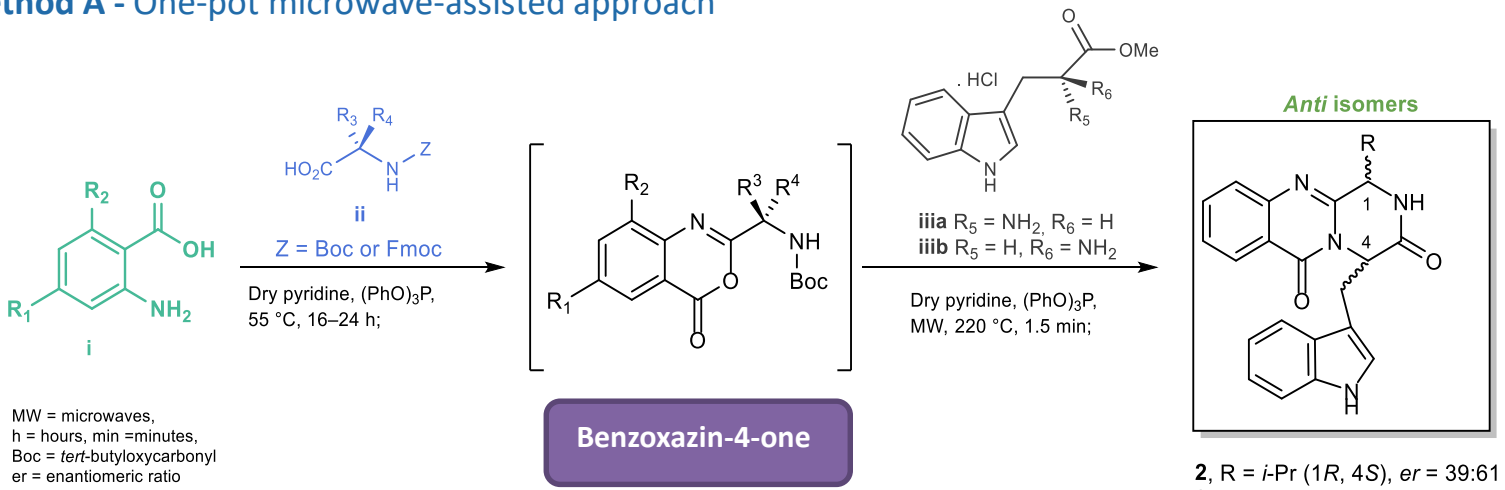
Three-component one-pot methodology promoted by microwave irradiation

Selective acylation of diketopiperazines with o-azido benzoyl chloride -> cyclization

Results and discussion

Synthesis of the pyrazinoquinazolinone alkaloids

Method A - One-pot microwave-assisted approach



MW = microwaves,
h = hours, min =minutes,
Boc = *tert*-butyloxycarbonyl
er = enantiomeric ratio

2, R = *i*-Pr (1*R*, 4*S*), *er* = 39:61
3, R = *i*-Pr (1*S*, 4*R*), *er* = 62:38
6, R = *i*-Bu (1*R*, 4*S*), *er* = 50:50
7, R = *i*-Bu (1*S*, 4*R*), *er* = 31:69

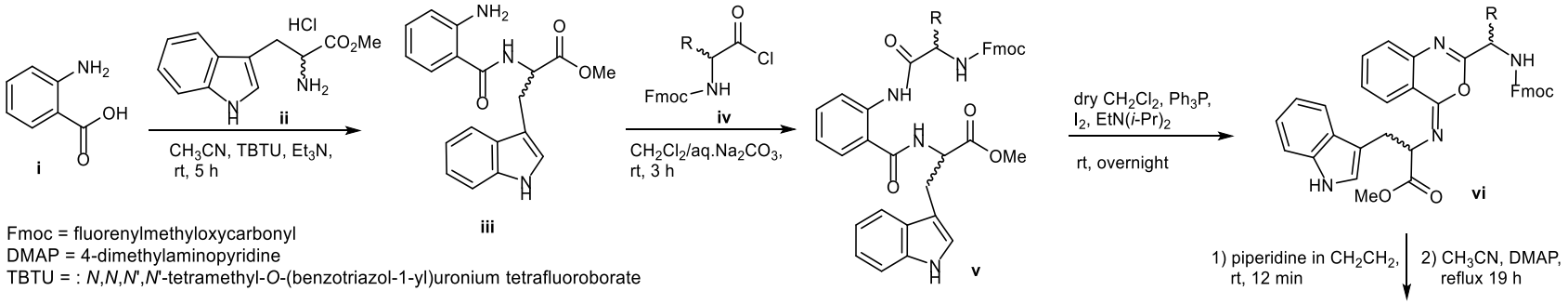
One-pot reaction → **Attempts to obtain the syn isomers failed** → **Isomerization to the anti-isomers**

Long, S.; Resende, D.; Kijjoa, A.; Silva, A.; Pina, A.; Fernández-Marcelo, T.; Vasconcelos, M.; Sousa, E.; Pinto, M., Antitumor Activity of Quinazolinone Alkaloids Inspired by Marine Natural Products. *Mar. Drugs* **2018,** *16* (8), 261.

Results and discussion

Synthesis of the pyrazinoquinazolinone alkaloids

Method B - Mazurkiewicz-Ganesan approach

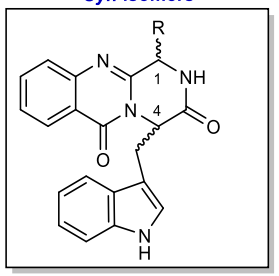


Fmoc = fluorenylmethyloxycarbonyl
DMAP = 4-dimethylaminopyridine
TBTU = : *N*,*N*,*N*',*N*'-tetramethyl-*O*-(benzotriazol-1-yl)uronium tetrafluoroborate

Multi-step approach

***Syn* isomers**

***Syn* isomers**



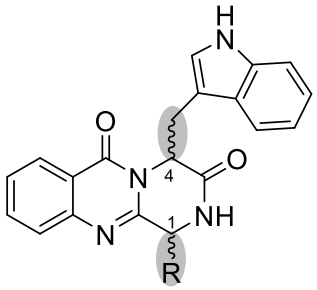
1, R = *i*-Pr (1*S*, 4*S*), *er* = 40:60
4, R = *i*-Pr (1*R*, 4*R*), *er* = 63:37
5, R = *i*-Bu (1*S*, 4*S*), *er* = 67:33
8, R = *i*-Bu (1*R*, 4*R*), *er* = 46:54

Long, S.; Resende, D.; Kijjoa, A.; Silva, A.; Pina, A.; Fernández-Marcelo, T.; Vasconcelos, M.; Sousa, E.; Pinto, M., Antitumor Activity of Quinazolinone Alkaloids Inspired by Marine Natural Products. *Mar. Drugs* **2018,** *16* (8), 261.

Results and discussion

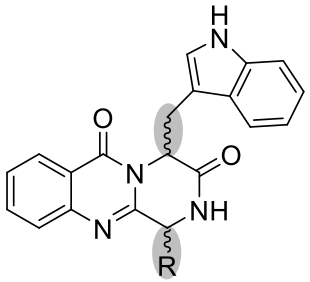
Molecular modifications

1st series
C1 and C4 stereochemistry



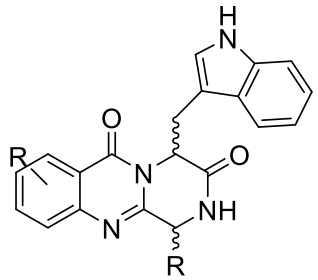
1, R = *i*-Pr, (1*S*, 4*S*)
2, R = *i*-Pr, (1*R*, 4*S*)
3, R = *i*-Pr, (1*S*, 4*R*) - Fiscalin B
4, R = *i*-Pr, (1*R*, 4*R*)
5, R = *i*-Bu, (1*S*, 4*S*)
6, R = *i*-Bu, (1*R*, 4*S*)
7, R = *i*-Bu, (1*S*, 4*R*)
8, R = *i*-Bu, (1*R*, 4*R*)

2nd series
C1 side chain and stereochemistry



9, R = *s*-Bu, (1*R*, 4*S*)
10, R = *s*-Bu, (1*S*, 4*R*)
11, R = $(CH_2)_2SCH_3$, (1*R*, 4*S*)
12, R = $(CH_2)_2SCH_3$, (1*S*, 4*R*)
13, R = $CH_2C_6H_4OCH_2C_6H_5$, (1*S*, 4*S*)
14, R = $CH_2C_6H_4OCH_2C_6H_5$, (1*R*, 4*S*)
15, R = $CH_2C_6H_4OH$, (1*S*, 4*S*)
16, R = $CH_2C_6H_4OH$, (1*R*, 4*S*)
17, R = CH_3 (1*R*, 1*R*)

3rd series
Substitution at Anthranilic moiety



18-29

Long, S.; Resende, D.; Kijjoa, A.; Silva, A.; Pina, A.; Fernández-Marcelo, T.; Vasconcelos, M.; Sousa, E.; Pinto, M., Antitumor Activity of Quinazolinone Alkaloids Inspired by Marine Natural Products. *Mar. Drugs* **2018,** *16* (8), 261.

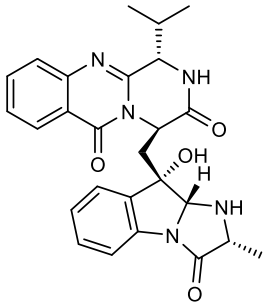
Long, S.; Resende, D. I. S. P.; Kijjoa, A.; Silva, A. M. S.; Fernandes, R.; Xavier, C. P. R.; Vasconcelos, M. H.; Sousa, E.; Pinto, M. M. M., Synthesis of New Proteomimetic Quinazolinone Alkaloids and Evaluation of Their Neuroprotective and Antitumor Effects. Molecules 2019, 24 (3), 534.

Results and discussion

Antibacterial Activity

Antibiotic Resistance → Research & Development of new treatments and antibiotic alternatives to address this important unmet medical need

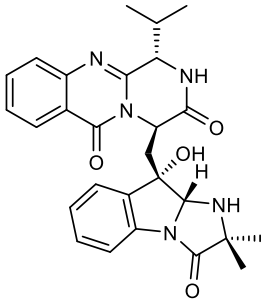
Natural fumiquinazolines with antibacterial activity



Neofiscalin A

Antibacterial activities against MRSA and VRE (MIC = 8 µg/mL)

Reduced the metabolic activity of the biofilms by approximately 50%. (200 µM)



Fiscalin C

Synergistic activity against MRSA when combined with oxacillin

MRSA - methicillin-resistant *Staphylococcus aureus*; VRE - vancomycin-resistant *Enterococcus,*

Bessa, L. J.; Buttachon, S.; Dethoup, T.; Martins, R.; Vasconcelos, V.; Kijjoa, A.; da Costa, P. M., Neofiscalin A and fiscalin C are potential novel indole alkaloid alternatives for the treatment of multidrug-resistant Gram-positive bacterial infections. *FEMS Microbiol. Lett.* **2016,** *363* (15), 1-5.

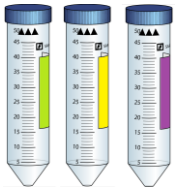
Results and discussion

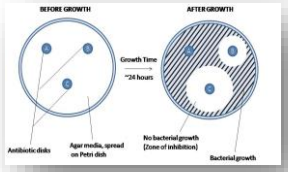
Antibacterial Activity - Bacterial growth inhibitory effect

Kirby-Bauer disk diffusion method

16 derivatives
of 1st and 2nd series

11 derivatives
3rd series





Gram-positive/Gram-negative
Clinical resistant strains

- *Escherichia coli* ATCC 25922 (G-)
- *Pseudomonas aeruginosa* ATCC 27853 (G-)
- *Staphylococcus aureus* ATCC 29213 (G+)
- *Staphylococcus aureus* 40/61/24 and 66/1
- *Enterococcus faecalis* ATCC 29212 (G+)
- *Enterococcus faecalis* A5/102 and B3/101
- Synergistic effect with vancomycin & oxacillin

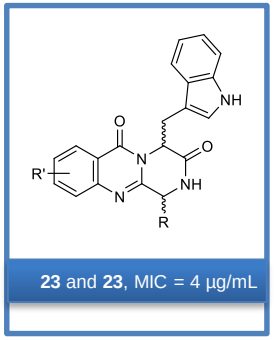
Gram-negative strains

In the range of concentrations tested, none of the compounds was active against Gram-negative bacteria.

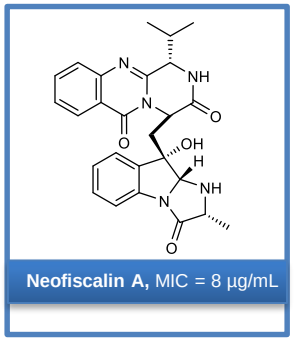
Gram-positive strains

Compounds **18**-**23** and **26**-**27** exhibited a bacterial growth inhibition of 4-32 µg/mL against *S. aureus* ATCC 29213
Compounds **22** and **23** exhibited a bacterial growth inhibition of 8 µg/mL against *S. aureus* 40/61/24 and 66/1 (MRSA)

Two Hit compounds:



23 and **23**, MIC = 4 µg/mL

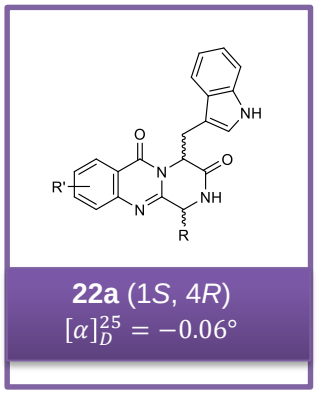


Neofiscalin A, MIC = 8 µg/mL

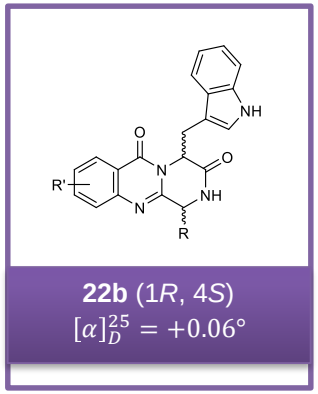
Results and discussion

Antibacterial Activity - Bacterial growth inhibitory effect

Looking for biological enantioselectivity…



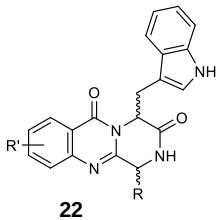
22a (1*S*, 4*R*)
$[\alpha]_D^{25} = -0.06°$



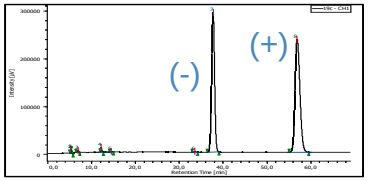
22b (1*R*, 4*S*)
$[\alpha]_D^{25} = +0.06°$

Chiral chromatography

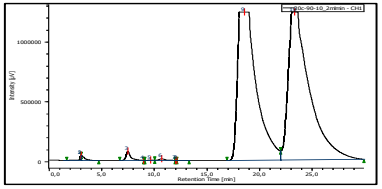
Chemical structure	Analytical column	Semipreparative column



22



(k_1 =5.60, α =1.75 *Rs* =11.69)



[a] Flow rate: 0.5 mL/min, loop 20 µL, detection: 254 nm, column: Lux® 5 µm Amylose-1, (250 x 4.6 mm), mobile phase: hexane:EtOH (90:10,v/v). [b] Flow rate: 2.0 mL/min, loop 200 µL, loading *ca*. 1.5 mg/mL in hexane:EtOH (50:50, v/v), detection 254 nm, column: amylose *tris*-3,5-dimethylphenylcarbamate coated with Nucleosil (200 mm x 7 mm); mobile phase: hexane: EtOH (90:10, v/v)

Antibacterial activity of enantiopure **22a** and **22b** on *S. aureus* strains

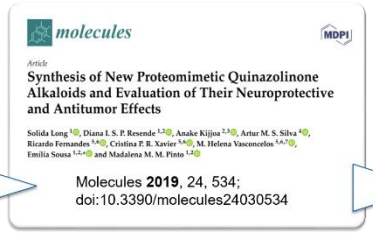
	S. aureus ATCC 29213		S. aureus 40/61/24		S. aureus 66/1 (MRSA)	
	MIC	MBC	MIC	MBC	MIC	MBC
22a	4	>64	4	>64	4	>64
22b	>64	>64	ND	ND	ND	ND

MIC = minimal inhibitory concentration, MBC = minimal bactericidal concentration VRE= vancomycin-resistant *Enterococcus,* MRSA= methicillin-resistant *Staphylococcus aureus*; ND = not determined, MIC and MBC are expressed in µg/mL.

Results and discussion

Biological Activity – Neuroprotection and antitumor activity

Neuroprotection capacity


molecules

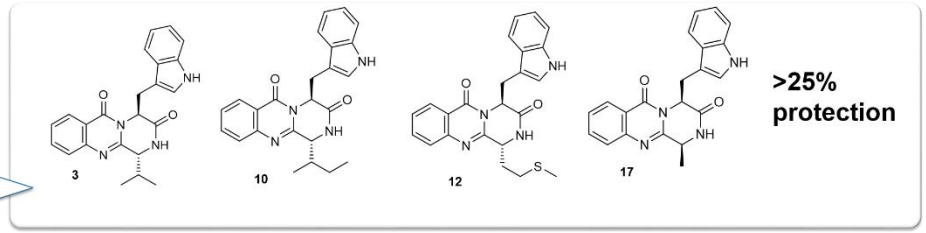
MDPI

Article

Synthesis of New Proteomimetic Quinazolinone Alkaloids and Evaluation of Their Neuroprotective and Antitumor Effects

Solida Long [1], Diana I. S. P. Resende [1,2], Anake Kijjoa [2,3], Artur M. S. Silva [4], Ricardo Fernandes [5,6], Cristina P. R. Xavier [5,6], M. Helena Vasconcelos [5,6,7], Emília Sousa [1,2,*] and Madalena M. M. Pinto [1,2]

Molecules **2019**, 24, 534; doi:10.3390/molecules24030534


3 10 12 17

>25% protection

Antitumor activity


marine drugs

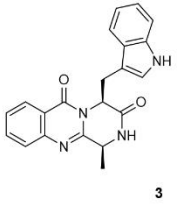
MDPI

Article

Antitumor Activity of Quinazolinone Alkaloids Inspired by Marine Natural Products

Solida Long [1], Diana I. S. P. Resende [1,2], Anake Kijjoa [2,3], Artur M. S. Silva [1], André Pina [4,5], Tamara Fernández-Marcelo [5], M. Helena Vasconcelos [5,6,7], Emília Sousa [2,3,*] and Madalena M. M. Pinto [1,2]

Mar. Drugs **2018**, 16, 261; doi:10.3390/md16080261


3

- **NCI-H460 (non-small cell lung cancer):** GI_{50} = 7.62 ± 0.7 μM (Doxorubicin: GI_{50} = 0.0124 ± 0.0018 μM)
- **BxPC3 (human pancreatic adenocarcinoma):** GI_{50} = 17.34 ± 1.7 μM (Gemcitabine: GI_{50} = 0.20 ± 0.08 μM)
- **PANC1 (human pancreatic adenocarcinoma):** GI_{50} = 10.06 ± 0.8 μM (Gemcitabine: GI_{50} = 0.73 ± 0.22 μM)

Conclusions

- Four series of **37** derivatives of fumiquinazoline
- Two synthetic methodologies: a three-component one-pot microwave-assisted approach and a multistep Mazurkiewicz–Ganesan approach.
- **Antitumor** Activity**:** six new analogues were found to exhibit tumor cell growth inhibitory activity.
- Four compounds showed potential for **neuroprotection** in a PD in vitro model.
- Two derivatives exhibited a potent **antibacterial** activity against *S. aureus* strains (MIC = 4-8 µg/mL) and isolation of the enantiomers revealed that only (1*S*, 4*R*) was active, indicating that stereochemistry is vital for the referred activity.

Acknowledgments

We thank the UID/Multi/04423/2019 through national funds provided by FCT-Foundation for Science and Technology and European Regional Development Fund (ERDF), in the framework of the program PT2020. This research was developed under Project No. 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, and Solida Long thanks Erasmus-Lotus+ program (LOTUS+, LP15DF0205).