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

Marine-derived fungi as a source of potential antimicrobial adjuvants

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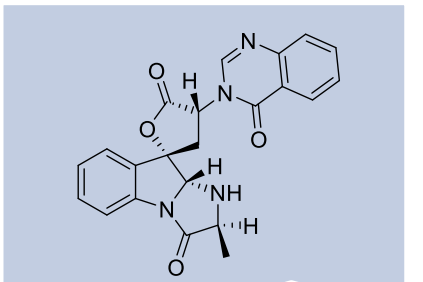
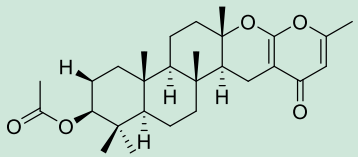
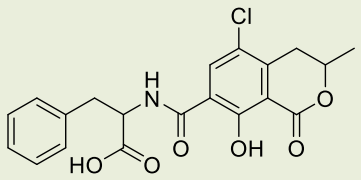
Marine-derived fungi as a source of potential antimicrobial adjuvants



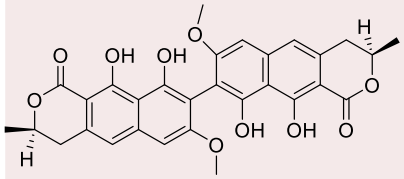
Alkaloids



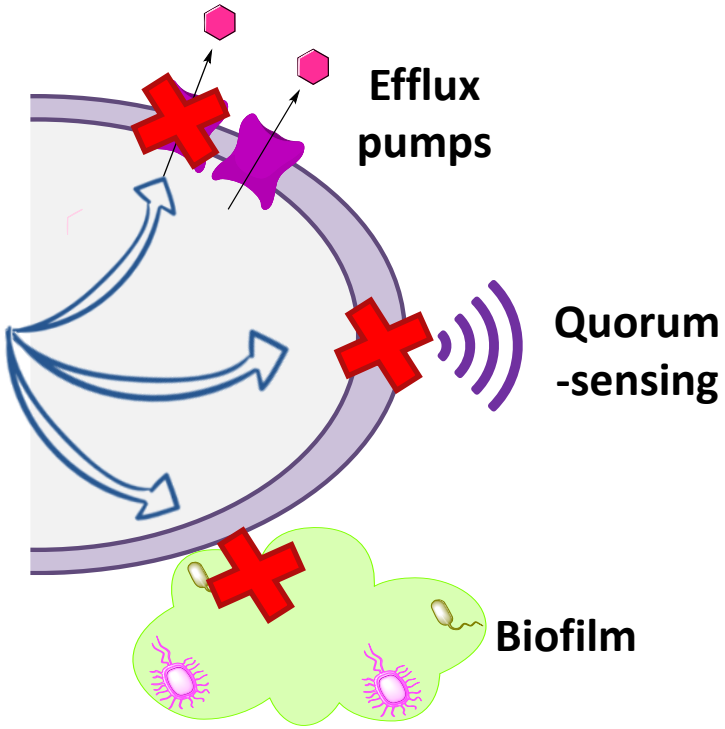
Meroditerpenes



Mycotoxins



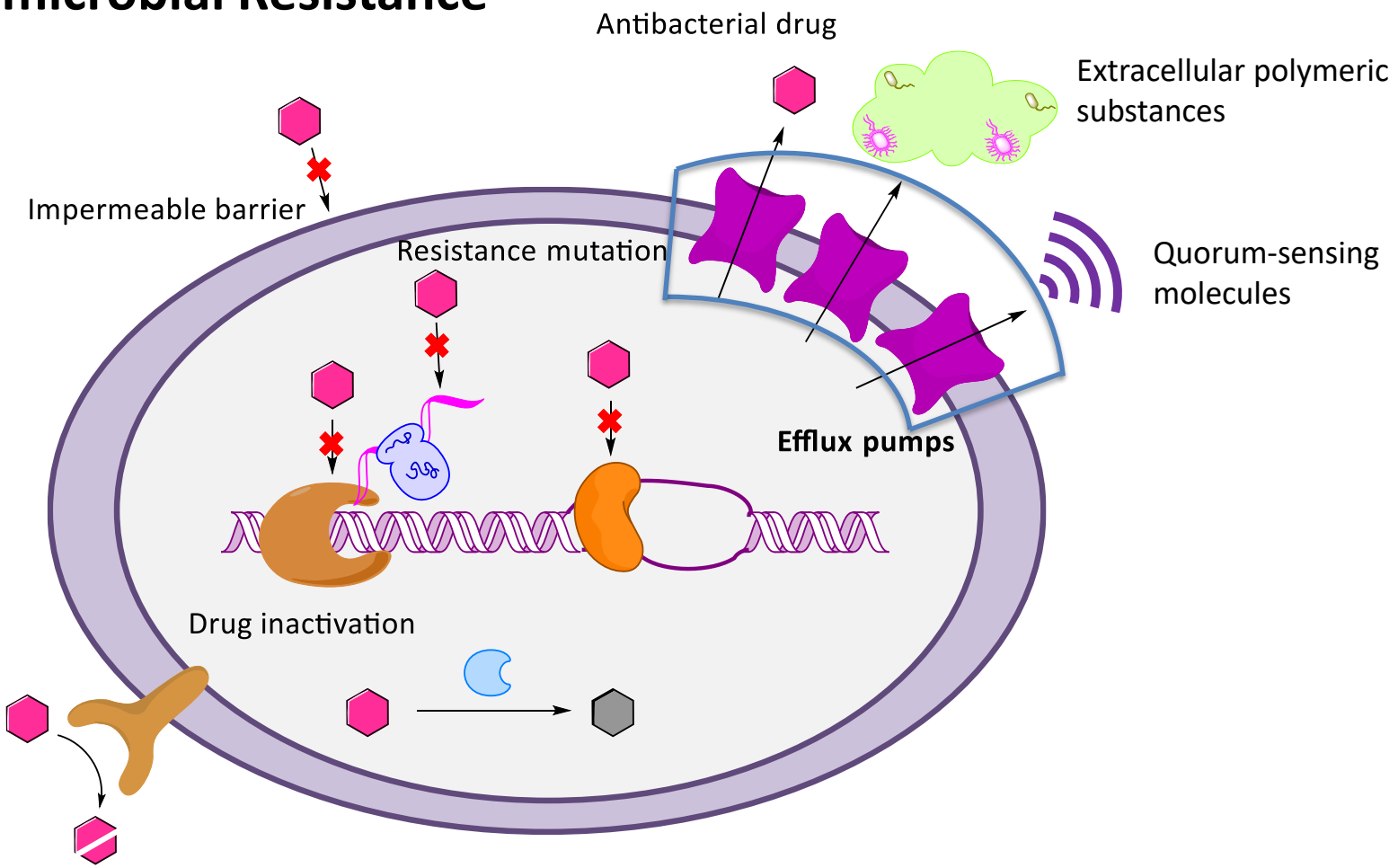
Dimeric naphthopyranones



Nature has always played an important role in therapeutics as a provider of bioactive compounds. Specifically, the marine environment is a rich, but underexplored, source of potential bioactive compounds. Recently, there has been an increased interest in marine microorganisms, namely bacteria and fungi, capable of producing secondary metabolites with new scaffolds.
In our efforts to discover compounds with potential to be used as antimicrobial agents and/or adjuvants, we turned our attention to compounds isolated from marine-derived fungi (*Aspergillus* and *Neosartorya* genera), presenting different scaffolds, some of which had already shown potential as antibacterial agents. Therefore, the aim of this study was to test nineteen metabolites from marine-derived fungi for their potential as inhibitors of bacterial efflux pumps, one of the most worrisome antimicrobial resistance mechanisms, and of biofilm formation and quorum-sensing, related resistance and virulence mechanisms, . Results have shown two compounds were effective as Gram-positive efflux pump inhibitors, and three displayed the same activity for the Gram-negative strain tested. Docking studies were useful for molecular visualization of the compounds in the predicted binding sites. Moreover, eight compounds were able to inhibit biofilm formation in the strains tested, and four inhibited quorum-sensing in the models chosen. Cytotoxicity studies were performed in NIH/3T3 cell line, and three compounds could be safely used as antibacterial, efflux pump inhibitors and/or biofilm formation inhibitors.
The outcomes of this study highlight the potential that lies in the sea, and the opportunities for finding new therapies, or inspiration for new molecules.

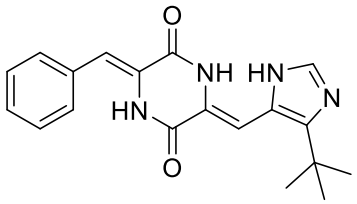
Keywords: antimicrobial activity; biofilm inhibition; efflux pump inhibition; marine-derived fungal metabolites; quorum-sensing inhibition

Antimicrobial Resistance

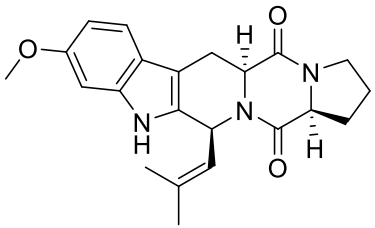


Adapted from Allen, H. K., *et al*. (2010). Nat Rev Micro 8(4): 251-259; Durães, F., *et al*. (2021). Antibiotics 10(5): 600.

Marine-derived fungi – a source of efflux pump inhibitors

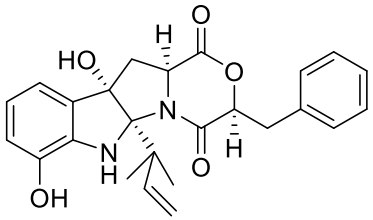


Halimide

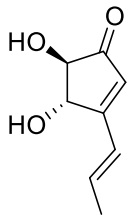


Fumitremorgin C

Metabolites from *Aspergillus* sp. with promising modulation of P-glycoprotein



Shornephine A



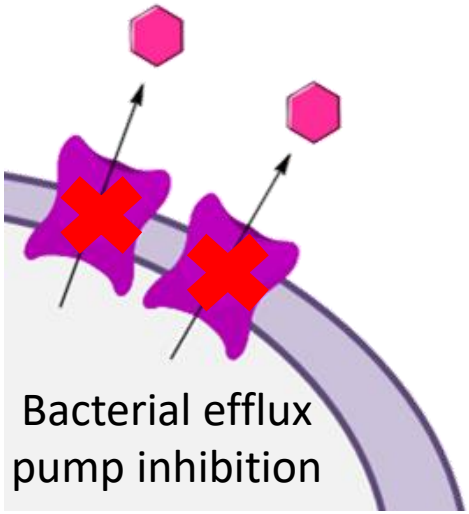
Terrein

Are metabolites from marine-derived fungi also able to inhibit bacterial efflux pumps?

Long, S., *et al*. (2016). Molecules (Basel, Switzerland) 21(7): 892.

Aims

19 compounds isolated from marine derived-fungi



Related mechanisms

Inhibition of biofilm formation

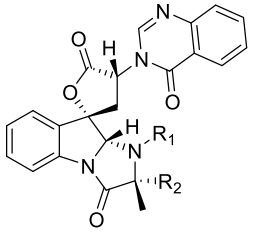
Inhibition of quorum-sensing

Cytotoxicity (NIH/3T3 cell line)

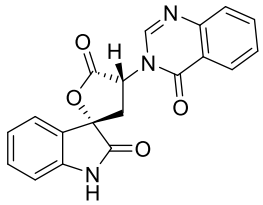
Durães, F., *et al*. (2021). Marine Drugs 19(9): 475.

Compounds used

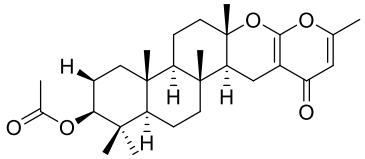
Compounds isolated from *Neosartorya siamensis*



Tryptoquivaline F (**1**): $R_1 = R_2 = H$
Tryptoquivaline H (**2**): $R_1 = OH$; $R_2 = H$
Tryptoquivaline L (**3**): $R_1 = OH$; $R_2 = CH_3$
Tryptoquivaline O (**4**): $R_1 = H$; $R_2 = CHO$

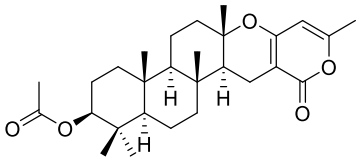


3′-(4-oxoquinazolin-3-yl)spiro(1*H*-3,5′-oxolone)-2,2′-dione (**5**)

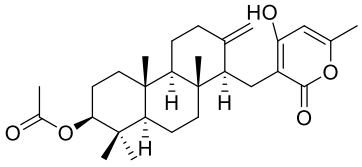


Chevalone C (**6**)

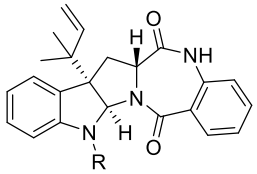
Compounds isolated from *Neosartorya takakii*



Chevalone B (**7**)



Aszonapyrone A (**8**)

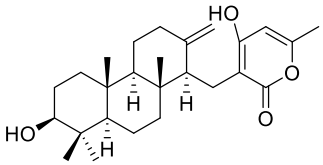


Aszonalenin (**9**): R = H
Acetyl aszonalenin (**10**): R = Ac

Gomes, N. M., *et al*. (2014). Marine Drugs 12(2): 822-839; Zin, W. W., *et al*. (2015). Marine Drugs 13(6): 3776-3790.

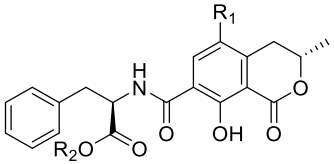
Compounds used

Compound isolated from *Neosartorya laciniosa*

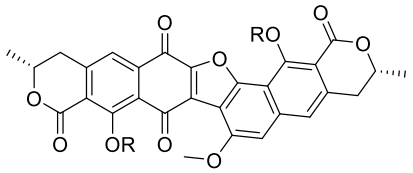


Aszonapyrone B (**11**)

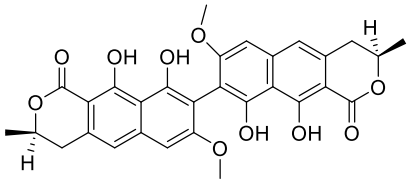
Compounds isolated from *Aspergillus elegans*



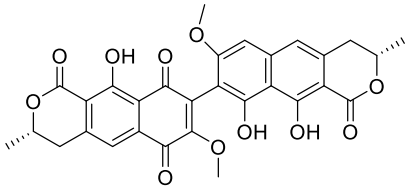
Ochratoxin A (**12**): R_1 = H; R_2 = Cl
Ocratoxin A methyl ester (**13**): R_1 = CH_3; R_2 = Cl
Ochratoxin B (**14**): R_1 = R_2 = H



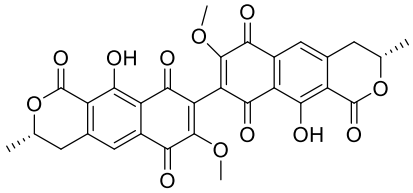
Rubrosulphin (**15**): R = H
Rubrosulphin diacetate (**16**): R = Ac



Vioxanthin (**17**)



Viomellein (**18**)

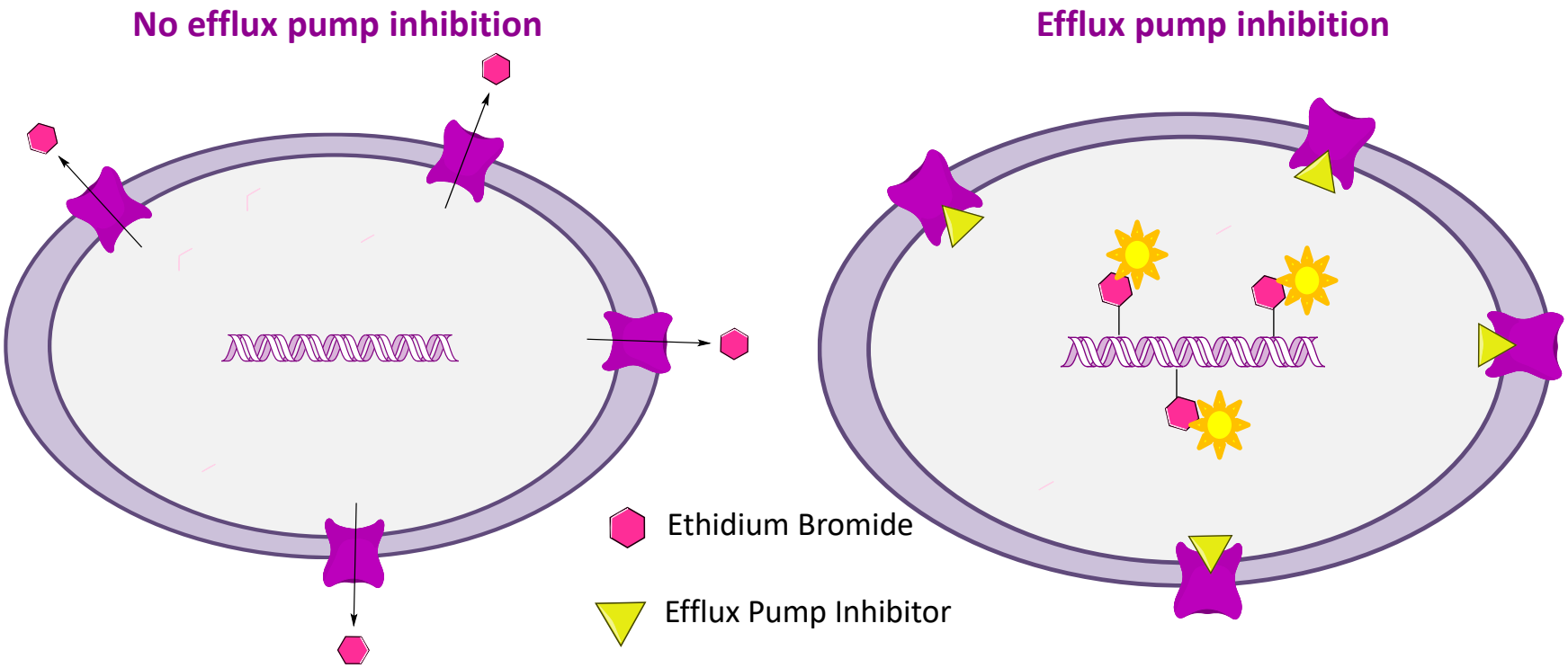


Xanthomegnin (**19**)

Kumla, D., *et al*. (2021). Phytochemistry 181: 112575; Eamvijarn, A., *et al*. (2013). Tetrahedron 69(40): 8583-8591.

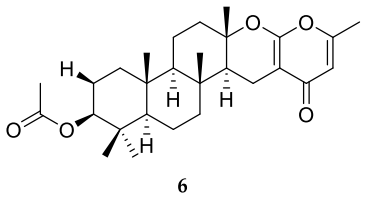
Inhibition of bacterial efflux pumps

The inhibition of efflux pumps was accessed through the real-time ethidium bromide accumulation assay, in *Staphylococcus aureus* 272123 and *Salmonella enterica* serovar Typhimurium SL1344 (*acrA* gene deleted)



Inhibition of bacterial efflux pumps

S. aureus 272123 (positive control: reserpine)



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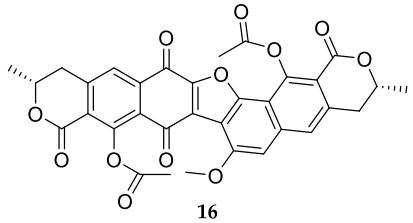

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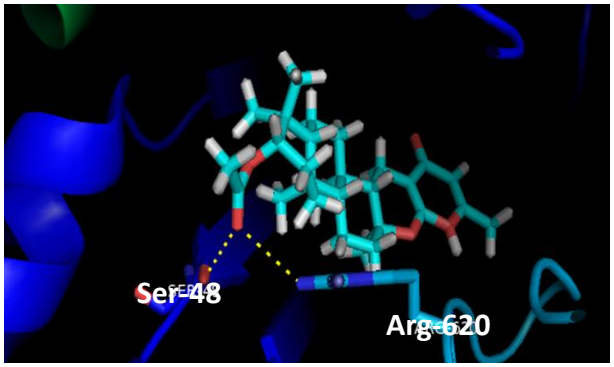
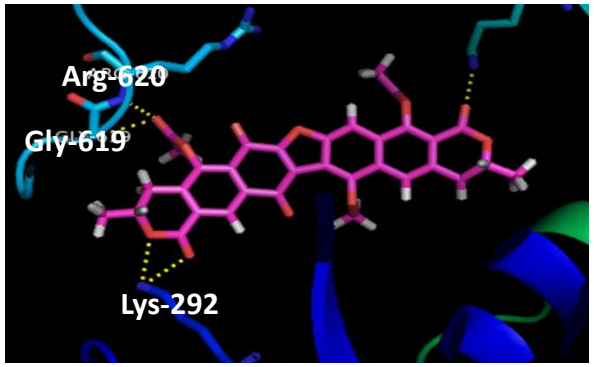


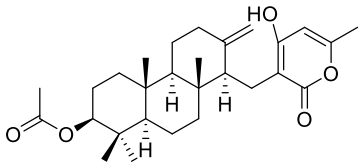


Molecular visualization in the binding core region of a NorA homology model

Zárate, S. G., *et al*. (2019). Antibiotics (Basel) 8(1).

Inhibition of bacterial efflux pumps

S. enterica serovar Typhimurium SL1344 (positive control: CCCP)



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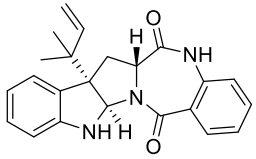

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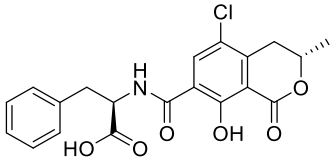

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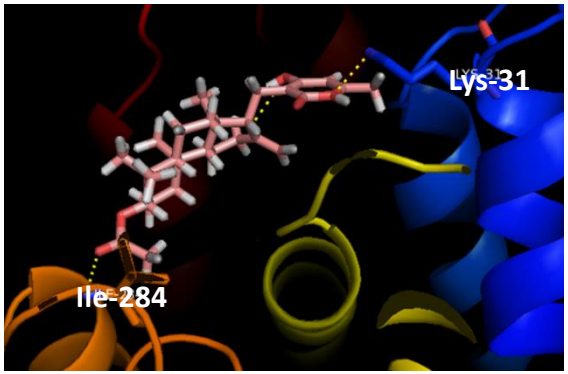
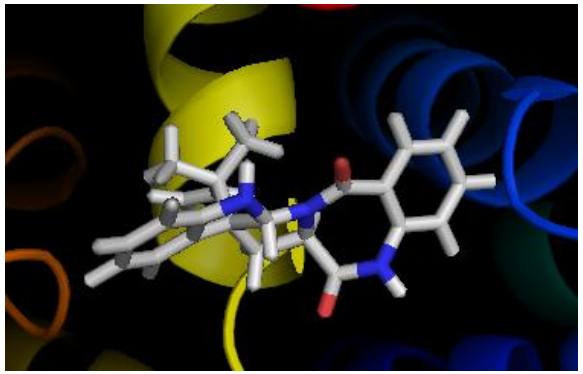
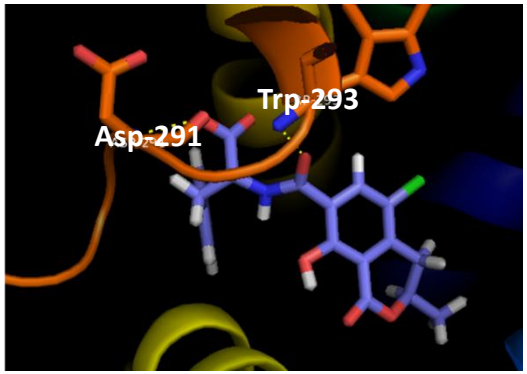




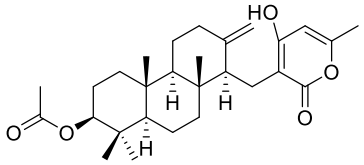


Molecular visualization in the substrate binding domain of AcrB (PDB:4DX5)

Aron, Z. and T. J. Opperman (2018). Research in Microbiology 169(7): 393-400.

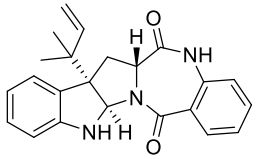
Inhibition of biofilm formation

S. aureus ATCC 29213



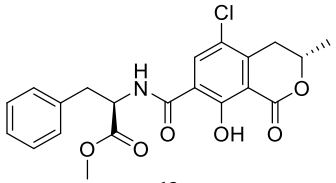
8

72% (9 μM)



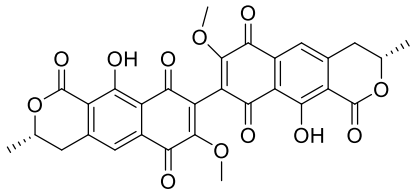
9

63% (100 μM)



13

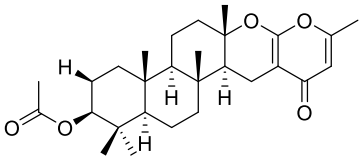
88% (10 μM)



19

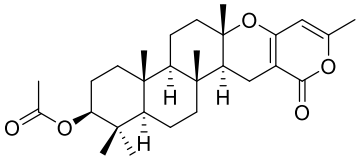
96% (100 μM)

S. aureus 272123



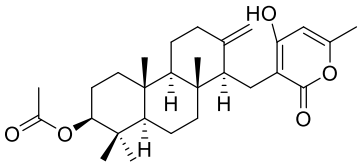
6

78% (12.5 μM)



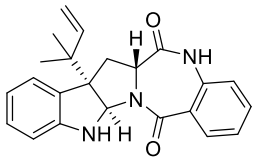
7

86% (100 μM)



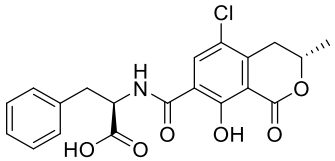
8

93% (6.25 μM)



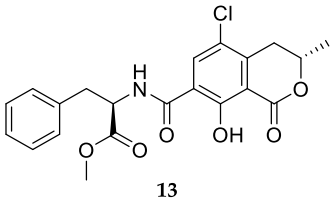
9

93% (6.25 μM)



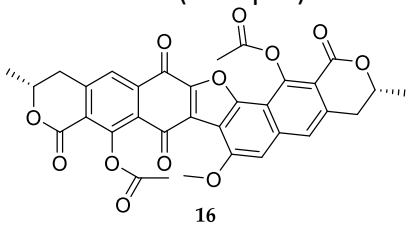
12

81% (100 μM)



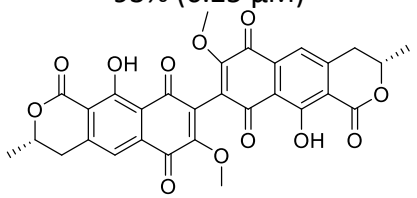
13

98% (25 μM)



16

93% (100 μM)

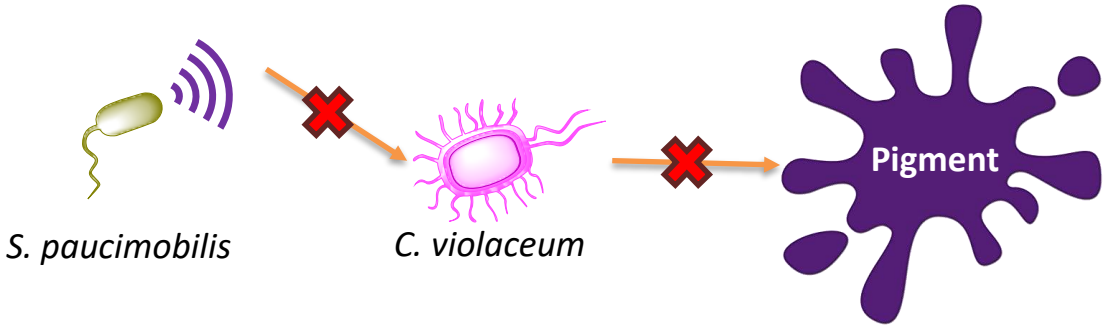


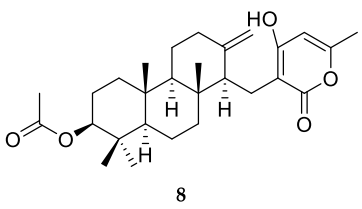
19

84% (50 μM)

Inhibition of quorum-sensing

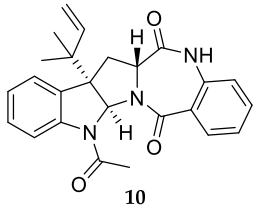
The model used was the sensor strain *Chromobacterium violaceum* CV026 (sensor strain) and *Sphingomonas paucimobilis* Ezf 10-17 (strain producer of quorum-sensing molecules)





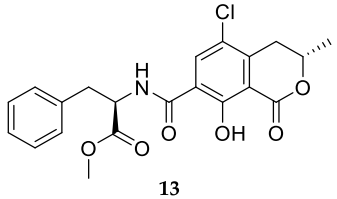
8

30 mm



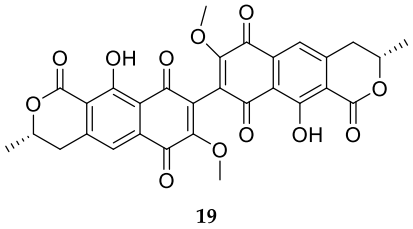
10

32 mm



13

31 mm

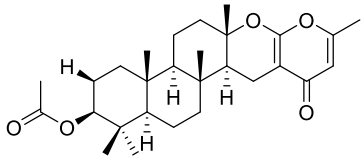


19

42 mm

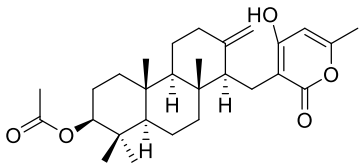
Cytotoxicity

MTT assay: Mouse embryonic fibroblast cell line – NIH/3T3



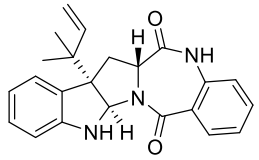
6

IC_{50} = 30.95 ± 0.13 µM



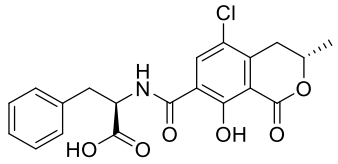
8

IC_{50} = 25.02 ± 2.37 µM



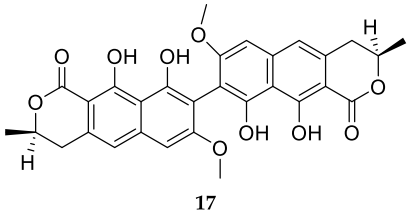
9

IC_{50} = 16.74 ± 1.40 µM



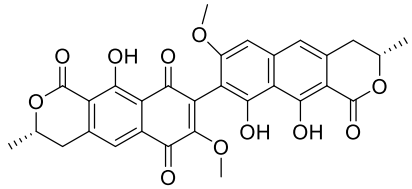
12

IC_{50} = 80.02 ± 3.66 µM



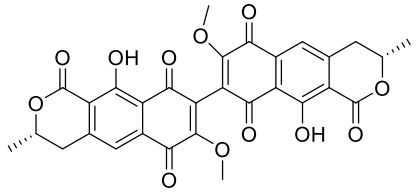
17

IC_{50} = 34.50 ± 3.14 µM



18

IC_{50} = 16.71 ± 1.52 µM



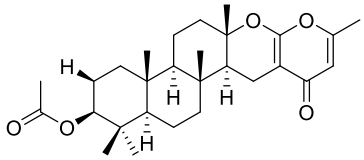
19

IC_{50} = 32.44 ± 3.22 µM

IC_{50} – Half-maximal inhibitory concentration

Cytotoxicity

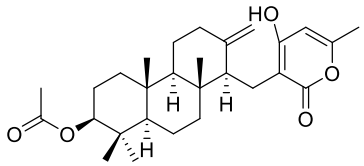
MTT assay: Mouse embryonic fibroblast cell line – NIH/3T3



6

IC_{50} = 30.95 ± 0.13 µM

✓ Efflux pump inhibitor
Biofilm formation inhibitor

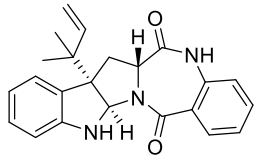


8

IC_{50} = 25.02 ± 2.37 µM

✗ Efflux pump inhibitor

✓ Biofilm formation inhibitor
Quorum-sensing inhibitor

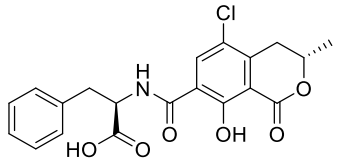


9

IC_{50} = 16.74 ± 1.40 µM

✗ Efflux pump inhibitor

✓ Biofilm formation inhibitor

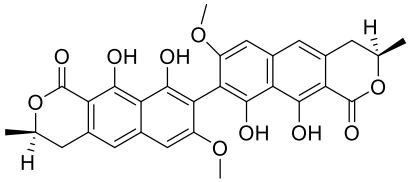


12

IC_{50} = 80.02 ± 3.66 µM

✗ Biofilm formation inhibitor

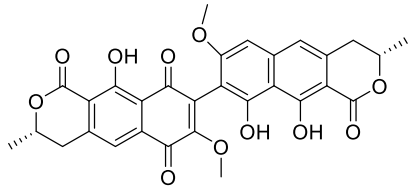
✓ Efflux pump inhibitor



17

IC_{50} = 34.50 ± 3.14 µM

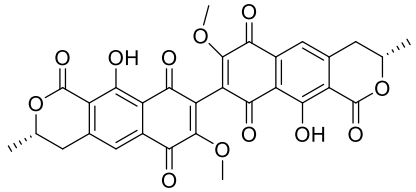
✓ Antibacterial
(MIC = 6.25 µM for *S. aureus* 272123)



18

IC_{50} = 16.71 ± 1.52 µM

✓ Antibacterial
(MIC = 6.25 µM for *S. aureus* 272123)



19

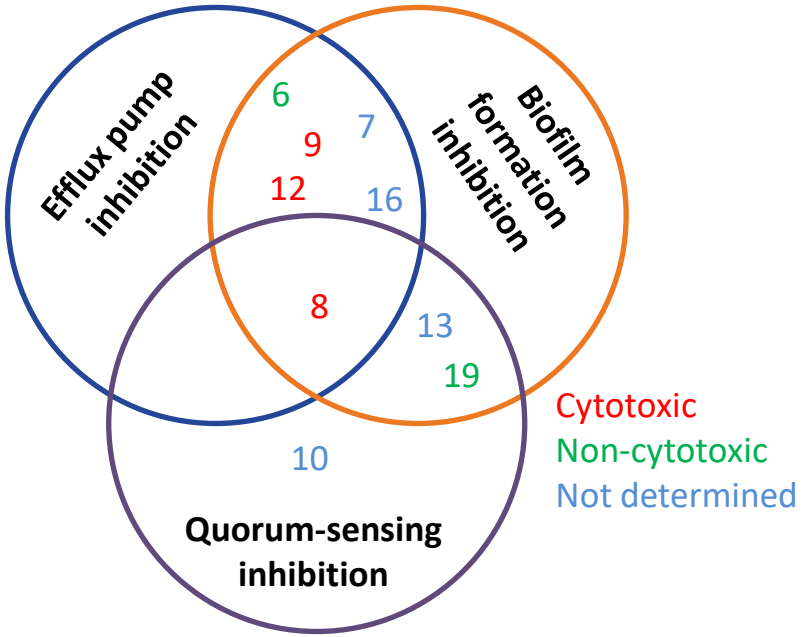
IC_{50} = 32.44 ± 3.22 µM

✗ Biofilm formation inhibitor

✓ Quorum-sensing inhibitor

Conclusions

Metabolites isolated from marine-derived fungi presented activity



Templates for the synthesis of derivatives with improved safety profiles

Inspiration for new substitution patterns

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 and structured program of R&D&I ATLANTIDA (NORTE-01-0145-FEDER-000040), supported by NORTE2020, through ERDF, and CHIRALSINTESE_APSFCT_IINFACTS_2021. FD thanks FCT for his PhD grant (SFRH/BD/144681/2019).