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New chalcone analogues with potential bacterial efflux pump inhibitory effect

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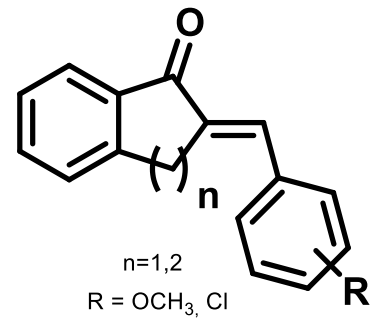
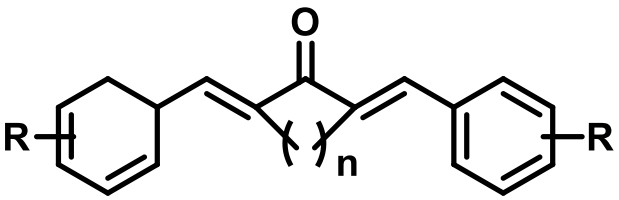
New chalcone analogues with potential bacterial efflux pump inhibitory effect

Synthesis of chalcone analogues



n=1,2

R = OCH_3, Cl



n=0,1,2

R = OCH_3, CH_3, NHR, F, Cl, Br

Antimicrobial activity evaluation

1. Screening for antibacterial potential

1 promising compound

(MIC = 32 µg/mL, *S. aureus* ATCC 29213)

2. Synergic effect evaluation

37 promising compounds

(decreased MIC of cefotaxime or vancomycin)

3. Efflux Pump Inhibition

9 promising compounds

(inhibited the efflux system of *S.* Typhimurium or *S. aureus)*

Abstract:

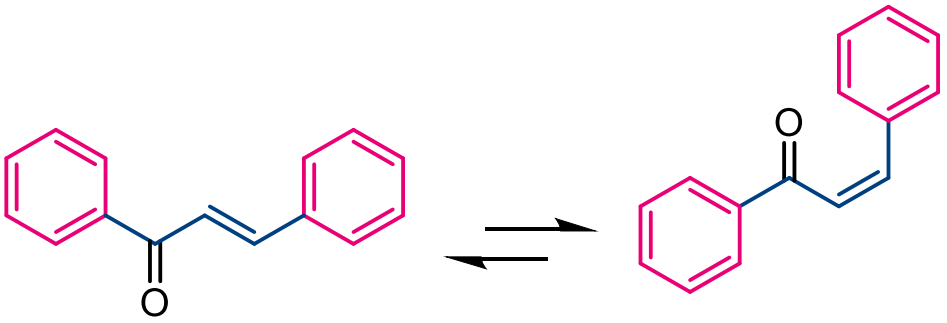
Chalcones comprise a class of natural products possessing two aromatic rings linked by an enone. These compounds **show extensive biological activities**, including antimicrobial activity in multidrug resistant bacteria [1].
Considering the potential of these compounds as antimicrobial agents**, a library of chalcones and their analogues was synthesized,** and the minimum inhibitory concentrations were determined by broth microdilution methods. Antibacterial activity was accessed for reference strains (*Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853). Multidrug-resistant isolates, ESBL *E. coli* SA/2, MRSA *S. aureus* 66/1 and VRE *E. faecalis* B3/101, were used for the synergy assay with antibiotics, cefotaxime, oxacillin and vancomycin, respectively. **Most of the compounds displayed promising results in combination with antibacterial drugs, while not displaying relevant antimicrobial activity**. Further insights into the mechanisms through which these compounds could exert synergistic activity were pursued, and their potential to inhibit bacterial efflux pumps was investigated in Gram positive (*S. aureus* MRSA 272123) and Gram negative (a mutant strain of *Salmonella enterica* Typhimurium expressing the AcrAB-TolC efflux system with the *acrA* gene inactivated) bacteria. The assay is based on the intracellular accumulation of the efflux pump substrate ethidium bromide measuring the increase in fluorescence in the presence of a potential efflux pump inhibitor [2]. By this approach, **some chalcone analogues displayed promising activity as bacterial efflux pump inhibitors.**

Keywords: Chalcone analogues, Antibacterial activity, Synergic effetc, Efflux pump

[1] Singh, P. et al., Eur. J. Med. Chem., 2014. 85: p. 758-777; [2] Nové, M. et al., Microorganisms, 2020. 8(4): p. 566.

Introduction: Chalcone and chalcone analogues

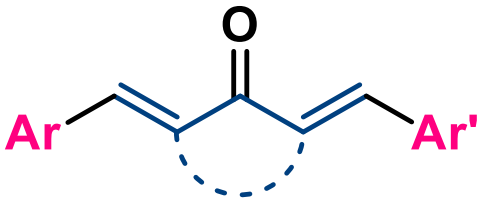
Chemistry of natural and synthetic chalcone



(*E*)-chalcone (*Z*)-chalcone

- Two aromatic rings
- three carbon bridge
- **Enone linker**

Chemistry of chalcone analogues: diarylpentanoids



Diarylpentanoids

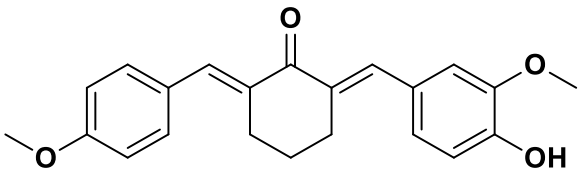
C5-curcuminoids

- Two aromatic rings
- Five carbon bridge
- **Enone linker**

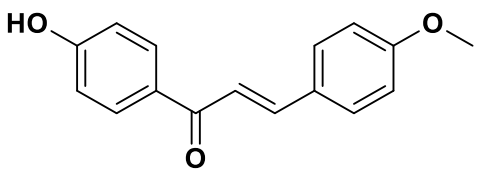
Introduction: Chalcone and Chalcone analogues

Biological activities of chalcone and chalcone analogues

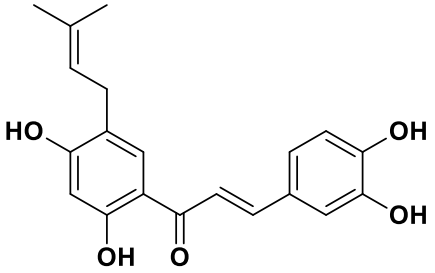
Anti-inflammatory



Antitubercular

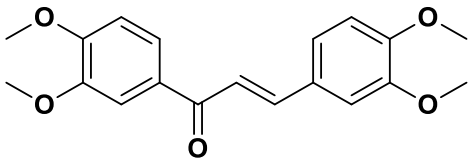


Antioxidant

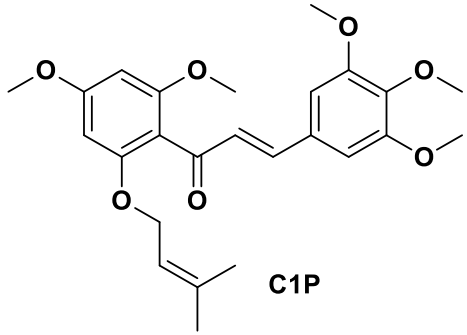


Broussochalcone

Anti-malarial

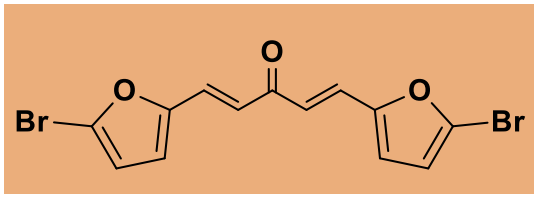


Antitumor



C1P

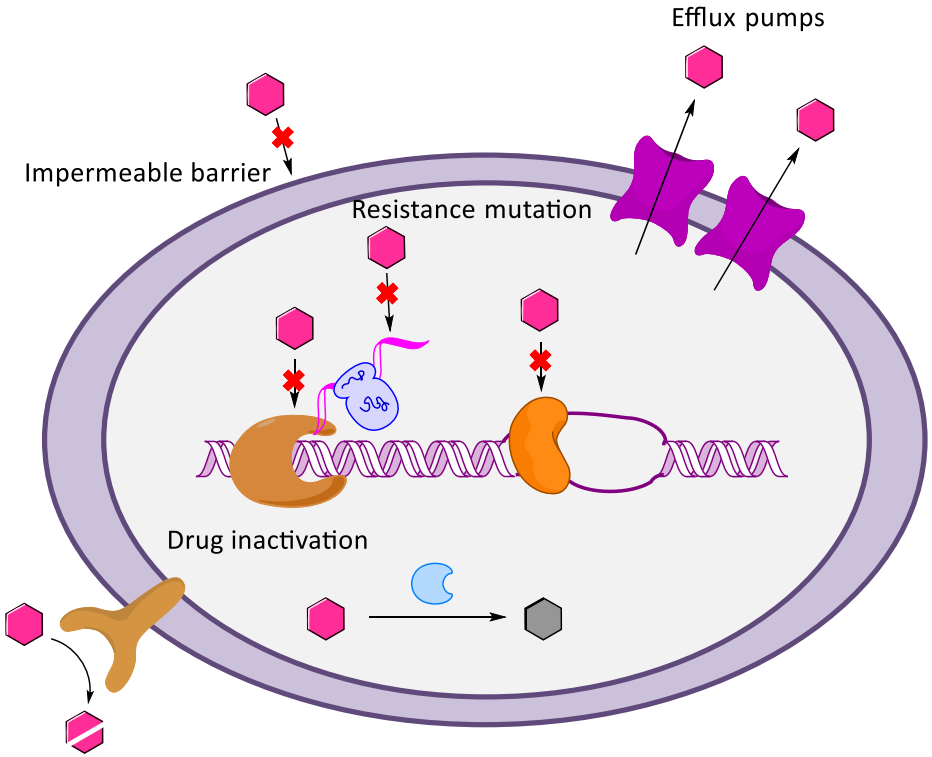
Antibacterial



Singh, P. et al., Eur. J. Med. Chem., 2014. 85: p. 758-777; Liang, G. et al., 2008, Chem. Pharm. Bull. 56(2): 162-167; Leão, M. et al., Life sciences, 2015. **142**: p. 60-65: Aluwi, M. et al., Bioorg. Med. Chem. Lett, 2016. **26**(10): p. 2531-2538.

Introduction: Antimicrobial Resistance Mechanisms

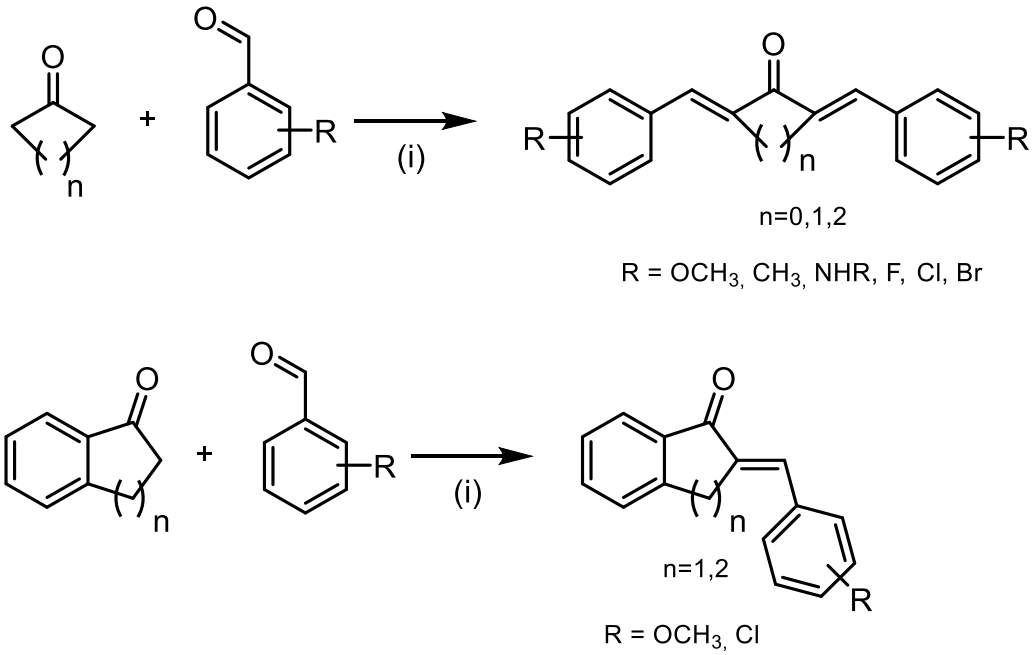
Bacteria can develop multiple mechanisms of antimicrobial resistance



This study will focus on the inhibition of bacterial efflux pumps

Duraes, D. et al., Curr. Med. Chem., 25 (2018) 6030-6069.

Results and Discussion: Synthesis

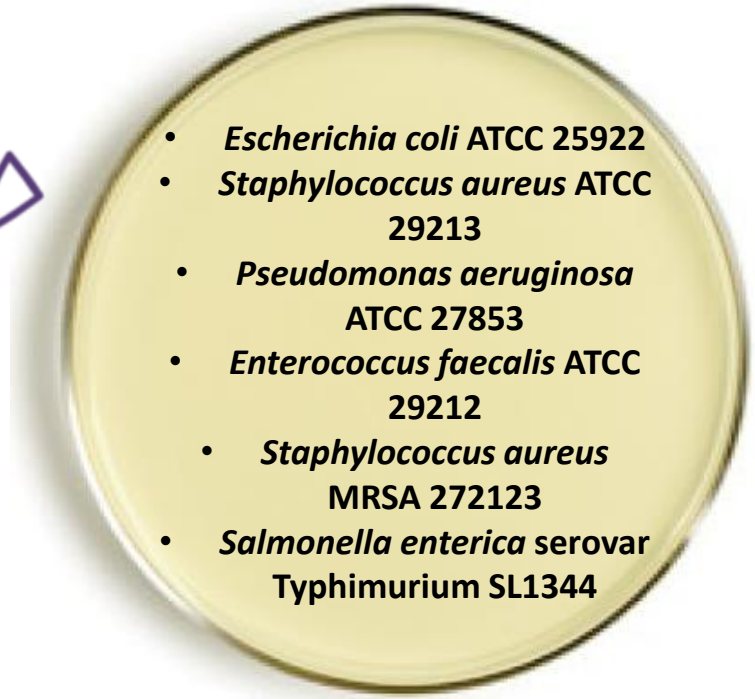


Scheme 1: Synthesis of diarylpentanoids. Reaction conditions: (i) NaOH, CH_3OH, 60 ºC.

A total of 46 derivatives were synthesized

Results and Discussion: Antimicrobial activity

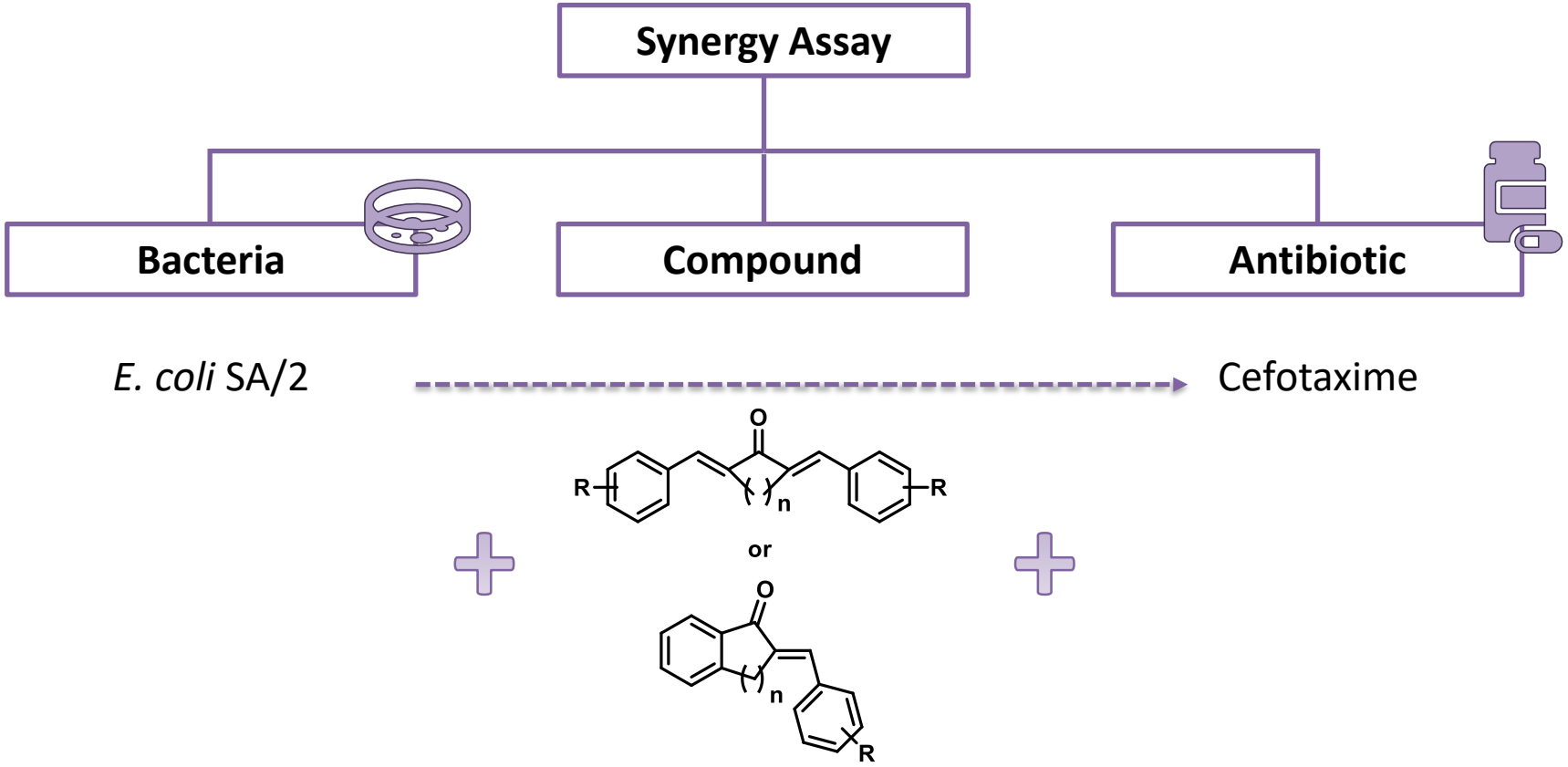
46 chalcone analogues were screened for their antibacterial potential



45 compounds did not display minimum inhibitory concentration (MIC) at the tested concentrations

One compound presented MIC = **32 µg/mL** for *S. aureus* ATCC 29213

Results and Discussion: Synergy with antibiotics



Results and Discussion: Synergy with antibiotics

46 compounds tested

E. coli SA/2 + Cefotaxime

MIC: 512 µg/ml

18 compounds **decreased** MIC of cefotaxime

- **2-fold** 13 compounds
- **4-fold** 4 compounds
- **8-fold** 1 compound

E. faecalis B3/101 + Vancomycin

MIC: 1024 µg/ml

19 compounds **decreased** MIC of vancomycin

- **2-fold** 15 compounds
- **4-fold** 3 compounds
- **16-fold** 1 compound

Mechanism?

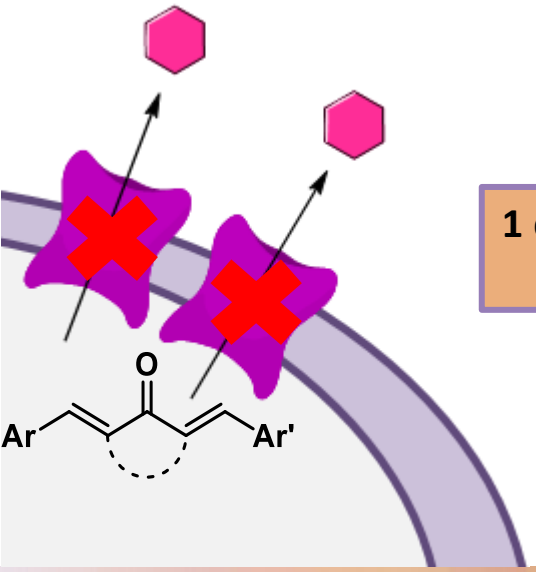
Results and Discussion: Efflux Pump Inhibition

29 compounds tested

Strains tested:
S. aureus MRSA 272123
S. Typhimurium SL1344

Ethidium bromide accumulation assay
(Positive control: reserpine)

Increased fluorescence



1 compound **inhibited the efflux system** of *S.* Typhimurium

8 compounds **inhibited the efflux system** of *S. aureus*

Conclusions

- **46 chalcone analogues** were synthesized

- All compounds were screened for their antibacterial potential as well as their synergic effect:
  - **One compound presented** MIC **= 32 µg/mL** for *S. aureus* ATCC 29213
  - **18** compounds **decreased** MIC of cefotaxime
  - **19** compounds **decreased** MIC of vancomycin

- The potential to inhibit bacterial efflux pumps of **29** chalcone analogues was investigated:
  - **1** compound **inhibited the efflux system** of *S.* Typhimurium
  - **8** compounds **inhibited the efflux system** of *S. aureus*

Acknowledgments

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