



# Methoxy-substituted hydroxychalcone reduces biofilm production, adhesion, and surface motility of *Acinetobacter baumannii* by inhibiting *ompA* gene expression

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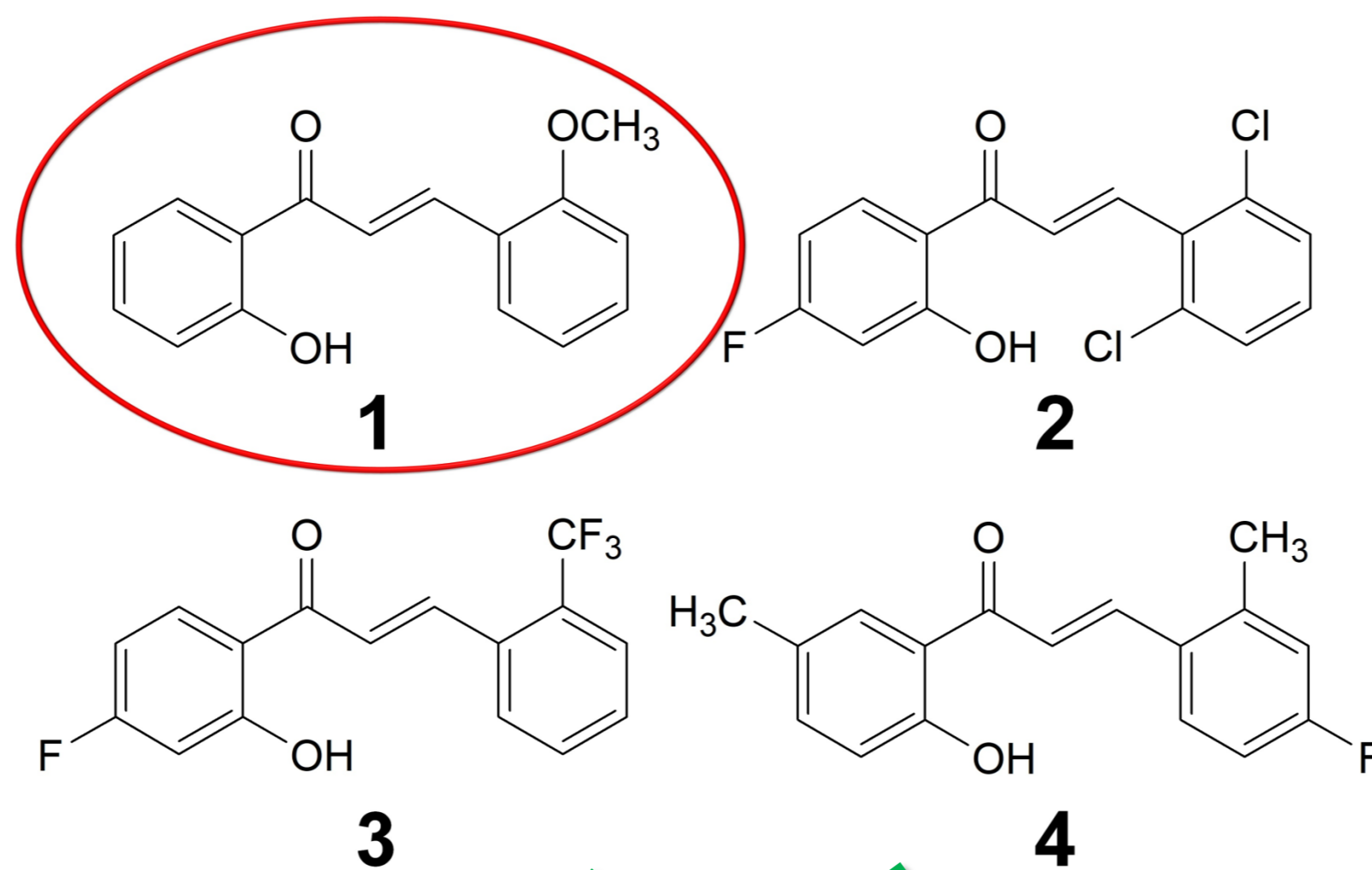
## INTRODUCTION

- Carbapenem-resistant *Acinetobacter baumannii* is recognized as a top priority pathogen for development of new therapeutic strategies by WHO and CDC.<sup>1</sup>
- Outer membrane protein A (OmpA) is a major virulence factor in *A. baumannii*, involved in adhesion and invasion of host cells, cytotoxicity, motility, biofilm production, OMVs biogenesis, immune evasion, and AMR.<sup>2</sup>
- Targeting virulence is a novel therapeutic strategy that provides possibility to disarm pathogens, while minimally affecting their growth, thereby slowing down the selection of resistant mutants.<sup>3</sup>
- Chalcones are compounds that shown plenty of potent antimicrobial and antivirulence activities in multiple studies.<sup>4</sup>



- 1) Select chalcone with the most potent antibiofilm activity against *A. baumannii*.
- 2) Determine its crystallinity and thermal properties.
- 3) Investigate its activity against biofilm-related virulence factors gene expression in *A. baumannii*.
- 4) Confirm its antivirulence activity through the evaluation of phenotypic features associated with the affected genes.

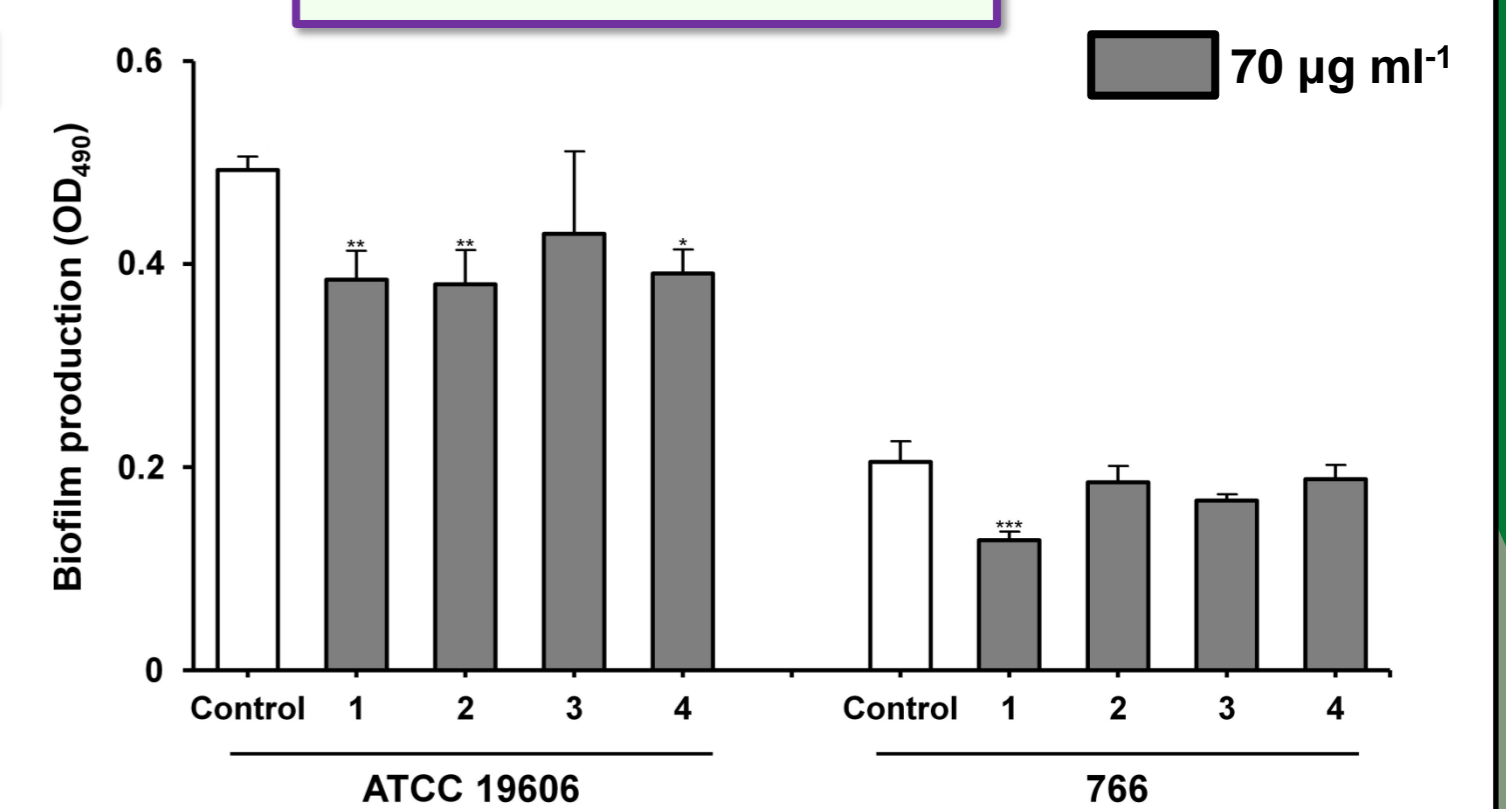
Hydroxychalcones were obtained by base-catalyzed Claisen-Schmidt condensation and the structures were verified by FTIR, <sup>1</sup>H NMR, <sup>13</sup>C NMR and ESI-MS.



## *A. baumannii* strains used in the study

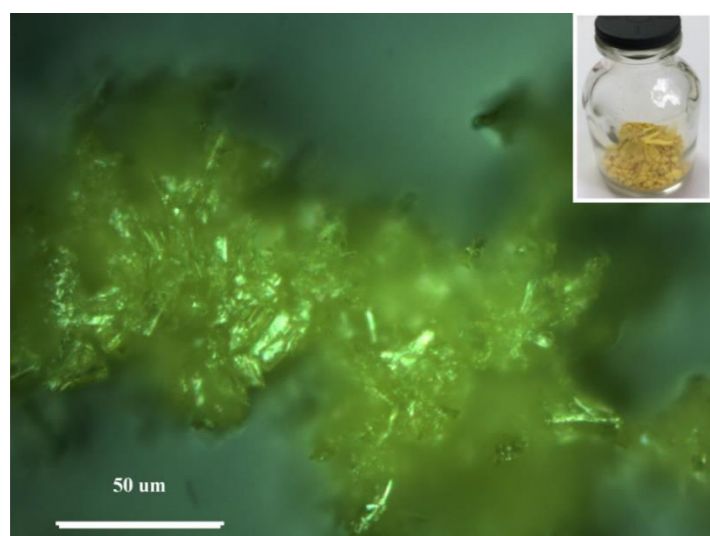
Strain	Origin	Sample
ATCC 19606	France	CSF
766	KBC Zvezdara, Belgrade	wound

Only compound 1 displayed significant antibiofilm activity against both strains

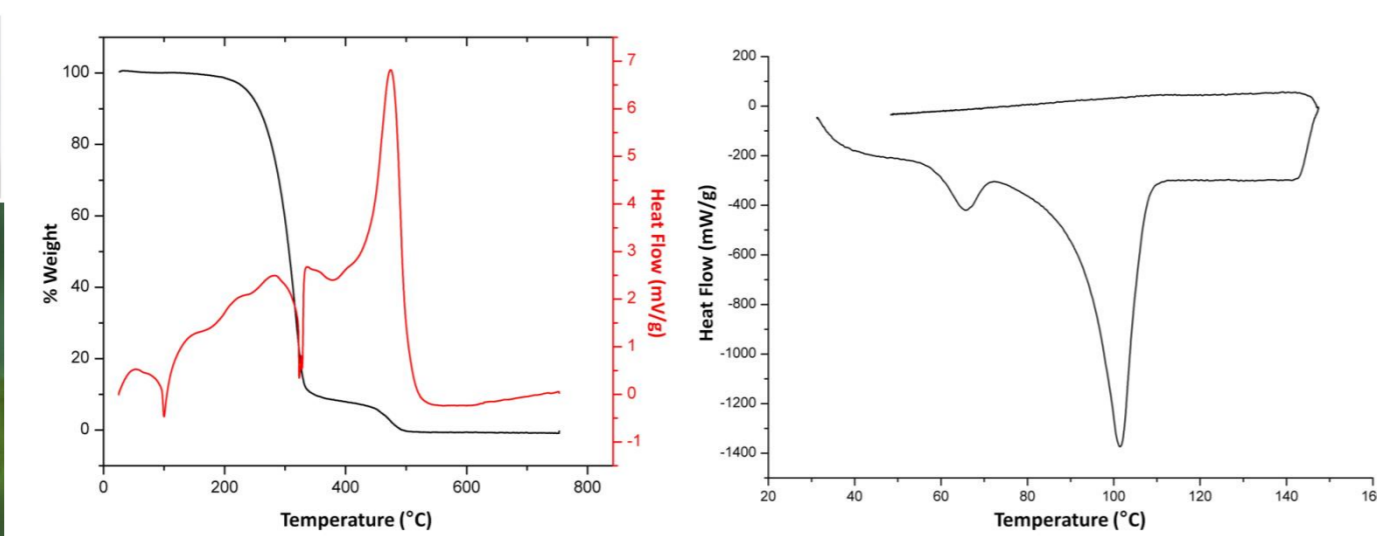


## Biofilm production

Optical microscopy revealed highly crystalline nature of compound 1. TGA/DTA revealed that it is stable up to 250°C, whereas DSC detected melting temperature at 101.4°C.

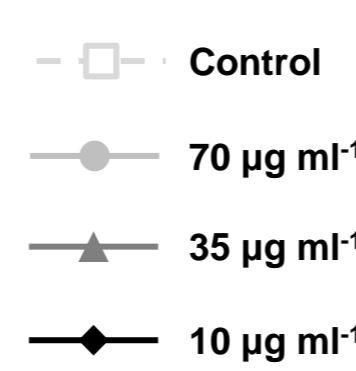


Microscopy



TGA/DTA

DSC

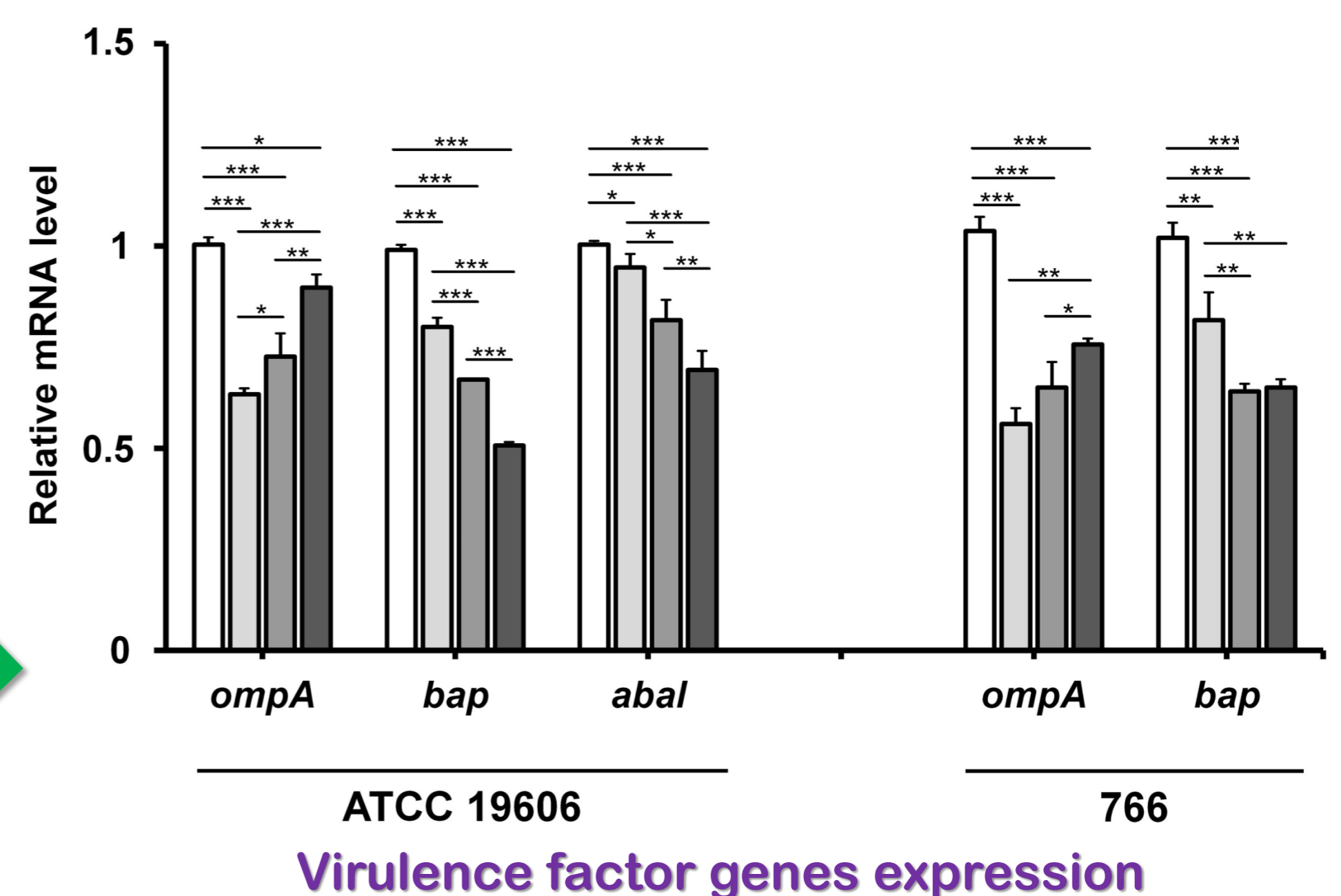
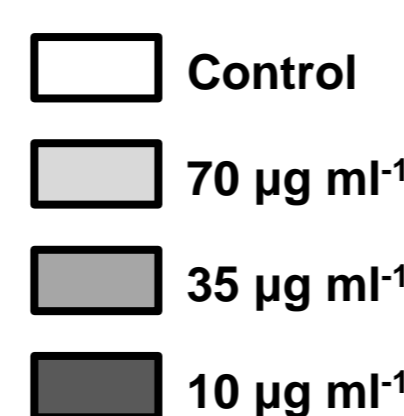


## Cell growth curves

Tested sub-MICs do not inhibit cell growth, so compound 1 could be considered as a suitable antivirulence drug candidate

Fibronectin- and collagen-mediated adhesion is significantly inhibited by compound 1. ATCC 19606 cells were almost completely deprived of its binding ability at 70 µg ml<sup>-1</sup>. Production of AHLs is dose-dependently reduced in ATCC 19606. Surface motility is substantially inhibited in 766 at 70 and 35 µg ml<sup>-1</sup>. The motility was not exhibited by ATCC 19606.

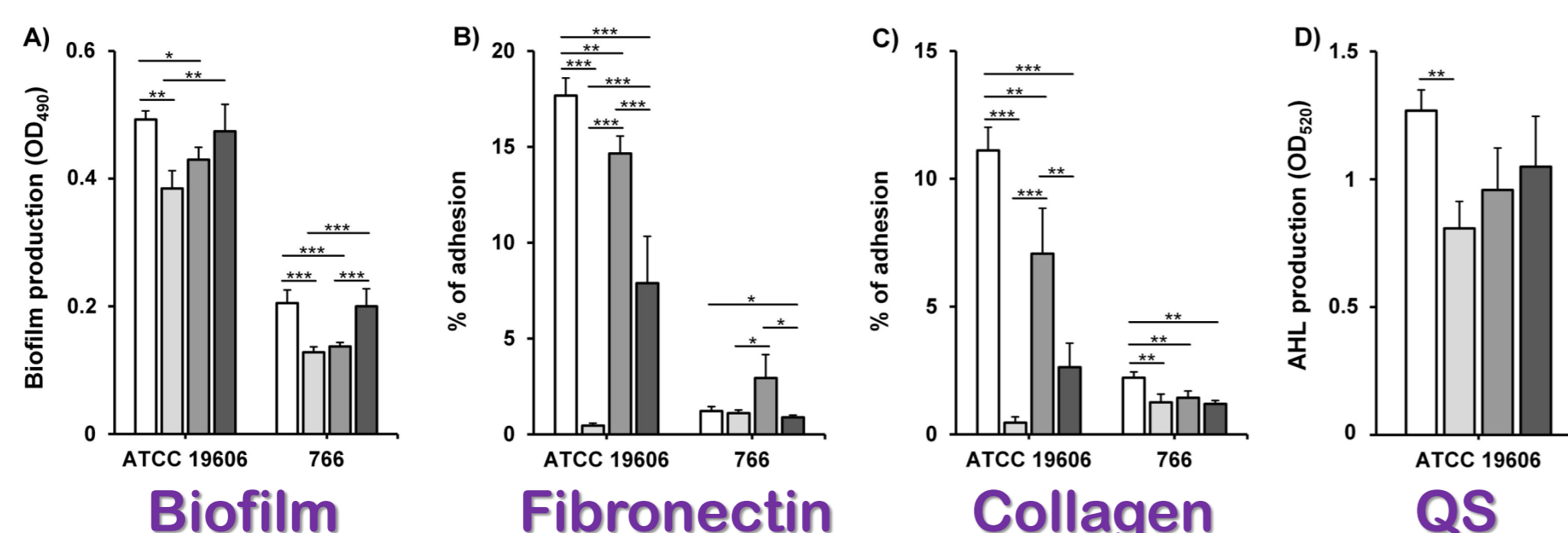
The mRNA expression of all examined virulence factor genes is significantly downregulated by compound 1 at all tested concentrations. Dose-dependent inhibition of *ompA* expression was achieved in both strains. The expression of *abal* was not detected in 766.



## Virulence factor genes expression

## Conclusions:

- ✓ Methoxy-substituted hydroxychalcone (compound 1) exhibits the most potent antibiofilm activity against *A. baumannii*.
- ✓ Gene expression of virulence factors OmpA, Bap, and Abal is significantly inhibited by methoxy-substituted hydroxychalcone.
- ✓ The expression of *ompA*, whose protein product is associated with numerous virulence traits, is downregulated by 1.58-fold to 1.85-fold at 70 µg ml<sup>-1</sup> → this is confirmed through the inhibition of ECM-mediated adhesion and surface motility.
- ✓ Methoxy-substituted hydroxychalcone can be considered as an appropriate antivirulence drug candidate against *A. baumannii*.

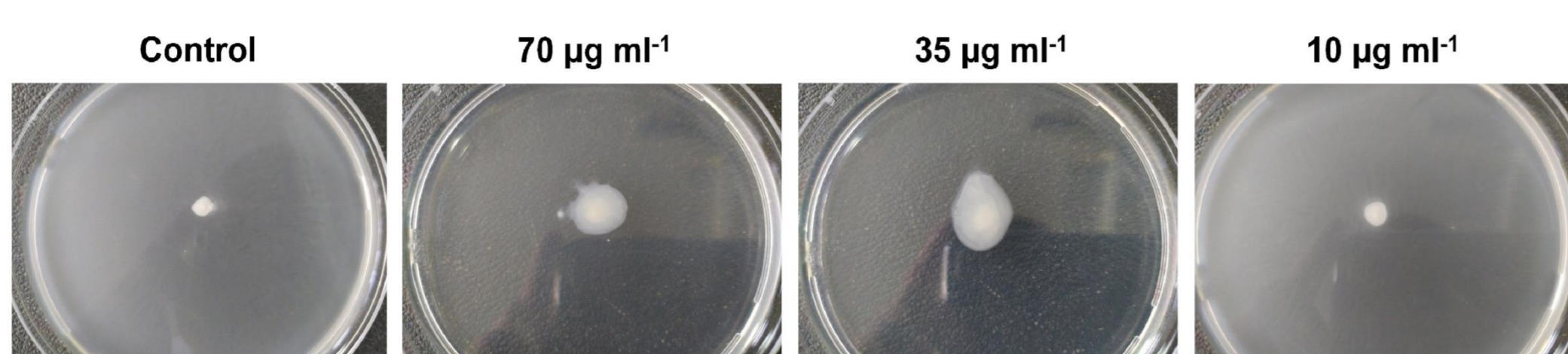


Biofilm

Fibronectin adhesion

Collagen adhesion

QS



Surface motility