Hamamelitannin analogues potentiate antibiotics in the fight against biofilm-related *Staphylococcus aureus* infections

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ABSTRACT

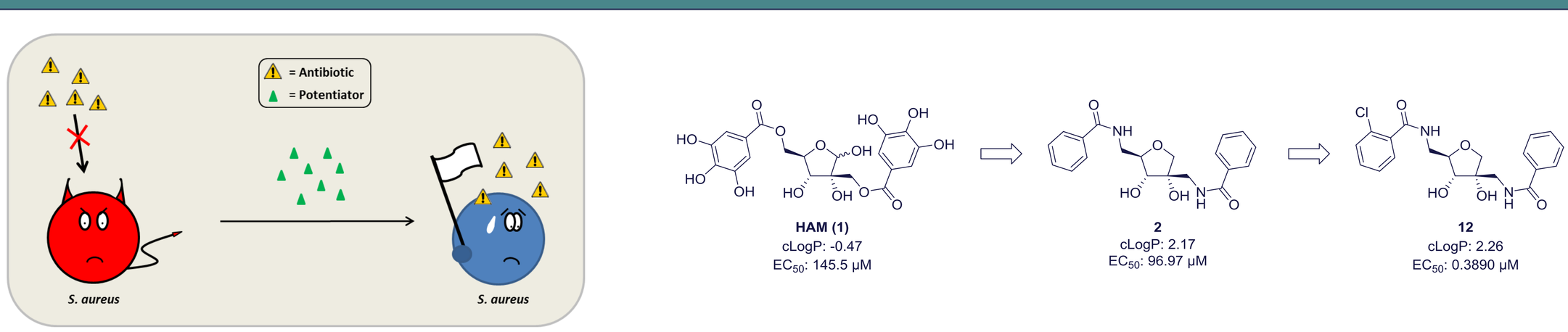
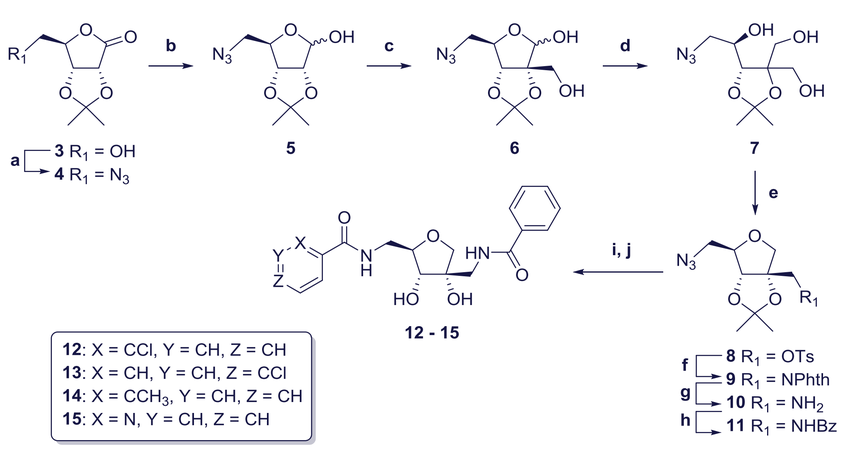


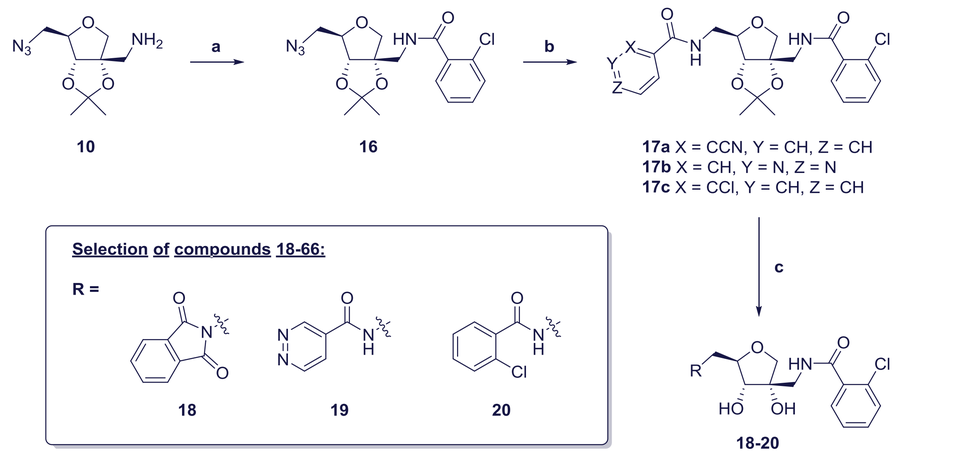
Figure 1: Identification of hamamelitannin analogue **12**, with exceptional antibiofilm activity in *Staphylococcus aureus*.

SYNTHETIC ORGANIC CHEMISTRY

In this poster we present a practical synthetic route that allows preparing a library of HAM derivatives with two non-identical aromatic moieties. A selection is given in Scheme 1 and 2.



Scheme 1. Reagents and conditions: **a)** i) MsCl, Et_3N, DCM, rt, 3h, ii) NaN_3, DMF, 60 °C, 16h, 94% (2 steps); **b)** DIBAlH, DCM, -78 °C, 4h, 98%; **c)** aq. CH_2O, K_2CO_3, MeOH, 50 °C, 24h, 84%; **d)** $NaBH_4$, MeOH, 0 °C, 16h, 93%; **e)** i) TsCl, pyridine, rt, 3h, ii) 60 °C, 16h, 72%; **f)** K phthalimide, NaI, DMF, 90 °C, 16h, 86%; **g)** $N_2H_4.H_2O$, EtOH, reflux, 4 h, 93%; **h)** BzCl, Et_3N, DCM, 0 °C, 3h, 99%; **i)** i) PMe_3, THF, H_2O, ii) RCO_2H, EDC.HCl, DIPEA, HOBt, DMF, rt, 16h; **j)** 35% TFA in H_2O.



Scheme 2. Reagents and conditions: **a)** 2-chlorobenzoic acid, EDC.HCl, DIPEA, HOBt, DMF, rt, 16h, 69%; **b)** i) PMe_3, THF, H_2O, ii) RCO_2H, EDC.HCl, DIPEA, HOBt, DMF, rt, 16h; **c)** 35% TFA in H_2O.

BIOLOGICAL EVALUATION & X-RAY STRUCTURE

Compound	EC_{50} (µM)	
	Pretreatment	Co-treatment
1 (HAM)	145.5	165.1
2	96.97	93.55
12	0.3890	7.976
13	18.01	21.39
14	13.67	52.62
15	70.42	104.4
18	0.2600	1.268
19	3.376	98.93
20	5.357	28.42

Table 1: selection of HAM analogues with *in vitro* results.

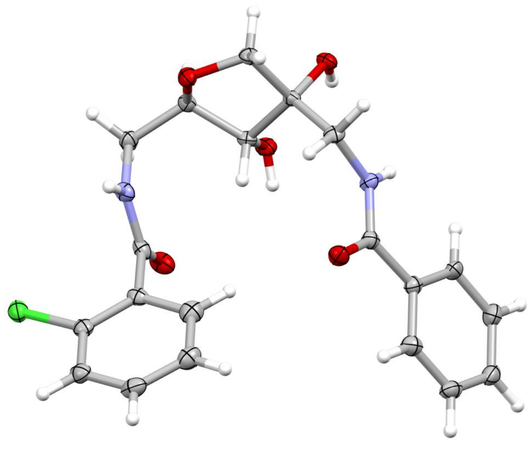


Figure 2: asymmetric unit of the crystal structure of **12.**

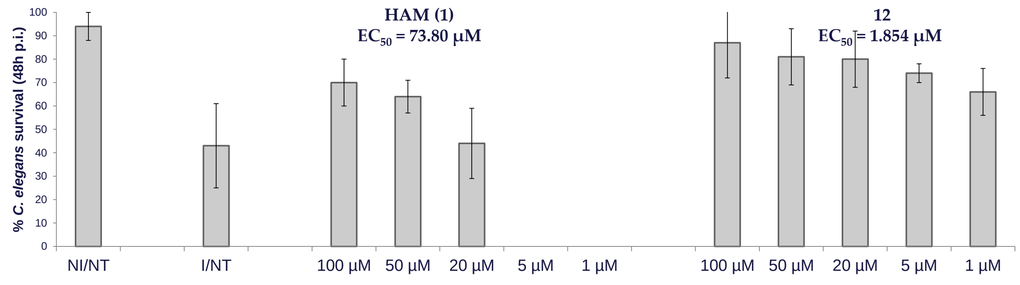


Figure 3: *C. elegans* survival after 48h for uninfected (NI) and infected (I) nematodes receiving no treatment (NT), treatment with HAM, or treatment with **12**. EC_{50} values are expressed as the concentration needed to increase survival by 50%.

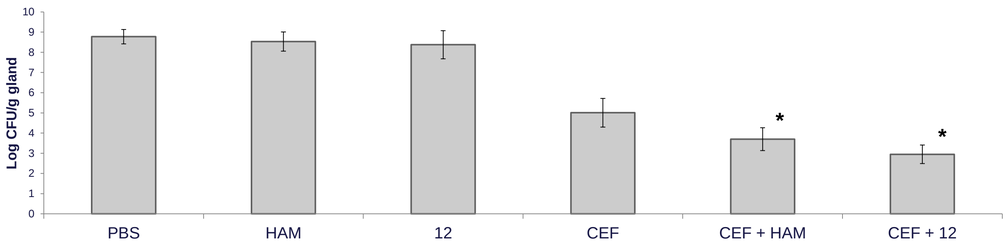


Figure 4: Log(CFU)/g mammary gland (average ± sd) of mice infected with *S. aureus*, reveiving no treatment, treatment with potentiator, or treatment with cefalexin (CEF) and potentiator.

RESULTS & CONCLUSIONS

- We developed a **practical synthetic route** that allows preparing a library of HAM analogues.
- Several compounds show promising activity on biofilm susceptibility (Table 1). 5-*ortho*-chlorobenzamide **12** emerges as one of the strongest potentiators (**EC_{50} = 0.389 µM**).
- The potentiators were **not cytotoxic** up to a concentration of 128µM (highest concentration tested) in MRC-5 lung fibroblast cells, suggesting acceptable therapeutic windows (not shown).
- ***Ortho*-substitution** of the benzamide increases activity. Twisting the benzamide group out of the plane (Figure 2) tends to improve interaction with the target.
- HAM analogues potentiate the activity of several classes of antibiotics (not shown).
- The most potent analogues (including **12**) significantly **enhance survival of infected *Caenorhabditis elegans*** (Figure 3).
- In a **mouse mastitis model**, significantly less CFU are present after treatment with the combination of cefalexin (CEF) and **12**, compared to treatment with antibiotic alone (Figure 4).
- In all cases, **MIC values were higher than 500 µM**, the highest concentration tested (not shown).

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