Contents

1 Results and discussion: HSL analogue-ciprofloxacin conjugates

1.1 Biological testing

This work

All conjugates were tested for growth inhibition (MIC), biofilm formation inhibition and activity against nascent (24 h) and established (48 h) biofilms in *P. aeruginosa*.

The conjugates shown in **??** were tested, as well as BHL **19**, HHQ **21**, PQS **22**, ciprofloxacin **24**, methyl ciprofloxacin **151**, the alkynyl ciprofloxacin derivative **68**, the *tert*-butyl ester ciprofloxacin derivative **198**, the carboxylic acid ciprofloxacin derivative **199**, trimethoprim **25** and the alkynyl trimethoprim derivative **71**.

Cultures were grown in the presence of the compounds at a range of 6 concentrations from 25 to 0.125 μM. MICs were calculated by fitting a modified Gompertz function.[1] An example of the fitting is shown in **??**.

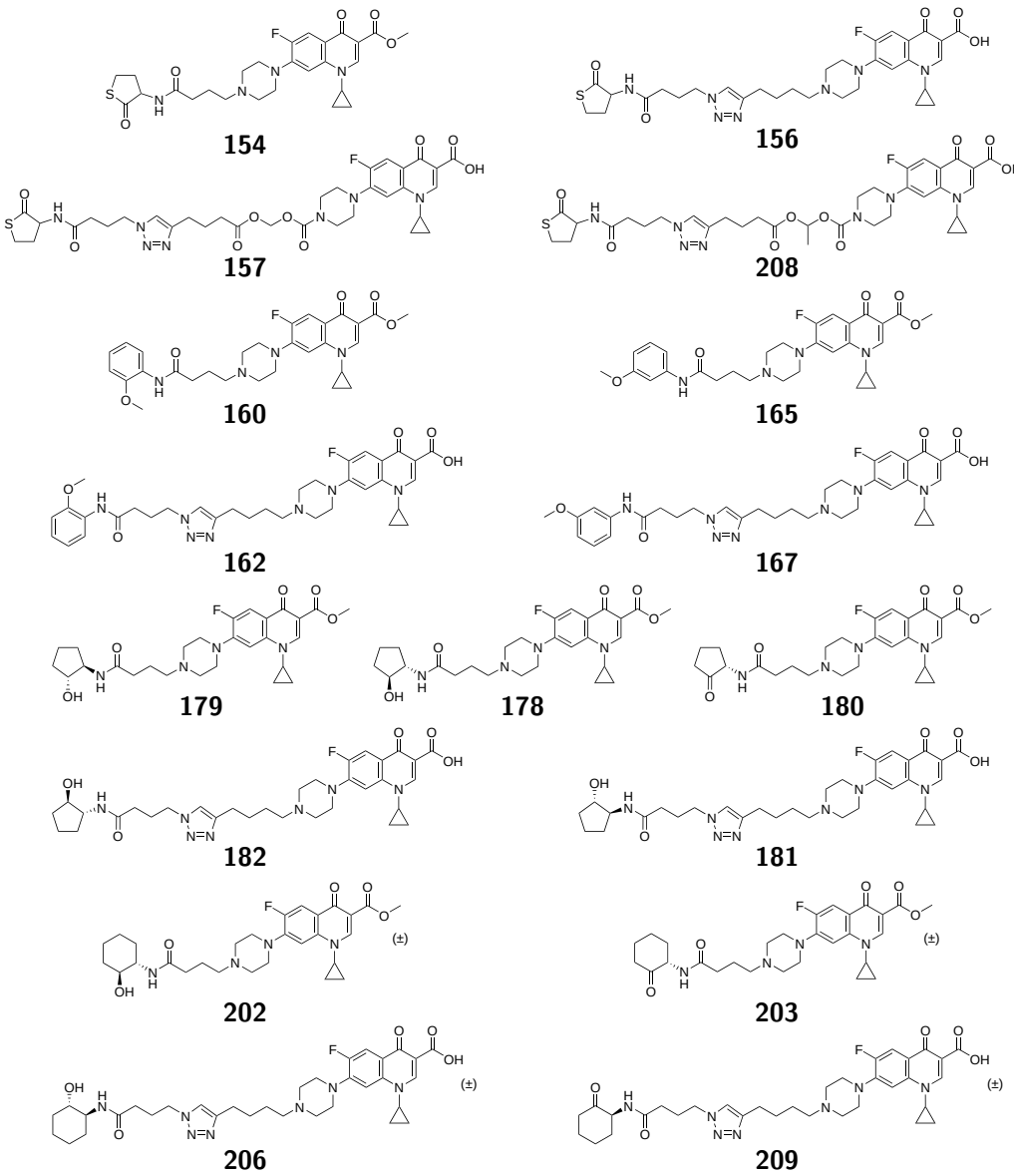

Figure 1

1.1.1 Antibacterial activity

1.1.1.1 YM64

In YM64 at 5 h several of the HSL analogue-ciprofloxacin conjugates showed activity at the highest concentration (see Figure 2 and Figure 3). Conjugates **162** and **167** showed similar activity to ciprofloxacin **24** and the cleavable conjugate **157** showed better activity (see Figure 2). The activity of the cleavable conjugate **157** was even more pronounced at 24 h (see Figure 4).

It should be noted that the highest concentration tested was 25 μM in this set of assays as opposed to 2 μM in the previous set (see **??**), but oddly all compounds including ciprofloxacin **24** showed less activity. This is thought to be due to a change in the plate seals used (see **??**).

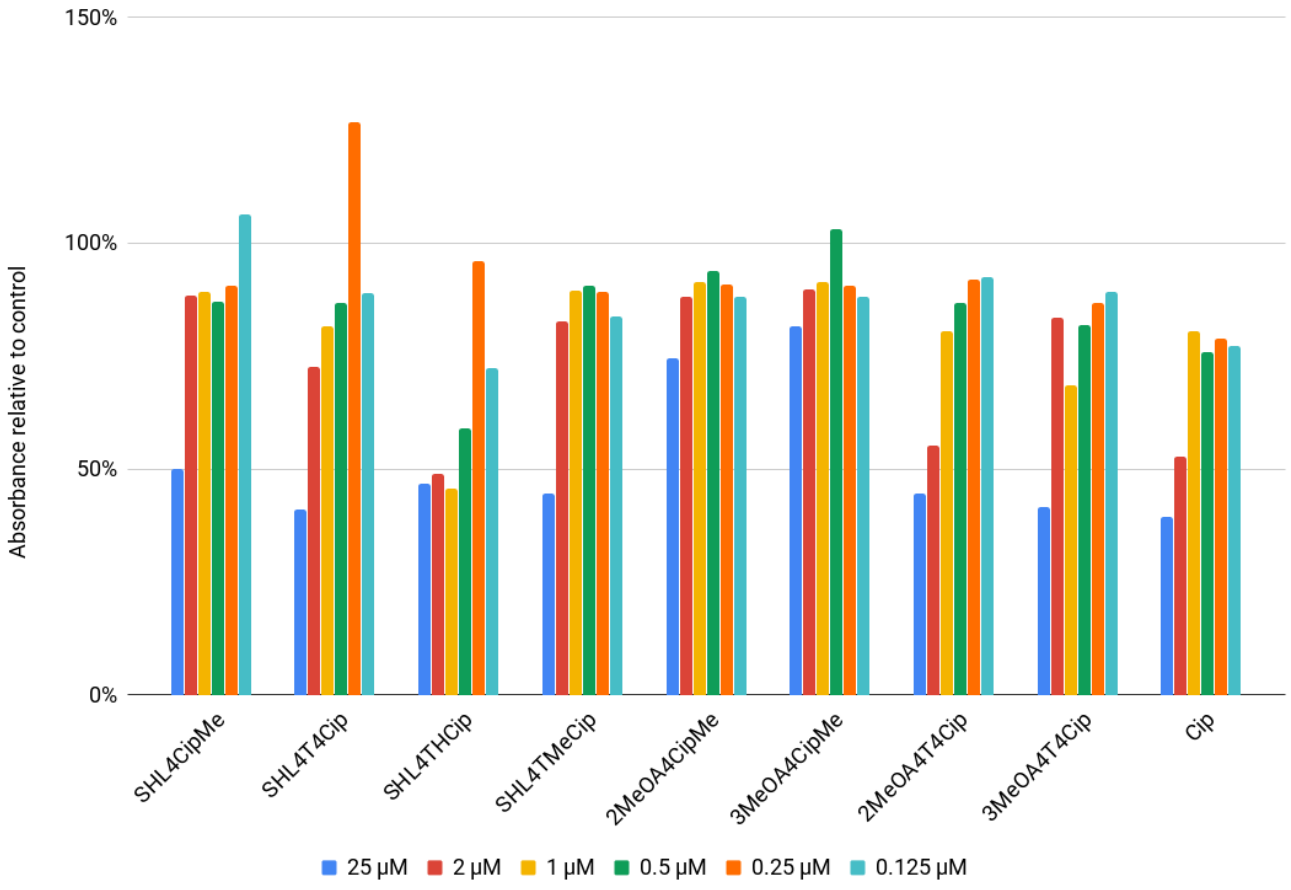

Figure 2: YM64 OD readings at 5 h for the HCTL, 2-methoxybenzene and 3-methoxybenzene HSL analogue-ciprofloxacin conjugates.

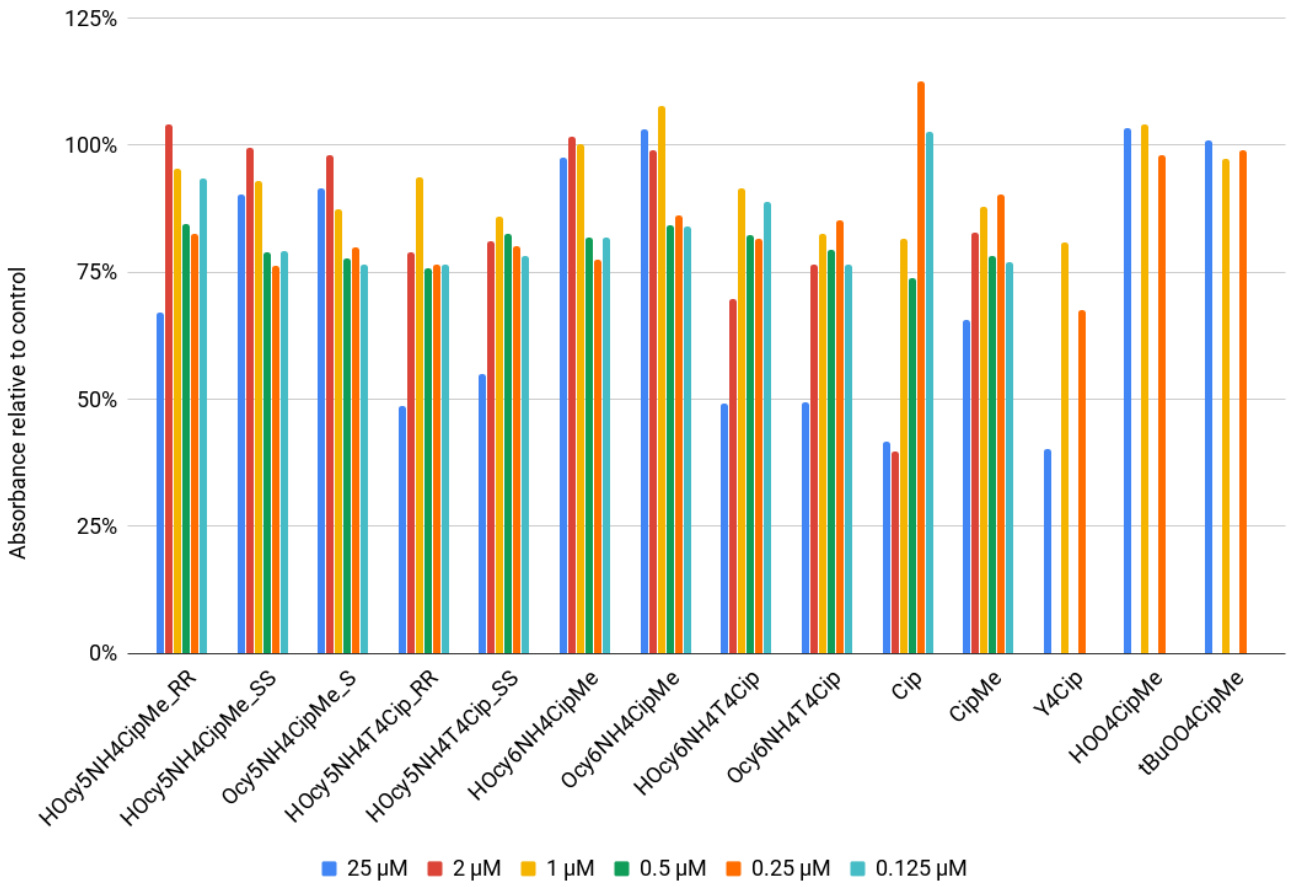

Figure 3: YM64 OD readings at 5 h for the alcohol and ketone HSL analogue-ciprofloxacin conjugates.

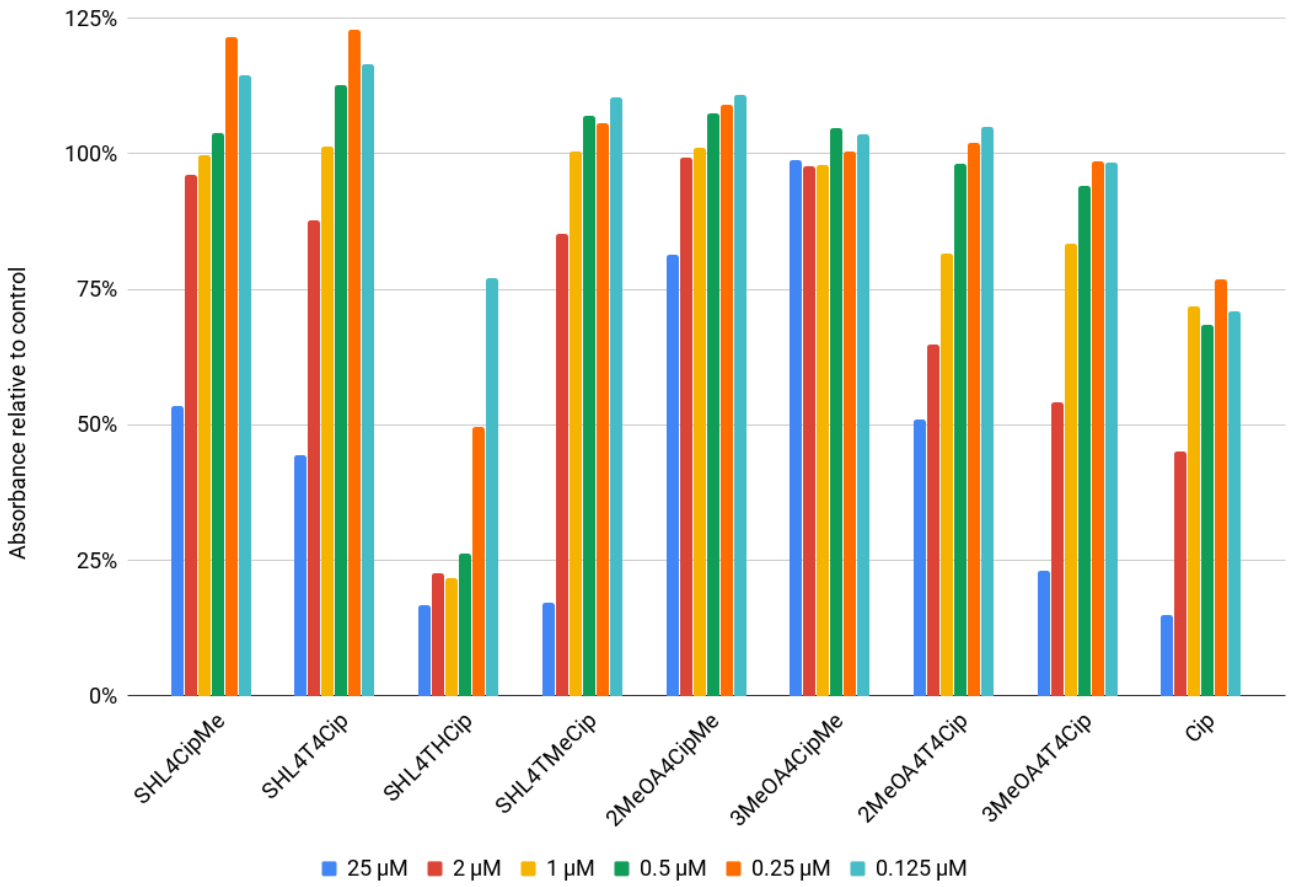

Figure 4: YM64 OD readings at 24 h for the HCTL, 2-methoxybenzene and 3-methoxybenzene HSL analogue-ciprofloxacin conjugates.

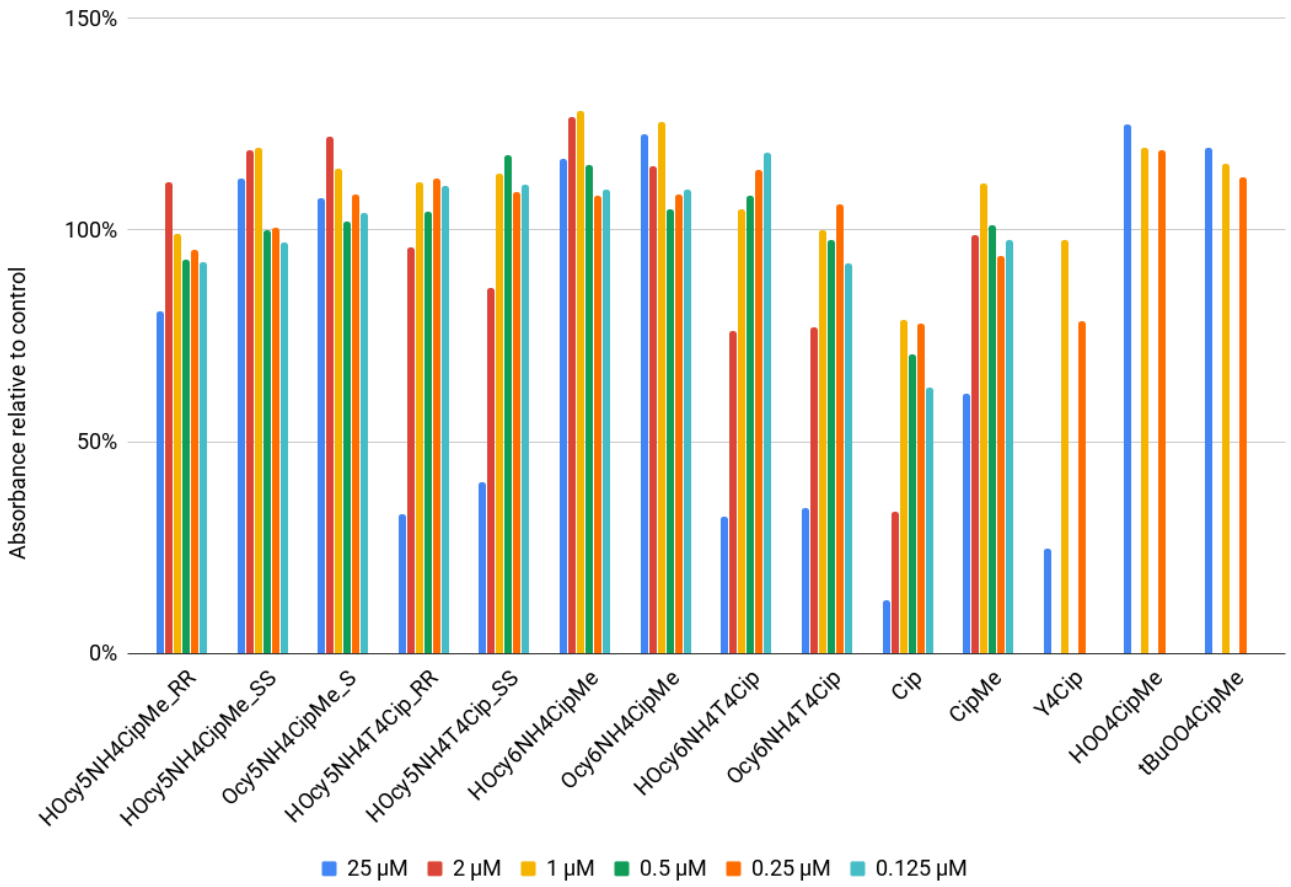

Figure 5: YM64 OD readings at 24 h for the alcohol and ketone HSL analogue-ciprofloxacin conjugates.

1.1.1.2 PAO1

In PAO1 at 5 h conjugates **157**, **162**, **167** showed activity at the highest concentration (see Figure 6). The cleavable conjugate **157** showed similar activity to ciprofloxacin **24**. At 24 h conjugate **167** still showed some activity, and cleavable conjugate **157** showed similar activity to ciprofloxacin **24** (see Figure 8).

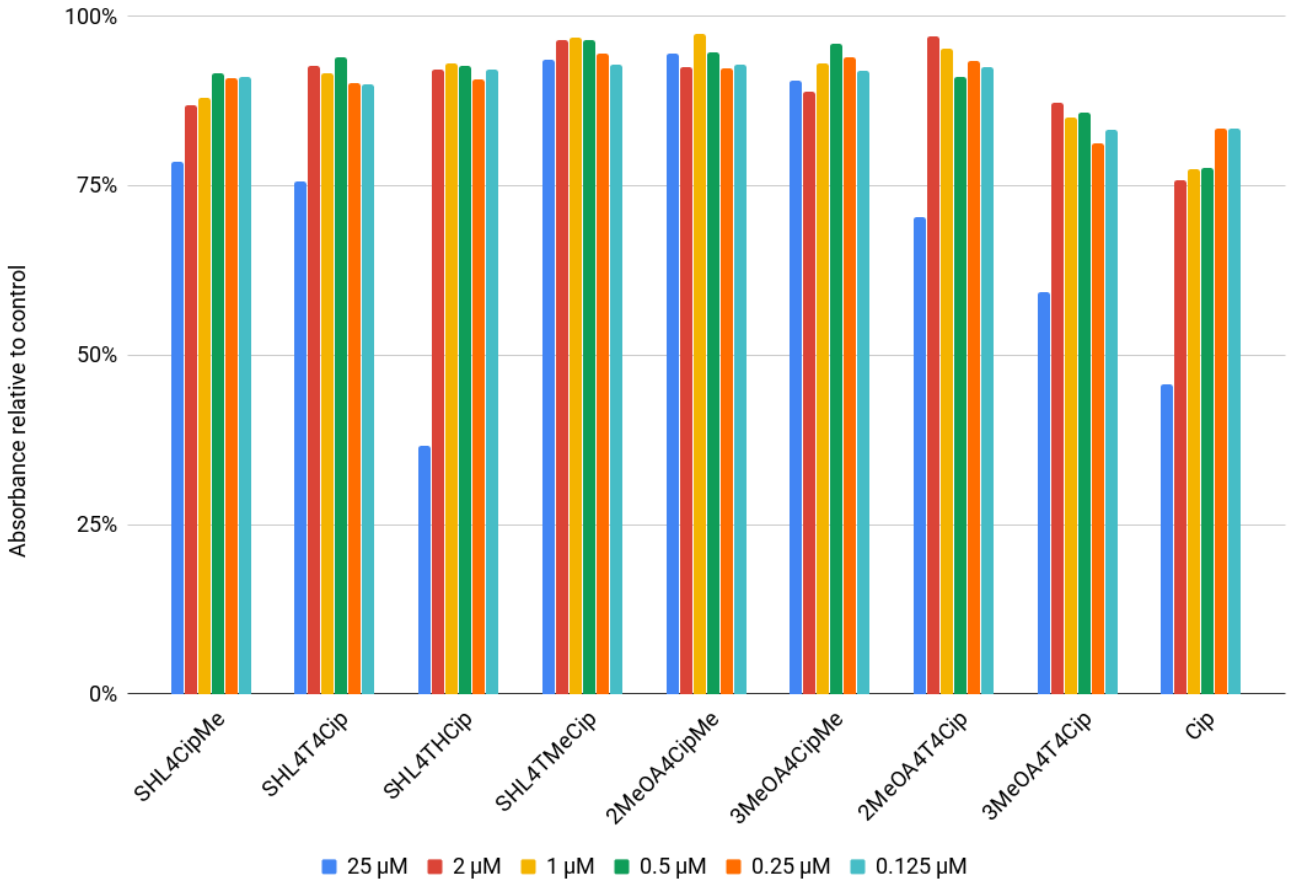

Figure 6: PAO1 OD readings at 5 h for the HCTL, 2-methoxybenzene and 3-methoxybenzene HSL analogue-ciprofloxacin conjugates.

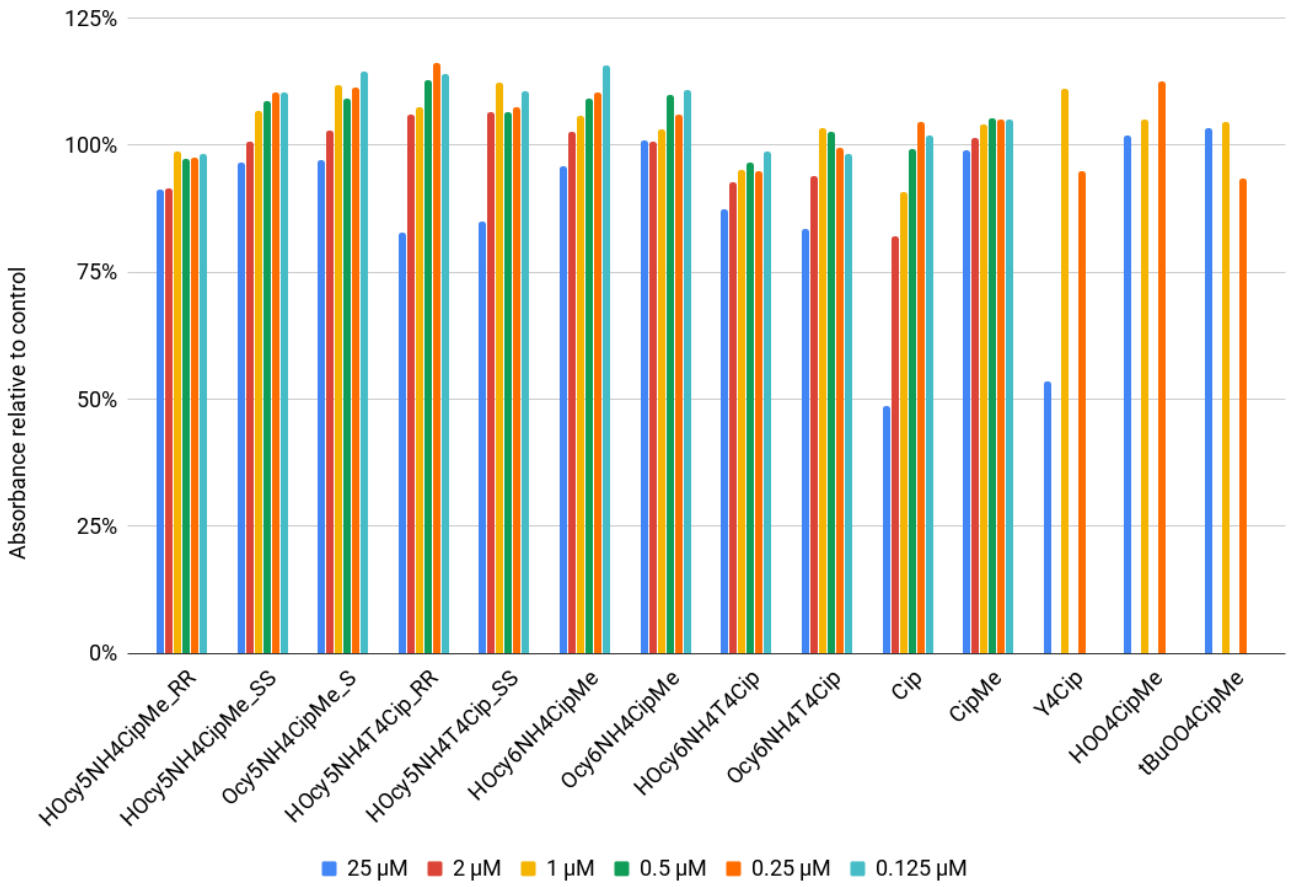

Figure 7: PAO1 OD readings at 5 h for the alcohol and ketone HSL analogue-ciprofloxacin conjugates.

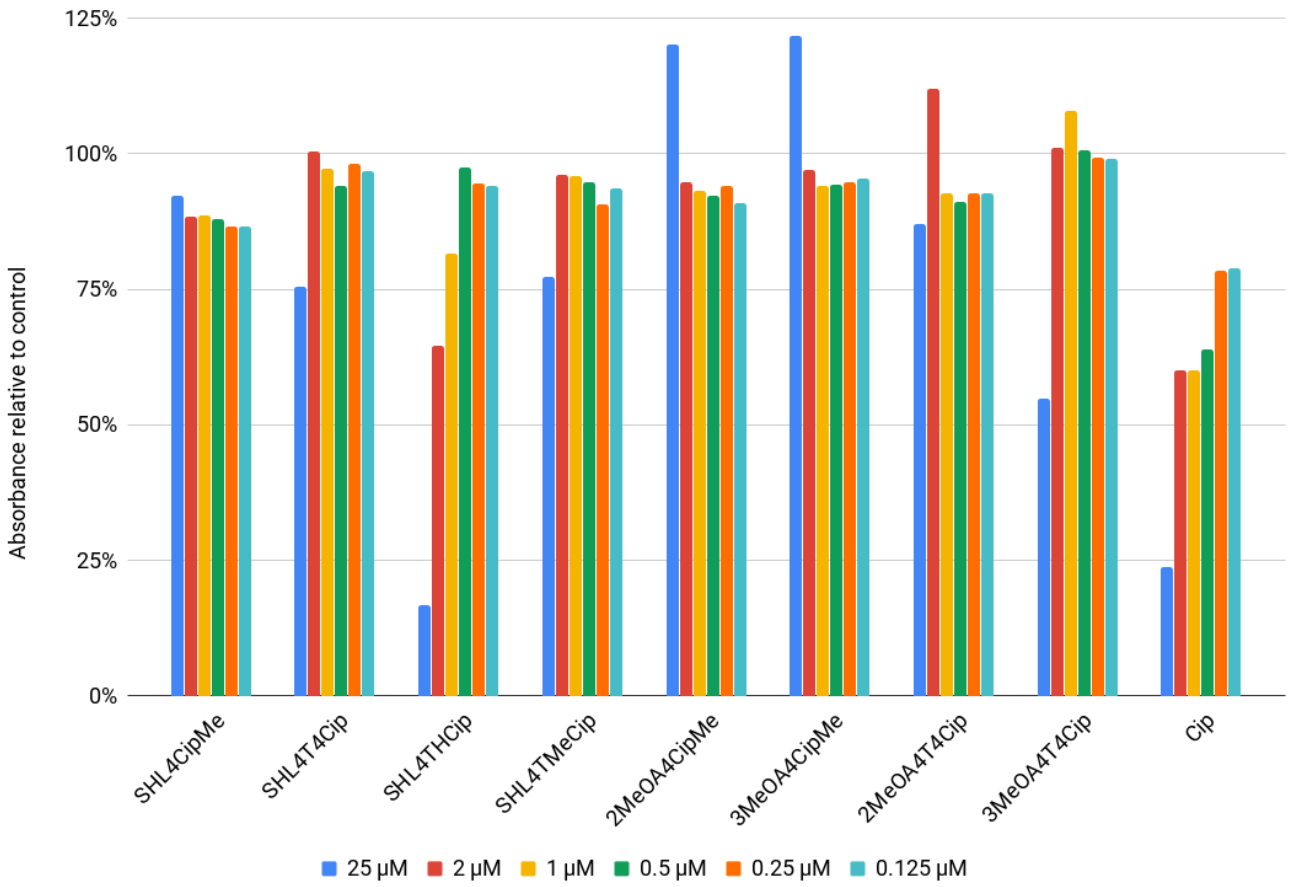

Figure 8: PAO1 OD readings at 24 h for the HCTL, 2-methoxybenzene and 3-methoxybenzene HSL analogue-ciprofloxacin conjugates.

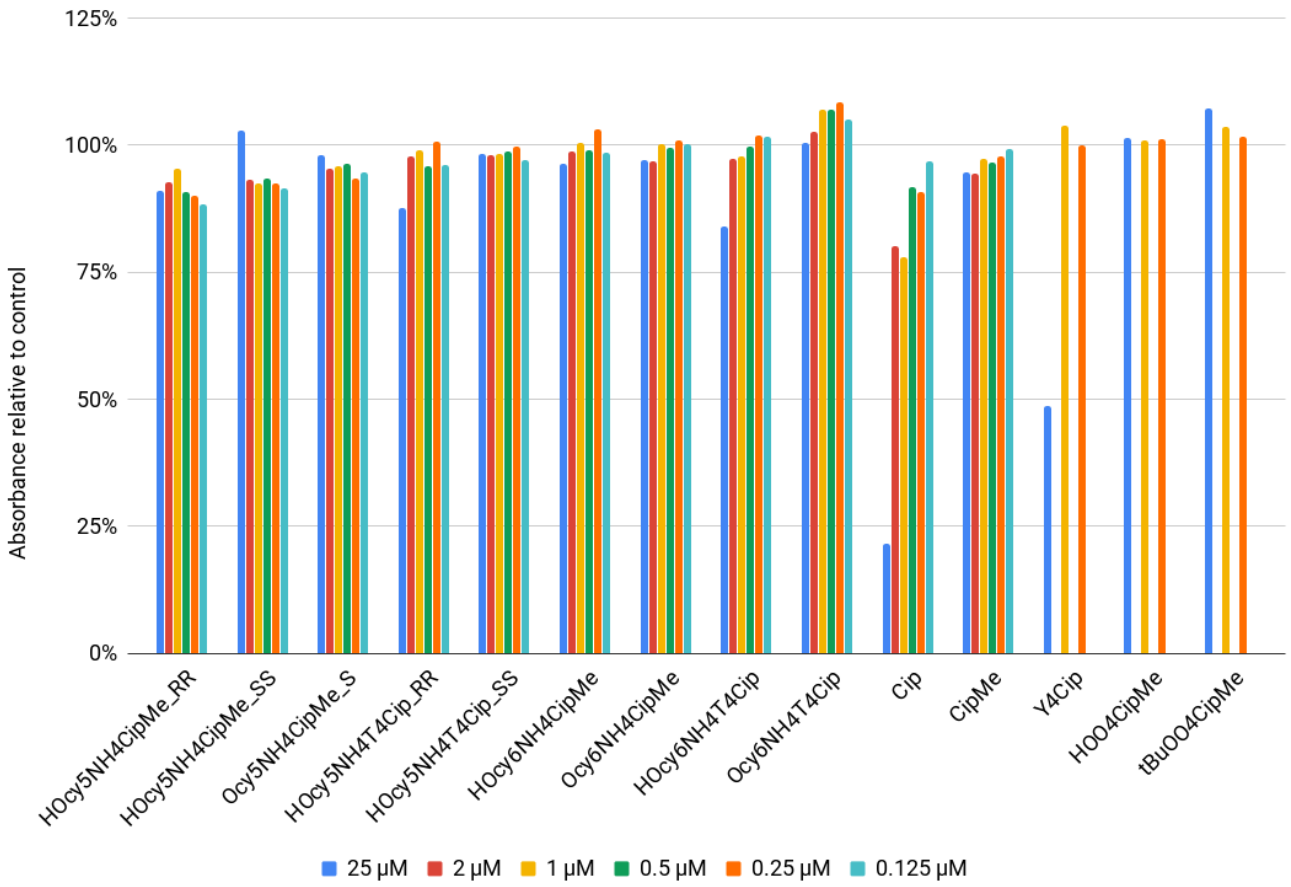

Figure 9: PAO1 OD readings at 24 h for the alcohol and ketone HSL analogue-ciprofloxacin conjugates.

Approximate MICs for the more active compounds are shown in **??**

Compound	**YM64 - 5 h**	**YM64 - 24 h**	**PAO1 - 5 h**	**PAO1 - 24 h**
SHL4THCip **157**				0.0455 ±
2MeOA4T4Cip **162**	0.0406 ±	0.0391 ±		
3MeOA4T4Cip **167**		0.0364 ±		
Cip **24**				

Table 1: .

1.1.2 Determination of anti-biofilm activity

Biofilm growth was measured using crystal violet staining.[2]

1.1.3 Effect on biofilm formation

1.1.4 Biofilm disruption

2 References

[1] R. Lambert and J. Pearson. Susceptibility testing: accurate and reproducible minimum inhibitory concentration (MIC) and non-inhibitory concentration (NIC) values. *Journal of Applied Microbiology*, 88(5):784–790, 2000.

[2] G. A. O'Toole and R. Kolter. Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. *Molecular Microbiology*, 30(2):295–304, 1998.

Todo list