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1 Results and discussion: autoinducer-antibiotic conjugates

1.1 Biological testing

1.1.1 Autoinducer-antibiotic conjugates

The eight triazoles made in **??** (see Figure 1) were tested for antibacterial and anti-biofilm activity in *P. aeruginosa* PAO1[1] and YM64.[2]

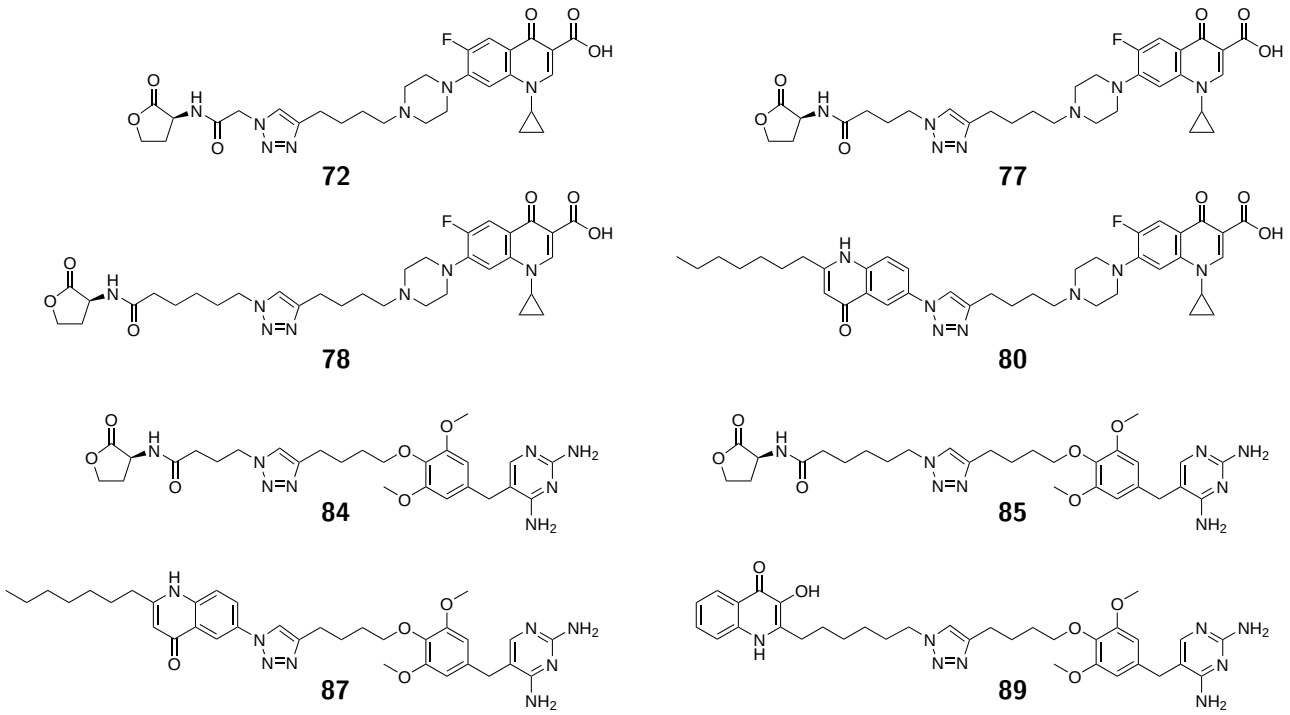


Figure 1

In YM64 at 5 h the HSL-ciprofloxacin conjugates **72**, **77** and **78** showed slight activity at the highest concentration, but not as much as ciprofloxacin **24**. This activity was not visible by 24 h (see Figure 3) and the compounds had no effect on biofilm formation (see Figure 4).

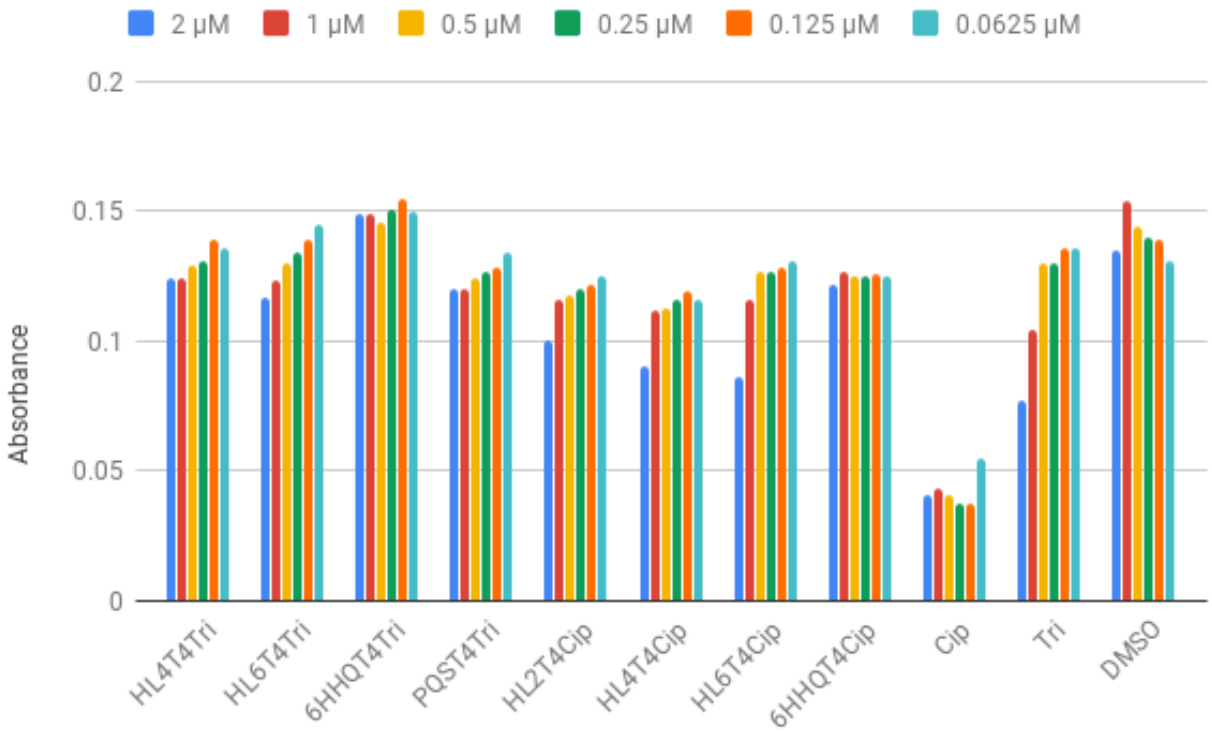


Figure 2: YM64 5 h.

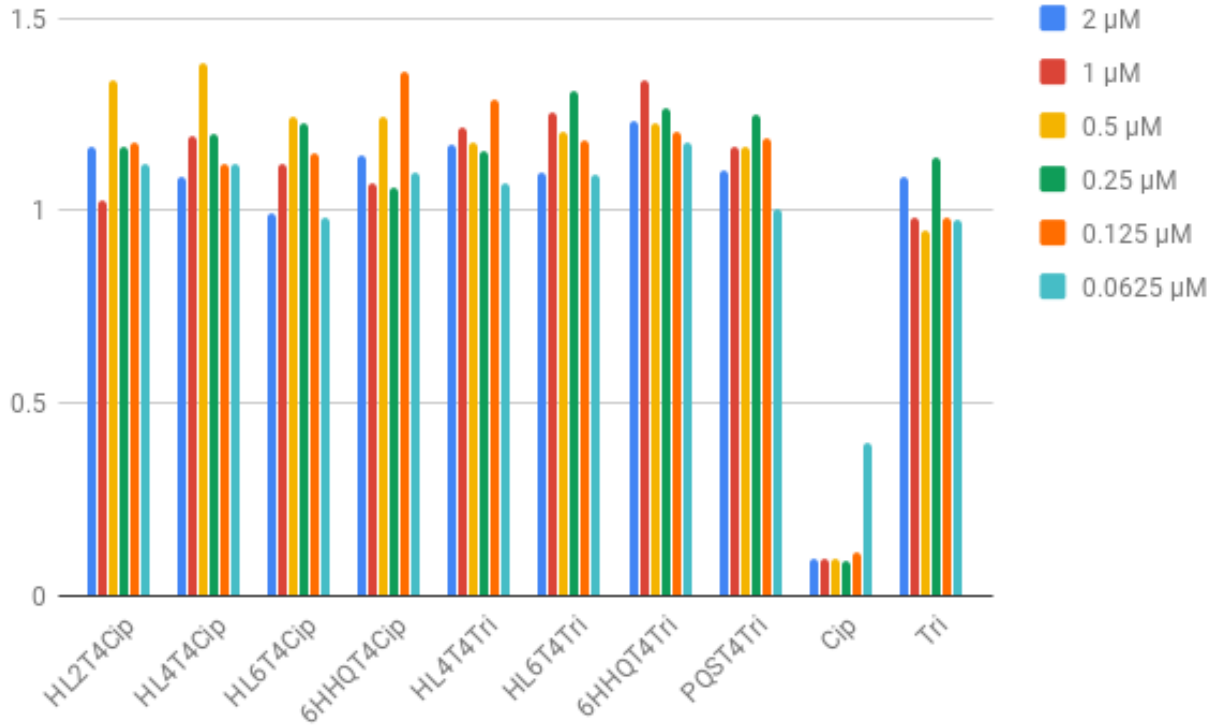


Figure 3: YM64 24 h.

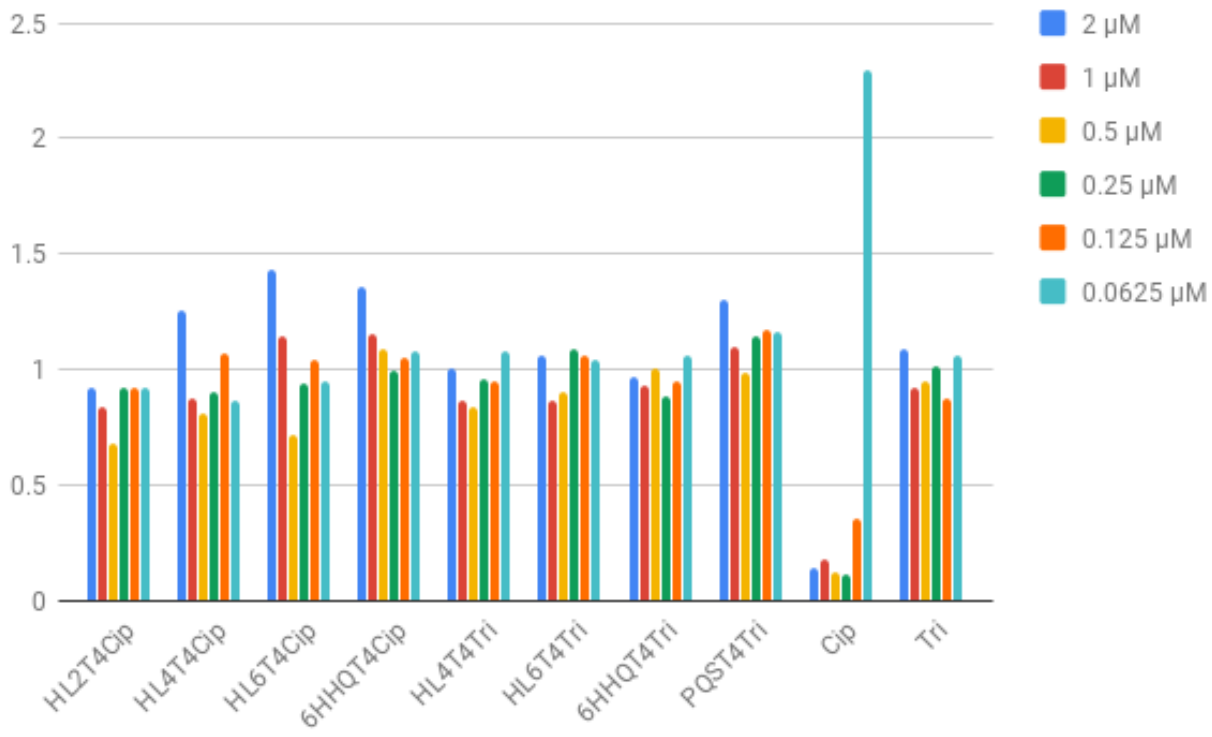


Figure 4: YM64 biofilms 24 h.

In PAO1 **78** showed similar activity to ciprofloxacin **24** at the highest concentration (see Figure 5), but not at lower concentrations. All other compounds did not show activity, and again there was no activity at 24 h or against biofilms.

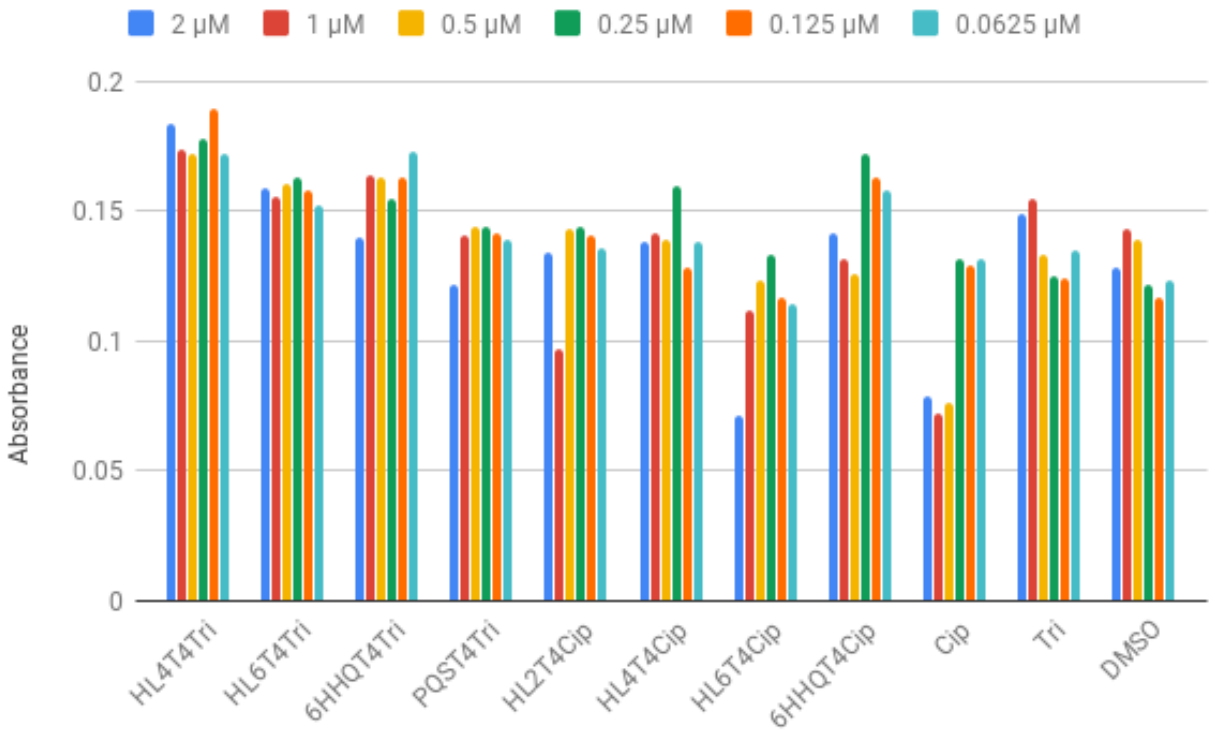


Figure 5: PAO1 5 h.

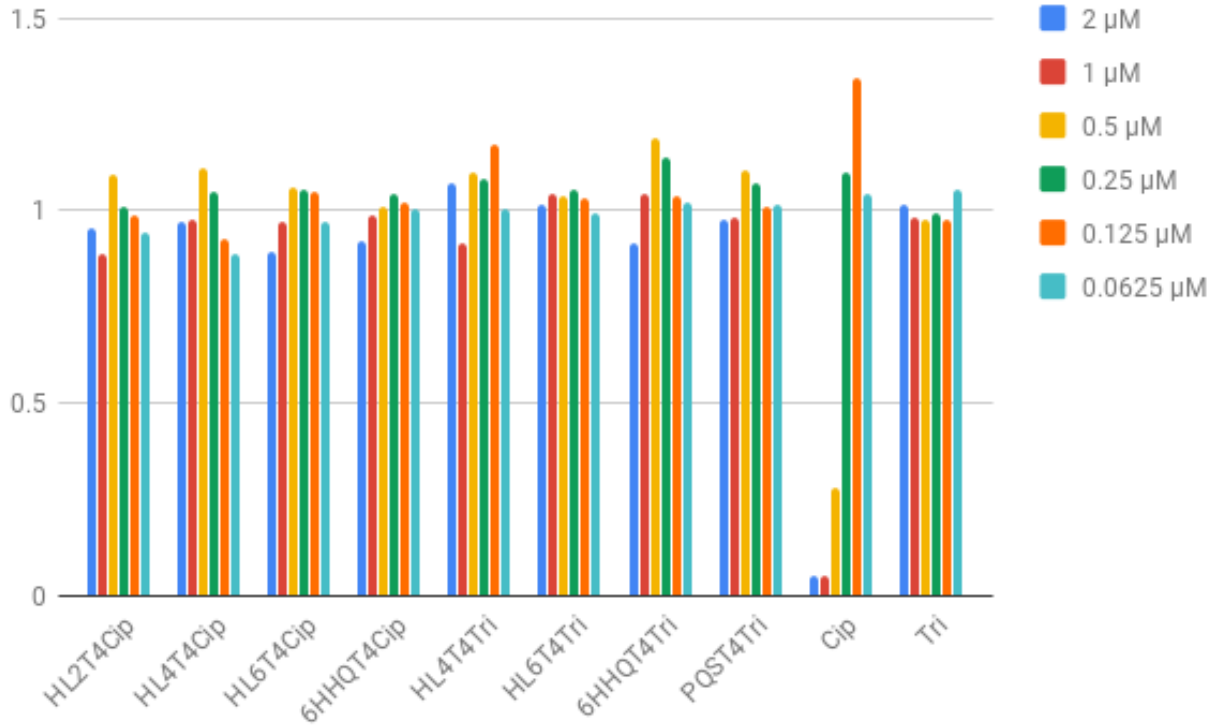


Figure 6: PAO1 24 h.

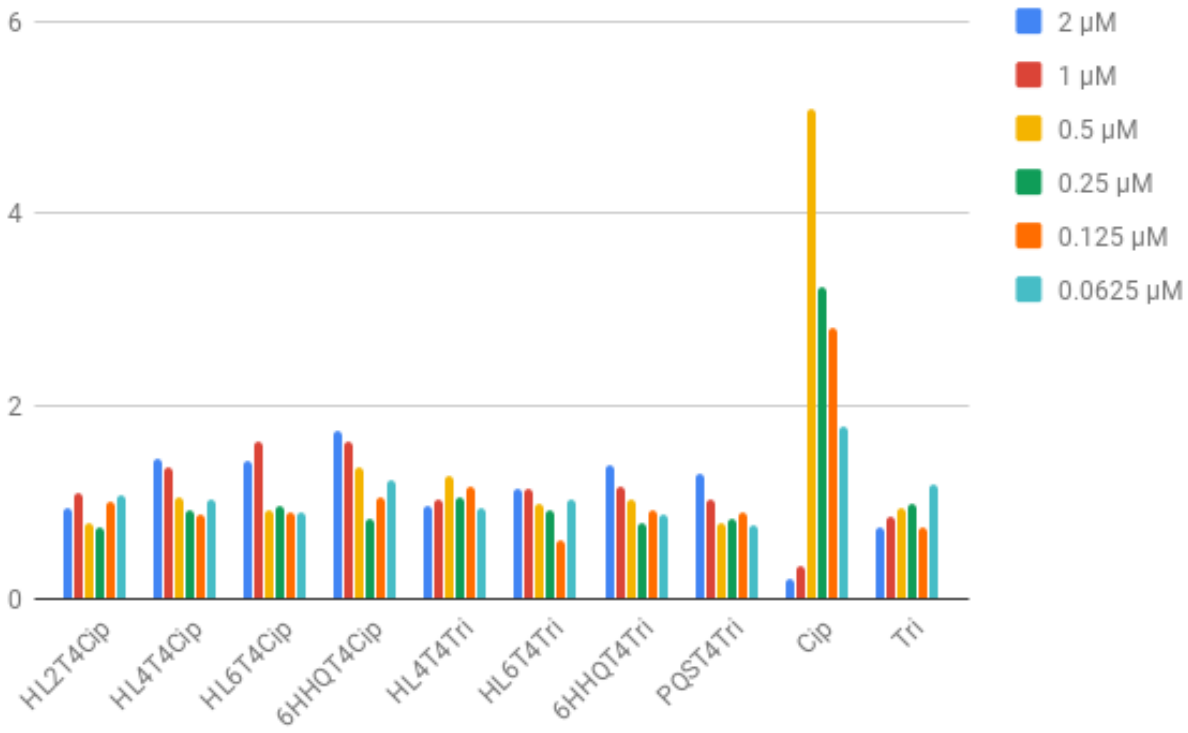
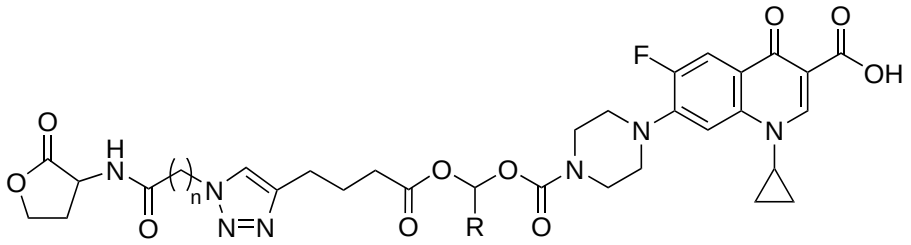


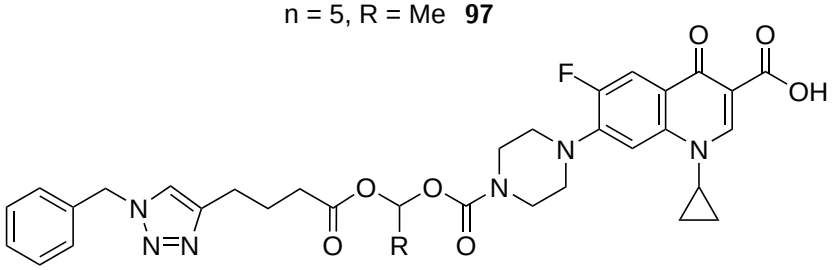
Figure 7: PAO1 biofilms 24 h.

1.1.2 Cleavable HSL-ciprofloxacin conjugates

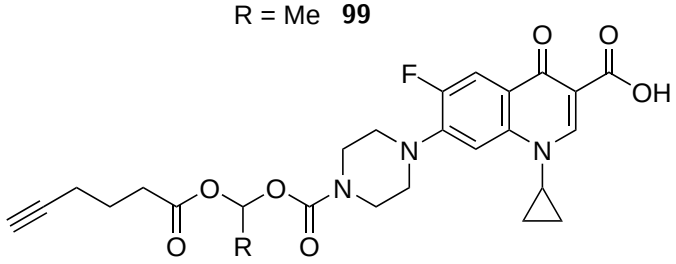
The eight cleavable HSL-ciprofloxacin conjugates, two controls and two alkynes described in **??** (see Figure 8) were tested for antibacterial and anti-biofilm activity in *P. aeruginosa* YM64.



n = 1, R = H **92**
n = 3, R = H **93**
n = 5, R = H **94**
n = 1, R = Me **95**
n = 3, R = Me **96**
n = 5, R = Me **97**



R = H **98**
R = Me **99**



R = H **90**
R = Me **91**

Figure 8

Here there was more success, although the activity was still not as high as for ciprofloxacin **24**. The HSL-ciprofloxacin conjugates with *N*-(acetoxymethoxycarbonyl) linkers (R = H) showed activity at high concentrations. A longer linker seems to give higher activity; **93** and **94** showed activity comparable with ciprofloxacin **24** at high concentrations. Unfortunately the control **98** and alkyne **90** with *N*-(acetoxymethoxycarbonyl) linkers (R = H) showed higher activity than the conjugates, indicating that the HSL head wasn't contributing to the activity of the conjugates.

The conjugates with an *N*-(acetoxyethoxycarbonyl) linker (R = Me) did not show any activity. This suggests that they either didn't enter cells or weren't suitable substrates for esterases. The *N*-(acetoxyethoxycarbonyl) linked alkyne (R = Me) did show some activity, indicating that maybe it could penetrate cells more easily than the conjugates due to its lower molecular weight and/or lower polarity.

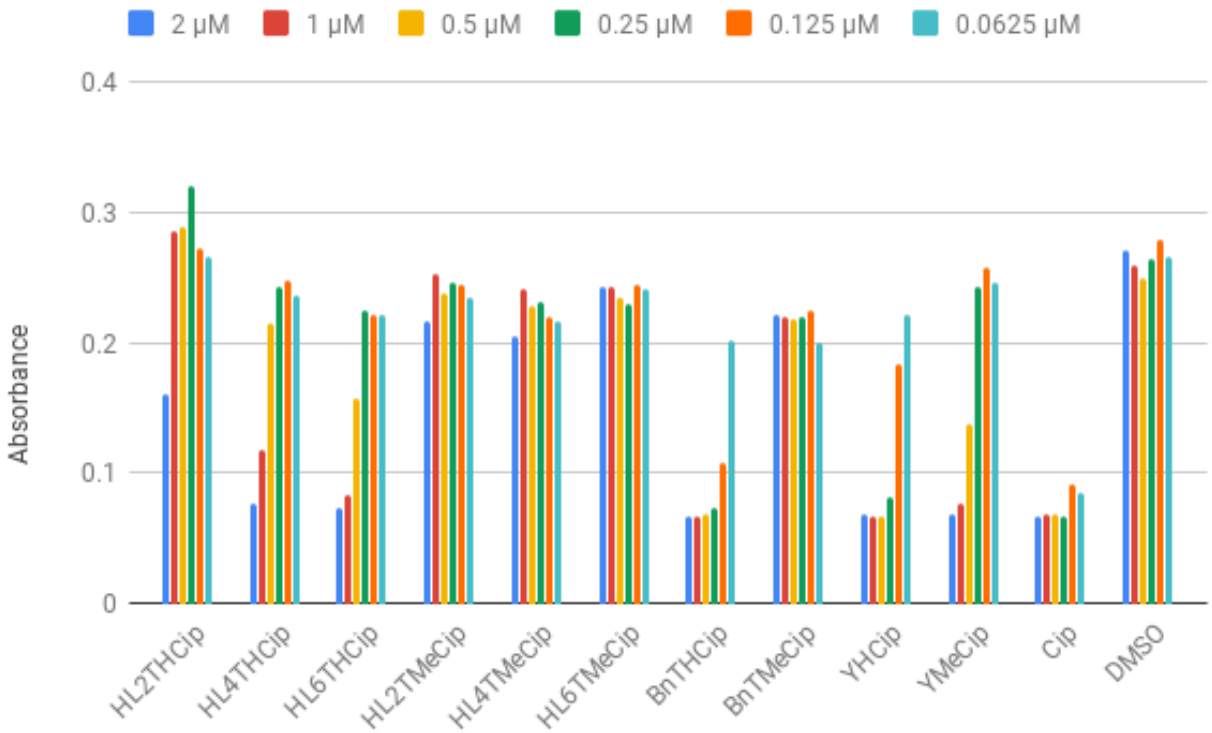


Figure 9: YM64 5 h cleavable conjugates.

1.2 Biological testing

Compounds were tested against *P. aeruginosa* PAO1[?] and YM64.[?]

The compounds were tested in PAO1, and YM64 which is missing 4 main efflux pumps. Antibacterial activity was measured by measurement of turbidity at 595 nm at 5 h and 24 h. The compounds were tested at 6 concentrations between 2 and 0.0625 μM in LB in 96-well plates.

1.2.1 Antibiotic susceptibility

Make overnight cultures (in 10 ml LB @ 37 oC). Dilute 1/100 to make 20 ml (200 ul culture, 19.8 ml LB) per 2 plates. Add 99 ul diluted culture to all wells except blanks and 'Just LB' wells (96-well plate, flat-bottomed wells). Remember to make 2 if doing biofilm inhibition testing after! Add 100 ul LB to the 'Just LB' wells. Add 1 ul compound solutions from the master plates. Stick on adhesive cover (remove edges, DO NOT USE A LID). Record OD Place the plate in shaker @ 37 oC, 100 rpm, Record the OD every 1h for 8h, then at 16h, 24h and 48h.

1.2.2 Quantification of biofilms

Prepare two plates according to steps 1-6 of 'MIC testing'. Incubate one for 24 h and one for 48 h (this can be the one from the MIC test), both @ 37 oC, 100 rpm. Record OD Aspirate out culture (don't touch sides). Add 120 ul water then aspirate again. Repeat x3. Add 120 ul 0.1 Aspirate out crystal violet. Add 120 ul water then aspirate again. Repeat x3. Add 30 Vortex plate. Read plate.

1.2.3 Biofilm inhibition

1.2.4 Biofilm dispersal

Make overnight cultures (toothpick in 10 ml LB @ 37 oC). Dilute 1/100 to make 20 ml (200 ul culture, 19.8 ml LB) per 2 plates. Add 99 ul diluted culture to all wells except the 'Just LB' wells (96-well plate, flat-bottomed wells). Make 2 plates per master plate per strain. Add 100 ul LB to the 'Just LB' wells. Shake for 24 h or 48 h @ 37 oC, 100 rpm. Record OD Add 1 ul compound solutions from the master plates. Record OD Shake for 24 h @ 37 oC, 100 rpm. Record OD Do steps 2-9 of 'Biofilm inhibition testing'.

2 References

[1] C. K. Stover, X. Q. Pham, A. L. Erwin, S. D. Mizoguchi, P. Warrener, M. J. Hickey, F. S. L. Brinkman, W. O. Hufnagle, D. J. Kowalik, M. Lagrou, R. L. Garber, L. Goltry, E. Tolentino, Y. Yuan, L. L. Brody, S. N. Coulter, K. R. Folger, A. Kas, K. Larbig, R. Lim, K. Smith, D. Spencer, G. K. Wong, Z. Wu, I. T. Paulsen, J. Reizer, M. H. Saier, R. E. W. Hancock, S. Lory and M. V. Olson. Complete genome sequence of *Pseudomonas aeruginosa* PAO1, an opportunistic pathogen. *Nature*, 406:959–964, 2000.

[2] Y. Morita, Y. Komori, T. Mima, T. Kuroda, T. Mizushima and T. Tsuchiya. Construction of a series of mutants lacking all of the four major *mex* operons for multidrug efflux pumps or possessing each one of the operons from *Pseudomonas aeruginosa* PAO1: MexCD-OprJ is an inducible pump. *FEMS Microbiology Letters*, 202:139–143, 2001.

Todo list