Contents

1 Autoinducer analogue-ciprofloxacin conjugates

1.1 Inspiration

Following on from the library of compounds based on *P. aeruginosa* autoinducers, a series of conjugates based on *analogues* of C_4-HSL were planned. This strategy was inspired by a paper[1] and patent[2] by Ganguly et al., who synthesised and characterised a conjugate **105** of methyl ciprofloxacin with homocysteine thiolactone (see Figure 1). Homocysteine thiolactone is an analogue of homoserine lactone with the ring oxygen replaced by sulfur, and has been used as the head group in several other known quorum sensing modulators.[3–10]

show compounds it's in and describe?

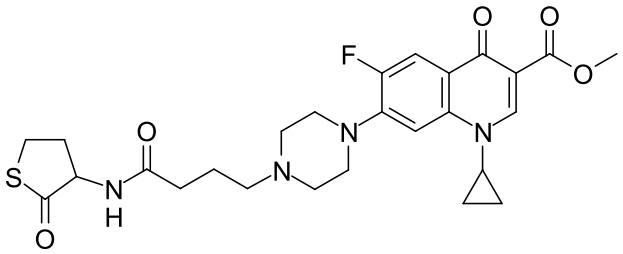
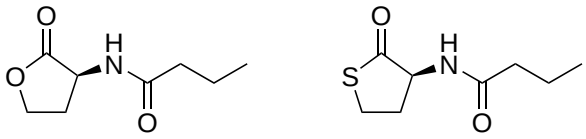


Figure 1: The HCTL-CipMe conjugate **105** studied by Ganguly et al.[1,2]

As part of their characterisation of the HCTL-CipMe conjugate **105**, Ganguly et al. found the MIC of the conjugate in *P. aeruginosa* under standard planktonic conditions. The MIC was found to be ten times higher for the conjugate vs. ciprofloxacin (50 vs. 5 μm), indicating that the conjgate was less effective than ciprofloxacin under planktonic conditions.

Ganguly et al. then investigated the effect of the conjugate on biofilms. The conjugate and ciprofloxacin were first added to dilute *P. aeruginosa* liquid culture at 25 μm. As expected, the culture failed to grow and form biofilm in the presence of ciprofloxacin, but did grow in the presence of the conjugate **105**. They then incubated cultures for 24 h, to allow biofilms to grow, before adding the compounds. In contrast, they found that the conjugate **105** disrupted the biofilm more effectively than ciprofloxacin. When the biofilm was grown for 48 or 72 hours the conjugate had similarly disruptive effects, whereas ciprofloxacin 'did not show any significant antibacterial activity'.

These results are exciting as they hint that an autoinducer conjugate might be able to combat an established *P. aeruginosa* infection more effectively than the unmodified antibiotic. Ganguly et al. suggest that their conjugate is more effective than ciprofloxacin in penetrating biofilms, and/or better at avoiding being pumped out by multidrug efflux pumps. They posit that this could be due to the thiolactone head, as they also showed that unconjugated C_4-HCTL **158** (see Figure 2) has 'either enhanced uptake or functional activity' when compared with C_4-HSL **2**.



2 **158**

Figure 2: C_4-HSL **2** and C_4-HCTL **158**. Note that Ganguly et al. tested the *S* enantiomer of C_4-HCTL **158**, but used a racemic mixture in their HCTL-CipMe conjugate.

While the results found by Ganguly et al. show promise, they only test one conjugate, and do not include controls to show that the HCTL group specifically is necessary for the enhanced effect. It was therefore decided to build on this work by synthesising a series of ciprofloxacin conjugates with head groups known as part of quorum modulators.[11,12]

read these again, put ones I didn't do in further work

check Boursier2018 for print publication details

1.2 Library design

[13]

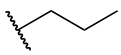
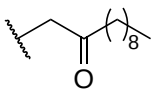
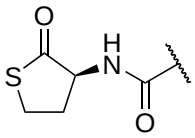
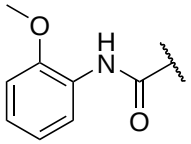
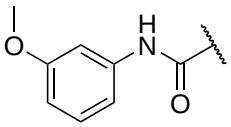
Head group		
	Partial agonist and antiagonst against LasR.[8] Shown to increase biofilm formation in *P. aerugi-nosa*.[1]	Strong agonist against LasR, comparable activity to native ligand.[5,6,8,14]
	Not yet studied	Not yet studied
	Partial agonist[12]	Strong antagonist[12]

Table 1: Click reactions attempted.

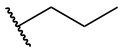
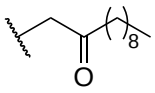
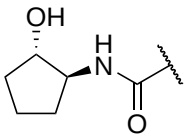
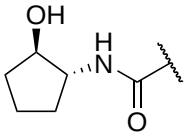
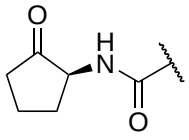
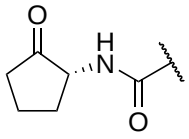
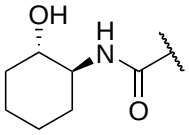
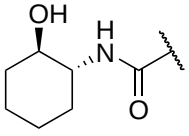
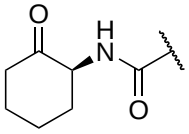
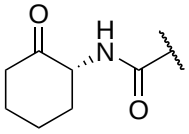
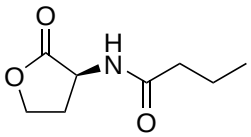
Head group		
		
		
		
		
		
		
		
		

Table 2: Click reactions attempted.

Discuss which AHL analogues were picked + why. Might as well make other enan of HOcy5



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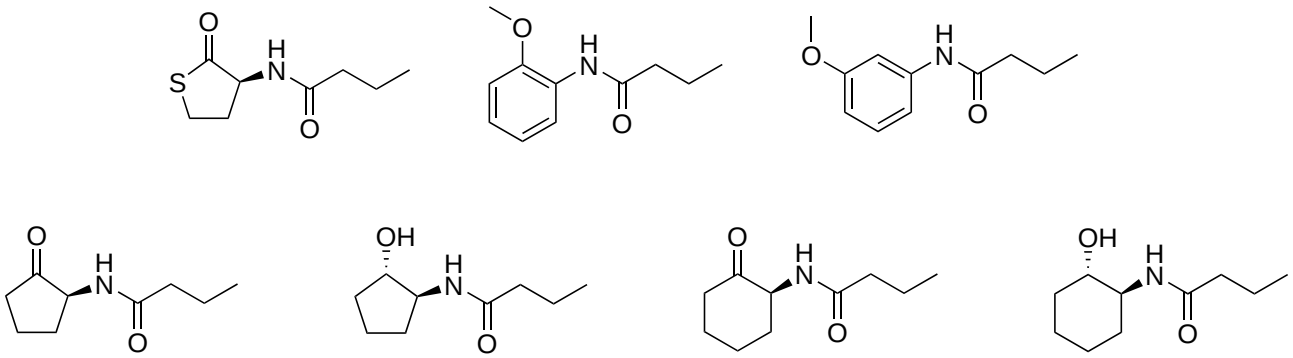


AHL analogues

Figure 3

Introduce initial strategy of making bromide then azide, and diverting down the two different paths to make directly linked or triazole linked products.

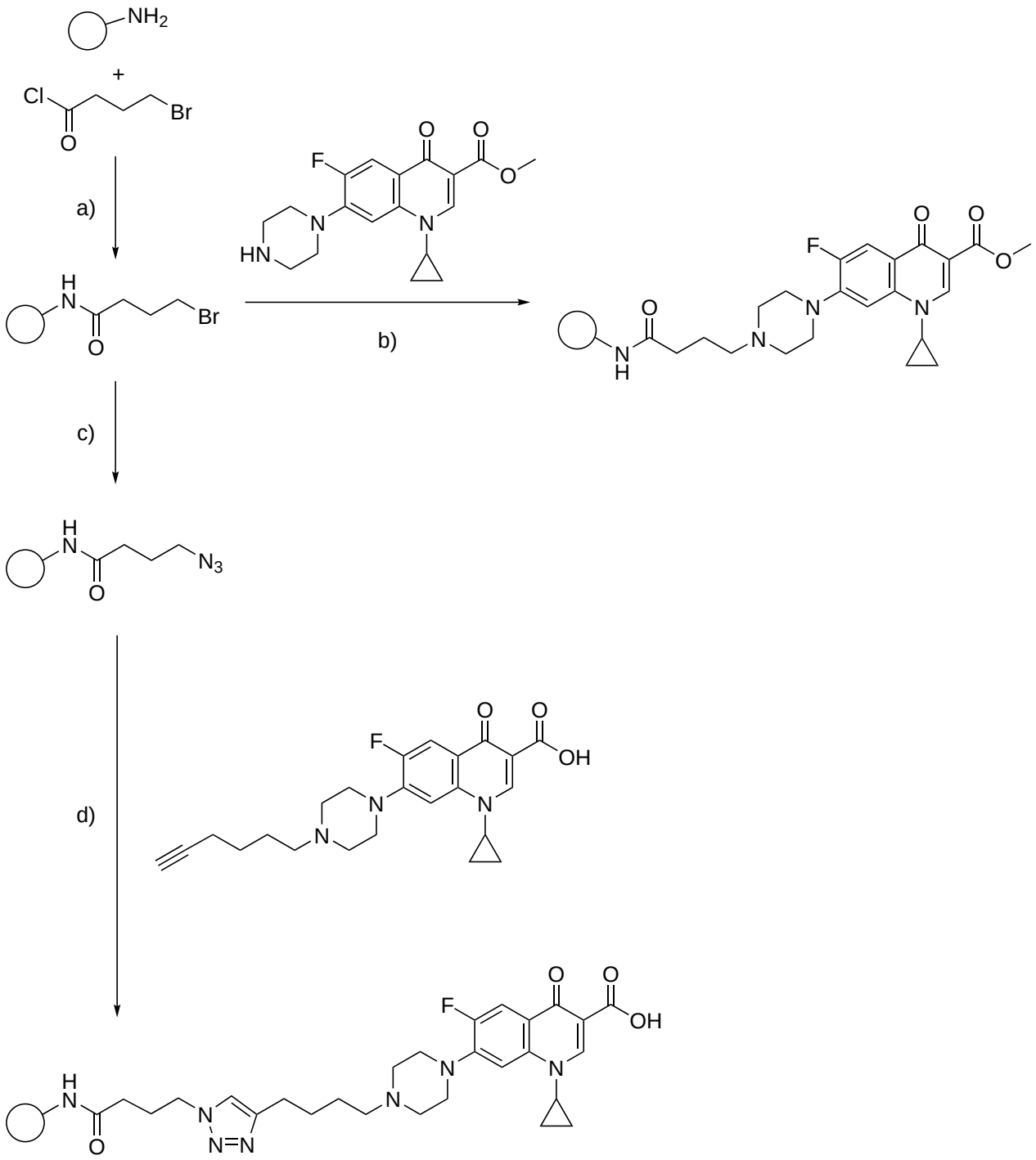
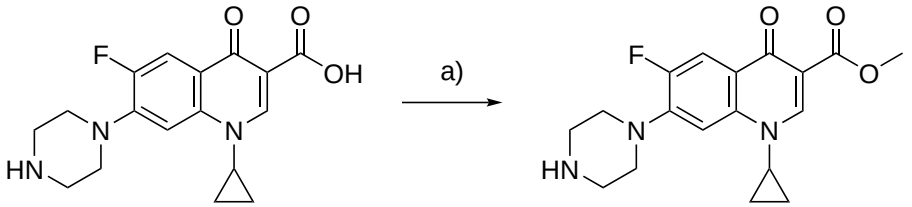


Figure 4

1.3 Synthesis of the homocysteine thiolactone derivatives

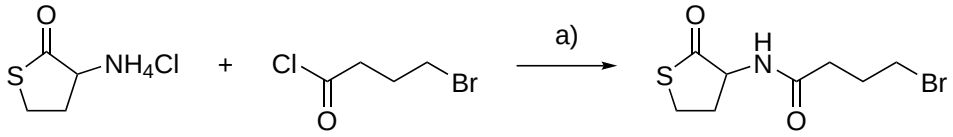
Methyl ciprofloxacin **102** was synthesised from ciprofloxacin **28** and MeOH in very good yield using *para*-toluenesulfonic acid as a catalyst.[15]



28 **102**

Scheme 1: Synthesis of methyl ciprofloxacin **102**. a) *p*-TSA, MeOH, 72 h, reflux, 83.3 %.

Br-C_4-HCTL **104** was synthesised using the Schotten-Baumann conditions employed previously for the HSL derivatives **59** and **62**. Br-C_4-HCTL **104** was isolated in markedly higher yield than that achieved by Ganguly *et al.*[1] (87.9 % vs. 25.0 %). It is possible that this was due to CH_2Cl_2 being used for the extraction, whereas Ganguly *et al.* used EtOAc.



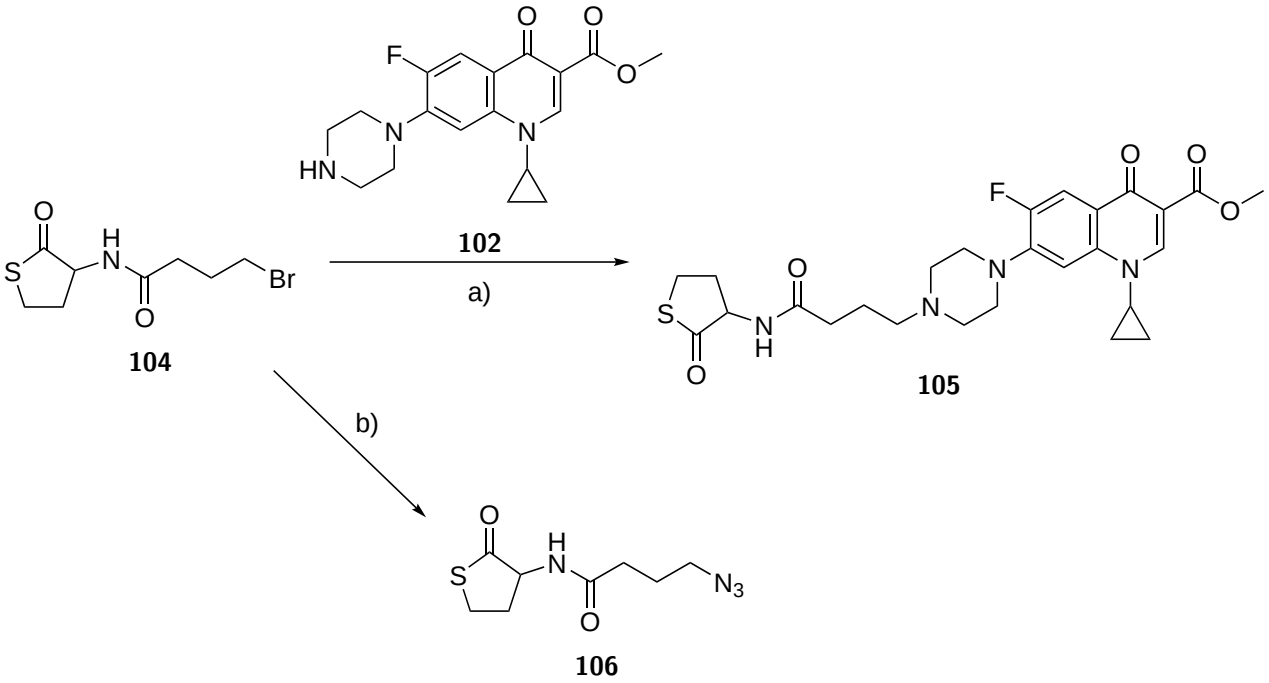
103 **58** **104**

Scheme 2: Synthesis of Br-C_4-HCTL **104**. a) $NaHCO_3$, CH_2Cl_2, H_2O, 0 °C, 1 h, 87.9 %.

The HCTL-CipMe conjugate **105** was synthesised using the procedure outlined by Ganguly *et al.*[1] Monitoring by LCMS showed slow conversion to the product. Br-C_4-HCTL **104** was presumably consumed by side reactions as 4 eq. were required to reach full conversion. Ganguly *et al.* do not quote a yield for comparison[1,2] but it is hoped that the 12.2 % achieved herein could be improved upon. The side reactions led to the production of an unidentified brown, viscous contaminant which made purification by flash column chromatography (as was used by Ganguly *et al.*) challenging. Preparatory HPLC on a partially purified sample gave enough pure HCTL-CipMe conjugate **105** for biological testing.

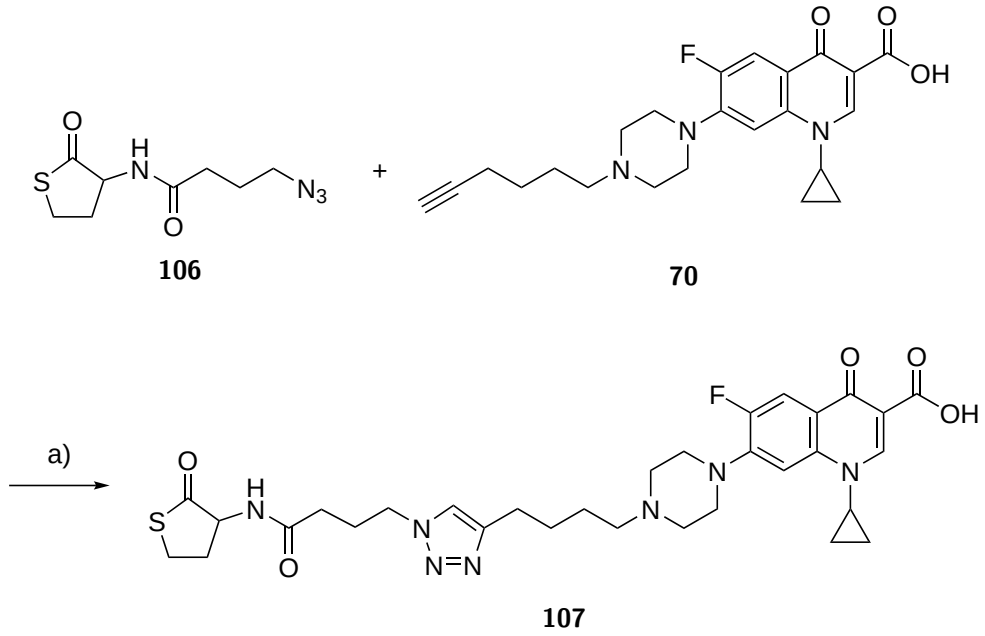
Future optimisation of the synthesis could focus on different routes to the product, e.g. the peptide coupling described in **??**, or different purification methods, e.g. using just preparatory HPLC, or reverse phase flash column chromatography.

Br-C_4-HCTL **104** was also converted into N_3-C_4-HCTL **106**, by an S_N2 reaction with sodium azide which proceeded in excellent yield.



Scheme 3: Synthesis of the HCTL-CipMe conjugate **105**, N_3-C_4-HCTL **106**, and the HCTL-Cip triazole conjugate **107**. a) K_2CO_3, acetonitrile, reflux, 24 h, 12.2 %. b) NaN_3, acetonitrile, reflux, 1.5 h, 89.3 %.

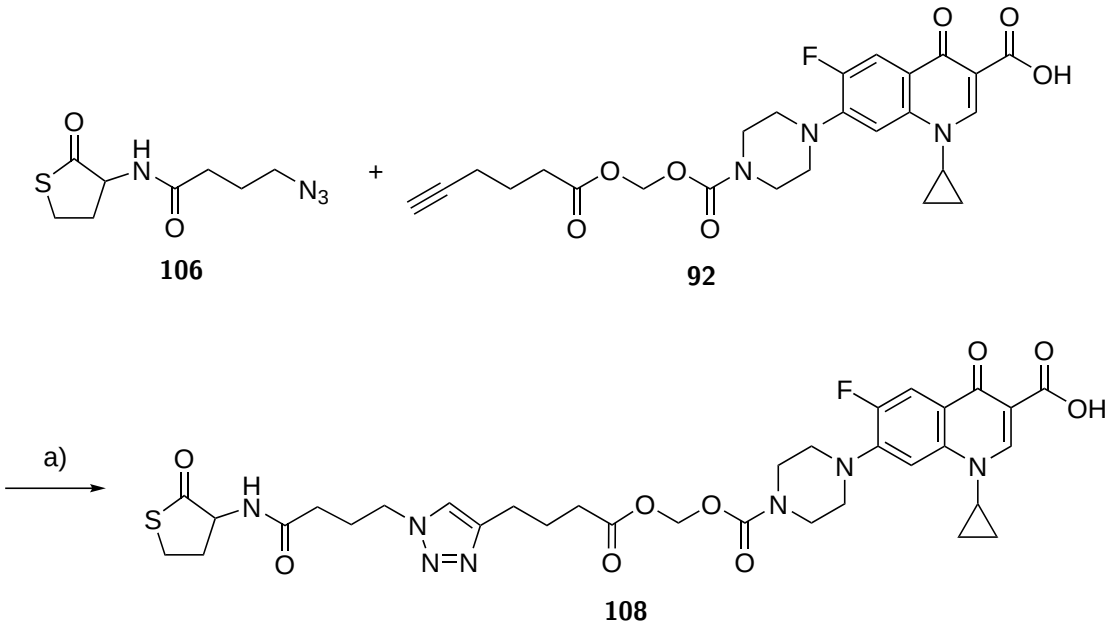
N_3-C_4-HCTL **106** was then subjected to the click reaction conditions optimised previously (see **??**). The reaction proceeded very slowly at first, until it was realised that the azide did not dissolve in the reaction solvent and formed a single solid clump. DMSO was added as a co-solvent, and the reaction began to proceed, albeit still slowly. It is possible that the sulfur atom coordinates to the copper, thus inhibiting its catalytic ability. Nonetheless the HCTL-Cip triazole conjugate **107** was eventually isolated in good yield (see Scheme 4).



Scheme 4: Synthesis of the HCTL-Cip triazole conjugate **107**. a) $CuSO_4$, THPTA, sodium ascorbate, H_2O, *t*-BuOH, DMSO, r.t., 7 d, 70.6 %.

A cleavable conjugate **108** was also synthesised from N_3-C_4-HCTL **106** by reaction with a cleavable alkyne-

Cip derivative **92** synthesised previously by Prof. Eddy Sotelo-Perez (see **??**). Conditions developed by Prof. Sotelo-Perez were used, but again the reaction proceeded very slowly. The disappointing yield is, however, most likely due to losses during purification.



Scheme 5: Synthesis of the cleavable HCTL-Cip triazole conjugate **108**. a) CuI, DIPEA, CH_2Cl_2, r.t., 3 h, 5.0 %.

2 References

[1] K. Ganguly, R. Wu, M. Ollivault-Shiflett, P. M. Goodwin, L. A. Silks and R. Iyer. Design, synthesis, and a novel application of quorum-sensing agonists as potential drug-delivery vehicles. *Journal of Drug Targeting*, 19(7):528–539. 2011.

[2] R. Iyer, K. Ganguly and L. A. Silks. Synthetic analogs of bacterial quorum sensors. Los Alamos National Laboratory. 2012.

[3] A. Eberhard, C. A. Widrig, P. Mcbath and J. B. Schineller. Analogs of the autoinducer of bioluminescence in Vibrio fischeri. *Archives of Microbiology*, 146(1):35–40. 1986.

[4] A. L. Schaefer, B. L. Hanzelka, A. Eberhard and E. P. Greenberg. Quorum sensing in Vibrio fischeri: probing autoinducer-LuxR interactions with autoinducer analogs. *Journal of Bacteriology*, 178(10):2897–2901. 1996.

[5] L. Passador, K. D. Tucker, K. R. Guertin, M. P. Journet, A. S. Kende and B. H. Iglewski. Functional analysis of the Pseudomonas aeruginosa autoinducer PAI. *Journal of Bacteriology*, 178(20):5995–6000. 1996.

[6] K. M. Smith, Y. Bu and H. Suga. Library Screening for Synthetic Agonists and Antagonists of a Pseudomonas aeruginosa Autoinducer. *Chemistry & Biology*, 10(6):563–571. 2003.

[7] S. R. Chhabra, P. Stead, N. J. Bainton, G. P. Salmond, G. S. Stewart, P. Williams and B. W. Bycroft. Autoregulation of carbapenem biosynthesis in Erwinia carotovora by analogues of N-(3-oxohexanoyl)-L-homoserine lactone. *The Journal of Antibiotics*, 46(3):441–454. 1993.

[8] C. E. McInnis and H. E. Blackwell. Thiolactone modulators of quorum sensing revealed through library design and screening. *Bioorganic & Medicinal Chemistry*, 19(16):4820–4828. 2011.

[9] G. D. Geske, J. C. O. Neill, D. M. Miller, M. E. Mattmann and H. E. Blackwell. Modulation of Bacterial Quorum Sensing with Synthetic Ligands : Systematic Evaluation of N-Acylated Homoserine Lactones in Multiple Species and New Insights into Their Mechanisms of Action. *Journal of the American Chemical Society*, 129(44):13613–13625. 2007.

[10] J. C. A. Janssens, K. Metzger, R. Daniels, D. Ptacek, T. Verhoeven, L. W. Habel, J. Vanderleyden, D. E. De Vos and S. C. J. De Keersmaecker. Synthesis of N -Acyl Homoserine Lactone Analogues Reveals Strong Activators of SdiA , the Salmonella enterica Serovar. *Applied and Environmental Microbiology*, 73(2):535–544. 2007.

[11] W. R. J. D. Galloway, J. T. Hodgkinson, S. D. Bowden, M. Welch and D. R. Spring. Quorum sensing in Gram-negative bacteria: small-molecule modulation of AHL and AI-2 quorum sensing pathways. *Chemical Reviews*, 111(1):28–67. 2011.

[12] J. T. Hodgkinson, W. R. J. D. Galloway, M. Wright, I. K. Mati, R. L. Nicholson, M. Welch and D. R. Spring. Design, synthesis and biological evaluation of non-natural modulators of quorum sensing in Pseudomonas aeruginosa. *Organic & Biomolecular Chemistry*, 10(30):6032. 2012.

[13] K. M. Smith, Y. Bu and H. Suga. Induction and Inhibition of Pseudomonas aeruginosa Quorum Sensing by Synthetic Autoinducer Analogs. *Chemistry & Biology*, 10(1):81–89. 2003.

[14] M. E. Boursier, D. E. Manson, J. B. Combs, E. Helen and H. E. Blackwell. A comparative study of non-native N-acyl L-homoserine lactone analogs in two Pseudomonas aeruginosa quorum sensing receptors that

share a common native ligand yet inversely regulate virulence. *Bioorganic & Medicinal Chemistry*, pages 1–17. 2018.

[15] K. Sachin, E.-M. Kim, S.-J. Cheong, H.-J. Jeong, S. T. Lim, M.-H. Sohn and D. W. Kim. Synthesis of N_4'-[^{18}F]fluoroalkylated ciprofloxacin as a potential bacterial infection imaging agent for PET study. *Bioconjugate Chemistry*, 21(12):2282–2288. 2010.

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