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Design, synthesis, and evaluation of transition-state analogs as inhibitors of the bacterial quorum sensing autoinducer synthase CepI

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ABSTRACT

Quorum sensing is a bacterial signaling system that involves the synthesis, secretion and detection of signal molecules called autoinducers. The main autoinducer in Gram-negative bacteria are acylated homoserine lactones, produced by the LuxI family of autoinducer synthases and detected by the LuxR family of autoinducer receptors. Quorum sensing allows for changes in gene expression and bacterial behaviors in a coordinated, cell density dependent manner. Quorum sensing controls the expression of virulence factors in some human pathogens, making quorum sensing an antibacterial drug target. Here we describe the design and synthesis of transition-state analogs of the autoinducer synthase enzymatic reaction and the evaluation of these compounds as inhibitors of the synthase CepI. One such compound potently inhibits CepI and constitutes a new type of inhibitor against this underdeveloped antibacterial target.

Quorum sensing is a bacterial signaling system that functions through the production, secretion, and detection of small signal molecules called autoinducers (AI).[1–3] As bacterial cell population increases, the concentration of autoinducer rises and engages its cognate receptor. The autoinducer-receptor complex acts as a transcriptional regulator allowing for coordinated changes in gene expression at high cell density. The main class of autoinducers in proteobacteria are acylated homoserine lactones (AHLs). The acyl group of AHL structures varies according to species, with lengths between 4 and 18 carbons and variable oxidation states at the beta position.[4] AHLs are produced by the LuxI family of AHL synthases and are detected by the LuxR family of AHL receptors, named after the AI synthase/receptor pair of the marine bacterium *Vibrio fischeri,* whose lux bioluminescence operon is controlled by quorum sensing (Fig. 1).[5]

Quorum sensing has been shown to control expression of virulence factors in bacteria that are human pathogens.[6] A well-studied example is *Pseudomonas aeruginosa,* a cause of acute and persistent infections in burn victims, cystic fibrosis patients, and a prominent hospital-acquired infection.[7] *Pseudomonas aeruginosa* has two AHL synthase/receptor quorum sensing systems, LasI/LasR and RhlI/RhlR, which control expression of its pathogenic phenotypes.[8] These include biofilm formation, antibiotic resistance, motility, and production of the rhamnolipid surfactant, which correlates with disease progression in cystic fibrosis patients.[9]

Small-molecule inhibition of quorum sensing is an active area of medicinal chemistry research to find drugs to suppress expression of virulence factors that are under quorum sensing control.[10,11] Numerous potent AHL receptor antagonists that block signal detection have been developed.[12,13] Some receptor antagonists have been shown to suppress virulence and improve host outcomes in infection models, validating quorum sensing as an antibacterial drug target.[14–17]

An alternative approach to block AHL quorum sensing is to inhibit

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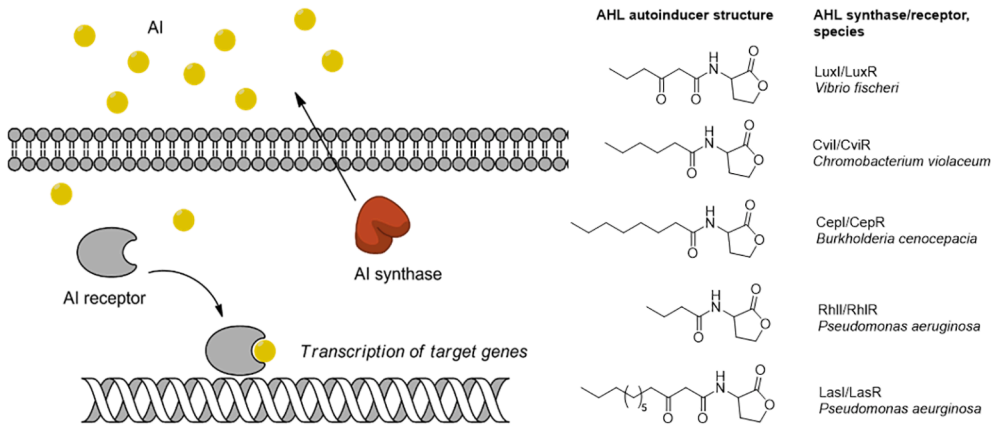


Fig. 1. Left: Autoinducer synthases produce AI signal molecules which accumulate in the environment at levels proportional to bacterial cell density. At a threshold concentration, the autoinducers bind their receptors which induces DNA binding and transcription of genes controlled by quorum sensing. Right: Structures of AHL autoinducers from various bacterial species.

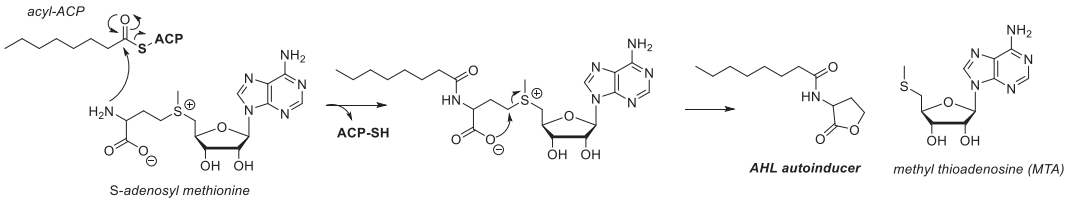


Fig. 2. The enzymatic reaction of AHL synthases.

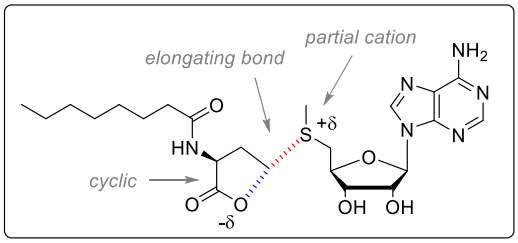


Fig. 3. Lactonization transition state of AHL synthases.

the AHL synthase enzyme and signal production. Synthases catalyze acyl group transfer from acylated acyl carrier protein (acyl-ACP) to the amino group of *S*-adenosylmethionine (SAM). The enzyme subsequently catalyzes an intramolecular S_N2 lactonization, releasing *S*-methyl thioadenosine (MTA) and the AHL autoinducer (Fig. 2).[18,19]

In contrast to the extensive work on AHL receptor antagonists, few AHL synthase inhibitors have been discovered. These can be divided into three classes. First, several inhibitors are autoinducer analogs. These include J8-C8, an acyl-aminocyclohexenone which was co-crystallized with its target TofI along with MTA, the AHL synthase byproduct.[20] AHL analogs with unnatural (*R*) stereochemistry and long acyl tails have been shown to inhibit the AHL synthase RhlI.[21] It was proposed that these compounds bind a cryptic pocket on the synthase. Second,

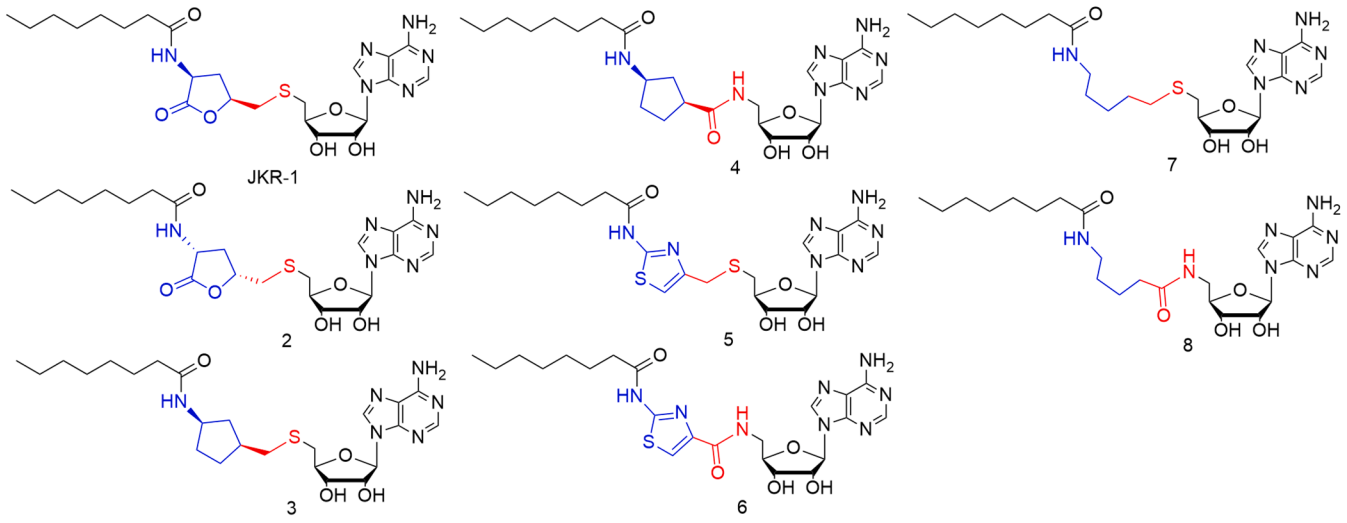
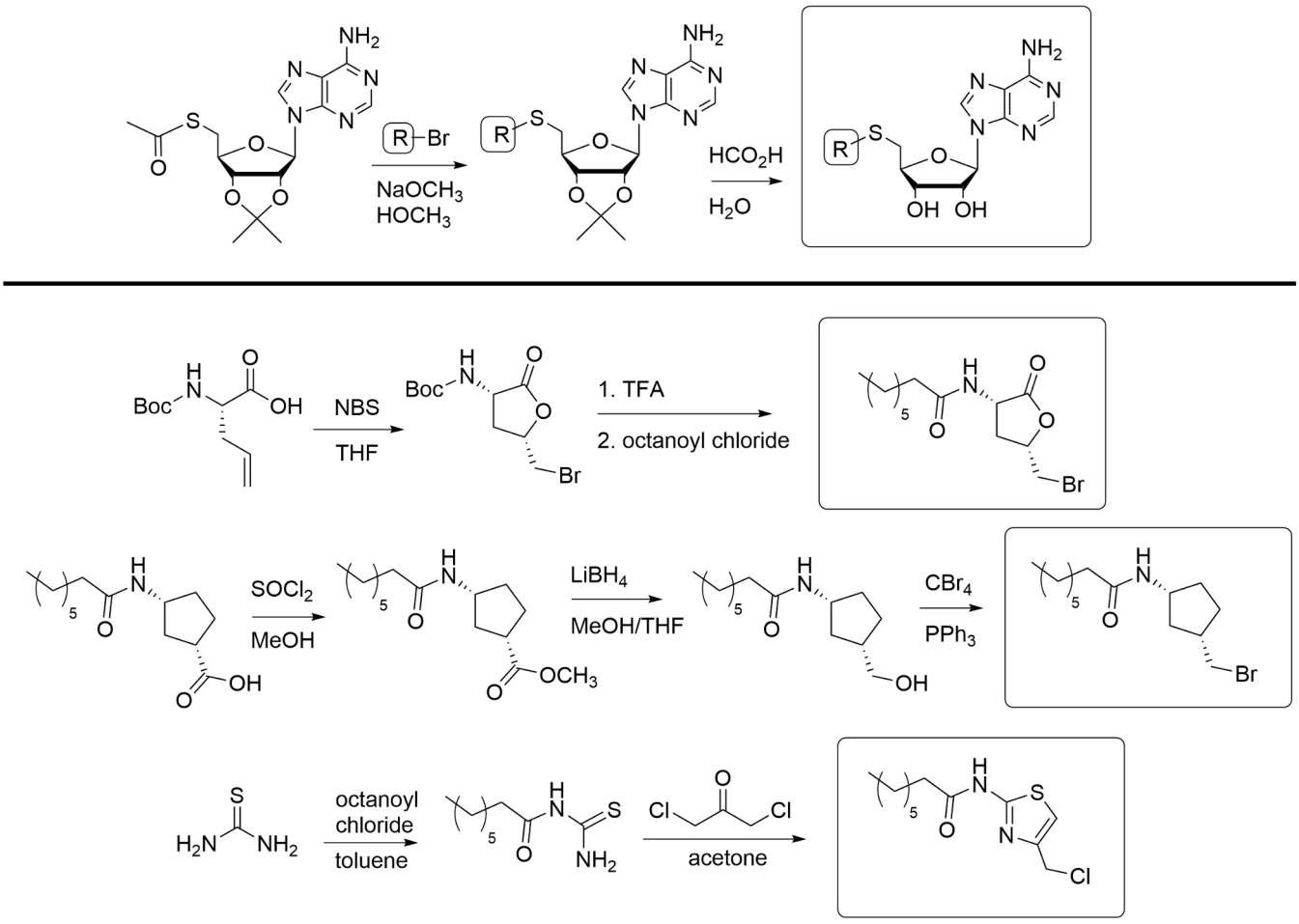


Fig. 4. Analogs of the lactonization transition state as target structures for AHL synthase inhibitors.



Scheme 1. Synthesis of thioether analogs by coupling alkyl halides with 5′ thioacetyl adenosine acetal.

bisubstrate analogs with adenosyl and acyl tail moieties joined by linker groups have been shown to be inhibitors of the synthase TofI.[22] These molecules were also attached to beads and used as affinity reagents for AHL synthase enzymes. Finally, some AHL synthase inhibitors bear no structural resemblance to any molecule along the synthase reaction coordinate. These include *N*-hydroxyindole derivatives as inhibitors of AHL synthases BmaI1 and YspI discovered in a novel high-throughput screening assay[23] and substituted diketopiperazines as inhibitors of the AHL synthase CepI.[24]

Our approach to develop inhibitors of AHL synthase enzymes was to design transition state analogs of the lactonization step of the enzymatic reaction. The theory of tight binding between enzymes and transition state analogs is well-established and has guided the design of many inhibitors.[25,26] The lactonization step of this reaction is particularly amenable to the design of transition state mimics. It is a 5-*exo-tet* cyclization, with the carboxylate displacing the sulfonium group. We designed transition state analogs to capture the cyclic nature of the closing lactone ring, the lengthening of the C—S bond as MTA departs, and the neutralization of the carboxylate and sulfonium charges (Fig. 3).

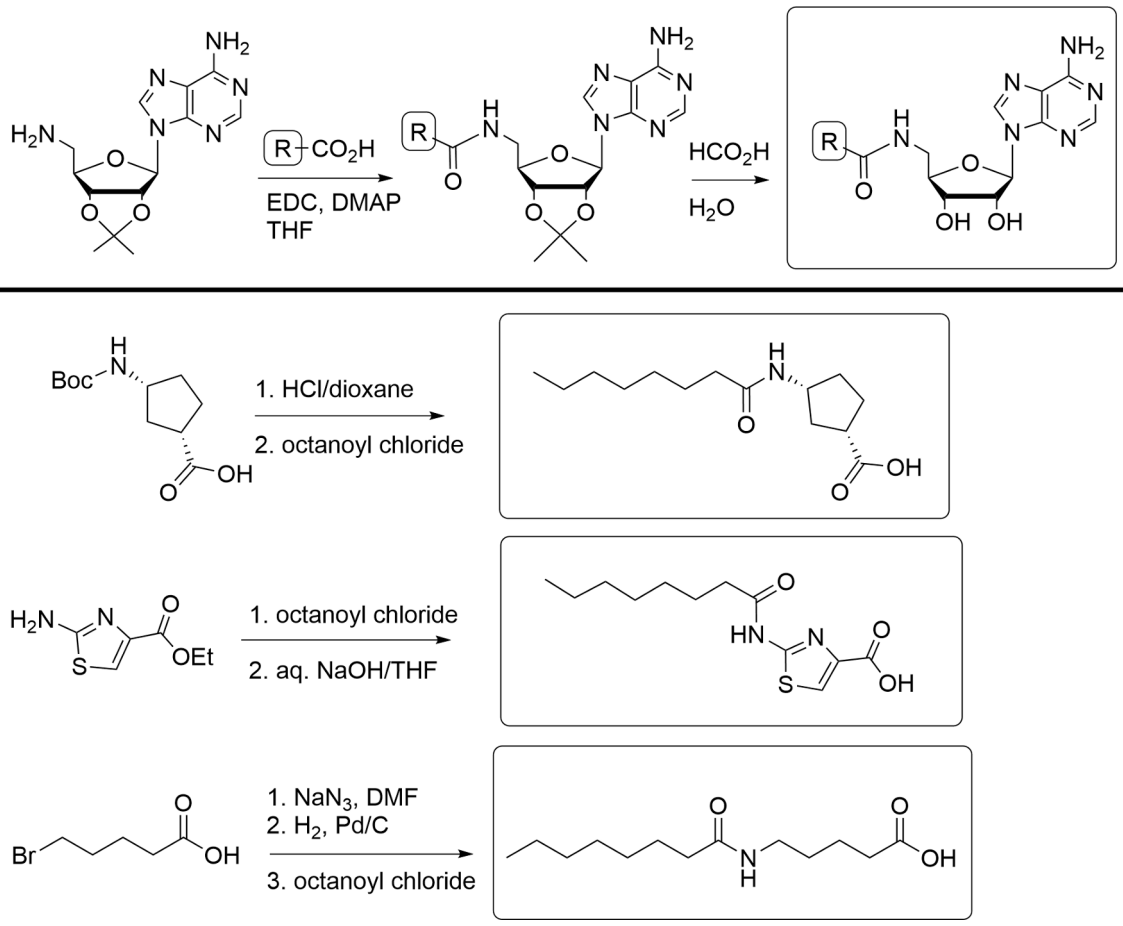
We designed a set of lactonization transition state analogs with lactone, cyclopentyl, and thiazole rings to mimic the nascent lactone of the enzymatic reaction and provide diversity in ring geometry. The rings were joined to adenosine by thioether or amide linkages to mimic the lengthening bond between the lactone ring and departing MTA. The two linker types allow some diversity of the polarity and degree of conformational freedom of this linker. The lactone, cyclopentyl, and thiazole rings all contain an exocyclic amine acylated with an octanoyl group, matching the acyl tail that our target enzyme CepI incorporates into its AHL product. We also made the corresponding acyclic amide and thioether (which was previously shown to inhibit the synthase TofI)[22] as control compounds (Fig. 4).

The thioether compounds **JKR-1**, **2**, **3**, **5** and **7** were all prepared from 5′ thioacetyladenosine acetal, prepared by Mitsunobu reaction of protected adenosine and thioacetic acid.[27] The 5′ thiol was liberated by basic methanolysis and coupled to suitable halides (Scheme 1).

The bromides leading to **JKR-1** and compound **2** were prepared from (*S*) or (*R*) Boc-allylglycine[28] which undergo diastereoselective bromolactonization with NBS.[29] The Boc-bromolactone was deprotected, acylated with octanoyl chloride then coupled to thioadenosine. The synthesis of cyclopentyl analog **3** began with commercially available (1*S*,3*R*)-Boc-3-aminocyclopentane carboxylic acid which was similarly deprotected and acylated with octanoyl chloride. The acid was esterified and subsequently reduced with methanolic $LiBH_4$[30] and brominated under Appel conditions before coupling to thioadenosine. The synthesis of thiazole analog **5** began with acylation of thiourea,[31] cyclization with 1,3 dichloroacetone[32] followed by coupling with thioadenosine. The linear analog **7** is a known compound and was prepared by the published sequence.[22]

The amide-linked analogs **4**, **6** and **8** were all prepared by EDC coupling of appropriate carboxylic acids with 5′ aminoadenosine acetonide (Scheme 2).[33]

The carboxylic acid of the thiazole analog **4** was prepared by acylation of ethyl aminothiazole carboxylate with octanoyl chloride followed by ester hydrolysis. The synthesis of cyclopentyl analog **6** was prepared from the carboxylic acid shown in Scheme 1. The linear amide-linked analog **8** was prepared from bromovaleric acid by treatment with



Scheme 2. Synthesis of amide-based analogs by coupling carboxylic acids with 5′ aminoadenosine acetonide.

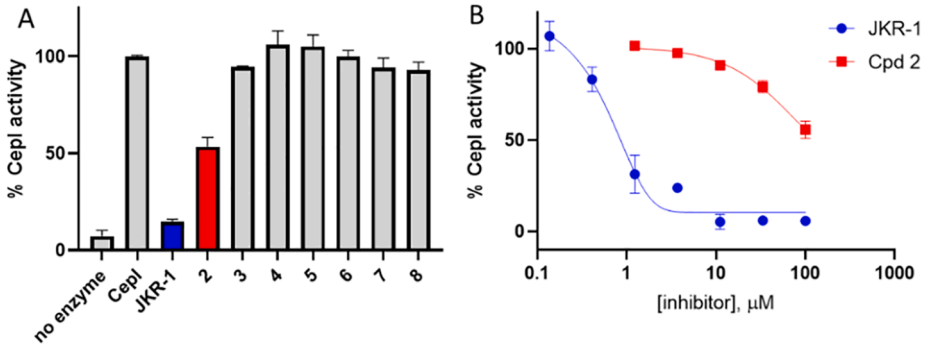


Fig. 5. A) Assay of each compound at 100 μM for CepI inhibition. B) Titration of **JKR-1** and compound **2** against CepI.

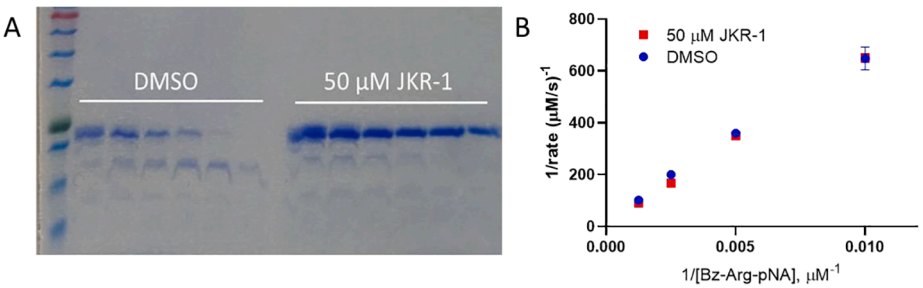


Fig. 6. A) Proteolytic digestion of CepI (5 μM) with Trypsin (0.1 μM) for 2.5, 5, 10, 20, 40 and 80 min in the presence or absence of **JKR-1** (50 μM). B) Trypsin (1 μg mL^{-1}) assay with the colorimetric substrate Bz-Arg-pNA in the presence and absence of 50 μM **JKR-1**.

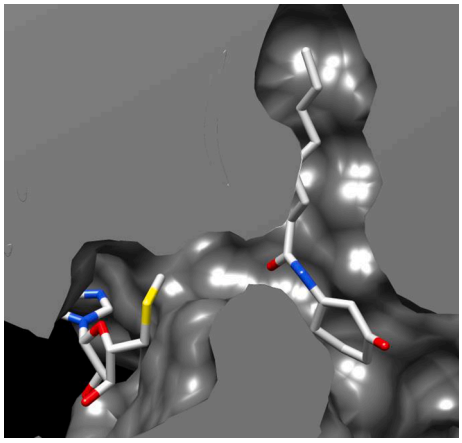


Fig. 7. X-ray crystal structure (from Ref. 20, PDB ID 3P2H) of the synthase TofI bound to MTA which occupies the adenosyl pocket (left) and the autoinducer analog J8C8 which occupies the acyl pocket (right).

sodium azide, reduction to the amine, and subsequent acylation with octanoyl chloride.

We tested these transition state mimics as inhibitors of CepI, the autoinducer synthase from *Burkolderia cenocepecia*.[34] We screened all compounds in Fig. 4 against CepI at 100 μM using the standard DCPIP assay to detect liberated ACP thiol, with 500 μM SAM and 15 μM octanoyl ACP. We found that **JKR-1** and compound **2**, the two lactone-based structures, showed inhibition of CepI under these conditions (Fig. 5A). We then titrated both these compounds against CepI under the same assay conditions and found that **JKR-1** has an IC_{50} of 1.0 ± 0.3 μM and **2** has an IC_{50} of ~100 μM (Fig. 5B).

We sought additional independent evidence that **JKR-1** binds to CepI. One method to do so is to measure if a protein's susceptibility to proteolysis is altered by ligand binding. Indeed, we observed that addition of **JKR-1** protected CepI from proteolysis by trypsin (Fig. 6A). We tested whether **JKR-1** inhibits trypsin itself using a standard colorimetric trypsin assay and confirmed that it does not (Fig. 6B). The suppression of CepI proteolysis by trypsin conferred by **JKR-1** suggests that **JKR-1** binds CepI and affects its structure.

JKR-1 is a new, potent inhibitor of the quorum sensing autoinducer synthase enzyme CepI. Among the set of molecules described here, **JKR-1** is the closest mimic of the "late" lactonization transition state: where the lactone ring is formed, MTA is departing, and the carboxylate and sulfonium charges are neutralized. **JKR-1** shares the same (*S*) stereochemistry at the amino group position as the SAM substrate, which may be why it proved to be a more potent inhibitor than its stereoisomer **2**.

It is unclear why **JKR-1** showed inhibition of CepI while other closely related molecules (notably compound **3**) did not. The crystal structure of the AHL synthase TofI bound to the autoinducer analog J8C8 and reaction byproduct MTA shows that there are distinct binding pockets for the SAM and acyl-ACP substrates.[20] These two pockets join at a junction where both the acyl transfer and lactonization chemical steps occur (Fig. 7).

Our transition state analog **JKR-1** contains both adenosyl and acyl groups, therefore it likely binds both the adenosyl and acyl tail pockets through this junction. The remainder of the compounds reported here may have failed to inhibit CepI if there is a strict geometric requirement to link both pockets through this junction. **JKR-1** may thus add key information on how to effectively do so. This information may help lead to potent, cell permeable AHL synthase inhibitors that are useful to block quorum sensing behaviors in bacteria, and ultimately test whether such inhibition mitigates bacterial infections in model systems.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.127873.

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