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Highly Potent, Chemically Stable Quorum Sensing Agonists for *Vibrio Cholerae*

Lark J. Perez^{a,§}, Theodora K. Karagounis^a, Amanda Hurley^b, Bonnie L. Bassler^{b,c}, and Martin F. Semmelhack^a

Martin F. Semmelhack: mfshack@princeton.edu

^aDepartment of Chemistry, Princeton University, Washington Road, Princeton, NJ 08544 (USA)

^bDepartment of Molecular Biology, Princeton University, Washington Road, Princeton, NJ 08544 (USA)

^cHoward Hughes Medical Institute Chevy Chase, MD 02815

Abstract

In the *Vibrio cholerae* pathogen, initiation of bacterial quorum sensing pathways serves to suppress virulence. We describe herein a potent and chemically stable small molecule agonist of *V. cholerae* quorum sensing, which was identified through rational drug design based on the native quorum sensing signal. This novel agonist may serve as a useful lead compound for the control of virulence in *V. cholerae*.

Introduction

Bacterial cells communicate with one another by producing, releasing, and detecting extracellular signaling molecules called autoinducers. This signaling process, termed quorum sensing (QS), enables groups of bacteria to synchronously modulate their behavior as a function of cell-population density.^{1–4} Specifically, autoinducers accumulate at high cell densities, and their detection drives synchronous group-wide changes in gene expression. QS-mediated behaviors are crucial in bacterial infection; accordingly, small-molecule modulators of QS pathways represent promising lead compounds en route to novel anti-bacterial therapeutics.^{5–11}

Typically, in pathogenic bacteria that cause persistent infection, the accumulation of autoinducers at high cell densities triggers QS-mediated virulence factor production and biofilm formation.^{12–15} By contrast, *Vibrio cholerae*, which causes the acute intestinal disease cholera, displays an unusual QS profile: at high population density, QS initiates a cascade that *suppress* virulence factor production (Figure 1).^{16–20} The distinct QS behavior exhibited by *V. cholerae* is attributed to its life cycle. Successful infection by *V. cholerae* leads to profuse diarrhea, which washes huge numbers of bacteria from the human intestine into the environment. Thus, at low cell density, the expression of genes for virulence and biofilm formation promotes establishment of infection in the host, while at high cell density, autoinducer-dependent repression of these traits promotes dissemination. The consequence

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Correspondence to: Martin F. Semmelhack, mfshack@princeton.edu.

[§]current address: Department of Chemistry and Biochemistry, Rowan University, Glassboro, NJ 08028 (USA)

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of this QS behavioral profile is that potent small-molecule agonists of QS could conceivably be used to repress virulence in *V. cholerae*. The development of an effective QS agonist for *V. cholerae* as a therapeutic agent is anticipated to have significant global ramifications, particularly in developing regions, which are especially vulnerable to the devastating effects of this pathogen.

Our strategy is to develop “pro-QS” molecules (agonists) that induce *V. cholerae* cells to exhibit high cell density behaviors – most notably repression of genes encoding virulence factors and those required for biofilm formation – regardless of their actual cell density. Previous work supports the feasibility of this strategy.^{20,21} Using an infant mouse model, we found that a *V. cholerae* mutant genetically locked in the high cell density state was severely defective in colonization.²⁰ Furthermore, commensal *Escherichia coli* engineered to produce the *V. cholerae* autoinducer protected mice from *V. cholerae* infection, indicating that a pro-QS molecule can function as a therapeutic in vivo.²¹ The strategy may also be applicable to other human pathogens from the vibrio genus, such as *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, and *Vibrio vulnificus* because, in these species, QS also represses virulence and biofilm traits at high cell density.^{18,22–27}

The small molecule CAI-1 [(*S*)-3-hydroxytridecan-4-one] is the primary QS signal in *V. cholerae* (Figure 2).²⁸ This compound is produced by the CqsA synthase^{29,30} and detected by the membrane-spanning receptor, CqsS.³¹ While no structural information is available for CqsS, significant understanding of the ligand-receptor interaction exists from studies of modified ligands and CqsS mutants.^{32,33} Focused libraries of CAI-1 analogs have revealed a pattern of signal specificity based on the fatty acid “tail” (R) and variations both in the heteroatom (X) and the carbon structure of the “head” group (Figure 2, general structure). Specifically, these studies show that: (a) agonist activity is highly sensitive to variations in chain length and incorporation of conformational restrictions in the tail; (b) the ester series analogs (Y=O) are comparably active and provide a convenient platform for synthesis; and (c) attachment of phenyl substituents in the head group gives rise to the first antagonists of QS in *V. cholerae*.^{34,35} While several CAI-1 analogs have been identified with activity comparable to that of the native signal (CAI-1, EC₅₀ = 30–40 nM), we aimed to expand the range of target structures to achieve higher potency and improved chemical stability.³⁰

We recently described the mechanism of biosynthesis of CAI-1, which revealed a chemically delicate intermediate (Ea-CAI-1, Figure 2) that is an order of magnitude more potent as a CqsS agonist compared to the native and longer-lived signal, CAI-1.²⁹ The structure of Ea-CAI-1 provides a new template on which to design effective agonists, with the goal of identifying molecules that maintain the high activity of Ea-CAI-1 while possessing the chemical stability of a useful probe or drug candidate. We describe herein the synthesis and evaluation of a series of Ea-CAI-1 analogs. These analogs incorporate variations in the tail portion of the molecule as well as new heterocyclic head groups that mimic the ene-amino ketone moiety of the parent compound.

Results and Discussion

We first sought to prepare a series of Ea-CAI-1 analogs of varying tail lengths through adaptation of our previously described route to CAI-1 derivatives.³⁶ Synthesis of these analogs required development of an effective enamine protecting group strategy. Deprotection of the ene-amino ketone functionality (**2–5**) is significantly complicated by the high propensity of the product (Ea-CAI-1) to undergo hydrolysis to the α -diketone **12**. As shown in Scheme 1, efforts to employ a *t*-Boc protecting group strategy (**2**, R¹ = *t*Bu) were unsuccessful; this functionality could not be removed under standard conditions. Similarly, attempts to reductively remove the Cbz group of **3** (R¹ = Bn) through exposure to H₂ and Pd

led to two major products, which were tentatively identified as aminoketone **13** and N-Cbz analog **14**; these compounds presumably arise via preferential reduction of the alkene unit prior to benzyl cleavage. Although the *N*-Teoc protecting group (**4**) could be removed by treatment with excess TASF in DMSO, this transformation was very slow and yielded the desired adduct along with inseparable silyl-byproducts. The *N*-Alloc protecting group (**5**, R = allyl) was most effective; upon exposure to Pd(PPh₃)₄ and Et₂NH, **5** was rapidly deprotected³⁷ to deliver Ea-CAI-1 in good yield. Importantly, the product was isolated in pure form after passage through a short plug of silica gel deactivated with Et₃N. Using this Alloc protecting group strategy, we successfully prepared a series of tail-length modified Ea-CAI-1 analogs (**6–11**).

The “tail-modified” analogs (**6–11**) were evaluated in a CqsS agonist bioassay (Table 1). Consistent with our earlier findings in the context of CAI-1 analogs,³⁵ the *V. cholerae* CqsS receptor was somewhat promiscuous with respect to variations in tail length. The parent compound (Ea-CAI-1, n = 8) and the analog bearing a one-carbon truncated tail (**9**, n = 7) show low nM activity and full activation (entries 4 and 5). Other analogs with shorter (**6–8**) or longer (**10**) tail lengths were 1–2 orders of magnitude less potent, but nonetheless promoted full activation (entries 1–3 and 6). Only a very long tail gave an analog (**11**, n = 11) that was much less active, even at high concentrations. Interestingly, we do not observe direct correlation between maximum %response and EC₅₀. Presumably, small perturbations in the structure of the ligand or binding site can disrupt the balance between kinase and phosphatase activity (see Figure 1). In all cases, the Ea-CAI-1 analogs were more active by an order of magnitude compared to the related examples from the native CAI-1 series, reported earlier.^{35, 36} These encouraging results prompted us to pursue the synthesis and evaluation of a broader range of Ea-CAI-1 analogs. In particular, we sought to explore the impact of replacing the enamino ketone “head” domain with more stable functionality, and of installing greater structural diversity onto the “tail” sector.

We first examined the effects of head group variation on agonist activity. We postulated that the stereoelectronic features of the ene-amino ketone motif of the parent compound could be mimicked through substitution with more stable isoxazole or acylpyrrole functional motifs (Scheme 2).³⁸ A series of analogs was synthesized and evaluated for agonist activity (see Scheme 2 and Supporting Information for details). Neither of the isoxazole analogs showed significant activity (**15** and **16**, EC₅₀ > 8000 nM). However, promising results were obtained with the series of analogs bearing acyl pyrrole head groups; importantly, these motifs retain both ketone and α-heteroatom hydrogen bond donor functionality. For the initial analysis, compounds **17** and **18** were prepared as a readily separable mixture of isomers for biological evaluation (Scheme 2). While both analogs elicit the full level of QS response, the 3-acyl isomer **18** exhibits an EC₅₀ >1000-fold more potent than the 2-acyl isomer. The EC₅₀ value of 4.2 nM identifies **18** as comparable in activity to the parent compound, Ea-CAI-1. Incorporation of additional substituents onto the 3-acyl pyrrole (**19** and **20**) resulted in lower agonist activity, as indicated by the higher EC₅₀ and lower % response.

The 3-acylpyrrole heterocyclic motif has therefore been identified as a viable, chemically stable bioisostere of the native Ea-CAI-1 head group.³⁹ Having identified a suitable replacement for the delicate enamino ketone moiety, we next probed the effect of variations in the tail structure on CqsS agonist activity (Table 2). We observed that analogs bearing a C9 or a C10 fatty acid tail were most potent and provided full activation (entries 4 and 5); these findings are comparable to the structure-activity relationships previously observed in the α-enamino ketone series. The other tail lengths were less potent and gave no more than 50% activation.

Further probing of the effect of tail structure involved introduction of simple *E*-alkene units and benzene rings, with the goal of examining the effects of conformational restriction and steric perturbation within the acyl tail (Figure 3).^{40,41} In all cases in which significant activity was observed (EC₅₀ values <5000 nM), the 3-acyl pyrrole analogs were much more potent than the corresponding 2-acyl pyrrole analogs. Introduction of a benzene ring in the 3-acylpyrrole series produced highly active analogs; compounds **26** and **29** show activities only 3- to 10-fold lower than the best agonists (Ea-CAI-1 and acylpyrrole **18**). This result suggests substantial steric flexibility in the presumed hydrophobic binding site for the tail group and expands the design opportunities for further exploration of the tail structure. It should be noted, however, that the maximum percent activation is lower for these benzyl analogs (54–75%). In our previous SAR work, focused on the structure of the fatty acid tail of CAI-1, we had observed that the conformational restriction provided by incorporation of an *E*-alkene unit conferred enhanced potency over the corresponding fully saturated analog. Similarly, in the 3-acylpyrrole Ea-CAI-1 series, incorporation of an *E*-alkene moiety (**32**) leads to one of the most potent agonists of *V. cholerae* QS yet discovered; compound **32**, with an EC₅₀ of 3.8 nM, is slightly more potent than both Ea-CAI-1 (4.1 nM) and acylpyrrole **18** (4.2 nM). Interestingly, incorporation of a biphenyl unit in the acyl chain leads to essentially inactive analogs (**34** and **35**).

Having identified a stable and highly potent lead compound (**18**), we examined its effect on the QS circuit in *V. cholerae*. In vivo, HapR is a transcription factor that functions as one of two master QS regulators in *V. cholerae*. Agonism of CqsS leads to increased production of HapR and HapR, in turn, drives the high-cell density gene expression program, which leads to repression of the expression of genes required for virulence and biofilm formation.¹ Figure 4 shows that production of HapR increased in response to 1 μM of the 3-acyl pyrrole **18** to a level similar to that achieved from exposure to 10 μM of the native ligand, CAI-1. Conversely, production of TcpA, the toxin-co-regulated pilus protein subunit A, and a major *V. cholerae* virulence factor is repressed by both CAI-1 and compound **18**. These results are consistent with the heterocyclic analog **18** acting on CqsS in a manner similar to CAI-1.

Given the possibility for further development of the 3-acylpyrrole **18** as a potential anti-virulence agent, we preliminarily investigated the cytotoxicity of this analog in bacteria and in a murine 3D3 fibroblast cell line. Consistent with our proposal that the molecule functions by influencing QS-controlled behaviors and not by altering growth, we observed that **18** is not toxic to bacteria. No decrease in OD₆₀₀ occurs when *V. cholerae* is treated with **18** at concentrations up to 25,000 nM (i.e. levels more than 5000-fold higher than the EC₅₀ for **18**, see Supporting Information). Likewise, **18** was tolerated by murine 3T3 fibroblast cells at concentrations up to 23,000 nM, as assessed by optical microscopy (see Supporting Information).

Despite the high activity of this class of molecules with respect to the *V. cholerae* CqsS receptor, none of the 3-acyl pyrrole analogs showed significant activity against the *Vibrio harveyi* CqsS receptor (data not shown). This result is perhaps surprising, given the high homology between these two QS receptors; however, it is consistent with our earlier observations that, compared to the *V. cholerae* receptor, the *V. harveyi* receptor is less tolerant of structural perturbations to the cognate ligand.³²

Conclusion

Ea-CAI-1, a delicate biosynthetic intermediate and the most potent natural agonist of QS in *V. cholerae*, was used as a starting point in designing chemically stable analogs with high potency in activating the CqsS receptor. Several derivatives of the 3-acylpyrrole structure have activities equivalent to Ea-CAI-1 and are promising leads for developing new

therapeutics to control *V. cholerae* infection. The 3-acylpyrrole with the C9 alkyl tail (**18**) functions analogously to the native signal, CAI-1, in regulating production of the transcription factor HapR and the virulence factor, TcpA. Compound **18** also does not inhibit bacterial growth nor does it show toxicity toward a mammalian cell line, and is therefore a promising candidate for further development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Notes and References

1. Ng W-L, Bassler BL. *Annu. Rev. Genet.* 2009; 43:197–222. [PubMed: 19686078]
2. Bassler BL. *Cell.* 2002; 109:421–424. [PubMed: 12086599]
3. Fuqua WC, Winans SC, Greenberg EP. *Annu. Rev. Microbiol.* 1996; 50:727–751. [PubMed: 8905097]
4. Waters CM, Bassler BL. *Annu. Rev. Cell Dev. Biol.* 2005; 21:319–346. [PubMed: 16212498]
5. Cegelski L, Marshall GR, Eldridge GR, Hultgren SJ. *Nat Rev Micro.* 2008; 6:17–27.
6. Clatworthy AE, Pierson E, Hung DT. *Nat Chem Biol.* 2007; 3:541–548.
7. Geske GD, O'Neill JC, Blackwell HE. *Chem. Soc. Rev.* 2008; 37:1432. [PubMed: 18568169]
8. Hentzer M, Wu H, Andersen JB, Riedel K, Rasmussen TB, Bagge N, Kumar N, Schembri MA, Song Z, Kristoffersen P, et al. *EMBO J.* 2003; 22:3803–3815. [PubMed: 12881415]
9. Njoroge J, Sperandio V. *EMBO Mol Med.* 2009; 1:201–210. [PubMed: 20049722]
10. Rasko DA, Sperandio V. *Nature Reviews Drug Discovery.* 2010; 9:117–128.
11. Swem LR, Swem DL, O'Loughlin CT, Gatmaitan R, Zhao B, Ulrich SM, Bassler BL. *Molecular Cell.* 2009; 35:143–153. [PubMed: 19647512]
12. Kovacicova G, Skorupski K. *Mol. Microbiol.* 2002; 46:1135–1147. [PubMed: 12421317]
13. Liu Z, Miyashiro T, Tsou A, Hsiao A, Goulian M, Zhu J. *Proc. Natl. Acad. Sci. USA.* 2008; 105:9769–9774. [PubMed: 18606988]
14. Schuster M, Lostroh CP, Ogi T, Greenberg EP. *J. Bacteriol.* 2003; 185:2066–2079. [PubMed: 12644476]
15. Smith RS, Iglewski BH. *Current Opinion in Microbiology.* 2003; 6:56–60. [PubMed: 12615220]
16. Nadell CD, Xavier JB, Levin SA, Foster KR. *PLoS Biol.* 2008; 6:e14. [PubMed: 18232735]
17. Hammer BK, Bassler BL. *Mol. Microbiol.* 2003; 50:101–104. [PubMed: 14507367]
18. Miller MB, Skorupski K, Lenz DH, Taylor RK, Bassler BL. *Cell.* 2002; 110:303–314. [PubMed: 12176318]
19. Zhu J, Mekalanos JJ. *Developmental Cell.* 2003; 5:647–656. [PubMed: 14536065]
20. Zhu J, Miller MB, Vance RE, Dziejman M, Bassler BL, Mekalanos JJ. *Proc. Natl. Acad. Sci. USA.* 2002; 99:3129–3134. [PubMed: 11854465]
21. Duan F, March JC. *Proc. Natl. Acad. Sci. USA.* 2010; 107:11260–11264. [PubMed: 20534565]
22. Defoirdt T, Bossier P, Sorgeloos P, Verstraete W. *Environ Microbiol.* 2005; 7:1239–1247. [PubMed: 16011761]
23. Gode-Potratz CJ, McCarter LL. *J. Bacteriol.* 2011; 193:4224–4237. [PubMed: 21705592]
24. Roh JB, Lee MA, Lee HJ, Kim SM, Cho Y, Kim YJ, Seok YJ, Park SJ, Lee KH. *J. Biol. Chem.* 2006; 281:34775–34784. [PubMed: 16971386]
25. Shao CP, Lo HR, Lin JH, Hor LI. *J. Bacteriol.* 2011; 193:2557–2565. [PubMed: 21398530]

26. Wang Q, Liu Q, Ma Y, Rui H, Zhang Y. *J. Appl. Microbiol.* 2007; 103:1525–1534. [PubMed: 17953563]
27. Ng W-L, Perez L, Cong J, Semmelhack MF, Bassler BL. *PLoS Pathog.* 2012; 8:e1002767. [PubMed: 22761573]
28. Higgins DA, Pomianek ME, Kraml CM, Taylor RK, Semmelhack MF, Bassler BL. *Nature.* 2007; 450:883–886. [PubMed: 18004304]
29. Wei Y, Perez LJ, Ng W-L, Semmelhack MF, Bassler BL. *ACS Chem. Biol.* 2011; 6:356–365. [PubMed: 21197957]
30. Kelly RC, Bolitho ME, Higgins DA, Lu W, Ng W-L, Jeffrey PD, Rabinowitz JD, Semmelhack MF, Hughson FM, Bassler BL. *Nat Chem Biol.* 2009; 5:891–895. [PubMed: 19838203]
31. Henke JM, Bassler BL. *J. Bacteriol.* 2004; 186:6902–6914. [PubMed: 15466044]
32. Ng W-L, Perez LJ, Wei Y, Kraml C, Semmelhack MF, Bassler BL. *Mol. Microbiol.* 2011; 79:1407–1417. [PubMed: 21219472]
33. Ng W-L, Wei Y, Perez LJ, Cong J, Long T, Koch M, Semmelhack MF, Wingreen NS, Bassler BL. *Proc. Natl. Acad. Sci. U.S.A.* 2010; 107:5575–5580. [PubMed: 20212168]
34. Perez LJ, Ng W-L, Marano P, Brook K, Bassler BL, Semmelhack MF. *J. Med. Chem.* 2012; 55:9669–9681. [PubMed: 23092313]
35. Bolitho ME, Perez LJ, Koch MJ, Ng W-L, Bassler BL, Semmelhack MF. *Bioorg. Med. Chem.* 2011; 19:6906–6918. [PubMed: 22001326]
36. Ng W-L, Perez LJ, Wei Y, Kraml C, Semmelhack MF, Bassler BL. *Mol. Microbiol.* 2011; 79:1407–1417. [PubMed: 21219472]
37. Genêt JP, Blart E, Savignac M, Lemeune S, Lemaire-Audoire S, Bernard JM. *Synlett.* 1993; 1993:680–682.
38. Bourbeau MP, Rider JT. *Org. Lett.* 2006; 8:3679–3680. [PubMed: 16898790]
39. Meanwell NA. *J. Med. Chem.* 2011; 54:2529–2591. [PubMed: 21413808]
40. Ferenczy GG, Keserü GM. *Drug Discov. Today.* 2010; 15:919–932. [PubMed: 20801227]
41. Freire E. *Drug Discov. Today.* 2008; 13:869–874. [PubMed: 18703160]

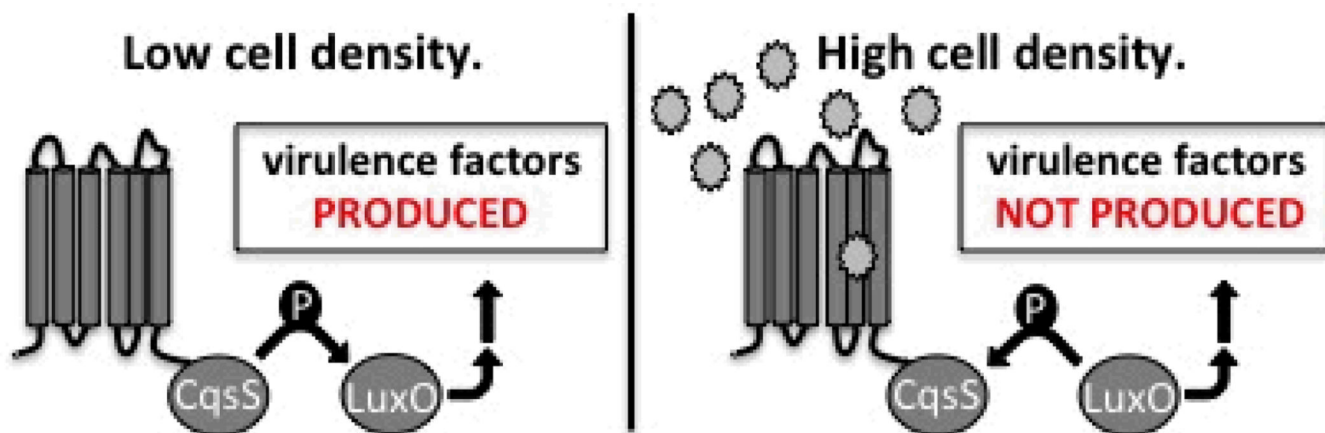


Figure 1.

Simplified schematic of quorum sensing in *V. cholerae*. In the absence of an agonist (i.e. at low cell density), CqsS acts as a kinase and virulence factors are expressed. When CqsS binds an agonist (stars), such as at high cell density with higher concentrations of the native agonist CAI-1 and Ea-CAI-1, CqsS acts as a phosphatase, repressing the expression of virulence factors.

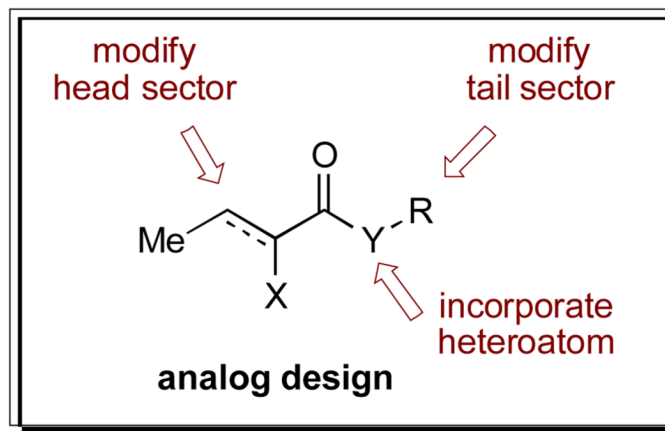
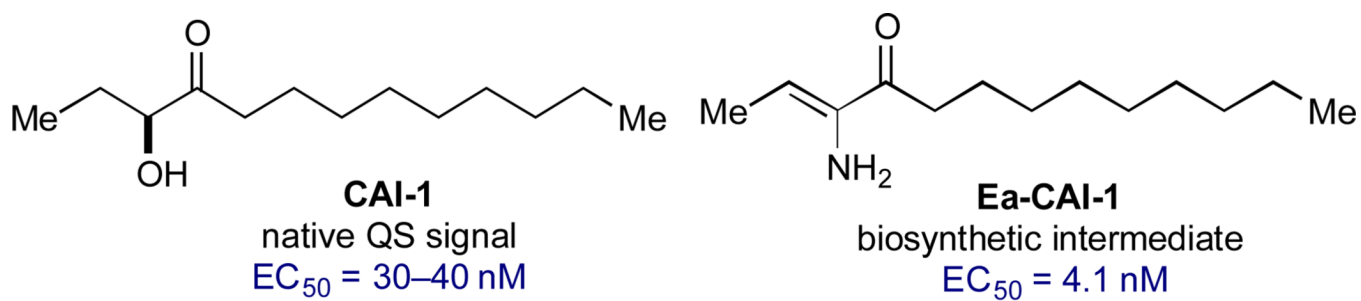
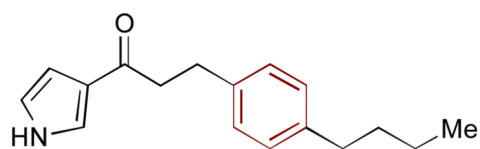
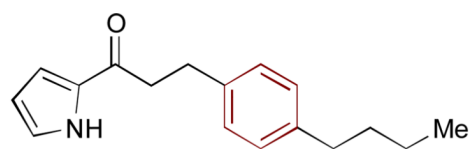


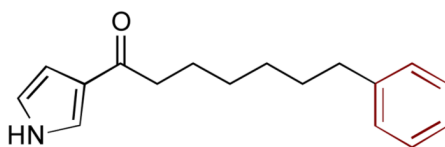
Figure 2.
CAI-1 Structure and CAI-1-Based Agonist Design.



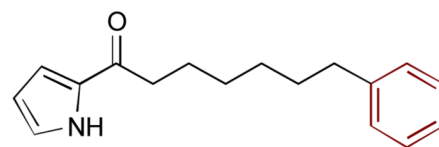
26
 $EC_{50} = 11 \text{ nM}$
 64% response



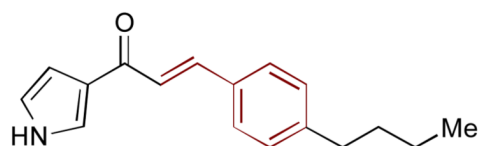
27
 $EC_{50} = 2000 \text{ nM}$
 75% response



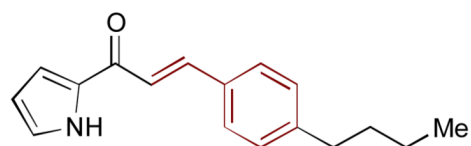
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 $EC_{50} = 36 \text{ nM}$
 75% response



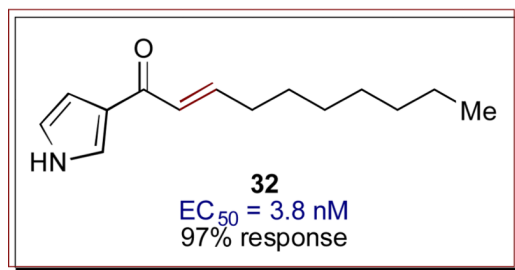
29
 $EC_{50} > 5000 \text{ nM}$



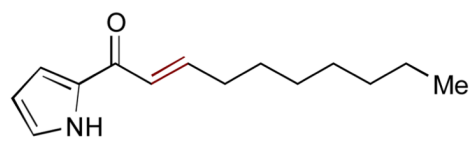
30
 $EC_{50} = 88 \text{ nM}$
 54% response



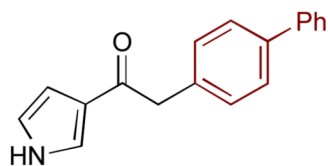
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 45% response



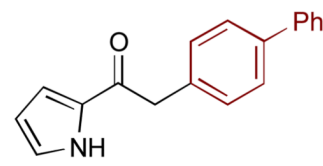
32
 $EC_{50} = 3.8 \text{ nM}$
 97% response



33
 $EC_{50} = 290 \text{ nM}$
 109% response



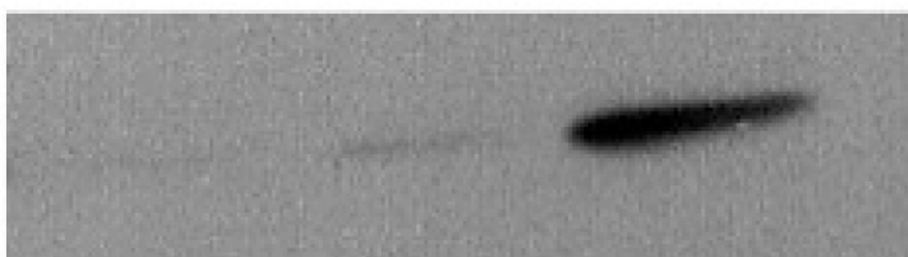
34
 $EC_{50} > 5000 \text{ nM}$



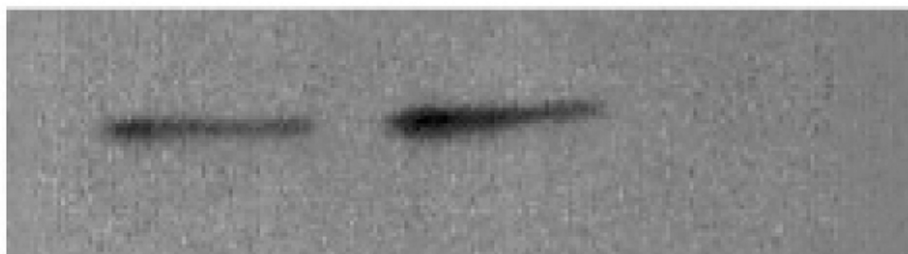
35
 $EC_{50} > 5000 \text{ nM}$

Figure 3.
 Modified tail structures in the 2- and 3-acylpyrrole motif.

18 (1 μ M)
CAI-1 (10 μ M)
DMSO

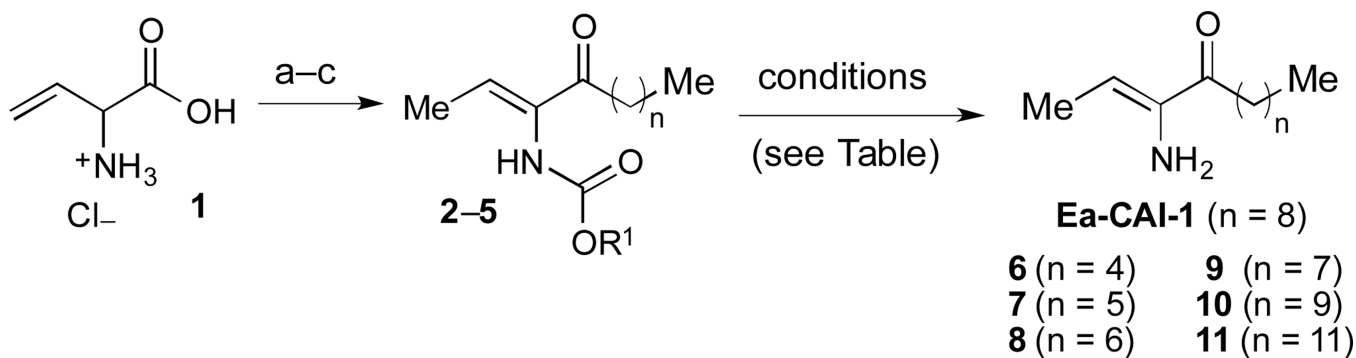


TcpA

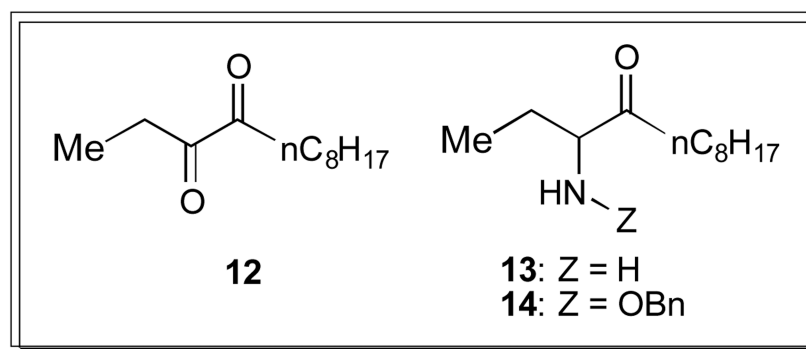


HapR

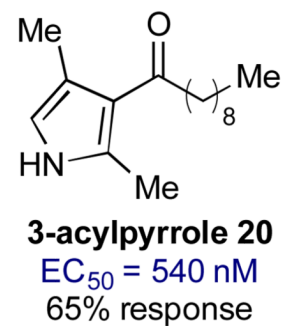
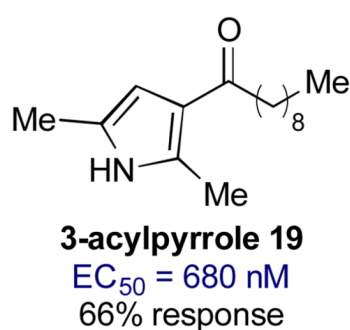
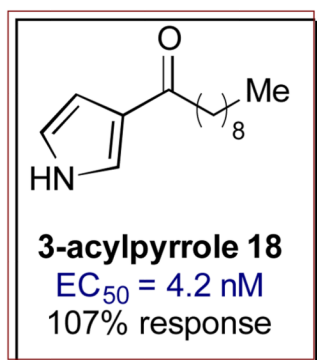
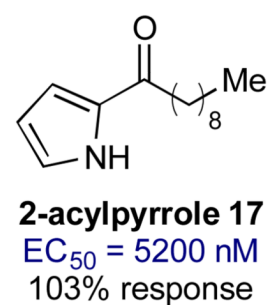
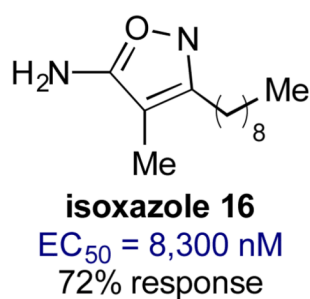
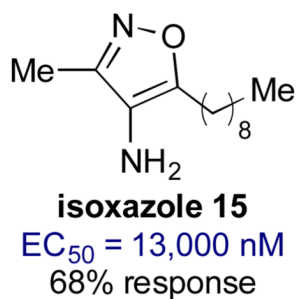
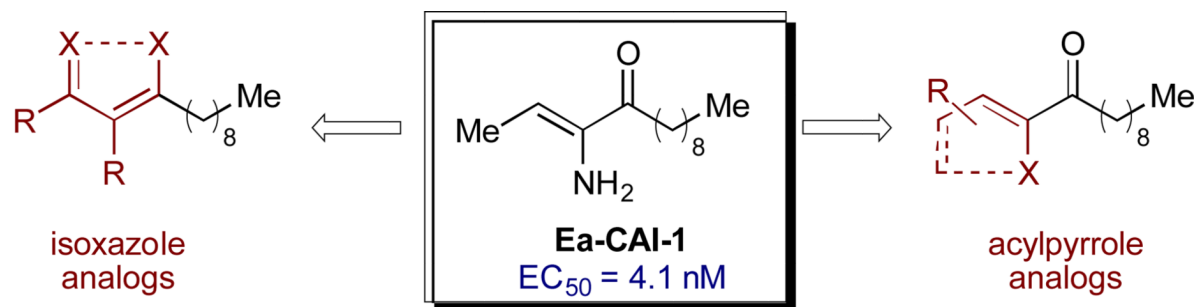
Figure 4.
CAI-1 and Compound **18** repress production of the *V. cholerae* toxin-correlated pilus TcpA and active production of HapR DMSO is used as the control see supporting Information.



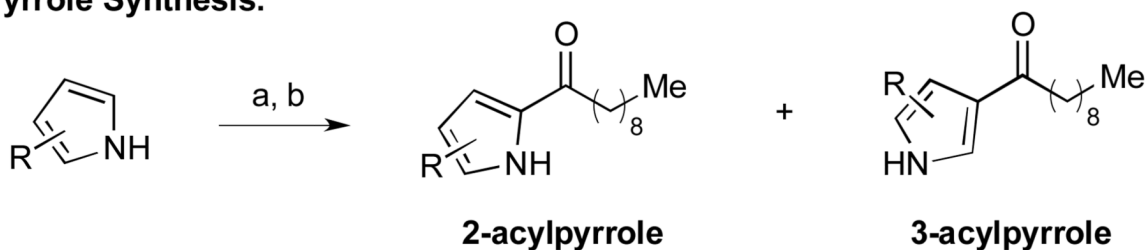
R ¹	conditions	yield
2 , <i>t</i> -Bu	TFA, CH ₂ Cl ₂ or 1 N HCl	0%
3 , Bn	H ₂ , Pd, EtOH	0%
4 , (CH ₂) ₂ TMS	TASF, DMSO	N/A [†]
5 , allyl	Pd(PPh ₃) ₄ , Et ₂ NH, THF	34–67%

**Scheme 1.**

Synthesis of Ea-CAI-1 and tail length-modified analogs. Reagents and conditions: (a) Amine production (see supporting Information for details); (b) HNMe(OMe)•HCl, EDC, HOBT, Et₃N, THF (42–58% from **1**); (c) R²MgX (71–93%).[†]These conditions led to quantitative consumption of the starting material and slow formation of the desired product along with inseparable silyl byproducts.



Pyrrole Synthesis:

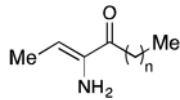


Scheme 2.

Variation of the Ea-CAI-1 head group: isoxazole and acylpyrrole analogs. Reagents and conditions: (a) MeMgBr, Et₂O; (b) *n*-C₉H₁₉COCl. see Supporting Information for details on the synthesis of the acylpyrrole and isoxazole analogs.

Table 1

Effect of Ea-EAI-1 tail length on QS agonist activity.

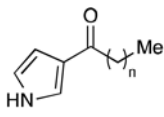
Entry		EC ₅₀ (nM) ^a	% Response ^b
1	6 , n = 4	590	100
2	7 , n = 5	290	120
3	8 , n = 6	40	109
4	9 , n = 7	2.6	112
5	Ea-CAI-1 , n = 8	4.1	100
6	10 , n = 9	170	105
7	11 , n = 11	>5000	38

Values were determined using the CqsS agonist bioassay.

^aAll EC₅₀ values are the mean of triplicate analyses.^bPercent maximal bioluminescence with respect to Ea-CAI-1, which is set at 100%. For more information, see Supporting Information.

Table 2

Effect of 3-acylpyrrole tail on QS agonist activity.

Entry		EC ₅₀ (nM) ^a	% Response ^b
1	21 , n = 4	1200	14
2	22 , n = 5	740	43
3	23 , n = 6	51	50
4	24 , n = 7	8.2	101
5	18 , n = 8	4.2	107
6	25 , n = 9	300	29

Values were determined using the CqsS agonist bioassay.

^a All EC₅₀ values are the mean of triplicate analyses.^b Percent maximal bioluminescence with respect to Ea-CAI-1, which is set at 100%. For more information, see Supporting Information.