Development of Peptide-Based Quorum Sensing Modulators that Attenuate *Streptococcus pneumoniae* Pathogenicity

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Introduction

The discovery of bacterial communication changed our general perception of microscopic organisms. Bacteria use signaling molecules (sometimes referred to as pheromones) released into the environment as a chemically-based language which allows for both intraspecies and interspecies communication.[1] The release and detection of the signaling molecule(s) allows bacteria to measure the number of bacteria within a population. This phenomenon is known as quorum sensing (QS).[2] QS is a process in which the concentration of signaling molecules enables a single cell to sense the overall bacterial population and allows bacteria to change their behavior once a threshold concentration is reached. When the threshold signaling molecule concentration is reached, bacteria coordinate their behavior through synchronization and the establishment of group behaviors such as the formation of biofilms, induction of competence, and release of virulence factors (REF).[2,3] This cooperation is important for pathogenic bacteria to communicate together in order to establish infection and to escape the host's immune response.[3] Bacteria are omnipresent in nature, and different bacterial species use unique molecules to communicate with each other

Streptococcus *pneumoniae*

- *Streptococcus pneumoniae* is a gram-positive bacteria that inhabits the nasopharynx of human and cause numerous disease.
- *S. pneumonia*e acquires antibiotic resistant genes through genetic transformation
- Competence in *S. pneumoniae* is initiated by the competence stimulating peptide (CSP)-mediated quorum sensing (QS) circuit
- *S. pneumoniae* strains can be divided into two main specificity groups, Group 1 and Group 2, based on the CSP signal (CSP1 or CSP2) and associated receptor (ComD1 or ComD2) they produce.[4]
- In this study we systematically altered the sequence of CSP2 to find analogs that can modulate the QS response and attenuate the pathogenicity of *S. pneumoniae*.

CSP Pathway

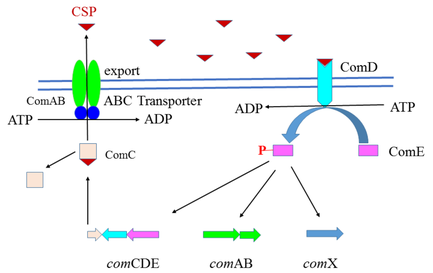


Figure: Signaling pathway of CSP induced competence in *S.pneumoniae*

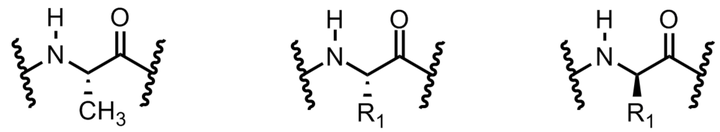
QS Modulators

Alanine Scanning

CSP2	EMRISRIILDFLFLRKK
CSP2-E1A	**A**MRISRIILDFLFLRKK
CSP2-M2A	E**A**RISRIILDFLFLRKK

D - Amino Acid Scaning

CSP2	EMRISRIILDFLFLRKK
CSP2-D-E1	eMRISRIILDFLFLRKK
CSP2-D-M2	EmRISRIILDFLFLRKK

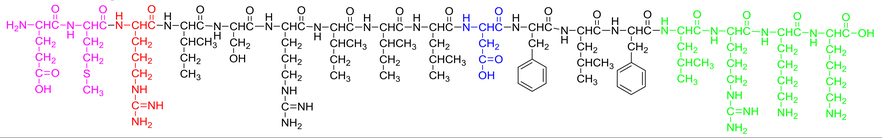


Structure Activity Relationship of CSP2

Name	EC_{50} /IC_{50} (nM) ComD-1	ComD-2	Name	EC_{50} /IC_{50} (nM) ComD-1	ComD-2
			CSP2	1654	**50.7**
CSP2-E1A	>1000	>1000	CSP2-e1	--	>1000
CSP2-M2A	--[a]	897	CSP2-m2	--	>1000
CSP2-R3A	--	--	CSP2-r3	--	--
CSP2-I4A	--	170	CSP2-i4	--	--
CSP2-S5A	--	148	CSP2-s5	--	550
CSP2-R6A	>1000	116/	CSP2-r6	830	182
CSP2-I7A	>1000	250/	CSP2-i7	452	240
CSP2-I8A	--	--	CSP2-i8	>1000	249
CSP2-L9A	--	30.1	CSP2-l9	>1000	111
CSP2-D10A	>1000	319	**CSP2-d10**	513	**2.86**
CSP2-F11A	--	164	CSP2-f11	--	270
CSP2-L12A	--	>1,000	CSP2-l12	--	345
CSP2-F13A	--	505	CSP2-f13	--	23.8
CSP2-L14A	--	175	CSP2-l14	>1000	54.2
CSP2-R15A	--	24.3	CSP2-r15	>1000	41.2
CSP2-K16A	--	80.5	CSP2-k16	>1000	38.6
CSP2-K17A	--	--	CSP2-k17	--	48.2

Table 1: EC_{50}/IC_{50} values of the alanine and D-amino acid scan analogs of CSP2 against ComD2 receptor.

[a] EC50/IC50 not determined due to the low induction, the analog shows in initial screening.

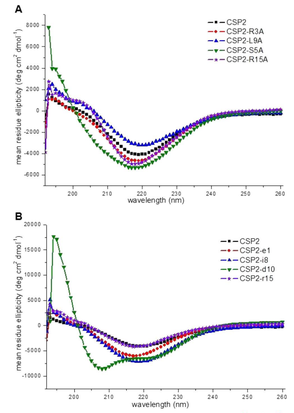


Second Generation CSP2

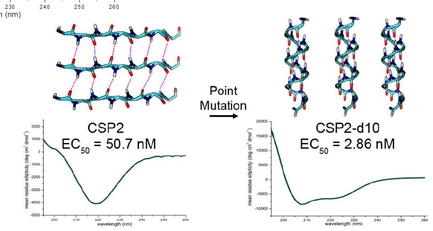
Name	EC_{50} /IC_{50} (nM) ComD-2
CSP2	**50.7**
CSP2-des-K17	44.4
CSP2-desK17K16	21.8
CSP2- desK17K16R15	42.3
CSP2- desK17K16R15L14	77.8
CSP2-E1Ad10	**56.5**

- We made more than 15 second generation analogs
- We were able to rational design to make 1st good inhibitor(CSP2-E1Ad10).[5]
- C- terminus is dispensable
- This result gives us a new window to make more rational analogs.

Circular Dichroism



CD spectra of select CSP2 analogs in membrane mimicking conditions. (A) CD spectra of select alanine-scanning analogs. (B) CD spectra of select d-amino acid scanning analogs. CSP2 and most of its analogs adopt a β-sheet conformation. However, CSP2-d10, the most active CSP2 analog identified, adopts an α-helix conformation.[5]



Summary

- Alanine and D-amino acid scanning shows correlation of specific amino acid with receptor.
- N-terminus is very critical for activating the receptor.
- CSP2-d10 and CSP2-E1Ad10 are able to turn on and off QS respectively.
- The CD analysis results suggest that α-helix is required for effective binding to the ComD2 receptor
- Suggests design of third generation peptides.

References

1) Miller, B. M.; Bassler, L.B. *Annu. Rev. Microbiol.* **2001,** *55*, 165.
2) Dunny, M. G. *Annu. Rev. Microbiol.* **1997,** *51*, 527.
3) Waters, C. M.; Bassler, L. B. *Annu. Rev. Cell Dev. Biol.* ***2005,*** *21*, 319
4) Zhu, L.; Lau, W. G. *PLoS Pathog.* **2012,** *7*, 1.
5) Yang, Y.; Koirala, B.; Sanchez, L.; Phillips, R. N.; Hamry, R. S.; Tal-Gan, Y. *ACS Chem. Biol.* **2017,** *12*, 1141-1151

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