In vitro activity of BV200 anti-virulent small molecules against a large and geographically diverse panel of *S. aureus* isolates from skin and lung infections

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Background

- BV200 is a novel series of anti-virulent small molecules designed to block *Staphylococcus aureus* quorum sensing (QS) system by selectively inhibiting the key transcriptional regulator AgrA.
- BV200 attenuates *S. aureus* virulence in murine skin and pneumonia infection models by inhibiting the production of a broad range of virulence factors including *δ*-toxin.
- A panel of 150 *S. aureus* isolates from lung and skin infections originating from 13 low- & medium-income countries (LMICs) was assembled and used to assess the activity spectrum of 3 lead molecules.

Objectives of the study

- **To develop an HPLC method to quantify *δ*-toxin production.**
- **To evaluate the activity of BV200 leads on a large panel of *S. aureus* strains**

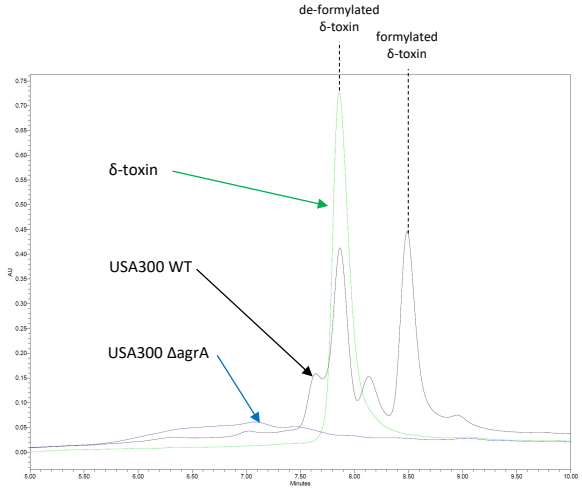


Figure 1: Chromatogram overlay (commercial δ-toxin, supernatant of USA300 WT & USA300 *Δ*agrA)

Methods

- A HPLC method was adapted[1] & implemented to quantify the production of δ-toxin, a PSM whose expression is directly regulated by AgrA.
- Both formylated and non-formylated δ-toxin peaks considered for quantification in *S. aureus* supernatants
- As expected, the USA300*Δ*agrA strain does not secrete quantifiable level of δ-toxin

[1] Quave C. L. & Horswill, A. R. *Quorum Sensing: Methods and Protocols*. Methods in Molecular Biology, vol. 1673, 363-370 (2018)

Strain Panel – Geography & Analysis

Geography & origin

- 60% of the 150 strains (acquired from JMI) originated from Asia and 40% from South America
- Strains isolated from skin infections (61%) and pneumonia (39%)

Table 1: geographic repartition

Isolation date	Strain Country	Number of strain
2018-2020	Argentina	10
	Brazil	10
	Colombia	10
	Costa Rica	10
	Malaysia	10
	Mexico	10
	Panama	10
	Philippines	10
	Thailand	10
	Turkey	10
	Vietnam	10
2013	China	20
	India	20

Strains analysis

- For all strains, Agr type and the sequence of AgrA was determined. The Agr type could not be determined for 6 strains (“ND”)
- 29 strains do not express (15) or at very low level (14) δ-toxin (<LOQ)

Table 2: Main characteristics of the strains

Strains (n=150)	
MRSA / MSSA	50.7 / 49.3 %
Skin / lung	61.3 / 38.7 %
Agr type I	50.7 %
Agr type II	29.3 %
Agr type III	14.7 %
Agr type IV	1.3 %
Agr ND	4 %
Mutation AgrA (excl. K136R)	12%

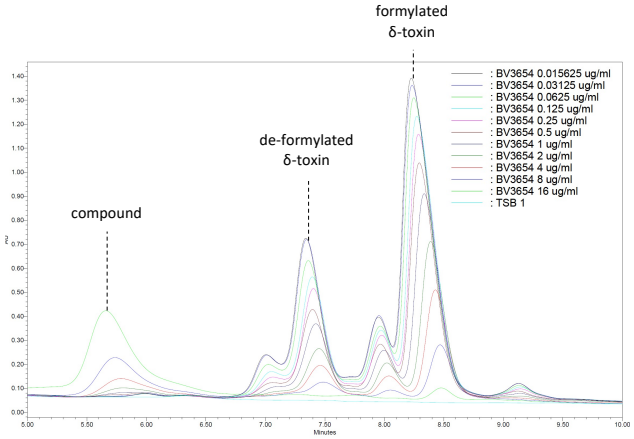
BV200 Lead molecules markedly reduce the production of δ-toxin from *S. aureus*

Results

1) Method development with BV3654

→ No peak interferences between δ-toxin and the compounds

→ HPLC quantification correlates with activity measured in our reporter gene assay – IC_{50} (BV3654) = 0.9μg/mL (both reporter assay & HPLC) (HPLC)

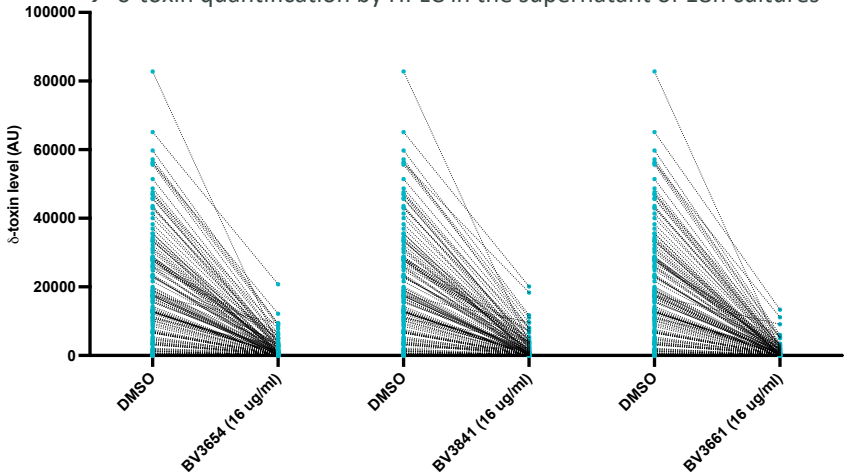


2) Activity of the lead molecules

→ 121 out of 150 strains have detectable expression of δ-toxin

→ exposed to DMSO or **16 µg/ml BV3654, BV3481 or BV3661**.

→ δ-toxin quantification by HPLC in the supernatant of 18h cultures



Conclusion

BV200 leads exhibited potent *in vitro* activity against the panel of *S. aureus* isolates with a median δ-toxin expression reduced by more than 10-fold and at least 5-fold reduction of δ-toxin expression in >90% of the strains independently of the Agr type.

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