

Identification of Benzyloxy Carbonimidoyl Dicyanide Derivatives as Novel Type III Secretion System Inhibitors via High-Throughput Screening

Yi-Nan Ma^{1†}, Liang Chen^{1,2†}, Nai-Guo Si², Wen-Jun Jiang¹, Zhi-Gang Zhou³, Jun-Li Liu², Li-Qun Zhang^{1*}

¹Department of Plant Pathology and MOA Key Laboratory of Pest Monitoring and Green Management, China Agricultural University, Beijing, China;

²State Key Laboratory of the Discovery and Development of Novel Pesticide, Shenyang Sinochem Agrochemicals R&D Co., Ltd, Shenyang, China.

³China-Norway Joint Lab on Fish Gut Microbiota, Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, PR China.

***Corresponding author:**

Li-Qun Zhang,

zhanglq@cau.edu.cn

†These authors have contributed equally to this work

Table S1. Primers used in this study.

Primer	Sequence (5'-3')	Description
<i>penAC</i> -F	ATGAATTCGGCAAGAAATCCATG GGCTCC	Amplifies <i>penAC</i> without the 5' signal peptide sequence.
<i>penAC</i> -R	ATGTCGACGCCCCTGACAGCGG GGGCTG	
Aave-3502sig-F	ATCATATGCCGCACGTTTCATTGG CATG	Amplifies the 5' signal peptide sequence of <i>Aave_3502</i> .
Aave-3502sig-R	ATGAATTCCCTGCCTGGGTCGAA TGCCGGA	
<i>penAC</i> -dF	ATGGCAAGAAATCCATGGGCTCC	<i>penAC</i> mutant detection.
<i>penAC</i> -dR	ATGCCCCTGACAGCGGGGGCTG	
<i>hrcC</i> -dF	GGATCTCGGTCTTCTTGACGAGG	<i>hrcC</i> mutant detection.
<i>hrcC</i> -dR	ATCAAGGGATTGAGCCGCGAC	

Table S2. The three equivalent structural and functional elements of class A β -lactamase.

Strain	Ambler class	Ser ⁷⁰ -X-X-Lys ⁷³	Ser ¹³⁰ -X-Asn ¹³²	Lys ²³⁴ -Thr/Ser-Gly
<i>Bacillus licheniformis</i> BlaP	A	---S T F K---	---S D N---	---K T G---
<i>Escherichia coli</i> pC15-1a TEM-1	A	---S T F K---	---S D N---	---K S G---
<i>Escherichia coli</i> HB101 SHV-1	A	---S T F K---	---S D N---	---K T G---
<i>Serratia marcescens</i> S6 SME-1	A	---S S F K---	---S D N---	---K T G---
<i>Acidovorax citrulli</i> PenAC	A	---S T I K---	---S D N---	---K T G---
<i>Bacillus subtilis</i> PenP	A	---S T Y K---	---S D N---	---K S G---
<i>Staphylococcus aureus</i> PC1	A	---S T S K---	---S D N---	---K S G---
<i>Pseudomonas aeruginosa</i> PAO1 AmpC	C	---S V S K---	---Q V F---	---L L N---
<i>Bacillus cereus</i> BcII	B	---Q L N K---	---F K K---	---C L V---
<i>Acinetobacter baumannii</i> OXA-58	D	---D G Q N---	---G E A---	---S G W---

Table S3. OD₆₀₀ indicating the minimal inhibitory concentration (MIC) of *A. citrulli*Δ*penAC*(pZAC-3502sig-*penAC*) treated with BCD derivatives.

BCD derivative	Concentration (μg/mL)									MIC (μg/mL)
	250	200	150	100	75	50	25	12.5	6.25	
BCD01	0.053	0.062	0.058	0.049	0.062	0.616	0.681	0.698	0.700	75
BCD02	0.079	0.099	0.054	0.088	0.085	0.099	0.641	0.660	0.667	50
BCD03	0.078	0.056	0.059	0.080	0.577	0.638	0.662	0.677	0.733	100
BCD04	0.089	0.058	0.091	0.095	0.063	0.628	0.683	0.769	0.723	75
BCD05	0.081	0.061	0.071	0.079	0.074	0.051	0.558	0.621	0.639	50
BCD06	0.032	0.033	0.035	0.039	0.049	0.058	0.509	0.588	0.614	50
BCD07	0.039	0.038	0.043	0.043	0.041	0.540	0.626	0.618	0.685	75
BCD08	0.055	0.058	0.062	0.040	0.043	0.051	0.598	0.656	0.639	50
BCD09	0.040	0.058	0.062	0.055	0.042	0.052	0.522	0.595	0.629	50
BCD10	0.071	0.069	0.054	0.057	0.557	0.593	0.652	0.690	0.689	100
BCD11	0.031	0.036	0.038	0.056	0.058	0.639	0.646	0.613	0.672	75
BCD12	0.089	0.062	0.079	0.078	0.581	0.609	0.640	0.643	0.688	100
BCD13	0.035	0.036	0.047	0.043	0.057	0.639	0.648	0.644	0.736	75
BCD14	0.092	0.094	0.092	0.047	0.054	0.349	0.636	0.703	0.754	75

BCD01-14 are annotated in Table 2 and Figure 2B.