

Cycloimidamicins, Novel Natural Lead Compounds for Translation Inhibition in *Pseudomonas aeruginosa*

Yoshimasa Ishizaki¹, Ryuichi Sawa², and Masayuki Igarashi¹

¹Laboratory of Microbiology and ²Laboratory of Molecular Structure Analysis, Institute of Microbial Chemistry (BIKAKEN)



Background: *Pseudomonas aeruginosa* – A Critical Priority Pathogen

Priority Pathogens Lists for R&D of New Antibiotics (Excerpt: Gram-negative bacteria only)

Pathogen	WHO (2017)	CDC (2019)	AMED (2021)
MDR <i>Acinetobacter</i>	Critical	Urgent (Carbapenem-R)	Priority1
MDR <i>Pseudomonas aeruginosa</i>	Critical	Serious (MDR)	Priority1
Carbapenem-R <i>Enterobacterales</i>	Critical	Urgent (Carbapenem-R)	Priority1
Ceph-R (ESBL+) <i>Enterobacterales</i>	Critical	Serious Ceph-R (ESBL+)	Priority1
Drug-R <i>Neisseria gonorrhoeae</i>	High	Urgent (drug-R)	Priority1

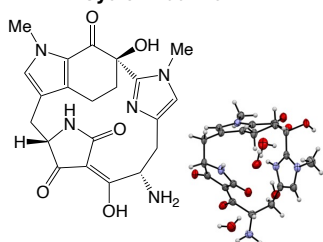
Data from AMED (<https://id3catalyst.jp/apid/en/list.html>)

Multidrug-resistant *Pseudomonas aeruginosa* (MDR-PA) has emerged as a **global priority pathogen** due to its **high resistance to antibiotics** and associated **high mortality rates**. The WHO and CDC identify MDR-PA as a critical and serious threat in their antimicrobial resistance (AMR) programs.

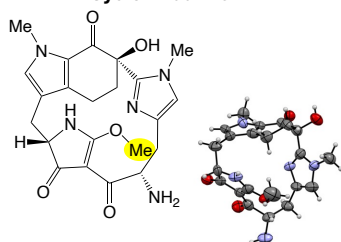
In **Japan**, MDR-PA infection has been a **notifiable disease**, underlining its recognized public health importance domestically as well.

Screening, Isolation, and Structure Determination of Cycloimidamicin A and B.

Cycloimidamicin A



Cycloimidamicin B



Cycloimidamicins **A** and **B** were isolated from the culture of a rare actinomycete strain, *Lentzea* sp. MM249-143F7, during a screening based on antibacterial activity against *P. aeruginosa*.

Their structures were determined by X-ray crystallography (crystal structure and absolute configuration) and ¹H/¹³C NMR spectroscopy (including 2D NMR for interatomic correlations). The absolute structures and ORTEP diagrams are shown on the left.

View Publication



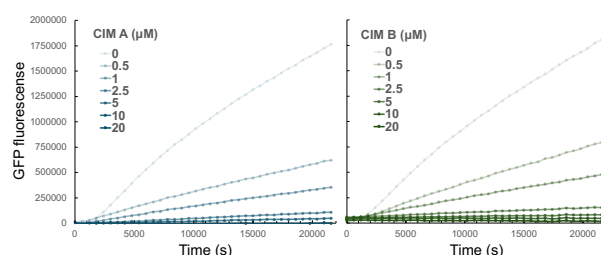
Biological activity of Cycloimidamicin A and B

Antibacterial activity of drugs against Gram-negative bacterial strains (microbroth dilution).

Test organisms	MIC (μg/ml)						
	CIM A	CIM B	DKB	MEPM	LVFX	CP	CL
<i>Pseudomonas aeruginosa</i> A3	64	8	0.25	1	0.063	8	4
<i>Escherichia coli</i> K-12	32	4	0.25	0.016	0.004	0.063	4
<i>Klebsiella pneumoniae</i> PCI 602	64	16	0.5	0.125	0.008	0.5	4

CIM A; cycloimidamicin A, CIM B; cycloimidamicin B, DKB; dibekacin, MEPM; meropenem, LVFX; levofloxacin, CP; chloramphenicol, CL; colistin.

Although Cycloimidamicins still have room for improvement in terms of antibacterial activity, they are relatively effective against *P. aeruginosa* compared with some other classes of antibiotics. In addition, Cycloimidamicins A and B are both shown to **effectively inhibit *E. coli* translation** in vitro in the same manner.

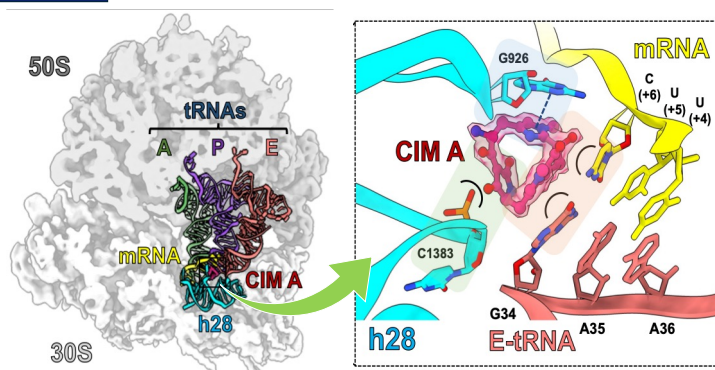


Effect of Cycloimidamicins A (CIM A) and B (CIM B) on GFP translation using an *E. coli* in vitro translation system.

Mode of inhibition of Cycloimidamicins A in bacterial translation

The mode of inhibition of Cycloimidamicin A (CIM A) on the *E. coli* ribosome was revealed by directly observing in vitro translation in the presence of CIM A, using cryo-electron microscopy. This novel approach is called **the DARC method**. In these observations, **CIM A interfered with actively translating ribosomes through interactions with rRNA (helix 28), mRNA, and tRNA**. Moreover, ribosomes were stalled at the same position on the mRNA, suggesting that **the effect of CIM A is highly sequence-specific**. We are currently analyzing the sequence-specificity of Cycloimidamicins and seeking to understand the biological significance of this unique inhibition mechanism.

This experiment is a collaboration with Drs. Takeshi Yokoyama at Hokkaido university and Yoshikazu Tanaka at Tohoku university.



Direct visualization of the CIM A binding site on the *E. coli* 70S ribosome.

Conclusion and perspectives

We introduced structurally unprecedented compounds, Cycloimidamicins A and B, isolated from the culture of an actinomycete strain. These compounds exhibit antibacterial activity against Gram-negative bacteria, including *P. aeruginosa*, through a unique mechanism of translation inhibition.

Our findings highlight that (1) actinomycetes remain a highly attractive source for drug screening in terms of structural novelty and biological activity, and (2) natural products continue to reveal new biology and contribute to the advancement of science and human health.

We will continue to explore nature for useful compounds that promote health and advance scientific discovery.