

# Progress on Compound 14 : A Potential *E. coli* Bam Inhibitor

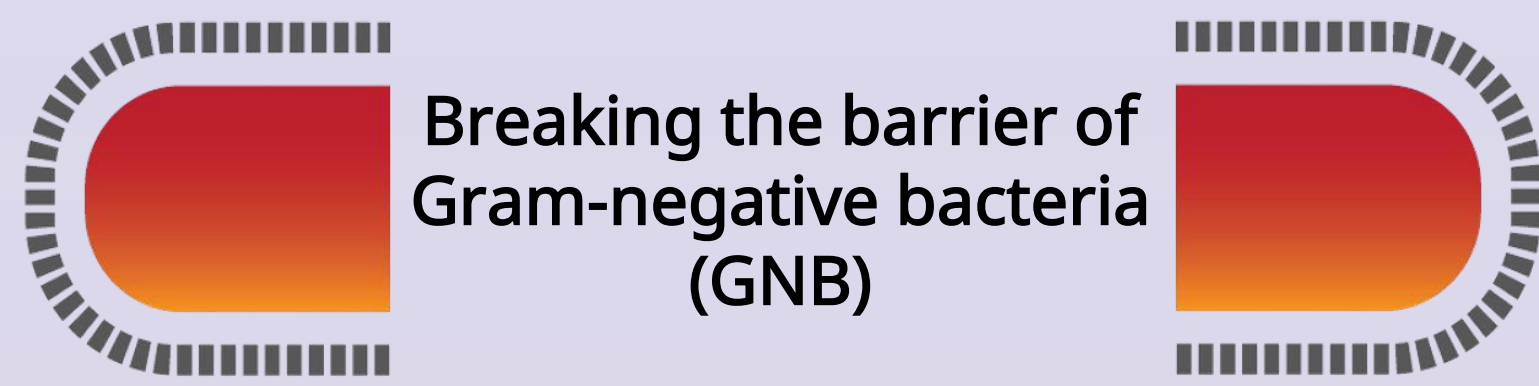
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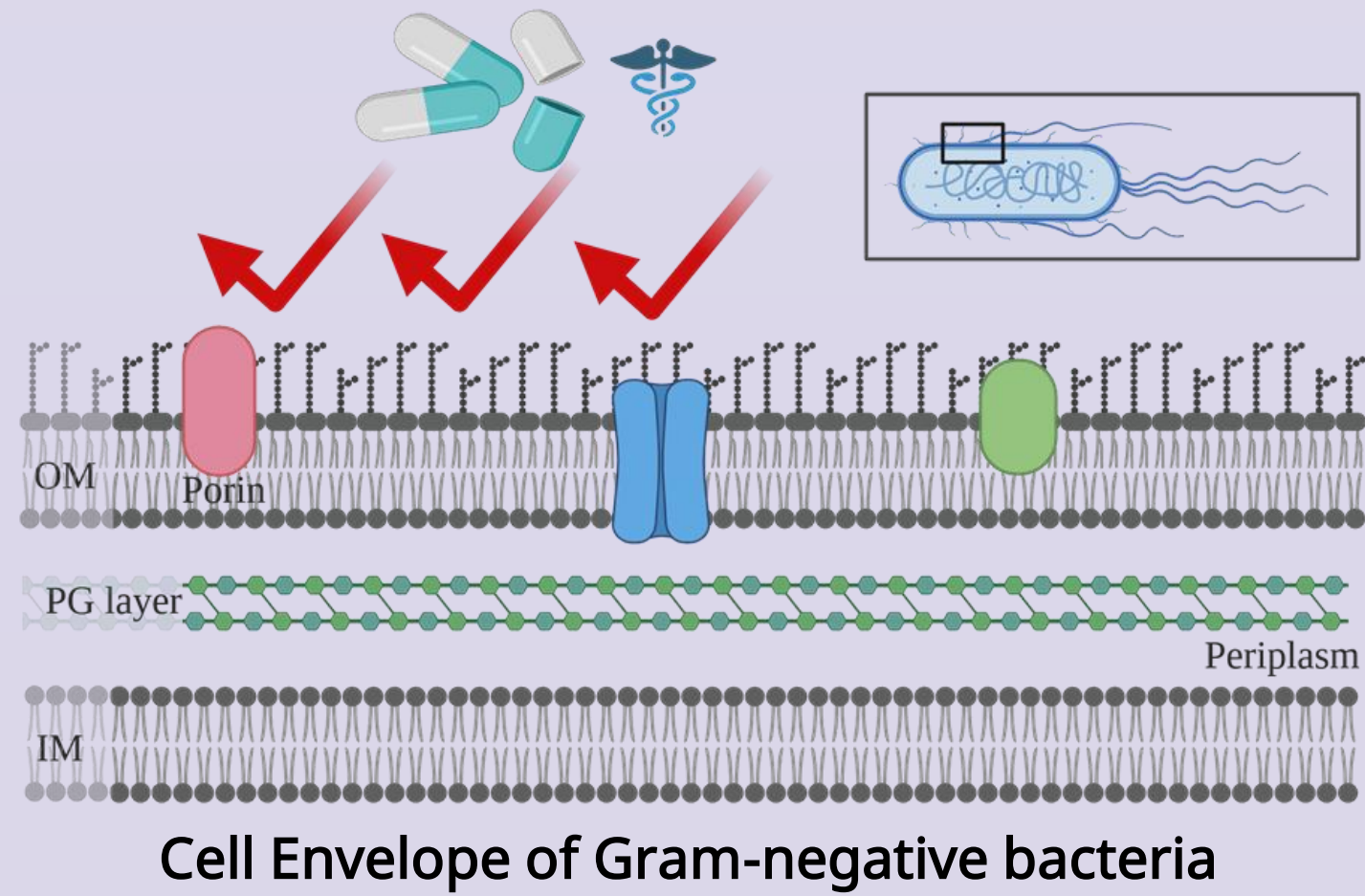
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## 1. BREAKthrough (EU ITN) Project

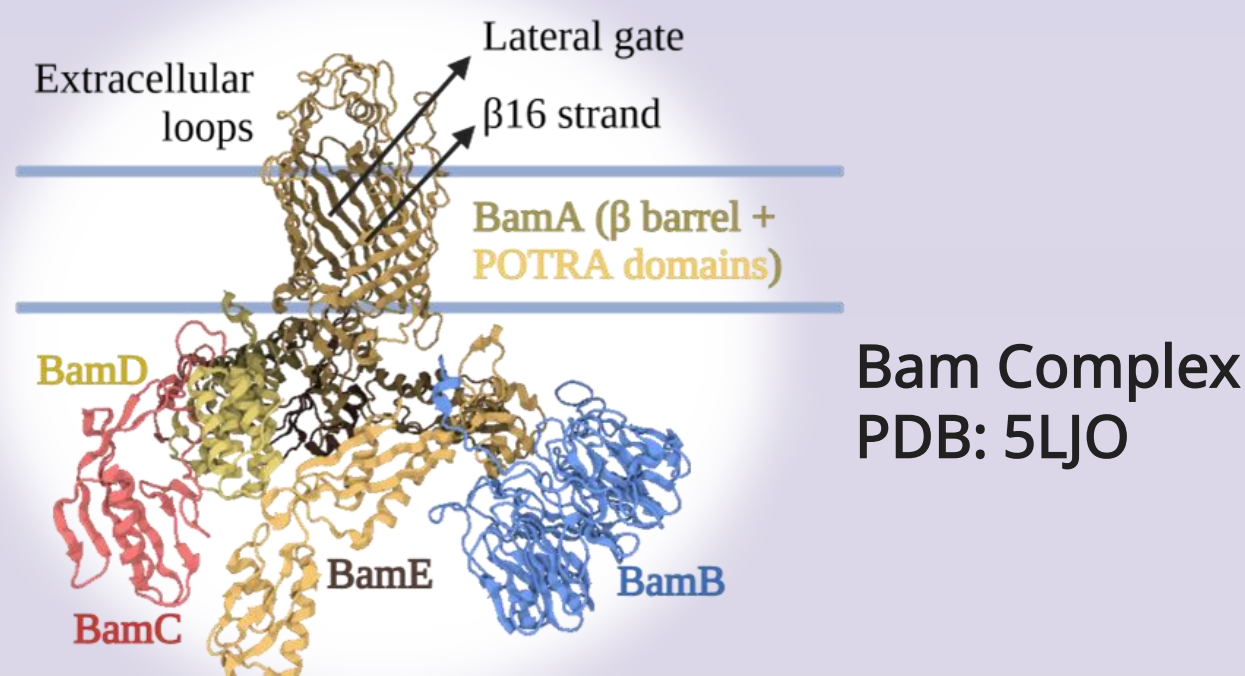


Breaking the barrier of Gram-negative bacteria (GNB)



Cell Envelope of Gram-negative bacteria

- Asymmetric outer membrane (OM) prevents large or hydrophobic compounds (cpds) from entering the cell
- Aims to permeabilize the OM using new/existing cpds & inhibit essential OM protein machineries like Bam complex

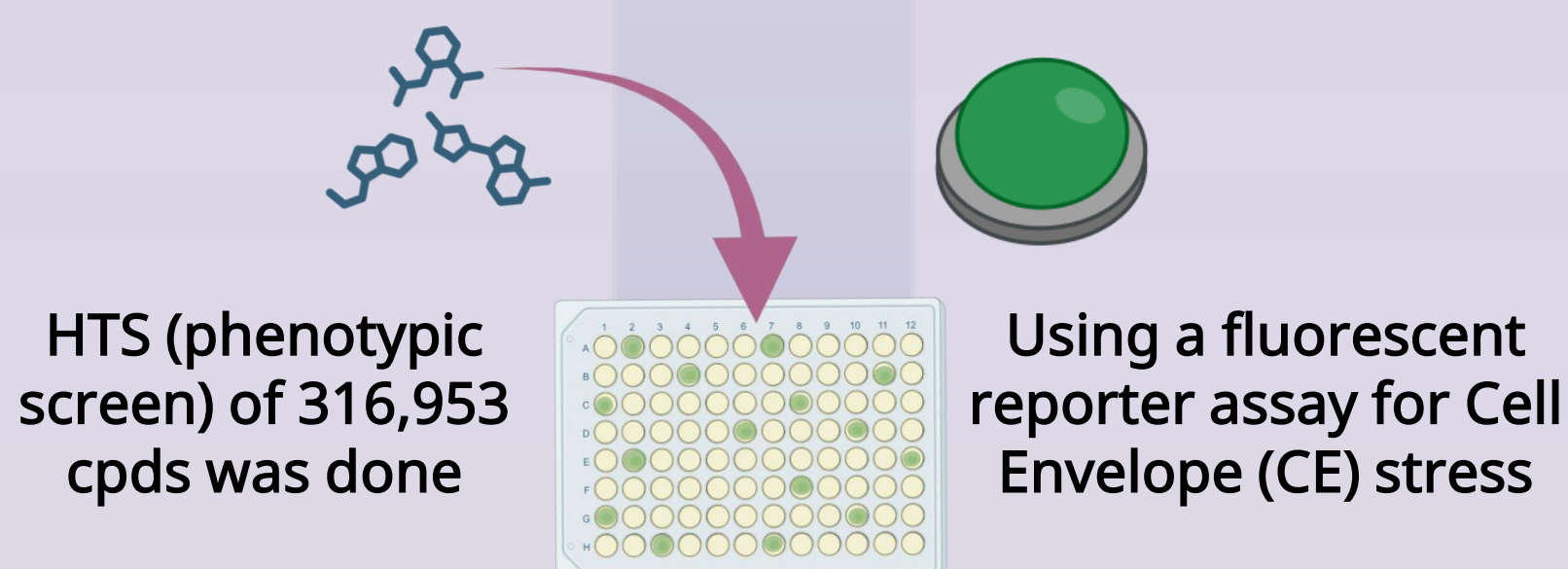


Bam Complex PDB: 5LJO

- Bam Complex comprising BamA (core subunit) is essential machinery for  $\beta$ -barrel assembly and OM integrity. BamA is surface exposed, essential, highly conserved in GNB and has no close human homologue

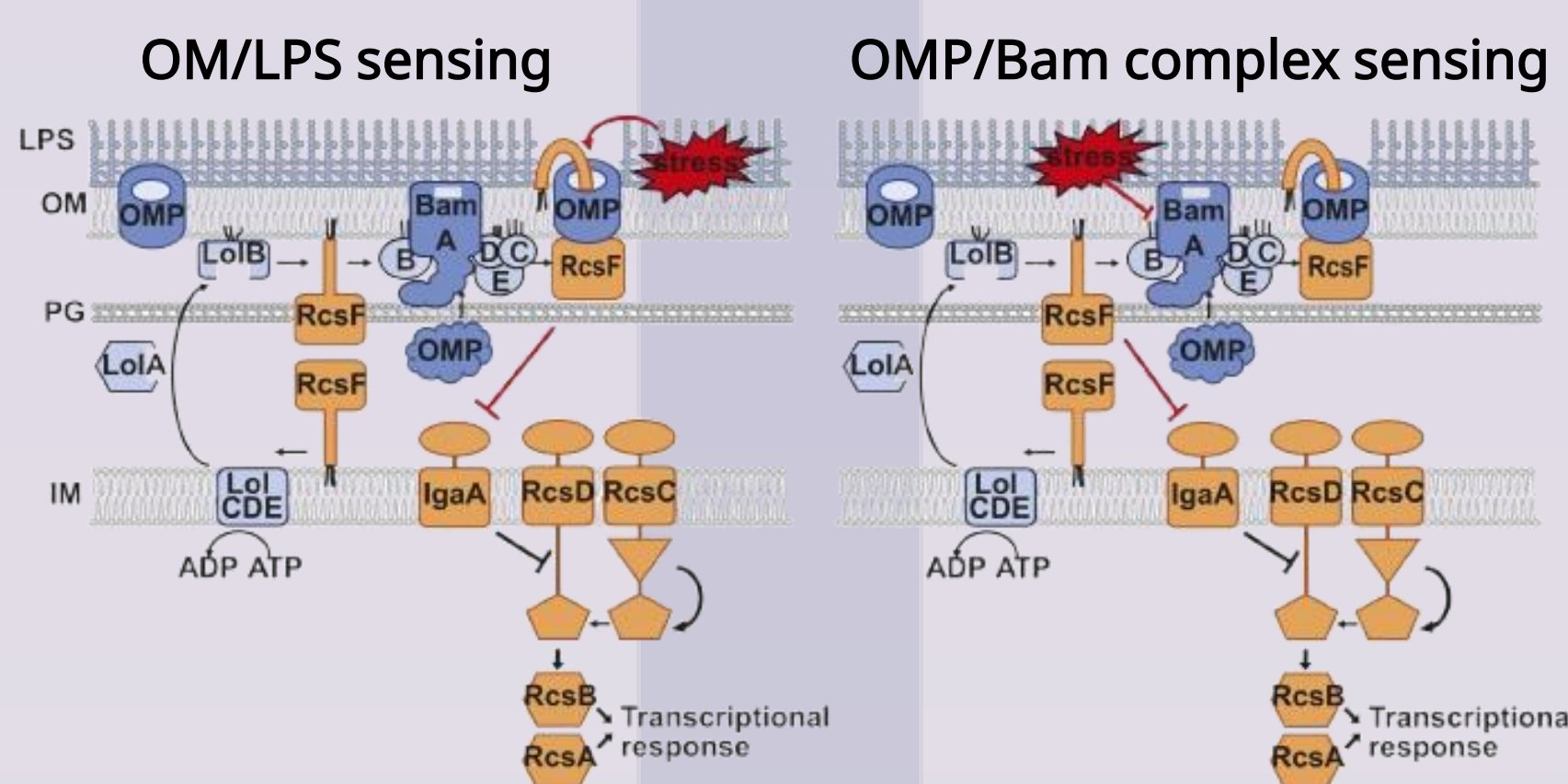
## 2. Background

### A. High Throughput Screen (HTS)<sup>1</sup>



### B. Two hit cpds including CPD 14 interfered with Bam activity

- Hit cpds were re-assayed for induction of Rcs OM stress
- Rcs stress - activated upon defects in LPS, Bam complex, peptidoglycan etc<sup>2</sup>



- Effect of BamA depletion and defects in OMP biogenesis ( $\Delta$ bamB,  $\Delta$ degP,  $\Delta$ surA) were more pronounced in presence of CPD 14

- Inhibition of autotransporter secretion *via* Bam complex
- Inhibition of OMP folding in proteoliposome reconstituted Bam complex

## 3. Goal : Improve CPD 14

- Lead CPD 14 has relatively high MIC (minimal inhibitory concentration) value. Precipitates at high concentrations

## 4. Results

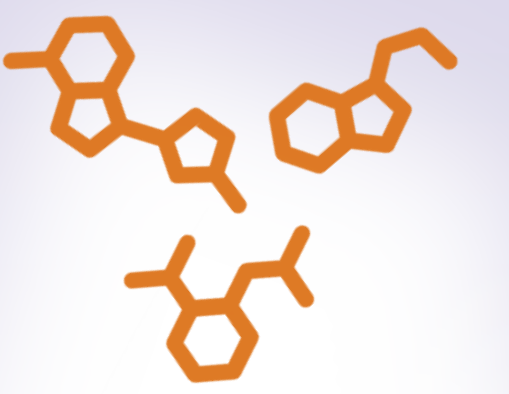
### A. Several CPD 14 derivatives outperformed lead CPD 14

Cpds	Antimicrobial Activity ( $\mu$ g/mL)									Fold change Rcs stress	Membrane Toxicity ( $\mu$ M)
	<i>E. coli</i> 25922	<i>A. baumannii</i> 19606	<i>P. aeruginosa</i> 27853	<i>K. pneumoniae</i> 13883	<i>S. aureus</i> 29213	<i>E. faecium</i> 19434	<i>E. faecalis</i> 29212	<i>E. coli</i> Top10F <sup>+</sup>	<i>B. subtilis</i> 168		
14-8	128	128	$\geq 128$	$\geq 128$	128	128	128	25	100	6	-
14-9	16	32	32	64	32	32	32	25	25	7	-
14-10	32	64	32	$\geq 128$	128	128	$\geq 128$	12.5	100	5	-
14-11	16	32	32	32	16	16	32	25	25	6	-
14-12	16	32	16	32	16	16	16	25	25	6.4	-
14-14	$\geq 128$	$\geq 128$	$\geq 128$	$\geq 128$	$\geq 128$	$\geq 128$	$\geq 128$	$> 200$	$> 200$	0	-
14-16	64	128	128	$\geq 128$	128	128	128	25	100	5.3	-
14-17	32	64	64	$\geq 128$	128	128	128	25	100	6.7	-
14-18	32	64	64	64	32	32	64	25	50	4.4	-
14-19	$\geq 128$	$\geq 128$	$\geq 128$	$\geq 128$	$\geq 128$	$\geq 128$	$\geq 128$	$> 200$	$> 200$	0	-
CPD14	64	128	64	512	256	128	128	25	200	7.5	-
14-20	$\geq 512$	256	512	$\geq 512$	$\geq 512$	$\geq 512$	$\geq 512$	$> 200$	$> 200$	0	-
14-25	256	256	128	512	256	128	32	100	400	16.3	-
14-28	32	128	256	512	128	128	128	25	$> 25$	7.2	-
14-29	16	64	64	32	32	32	32	12.5	25	15.6	-
14-30	2	8	128	8	8	8	8	12.5	12.5	3.7	-
14-32	32	128	128	128	64	64	64	12.5	25	9.9	Toxic at 100
14-33	32	128	128	128	64	64	64	6.25	12.5	10.7	Toxic at 100
14-35	16	32	64	32	32	32	32	200	200	9.6	-
14-36	64	128	256	128	128	128	128	25	100	6	-
14-37	8	64	32	32	32	16	32	25	25	7.8	-
14-38	4	8	32	16	8	8	8	12.5	6.25	5.13	-
14-40	4	8	32	8	8	8	8	12.5	6.25	15.8	Toxic at 100
14-41	4	8	16	8	8	8	8	6.25	6.25	3.2	-
14-43	8	32	128	256	128	32	32	25	100	6.5	-
14-44	64	256	256	256	256	256	256	25	100	2.98	-
14-45	64	256	512	256	256	256	256	12.5	25	8.9	-
14-46	512	256	512	$\geq 512$	$\geq 512$	$\geq 512$	512	$> 200$	$> 200$	0	-

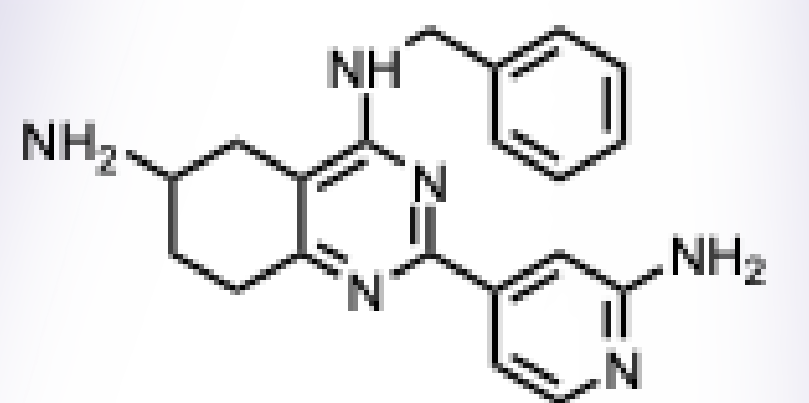
- Gram-negative
- Gram-positive
- Lead CPD 14
- Selected Derivatives

#### Selection Criteria

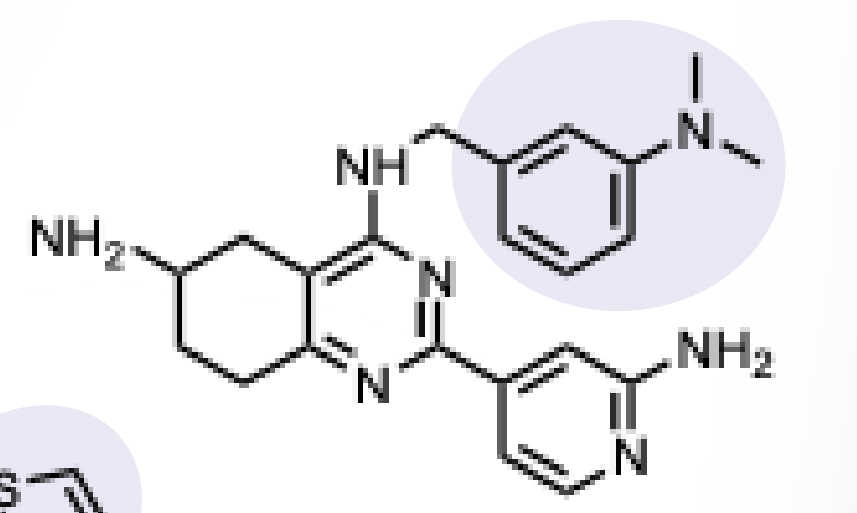
- Lower MIC & High OM Rcs stress than lead CPD 14
- Specific to GNB
- Not membrane toxic



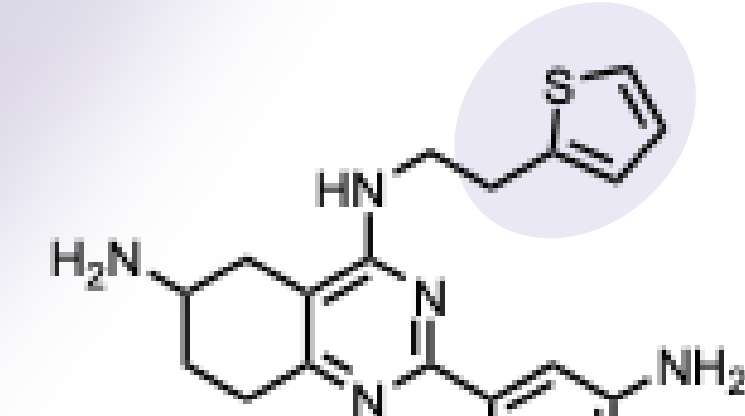
Original CPD 14



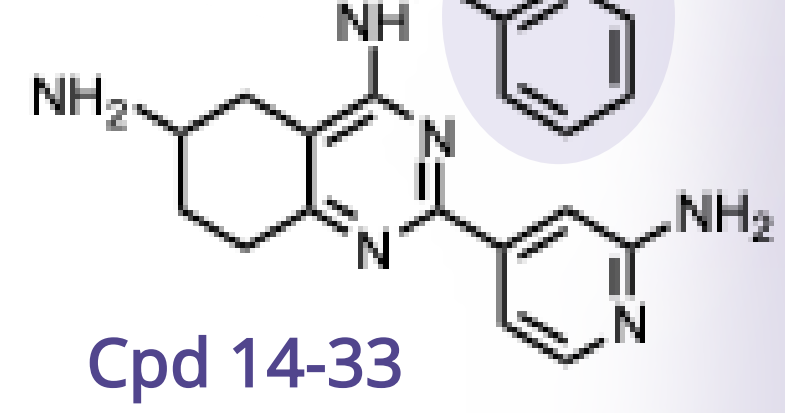
Selected Derivatives CPD 14



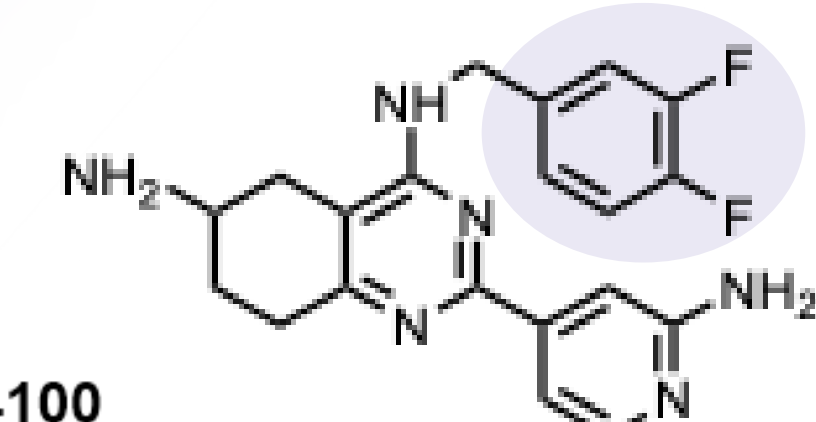
Cpd 14-43



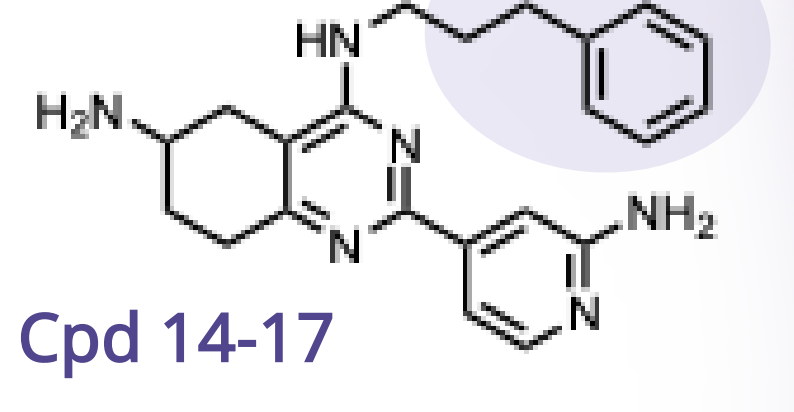
Cpd 14-28



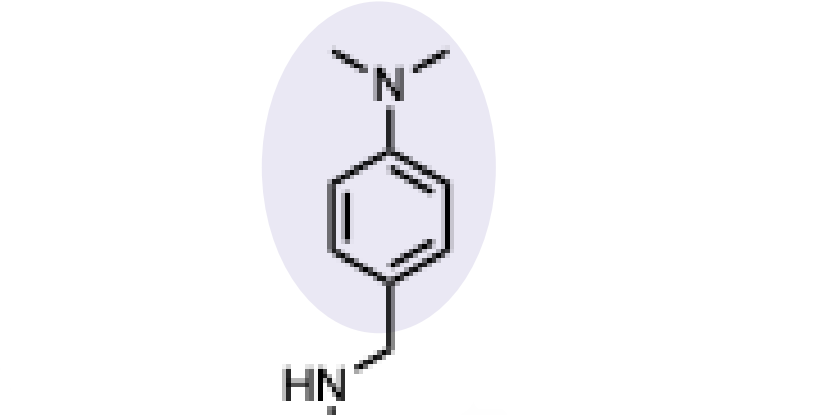
Cpd 14-33



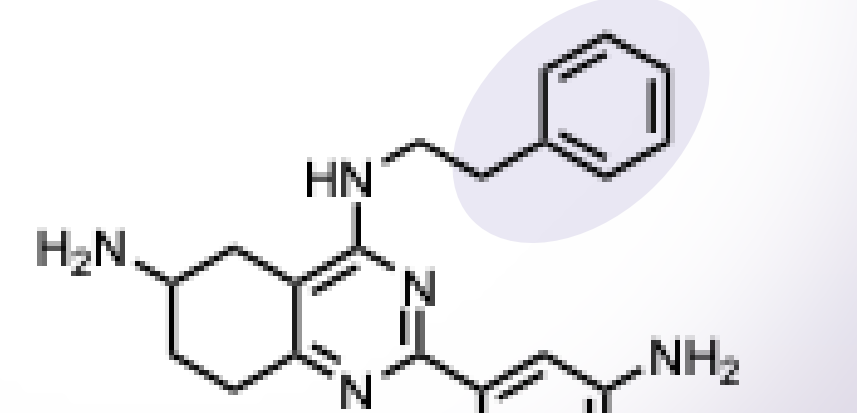
Cpd 14-32



Cpd 14-17

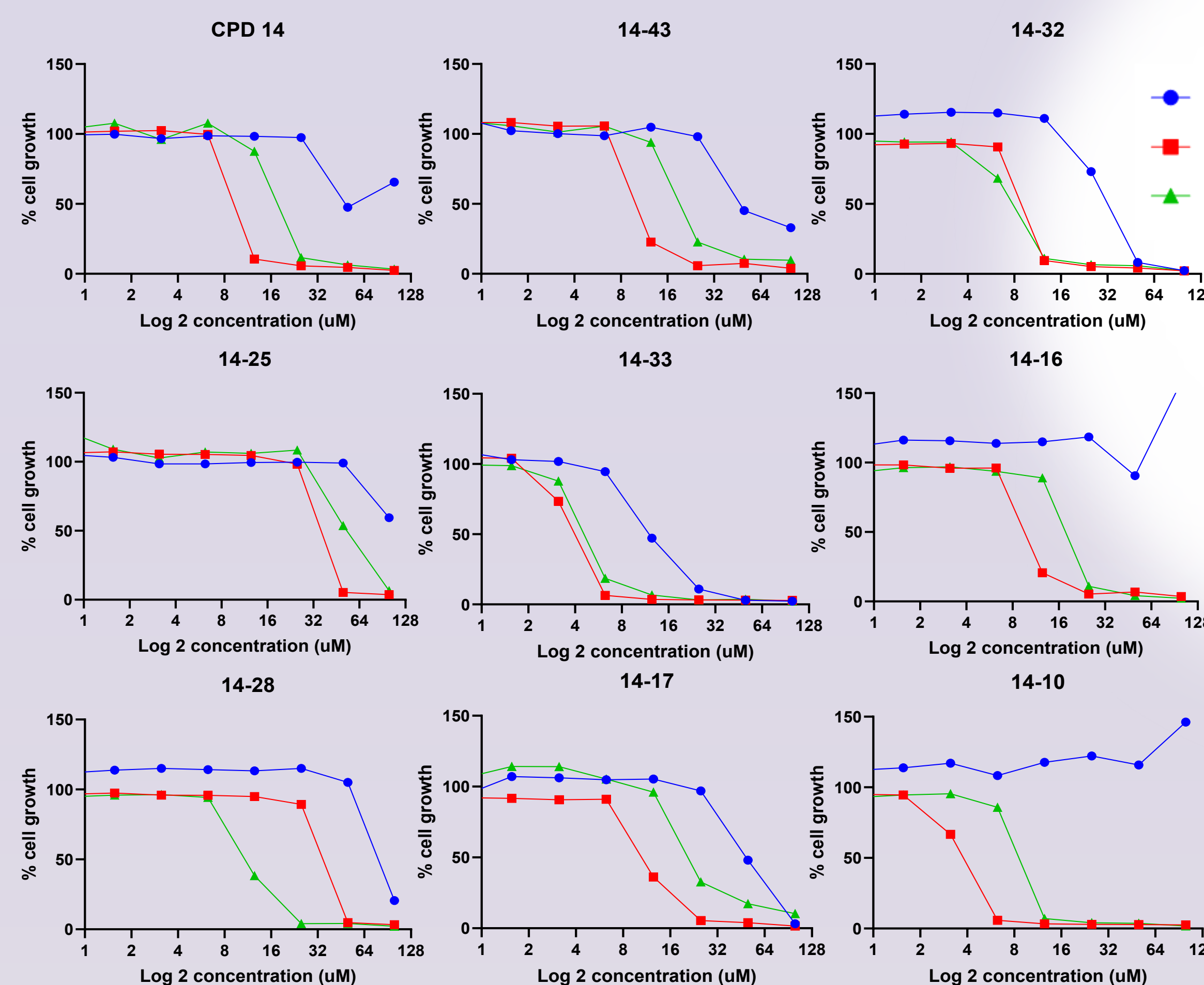


Cpd 14-10



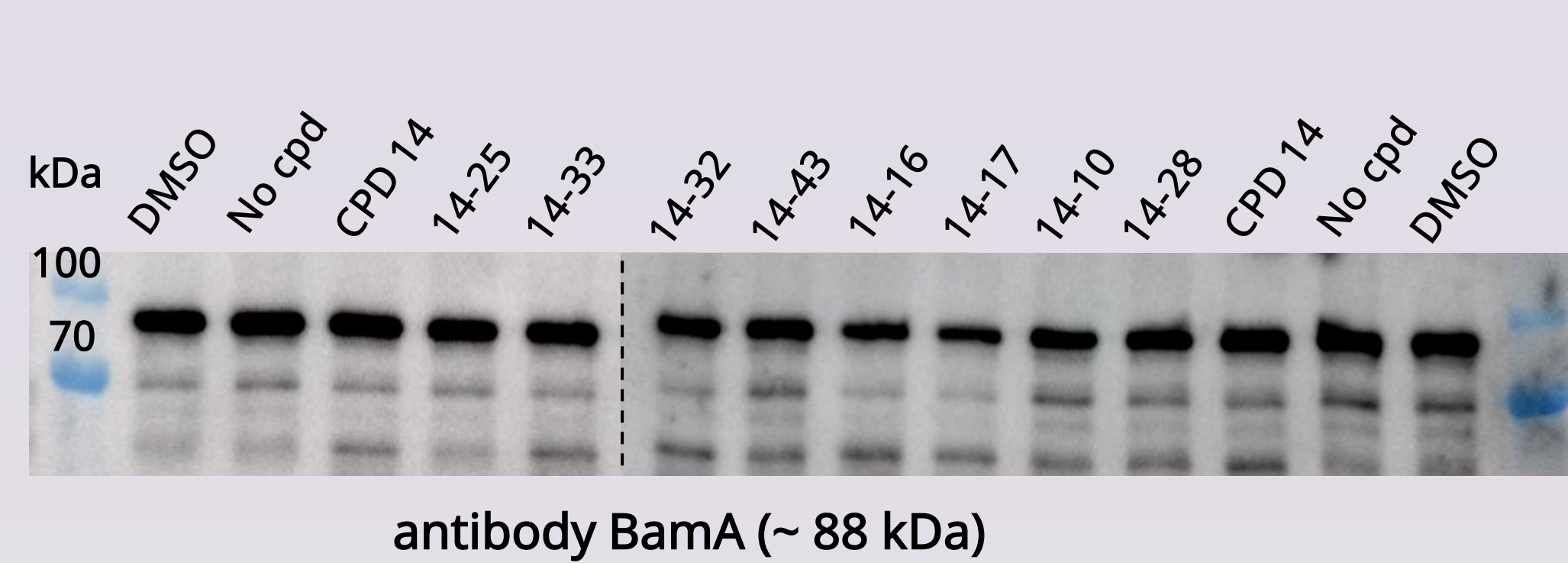
Cpd 14-16

### B. BamA depleted and $\Delta$ BamB strains exhibited enhanced sensitivity in the presence of selected derivatives

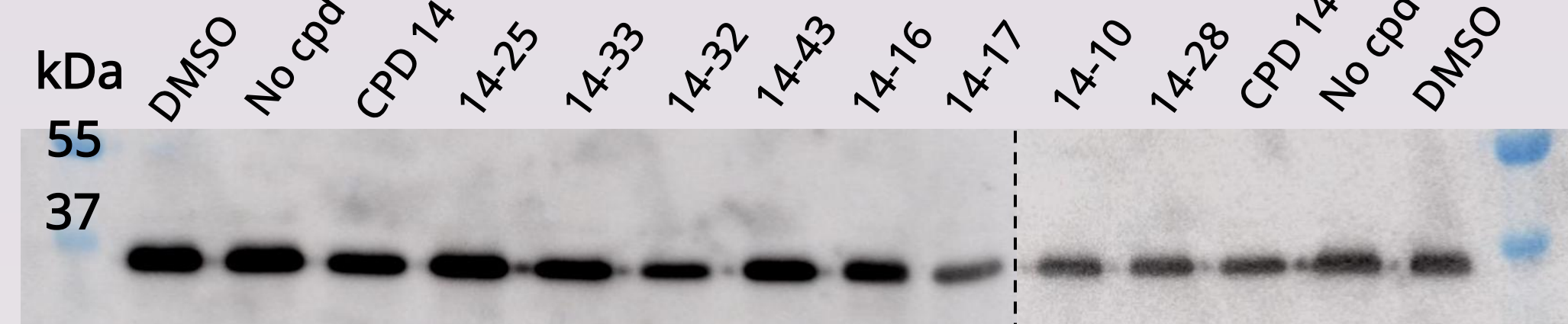


### D. Three derivatives lower porin levels at 0.5x $EC_{50}$ \* value

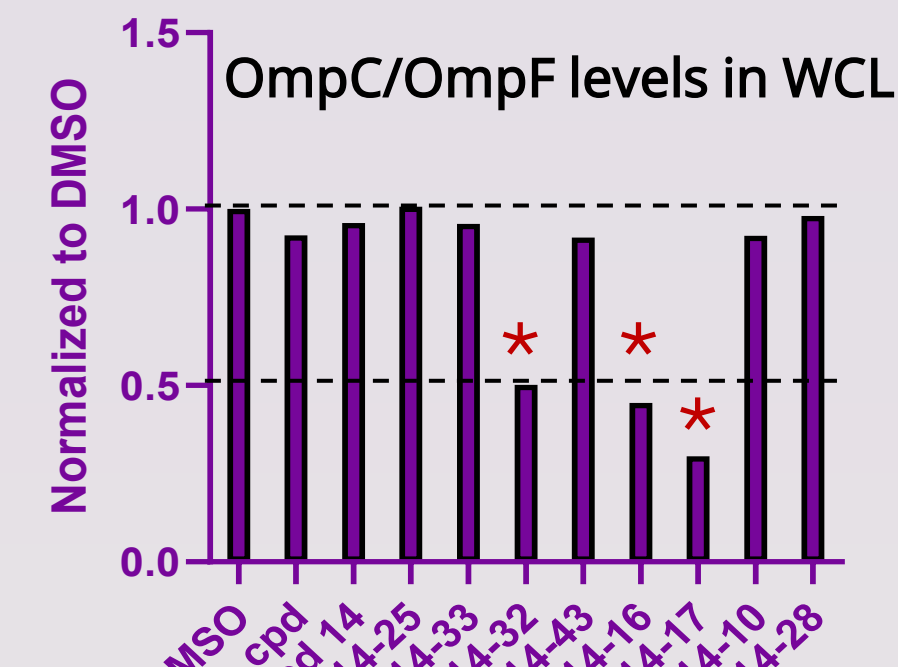
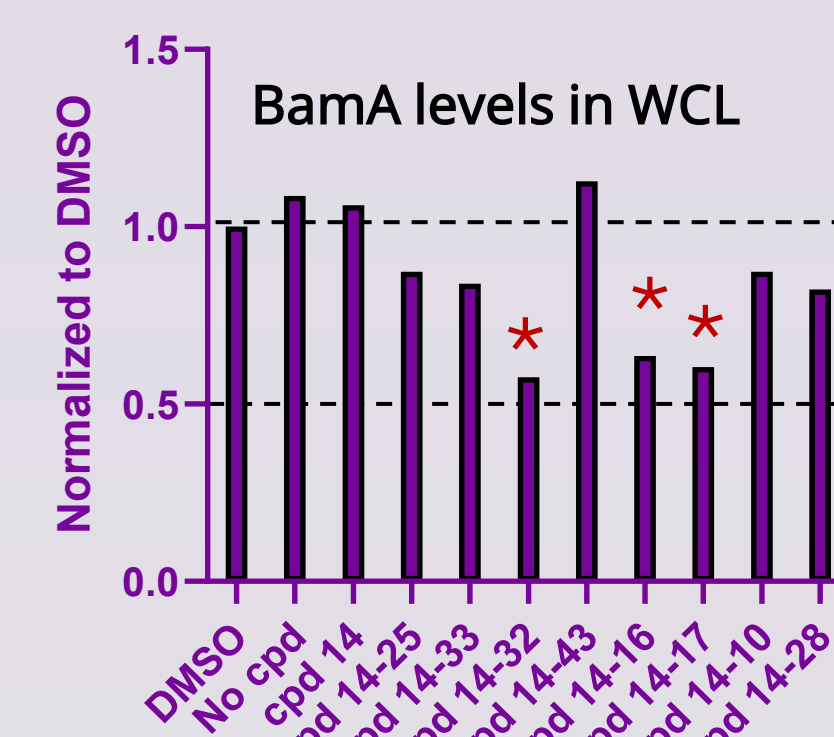
\*  $EC_{50}$  is the concentration of the cpd that induces activity halfway between baseline and maximum possible effect



antibody BamA (~ 88 kDa)



antibody OmpC/OmpF (~ 37 kDa)



## 5. Conclusion

- Derivatives of CPD 14 were made that have improved properties (lower MIC, high OM stress, specific to GNB and not showing generic membrane toxicity) than the original CPD 14

- The improved derivatives probably act on Bam complex in a similar way as the original CPD 14 (enhanced activity in BamA depletion and  $\Delta$ BamB strain and lower porin levels in the OM)

- The modified handle seems to play a role in the inhibitory function

## 7. References

- Steenhuis M, Luirink J. Combining Cell Envelope Stress Reporter Assays in a Screening Approach to Identify BAM Complex Inhibitors. *ACS Infect Dis* (2021)
- M. Tata, A. Konovalova, High-throughput suppressor screen demonstrates that RcsF monitors outer membrane integrity and not Bam complex function *PNAS* (2021).

## Contact Me !

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