Synthesis and Assessment of Prodrug Activity Against Gram-Positive Bacteria

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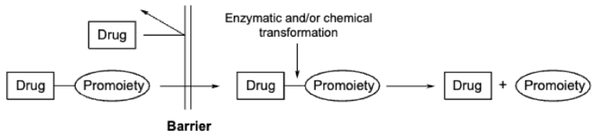
Background

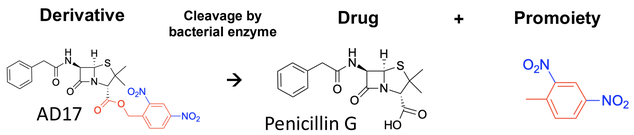
As antibiotic resistance continues to pose a significant threat, the development of new antibiotics is more important than ever. However, one barrier in transforming an active parent compound into a viable pharmaceutical drug is optimization of the drug's pharmacokinetic properties, including stability, permeability, or targeted delivery to pathogen. Effective prodrug modification can accomplish this goal. The goal of our project is to categorize novel prodrug structures for addition to new antibiotics with difficult properties. Penicillin G was used as the base compound. Penicillin G is a well-known antibiotic with a single acidic site, providing a convenient parent compound for synthesis and well-established MIC values for testing. We determined that, consistent with hypothesis, prodrug structures with increasing enzymatic triggers increased antibiotic efficacy.

Prodrugs

Prodrugs are inactive or chemically modified derivatives of a parent compound that are converted to their active form *in vivo*, usually through enzymatic cleavage.



Rautio, J. (2010). Prodrug Strategies in Drug Design. In Prodrugs and Targeted Delivery



Minimum Inhibitory Concentration (MIC) of Compounds in *B. anthracis* Sterne

Compound	MIC (μM)
PCN	240
EK36	>240
ER30	>240
EK38	>240
EK40	>240
AD21	>240
AD62	>240
EK35	120
EK30	60
AD99	60
AD11	60
AD125	60
ER26	30
AD53	30
AD68	30

EK, ER –earlier compound synthesis

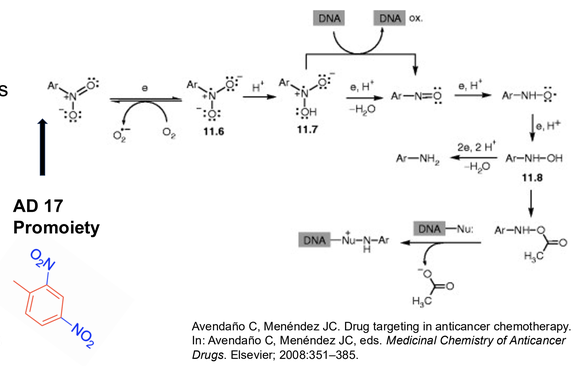
AD—later compound synthesis

AD104	30
AD126	30
ER25	15
EK31	15
ER31	15
AD74	15
AD101	15
AD103	15
AD110	15
ER29	7.5
AD16	7.5
AD17	7.5
AD23	7.5
AD75	7.5

Hypothesis

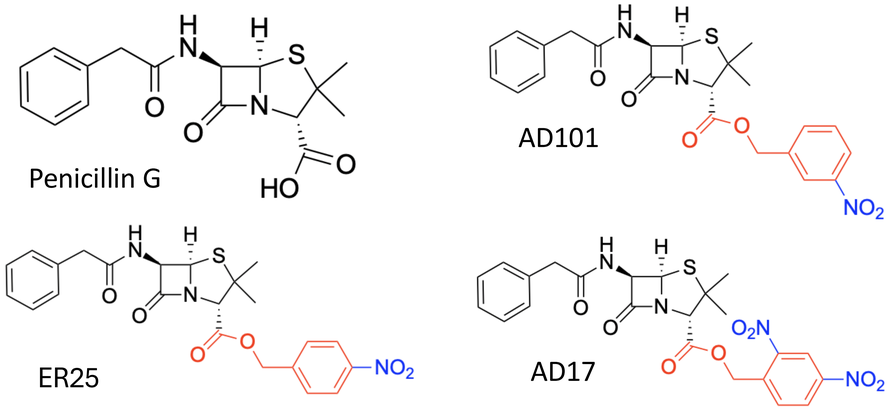
Mechanism for "Triggers" for Enzymatic Activation

- We hypothesize that increasing number of enzymatic "triggers" such as $–NO_2$ or –Ph, provide multiple sites for bacterial enzymatic cleavage by enzymes such as nitroreductases or esterases.
- Additionally, these prodrugs increase compounds lipophilicity and may increase permeability through the bacterial cell membrane prior to bacterial cleavage and activation.
- Figure shows AD17's prodrug cleavage and activation of drug via nitroreductase enzymes



Avendaño C, Menéndez JC. Drug targeting in anticancer chemotherapy. In: Avendaño C, Menéndez JC, eds. *Medicinal Chemistry of Anticancer Drugs*. Elsevier; 2008:351–385.

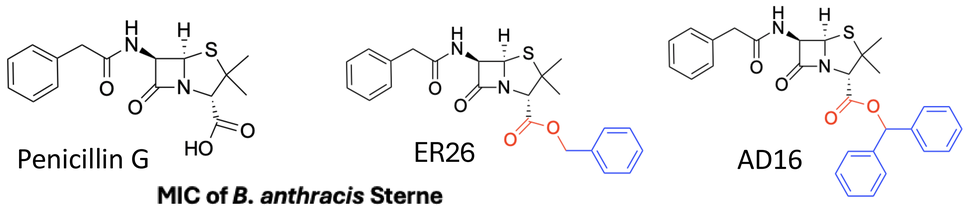
Nitro-substituted Benzyl Ester Series



MIC of *B. anthracis* Sterne

Compound	MIC (μM)	Ester Promoiety
Penicillin G	240	--
ER25	15	p-nitrobenzyl ester (one nitro group)
AD101	15	m-nitrobenzyl (one nitro group)
AD17	7.5	dinitrobenzyl ester (2 nitro groups)

Benzylic Ester Series



MIC of *B. anthracis* Sterne

Compound	MIC (μM)	Ester promoiety
Penicillin G	240	--
ER26	30	benzyl (1 phenyl ring)
AD16	15	benzhydryl (2 phenyl rings)

Data in Other Gram-positive Bacteria

Preliminary data of select compounds tested in *Bacillus cereus* showed similar trends to the closely related *B. anthracis:*

MIC of *B. cereus*

Compound	MIC (μM)
Penicillin G	500
AD17	120

However, data in vancomycin-resistant Enterococcus faecalis showed variation from the trend observed in *Bacillus:*

MIC of vancomycin-resistant *E. faecalis*

Compound	MIC (μM)
Penicillin G	7.5
AD17	>30
AD101	7.5

This may be due to VRE's already higher susceptibility to Penicillin G, or due to other strain specific differences, possibly in bacterial enzymes.

Conclusions

In *B. anthracis* Sterne, the penicillin prodrug moieties with increasing enzymatic triggers increased efficacy of the parent compound, Penicillin G. This same trend was not observed in *E. faecalis*, potentially due to increased penicillin susceptibility or strain specific differences in bacterial enzymes.

Future Directions

- Resynthesize hydrolyzed compounds and complete testing of these series in *B. cereus*, *E. faecalis*, and other gram-positive bacteria
- Synthesize new prodrugs with varying "triggers" to see if our trend holds
- Identify successful prodrugs for synthesis of novel antibiotics

Acknowledgments

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