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COMMUNICATION

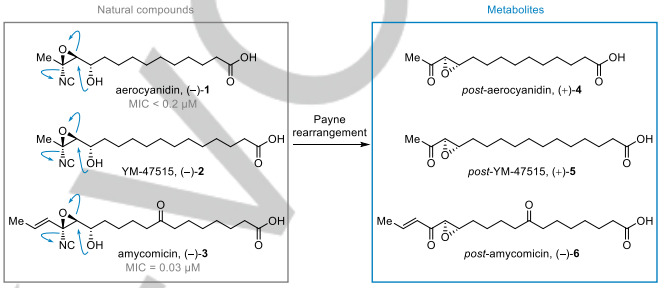
Epoxy isonitriles, a unique class of antibiotics – Synthesis of their metabolites and biological investigations

Guillaume Ernouf,[a] Ingrid K. Wilt,[a] Sara Zahim,[a] and William M. Wuest*[a][b]

Abstract: Epoxy isonitrile containing natural products often possess specific and potent antibacterial activity against Gram-positive pathogens. This scaffold, however, is extremely labile under acidic and basic conditions, undergoing a Payne rearrangement to produce a stable epoxy ketone metabolite and releasing hydrogen cyanide. We synthesized and performed biological assays with epoxy ketone containing metabolites and identified that the epoxy isonitrile moiety is pertinent for biological activity. Serendipitously, we discovered an α,β-unsaturated epoxy ketone analogue that exhibited moderate activity against *S. aureus*.

Natural products play an important role in the pharmaceutical industry, especially for antimicrobial drug discovery.[1] Due to the widespread resistance of antibiotics,[2] there is an urgent need for new and improved antimicrobials against multi-resistant bacteria. Selective or narrow-spectrum antibiotics may act as a means of combating emerging bacterial resistance. Ideally, these treatments would only target pathogenic bacteria and allow for the commensal bacteria to survive, which would both aid a host in fighting off infection and prevent rapid evolution of resistance. Despite their highly potent and selective antimicrobial activity, epoxy isonitrile natural products have attracted the attention of few synthetic chemists and pharmacologists, mainly due to their complex frameworks. Isolated by Sykes *et al.* in 1987, the epoxy isonitrile containing aerocyanidin[3] exhibits a highly potent activity against Gram-positive pathogens. A decade later, an elongated analogue of aerocyanidin, YM-47515,[4] was isolated, and very recently Clardy *et al.*, identified amycomicin through bacterial co-culturing with *Streptomyces* 84 *coelicolor* M145, which displays a very selective profile for *S. aureus*.[5]

In all cases, the epoxy isonitrile moiety proves to be labile to mildly basic and acidic conditions causing considerable difficulty during the isolation and purification process. Exposure to pH less than 4 and greater than 8 triggers a Payne rearrangement of the natural compound to produce a stable epoxy ketone metabolite (Scheme 1). Due to the lability of the epoxy isonitrile moiety, synthesis of such scaffolds are particularly challenging and examples are scarce in the literature.[6] Despite the successful synthesis of cyclic epoxy isonitrile trichoviridin in 1996,[7] attempts by Baldwin *et al.* to produce aerocyanidin were fruitless, resulting in only the desepoxy form of the natural product.[8]



Scheme 1. Under mildly basic or acidic conditions, epoxy isonitrile scaffolds of aerocyanidin, YM-47515, and amycomicin undergo a Payne rearrangement to produce stable epoxy ketone metabolites. MICs reported for *S. aureus*.[3,5]

The specific bioactivity exhibited by this class of natural products stimulated our desire to better understand its mode of action. While these potent antibacterial agents exhibit tremendous activity and selectivity against *S. aureus* (MIC < 0.05 μM), the instability of the epoxy isonitrile scaffold and release of toxic hydrogen cyanide upon exposure to mildly acidic or basic conditions render it an unusable antibiotic.[3,9] Upon comparison to inorganic sodium cyanide, however, aerocyanidin was shown to be much more potent,[3] leading us to believe it might be acting as a prodrug. If the epoxy isonitrile is acting as a prodrug, the epoxy ketone metabolite resulting from the Payne rearrangement would therefore retain activity and simultaneously decrease its toxicity associated with the release of hydrogen cyanide.

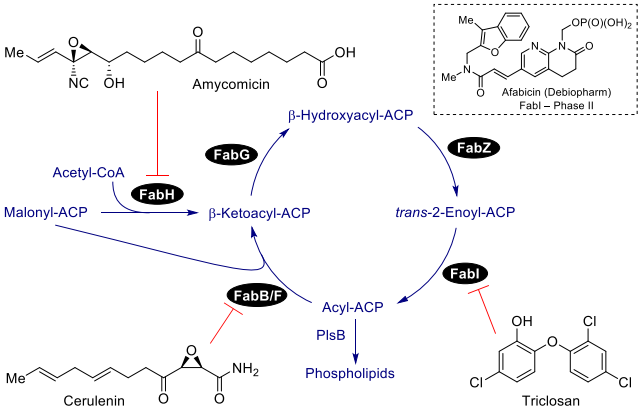


Figure 1. Known inhibitors of fatty acid biosynthesis.

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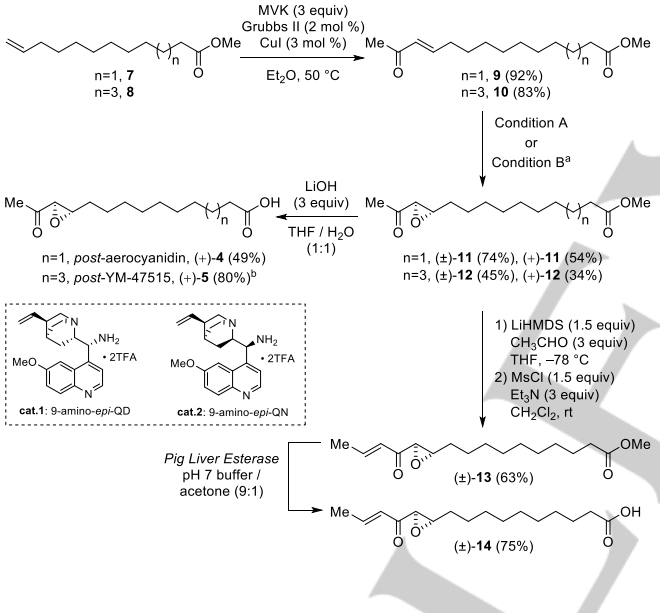
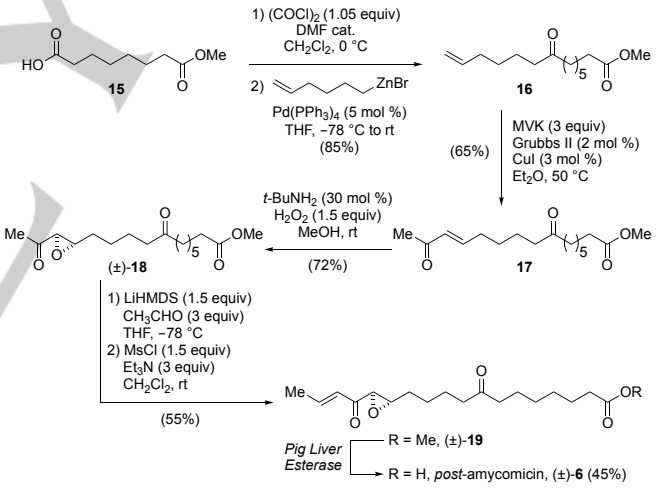
Additionally Clardy *et al.* recently demonstrated that amycomicin inhibits fatty acid biosynthesis through inhibition of FabH.[5] The chemotype similarities between epoxy ketones (+)-**4**, (+)-**5**, (–)-**6** and cerulenin, a known FabB/F inhibitor, led us to hypothesize that epoxy isonitrile metabolites may be targeting β-ketoacyl-ACP synthases through a covalent adduct mechanism akin to cerulenin.[16] Indeed lipid biosynthesis is a target of interest for antibacterial agents as metabolism of fatty acids is often unique to each species of bacteria.[17] Despite controversial interest of targeting such a biological pathway,[18] several natural products including cerulenin and phomallenic acid C[19] are encountered in the literature. Other synthetic inhibitors are also currently in development to fight MRSA infections including CG400549[20] and Afabicin[21] (FabI) confirming the potential of this pathway for developing narrow-spectrum antibiotics. (Figure 1).[18]

Motivated by the potential of this approach, we began synthetic investigations of epoxy ketone metabolites of isonitrile containing natural products. We sought to determine if the epoxy isonitrile scaffold was acting as a prodrug, focusing on aerocyanidin and its elongated analogue as well as amycomicin, with the goal of creating new, potent, selective, and less toxic antibiotics.



Scheme 2. Synthesis of *post*-aerocyanidin, *post*-YM-47515, and *post*-amycomicin prototype. [a] Condition A: Alkene (1 equiv), H_2O_2 in H_2O 30% (w/w) (1.5 equiv), *t*-$BuNH_2$ (30 mol %), MeOH, rt, 16 h; Condition B: Alkene (1 equiv), H_2O_2 in H_2O 30% (w/w) (1.5 equiv), cat.1 (30 mol %), dioxane, 50 °C, 16 h. [b] (–)-11 was obtained using the same conditions with cat.2 (55%); [b] PLE was used since LiOH hydrolysis gave lower yield.

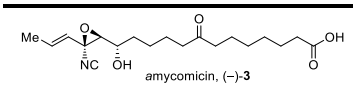
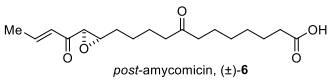
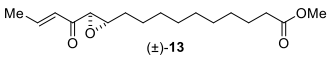
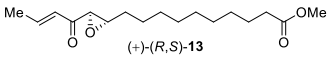
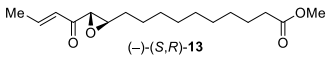
The synthesis of *post*-aerocyanidin (+)-**4** and *post*-YM-47515 (+)-**5** commenced from terminal alkenes **7** and **8** respectively prepared from commercially available 10-undecenoic acid[10] and 11-bromo-undecan-1-ol[11] in six steps. Subsequent cross-metathesis with the methyl vinyl ketone (MVK) afforded the α,β-unsaturated ketones **9** and **10**.[12] Organocatalyzed asymmetric epoxidation[13] of the α,β-unsaturated ketones **9** and **10** in the presence of cinchona primary amine catalyst yielded optically enriched epoxides (+)-**11** and (+)-**12** in moderate yields. From there, *post*-aerocyanidin (+)-**4** and *post*-YM-47515 (+)-**5** were obtained by rapid treatment with lithium hydroxide (THF:H_2O (1/1), rt, 30 min). It is worth noting that the utilization of sodium hydroxide led to slower reaction time and partial degradation of the epoxy ketone. To test the feasibility of our approach to access *post*-amycomicin (–)-**6**, we envisaged transforming the methyl ketone (±)-**11** to the enone (±)-**14**. After aldolization of (±)-**11** and acetaldehyde (LiHMDS, THF, –78 °C), the resulting diastereoisomeric mixture of alcohols (1:1) was treated with methanesulfonyl chloride in CH_2Cl_2 in the presence of Et_3N to afford the enone (±)-**13** in 85% yield over two steps containing the α,β-unsaturated ketone epoxy moiety found in amycomicin. Attempts to deprotect methyl ester (±)-**13** with hydroxide sources were unfruitful and lead to complete degradation of the substrate. However, hydrolysis in the presence of pig liver esterase (PLE) smoothly proceeded at 37 °C giving rise to the desired product (±)-**14** (75%) (Scheme 2).[14] With model product (±)-**14** in hand, we set about the coupling reaction toward the synthesis of *post*-amycomicin (±)-**6**. Suberic acid monomethyl ester **15** was converted to the corresponding acyl chloride and treated with a solution of hex-5-enyl zinc bromide in the presence of tetrakis(triphenylphosphine) palladium to provide the ketone **16** in 85% yield over 2 steps.[15] Subsequent cross-metathesis and stereoselective epoxidation afforded the desired methyl ketone (±)-**18**. Enone (±)-**19** was then obtained following the same sequence and final enzymatic hydrolysis resulted in desired *post*-amycomicin (±)-**6** (Scheme 3).[14]



Scheme 3. Synthesis of *post*-amycomicin (±)-**6**.

Having obtained all rearranged natural products, we began biological assays with *S. aureus* to test our prodrug hypothesis. Unfortunately, none of the epoxy ketone metabolites exhibited notable activity against *S. aureus* (Table 1 and Table S4) undermining our postulate. Notably, during the course of our study, Clardy observed complete loss of activity of amycomicin at pH > 8, which hints at the formation of an inactive rearranged product (–)-**6**.[5] We then tested the intermediates in our synthesis of the metabolites and serendipitously found that the amycomicin model substrate, enone (±)-**13**, was active against hospital-acquired methicillin-resistant *S. aureus* (HA-MRSA) and community-acquired methicillin-resistant *S. aureus* (CA-MRSA) albeit at modest concentrations.

Table 1. Growth inhibition of amycomicin **3**, *post*-amycomicin **6** and enone **13**.

	IC_{50} (μg/mL)[a],[b],[c]				
compound	*S. aureus* (CA-MRSA) (USA300)	*S. aureus* (HA-MRSA) (ATCC 33592)	*S. aureus* (MSSA) (Newman)	*E. faecalis* (ATCC 51575)	*P. aeruginosa* (PAO1)
 amycomicin, (–)-**3**	0.011[d]	-	-	>1[d]	>1[d]
 *post*-amycomicin, (±)-**6**	>160	-	-	-	-
 (±)-**13**	29	43	32	53	51
 (+)-(*R*,*S*)-**13**	32	46	35	52	55
 (–)-(*S*,*R*)-**13**	20	36	27	50	34

[a] Quaternary ammonium cation (12,3,2,3,12) was included as a positive control.[22] [b] No inhibition observed against *B. subtilis* (ATCC 6633) and *E. coli* (MC4100). [c] These assays were realized in triplicate and over 16 h. [d] MICs reported in ref. 5

Interestingly, methyl ester and the α,β-unsaturated ketone were essential to preserve the bioactivity since neither carboxylic acid (±)-**14** nor methyl ketone (±)-**11** were active (Table S4). To identify if a single enantiomer was more active, we then prepared the two optically enriched enantiomers (–)-**13** and (+)-**13** from enone **9** using both cinchona primary amine catalysts.[13]

We then measured the IC_{50} of **13** against various strains of Gram-positive (*S. aureus*, *E. faecalis*, *B subtilis*) and Gram-negative (*E. coli*, *P. aeruginosa*) pathogens and observed compound (–)-**13** was in all cases the most active enantiomer. The increased activity observed by only enantiomer (–)-**13** may indicate growth inhibition is occurring through substrate interaction with an enzyme active site. Although possessing similar chemotypes, further studies are necessary to determine if enone (–)-**13** bioactivity is occurring through a novel mechanism or if it acts in a similar manner to cerulenin targeting FabB/F.

In conclusion, we herein describe the first total synthesis of epoxy ketone natural product metabolites and demonstrate that they are inactive as prodrugs. These findings suggest that the epoxy isonitrile moiety is crucial for maintaining potency and selectivity of aerocyanidin, YM-47515, and amycomicin against *S. aureus*. Through these studies, we serendipitously discovered an enantiopure α,β-unsaturated epoxy ketone (–)-**13** that possessed modest antibacterial activity against *S. aureus*.

Experimental Section

A full description of the experimental details can be found in the supporting information.

Acknowledgements

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Keywords: *Staphylococcus aureus* • epoxy isonitrile • natural product

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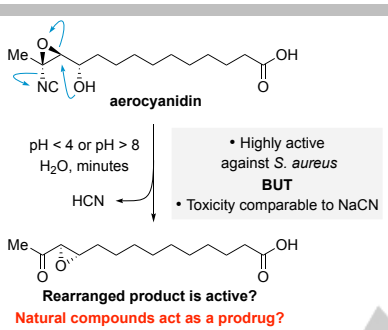

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Epoxy isonitriles, a unique class of antibiotics – Synthesis of their metabolites and biological investigations