Antibiotics

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Inhibitors of Sterol Biosynthesis as *Staphylococcus aureus* Antibiotics**

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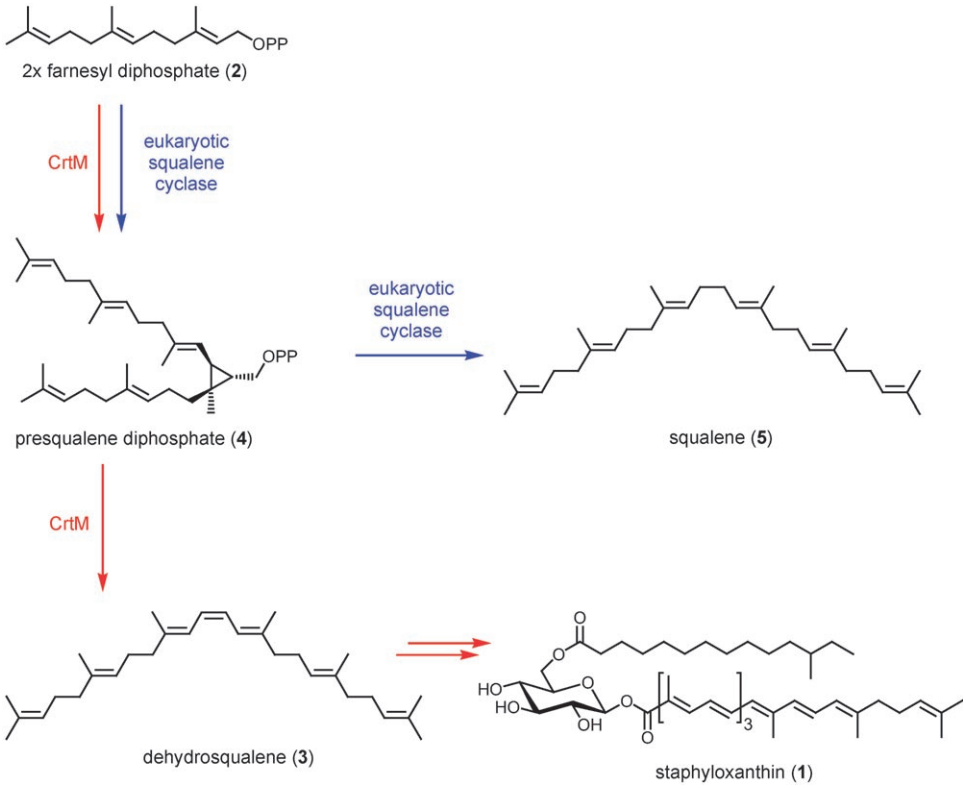
One of the truisms in the medicinal chemistry of antibiotics is that there can never be enough scaffolds and structures, whether of natural or synthetic origin. No matter how successful a new antibiotic is upon clinical introduction, the inevitable selection for resistant microbes will limit its useful lifetime.[1] Resistance to all major classes of antibiotics has been well chronicled, and one of the bacterial pathogens best known for its resistance is *Staphylococcus aureus*. With high global mortality in human infections, *S. aureus* has been described as a professional pathogen that has overcome every antibiotic campaign. Methicillin-resistant *S. aureus* (MRSA) infections are particularly problematic in both community and clinical settings.[2]



Scheme 1. The role of CrtM in staphyloxanthin biosynthesis. The *S. aureus* squalene cyclase CrtM catalyzes the formation of dehydrosqualene from two molecules of farnesyl diphosphate. Dehydrosqualene is converted subsequently into staphyloxanthin (**1**).

The *aureus* designation (Latin for golden) comes from the pigment staphyloxanthin (**1**),[3] a glycosylated carotenoid whose biosynthetic pathway proceeds through the same early steps as sterol biosynthesis. A recent anti-MRSA drug-discovery effort by Liu et al.[4] focuses on one step in particular: the head-to-head condensation of two molecules of farnesyl diphosphate (**2**) under the catalysis of the *S. aureus* enzyme CrtM to give the C_{30} hydrocarbon dehydrosqualene (**3**; Scheme 1). This transformation parallels the eukaryotic pathway to cholesterol, including the intermediacy of the cyclopropane-containing molecule presqualene diphosphate (**4**).[5] However, a distinction occurs in the last step of catalysis: The eukaryotic squalene synthase[6] uses an NADPH-dependent reduction to quench an allylic cation and yield squalene (**5**; NADPH is the reduced form of nicotinamide adenine dinucleotide phosphate), whereas the staphylococcal enzyme probably abstracts a proton to yield the central olefin in dehydrosqualene (**3**). Subsequent enzymatic dehydrogenation steps create the conjugated chromophore responsible for the golden color of the end product staphyloxanthin (**1**).[3]

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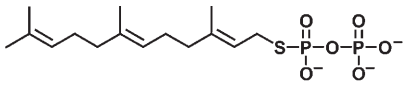
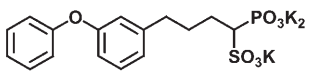
Staphyloxanthin (**1**) functions as a virulence factor for *S. aureus*. The conjugated polyene system is thought to quench reactive oxygen species, such as superoxide, peroxide, and hypochlorite, that are produced by the white cells of vertebrate hosts as they try to kill the staphylococci during the inflammatory response.[7] Thus, although staphyloxanthin biosynthesis does not appear to confer a growth advantage on *S. aureus*, it increases survival during vertebrate infections and therefore qualifies as a virulence factor.

In the search for the next generation of antibiotics, recent efforts have targeted virulence rather than essential gene functions.[8] A team of investigators, including structural biologists, chemists, and microbiologists, discovered recently that inhibition of the *S. aureus* dehydrosqualene synthase reduces bacterial survival during infections, offering a proof-of-principle for such a virulence-targeted approach.[4]

The study was based on the determination of the X-ray crystal structure of the *S. aureus* dehydrosqualene synthase CrtM after its heterologous expression in *E. coli* and subsequent purification and crystallization. As prenyltransferases are involved in terpene and sterol biosynthesis and the posttranslational S-prenylation of proteins, many of these enzymes have been studied in mechanistic detail. The eukaryotic enzyme squalene synthase, a potential drug target for cholesterol-lowering therapy, has been the object of several medicinal chemistry campaigns to identify potent and specific small-molecule inhibitors. On the basis of these efforts, a variety of known inhibitors could be resynthesized and tested as inhibitory ligands for *S. aureus* CrtM. One of these molecules, the sulfur-containing farnesyl analogue **6** (Scheme 2), could be cocrystallized with CrtM. The X-ray structure of the complex shows two molecules of **6** in the active site, which probably define the orientation of the prenyl side chains of the natural CrtM intermediate presqualene diphosphate.[4]



farnesyl thiodiphosphate (**6**)



BPH-652 (**7**)

Scheme 2. Chemical structures of CrtM inhibitors.

Evaluation of several inhibitors, including other farnesyl diphosphate analogues and amine-containing hydrocarbons that had been prepared previously as mimics of cationic intermediates in the squalene/dehydrosqualene synthase reaction, led to the observation that phosphonosulfonate scaffolds are submicromolar inhibitors of CrtM and could also be cocrystallized. The biarylether phosphonosulfonate **7** (Scheme 2) was chosen for further evaluation for several reasons: It had a K_i value of 1.5 nM against CrtM, it inhibited staphyloxanthin production when administered to live *S. aureus* ($IC_{50} = 110$ nM), and it had already progressed through preclinical toxicology and into human clinical studies as a cholesterol-lowering agent without significant adverse effects. Liu et al. found that **7** had no effect on the growth of three human cell lines in serum, a cholesterol-rich medium. Although **7** caused *S. aureus* colonies to lose their golden color, it did not inhibit the growth of *S. aureus*, as staphyloxanthin is not essential for growth.[4]

The subsequent evaluation of CrtM as a virulence factor involved a model of systemic infection in mice. When 10^8 colony-forming units of wild-type *S. aureus* or a *crtM* knockout were inoculated intraperitoneally (i.p.), the *crtM*-deficient strain was successfully cleared 72 h later, whereas the wild-type strain, as expected, was far more resistant to the oxidant-based defenses of the mice. With this result as a backdrop, mice were treated with **7** for four days and infected i.p. on the second day; bacteria remaining in the kidney were assessed at the end of the four days. The treatment worked successfully, and the authors conclude that "on average this result corresponds to a 98% decrease in surviving bacteria in the treatment group".[4]

What lessons should be taken from this report? First, the principle that the inhibition of a virulence factor in a bacterial pathogen can have a dramatic effect on bacterial survival in a vertebrate host has been proven. (However, since *S. aureus* is famously difficult to defeat, it will be instructive to see whether repeated passaging of the staphyloxanthin-deficient *S. aureus* strain leads to compensatory mutations that restore evasion of oxidative host defenses.) A second lesson is that prior medicinal chemistry studies on mammalian squalene synthases were of great utility in this antibiotic drug development program. These efforts have produced a molecular inventory of inhibitors that served as valuable starting points for the evaluation of selectivity for the bacterial enzyme over the host enzyme, ability to penetrate into *S. aureus* cells, and lack of toxicity in mammalian cells. The definition of a new target is only the beginning of an antibacterial-development program; however, the existence of compounds that have already been tested in humans lends much confidence to the effort.

This story raises the broader question of the utility and advisability of narrow-spectrum versus broad-spectrum antibiotics. It is likely that inhibitors of staphyloxanthin biosynthesis would be restricted to the treatment of *S. aureus*. Is this pathogen an important enough target to warrant the development of antibiotics specifically tailored to it? Probably: Given the high mortality associated with *S. aureus* human infections, three of the antibiotics approved recently by the Food and Drug Administration (FDA; quinupristin/dalfopristin, linezolid, and daptomycin) share MRSA as a primary target.[9] Furthermore, combination therapies may become more prevalent in the face of infections by multidrug-resistant bacteria; therefore, an inhibitor of staphyloxanthin biosynthesis might become a useful agent in such an antibacterial cocktail.

The recommendations of a US National Research Council committee in 2006 included the development of narrow-spectrum antibiotics to minimize the perturbation of normal microbial flora and to minimize resistance development.[10] Although ecologically sound, such a discovery and develop-

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ment strategy will have its own challenges, including real-time diagnostic tests for rapid pathogen identification and a change in mindset about the acceptable market size for a new antibacterial agent. A breakthrough antibiotic targeted against virulence would advance such a debate.

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[1] C. T. Walsh, *Antibiotics: Actions, Origins, Resistance*, ASM, Washington, **2003**.
[2] R. M. Klevens, M. A. Morrison, J. Nadle, S. Petit, K. Gershman, S. Ray, L. H. Harrison, R. Lynfield, G. Dumyati, J. M. Townes, A. S. Craig, E. R. Zell, G. E. Fosheim, L. K. McDougal, R. B. Carey, S. K. Fridkin, *JAMA J. Am. Med. Assoc.* **2007**, *298*, 1763.
[3] A. Pelz, K. P. Wieland, K. Putzbach, P. Hentschel, K. Albert, F. Götz, *J. Biol. Chem.* **2005**, *280*, 32493.
[4] C. I. Liu, G. Y. Liu, Y. Song, F. Yin, M. E. Hensler, W. Y. Jeng, V. Nizet, A. H. Wang, E. Oldfield, *Science* **2008**, *319*, 1391.
[5] P. A. Edwards, J. Ericsson, *Annu. Rev. Biochem.* **1999**, *68*, 157.
[6] J. Pandit, D. E. Danley, G. K. Schulte, S. Mazzalupo, T. A. Pauly, C. M. Hayward, E. S. Hamanaka, J. F. Thompson, H. J. Harwood, Jr., *J. Biol. Chem.* **2000**, *275*, 30610.
[7] A. Clauditz, A. Resch, K. P. Wieland, A. Peschel, F. Götz, *Infect. Immun.* **2006**, *74*, 4950.
[8] D. T. Hung, E. A. Shakhnovich, E. Pierson, J. J. Mekalanos, *Science* **2005**, *310*, 670.
[9] R. H. Drew, *Pharmacotherapy* **2007**, *27*, 227.
[10] Committee on New Directions in the Study of Antimicrobial Therapeutics, *Treating Infectious Diseases in a Microbial World*, National Academies Press, Washington, **2006**.

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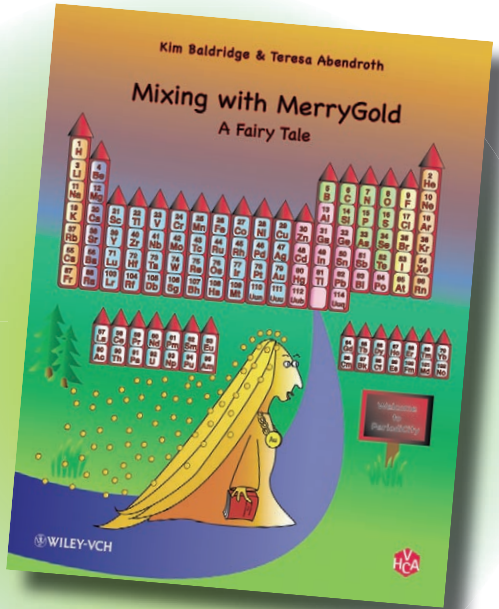

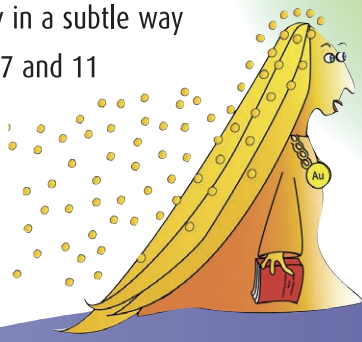

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