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Platensimycin, a New Antibiotic and "Superbug Challenger" from Nature

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Multi-resistant "superbugs" have become a physician's nightmare, particularly in hospitals, where antibiotics are heavily used.[1] Bacterial infections increasingly evade standard treatment as resistance to existing antibiotics is spreading throughout the world. Reports of escalating treatment costs and—often fatal—therapy failures are on the rise. Of particular concern are infections by Gram-positive pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA),[2] vancomycin-resistant enterococci (VRE),[3] and penicillin-resistant *Streptococcus pneumoniae* (PRSP).[4] Regardless of their historic titles, they all have acquired resistance to multiple antibiotic classes. As bacteria can replicate in less than half an hour, they are able to swiftly mutate and outsmart the antibiotic pressure by clever mechanisms that quickly spread through their microbial populations and help select for resistant organisms. Evolving resistance calls for new, effective, and safe antibacterial drugs without cross-resistance to antibiotics in clinical use. Only the persistent discovery and development of new antibiotics will guarantee future therapy.

New classes of antibiotics that address novel and valid targets are urgently needed

The established antibiotics use only a limited array of mechanisms.[5] Therefore, new structural classes of antibiotics that address novel and valid targets are urgently needed. In most cases, newly discovered antibiotics address known targets at diverse binding sites or with new binding modes (such as ketolides). The discovery of a completely new structural class that acts on an unexploited target, is an extraordinary event. Within the last few decades, the introduction of daptomycin into clinical therapy has remained the only example of this. Thus, the discovery of platensimycin (**1**, Figure 1), the first member of a novel class of natural antibiotics appears in a time of need and is of great significance.

The discovery of platensimycin—classical extract screening in tandem with RNA silencing

Scientists from Merck Research Laboratories in Rahway, New Jersey, one of the few remaining hotbeds of antibacterial drug discovery, have detected this promising candidate—a payback for Merck's consistency and commitment to natural product research.[6] In the May 18th issue of Nature, Wang et al.[7] report the discovery of a new antibiotic, platensimycin, from screening natural product extracts. While most companies have abandoned empirical extract screening, Merck's researchers revived and combined it with new RNA gene-silencing techniques. In the search for novel FabF/H inhibitors, they determined minimal inhibitory concentrations (MICs) in purpose-built *S. aureus*, which expressed less FabF/H enzyme than the corresponding wild-type bacteria. This innovative whole-cell mechanism-based screening approach allowed a most sensitive readout of Merck's natural product library.[8] With it, platensimycin was discovered from fermentation extracts of *Streptomyces platensis*, a soil bacterium collected in South Africa.

Platensimycin is the first member of a novel class of natural antibiotics

Platensimycin comprises two structural entities, the uncommon polar 3-amino-2,4-dihydroxybenzoic acid head group and a unique lipophilic tetracycle which are linked by a flexible propionamide chain. Though diterpenes are not common in bacteria,[9] and it seems risky to speculate on the biosynthesis of novel natural products without feeding experiments, the carbon skeleton and the oxidation pattern of platensimycin's lipophilic C_{17} unit might have a diterpenoid background ($C_{17}=C_{20}-C_3$) reminiscent of familiar kauranes (C_{20}) from plants (Figure 2).[10] While *N*-formyl-3-amino-2-hydroxybenzoic acid is known as a bacterial metabolite from antimycins,[11] the exact substitution pattern of platensimycins' relevant benzoic acid group, to our knowledge, has not yet been observed in nature. In a sequence

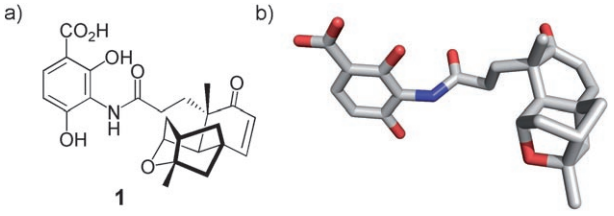

Figure 1. a) Structure of platensimycin ($C_{24}H_{27}NO_7$, $M_w=441.47$); b) active conformation of platensimycin bound to its target enzyme FabF.

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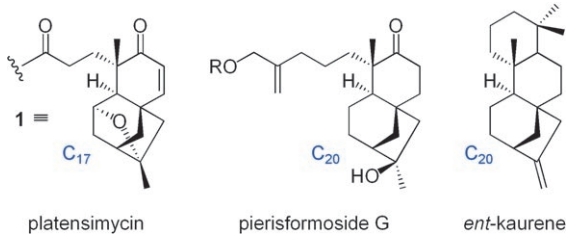


Figure 2. Structural similarity of platensimycin's lipophilic C_{17} unit and diterpenoids. *ent*-Kaurene is common in plants and fungi.[11] Pierisformoside G is an *O*-glucoside from *Pieris formosa*.[10] Platensimycin's C_{17} unit could be the bacterial degradation product of a related diterpene from plants or fungi.

of professional biochemical, pharmacological, and structural studies, Wang and colleagues characterized this new antibiotic weapon.[7]

Platensimycin has potent antibiotic properties

Platensimycin has potent antibacterial activity against Gram-positive pathogens including multiresistant strains of staphylococci and enterococci. Despite good permeability through bacterial membranes, it is not active against wild-type Gram-negative bacteria such as *Escherichia coli*. Efflux phenomena rather than specificity are responsible for this limitation.

Platensimycin exhibits efficacy in a murine model of a disseminated *S. aureus* infection (sepsis), albeit under special conditions. To achieve efficacy in vivo, the authors had to apply the drug by continuous parenteral infusion in a high dose range of 120–180 mg kg^{-1} per day. Limited plasma stability, fast clearance from the body, or both, could be the underlying cause. Although plasma levels slightly below MIC (in the presence of serum) were achieved, all in all, MIC and exposure data correlated well with the reduction of bacterial load and in vivo efficacy. Lack of antifungal activity and low mammalian cell toxicity are clear signs for platensimycin's marked selectivity. So far, no toxic effects have been observed in mice. Due to a unique mode of action, unexploited by established antibiotics, this new agent does not exhibit cross-resistance to drugs in clinical use. Yet, the pharmacokinetic profile of platensimycin does not seem to be optimal.

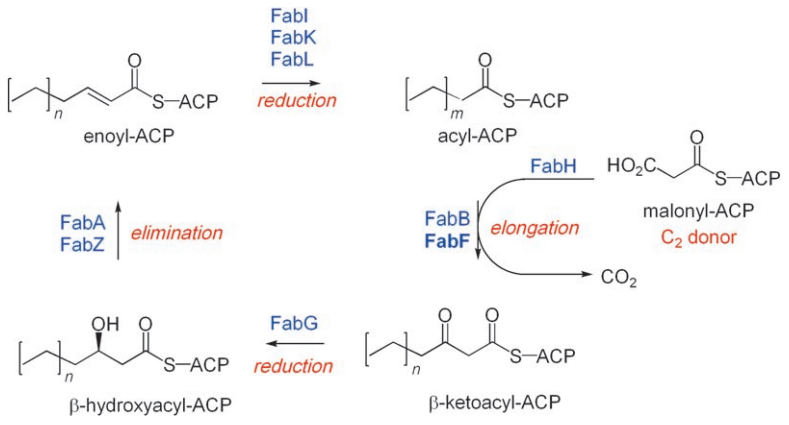
Platensimycin is a selective inhibitor of bacterial fatty acid biosynthesis

A standard method for identifying or confirming the mode of action of an antibacterial agent is to examine its effect on the key bacterial macromolecular biosynthetic pathways in the presence of specific radiolabeled precursor molecules in whole cells.[5] In this selectivity experiment, platensimycin does not hamper the biosynthesis of bacterial DNA, RNA, cell wall, or protein, but shows selective inhibition of lipid biosynthesis in *S. aureus* and *S. pneumoniae*. Fatty acid biosynthesis (Fab) is an essential metabolic process for all living organisms. Fatty acids, long, even-numbered, aliphatic carboxylic acids (up to C_{20}), are important components of cell membranes and cell envelopes. Nature creates them by repetitive addition of C_2 units in consecutive biosynthetic cycles. Since fatty acid biosynthesis is organized differently in bacteria and humans, it is an attractive target area for antibacterial drug discovery.[12] Platensimycin exerts its antibiotic effect exclusively through blocking bacterial fatty acid biosynthesis. Its molecular target is the bacterial β-ketoacyl-(acyl-carrier-protein) synthase (FabF), one of the key enzymes in bacterial fatty acid biosynthesis. FabF catalyzes the crucial C–C bond formation in the chain-elongation step (Scheme 1).

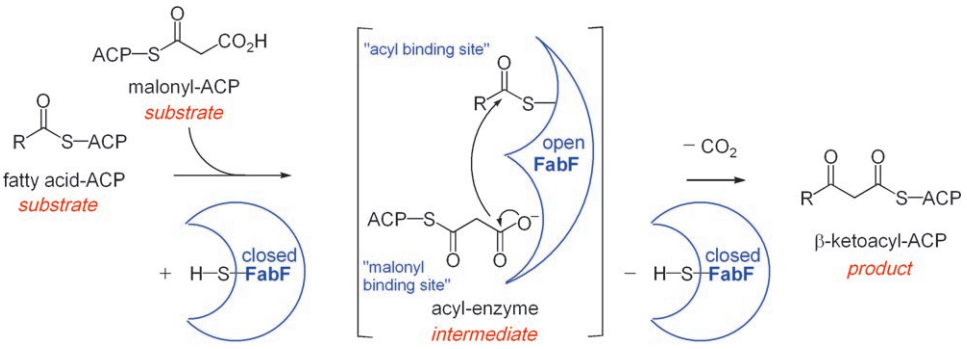
The acyl-carrier-protein (ACP) transfers the growing fatty acid to the active site cysteine of FabF, building a transitory acyl-enzyme intermediate. By forming this intermediate, FabF swings from a closed into an open conformation and a gate-keeper amino acid (Phe^{400}) opens the malonyl binding subsite. The substrate (and C_2 source), malonyl-ACP, is bound, and following decarboxylation, the resulting activated C_2 unit is inserted into the acyl-enzyme intermediate to afford the elongated β-ketoacyl-ACP product (Scheme 2).

Wang and colleagues demonstrate that platensimycin competes with malonyl-ACP for the malonyl binding site of FabF, whereby platensimycin's benzoic acid occupies the oxyanion hole and mimics the natural malonate substrate. For proof, they prepared a mutated *E. coli* FabF enzyme, in which the active site cysteine was replaced by glutamine. X-ray crystallographic structure analysis revealed that this mutated FabF enzyme adopts a conformation that mimics the short-lived acyl enzyme intermediate and perfectly binds platensimycin and blocks the malonate binding site.

Several inhibitors of diverse steps of the bacterial fatty acid biosynthetic pathway have already been described (Table 1). In contrast to most of these compounds, platensimycin addresses a

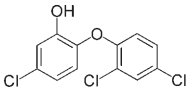
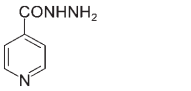
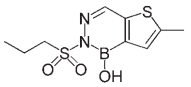
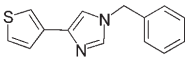
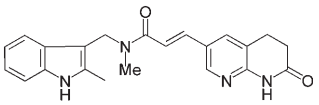
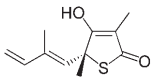
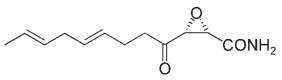
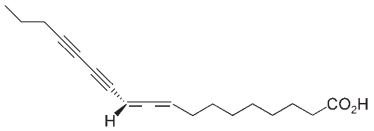
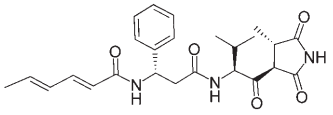


Scheme 1. Bacterial fatty acid biosynthesis.



Scheme 2. FabF catalyzes C–C bond formation in the chain-elongation step of bacterial fatty acid biosynthesis.

Table 1. Inhibitors of bacterial fatty acid biosynthesis: selected structural classes.

Compound	Structure	Fab Target
triclosan		FabI[13]
isoniazid		FabI[14]
diazaborines		FabI[15]
imidazoles		FabI[16]
naphthyridones		FabI/K[17]
thiolactomycin		FabH FabF/B[8,18]
cerulenin		FabF/B[8,18]
phomallenic acid C		FabH/F[8]
moiramide B		ACC[a][19]

[a] ACC = acetyl-CoA carboxylase

broad-spectrum target that is highly conserved among clinically important pathogens. For this reason, platensimycin is an important new antibiotic.

Conclusions

The discovery of platensimycin by Wang and colleagues is an exceptional achievement in several ways. It demonstrates that even today, striking new antibiotic classes can be discovered from ordinary sources when intelligent assay setups are used. This discovery is an instructive example of the value of natural products as guideposts for unexplored targets. It could evoke renewed interest in natural product research and the essential targets of the bacterial fatty acid biosynthetic pathway. Indeed, the new antibiotic platensimycin, with its promising mode of action, has the potential to challenge the superbugs, but it is still far from being a drug. Nonetheless, the proficient biochemical, pharmacological, and structural studies by Wang and colleagues provide a solid basis for optimizing its total profile by structural variations. It could be rewarding for medicinal chemistry to explore those parts of platensimycin that are not involved in FabF binding. Improving the pharmacokinetics might be one of the next goals. Further studies probing the spontaneous frequency of resistance of this new class will be decisive. It will require Merck's continuing commitment and considerable effort to progress this new natural product towards a valid drug. In the patients' interest, it will be a challenge worth accepting.

Keywords: antibiotics · biosynthesis · fatty acids · natural products · terpenoids

[1] H. Pearson, *Nature* **2002**, *418*, 469.
[2] a) J. F. Barrett, *Expert Opin. Ther. Targets* **2004**, *8*, 515–519; b) C. D. Salgado, B. M. Farr, D. P. Calfee, *Clin. Infect. Dis.* **2003**, *36*, 131–139.
[3] L. M. Deshpande, G. J. Moet, D. J. Biedenbach, R. N. Jones, *44th Interscience Conference on Antimicrobial Agents and Chemotherapy*, Washington, DC, USA, **2004**, abstract C2–1360 (SENTRY antimicrobial surveillance program).
[4] a) K. A. Gordon, D. J. Biedenbach, R. N. Jones, *Diagn. Microbiol. Infect. Dis.* **2003**, *46*, 285–289; b) C. G. Whitney, M. M. Farley, J. Hadler, L. H. Harrison, C. Lexau, A. Reingold, L. Lefkowitz, P. R. Cieslak, M. Cetron, E. R. Zell, J. H.

Jorgensen, A. Schuchat, *N. Engl. J. Med.* **2000**, *343*, 1917 – 1924.
[5] F. von Nussbaum, M. Brands, B. Hinzen, S. Weigand, D. Häbich, *Angew. Chem.* **2006**, *118*, 5194 – 5254; *Angew. Chem. Int. Ed. Engl.* **2006**, *45*, 5072 – 5129.
[6] S. B. Singh, J. F. Barrett, *Biochem. Pharmacol.* **2006**, *71*, 1006 – 1015.
[7] J. Wang, S. M. Soisson, K. Young, W. Shoop, S. Kodali, A. Galgoci, R. Painter, G. Parthasarathy, Y. S. Tang, R. Cummings, S. Ha, K. Dorso, M. Motyl, H. Jayasuriya, J. Ondeyka, K. Herath, C. Zhang, L. Hernandez, J. Allocco, Á. Basilio, J. R. Tormo, O. Genilloud, F. Vicente, F. Pelaez, L. Colwell, S. H. Lee, B. Michael, T. Felcetto, C. Gill, L. L. Silver, J. D. Hermes, K. Bartizal, J. Barrett, D. Schmatz, J. W. Becker, D. Cully, S. B. Singh, *Nature* **2006**, *441*, 358 – 361.
[8] K. Young, H. Jayasuriya, J. G. Ondeyka, K. Herath, C. Zhang, S. Kodali, A. Galgoci, R. Painter, V. Brown-Driver, R. Yamamoto, L. L. Silver, Y. Zheng, J. I. Ventura, J. Sigmund, S. Ha, A. Basilio, F. Vicente, J. R. Tormo, F. Pelaez, P. Youngman, D. Cully, J. F. Barrett, D. Schmatz, S. B. Singh, J. Wang, *Antimicrob. Agents Chemother.* **2006**, **50**, 519 – 526.
[9] T. Dairi, Y. Hamano, T. Kuzuyama, N. Itoh, K. Furihata, H. Seto, *J. Bacteriol.* **2001**, *183*, 6085 – 6094.
[10] L. Q. Wang, G. W. Qin, S. N. Chen, C. J. Li, *Fitoterapia* **2001**, *72*, 779 – 787.
[11] *Römpp Encyclopedia—Natural Products* (Eds.: W. Steglich, B. Fugmann, S. Lang-Fugmann), Georg Thieme, Stuttgart, **2000**.
[12] a) R. J. Heath, C. O. Rock, *Curr. Opin. Invest. Drugs* **2004**, *5*, 146 – 153; b) D. J. Payne, P. V. Warren, D. J. Holmes, Y. Ji, J. T. Lonsdale, *Drug Discovery Today* **2001**, *6*, 537 – 544; c) J. W. Campbell, J. E. Cronan, Jr., *Annu. Rev Microbiol.* **2001**, *55*, 305 – 332.
[13] R. J. Heath, Y. T. Yu, M. A. Shapiro, E. Olson, C. O. Rock, *J. Biol. Chem.* **1998**, *273*, 30316 – 30320.
[14] A. Banerjee, E. Dubnau, A. Quemard, V. Balasubramanian, K. S. Um, T. Wilson, D. Collins, G. de Lisle, W. R. Jacobs, Jr., *Science* **1994**, *263*, 227 – 230.
[15] C. Baldock, J. B. Rafferty, S. E. Sedelnikova, P. J. Baker, A. R. Stuitje, A. R. Slabas, T. R. Hawkes, D. W. Rice, *Science* **1999**, *274*, 2107 – 2110.
[16] D. A. Heerding, G. Chan, W. E. DeWolf, A. P. Fosberry, C. A. Janson, D. D. Jaworski, E. McManus, W. H. Miller, T. D. Moore, D. J. Payne, X. Qiu, S. F. Rittenhouse, C. Slater-Radosti, W. Smith, D. T. Takata, K. S. Vaidya, C. C. Yuan, W. F. Huffman, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2061 – 2065.
[17] M. A. Seefeld, W. H. Miller, K. A. Newlander, W. J. Burgess, W. E. DeWolf, Jr., P. A. Elkins, M. A. Head, D. R. Jakas, C. A. Janson, P. M. Keller, P. J. Manley, T. D. Moore, D. J. Paine, S. Pearson, B. J. Polizzi, X. Qiu, S. F. Rittenhouse, I. N. Uzinskas, N. G. Wallis, W. F. Huffman, *J. Med. Chem.* **2003**, *46*, 1627 – 1635.
[18] A. C. Price, K. H. Choi, R. J. Heath, Z. Li, S. W. White, C. O. Rock, *J. Biol. Chem.* **2001**, *276*, 6551 – 6559.
[19] C. Freiberg, N. A. Brunner, G. Schiffer, T. Lampe, J. Pohlmann, M. Brands, M. Raabe, D. Häbich, K. Ziegelbauer, *J. Biol. Chem.* **2004**, *279*, 26066 – 26073.

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