Ironing out New Antibiotic Mechanisms with Xanthocillin X

Alexander V. West and Christina M. Woo

Xanthocillin X, an isonitrile antibiotic, functions by direct inhibition of iron-bound heme, which may represent a new target for further antibacterial development.

Antibiotic resistance is a growing health crisis, as the rate of antibiotic resistance is developing more rapidly than the approval of new antibiotic therapies.1−3 Even more troubling is the emergence of multi-drug-resistant pathogens and “super bugs” that are resistant to many common antibiotics used in the clinic.4 To address this health crisis, new antibiotics that have unique targets are needed, so that a strain of bacteria that is resistant to one antibiotic will likely be sensitive to a novel antibiotic treatment. In this issue of ACS Central Science, Hübner and colleagues uncover a new antibiotic mechanism of action that could meet this demand.5 Unique natural products are a promising source of new antibiotic discovery, but without an understanding of their mechanism, the modification and optimization of those compounds are challenging.6 Several hundred isonitrile antibacterial natural products have been discovered since the first isolation of Xanthocillin X (Xan) in 1948; yet, a cellular target for these compounds has been missing until now.7

Hübner and team discovered heme biosynthesis as a novel antibacterial target of Xan. The authors began with an evaluation of Xan’s effectiveness against several strains of bacteria and found that it had broad spectrum activity including against the most challenging Gram-negative bacteria strains. Excitingly, Xan was most effective against Acinetobacter baumannii, a clinically relevant pathogen identified as a high-priority health threat by the WHO.8 Previous studies identified copper binding and disruption of copper-dependent enzymes as a mechanistic target of isonitrile compounds.9 To see if Xan acts similarly, the authors began by testing its ability to bind metals in solution. They found that Xan exclusively bound to copper(II) and that an inactive Xan analogue (XanDME) did not bind to copper(II). These observations suggested that copper binding was involved in Xan’s antibacterial mechanism; however, a key control experiment showed that XanDME was being quickly exported from the bacteria and regained antibiotic activity in a mutant strain of A. baumannii with two efflux pumps knocked out. This suggested that copper chelation was not essential to Xan or XanDME’s activity and that these compounds were likely proceeding through another, unreported mechanism.

The authors next evaluated whether Xan has a protein target using a chemical proteomics approach to identify proteins that it binds to or covalently labels in A. baumannii. The authors identified several covalent protein targets using an alkyne-modified Xan probe (XP); however, none of the identified proteins were essential for cellular function.

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A similar set of photoaffinity labeling experiments to identify binding partners of Xan was performed with a photoaffinity probe (XPP). Although many proteins were labeled by XPP, none of these proteins were essential for cellular viability. Therefore, the protein interactions formed by Xan were clearly not linked to its lethality in *A. baumannii*.

At this stage, the authors turned to a classical method of target identification in bacteria: raising resistant mutants. This is a common approach for studying an antibiotic’s mechanism, but it is not always feasible, as resistance depends on the growth conditions and mechanism of action. Fortunately, the authors were able to generate nine resistant colonies for comparison to negative controls. Amazingly, all of the resistant colonies contained a mutation in *hemB*, which is a gene encoding porphobilinogen synthase (PbgS). PbgS is an essential enzyme that catalyzes the first step in the biosynthesis of tetrapyrroles found in heme. Investigation of the resistant P241S mutation revealed that the mutant enzyme had reduced activity compared to the wild type enzyme. Interestingly, they found that Xan did not directly inhibit PbgS (wt), which explains why PbgS did not appear in their chemoproteomics studies.

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Reduced heme synthesis from a less active PbgS mutant, along with increased heme degradation from upregulated heme oxygenases, pointed to the heme biosynthetic pathway as a target of Xan.

To further investigate the resistance mechanism, Hübner and team performed a global proteome analysis to survey all of the proteins in the resistant *A. baumannii* strains. They found that the three resistant strains they analyzed had increased levels of multiple heme oxygenases, which catalyze heme degradation in cells. Reduced heme synthesis from a less active PbgS mutant, along with increased heme degradation from upregulated heme oxygenases, pointed to the heme biosynthetic pathway as a target of Xan.

With the heme biosynthetic pathway as the target, Hübner et al. investigated whether Xan directly targets heme itself and found that it does. Xan binds to heme through the isonitrile functional groups, suggesting that its antibacterial effects are a result of the direct interaction between the compound and heme cofactor. In the experiments that followed, the authors showed that Xan interacts selectively to iron-bound heme and that binding to heme sequesters the amount of regulatory heme in cells and destabilizes heme regulation. As a result, the porphyrin precursor of heme builds up, which results in the accumulation of reactive oxygen species that leads to cell death.

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mammalian cells, and undesirable toxicity in vivo may be observed. Xan also has varying degrees of potency across several bacterial strains that rely on heme, and why it is much more effective against *A. baumannii* than other species warrants further investigation. Nonetheless, the systematic investigation of mechanistic candidates using multiple approaches and techniques sets a strong precedent for similar mechanistic efforts with other novel antibacterial agents and reveals isonitrile compounds that target heme biosynthesis as an exciting new avenue for antibiotic development in the future.

**Author Information**

**Corresponding Author**
Christina M. Woo; orcid.org/0000-0001-8687-9105; Email: cwoo@chemistry.harvard.edu

**Author**
Alexander V. West

Complete contact information is available at: https://pubs.acs.org/10.1021/acscentsci.1c00130

**Notes**
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