Journal Club

Strapline: AMPLIFYING DIVERSE VOICES

A xanthone-derived antibiotic with a multifaceted mode of action

Bacterial resistance to available antibiotics continues to pose a great challenge to the successful treatment of infections. In 2019, there were close to five million annual deaths associated with antimicrobial resistance (AMR) globally, and this has been predicted to rise to 10 million deaths by 2050 if nothing is done to mitigate this "silent pandemic"[1]. In addition to the misuse of currently available antibiotics, the issue of AMR has been further complicated by the paucity of new antimicrobials with unique mechanisms of action. Hence, the development of novel antimicrobials, for example from natural products, for example from natural products, with broad-spectrum activity against Gram-negative and Gram-positive pathogenic bacteria is needed. Currently, only a small proportion of new antibiotics in development are effective against Gram-negative bacteria, highlighting the urgent need for the development of new antimicrobials with high potency against these resistant strains. Towards this goal, in a recent study published in *Science Advances*, Li and colleagues synthesized a broad-spectrum xanthone derivative with multiple mechanisms of action and reduced risk of resistance.

Xanthones (9H-xanthen-9-one) are natural products that are produced by plants, fungi, and algae, with oxygen-containing dibenzo-γ-pyrone heterocyclic scaffold[2]. One of the most studied xanthones is α-mangostin, a natural product from the pericarp, bark, or dried sap of mangosteen. Previously, α-mangostin has been shown to have good antibacterial activity against Gram-positive bacteria but low activity against Gram-negative bacteria. This xanthone is also associated with high production cost and high hemolytic activity. Furthermore, little is known about the mechanism of action of this xanthone against bacterial pathogens. Consequently, Li *et al.* aimed to design xanthone derivatives with potent activity against both Gram-positive and negative bacteria. Due to the limited understanding of the mode of action of xanthones, Li *et al.* also set out to determine the mechanisms of action of these xanthone derivatives.

Using a rational design approach, which allows for systematic modification of the hydrophobic core of α-mangostin based on hypothesised activity, Li *et al.* synthesised numerous derivatives of α-mangostin by adding hydrophobic chains to carbon 1 (C1) and high pKa cationic groups to C3 and C6 positions. These modifications were made to enhance uptake and improve the antibacterial activity of these derivatives against both Gram-positive and Gram-negative bacteria. Unlike the conventional method of randomly synthesizing structurally related derivatives, this targeted approach is faster and cost-effective. Broth microdilution assay, as recommended by Clinical and Laboratory Standards Institute (CLSI), was then used to assess the antibacterial efficacies of these newly synthesized xanthone-derivatives against priority pathogens including *Staphylococcus aureus* (methicillin-susceptible and resistant strains), *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumoniae,* and *Escherichia coli* strains. Additionally, rabbit erythrocyte was used to assess the hemolytic activity of the

derivatives, while Human hepatoma cells and mouse fibroblasts were used to assess cytotoxicity. *In vivo* efficacy was also determined with female C57Bl/6 mice. Modes of action were determined with SYTOX Green uptake assay, 3,3'-Dipropylthiadicarbocyanine iodide [$DiSC_3(5)$] assay, mobility shift assay, molecular docking, and transcriptomics. Lastly, the rate of resistance evolution to xanthone-derived antibiotics by *S. aureus* and *P. aeruginosa* was assessed with a serial passage resistance induction assay.

Of all the 45 xanthone derivatives that were synthesized, XT17 and XT18 showed the best activity against both Gram-positive and Gram-negative bacteria with minimum inhibitory concentration values ranging between 0.39 - 3.125 µg/ml. Nonetheless, XT17 had a lower hemolytic activity compared to XT18 and the best in vivo antibacterial activity against both *S. aureus* and *P. aeruginosa* in a corneal mouse model, giving at least 2.8 log CFU bacterial reduction per cornea. XT17 showed little or no cytotoxicity *in vitro* and was well tolerated in the mouse model. Interestingly, XT17 also showed better killing against *S. aureus* and *P. aeruginosa* compared to vancomycin and ciprofloxacin, respectively. The authors showed that there was slower resistance evolution to XT17 by both species compared to norfloxacin (*S. aureus*) and ciprofloxacin (*P. aeruginosa*) over a 20-day passage *in vitro*. XT17 neither caused a disruption in the membrane of *S. aureus* and *P. aeruginosa* nor permeabilized the membrane but strongly bound to lipoteichoic acid on the Gram-positive cell wall and lipopolysaccharide on the Gram-negative outer membrane which enhanced its uptake. Transcriptomics analysis of bacterial cells treated with XT17 showed that the compound influenced the bacterial transcription process, DNA replication, DNA repair, recombination, and signal transduction pathways. Lastly, XT17 was shown to have a strong affinity with and inhibit DNA gyrase in a similar manner as ciprofloxacin.

This study provides an insight into the mechanism of action of xanthones, improving the understanding of how these unique compounds work. Additionally, the cheap, simple, and easily scalable targeted rational design approach used in this study would make it possible for scientists to design novel derivatives from other xanthones and similar plant-based natural products with desired activities. Hence, this approach would enhance the discovery of new antibiotics, especially against Gram-negative bacterial pathogens. Furthermore, the presence of multiple modes of action of xanthone derivatives and their low resistance evolution risk, compared to some mainline antibiotics, make them compounds of high interest. However, more experiments are needed to identify the other intracellular targets of XT17, outside DNA gyrase, as transcriptomics data show that it inhibits multiple targets. Additionally, Li et al. did not show if there was any cross-resistance between XT17 and ciprofloxacin, due to their similar mechanism of action.

In conclusion, this study has introduced a cost-effective approach to synthesizing xanthone derivatives which could be applied to other natural products, including many polyphenols, to generate desirable properties. The authors have also shown that xanthones are interesting antimicrobial compounds with unique and multiple mechanisms of action and might pave the way for a new generation of antibiotics with reduced risk of resistance.

Author name: Oluwatosin Q. Orababa (Corresponding author)
Affiliation: School of Life Sciences, Gibbet Hill campus, University of Warwick, Coventry CV4 7AL, United Kingdom
email: Oluwatosin.orababa@warwick.ac.uk

Author name: Raphael P. Galleh
Affiliation: Biomedical Science, Institute of Science and Environment, University of Cumbria, Cumbria, United Kingdom
Email: raphael.galleh@cumbria.ac.uk

Original Article: [1]

1. Li, H. *et al.* Development of xanthone derivatives as effective broad-spectrum antimicrobials: Disrupting cell wall and inhibiting DNA synthesis. Science Advances. https://doi.org/10.1126/sciadv.adt4723 (2025)

Other References

1 Murray, C. J. *et al. The Lancet* **399**, 629-655 (2022).
2 Badiali, C. *et al. Plants* **12**, 694 (2023).

Competing interests:

The authors declare no competing interests