Effective method to avoid activating a cellular detoxification receptor to overcome drug resistance and toxicity

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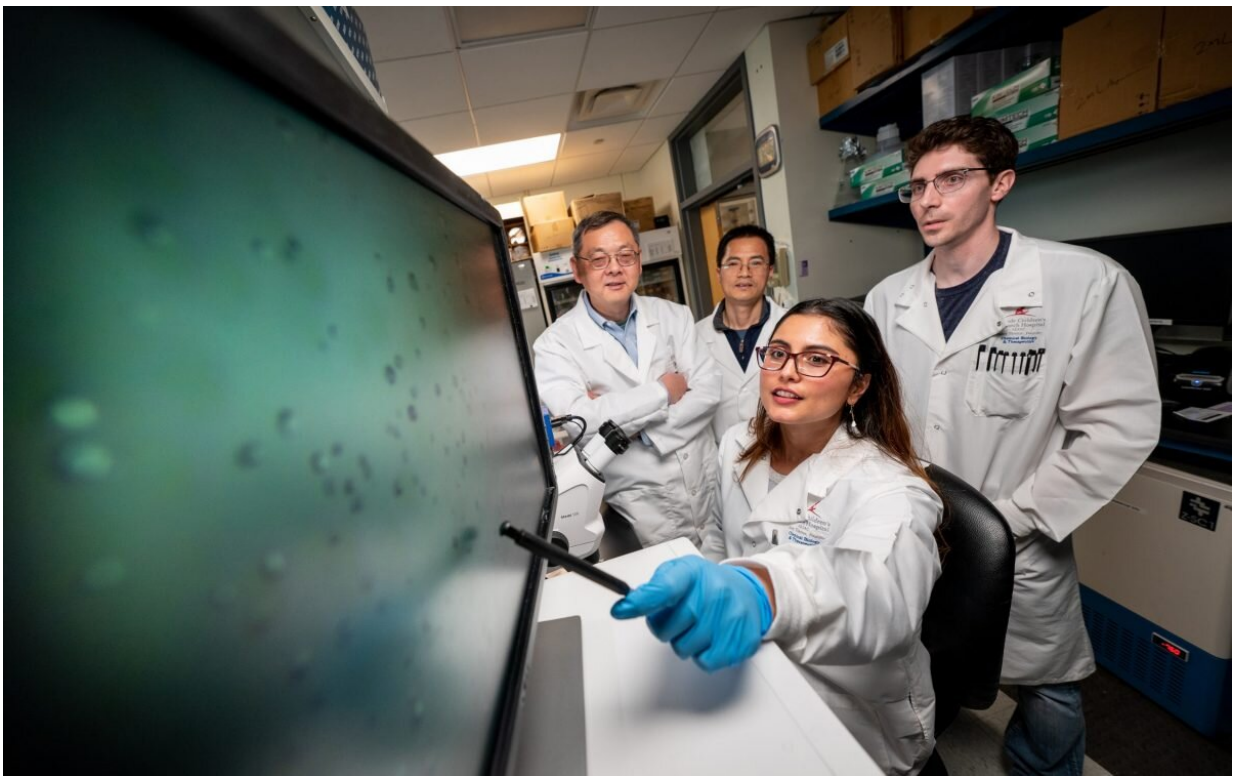

(L to R) Co-corresponding author Taosheng Chen, Ph.D., PMP, first author Wenwei Lin, Ph.D., Shyaron Poudel and co-corresponding and co-first author Andrew Huber, Ph.D., all of the St. Jude Department of Chemical Biology and Therapeutics. Credit: St. Jude Children's Research Hospital

Scientists at St. Jude Children's Research Hospital have demonstrated how drug makers can avoid two key problems: toxicity and resistance. The researchers made slight changes to a small molecule to reduce its metabolism and elimination by the cellular detoxification network regulated by the pregnane X receptor (PXR). This research provides a framework to develop solutions to the long-standing issue of how to evade detoxification networks using medicinal chemistry. The findings were published today in *Proceedings of the National Academy of Sciences*.

The researchers were able to change a drug that normally binds well to the detoxification receptor PXR into a drug that bound poorly. The altered drug stretched out the binding region of PXR, making binding energetically unfavorable. Structural modification of the drug lowered the levels of PXR-induced enzymes—indicating this approach could be used to evade detection of a drug by the detoxification network in drug development. The potential implications of the research are vast, because many drugs interact with PXR, and over half of all clinically approved drugs in the U.S. are metabolized by the PXR-induced enzymes.

"This is a major breakthrough in a very challenging field," said co-corresponding author Taosheng Chen, Ph.D., PMP, St. Jude Department of Chemical Biology and Therapeutics. "The challenge is that evolutionarily the human body wants to destroy foreign compounds it detects, including drugs. This is a big problem for drug discovery and development. That's why we are very excited that we discovered a structural feature that we can apply from one chemical scaffold to another to avoid being detected by the detoxification network."

"Before this, PXR was seen as a challenging problem that could not be solved because there was no way to study a structure-activity relationship for such a promiscuous detoxification receptor," Chen said. "This is a

significant change for the field, showing that it is, indeed, possible. The takeaway message is that we showed drug toxicity and resistance can be prevented or overcome using a systematic approach."

Avoiding drug resistance and toxicity

When a drug molecule enters certain cells in the liver and intestine, proteins in the detoxification network are activated to metabolize the drug. This has two major consequences. The first is drug resistance—a higher dose of drug must be administered to reach its therapeutic target. The second is toxicity. When many drugs are metabolized, the resulting molecule(s) might still be biologically active, often in a toxic way, sometimes causing side effects.

"When you see commercials for a drug and the longest part of the commercial is the list of side effects, many of those side effects are from metabolic events caused by the detoxification network," said co-corresponding and co-first author Andrew Huber, Ph.D., St. Jude Department of Chemical Biology and Therapeutics. "We found a way to avoid those metabolic events more effectively, which we hope will shorten that list of side effects."

A team-driven strategy of structural mix-and-match solutions

The scientists hoped they could compare multiple structures of drugs bound to PXR and then identify ways to reverse engineer compounds that avoid binding to PXR. The team took two series of molecules that bind PXR (i.e., PXR ligands), one large (weaker binders) and one small (stronger binders), and compared them to see if they could find a way to disrupt PXR binding of the smaller molecule. PXR is known to bind to many molecules, though the reasons why some drugs bind well and some

bind poorly was only partially understood.

One of the major drugs that binds PXR is rifampicin, which is used to treat the respiratory disease tuberculosis. Rifampicin is also one of the largest molecules that binds to PXR. There are multiple variants of rifampicin, so the scientists compared the ones that highly activate PXR to those that don't. They found specific parts of the structure on poor activators that made them extra "bulky" compared to potent activators. Like overloading a pocket in a pair of pants, putting too large of an object into PXR affects its function. These bulky regions stretched out the binding pocket of PXR, incurring an energetic penalty that made binding unfavorable.

To see if they could prevent binding to PXR, the scientists took one of the smallest PXR ligands, and added a "bulky" region to it. Normally, the small PXR ligand binds PXR tightly and is a strong activator. With just one bulky region added, the altered small molecule became much less effective at binding and activating PXR. The St. Jude group also showed they could do the reverse—removing bulky regions from the large molecules so that they bound and activated PXR more efficiently.

"We showed you could make a drug in a smarter way that interacts less favorably with PXR, so it will limit drug-drug interactions, reduce clearance and metabolizing," said co-first author Wenwei Lin, Ph.D., St. Jude Department of Chemical Biology and Therapeutics. "This type of approach will enhance drug efficacy, which is important for certain patients—like those with cancer. They need to receive treatment over a long time. Reducing drug resistance will be particularly beneficial in these patients, because using a lower dose over an extended period can reduce future health complications."

The discovery required a mix of scientists with many different skills: from pharmaceutical development to biochemistry to X-Ray

crystallography. The team credits their collaboration for giving them the ability to tackle PXR in a way no one in industry or academia had attempted.

More information: Wenwei Lin et al, Structure-guided approach to modulate small molecule binding to a promiscuous ligand-activated protein, *Proceedings of the National Academy of Sciences* (2023). DOI: 10.1073/pnas.2217804120

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