GSK's topoisomerase in the hole

By Kai-Jye Lou, Staff Writer

Fluoroquinolone antibiotics work by blocking bacterial topoisomerase IIA, but bacterial resistance to this class of drugs is rising faster than new treatments can be developed.[1–3] Researchers at **GlaxoSmithKline plc** now have generated a new class of antibacterial compounds that can block the activity of bacterial topoisomerase IIA via a mechanism that is distinct from that of fluoroquinolones.[4]

Bacterial topoisomerase IIA is needed to carry out DNA replication: it works to unwind DNA, cleave the double strands and ligate the broken strands back together. Mutations in the two types of bacterial topoisomerase IIA—DNA gyrase and topoisomerase IV—can cause resistance to fluoroquinolones.

The GSK group, led by Michael Gwynn, director of the Antibacterial Discovery Performance Unit, set out to identify and synthesize new inhibitors of the two types of topoisomerase IIA. In a panel of Gram-positive and Gram-negative bacteria, the team showed that a newly synthesized compound had broad-spectrum antibacterial activity and was not significantly affected by mutations in DNA gyrase and topoisomerase IV that affect the fluoroquinolones. The compound, GSK299423, was derived from the piperidinylalkylquinoline chemical series.

The group generated high-resolution crystal structures of GSK299423 in complex with synthetic strands of DNA and DNA gyrase from *Staphylococcus aureus*. The researchers compared those structures with ciprofloxacin complexed to the targets. They found that GSK299423 binds midway between two active sites on the DNA gyrase at a location that is near to but distinct from the two binding sites for fluoroquinolones.

Ciprofloxacin is a generic, broad-spectrum fluoroquinolone.

Results were published in *Nature*.

"Our findings open up a basis for more rational approaches to optimizing and developing compounds against these clinically validated targets," said Gwynn, who was a corresponding author on the paper. "Our work provides a structure-based platform for developing new classes of antibacterial compounds against type IIA topoisomerases that could be effective against strains that are resistant to fluoroquinolones. The structural data contained in our study also could provide the scientific community with insights on new ways to attack this target."

"The approach described in the paper goes back to validated targets and looks for new ways to target them," said Deborah Hung, an assistant professor in the Department of Microbiology and Molecular Genetics at **Harvard Medical School** and the Department of Molecular Biology at **Massachusetts General Hospital**.

"It's exciting to see structural data appearing for this target," said Erin Duffy, VP of discovery research at **Rib-X Pharmaceuticals Inc.** "The structures described in this work complement two recent reports describing the atomic-level details of the binding action of quinolone antibiotics. By having the structural data and details of what the drug binding sites look like, much of the guesswork for improving target potency is removed, and new opportunities for overcoming target-based resistance and broadening spectrum are available."

In 2009, researchers at the **University of London** published the crystal structures of moxifloxacin and clinafloxacin in complex with bacterial topoisomerase IV in *Nature Structural and Molecular Biology*.[5] And last month, Gwynn and colleagues at GSK published a higher-resolution crystal structure of moxifloxacin in complex with topoisomerase IV in the same journal that highlights the mechanisms of fluoroquinolone inhibition and resistance.[6]

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>
> *—Erin Duffy, Rib-X Pharmaceuticals Inc.*

Bayer AG markets moxifloxacin, a fluoroquinolone antibiotic, under multiple brand names. Clinafloxacin was originally being developed by Warner-Lambert Co., now part of **Pfizer Inc.**, but was discontinued due to phototoxicity.

Rib-X's platform uses the 3D structure of another validated target—the bacterial ribosome—to aid the rational design of new classes of antibiotics with potentially new mechanisms of action. The company's lead compound, delafloxacin (RX-3341), is a broad-spectrum fluoroquinolone antibiotic that has successfully completed Phase II trials to treat acute bacterial skin and skin structure infections (ABSSIs) and lung infections. Rib-X in-licensed the compound from **Wakunaga Pharmaceutical Co. Ltd.** and is planning to start an exploratory Phase II trial in 1Q11 of delafloxacin in ABSSIs to establish objective endpoints. The company's most advanced in-house compound, radezolid (RX-1741), is an oxazolidinone antibiotic that has completed Phase II trials to treat skin infections and pneumonia.

Michael Pucci, senior director of antimicrobial drug discovery at **Achillion Pharmaceuticals Inc.**, said the results reported in *Nature* reinforce the idea of "searching for new ways to inhibit well-validated targets that won't be subject to cross-resistance with existing drugs." Achillion's ACH-702, a topical isothiazoloquinolone that blocks DNA gyrase and topoisomerase IV, is in preclinical development for bacterial infections.

"The structural data from GSK show that the binding sites for quinolone antibiotics and these new compounds are close to each other but not overlapping," added Atul Agarwal, senior director of computational chemistry and informatics at Achillion. "They show that by occupying two distinct binding pockets, the novel bacterial topoisomerase inhibitor and quinolones are not cross-resistant to each other."

He said the new inhibitors described in *Nature* appear to bind to and stabilize the DNA-topoisomerase complex prior to the DNA cleavage step, whereas fluoroquinolones bind to already-cleaved DNA strands to stabilize the complex.

Both Pucci and Agarwal wanted to see resistance induction studies that evaluate the ease with which bacteria could develop resistance to compounds that exploit this new mechanism.

Pucci added that because the new compounds appear to interact with a larger number of topoisomerase amino acids than the fluoroquinolones, "it would theoretically be easier to build target specificity in such antibiotics, which would reduce off-target activity and, thereby, side effects. But it also presents more candidate amino acids for mutation and could thus increase the possibility of resistance development."

Both delafloxacin and ACH-702 have shown greater potency against fluoroquinolone-resistant strains of bacteria than marketed fluoroquinolone antibiotics.

Rational design

Despite the undetermined potential for resistance, GSK's Gwynn said that developing compounds against validated targets like topoisomerase IIA could have fewer surprises than going after newer targets that are being identified via genomic approaches.

"We know a lot about these existing targets and already know that a good spectrum of activity could be achieved by inhibiting their activity," he told *SciBX*.

Gwynn also noted that structural information could "make the drug development process more efficient as it will provide a rational basis on how to improve and optimize the properties of a compound and its activity against its target. It will also provide information on which compounds in a series are worth synthesizing and pursuing for further evaluation."

He said the group at GSK is optimizing compounds that exploit the new mechanism of action.

"Through our early-stage hits, we have also identified other points on the protein that could potentially be targeted," he added. "We are currently optimizing the class and making further derivatives to identify a lead candidate against Gram-positive and Gram-negative infections. We are also building in the pharmacokinetic, safety and structural features needed to produce a good clinical candidate. We could have a compound that we could test in the clinic within 18 months."

GSK declined to disclose the patent status associated with the findings described in *Nature*. The work is unavailable for licensing.

Lou, K.-J. *SciBX* 3(34); doi:10.1038/scibx.2010.1031
Published online Sept. 2, 2010

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