New drug-screening method yields long-sought anti-HIV compounds

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Scientists at The Scripps Research Institute have used a powerful new chemical-screening method to find compounds that inhibit the activity of human immunodeficiency virus (HIV), the virus that causes AIDS. Unlike existing anti-HIV drugs, the compounds bind to a protein called "nucelocapsid," which is unlikely to mutate into drug-resistant forms.

"Most of the nucleocapsid-inhibiting compounds that have been identified to date are very toxic, but our screening method identified inhibitors that are less toxic and thus more likely to lead to useful drugs," said Scripps Research Associate Professor Bruce Torbett. Torbett is the senior author of the new report, which appears in the June 14, 2012 print issue of the Journal of Medicinal Chemistry.

HIV's nucleocapsid protein binds to the viral genome to package and protect it, and plays a key role in the assembly of new virus copies, as well as in the reverse transcription of the viral genome into DNA. It has long been a target of HIV drug developers because it grabs hold of the viral genome using protein structures—known as zinc knuckles—that can't change much without losing their functionality. It thus is thought to have little room to mutate into drug-resistant forms, in contrast with other HIV proteins.

Screening Out Toxicity

However, despite almost two decades of research, there are still no FDA-

approved drugs that target HIV's nucleocapsid protein and its zinc knuckle structures. One reason is that similar structures exist on many healthy cellular proteins; thus compounds that target them are apt to have unwanted side effects. "When researchers have targeted these nucleocapsid zinc knuckles in the past, they've usually ended up producing toxicity," Torbett said.

To increase the chances of finding safe compounds, Torbett and his colleagues—postdoctoral researcher Sebastian Breuer, the study's first author, and Max Chang and Jinyun Yuan, also postdoctoral researchers—began with the Maybridge HitFinder Collection, a library of 14,400 chemical compounds from which many broadly toxic molecules have already been excluded. The Scripps Research Molecular Screening Center maintains the latest robotic equipment for quickly applying chemical tests to such libraries. With the help of screening expert Scripps Research Professor Hugh Rosen, Screening Center Staff Scientist Steven Brown, and Research Assistant Jacqueline Lohse, Breuer applied a special combination of screening tests to the Maybridge library to rapidly zero in on effective and safe nucleocapsid-inhibiting compounds.

The first screening test employed a technique known as fluorescence polarization to measure the ability of each compound in the library to displace the binding of the viral genome to the nucleocapsid protein. (The study focused on the virus type HIV-1, which accounts for the vast majority of HIV infections outside West Africa.) The second test, using differential scanning fluorimetry, was applied to the 101 compounds that passed the first test; it identified those that perform the displacement by binding strongly to the nucleocapsid protein rather than to the viral genome.

After eliminating the weaker and more toxic candidates with further tests, Breuer, Torbett, and their colleagues ended up with 10 compounds.

Detailed analyses of these yielded two that were sufficiently powerful at inhibiting viral infectivity in cell culture tests, without being unacceptably toxic.

"We went very quickly from having a concept to having these two inhibitors with demonstrated anti-HIV activity in cells," said Torbett.

Searching for the 'Sweet Spot'

With his Scripps Research colleagues M. G. Finn and Valery Fokin, Torbett now plans to evaluate compounds that are closely related to the two inhibitors to see if the scientists can find any that are even more safe and effective. Torbett and colleagues also plan to apply the same combination-screening method to larger compound libraries to identify entirely new nucleocapsid-inhibiting compounds.

To gain a better understanding of how these inhibitors work, Torbett is also collaborating with Scripps Research structural biologists, including David Stout and Arthur Olson, and virologist John Elder to perform X-ray crystallography studies of the inhibitors in combination with the HIV nucleocapsid protein.

"The overall goal here is to find a 'sweet spot' on the nucleocapsid protein that can be targeted effectively by a small-molecule drug without causing toxicity," Torbett said.

Provided by The Scripps Research Institute