Barking up the right tree

By Michael J. Haas, Senior Writer

Researchers at **Stanford University** have developed a simple, scalable method for synthesizing the HIV drug candidates prostratin and its derivative 12-deoxyphorbol-13-phenylacetate that obviates the need to isolate prostratin from plant sources. The method, reported in *Science* by a team led by Paul Wender, professor of chemistry at Stanford, paves the way for the commercial development of these compounds to treat latent HIV infection.[1]

There are no marketed HIV treatments that go after latent virus reservoirs—copies of the virus that lie dormant in host cells for years before becoming active. Most compounds that activate latent virus and make it susceptible to antiretroviral therapy are also plagued by side effects such as oncogenicity, T cell depletion and toxicity that make them unlikely adjuvants to HIV combination therapy.[2,3]

There are, however, two exceptions: prostratin and 12-deoxyphorbol-13-phenylacetate (dPP), which do not exhibit such side effects. Instead, they have another problem—they can only be obtained from plant sources in small quantities that are insufficient for preclinical or clinical development.

> **"We haven't pushed the limits of scalability, but we have more than enough compound for preclinical development purposes."**
> *—Paul Wender, Stanford University*

The Stanford method starts with one of two inexpensive, commercially available materials. One is oil from the seeds of the croton plant *Croton tiglium*. The other is oil from the seeds of the Barbados nut, *Jatropha curcas*, which is a source of biodiesel. Depending on which plant source is used—from *J. curcas* or *C. tiglium*—the synthesis has four or five steps, respectively. The four-step procedure gives overall yields of 40% for prostratin and 32% for dPP, whereas the five-step procedure yields 16% for prostratin and 13% for dPP.

Wender, who is a consultant to biotech and pharma companies on process and medicinal chemistry, said the synthesis can be easily scaled up to produce enough material for commercialization.

"This takes a major obstacle off the table by providing a reliable source of material" for development purposes, Wender told *SciBX*.

He noted the team stuck to a small scale in this study, in part because of cost considerations. "We haven't pushed the limits of scalability, but we have more than enough compound for preclinical development purposes," said Wender.

Wender also said the method is amenable to making derivatives of prostratin and dPP. The approach will allow researchers "not only to advance these leads but also to explore other variants that could be clinically superior," he said.

Philippe Gallay, associate professor of immunology at **The Scripps Research Institute**, agreed. "If prostratin is found to be safe *in vivo*, it will allow clinicians to test the possibility of eradicating all HIV reservoirs" in patients, he said.

Ahead of the curve

The method reported by Wender's team is important because prostratin and dPP have long been potential drug candidates for treating latent HIV.

In 1992, researchers at the National Cancer Institute's **Frederick Cancer Research and Development Center**, the **Southern Research Institute** and **Brigham Young University** showed that prostratin activated latent HIV but lacked the tumor-promoting activity of structurally similar compounds.[4]

Wender noted that preliminary evidence of prostratin's safety in humans does exist. The prostratin in that study was isolated from the bark of the mamala tree. Traditional Samoan healers use the bark to treat hepatitis without acute side effects, Wender said.

A 2003 study by a team at NCI showed that dPP, a non–tumor-promoting derivative of prostratin, was an even more potent activator of latent HIV than the parent molecule.[5]

The mechanism by which prostratin and dPP activate latent HIV is not fully understood. But a recent study by researchers at the **University of California, San Francisco** found that the transcription factor NF-κB plays a role in prostratin's activation of latent HIV.[6] That study and another by researchers at the **Mayo Clinic College of Medicine** identified specific isoforms of protein kinase C (PKC) that are also involved in the activation process.[6,7]

A third study showed that prostratin-induced PKC isoforms also inhibit viral entry by downregulating the expression of CD4 and CXC chemokine receptor 4 (CXCR4) on T cells.[8] That study was conducted by researchers at the **AIDS Research Alliance**, **Geneva University** and **Geneva University Hospitals**.

Hurdles jumped, others remain

Company and institution representatives contacted by *SciBX* agreed that the synthetic method removes a major obstacle in developing prostratin to treat latent HIV—but said other hurdles lie ahead.

Youssef Bennani, VP of drug innovation at **Vertex Pharmaceuticals Inc.**, said the authors had a "chemistry-enabling paradigm. There have been very few novel therapeutic developments in the area of latent HIV reservoirs."

According to Bennani, "most active natural products are generally structurally complex and synthetically inaccessible at larger, produc-

tion-scale levels. The ability to provide a scalable synthetic route to additional analogs opens wide the possibility of discovering more potent and safer drugs" to treat HIV.

Partners Vertex and **GlaxoSmithKline plc** market the HIV protease inhibitor Lexiva fosamprenavir.

"Latent HIV reservoirs are the reason HIV/AIDS is an incurable disease, so any progress in this area is worth watching," said Amy Espeseth, director of RNAi therapeutics at **Merck & Co. Inc.**'s Merck Research Laboratories.

Espeseth also agreed with Wender and Bennani that the method could lead to derivatives of prostratin and dPP that are more effective than either compound.

She did say the synthesis method reported in *Science* did not remove all obstacles from prostratin's path because "the experimental models for studying latency are extremely challenging."

Safety and efficacy aside, Bennani thinks prostratin might face another hurdle. "Scale-up into manufacturing still remains a significant challenge" because the key constituent in croton oil, one of the two suggested starting materials, is phorbol, a known tumor producer, he said.

Wender disagreed that croton oil presented a particular safety issue for scale-up. He said safe handling practices for croton oil have been part of its commercial production for decades.

"Almost all chemicals require special handling when used in bulk" as part of standard manufacturing procedures, said Wender. "Croton oil is not any more demanding than many others."

He said his team is screening prostratin and dPP derivatives for activity and developing structure-activity relationships.

"We are collaborating with others on the basic science connected to preclinical development," he said. "We are going to clear the runway to make this attractive to potential partners."

Stanford has applied for a patent on the method reported in *Science*.

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COMPANIES AND INSTITUTIONS MENTIONED

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Brigham Young University, Provo, Utah
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Geneva University, Geneva, Switzerland
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Mayo Clinic College of Medicine, Rochester, Minn.
Merck & Co. Inc. (NYSE:MRK), Whitehouse Station, N.J.
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