Research team achieves breakthrough in the production of an acclaimed cancer-treating drug

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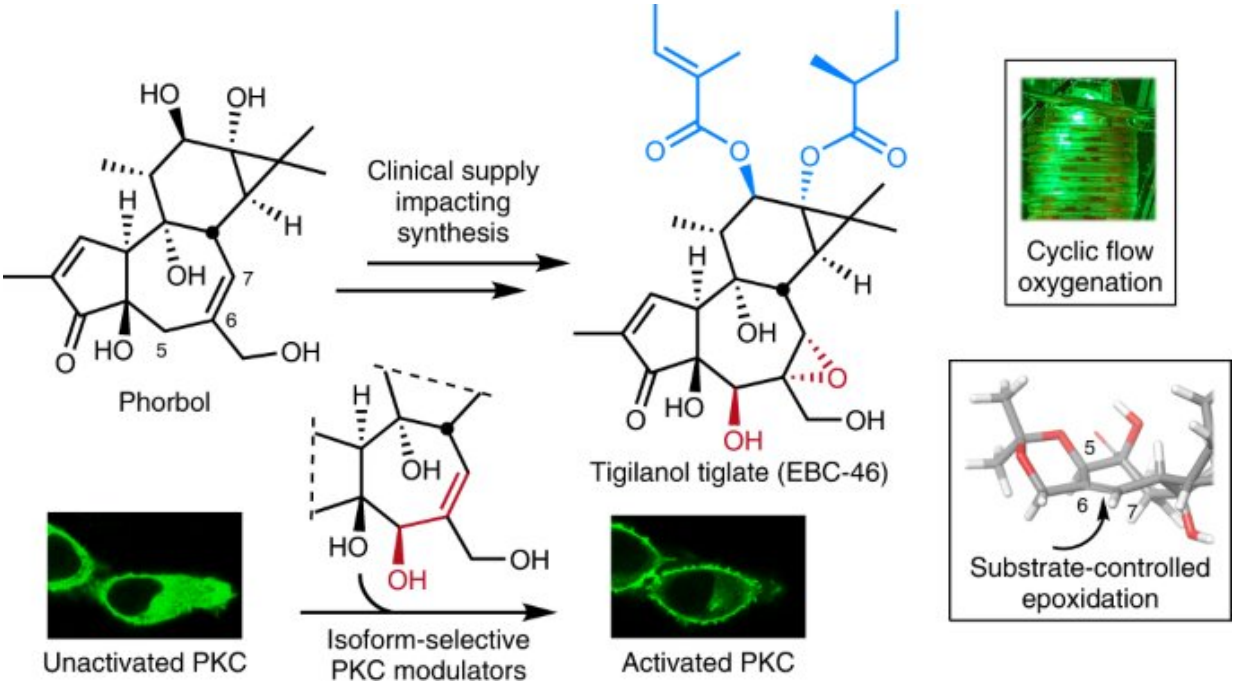

Credit: Paul A. Wender et al, *Nature Chemistry* (2022). DOI: 10.1038/s41557-022-01048-2

Stanford University researchers have discovered a rapid and sustainable way to synthetically produce a promising cancer-fighting compound right in the lab. The compound's availability has been limited because its only currently known natural source is a single plant species that grows

solely in a small rainforest region of Northeastern Australia.

The compound, designated EBC-46 and technically called tigilanol tiglate, works by promoting a localized immune response against tumors. The response breaks apart the tumor's blood vessels and ultimately kills its cancerous cells. EBC-46 recently entered into human clinical trials following its extremely high success rate in treating a kind of cancer in dogs.

Given its complex structure, however, EBC-46 had appeared synthetically inaccessible, meaning no plausible path seemed to exist for producing it practically in a laboratory. However, thanks to a clever process, the Stanford researchers demonstrated for the first time how to chemically transform an abundant, plant-based starting material into EBC-46.

As a bonus, this process can produce EBC-46 "analogs"—compounds that are chemically similar, but which could prove even more effective and potentially treat a surprisingly wide range of other serious maladies. These diseases, which include AIDS, multiple sclerosis, and Alzheimer's disease, all share biological pathways impacted by EBC-46's target, a key enzyme called protein kinase C, or PKC.

"We are very excited to report the first scalable synthesis of EBC-46," said Paul Wender, the Francis W. Bergstrom Professor in the School of Humanities and Sciences, professor of chemistry and, by courtesy, of chemical and systems biology at Stanford, and corresponding author of a study describing the results in the journal *Nature Chemistry*. "Being able to make EBC-46 in the lab really opens up tremendous research and clinical opportunities."

Co-authors of the study are Zachary Gentry, David Fanelli, Owen McAteer, and Edward Njoo, all of whom are Ph.D. students in Wender's

lab, along with former member Quang Luu-Nguyen.

Wender conveyed the immense satisfaction the research team felt over the EBC-46 synthesis breakthrough. "If you were to have visited the lab the first few weeks after they succeeded," said Wender, "you would've seen my stellar colleagues smiling from ear to ear. They were able to do something many people had considered impossible."

PhD students Edward Njoo, David Fanelli, Zach Gentry, and Owen McAteer. These researchers achieved the synthesis of the cancer-fighting compound EBC-46. Credit: Paul Wender

From a remote region

Tigilanol tiglate initially turned up through an automated drug candidate screening process by QBiotics, an Australian company. In nature, the compound appears in the seeds of the pink fruit of the blushwood tree, Fontainea picrosperma. Marsupials such as musky rat-kangaroos that eat blushwood fruit avoid the tigilanol tiglate-rich seeds, which when ingested trigger vomiting and diarrhea.

Injecting far smaller doses of EBC-46 directly into some solid tumors modifies the cellular signaling by PKC. Specifically, EBC-46 is proposed to activate certain forms of PKC, which in turn influence the activity of various proteins in the cancerous cells, attracting an immune response by the host's body. The resulting inflammation makes the tumor's vasculature (blood vessels) leaky, and this hemorrhaging causes the tumorous growth to die. In the case of external, cutaneous malignancies, the tumors scab up and fall off, and ways of delivering EBC-46 to internal tumors are being investigated.

In 2020, both the European Medicines Agency and the Food and Drug Administration in the United States approved an EBC-46–based medication, sold under the brand name Stelfonta, to treat mast cell cancer, the most common skin tumors in dogs. A study showed a 75% cure rate after a single injection and an 88% rate following a second dose. Clinical trials have since commenced for skin, head and neck, and soft tissue cancers in humans.

Based on these emerging research and clinical needs coupled with the source seeds' geographical limitations, scientists have considered setting up special plantations for blushwood trees. But doing so presents a host of issues. For starters, the trees require pollination, meaning the right sort of pollinating animals must be on hand, plus trees must be planted in appropriate densities and distances to aid pollination. Furthermore, seasonal and climate variations affect the trees, along with pathogens. Setting aside plots for blushwood trees further poses land use problems.

"For sustainable, reliable production of EBC-46 in the quantities we need," Wender said, "we really need to go the synthetic route."

Making EBC-46 from scratch

A good starting point for making EBC-46, Wender and colleagues realized, is the plant-derived compound phorbol. More than 7,000 plant species produce phorbol derivatives worldwide and phorbol-rich seeds are commercially inexpensive. The researchers selected Croton tiglium, commonly known as purging croton, an herb used in traditional Chinese medicine.

The first step in preparing EBC-46, Wender explains, is similar to an everyday experience. "You buy a sack of these seeds, and it's not unlike making coffee in the morning," said Wender. "You grind up the seeds and run some hot solvent through them to extract the active ingredient," in this case a phorbol-rich oil.

After processing the oil to yield phorbol, the researchers then had to figure out how to overcome the previously insurmountable challenge of bedecking a part of the molecule, called the B ring, with carefully placed oxygen atoms. This is required to enable EBC-46 to interact with PKC and modify the enzyme's activity in cells.

To guide their chemical and biological studies, the researchers relied on instrumentation at the Stanford Neuroscience Microscopy Service, the Stanford Cancer Institute Proteomics/Mass Spectrometry Shared Resource, and the Stanford Sherlock cluster for computer modeling.

With this guidance, the team succeeded in adding extra oxygen atoms to phorbol's B ring, first via a so-called ene (pronounced "een") reaction conducted under flow conditions, where reactants mix as they run together through tubing. The team then introduced other B ring groups in

a stepwise, controlled manner to obtain the desired spatial arrangements of the atoms. In total, only four to six steps were required to obtain analogs of EBC-46 and a dozen steps to reach EBC-46 itself.

Wender hopes that the far broader availability of EBC-46 and its PKC-influencing cousin compounds afforded by this breakthrough approach will accelerate research into potentially revolutionary new treatments.

"As we learn more and more about how cells function, we're learning more about how we can control that functionality," said Wender. "That control of functionality is particularly important in dealing with cells that go rogue in diseases ranging from cancer to Alzheimer's."

Wender is also a member of Stanford Bio-X and the Stanford Cancer Institute, and a fellow of Sarafan ChEM-H.

More information: Paul A. Wender et al, Practical synthesis of the therapeutic leads tigilanol tiglate and its analogues, *Nature Chemistry* (2022). DOI: 10.1038/s41557-022-01048-2

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