

## Simple chemical compounds, powerful anti-tumor drugs

By Leslie Mertz

In a laboratory at Wayne State University, a research team is making – and then tweaking – the structures of common compounds, called polyamines, that occur naturally in the body. These slight structural alterations are generating an amazing range of powerful drugs that fight cancer or battle numerous human parasites.

“The compound shown here is active against lung cancer, and it works in a way similar to the antitumor agent taxol. This related compound is an excellent trypanoside (anti-parasitic agent) and also works well against *Microsporidia*, a serious infection that occurs in AIDS patients. The drug industry is looking at that one for solid tumors, brain tumors and other types of tumors,” said Patrick Woster, as he sat at his desk and pointed to schematic drawings of various chemical compounds that he and his students have made. Woster, a professor of medicinal chemistry in the WSU Department of Pharmaceutical Sciences, leads the research team.

Woster’s work centers around polyamines, which are required for normal cell growth and division. “It has also long been known that the cellular requirements for polyamines are increased in rapidly dividing cell types, such as tumor cells and parasitic organisms,” he said. “The faster a cell divides, the more polyamines it’s going to need. The basis of our work is the hypothesis that if cells are deprived of polyamines in some way, then the cells that are dividing the fastest are going to die the quickest.”

The research team is proving the idea correct. Dr. Woster’s group of graduate students and postdoctoral researchers has

made dozens of slightly different polyamines with very different functions.

### The critical pathway

“Basically, polyamines are just polymers of two, three or four amino groups spaced by a certain length of carbon chain between them,” Woster said. (Amino groups are nitrogen and hydrogen assemblages depicted as  $\text{NH}_2$ .) Although scientists have not discovered all of the ways that polyamines are involved in cell growth and division, they do understand the pathway that makes them, he said. “We’re trying to chemically disrupt this pathway.” By shutting down this pathway, fast-dividing cells wouldn’t have access to the polyamines necessary for their survival.

In humans, polyamines originally come from the amino acid called ornithine. Each step of the pathway produces one of three different polyamines. In the first step, a specific enzyme converts ornithine into the polyamine known as putrescine. In the second step, two enzymes work together to change the putrescine into a separate polyamine called spermidine. Finally, with the help of two enzymes, the spermidine transforms into the polyamine called spermine. These three polyamines – putrescine, spermidine and spermine – are the commonly occurring varieties in humans. A back-conversion pathway uses two other enzymes to reverse the process.

For Woster’s work, he is concentrating on two of the enzymes critical to the pathway: AdoMet-DC and SSAT. AdoMet-DC, short for *S*-adenosylmethionine decarboxylase, is required for the generation of both spermidine and spermine in the forward

pathway. SSAT, or spermidine/spermine-*N*<sup>1</sup>-acetyltransferase, is necessary for the reverse pathway.

“We became very interested in AdoMet-DC, because the pathway uses it twice. You need it to make both spermidine and then spermine,” Woster said. His research group, along with long-time collaborator Robert Casero Jr. at Johns Hopkins Oncology Center in Baltimore, have synthesized and tested series of nucleosides (chemical compounds found in DNA) in hopes of inactivating AdoMet-DC. The synthesized compounds were similar to the naturally occurring compound S-adenosylmethionine, but not identical. In essence, this allowed the synthesized compounds to trick the enzyme into binding with them, and a subsequent reaction then inactivates the enzyme. Such compounds are known as “suicide substrates,” since the enzyme uses its own mechanism to kill itself. Without AdoMet-DC, spermidine and spermine decrease, and the polyamine pathway grinds to a halt.

Through their work, they confirmed that even small changes in these nucleoside-like inhibitors could have drastic effects on their function. They even found that mirror images (called enantiomers) of the same compound could have different potency against AdoMet-DC. Because these nucleosides are transported into parasites, but not into human cells, they act as parasite-specific toxins.

### **In the back door**

As they continued to study ways to shut down polyamine production, Woster began to focus on the reverse pathway. Here, the crucial player is the enzyme SSAT, which normally exists in the cell in very low quantities. SSAT’s job is to make a chemical change called an acetylation (an

addition of a -COCH<sub>3</sub> group) to spermine and start the reverse pathway. As part of the cell’s normal system to regulate its polyamine level, the cell recognizes any acetylated polyamines, and either oxidizes them to produce a shorter polyamine, or transports them out of the cell. Woster’s notion was to trigger a massive production of SSAT in a target cell. The high level of SSAT would acetylate most or all of the polyamines in the cell and, in turn, encourage the transporter system to eliminate or substantially reduce the polyamines. Without the required polyamines, the cell would die.

To test this hypothesis, they synthesized dozens of different polyamines designed to induce SSAT production. For some of the so-called polyamine analogs, Woster’s research group lengthened the carbon chains between the amino groups. For others, they added a chemical group to one or both of the polyamine’s ends, or added a different group to each end.

“In order to make the selective functionalizations we needed, we had to go through a lot in terms of the synthesis,” he explained. To add one chemical group to a specific site, for instance, a researcher has to chemically protect every other reactive site, so that the added group attaches in the proper location. The researcher repeats the steps for every added chemical group. He noted, “It’s kind of a pain to get these analogues properly put together, but I have very good students who work very hard on that.”

Now, of the more-than-100 polyamine analogs synthesized in Woster’s lab, nearly all have shown at least preliminary effectiveness in treating human cancer, parasite infections or other health conditions.

## Anti-tumor benefits

“One of the compounds that we’ve developed, called IPENSpm, causes a G<sub>2</sub>/M cell-cycle arrest.” In other words, the analog prohibits the cell from dividing by disrupting a critical phase of cell division known as tubulin polymerization. “The only other compound that’s known to do that is taxol, and, as you know, taxol is a red-hot cancer drug right now. So, here we have a reasonably simple molecule that has the same type of activity as taxol.”

Woster and his research team have spent a good deal of time investigating an analog called CPENSpm that has a three-membered-ring on one end. “We have found that it is active against a wide spectrum of breast tumor lines, as well as prostate, melanoma and lung cancer. The Johns Hopkins group has tested it on mouse tumors, and I’m told that it looks very promising.”

Continuing their studies, Woster wanted to see what would happen if they put a small chemical group on one side of a polyamine and a larger seven-membered-ring group on the other end. They made the compound, called CHENSpm, and tested it on two different types of lung tumor cells, including a large and particularly virulent tumor cell known as H157. “When we made the seven-membered ring, it wiped out the large-cell lung carcinoma. Even at small concentrations of just 1 micromolar, 99 percent of the tumor cells are dead.”

## The parasite connection

As the next step, Woster began studying analogs with even longer carbon chains. “When we made analogues with a seven-membered central chains and put them in with tumor cells, they did nothing: The

tumor cells continued to grow happily,” he said. Nevertheless, he found a use for these longer-chain compounds. “It turns out that these are good anti-parasitic agents. In fact, they kill the parasitic trypanosomes that cause African sleeping sickness, and also *Microsporidia*, which is an opportunistic infection that poses problems in patients who are immunocompromised.” He noted that a local high school student, Benjamin Wei, volunteered to work in his lab in the summer of 2000 and performed the synthetic work on one of these compounds, which turned out to be particularly effective.

They are also conducting investigations on an analog that works against both malaria and *Leishmania*. The latter parasite, which is transmitted through the bite of a sand fly, made news during the Gulf War when American soldiers became infected while serving in the Middle East.

Woster was a bit surprised by the effectiveness of polyamine analogs against these infections, but also very encouraged. “You don’t think about parasites much in this country, but if you look at (medical) newsletters from Third World countries, you see that people are dealing with really horrendous problems. The only effective drug for late-stage trypanosomiasis is an arsenic-containing preparation that kills 5 percent of the patients that take it. To prevent infection, they actually try to get rid of the insects that transmit the diseases by soaking rags in pesticides and hanging them over their windows at night. It really breaks your heart to see how bad it is.”

“Once we found out that these compounds worked, we started pursuing it, because what these countries really need is a drug that’s very simple to make, that can be made in large quantities and that can then be distributed at low cost to the people, who are often very poor.”

Unfortunately, he said, economics enters the picture. Drug development is a long and expensive process, and one that undeveloped nations cannot afford. He added that drug companies do not typically undertake this type of research unless they can justify it financially. “What we try to do on this end is get organizations or companies interested in picking up these compounds and developing them, but with drugs that are primarily for use in Third World countries, it’s very difficult to get that kind of sponsorship.”

### **The future**

Already, Woster’s lab has synthesized more than 100 polyamine analogs. Now, he hopes to begin to understand the details of how they work. “It appears that all of our compounds have a common endpoint: They cause apoptosis,” he said. “Apoptosis is a process whereby the DNA of the cell is broken down, and the cells essentially commit suicide. We have found that our

compounds can cause this to happen.” For the SSAT-inducing analogs, he believes apoptosis results from peroxide and lethal hydroxyl radicals that are generated as byproducts when their polyamine analogs interact with the enzyme. “We don’t know how the other analogs with the big rings work, but we do know that they cause the same results.”

By understanding the exact mechanism, he hopes to tailor the analogs for specific uses. “At first, making these analogs was sort of a crap shoot, but after we made the first few dozen, we started to get an idea of what was required structurally, and began making more informed choices,” he said. “Already, we’re getting to the point where we can start using little tricks to make compounds with different types of activity.”

All of the work builds upon itself, he commented. “Information like this is only going to help us make better drugs in the future.”

### **Biography**

*Dr. Patrick Woster earned his B.S. degree in pharmacy from the University of Nebraska Medical Center and his Ph.D. in medicinal chemistry from the University of Nebraska - Lincoln. He joined Wayne State University’s Eugene Applebaum College of Pharmacy and Health Sciences in 1998.*