Behind the Scenes in Pharmacognosy: The Klausmeyer Legacy

Editor's note: Our "Behind the Scenes" column regularly features the work of our Society's diverse members. Sadly, ASP member Mr. Paul Klausmeyer passed away this January (a memoriam to him may be found in this issue). Mr. Klausmeyer worked as a natural products chemist in the lab of Mr. McCloud at the National Institutes of Health (NIH) National Cancer Institute (NCI) in Frederick, Maryland, for 13 years carrying out analysis, isolation, purification, and identification of bioactive natural products as a part of the Developmental Therapeutics Program (DTP) anticancer drug discovery program. In this issue's column, Mr. McCloud describes the work Mr. Klausmeyer did leading to a recent Journal of Natural Products (JNP) *publication. Please read the full article entitled, "Histone Deacetylase Inhibitors from* Burkholderia thailandensis*," in* J Nat Prod. *2011, 74, 2007-2011. (doi.org/10.1021/np200532d.)*

By Mr. Tom McCloud and Dr. Amy Keller

How did you and your lab become interested in working with natural compounds that inhibit histone deacetylases (HDAC)?

The hypoxia inducible factor (HIF)-1α signaling pathway is considered a promising target for anticancer chemotherapeutic intervention, so a robust, high-throughput in vitro screen (HTS) for the detection of HIF-1α inhibitors was developed by the Screening Technologies Branch, NCI-Frederick. Utilizing a U251-HRE human glioma tumor cell line containing a luciferase reporter, and run under hypoxic conditions, greater than 60,000 extracts from the NCI Natural Products Repository in Frederick were assayed. The data indicated both HIF inhibiting and elevating activities existed in these crude extracts. The first priority was dereplication of these crude extracts giving inhibition of HIF-1α activity, which was a major assignment for Mr. Klausmeyer, who isolated and identified several small molecules of varying chemotypes that elicited a decrease of HIF-1α activity. We have previously published on some of these, listed below.

Mr. Klausmeyer receiving an award certificate for one of the winning posters at the 2008 NCI-Frederick Spring Research Festival by Dr. Howard Young, Head of the Cellular and Molecular Immunology Section, NCI-Frederick, August 6, 2008. The poster entitled, "HIF-1α Active Components of the Unstudied Plant, *Crossosoma bigelovii,"* by Paul Klausmeyer, et.al., was later presented at the 4th Interim ASP Meeting, Oxford, Mississippi, in 2008. This was later published in full form (see publication list).

MR. JON SUMMERS.

Initially puzzling at the time was a small subset of extracts which, rather than inhibiting HIF-1α, produced a 500% induction of expression as determined from the luciferase reporter. In order to more fully understand the performance of the screen, several of these HIF-high extracts were examined. One of these extracts was found to contain the known class 1 HDAC inhibitor FK-228, already under development as an anticancer drug (NOTE: has now been approved for clinical use as Istodax, Celgene Corp.). Since there are several families of HDACs, and interest in exploiting them as targets for new anticancer drugs, I felt it was worthwhile to search for a series of compounds with HDAC activity but different specificity from FK-228. Paul's manuscript on *Burkholderia* derived from that exploration.

What specifically was Mr. Klausmeyer's role in the research described, and what was it like to work with him?

Paul was an accomplished isolations chemist. The techniques he developed permitted 'high throughput dereplication' to be done in my lab, the Natural Products Support Group at NCI-Frederick. Being able to perform high throughput dereplication in a lab working in support of a high throughput screening operation is extremely important, as the number of 'hits' can quickly become unmanageable. The techniques for examining crude extracts including HPLC/multidetector analysis, creating a 96-well fraction plate and subsequently a 284-well retest plate, in four dilutions for testing, produced data which allowed for prioritization of biologically active extracts. Paul was given a list of crude extract hits from several different screens, and every couple of weeks we would go over the results of his dereplication studies, both chemical and biological data, and decide which 'hits' were worth spending more time on, and which were to be dropped. Both Paul and I were early risers. When I arrived at 7am, the coffee-

continued on page 8

continued from page 7

pot was already going and Paul was at work in the lab. He knew what had to be done, and went about it with very little additional guidance from me.

Could you provide a brief explanation of the work and results in your own words? In what way are the data in your paper new?

In the paper being featured, the compounds produced by *Burkholderia* have biological activity as HDAC inhibitors, and one is a new structure only slightly different from known structures, but unfortunately is another class 1 inhibitor with about the same specificity toward HDACs as FK-228, so it is not likely worthy of development as a new anticancer drug. The length of time from elucidation of structure to submission of manuscript exceeded 3 years, mostly due to the time spent in the NIH legal system in determining whether they wanted to file a patent. Getting this information into the scientific literature we hope will enable a fuller understanding of HDAC-interacting compounds and lead to more potent and more specific anticancer chemotherapeutics.

What impact does this research have on natural product science and health research in general?

This present publication, as with many others in the *JNP,* continue to demonstrate the wealth of yet-to-be-discovered biologically active molecules with drug potential to be found in plants, marine organisms, and microbes. The crude extracts are available in the DTP Natural Products Repository. It is only a matter of development of new screens for new targets, followed by testing, isolation, and structure elucidation, to find previously unknown compounds.

Fishing at the summer picnic which was held at Paul's farm this past summer, July 12, 2012. (There is a pond on the property). From left to right: Mr. Jerell Thompson, Mr. James Whitt and standing with the fish, Mr. Klausmeyer.

MR. TOM MCCLOUD.

More for interested readers:

Klausmeyer P, McCloud TG, Uranchimeg B, Melillo G, Scudiero DA, Cardellina II JH, Shoemaker RH. Separation and SAR Study of HIF-1α Inhibitory Tubulosines from *Alangium cf. longiflorum. Planta Med.* **2008**, 74:258-263.

Klausmeyer P, Zhou Q, Scudiero DA, Uranchimeg B, Melillo G, Cardellina II JH, Shoemaker RH, Chang CJ, McCloud TG. Cytotoxic and HIF-1α Inhibitory Compounds from *Crossosoma bigelovii. J Nat Prod.* **2009**, 72:805-812.