Behind the Scenes in Pharmacognosy

By Dr. Amy Keller

This summer, the Journal of Natural Products *published work from ASP member Dr. Tomofumi Miyamoto and colleagues at Kyushu University, Fukuoka, Japan, entitled, "Structure, Synthesis, and Biological Activity of a C-20 Bisacetylenic Alcohol from a Marine Sponge* Callyspongia *sp." Dr. Miyamoto discusses his lab's work on marine sponges and explains the origin of their futsal team name. Please read the full article in the* Journal of Natural Products*, 2013;76: 1337−1342 dx.doi.org/10.1021/np400297p.*

1. How did you become interested in working with bioactive compounds from marine sponges, and how did you come to focus on compounds that targeted the lymphatic system in the context of cancer?

First, I am interested in the chemical defenses (allelochemicals) of marine opisthobranchs. The order Nudibranchia comprises shell-less mollusks dressed in a colorful coat; their allelochemicals are often derived from the sponges they prey on. Some allelochemicals have shown a potent bioactive property against mammalian cell lines.[1] Our interest moved to the bioactive compounds of marine sponges. These organisms are much more abundant than those of marine mollusks. Since 2000, we have been applying molecular targeted screening against natural resources such as marine invertebrates and medicinal plants (crude drugs), and one of our molecular targets is angiogenesis and related factors in the endothelial cell proliferation. Some of them showed a good potential for lead compounds. Recently, we have focused on compounds that inhibit new lymphatic vessels, as therapeutics targeting tumor-associated lymphangiogenesis have not been established yet. In addition, we can use a lymphatic cell line (TR-LE), which was established by co-workers Drs. Koizumi and Saiki in 2006. This is our second paper about anti-lymphangiogenic compounds from natural resources.[2]

2. Who in your laboratory carried out the research?

This work was a collaborative effort among several research groups, however, the cell proliferation assay, bioassay-guided isolation, structure determination, and synthetic study, have been done by Dr. Takayuki Shirouzu. Dr. Shirouzu was a Ph.D. candidate of natural products chemistry and this work was part of his doctoral thesis; now he is a corporate researcher. One of our collaborators, Dr. Watari, who graduated from our lab, now an assistant professor of Dr. Ono's lab, Department of Pharmaceutical Oncology, assisted with the lymphangiogenic and cell cycle studies.

Dr. Rob van Soest, now in his new office at the Naturalis Biodiversity Center in Leiden, The Netherlands, continually helped us to identify marine sponges. Unfortunately, Dr. Shirouzu is not a good swimmer and is afraid of marine diving, so Dr. Chiaki Tanaka and I gathered marine sponges for him.

THE MIYAMOTO TEAM. Front left: Dr. Takayuki Shirouzu, holding synthetic reagents; front right: Dr. Chiaki Tanaka, holding marine sponge; back left: Dr. Kosuke Watar, holding a microplate; back right: Dr. Miyamoto, holding a copy of the manuscript.

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3. 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?

When Dr. Shirouzu isolated and determined the bisacetylenic alcohol, he and I were disappointed at the result because the compound was known and isolated from the same species. However, our compound was an isolated racemic mixture. The (-)-enantiomer had not been reported, and the anti-lymphangiogenic activity

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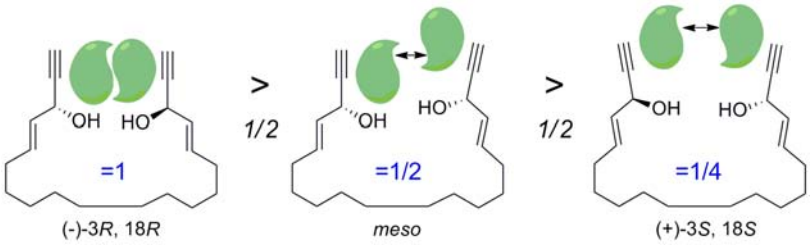
The natural products which have both anti-inflammatory and anti-lymphangiogenic activities might be good candidates for novel anti-cancer and anti-metastatic agents.



Plausible mechanisms of action of acetylenic alcohols.

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of bisacetylenic alcohol had not been examined. We separated the racemate using chiral-phase HPLC and evaluated the biological activity of each enantiomer; we found that these enantiomers showed different properties. To confirm this result and investigate the structure-activity relationships of bisacetylenic alcohols, we synthesized 11 derivatives and clarified the essential structure requirements for antiproliferative activity. Although the anti-lymphangiogenic activity of bisacetylenic alcohol was irrelevant, we are interested in the mechanism of the action of bisacetylenic alcohol, and we proposed a mechanism of action involving the stabilization of protein dimers. We are preparing a photo-affinity probe of bisacetylenic alcohol to identify the target molecule and to establish our plausible mechanism of action.

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

Recently, the close association of inflammation, angiogenesis, lymphangiogenesis and cancer progression, and metastasis in the tumor microenvironment has been highlighted. A lot of anti-inflammatory natural products have been reported, and several types of anti-angiogenic compounds have been found from natural resources. Furthermore, Dr. Watari has presented evidence that proinflammatory cytokine, IL-1ß can induce lymphangiogenesis and that this activity is mediated by the up-regulation of lymphangiogenic factors together with the recruitment and activation of macrophages.[3] Hence, the natural products which have both anti-inflammatory and anti-lymphangiogenic activities might be good candidates for novel anti-cancer and anti-metastatic agents.

5. What is a favorite nonscientific activity of your lab?

We enjoy playing tennis and futsal for recreation. "Anny" is the name of our futsal team. "Anny" is derived from Dr. Miyamoto's pet's name (a border collie). He mentions that the name of "Anny" is also the nickname of "Anakin Skywalker (Darth Vader)", but he accidentally spelled it "Annie" instead of "Anny."

The futsal uniform of team "Anny."

DR. TOMOFUMI MIYAMOTO]

6. What is your lab's motto?

"Love nature, enjoy nature, and learn from nature!" We remind ourselves that the improvement of structure determination is the most important and fundamental technique for carrying out our research.

7. What is your greatest extravagance in the lab?

GC-MS (Shimadzu QP5050) is the greatest extravagance in my lab, but the spectrometer was installed a decade ago. The NMR spectrometer (Varian INOVA 600) and ESITOFMS (Bruker micrOTOF) are communally owned equipment, but helium gas is mostly unavailable and is an expensive consumption when running the NMR and MS spectrometers in the last six months. ■

1. *Progress in Molecular and Subcellular Biology Subseries Marine Molecular Biotechnology*, Eds. Cimino, G., Gavagnin, M. Springer-Verlag, **2006**, Berlin, Heidelberg.

2. Jeong D, et al. Studies on lymphangiogenesis inhibitors from Korean and Japanese crude drugs. *Biol Pharm Bull.* **2013**, 36, 152-157.

3. Watari K, et al. Role of macrophages in inflammatory lymphangiogenesis: enhanced production of vascular endothelial growth factor C and D through NF-kappaB activation. *Biochem Biophys Res Commun.* **2008**, 377, 826-831.

Natural products drug discovery and development is very much alive and well...