3-2-2021

Neglected Nematodes: The Neurolenin Solution

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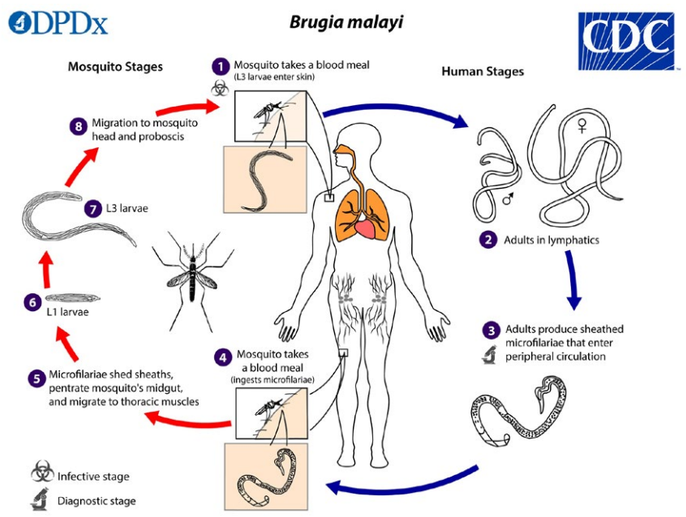
The Neurolenin Solution

Kevin Shea is a Professor of Chemistry at Smith College, Massachusetts. His research focuses on synthesising novel organic molecules and the development of pedagogical innovations for the instruction of organic chemistry. Steven A. Williams is Smith College's Gates Professor of Biological Sciences, and his research focus is the application of molecular biology to the study of neglected tropical diseases. Together, they investigate natural product isolation and derivatisation of neurolenin compounds for the treatment of lymphatic filariasis, a neglected tropical disease. Alongside this, they pilot a Course-based Undergraduate Research Experience for chemistry students at Smith College.

Over 1 billion people worldwide are affected by Neglected Topical Diseases (NTDs). This group of pathogens encapsulates viruses, bacteria and parasites, and disproportionately affects the world's poorest and most vulnerable communities in Africa, Asia and Latin America. Lymphatic Filariasis (LF) is an NTD caused by a parasitic nematode infection. The larvae of these filarial nematode worms are transmitted via mosquito bites and disrupt the functioning of the infected individual's lymphatic system. LF can be caused by three types of filarial worms, *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*, which are transmitted by the Culex, Anopheles and Aedes mosquito families. Symptomatic infection manifests in the form of lymphoedema and elephantiasis, in which patients experience highly debilitating and stigmatising enlargement of the limbs and genitals.

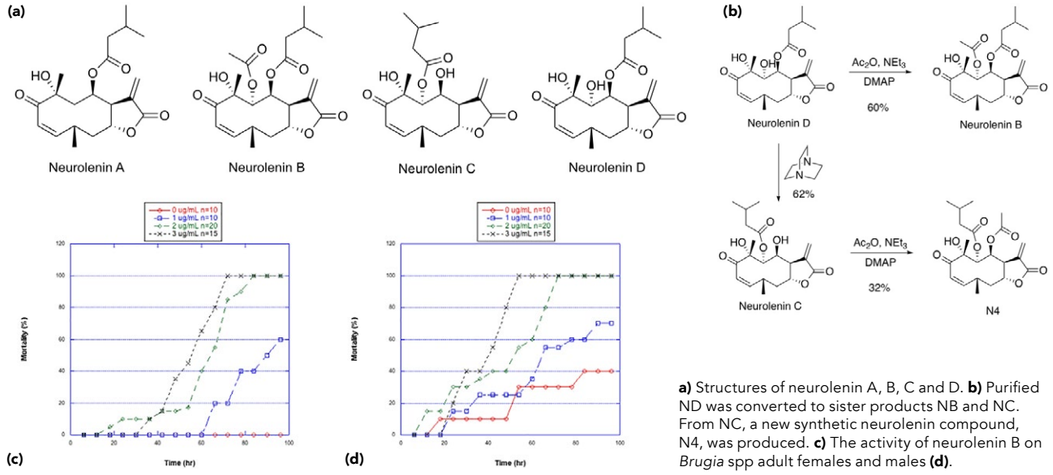
The World Health Organisation (WHO) estimates that 120 million people currently live with chronic LF. While the disorder is technically preventable through annual chemotherapy, most WHO-approved medications are ineffective against adult nematodes, meaning those with a chronic infection may need five to ten years of treatment. New medications that are filaricidal at all nematode life stages are essential if eradication of this disease is to be achieved. In recent years, scientists have looked towards the plant world for a solution.



DISCOVERING NEUROLENINS

Neurolaena lobata is a perennial flowering plant found in Latin America and the Caribbean. While its primary uses are founded in traditional medicine, a 2005 study showed that *N. lobata* leaf extracts exhibit filaricidal activity *in vitro*. The most broadly active *N. lobata* molecule is neurolenin B (NB), which displays anti-malarial and anti-viral properties. With the help of the Filariasis Research Reagent Resource Center, and many talented Smith College research students, Prof Kevin Shea and Prof Steven A. Williams investigated NB and its various synthetic and non-synthetic sister compounds as potential treatments for LF.

The Shea lab research team began by extracting pure neurolenin D from dried *N. lobata* leaves. ND, like its sister compounds neurolenin A, B and C, is an α,β-unsaturated carbonyl compound, and thus exhibits a conjugated alkene-carbonyl system. After successfully purifying ND, the team conducted several reactions (acetic anhydride, triethylamine and DMAP; bicyclic amine base DABCO, respectively) on the molecule to convert it to its sister products NB and NC. Following the success of these reactions, the Shea lab progressed to forming synthetic neurolenins. Using acetic anhydride, triethylamine and DMAP, they produced a new synthetic neurolenin compound, N4, from NC. They also treated ND with commercially available anhydrides, producing novel neurolenin esters N6, N7, and N8, all with differing lengths of ester carbon chains. N9, a doubly propionic ester, in which both the secondary and tertiary alcohols of ND had undergone esterification, was also produced, though in a low yield. Subsequently, using a hypervalent iodine reagent that oxidises secondary alcohols to ketones (Dess-Martin periodinane), the researchers were able to convert ND into the ketone N10. After producing several synthetic molecules by modifying neurolenin alcohol groups, they then investigated the activity of the alkene bonds in NB and ND by conducting hydrogenation reactions, and successfully generated the saturated alkanes N11 and N12. Once they had produced a variety of non-synthetic and synthetic molecules, the Williams lab set about testing each compound's filaricidal efficacy on nematode worms.



a) Structures of neurolenin A, B, C and D. **b)** Purified ND was converted to sister products NB and NC. From NC, a new synthetic neurolenin compound, N4, was produced. **c)** The activity of neurolenin B on *Brugia* spp adult females and males **(d)**.

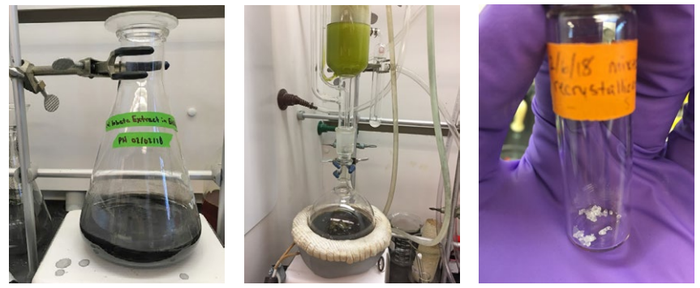
DETERMINING FILARICIDAL EFFICACY

The Williams research team tested the neurolenins on *Brugia* spp nematodes, by culturing the filarial worms in six-well plates, and differentially dosing each well with each of the compounds described previously. Nematode responses were recorded every 6 hours over a 104-hour period, and the filarial worms were declared dead on becoming immobile. In the first assay, wells were dosed with increasing (0, 1, 2, 3 ug/mL) doses of neurolenin B. All doses besides the control (0 ug/mL) achieved 100% nematode mortality within 72 hours, and NB filaricidal efficacy was confirmed. In the second assay, nematode response to 3 ug/mL dosing of neurolenins D, C, 6, 7, 8, 9, 10, 11, and 12 were recorded.

By comparing 7 neurolenins and neurolenin derivatives, Prof Shea and Prof Williams were able to pinpoint molecular groups directly responsible for Brugia spp mortality.

Both ND and NC achieved 90% mortality after 96 hours, showing a good, but slightly lower filaricidal efficacy than NB. As ND and NC are constitutional isomers of each other, the mirrored nematode response to both molecules was anticipated. In a similar manner, N4 achieved 100% morality after 78 hours. As N4 itself is a constitutional isomer of the highly filaricidal NB, this was an unsurprising, though promising, result.

The neurolenin esters N6, N7, and N8 all reached 100% mortality within 66-72 hours. Although these three compounds demonstrate strong antifilarial activity, no significant difference in activity was distinguishable between them, indicating that the length of the ester carbon chain does not determine lethality. The neurolenin ketone, N10, showed comparable activity to ND and NC, achieving 100% mortality within 80 hours. The lack of difference in activity between ND, NC and N10 is notable, although neither the ketone (N10) nor the alcohol compounds (NC, ND) are as effective as those that carry an ester at the same position (B, 6,7,8). The doubly propionic esterified neurolenin, N9, and the hydrogenated molecules N11 and N12 showed little antifilarial activity, indicating that removal of the tertiary alcohol group (N9) or removal of both C=C double bonds (N11,



Students isolated and purified neurolenin compounds from *N. lobata* leaves, using Soxhlet extraction.

N12) is directly correlated to abolition of filaricidal behaviour, suggesting that both groups are essential components to ensure biological interaction with *Brugia* spp. By comparing these 7 neurolenins and neurolenin derivatives, the Shea and Williams labs were able to pinpoint molecular groups directly responsible for *Brugia* spp mortality; the most effective compounds contain two ester chains in addition to one tertiary alcohol (N4, N6, N7, N8 and NB), while the compounds without an alcohol group (N9) or any conjugated alkenes (N11, N12) exhibit no activity. Neurolenins B and 4 remain the most effective agents.

LESSONS LEARNED: APPLYING RESEARCH TO EDUCATION

Prof Shea then applied their research findings to his teaching at Smith College. Along with colleagues David Gorin and Kerry Barnett, he piloted an undergraduate-level research project for second-semester organic chemistry students, focused on the isolation and derivatisation of neurolenins.

Course-based Undergraduate Research Experiences (CUREs) are currently employed as a teaching device at many universities across the United States, both in introductory and advanced courses. A central pillar of CURE programmes is to create a 'true sense of discovery', in which neither the student nor instructors know the outcome of experiments. This type of course helps foster student independence, encourages student-work ownership and promotes high-level scientific communication between teachers and students. Prof Shea utilised the CURE format in the design of his own 12-week Organic Chemistry laboratory series for second-semester undergraduate chemistry students. During the first 4 weeks, students attended weekly 3-hour practical sessions, where they isolated, purified and identified neurolenin compounds from *N. lobata* leaves, using Soxhlet extraction, charcoal purification, flash chromatography and NMR spectroscopy. Alongside these lab sessions, students were asked to use literature sources and lecture material from first-semester Organic Chemistry classes to design a set of experimental protocols for the production of synthetic neurolenins. In week 6, the students presented their proposed reactions to the class, in the form of a student symposium, aimed at nurturing inter-student collaboration. The students then used the remaining 6 weeks of the course to carry out their planned reaction schemes.

Throughout this period, Prof Shea, Prof Gorin, and Prof Barnett reviewed the students' ideas, advising against hazardous or overly complex reactions. While some reactions were extremely successful, others failed to produce anticipated results. This helped develop the students' understanding of the authentic research process and environment; in science, things rarely go 'to plan'. At the end of the 12-week course, the students presented their findings at a public Smith College poster session and wrote up their results in a formal lab report. Students in the CURE lab sections performed as well in the lecture portion of the course as students in traditional lab sections where lecture and lab content were coordinated. Significant gains were made in student attitude, confidence, and perceived overall understanding of the course content. Students also reported having a stronger sense of learning community. All of these metrics demonstrate that CURE-based programmes can provide a transformative educational experience.

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HOPE FOR THE FUTURE

Over the course of their studies, Prof Shea and Prof Williams have not only produced successful antiparasitics that pave a route towards drug development for lymphatic filariasis, but they have also demonstrated the effective translation of their research into an exemplary study course that fosters both students' independence and education. Looking to the future, the team hopes to utilise their research to determine the efficacy of neurolenins against additional neglected tropical diseases, alongside inspiring other educational establishments to take up the mantel and employ CURE-based learning in their curriculums.

Students participating in the CURE programme (top row) and students from the Shea lab (bottom row).

Behind the Research

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Research Objectives

Prof Shea and Prof Williams are working to develop a treatment for lymphatic filariasis, a disease transmitted to humans by mosquitos. This research project has also been used in an intermediate organic chemistry teaching lab.

Detail

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Bio

Kevin Shea is a professor of chemistry at Smith College in Northampton, Massachusetts. He was an undergraduate at WPI and received his PhD in synthetic organic chemistry at MIT. His research interests are in the synthesis of novel organic molecules and chemical education focused on teaching organic chemistry.

Steven A. Williams is the Gates Professor of Biological Sciences and Biochemistry at Smith College and on the faculty of the Graduate Program in Molecular and Cellular Biology at the University of Massachusetts. He received his PhD in Genetics from the University of California, Davis. His research interests are in the application of molecular biology and genetics to the study of neglected tropical diseases.

Funding
This work has been supported through funding from Smith College, the Howard Hughes Medical Institute's Undergraduate and Graduate Science Education Program, the Camille and Henry Dreyfus Foundation Special Grant Program and the Blakeslee Fund for Genetics Research at Smith College.

Collaborators

- Kerry Barnett, Assistant Professor of Chemistry, University of Central Arkansas
- David Gorin, Associate Professor of Chemistry, Smith College
- Susan Haynes, Research Technician, Smith College
- Shea Lab Research Students: Catherine McGeough, Megan Neubig, Peyton Higgins, Anisha Tyagi, Urvi Savant
- Williams Lab Research Students: Saira Huq, Kristine Trotta, Meghna Purkayastha, Lizzette Perez Perez, Lydia D'Angelo
- The Filariasis Research Reagent Resource Center (FR3)

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Personal Response

What neglected tropical diseases do you hope to test neurolenins on next?

"Lymphatic filarial parasites belong to a much wider group of nematode parasites that infect hundreds of millions of the world's poorest people. These diseases include the soil-transmitted helminth (STH) parasites, hookworm, whipworm and roundworm, which together cause over 1.5 billion infections worldwide and are a major contributor to the devastating cycle of disease and poverty. Major impacts of STH infections in children include anemia and concomitant delays in mental and physical development. These STH parasites will be the next targets for neurolenin testing."

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