Alternative antimalarials

With a background in chemistry, Dr Adrian Martin Pohlit discusses his motivations to derive new drug leads from natural products in the Amazon.

What inspired you to pursue research on natural products within the context of malaria?

While studying for my PhD thesis at the University of São Paulo, Brazil, under the supervision of the late Professor Helena Maria Carvalho Ferraz, I became interested in the chemistry related to the total synthesis of artemisinin (an antimalarial isolated from the leaves of Artemisia annua); semi-synthesis of derivatives of artemisinin (more active and druggable than artemisinin and, at the time, being introduced all over the world in clinical practice as antimalarials); and also analogues containing the artemisinin pharmacophore. I began to read voraciously on the chemistry and antimalarial activity of these different compounds. As a synthetic chemist, the challenge was to come up with something new, artemisinin-like in terms of chemical structure and with antimalarial activity, to obviate the necessity of having to harvest A. annua and isolate artemisinin.

I took a job as a chemistry professor at the Federal University of Amazonas (UFAM), Manaus, Brazil, and began to study plants that could be abundant sources of the precursors. I had envisioned. The Amazon region has a rich tradition of medicinal plant use in the treatment of many diseases, especially malaria. So it was here that I learnt to work with natural products and discovered the richness and diversity of locally used antimalarial plants, many of which were actually brought by migrants from other parts of Brazil or the world.

A. annua is, to my mind, the traditionally used antimalarial plant to beat, and the history of the development of artemisinin derivatives is the proof of concept that a natural product can be transformed into an affordable, effective drug used worldwide. It is this basic tenet that I hold in moulding my approach to antimalarial drug discovery (and I am not alone in this approach).

Can you describe the advantages of training students from local graduate schools in the multidisciplinary research conducted in your lab?

It is important to have professionals who are trained in conducting research on relevant aspects of the knowledge of the region’s plants and other organisms to exploit the wealth of accumulated genetic material present. Human resources and education are the key ingredients to any scientific study. We would like to attract green industries to this region to become involved in the commercialisation of products in a sustainable way and one that would otherwise research, develop and manage biodiversity in a rational manner. Medicinal plants have an enormous potential, though many products from the Amazon region are plant materials, crude extracts and essential oils that are accepted in many foreign markets. Many of these products are not sustainably harvested, despite the existence of technology for cultivation.

What is the next step in translating your work on ellipticine, a polyaromatic small molecule hemozoin-formation inhibitor, into a form suitable for clinical use?

We are looking for collaborators to help synthesise more ellipticine derivatives. The great thing is that ellipticine and its derivatives are relatively straightforward to synthesise and a number of syntheses have been published or are no longer covered by patents. We (and others) have shown that derivatives are more active in vitro than ellipticine. So, we need help from partners to synthesise ellipticine derivatives which we could then test and help optimise.

Do you believe your approach to derivatising natural antimalarials from the Amazon will prove successful?

What we argue is that people who use plants in traditional settings have helped identify the natural substances (quinine, artemisinin) that work for them in their infusions, decoctions, etc. and historically, knowing that quinine and artemisinin worked in clinic, allowed synthetic and medicinal chemists to develop the quinoline and artemisinin derivative classes of antimalarials, now the basis of antimalarial therapy worldwide. Quinolines are purely synthetic compounds inspired by quinine’s structure. Artemisinin derivatives are made from artemisinin, which comes from A. annua. Both approaches are valid, and human use of plants gave us, and will further provide us with, these molecules from which to develop antimalarials.

What does the future hold for your research?

We are now actively screening plant extracts of antimalarial plants used in the Amazon region according to ethnobotanic literature sources as part of graduate student projects. We plan to continue to study the chemical composition of active extracts in search of active antimalarial compounds. We will also be looking at the synergism of compounds known not to be antimalarial themselves, but that are known to enhance the antimalarial activity of other compounds.
Traditional remedies as novel drug leads

Working with plant extracts long known to have antimalarial properties, a group at the Brazilian National Institute of Amazonian Research is searching for new compound leads for development into pharmaceuticals.

CONSIDERED ONE OF the biggest global killers, malarial infection was responsible for an estimated 627,000 deaths worldwide in 2012. The disease is caused by Plasmodium parasite species carried by Anopheles mosquitoes and spread through an infected mosquito’s bite. There is a growing need for alternative therapeutics for malaria due to the rapid emergence of resistance to current drugs. The two most commonly used pharmaceutical groups to treat malaria, quinolines and artemisinins, were originally inspired by molecules isolated from natural products that had been long used by local populations to treat the disease. In fact, natural products have been the source, directly or indirectly, of two-thirds of all drugs introduced in the past 30 years.

ETHNOPHARMACOLOGICAL STUDIES

The INPA team employs natural products from plants traditionally used for malarial treatments in the Amazon. Pohlit’s potential drug targets are those that are verifiably found in the plant part that has been locally used to treat malaria or malarial symptoms. As an example, this could be carapanaúba bark or peroba tree (Aspidosperma species), from which members of Pohlit’s group isolated ellipticine.

Once a candidate has been identified, studies must be carried out to determine its antiplasmodial efficacy. First, in vitro screens are performed. Blood stage antiplasmodial assays are regularly used with cultured human malaria parasites, involving simple counting of parasites in the blood after the drug has been administered. Antiplasmodial activity can then be further characterised in vivo with rodent models using the rodent malarial species, P. berghei. In these latter stages of activity characterisation, data on the effects of drug metabolism and pro-drug compound existence (ie. a precursor of the active compound) can be further understood. As a result, the team is afforded a better understanding of the natural compound’s potential as a future drug.

However, in antiplasmoidal research more focus is now being placed on the mosquito infective stage, when the parasite is in its sexual form, and the liver stage, which is soon after plasmodial infection in the human. Pohlit’s team is therefore looking to collaborate with other groups to expand their repertoire of assays to screen for drug effects on these stages.

ELUCIDATING ELLIPTICINE EFFECTS

The aforementioned ellipticine is isolated from Aspidosperma species and derivatives have previously been used in cancer chemotherapies. “There is evidence that ellipticine acts by another mechanism that is specific to the malaria parasite (inhibition of haemozoin formation by the parasite), not present in human cells,” describes Pohlit. His screens identified the agent to be highly active against P. berghei in the rodent model and, in terms of animal survival and parasite suppression, had similar antiplasmodial activity as the routinely used chloroquine. Therefore, ellipticine has significant potential for being developed into an antimalarial drug.

Pohlit and colleagues have since built on this work by preparing their own simple derivatives, including a nitro derivative which had greater in vitro antiplasmodial activity than ellipticine. Further to this, olivacine, a structurally related isomer of ellipticine, displayed similar in vivo properties to ellipticine. This opens up both olivacine and ellipticine as part of a novel class of structurally simple antimalarials.
A SUSTAINABLE AMAZON

An important strand of Pohlit’s research into antimalarials develops alongside the experimental aspects. The project has involved training large numbers of PhD and Master’s students from Amazonian biotechnology, chemistry and pharmacology graduate schools. In particular, training of the graduate students in laboratory procedures took place during the 10 years required to set up the in vitro screening lab in Manaus. Importantly, this involved bringing in experts and incorporating visits to labs nationally and internationally, which was essential to his group’s success: “Each compound that is isolated has a rather large number of unknown or undetermined properties, so a person must be trained to be able to handle many unknowns and still conduct research,” Pohlit details.

Projects such as NOSSAPLAM seek to build local capacity and technological knowledge for maximum benefit of the region’s research. Such an approach is also expected to allow industry to develop more sustainably and provides high-quality training in the local area: “We are interested in having trained personnel hired as staff at our local institutions to further the research efforts of our labs and the ambitions of the fledging industries to further the research efforts of our labs and the ambitions of the fledging industries interested in commercialising medicinal plants, phytochemicals, botanicals and other products from this region,” Pohlit explains. Although Brazil has a rich and largely unexplored selection of molecules with possible therapeutic activities, its role in this area is as a plant supplier. Potentially, if capacity and capability increases, research on plant-derived substances could be improved and more optimal drugs produced, while still valuing the biodiversity of the local area. “The approach involves the training of local human resources in aspects of applied chemistry and related fields that will hopefully contribute to local development and capacity building in the area of drug discovery in this region,” summarises Pohlit.

FUTURE DRUGS

Ellipticine was just one example that Pohlit identified as having antiplasmodial activity during the project’s duration. Additionally, aspidospermine (from an Aspidosperma species) and neospergulide (from a Picrolema species), chemical components from some of the most widely used antimalarial plants in the Amazon, and 4-norlidiacatechol were studied, but all need additional work to position them in terms of drug lead potential. Also, few tests have been carried out on these substances’ action on P. vivax, the most common Plasmodium species in the Amazon region. Increased screening facilities will be required in malaria endemic areas to support the drug discovery process from the lead compounds that Pohlit has identified to a marketable drug. He is continuing the screening of plant extracts identified by ethnobotanic literature as graduate student work and, through this, the team expects many more lead compounds to be found. Building on this, the group will also start investigating the potential of some compounds to act synergistically with antiplasmodial substances to increase their antimalarial activity.

Pohlit and colleagues have confidence in ellipticine, olivine and their derivatives as potential future antimalarials, of which syntheses already exist. This perfectly positions the new class of drugs to be scaled up for production by a pharmaceutical company. Collaboration with synthetic chemists is necessary to gain a deeper understanding of the medicinal chemistry of these compounds. Soon, these natural products could be passed on to local companies for sustainable development into marketable drugs.