Structured design for drug discovery

Principal Investigators Drs Larissa Podust and William Roush discuss their progress in developing new inhibitors that will result in better treatment options for Chagas disease

What led to the formation of the Podust Lab?

LP: My interest in neglected tropical diseases began about 10 years ago when one of the cytochrome P-450 enzymes I had been analysing, sterol 14α-demethylase (CYP51), was identified as a therapeutic target in Trypanosoma cruzi, the parasite that causes Chagas disease.

Could you describe the underlying mechanisms that need to be understood for effective treatments to be developed for patients suffering with chronic Chagas disease?

LP: Most T. cruzi parasites in chronic infection reside intracellularly, largely in the heart, gut and skeletal muscles, so anti-T. cruzi drugs must be lipophilic enough to penetrate the cell membrane, and reach deep tissues. It’s a balancing act, though, because poor water solubility leads to ineffective drug absorption.

Do anti-fungal drugs work against CYP51?

LP: The antifungal drug posaconazole (Noxafil, Merck) has superior anti-T. cruzi potency in a mouse model of Chagas disease, and a record of alleviating chronic Chagas disease in humans. It has favorable drug metabolism and pharmacokinetic properties, including oral availability, long terminal half-life and large distribution volume. And yet the majority of azole antifungals in clinical use, and those in development, are not curative in animal models of T. cruzi infection. Not only that, scientific meeting reports and a recent announcement from the Drugs for Neglected Diseases Initiative (DNDi) about recently completed clinical trials of posaconazole and ravuconazole (Eisai, Tokyo) conclude that neither drug is superior to benznidazole. This means that the quest for an anti-Chagas cure must continue.

Could you provide an overview of your project to design a new series of 4-aminopyridyl-based inhibitors targeting T. cruzi CYP51?

WR: The project began with the discovery of an initial non-azole hit compound, LP10, from high-throughput screening at the University of California, San Francisco (UCSF). We used LP10 as a starting point to evolve more potent CYP51 inhibitors with substantially improved drug-like properties. We use structure-based design techniques guided by co-crystal structures and homology models of CYP51-bound inhibitors.

What arose from your structure-activity and structure-property relationship analyses of the features that enhance the biochemical and cell-based activity and microsomal stability of the LP10 series of CYP51 inhibitors?

LP: The half-maximal effective concentration of some members of the new scaffold series improved by orders of magnitude compared with the initial non-azole hit. And as the potency of hits improved, so did the resolution and quality of the co-crystal structures. Now we use these high-resolution structures to identify site-directed interventions for further lead improvements. To speed up the testing cycle of compounds in vivo, we adopted a four-day mouse model utilising a T. cruzi strain expressing firefly luciferase (a gift from Drs Barbara Burleigh, Harvard School of Public Health, and Ana Rodriguez, New York University). While the parental compound LP10, which previously demonstrated potency in a 30-day mouse model, is only weakly active in this new, more stringent model, the bar is set high in order to accelerate compound prioritising for longer-term dosing studies and ensure success of hit-to-lead optimisation. It is more likely that targeted inhibitors, optimised by structure-based drug design and pharmacokinetics parameters, will be more effective in human T. cruzi infections than the antifungal agents.

Looking back on your research into Chagas disease, is there a particular achievement of which you are most proud?

LP: A major accomplishment was establishing a Chagas disease drug discovery pipeline, distinct from other efforts in the field, which is based on de novo, structure-aided medicinal chemistry. Funded by a National Institutes of Health R01 grant, my team has assembled the drug leads, methodologies and tools necessary to successfully prosecute each step of the lead optimisation process on the way to ultimately achieving parasitological cure in humans.

WR: Our most significant achievement is the identification of a new CYP51 inhibitor series. It has the potential to become a new class of anti-Chagas drugs. We have published results showing CYP51 inhibitors with picomolar activity (<10⁻⁹ M) against T. cruzi amastigotes in cultured mouse myoblasts. Encouragingly, these molecules have very promising in vitro pharmacokinetic properties, including microsome stability and lack of inhibition of human metabolic CYPs.
Scaffolding a cure for Chagas disease

Researchers at the Center for Discovery and Innovation in Parasitic Diseases, San Francisco, and the Scripps Research Institute, Florida, are combining their expertise in structural biology, medicinal chemistry, parasite and host-parasite biology to find a safe, durable therapy to combat Chagas disease.

**CHAGAS DISEASE AFFECTS** about 8 million people in South America, where it is the leading cause of heart failure. In Brazil alone decreased earnings capacity and lost productivity due to Chagas exact a cost of more than US $1.3 billion a year. International travel, infected blood transfusions, co-infection with HIV and migration of the ‘kissing bug’ insect vector that spreads Trypanosoma cruzi, the parasite that causes the disease, all help to drive up the number of cases and push the incidence outside the historic range. This neglected tropical disease is now seen in Europe, North America and Asia and seems set to become an urgent public health issue in countries far beyond its source in South America.

Though it can prove fatal at any stage, Chagas disease can be asymptomatic in both the acute and chronic stages, so infected people may not seek treatment in a timely fashion. In addition, although in the past antiparasitic treatment was not recommended for chronic patients, the standard of practice has changed, and treatment is now recommended for all acute and chronic patients. Another important issue is that treatment itself can be unsafe. The only available drugs, nifurtimox and benznidazole, developed more than 40 years ago, both carry the risk of grave side effects. Against the initial acute stage these drugs are about 80 per cent effective, but in the much longer chronic stage their efficacy is controversial. To complicate things further, some T. cruzi strains have developed resistance to them. Taking all factors into account, it is clear that new ways of treating Chagas disease must be discovered.

Dr Larissa Podust is aiming at sterol 14α-demethylase (CYP51), an enzyme in the cytochrome P450 family, as T. cruzi’s weak point. At her laboratory in the Center for Discovery and Innovation in Parasitic Diseases (CDIPD), at the University of California, San Francisco, she studies enzyme reactions in the neglected infectious diseases that afflict poorer communities across the world, and generates candidate compounds to treat them.

**TARGETING CYP51**

In a shift in anti-Chagas disease strategy, the azole drugs fluconazole, ketoconazole and posaconazole have been used to target CYP51 in T. cruzi-infected mammalian cells. Originally developed for pathogenic fungal infections, these drugs have been shown to react with the same cellular target in trypanosome parasites. “A popular approach in drug discovery is to piggyback on industry research directed at other diseases, so we decided to capitalise on the fact that CYP51 is an important therapeutic target in both fungal and parasitic infections due to its role in the biosynthesis of ergosterol, an essential component of cell membranes in fungi and protozoa, including T. cruzi,” observes Podust.

Indeed, the similarity between sterols and their biosynthetic pathways in both systems has led to several recent clinical trials of azole antifungal agents for treating Chagas disease patients. Results thus far have been less than optimal, though, with only low efficacy over a longer time frame. Further trials, at different doses or in combination with benznidazole, are thus indicated. But the strategy of using azole chemotypes against Chagas disease is further complicated because resistance to azoles has emerged in T. cruzi cell cultures and infected mice. Accordingly, Podust decided to attack the problem from a different angle, with entirely new chemical scaffolds.

**TEAM EFFORT**

Podust obtained funding from the National Institutes of Health (NIH) for a collaborative project to develop candidate CYP51 inhibitors with improved properties and new non-azole chemotypes. The collaboration, between Podust and Dr Jair Lage Siqueira-Neto, also at CDIPD, co-Principal Investigator Dr William Roush from the Scripps Research Institute in Florida, and Dr Claudia Calvet at Oswaldo Cruz Foundation (Fiocruz) in Rio de Janeiro, aims not only to find a drug to cure Chagas disease, but one practical for use in the typical Chagas disease patient. “Our goal was to build a scaffold with the key features of clinical drug candidates: potency against the therapeutic target, oral bioavailability, long terminal half-life and high tissue tropism,” Podust explains. “By optimising each of these attributes, we hope to discover a safe, affordable drug for Chagas disease that T. cruzi won’t become resistant to later on.”
The collaboration aims not only to find a drug to cure Chagas disease, but one practical for use in the typical Chagas disease patient.