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.

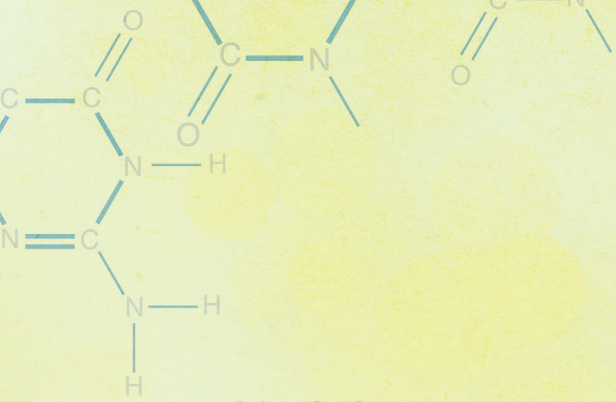
What arose from your structure-activity and structure-property relationship analyses of the features that enhance the biochemical and cell-based activity and microsomal stability of the LP10 series of CYP51 inhibitors?

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 AFFLICTS 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.

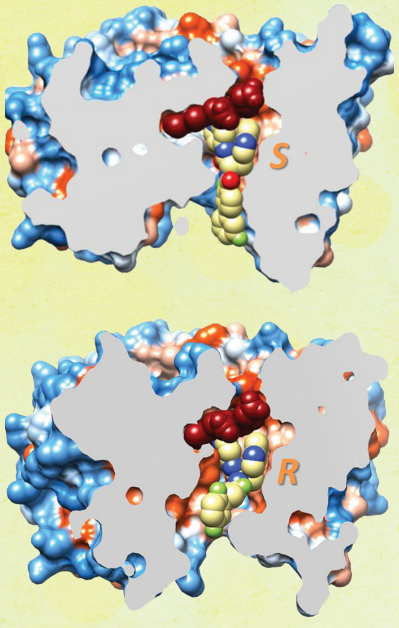
Dr Larissa Podust is aiming at sterol 14α-demethylase (CYP51), an enzyme in the cytochrome P450 family, as *T. cruzi*'s weak point. At her laboratory in the Center for Discovery and Innovation in Parasitic Diseases (CDIPD), at the University of California, San Francisco, she studies enzyme reactions 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."



Slice through the CYP51 target shows *S*- (top) and *R*- (bottom) stereoisomers of LP10 derivatives (yellow spheres) interacting with the hydrophobic surface of the CYP51 active site (orange). Hydrophilic protein surface is coloured blue. Haem is shown in dark red van der Waals spheres.

STRUCTURE-AIDED DESIGN OF CYP51 INHIBITORS FOR TREATMENT OF PARASITIC INFECTIONS

OBJECTIVES

To discover and develop new therapies for Chagas disease based on the inhibition of parasite sterol biosynthesis.

KEY COLLABORATORS

Principal Investigators: **Dr William Roush**, Scripps Research Institute, Florida, USA • Team members: Professor **James McKerrow**; **Dr Jair Lage Siqueira-Neto**, University of California, San Francisco (UCSF), USA • Former postdoctoral trainee **Dr Claudia Calvet**, Fiocruz in Rio de Janeiro, Brazil

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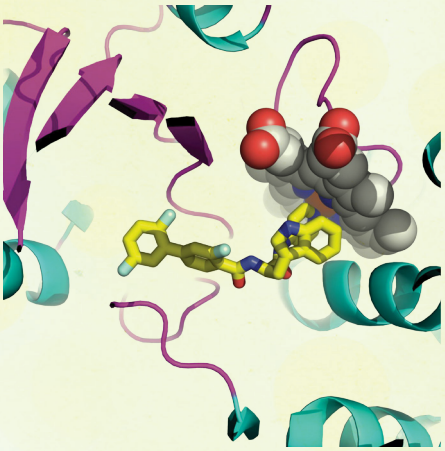
LARISSA PODUST received her PhD in Chemistry from Novosibirsk State University, Russia, and subsequently completed postdoctoral training, first in the enzymology of DNA replication at the University Zurich-Irchel, and then in protein X-ray crystallography at Vanderbilt University. She is currently Adjunct Assistant Professor at the UCSF School of Medicine, California. Her research interests are primarily focused on neglected tropical diseases and specifically Chagas disease. Podust has contributed 69 depositions into the Protein Data Bank (PDB) of protein crystal structures from 15 different organisms. She has co-authored 55 peer-reviewed publications.

WILLIAM ROUSH received his PhD in Chemistry and postdoctoral training from Harvard University. He held faculty positions in Chemistry at MIT, Indiana University and the University of Michigan before assuming his present positions as Professor of Chemistry and Executive Director of Medicinal Chemistry at the Scripps Research Institute, Florida. In addition to his longstanding interests in the synthesis of natural products and development of new synthetic methodology, he has focused on a range of medicinal chemistry topics including the development of cysteine protease inhibitors and CYP51 inhibitors targeting *T. cruzi*. He has co-authored more than 300 peer-reviewed publications.

CDIPD CENTER FOR DISCOVERY AND INNOVATION IN PARASITIC DISEASES

The collaboration aims not only to find a drug to cure Chagas disease, but one practical for use in the typical Chagas disease patient

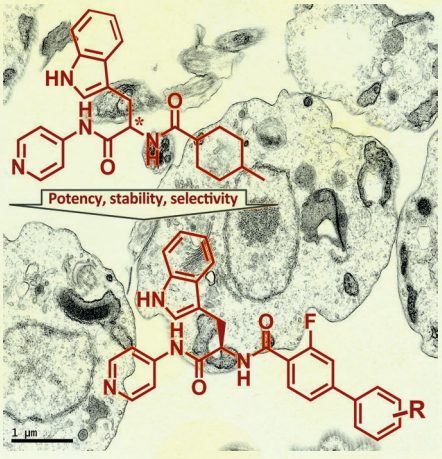


LP10 derivative (yellow) bound in the active site of *T. cruzi* CYP51 in the 2.8 Å resolution x-ray structure. Protein is represented by the ribbon; haem group is shown in van der Waals spheres.

The CDIPD undertakes interdisciplinary research in neglected tropical diseases, aiming to translate the findings to drug discovery. The Centre educates scientists from areas of the world where these diseases are endemic. The laboratory of James McKerrow, Director of the Centre, specialises in the biology of host-parasite interactions, applying expertise that ranges from cellular immunology to structural biology. Siqueira-Neto's laboratory is focused on parasite biology, developing screening assays and animal models of kinetoplastid diseases. Calvet, an alumna of the CDIPD, specialises in ultra-structural cellular analysis. At the Scripps Research Institute, Roush's laboratory applies medicinal chemistry and molecular modelling to design and synthesise new compounds and improve their structure, properties and activity. Using high throughput screening, Podust and her collaborators discovered a promising lead compound for a new series of non-azole CYP51 inhibitors, LP10, which they have refined and optimised over the course of their studies: "Our work is focused on developing a novel set of leads that use a pyridine ring to coordinate to the haem group," Podust explains.

HIT OPTIMISATION

While the first set of drug candidates were moderately potent, they had low stability in liver microsome preparations and poor selectivity against human metabolic cytochrome P450s. With further support from the NIH, FIOCRUZ and Brazil's Conselho Nacional de Desenvolvimento Cientifico e Tecnologico, the team followed an iterative approach toward molecular design, folding in rounds of analysis of structure-activity and structure-property relationships, compound synthesis, testing and evaluation. First, Podust, Roush and their colleagues pursued the LP10 *S*-enantiomer to design CYP51 inhibitors. Having carried out a detailed review of the features and binding modes of the *S*-enantiomer in a co-crystal structure of CYP51, the team switched to the *R*-enantiomer as the basis for a second-generation lead compound. They have now developed an *R*-enantiomer series of LP10 leads that has more than 1,000 times the potency of the first generation inhibitors against *T. cruzi*-infected cells, much improved stability in microsome preparations and an acceptable level of specificity against human CYPs. Rounds of molecular modelling and inhibitor synthesis to improve stability and selectivity followed, ultimately leading to enhanced binding to *T. cruzi* CYP51 and new high resolution X-ray structures for this therapeutic target. In short, they have successfully tailored inhibitors to specifically target human *T. cruzi* infection. While antifungal drugs like posaconazole and ravuconazole showed efficacy against *T. cruzi in vitro* and in some experimental models, they were never designed specifically for the *T. cruzi* CYP51 target. "We look forward to opportunities to discuss our results with the drug development and tropical disease communities," states Roush, who notes that the LP10 series is continuing to evolve.



LP10 hit (top) and its increasingly optimised derivatives (bottom). In the background is an electron micrograph of the unicellular *T. cruzi* organism (epimastigote form). Courtesy of Iosune Ibiricu Urriza.