Drug Discovery

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A Quinoline Carboxamide Antimalarial Drug Candidate Uniquely Targets Plasmodia at Three Stages of the Parasite Life Cycle

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Malaria remains a global health problem. Continued resistance to the currently available antimalarial drugs and developing resistance to artemisinins reinforces the need to discover and develop new antimalarial therapies. Malaria is a disease that is challenging to treat and even more difficult to eliminate because of the complicated life cycle of the most lethal parasite, *Plasmodium falciparum*. Following a bite from an infected mosquito, parasites quickly enter the human host and invade liver cells, and after a development period of around 6 days, they go on to infect red blood cells. After multiplication in the red blood cells, the malaria parasites then change to a stage that can be taken up by mosquitoes to facilitate transmission to another uninfected human host (Figure 1).

The majority of currently used antimalarials operate through mechanisms that only target one or two stages of this complex life cycle. Now, an international collaboration of scientists, led by Ian Gilbert and Kevin Read at the University of Dundee, have revealed a new drug, DDD107498 (**4**), which has low nanomolar activity in assays modeled to represent each of these stages of the *Plasmodium* life cycle, including the liver, blood, and mosquito-borne stages (Figure 1).[1] As a result of this unique multistage-targeting feature, this novel drug class could have potential to be used for disease treatment, transmission blocking, and chemoprevention. The development team say that their discovery could pave the way to a single-dose cure or preventative medicine for as little as $1 (£0.64) per treatment.

Diverse strategies are available for the discovery of novel antimalarial drugs, including improving existing drugs, rational target-based drug design, and more recently library screening against the parasite in what is called "phenotypic screening" or "whole-cell assay".[2,3] The hit molecule (**1**) that led to DDD107498 was discovered in a screen of over 4700 compounds against malaria-causing parasites. It was shown

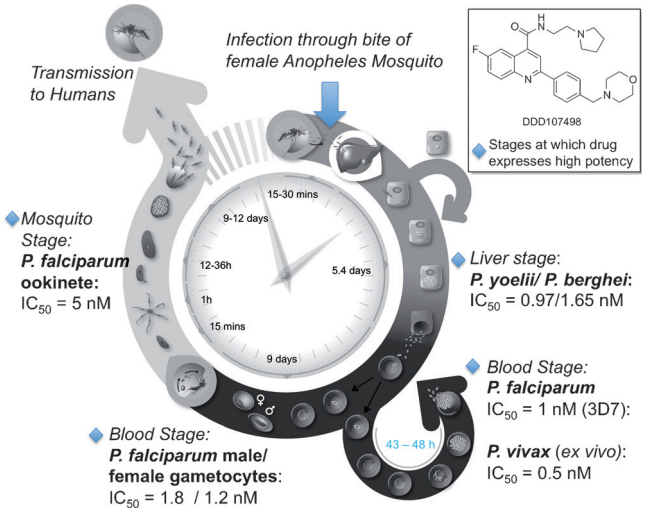

Figure 1. Life cycle of the malaria parasite, highlighting stages at which the protein synthesis inhibitor DDU107498 expresses nanomolar potency.

that the 2,6-disubstituted quinoline-4-carboxamide scaffold **1** (Scheme 1) was effective against malaria parasites but had suboptimal pharmaceutical properties. Based on this chemical starting point, the Dundee team were able to improve the qualities of the starting molecule within just a few simple rounds of medicinal chemistry optimization to give the candidate DDD107498 (**4**). The initial hit molecule already showed nanomolar potency against cultures of *Plasmodium falciparum* but the calculated lipophilicity and metabolic stability were poor, thus indicating that this molecule would have poor activity following oral administration. Replacement of the lipophilic 6-bromo substituent with a fluorine atom provided analogue **2**, which has slightly reduced potency but improved metabolic stability. Solubility and metabolic stability were significantly improved by replacing the 3-amino pyridine group with an extended pyrrolidine amide side chain to provide **3**; this drive to improve the drug-like properties of the template came at the expense of antimalarial potency, with an almost 6-fold drop in potency. However, the rewards of this strategy of improving drug-like properties were finally realized when replacement of the methyl group in **3** with

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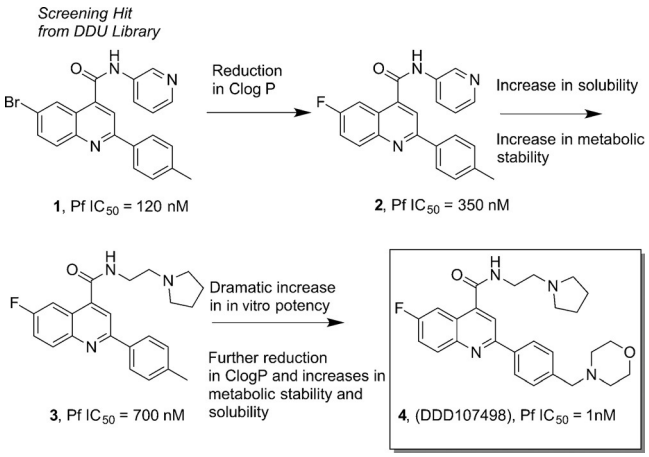

Scheme 1. Medicinal chemistry optimization of the initial Dundee drug discovery (DDU) hit molecule **1** to provide candidate **4**. Pf = *Plasmodium falciparum*.

a benzyl morpholine functional group (to provide **4**) gave a remarkable jump in antimalarial potency to a half maximal inhibitory concentration (IC_{50}) value of 1 nM, a reduction in ClogP with a concomitant increase in solubility, and an increase in mouse metabolic stability.

The outstanding properties observed in the initial medicinal chemistry optimization phase were extended to in vivo studies in animals to reveal excellent oral bioavailability and a long plasma half-life (both prerequisite for the target product profile of the Medicines for Malaria Venture (MMV) and their aim to achieve single-dose cures for malaria or effective preventative treatment).[4] DDD107498 exhibited outstanding oral antimalarial potency in a humanized mouse model of malaria (a model where human parasite-infected red blood cells can be grown in mice[5]), with potencies exceeding that of most currently used antimalarials. As part of the preclinical development program, this molecule was shown to have a high safety window with low risk of P450 inhibition or induction and was non-mutagenic in genotoxicity studies. Rodent toxicology studies also gave no concerns, thus enabling progression to full preclinical safety evaluation.

It is interesting to note that a similar starting point (**5**; Figure 2) was published by Calderon et al. within the GSK Tres Cantos Antimalarial Set (TCAMS) collection of hit molecules, thus demonstrating the benefits that can be derived from starting with high-quality libraries.[6] More generally, for the drug discovery community, this highlights the availability of chemical starting points within the public domain that have the potential for rapid advancement to

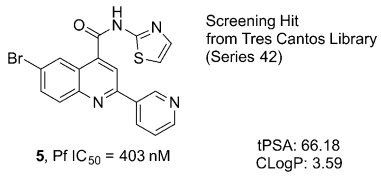

Figure 2. Related quinoline carboxamide hit molecule **5**, previously identified by Calderon et al.[6]

a candidate molecule with such good all-around properties through a few rounds of precision medicinal chemistry optimization.

The development team also determined the molecular target by exposing the malaria parasite to sublethal concentrations of drug ($5 \times IC_{50}$) until resistance developed. Comparison of the genomes of resistant parasites with the genomes of drug-sensitive lines led to the identification of the key gene that had changed to confer resistance. It was established that the quinoline carboxamide (**4**) targets translation elongation factor 2 (eEF2), which drives GTP-dependent ribosome translocation along mRNA. Protein synthesis is essential for parasite development, which explains the activity of the compound at multiple life-cycle stages and highlights protein synthesis as an excellent target for achieving broad-stage antimalarial activity. Defining the mechanism of action prior to entry into the clinic will be invaluable for trying to mitigate resistance in the field once the drug is deployed.

This project underlines the power of phenotypic drug discovery (whole-cell assays), which has often been ignored or given lower priority by pharmaceutical researchers working in other disease areas in favor of highly selective target-based approaches. The beauty of the phenotypic approach is that after efficient medicinal chemistry optimization, the result is a drug class that is proven to gain access to the intraparasitic drug target. Furthermore, through the application of precise genetic approaches, the drug target can be identified retrospectively. This pattern of antimalarial drug discovery has been championed by other academic groups and major pharmaceutical research groups, including teams at Novartis, GSK, and AstraZeneca.[7–9] This tactic accelerates target validation and identification and provides information for subsequent target-based approaches to drug discovery.

To prevent rapid resistance to this class of drug from developing, it will be important to select an appropriate drug partner for combination deployment. Although it has multistage activity, this compound kills the parasite relatively slowly and it has a long elimination profile, both of which are potential drivers of resistance. Depending on how the development team and MMV decide to progress with **4**, it may be sensible to consider combining it with a fast-killing drug such as an endoperoxide[10] or one of the new PfATP4[8] inhibitors that are also rapidly moving through preclinical and clinical development. The program has now moved from its academic origins to the Merck Serono portfolio, where the compound is currently undergoing late-stage preclinical safety testing in readiness for clinical evaluation and eventual registration. Regulatory registration would represent a huge success and would deliver a drug that could prove invaluable for malaria eradication. This project underlines the important role and impact that academic drug discovery can have when supported by well-funded, highly experienced, and focused product-development partnerships with an organization that has close links to industry (in this case, the MMV), which is vital for developing antimalarials all the way to the clinic.

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