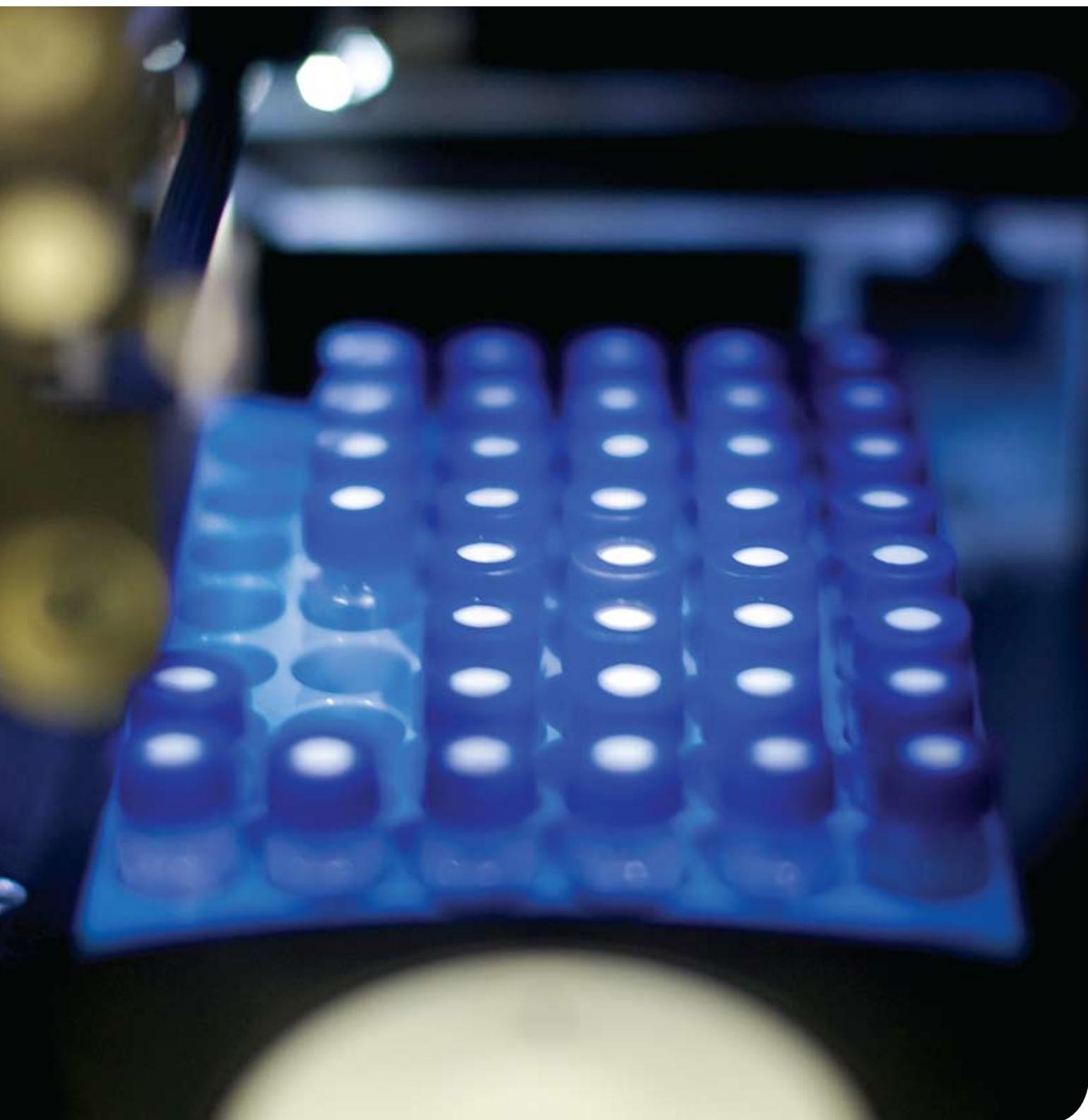
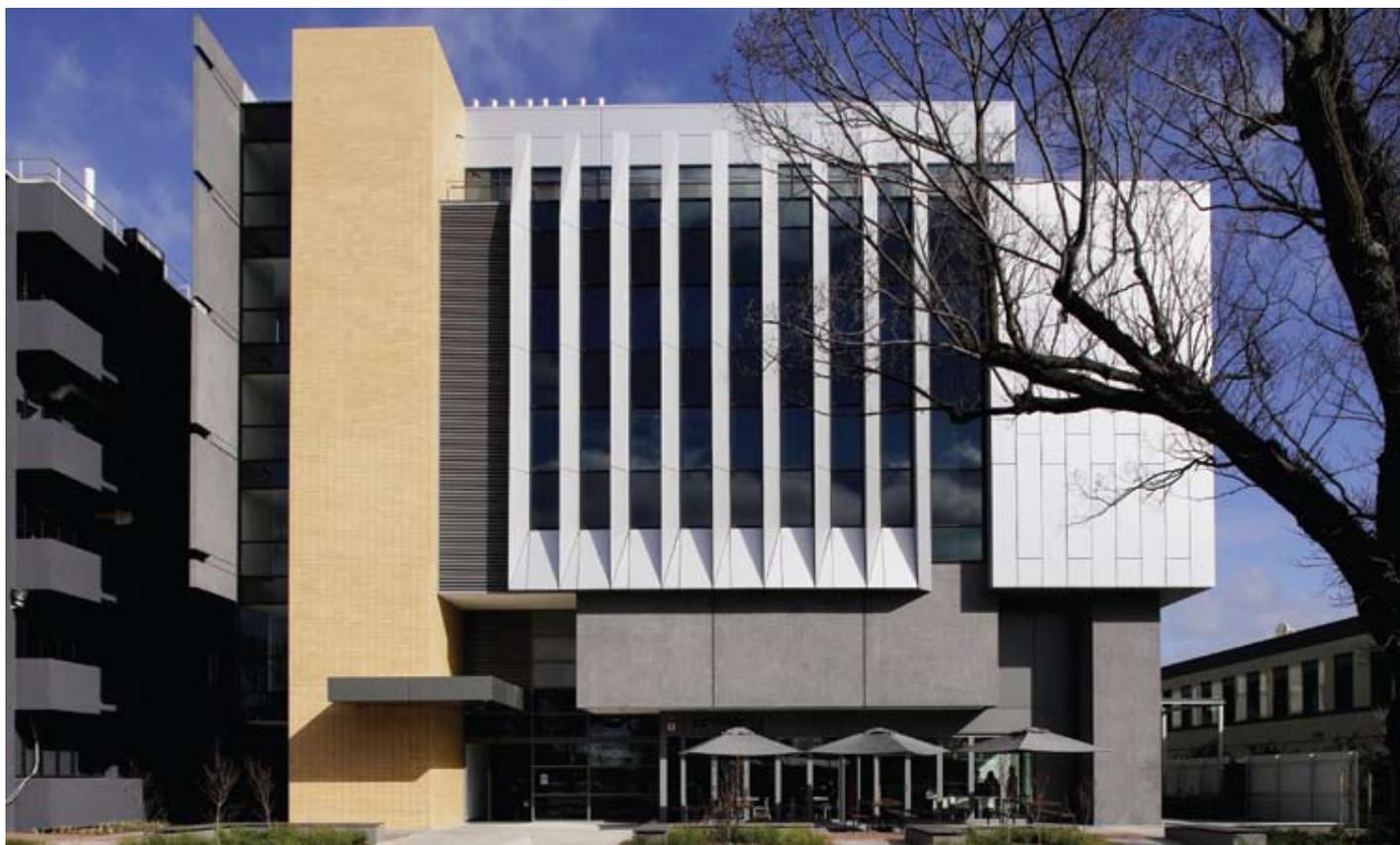


Centre for Drug Candidate Optimisation



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Experience / Capability / Insight



The Centre for Drug Candidate Optimisation (CDCO) is an innovative research centre that provides collaborative ADME lead optimisation in support of emerging drug discovery programs.

Lead optimisation facilitates the informed design, identification and selection of drug candidates through an integrated assessment of their chemical, metabolic, biopharmaceutical and developmental properties.

The CDCO is one of the four themes of the Monash Institute of Pharmaceutical Sciences (MIPS) at Monash University. MIPS is a leading centre of pharmaceutical science that drives both basic and applied research and comprises the largest and most experienced group of pharmaceutical scientists in Australia.

Equipped with state of the art instrumentation and employing highly qualified and experienced scientists, the CDCO offers contemporary approaches and methodologies to address key issues associated with candidate selection and progression from drug discovery to development. Collaborative programs are commercially focused and specifically designed to meet the needs of individual for-profit and not-for-profit organisations.

Our mission

- to undertake collaborative ADME lead optimisation research with emerging biotechnology and pharmaceutical companies and not-for-profit research institutes to produce optimised, high quality drug candidates for subsequent pre-clinical and clinical development
- to develop and maintain fully validated scientific platforms, and to utilise innovative study designs, to provide high impact, decision-quality data to support lead optimisation programs
- to work flexibly with partners in a professional and timely manner and to add pharmaceutical and economic value to partners' pre-existing chemistry and biology discoveries
- to provide a stimulating working and training environment for research staff and, where possible, engage the broader biomedical research community.

The commercial attractiveness of new drug candidates is enhanced by:

- decreasing risks associated with costly development programs
- providing an improved intellectual property position
- improving licensing opportunities
- increasing the probability of successful progression through clinical trials.

Bioanalysis

Rapid, specific and quantitative analysis of drug candidates in biological samples supports all aspects of ADME lead optimisation at the CDCO. LC-MS and LC-MS/MS offer the flexibility to develop specific and rapid methods for the analysis of candidate drugs and their metabolites in complex biological matrices.



LC-MS and LC-MS/MS instrumentation

LC-MS and LC-MS/MS instrumentation to support lead optimisation studies includes:

- single quadrupole mass spectrometers, which are well suited for samples requiring high specificity in a complex matrix, rapid throughput and moderate sensitivity
- triple quadrupole mass spectrometers that provide high specificity and sensitivity, enabling the detection of trace levels of compounds in complex biological matrices; these instruments are used primarily for quantitative analysis of plasma samples from pharmacokinetics studies; MS/MS is utilised for the confirmation of metabolite structure
- time-of-flight (TOF) mass spectrometers that provide highly accurate mass determination, particularly suited for identification of metabolites resulting from *in vitro* and *in vivo* biotransformation reactions
- HPLC and UPLC systems equipped with plate-based sampling compatibility to enable high throughput method development and validation, sample preparation and processing.

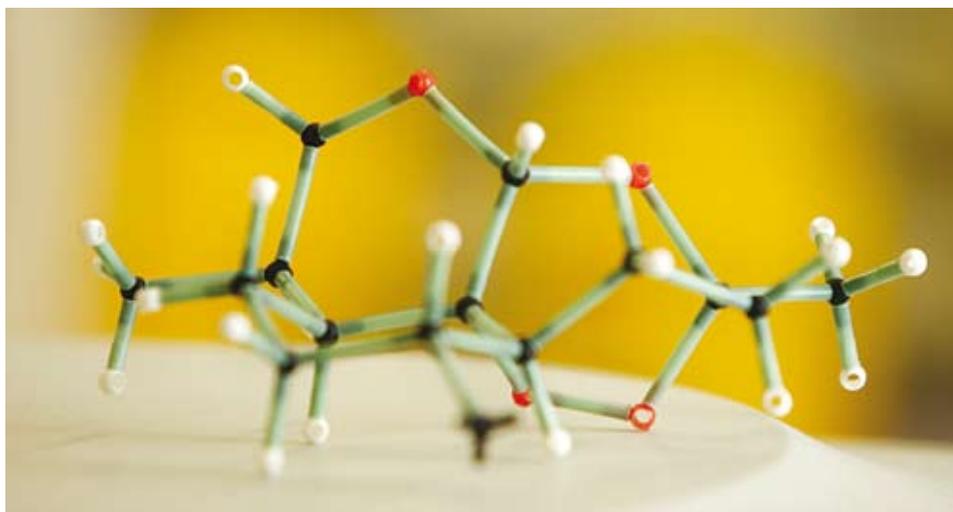
Sample preparation

Biological samples such as plasma and urine contain multiple components with characteristics similar to the drug or metabolite of interest. The challenge of a bioanalytical method is to detect, identify and quantify the compounds of interest without having to rely on complex sample preparation procedures prior to analysis.

The high specificity of the CDCO's LC-MS instrumentation means that minimal sample preparation is required. Combining this feature with the use of short, narrow-bore analytical columns and generic gradient conditions minimises the time required for assay development and sample run times.

Physicochemical profiling

Assessing the physicochemical properties of a drug candidate in early discovery provides critical information regarding potential problems with oral absorption, disposition and product formulation. Physicochemical profiling has become an integral part of drug discovery, leading to property optimisation and rank ordering for 'drug-like' characteristics.



Initial physicochemical profiling at the CDCO is conducted *in silico* to provide rapid and cost effective prospective data on a structural series. Once compounds have been synthesised, high throughput physicochemical screening methods are utilised that only require minimal quantities of material.

Specific physicochemical assays utilised by the CDCO include:

- computational estimates of pKa, Log P / Log D and polar surface areas, along with other molecular surface properties related to ADME processes
- rapid and automated measurements of aqueous solubility under physiologically relevant conditions using either high throughput methods or equilibrium solubility measurements
- Log P / Log D determination using rapid chromatographic techniques or traditional 'shake flask' methods
- experimental determination of ionisation constants (pKa) using potentiometric titration
- solution stability under accelerated conditions and assessment of impurity profiles.

Salt selection and solid state characterisation

Rapid identification and selection of the optimal salt form for basic and acidic candidate drugs is a key development step in their early pre-clinical assessment. The systems and methods at the CDCO have been adapted to a small scale, and the salts selected for advancement are assessed for their physicochemical, biopharmaceutic, solid state and stability characteristics.



Drug metabolism and metabolite profiling

Rapid metabolism is a major limiting feature of many new drug candidates and can lead to low oral bioavailability, a short *in vivo* half-life, or the production of potentially active or toxic metabolites.



The CDCO offers a range of different assays to assess the metabolism of compounds during early lead identification through to candidate selection and optimisation:

- assessment of the *in vitro* biotransformation of parent compound using microsomes, S9 or cytosolic fractions, or hepatocytes to assess all of the key metabolic transformations
- comparison of metabolite profiles using microsomes from multiple species
- examination of the kinetics of metabolite formation and the potential saturation of metabolic processes
- screening for potential inhibition of the most common cytochrome P450 (CYP) isoforms, which can lead to drug–drug interactions *in vivo*
- reaction phenotyping using cDNA-expressed human cytochrome P450 or UGT isoforms
- detection and identification of metabolites in plasma, urine and bile following *in vivo* administration to laboratory animals, or *in situ* using isolated perfused organ models.

Major metabolites

The data and information generated from these studies can be used to identify major metabolites and metabolising enzymes, identify metabolically labile functional moieties within a structural series, and provide a basis for structural modifications to reduce metabolic lability. With some necessary assumptions, *in vitro* studies can also be used to predict *in vivo* clearance and provide inter-species metabolic profiling to guide species selection for initial toxicity testing.

Drug–drug interactions

Determining the potential for enzyme inhibition/induction has become a major focus of regulatory agencies worldwide due to the potential for serious adverse drug–drug interactions resulting from these processes.



The CDCO uses a staged approach to assess the potential for enzyme inhibition:

Tier 1

- early stage screens are conducted using a substrate-specific approach, but utilise only a single concentration of test inhibitor to provide rapid information during early-stage discovery

Tier 2

- more advanced assessments are conducted during later stage discovery using CYP-specific substrates to determine *in vitro* inhibition constants (ie IC_{50} or K_i) and the mode of inhibition

Tier 3

- mechanism-based or time-dependent inhibition is assessed based on structural alerts or results from the Tier 1 / Tier 2 assays
- the potential for enzyme induction is assessed using *in vitro* and *in vivo* systems.

Bioavailability and pharmacokinetics

Pharmacokinetics characterises the absorption, distribution, metabolism and excretion (ADME) properties of a drug candidate, allowing identification of limiting features in relation to the desired route of administration. Coupled with the results from *in vitro* screens, *in vivo* ADME data enables the early identification of compound liabilities, providing a basis for structural modifications or for the early initiation of development strategies to overcome these problems.



ADME properties

The CDCO examines ADME properties for selected candidates within a chemical series after intravenous and/or oral administration to small laboratory animals such as rats or mice. A number of formulation approaches are used to facilitate solubilisation in vehicles suitable for intravenous administration. LC-MS and LC-MS/MS are used for the quantitation of compounds in plasma or other biological fluids such as urine or bile, providing rapid method development and high specificity and sensitivity. These methods also typically enable an initial indication of *in vivo* metabolites. Early assessment of *in vivo* ADME properties provides critical data for the validation of *in vitro* screens (based on physicochemical and metabolic profiles) and establishment of *in vitro* / *in vivo* correlations.

Culex™ ABS

The CDCO uses the Culex™ ABS system to collect blood and biological samples from conscious and free moving rats, greatly minimising animal stress. Utilisation of the Culex™ ABS greatly enhances the conduct of *in vivo* studies, providing overnight sampling, flexible study design, high quality data and reduced animal handling.

In addition to the methods described above, the CDCO uses a number of other approaches to characterise individual processes that underpin the exposure profile, including:

- the stability of a compound in blood or plasma
- the binding of drug to plasma proteins and the distribution into red blood cells
- absorption across the gastrointestinal mucosa using *in situ* intestinal perfusion models
- clearance of drug by the liver using an isolated perfused liver preparation
- *in vivo* studies to determine the role of cytochrome P450-mediated metabolism using specific and non-specific enzyme inhibitors.

In vitro assessment of drug permeability

Permeability across biological membranes is a key factor in the absorption and distribution of drugs. Poor permeability, which can arise due to a number of structural features as well as membrane-based efflux mechanisms, can lead to poor absorption across the gastrointestinal mucosa or poor distribution throughout the body.

Two approaches are used by the CDCO to assess the permeability of new drug candidates:

PAMPA

The parallel artificial membrane assay measures the ability of a compound to passively permeate across an artificial lipid bilayer. This system is non-cell based, rapid and useful for the identification of compounds whose permeability is likely to be limited by their structural properties.

Cell monolayers

Caco-2 cell monolayers are used to determine an apparent permeability coefficient, which allows the ranking of compounds as highly permeable, moderately permeable or poorly permeable relative to well characterised drug standards. MDCK cells are also utilised as a model for membrane permeation studies due to their ease and speed of culturing. This cell line can be transfected with human P-glycoprotein (MDCK-MDR1) and, when used with the wild-type MDCK cell line, can provide insight into the importance of efflux mechanisms *in vitro* at an early stage of the drug discovery process.



Blood-brain barrier permeation

A major limitation of many potential central nervous system (CNS) drug candidates is their inability to cross the lining of the endothelial cells separating the blood from the brain, typically referred to as the blood-brain barrier (BBB).



Tier 1

Physicochemical and molecular surface properties of a candidate compound are predicted *in silico* or measured. PAMPA (described above) offers a high throughput measure of intrinsic permeability, which is one factor governing a compound's ability to cross the BBB, and MDCK cells are used to assess the importance of Pgp in limiting brain permeation.

Tier 2

The brain to plasma ratio is determined following intravenous administration to mice. For compounds with a low brain/plasma ratio, or those that have shown to be substrates *in vitro*, additional studies can then be conducted in P-glycoprotein deficient (knockout) mice to determine whether P-glycoprotein is a major factor hindering brain uptake *in vivo*.

Neglected disease drug discovery projects

Malaria is a devastating tropical disease caused by the mosquito borne parasite *Plasmodium falciparum*, killing between one and two million people annually. There are approximately 300 to 500 million new clinical cases each year, with the majority of victims being children under five years of age and pregnant women. Approximately 90% of malaria cases are found in sub-Saharan Africa, but malaria also remains a significant problem in other parts of Africa, Asia and South America.

Medicines for Malaria Venture

There is an urgent need to discover new and effective drugs at affordable prices to combat the existing drug resistance and provide effective therapies for poor, disease endemic countries. The Medicines for Malaria Venture (MMV, www.mmv.org) was founded in 1999 as a non-profit organisation focused on the discovery and development of new, affordable antimalarial drugs. MMV was among the first public-private partnerships established to tackle a major world disease, arising from discussions between the World Health Organization (WHO), the International Federation of Pharmaceutical Manufacturers and Associations, and other key organisations. MMV receives funding from the Bill and Melinda Gates Foundation, Rockefeller Foundation, World Bank, ExxonMobil, WHO, BHP Billiton, Wellcome Trust, various governments and others. The CDCO is a collaborative partner in four MMV-funded projects.

Synthetic peroxide drug discovery project

The aim of this project was to design a new class of synthetic, peroxide-based, orally active antimalarial drugs that have a low cost of goods, are more potent than the currently available artemisinin derivatives and have a better pharmacokinetic profile, enabling a three-day treatment regimen. The partners for this project include the University of Nebraska (medicinal chemistry), the Swiss Tropical Institute, Basel (*in vitro* / *in vivo* biological evaluation), CDCO / Monash University (ADME and lead optimisation) and F. Hoffman-LaRoche, Basel.

Lead optimisation played a critical role in identifying the limiting properties of early compounds and guiding the medicinal chemistry program. *In vitro* metabolism studies in hepatic microsomes and hepatocytes allowed the selection of compounds best suited to progress into studies in rats for assessment of the ADME properties. A formal selection matrix approach was developed for selection of

a candidate compound for progression into pre-clinical development, leading to clinical assessment. The results of these initial studies leading to the selection of a clinical candidate were recently published (Vennerstrom et al, *Nature*, 430, 900-904, 2004).

Using a rational design approach that integrated data from *in vitro* and *in vivo* assays, the project team was able to identify a new series of compounds with greatly improved properties over the existing peroxide antimalarials. The current series of compounds provides a single dose oral cure in mouse models of malaria, and greatly exceeds the efficacy of comparator compounds in this model. These new compounds also show prophylactic activity, a feature that has not previously been demonstrated with peroxide antimalarials. A clinical development candidate has recently been selected and preclinical testing is being undertaken to support Phase 1 testing in 2009.



Photo courtesy of Anna Wang, Medicines for Malaria Venture.

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