DRUG DISCOVERY SERVICES

HOLISTIC DRUG DISCOVERY AND DEVELOPMENT (H3D) CENTRE

H3D
PIONEERING WORLD-CLASS DRUG DISCOVERY IN AFRICA

UNIVERSITY OF CAPE TOWN
IYUNIVESITHI YASEKAPA • UNIVERSITEIT VAN KAAPSTAD

technology innovation AGENCY
Innovating Tomorrow Together

ADME

Absorption, Distribution, Metabolism & Excretion

The H3D ADME team routinely conducts *in vitro* studies on small drug-like molecules to understand the drug properties and to obtain decision-making information for progressing drug candidates for *in vivo* evaluation.

Aqueous Kinetic Solubility

Solubility plays an important role in the oral absorption of a drug. Solubility is defined as the maximum amount of a substance that will be dissolved in an amount of solvent at a specified temperature and pH.

Lipophilicity (LogD)

Lipophilicity measures the extent of distribution of a substance in an aqueous (hydrophilic) vs. hydrophobic (lipophilic) phase. Lipophilicity impacts absorption, distribution, metabolism and excretion of compounds.

Permeability (PAMPA)

Permeability, like solubility, also drives the oral absorption of drugs. Parallel artificial membrane permeability assay (PAMPA) is an *in vitro,* non-cell-based model of passive, transcellular permeation used as a screening tool for the evaluation of a test compound's permeability across various experimental membranes.

Metabolic Stability Assays

The liver is the main organ involved in drug metabolism. Subcellular fractions of the liver, such as microsomes, can provide a good estimate of *in vivo* hepatic clearance. Data is reported as the % remaining following a 30-minute incubation, *in vitro* half-life, and *in vitro* clearance. The same experiment format can be used to identify metabolites that contribute to the observed clearance, thereby allowing targeted medicinal chemistry efforts to improve metabolic stability. H3D offers microsomal stability turnover assay in human, rat and mouse microsomes.

Plasma Protein Binding (PPB)

The plasma protein binding assay is used to determine the binding of a compound to proteins in plasma. The fraction unbound measured in this experiment is used to calculate free concentrations from *in vivo* experiments, thus allowing a better understanding of pharmacokinetic parameters as well as pharmacokinetic-pharmacodynamic (PK/PD) relationships. H3D routinely performs Human PPB assays.

DMPK

Drug Metabolism and Pharmacokinetics

The H3D Drug Metabolism and Pharmacokinetics (DMPK) team routinely conducts *in vivo* PK (pharmacokinetics) evaluation studies. DMPK studies are important for estimating the basic PK parameters of a compound and associated optimization of compounds for human use or consumption.

IV and PO (oral) PK in mice and rats

IV and PO (oral) dosing studies are performed in mice and rats to determine pre-clinical PK parameters and bioavailability. These data are combined and expanded upon using the studies outlined below as well as additional studies described in this brochure.

Dose Fractionation Studies

Dose fractionation studies are used to evaluate the differences in dose relative to a compound's efficacy and toxicity and are key to eventual determination of the drug dosing regimen of a given drug candidate. This is because only a certain fraction of each dose for any given drug is absorbed and able to become pharmacologically active. It is very important to carefully evaluate these parameters before moving onto experimental design of human clinical trials.

**Note: DMPK team currently has ethical approval to conduct PK studies in mice and rats for novel compounds in the infectious disease areas of malaria, tuberculosis and antimicrobial resistance. Compounds needs a selectivity index of >10 to be progressed to in vivo pharmacokinetic evaluation. Prior to commencing with in vivo pharmacokinetic studies we routinely require an initial consult with the client to understand the requirements and study design*

AMR Biology

Antimicrobial Resistance

H3D has an AMR Biology Suite which houses the facilities for screening of antimicrobial peptides (AMP's), small molecules and natural products against the bacterial ESKAPE pathogens. Additional capabilities include resistance determination and mechanism of action studies for active, novel compounds.

AMR Screening Assay

The percentage inhibition at a single concentration and/or the minimum inhibitory concentration (MIC) of test material can be determined against the following strains:

- ***E**scherichia coli 25922*
- ***S**taphylococcus aureus 25923*
- ***K**lebsiella pneumoniae BAA-1705*
- ***A**cinetobacter baumannii 19606*
- ***P**suedomonas aeruginosa 27853*
- ***E**nterobacter cloacae 700323*

Minimum Bactericidal Concentration (MBC) Assay*

This assay enables the characterization of compounds as either bacteriostatic or bactericidal for a specific strain. The $MBC_{99.9}$ is the lowest concentration at which 99.9 % of viable bacteria are killed.

AMR Clinical Strain Panel*

We maintain a collection of clinically isolated ESKAPE pathogens with various resistance profiles, representative of the African population. These strains are available for screening in a Single-Point and/or Dose Response assay. The results from phenotypic screening assays can inform a project on the specificity and selectivity of the samples against these strains.

PMBN Assay*

The cell envelope of Gram-negative bacteria is a physical barrier that provides inherent resistance to antimicrobials by selectively preventing cell entry. Polymyxin B nonapeptide (PMBN) is a membrane permeabilizer. Test compounds can be evaluated in the presence of PMBN to determine if lack of or reduced permeation plays a role in the activity observed.

**Note: These assays are only performed on compounds with an MIC $<$10 µM or µg/ml.*

AMR is a global health threat. The ESKAPE pathogens are a group of highly aggressive and resistant organisms and are the leading cause of nosocomial infections globally. Novel therapies need to be identified and developed to combat this threat.

TB Biology

Tuberculosis

H3D has a dedicated TB biology team housed at the Institute of Infectious Disease and Molecular Medicine (IDM) with access to BSLII and BSLIII facilities.

TB MIC Screening Assay

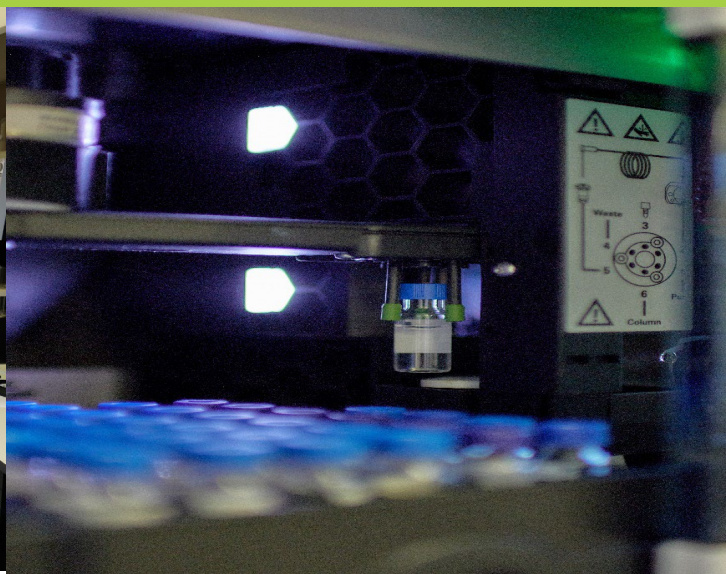
This assay tests a range of compound concentrations to determine the minimum inhibitory concentration (MIC) required to inhibit the growth of *Mycobacterium tuberculosis H37Rv in vitro*.

TB Mutant Screening

TB is currently treated with several antimicrobial drugs simultaneously; and these include isoniazid, rifampin, pyrazinamide, and ethambutol. Investigating a compound's activity and efficacy in relation to these established treatments is critical to developing a competitive drug candidate for treating TB.

Screening-based approaches against a panel of both mutant and single drug resistant (SDR) *Mtb* strains are available in-house to assist in delineating a drug candidates' mode of action.

The TB biology team routinely conducts whole-cell and target-based screening, biology triaging, target identification and validation studies for the TB projects. The BSLIII Drug Discovery lab houses the TB screening platform and bench space for assay development and target ID/mechanism of action work.



MALARIA BIOLOGY

H3D has a well-established malaria biology lab which screens thousands of small molecules and natural product extracts for anti-plasmodial activity each year. In addition to the routine screening, the biology lab is one of the few labs globally with the expertise to perform *in vivo* malaria efficacy studies in the humanised mouse model.

Malaria IC50 Screening Assay

The NF54 and Dd2 strains are two of the most commonly used *Plasmodium falciparum* strains in labs worldwide and are used by H3D for many malaria biology assays. The following drug-sensitive and resistant strains are available for single-point inhibition* and/or dose-response screening at a relevant concentration:

- **NF54**: Wild-type drug-sensitive strain of *P. falciparum* used as the primary screening assay for anti-plasmodial activity.
- **Dd2:** Chloroquine-resistant strain of *P. falciparum*.
- **K1:** Multidrug-resistant strain of *P. falciparum*.
- **3D7:** Chloroquine-sensitive clone of *P. falciparum* used to generate the parasites in the NSG humanised mouse model for malaria.

**Note: Single-point assays will only be run for libraries of 40 or more compounds*

Parasite Reduction Ratio

This assay is used to determine killing kinetics of new compounds, to assess both the onset of activity and the rate of kill for new compounds.

Stage Specificity

The entire malarial life cycle contains 14 stages between the human and mosquito hosts; stage-specific assessment of candidate drug inhibitory effects is necessary to clarify which symptomatic stage or stages a compound has potential for clinically-relevant activity in humans.

NSG/SCID Studies

The NSG/SCID *in vivo* antimalarial efficacy study is a highly specialized and unique study in which new anti-plasmodial compounds can be properly evaluated in a live model using human species of malaria. This is accomplished by using SCID (severe combined immunodeficiency) mice that have been infused with human blood, and subsequently can be studied as an experimental model of functional human malaria

**Note: Prior to a NSG study it is a prerequisite that the highest oral dose planned for the NSG study should be administered in healthy mice to ensure no clinical signs are observed prior to commencing the NSG study*

ENZYMOLOGY

H3D has established biochemical assays that utilise recombinant protein expressed and purified in-house for several high priority targets. These miniaturised inhibition assays are carried out in 384-well plates in either single-point inhibition* or dose-response format.

Mycobacterium Tuberculosis Caseinolytic Peptidase ClpP1P2

In vitro ClpP1P2 peptidase inhibition assays, performed using purified recombinant Clp subunits P1 and P2, reconstituted to form the active ClpP1P2 complex in the presence of BZ-Leu-Leu.

Plasmodium Kinases

In vitro kinase inhibition assays, single point inhibition or dose response assays, are available for 3 validated *Plasmodium* kinases:

- ***Pv*PI4Kβ:** *P. vivax* phosphatidylinositol 4-kinase beta
- ***Pf*PKG:** *P. falciparum* cGMP-dependent protein kinase
- ***Pf*CLK3:** *P. falciparum* cyclin-dependent like protein kinase 3

**Note: Single-point assays will only be run for libraries of 40 or more compounds*

TOXICOLOGY

Cytotoxicity IC50 screening

To ensure that a compound is non-toxic to the host, H3D can test for cytotoxicity in single-point inhibition* or dose-response assays against various mammalian cell lines:

- **HepG2:** Human hepatoblastoma cell line
- **Vero:** Derived from monkey kidney cells
- **CHO:** Chinese Hamster Ovarian cells
- **L6:** Cells from the thigh muscle of the brown rat

**Note: Single-point assays will only be run for libraries of 40 or more compounds*

Hemolysis

The hemolysis assay determines whether compounds may be toxic to red blood cells via sufficiently damaging to the outer membrane, which causes the cells to burst.

Crabtree Effect/Glu-gal Assessment

This assay identifies whether activity against cells might be linked to disruption of mitochondrial membrane potential via disruption of the electron transport chain and is useful in elucidating the potential mechanism of action of the compound.

CONTACT US

H3D provides all of the assays as a fee of service to the broader research community. We are continually updating our platforms and validating new assays. Please visit the H3D website for the latest information and contact us for an obligation-free quotation.

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