Drug Discovery for Malaria

I. Introduction to Drug Discovery
II. Application to Identification of New Antimalarial Agents

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CWHM Mission: Discover and advance to the clinic affordable new therapies for unmet medical needs with an emphasis on orphan diseases, neglected diseases, and diseases of poverty
Research & Development Process

Drug Discovery: 3-7 years

Target Discovery
- Disease identification & therapeutic needs
- Target identification
- Target validation

Lead Discovery
- Assay development
- Hit identification
- High-throughput screening (HTS)
- Structure-activity relationships (SAR)
- Medicinal chemistry "hit-to-lead"
- Mechanism of action (MOA)

Lead Optimization
- Medicinal chemistry optimization
- Pharmacodynamics (PD) – drug interaction with the target
- Pharmacokinetics (PK) – getting the drug to the target
- Absorption, distribution, metabolism, and excretion (ADME)
- Efficacy in animal models

Preclinical Development
- Safety & Toxicology
- Process Chemistry & Scale-up
- Formulation & stability
- ADME & Pharmacology
- Patents filed
- Regulatory package

Investigational New Drug (IND) Application

Drug Discovery: 3-7 years
Development: 7-10 years
Research & Development Process

Development: 7-10 years

Phase I Clinical Trials
- Determine drug safety
- “Is the drug safe to test?”
- 10-100 healthy volunteers
- Unexpected side effects may be identified

Phase II Clinical Trials
- Determine drug efficacy
- “How well does the drug work?”
- 100-500 patients
- Most drugs fail in Ph II due to lack of sufficient efficacy

Phase III Clinical Trials
- Confirm findings in large patient population
- 1000-5000 patients
- Greater chance to find rare side effects
- Compare to “gold standard” treatment

Phase IV
- Monitor long-term safety
- Patient population more heterogeneous
- Adverse reactions may lead to withdrawal of drug

FDA Review & Approval: New Drug Application (NDA)

• Overall R&D process takes 10-17 years
• Overall average cost of a new drug ~$1B
  ~10,000 compounds synthesized
  ~100 tested in animals
  ~10 reach Phase I clinical trials
  ~1 reaches the market
Drug Discovery: 3-7 years

Development: 7-10 years

Translational Drug Discovery
Selecting a Drug Target

- Understanding the biology relevant to the disease of interest is critical to success
- This is not always well understood at the beginning of a drug discovery project
- Not all targets will produce the desired biological effect or to a sufficient degree to be efficacious
- Historically, many drugs have been developed where the drug target or biological mechanism was not known

Figure 1 | Therapeutic target classes. All current therapeutic targets can be subdivided into seven main classes, wherein enzymes and receptors represent the largest part. Adapted with permission from REF. 1 © American Association for the Advancement of Science (2000).
Lead Discovery

- Bioassays must be developed in order to identify and prioritize “hits” for medicinal chemistry optimization
- Some assays may be used to eliminate certain compounds for further consideration
  - Off-target selectivity, cytotoxicity, etc.

Definitions:
- Hit – a compound that has some activity against the chosen biological target
- Lead – a compound that has some activity against the chosen biological target, belongs to a class of compounds that show promise to lead to a drug, but is not good enough to be the drug itself
- Testing Funnel – a set of assays used to filter compounds to identify promising leads
Finding a Hit or Lead Compound

Figure 4 | Hit-identification strategies. The most commonly applied hit-identification strategies today range from knowledge-based approaches, which use literature- and patent-derived molecular entities, endogenous ligands or biostructural information, to the purely serendipity-based ‘brute-force’ methods such as combinatorial chemistry and high-throughput screening. The amalgamation of both extremes is anticipated to deliver more high-content chemical leads in a shorter period of time.
High Throughput Screening (HTS)

100’s to 1,000,000’s of compounds

HTS Assay

Hits

Screening hits are potential leads that need to be validated!
Hit Triage: Validating the hit

- **Is the hit real?** Need to validate!

- **Confirm identity**
  - Resynthesize or purchase pure compound and retest

- **Dose response curve (IC$_{50}$ determination)**

- **Orthogonal testing**

- **Secondary testing**

- **Confirm mechanism of action**
Selecting the Lead

• This is a process—multiple hits/leads may be selected initially for followup (Hit-to-Lead phase)
  – Don’t know which hit/lead will be result in a drug

• Is the hit drug-like?
• Is there good potential for optimization?
• Selectivity, solubility, toxicity, etc.
• Is it patentable?
• Ease of synthesis?
• Are there related compounds with SAR that makes sense?
Drug design is the application of medicinal chemistry towards the optimization of PD and PK

- **Pharmacodynamics (PD)** refers to what the drug does to the body
  - E.g., optimization of interactions of a drug with its target

- **Pharmacokinetics (PK)** refers to what the body does to the drug
  - E.g., optimization of the ability of a drug to reach its target
Optimizing Pharmacodynamics

**Aim** - To optimize binding interactions with target

**Reasons**
- To increase activity and reduce dose levels
- To increase selectivity and reduce side effects

Diagram shows a drug binding to a macromolecular target in an induced fit process, with binding sites, regions, groups, and bonds highlighted.
Types of Binding Interactions

- Electrostatic or ionic bonds (20-40 kJ/mol)
- Hydrogen bonds (16-60 kJ/mol)
  - Hydrogen bond acceptor (HBA)
  - Hydrogen bond donor (HBD)
- Van der Waals interactions (2-4 kJ/mol)
- Dipole-dipole/ion-dipole interactions
- Repulsive interactions
- Hydrophobic interactions
Structure-Activity Relationships (SAR)

- Relationship between the chemical structure of a molecule and its biological activity
- Determining the SAR allows for optimization of potency and reduction of off-target toxicity
- Typically specific chemical groups are modified, replaced or added to determine the SAR
A pharmacophore is a model which describes important characteristics for binding to a target.
Pharmacokinetics: Access to the target

- **Aqueous Solubility** – drug needs to dissolve
- **Absorption** – drug needs to reach blood stream
- **Distribution** – drug needs to reach the target
- **Metabolism** – need drug stability to maintain sufficient concentration to affect target over time (half-life)
- **Excretion** – elimination of drug in a reasonable time period
Pharmacokinetics & Drug Design

- Drugs must be sufficiently polar to be soluble in aqueous conditions
- Drugs must be sufficiently polar to interact with molecular targets
- Drugs must be sufficiently ‘greasy’ to cross cell membranes
- Drugs must be sufficiently ‘greasy’ to avoid rapid excretion
- **Drugs must have a balance of both hydrophilic and lipophilic characteristics**
Pharmacokinetic (PK) Parameters

- **Clearance (CL)** – rate at which a compound is cleared from the body
- **Half-life (t_{1/2})** – period of time in which half of the compound has been eliminated from the plasma
- **Volume (V)** – represents how widely the compound is distributed to the tissues
- **Bioavailability (BA or %F)** – % of compound that reaches the blood stream from the oral dose

**PK Parameters (rat)**
- CL 18 mL/min/kg
- T_{1/2} 0.4 hour
- Vdss 0.6 L/kg
- BA 20%

**Diagram:**
- IV (1 mg/kg)
- PO (2.5 mg/kg)
Many Hurdles for Preclinical Drug Discovery

- Find right target
- Develop assays
- Find a lead
- Optimize potency
- Increase selectivity
- Needs to be absorbed
- Reduce metabolism
- Getting to the target tissue
- Low toxicity
- Efficacious in animal model
- Not too difficult to synthesize
- Stable to long-term storage
- Crystalline compound
- Aqueous solubility
- Etc...

Figure 2 | Don’t panic… Turning an organic compound into a high-content chemical lead series is a challenging and sometimes extremely complex endeavor, as numerous hurdles beyond activity and selectivity have to be overcome. It is vital to identify high-quality actives, or ‘hits’, as the molecular starting point is crucial in determining the later potential for success. Hit discovery and lead generation is therefore far more than just the identification of active compounds; it is the multi-disciplinary process of selecting the most promising lead candidates from rigorously assessed molecular series.
Malaria – The Need for New Antimalarials

“Malaria disproportionately affects poor people who cannot afford treatment or have limited access to health care, trapping families and communities in a downward spiral of poverty.”

– World Health Organization [website](#)

- ~225 million cases/yr
- ~1 million deaths/yr
- In Africa:
  - $1.8B healthcare costs
  - Loss of $12B GDP
- Resistance to most existing drugs is widespread

Alonso & Tanner, Nature Medicine 2013, 19, 150-155
Plasmodium Life Cycle

Four Species
P. falciparum
P. vivax
P. ovale
P. malariae
Malaria Medicine Cabinet

Goal: Identify antimalarial drug with novel chemotype and mechanism of action
### Potential Targets for New Malaria Drugs

**Table 1.** Drugs and possible drug targets for antimalarials against blood-stage and liver-stage parasites. ND, life cycle stage not determined; BS, blood stage; LS, liver stage; DHFR, dihydrofolate reductase; DHPS, dihydropteroate synthase; SHMT, serine hydroxymethyltransferase.

<table>
<thead>
<tr>
<th>Location/function</th>
<th>Drug</th>
<th>Life stage/target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apicoplast</td>
<td>Ciprofloxacin</td>
<td>BS and LS*/DNA replication</td>
</tr>
<tr>
<td></td>
<td>Clindamycin/doxycycline</td>
<td>BS and LS/protein translation</td>
</tr>
<tr>
<td></td>
<td>Fosmidomycin</td>
<td>BS and LS*/isoprenoid biosynthesis</td>
</tr>
<tr>
<td></td>
<td>Rifampicin</td>
<td>BS and LS*/RNA transcription</td>
</tr>
<tr>
<td></td>
<td>Fatty acid synthesis inhibitors</td>
<td>LS/fatty acid biosynthesis</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>Atovaquone</td>
<td>BS and LS/cytochrome bc&lt;sub&gt;1&lt;/sub&gt; complex</td>
</tr>
<tr>
<td>Parasite cytoplasm</td>
<td>Pyrimethamine/chlorproguanil</td>
<td>BS/DHFR</td>
</tr>
<tr>
<td></td>
<td>Sulfadoxine</td>
<td>BS/DHPS</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>BS/SHMT</td>
</tr>
<tr>
<td>Endoplasmic reticulum</td>
<td>None</td>
<td>BS and LS*/plasmepsin V</td>
</tr>
<tr>
<td>Food vacuole</td>
<td>Chloroquine/amodiaquine</td>
<td>BS/heme polymerization</td>
</tr>
<tr>
<td></td>
<td>Mefloquine/quinine</td>
<td>BS/heme polymerization</td>
</tr>
<tr>
<td></td>
<td>Artemisinin†</td>
<td>BS and gametocyte/heme polymerization†</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>BS/falcipains</td>
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<tr>
<td></td>
<td>None</td>
<td>BS/plasmpesins</td>
</tr>
<tr>
<td>Parasitophorous vacuole</td>
<td>None</td>
<td>BS and LS*/putative translocon</td>
</tr>
<tr>
<td>Merozoite invasion</td>
<td>None</td>
<td>BS/rhomboid 4, subtilisin 2</td>
</tr>
<tr>
<td>Merozoite egress</td>
<td>None</td>
<td>BS and LS/DPAP3, SERA5, subtilisin 1</td>
</tr>
<tr>
<td>Unknown</td>
<td>Primaquine</td>
<td>LS and gametocytes/drug target not known</td>
</tr>
</tbody>
</table>

*No published data to confirm drug action. †Exact drug target is not known. ‡Possible drug target.

Russo et al., *Nature* 2010, 463, 632
Boddey et al., *Nature* 2010, 463, 627

Kappe et al, 2010 Science 328: 862
## *Plasmodium* Aspartic Proteases

<table>
<thead>
<tr>
<th>Asp Protease</th>
<th>Function(s)</th>
<th>Potential for Drug Discovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasmepsins I-IV</td>
<td>Degrade hemoglobin in red blood cells for food source</td>
<td>Poor—functionally redundant with each other and falcipains</td>
</tr>
<tr>
<td>Plasmepsin V</td>
<td>Regulates export of hundreds of parasite proteins; potential role in both BS and LS</td>
<td>Attractive but challenging — essential gene, but limited tools/assays</td>
</tr>
<tr>
<td>Plasmepsins VI-X</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Signal Peptide Peptidase</td>
<td>Degrade unstable proteins; potential role in multiple life stages</td>
<td>Attractive but challenging — essential gene, but limited tools/assays</td>
</tr>
</tbody>
</table>
Aspartic Proteases are Druggable Targets

- Renin for hypertension
- β-secretase (BACE) for Alzheimer’s Disease
- HIV-1 Protease Inhibitors
  - Ten FDA-approved marketed drugs, mostly based on transition-state mimics
  - Structure-based drug design (SBDD) played major role in HIV-1 protease drug discovery
  - Major challenges in protease inhibitor drug development: high MW, poor absorption, cell permeability, poor aqueous solubility, high hepatic clearance

Plasmodium falciparum has at least 11 aspartic proteases
How do we identify a new antimalarial drug lead?

**Phenotypic Approach**
- Identifies compounds that kill the parasite
- Compounds active in cells—more “drug-like”
- Source for most antimalarial drugs
- Target is usually not known making optimization empirical

**Target-based Approach**
- Inhibition of a specific protein target will lead to parasite death (presumably)
- High throughput tools for optimization: enzyme assays, structure-based drug design
- Potent inhibitors may not have good cell/in vivo potency

**Hybrid Approach**
- Identify target of a phenotypic hit
- Use tools of target based drug discovery to optimize
Thousands of chemical starting points for antimalarial lead identification

Francisco-Javier Gamo, Laura M. Sanz, Jaume Vidal, Cristina de Cozar, Emilio Alvarez, Jose-Luis Lavandera, Dana E. Vanderwall, Darren V. S. Green, Vinod Kumar, Samiul Hasan, James R. Brown, Catherine E. Peishoff, Lon R. Cardon & Jose F. Garcia-Bustos

Malaria is a devastating infection caused by protozoa of the genus *Plasmodium*. Drug resistance is widespread, no new chemical class of antimalarials has been introduced into clinical practice since 1996 and there is a recent rise of parasite strains with reduced sensitivity to the newest drugs. We screened nearly 2 million compounds in GlaxoSmithKline’s chemical library for inhibitors of *P. falciparum*, of which 13,533 were confirmed to inhibit parasite growth by at least 80% at 2 μM concentration. More than 8,000 also showed potent activity against the multidrug resistant strain Dd2. Most (82%) compounds originate from internal company projects and are new to the malaria community. Analyses using historic assay data suggest several novel mechanisms of antimalarial action, such as inhibition of protein kinases and host–pathogen interaction related targets. Chemical structures and associated data are hereby made public to encourage additional drug lead identification efforts and further research into this disease.
Strategy: Mine phenotypic screening databases for drug-like aspartic protease inhibitors as good lead compounds for optimization as drugs

Target-based Approach
- Renin inhibitors
- HIV Protease inhibitors
- BACE inhibitors
- Cathepsin D/E inhibitors

Phenotypic Approach
- GSK TCAMS
- SJCRH collection
- Novartis’ “Malaria Box”
- Scientific literature

Pf Asp Protease inhibitors?

Lead compounds for optimization as new antimalarial agents
Inhibition of the Asp Protease Mechanism

Substrate Protein → Transition-State Intermediate → Hydrolysis Products

Ritonavir
Known Inhibitors of Asp Protease β-Secretase

Pharmacia/Elan

Merck
*J. Med. Chem.* 2008, 6259

Wyeth
*J. Med. Chem.* 2010, 1146

Wyeth 44

= key catalytic Asp residue-binding moiety

Blue structures used in substructure search of the Tres Cantos Antimalarial Set (TCAMS)
Antimalarial Aspartic Protease Inhibitors in TCAMS

Are these plasmepsin inhibitors?
Are they good drug-like starting points?

Lipinski’s Rule of Five properties of “Drug-like” molecules
- 5 or less hydrogen bond donors
- 10 or less hydrogen bond acceptors
- Molecular weight under 500
- Partition coefficient log P less than 5 (lipophilicity)

Aminohydantoins – Med Chem Strategy

Aminohydantoin-Aspartic Protease BACE Binding Mode

1. Prepare R1/R2/R3 analogs to develop SAR
2. Derivatize X to confirm aspartic protease target

X = NH, O, S

4 (TCMDC-136879)
35 examples in TCAMS
IC$_{50}$ range 140-1500 nM

“Hit Compound”

ACS Med Chem Lett 2014, 5, 89-93
Evidence for Asp Protease Mechanism of Action

Aminohydantoin/BACE Binding Mode

CWHM-117
MW 393
cLogP 3.93
3D7 IC$_{50}$ = 309 nM
PM-II IC$_{50}$ = 4 nM
PM-IV IC$_{50}$ = 15 nM

CWHM-209
MW 411
cLogP 4.75
3D7 IC$_{50}$ > 5 µM
PM-II IC$_{50}$ > 10 µM

CWHM-208
MW 394
cLogP 3.86
3D7 IC$_{50}$ > 10 µM
PM-II IC$_{50}$ > 10 µM
PM-IV IC$_{50}$ > 10 µM

3INH. Malamas et al., J. Med. Chem., 2010, 53, 1146
SAR and Selectivity

3D7 = 7.92 µM  
PM2 = 0.487 µM  
BACE = 7.92 µM [25x]

3D7 = 0.463 µM  
PM2 = 0.004 µM  
BACE = 12.0 µM [3000x]

3D7 = 6.88 µM  
PM2 = 1.01 µM  
BACE = 1.68 µM [2x]

3D7 = 0.750 µM  
PM2 = 0.013 µM  
BACE = 18.1 µM [1400x]

Aminohydantoin/BACE Binding Mode

3INH. Malamas et al., J. Med. Chem., 2010, 53, 1146
# Identification of a Lead Compound(s)

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>cLogP</td>
<td>4.0</td>
<td>2.1</td>
<td>4.3</td>
<td>2.6</td>
</tr>
<tr>
<td>Pf 3D7 IC$_{50}$ (µM, 72h)</td>
<td>0.463</td>
<td>0.459</td>
<td>0.383</td>
<td>0.463</td>
</tr>
<tr>
<td>Pf Dd2 IC$_{50}$ (µM, 72h)</td>
<td>0.480</td>
<td>0.526</td>
<td>0.367</td>
<td>0.442</td>
</tr>
<tr>
<td>Pf 3D7 IC$_{50}$ (µM, 48h)</td>
<td>0.751</td>
<td>0.404</td>
<td>0.339</td>
<td>0.571</td>
</tr>
<tr>
<td>HepG2 IC$_{50}$ (µM)</td>
<td>9.4</td>
<td>&gt;50</td>
<td>8.0</td>
<td>30</td>
</tr>
</tbody>
</table>

### Pharmacokinetics (PK) in Rat
- Half-life = 2.9 h
- Bioavailability = 16%
Orally efficacious in mice infected with *Plasmodium falciparum*
- 89% suppression of parasitemia achieved at 100 mpk (qd)
- Full suppression achieved at 300 mpk (qd)

**Goal is to improve potency another 10-fold**
Aspartic protease inhibitors represent novel antimalarial chemotypes and mechanism of action

- Potent and Selective
- Orally Efficacious
- Need 10x more potency