Antimalarial drug development through product development partnerships

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GHIT-PDPs Webinar Series:
Advancing innovations for neglected disease during and beyond the pandemic
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東京大学
THE UNIVERSITY OF TOKYO

Screening platform: 2018-2020

"High throughput screening of DDI compound library against the asexual blood stage of *Plasmodium falciparum*"

Hit-to-Lead platform: 2021-2023

"Hit-to-Lead development of DDI series for antimalarial development"

Jeremy Burrows

東京大学
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Drug Discovery Initiative DDI
創薬機構

GHIT Fund
Global Health Innovative Technology Fund

Norio Shibata
Nagoya Institute
of Technology

Paul Willis

James Duffy

MMV
Medicines for Malaria Venture

Tomo NOZAKI
Who am I?

Professor
Department of Biomedical Chemistry
Vice Dean
Graduate School of Medicine,
The University of Tokyo

M.D., Ph.D. (Keio University School of Medicine)
Specialty: Molecular parasitology (*Entamoeba, Plasmodia, Trypanosoma,* and *Leishmania*)
Major interests: Metabolism, Pathogenesis, Evolution, and Drug development

My dream when I was a medical student: a doctor in Amazon

Tomo NOZAKI
Who am I?

Major achievements

Metabolism

Cysteine/methionine, FeS, NADH, CoA

Adv Parasitol, 60, 1-99, 2005; Clin Microbiol Rev, 20, 164-187, 2007; Adv Parasitol, 83, 1-92, 2013; Biochimie 95, 309-319, 2013; Curr Opin Microbiol 20C:118-124, 2014; Front Microbiol 9:2902, 2018; Front Cell Inf Microbiol 11:639065, 2021

Pathogenesis

Vesicular traffic, proteases, phago(& trogo)cytosis, lipid transport

Nature 433, 865-868; Nat Commun 8,101, 2017; PLoS Pathog, 14:e1006882, 2018.; PLoS Pathog, 17:e1009551, 2021. PLoS Pathog., 17:e1008909, 2021

Evolution

Mitochondria under anaerobic environment

Proc Natl Acad Sci USA, 106, 21731–21736; Proc Natl Acad Sci USA 112:E2884-90, 2015; Trends Parasitol. 34:1038-1055, 2018.

Drug development

Target identification and anti-amebic drug lead identification

FEBS J 275, 548-560, 2008; IUBMB Life, 61, 1019-1028, 2009; J Antimicrob Chemother 66, 2045-2052, 2011; Front Microbiol 6, 962, 2015.; Parasitol Int 102432, 2021

How we started MMV-UTokyo collaboration: background

When we wanted to start our drug discovery campaign,....

- A majority of screening projects were conducted by pharmaceutical companies, using their own libraries.
- There were very few projects from Japanese academia. Only a limited set of their institution-owned natural compounds were tested against malaria and other parasitic diseases.

Why not use academia-owned chemical libraries for malaria drug discovery.

How we started MMV-UTokyo collaboration

- ✓ We have expertise in molecular parasitology, and we are keen to develop medicines for malaria and other parasitic diseases.
- ✓ We have excellent target (enzyme)-based and cell-based (phenotypic) assays.
- ✓ Structurally-defined chemical and natural microbial culture broth libraries are readily available.

So, ...

- ✓ We need external help for chemistry, preclinical development including safety testing, PK/PD, and ADME.
- ✓ We need a compass for directions and good collaborative network for the above.

Then, ...

- ✓ We started searching potential collaborators and funding sources.

Our MMV-UTokyo collaboration

We aim at:

Discovery and optimization of new compounds that are efficacious against *Plasmodium falciparum* and have new scaffold and novel mechanism of action

Desired properties include:

- Fast acting for treatment
- Long duration for prophylaxis
- Oral dose
- Efficacious against drug resistant field strains
- Targeting multiple lifecycle stages
- Low propensity of raising drug resistance
- Cheap
- Applicable for vulnerable populations (children and women of child-bearing potential)

Burrows, J. N. *et al. Malar. J.* **16,** 26 (2017)

from a Japan-made structurally defined chemical library

Objective

Discover new
mechanism of
from a Japan-

Compou

The compounc
The University
- 210,150 con

Assay

P. falciparum erythrocytic stage
3D7 386 well plates
LDH/SYBR Green assays

Components of Chemical Library (March 2019)

Validated Compound Library
Known bioactives for checking assay systems or repositioning

Core Library
9,600 diverse compounds for pilot screening
Some derivatives of each core compound are prepared. A relatively lipophilic 2,400 subset is chosen for cellular assays.

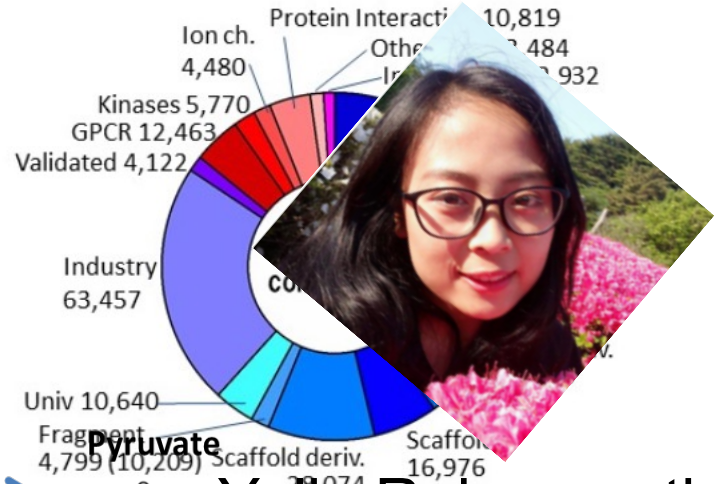
Advanced Core Library
Another 22,400 diverse set after pilot screening

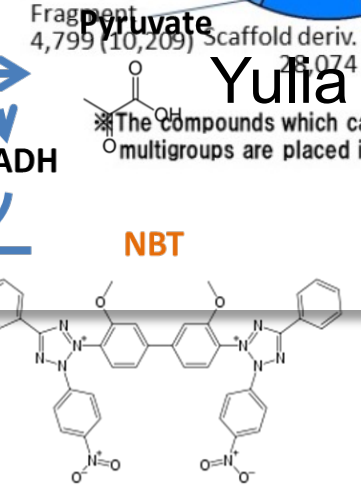
Full Library
General 210,000 samples

Fragment / Scaffold Library
Collection as partial structures of drug candidates

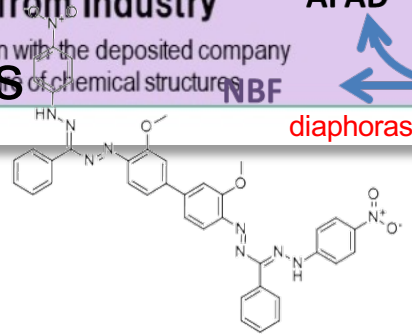
Focused Library
Candidates predicted to bind to ligands of target proteins

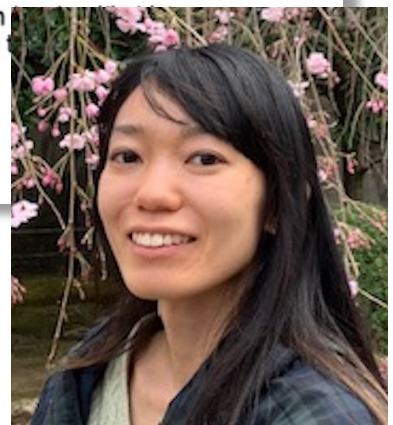
Samples from Industry
For collaboration with the deposited company
Unique properties of chemical structures





※The compounds which can
multigroups are placed in





Yulia Rahmawati

Haruka Ohno

Natsuki
Watanabe

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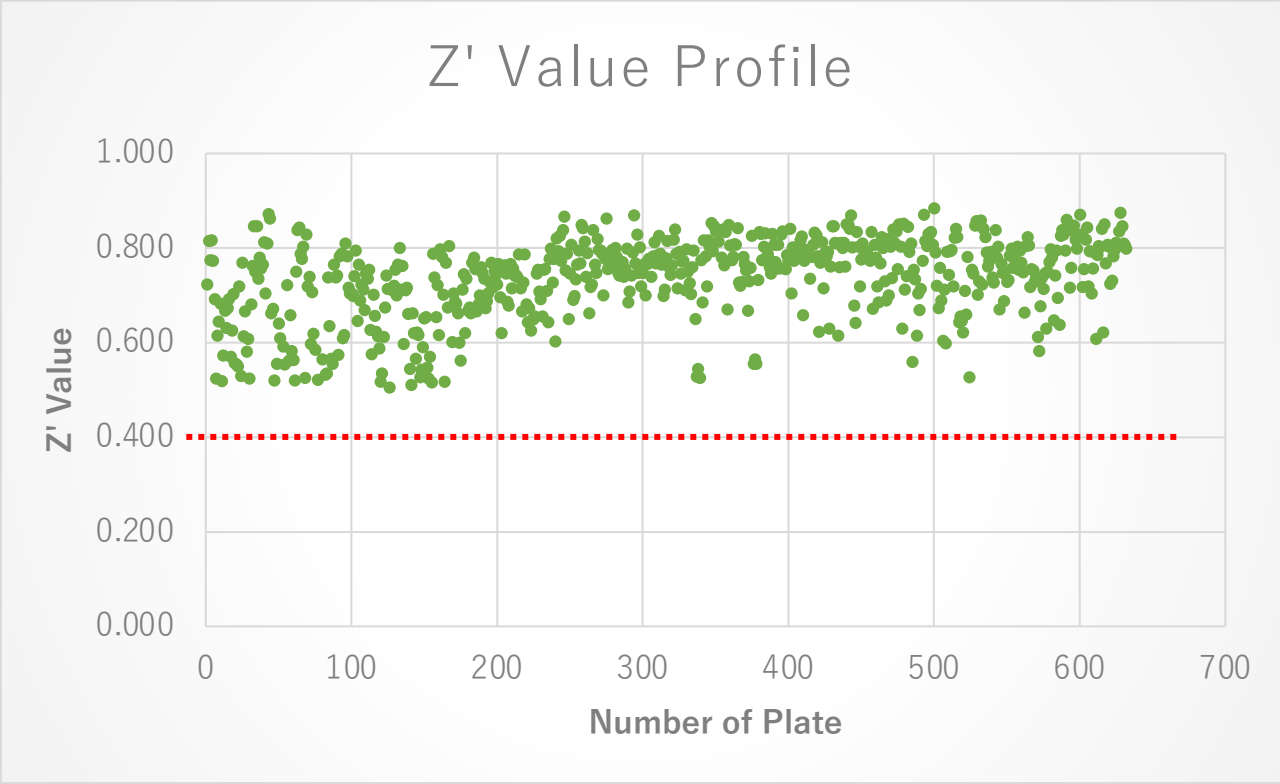
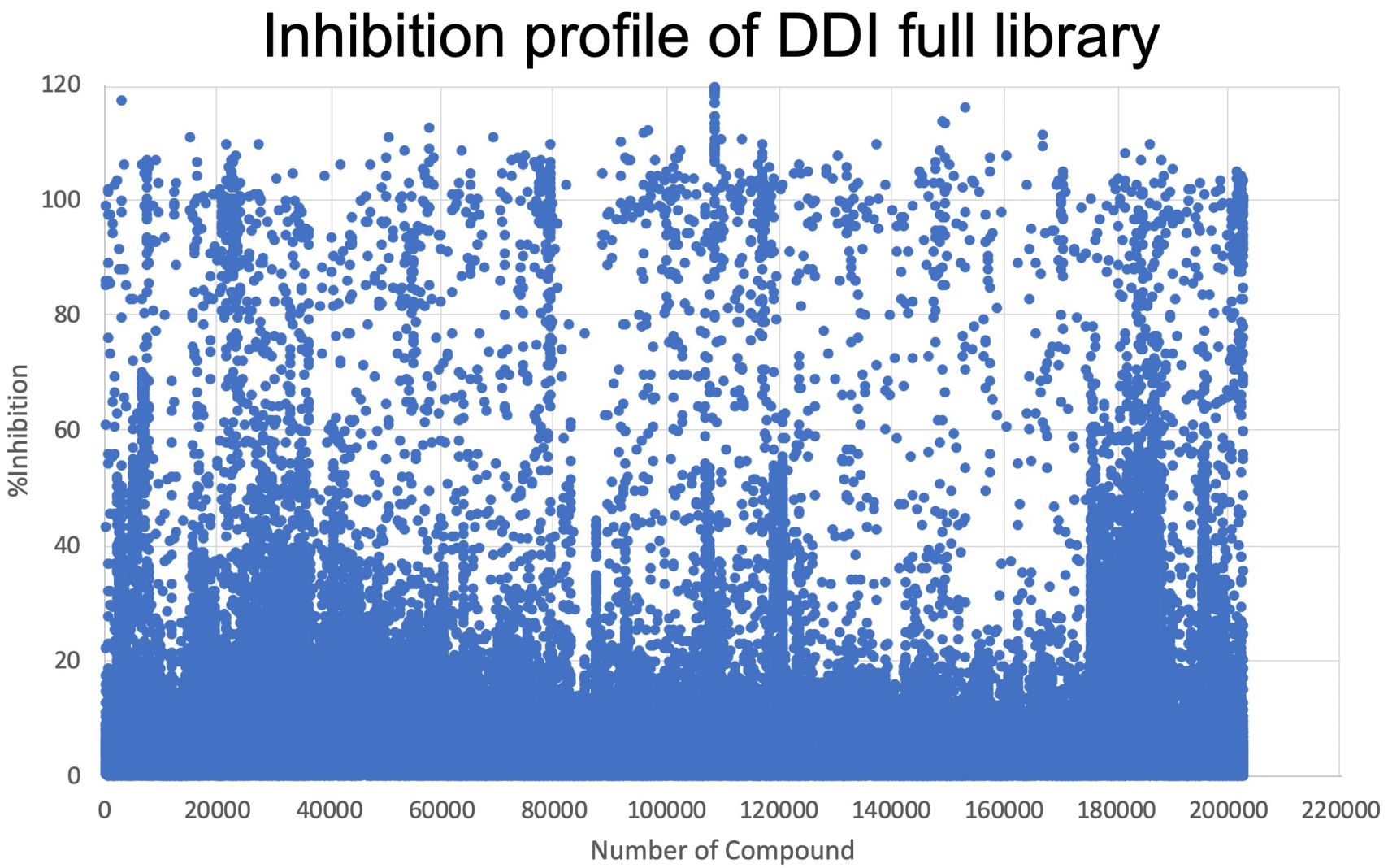
Assay evaluation and validation



Z’ value : 0.76 ± 0.07
Excellent assay

S/B value : mostly > 3

Inhibition profile and hit rate

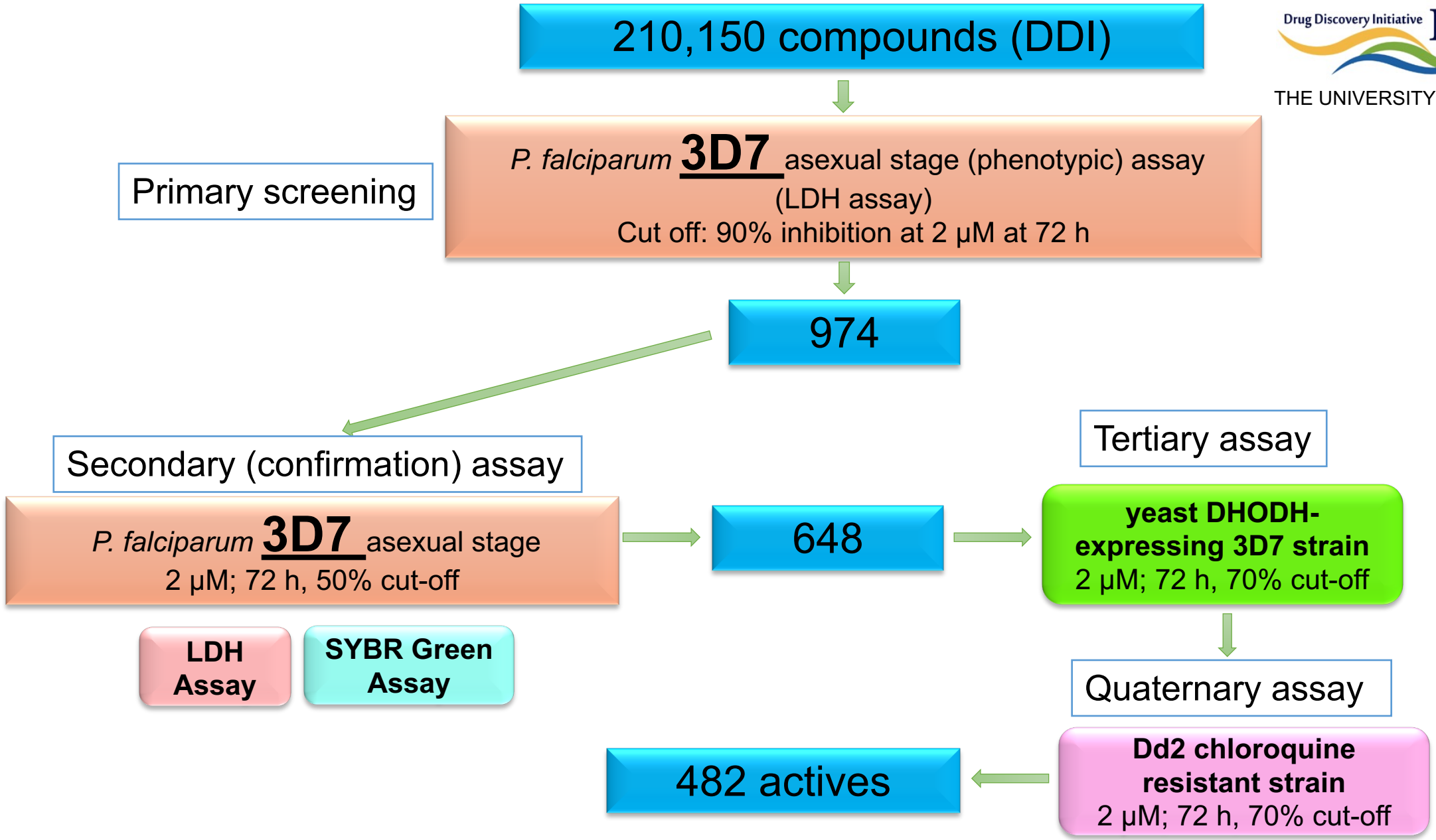


Number of actives

% Inhibition (2 μM)	Number of compounds
≧30	5,831
≧50	2,505
≧70	1,580
≧80	1,307
≧90	974
Total number	210,150

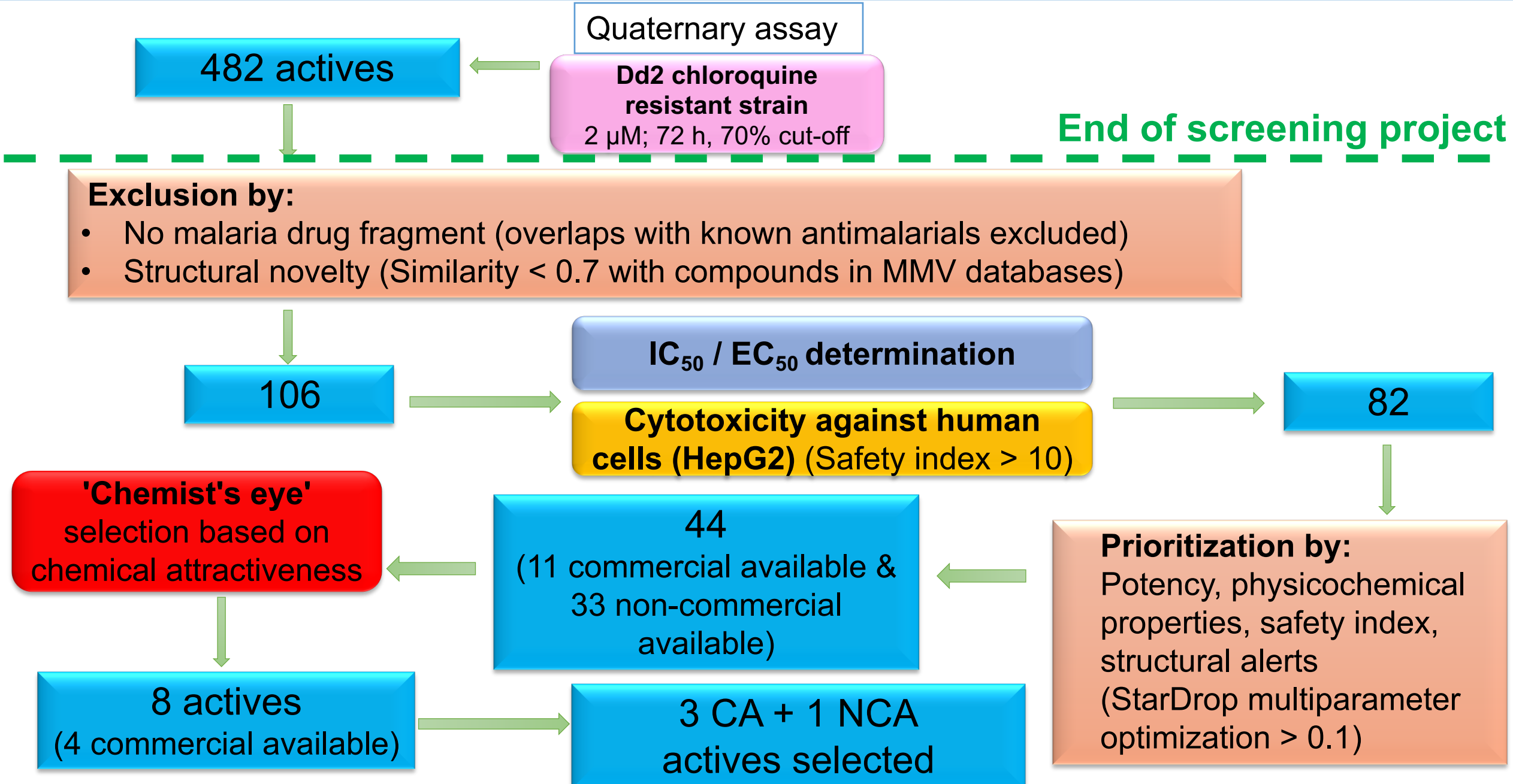
Active rate (>90% inhibition at 2 μM): 0.5%
(>50%) : 1.2%

Screening cascade

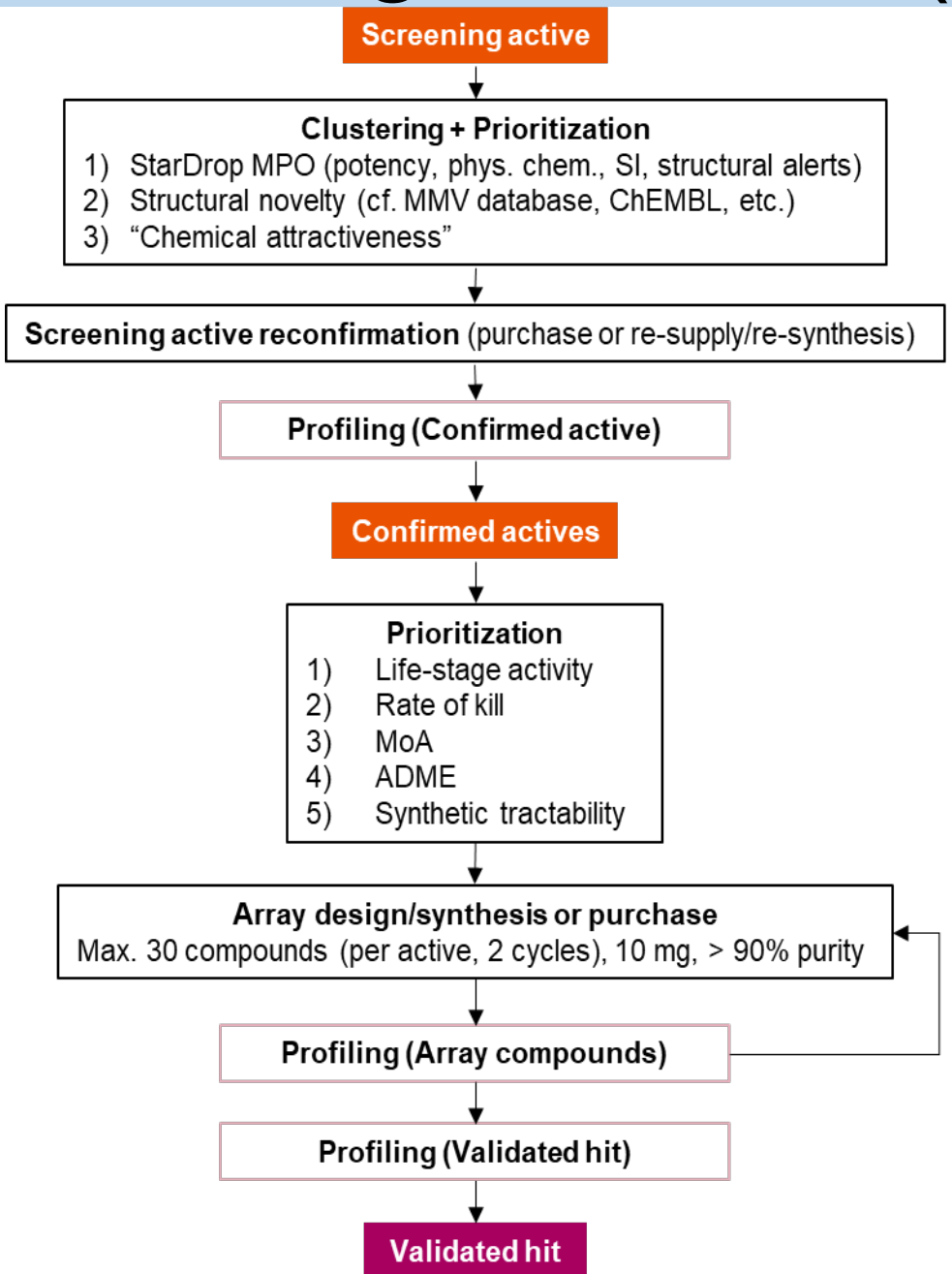


Drug Discovery Initiative DDI
THE UNIVERSITY OF TOKYO

Screening cascade



Screening cascade (after active discovery)



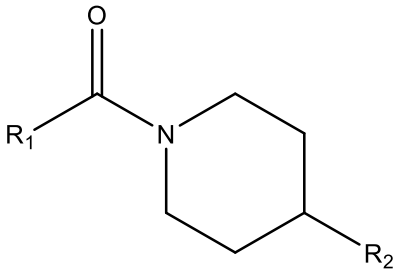
Active and Hit profiling

Assay	Confirmed active	Array compounds	Validated hit
Pf Asexual blood stage	x	x	x
Lifecycle profile[1]	x	-	x
Rate of kill[2]	x	-	x
Hit deconvolution[3]	x	-	x
ADME I[4]	x	x	x
ADME II[5]	-	-	x
Cytotoxicity	x	x	x
hERG	x	-	x
Resistance panel[6]	-	-	x

[1] = Liver stage (Pb, Pf, Pv), Transmission blocking (gametocytes, DGFA)
[2] = Confimed active: High throughput rate of kill assay (e.g. 12 h vs 72 h incubation); Validated Hit: Full time course PRR
[3] = Drug resistant strain (Dd2 or K1), yDHODH (mtETC inhibitors), Dd2-mutants (Carl/PI4K/ACL), pH-based assays (PfATP4, etc.)
[4] = LogD, kinetic sol., human liver microsomes, rat hepatocytes
[5] = Albumax binding, human plasma protein binding, Caco-2 permability, human plasma stability, CYP inhibition (5 isoforms)
[6] = Lab-adapted field isolates and drug-resistant mutants

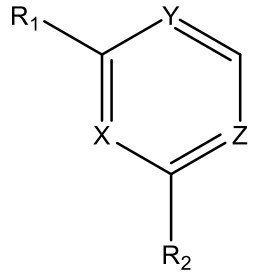
Three hit-to-lead candidates

Series 1



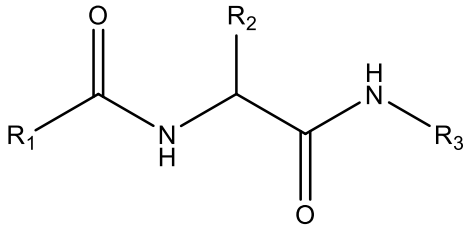
T-117040 (MMV1804220)

Series 2



T-132522 (MMV1804245)

Series 3



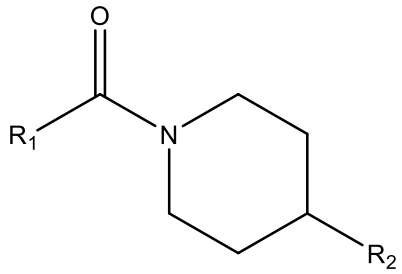
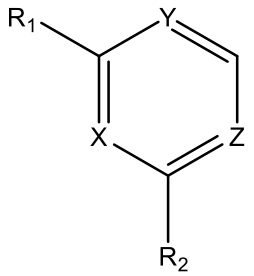
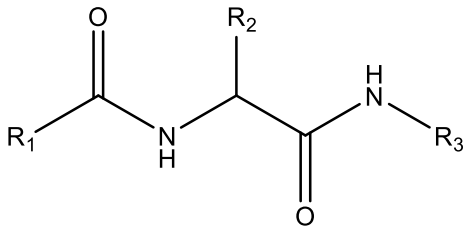
T-210671 (MMV1804223)

- Good lipophilicity (eLogD: 2.72-3.53) as starting actives
- Reasonable kinetic solubility (46~190 μM)
- Series 1-3 inhibit none of the following known targets: DHODH, electron transport chain including bc1 complex, ACS, CARL, PI4K, and ATP4

Profiling of three hit-to-lead candidates

	Series 1 **T- 117040 (MMV1804220)**	Series 2 **T-132522 (MMV1804245)**	Series 3 **T-210671 (MMV1804223)**
IC50 3D7 asexual stages (LDH 72 hr)	0.75 µM	1.3 µM	0.23 µM
3D7 rate of kill	Slow	**Fast**	**Fast**
IC50 drug resistant Dd2 (SYBR Green 72 hr)	1.58 µM	0.52 µM	0.16 µM
IC50 *P. berghei* liver activity	**0.11 µM**	9.77 µM	**0.041 µM**
% inhibition @ 1 µM Pf male and female gamete formation	NA	**43-96%**	0-3.9%
IC50 human primary hepatocytes 96 hr	>10 µM	>10 µM	>10 µM
hERG K^+ CHO (patch clamp)	>30 µM	1.26 µM	>30 µM
Metabolism by human microsomes CLint	**17 µL/min/mg**	270	116
Metabolism by rat hepatocytes CLint	44.5 µL/min/10^6 cells	95.6	**9**

Advantages and issues of three hit-to-lead candidates

	Series 1	Series 2	Series 3
			
	T-117040 (MMV1804220)	**T-132522 (MMV1804245)**	**T-210671 (MMV1804223)**
Strengths	• Liver stage activity • Metabolic stability	• Fast kill • Sexual stage activity • De-risked potential toxicity issue	• Fast kill • Liver stage activity
Issues	• Improve potency • Define SAR	• Need to identify minimal pharmacophore	• Peptide like (Oral absorption?) • Complex synthesis (3 chiral centers) • Address liabilities in new molecules
Number of derivatives synthesized	20	3	13

Summary

- ✓ U Tokyo/MMV screened DDI library, Japanese academia-owned chemical library of 210k compounds, and identified 3-4 candidates to advance to "hit-to-lead".
- ✓ Structural optimization (based on potency, physicochemical properties, stability, and SAR) and profiling of the improved actives (the rate of kill, multiple life cycle stages, mechanism of action, and ADME) are under way.

Future perspective and requests from academia

- ✓ We have directly gained and will gain knowledge and expertise on antimalarial (& other IDs) drug development from MMV and also indirectly via liaison with experts in pharmaceutical industries and other international partners.
- ✓ We, parasitologists in academia, need to create a broader network covering natural and synthetic chemistry, safety, and even regulatory sciences.
- ✓ MMV bridges a gap between academia and industries (also within academia) by promoting networking and helping us be connected with experts with necessary disciplines.
- ✓ MMV also provides young researchers an opportunity for exposure to the outside of the lab, and lots of experience, encouragement, and excitement for realization of their research.

- ✓ We are looking forward to good progress of our structurally optimized initial leads toward Lead Optimization in 1.5 years, and Preclinical/Clinical platforms in 3-4 years.

- ✓ It would be nice if GHIT Fund also aids in connecting us with pharmaceutical industries and even contract research organizations (CROs).

Acknowledgements

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科研費 KAKENHI

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