

Metabolomics helps to unravel the mode of action of novel anti-malarial compounds

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Malaria: we need new drugs



P. falciparum

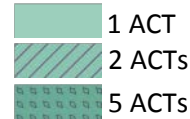


Anopheles
Mosquito

>200 million cases of malaria and an estimated 429000 deaths in 2015 (WHO 2016)

- *P. falciparum* has developed resistance to the first line of malaria treatment, Artemisinin-based Combination Therapies (ACTs) in many countries in South-East Asia^{1,2}
- Resistance has also been reported from Africa³
- We need new anti-malarials to combat the on-going threat of anti-malarial drug-resistance

High ACT failure rate (>10%)

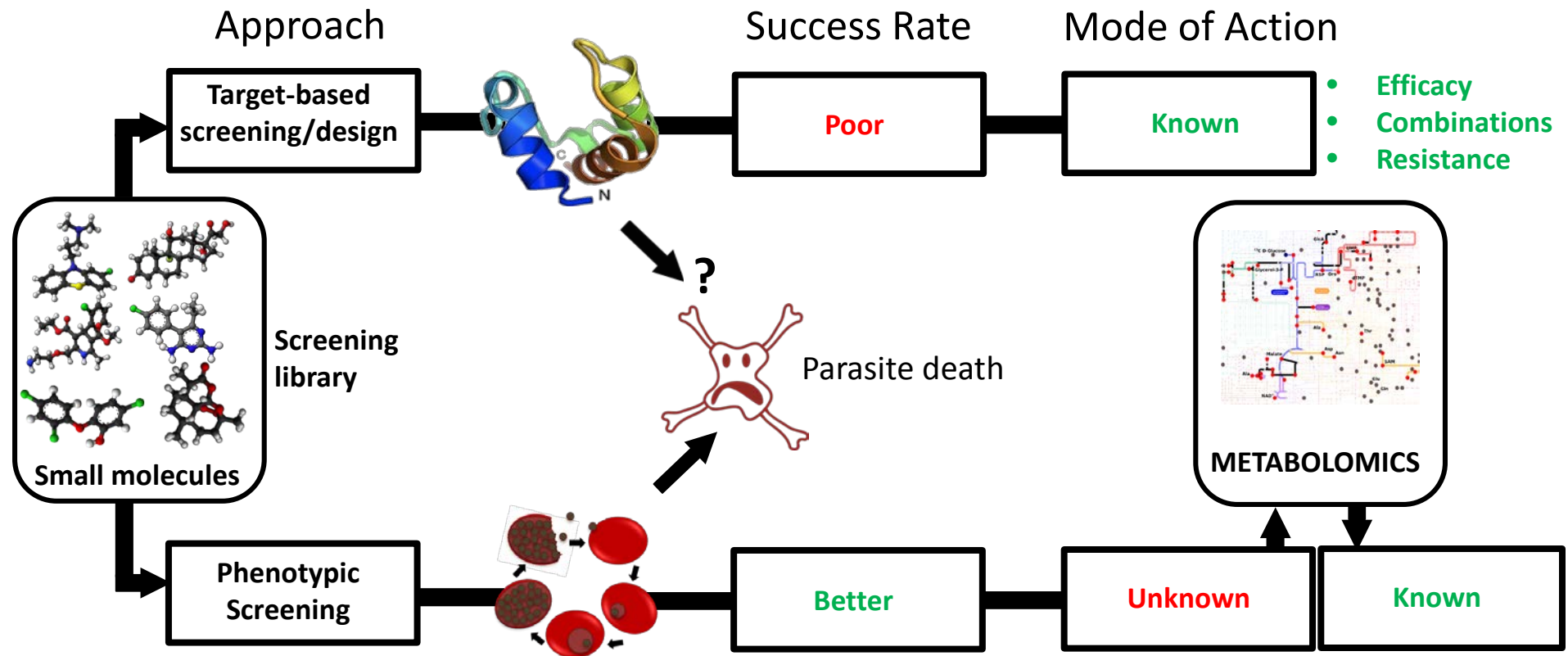


1. Dondorp AM, et al. 2009 N Engl J Med; 361:455-467

2. Ashley EA, et al. 2014 N Engl J Med; 371:411-423

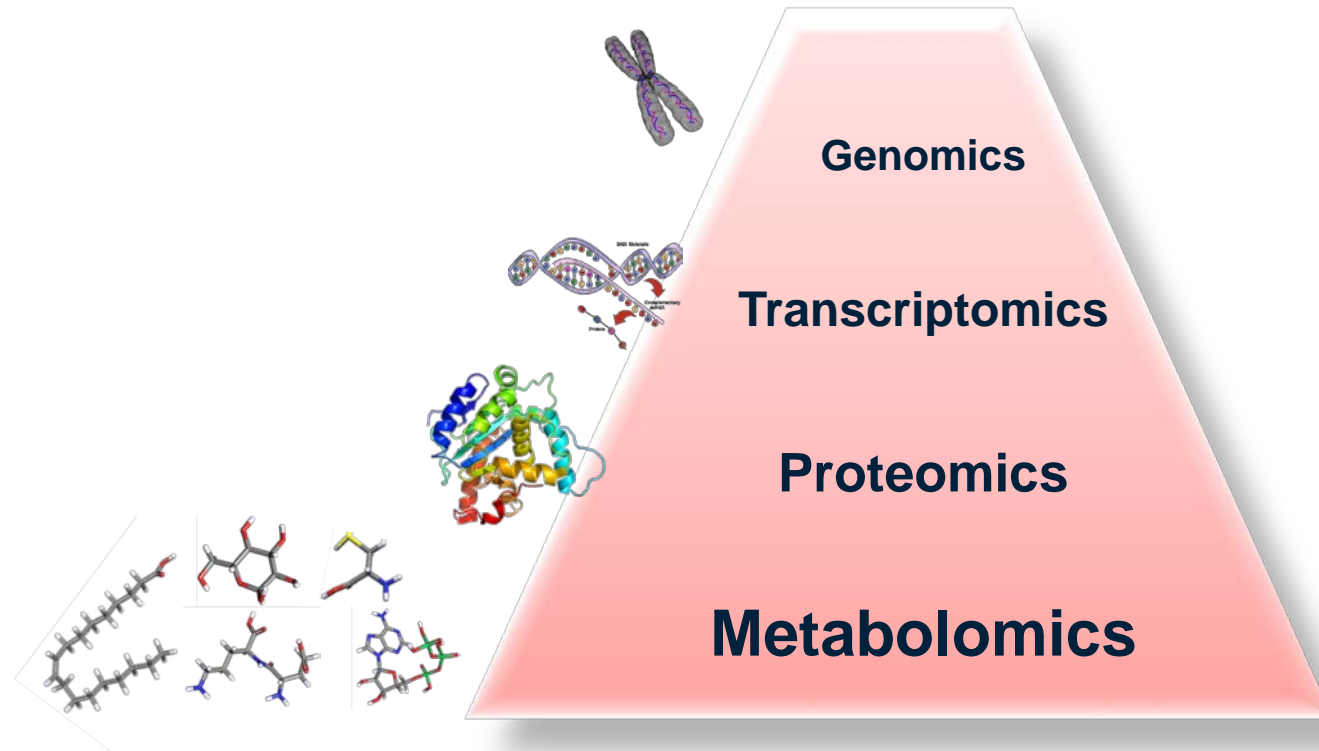
3. Feng Lu, et al. 2017 N Engl J Med; 376:991-993

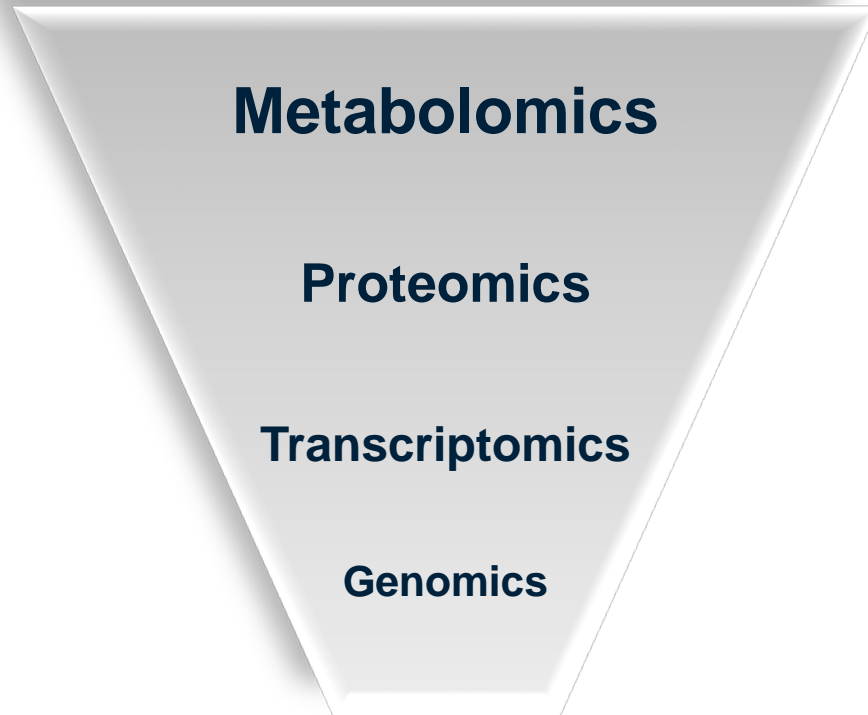
Anti-parasitic drug discovery approaches



Drug Discovery

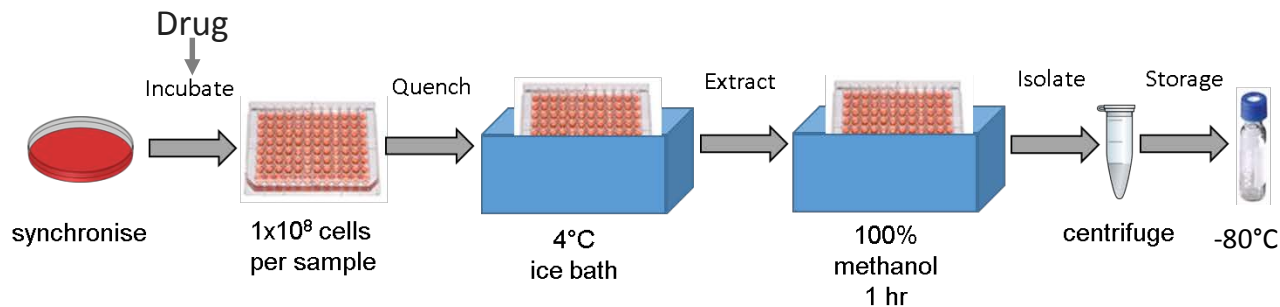
Systems Pharmacology



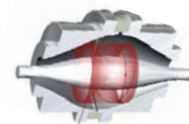
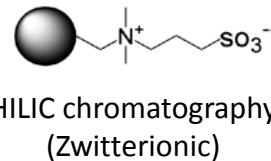


Metabolomics Methodology

Incubation/ Extraction

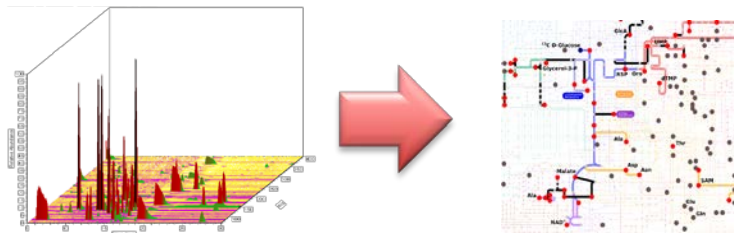


LC-MS: HILIC-Orbitrap (Untargeted)

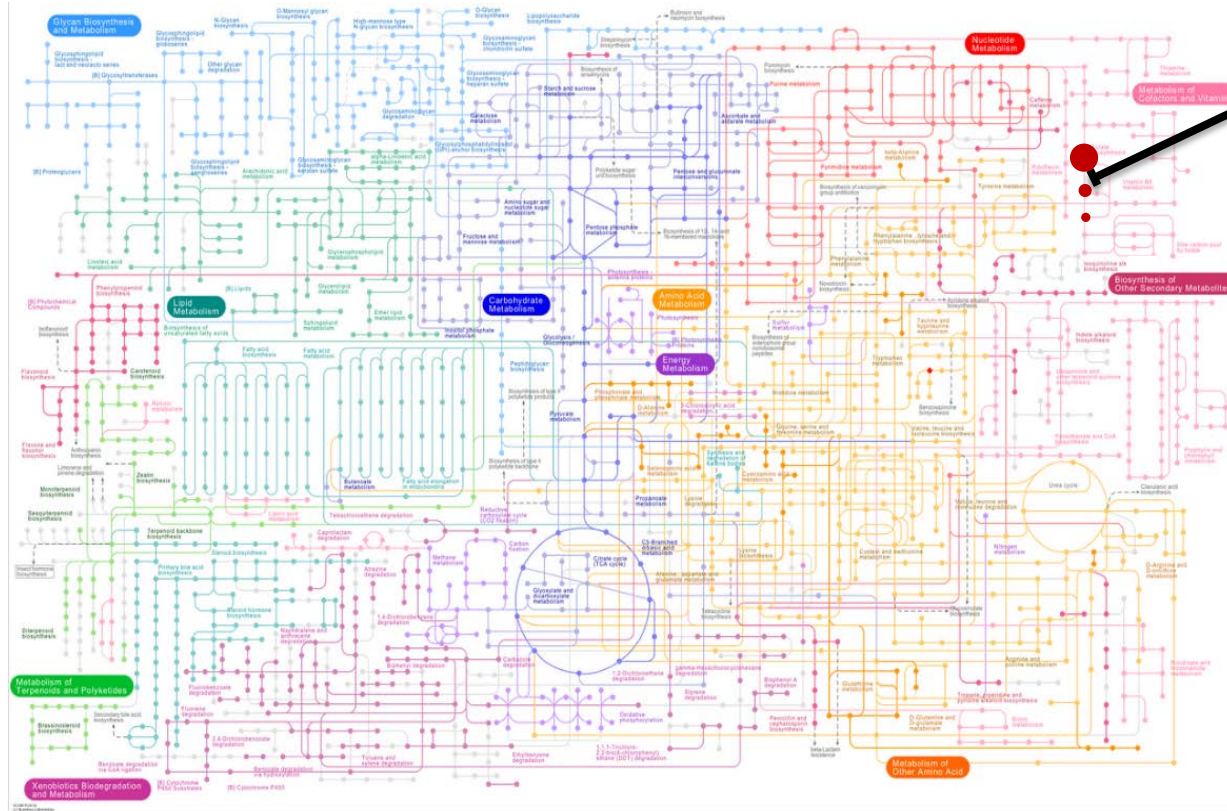


High resolution Accurate
mass MS

Data Analysis: IDEOM workflow



Using Metabolomics to study mode of drug action



Drug



Compounds used in this study

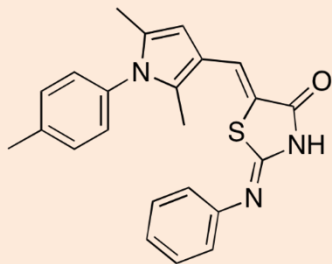
Novel anti-malarials

- Potent activity
- No known target

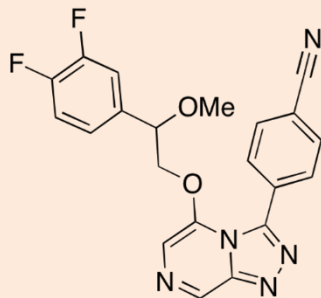


<http://opensourcemalaria.org>

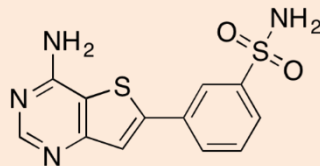
OPEN SOURCE MALARIA
Looking for New Medicines



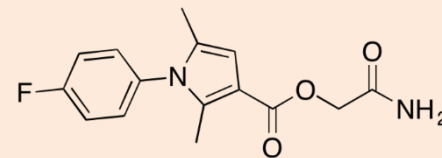
OSM-S-37



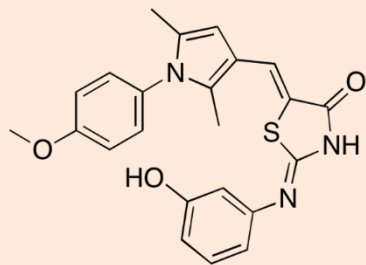
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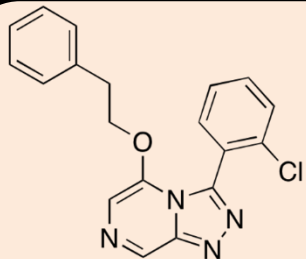
OSM-S-106



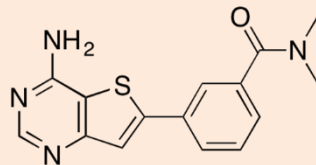
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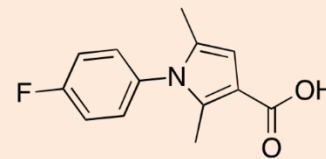
OSM-S-112



OSM-S-291



OSM-S-133

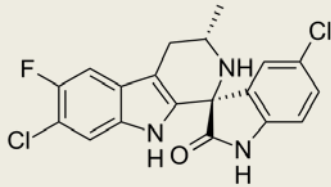


OSM-S-4

Inactive

Compounds used in this study

PfATP4 inhibitors



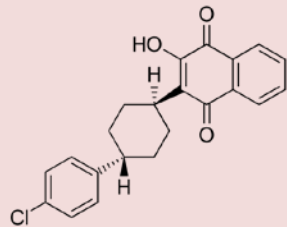
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?

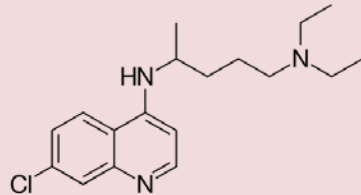
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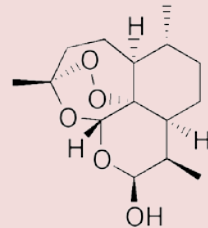
MMV390482



Atovaquone



Chloroquine

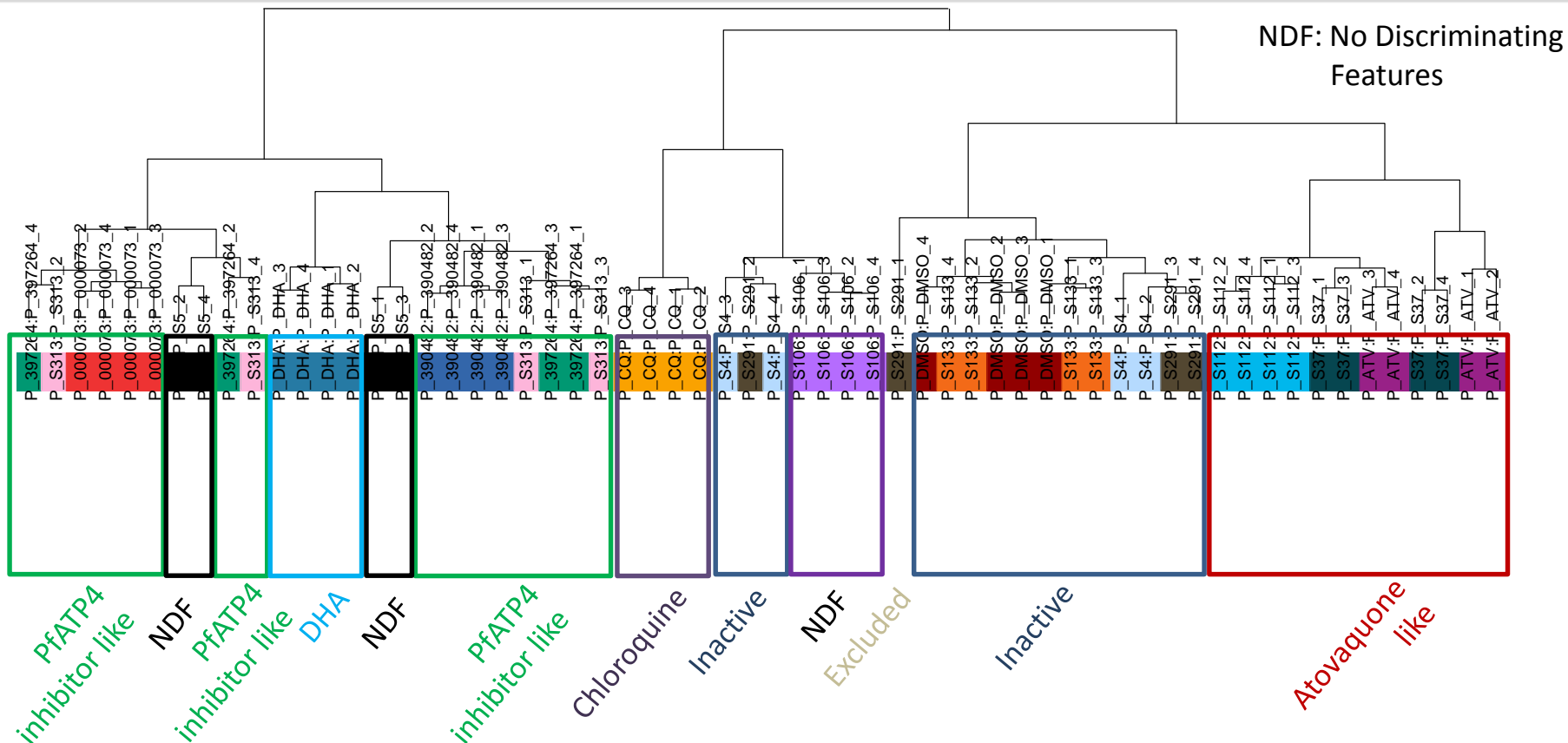


Dihydroartemisinin

Known anti-malarials

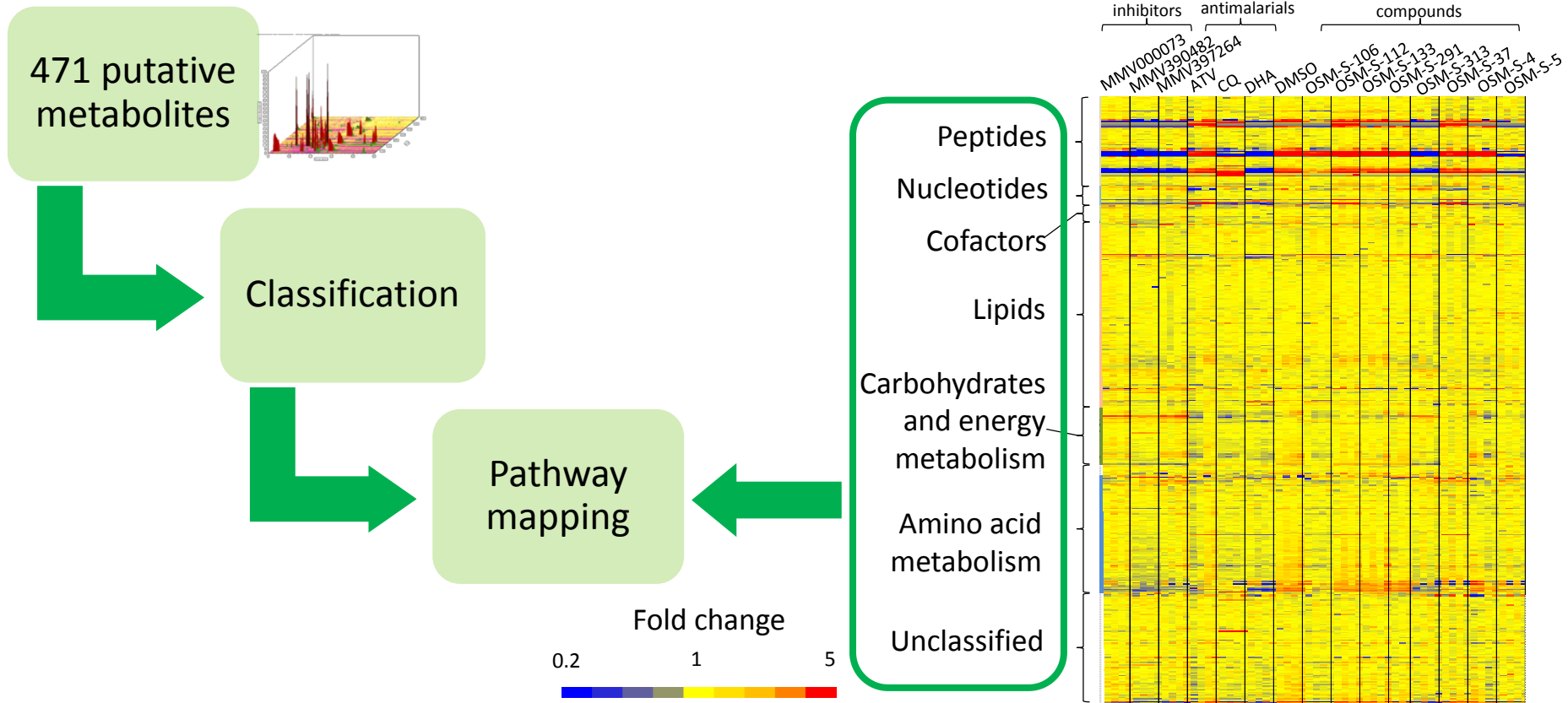
Results: Multivariate analysis

Unsupervised Hierarchical Clustering



Results: Multivariate analysis

Heat map



Compounds used in this study

Novel anti-malarials

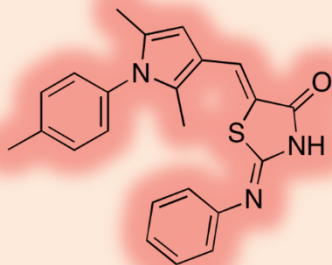
- Potent activity
- No known target



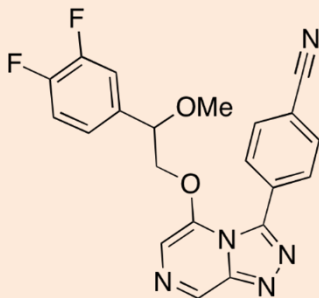
<http://opensourcemalaria.org>

OPEN SOURCE MALARIA

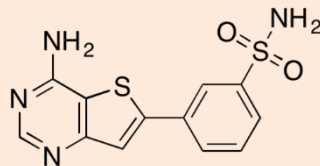
Looking for New Medicines



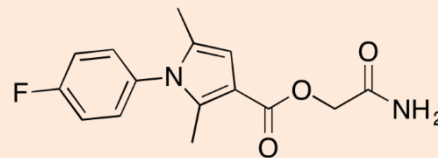
OSM-S-37



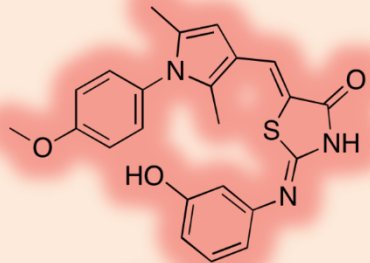
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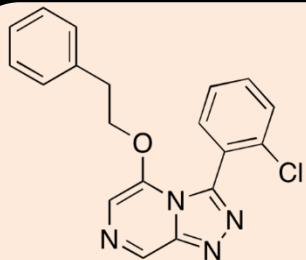
OSM-S-106



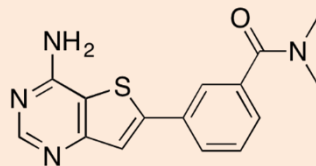
OSM-S-5



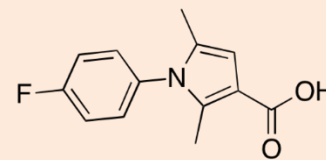
OSM-S-112



OSM-S-291



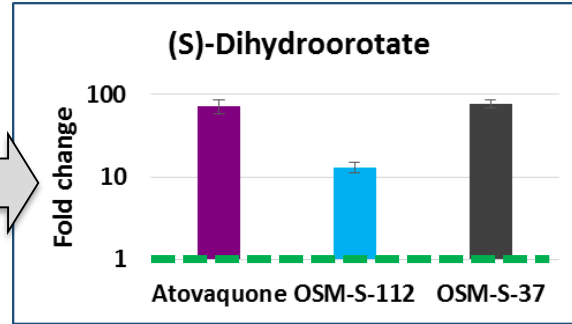
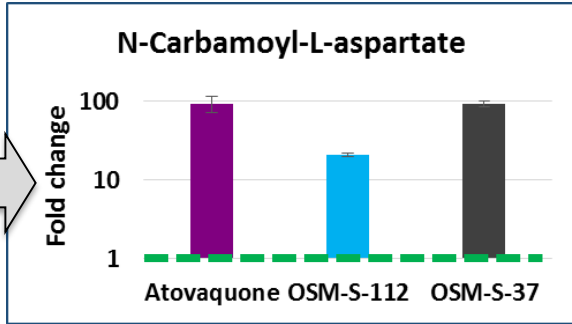
OSM-S-133



OSM-S-4

Inactive

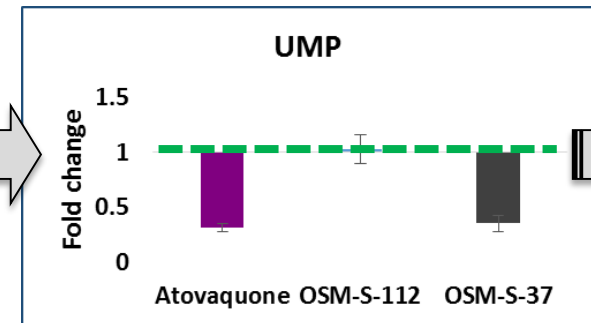
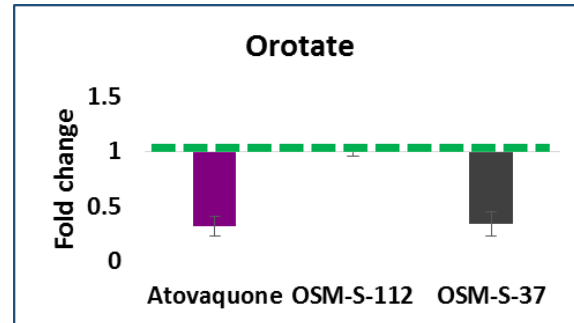
MoA of OSM-S-112 and OSM-S-37 Similar to Atovaquone






Pyrimidine biosynthesis is affected in parasites treated with OSM-S-112 and OSM-S-37

OSM-S-37 >> OSM-S-112

 DHODH 



 DMSO control
 DHODH Dihydroorotate dehydrogenase
 Inhibition

Compounds used in this study

Novel anti-malarials

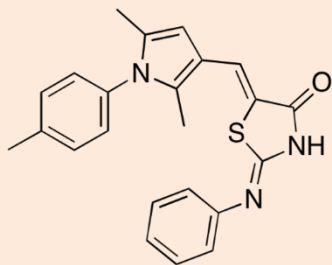
- Potent activity
- No known target



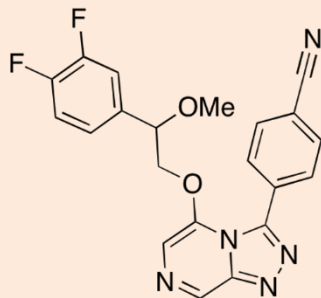
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OPEN SOURCE MALARIA

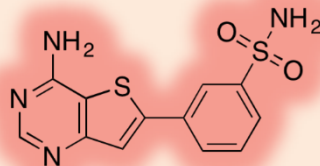
Looking for New Medicines



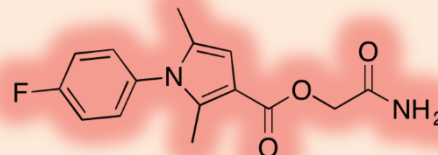
OSM-S-37



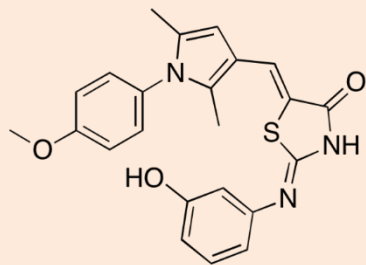
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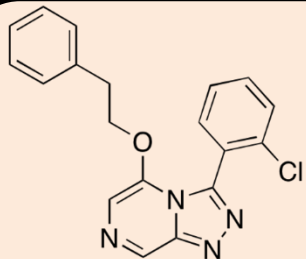
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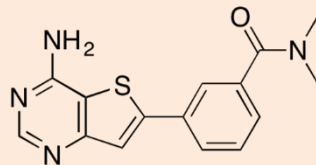
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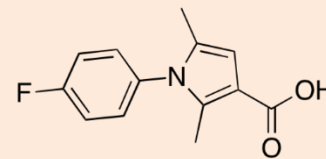
OSM-S-112



OSM-S-291



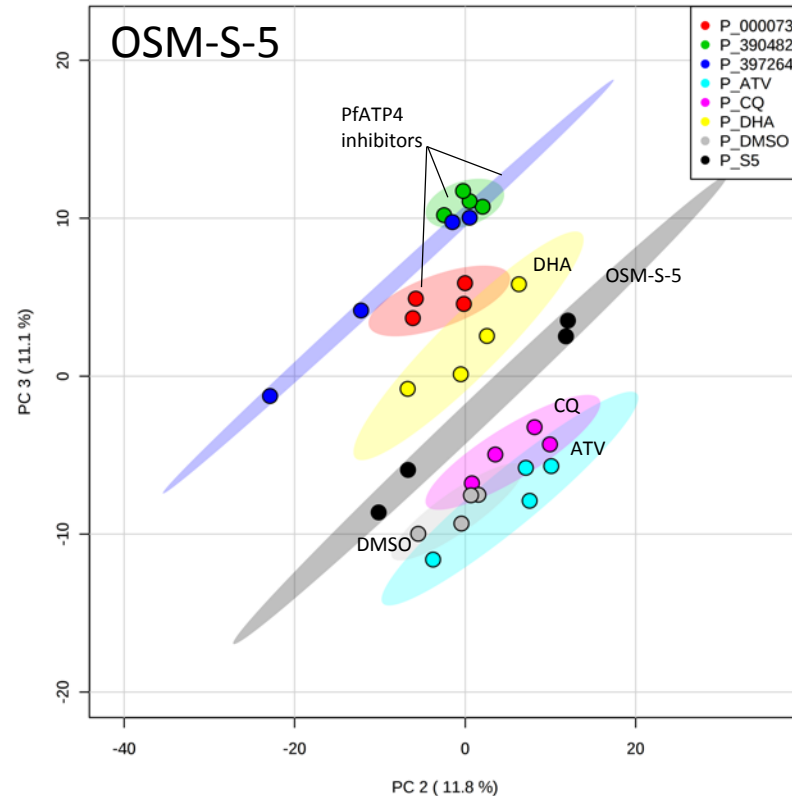
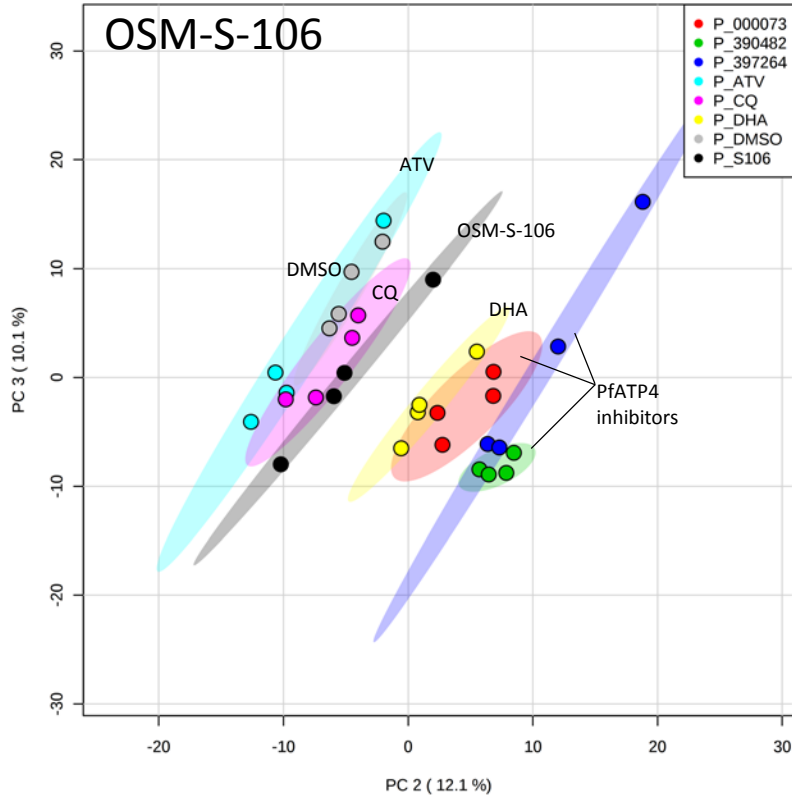
OSM-S-133



OSM-S-4

Inactive

MoA of OSM-S-106 and OSM-S-5 Unknown



- Multivariate analysis (PCA)
- No unique changes
- MoA requires further investigation
- e.g. enriched (>90%) parasite cultures

Compounds used in this study

Novel anti-malarials

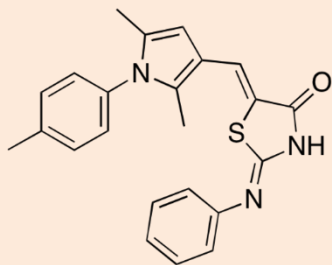
- Potent activity
- No known target



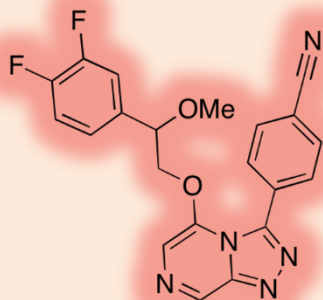
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OPEN SOURCE MALARIA

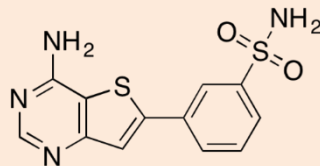
Looking for New Medicines



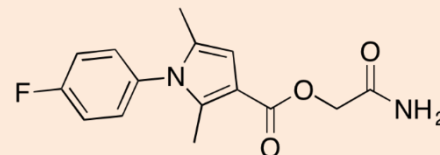
OSM-S-37



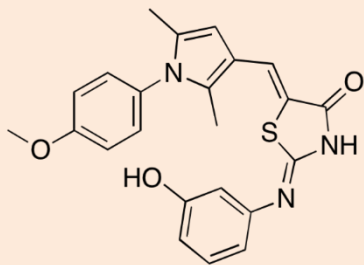
OSM-S-313



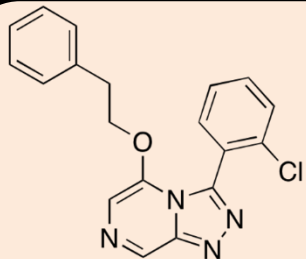
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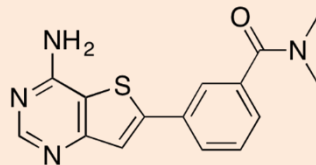
OSM-S-5



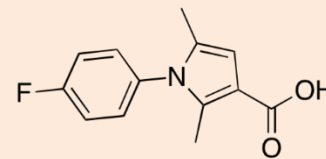
OSM-S-112



OSM-S-291



OSM-S-133

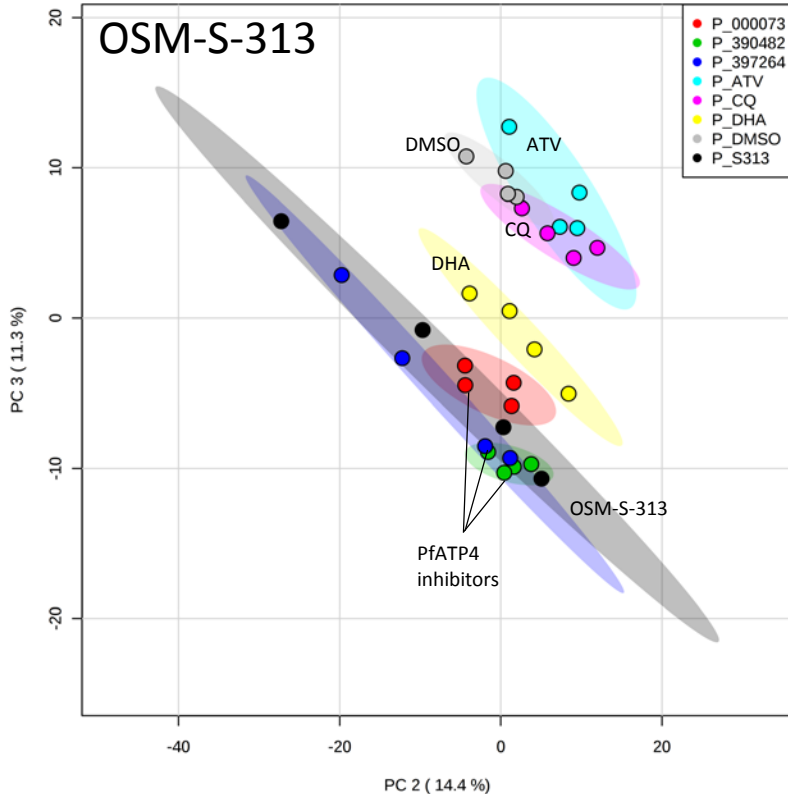


OSM-S-4

Inactive

MoA of OSM-S-313

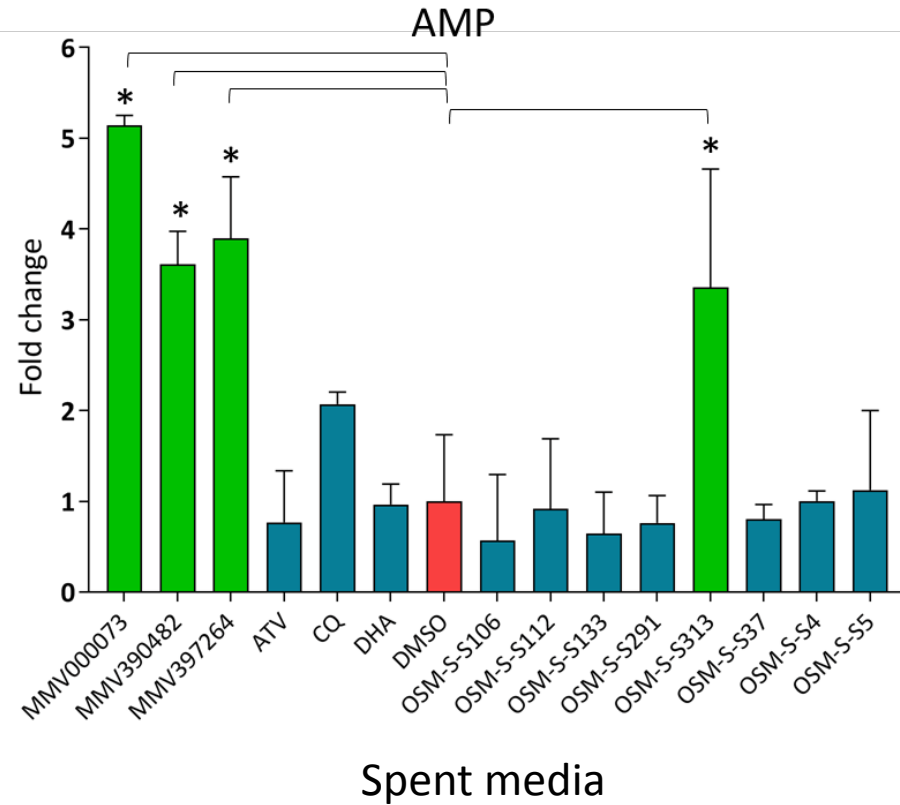
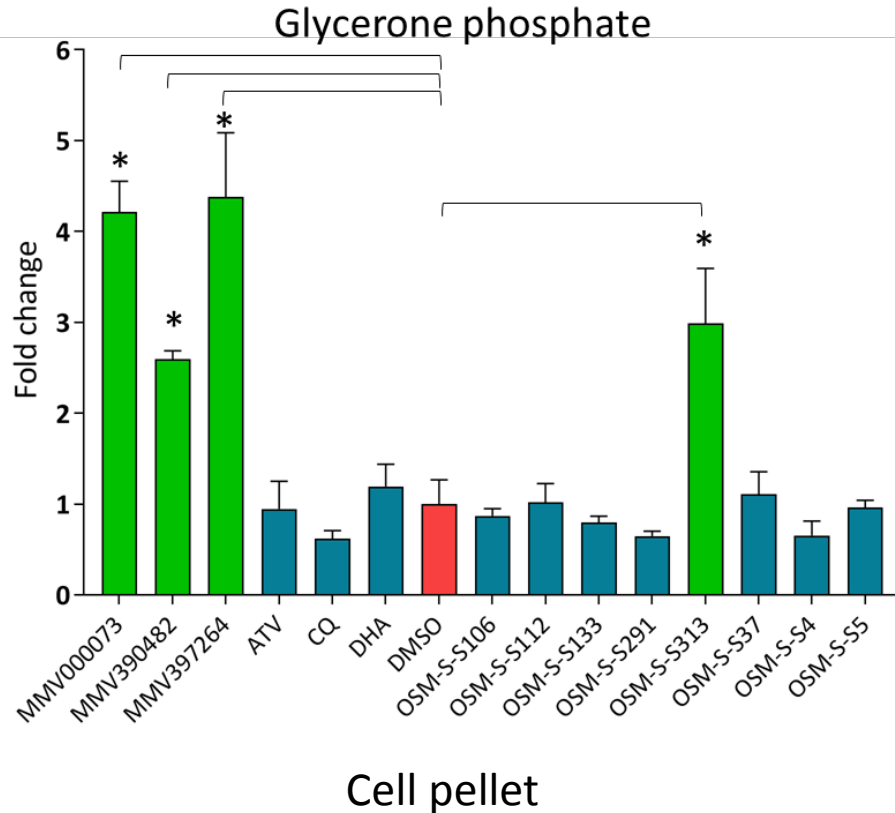
Similar to MMVPfATP4 inhibitors



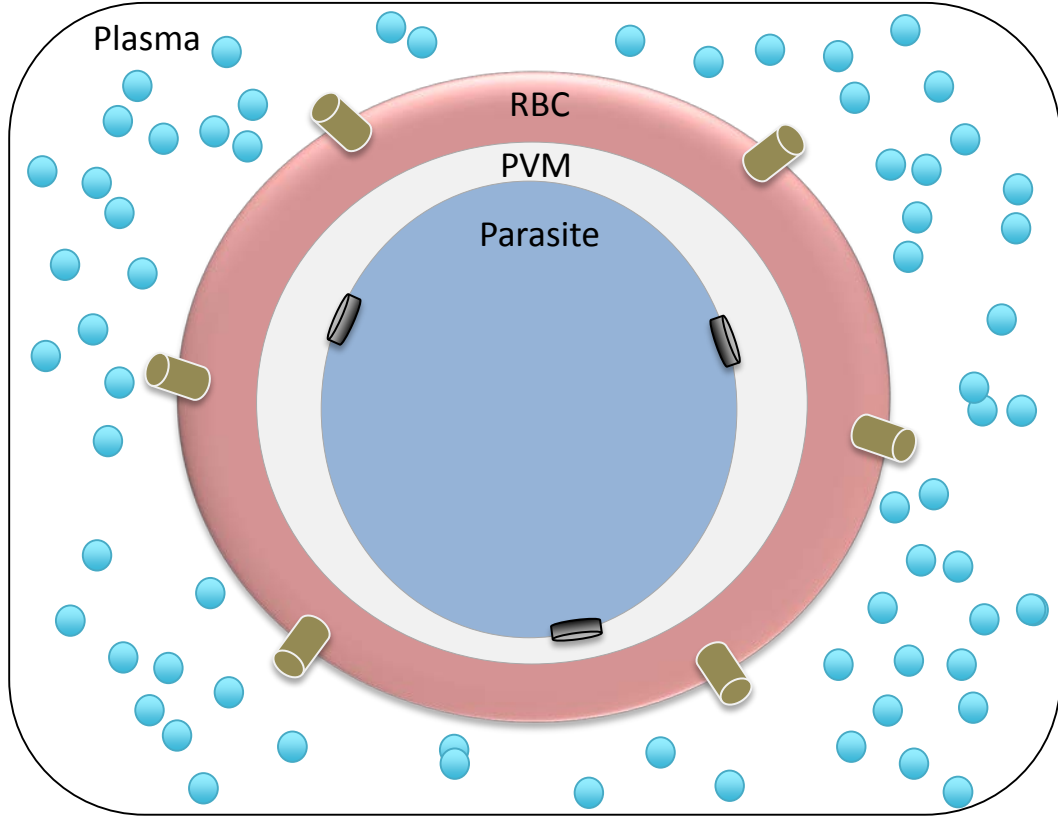
Multivariate analysis (PCA) showed that parasites treated with OSM-S-313 cluster together with parasites treated with the MMV PfATP4 inhibitors

MoA of OSM-S-313

Similar to MMVPfATP4 inhibitors



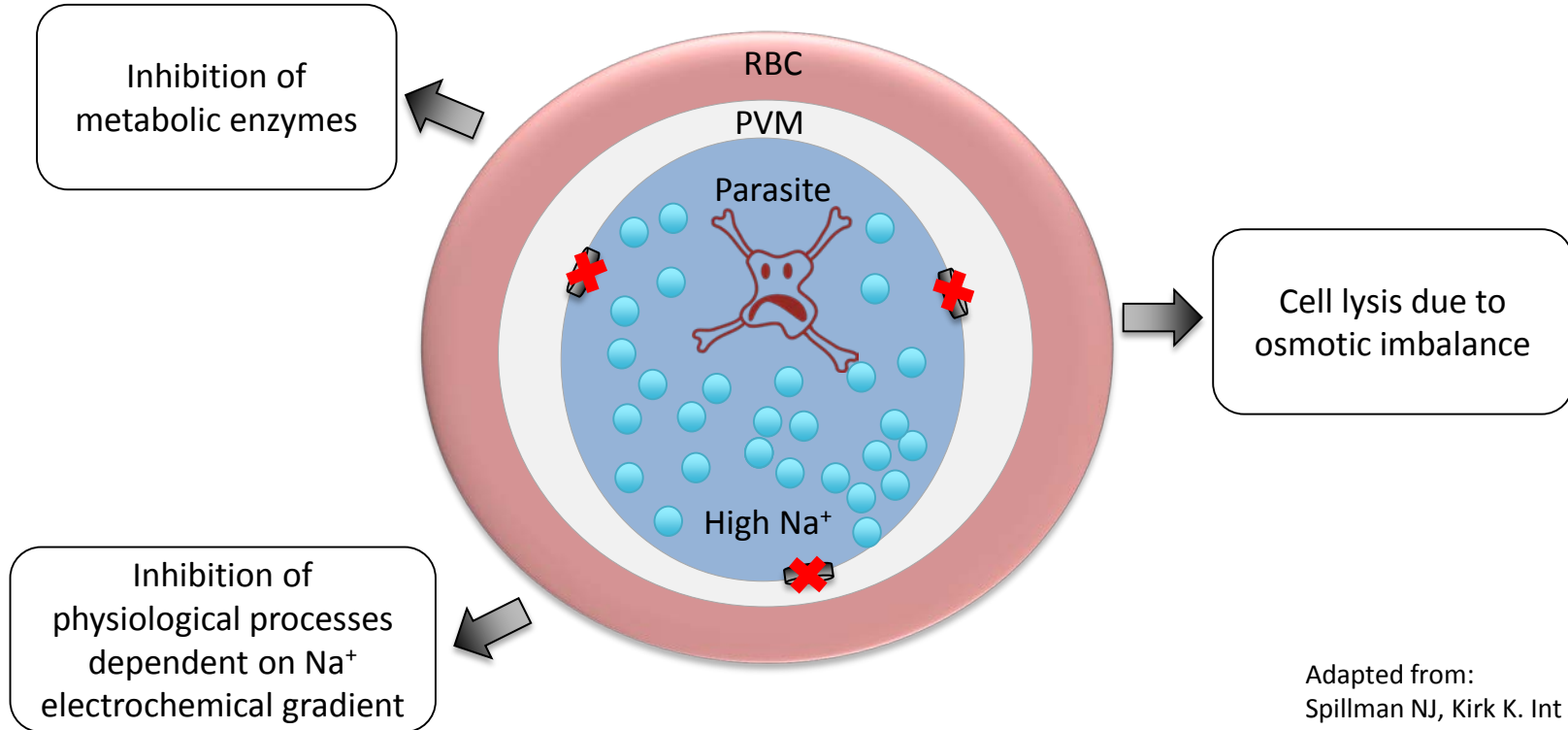
PfATP4 maintains Na⁺ homeostasis in the malaria parasite



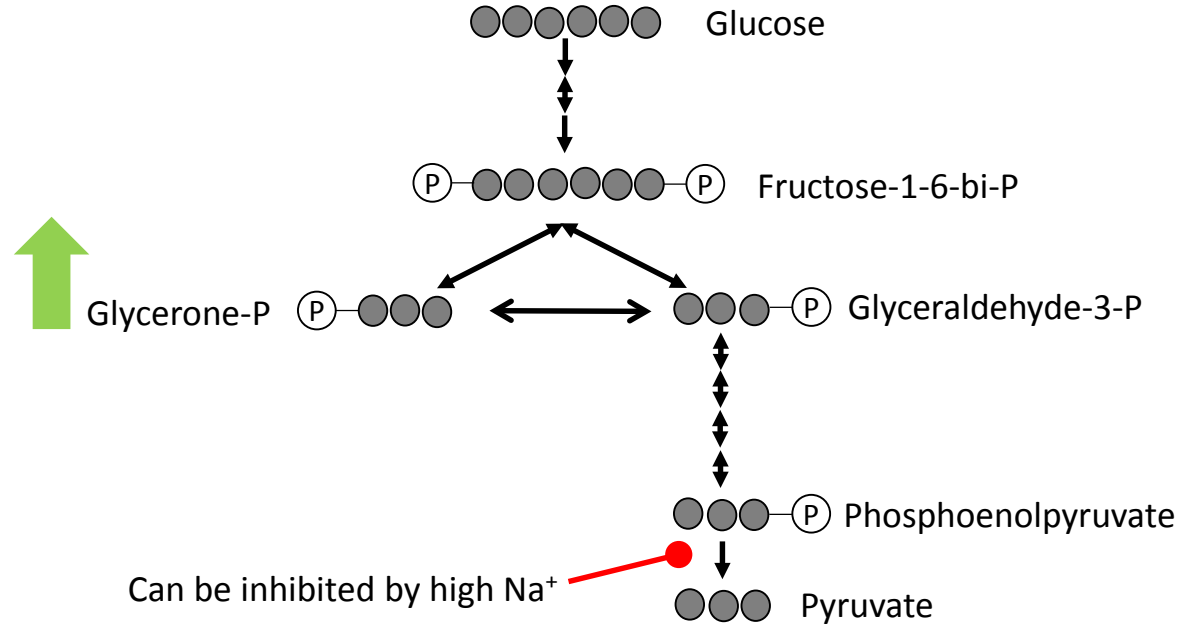
- Na⁺
- NPPs
- PfATP4

Adapted from:
Spillman NJ, Kirk K. Int J Parasitol Drugs
Drug Resist. 2015;5: 149–162

Disruption of Na⁺ regulation: Potential mechanisms of parasite killing

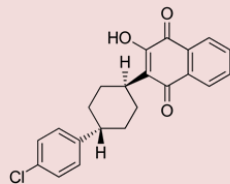
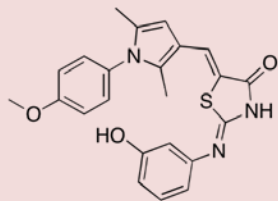
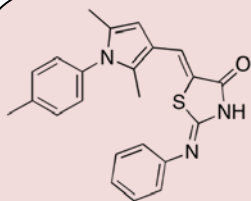


High Na⁺ concentration can inhibit glycolysis



AMP This can prevent re-phosphorylation of adenylates

Summary

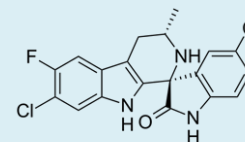
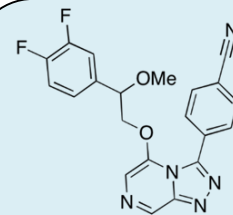


OSM-S-37

OSM-S-112

Atovaquone

Pyrimidine synthesis



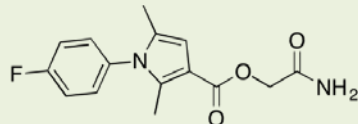
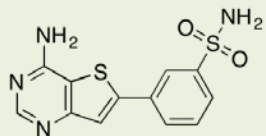
MMV397264

MMV390482

OSM-S-313

MMV000073

Glycolysis

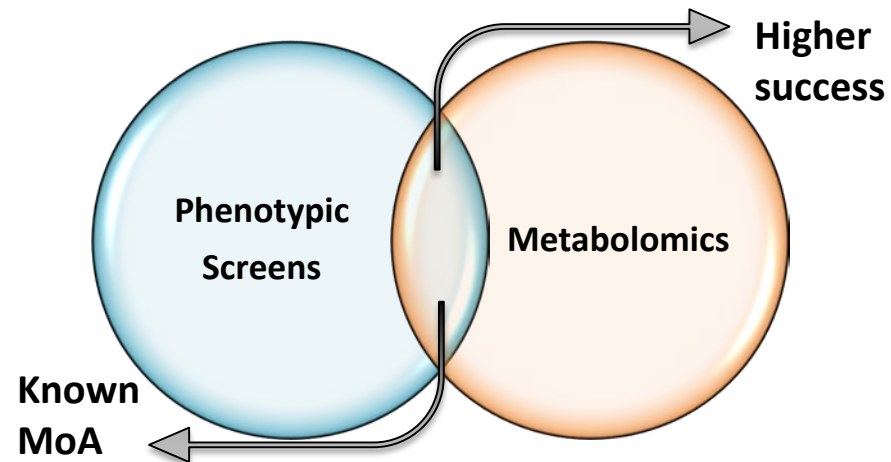


OSM-S-106

OSM-S-5

Unknown

Unknown



Acknowledgements



 @CreekLab



Darren Creek
Carlo Giannangelo
Anna Sexton
Amanda Peterson
Dovile Anderson

Ghizal Siddiqui
Katherine Ellis
Matthew Challis
Tom Kralj
Amanda De Paoli



Matthew Todd



Jeremy Burrows