

60 anos do Prof. Glaucius Oliva

Rafael V. C. Guido



**"30 anos de Cristalografia de Proteínas"
no IFSC/USP**



anos

EESC - USP 1977

CIBFar 2019

Logos of partner institutions: IFSC, USP, CNPq, EESC, INBEQ&DI, SBBq, EMS, hhmi, and others.





Graduação em Farmácia-Bioquímica 1998 – 2002 (UNESP – Araraquara)

CIBFar





RAFAEL VICTÓRIO CARVALHO GUIDO

Planejamento de inibidores da enzima gliceraldeído-3-fosfato desidrogenase de *Trypanosoma cruzi*: biologia estrutural e química medicinal.



Tese apresentada ao Programa de Pós-Graduação em Física do Instituto de Física de São Carlos, da Universidade de São Paulo, para a obtenção do título de Doutor em Ciências.

Área de concentração: Física Aplicada - opção Física Biomolecular.

Orientador: Prof. Dr. Glaucius Oliva



DOCENTE NO INSTITUTO DE FÍSICA DE SÃO CARLOS



| | |
|-----------------------------|------------|
| Professor Doutor, MS-3.1 | 2010-2014 |
| Professor Doutor, MS-3.2 | 2014-2019 |
| Professor Associado, MS-5.1 | 2019-atual |



Pesquisador Associado
Coordenador:
Prof. Dr. Glaucius Oliva



INBEQMeDI

*Instituto Nacional de Biotecnologia e
Química Medicinal em Doenças Infecciosas*

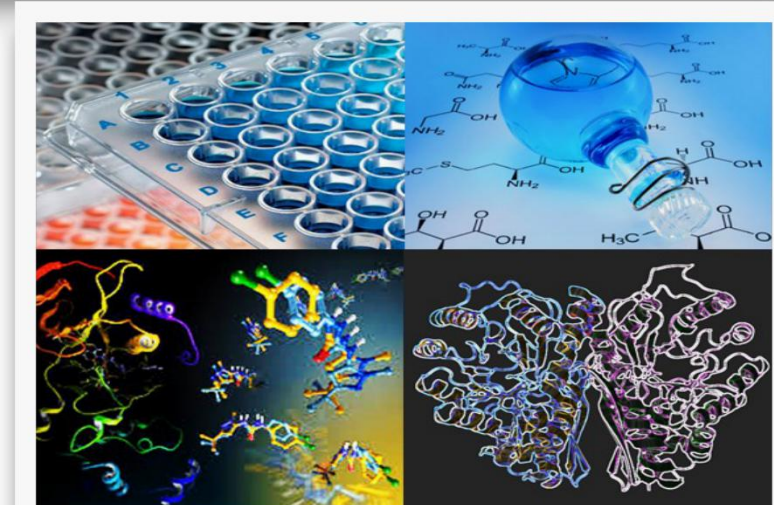
Pesquisador Associado
Coordenadores:
Prof. Dr. Glaucius Oliva
Prof. Richard C. Garratt



Descoberta de Compostos Bioativos para Doenças Infecciosas

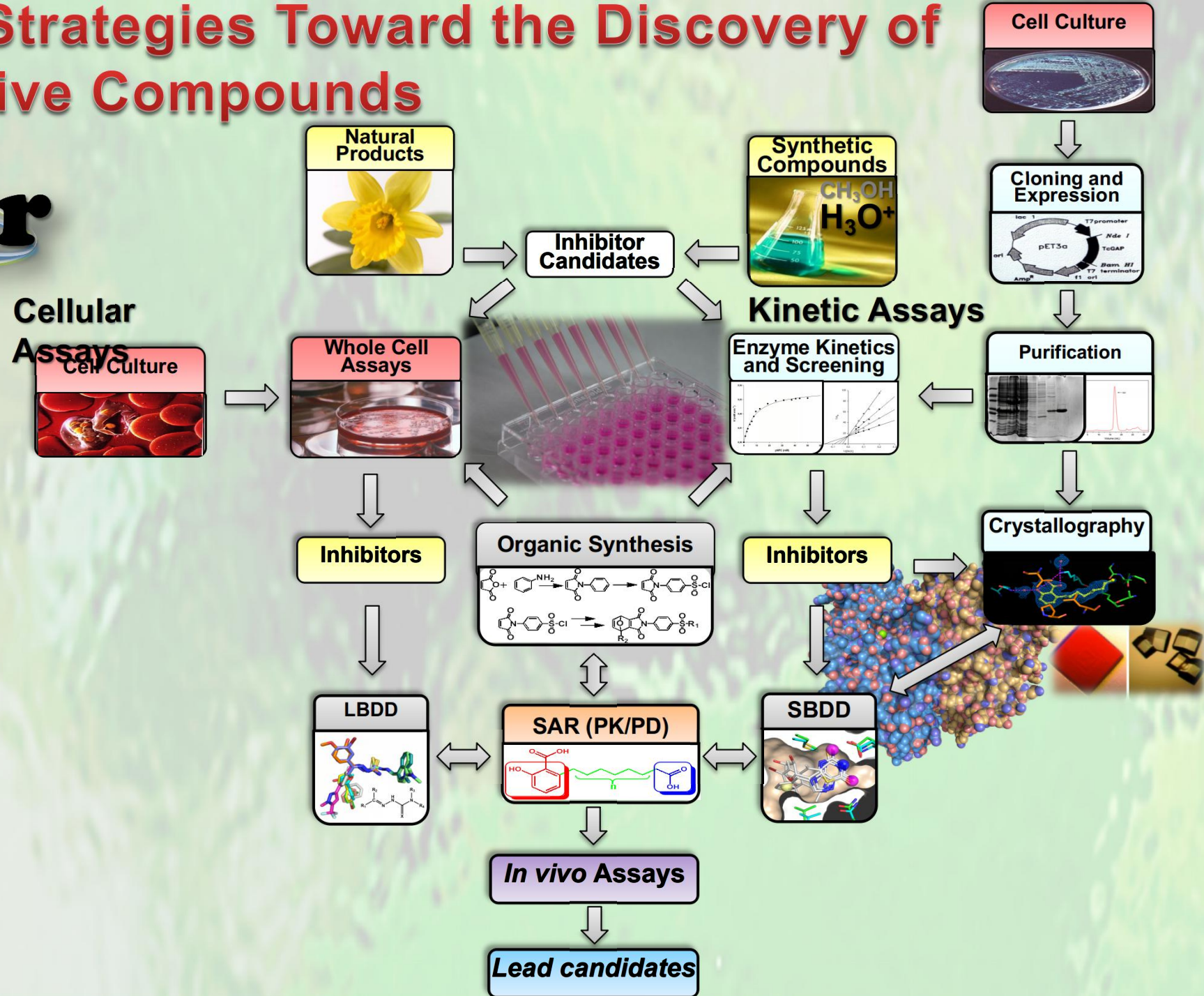
Integração da Biologia Estrutural com a Química Medicinal:

- elucidação e compreensão das bases moleculares responsáveis pela estrutura, função e reconhecimento molecular de alvos biológicos:



Integrated Strategies Toward the Discovery of New Bioactive Compounds

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Malaria is a devastating infectious disease that is characterized by intermittent high fevers and, in the case of cerebral malaria, neurological complications, such as brain injury and coma.



Global Impact (2017)

Cases: 203–262 millions/year (219 millions)

Mortality: 235.000–639.000/year (435.000/year)

435.000 deaths / 518.400 min \approx 1 death/min!

60% of deaths occurring in children under the age of 5 years

People at risk: > 3.4 billions

Causative Agent

Protozoan parasites of the genus *Plasmodium*

Treatment

First-line treatment for *P. falciparum* infections

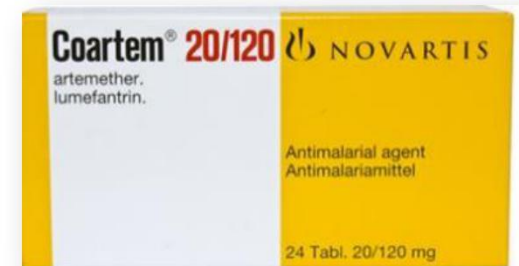
(in regions where chloroquine resistant parasites are present):

Combination therapy of artemisinin derivatives with partner drugs (ACT)

- artemether–lumefantrine (Coartem; Novartis)
- amodiaquine–artesunate (Coarsucam; Sanofi-Aventis)

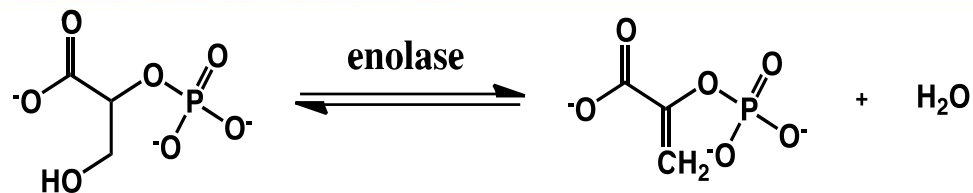
Distribution

91 countries on the tropical e subtropical regions of the planet





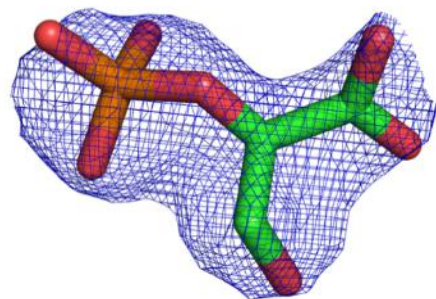
P. falciparum Enolase 3D structure



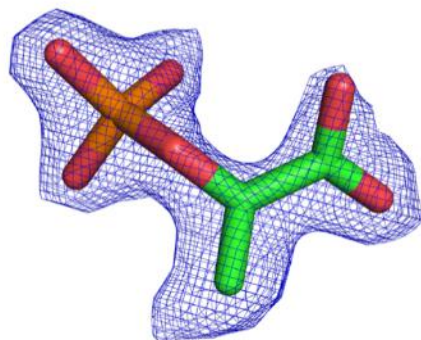
Superposition
APO / PEP+Mg²⁺



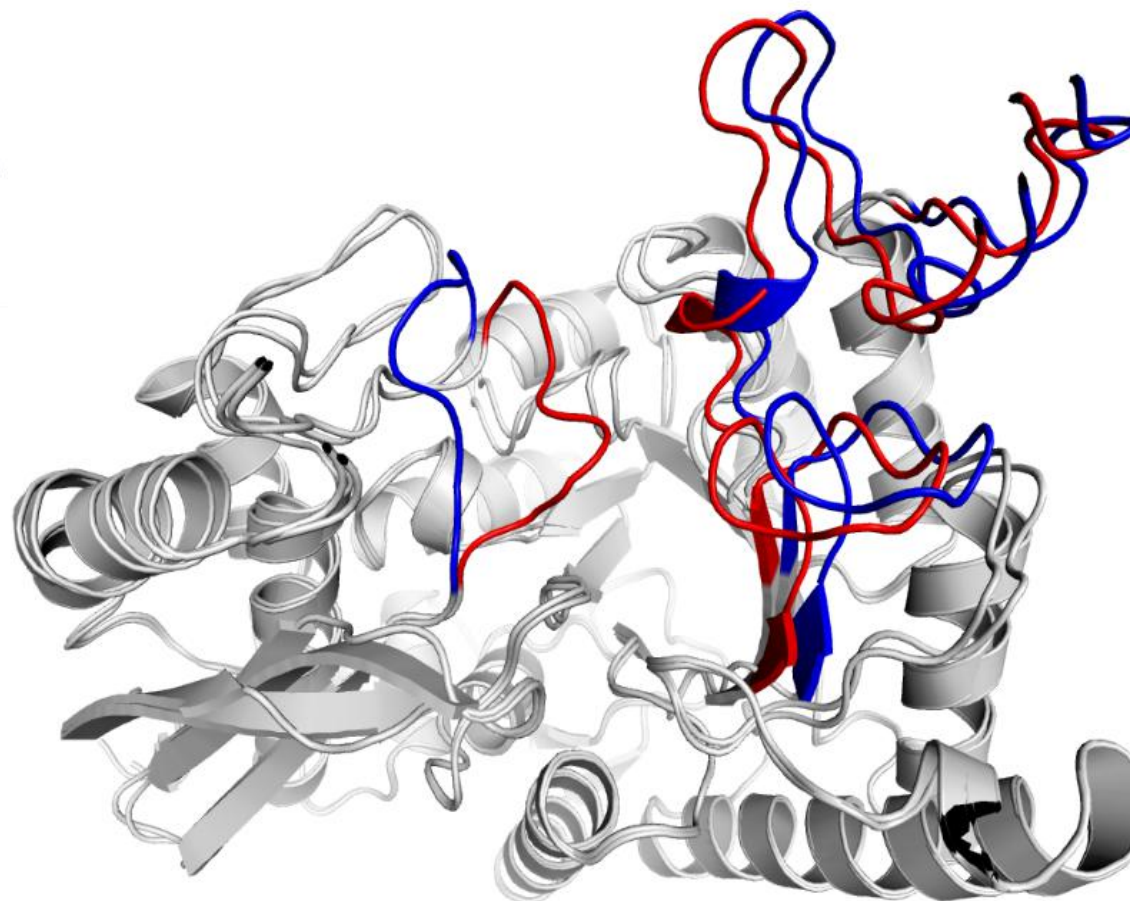
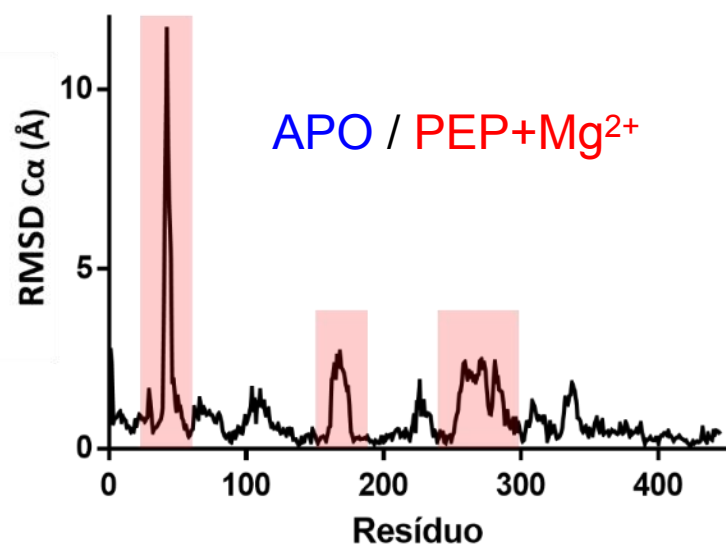
Dr. Fernando Maluf



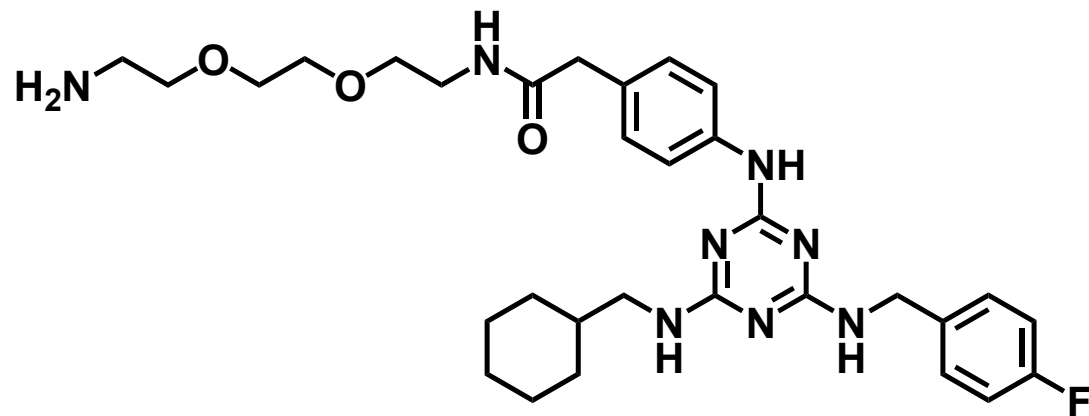
2PG



PEP

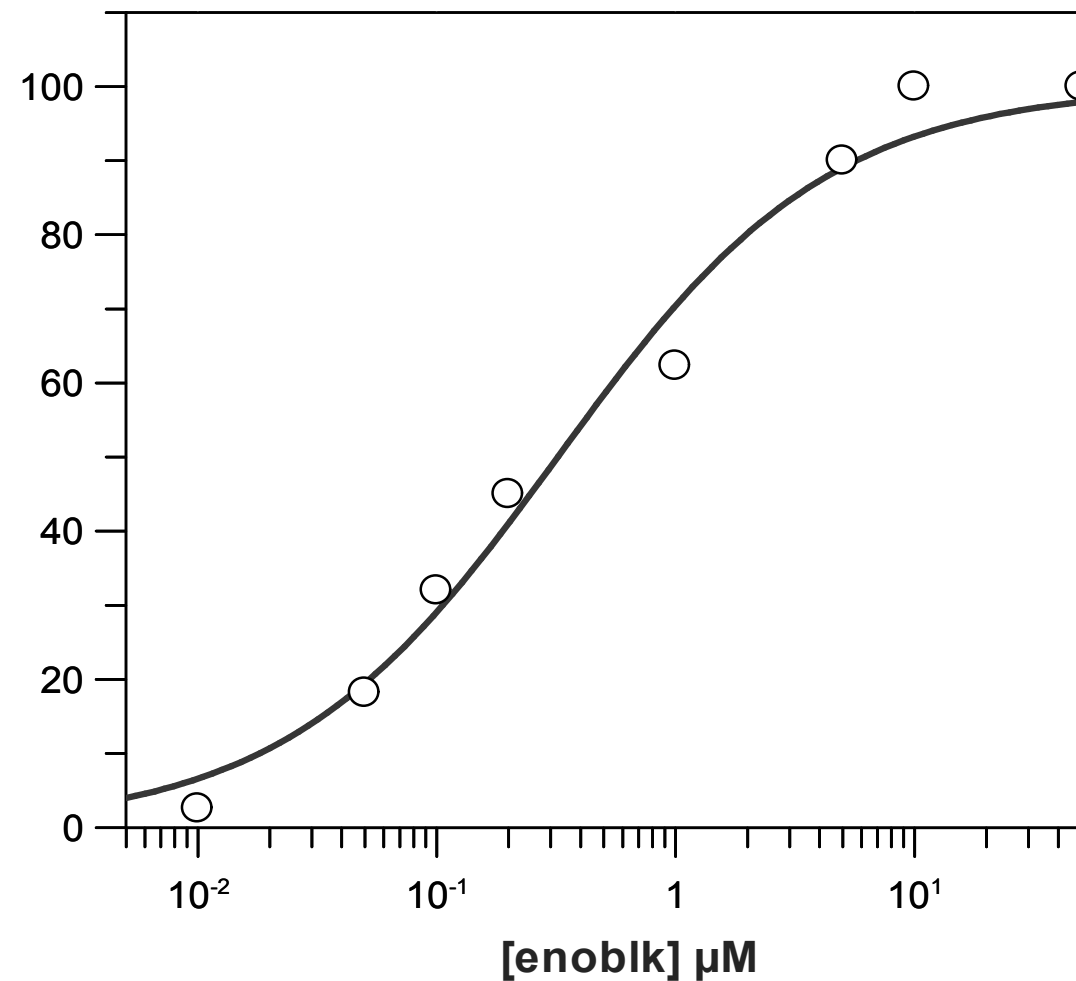


Pt-Enolase: Inhibitor discovery



$IC_{50} = 0.32 \pm 0.04 \mu M$

% Inhibition

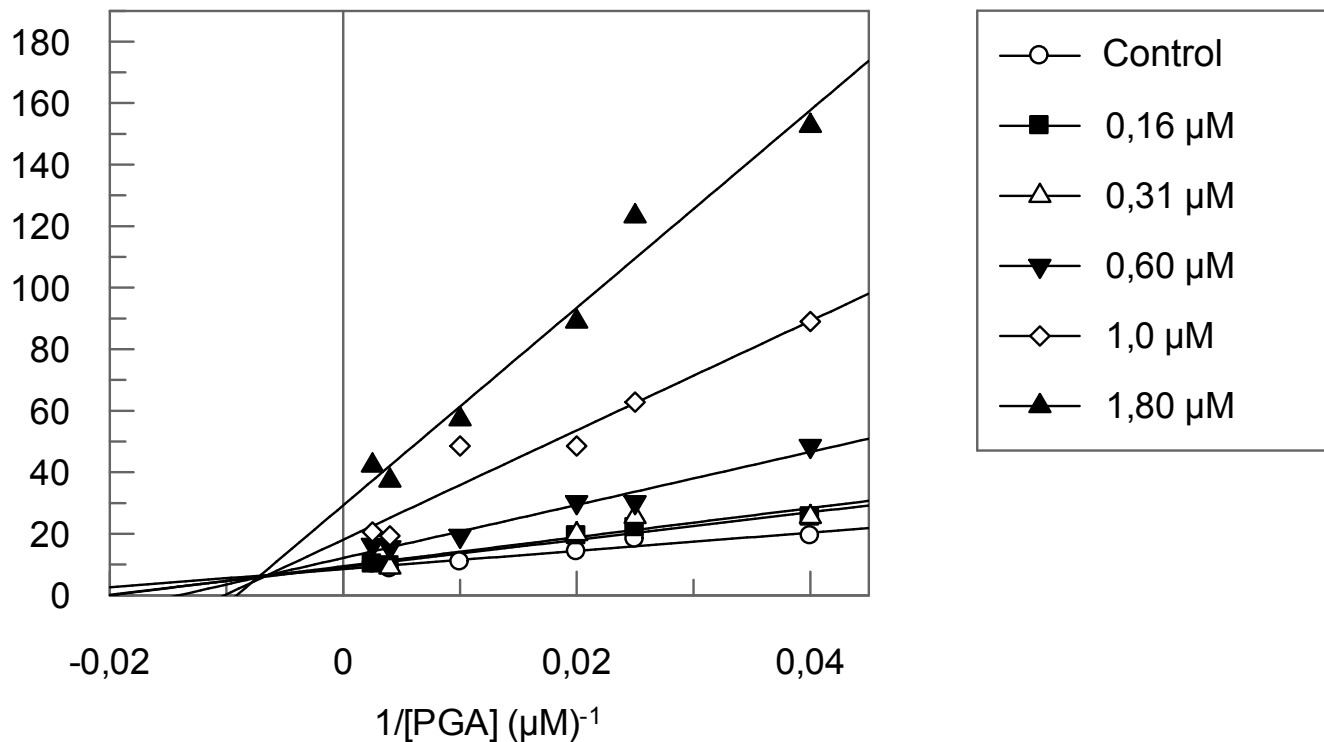
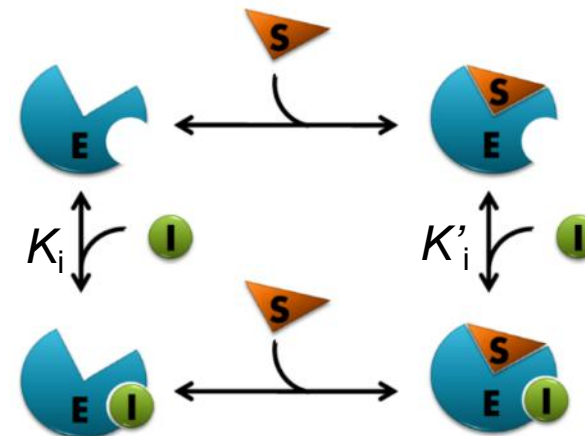
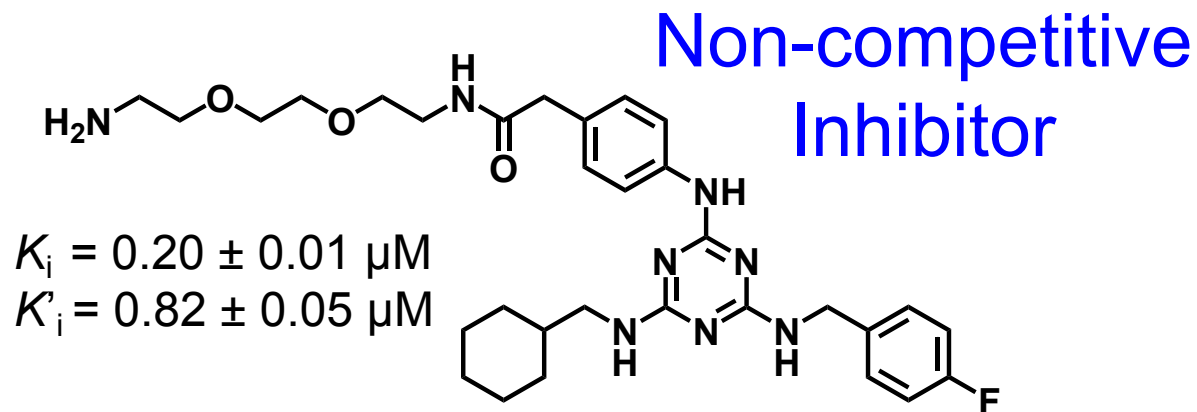


Dr. Fernando Maluf

Dr. Lorena Souza



Pf-Enolase: Mechanism of Inhibition

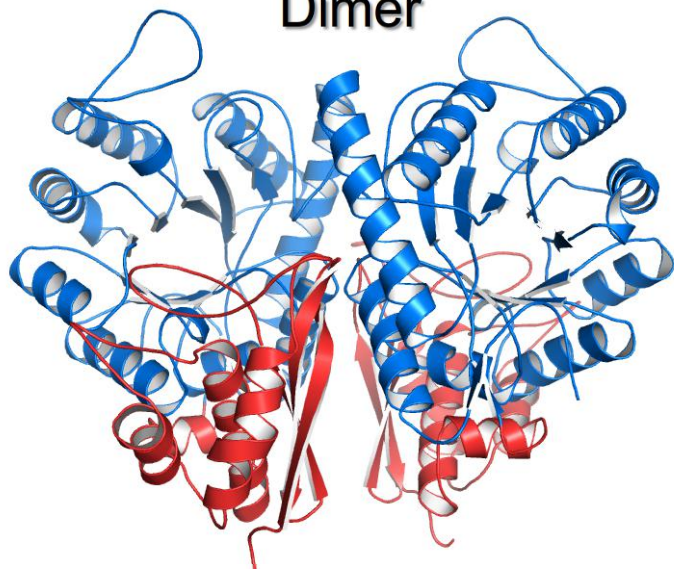




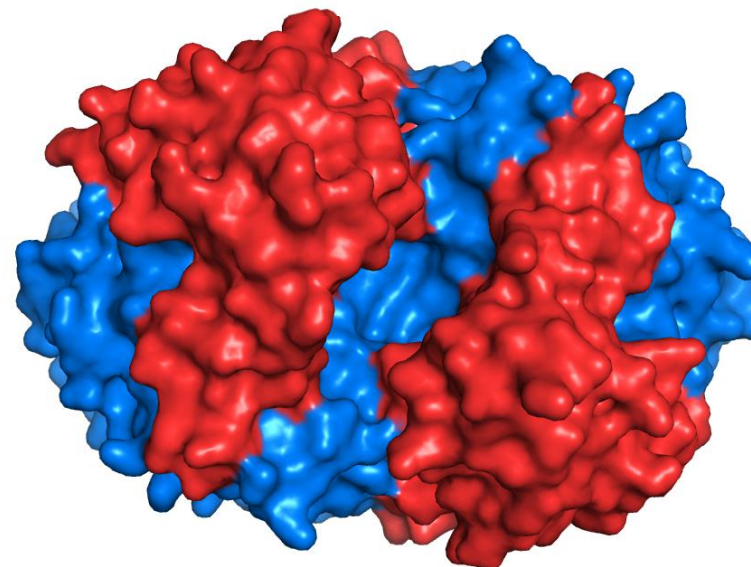
Cristallographic complex: *Pfenolase-Enoblock*

CIBFar

Dimer

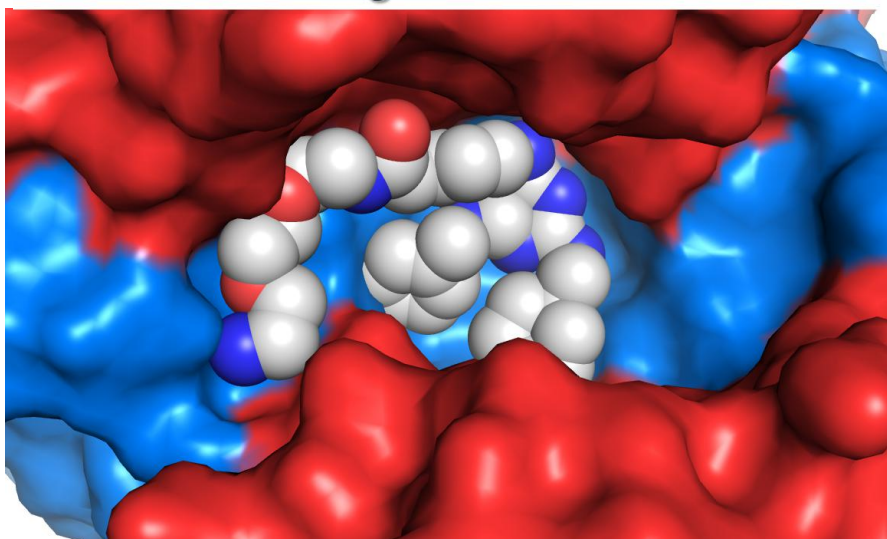


Dimer interface

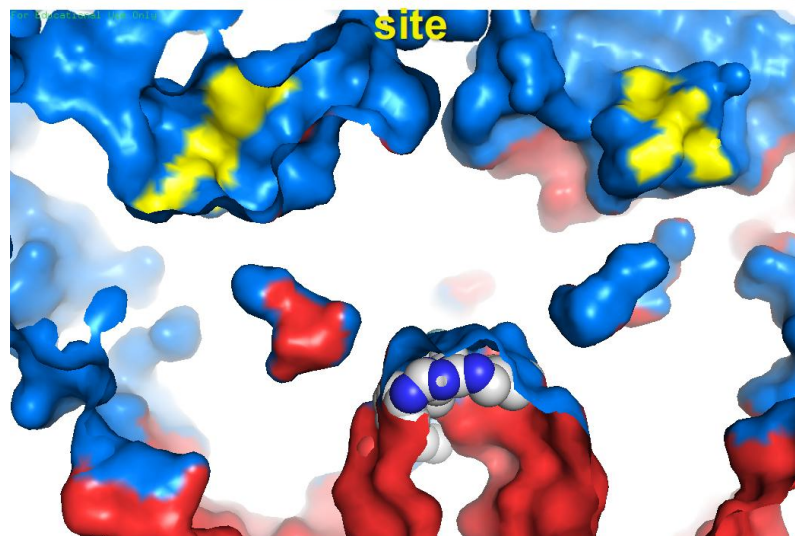


Pfenolase-ENOblock

Inhibitor binding site: Dimer interface



Inhibitor binding site / catalytic binding site

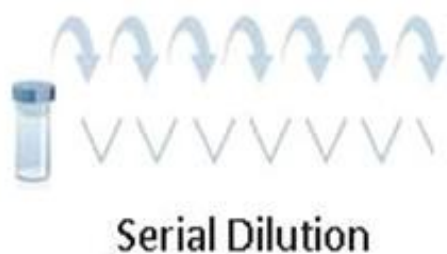


| | ENOblock Difratômetro Micromax-007 R-AXIS IV ++ |
|---------------------------------|---|
| <i>Data Process</i> | |
| Resolution (Å) | 89,61 – 1,80 |
| Space Grup | P2 ₁ |
| Cell Parameters (Å) | a=59,9 b=77,4 c=90,19 |
| Cell Parameters (°) | $\alpha=90,00$ $\beta=96,51$ $\gamma=90,00$ |
| Total number reflections | 61.761 |
| Unique number reflections | 9.392 |
| Multiplicity | 2,1 (2,0) |
| Completeness (%) | 89,9 (89,8) |
| R _{merge} (%) | 6,7 (48,0) |
| $\langle I \rangle / \sigma(I)$ | 10,9 (2,1) |
| <i>Refinement</i> | |

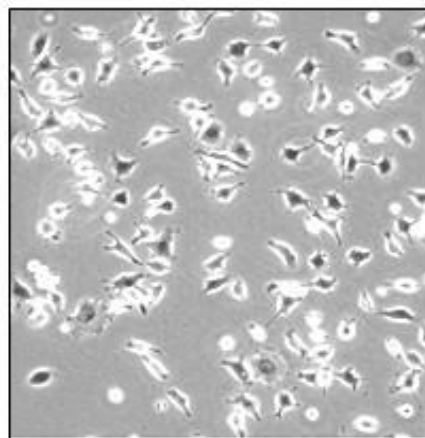


In vitro

Inhibitory activity and toxicity evaluation



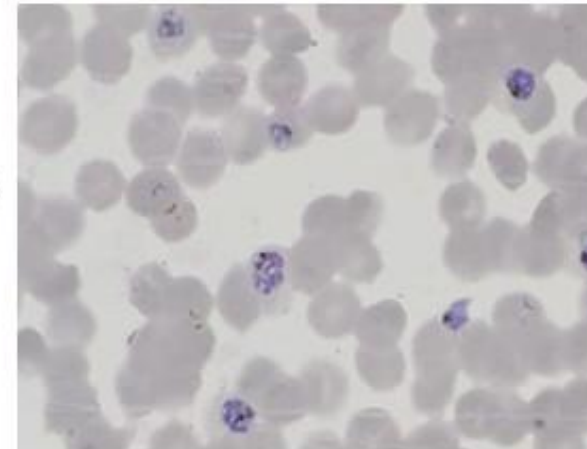
In vitro cytotoxicity
MDL₅₀



HepG2 e BGM
MTS



Antiplasmodial activity
IC₅₀



3D7 e K1
SYBR GREEN



Dr. Anna Caroline
C. Aguiar



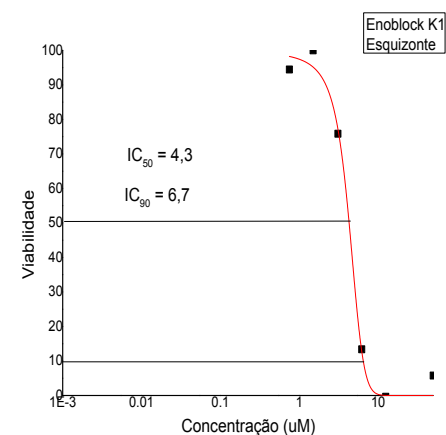
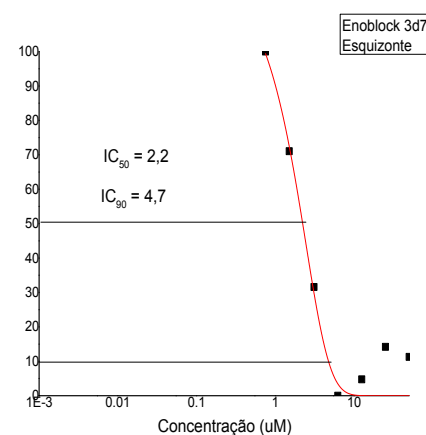
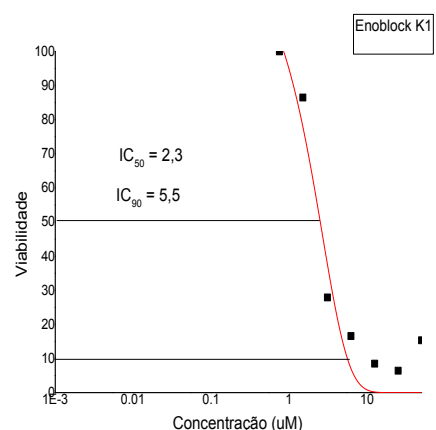
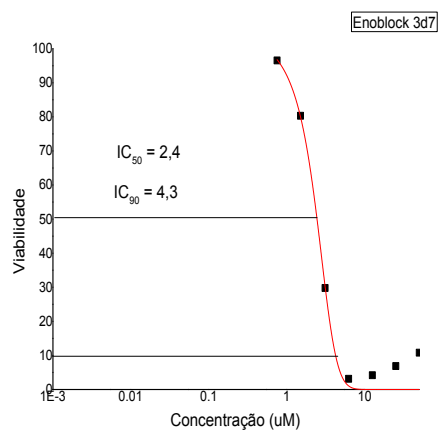
Enoblock: In vitro activity

| ENOBLOCK | <i>Plasmodium falciparum</i> IC ₅₀ (µM) | | Cytotoxicity IC ₅₀ (µM) | | |
|----------|---|-----------------|---------------------------------------|--------------|-----------|
| | <i>Ring</i> | <i>Schizont</i> | <i>BGM</i> | <i>HepG2</i> | <i>SI</i> |
| 3D7 | 2.2 ± 0.1 | 2.5 ± 0.3 | > 750 | > 750 | > 375 |
| K1 | 1.9 ± 0.2 | 3 ± 1 | > 750 | > 750 | > 375 |



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C. Aguiar

| ENOBLOCK | <i>Plasmodium falciparum</i> IC ₉₀ (µM) | |
|----------|---|-----------------|
| | <i>Ring</i> | <i>Schizont</i> |
| 3D7 | 4.3 ± 0.2 | 4.7 ± 0.3 |
| K1 | 5.5 ± 0.2 | 6.7 ± 0.8 |





In vivo antiplasmodial activity (*P. berghei*)



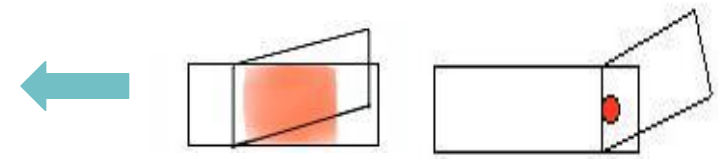
Dr. Anna Caroline C. Aguiar



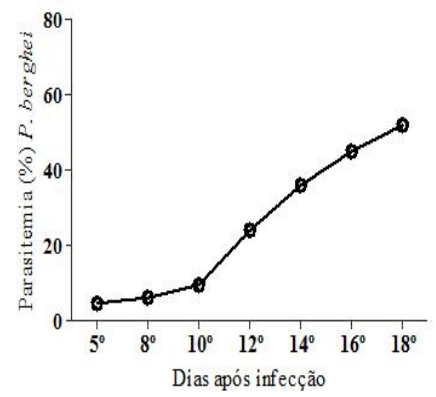
Infection: 5×10^5 iRBC *P. berghei* (strain NK65)



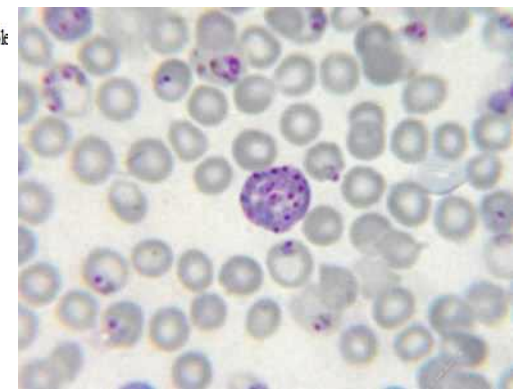
VO treatment for 3 days (100 mg/Kg)



Blood smear



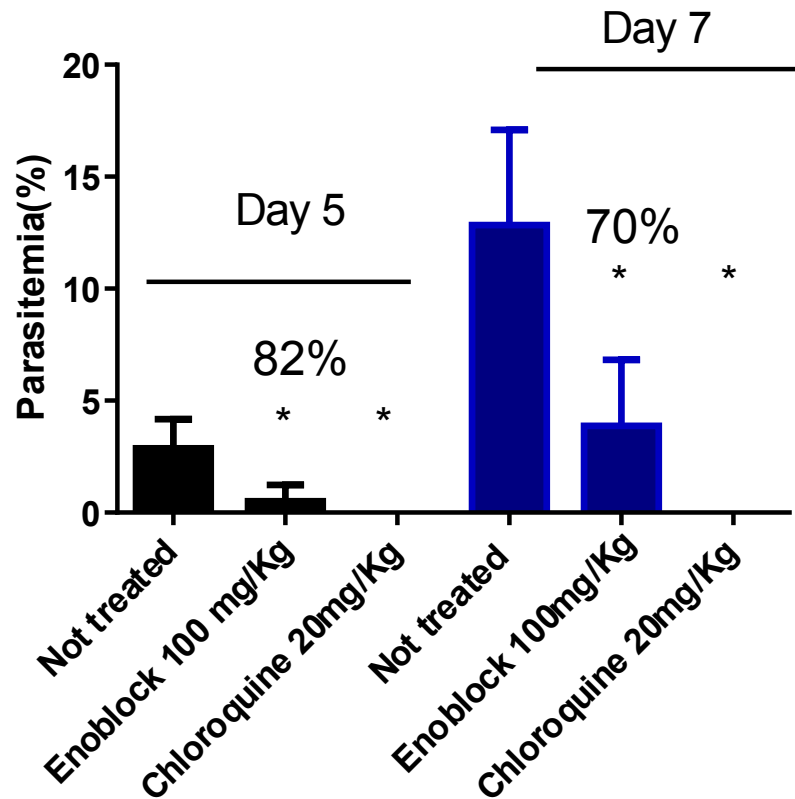
Parasitemia evaluation



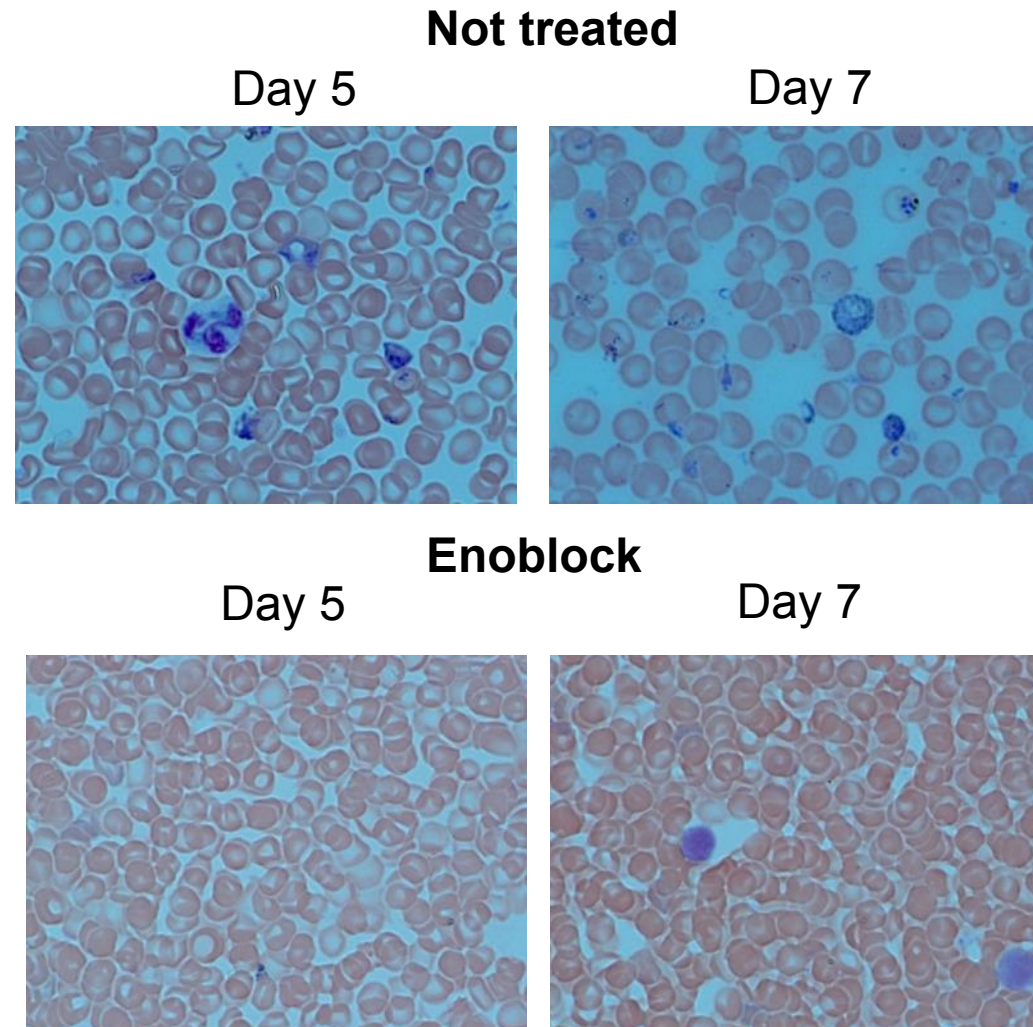


Enoblock: Proof of concept (in vivo)

Plasmodium berghei parasitemia reduction after oral treatment with Enoblock (100 mg/Kg, during 3 consecutive days)



*p<0.05



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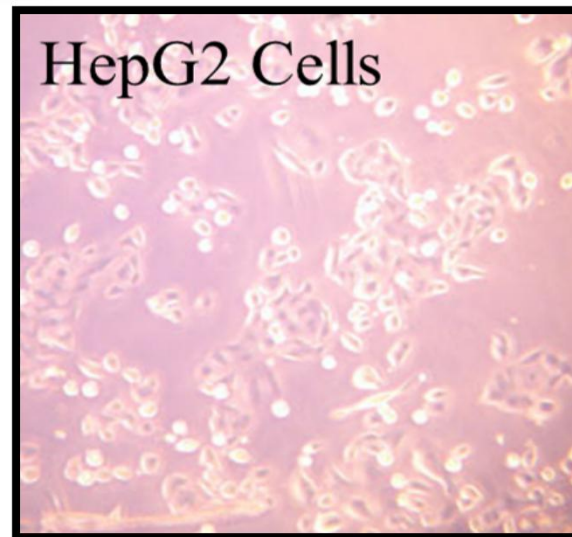
MMV 12/0103
Long duration
heterocycles_(Aza)Benzimidazole

Prof. Luiz Carlos Dias (Unicamp) and
Prof. Rafael Guido (USP)

Defeating Malaria Together



- **Inhibitory activity evaluation (SYBR Green assay)** against sensitive and resistant strains of *P. falciparum*
 - 140 compounds tested in 2018
- **Generation of resistant clones** to unravel the mode of action
- **Cytotoxicity testing** (HepG2 cells)





- Schizont maturation test in *P. vivax* and *P. falciparum* Brazilian isolates

Center of Excellence for MMV projects in Porto Velho at Rondonia Tropical Medicine Center in cooperation with Dr. Dhelio B. Pereira

40 compounds/year from MMV projects

USP-CEPEM-MMV Team



Prof. Rafael Guido



Dr. Dhelio B. Pereira



Prof. Glaucius Oliva



Dr. Anna Caroline Aguiar Me.



Guilherme Souza



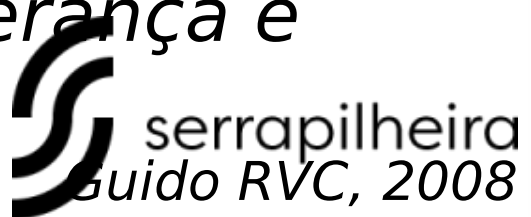
Acknowledgments

Ao Prof. Dr. Glaucius Oliva
pela
confiança, suporte,
orientação,
exemplo de liderança e
amizade.

2017



2018



Dr. Paul Willis
Dr. Javier Gamo

UNICAMP

- Prof. Luiz Carlos Dias
- Prof. Carlos R. D. Correia
- Prof. Jose Luiz Costa

FCF-USP

- Prof. Celia R. S. Garcia

UNIFESP

- Prof. Fabio C. Cruz