

60 anos do Prof. Glaucius Oliva

Rafael V. C. Guido

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





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

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Doutorado em Física Biomolecular IFSC (2002 – 2008)

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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

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



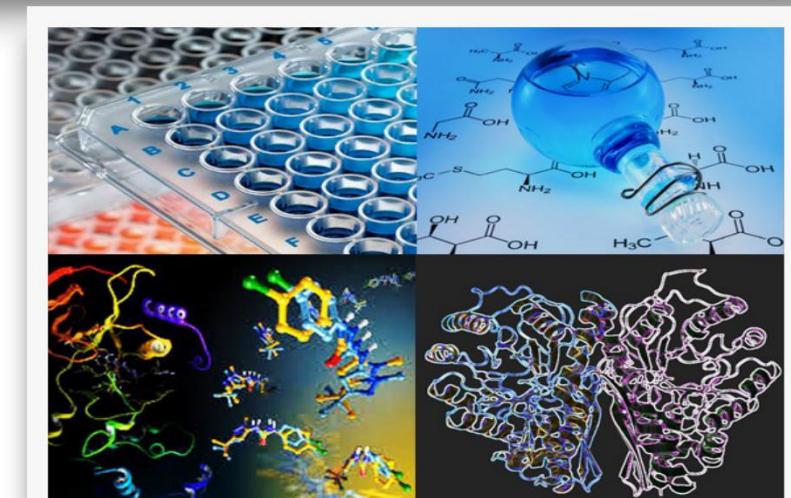
INBEQMeDI

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

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Pesquisador Associado
Coordenador:
Prof. Dr. Glaucius Oliva

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



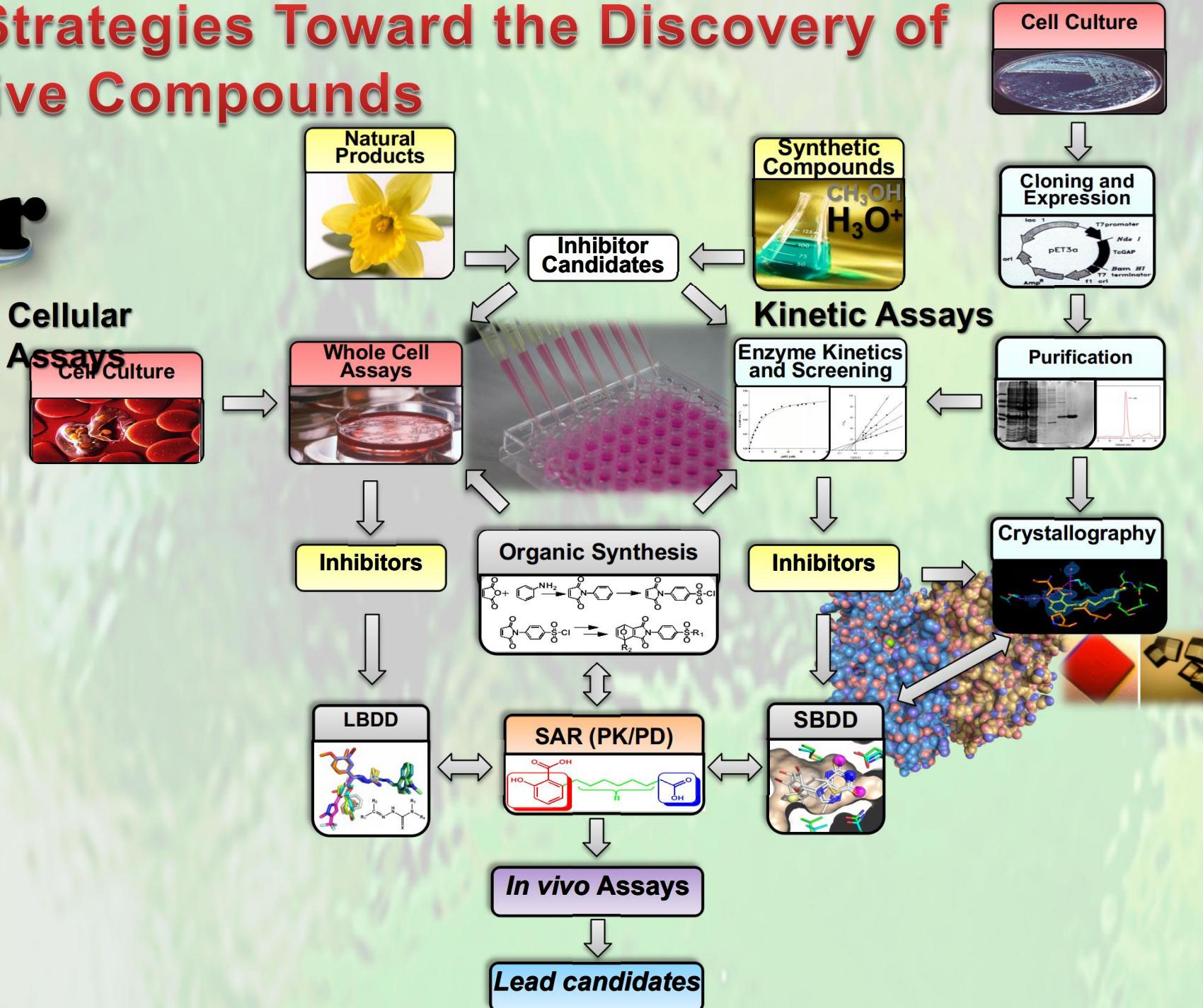
Descoberta de Compostos Bioativos para Doenças Infecciosas

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

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

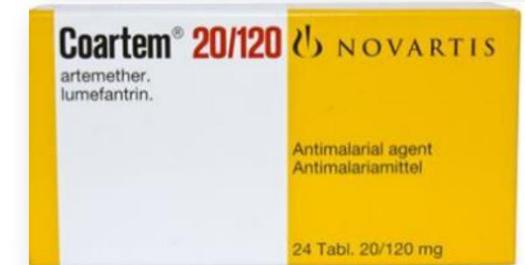
(in regions where chloroquine resistant parasites are present):

Combination therapy of artemisinin derivatives with partner drugs (ACT)

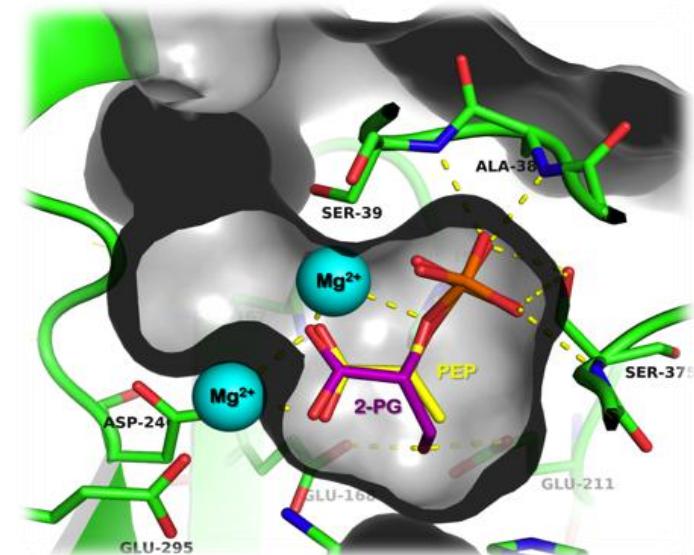
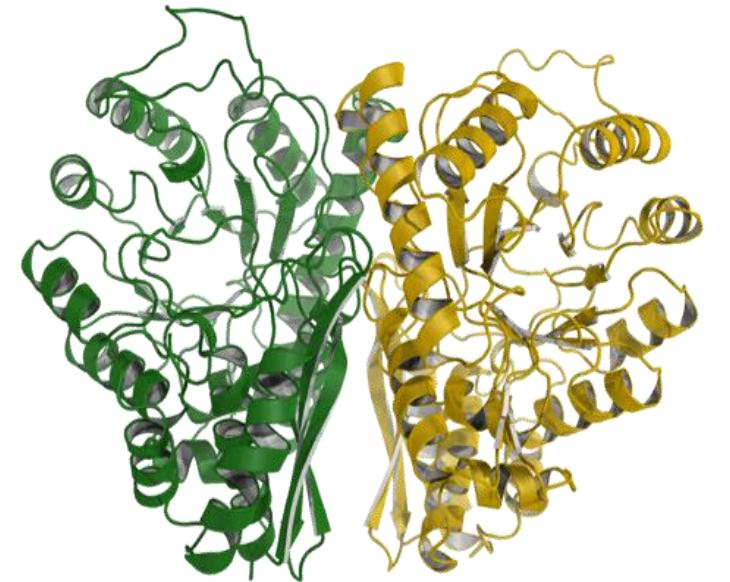
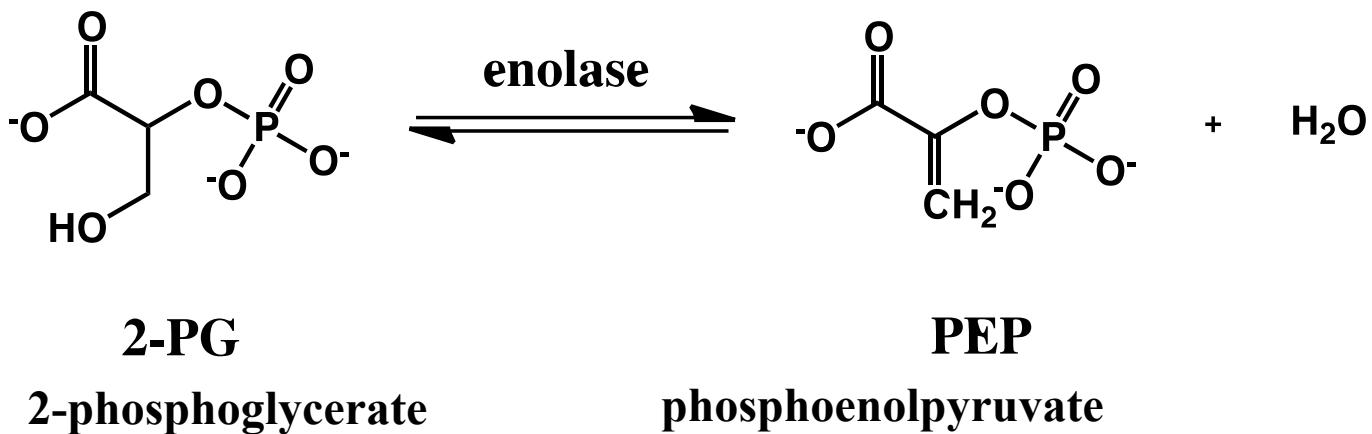
- artemether–lumefantrine (Coartem; Novartis)
- amodiaquine–artesunate (Coarsucam; Sanofi-Aventis)

Distribution

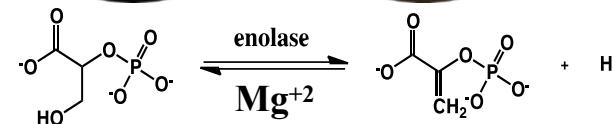
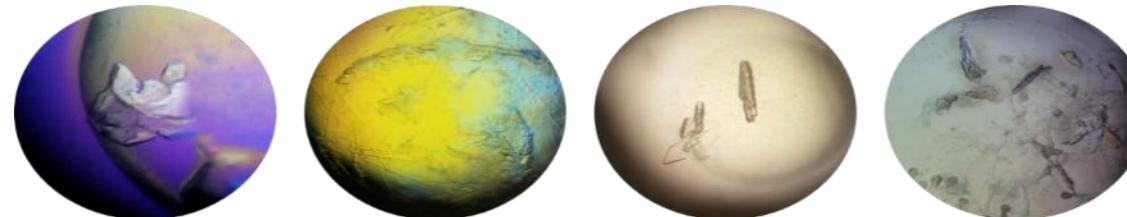
91 countries on the tropical and subtropical regions of the planet



- Glycolytic enzymes play important roles in Plasmodium biology
- Intra-erythrocytic stages of *P. falciparum* lack the functional tricarboxylic acid cycle
- Enzymes of the glycolytic pathways are attractive targets for antimalarial drug discovery and development
- Enolase (E.C. 4.2.1.11), the ninth enzyme of the glycolytic pathways, is a dimeric enzyme of 100 KDa molecular mass and magnesium dependent



Crystallization, X-ray Crystallography and Structure Determination



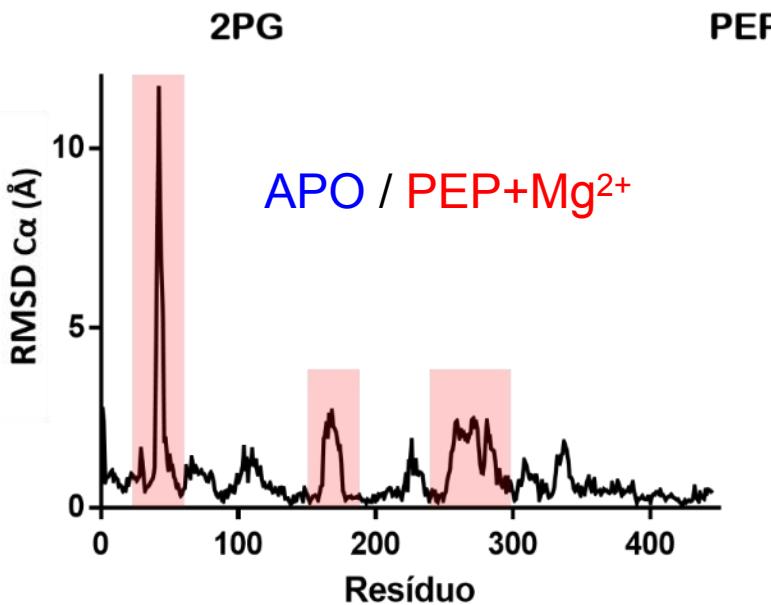
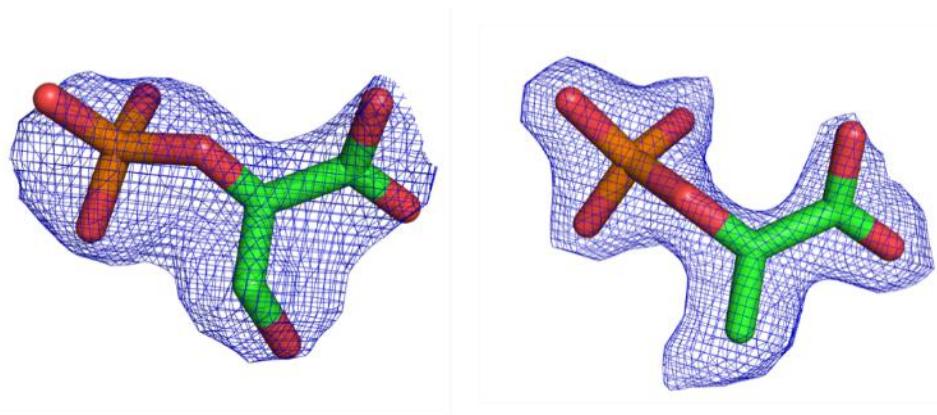
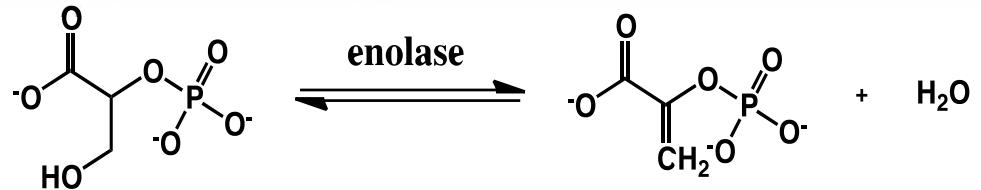
	APO	2-PG	PEP	PEP+MG
Coleta				
Fonte	Micromax-007	Micromax-007	Micromax-007	MX2 - LNLS
Detector	R-AXIS IV ++	R-AXIS IV ++	R-AXIS IV ++	PILATUS 2M
$\Delta\phi$	0,5	0,5	0,5	0,2
Imagens	391	326	230	1800
Processamento				
Resolução (Å)	89,64 – 1,60	77,78 – 1,88	77,53 – 1,80	47,74 – 1,80
Grupo espacial	P2 ₁	P2 ₁	P2 ₁	P2 ₁
Parâmetros de cela - a,b,c (Å)	60,56; 77,46; 90,18	60,62; 77,78; 90,16	60,65; 77,53; 90,09	61,12; 77,01; 87,42
Parâmetros de cela (°)	$\alpha=\gamma=90,00$ $\beta=96,28$	$\alpha=\gamma=90,00$ $\beta=96,01$	$\alpha=\gamma=90,00$ $\beta=95,72$	$\alpha=\gamma=90,00$ $\beta=95,55$
Nº reflexões totais	376.538	222.422	178.424	422.277
Nº reflexões únicas	108.746	65.086	74.565	73.934
Multiplicidade	3,5 (2,8)	3,4 (3,5)	2,4 (2,3)	5,7 (4,5)
Completeza (%)	99,80 (99,8)	95,20 (90,9)	96,7 (100)	98,8 (97,4)
R _{merge} (%)	9,0 (52,4)	9,2 (66,4)	6,4 (41,1)	7,5 (49,0)
$\langle I \rangle / \sigma(I)$	9,3 (2,3)	12,1 (2,1)	10,9 (2,1)	13,5 (2,1)
Mosaicidade média	0,40	0,28	0,20	0,47
Refinamento				
R _{work} ⁹² (%)	17,77	18,95	17,0	19,50
R _{free} ⁹² (%)	21,82	23,85	21,5	23,6

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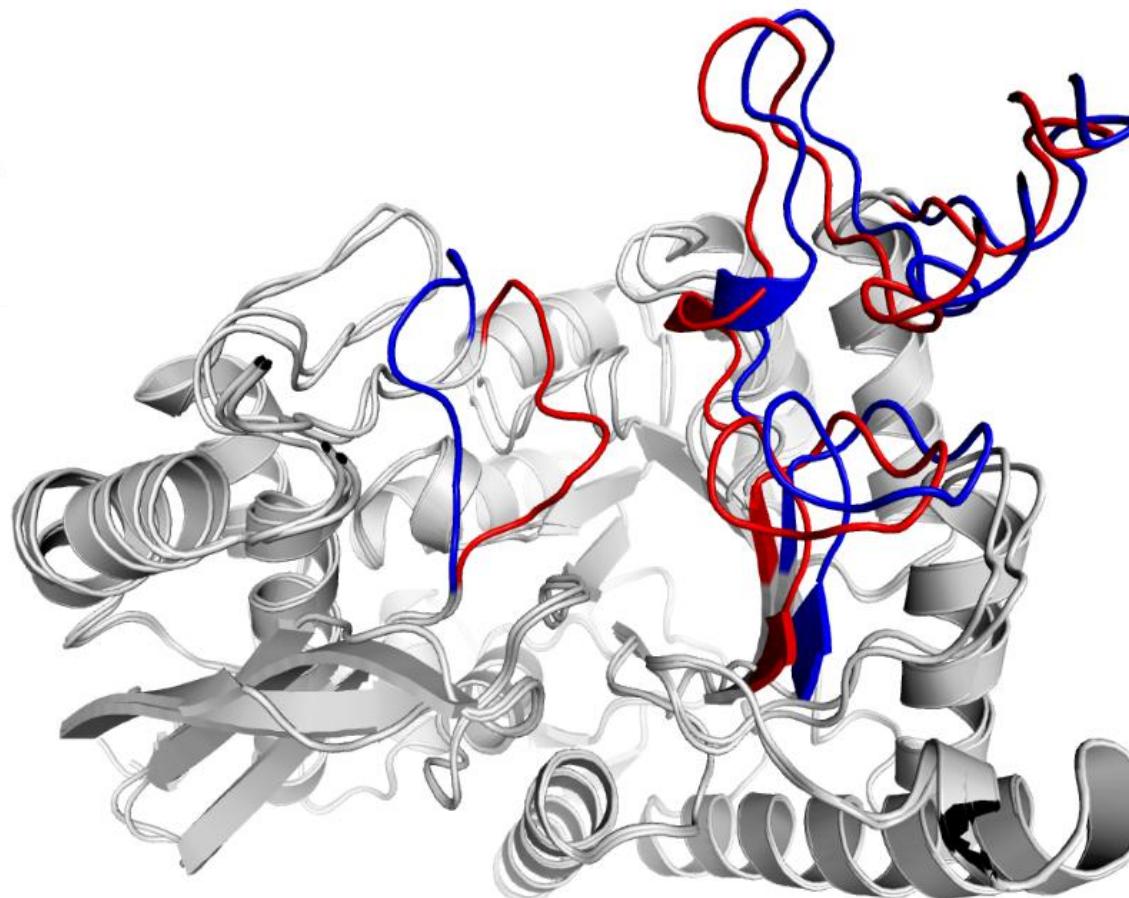


P. talcifarum Enolase 3D structure

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Superposition
APO / PEP+ Mg^{2+}

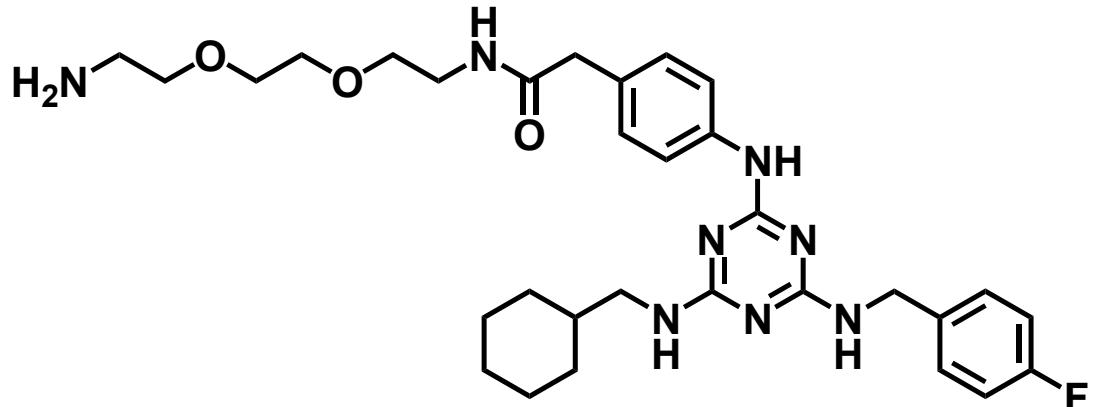


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Maluf



Pt-Enolase: Inhibitor discovery

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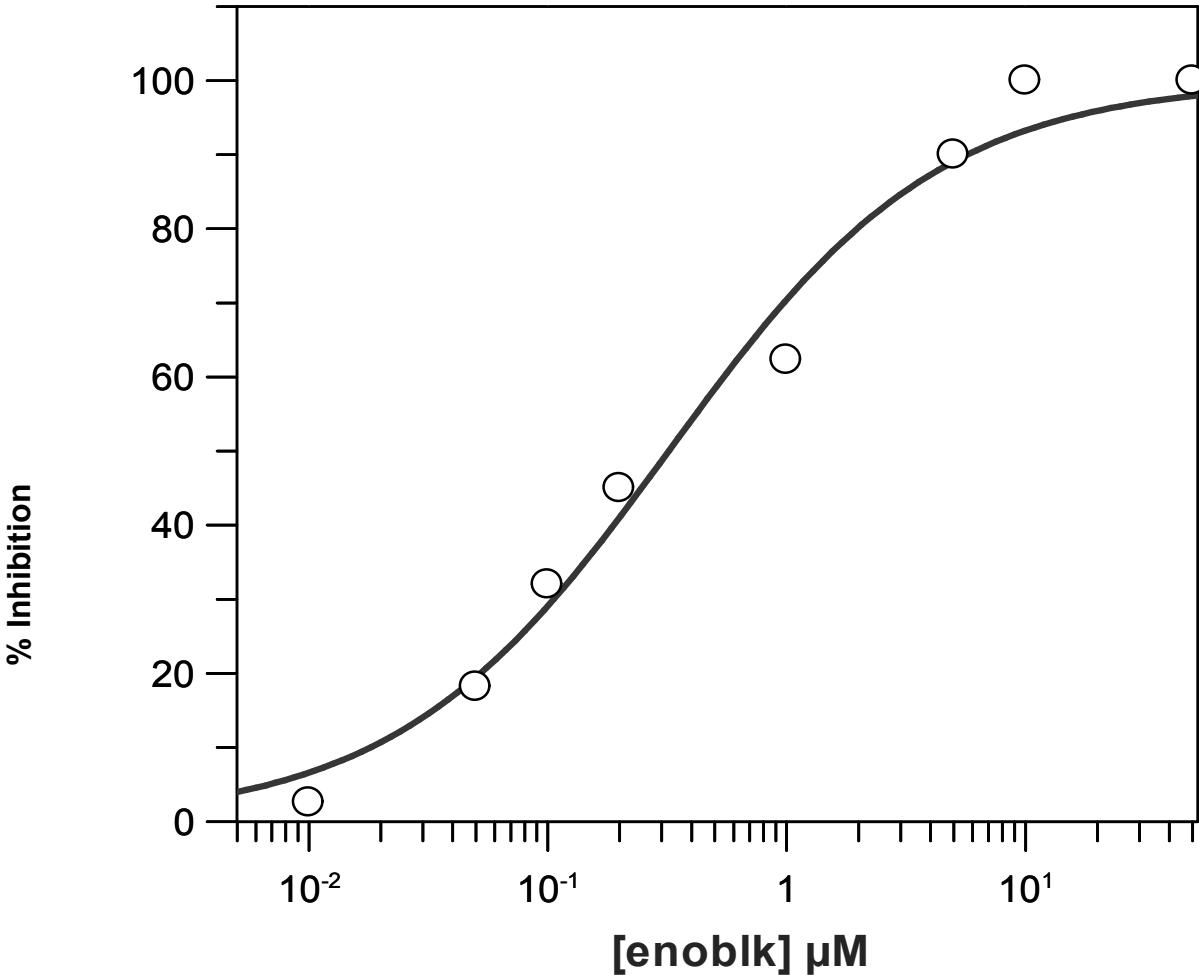


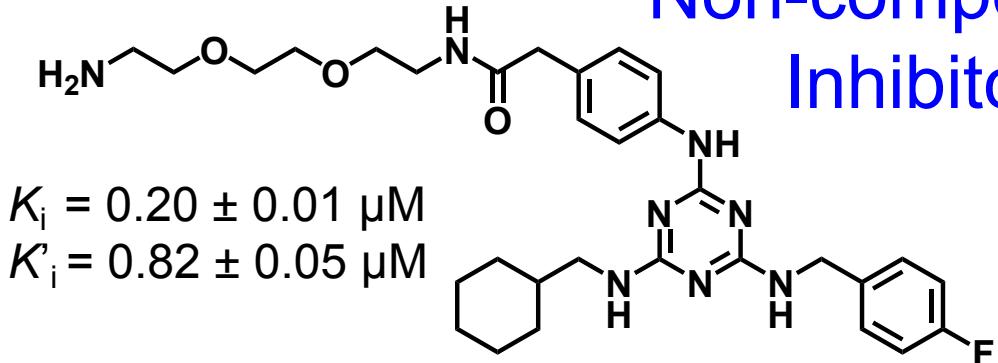
$IC_{50} = 0.32 \pm 0.04 \mu M$



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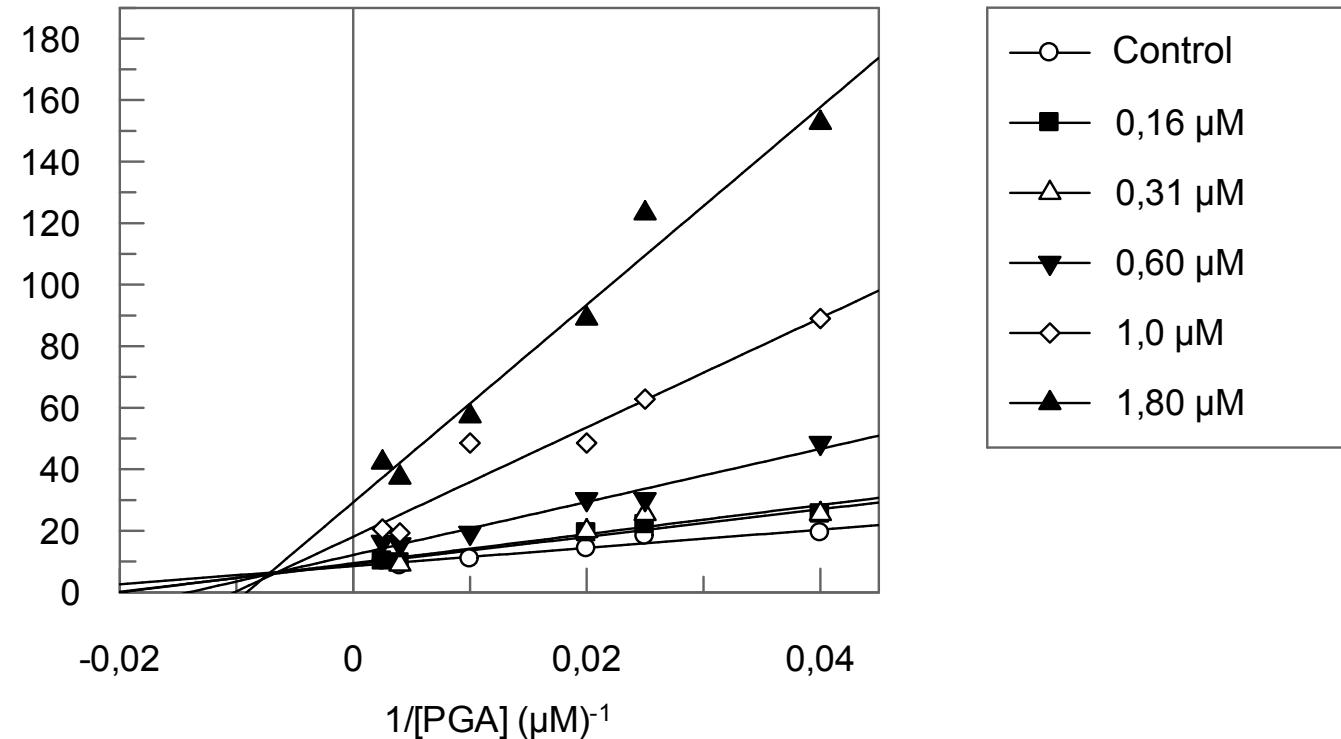
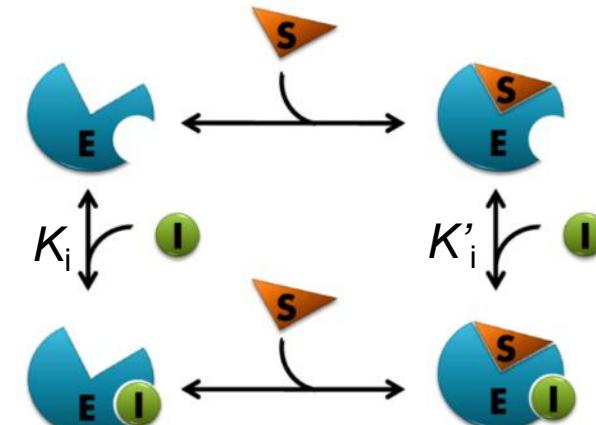
Dr. Lorena Souza





$$K_i = 0.20 \pm 0.01 \mu\text{M}$$

$$K'_i = 0.82 \pm 0.05 \mu\text{M}$$



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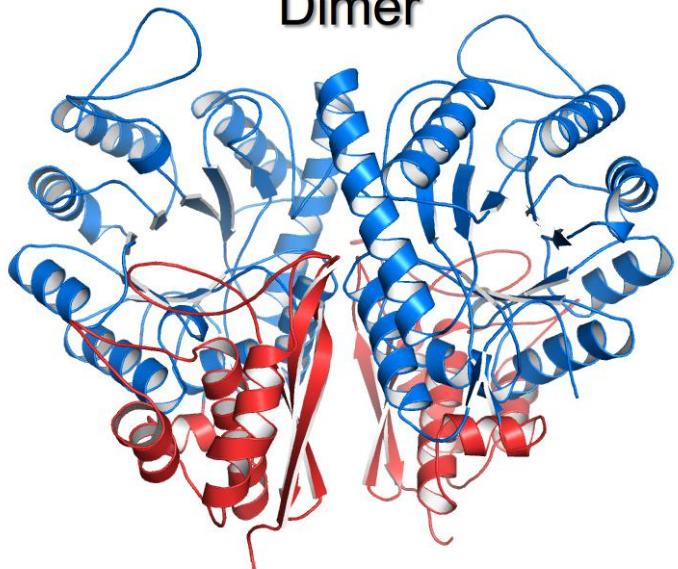
Dr. Lorena Souza



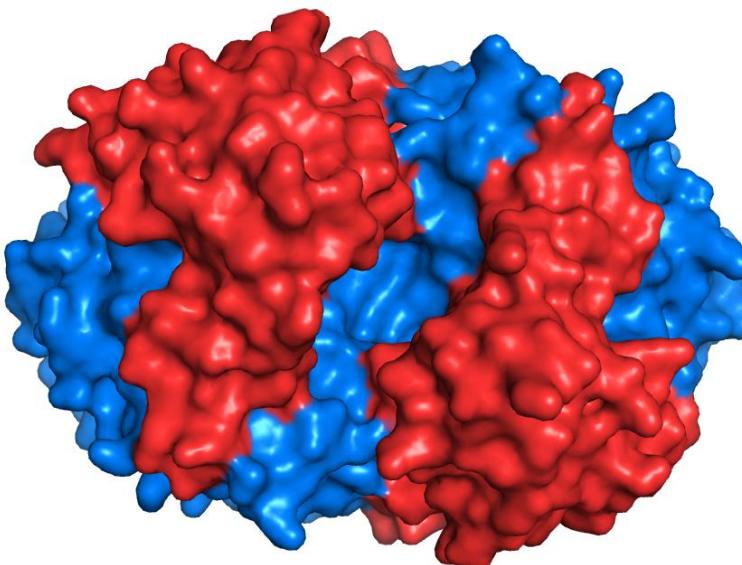
Cristallographic complex: Pfenolase-Enblock

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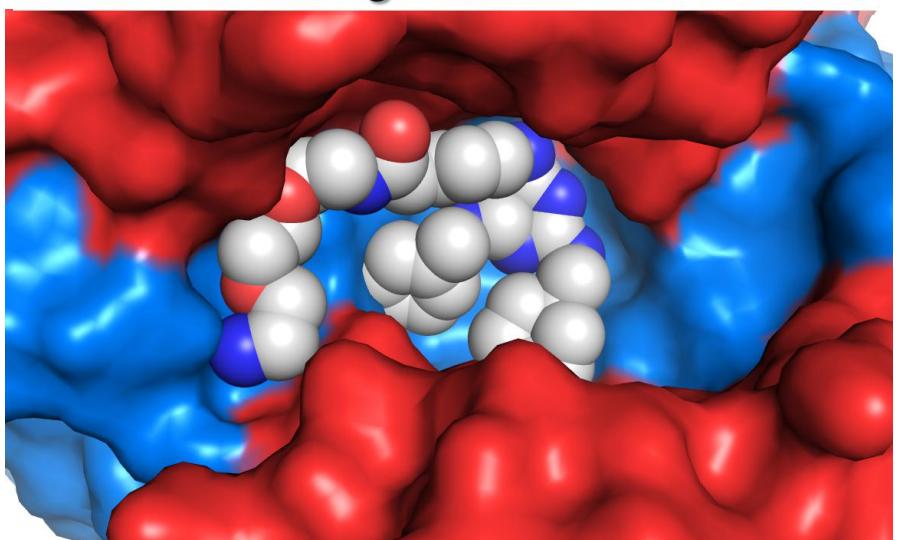
Dimer



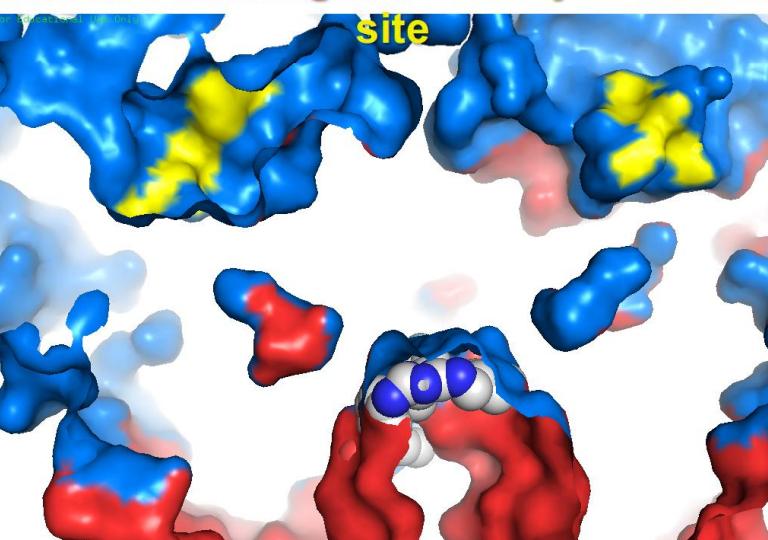
Dimer interface



Inhibitor binding site: Dimer interface



Inhibitor binding site / catalytic binding



Pfenolase-ENBlock

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 (°)	α=90,00 β=96,51 γ=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)
<(I)/σ(I)>	10,9 (2,1)
<i>Refinement</i>	



In vitro antiplasmodial activity

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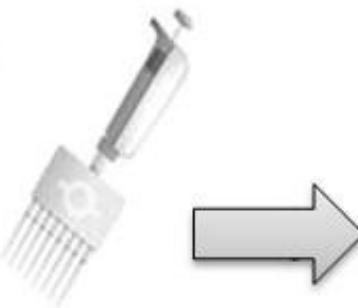
In vitro Inhibitory activity and toxicity evaluation



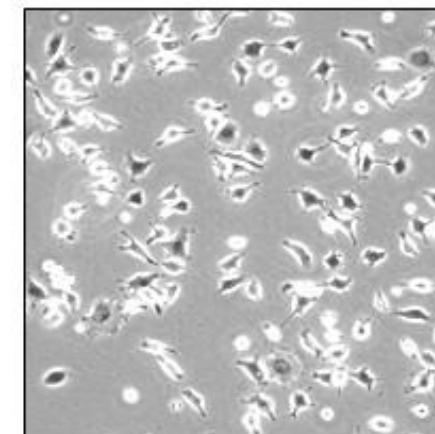
Dr. Anna Caroline
C. Aguiar



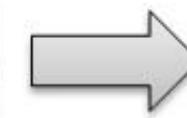
Serial Dilution



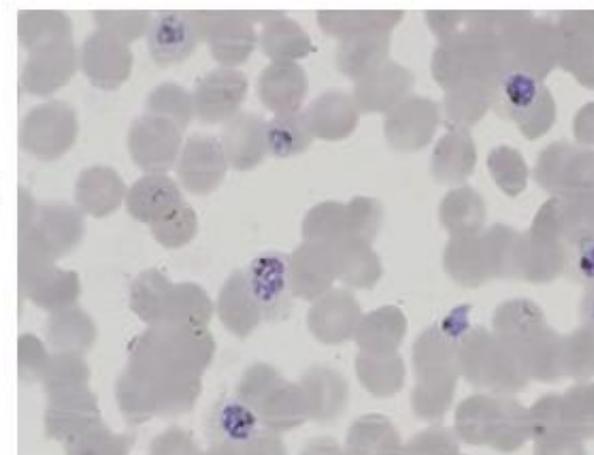
In vitro citotoxicity
 MDL_{50}



HepG2 e BGM
MTS



Antiplasmodial activity
 IC_{50}



3D7 e K1
SYBR GREEN



Enoblock: In vitro activity

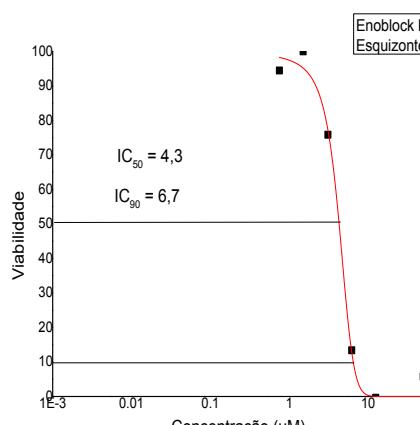
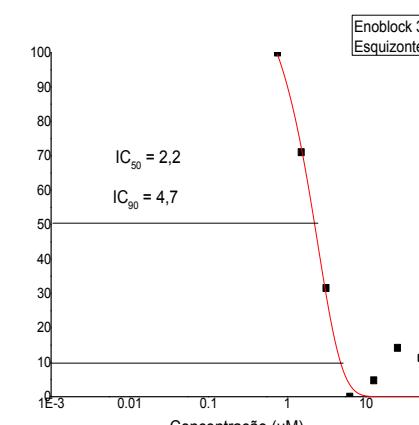
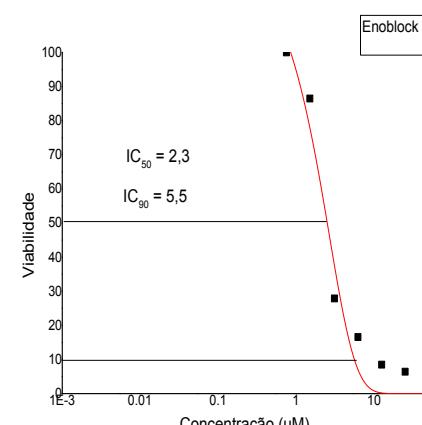
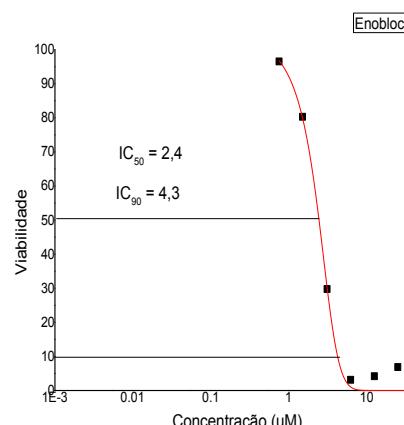
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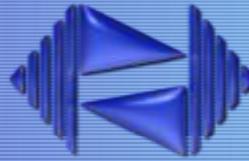
ENOBLOCK	<i>Plasmodium falciparum</i> IC_{50} (μM)		<i>Cytotoxicity</i> IC_{50} (μM)		
	<i>Ring</i>	<i>Schizont</i>	<i>BGM</i>	<i>HepG2</i>	<i>SI</i>
3D7	2.2 ± 0.1	2.5 ± 0.3	> 750	> 750	> 375
K1	1.9 ± 0.2	3 ± 1	> 750	> 750	> 375



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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*)

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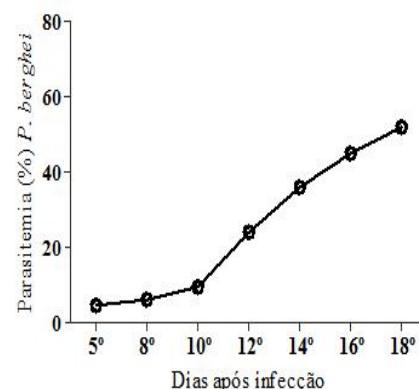
Infection: 5×10^5 iRBC *P. berghei* (strain NK65)



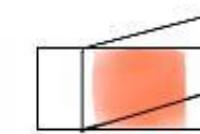
VO treatment for 3 days
(100 mg/Kg)



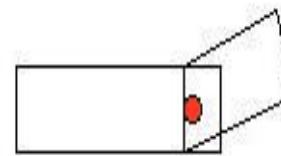
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Parasitemia evaluation



Blood smear



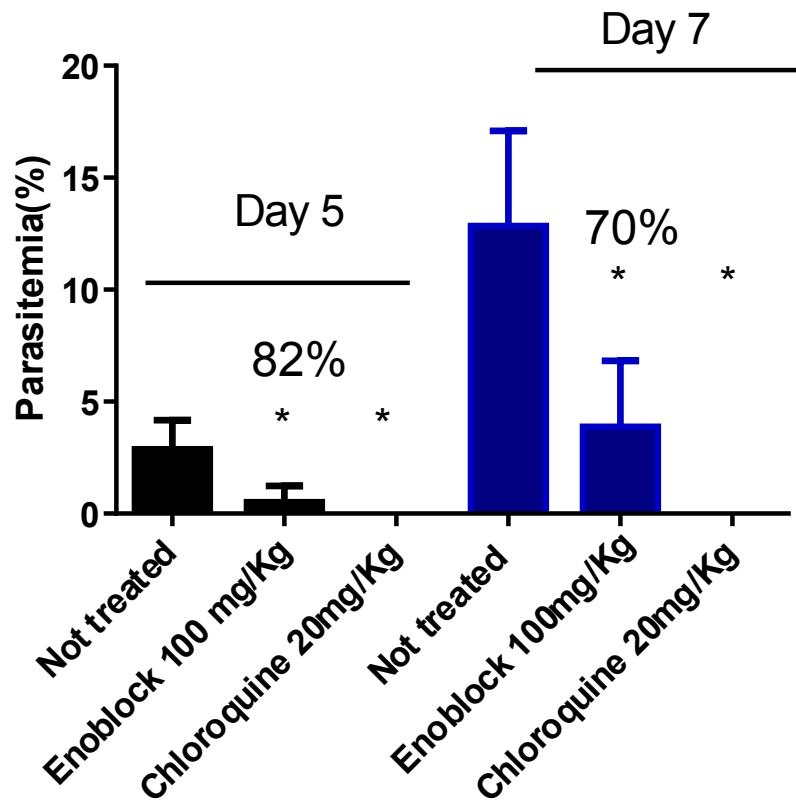
(Peters, 1965)



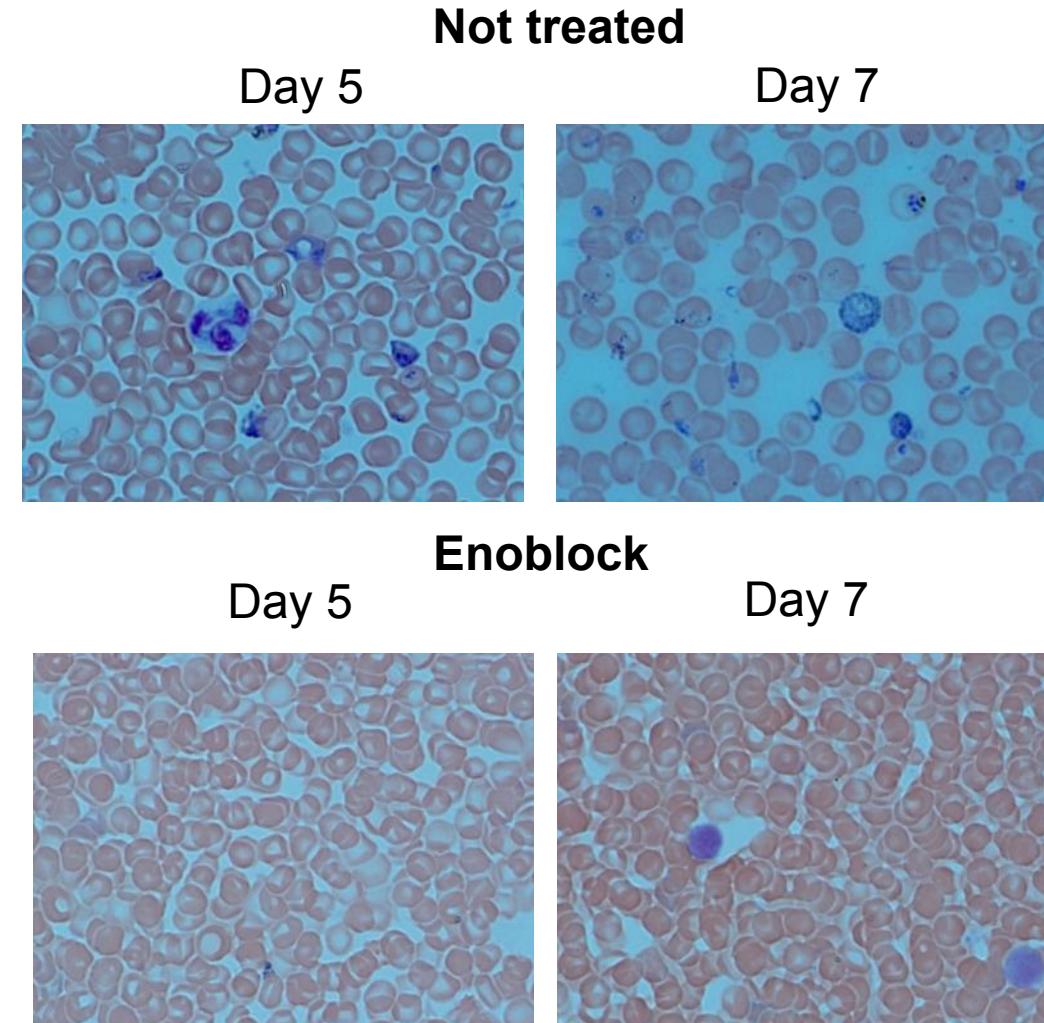
Enoblock: Proof of concept (in vivo)

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



* $p < 0.05$



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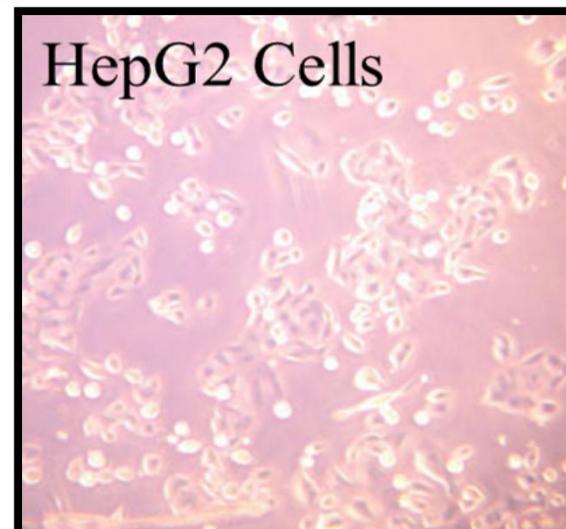


MMV 12/0103
*Long duration
heterocycles_(Aza)Benzimidazole*

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



- **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

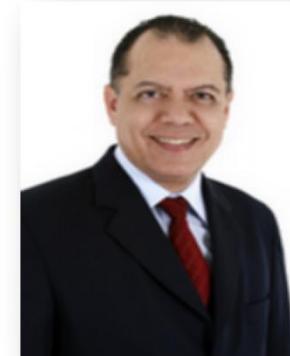
USP-CEPEM-MMV Team



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

Collaborators

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

FCF-USP

- Prof. Celia R. S. Garcia

UNIFESP

- Prof. Fabio C. Cruz



Dr. Paul Willis
Dr. Javier Gamo

