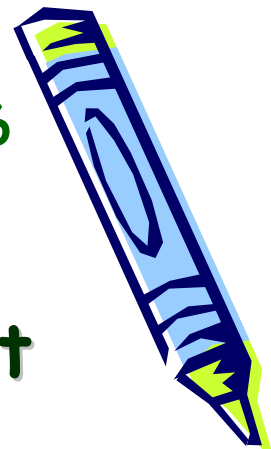




CONFERENCE

9th August 2006



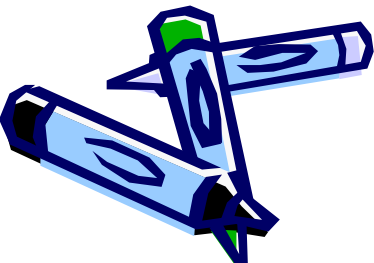
New Lead-Compounds for the Treatment of Neglected Disease

Dra Lídia Moreira Lima
(Professora Adjunta, FF-UFRJ)
lidialima@ufrj.br/lidia@pharma.ufrj.br

V Simpósio de Farmácia e
III Mostra de Trabalhos UNIVIX

UNIVIX

Faculdade Brasileira
Na prática, o melhor aprendizado.



INTRODUCTION:

Definitions and examples



"The so-called neglected diseases form a group because they affect almost exclusively poor and powerless people living in rural parts of low-income countries. While they cause immense suffering and often life-long disabilities, these diseases rarely kill and therefore do not receive the attention and funding of high-mortality diseases like AIDS, tuberculosis and malaria".

Edited by Mary Kay Kindhauser
World Health Organization
Geneva, 2003

Neglected Diseases: Onchocerciasis; Leprosy; Guinea worm disease; Lymphatic filariasis; Schistosomiasis; African trypanosomiasis; Chagas' disease; Dengue and Dengue haemorrhagic fever; Leishmaniasis, Tuberculosis; Malaria



INTRODUCTION:

Table: New chemical entities (NCEs) approved between 1975 and 1999 by class (Trouiller *et al.*, 2002)

THERAPEUTICS AGENTS	APPROVED NCEs 1975-1999
Central Nervous System	211 (15.1%)
Cardiovascular	119 (12.8%)
Cytostatics (neoplasms)	111 (8.0%)
Respiratory (non-infectious)	80 (6.4%)
Anti-infectives and Antiparasitics	224 (16.1%)
HIV/AIDS	26 (1.9%)
Tuberculosis	3 (0.2%)
Tropical Diseases (total)	13 (0.9%)
Malaria	4 (0.3%)
Other therapeutic categories	579 (41.6%)
Total	1393 (100%)

Trouiller, P *et al.*, Lancet (2002) 359: 2188

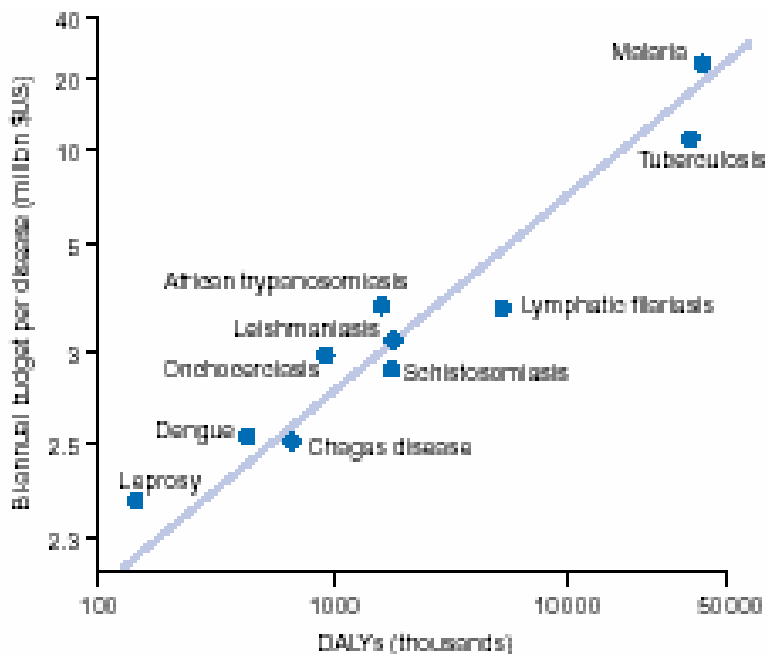
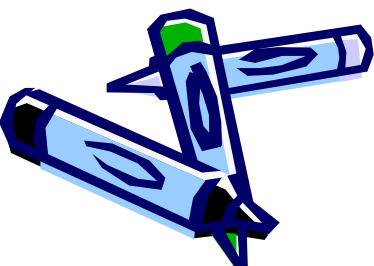


Fig. 1. Relationship between the research budget for diseases in the TOR portfolio and disease burden. In the TOR strategy, the higher the burden of a disease, the higher the investment in research and development related to that disease. The graph displays the relationship between the TOR investment (operation) for each of the ten targeted diseases in the 2002–2003 approved budget and the diseases burden in disability-adjusted life years (DALYs) [17] according to the 2000 estimates [16] (regression line: $\text{budget} = \text{DALYs}^{0.66} + 2,211,000$). This relationship and additional information on the TOR budget can be found at <https://www.who.int/dm/publications/publications/pd/budget.pdf>

Remme, JHF *et al.*, Trends in Microbiology (2002) 10: 435-440

INTRODUCTION:

PPPs “Public-private partnerships

US Walter Reed Army Institute of Research (WRAIR)
Tropical Disease Research (TDR)
Gates Foundation
Philanthropic Institutions
Academia
Governments
Industry



Medicines for Malaria Venture
Global Alliance for Tuberculosis
Drug Development (GATB)
Drugs for Neglected Diseases
Initiative (DNDi)
Institute for one World Health
(IOWH)

Antimalarial Portfolio: 21 projects in
various stages of clinical
development



Trouiller, P *et al.*, *Lancet* (2002)

359: 2188

Carey, J. *DDT* (2002)

9: 155-156

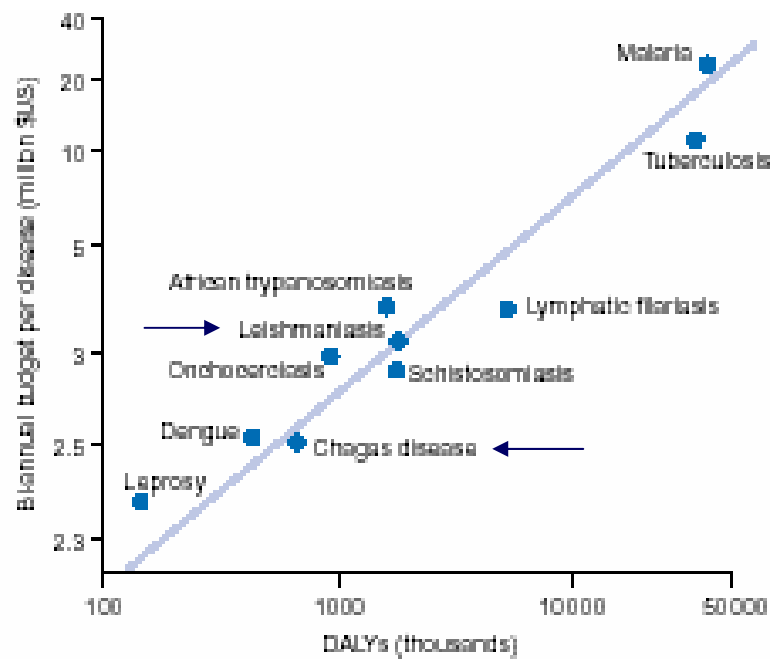
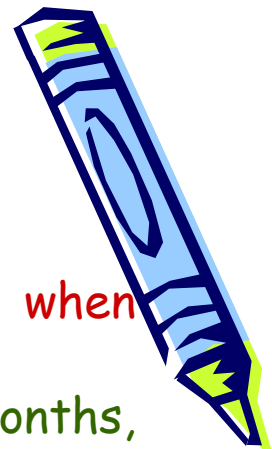


Fig. 1. Relationship between the research budget for diseases in the TDR portfolio and disease burden. In the TDR strategy, the higher the burden of a disease, the higher the investment in research and development related to that disease. The graph displays the relationships between the TDR investment (operation) for each of the ten targeted diseases in the 2002–2003 approved budget and the disease burden in disability-adjusted life years (DALYs) [17] according to the 2000 estimates [18] (regression line: $\text{budget} = \text{DALYs}^{0.66} + 2.211000$). This relationship and additional information on the TDR budget can be found at: <https://www.who.int/initiatives/publications/publications/pd/budget.pdf>

Remme, JHF *et al.*, *Trends in Microbiology*
(2002) 10: 435-440



INTRODUCTION: Leishmaniasis (forms, epidemiology, treatment)



The disease in human has four forms ranging in severity:

- **Visceral leishmaniasis (VL, also known as kala azar)** ⇒ fatal when untreated
- **Cutaneous leishmaniasis (CL)** ⇒ frequently self-cures within 3-18 months, although they cause serious disability and severe and permanent disfiguring scars
- **Mucocutaneous leishmaniasis (MCL)** ⇒ is a mutilating disease
- **Diffuse cutaneous leishmaniasis (DCL)** ⇒ is a long-lasting disease due to a deficient cellular-mediated immune response. Produces disseminated and chronic skin lesions resembling those of multibacillary leprosy.

Epidemiological Trends:

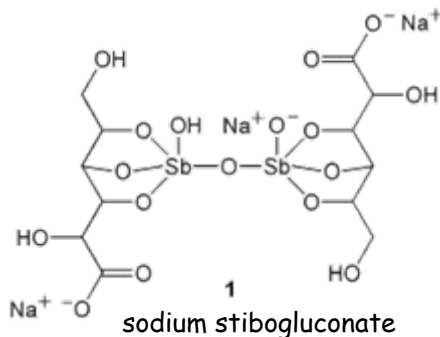
- Leishmaniasis is presently endemic in **88 countries**, placing **350 million people** at risk;
- WHO estimates that 12 million people are currently infected, with around 1.5-2 million new infections occurring each year.

• In several areas of the world, there is a clear and disturbing increase in the number of cases [CL in Brazil: 1998 → 21.800 cases; 2002 → 40.000 cases/ ↑46% in 4 years]
[VL in North-eastern Brazil: 1998 → 1840 cases; 2002 → 6.000 cases/ ↑69% in 4 years]





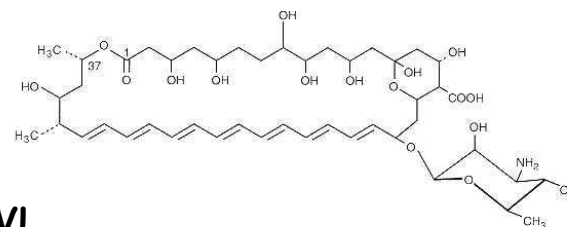
1 - Pentavalent antimonials (1945) e.g. Pentostam® (GSK); Glucantime® (Aventis).



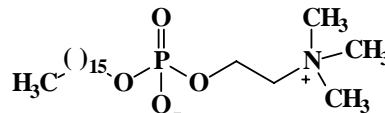
meglumine antimoniate

NC(=O)c1ccc(OCCCCOc2ccc(NC=O)cc2)cc1

Amphotericin B



3-Miltefosine⇒ approved in India (2002) for oral therapy of VL



Ouellette, M. et al. (2004) *Drug Resistance Updates* 7:257-266

Desjeux, P. (2004) *Comparative Immunology, Microbiology & Infectious Diseases* 27:305-318





INTRODUCTION:Chagas' Disease (epidemiology, mortality, treatment)

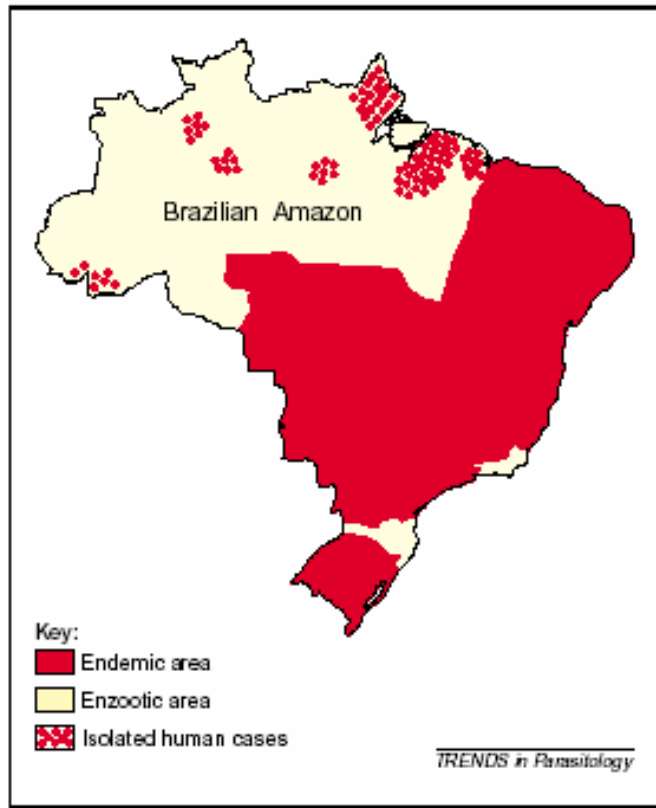


Fig. 1. Distribution of Chagas disease in Brazil: enzootic areas with isolated human cases or small outbreaks and endemic regions [6,8,15].

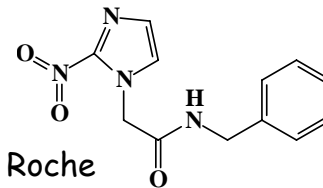
TRENDS in Parasitology Vol.18 No.4 April 2002

- *Trypanosoma cruzi* affects more than 25 million people annually in South America;
- Causes more than 45.000 deaths/year;
- 100.million people in Latin America are believed to be in risk of infection;

Treatment

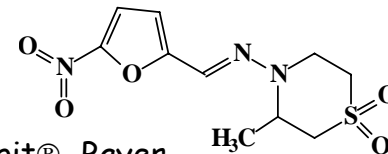
The target is the circulating form of the parasite, i.e. trypomastigote (only during the acute phase) and intracellular amastigotes

1- Benznidazole (launched in the 1970s)



Rochagan®, Roche

2- Nifurtimox (launched in the 1960s)



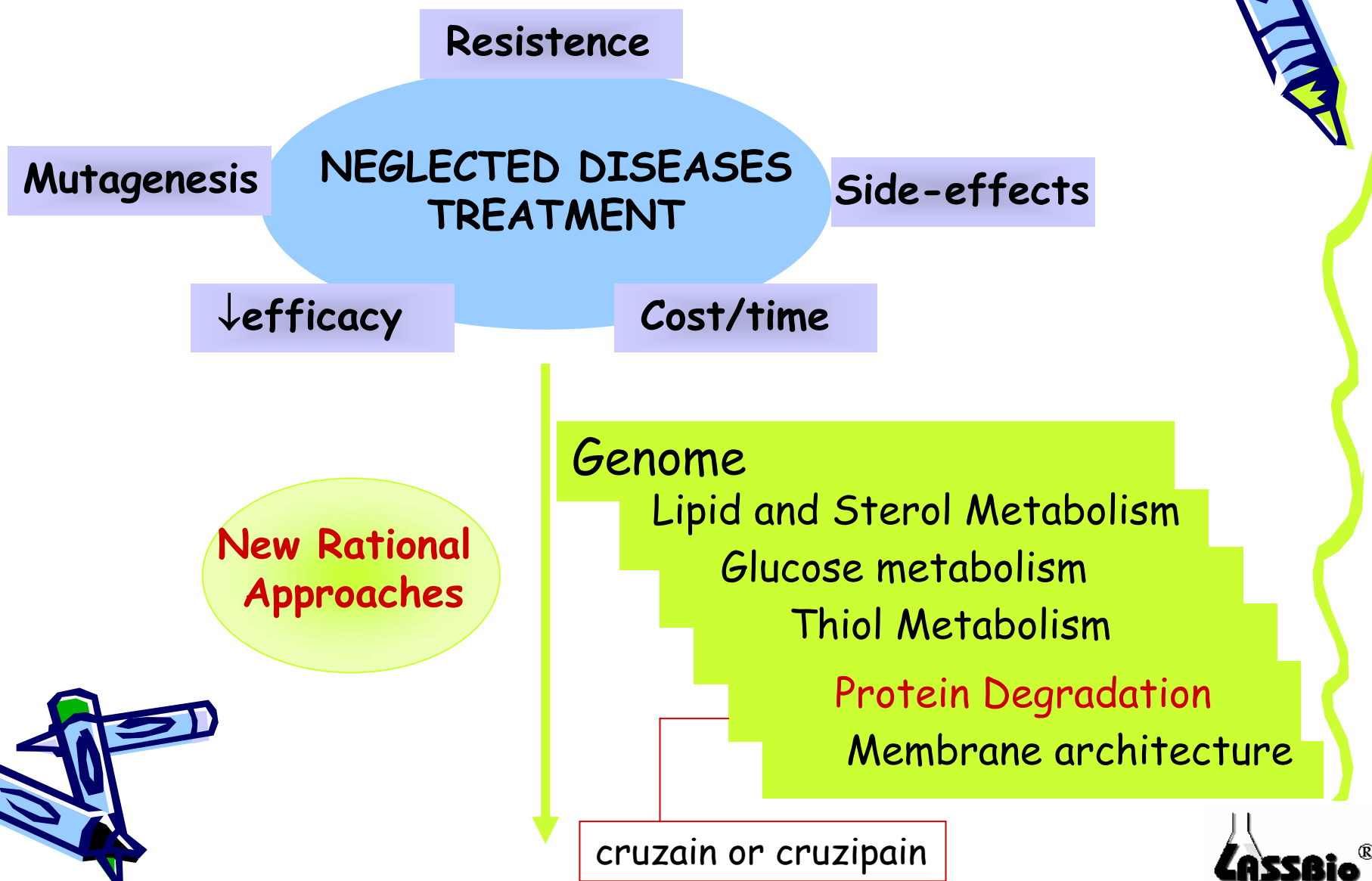
Lampit®, Bayer

Maya and coworkers described that the trypanocidal effect of benznidazole does not depend on oxygen radicals production as with nifurtimox

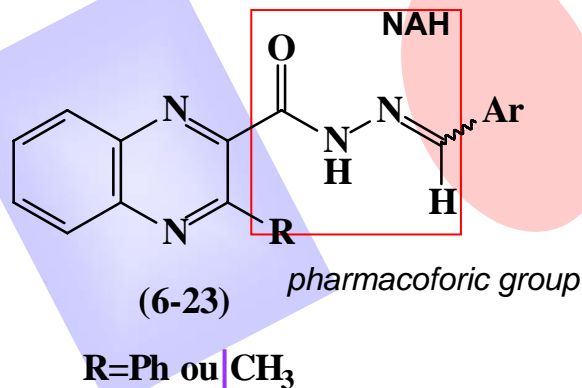
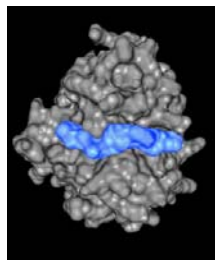
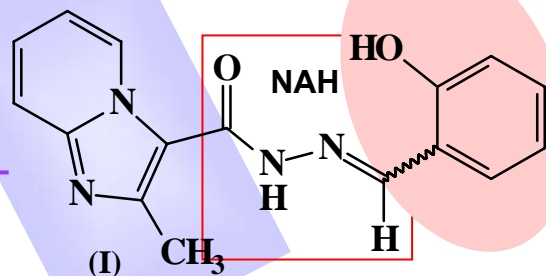
Maya, JD *et al.* (2003) *Biochemical Pharmacology* 65: 999-1006; Barret, MP *et al.* (2003) *Lancet* 362: 1469-1480; Brinen, LS *et al.* (2000) *Structures* 8: 831-840



INTRODUCTION: THERAPY (New Rational Approaches)



MOLECULAR DESIGN: New Cysteine Protease Inhibitors (i.e. cruzain)

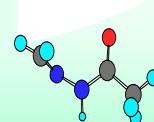
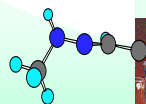
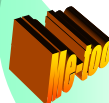
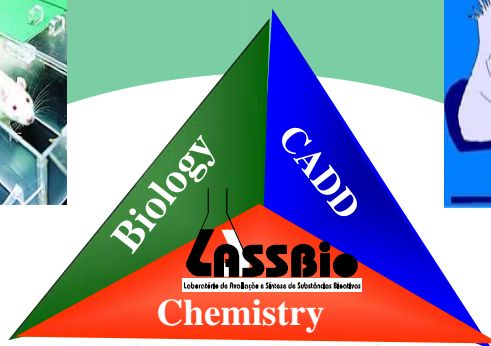
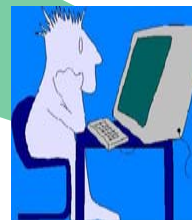


Bioisosterism

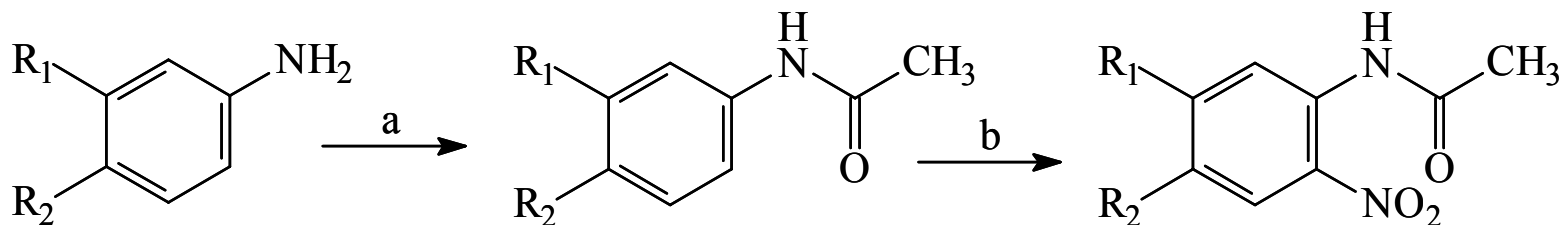
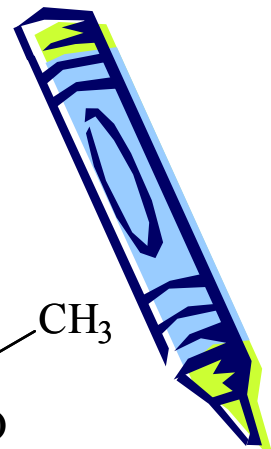
Cruzain or Cruzipain (*T.cruzi*)

Ribeiro, I. G. *et al.* (1998) *Eur. J. Med. Chem.*: 33, 225-235

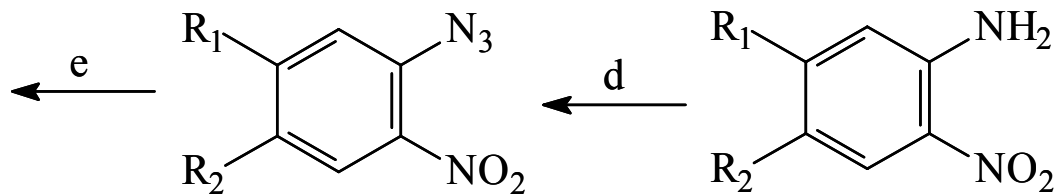
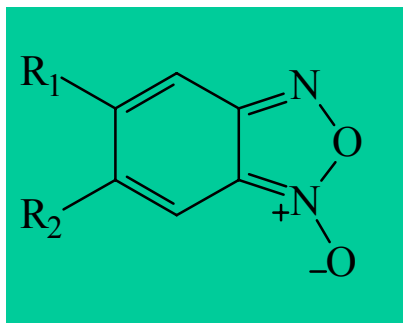
Ifa, D.R. *et al.* (2000) *J. Mol. Struct.-Theochem* 505: 11-17



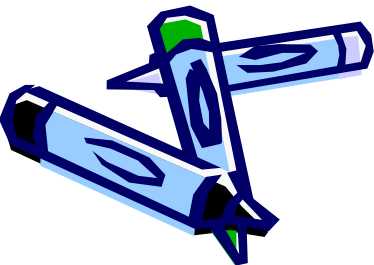
RESULTS AND DISCUSSION: Chemistry



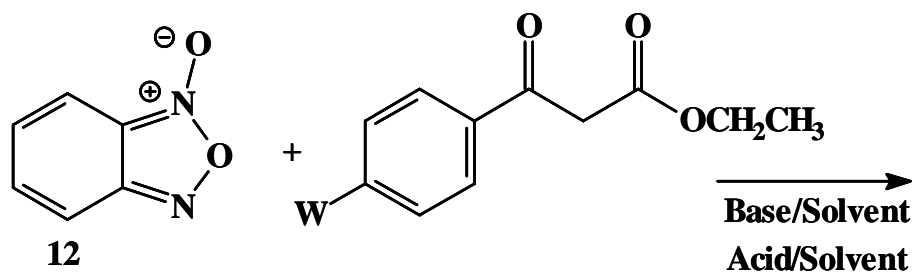
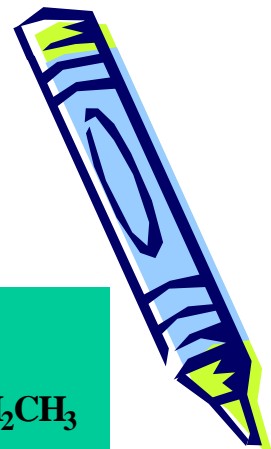
Ref1. Monge, A. *et al.*, *J. Med. Chem.* (1995) 38: 1786



Condições: **a)** Ac_2O , AcOH , refluxo, 15 min (72-87%); **b)** HNO_3 , H_2SO_4 , -4°C ? t.a. 30 min (64-91%); **c)** H_2SO_4 , 100°C , 15 min (71-93%); **d)** NaN_3 , NaNO_2 , AcONa , $\text{HCl}:\text{H}_2\text{O}$, 0°C 15 min (14-68%); **e)** PhCH_3 , refluxo, 2 h (47-88%)

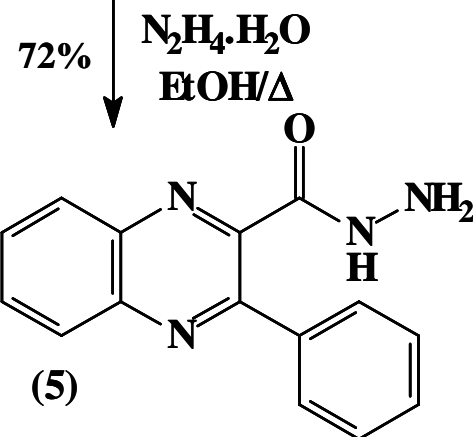
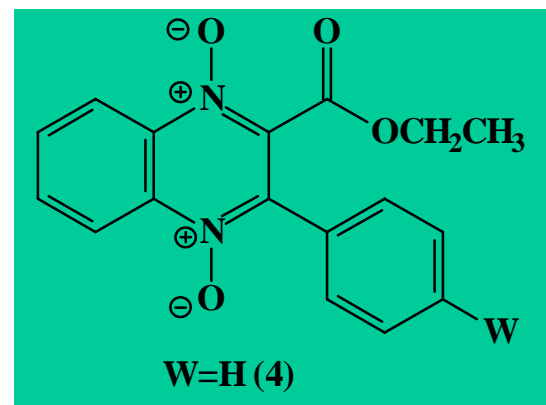
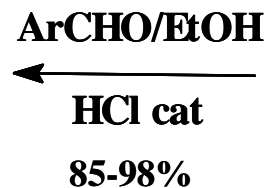
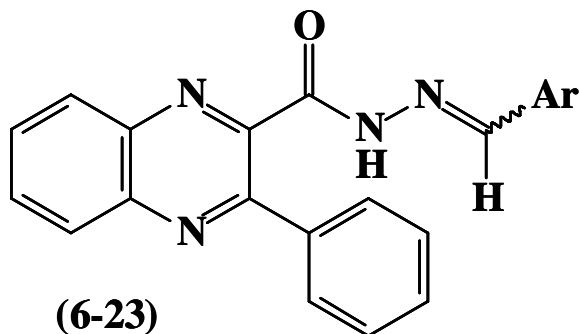


RESULTS AND DISCUSSION: Chemistry



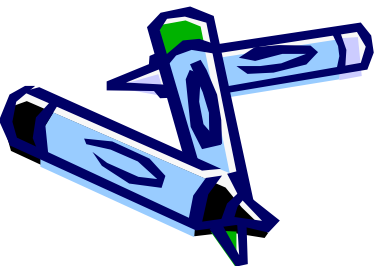
Ref2. Lima, L.M. *et.al. J. Heterocyclic Chem.* (2005) 42: 1381-1385

Ref3. Lima, P.C. *et.al. Eur. J. Med. Chem.* (2000) 35: 187-203



Mixture *E/Z* (1:1)

RMN ^1H , RMN ^{13}C , massas, IV, análise elementar (C,N,H)



RESULTS AND DISCUSSION:

Pharmacological Assay

Table 1: In vitro anti-trypanosomatid activity. As a first screening the ability of derivatives to inhibit the growth of the epimastigote form of *T. cruzi* (Tulahuen 2 strain)¹ was evaluated at 25 μ M and the IC₅₀ was determined for the most active compounds

Compounds (25 μ M)	% inhibition of epimastigote forms of <i>T. cruzi</i>	IC ₅₀ (μ M)
Nifurtimox®	100	10
LASSBio-1008	3	n.d.
LASSBio-1009	22	n.d.
LASSBio-1010	40	n.d.
LASSBio-1011	53	n.d.
LASSBio-1012	47	n.d.
LASSBio-1013	35	n.d.
LASSBio-1014	29	n.d.
LASSBio-1015	19	n.d.
LASSBio-1016	96	15,9

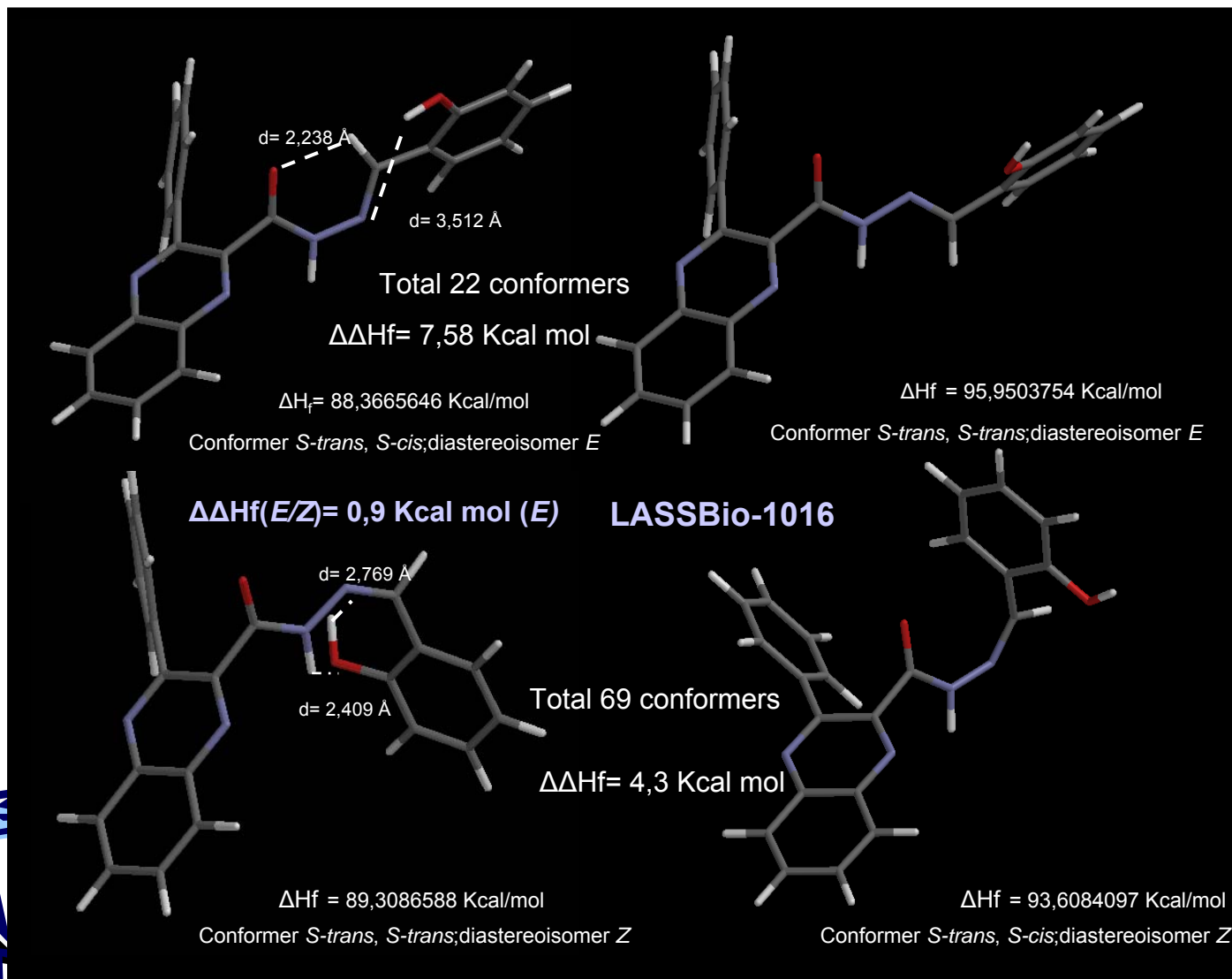
Compounds (25 μ M)	% inhibition of epimastigote forms of <i>T. cruzi</i>	IC ₅₀ (μ M)
LASSBio-1017	27	n.d.
LASSBio-1018	36	n.d.
LASSBio-1019	0	n.d.
LASSBio-1020	0	n.d.
LASSBio-1021	0	n.d.
LASSBio-1022	81	20,0
LASSBio-1023	0	n.d.
LASSBio-1024	4	n.d.
LASSBio-1025	0	n.d.

¹Denicola, A. *et al.*, (1993) *Arch. Biochem. Biophys.* 304: 279-286

RESULTS AND DISCUSSION:

Molecular Modeling (Spartan Pro 1.0.5)

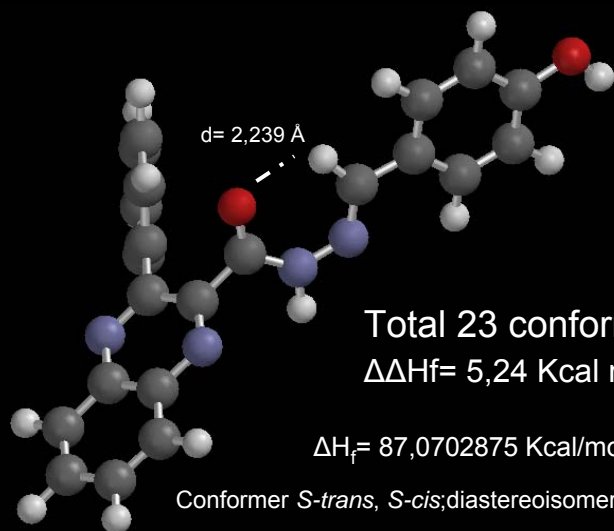
Conformers Distribution: Semi-Empirical Method (AM1)



RESULTS AND DISCUSSION:

Molecular Modeling (Spartan Pro 1.0.5)

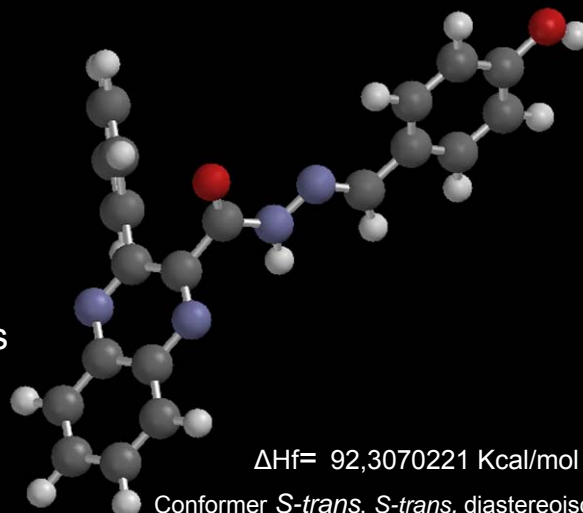
Conformers Distribution: Semi-Empirical Method (AM1)



Total 23 conformers
 $\Delta\Delta H_f = 5,24 \text{ Kcal/mol}$

$\Delta H_f = 87,0702875 \text{ Kcal/mol}$

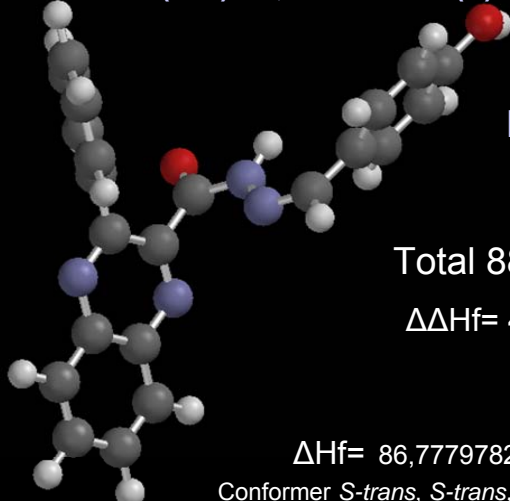
Conformer *S-trans*, *S-cis*; diastereoisomer *E*



$\Delta H_f = 92,3070221 \text{ Kcal/mol}$

Conformer *S-trans*, *S-trans*, diastereoisomer *E*

$\Delta\Delta H_f(E/Z) = 0,3 \text{ Kcal/mol (Z)}$



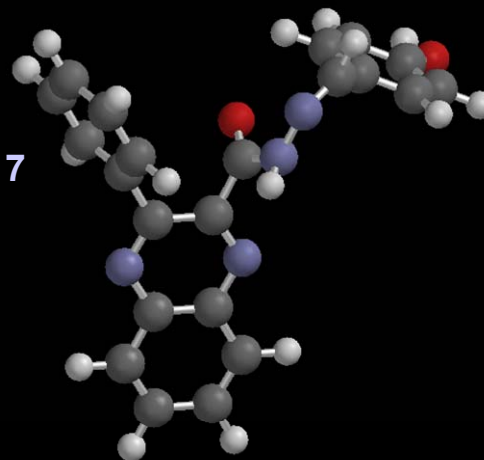
LASSBio-1017

Total 88 conformers

$\Delta\Delta H_f = 4,98 \text{ Kcal/mol}$

$\Delta H_f = 86,7779782 \text{ Kcal/mol}$

Conformer *S-trans*, *S-trans*, diastereoisomer *Z*



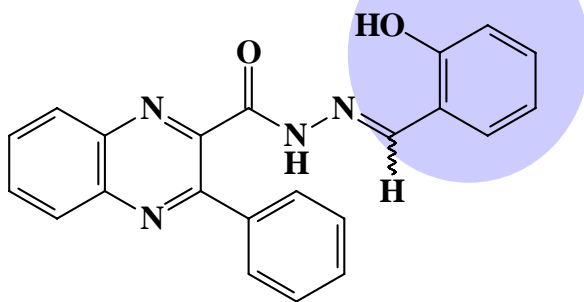
$\Delta H_f = 91,7533987 \text{ Kcal/mol}$

Conformer *S-trans*, *S-cis*, diastereoisomer *Z*

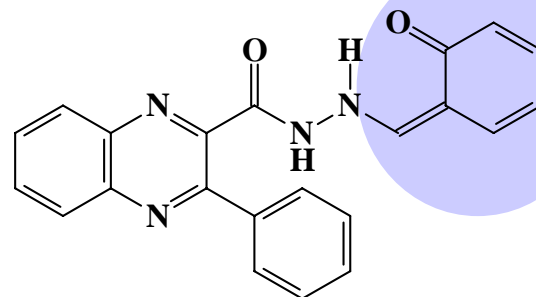
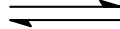
RESULTS AND DISCUSSION:

Molecular Modeling (Spartan Pro 1.0.5)

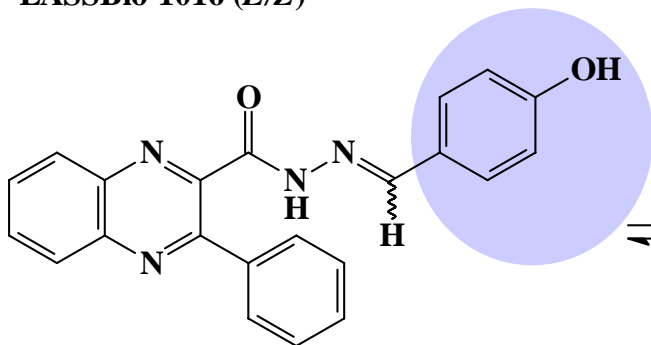
Tautomeric Species



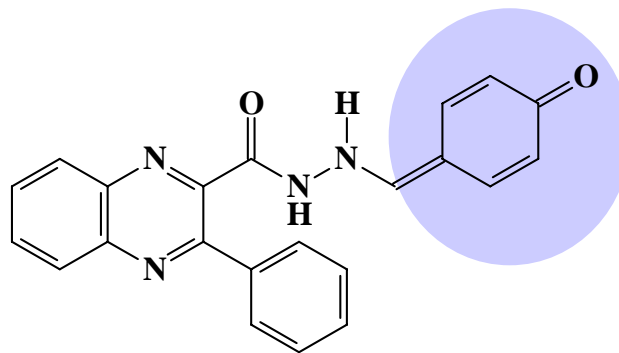
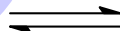
LASSBio-1016 (E/Z)



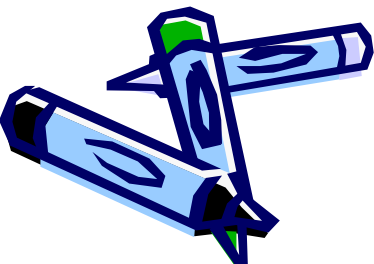
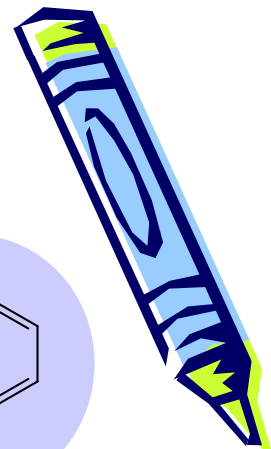
LASSBio-1016 (*iminoquinona*)



LASSBio-1017 (E/Z)

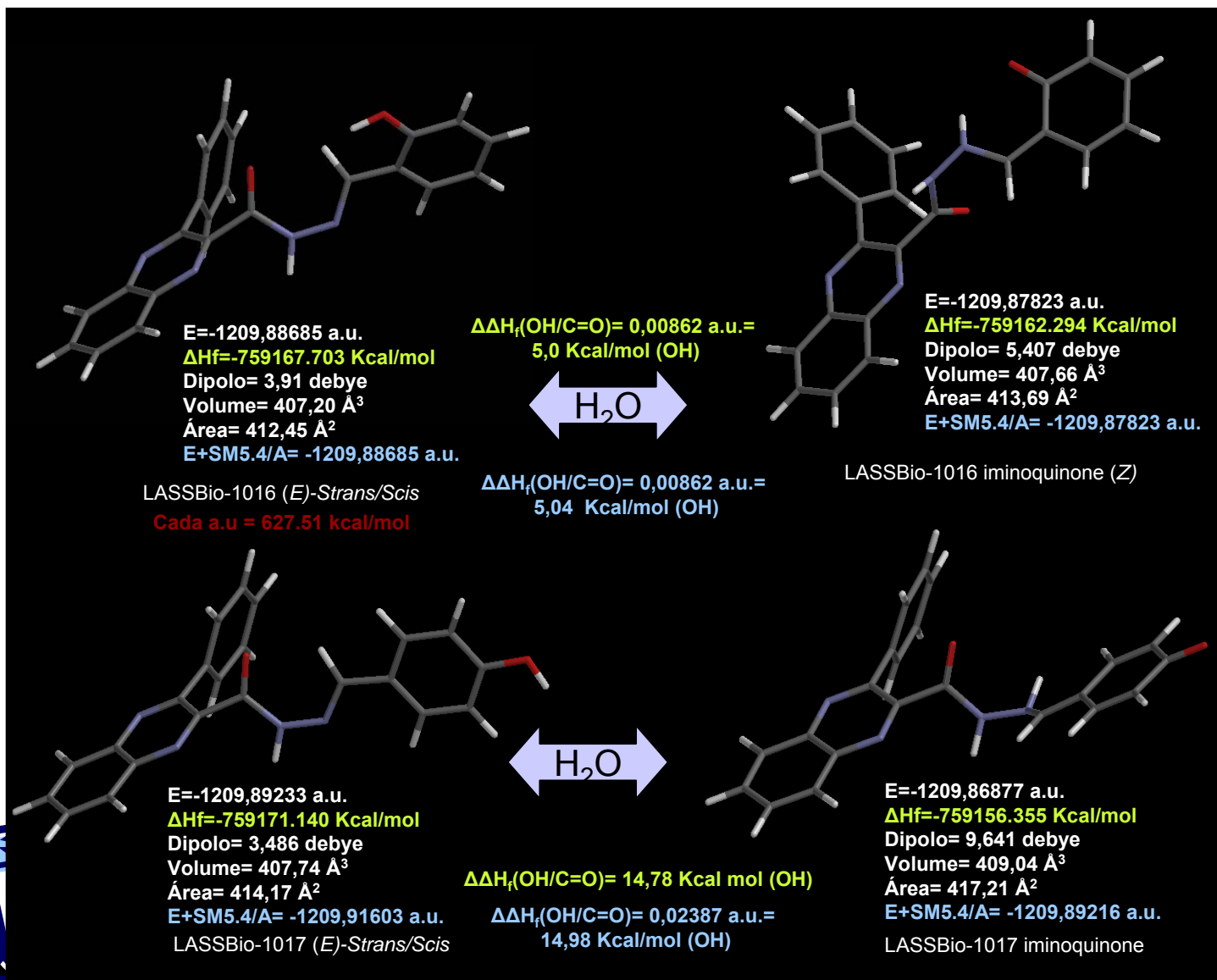


LASSBio-1017 (*iminoquinona*)



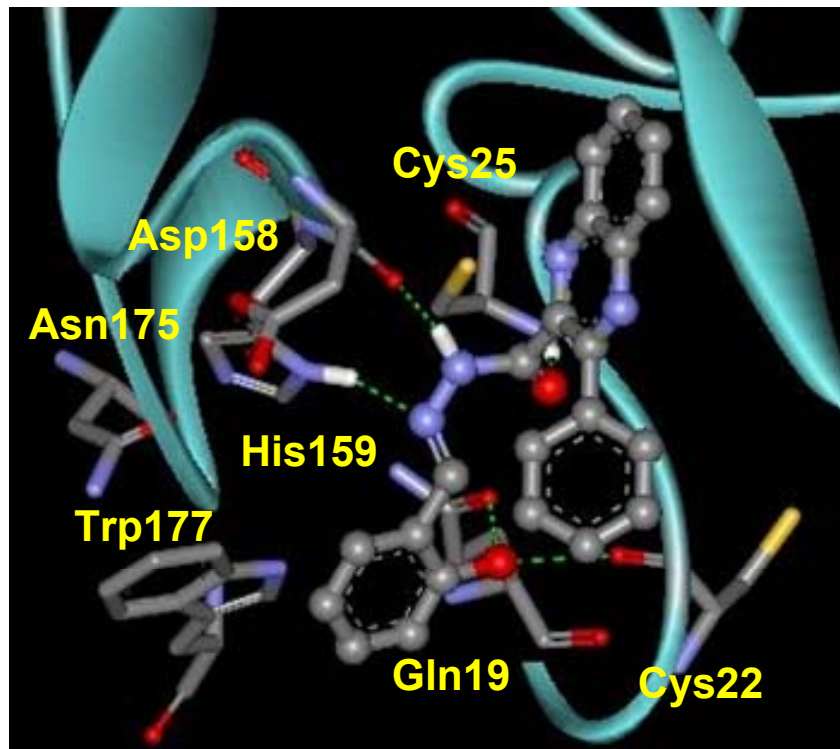
RESULTS AND DISCUSSION:

Molecular Modeling (Spartan Pro 1.0.5)

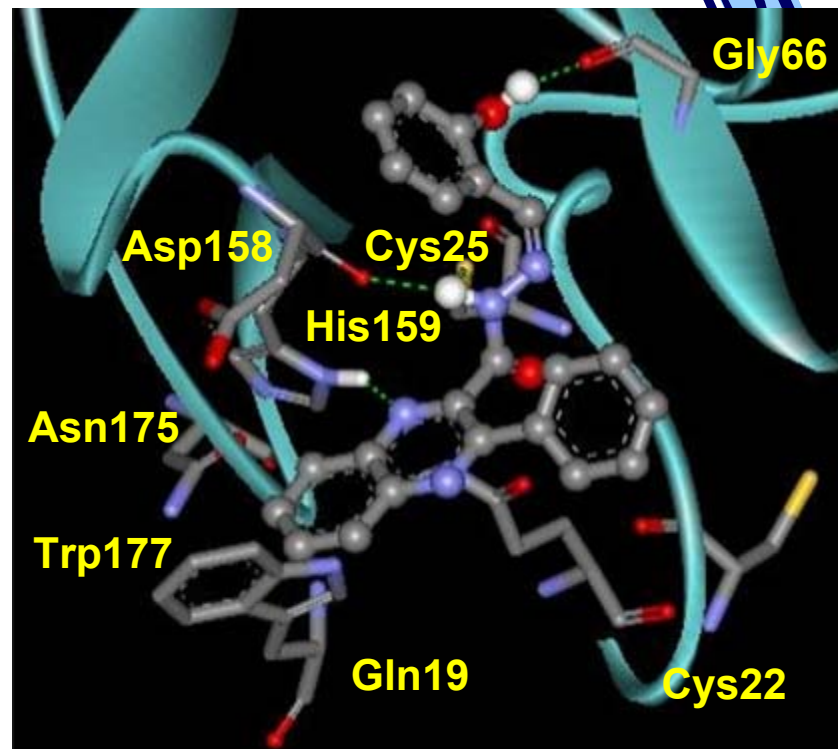


RESULTS AND DISCUSSION:

Docking Studies (FLE_xE)¹



LASSBio-1016(E)



LASSBio-1016(Z)

HYDROGEN BONDING INTERACTIONS:

LASSBio-1016(E)

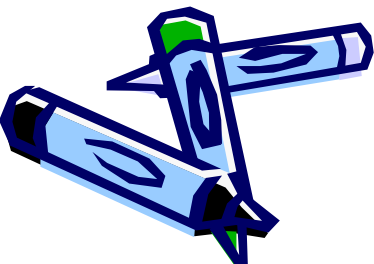
$\Delta G_{\text{binding}}$ (kJ/mol) = -27,796

LASSBio-1016(Z)

$\Delta G_{\text{binding}}$ (kJ/mol) = -25,838

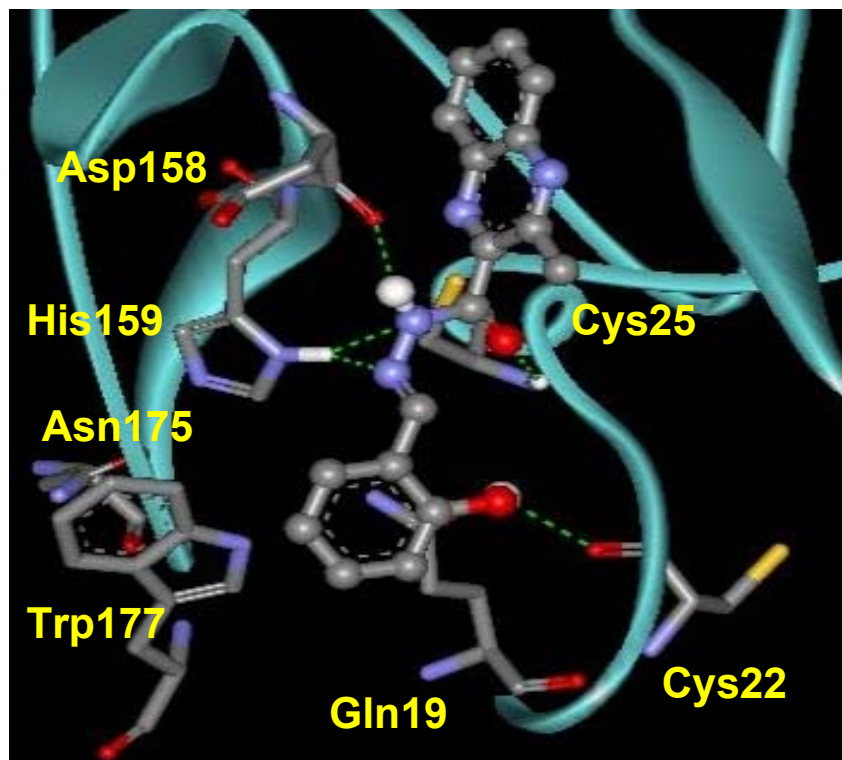
Gln19, Cys22, Cys25, Asp158, His159

Cys25, Gly66, Asp158, His159

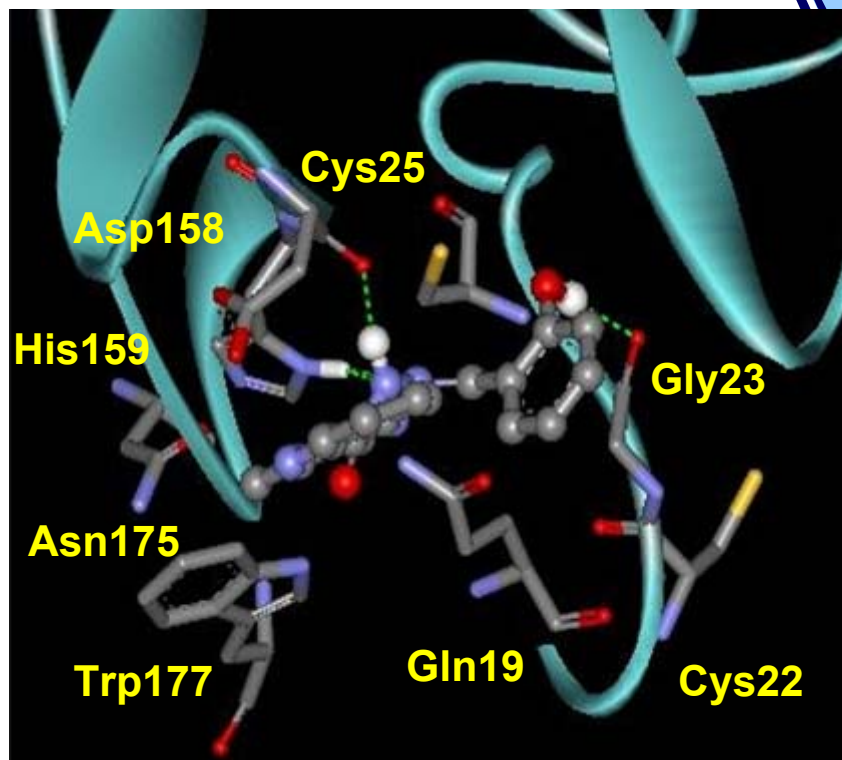


RESULTS AND DISCUSSION:

Docking Studies (FLExE)¹



LASSBio-1022(E)



LASSBio-1022(Z)

HYDROGEN BONDING INTERACTIONS:

LASSBio-1022(E)

$\Delta G_{\text{binding}}$ (kJ/mol) = -29,035

Gln19, Cys22, Cys25, Asp158, His159

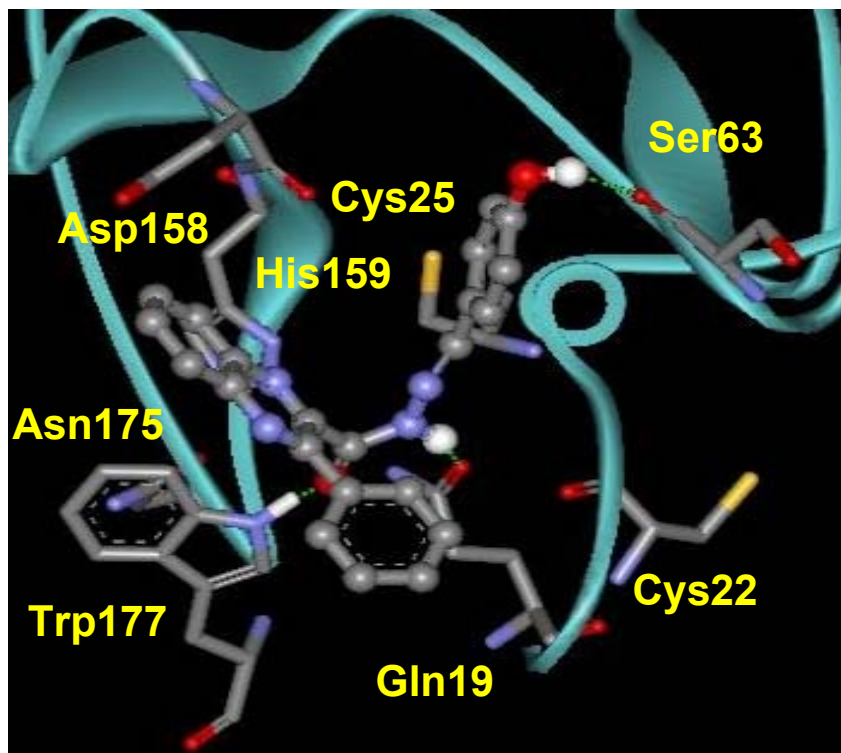
LASSBio-1022(Z)

$\Delta G_{\text{binding}}$ (kJ/mol) = -27,089

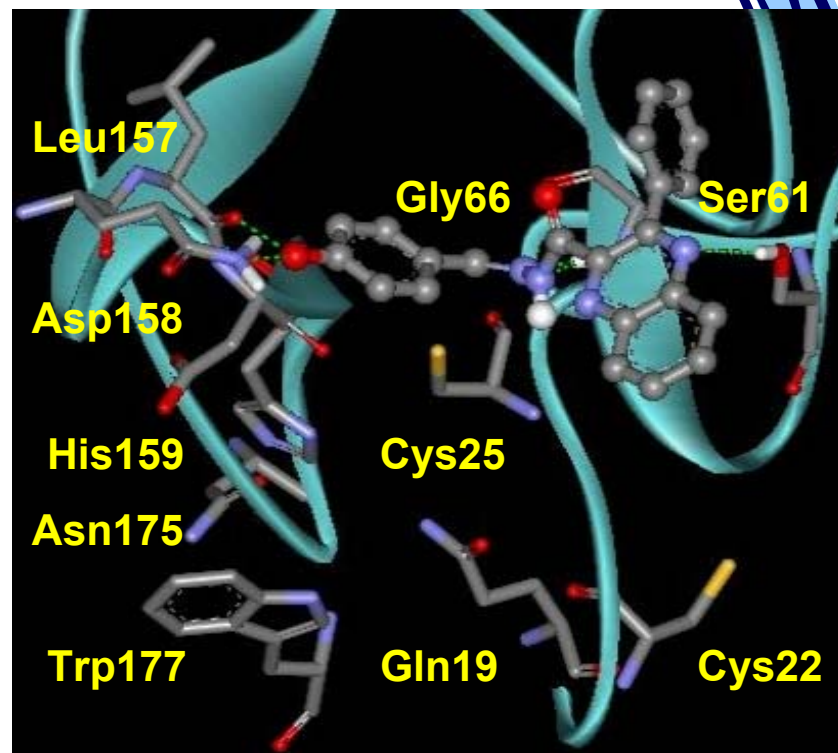
Gly23, Asp158, His159

RESULTS AND DISCUSSION:

Docking Studies (FLE_xE)¹



LASSBio-1017(E)



LASSBio-1017(Z)

HYDROGEN BONDING INTERACTIONS:

LASSBio-1017(E)

$\Delta G_{\text{binding}}$ (kJ/mol) = -19,245

Gln19, Ser64, His159

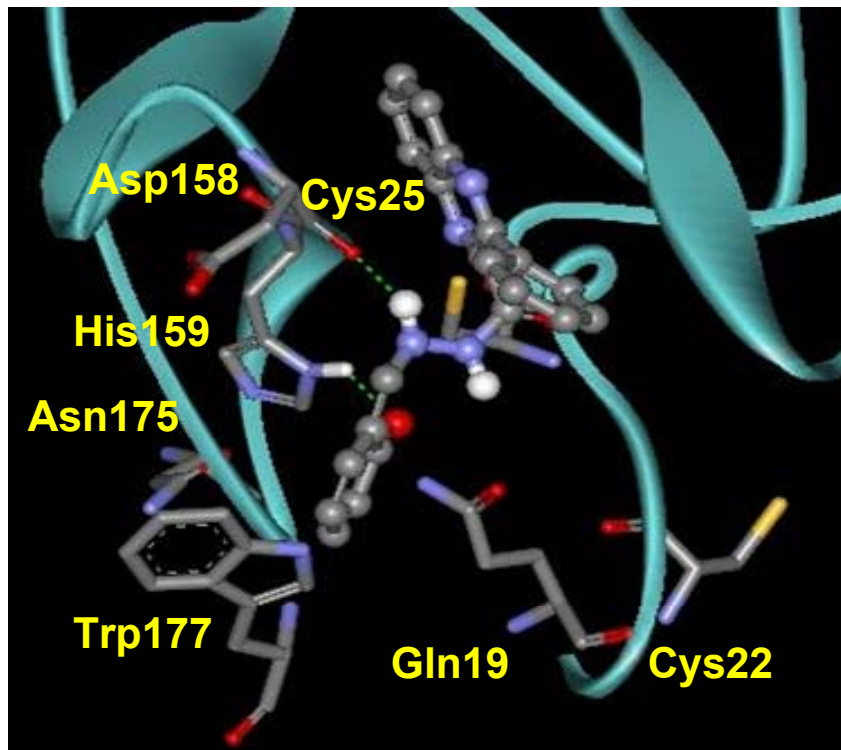
LASSBio-1017(Z)

$\Delta G_{\text{binding}}$ (kJ/mol) = -21,506

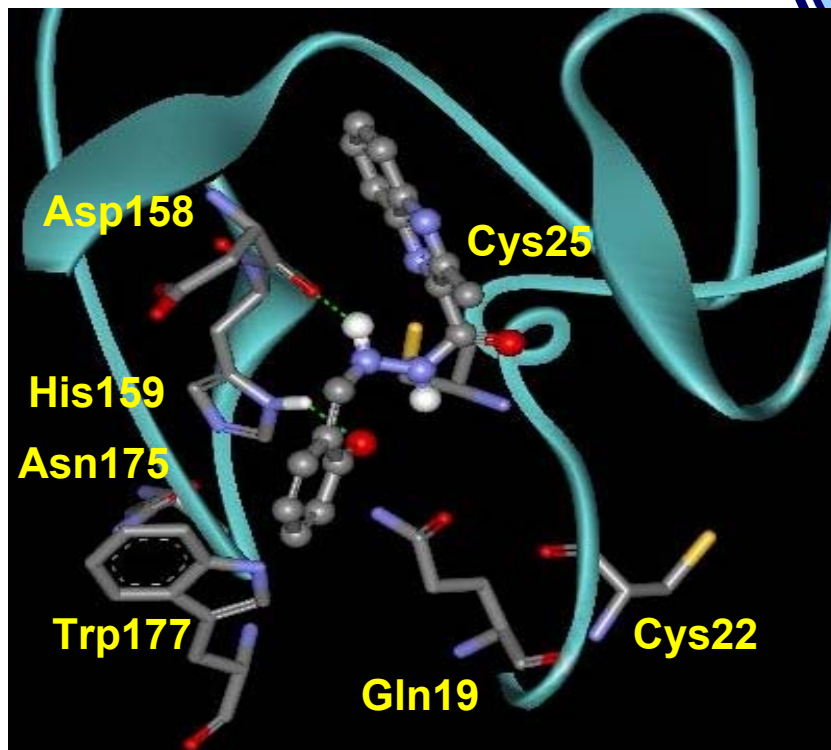
Ser61, Gly66, Gln156, Leu157

RESULTS AND DISCUSSION:

Docking Studies (FLE_xE)¹



LASSBio-1016 iminoquinona (Z)



LASSBio-1022 iminoquinona (Z)

HYDROGEN BONDING INTERACTIONS:

LASSBio-1016 iminoquinona (Z)

$\Delta G_{\text{binding}}$ (kJ/mol) = -27,566

Asp158, His 159

LASSBio-1022 iminoquinona (Z)

$\Delta G_{\text{binding}}$ (kJ/mol) = -27,089

Asp158, His159

RESULTS AND DISCUSSION: ANTILEISHMANIAL ACTIVITY

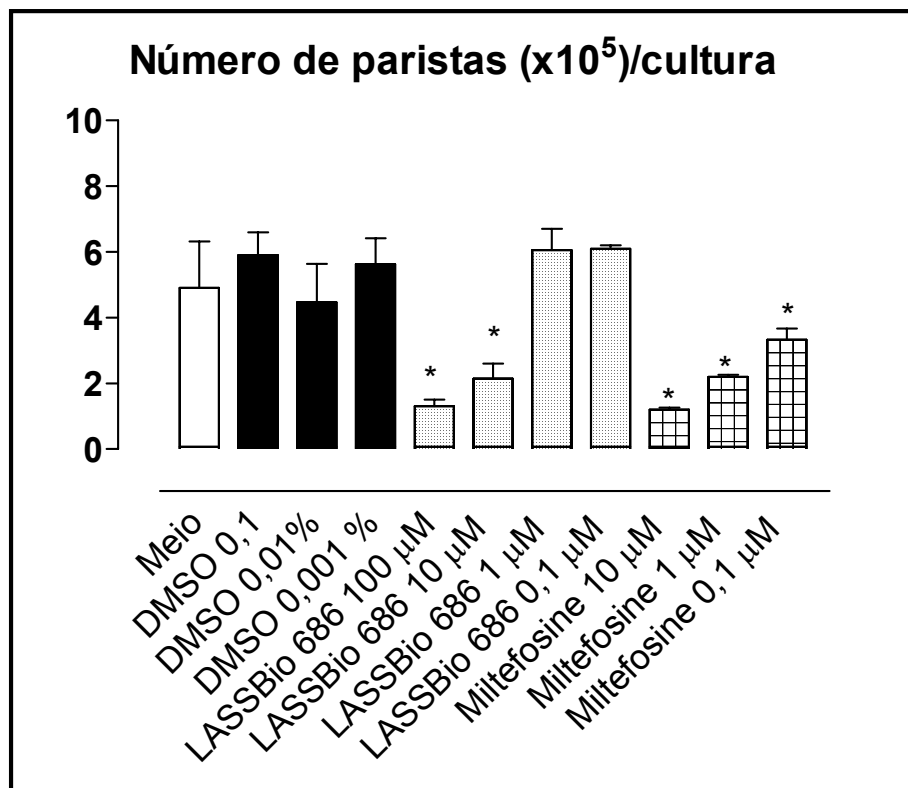


Figura 2. LASSBio 686 inibe a replicação da *L. major* de maneira concentração-dependente. Macrófagos peritoneais elicitados foram infectados com *L. major* e cultivados em meio DMEM completo, na ausência ou presença de LASSBio 686 (100 - 0,1 μ M) ou Miltefosine (10 - 0,1 μ M), por três dias em estufa de CO₂. Após este período os macrófagos recebiam meio Schneider's e seguido 3 dias era determinada a carga parasitária (número de parasitas extracelulares) após cultivo em estufa de BOD a 26 oC. Os valores foram considerados significativos quando $*P < 0,05$. Os resultados foram expressos como média \pm erro padrão da média de triplicatas.

RESULTS AND DISCUSSION: ANTILEISHMANIAL ACTIVITY

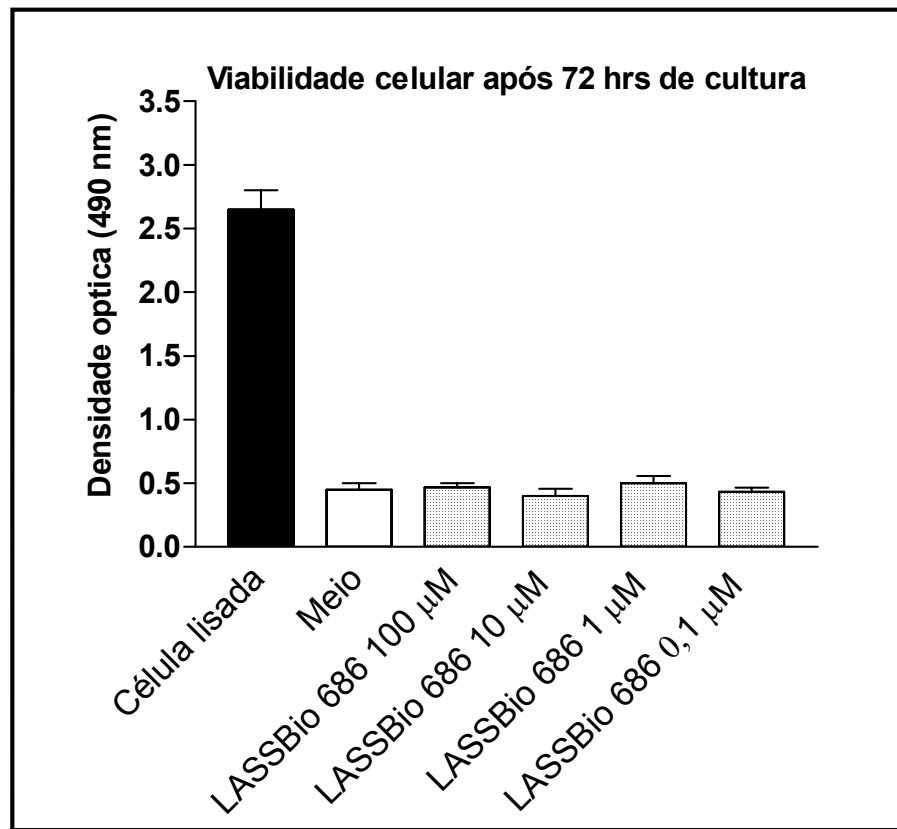


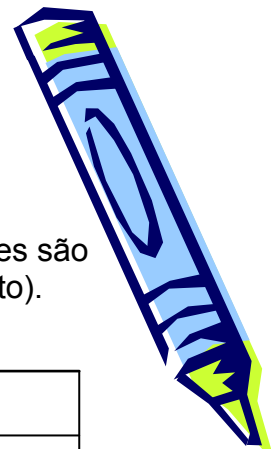
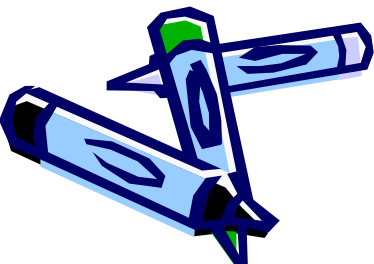
Figura 1. LASSBio 686 não possui efeito tóxico nas doses de 100 - 0,1 μ M. A sensibilidade de macrófagos peritoneais foi determinada através do ensaio de lactato desidrogenase LDH em cultura durante 72 horas de tratamento na presença de LASSBio 686 nas doses de (100 - 0,1 μ M). Após este período a viabilidade celular era determinada pelo método de LDH e expresso em densidade optica detectado a 460 nm. As culturas eram feitas em placas de 48 poços em triplicatas e a viabilidade celular era comparada ao padrão de morte obtido com amostras de células previamente lisadas.

RESULTS AND DISCUSSION: ANTILEISHMANIAL ACTIVITY

Tabela 1. Efeito do LASSBio 686 no crescimento de formas promastigotas de *L. Major*. Os valores são expressos como percentagem do crescimento do controle em meio (considerado 100 % do crescimento).

Concentração do Parasita	Crescimento do parasita (incorporação de timidina tritiada)			
	Tratamento			
	—	LASSBio 100 µM	LASSBio 10 µM	LASSBio 1 µM
10 ⁶	2537,5 ± 310	2211 ± 210 (12,8)	1782,5 ± 345 (29,7)*	2255,5 ± 276 (11,8)
5 x 10 ⁵	3866 ± 317	1852 ± 205 (52,1)*	1954 ± 346 (49,4)*	2588 ± 399 (33)*
10 ⁵	5861 ± 370	912,5 ± 221 (84,4)*	2861 ± 170 (51,1)*	4385 ± 515 (25,1)*
5 x 10 ⁴	3942 ± 339	3201 ± 532 (18,7)	4300 ± 1224 (0)	4705 ± 759 (0)
10 ⁴	1539 ± 104	2766 ± 59 (0)	1888 ± 71 (0)	1672 ± 725 (0)

* $P < 0,05$. Os resultados estão expressos como média ± erro padrão da média de triplicata. A % de inibição estão descritos nos parenteses.





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Collaborators

Dr Eliezer J. Barreiro (LASSBio, FF-UFRJ, BR)

Dr Carlos Alberto Manssour Fraga (LASSBio, FF-UFRJ, BR)

Dra Nelilma C Romeiro (LASSBio, FF-UFRJ, BR)

Dr Antonio Monge (FF-Universidad de Navara, Spain)

Dr Hugo Cerecetto (Universidad de la República, Uruguay)

Dra Mercedes González (Universidad de la República, Uruguay)

Dr José Osvaldo Previato (IBCCF-UFRJ, BR)

Dra Lúcia M. Previato (IBCCF-UFRJ, BR)

Dra Magna Suzana Alexandre Moreira (LFI-UFAL, BR)

Dra Marise Pinheiro Nunes (FIOCRUZ-IOC, BR)

Dra Elizabeth P. Sampaio (FIOCRUZ-IOC, BR)

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