Curso: Desarrollo de fármacos de base metálica: Técnicas biológicas de evaluación

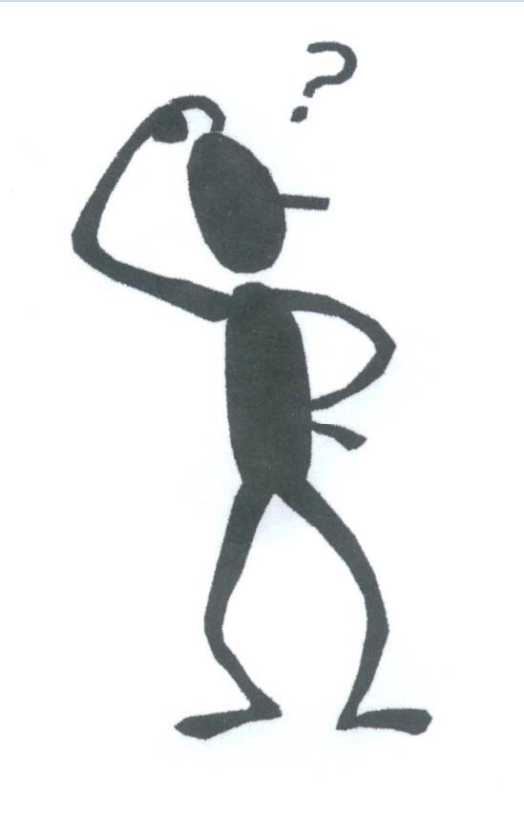
México DF 5-8 de setiembre de 2011

Módulo I. Pruebas de evaluación biológica

Ensaios de actividad biologica na pesquisa de novas drogas contra a tuberculose

Clarice Queico Fujimura Leite/Fernando Rogerio Pavan

What is tuberculosis ?



- Tuberculosies (TB) is a succesfull air borne, preventable and curable infectious disease.
- The main ethiological agent is *Mycobacterium tuberculosis* (MTB)

Current Global Status

Infected: 1.86 billion (32%)
New cases/yr: 8.7 million
1/3 of world population: infected by latent MTB

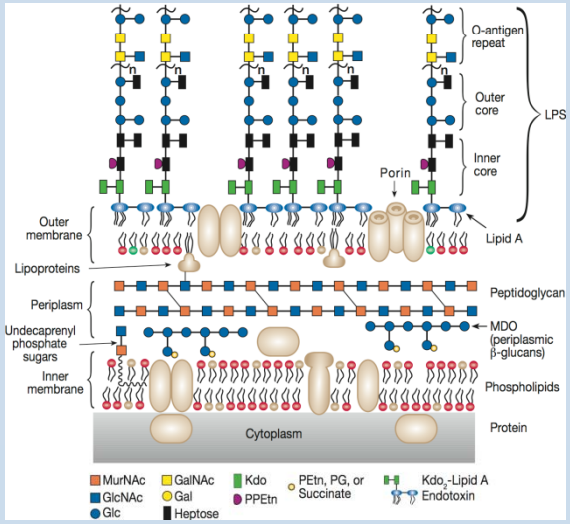
Deaths/yr: 1.7 million (5,000/day)
26% of avoidable deaths in developing world
456.000 people deatths due due MTB + HIV co-infetion

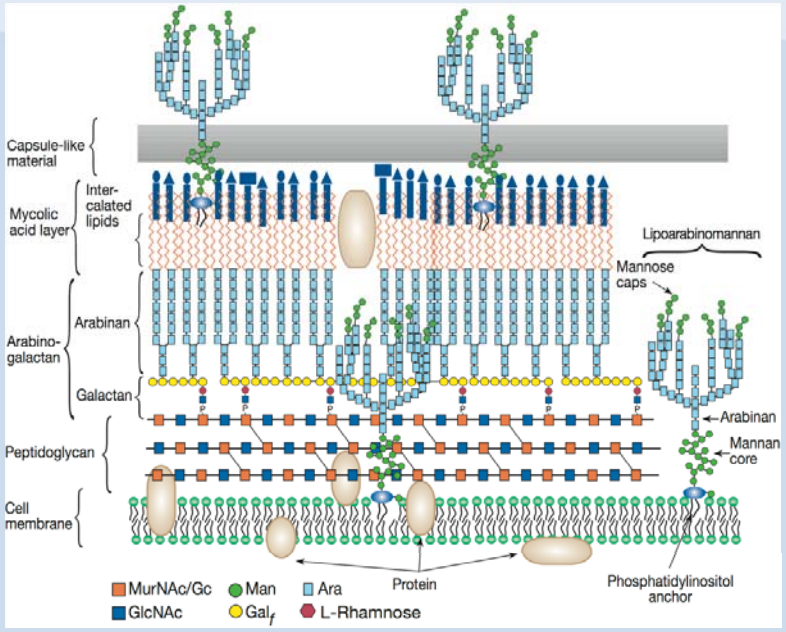
Drug resistance: primary: 10.4% MDR, acquired: 36%
MDR-TB, XDR-TB and TB/HIV: impossible the TB control

"No new drugs excepting rifabutin and rifapentine after rifampicin"

Bacterial Cell wall

Gram-negative

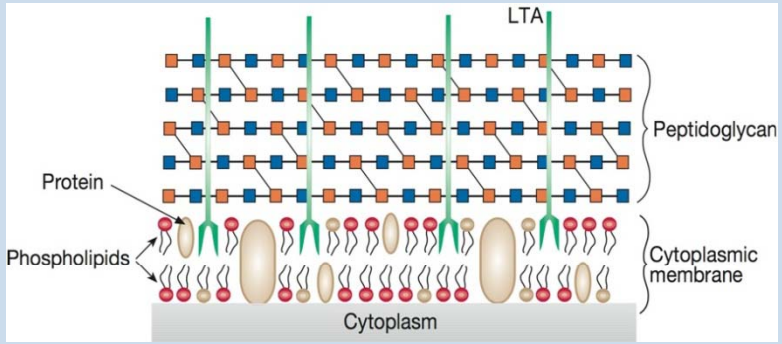




Mycobacterium

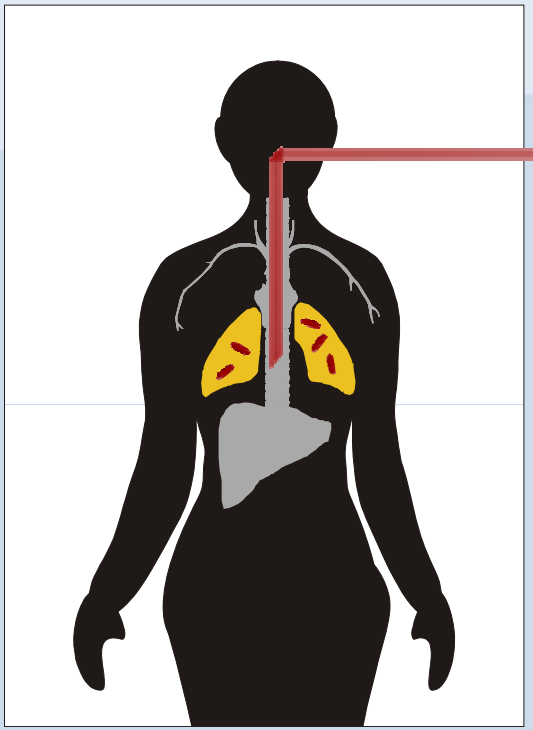
- **High resistance**
- **Slow growing**
- **Macrophage survive capacity**
- **Latence**

Gram-Positive

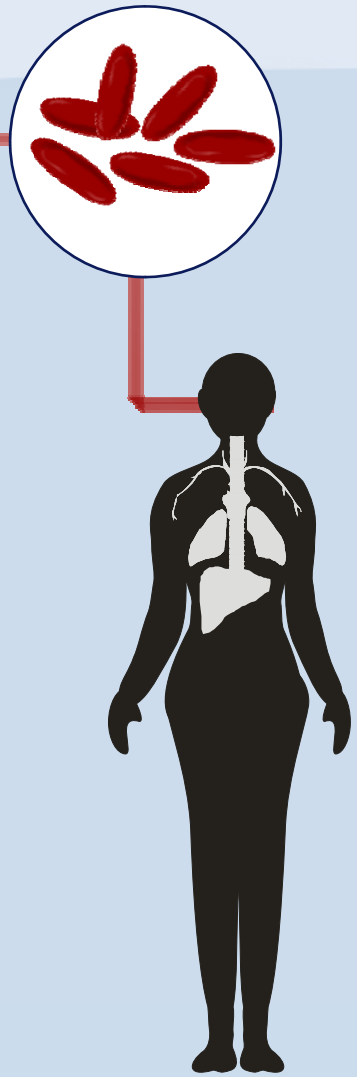


Essentials of Glycobiology
Varki A; Cummings RD; Esko JD; et al..
Capítulo 20, 2009.

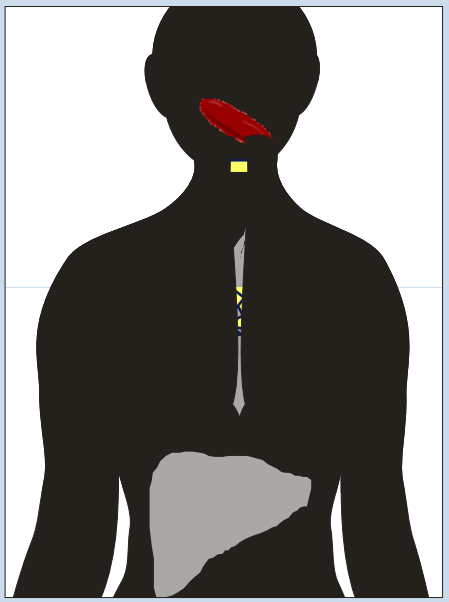
Transmition



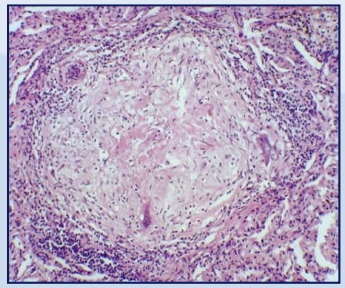
1.Indivíduo doente

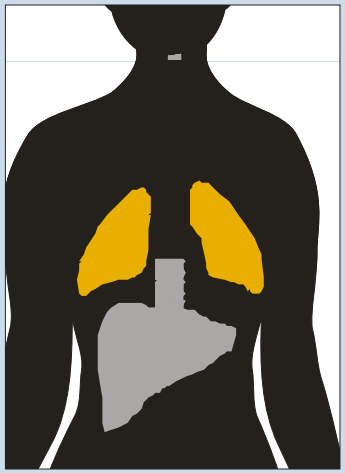


2.Inalação do bacilo pelo Hospedeiro



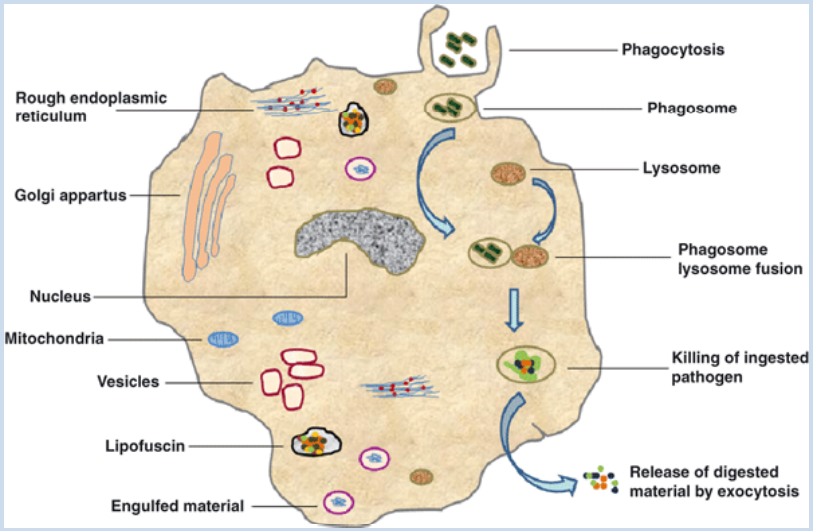
3.Migração dos bacilos para os pulmões

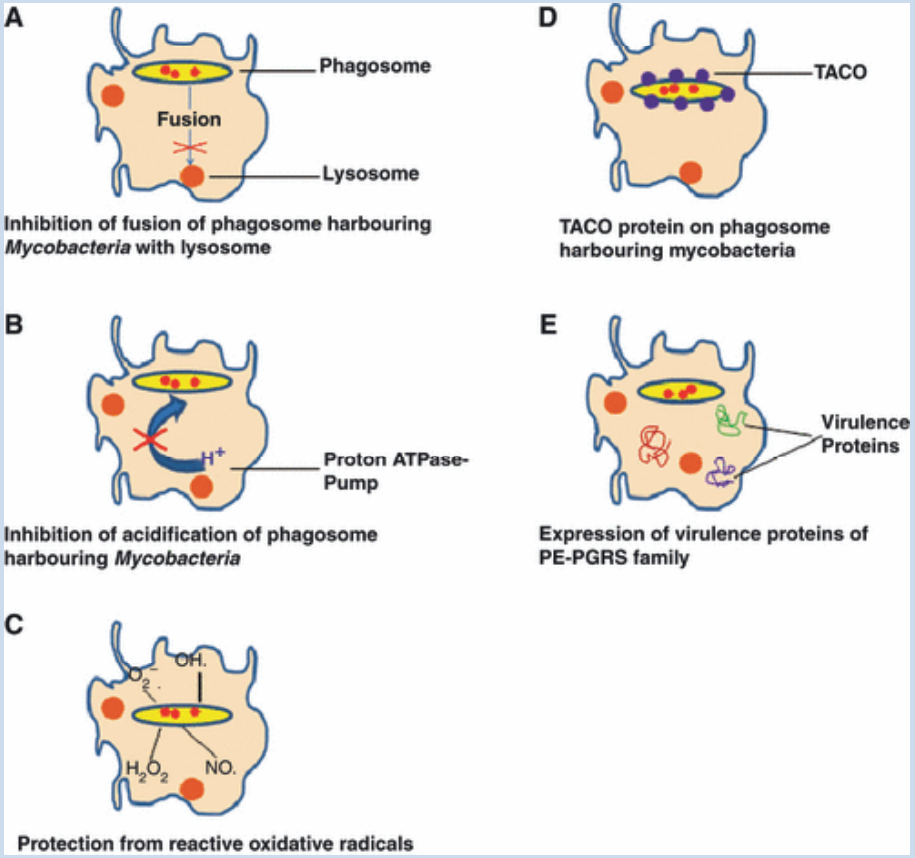




4. Bacilo nos pulmões
Formação do granuloma

M. tuberculosis Survive Mechanism – Intracellular Phatogen

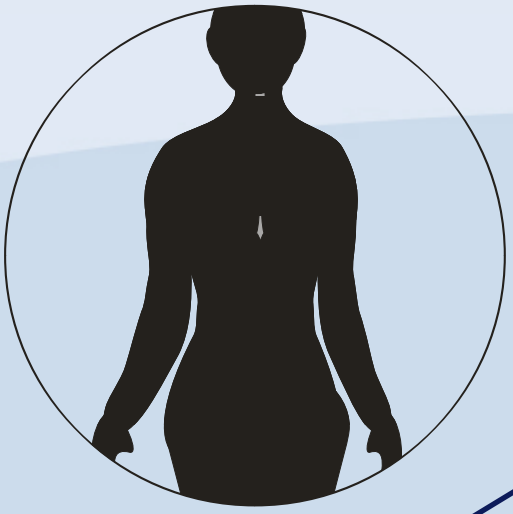


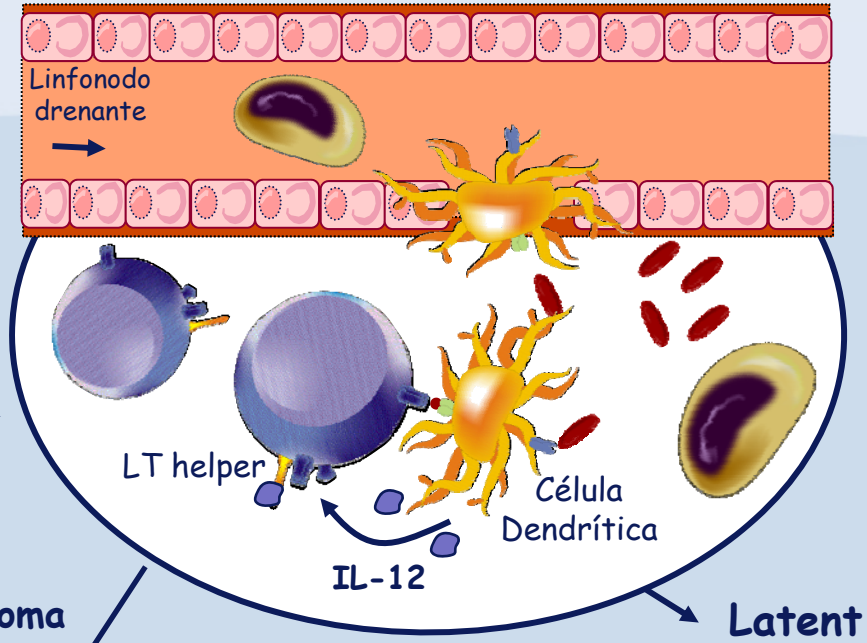


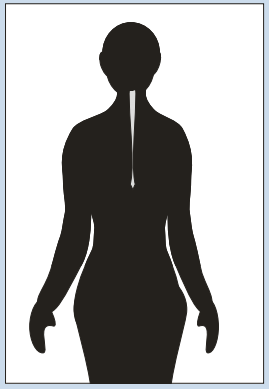
FEBS Journal

MEENA, L. S.; RAJNI. Survival Mechanisms of Pathogenic *Mycobacterium tuberculosis* $H_{37}Rv$. v. 227, n. 11, p. 2416-2427, 2010.

Infection, disease and mechanism of imunology

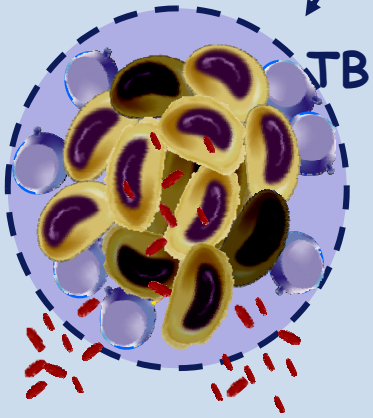






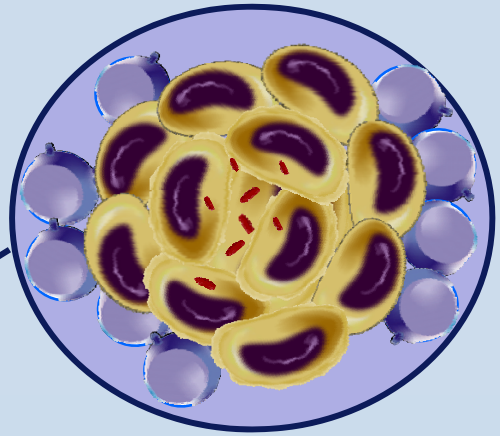
Cura espontânea ?

Granuloma



TB diseasea

Latent Infection



Reativação

Disseminação e
Transmissão

TB pós-primária

Efetive Tuberculose Control ?

Current treatment for TB

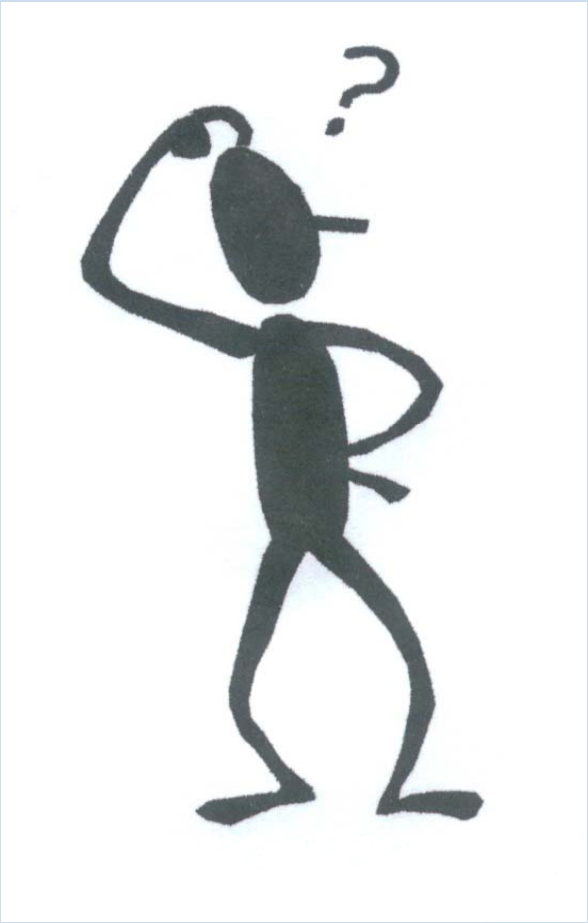
American Thoracic Society, CDC, WHO

- **2 months, daily (intensive phase)**
  - **Isoniazid (INH), 5 mg/kg po (300 mg)**
  - **Rifampin, 10 mg/kg po (600 mg)**
  - **Pyrazinamide, 15-30 mg/kg po (1-2 g)**

    and
  - **Ethambutol, 15-25 mg/kg po (2-5g)**

- **4 months daily (continuation phase)**
  - **Isoniazid (INH), 5 mg/kg po (300 mg)**
  - **Rifampin, 10 mg/kg po (600 mg)**

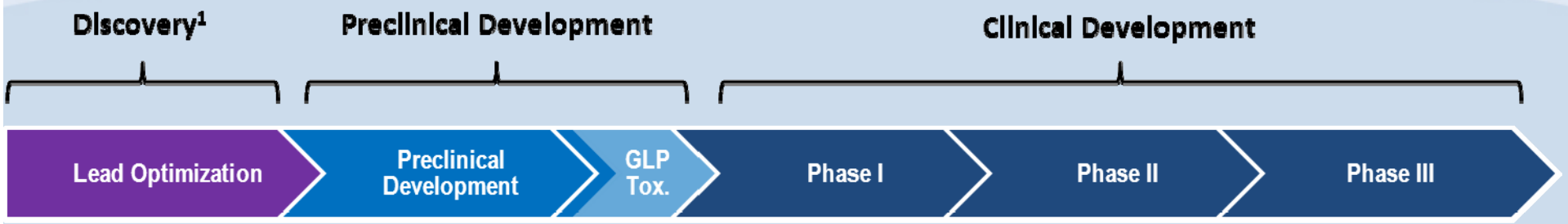
Why new drugs against MTB?
What approche use?



Impact of New Chemotherapy

1. **Reducing treatment duration**
   - **Improved compliance**
2. **Successful treatment of MDR/XDR-TB**
   - **Reduce transmission of MDR-TB**
3. **Cure latent TB infection**
   - **Reduce/eliminate disease reservoir**
4. **No Drug-Drug Interaction**
   - **Anti-Retrovirus treatment**
   - **Diabets**

Global TB Drug Pipeline



Lead Optimization	Preclinical Development	GLP Tox.	Phase I	Phase II	Phase III
Nitroimidazoles	CPZEN-45	BTZ043	AZD5847	Bedaquiline (TMC-207)	Gatifloxacin
Mycobacterial Gyrase Inhibitors	SQ641		PNU-100480	Delamanid (OPC67683)	Moxifloxacin
Riminophenazines	SQ609			PA-824	Rifapentine
Diarylquinoline	DC-159a			Linezolid	
Translocase-1 Inhibitor	Q201			SQ-109	
MGyrX1 Inhibitor				Rifapentine	
InhA Inhibitor				Novel Regimens[2]	
GyrB Inhibitor					
LeuRS Inhibitor					
Pyrazinamide Analogs					

[1] Ongoing projects without a lead compound series can be viewed at http://www.newtbdrugs.org/pipeline-discovery.php.

[2] Drug combination regimens: first clinical trial (NC001) of a novel TB drug regimen testing the three drug combination of PA-824, moxifloxacin, and pyrazinamide was initiated November 2010.

WORKING GROUP ON NEW TB DRUGS

www.newtbdrugs.org

Updated: July 18, 2011

Approaches to New TB Drugs

- **Drug-based** whole cell screening
  - optimize TB drugs
  - optimize non-TB antimicrobial classes
  - **novel synthetic**
  - **novel natural products**
    - Ethnomedical
- **Target-based** discovery
  - Target identification
  - **Screening (in silico, NMR, functional)**

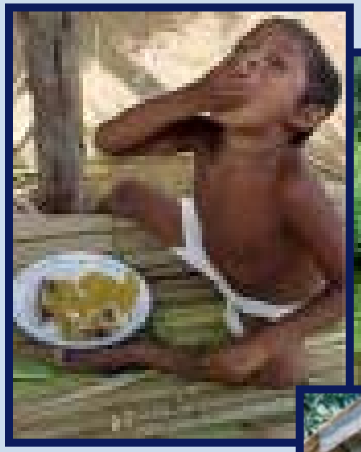
Why new compounds from Plants?

- Plants have provided many drugs in the past, and they remain a rich source of novel compounds

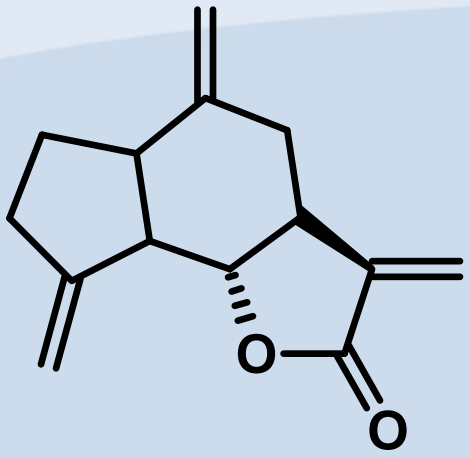
- The natural products have received considerable attention as potential anti-TB agents (Cantrell et al, 2001, Okunade et al, 2004, Coop & Pearce, 2007, Higuchi *et al*, 2008, Leite *et al*, 2008, Pavan *et al*, 2009)

There is a traditional knowledge in the world of how to use native plants to treat several diseases.

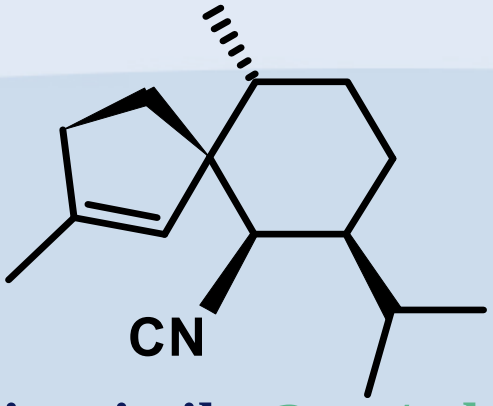
Many communities don't have access to synthetic medicines



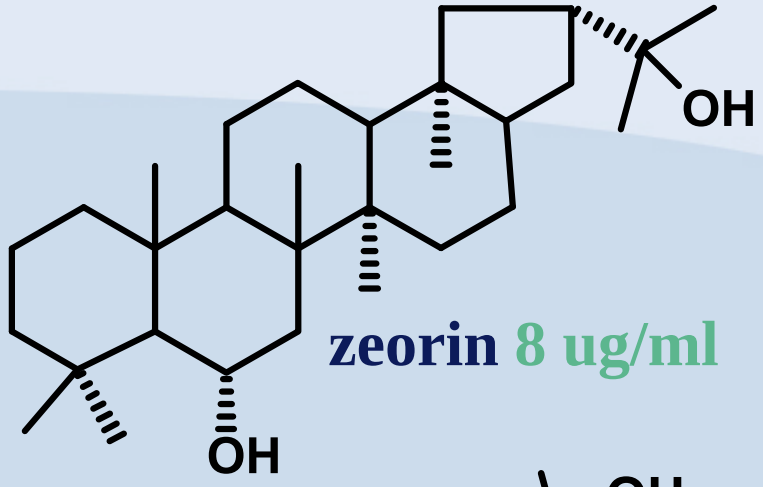
Natural Products with Anti-TB Activity



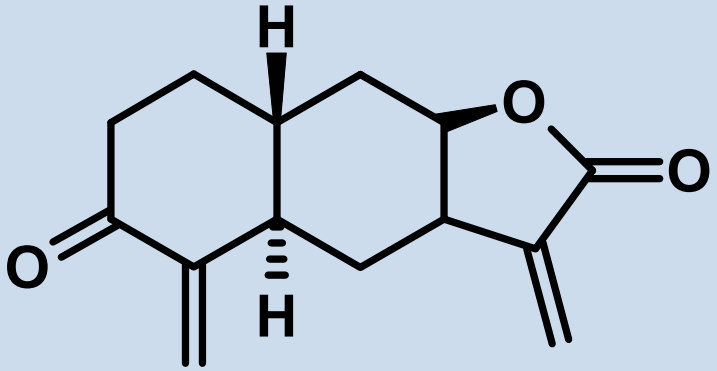
dehydrocostus lactone 2 ug/ml



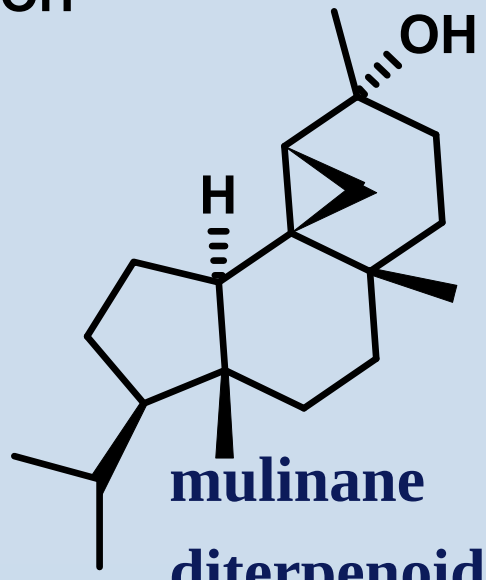
axisonitrile 2 ug/ml



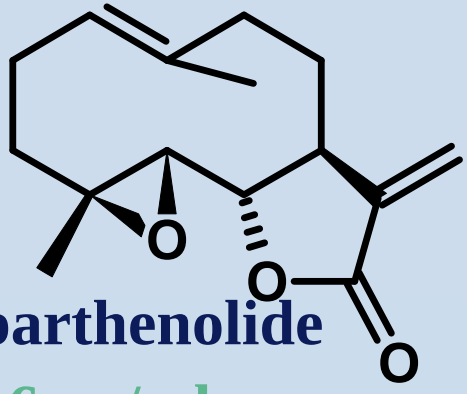
zeorin 8 ug/ml



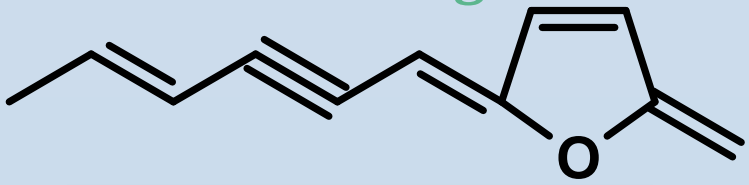
encelin 16 ug/ml



mulinane diterpenoid 20 ug/ml



parthenolide 16 ug/ml



matricaria ester 12.5 ug/ml

In vitro anti-Mycobacterium tuberculosis activity of some Brazilian "Cerrado" plants

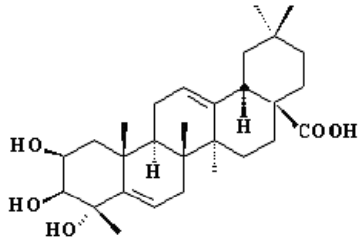
Fernando R. Pavan, Daisy N. Sato, Célio T. Higuchi, Adolfo C. B. Santos,Wagner Vilegas, Clarice Q. F. Leite. v. 19 (1B) 204-206, 2009.

Plants	Plant Part	$CHCL_3$ MIC (µg/mL)	MeOH MIC (µg/mL)
Leguminosae			
Indigofera suffruticosa	Leaf	1000	125
Indigofera truxilensis	Leaf	NR	500
Loganiaceae			
Strychnos pseudoquina	Leaf	125	4000
Malpighiaceae			
Byrsonima basiloba	Leaf	125	250
Byrsonima coccolobifolia	Leaf	NR	1000
Byrsonima crassa	Leaf	125	1000
Byrsonima crassa	Bark	2000	1000
Byrsonima fagifolia	Leaf	62.5	500
Melastomataceae			
Miconia cabuku	Leaf	250	31.2
Miconia rubiginosa	Leaf	250	31.2
Guapira noxia	Leaf	> 250	31.2
Neea theifera	Leaf	62.5	250
Vitaceae			
Cissus suscicaulis	Leaf	62.5	NR
Vochysiaceae			
Qualea grandiflora	Bark	62.5	1000
Qualea multiflora	Bark	125	500

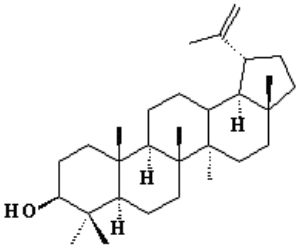
Byrsonima fagifolia Niedenzu Nonpolar Compounds with Antitubercular Activity

C. T. Higuchi, M. Sannomiya, F. R. Pavan, S. R. A. Leite, D. N. Sato, S. G. Franzblau, L. V. S. Sacramento, W. Vilegas and C. Q. F. Leite. doi:10.1093/ecam/nen077, 2008

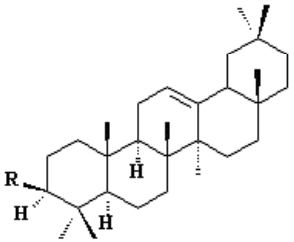
Samples	MIC (μg/mL)
Extracts	
80% MeOH (1)	500
MeOH (1)	250
$CHCl_3$ (2)	62,5
Enriched Fraction/compounds	
mixture of lupeol, α-and β-amyrin	31.25
mixture of lupeol, acetates of α- and β-amyrin	31.25
α-amyrin acetate	62.5
dotriacontane	62.5
bassic acid	2.5
isoniazid	0.03



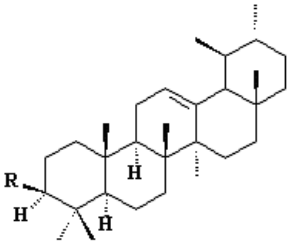
Bassic acid



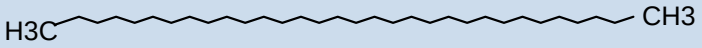
Lupeol



R=OH, β-amyrin
R=AcO, β-amyrin acetate



R=OH, α-amyrin
R=AcO, α-amyrin acetate



dotriacontane

Quim. Nova 1978, vol.1, no.1

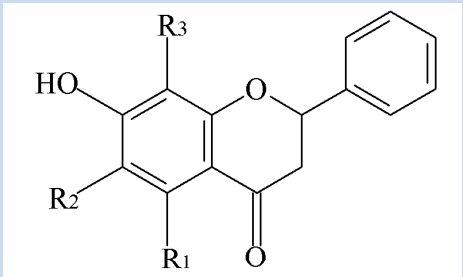
Evaluation of anti-*Mycobacterium tuberculosis activity of Campomanesia adamantium*

Fernando Rogério Pavan , Roberta Gomes Coelho, Isabel Duarte Coutinho, Neli Kika Honda, Claudia Andréa Lima Cardoso, *Wagner Vilegas, Sergio Roberto de Andrade Leite, Daisy Nakamura Sato, Clarice Queico Fujimura Leite.* *"in press", 2009.*

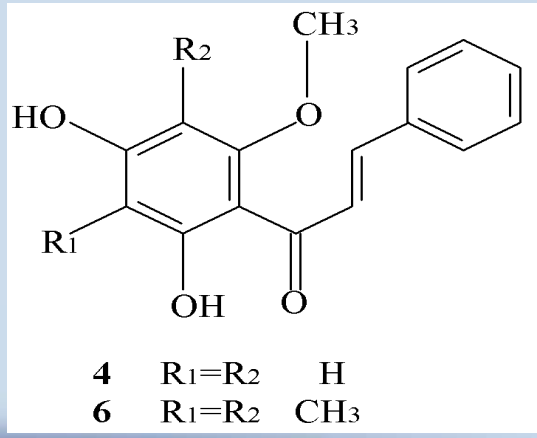
Samples	MICs (μg/mL)	Compounds (mg/g of fraction)
methanol extract	1000	
ethyl acetate extract	62.5	
F1	> 250	ND
F2	125	ND
F3	125	ND
F4	125	6 (34.7)
F5	62.5	5 and 6 (43.7 and 247.3)
F6	39.1	5 and 6 (123.7 and 330.0)
F7	39.1	5 and 6 (147.9 and 290.3)
F8	125	5 and 6 (349.0 and 147.6)
F9	62.5	1,2,3 and 4 (53.7, 175.0, 60.4 and 12.3)

Samples	MICs (μg/mL)
Compound 5	>250
Compound 6	62.5
Mixture 5 + 6 (2:8)	7.8
Mixture 5 + 6 (3:7)	15.6
Mixture 5 + 6 (1:1)	15.6
Mixture 5 + 6 (7:3)	31.2
Mixture 5 + 6 (8:2)	62.5

Compounds: 7-hydroxy-5-methoxy-6-C-methylflavanone **(1)**, 5,7-dihydroxy-6-C-methylflavanone **(2)**, 5,7-dihydroxy-8-C-methylflavanone **(3)**, 2´, 4´-dihydroxy-6´-methoxychalcone **(4)**, 5,7-dihydroxy-6, 8-di-C-methylflavanone **(5)**, 2',4'-dihydroxy-3',5'-dimethyl-6'-methoxychalcone **(6).**



1 R_1= OCH_3 R_2= CH_3 R_3= H
2 R_1= OH R_2= CH_3 R_3= H
3 R_1= OH R_2= H R_3= CH_3
5 R_1= OH R_2= CH_3 R_3= CH_3



4 R_1=R_2 H
6 R_1=R_2 CH_3

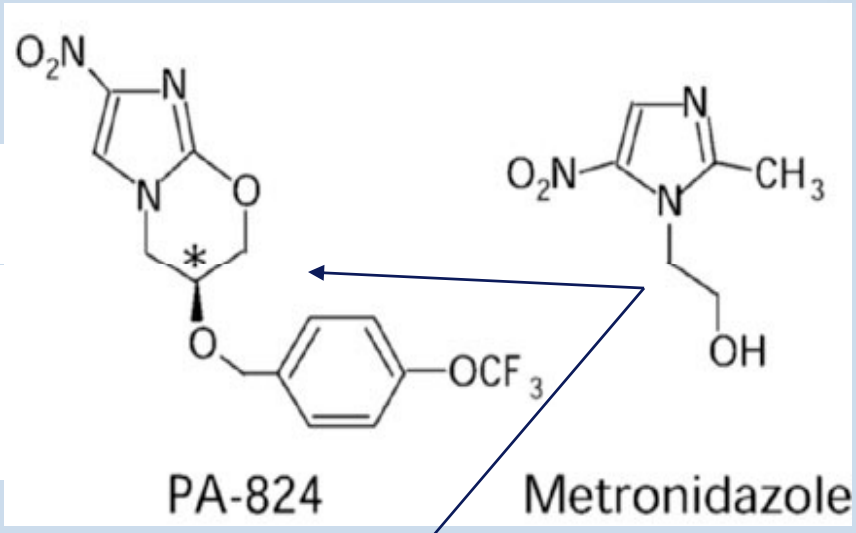
Approach: Utilize existing non-TB Drugs

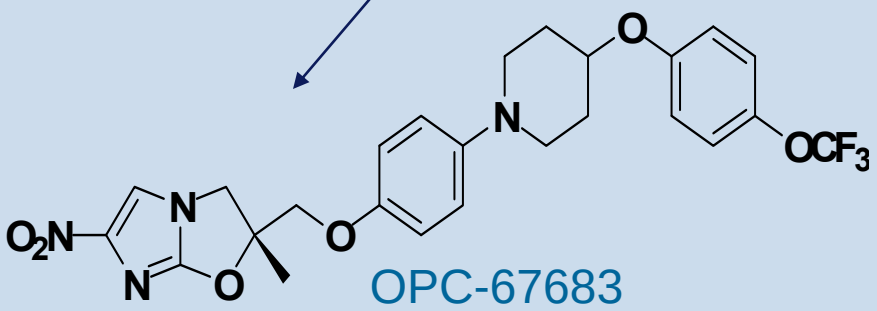
- Moxifloxacin
  - Goal: shorten duration of Tx
  - Current trial: multi-center phase III
- Linezolid
  - Goal: use for MDR-TB
  - Current trial: early bactericidal activity (EBA)
- Metronidazol
  - Goal: Shorten duration of Tx
  - Current trial: planned use in XDR

Approach: Optimize *non*-TB Drugs

Stage:Clinical

- Nitroaromatics
  - **PA-824**
    - In phase IIa
      - (EBA)
  - **OPC 67683**
    - In phase II trial



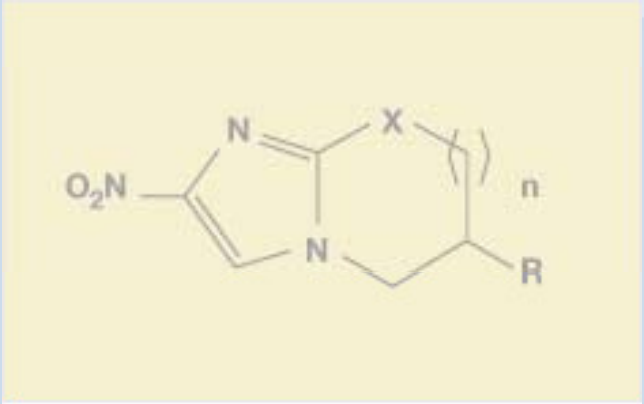


Approach: Optimize non-TB drugs

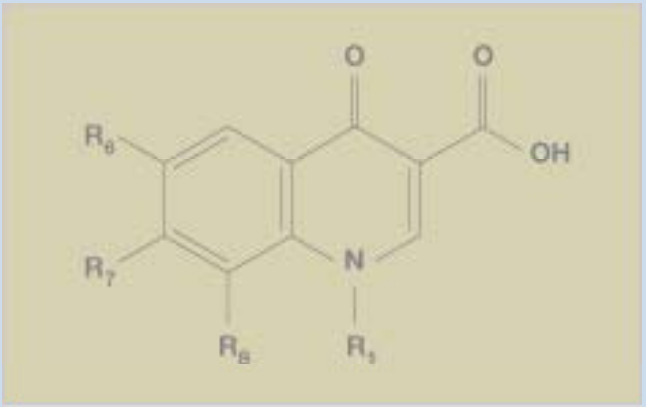
Stage:Discovery

Goal: improve therapeutic index

- Nitroaromatics *(Auckland/UIC, USP, U. Tenn)*
- Fluoroquinolones *(KRICT, Yonsei U.)*
- Oxazolidinones *(Pfizer/UIC)*
- Phenothiazines *(Penn/UIC, Salisbury, ITMH)*
- Beta-lactams
- Mefloquine *(UIC, CSU)*
- Pentamidine *(UNC/UIC/Immtech)*
- Macrolides *(UIC, terminated)*



Nitroimidazole Analogs
(University of Auckland, New Zealand)



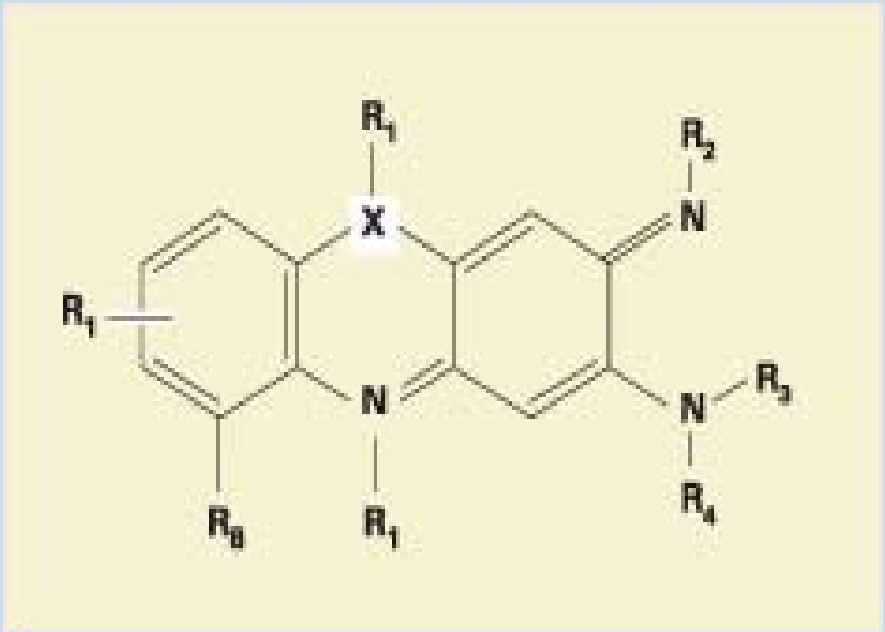
Quinolones
(KRICT / Yonsei University)

Approach: Optimize TB Drugs

Stage:Discovery

Goals:

- *Non-prodrug*
  - **Isoniazid (target-based)**
  - Pyrazinamide
- *Reduce toxicity/DDI*
  - **Riminophenazines**
    - **clofazimine**
  - Rifamycins
  - Ethionamide
- *Oral/pulmonary delivery*
  - **Capreomycin**
  - Aminoglycosides

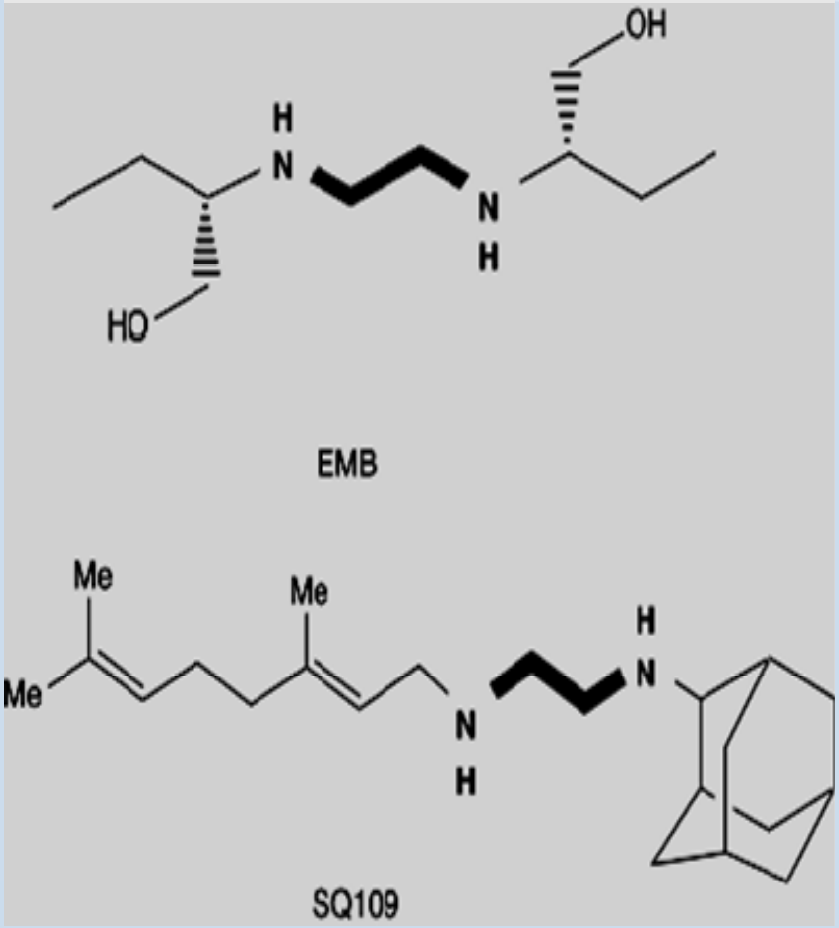


Riminophenazines
(Institute of Materia Medica)

Approach: Optimize TB Drugs

Stage: Clinical

- Optimize ethambutol
  - potency
  - bactericidal activity
- **SQ-109 (Sequella)**
  - *Approach*:
    - extensive analoging via combichem
  - *Status*:
    - Completed phase I trial
    - FDA fast track approval
  - *Other indications*:
    - anti-fungal?

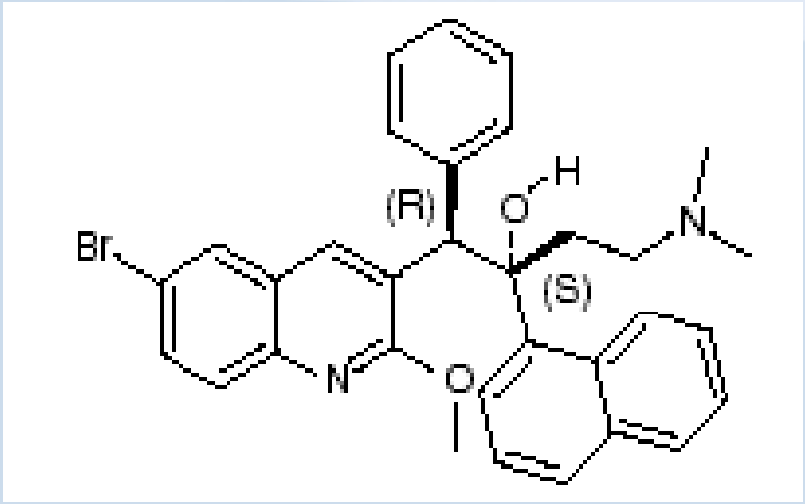


Approach: Find Novel Drugs

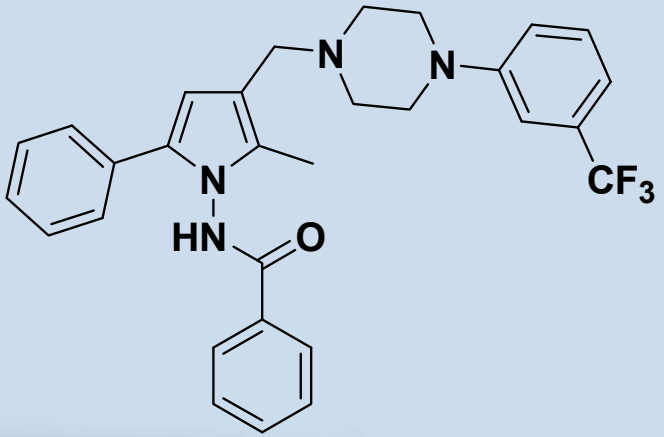
Stage:Clinical

TMC 207 (Tibotec)

- From HTS of compound library vs. *M. smegmatis*
- Novel target: ATP synthase
- Spectrum of activity: mycobacterial only
- Synergistic with PZA in mice – shortens Tx
- Status: Phase II clinical trial



LL 3858 (Lupin)



Why synthetic metallo-organic complexes?

Why synthetic metallo-organic complexes?

- For the first time in decades, there is now a pipeline of new synthetic compounds that are being tested on TB
- Within this growing pipeline of potential new TB drugs, seven are at various stages of clinical development

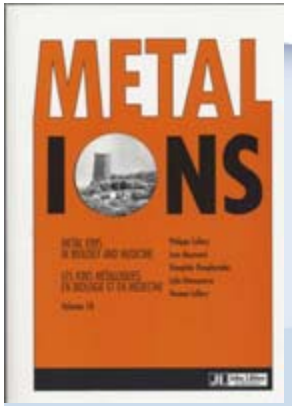
Tuberculosis (TB) clinical drug development programs

Compound	Development Stage	Sponsor / Coordinator
Gatifloxacin	Phase 3	European Commission; IRD; WHO/TDR; Lupin
Moxifloxacin	Phase 2/3	Bayer; TB Alliance; CDC; University College London; Johns Hopkins University
TMC 207 (Diarylquinoline)	Phase 2	Johnson & Johnson (Tibotec)
OPC 67683 (Nitroimidazole)	Phase 1 EBA	Otsuka Pharmaceutical
PA 824 (Nitroimidazole)	Phase 1	TB Alliance
LL 3858 (Pyrrole)	Phase 1	Lupin
SQ 109 (Diamine)	Phase 1	Sequella

(Melvin K. Spigelman JID, 2007)

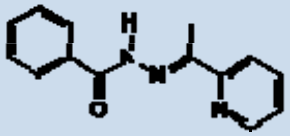
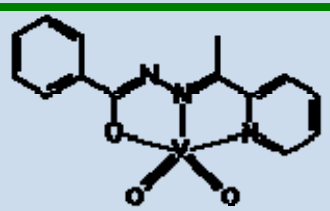
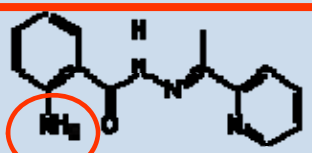
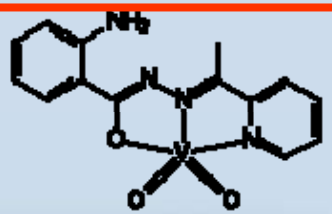
Why synthetic metallo-organic complexes?

1. Within this group, there are no complexes between metals and organic compounds
2. Medicinal Inorganic Chemistry is an area in continuous expansion
3. Many studies have shown an increase in the pharmacological activity of pure organic compounds when complexed with metals
4. We decided to make complexes of vanadium with thiosemicarbazone, semicarbazone and hydrazone derivatives as ligands
5. For each compound made, we determined its anti-TB activity and cytotoxicity



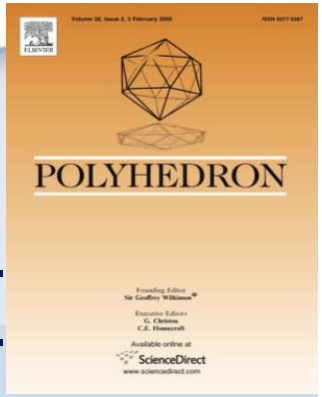
Synthesis and *anti-Mycobacterium tuberculosis* activity of Vanadium complexes with N,N,O-donor ligands

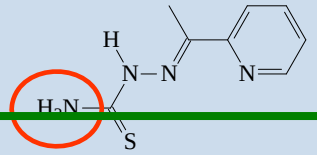
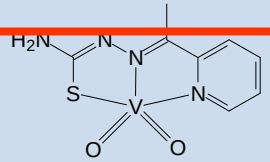
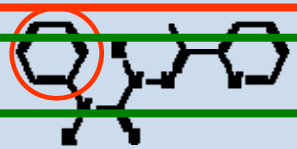
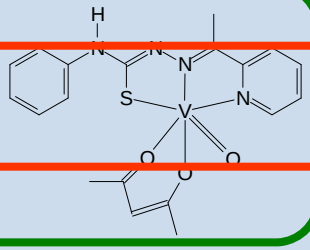
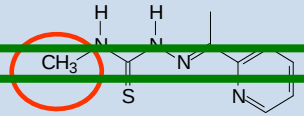
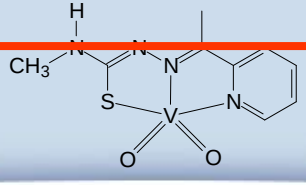
Pedro I. da S. Maia, Victor M. Deflon, Fernando R. Pavan, Clarice Q.F. Leite, Claudia C. Gatto, Sebastião S. Lemos, Alzir A. Batista. John Libbey Eurotext, Paris; v. 10, 197-203, 2008.

Compounds	Structures	REMA (MIC) (µg/mL)	IC_{50}
Hydrazones, Semicarbazones and Vanadium Complexes			
Hapbh		1.9	1.9
$[VO_2(apbh)]$		0.97	1.9
Hapah		15.6	1.9
$[VO_2(apah)]$		7.8	1.9

Vanadium complexes with thiosemicarbazones: Synthesis, characterization, crystal structures and *anti-Mycobacterium tuberculosis* activity

Pedro I. da S. Maia, Fernando R. Pavan, Clarice Q.F. Leite, Sebastião S. Lemos, Gerimário F. de Sousa, Alzir A. Batista, Otaciro R. Nascimento, Javier Ellena, Eduardo E. Castellano, Elke Niquet, Victor M. Deflon.v. 28, 398-406, 2009.



Compounds	Structures		REMA(MIC)	IC_{50}
	Ligands	Complexes	µg/mL	
Thiosemicarbazones and Vanadium Complexes				
Haptsc			31.3	156
$[VO_2(aptsc)]$			31.3	19.5
Happtsc			15.6	3,9
VO(acac)(apptsc)]			1.6	3.9
Hapmtsc			7.8	1.9
$[VO_2(apmtsc)]$			3.9	1.9

Target-based antibacterial drug discovery (vs phenotypic approach)

Pro

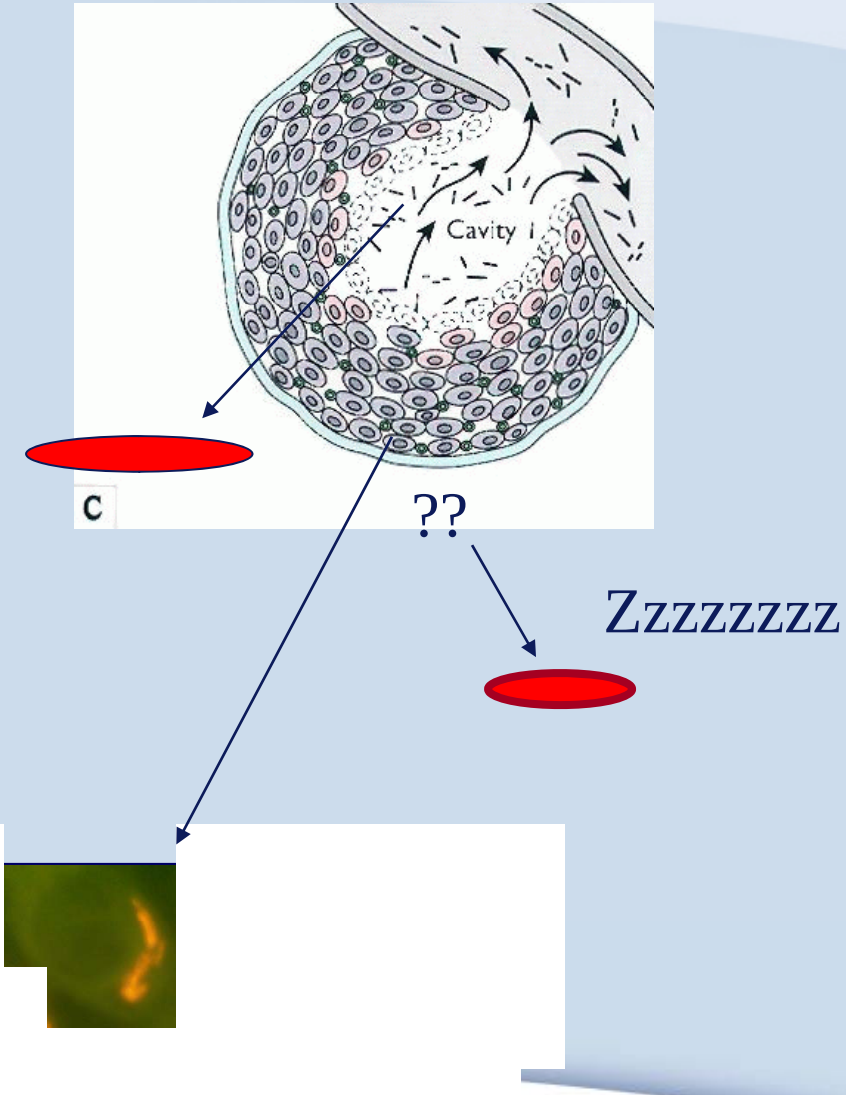
- Predict phenotype
- Selective
- Sensitivity
- **Rational approach to:**
  - **Improve potency**
  - Reduce toxicity?
  - Improve DMPK?

Con

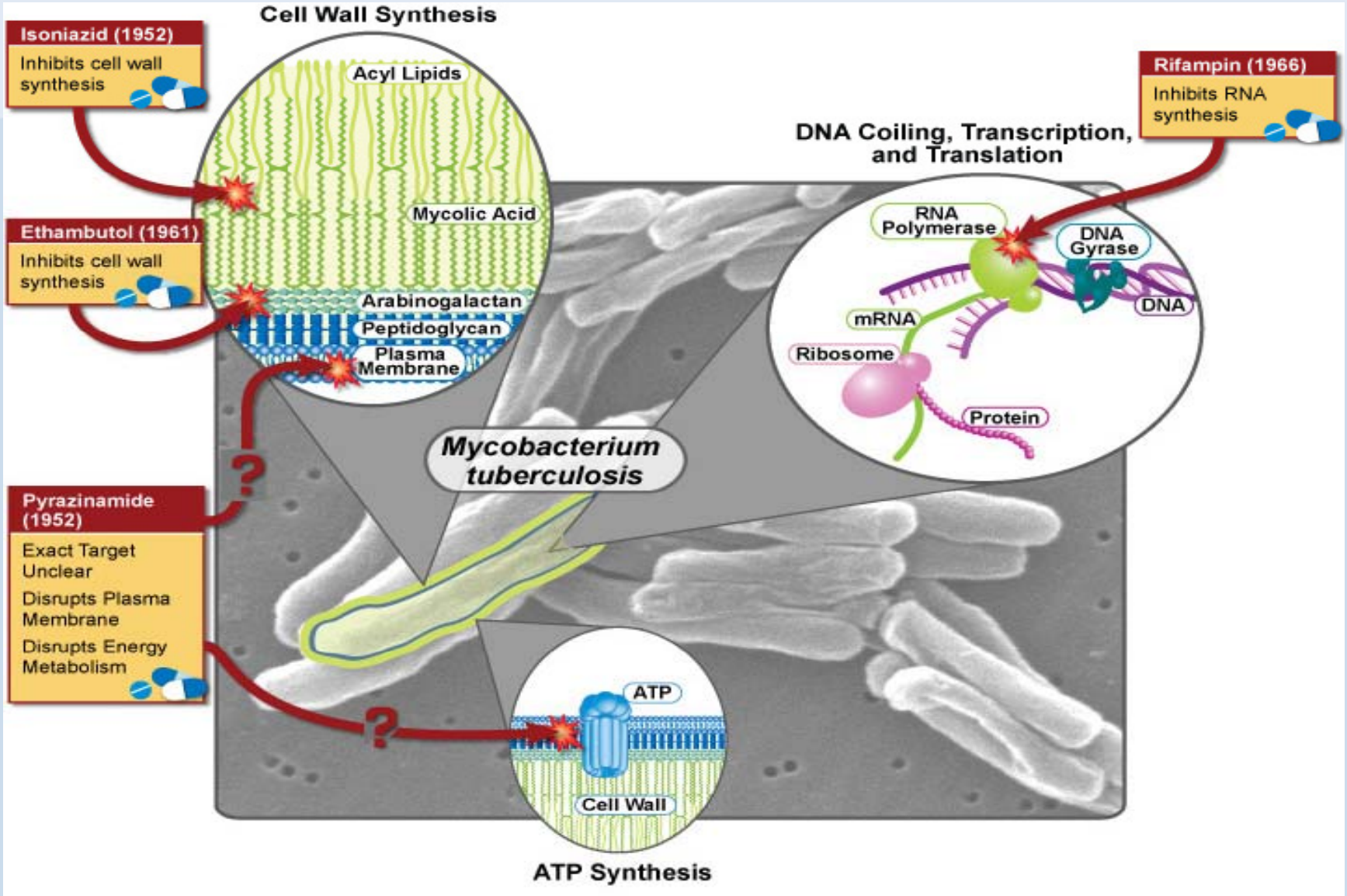
- No track record
- "Drugability" uncertain
- Single target may be undesirable – high rate of resistance?
- **Does not consider penetration into bacteria/efflux and/or metabolism**

Target validation

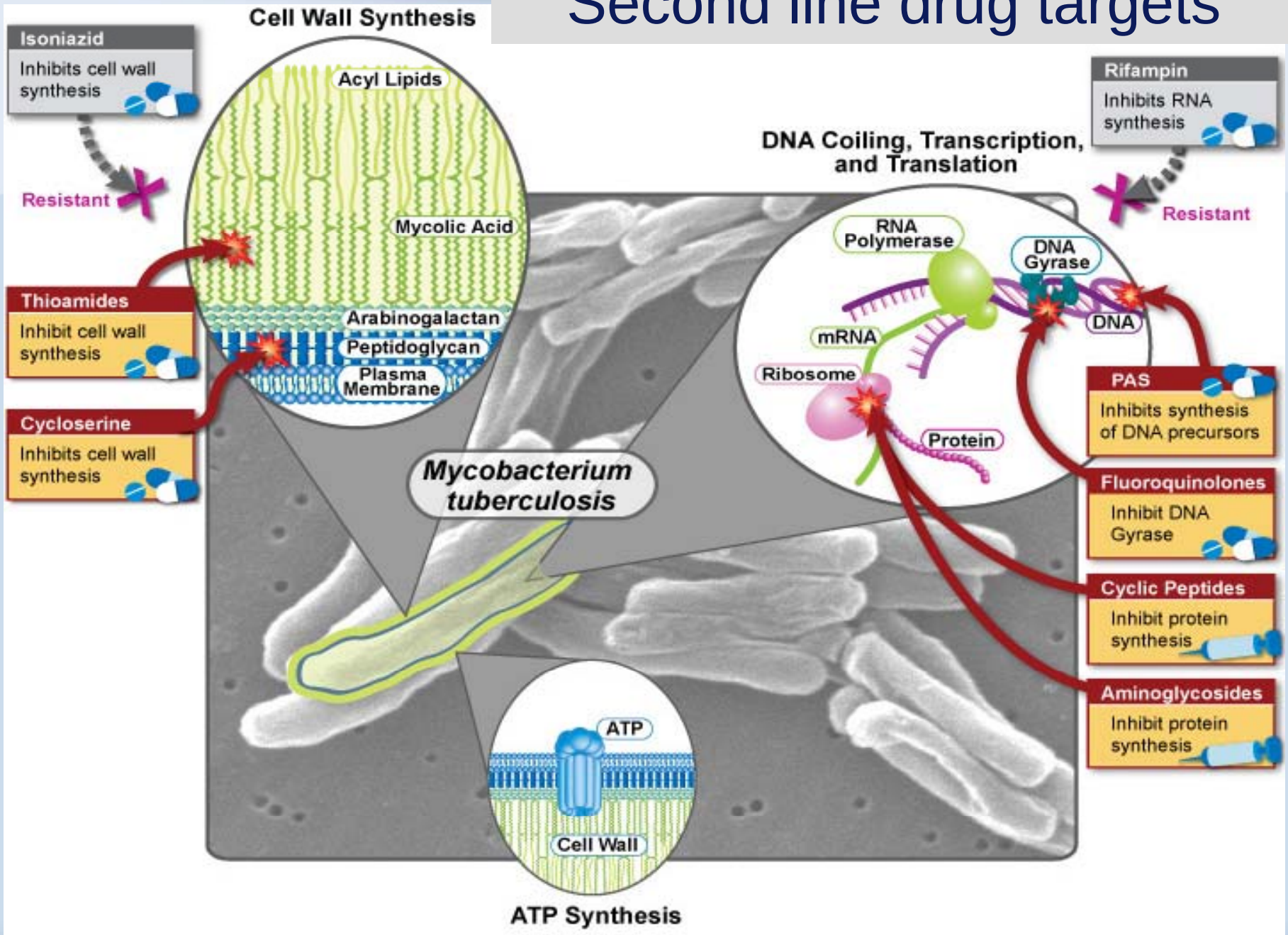
- **Essential for survival in host**
  - **(or virulence?)**
- Consequence of knock-down
  - (vs knock-out)
- Druggable in vivo



First line drug targets



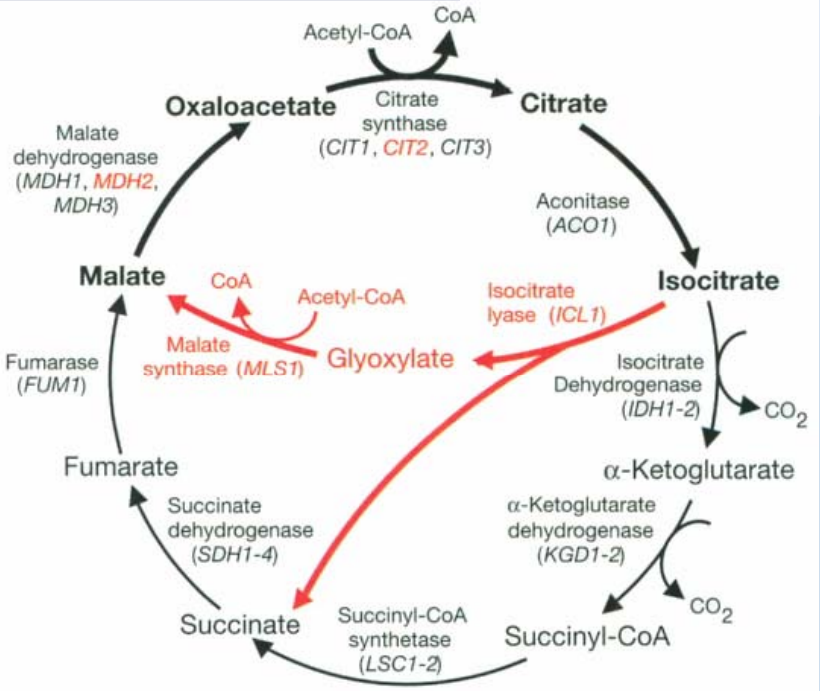
Second line drug targets

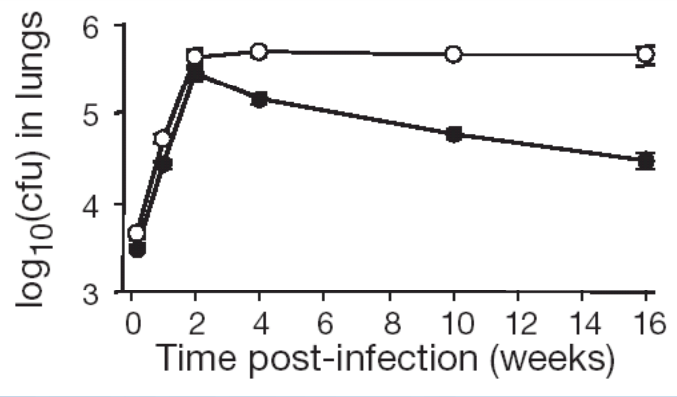


Target-based TB drug discovery

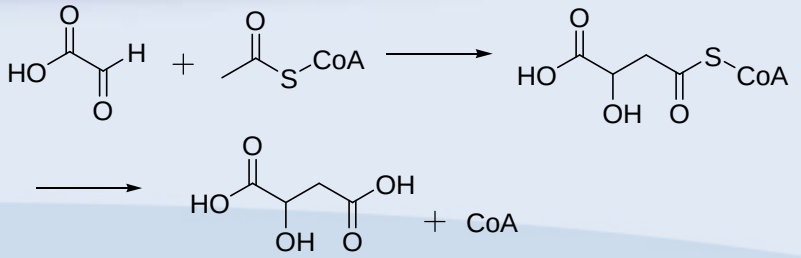
Glyoxylate shunt

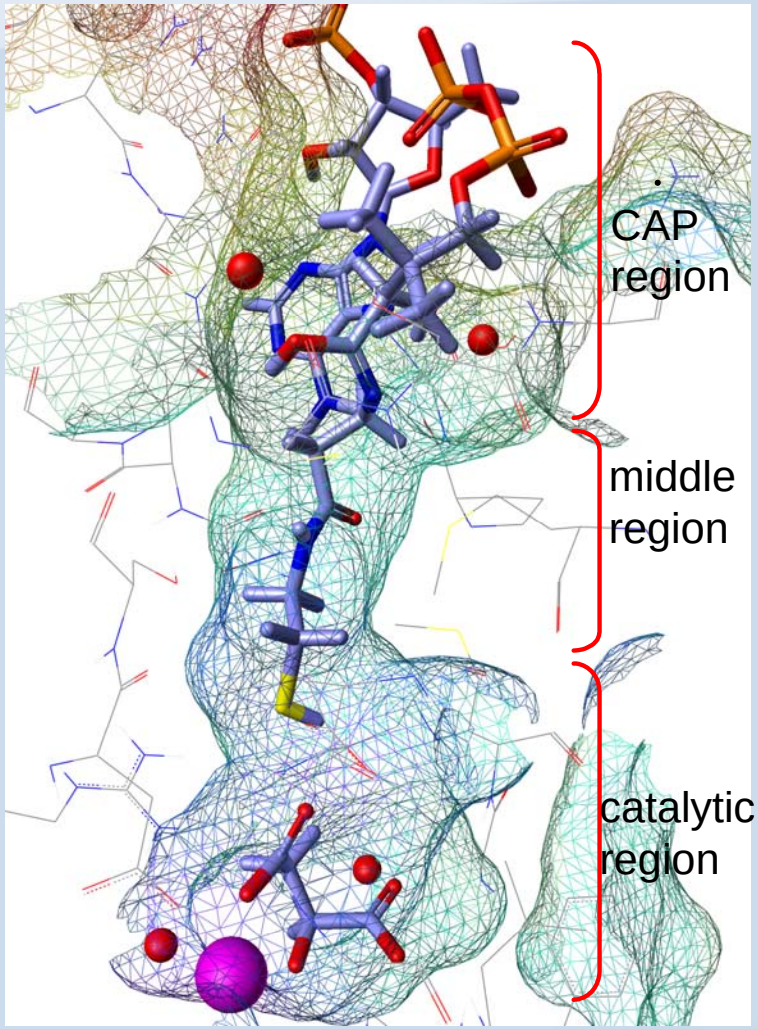
- Isocitrate lyase
- **Malate synthase**
  - No human counterpart
  - Essential for persistance in macrophages and mouse model
  - Crystal structures known
    - **In silico screening possible**





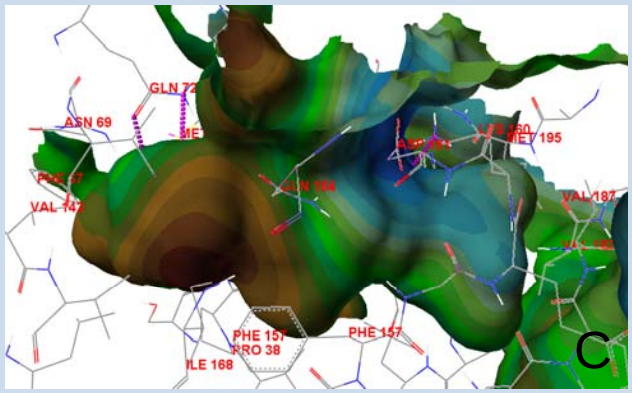
Malate synthase

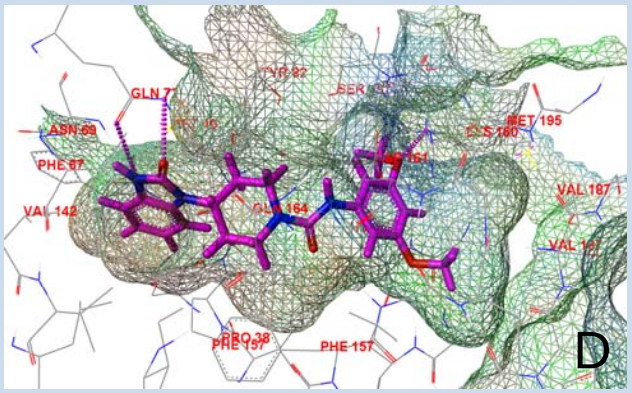


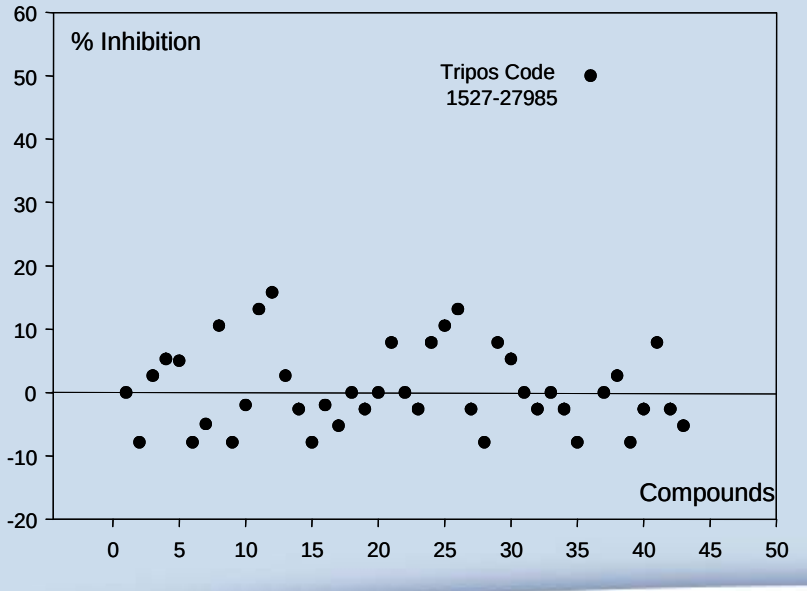


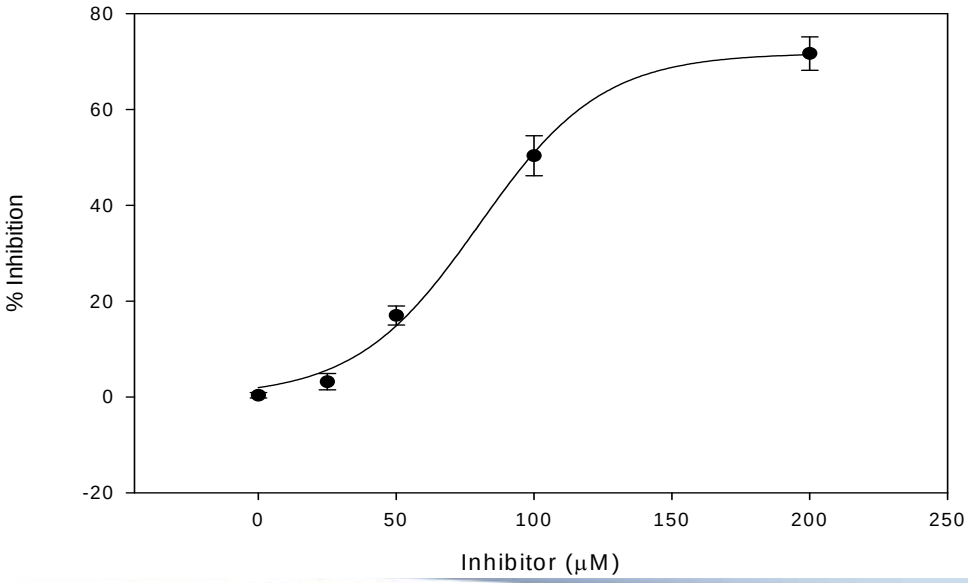
Compound	IC_{50} vs. MS	% inhibition at 500 uM vs. M. tb in	
	uM	NRP	Log
A	178	**91**	31
B	210	74	30

Pantothenate synthetase
Virtual screen to functional inhibition



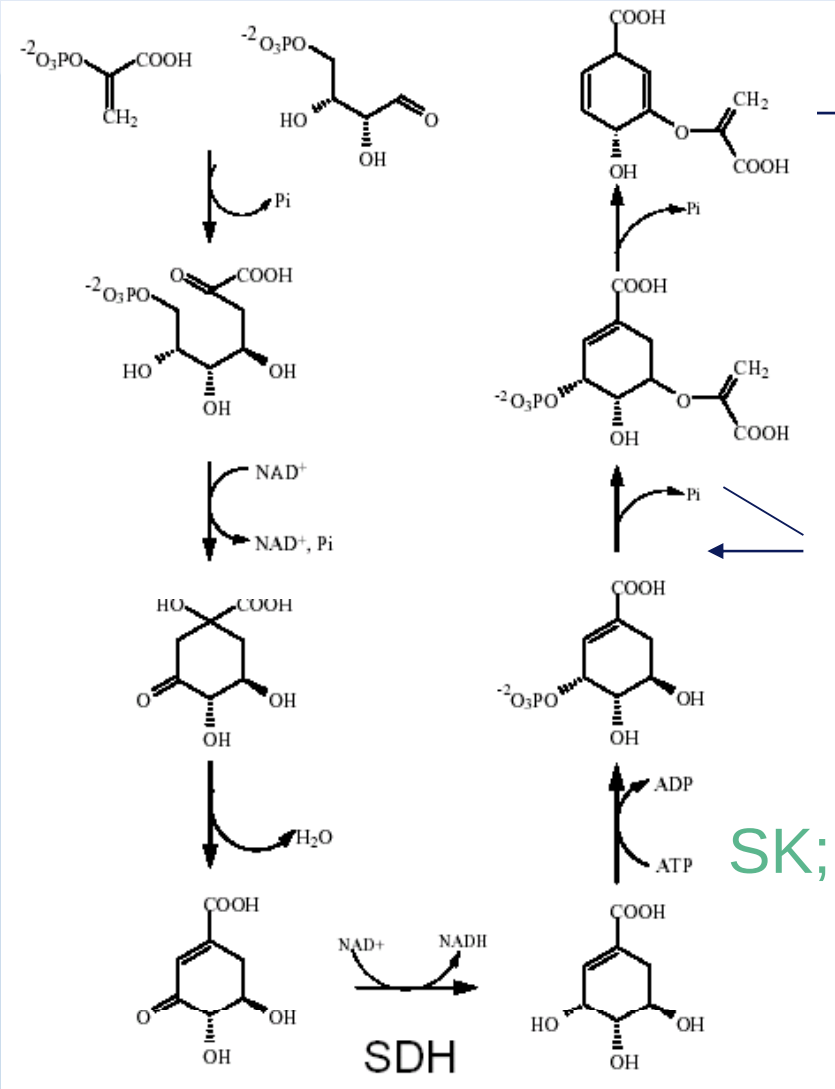






Shikimate Pathway – Shikimate kinase

- Synthesizes precursor to almost all aromatic compounds
- No human analogue.
- Glyphosate (Roundup ™) as a non-specific herbicide.
- Essential in M. tb
- Upregulated in non-replicating M. tb
- Crystal structure available



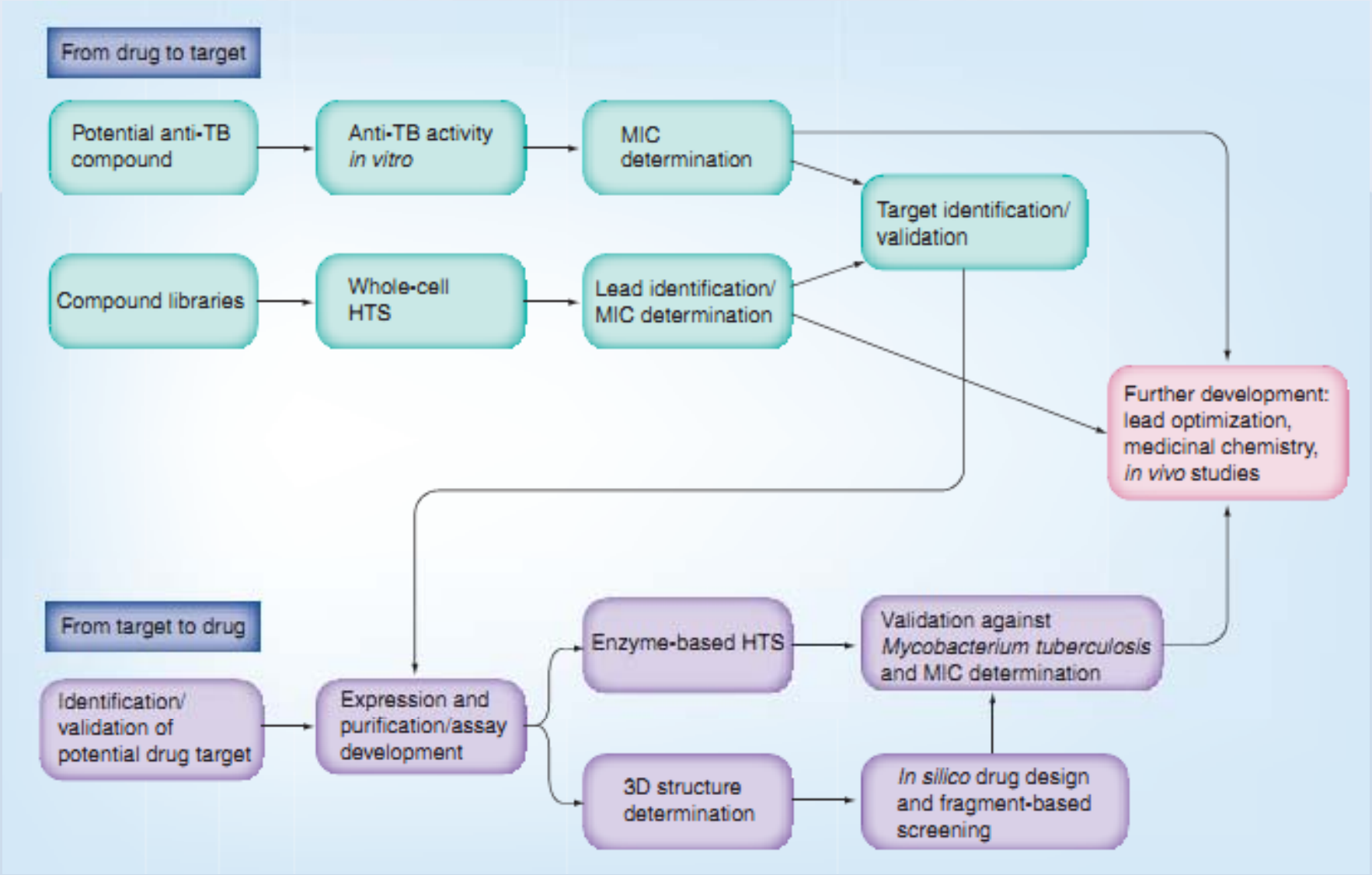
Phe

Trp

Tyr

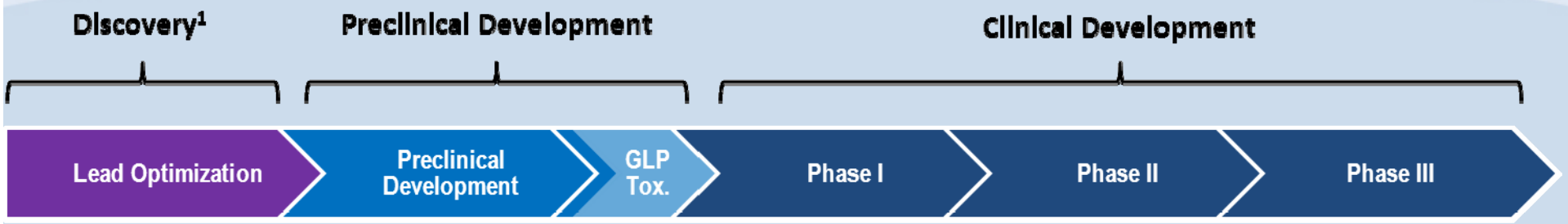
Glyphosate (PEP analog)

SK; Rv2539c



Sala and Hartkoom . Future Microbiol (2011) , 6: 617-633

Global TB Drug Pipeline



Lead Optimization	Preclinical Development	GLP Tox.	Phase I	Phase II	Phase III
Nitroimidazoles	CPZEN-45	BTZ043	AZD5847	Bedaquiline (TMC-207)	Gatifloxacin
Mycobacterial Gyrase Inhibitors	SQ641		PNU-100480	Delamanid (OPC67683)	Moxifloxacin
Riminophenazines	SQ609			PA-824	Rifapentine
Diarylquinoline	DC-159a			Linezolid	
Translocase-1 Inhibitor	Q201			SQ-109	
MGyrX1 Inhibitor				Rifapentine	
InhA Inhibitor				Novel Regimens[2]	
GyrB Inhibitor					
LeuRS Inhibitor					
Pyrazinamide Analogs					

[1] Ongoing projects without a lead compound series can be viewed at http://www.newtbdrugs.org/pipeline-discovery.php.

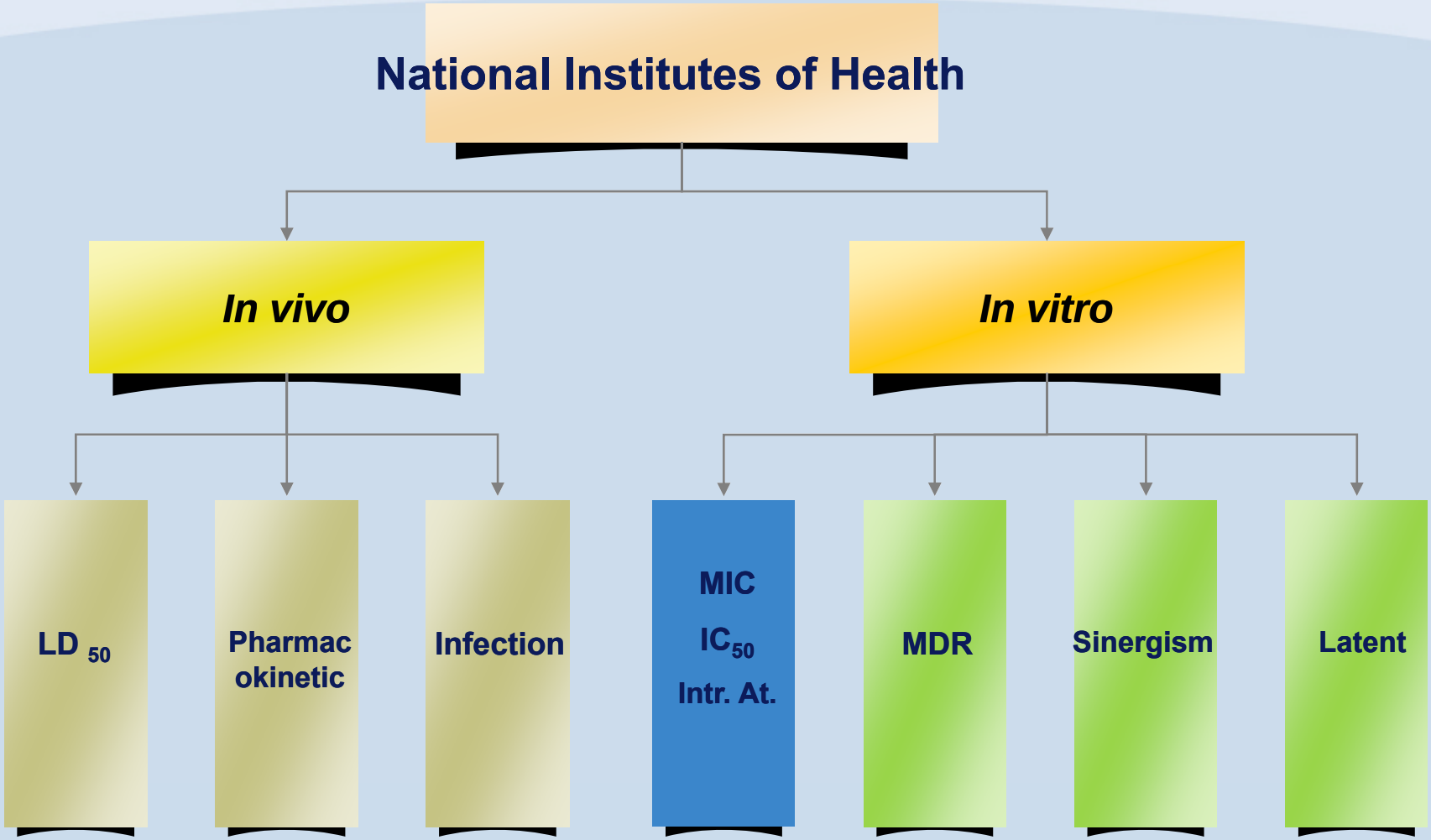
[2] Drug combination regimens: first clinical trial (NC001) of a novel TB drug regimen testing the three drug combination of PA-824, moxifloxacin, and pyrazinamide was initiated November 2010.

WORKING GROUP ON NEW TB DRUGS

www.newtbdrugs.org

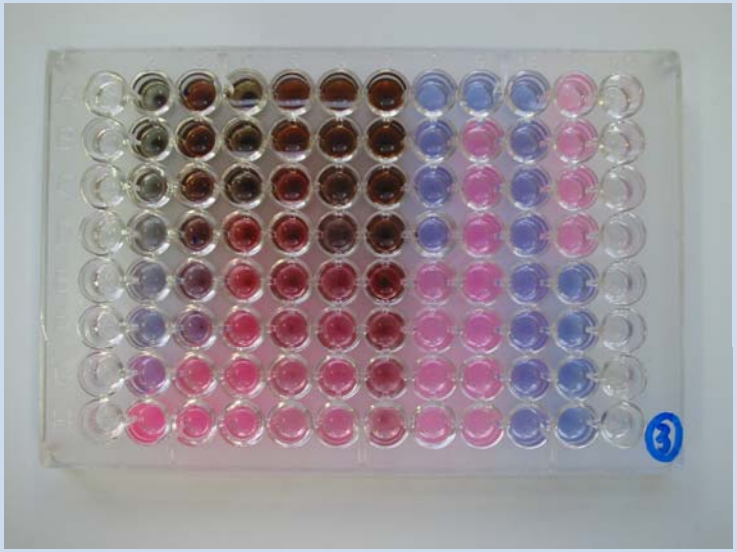
Updated: July 18, 2011

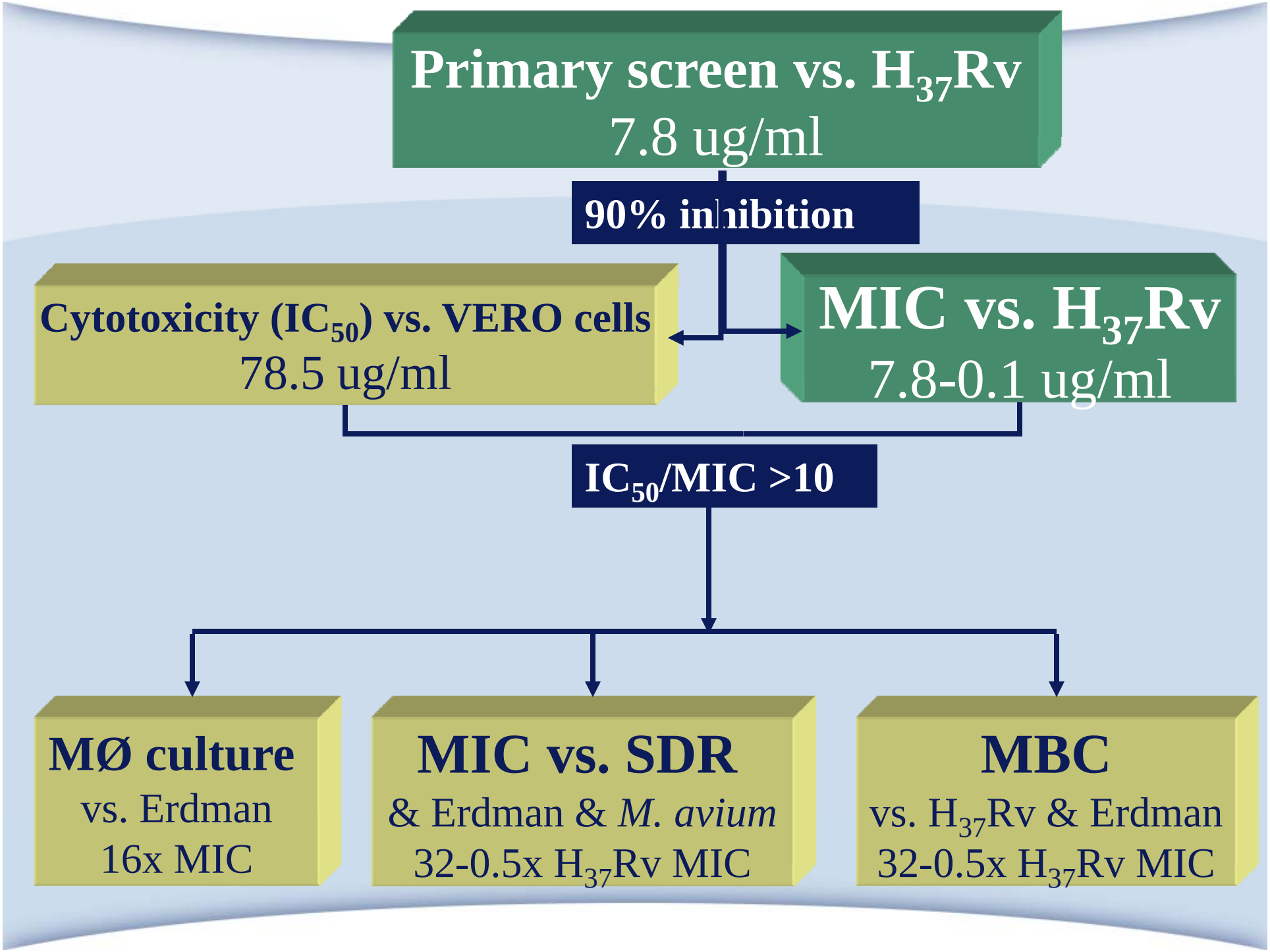
Pre-Clinical Research



Antimycobacterial activity *in vitro* Assay
Resazurin Microtiter Assay - REMA

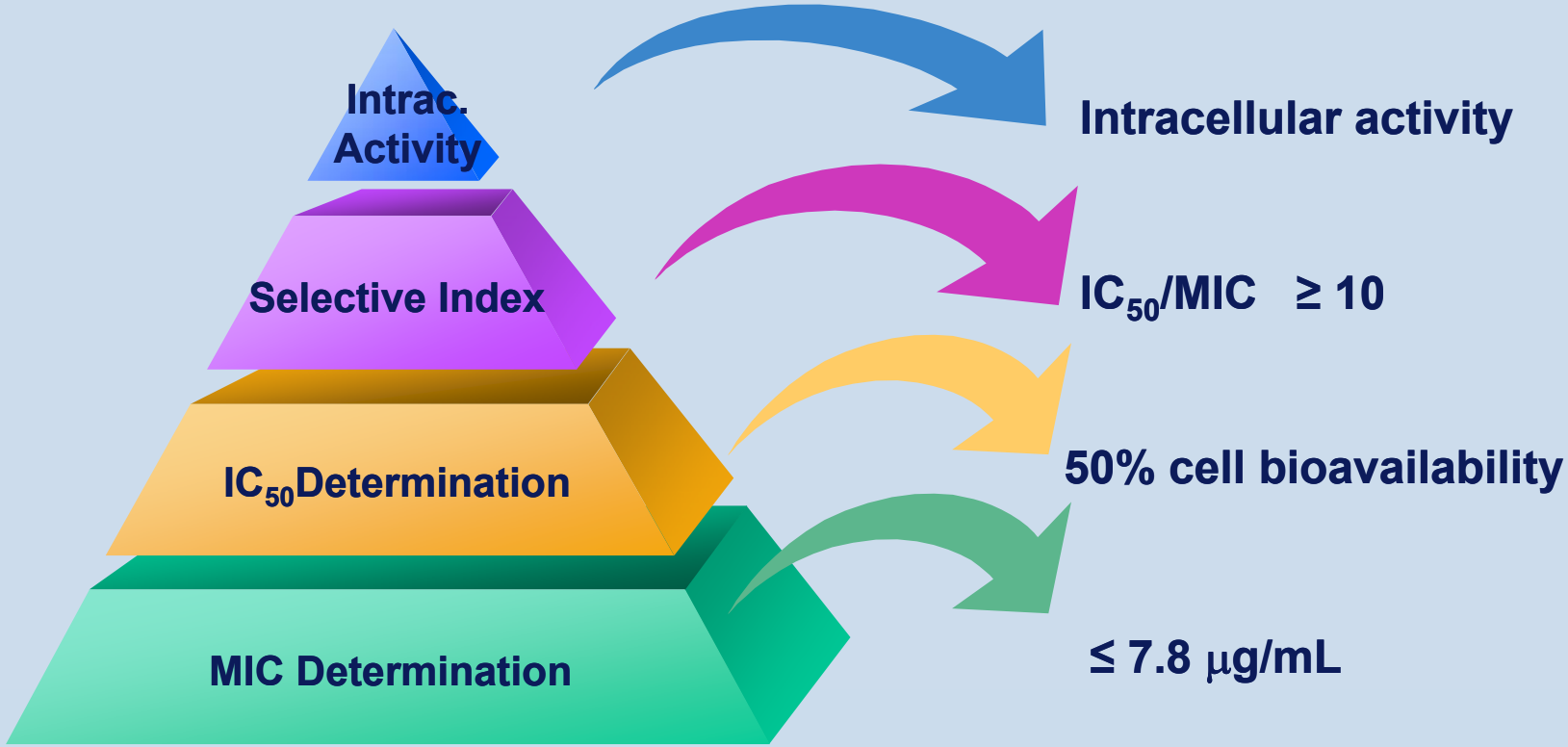
- *Mycobacterium tuberculosis* H37Rv
- 96-well format, 200 **uL**
- Small sample requirement
- Incubation: 6 day , 37°C
- Mycobacterial growth is determined by reduction of the blue dye (Resazurin), to the pink and fluorescent resofurin
- A change from blue to pink indicates bacterial cells growth
- The MIC is determined as the lowest drugs concentration that inhibits 90% of cell growth
- High-throughput anti-TB assay using microplate spectrophotometer or fluorimeter

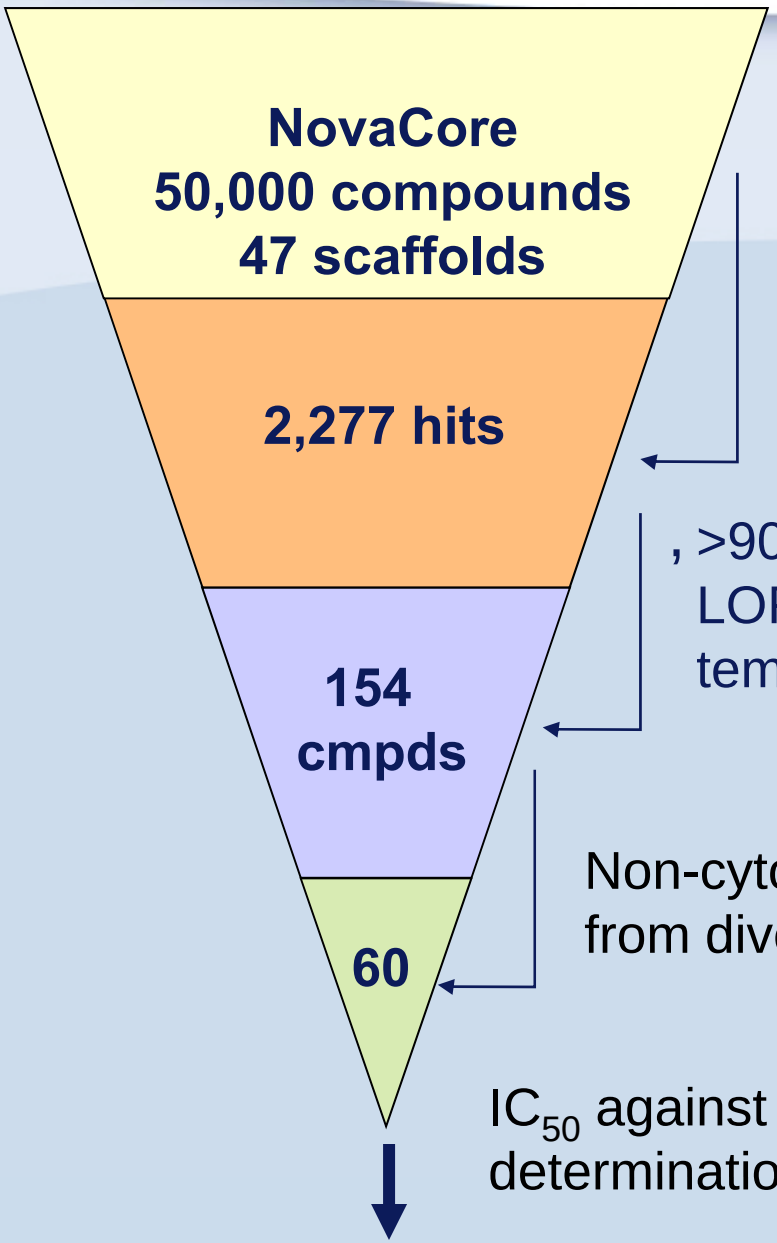



Primary screen vs. $H_{37}Rv$
7.8 ug/ml
90% inhibition
Cytotoxicity (IC_{50}) vs. VERO cells
78.5 ug/ml
MIC vs. $H_{37}Rv$
7.8-0.1 ug/ml
IC_{50}/MIC >10
MØ culture
vs. Erdman
16x MIC
MIC vs. SDR
& Erdman & *M. avium*
32-0.5x $H_{37}Rv$ MIC
MBC
vs. $H_{37}Rv$ & Erdman
32-0.5x $H_{37}Rv$ MIC

Screening of new anti-TB candidates

National Institute of Health (USA)





625 plates: Z' = 0.62

>90% inhibition at **30 uM** in luminescence assay - 18 days to complete

, >90% inhibition in MABA, active at 30 uM in LORA, selected representatives of all active template series

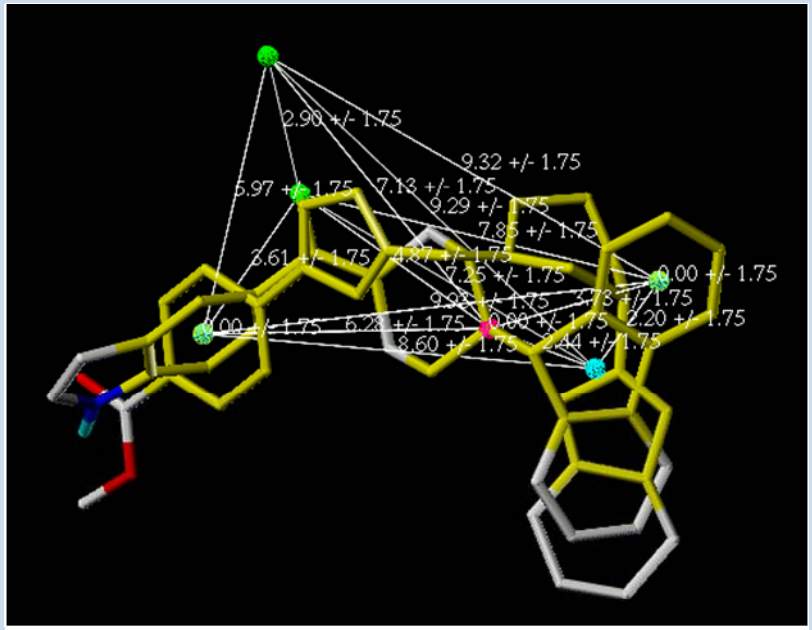
Non-cytotoxic against Vero cells (representatives from diverse template series)

IC_{50} against J774 and HepG2 cells, MIC determination with or without FBS (10%)

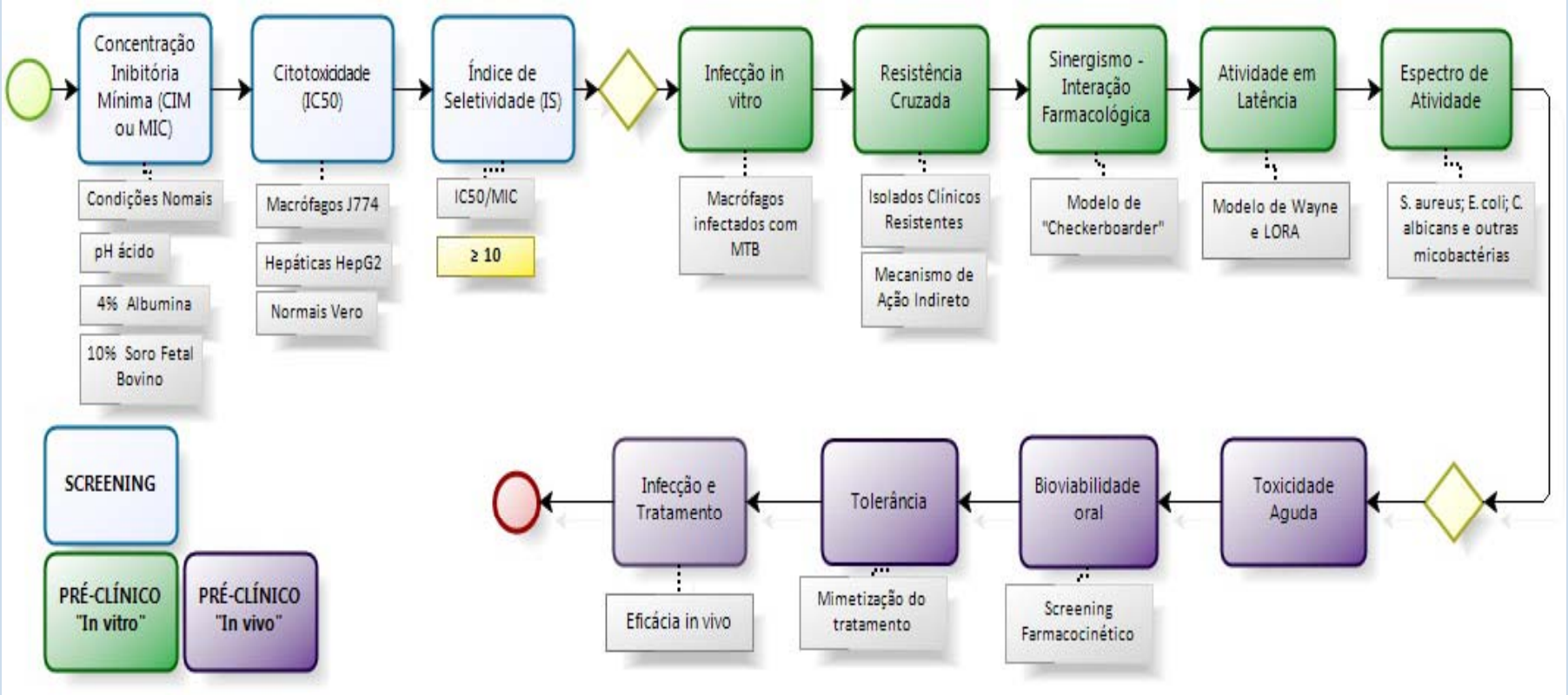
4 prioritized scaffolds

Identifying the molecular target of a phenotypic screening-derived lead

- Generate resistant mutant
  - Map mutation
  - Cross resistance
- Gene expression profiling
- Extended spectrum of activity - bioinformatic
- Overexpression library
- Transposon mutant library
- Pharmacophore matching to compound with known target



Pipeline: Search for New Drugs aganst Tuberculosis
Dr. Hugo David Laboratotry of Mycobacteriology



10 Pavan et al., An Approach to research for new drugs against TB, TUBERCULOSIS, accept, 2011.

Publication with CYTED Group

Publicações 2010 e 2011

- Fernando R. Pavan, Gustavo V. Poelhsitz, Marilia I.F. Barbosa, Sergio R.A. Leite, Alzir, A. Batista, Javier Ellena, Leticia S. Sato, Scott g. Franzblau, Virtudes Moreno, Dinorah, Gambino. Ruthenium (II) phosphine/diimine/picolinate complexes: Inorganic compounds as agentds against tuberculosis. **European Journal of Medicinal Chemistry**, 2011, in press.
- Gambino, Dinorah, Fernández, Mariana, Santos, Diego, Etcheverría, Gustavo A., Piro, Oscar E., Pavan,Fernando R., Leite, Clarice Q.F., Tomaz, Isabel, Marques, Fernanda Searching for gallium bioactive compounds: Gallium(III) complexes of tridentate salicylaldehyde semicarbazone derivatives. **Polyhedron**. , p.1360 - 1366, 2011
- Fernando R. Pavan F,*, Pedro I. da S. Maia b, Sergio R.A. Leite c, Victor M. Deflon b, Alzir A. Batista , Daisy N. Sato , Scott G. Franzblau, Clarice Q.F. Leite. Thiosemicarbazones, semicarbazones, dithiocarbazates and hydrazide/hydrazones: Anti – Mycobacterium tuberculosis activity and cytotoxicity **European Journal of Medicinal Chemistry** 45 (2010) 1898–1905
- Fernando R. Pavan, Gustavo Von Poelhsitz , Fabio B. do Nascimento , Sergio R.A. Leite , Alzir A. Batista , Victor M. Deflon , Daisy N. Sato , Scott G. Franzblau , Clarice Queico F. Leite. Ruthenium (II) phosphine/picolinate complexes as antimycobacterial agents. **European Journal of Medicinal Chemistry** 45 (2010) 598–601
- Tarallo, M. Belén, Urquiola, Carolina, Monge, Antonio, Costa, Beatriz Parajón, Ribeiro, Ronny R., Costa-Filho, Antonio J., Mercader, Roberto C., Pavan, Fernando R., Leite, Clarice Q.F., Torre, María H. Design of novel iron compounds as potential therapeutic agents against tuberculosis?. **Journal of Inorganic Biochemistry**. , v.104, p.1164 - 1170, 2010.
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Patentes

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