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UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO"

FACULDADE DE CIÊNCIAS FARMACÊUTICAS DE ARARAQUARA
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Screening of synthetic metallo-organic compounds with anti-TB activity

29-30/07/2009 - CYTED

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Why new drugs against *Mycobacterium tuberculosis*?

Current Global Status

Infected: 1.86 billion (32%)
New cases/yr: 8.7 million

Deaths/yr: 1.7 million (5,000/day)
26% of avoidable deaths in developing world
Brazil : reports the second-highest TB mortality and morbidity among all countries in the Americas

Drug resistance: ubiquitous (WHO/TB)
primary: 1.4% MDR, 10.4% SDR
acquired: 13% MDR, 36% SDR

"No new drugs excepting rifabutin and rifapentine after rifampicin"

Current treatment for TB

American Thoracic Society, CDC, WHO

- 2 months, daily (intensive phase)
  - Isoniazid (INH), 5 mg/kg
  - Rifampin, 10 mg/kg
  - Pyrazinamide, 15-30 mg/kg
- 4 months daily (continuation phase)
  - Isoniazid (INH), 5 mg/kg
  - Rifampin, 10 mg/kg

Impact of New Chemotherapy

1. Reducing Treatment Duration
   - Improved compliance
2. Successful treatment of MDR-TB
   - Reduce transmission of MDR-TB
     - Decrease cost of treatment
3. Cure latent TB infection
   - Reduce/eliminate disease reservoir

Ideal properties of new anti-TB drugs

- Good oral bioavailability and tolerability
- No drug-drug interations or antagonism with retro virus drugs
- Rapid bactericidal activity on extra and Intracellular bacilli (inside macrophages)
- Low toxicity : hepato, cardio, bone marrow, nefro, neuro, and genotoxicity

None Toxicity

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)

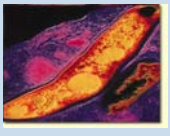
Why synthetic metallo-organic complexes?

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

How to evaluate biological activities?

Determination of *in vitro* antimycobacterial activity

- Target Bacterium
- ***Mycobacterium tuberculosis H37Rv (Lab. Level 3)***

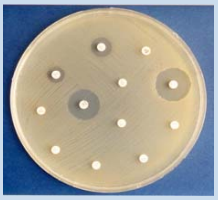


Mycobacterium tuberculosis

- Ancient pathogen, level 3 of danger
- Very slow growth
- Growth inside host macrophage
- Complex and lipids rich cell wall
- Resistant to many common antibiotics

Biological assays

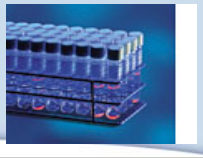
- Classic disk diffusion assay should be avoided because the mycobacteria grow slowly.
- Dilution methods in agar they are accomplished, for study of extracts and fractions, but the technique is difficult and slow (18 days to have resulted of CIM)



BACTEC System

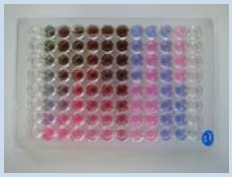
- Radiometric BACTEC 460 Assay : **is expensive method, the medium is a radioactive and each tube cost U$3.00**

- MGIT: Non radiometric



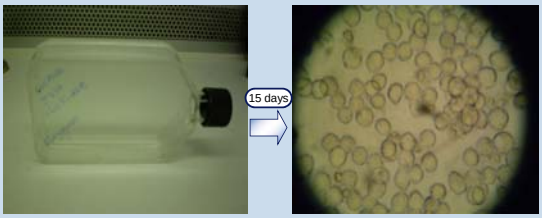
in vitro Antimycobacterial Activity Assay Resazurin Microtiter Assay - REMA

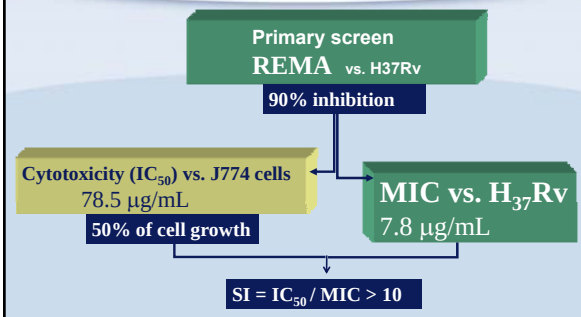
- *Mycobacterium tuberculosis* H37Rv
- 96-well format, 200 ul.
- Small sample requirement
- Incubation: 6 day , 37°C
- Mycobacterial growth is determined by reduction of the blue dye (Resazurin), to the pink and fluorescent resofurin
- A change from blue to pink indicates bacterial cells growth
- The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



Cytotoxicity Assay (IC_{50}) and IS

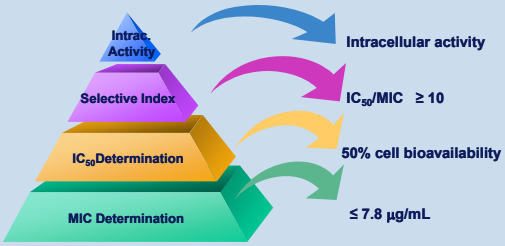
Determination of Intracellular activity



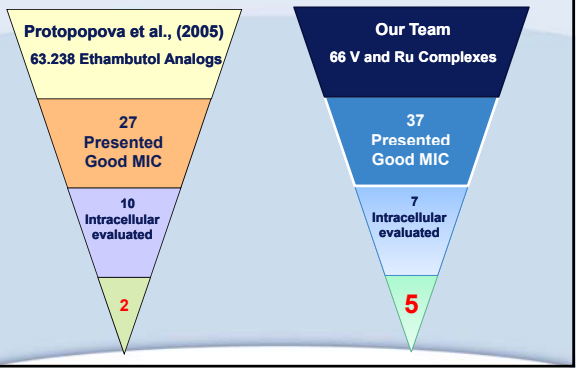


Screening of new anti-TB candidates

National Institute of Health (USA)



RESULTS



Synthesis, characterization, X-ray structure and in vitro antimycobacterial and antitumoral activities of Ru(II) phosphine/diimine complexes containing the "SpymMe2" ligand, SpymMe2 = 4,6-dimethyl-2 mercaptopyrimidine

Fábio B. do Nascimento, Gustavo Von Poelhsitz, Fernando R. Pavan , Daisy N. Sato, Clarice Q.F. Leite , Heloísa S. Selistre-de-Araújo, Javier Ellena, Eduardo E. Castellano, Víctor M. Deflon, Alzir A. Batista. v. 102, 1783-1789, 2008.

Compounds	Structures	MIC µg/mL	MIC µM
Free Phosphines and Diimines Ligands			
dppb		>50	>117,20
Bipy		25	160,10
Me-Bipy		25	135,70
HspymMe2		25	178,30

Compounds	Structures	MIC µg/mL	MIC µM
Ru(II) phosphine/diimine complexes			
[Ru(SpymMe2)(dppb)(bipy)]PF6		0,78	0,80
[Ru(SpymMe2)(dppb)(Me-bipy)]PF6		0,78	0,78
cis-[RuCl2(dppb)(bipy)]		3,90	5,17
cis-[RuCl2(dppb)(Me-bipy)]		6,25	7,99

Novel Iron Complexes with quinoxaline N^1, N^4 – dioxide derivatives: synthesis, characterization and Antimycobacterial Activity

M. Belén Tarallo, Carolina Urquiola, Antonio Monge, Fernando R. Pavan, Clarice Q.F. Leite, María H. Torre, Dinorah Gambino. John Libbey Eurotext, Paris; v. 10, 867-872, 2008.

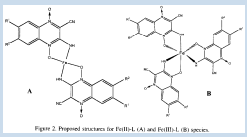
Compounds	Structures	REMA MIC (µg/mL)	IC50	IS
Iron Complexes with quinoxaline N^1, N^4 – dioxide derivatives				
Fe(II) – L1		3.9	125	32
Fe(III) – L1		7.8	156	20
Fe(III) – L2		3.9	156	40
Ligand				

Figure 2. Proposed structures for Fe(II)-L (A) and Fe(III)-L (B) species.

Research of new mixed-chelate copper complexe with quinoxaline N1,N4-dioxide derivatives and alanine as ligands, potential antimycobacterial agents

M. Belén Tarallo, Antonio J. Costa-Filho, Ernanni D. Vieira, Antonio Monge, Clarice Q.F. Leite, Fernando R. Pavan, Graciela Borthagaray, Dinorah Gambino, María H. Torre. "in press", 2009.

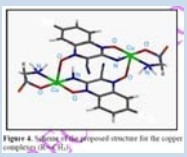
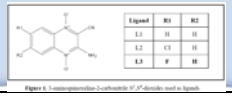
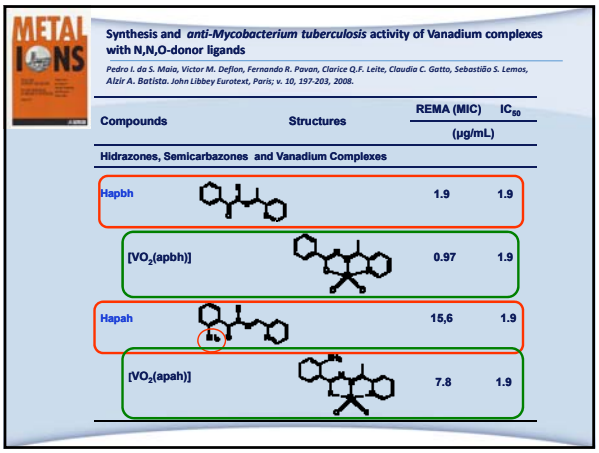
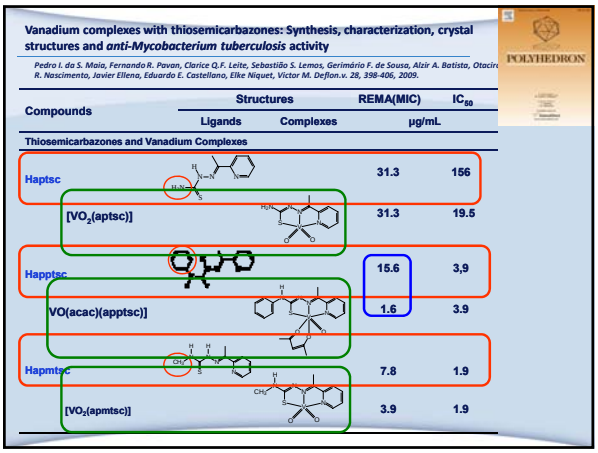
Compounds	Structures	REMA MIC (µg/mL)
mixed-chelate copper complexe with quinoxaline N1,N4-dioxide derivatives and alanine as ligands		
CuL1ala		7.8
CuL2ala		7.8
CuL3ala		7.8
Ligand		

Figure 4. Suggested proposed structure for the copper complexes (L1 and L2).


METAL IONS
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)
IC50
Hidrazones, Semicarbazones and Vanadium Complexes
Hapbh
1.9
1.9
[VO2(apbh)]
0.97
1.9
Hapah
15,6
1.9
[VO2(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.
POLYHEDRON
Compounds
Structures
Ligands
Complexes
REMA(MIC) µg/mL
IC50
Thiosemicarbazones and Vanadium Complexes
Haptsc
31.3
156
[VO2(aptsc)]
31.3
19.5
Happtsc
15.6
3,9
VO(acac)(apptsc)]
1.6
3.9
Hapmtsc
7.8
1.9
[VO2(apmtsc)]
3.9
1.9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