Research of New Drugs Against Tuberculosis

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What is “Mycobacterium tuberculosis”??

Mycobacterium tuberculosis

- Ancient pathogen level 3 of danger
- Acid-fast
- Very slow growth
- Complex cell wall
- Approx. 4,000 genes
- Aerobic/anaerobic
- Intracellular/extracellular
- Resistant to many common antibiotics
- No environmental reservoir
- Drying Resistance
Bacteria Cell wall

- Gram +
- Gram -
- BAAR

Mechanism of Transmission

1. Sick patient
2. Host bacilli inhalation
3. Lungs Bacilli migration
4. Bacilli in the lungs
   Granulome formation

Focus:
- Contact
- Mix population?
When and how appeared “Mycobacterium tuberculosis”? 

Origins of M. tuberculosis

M. tuberculosis

Air Transmission

M. bovis

Human Pathogen

Cattle

M. princips

First Mycobacterium

(Free in the nature)

Mycobacterial Origens

Fungos

Protistas

Vírus

Within 4,000 - 2,000 AC

8,000 a 3,000 AC

Human disease

After cow domestication

M. bovis

Man bone tuberculosis

M. princips

First Mycobacterium

(Free in the nature)
Tuberculosis: infectious disease
Agente: “Mycobacterium tuberculosis”

Ordem:
Actinomycetales
Family:
Mycobacteriaceae
Gênero:
Mycobacterium

Aerobic bacillus, non mobile, no spores, with persistent forms

“Mycobacterium tuberculosis Complex”

- M. tuberculosis
- M. bovis
- M. africanum
- M. ulcerans
- M. microti

The genome (DNA) of M. tuberculosis has totally determined also its chemical characteristic and virulence.

The M. microti is not pathogenic and the M. ulcerans cause skin lesion.

The M. bovis is a zoonose

The M. africanum is less pathogenic than M. tuberculosis

Current situation of “tuberculosis”??
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”

Over 95% of TB cases in the world are in developing countries
For this reason, we who live in the developing countries, feel
great responsibility for the search for new anti TB drugs

The “BCG strain” is a viable bacterium, originated of the
bovine bacillus
that was cultivated in glycerin potato middle
with ox bile, during 13 years and 230 cultivations
biweekly. The bacillus suffered a mutation becoming
Non virulent, maintaining the immunogenic properties

Rosenberg J. Vacinação BCG
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 (if primary resistance >4% in community)
  - Ethambutol, 15-25 mg/kg po (2-5g)
  - Streptomycin, 15 mg/kg im (1g)
- 4 months daily (continuation phase)
  - Isoniazid (INH), 5 mg/kg po (300 mg)
  - Rifampin, 10 mg/kg po (600 mg)

How to understand the phenomenon of “M. tuberculosis” resistance?

Human interference: introduction of the drugs
**Resistência natural em cepas selvagens do “M. tuberculosis”**

<table>
<thead>
<tr>
<th>DRUGS</th>
<th>CONCENTRATION IN THE MEDIUM (ug/ml)</th>
<th>RESISTANT MUTANTS FREQUENCY</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMP</td>
<td>40</td>
<td>1 in 10⁴</td>
</tr>
<tr>
<td>INH</td>
<td>0.2</td>
<td>1 in 10⁴</td>
</tr>
<tr>
<td>SM</td>
<td>4</td>
<td>1 in 10⁴</td>
</tr>
<tr>
<td>EMB</td>
<td>2</td>
<td>1 in 10⁵</td>
</tr>
<tr>
<td>ETH</td>
<td>20</td>
<td>1 in 10²</td>
</tr>
<tr>
<td>PZA</td>
<td>25</td>
<td>1 in 10²</td>
</tr>
</tbody>
</table>


**Where happens the natural resistance?**

Natural mutação
only in great bacilar population (cavitary: > 10⁸)

**Drugs association to avoid “M. tuberculosis” resistance**

“CROSSED FIRE”
10⁴ resistant bacilli to INH
10⁸ resistant bacilli to RMP

Fontes: Controle da Tuberculose, 3a Ed., CNTC/NUTES/MS, R.J.1992
Dalcolmo MP, Tese de Doutorado, 1999
First line drug targets

- DNA Cycling, Transcription, and Translation
- ATP Synthesis
- Mycobacterium tuberculosis
- ATP Synthesis

Second line drug targets

- DNA Cycling, Transcription, and Translation
- ATP Synthesis
- Mycobacterium tuberculosis
- ATP Synthesis

Mechanisms of action of the drugs

- Inhibitors of biosynthesis of acylcarnitines and protein synthesis (SM, Claritromicina, Linezolide)
- Inhibitors of processes realized by DNA (Rifampicina, Fluoroquinolonas)
- Inhibitors of dehydrofolate reductase or biosynthesis of siderophores (PAS)
- Inhibitors of biosynthesis of fatty acids (INH, PZA ETB, PA-824, OPC-67683)
- Inhibitors of biosynthesis of arabinogalactan and peptidoglycan (ETB, D-Ciclocerina, Amoxicilina, Clofazimina)
- Inhibitors of processes realized by DNA (Rifampicina, Fluoroquinolonas)
Genes associated with resistency

- Gene rpoB – RMP
- Genes katG, inhA, oxyR-ahpC, kasA – INH
- Genes rrs e rpsL – SM
- Gene pncA – PZA
- Genes embA, embB e embC – ETB
- Genes gyrA e gyrB – fluoroquinolonas

Why new drugs against *Mycobacterium tuberculosis*?
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

Approaches to New TB Drugs

- Ligand-based whole cell screening
  - Optimize non-TB antimicrobial classes
  - Optimize TB drugs
  - Novel synthetic
  - Novel natural products
  - Ethnomedical

- Target-based discovery
  - Target identification
  - Screening (in silico, NMR, functional)

- Random high throughput screening (HTS) of synthetic and natural products vs. *M. tuberculosis* (whole cell screening)

Ideal properties of new anti-TB drugs

- Rapid bactericidal activity on extra and intracellular bacilli (inside macrophages)
- Long tissue half-life and TB activity
- Good oral bioavailability and tolerability
- Low toxicity: hepato, cardio, bone marrow, nephro, neuro, and genotoxicity
- No drug-drug interactions or antagonism with retro virus drugs

None Toxicity
Reasons for delayed investigation

1. The search is expensive
2. The bacterium is hard to handle
3. The companies engaged in the development of new TB drugs perceived lack of commercial return
   - Over 95% of TB cases in the world are in developing counties
4. For this reason, we who live in the developing countries, feel responsible for the search for new anti-TB drugs

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>Development Stage</th>
<th>Sponsor/Coordinator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gatifloxacin</td>
<td>Phase 3</td>
<td>European Commission, IRD, WHO/TDR, Lupin</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>Phase 2/3</td>
<td>Bayer, TB Alliance, CDC, University College London, Johns Hopkins University</td>
</tr>
<tr>
<td>THC 207 (Diarylquinoline)</td>
<td>Phase 2</td>
<td>Johnson &amp; Johnson (Tibotec)</td>
</tr>
<tr>
<td>OPC 67683 (Nitroimidazole)</td>
<td>Phase 1 EBA</td>
<td>Otsuka Pharmaceutical</td>
</tr>
<tr>
<td>PA 824 (Nitroimidazole)</td>
<td>Phase 1</td>
<td>TB Alliance</td>
</tr>
<tr>
<td>LL 3958 (Pyrrrole)</td>
<td>Phase 1</td>
<td>Lupin</td>
</tr>
<tr>
<td>SQ 109 (Diamine)</td>
<td>Phase 1</td>
<td>Sequella</td>
</tr>
</tbody>
</table>

(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.

Methods to evaluate biological activities?
Determination of \textit{in vitro} antimycobacterial activity

- Target Bacterium
  - \textit{Mycobacterium tuberculosis H37Rv} (Lab. nivel 3)
  - \textit{Mycobacterium smegmatis}

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 MIC)

Sistema BACTEC

- Radiometric BACTEC 460 Assay: \textit{Método caro}, um único tubo de 4 ml, contendo meio radioativo, US$2.50
- MGIT: Non radiometric
Antimycobacterial activity in vitro
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

Primary screen vs. H37Rv
7.8 ug/ml
90% inhibition

Cytotoxicity (IC₅₀) vs. VERO cells
78.5 ug/ml

MIC vs. H37Rv
7.8-0.1 ug/ml

IC₅₀/MIC >10

MO culture vs. Erdman
16x MIC

MIC vs. SDR & Erdman & M. avium
32-0.5x H₃₇Rv MIC

MBC vs. H₃₇Rv & Erdman
32-0.5x H₃₇Rv MIC

Screening of new anti-TB candidates

Intracellular activity
IC₅₀/MIC ≥ 10
50% cell bioavailability
≤ 7.8 μg/mL

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


Compounds Structures MIC (μg/mL)

<table>
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<tr>
<th>Fre Phosphines and Diimines Ligands</th>
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<tbody>
<tr>
<td>Free Phosphines and Diimines Ligands</td>
</tr>
<tr>
<td>dppb</td>
</tr>
<tr>
<td>Bipy</td>
</tr>
<tr>
<td>Me-Bipy</td>
</tr>
<tr>
<td>HspymMe2</td>
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</tbody>
</table>

**Ru(II) phosphine/diimine complexes**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Structures</th>
<th>MIC (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Ru(SpymMe2)(dppb)(bipy)]PF6</td>
<td><img src="image1.png" alt="Image" /></td>
<td>0.78</td>
</tr>
<tr>
<td>[Ru(SpymMe2)(dppb)(Me-bipy)]PF6</td>
<td><img src="image2.png" alt="Image" /></td>
<td>0.78</td>
</tr>
<tr>
<td>cis-[RuCl2(dppb)(bipy)]</td>
<td><img src="image3.png" alt="Image" /></td>
<td>3.90</td>
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<tr>
<td>cis-[RuCl2(dppb)(Me-bipy)]</td>
<td><img src="image4.png" alt="Image" /></td>
<td>6.25</td>
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</table>

Novel Iron Complexes with quinoxaline N1, N4 – dioxide derivatives: synthesis, characterization and Antimycobacterial Activity.

M. Belén Tarallo, Carolina Urquiola, Antonio Monge, Fernando R. Pavan, Clarice Q.F. Leite, Maria H. Torre, Dinorah Gambino. 

Compounds Structures MIC (μg/mL)

<table>
<thead>
<tr>
<th>Iron Iron Complexes with quinoxaline N1, N4 – dioxide derivatives</th>
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<tbody>
<tr>
<td>Fe(II) L1</td>
</tr>
<tr>
<td>Fe(III) L1</td>
</tr>
<tr>
<td>Fe(III) L2</td>
</tr>
</tbody>
</table>

Ligand

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


Compounds Structures MIC (μg/mL)

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<th>mixed-chelate copper complex with quinoxaline N1,N4- dioxide derivatives and alanine as ligands</th>
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<tbody>
<tr>
<td>CuL1ala</td>
</tr>
<tr>
<td>CuL2ala</td>
</tr>
<tr>
<td>CuL3ala</td>
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</tbody>
</table>
### Synthesis and anti-Mycobacterium tuberculosis activity of Vanadium complexes with N,N,O-donor ligands

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Structures</th>
<th>REMA (MIC)</th>
<th>ICREMA (MIC)</th>
<th>IC50</th>
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<tbody>
<tr>
<td>Hidrazones</td>
<td><img src="image" alt="Structure Hidrazones" /></td>
<td>1.9</td>
<td>1.9</td>
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<tr>
<td>Semicarbazones</td>
<td><img src="image" alt="Structure Semicarbazones" /></td>
<td>0.97</td>
<td>1.9</td>
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<tr>
<td>Vanadium Complexes</td>
<td><img src="image" alt="Structure Vanadium Complexes" /></td>
<td>15.6</td>
<td>1.9</td>
<td></td>
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<tr>
<td></td>
<td><img src="image" alt="Structure Vanadium Complexes" /></td>
<td>7.8</td>
<td>1.9</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Structures</th>
<th>REMA (MIC)</th>
<th>ICREMA (MIC)</th>
<th>IC50</th>
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<tbody>
<tr>
<td>Thiosemicarbazones</td>
<td><img src="image" alt="Structure Thiosemicarbazones" /></td>
<td>31.3</td>
<td>19.5</td>
<td></td>
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<td></td>
<td><img src="image" alt="Structure Thiosemicarbazones" /></td>
<td>7.8</td>
<td>1.9</td>
<td></td>
</tr>
</tbody>
</table>

### Collaborators

**Our Group**
- Ph.D. Students
  - Ana Carolina Malagutti
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- Wagner Vilegas
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- Victor M. Deflon
Thanks a lot for your attention

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