Preliminary structure-activity relationships and biological evaluation of novel antitubercular indolecarboxamide derivatives against drug-susceptible and drug-resistant *Mycobacterium tuberculosis* strains


Celeste Alverez
Tuberculosis

- Bacterial infection caused by *Mycobacterium tuberculosis*
- Typically affects lungs
  - Can also affect kidneys, liver, spine, brain, any organs reached by lymph node or bloodstream
- 2 forms:
  - Latent – infected with TB, but *M. tuberculosis* is in inactive state, cause no symptoms, is not contagious, but can turn active
  - Active – active infection causing symptoms and is contagious
- Symptoms:
  - Cough lasting 3+ weeks, coughing up blood or mucus, chest pain, weight loss, loss of appetite, fatigue, fever, chills
- Spread to others through the air via cough, sneeze, or talking by someone with active infection
Epidemiology

- **US:**
  - 10,528 new TB cases in 2011
  - 529 deaths in 2009

- **Worldwide:**
  - One third of the world’s population is infected with TB
  - In 2011:
    - There were an estimated 8.7 million new cases
    - There were around 1.4 million TB-related deaths
  - TB is a leading killer of people infected with HIV
  - Incidence has been decreasing worldwide for several years
    - 2.2% per year between 2010 and 2011
Worldwide TB incidence 2011
Current Treatments

**Latent:**

<table>
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<tr>
<th>Drug</th>
<th>Duration</th>
<th>Dosing</th>
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<tbody>
<tr>
<td><em>Isoniazid</em></td>
<td>9 months</td>
<td>Daily or twice weekly</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>6 months</td>
<td>Daily or twice weekly</td>
</tr>
<tr>
<td>Isoniazid and Rifapentine</td>
<td>3 months</td>
<td>Once weekly</td>
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<tr>
<td>Rifampin</td>
<td>4 months</td>
<td>Daily</td>
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**Active:**

<table>
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<tr>
<th>Regimen type</th>
<th>Initial phase</th>
<th>Continuation phase</th>
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<tr>
<td>Preferred/Standard</td>
<td>Daily isoniazid, rifampin, ethambutol, and pyrazinamide for 8 weeks</td>
<td>Daily isoniazid and rifampin for 18 weeks or twice weekly for 18 weeks</td>
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<tr>
<td>Alternative</td>
<td>Daily isoniazid, rifampin, ethambutol, and pyrazinamide for 2 weeks, then twice weekly for 6 weeks</td>
<td>Twice weekly isoniazid and rifampin for 18 weeks</td>
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<tr>
<td>Alternative</td>
<td>3 times weekly isoniazid, rifampin, ethambutol, and pyrazinamide for 8 weeks</td>
<td>3 times weekly isoniazid and rifampin for 18 weeks</td>
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</table>
TB drug approval

- First line drug options have not changed in nearly 40 years with the last novel drug (Rifampin) being approved 50 years ago

**First-line TB drugs (drug-sensitive TB)**

**Second-line TB drugs (drug-resistant TB)**

In the Pipeline

Discovery

Diarylquinolines
InhA Inhibitors
LeuRS Inhibitors
Mycobacterial Gyrase Inhibitors
Pyrazinamide Analogs
Riminophenazines
Ruthenium (II) complexes
Spectinamides
Translocase-1 Inhibitors

Preclinical Development

CPZEN-45
DC-159a
Q201
SPR-10199
SQ609
SQ641

GLP Tox.

BTZ043
TBA-354

Phase I

AZD5847
*Bedaquiline (TMC-207)
Linezolid
Novel Regimens
*PA-824
Rifapentine
SQ-109
Sutezolid (PNU-100480)

Phase II

Delamanid (OPC-67683)
Gatifloxacin
Moxifloxacin
Rifapentine

Phase III

Chemical classes: fluoroquinolone, rifamycin, oxazolidinone, nitroimidazole, diarylquinolone, benzothiazinone

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

2 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 and completed in 2011 with promising results. The second clinical trial (NC002) of this regimen was launched in March 2012 and will test the efficacy of the regimen in drug-sensitive and multidrug-resistant patients. The third clinical trial (NC003) will evaluate PA-824, TMC-207, pyrazinamide and clofazimine in combinations and is scheduled to begin September 2012.
Drug Resistance

- Major problem resulting from non-compliance and limited access to drugs
- 2 classes:
  - Multidrug-resistant TB (MDR-TB) – resistant to at least isoniazid and rifampin
    - Treatment requires 18-24 months of combination therapy with second-line drugs (more toxic, expensive, and difficult to administer)
  - Extensively drug-resistant TB (XDR-TB) – resistant to isoniazid and rifampin, and one or more fluoroquinolone, and at least one of three injectable second-line drugs (i.e., amikacin, kanamycin, or capreomycin)
    - Especially deadly for those with compromised immune systems
New drug approved for resistant TB

- Bedaquiline (TMC207, *Sirturo*) approved in December 2013 for treatment of MDR-TB and XDR-TB when other treatments are ineffective
  - Accelerated approval after phase II
  - Has black-box warning – can cause arrhythmias by blocking hERG
HTS – identifying a lead

- 6800 compounds screened
  - All lead-like, conforming to Lipinski’s rule of 5
- Screened in a phenotypic screen against replicating (active) *M. tuberculosis* measuring bacterial viability

- Identified an indole-2-carboxamide as one of the hit series

  ![Indole-2-carboxamide](image)

- MIC = 0.93 μM, and low toxicity in Vero cells
- Target is MmpL3 – membrane protein in *M. tuberculosis* apart of the resistance, nodulation, and cell division family of membrane transporters
SAR approach

Substitution by hydrophilic or lipophilic groups

Alkylation of nitrogen atoms

Replacement by substituted or unsubstituted aryl and cycloalkyl groups

Replacement of the indole ring
Synthesis of analogs 3-25

1. NaOEt, EtOH, r.t. to 70°C, 1 h
2. NaOH, 70°C, 20 min
3. HCl, r.t.
4. EDC-HCl, HOBr, R-NH₂, TEA, DCM, r.t., 12-16 h

19-23

1. NaOH, THF/EtOH, r.t. 3-4 h
2. EDC-HCl, HOBr, R-NH₂, TEA, DCM, r.t., 12-16 h

24, 25

R₂ = 5-Cl, 4,6-dimethyl
Synthesis of analogs 26-44

X = CH, C, N
R₃ = H, 4,6-difluoro, 6-OME, 5-Cl

26-34

35, 36

37-40

41-44
<table>
<thead>
<tr>
<th>Compd</th>
<th>R</th>
<th>MIC (μM)</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (μM)</th>
<th>Compd</th>
<th>R</th>
<th>MIC (μM)</th>
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Compd R MIC (μM)

33, 34

37-40

41-44
**Efficacy against resistant strains**

<table>
<thead>
<tr>
<th>compd</th>
<th>V4207 (DS) $^a$</th>
<th>TF274 (XDR) $^b$</th>
<th>R506 (XDR) $^b$</th>
<th>KZN494 (MDR) $^c$</th>
<th>V2475 (MDR) $^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0.93</td>
<td>0.46</td>
<td>0.46</td>
<td>3.7</td>
<td>0.93–1.9</td>
</tr>
<tr>
<td>11</td>
<td>0.11</td>
<td>0.055</td>
<td>0.055</td>
<td>0.11</td>
<td>0.11</td>
</tr>
<tr>
<td>12</td>
<td>0.026</td>
<td>0.026</td>
<td>0.0067</td>
<td>NT$^e$</td>
<td>NT$^e$</td>
</tr>
</tbody>
</table>

Values shown indicate the lowest concentration of leading to at least 90% reduction of bacterial growth in a microplate Alamar Blue assay. Values an average of 3 measurements.
**Ex vivo results**

**Serum inhibition titration for compound 12.** BALB/c mice were dosed with two doses (100 and 300 mg/kg) 12, blood was collected at 15, 30, and 60 min and the serum separated 60 min later. Growth inhibition of serially diluted serum on H37Rv was determined using the Alamar Blue assay. Vehicle 0.5% carboxylmethyl cellulose; isoniazid 10 mg/kg as positive control.
Summary

- Alkylation not tolerated at amide or indole nitrogen
- Hydrogen bond donor necessary
- Hydrogen bond acceptor not tolerated

- Substitution of indole ring is necessary for activity
- 4,6-substitution is best
- EWG and EDG tolerated

- Larger cycloalkyl groups improve activity (more lipophilic and bulky)
- Aromaticity not tolerated
- Heteroatoms in ring not tolerated

- Additional heteroatoms not tolerated
- Substitution of heteroatoms well not tolerated
- 2-position substitution necessary for activity
Conclusion

- Compound 3 was identified by a HTS to be active against *M. tuberculosis*
- 3 of the analogs (12-14) are more active than the standard first line drug isoniazid as well as the new drug bedaquiline
- Compounds 3, 11, and 12 are effective against drug susceptible, MDR-TB, and XDR-TB
- 3, 11, and 12 display low toxicity
- 12 is orally active

- Promising novel class of compounds for the treatment of TB