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

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Celeste Alverez

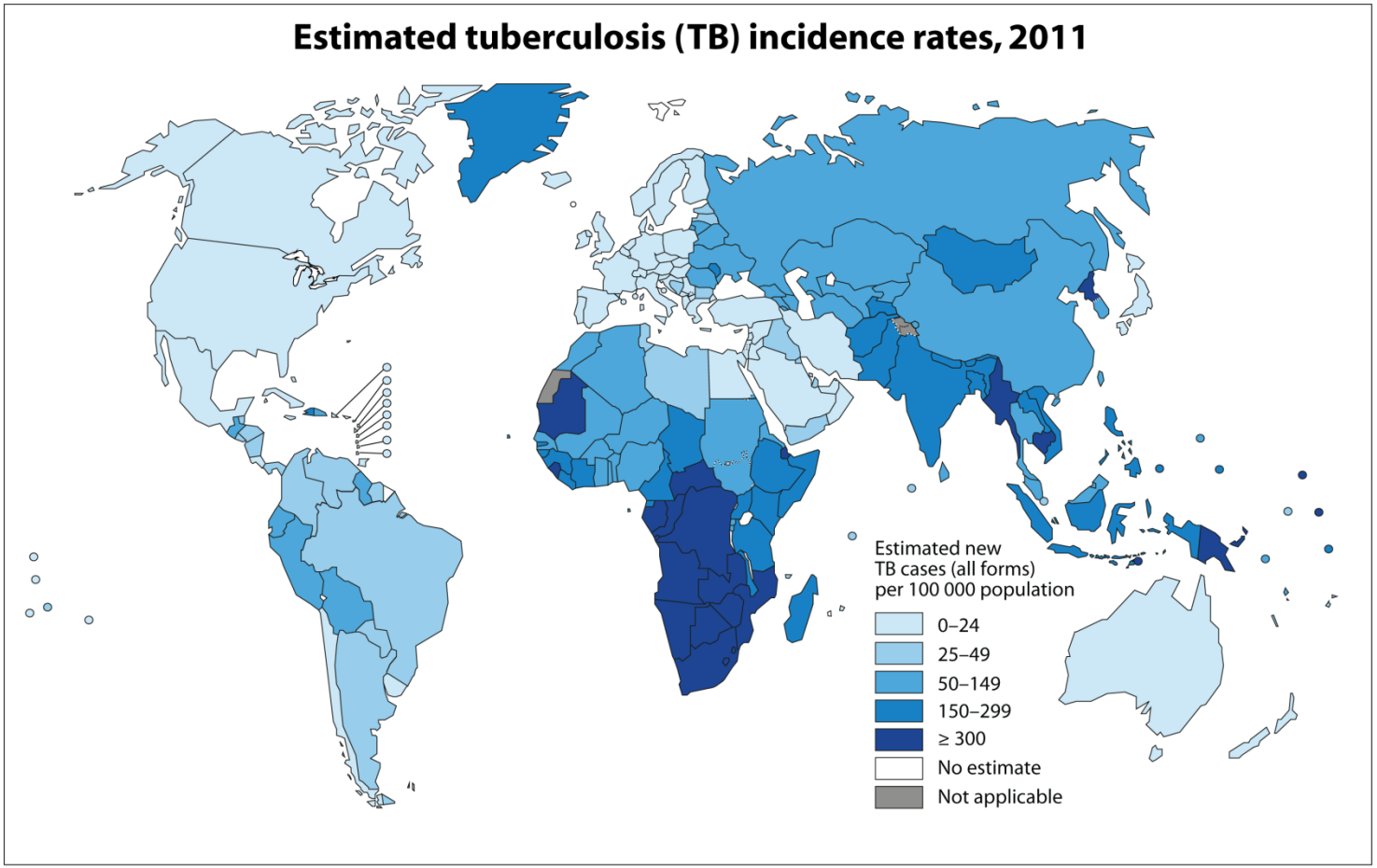
Tuberculosis

- 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



The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted and dashed lines on maps represent approximate border lines for which there may not yet be full agreement.

Source: *Global Tuberculosis Report 2012*. WHO, 2012.

World Health Organization

Current Treatments

- Latent:

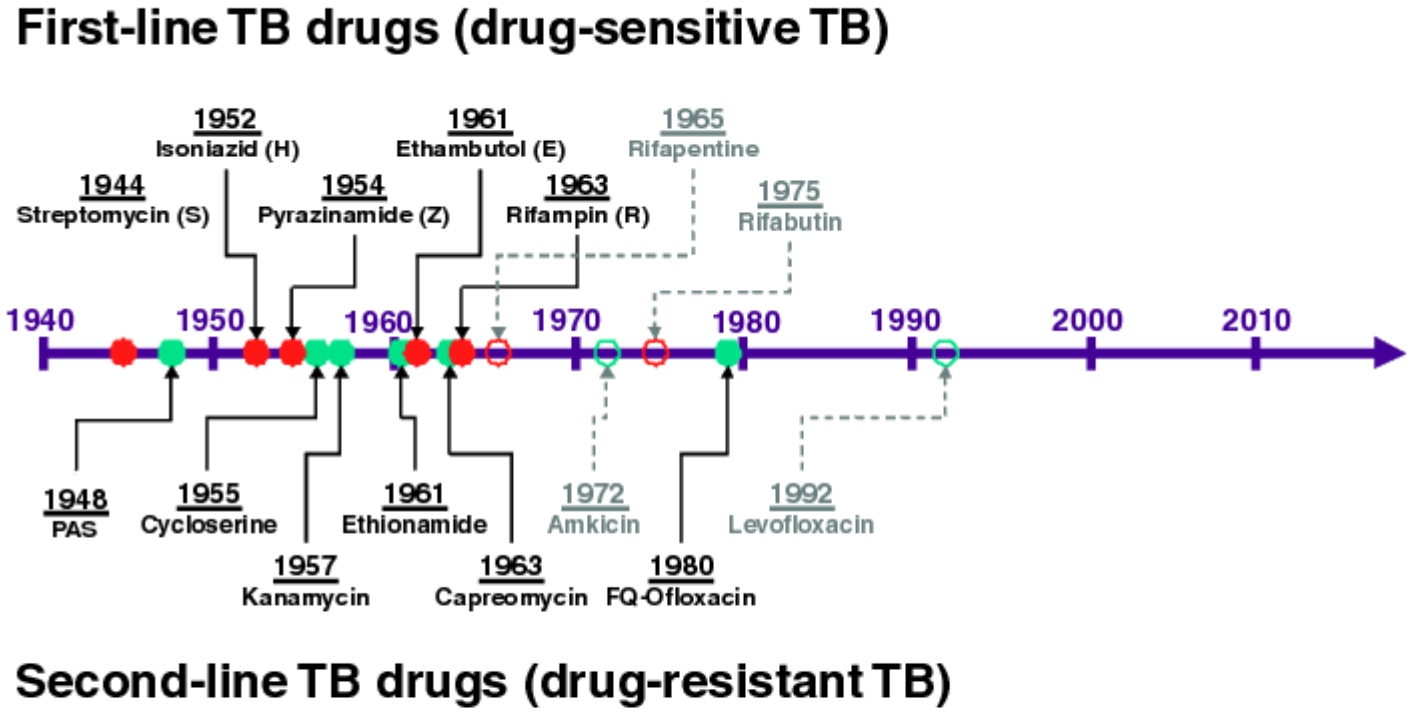
Drug	Duration	Dosing
*Isoniazid	9 months	Daily or twice weekly
Isoniazid	6 months	Daily or twice weekly
Isoniazid and Rifapentine	3 months	Once weekly
Rifampin	4 months	Daily

- Active:

Regimen type	Initial phase	Continuation phase
Preferred/Standard	Daily isoniazid, rifampin, ethambutol, and pyrazinamide for 8 weeks	Daily isoniazid and rifampin for 18 weeks or twice weekly for 18 weeks
Alternative	Daily isoniazid, rifampin, ethambutol, and pyrazinamide for 2 weeks, then twice weekly for 6 weeks	Twice weekly isoniazid and rifampin for 18 weeks
Alternative	3 times weekly isoniazid, rifampin, ethambutol, and pyrazinamide for 8 weeks	3 times weekly isoniazid and rifampin for 18 weeks

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



Giffin, R. And Robinson, S. *Addressing the Threat of Drug-Resistant Tuberculosis: A Realistic Assessment of the Challenge*; The National Academies Press; Washington D.C., 2009.

In the Pipeline

Discovery[1] | **Preclinical Development** | **Clinical Development**

Lead Optimization → **Preclinical Development** → **GLP Tox.** → **Phase I** → **Phase II** → **Phase III**

Lead Optimization

- 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.

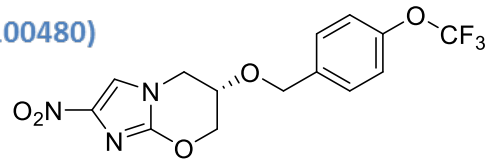
- BTZ043
- TBA-354

Phase I

- AZD5847
- *Bedaquiline (TMC-207)
- Linezolid
- Novel Regimens[2]
- *PA-824
- Rifapentine
- SQ-109
- Sutezolid (PNU-100480)

Phase II

- Delamanid (OPC-67683)
- Gatifloxacin
- Moxifloxacin
- Rifapentine



PA-824

Chemical classes: fluoroquinolone, rifamycin, oxazolidinone, nitroimidazole, diarylquinoline, 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.

WORKING GROUP ON NEW TB DRUGS

www.newtbdrugs.org

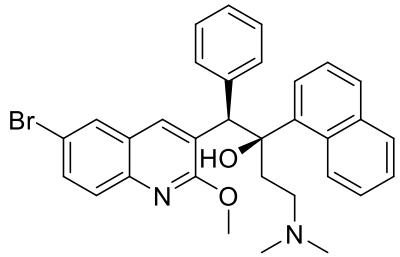
Updated: November 13, 2012

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

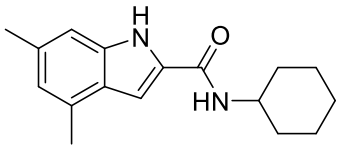
- Bedaquiline (TMC207, *Sirturo*) approved in December 2013 for treatment of MDR-TB and XDR-TB when other treatments are ineffective
  - Accelerated approval after phase II
  - Has black-box warning – can cause arrhythmias by blocking hERG



Bedaquiline

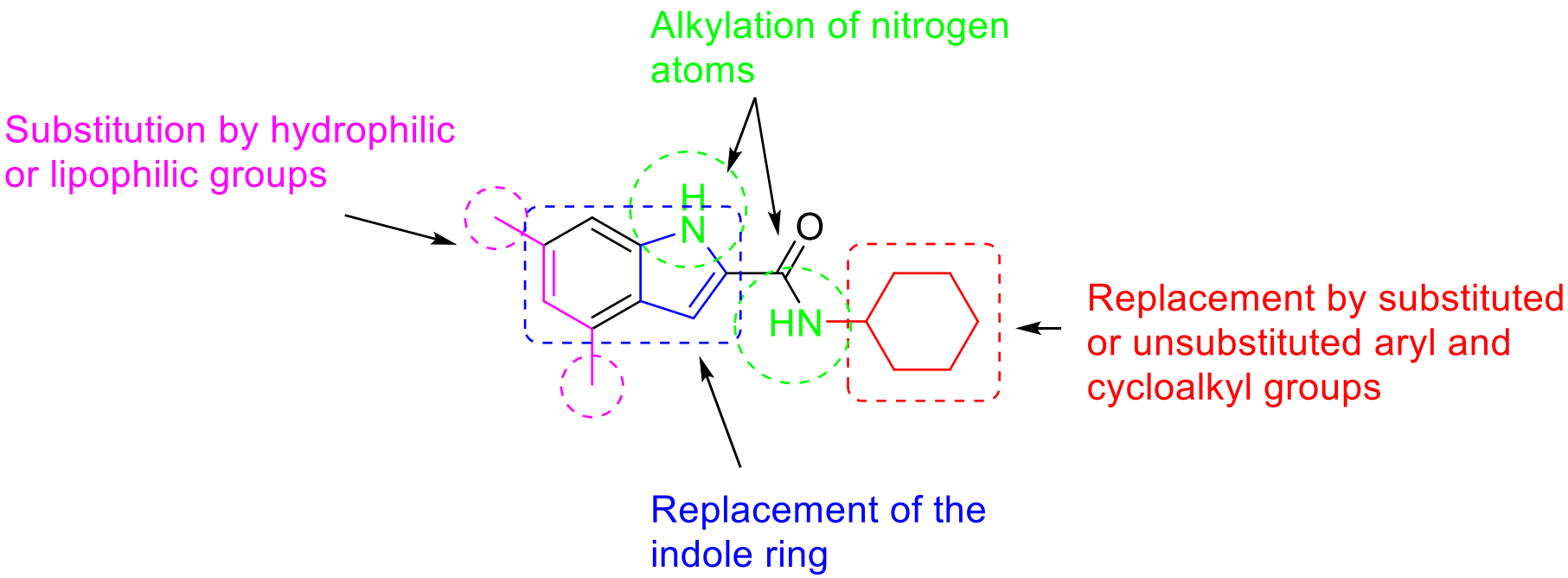
HTS – identifying a lead

- 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

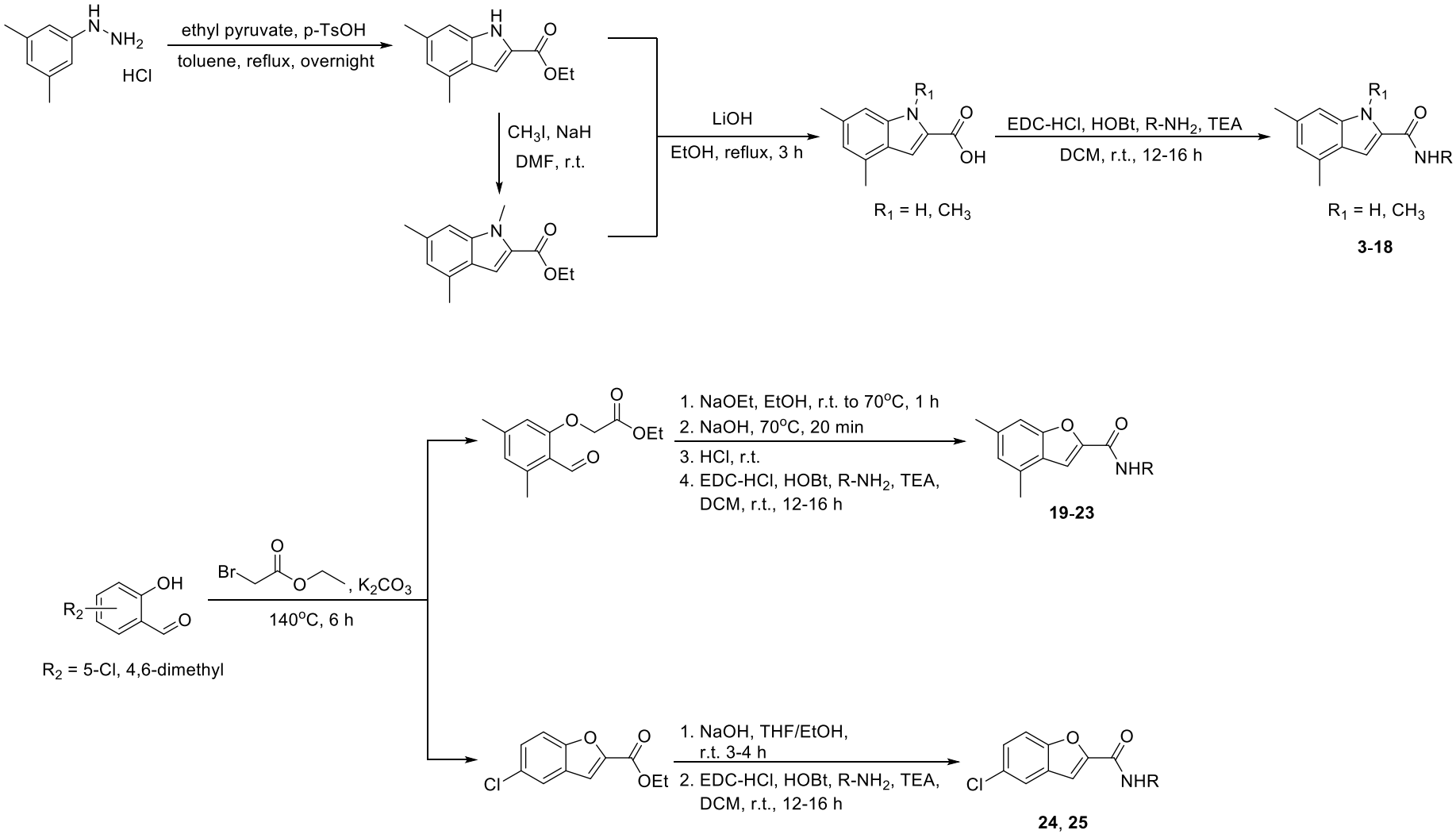


- MIC = 0.93 μM, and low toxicity in Vero cells
- Target is MmpL3 – membrane protein in *M. tuberculosis* apart of the resistance, nodulation, and cell division family of membrane transporters

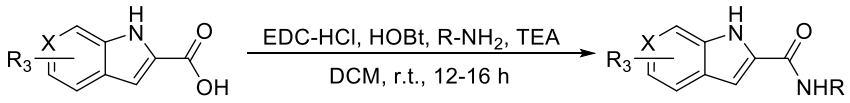
SAR approach



Synthesis of analogs 3-25



Synthesis of analogs 26-44



X = CH, C, N
R_3 = H, 4,6-difluoro, 6-OMe, 5-Cl

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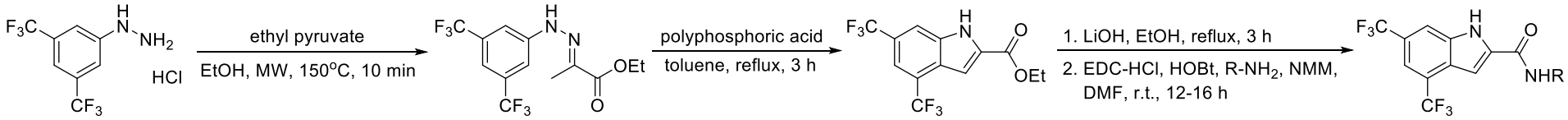
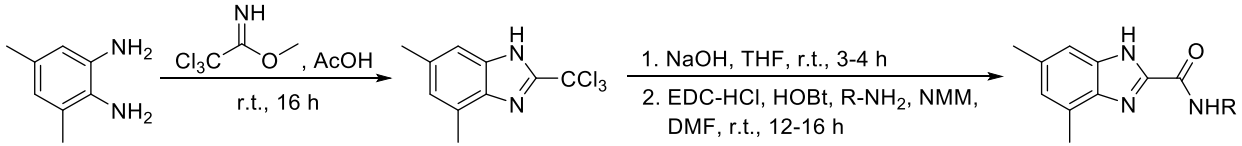
26-34



35, **36**



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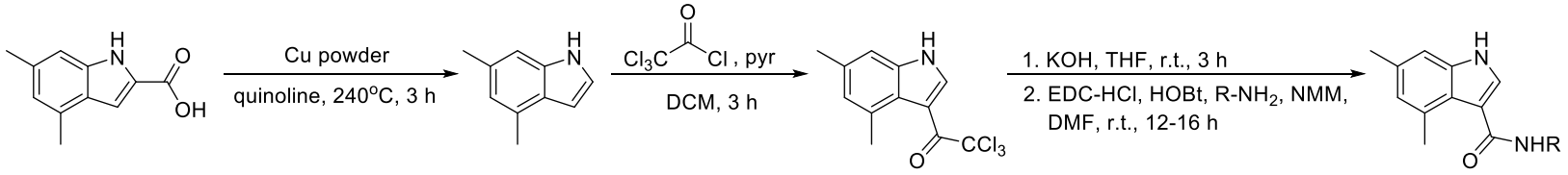

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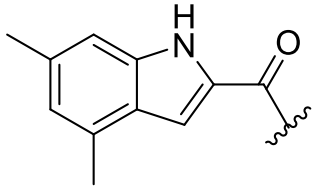

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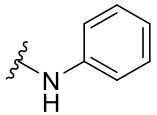
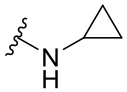
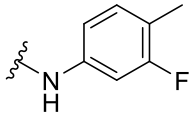
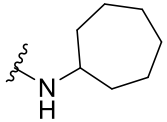
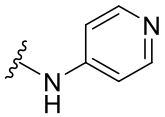
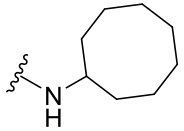
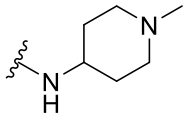
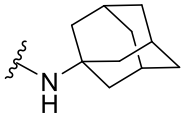
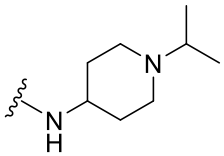
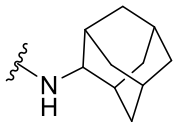
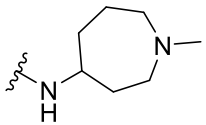
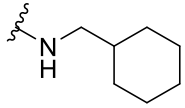
4		3.8	>200	10		561	-
5		1.7	>200	11		0.055	>200
6		240	>200	12		0.013	54
7		448	-	13		0.012	>200
8		204	-	14		0.012	>200
9		428	-	15		0.88	>200

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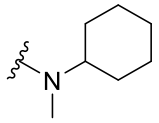
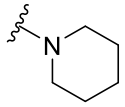
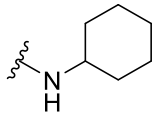
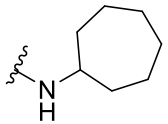
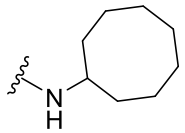
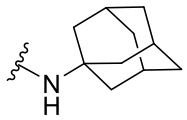
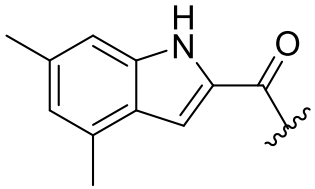
16		450
17		>499
18		450
19		56
20		27
21		3.1



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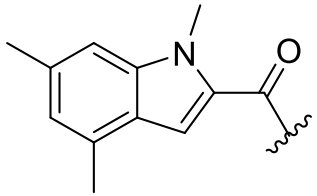

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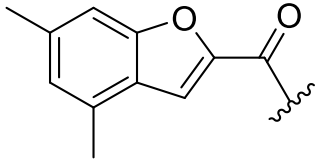

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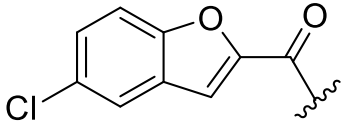

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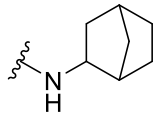
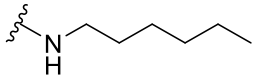
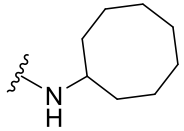
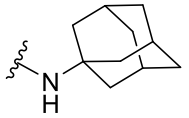
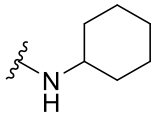
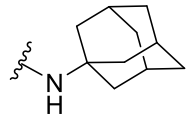
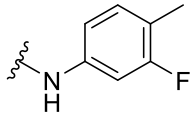
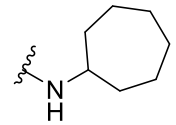
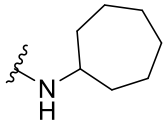
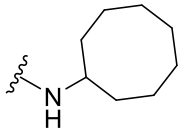
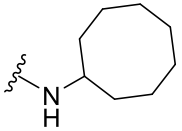
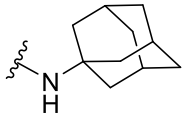
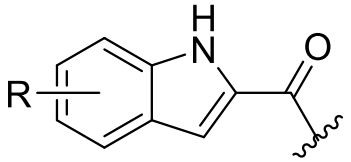
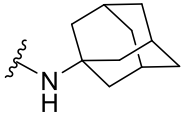
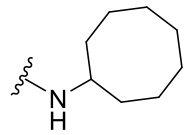
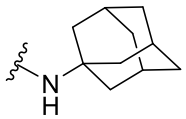
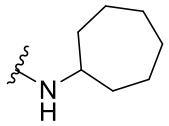
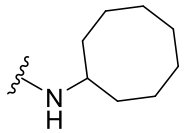
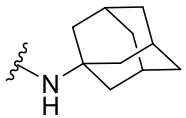
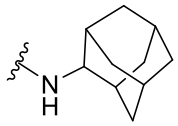
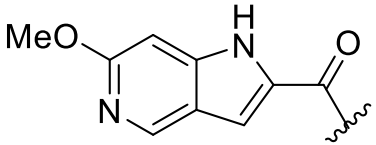
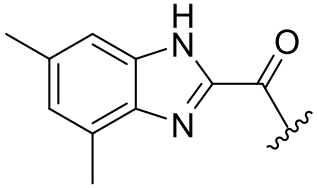
Compd	R	MIC (μM)
22		113
23		59
24		26
25		>388

Compd	X	R	MIC (μM)	Compd	X	R	MIC (μM)
26	H		>528	32	6-OH		13
27	H		477	35	4,6-bis (CF_3)		0.64
28	4,6-difluoro		0.86	36	4,6-bis (CF_3)		0.04
29	4,6-difluoro		0.10				
30	6-OMe		0.77				
31	5-Cl		0.38				

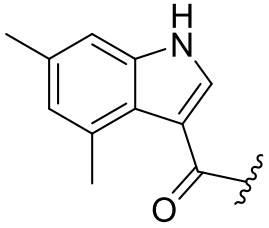
Compd	R	MIC (μM)
33		6.6
34		1.5
37		>224
38		1.7
39		0.39
40		1.5



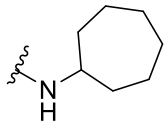
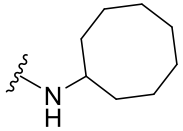
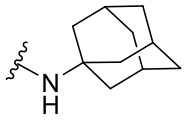
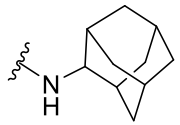
33, 34



37-40



41-44

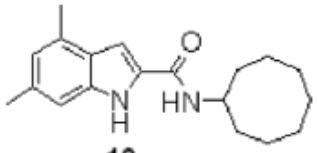
Compd	R	MIC (μM)
41		~200
42		~200
43		~200
44		~200

Efficacy against resistant strains

compd	MIC[d] (μM)				
	V4207 (DS)[a]	TF274 (XDR)[b]	R506 (XDR)[b]	KZN494 (MDR)[c]	V2475 (MDR)[c]
3	0.93	0.46	0.46	3.7	0.93−1.9
11	0.11	0.055	0.055	0.11	0.11
12	0.026	0.026	0.0067	NT[e]	NT[e]

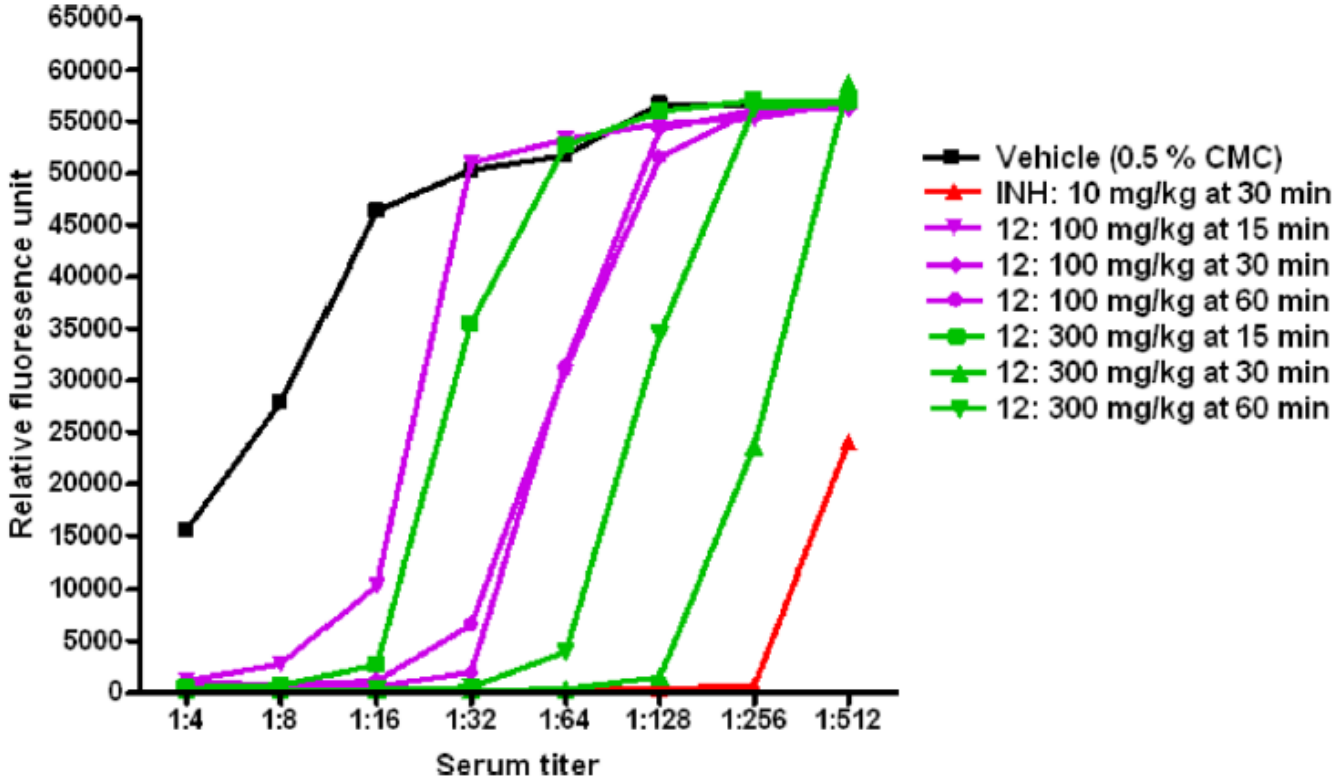
Values shown indicate the lowest concentration of leading to at least 90% reduction of bacterial growth in a microplate Alamar Blue assay.
Values an average of 3 measurements.

Ex vivo results



12

nNH = 2
nON = 3
MW = 298.4
TPSA = 44.89
ClogD = 5.32
nRot. bond = 2

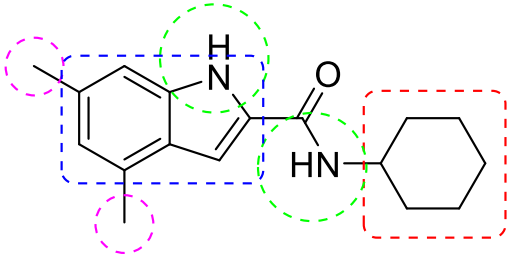


Serum inhibition titration for compound 12. BALB/c mice were dosed with two doses (100 and 300 mg/kg) **12**, blood was collected at 15, 30, and 60 min and the serum separated 60 min later. Growth inhibition of serially diluted serum on H37Rv was determined using the Alamar Blue assay. Vehicle 0.5% carboxylmethyl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