….from a therapeutic perspective:

How to better use PZA?

- Role in new drug regimens?
- Address weight banding issue
- Whether PZA-R translates into therapeutic outcome?
- Develop rapid diagnostics for PZA-R

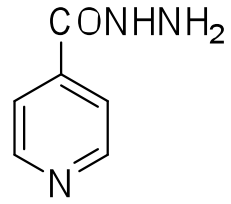
How to make a better PZA?

Discovery of a New Generation of Pyrazinamide

Working Group on New TB drugs:
"Essentiality of Pyrazinamide" Workshop
Bethesda
June 1, 2011

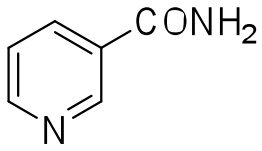
Background

Isoniazid

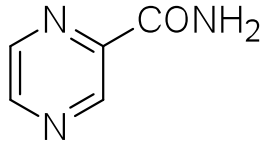


Chemical Formula: $C_6H_7N_3O$
Molecular Weight: 137.14
CLogP: -0.668

Nicotinamide



Pyrazinamide



Chemical Formula: $C_5H_5N_3O$
Molecular Weight: 123.11
CLogP: -0.67632

- Discovered in 1952 by testing limited nicotinamide analogs in mouse (Dessau, et al., Am. Rev. Tuberc., 1952, 65, 635)
- Pro-drug – requires activation by pyrazinamidase (PncA) to pyrazinoic acid
- Narrow spectrum – mycobacteria only
- Low, pH dependent activity – MIC ≥ 16 µg/ml (at pH 5.5)
- Potent *in vivo* efficacy against chronic but not acute infection in mice
- Dose-limiting toxicities – hepatotoxicity and arthralgia

Therapeutic Role of PZA – Current TB Treatment

Drug-sensitive TB

- Key component for 1st line therapy; responsible for shortening therapy from 9 to 6 months
- Unclear role in continuation phase

Drug-resistant TB

- Part of MDR therapy, although ~ 50% MDR isolates are PZA-resistant
- No rapid diagnostics/slow drug susceptibility test

Latent TB Infection

- PZA in combination with RIF can shorten therapy to 2 months, but with unacceptable hepatic toxicity

Therapeutic Role of PZA – Future TB Treatment

Synergistic with virtually all new drugs in development

- Demonstrated in mouse models: moxifloxacin, clofazimine, TMC207, PA-824, PNU100480, etc.
- To be confirmed in human EBA trials: TMC207/PZA and PA-824/PZA

Unique activity profile suggests niche environment

- Unconventional potency profile
  - More active under acidic conditions in vitro
  - More efficacious in mouse chronic infection model
- Further enhances bactericidal and sterilizing activity on top of almost any regimens

Will continue to play an important role in future TB regimens

Major Issues of PZA

Drug Resistance

- About 50% MDR clinical isolates are PZA-resistant
- Lack of rapid diagnostics/susceptibility tests

Toxicity Limitation

- Hepatic toxicity - narrow therapeutic window

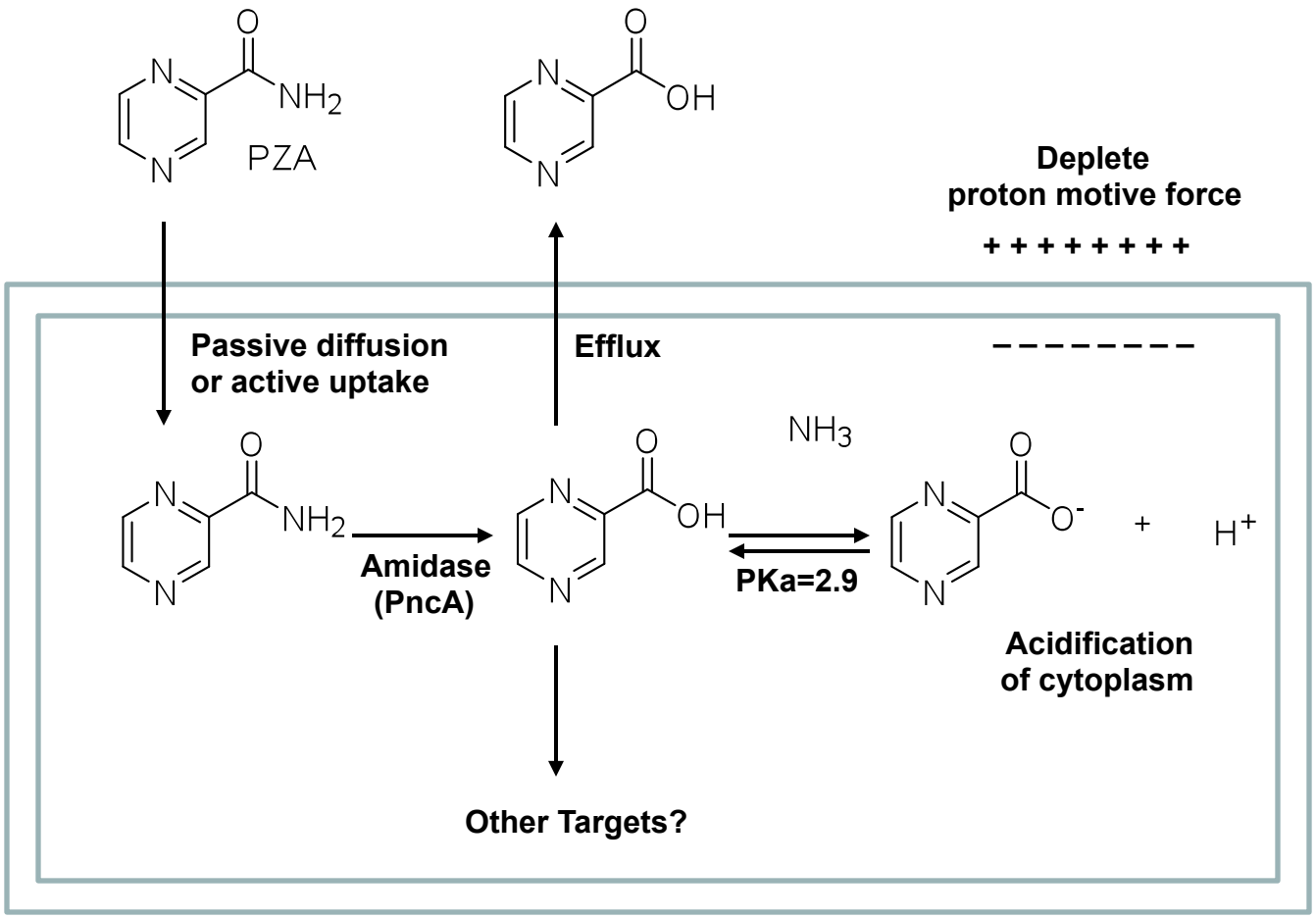
Weight Banding

- Currently 2-4 weight bands used
- Difficulty for compliance and development of FDC

PZA Program: Target Profiles

Profile	Ideal	Acceptable
Target population	Both PZA-S and PZA-R	PZA-S only
Mood of action	Same as PZA, but overcome PZA-R mechanisms	Same as PZA; cross-R with PZA
Efficacy	More potent than PZA, lower dose required	Same as PZA, but wider therapeutic window
Safety/Tolerability	Better than PZA	Same as PZA, but wider therapeutic window
PK/PD	Once daily dosing	Once daily dosing
Dose Regimen	Lower dose than PZA; no weight banding	No weight banding
DDI	No DDI with ARVs	Minor DDI with ARVs, but no dose adjustment needed
COGs	Same as PZA	Multiples of PZA

MoA of PZA – A Working Hypothesis



Some key publications:

Boshoff HI, Mizrahi V. J Bacteriol. 2000; 182(19): 5479-85.

Zhang Y ; Mitchison D, Int. J. Tuberc. Lung. Dis., 2003, 7: 6-21.

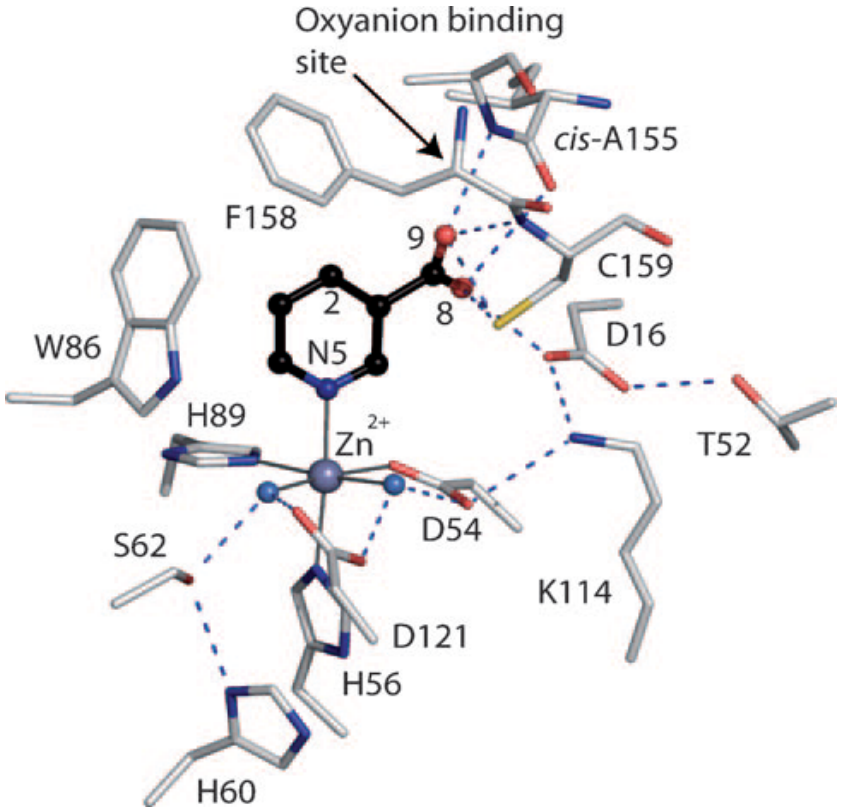
Ngo SC, et al., Antimicrob. Agents Chemother., 2007, 51: 2430.

Potential Approaches to Make a Better PZA?

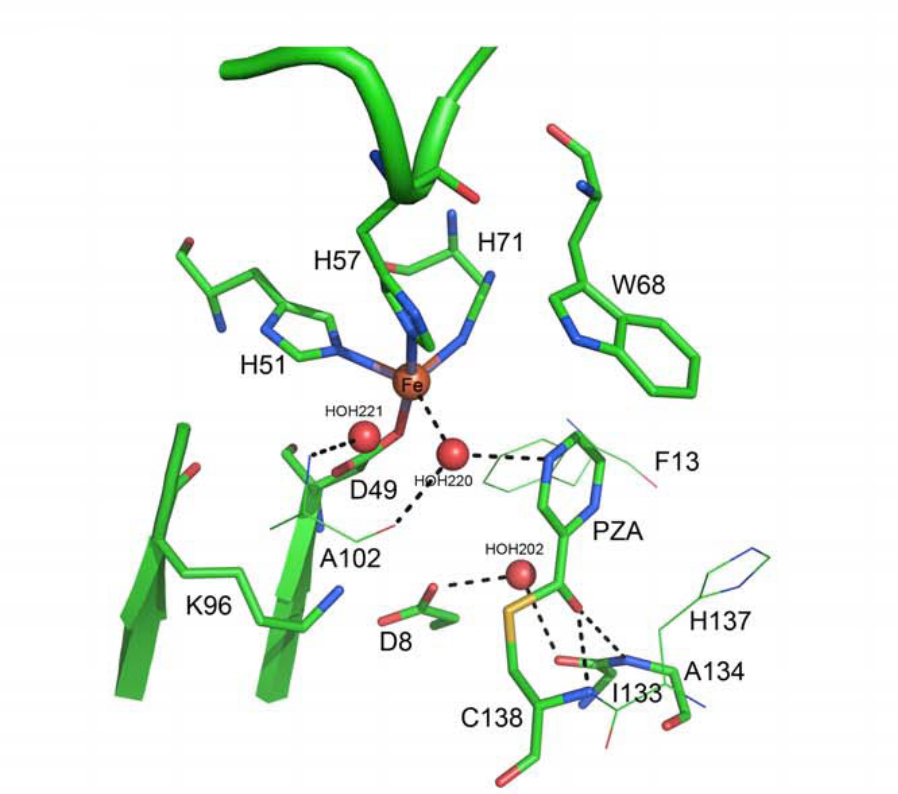
Approach	Associated Tool
▪ To enhance rate of PncA mediated hydrolysis	▪ PncA biochemical assays; kcat/Km determination
▪ To optimize structure of the acid – minimize efflux	▪ Mtb whole-cell assays at pH 5.2 and 6.7
▪ To overcome PncA mutation and PZA-R	▪ PncA knock-out and PZA-R mutant strains

- Mouse chronic infection model to assess efficacy

New Tools: Crystal Structure of PncA

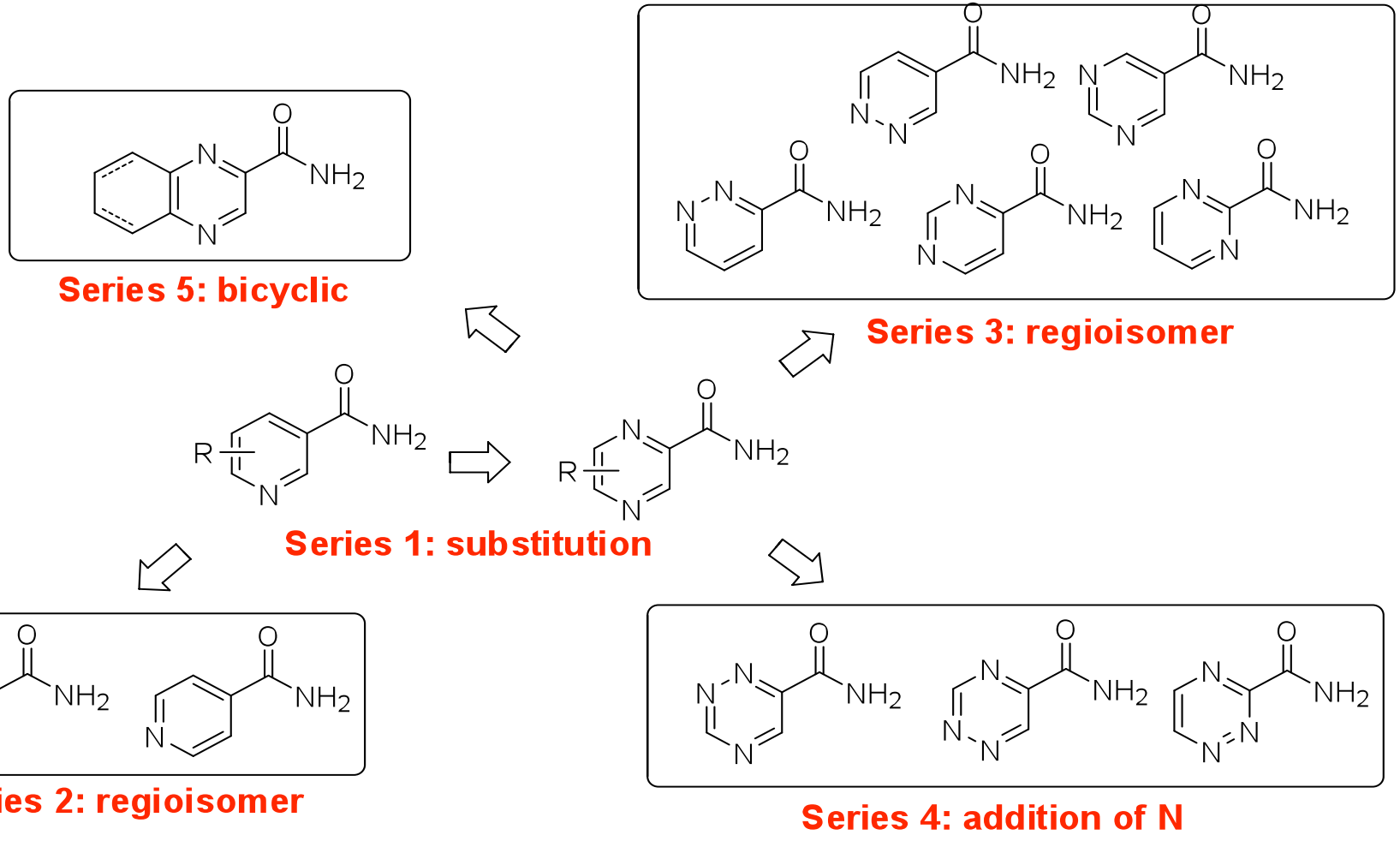


Specificity and mechanism of *A. baumanii* nicotinamidase: implications for activation of the front-line tuberculosis drug pyrazinamide.
Fyfe et al. Angew Chem Int Ed Engl. 2009; 48(48): 9176-9.



Model of the putative acyl-enzyme intermediate formed between PZA and the side chain of Cys138 of Mtb PncA.
Petrella et al, PLOS One. 2011 ; 6(1) : e15785

Initial Medicinal Chemistry Approaches



- Impact PncA activity, efflux and other target binding affinity

Progress to Date

More than 200 compounds made to date leading to three distinct groups of interest:

Group I – both pH and PncA dependent activity (PZA-like)

- 5 compounds
- All are equal or better PncA substrates than PZA (low Km, high kcat)
- pH dependent activity against WT but not active against PncA KO

Group II – pH dependent, but PncA independent activity

- 8 compounds
- not PncA substrates
- with pH dependent activity against both WT and PncA KO
- activated by an alternative amidase?

Group III – both pH and PncA independent activity

- 8 compounds with MIC < PZA

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