Grand Challenges
African Drug Discovery Accelerator

Drug Discovery and Development Course

Hit-to-Lead Med Chem Optimization Case Studies

Greg Basarab

Supported by

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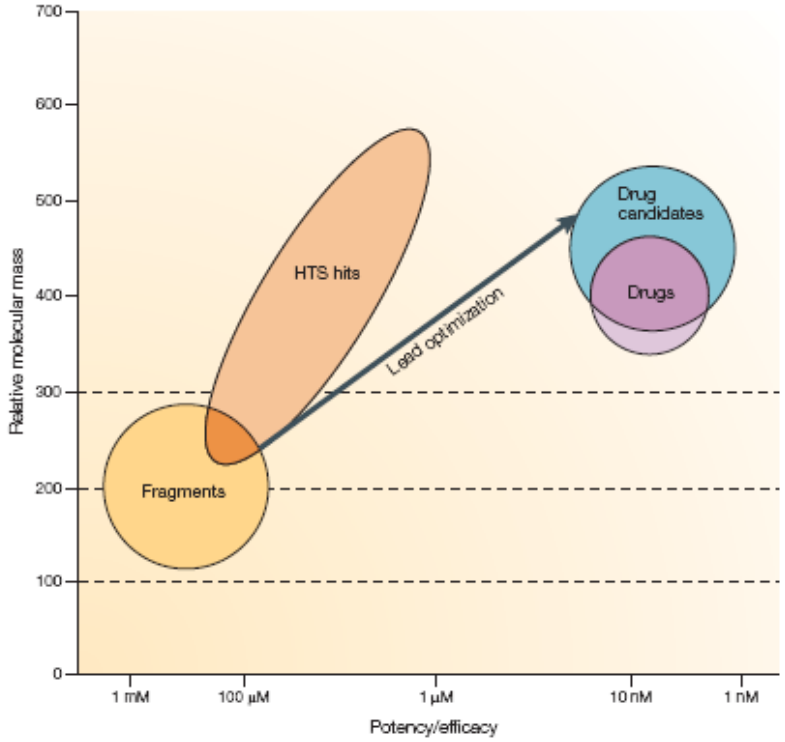
Learning Objectives

- Fragment-based lead generation
- Phenotypic Screening
- Considerations around hits and ligand efficiency (LE); lead optimization of ligand lipophilicity efficiency (LLE)
- Drug repurposing/repositioning

Program

- Fragment-based Hit ID and H2L
- Phenotypic Screening Hit ID and H2L
- Drug repurposing

Fragment Screening



- Fragment Screening and Hits
  - Medium throughput (100-2000 cmpds)
  - Weak potency
  - More MW 'headroom' for elaboration
  - Generally high ligand efficiency
  - Guidance from protein structure/molecular modeling
- Screening strategies
  - Enzyme inhibition
  - NMR methods (waterLOGSY, STD-NMR, ILOE, ^{1}H-^{15}N HSQC)
  - Isothermal titration calorimetry (ICT)/Microscale Thermophoresis (MST)
  - X-ray crystallography

The challenge inevitably becomes maintaining drug-like properties while designing potency improvements

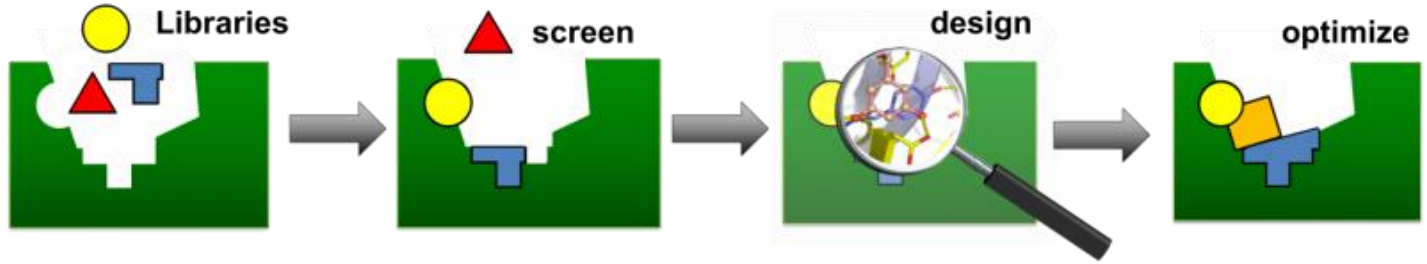
Congreve, M et al. H. *Drug Disc. Today*, 8(19), 2003, 876

Fragment Screening

Rule of three: begin small ⇒ compound growth will (generally) be inevitable

Lead-like molecules for FGLD – emphasis on ligand efficiency:

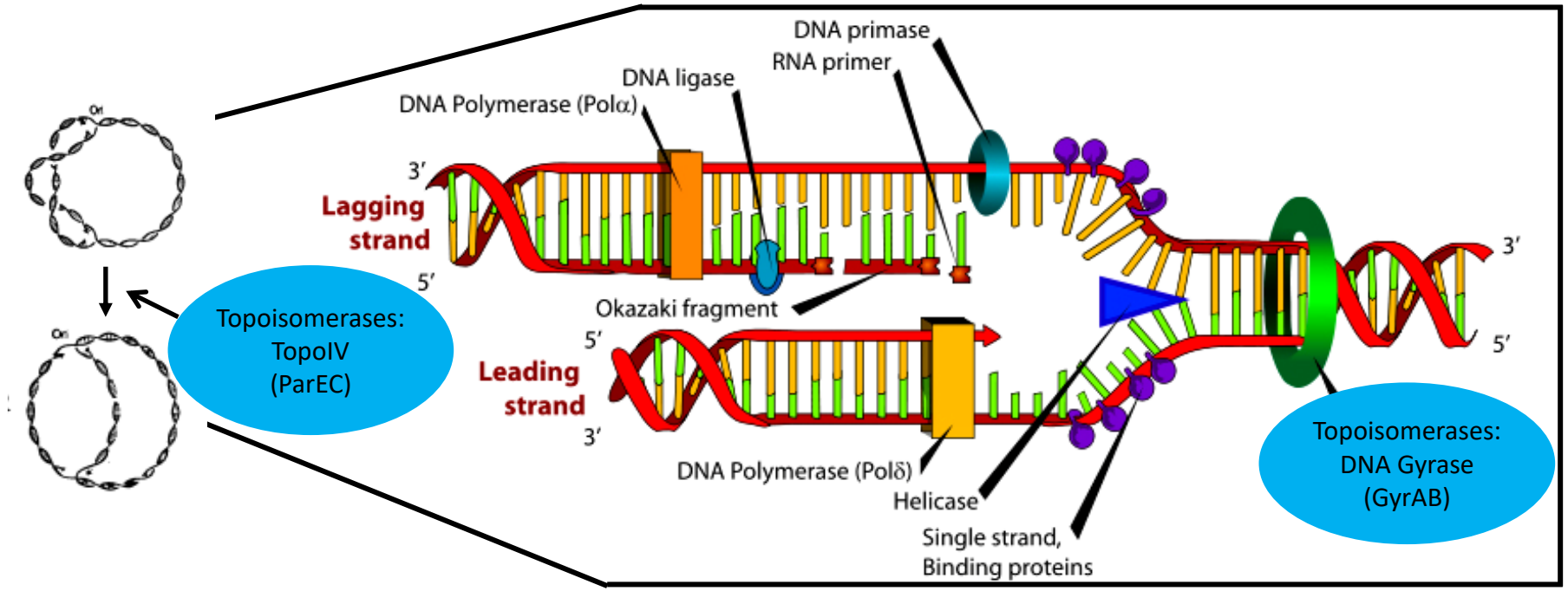
- **≤ 3 H-bond donors**
- **≤ 3 H-bond acceptors**
- **MW ≤ 300 Da**
- **LogP ≤ 3**
- **# rot bonds ≤ 3**
- **PSA ≤ 60 Å²**



Considerations of ligand growing and linking strategies

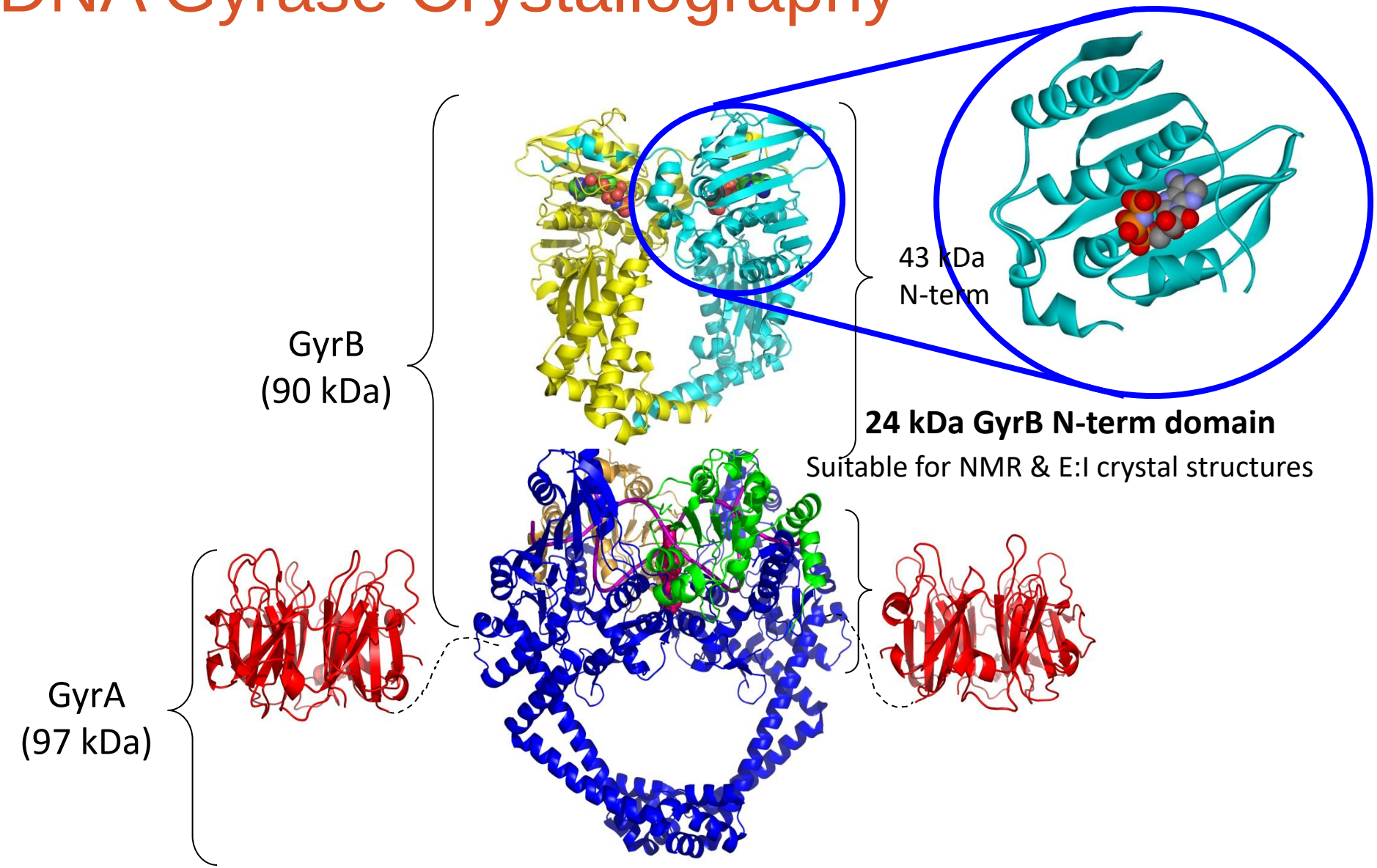
Jhoti et al. *Nat. Rev. Drug Disc.* 12(8), 2013, 644; Johnson et al. *J. Med. Chem.*, 2016.

Case History: Bacterial Topoisomerases



- DNA gyrase & Topo IV are among many enzymes essential for DNA replication & cell viability
- DNA gyrase ($GyrA_2B_2$) supercoils DNA ahead of replication fork to relax torsional strain
- Topo IV ($ParC_2E_2$) relaxes and decatenates DNA beyond replication fork
- Due to high homology – inhibitors of DNA gyrase and Topo IV crossover to one another

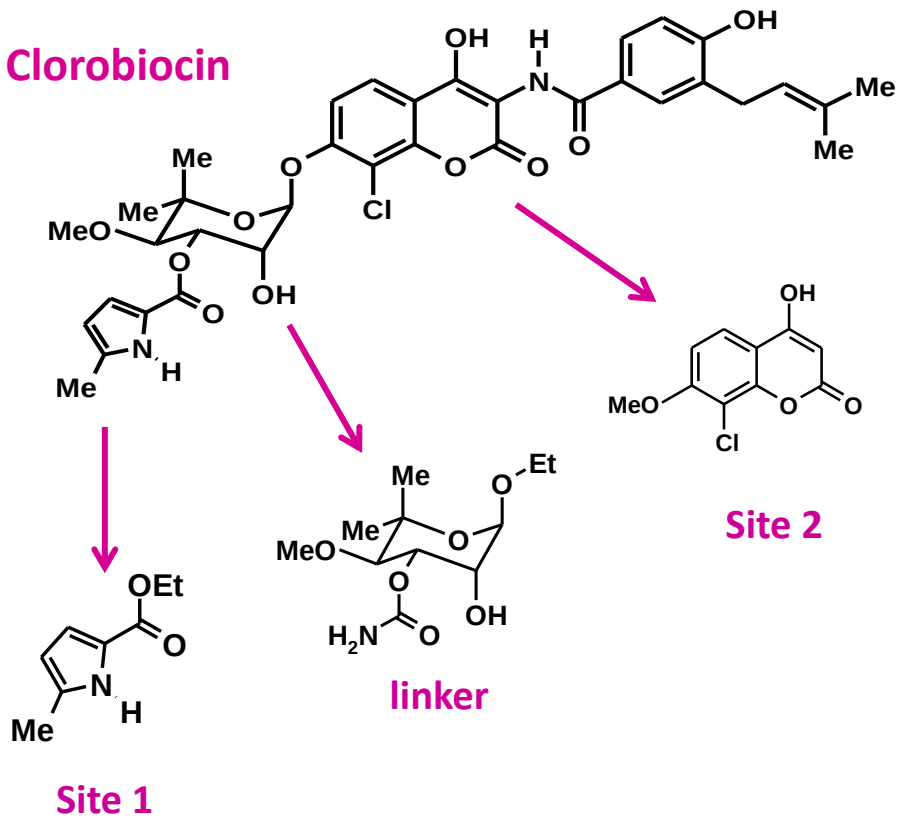
DNA Gyrase Crystallography

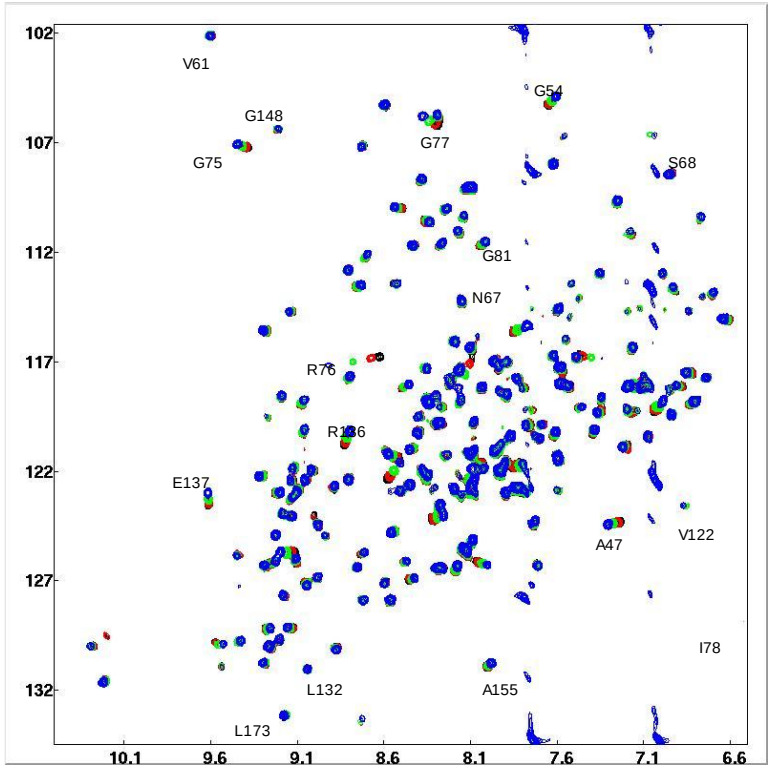


Wigley, et al., Nature, 1991; Bax et al., Nature, 2010; Corbett et al., PNAS, 2004

Fragment-based Hit Identification

- **Screened 960 small molecules for binding to *E. coli* GyrB-24 using NMR**
- **Fragments of known inhibitors provided the most potent hits with K_d values from 10 to 950 μM**





Eakin, A. et al *AAC 56(3)*, 1240, 2012

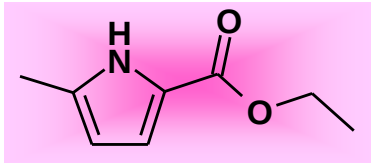
Two Fragments of Interest

Models: Pyrrole at Site 1 – key H-bond and water bridge to Asp81

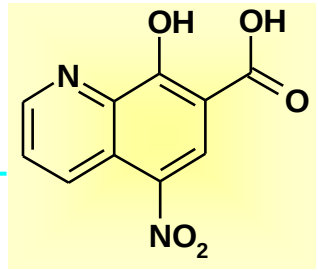
Quinoline acid @ Site 2 – key salt bridge with Arg 144, π-stack w/ Arg84

Site 1
Pyrrole Hit

K_d = 1070 μM
LE = 0.27



Linker



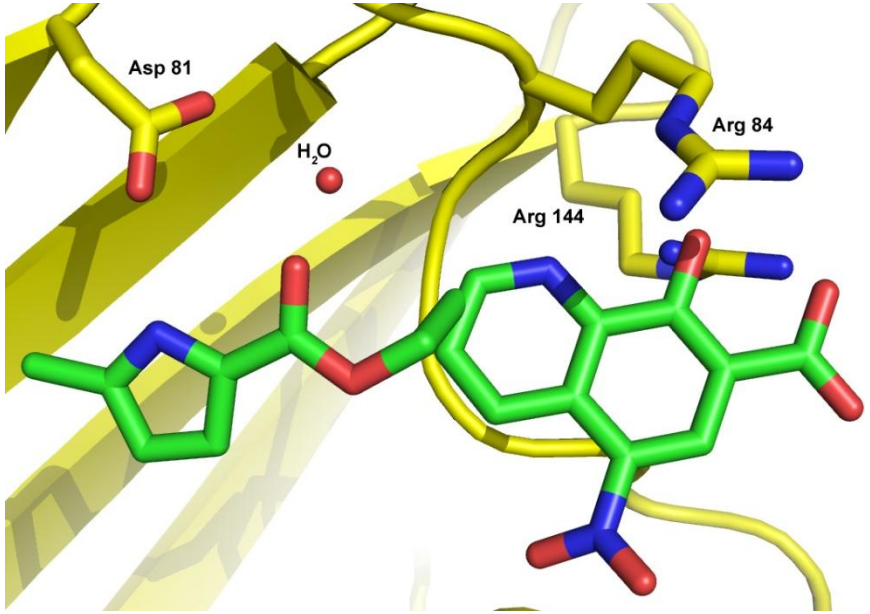
Site 2
Quinoline Hit

K_d = 5000 μM
LE = 0.22

Rule of 3 compliant?

- ✓ ≤ 3 H-bond donors (1)
- ✓ ≤ 3 H-bond acceptors (2)
- ✓ MW ≤ 300 Da (137)
- ✓ cLogP ≤ 3 (1.15)
- ✓ # rot bonds ≤ 3 (1)
- ✓ PSA ≤ 60 Å² (42)

Site 1 – adenine of ATP



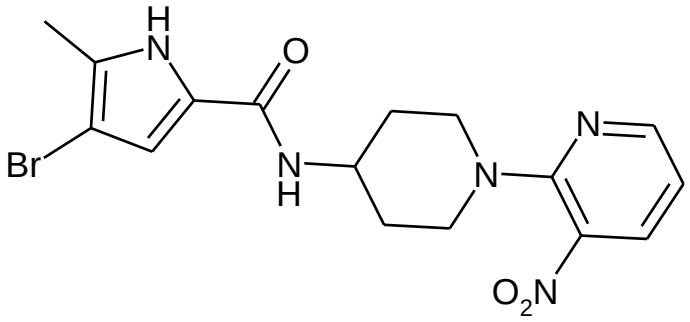
Site 2 – outside of ATP binding site

Rule of 3 compliant?

- ✓ ≤ 3 H-bond donors (1)
- ✓ ≤ 3 H-bond acceptors (5)
- ✓ MW ≤ 300 Da (234)
- ✓ cLogP ≤ 3 (0.19)
- ✓ # rot bonds ≤ 3 (2)
- ✓ PSA ≤ 60 Å² (116)

Eakin, A. et al *AAC 56(3)*, 1240, 2012

Best hit from library of pyrrole amides



ATPase IC_{50} (μM)

E. coli gyrase:	0.61	LE = 0.24
	K_i = 0.28	0.26
S. aureus gyrase:	1.4	0.23

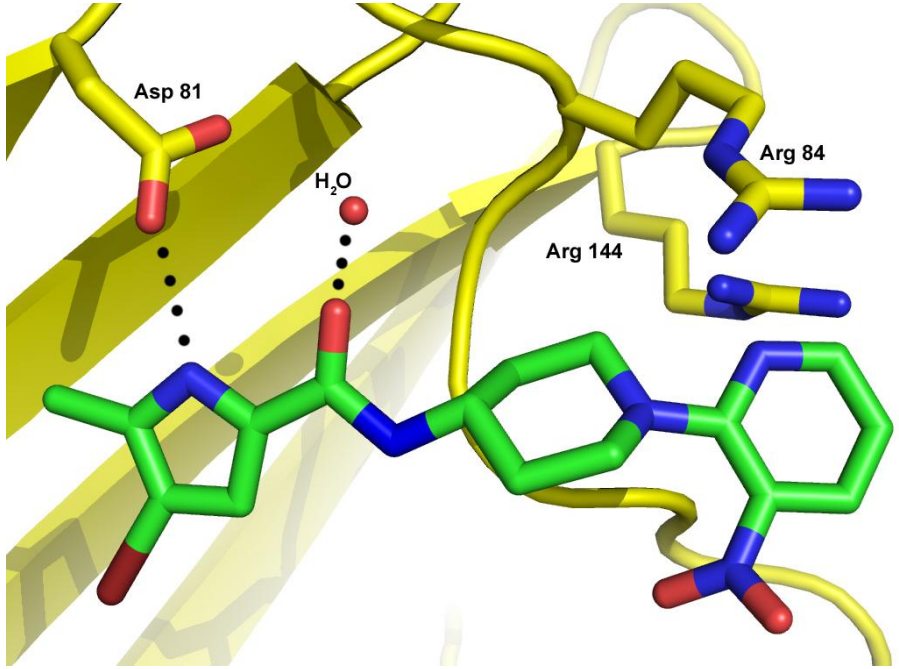
MIC (μg/ml)

S. pneumoniae:	>64
S. aureus	>64

Microsomal Clearance (mg/min/ml)

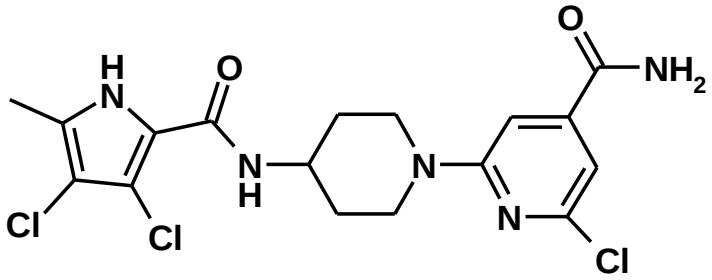
Human:	>100

GyrB:Pyrrolamide Crystal Structure



- **Pyrrole binds as predicted from NMR**
- **Linker and heterocycle extend into the Site 2 region**

Hit to Lead



Gyrase K_I (nM):

S. aureus = 19 *E. coli* = 9.6

LE = 0.30 LE = 0.32

MICs (μg/ml):

S. pneumoniae = 1

M. catarrhalis = 2

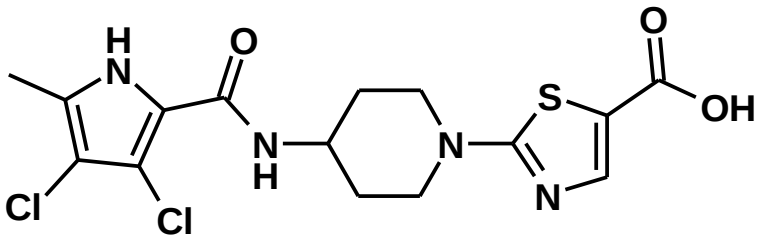
H. influenzae = 4

Physical Properties

MW = 431

logD = 3.4 μM

Solubility < 3 μM



Gyrase K_I (nM):

S. aureus = 18 *E. coli* = 4.4

LE = 0.30 LE = 0.33

MICs (μg/ml):

S. pneumoniae = 0.25

M. catarrhalis = 0.5

H. influenzae = 2

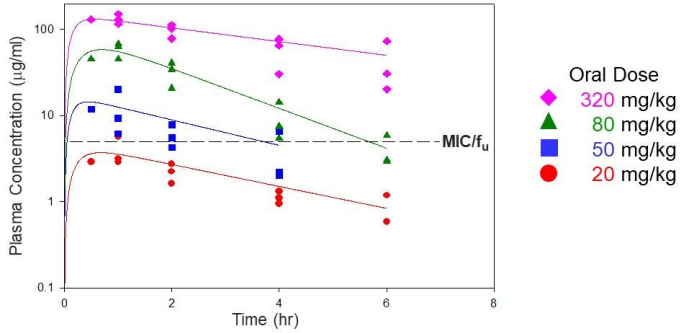
Physical Properties

MW = 403

logD = 0.02

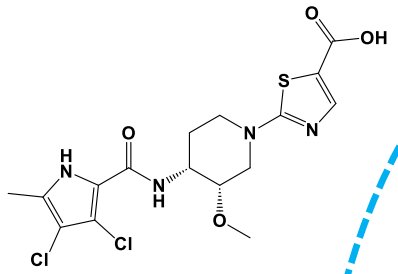
Solubility > 260 μM

Efficacy demonstrated on PO dosing
Mouse thigh model of *S. pneumonia* infection

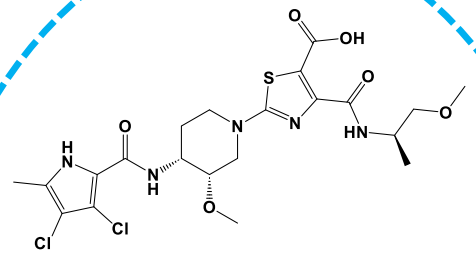


Eakin, A. et al *AAC 56(3)*, 1240, 2012

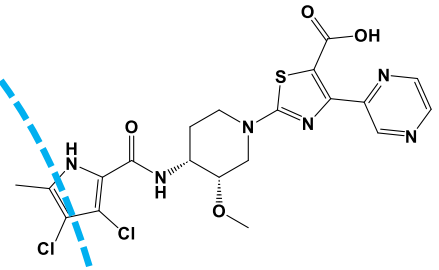
Development Candidate



(1)



(2)

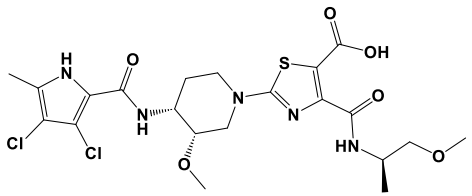


(3)

Gyr A Sau K_i (μM)	<10	<10	<10
ParE Eco K_i (μM)	240	73	54
WT Sau MIC (μg/ml)	0.32	0.036	0.031
MRSA MIC (μg/ml)	0.5	0.057	0.018
Spn MIC (μg/ml)	0.016	0.016	0.007
Solubility (μM)	ND	660	350
Rat Cl_u (ml/min/kg)	>1300	411	38
Bioavailability (%)	6.7	81	ND

Basarab, G. et al *J. Med. Chem. 57(14)*, 6060, 2014

Phase 1 IV PK Trial – AZD5099



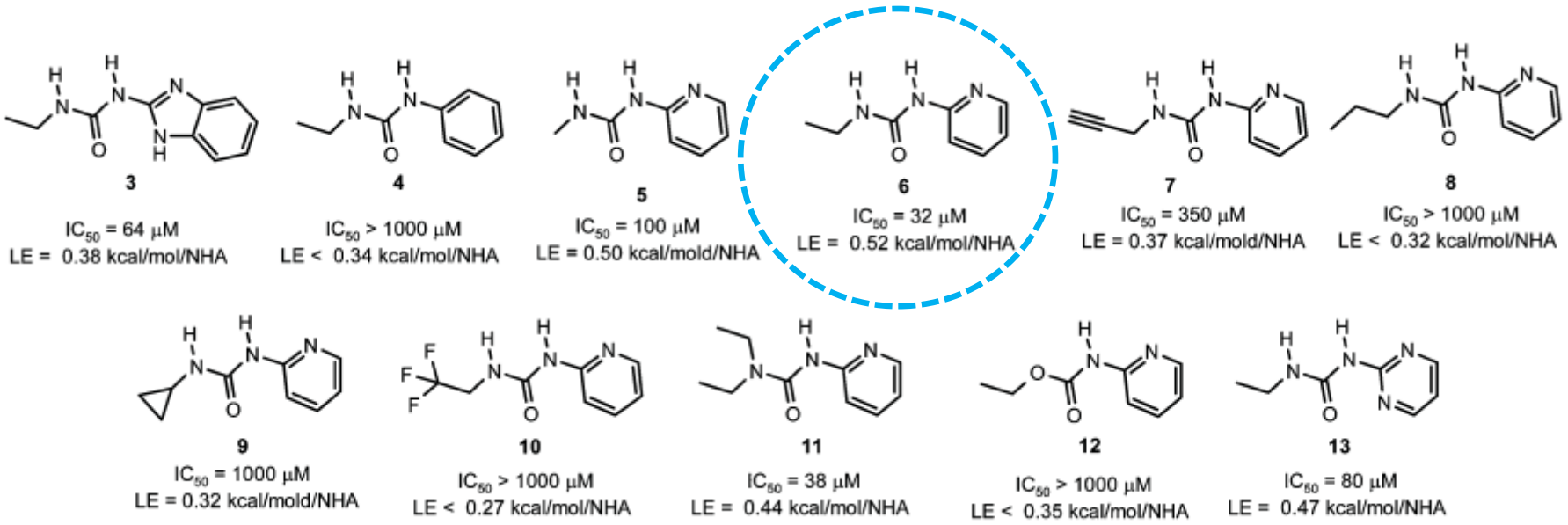
- no deaths, serious or severe adverse events, or events that called for discontinuation
- 10 (23.8%) active-treated volunteers reported at least one adverse event (diarrhea, headache, or dizziness)
- 7 (50%) placebo-treated volunteers reported at least one adverse event
- No safety concerns in clinical laboratory tests, vital signs, electrocardiograms, or physical examinations; no hypothermia or hyperthermia.

Product Vision: IV & oral step-down therapy for acute bacterial skin and skin structure infections and bacterial pneumonia caused by sensitive and drug-resistant Gram-positive and fastidious Gram-negative respiratory tract pathogens

Development stopped due to:

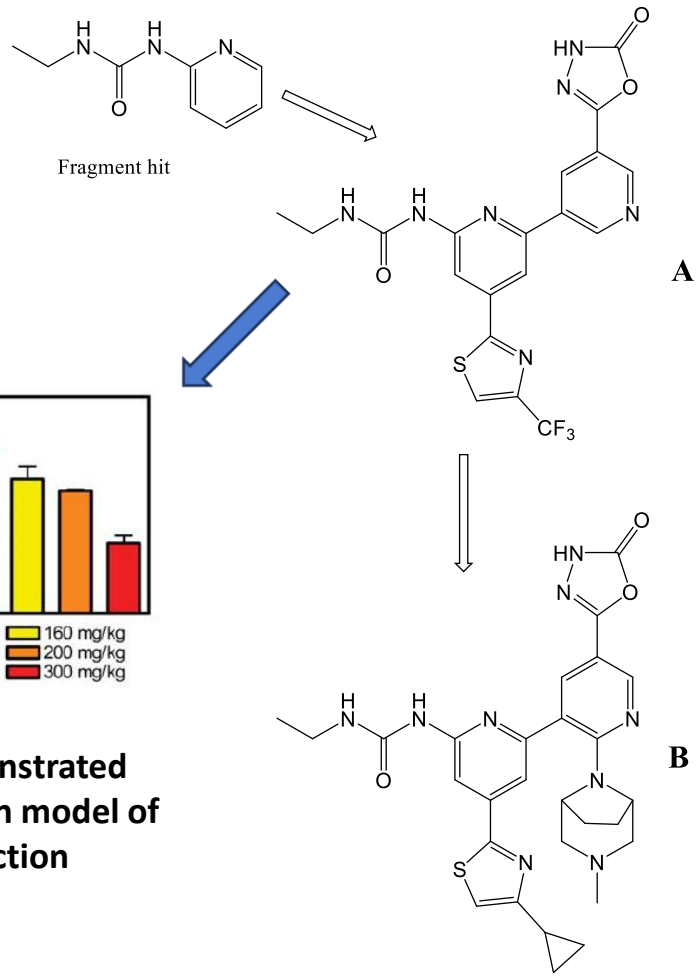
- High exposure variability in Phase 1 SAD study
- Swollen mitochondria signal in rat & dog liver tissue (28d GLP study)
- PK/PD magnitude decrease from continued mouse efficacy experiments
- Realignment of infectious disease commercial position

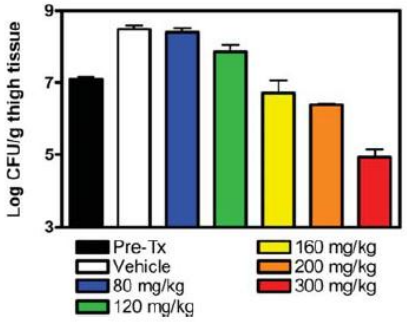
Second Fragment-Based Screen



Basarab, G. et al *J. Med. Chem. 56(21)*, 8712, 2013

Fragment hit to lead





Efficacy demonstrated in mouse thigh model of *S. aureus* infection

	A	B
GyrB *E. coli* IC_{50} (μM)	<0.01	0.002
ParE *E. coli* IC_{50} (μM)	0.23	0.003
WT *S. aureus* MIC (μg/ml)	0.04	<0.06
MRQR *S. aureus* MIC (μg/ml)	0.06	<0.06
MIC *S. pneumoniae* (μg/ml)	0.05	<0.06
MIC *E. coli* (μg/ml)	>64	0.57
MIC *A. baumannii* (μg/ml)	ND	0.5
MIC *P. aeruginosa* (μg/ml)	ND	1.1
Solubility (μM)	19	66
PPB human (%)	95.6	85.1
Rat Cl_u (ml/min/kg)	820	ND

Basarab, G. et al *J. Med. Chem. 56(21)*, 8712, 2013; Ho, S. W. et al. Eur. J. Med. Chem, 157, 610, 2018.

Phenotypic screening

- Uses physiological systems, e.g. cells, tissues or whole organisms
- Surveys compounds that act simultaneously on more than one target
- Assumes no knowledge of the molecular mode-of-action relying on empiricism
- Requires reverse genomics and proteomics to determine the target

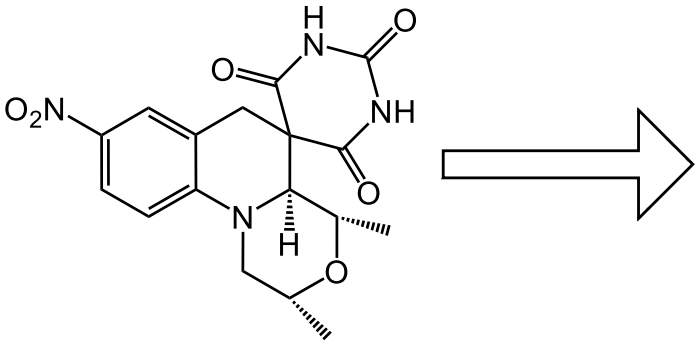
Phenotypic assays endpoints:

1) identify new drug candidates and their corresponding molecular mechanisms of action

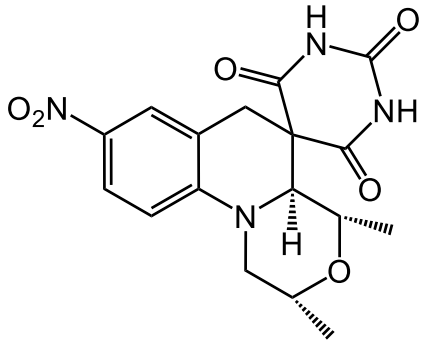
2) understand the underlying biology that will lead to identification of translation biomarkers

3) identify undesired effects related to toxicity of drug candidates

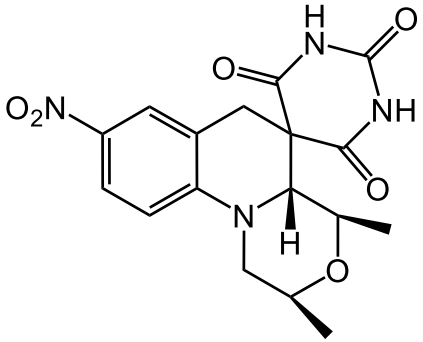
SPT Early History: Pharmacia-Upjohn



racemate
MIC 1.0 μg/ml *S. aureus*
4.0 *S. pneumoniae*



(-)-enantiomer
MIC 0.5 μg/ml *S. aureus*
2.0 *S. pneumoniae*



(+)-enantiomer
MIC >64 μg/ml *S. aureus*
>64 *S. pneumoniae*

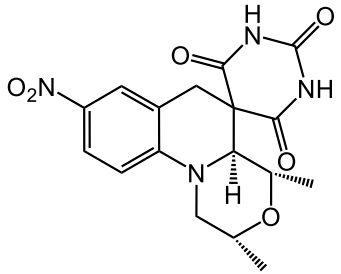
Drug Likeness
- ✓ ≤ 5 H-bond donors (2)
- ✓ ≤ 10 H-bond acceptors (6-9)
- ✓ MW ≤ 500 Da (374)
- ✓ cLogP ≤ 3 (-0.59)
- ✓ # rot bonds ≤ 10 (1)
- ✓ PSA ≤ 133 Å^2 (133)

Issues
- Nitro group
- High serum MIC shift

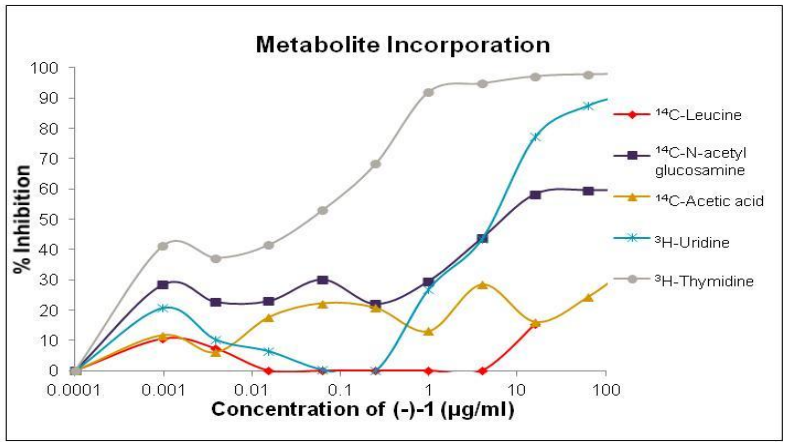
- PNU-286607 ID'd via phenotypic screening against *S. aureus* & *E. coli*
- Favorable PK properties – Cl_p = 12 ml/min/kg; Cl_u = 39 ml/min/kg, F = 95%
- Efficacy in mouse thigh model of *S. aureus* infection
- Activity resides in (-)-enantiomer

Miller, A. A., et al. *AAC*, 2806-12, 2008.

SPT Early History: Pharmacia-Upjohn



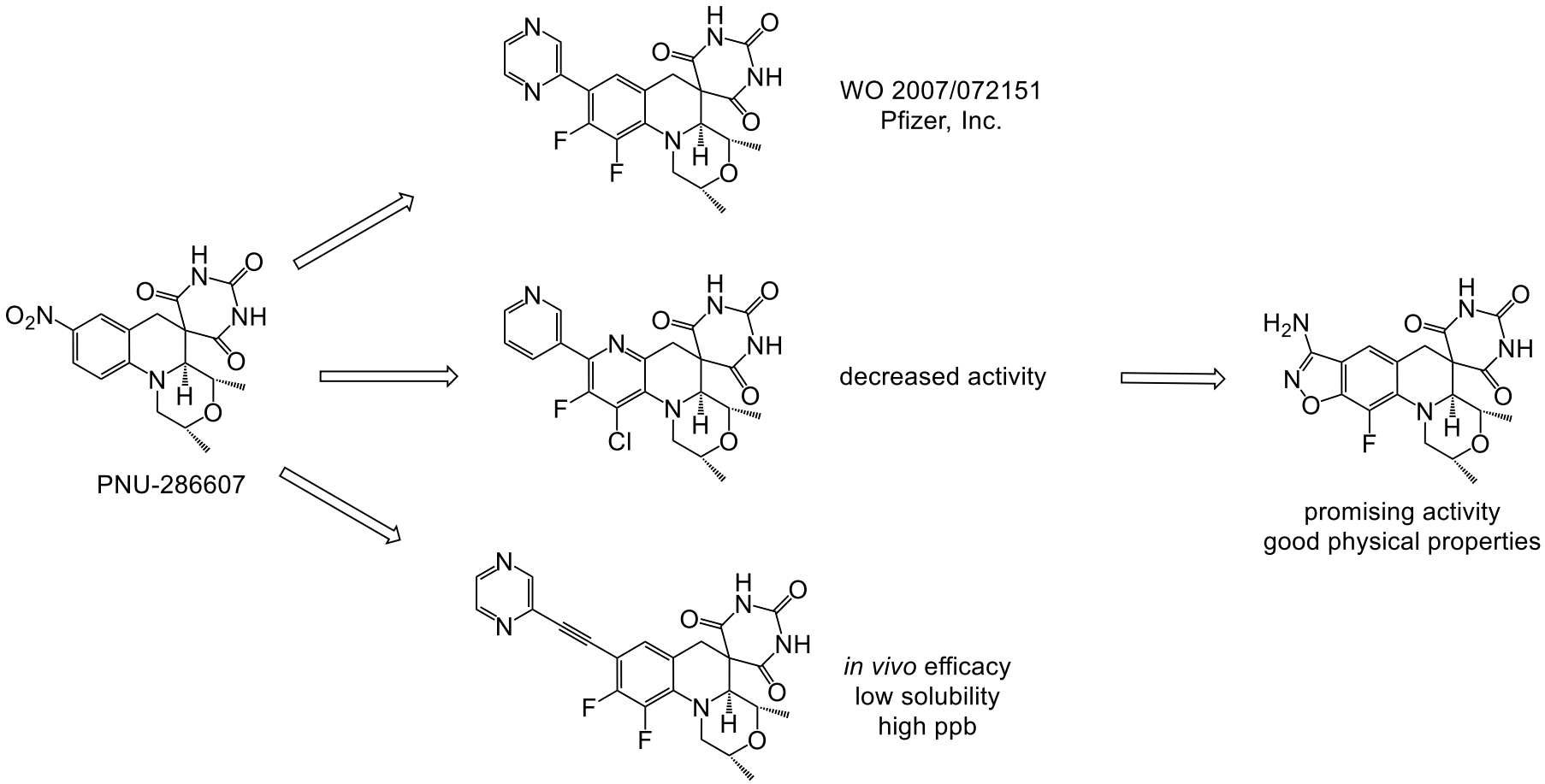
PNU-286607



- Labelled metabolite incorporation implicated DNA biosynthesis
  - Lab resistant strains ⇒ mutations in DNA gyrase
- Lab resistant strains ⇒ single point mutations in gyrB (Asp437) – near DNA cleavage site
- No cross-resistance to other DNA gyrase inhibiting antibacterials including ciprofloxacin & novobiocin

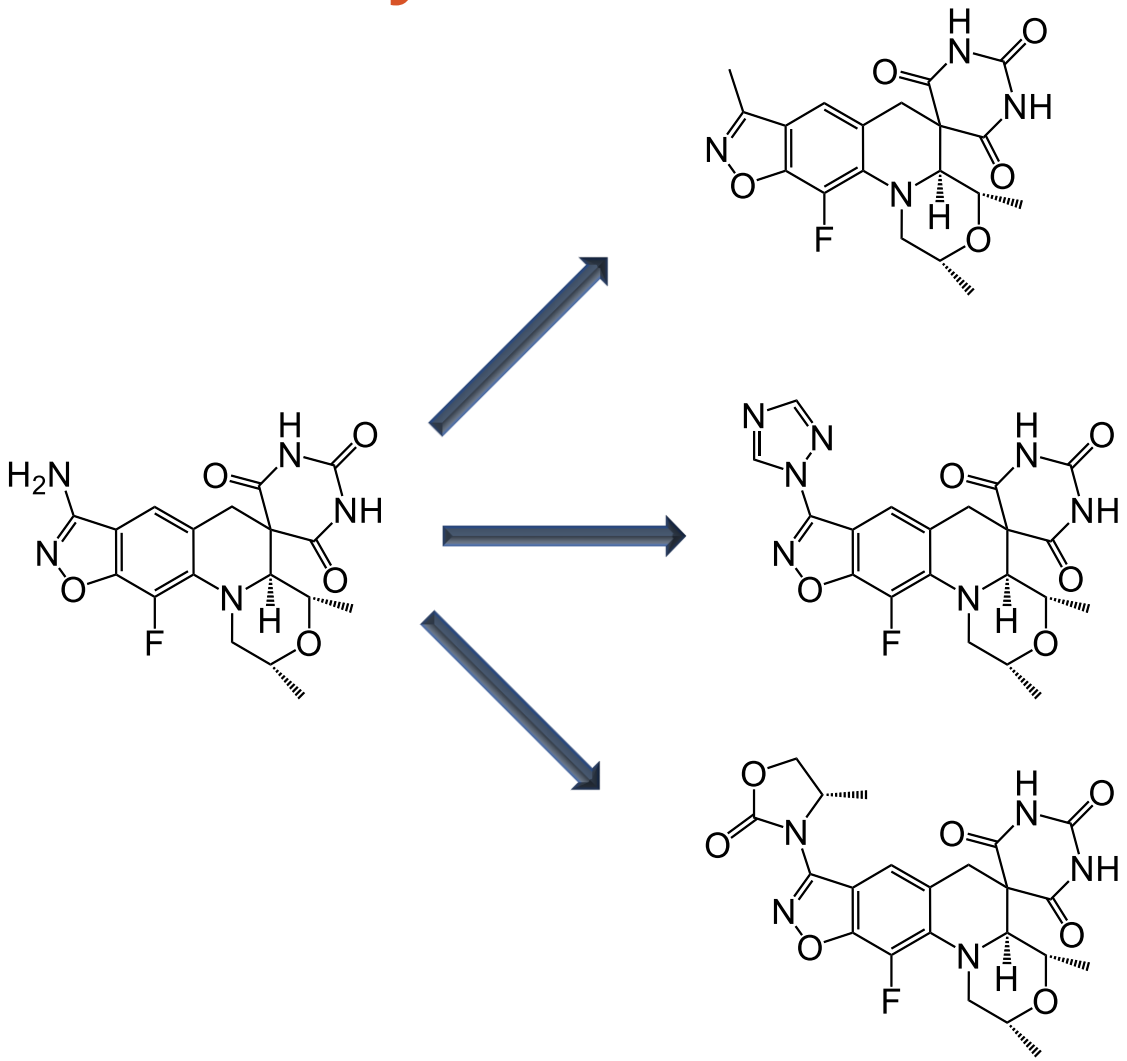
Basarab, G. et al *AAC. (2)*, e02192-21, 2022

Hit to Multiple Leads



Basarab, G.. et al. *Org. Lett* 16(24), 6456-59, 2014; Basarab, G. et al. ICAAC, 2012.

A Brief History of Time



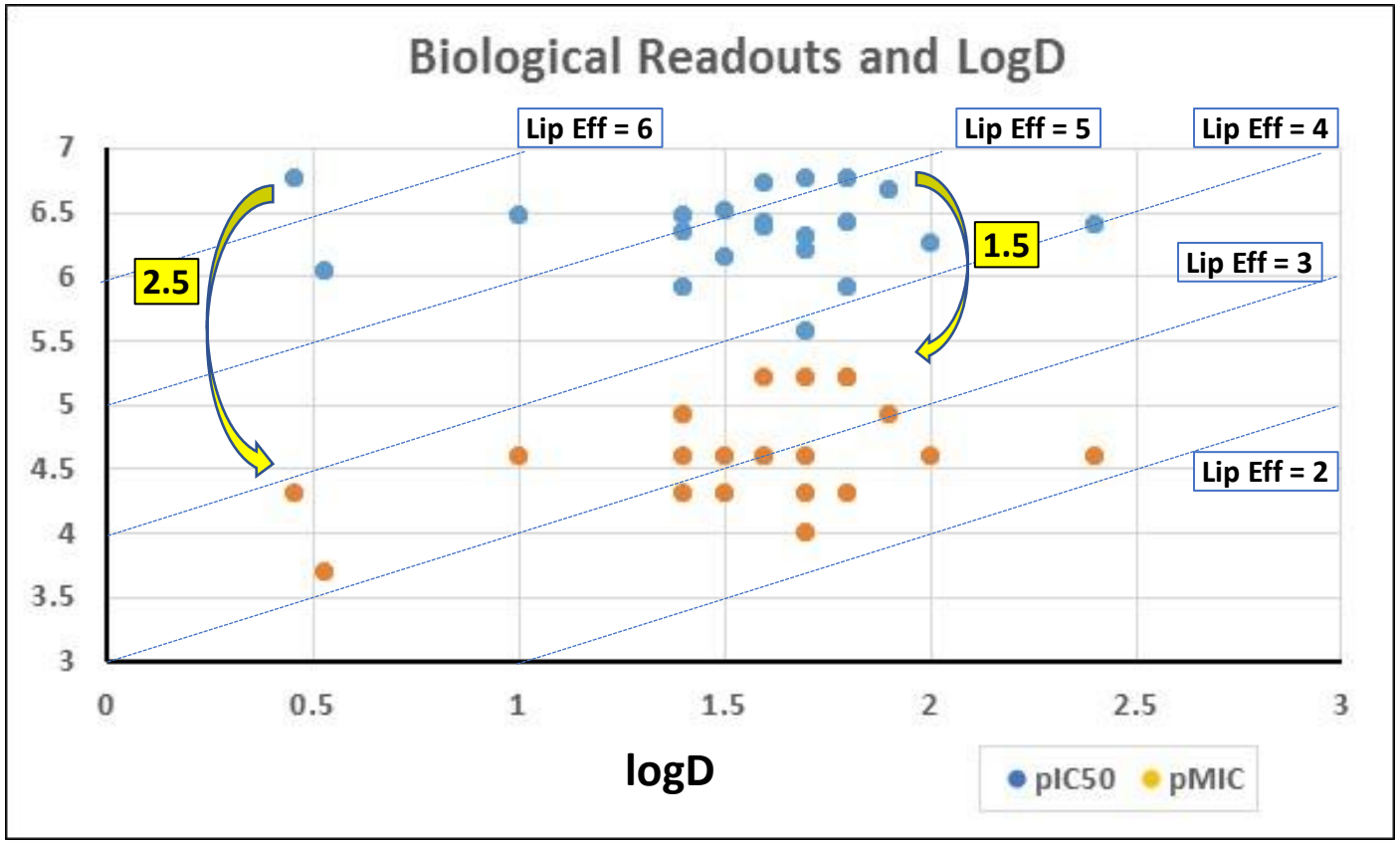
Oral MRSA agent	
Sau, Spn, Spy MICs (μM)	1.5, 6.2, 3.5
Sol (μM)	200
ppb (%free)	11
Rat/Dog Cl (ml/min/kg)	31/5.3
MLA genotoxicty (μM)	50
Erythrocyte IC_{50} (μM)	NT
In vivo efficacy	yes

IV/PO Gram-(+) agent	
Sau, Spn, Spy MICs (μM)	0.32, 0.45, 0.33
Sol (μM)	80
ppb (%free)	13
Rat/Dog Cl (ml/min/kg)	9.1/5.9
MLA genotoxicty (μM)	6.2
Erythrocyte IC_{50} (μM)	6
In vivo efficacy	yes

Zoliflodacin (PO *N.g.*, G+)	
Sau, Spn, Spy MICs (μM)	0.57, 0.43, 0.36
Sol (μM)	390
ppb (%free)	17
Rat/Dog Cl (ml/min/kg)	22/3.7
MLA genotoxicty	N
Erythrocyte IC_{50} (μM)	>100
In vivo efficacy	yes

Basarab, G. et al. *J. Med. Chem.* 57, 9078-95, 2014; Basarab, G. et al. *J. Med. Chem.* 58, 6264-82, 2015

LipE – Target and Whole Cell Activity



Note the use of LogD and not cLogP

LLE: pIC_{50} – logD
MIC-LLE: pMIC – logD

} Skew analog series to higher polarity

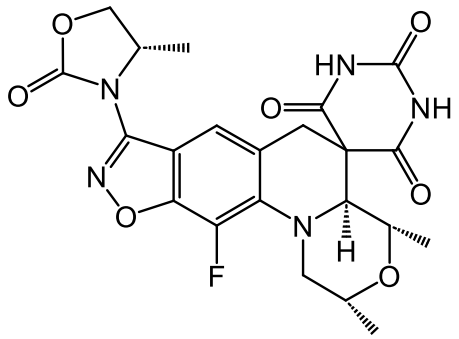
Lipinski ‘rule of five’

- ≤ 5 H-bond donors
- ≤ 10 H-bond acceptors
- MW ≤ 500 Da
- LogP ≤ 5

Others

Rot. Bonds <10

TPSA <140



MW = 487	Fraction sp^3 = 0.52
2 H-bond donors	LE = 0.19
8-12 H-bond acceptors	BEI = 0.014
cLogP = 0.76	SEI = 0.047
clogD = 1.6	LiPE = 6.0
Rot. Bonds = 1	PFI = 3.6
TPSA = 143	LELP = 4
Solubility = 390 μM	Dose # = 0.011 (by PK/PD) = 0.074
F = 46% mouse 34% rat 71-100% dog 58% monkey ??? human	

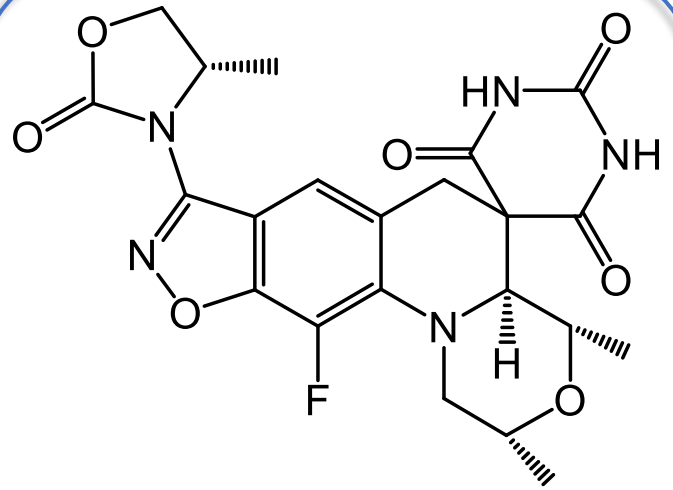
Oral Treatment for *N. gonorrhoeae*

Vision

- Oral single dose treatment of uncomplicated gonorrhea

Attributes

- Spiropyrimidinetrione (SPT): first-in-class non-quinolone topoisomerase chemotype and mode-of-action
- Highly potent against *Neisseria gonorrhoeae*
- Addresses evolving unmet medical need
- FDA endorsed Ph2/3 study design



zoliflodacin
AZD0914\ETX0914

Current Status

- Phase 1: PK, safety, tolerability in healthy volunteers, completed March 2014
- Phase 1: ADME w/ healthy volunteers, completed February 2015
- Phase 2: Uncomplicated gonorrhea in patients, completed December 2015
- Phase 3: Uncomplicated gonorrhea met endpoint (November 2023)
- NDA application in progress

Drug Repurposing/Repositioning/Modification

Investigation of existing or shelved/failed drugs for new therapeutic purposes or modification of an existing entity to better fit the new therapeutic purpose

Compound	Initial indication	Repurposing
Sildenafil	Angina	Erectile dysfunction
Gemcitabine	Antiviral	Cancer
Doxycylcline	Periodontic	Rosacea, antimicrobial
Minoxidil	Hypertension	Hair loss

Drugs get shelved drugs due to:

- Insufficient efficacy
- Commercial failure
- Re-evaluation of market potential
- Costs and difficulty of clinical trials

Compound	Primary disease	Repositioning
Acetylsalicylate	Mild analgesic	Antithrombotic
Amantadine	Influenza	Parkinson's disease
Bleomycin	Various cancers	Pleural effusion
Cycloserine	Tuberculosis	Urinary tract infections
Cyclosporin	Transplant rejection	Rheumatoid arthritis; Psoriasis
Eflornithine	Facial hirsutism	Sleeping sickness
Finasteride	Benign prostate enlargement	Male pattern baldness; Hersutism in women
Histrelin	Prostate cancer	Precocious puberty
Infliximab	Ulcerative colitis; Crohn's disease	Rheumatoid arthritis; Psoriasis
Interferon alfa	Hepatitis B and C	Various cancers
Retuximab	Various cancers	Rheumatoid arthritis
Bleomycin	Various cancers	Pleural effusion

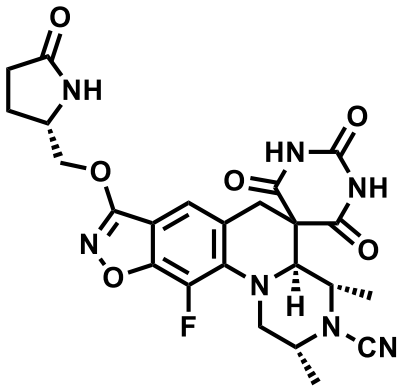
SPTs for *Mycobacterium tuberculosis*

Entasis (AstraZeneca spinoff) provided 24 SPTs for evaluation against *M. tuberculosis* (*Mtb*)

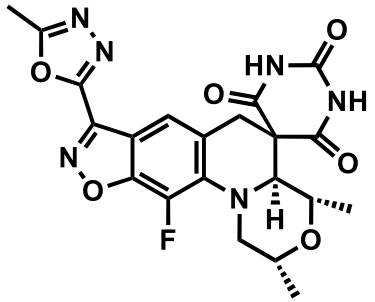
- **10 cmpds showed MICs < 10 μM across 5 media conditions**
- **5 cmpds mitigated issues associated with genotoxcity and cytotoxicity**
- **Optimization campaign initiated**

Basarab, G. et al *AAC. (2)*, e02192-21, 2022

Selective *M. tuberculosis* SPT

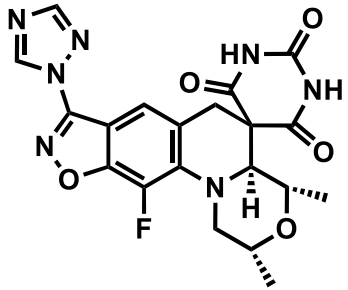


H3D-005867

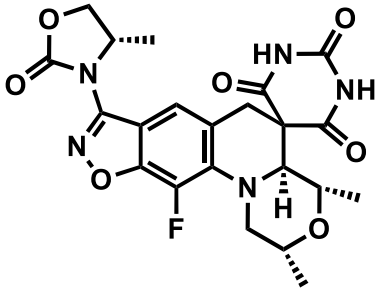


H3D-006621

Efficacious in mouse Mtb model



H3D-003387



Zoliflodacin

	Mtb ADC/GLU/TW IC_{50} (μM)	*S. aureus* IC_{50} (μM)	*Mtb* MIC (μM)	*S. aureus* MIC (μM)	*E. coli* MIC (μM)	HepG2 IC_{50} (μM)
H3D-005867	2.6	ND	0.49	>125	>125	>300
H3D-006621	2	ND	1.7	0.45	31	>300
H3D-003387	18	1.7	2.0	0.24	2.0	26
Zoliflodacin	32	4.3	7	0.14	1.4	>50

Govender, P. et al *J. Med. Chem.* ***65****(9)*, 6264, 2022

Grand Challenges
African Drug Discovery Accelerator

If we knew what we were doing, it would not be called research, would it?

Albert Einstein

Theoretical physicist, mathematician, lecturer, patent examiner, intellectual

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