Determining structure/activity relationships

Optimizing Efficacy and Safety

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Drug R&D

- The process of finding a new compound & developing it
  - Compound can be novel compound vs. novel target, novel compound against validated target, modification of existing compound
  - Progressing a lead to a drug candidate (it takes a team)
  - Simultaneously optimizing potency, pharmacokinetics & safety (preclinical toxicology assays)
- Preparing a compound for Regulatory Submission
  - Use of animal models for efficacy
  - Use of animal models for safety
  - IND (Investigational New Drug) builds to CTD (Common Technical Document)
  - Clinical Trials Phases (Ph I, II, III)
  - Adding data to the CTD and evolving it into the NDA
  - NDA review & approval by FDA

Developing a Hit to a Lead Series

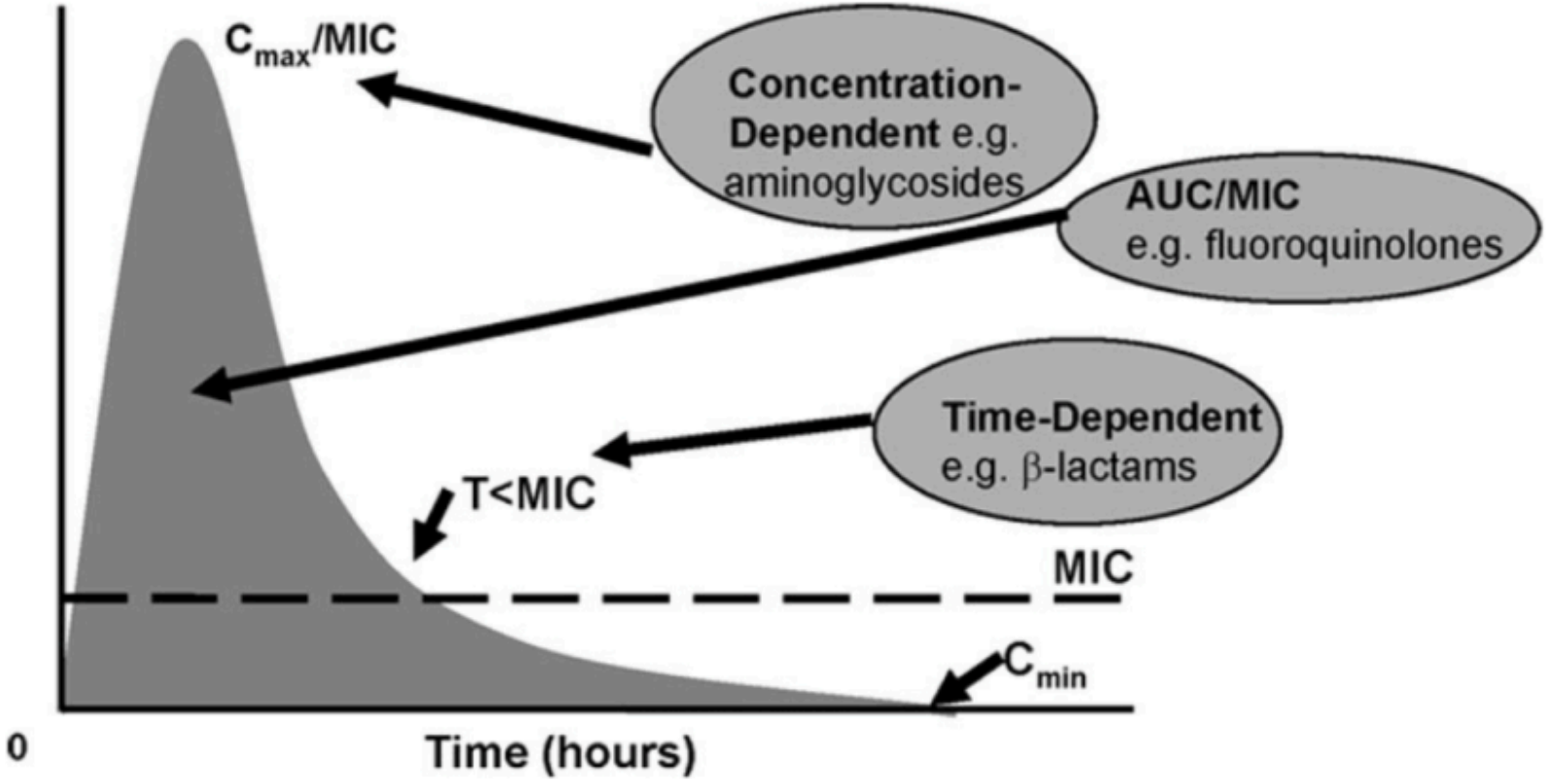
- In this section, we will walk through the steps involved in developing an initial hit into a lead and then to a clinical candidate
- Two key areas to be optimized:
  - **Efficacy** (MIC, reasonable PK & animal infection models)
  - **Safety** (in vitro tests, cell based tests, preliminary animal toxicology studies)
- Highlight parameters that need to be optimized to move a compound toward the myriad properties necessary for a potential clinical candidate (potency, DMPK, Safety)
- Rather than discussing generalities, we will employ a recent "real life" program as an example of the process & problems one can encounter
- Highlight some points that need to be addressed early in a program to insure that the chemical series being pursued has the potential to be developed to a therapeutic

Several Parameters for Hit to Lead Optimization

- Improvement of activity (e.g., target affinity to result in improved MICs) vs. target organisms
- Mitigation of resistance development (via target mutations, improved bacterial cell penetration, reduce efflux by pumps)
- Minimize serum protein binding
  - It is generally recognized that the protein bound fraction of an antimicrobial is microbiologically inactive and thus should be accounted for during pharmacokinetic interpretation
- Optimize pharmacokinetic & pharmacodynamic properties of lead compound

Pharmacokinetics/Pharmacodynamics

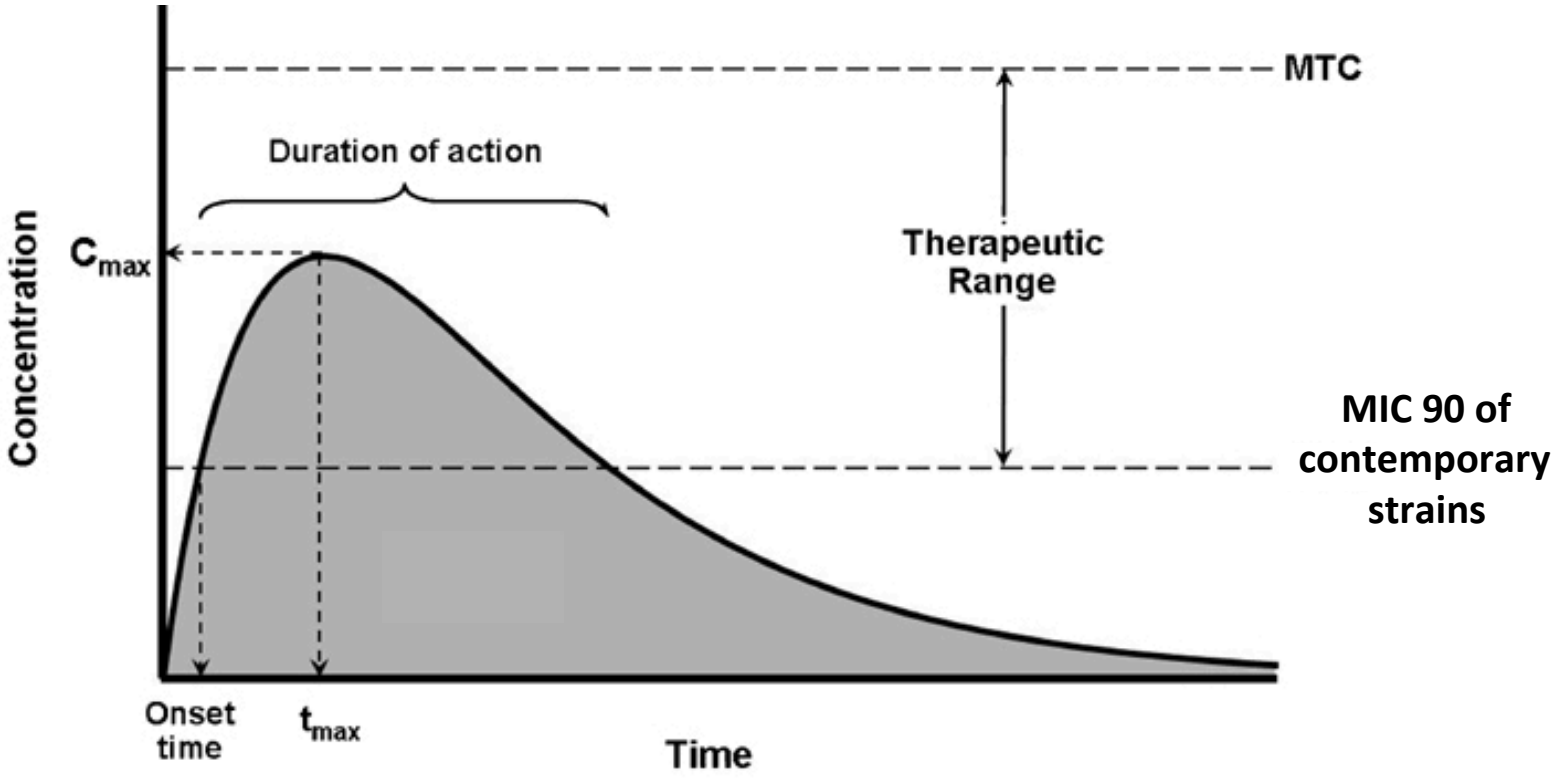
Different antibiotic classes are effective due to different PK/PD parameters (PK/PD "drivers")
Free antibiotic levels & rate of clearance dictate exposure



Therapeutic Index (TI)

TI or therapeutic range is ratio of MTD/MIC

- Difference between the effective MIC & the maximum tolerated concentration (Safety)
- Aim for a ~10-25 fold "window" if toxicology indicates a serious liability
- Becomes important when dosing humans (widely varying PK); must dose at safe levels



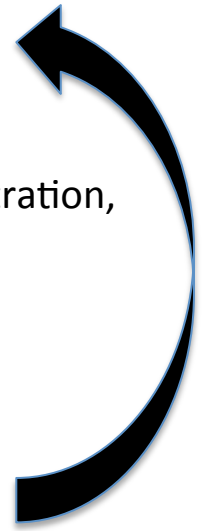
Antibiotic R&D

The Discovery Process

- Programs can start
  - from modification of existing compound to circumvent resistance, reduce toxicity, improve PK
  - Or, from a novel class identified by, e.g. an HTS, a SBDD or Fragments
- In case of a novel compound, initial "hits" are retested & IC50's evaluated
- Specificity- test for activity vs. yeast & mammalian cells
- Spectrum- Gram + and Gram -? Atypicals?
- Medicinal chemistry/DMPK/Toxicology input critical; some compounds have significant metabolism or toxicity liabilities. The most "potent" compound is not necessarily the best starting point
- Often investigate 2-3 chemical series initially

Antibiotic Preclinical Development

- Iterative rounds of medicinal chemistry & key biology testing:
  - Chemical design & limitations
    - Using structural data on target-compound interaction (if available)
    - Target engagement and inhibition (enzymology)
    - changing LogD, adding interactive groups, etc. to modify efficacy, cell penetration, solubility, PK etc.
  - Small MIC testing panels (usually 10-20 organisms)
  - Preclinical toxicology testing (in vitro hERG, mammalian cell tox)
  - Team data review meetings & discussions on next steps
  - Rinse, repeat cycle….



- Compounds that evolve and potentially meet criteria as possible drug candidates get more extensive work up:
  - Broader MIC panel (including MDR strains)
  - Animal Infections models with satellite PK (preliminary half life, Vd, elimination routes)
  - CEREP panel to test for off target pharmacological interactions

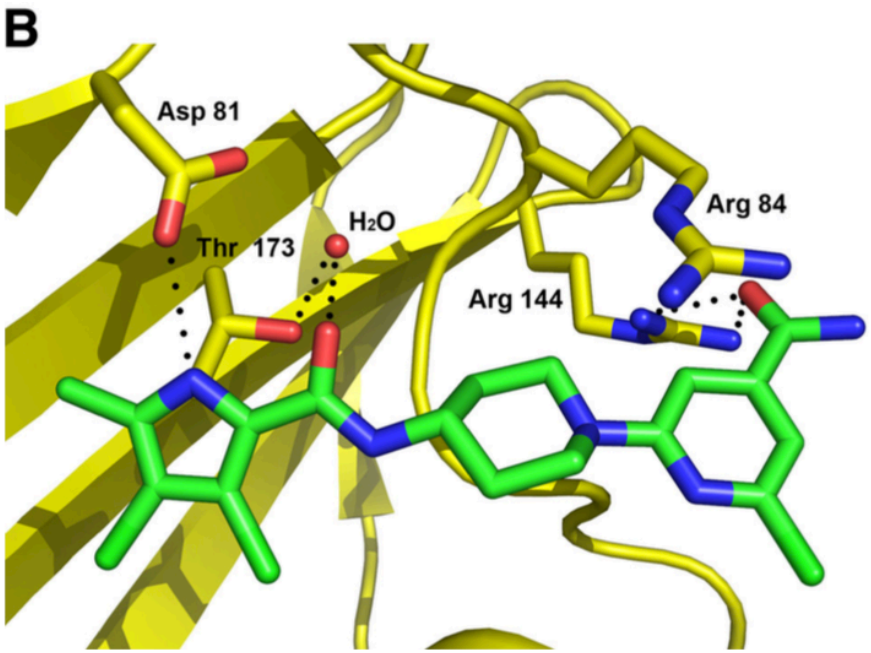
Novel DNA Gyrase Inhibitors

AstraZeneca effort to access new inhibitors for an established target (Gyrase B subunit)

NMR Fragment Screening

- Utilizing low-molecular-mass (generally 350-Da) compound "fragments" as chemical starting points rather than larger, elaborated compounds from an HTS library
- The screening library consisted of 1,000 diverse low-molecular-mass (100- to 370-Da) compounds
- Small binding compounds are linked together
- Results in compounds with greater specificity and ligand binding efficiency for the target.

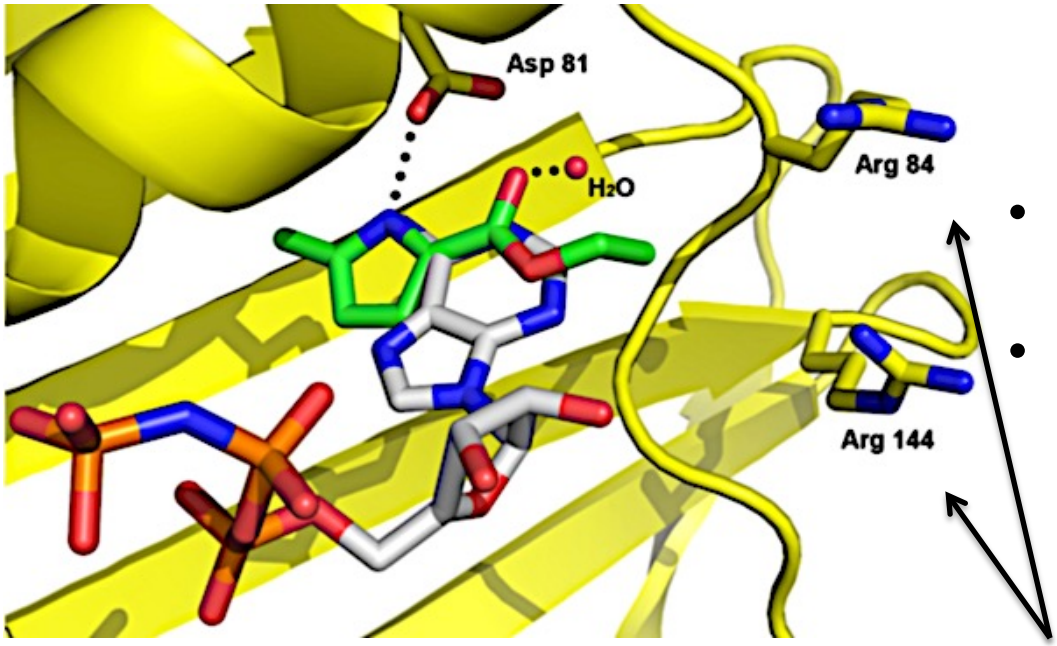
Structural Data Key



Identified pyrollamide class of gyrase inhibitors

Binding of Pyrollamide Fragment

Docking of initial pyrollamide fragment in *S. aureus* gyrase

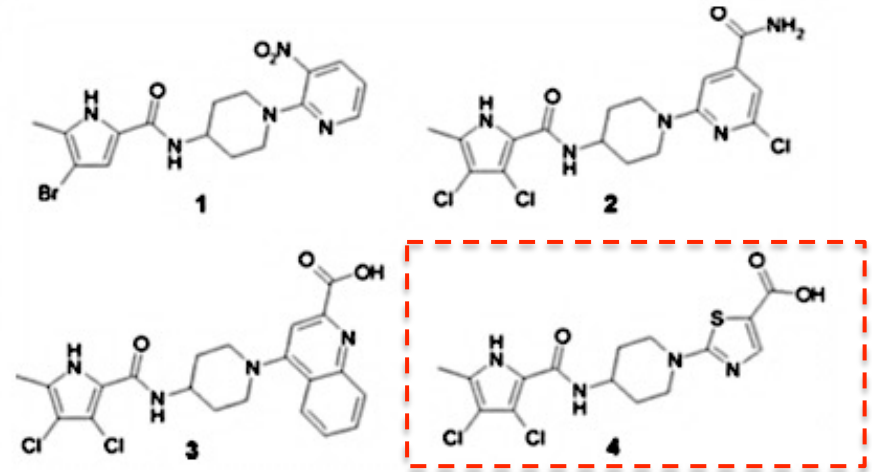


Another round of NMR screening

- Fragment made key interactions with ATP binding elements (Asp 81 & bound water)
- Second screen with the GyrB adenine pocket fully occupied
- Second-site binder was identified that appeared by NMR to be binding in a more distal region of the binding pocket (close to Arg84 and Arg144)

Elaborated Pyrrolamide DNA topoisomerase Inhibitors

- A number of “right side” groups were tested :
- Pyrrolamide gyrase inhibitors & structures obtained; additional contacts in ATP binding pocket



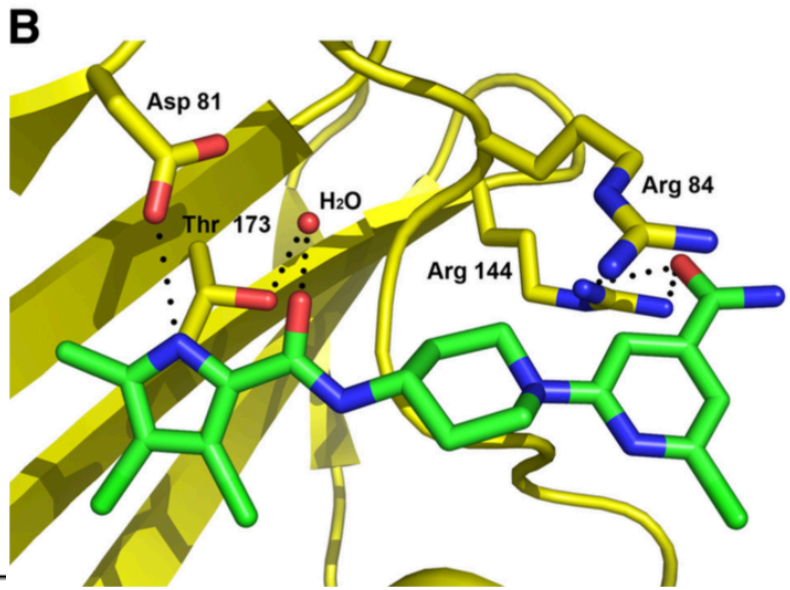


TABLE 2 Enzyme and antibacterial activities of selected pyrrolamide analogs

Compound	*E. coli* ATPase IC_{50} (nM)	MIC (μg/ml)					
		E. coli ARC523	*E. coli* ARC524[a]	*H. influenzae* KW20	*S. aureus* ARC516	*S. pneumoniae* ARC548	*E. faecium* ARC521
1	3,000	>64	>64	>64	>64	>64	>64
2	14	>64	2	64	2	1	1
3	0.9	8	<0.06	0.25	0.5	0.5	2
4	25	>64	0.25	2	8	0.5	2

[a] Strain ARC524 is equivalent to ARC523 with a Tn*10* insertion in *tolC*; efflux mutant.

Advancing a potential Hit to Lead series candidate

Antimicrobial potency is important, but so are physicochemical properties

- Due to a combination of suitable potency and desirable physical properties (e.g., solubility), pyrrolamide 4 was selected as the optimal series representative for further profiling-Lead series

Reasonably resistance low rate important

- Spontaneous resistance was measured in *S. aureus*, with an average frequency of approximately 2.5×10^{-9}

Mutant MIC increases are not large- a potent compound may retain activity against initial resistant mutants

- Resistant strains isolated above demonstrated a 4- to 8-fold increase in the MIC of pyrrolamide 4 relative to the parent strain

In vivo activity

- Testing of compound 4 in immunocompetent mouse lung infection efficacy study was completed successfully to establish series potential

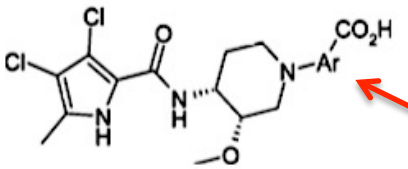
Pyrollamide Series Optimization

- Pilot studies established the series as having clinical candidate potential
- Further medicinal chemistry needed to improve potency and refine other properties
- Parameters are monitored during the optimization process
  - Bacterial target enzyme inhibition & MIC progression
  - Serum Protein binding (PPB-only the free fraction is active in vivo)
  - Solubility
  - LogD measure of lipophilicity at pH7.4
  - Toxicology assays periodic checks in representatives of a series (hERG, CEREP panels, mammalian cell toxicity)

Optimizing pharmacokinetics

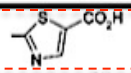
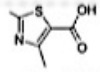
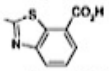
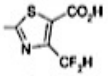
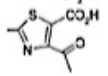
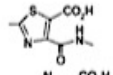
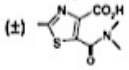
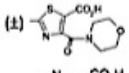
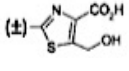
incorporating targeting & rat clearance data for optimization

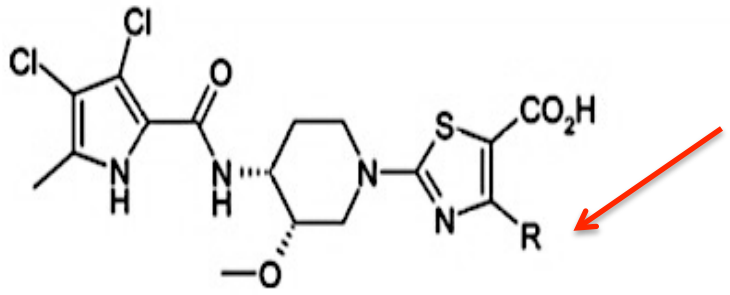
Gyrase & TopoIV target improved inhibition

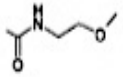
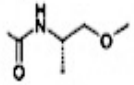
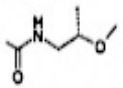
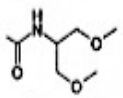
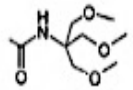
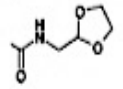


Measuring MIC/Fu

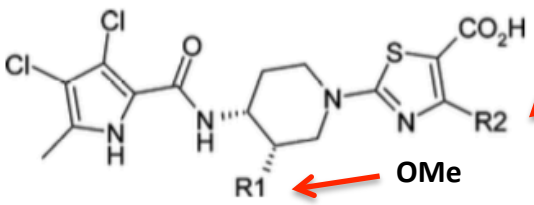
Rat Clearance measurements

Cmpd	Ar	LogD	PPB (% f_u)	Solubility (µM)	Sau GyrB IC_{50} (nM)	Eco ParE IC_{50} (nM)	MICs (µg/mL) Spn[a]	Spy[b]	MSSA[c]	MRQR[d]	MRQR[d] MIC/f_u	Hin[e]	Mca[f]	Eco[g]	Eco[g] *tolC*⁻	Rat Cl (mL/min/kg)
23		ND[h]	7.1	ND	<10	240	0.016	ND	0.32	0.5	7	0.18	0.031	64	0.18	52
47		1.2	1.8	450	<10	65	0.013	<0.008	0.031	0.053	2.9	0.072	0.008	>8	0.25	26
48		1.7	<1	<1	<10	14	0.032	ND	0.031	0.031	>31	0.031	<0.001	8	0.25	60
49		0.52	ND	690	<10	ND	0.063	0.13	2	2	-	1	0.063	>8	0.5	70
50		0.36	1.9	890	<10	ND	0.020	0.020	0.063	0.063	3.7	0.15	0.020	30	0.26	9.3
51		1.3	2.0	56	<10	72	0.016	0.013	0.063	0.063	3.15	0.14	0.011	18	0.30	14
52		-1.0	13	>1000	<10	420	4	ND	8	>8	>62	8	2	>8	2	ND
53		-0.99	14	200	<10	ND	2	ND	8	>8	>57	8	2	>8	4	ND
54		-0.27	2.9	ND	<10	ND	2	ND	>8	>8	17	8	1	>8	1	ND

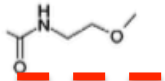
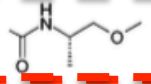
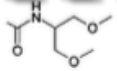


Cmpd	R	LogD	PPB (% f_u)	Solubility (μM)	Sau GyrB IC_{50} (nM)	Eco ParE IC_{50} (nM)	MICs (μg/mL) Spn[a]	Spy[b]	MSSA[c] Sau	MRQR[d] Sau	MRQR[d] MIC/f_u	Hin[e]	Rat Cl (mL/min/kg)
51		1.3	2.0	56	<10	72	0.016	0.013	0.063	0.063	3.15	0.14	14
61		0.85	4.3	280	<10	120	0.039	0.039	0.5	0.5	12	0.16	NT
62		1.2	2.7	880	<10	53	0.018	0.017	0.078	0.089	3.3	0.15	9.5
63		1.4	2.5	960	<10	73	0.016	0.014	0.036	0.057	2.3	0.13	14
64		ND[h]	2.7	ND	<10	43	0.016	<0.008	0.031	0.031	1.1	0.13	13
65		1.0	2.1	860	<10	51	0.016	0.016	0.050	0.049	2.3	0.10	18
66		1.9	4.7	720	<10	42	0.016	0.016	0.031	0.031	0.66	0.15	18
67		0.76	2.8	940	<10	36	0.040	0.020	0.063	0.10	3.6	0.10	33

PK Properties of Final Candidates in selected Animal Models



Oral bioavailability
F = fraction dose absorbed

Cmpd	R1	R2	pK_a	Mouse ppb (% f_u)	Mouse F (%)	Mouse Cl (mL/min/kg)	Rat ppb (% f_u)	Rat F (%)	Rat Cl (mL/min/kg)	Dog ppb (% f_u)	Dog F (%)	Dog Cl (mL/min/kg)
1	F	H	3.4	8.9	ND[a]	15.5	8.6	6.7	54	12	ND	3
23	OMe	H	3.6	ND	ND	99	ND	6.7	66	22	ND	26
62	OMe		4.4	5.85	57	57	3.8	34	9.5	8.2	100	3.0
63*	OMe		4.2	4.7	100	10	3.4	81	17	6.2	96	0.64
65	OMe		4.5	4.2	25	5.35	3.8	ND	18	12	29	1.6
73	OMe		5.4	7.4	ND	ND	7.15	ND	2.5	13	18	0.73
74	OMe		4.5	9.7	74	7.4	8.8	65	16	20	70	1.6
75	OMe		5.2	18	100	22	19	100	31	24	100	1.9

[a]ND = not determined.

Profiling of Compounds in an animal Infection Model to Select Candidate

- Neutropenic thigh mouse model with *S. aureus*
- PK data also collected to correlate efficacy with compound exposure (note: exposure does NOT necessarily correlate with dose!)
- For compound **63** pharmacokinetic properties and bioavailability were favorable across species, positioning the compound for both parenteral and oral administration.

Table 10. Efficacy of Pyrrolamides against *S. aureus* ARC516 in the Neutropenic Thigh Infection Model

compd	total daily dose/regimen	mean AUC μg·h/mL	mean free AUC μg·h/mL	mean delta log CFU[a]	MIC μg/mL
63	30 mg/kg q24	116	5.45	1.51	0.03
65	30 mg/kg q24	148	6.26	−0.16	0.03
74	30 mg/kg q24	204	19.8	1.54	0.03
75	30 mg/kg q6	37.6	6.7	−1.57	0.06

[a]Relative to the pretreatment inoculum.

MIC_{90s} with a battery of recent clinical isolates of target organisms

Table 11. MIC_{90}s of 63 and Comparators

pathogen	no. of strains	MIC_{90}s (μg/mL) 63	levofloxacin	azithromycin
S. aureus	200	0.06	32	>128
methicillin resistant	110	0.06	64	>128
E. faecalis	100	0.015	32	>128
vancomycin resistant	8[a]	0.015	64	>128
E. faecium	100	0.06	>128	>128
vancomycin resistant	50	0.06	>128	>128
H. influenzae	200	0.25	0.015	2
amoxicillin resistant	40	0.25	0.015	2

[a]Highest MIC for the number of indicated strains.

MIC_{90} = Concentration that inhibits 90% of the isolates being tested

Additional Preclinical Properties

- The frequencies of spontaneous resistance to **63** in multiple isolates of *S. pneumoniae* and *S. aureus* were all less than the detection limit ($<9.6 \times 10^{-10}$) at 4 and 8 times the concentration that prevented confluent bacterial growth, with no resistant variants emerging
- **63** showed no signs of mutagenicity (*at the highest concentrations tested in an Ames mutagenicity assay, an in vitro micronucleus assay using mouse lymphoma cells, and an in vitro mouse lymphoma TK assay. 1000 X window to inhibition of human topoisomerase*)
- Compound **63** showed no hERG inhibition or inhibition of other ion channels at the highest concentration tested (100 μM), representing a greater than 200-fold margin to predicted free Cmax
- Compound **63** showed no inhibition at the highest concentration of 50 μM across a series of five of the most prevalent human cytochrome P450 enzymes (*Cyp1A2, Cyp2C19, Cyp2C9, Cyp2D6, and Cyp3A4*), mitigating one mode of drug−drug interactions

To the clinic….

- Compound **63** went through & passed pivotal animal toxicity testing; established NOAEL (no adverse effect level) & MTD (maximum tolerated dose)
- IND (Investigational New Drug application) filed with FDA for initial Ph. I clinical studies (safety & preliminary human PK)
- Designated as **AZD5099** for oral & parenteral treatment of Gram-positive and fastidious Gram-negative bacteria.
- human volunteers - Ph. 1 SAD (single ascending dose) and MAD (multiple ascending dose) studies
- In man, **AZD5099** was dosed i.v. up to 500 mg per individual, but further clinical work was discontinued for a combination of factors:
  - High variability in exposure within a small group of healthy volunteers, which eroded confidence that efficacious exposures could be achieved within defined safety margins
  - Concerns related to mitochondrial changes observed in preclinical safety species.
- Project discontinued. *How depressing…..*

Summary

- *Briefly described some of the important PK/PD principles in optimizing a compound for progression to development*
  - Antimicrobial activity balanced against protein binding (*F* unbound), PK parameters such as AUC/MIC, Cmax, and clearance rates in animals
- *An example of employing Fragment based drug design against a classic target*
  - Optimization of the various parameters; balancing properties to achieve optimal effect
  - Animal model infections to test efficacy
  - Determining clearance in several species
  - In vitro toxicity testing
- Despite everything done in the discovery preclinical setting***, failure is not unusual***. The overall lesson: ***arriving at a single molecule that meets all the criteria is a rare event***.
- It is possible to approach established antibiotic targets with fundamentally new molecules

Extra Slides

Target Product Profile

- First: What is the therapeutic aim of your program?
- To:
  - Address infections with MDR Gram positives ?
  - Address serious Gram negative MDR pathogen infections (BP, IAI)?
  - Address skin & soft tissue infections? (SSTI)
  - Address community acquired bacterial pneumonia (CABP)?
  - Address complicated Urinary tract infections? (cUTI)?
- The aim sets the goals for:
  - The bacterial pathogens you must cover with your compound
  - The infected organs that must have reasonable drug levels (e.g., lung, kidneys) to kill/inhibit bacterial growth
    - Tissue concentrations, metabolism of compound
    - Routes of elimination (clearance)
- Recognize that antibiotics are used in high doses
  - 200 mg to 2-4 grams per day (e.g. BP medicines are 8-16 mg/day)
  - Toxicology “window” (therapeutic index) can be challenging- TI

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