Corramycin: Enlarging the Antibacterial Spectrum and Optimizing the Developability Profile of Corramycin, a Novel Class Natural Product Antibacterial

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Abstract

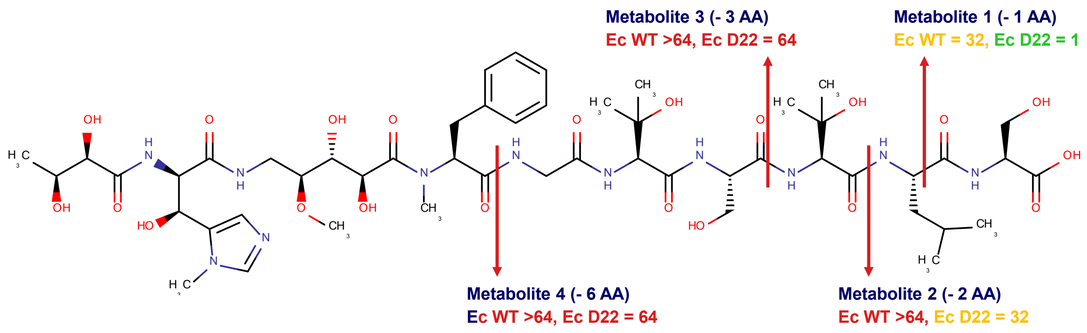
Corramycin is a novel zwitterionic peptide with antibiotic properties. However, being mainly active against *E. coli*, Corramycin interest as a starting point for a new antibacterial program was challenging despite a promising activity in an *E. coli* septicemia/peritonitis animal model. We postulated that Corramycin spectrum could be improved if bacterial penetration could be enhanced. Scanning Corramycin peptidic structure by replacement with alanine revealed several positions amenable to chemical optimization. Leucine replacement for an amino acid with a tert-butyl side chain in the penultimate position was a starting point to improve spectrum and metabolic stability. Grafting an amine or a catechol moiety at the C-terminus showed also a propensity to improve spectrum. Catechol vectorization proved to be the most fruitful approach, providing penetration owing to siderophore transporter(s) along with broader spectrum and higher potency against key Gram-negative bacteria.

During the course of the optimization, a very potent derivative (Corramycin 2) was shown to be prone to the selection of a resistant subpopulation in animal models. Corramycin 2 properties favored the uptake through a single inner membrane transporter, which led to the rapid appearance of resistance through invalidation of this transporter.

Careful design of the linker between the Corramycin C-terminus and the siderophore moiety proved critical to obtain potent molecules whose uptake was shared between several inner membrane transporters, and whose administration in animal models resulted in an efficacious treatment of infection while avoiding the selection of resistant bacteria.

The lessons learnt from this program of >800 derivatives showed the benefits of vectorization to enlarge spectrum and to improve efficacy of antibacterials, as well as the need of a careful molecular design to solve issues such as resistance risk and metabolic stability.

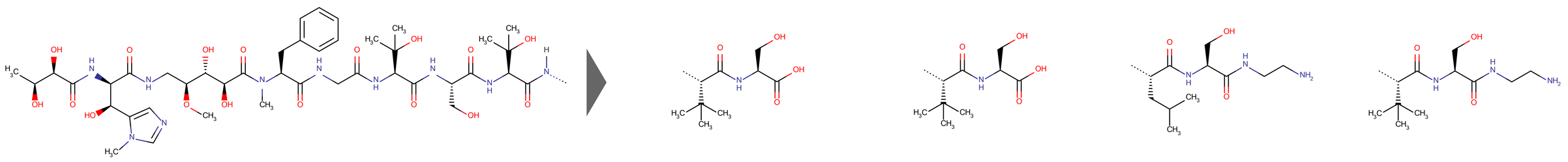
Native Corramycin as the starting point



MIC in µg/mL		Native Corramycin
E. coli ATCC 25922		4
K. pneumoniae ATCC 13883		16
A. baumannii ATCC 19606		> 32
Genotoxicity alert (micronucleus event at 500 µg/ml)		Pos (98)
Metabolic stability (% remaining in lung & kidney)		1h: 7 & 41; 4h: 1 & 6
In vivo activity: Log CFU reduction	Septicemia model with *E. coli* ATCC 35218	- 3.3 at 20mg/kg
	RTI model with *K. pneumoniae* ATCC 13883	- 1.2 at 80mg/kg

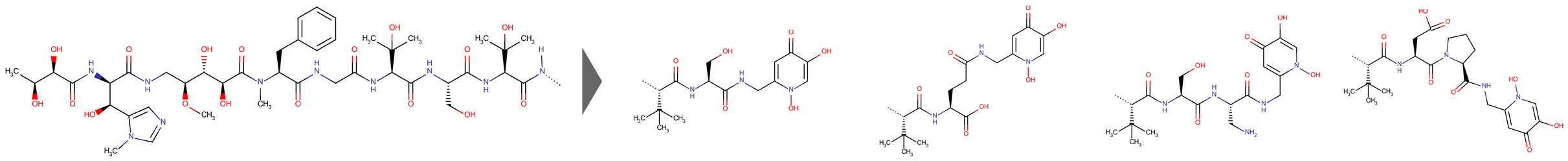
- No Phys-Chem warnings: good solubility (8.1 mg/ml at pH 4.5 and 9.1 mg/ml at pH 5.5) and good chemical stability
- No critical ADME alerts: no CYP inhibition/induction, good plasma stability and low protein binding (~70% in mouse and rat plasma); clean *in vitro* toxicity profile (cytotoxicity, hERG, CEREP, …) with the exception of a genotoxicity alert (possibly on-target)
- Stable in human, mouse and rat blood but unstable in mouse lung and kidney tissues. All metabolites are inactive or less active on *E. Coli* compared to the native Corramycin.

Stabilization as the first primary objective



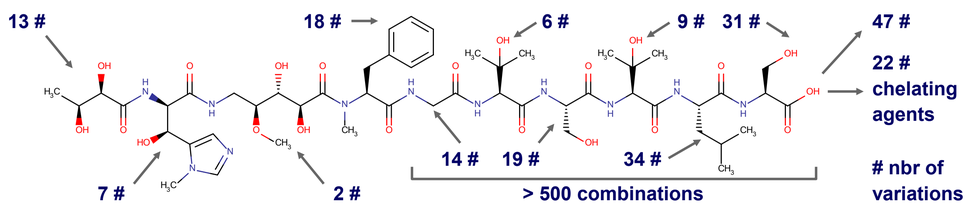
MIC in µg/mL		Corramycin 3	Corramycin 4 (+ Gly → Ala)	Corramycin 5	Corramycin 6 (+ Phe → pF-Phe)
E. coli ATCC 25922		2	4	2	0.125
K. pneumoniae ATCC 13883		2	32	8	0.25
A. baumannii ATCC 19606		> 32	> 32	> 32	> 8
Genotoxicity alert (micronucleus event at 500 µg/ml)		Pos (66)	–	Pos (9)	Neg
Metabolic stability (% remaining in lung & kidney)		1h: 105 & 104; 4h: 104 & 72	1h: –; 4h: –	1h: –; 4h: –	1h: 127 & 105; 4h: 138 & 92
In vivo activity: Log CFU reduction	Septicemia model with *E. coli* ATCC 35218	- 5.9 at 10mg/kg	- 3.0 at 20mg/kg	- 4.1 at 20mg/kg	- 5.6 at 3mg/kg
	RTI model with *K. pneumoniae* ATCC 13883	- 3.0 at 80mg/kg	–	- 1.0 at 30mg/kg	- 1.7 at 3mg/kg

Spectrum enlargement as the second objective



MIC in µg/mL		Corramycin 7	Corramycin 8	Corramycin 9 (+ Phe → pF-Phe)	Corramycin 2
E. coli 25922		0.031	0.031	0.031	0.015
K. pneumoniae 13883		0.125	0.25	0.125	0.031
A. baumannii 19606		1	0.5	0.5	0.031
Genotoxicity alert (micronucleus event at 500 µg/ml)		Neg	Neg	Pos (17)	Neg
Metabolic stability (% remaining in lung & kidney)		1h: 84 & 99; 4h: 73 & 84	1h: 100 & 97; 4h: 99 & 103	1h: 96 & 97; 4h: 107 & 95	1h: 100 & 103; 4h: 80 & 91
In vivo activity: Log CFU reduction	Septicemia model with *E. coli* ATCC 35218	–	–	- 3.8 at 1mg/kg	- 3.9 at 1mg/kg
	RTI model with *K. pneumoniae* ATCC 13883	-3.2 at 30 mg/kg	-3.0 at 10mg/kg	- 3.0 at 1mg/kg	- 3.3 at 1mg/kg

- MedChem effort led to a potential candidate with the best possible profile applying multi-parametric optimization
- Main parameters optimized were synthetic efficiency, potency on wild type strains and relevant mutants, spectrum, genototoxicity, chemical and metabolic stability… without structural information (target not cracked)
- Unsuccessful strategies to shorten/simplify the chemical structure
- 3 main metabolites identified which are all inactive



Conclusion

Considering its novel structure, its novel mode of penetration, its *in vivo* efficacy, the antibacterial natural product Corramycin constitutes an attractive starting point for a lead optimization program despite several challenges. This optimization effort (described in poster # 3417) led to a candidate named Corramycin2 (see also poster # 5431) that has the potential to afford a new class of antibacterials able to address severe nosocomial infections caused by multidrug-resistant Enterobacteriaceae and Acinetobacter bacteria.

See also other posters/presentation

- Poster #3472 Corramycin: Biosynthetic Clusters Of A Promising Antibacterial Scaffold From Myxobacteria Including The Biosynthetic Pathway For A Previously Undescribed Hydroxyl-n-methyl-histidine
- Poster #3417 Corramycin: Enlarging the Antibacterial Spectrum and Optimizing the Developability Profile of Corramycin, a Novel Class Natural Product Antibacterial
- Poster #5431 Corramycin2, a new class potent antibacterial candidate with a novel mode of penetration into bacteria, a novel mechanism of action and compelling activities in animal models of infection