Design, synthesis and biological activity of novel dual ATP-competitive inhibitors of DNA gyrase and topoisomerase IV

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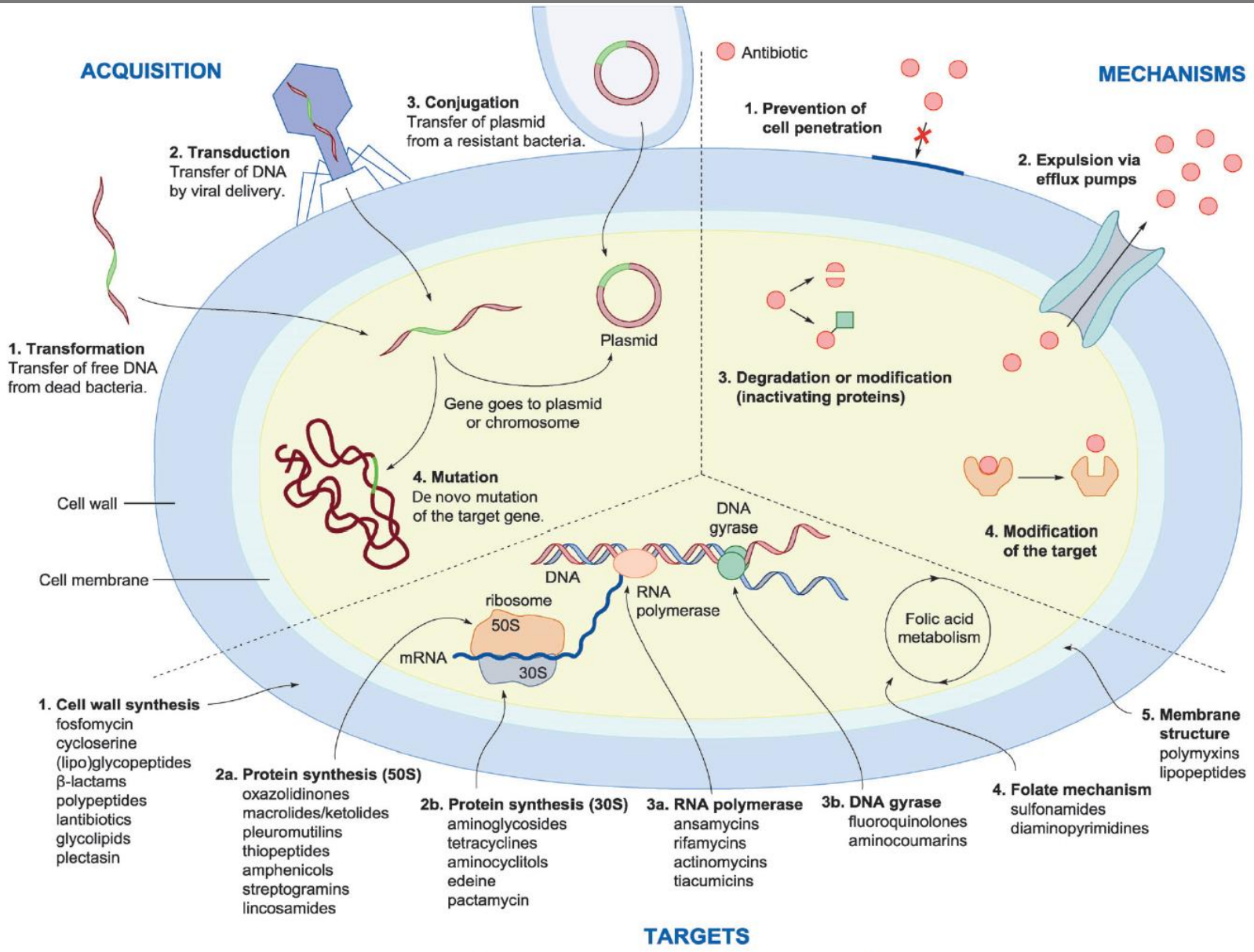


Figure 1. The four resistance acquisition pathways, the four main mechanisms of resistance, and the five main targets for antibiotics.

Chellat, M.F.; Raguž, L.; Riedl, R. Targeting antibiotic resistance, *Angew. Chem. Int. Ed.* **2016**, *55*, 2-30.

Multitargeting in bacteria - drugs

Why?

- not subject to high-level target-based resistance by single genetic changes in the host

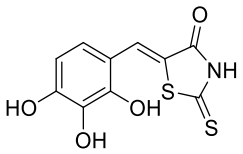
Does it work?

- β-lactams – PBPs
- macrolides, lincosamides, tetracyclines, aminoglycosides, oxazolidinones – multiple copies of rRNA genes
- fluoroquinolones – topoisomerases (DNA gyrase and topoisomerase IV)

Silver, L. L. Challenges of Antibacterial Discovery, *Clin. Microbiol. Rev.* **2011**, *24*, 71-109.

Multitargeting in bacteria – drug discovery

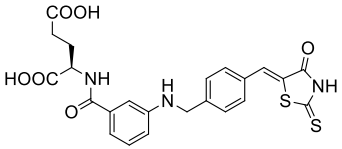
- MurC-F ligases - ATP-competitive inhibitors, substrate/product analogues



5a

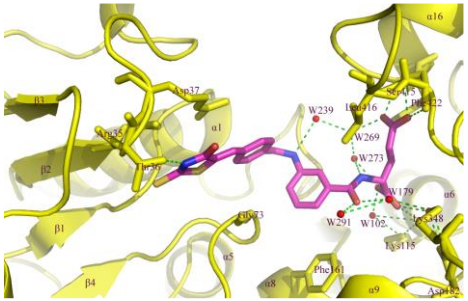
IC_{50} (MurD) = 2 μM
IC_{50} (MurE) = 6 μM
IC_{50} (MurF) = 2 μM

Tomašič, T. et al., *ChemMedChem* **2010**, *5*, 286-295



IC_{50} (*E. coli* MurD) = 8.2 μM
IC_{50} (*S. aureus* MurD) = 6.4 μM
IC_{50} (*E. coli* MurE) = 180 μM
IC_{50} (*S. aureus* MurE) = 17 μM

MIC (MRSA) = 8 μg/mL

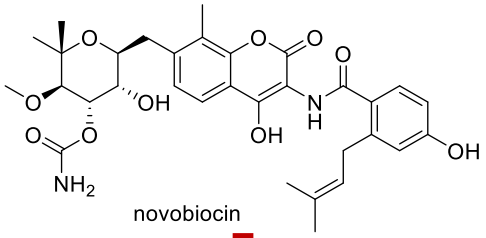


Tomašič, T. et al., *ACS Med. Chem. Lett.* **2012**, *3*, 626-630.
Tomašič, T. et al., *J. Med. Chem.* **2011**, *54*, 4600-4610.

- DNA gyrase/topoisomerase IV
- dual inhibitors of Gram-positive DNA polymerases
- β-ketoacyl-ACP synthases of FasII
- hybrid molecules: dual pharmacophore, multiple targets

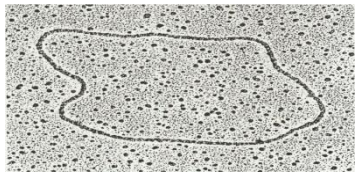
Silver, L. L. Challenges of Antibacterial Discovery, *Clin. Microbiol. Rev.* **2011**, *24*, 71-109.

DNA gyrase and topoisomerase IV

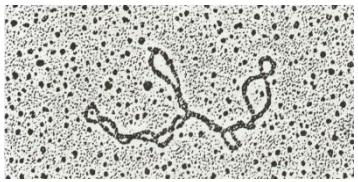


first-in-class agent
Gram positive spectrum
bactericidal against MRSA

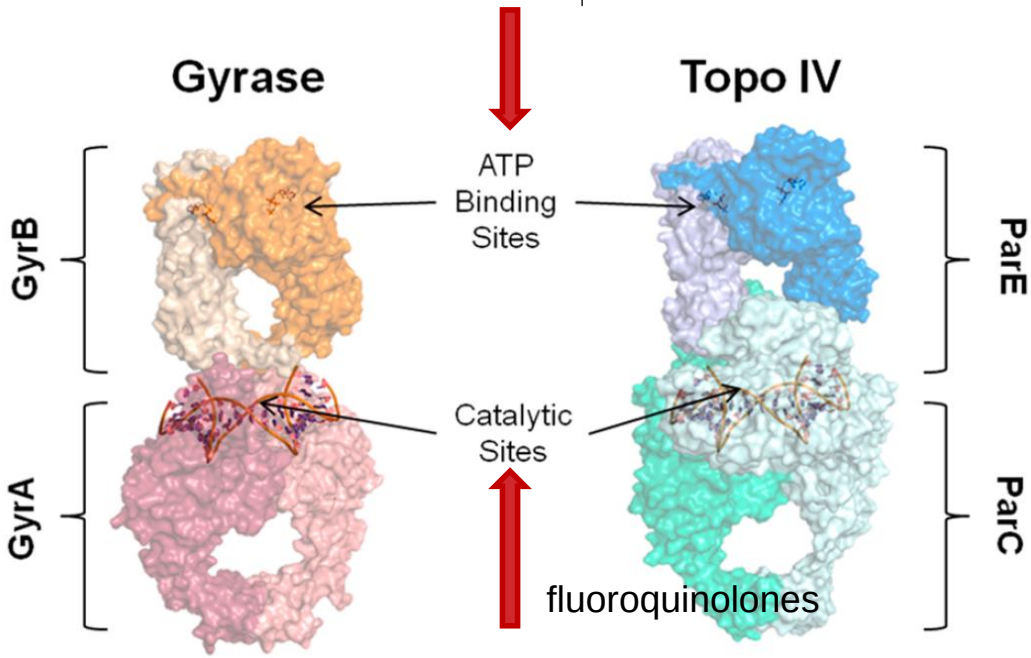
relaxed

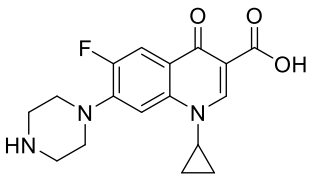


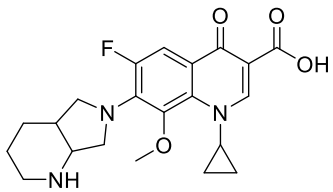
DNA gyrase ⇅ *topoIV*

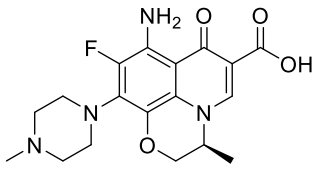


supercoiled

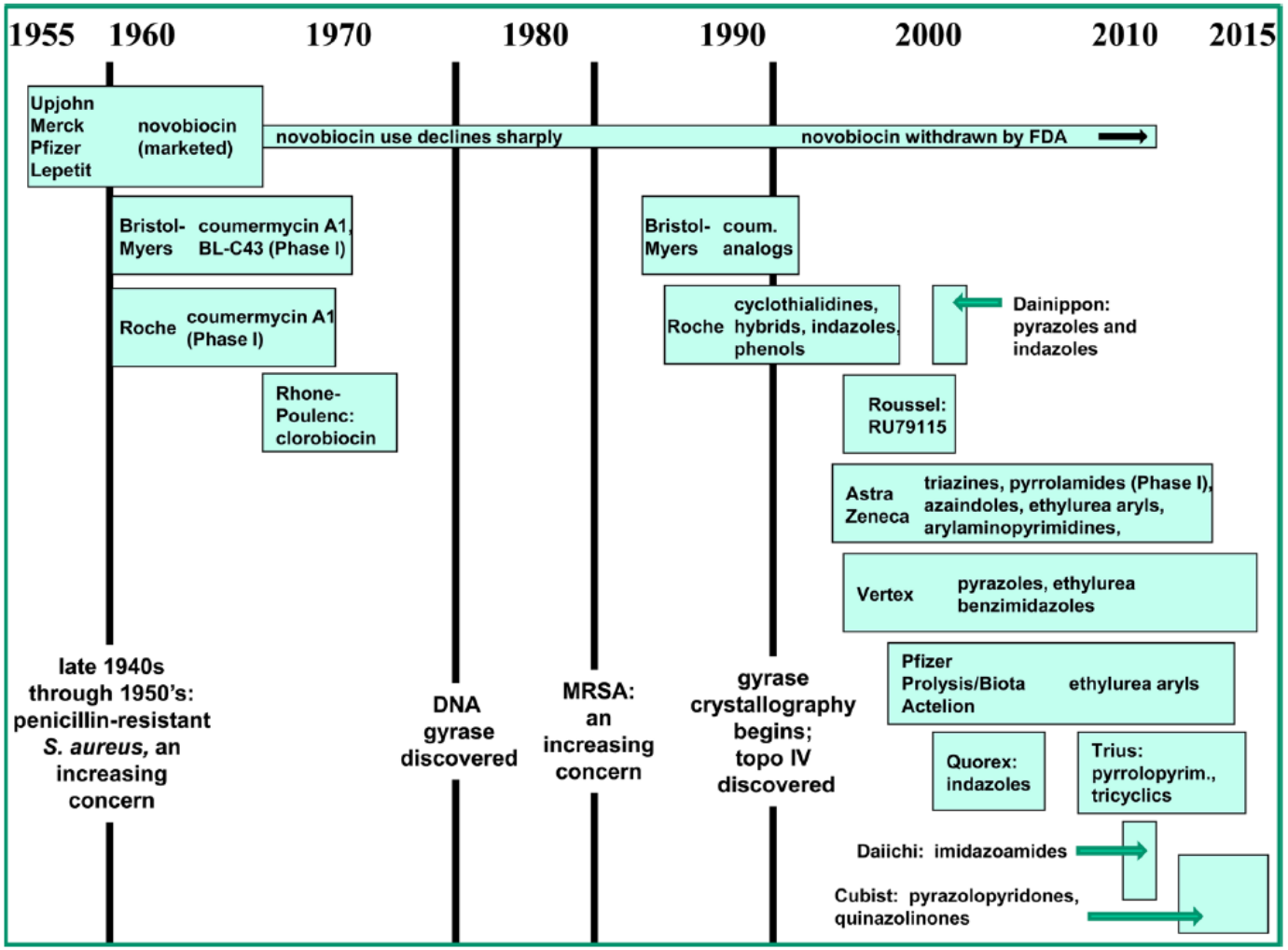



ciprofloxacin


moxifloxacin

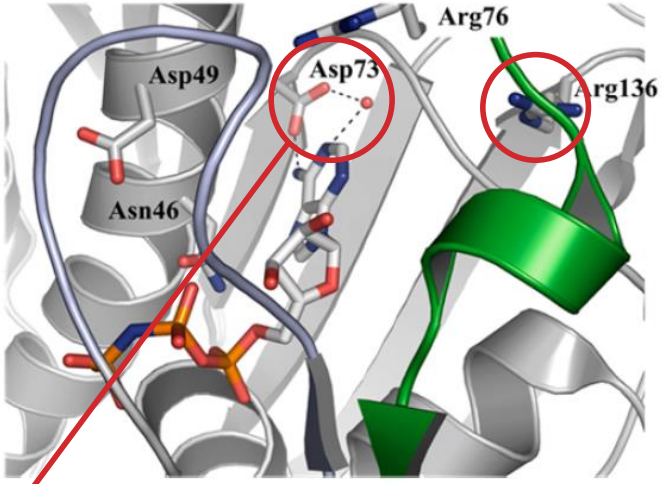

antofloxacin

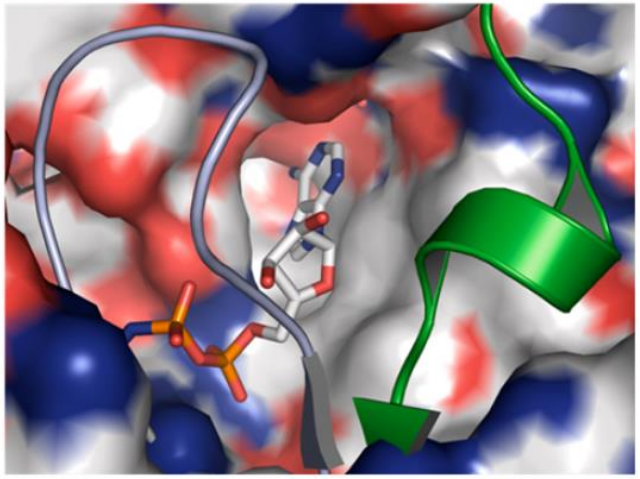
GyrB/ParE inhibitors

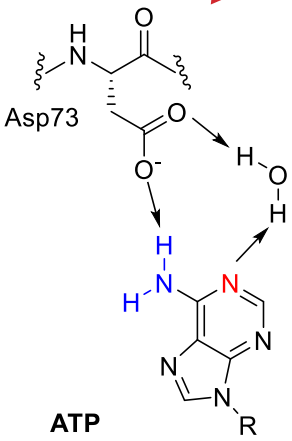


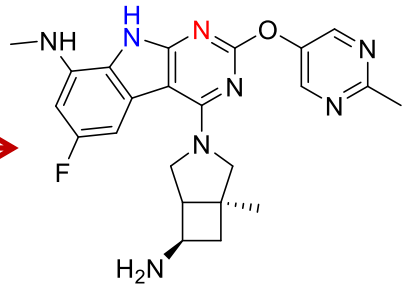
Bisacchi, G. S.; Manchester, J. I. *ACS Infect. Dis.* **2015**, *1*, 4-41.

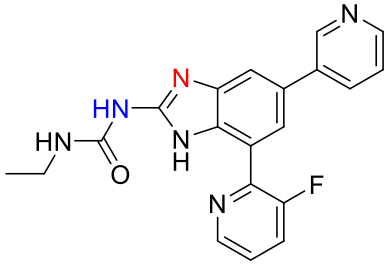
GyrB and ParE

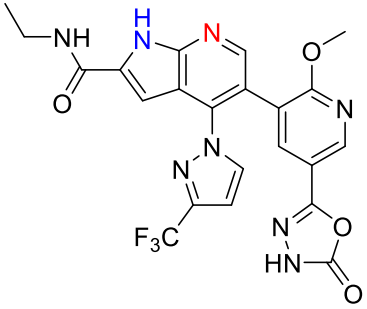








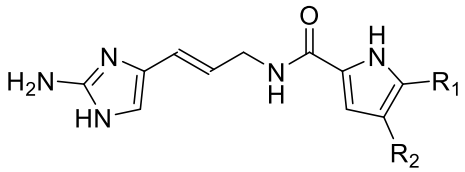




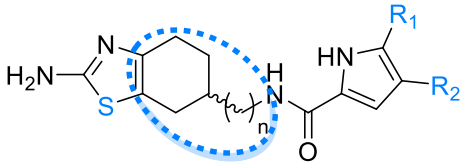
Bisacchi, G. S.; Manchester, J. I. *ACS Infect. Dis.* **2015**, *1*, 4-41.
Tomašić, T.; Mašič, L. P. *Curr. Top. Med. Chem.* **2014**, *14*, 130-151.
Mayer, C.; Janin, Y. L. *Chem. Rev.* **2014**, *114*, 2313-2342.

Marine alkaloids

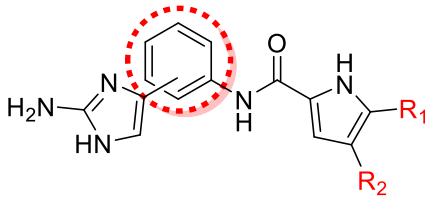
Agelas oroides



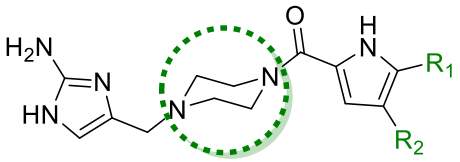
clathrodin (R_1 = R_2 = H)
hymenidin (R_1 = H, R_2 = Br)
oroidin (R_1 = R_2 = Br)



n = 0, 1
type **A** analogues



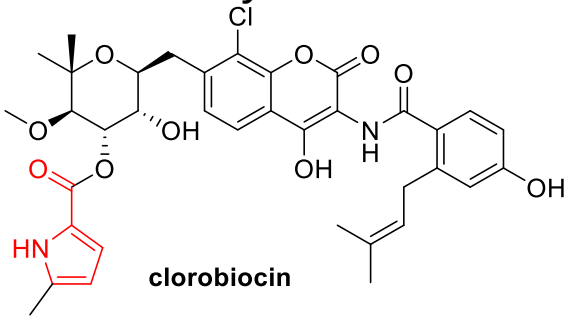
type **B** analogues



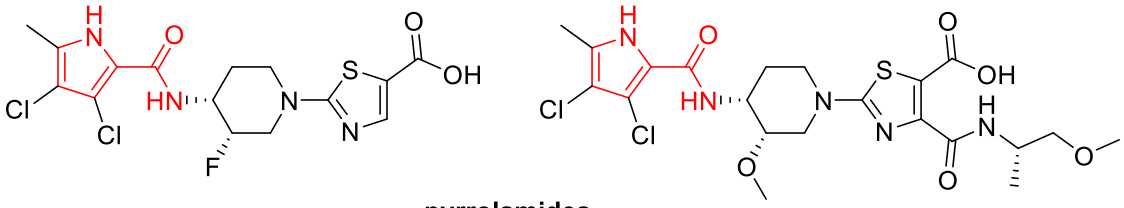
type **C** analogues

Hodnik, Ž. et al. *Eur. J. Med. Chem.* **2013**, *70*, 154-164.
Zidar, N. et al. *Eur. J. Med. Chem.* **2014**, *74*, 23-30.
Tomašič, T. et al. *MedChemComm* **2015**, *6*, 105-110.

- similarity to known DNA gyrase B inhibitors - pyrrolamides



clorobiocin

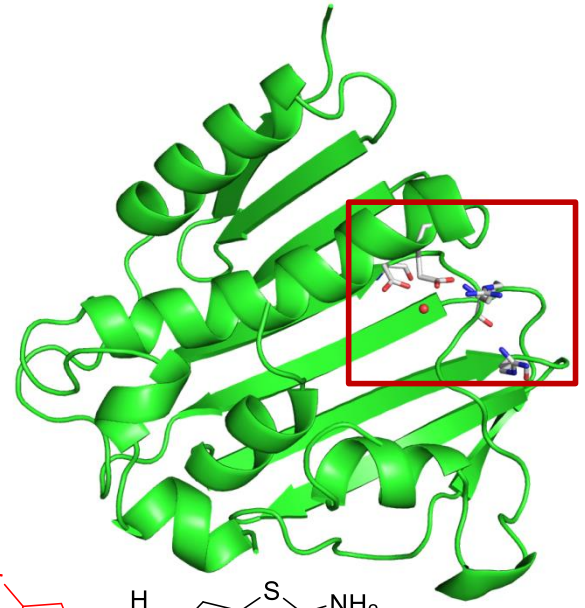


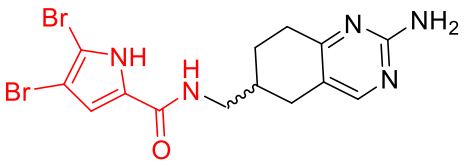
pyrrolamides

Sherer, B. A. et al. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 7416-7420.

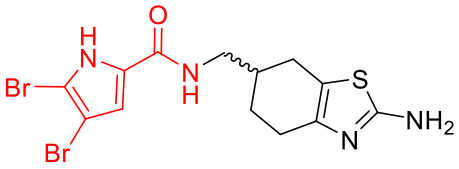
Discovery of novel GyrB inhibitors

- virtual screening
  - library of oroidin analogues (>120 compounds)
  - ATP-binding site of *E. coli* GyrB (PDB: 4DUH)
  - 20 compounds tested *in vitro*
  - 5 active compounds

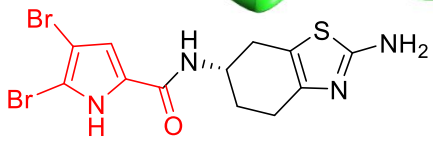




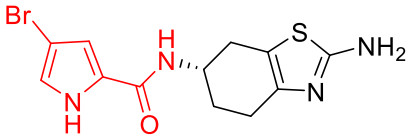
E. coli GyrB IC_{50} = 170 μM



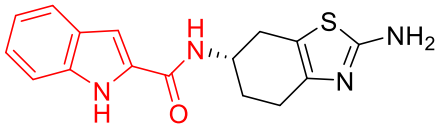
E. coli GyrB IC_{50} = 120 μM



E. coli GyrB IC_{50} = 12 μM



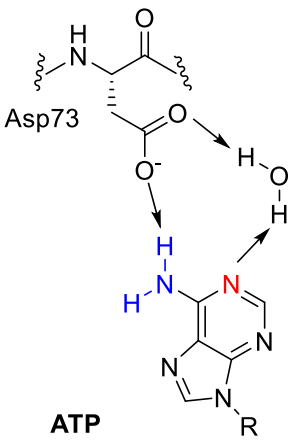
E. coli GyrB IC_{50} = 520 μM

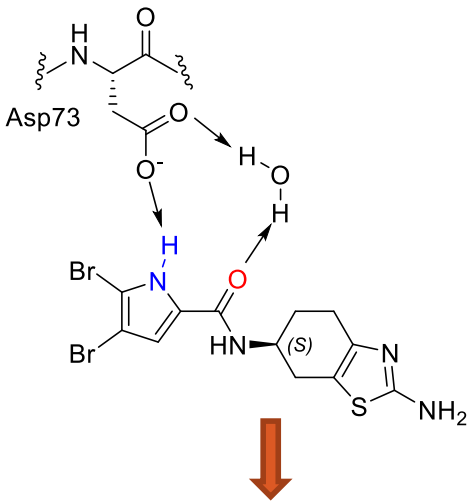


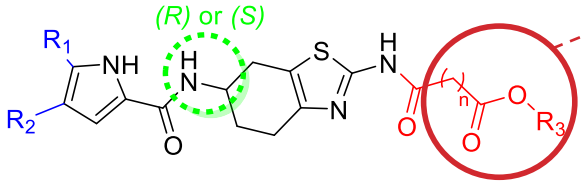
E. coli GyrB IC_{50} = 21 μM

Tomašič, T. et al. *J. Med. Chem.* **2015**, *58*, 5501-5521.

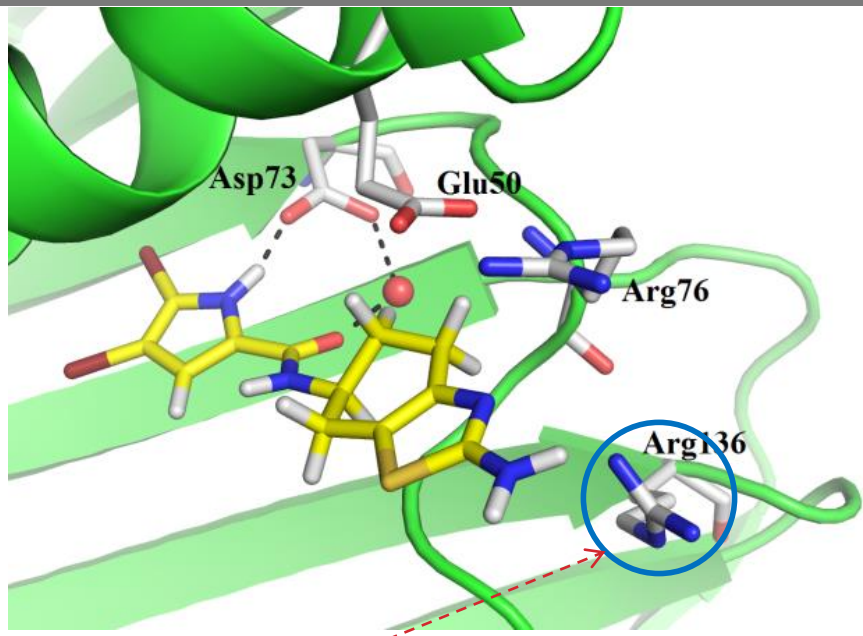
Design

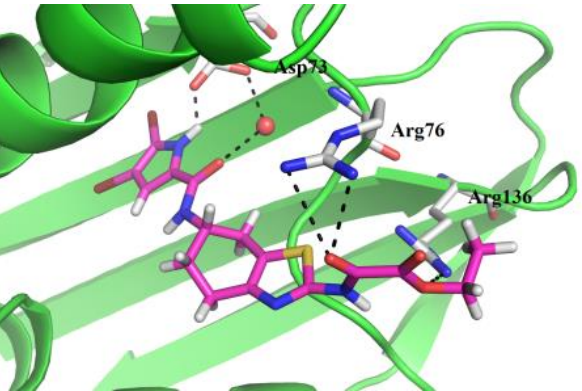


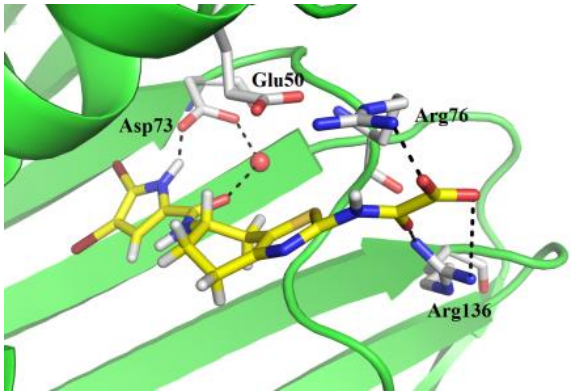


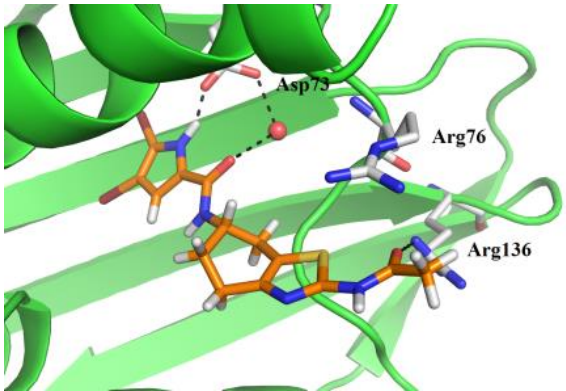


$R_1 = R_2 = Cl, Br$
$R_3 = Me, Et, H$
$n = 0\text{-}2$

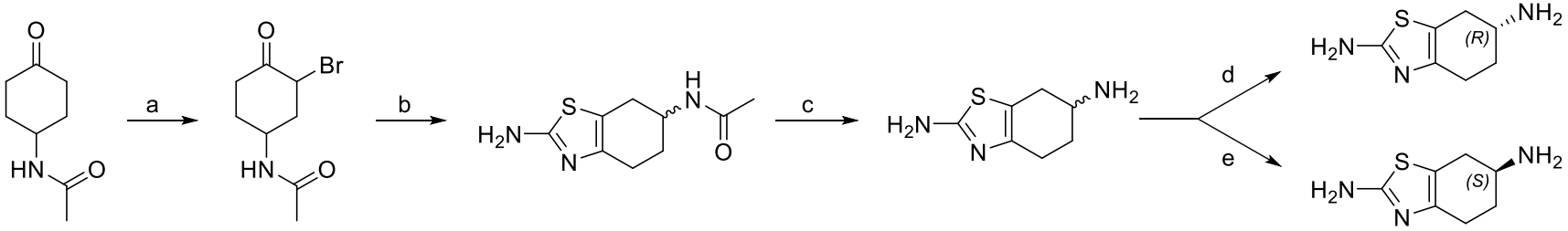




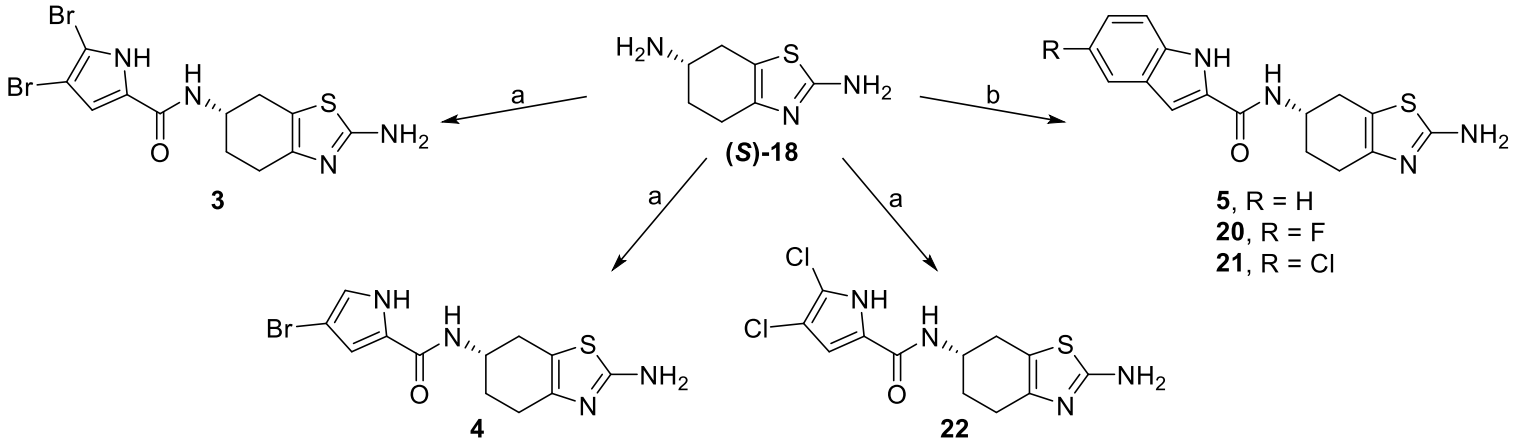




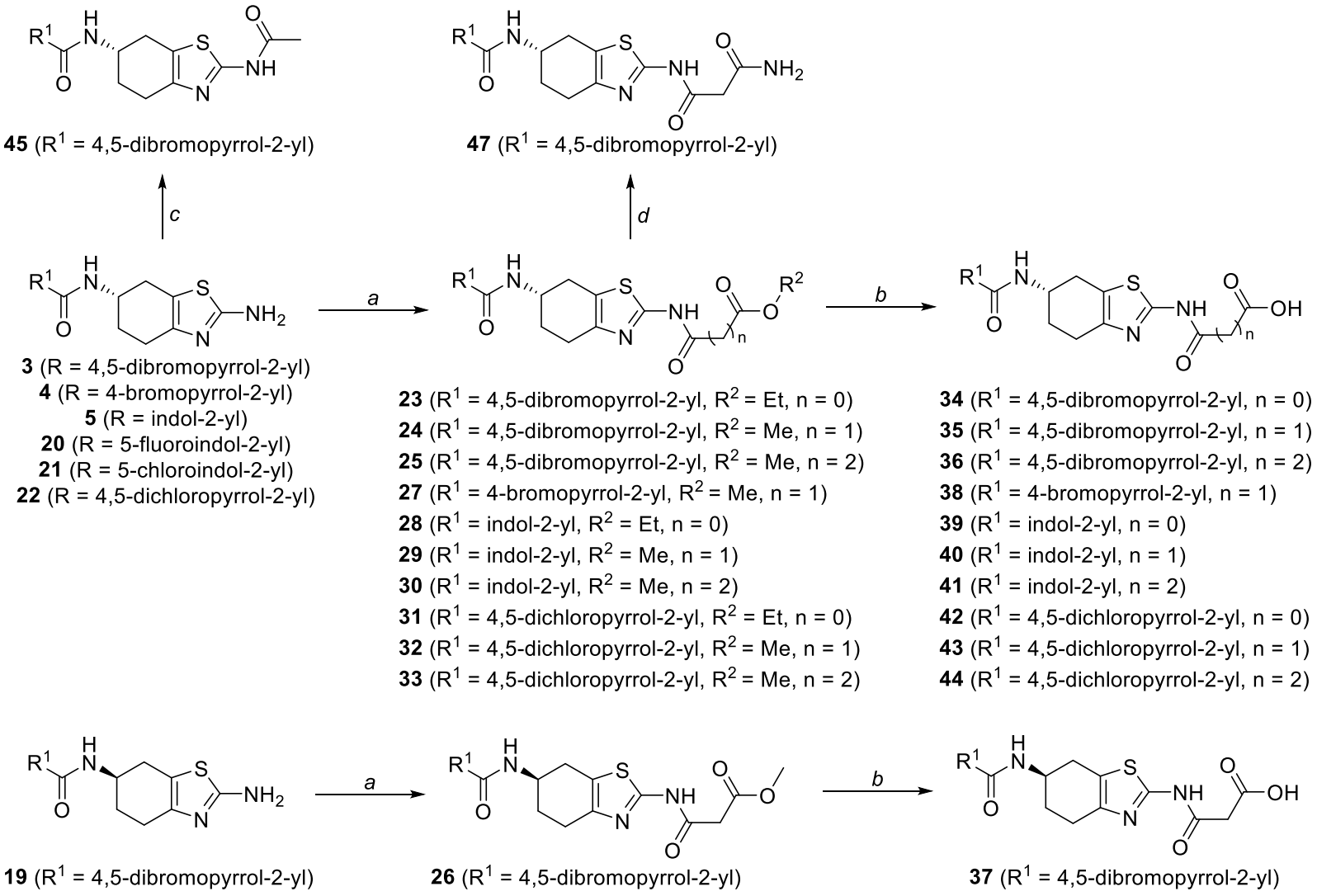
Synthesis



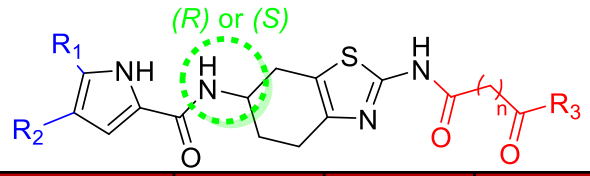
Scheme 1. Reagents and conditions. a) Br_2, AcOH, 60°C, 3 h; b) thiourea, AcOH, reflux, 3 h; c) concentrated HBr, reflux; 18 h; d) (*i*) D-(-)-tartaric acid, EtOH, reflux, 3 h; (*ii*) 10 M NaOH, r.t.; e) (*i*) L-(+)-tartaric acid, EtOH, reflux, 3 h; (*ii*) 10 M NaOH, r.t.



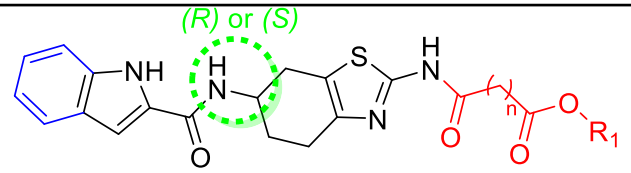
Scheme 2. Reagents and conditions: (*a*) 2,2,2-trichloro-1-(4,5-dibromo-1*H*-pyrrol-2-yl)ethanone or 2,2,2-trichloro-1-(4-bromo-1*H*-pyrrol-2-yl)ethanone or 2,2,2-trichloro-1-(4,5-dichloro-1*H*-pyrrol-2-yl)ethanone, Na_2CO_3, DMF, 35 ºC, 2-3 h; (*b*) 1*H*-indole-2-carboxylic acid or 5-fluoro-1*H*-indole-2-carboxylic acid or 5-chloro-1*H*-indole-2-carboxylic acid, TBTU, Et_3N, DMF, r.t., 2.5 h.



Scheme 3. Reagents and conditions: (*a*) ethyl oxalyl chloride (for **23**, **28** and **31**) or methyl malonyl chloride (for **24**, **26-28** and **32**) or methyl succinyl chloride (for **25**, **30** and **33**), Et_3N, 1,4-dioxane, r.t., 24 h; (*b*) 1 M NaOH, MeOH/H_2O, r.t., 24 h; (*c*) acetyl chloride, Et_3N, 1,4-dioxane, r.t., 24 h; (*d*) $NH_{3(g)}$, EtOH, r.t., 2 h.

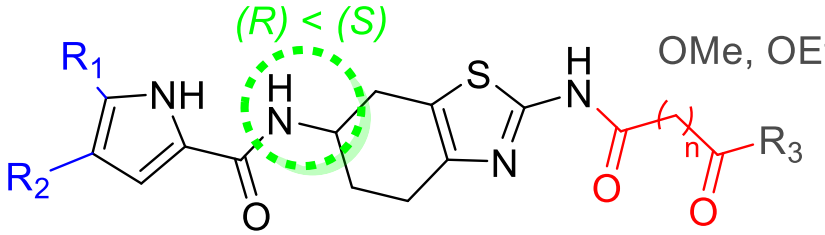


					DNA gyrase IC_{50} [μM]		topoisomerase IV IC_{50} [μM]	
	R_1	R_2	R_3	*n*	*E. coli*	*S. aureus*	*E. coli*	*S. aureus*
(*S*)-23	Br	Br	OEt	0	0.10	80	n.a.	180
(*S*)-24	Br	Br	OMe	1	0.096	110	86	n.a.
(*S*)-25	Br	Br	OMe	2	0.093	n.a.	n.a.	n.a.
(*R*)-26	Br	Br	OMe	1	9.1	n.a.	n.a.	n.a.
(*S*)-34	Br	Br	OH	0	0.058	120	200	78
(*S*)-35	Br	Br	OH	1	0.069	86	74	76
(*S*)-36	Br	Br	OH	2	0.049	270	n.a.	110
(*R*)-37	Br	Br	OH	1	0.50	n.a.	n.a.	n.a.
(*S*)-47	Br	Br	NH_2	1	1.7	n.a.	n.a.	n.a.
(*S*)-31	Cl	Cl	OEt	0	0.40	320	300	290
(*S*)-32	Cl	Cl	OMe	1	0.40	n.a.	n.a.	n.a.
(*S*)-33	Cl	Cl	OMe	2	0.89	n.a.	n.a.	n.a.
(*S*)-42	Cl	Cl	OH	0	0.59	300	n.a.	170
(*S*)-43	Cl	Cl	OH	1	0.13	n.a.	n.a.	n.a.
(*S*)-44	Cl	Cl	OH	2	0.30	10	n.a.	n.a.



	R_1	*n*	DNA gyrase IC_{50} [μM] *E. coli*	DNA gyrase IC_{50} [μM] *S. aureus*
(*S*)-28	Et	0	10 μM	n.a.
(*S*)-29	Me	1	35 μM	n.t.
(*S*)-30	Me	2	9.1 μM	n.a.
(*S*)-39	H	0	7.7 μM	n.a.
(*S*)-40	H	1	7.6 μM	n.a.
(*S*)-41	H	2	8.1 μM	n.a.

structure-activity relationship (*E. coli* DNA gyrase)

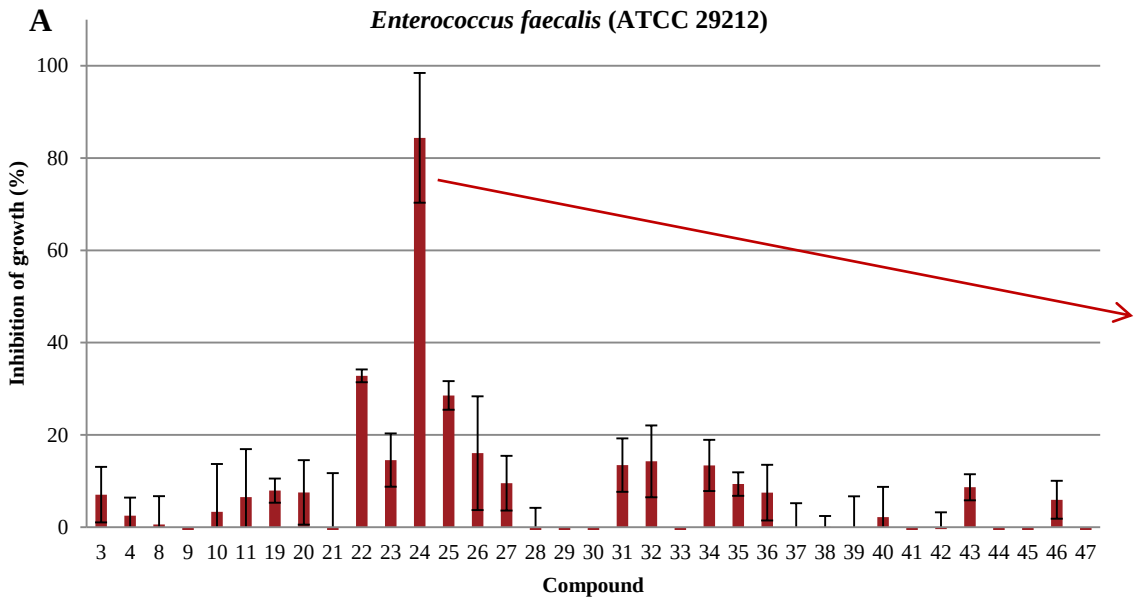


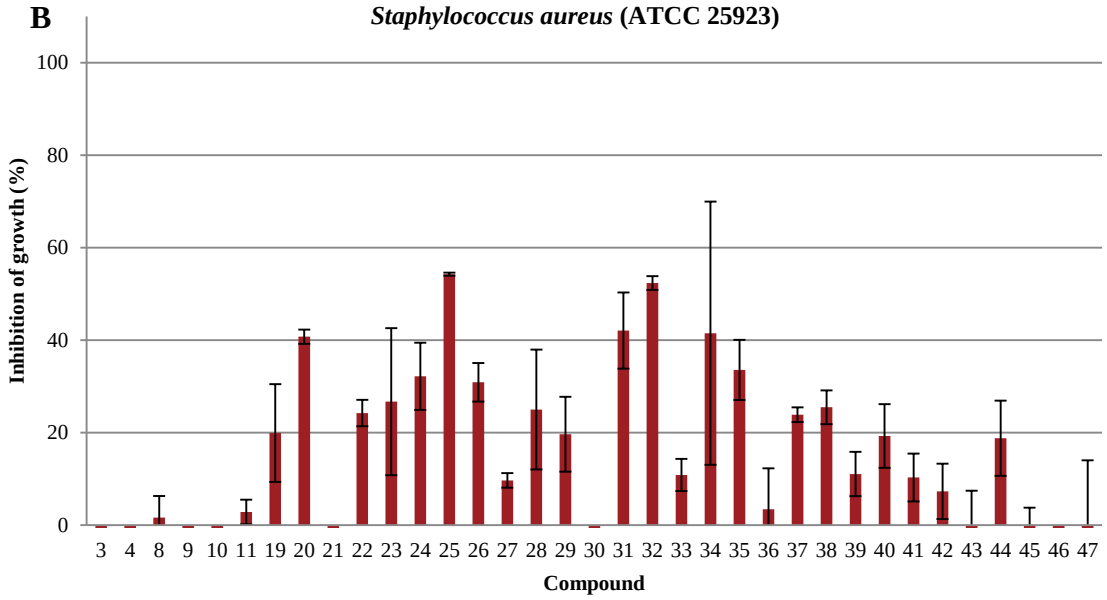
OMe, OEt, OH > NH_2

4,5-dibromopyrrole > 4,5-dichloropyrrole > indole > 4-bromopyrrole

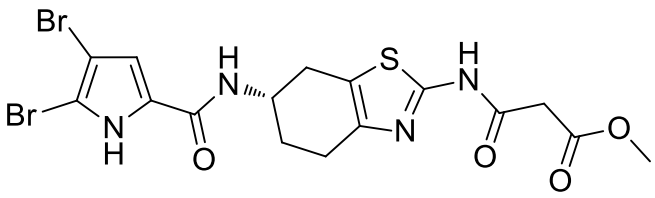
n=0 = n=1 = n=2

Tomašič, T. et al. *J. Med. Chem.* **2015**, *58*, 5501-5521.

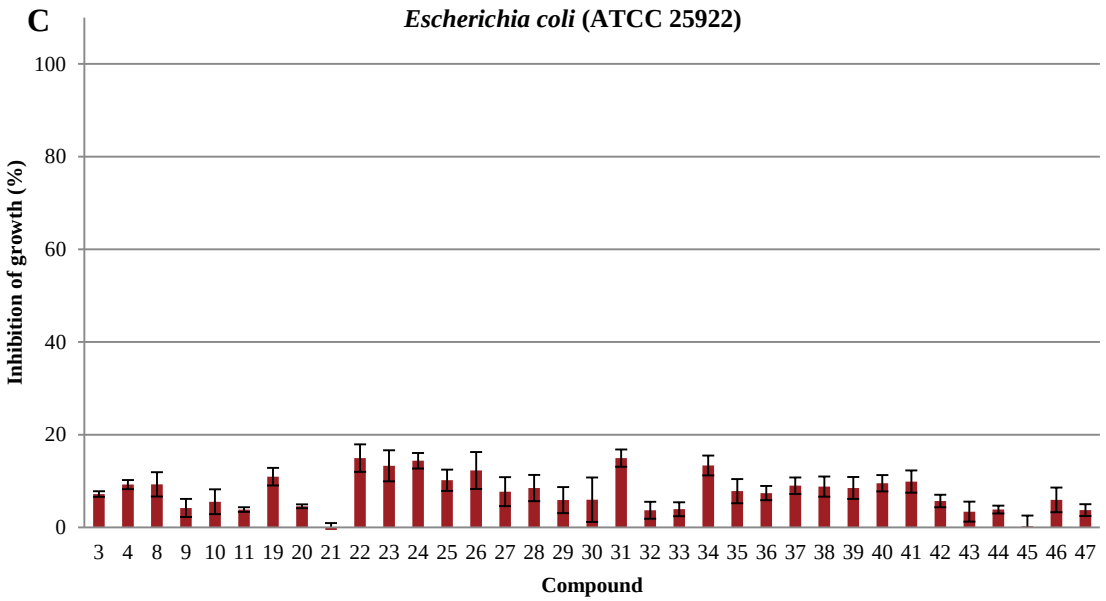


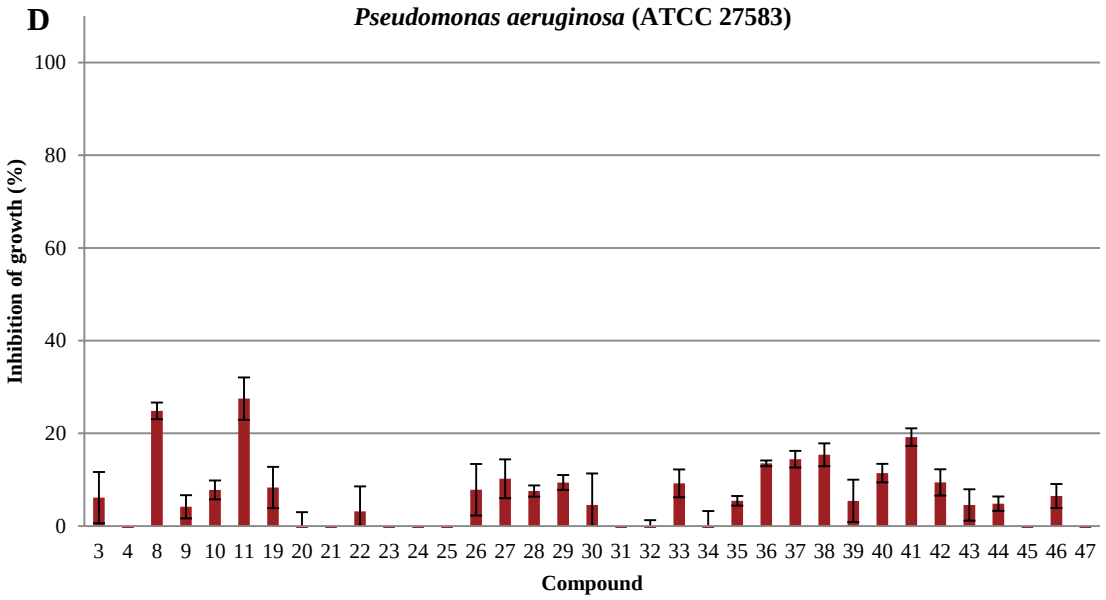


- weak antibacterial activity against Gram positive strains at 50 μM

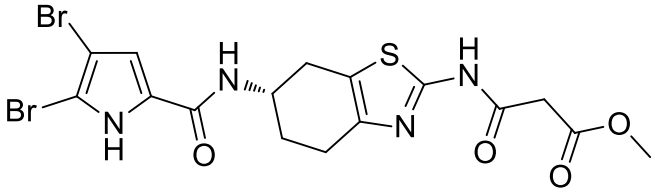


- *E. coli* DNA gyrase
  IC_{50} = 0.096 μM
- *S. aureus* DNA gyrase
  IC_{50} = 110 μM
- *E. coli* topoIV
  IC_{50} = 86 μM
- *S. aureus* topoIV
  inactive

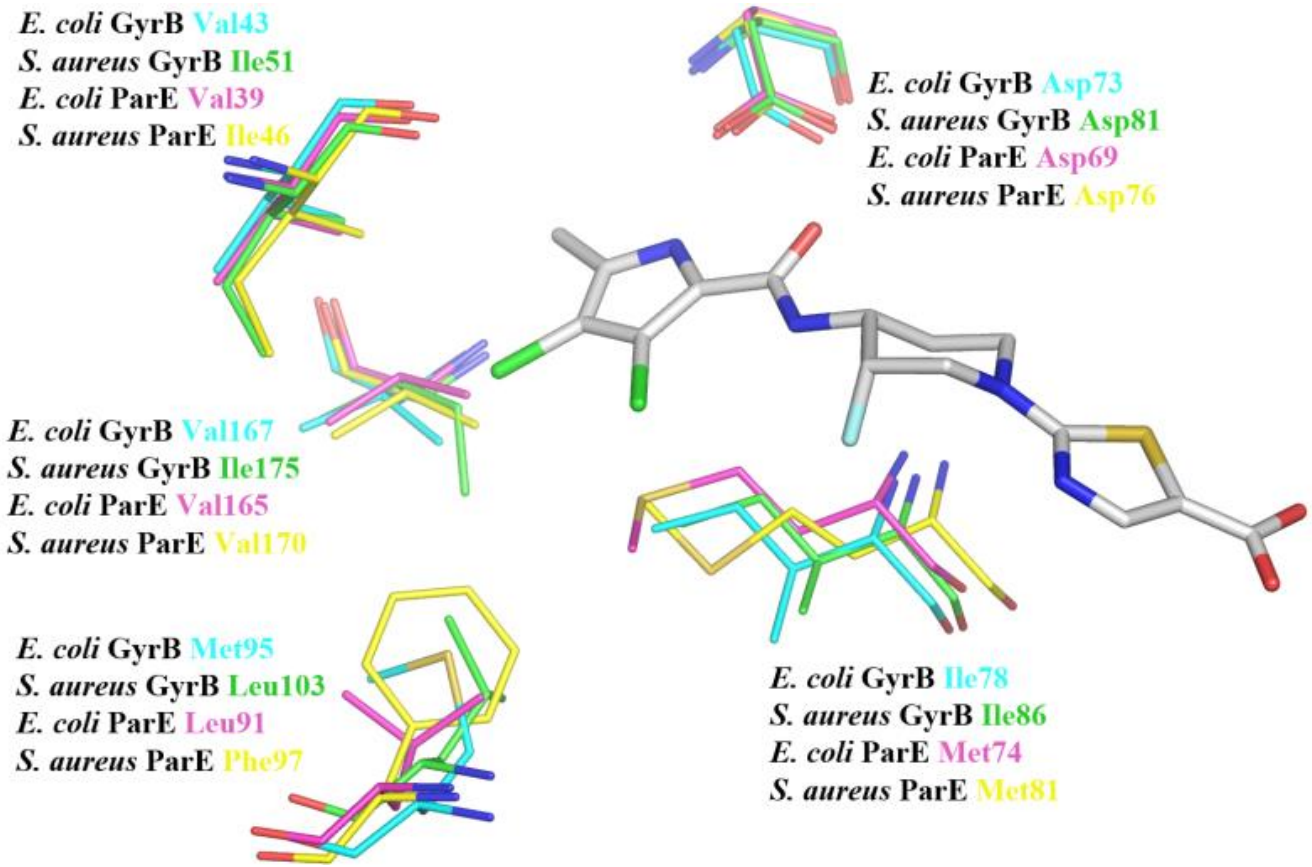




- inactive against Gram negative strains

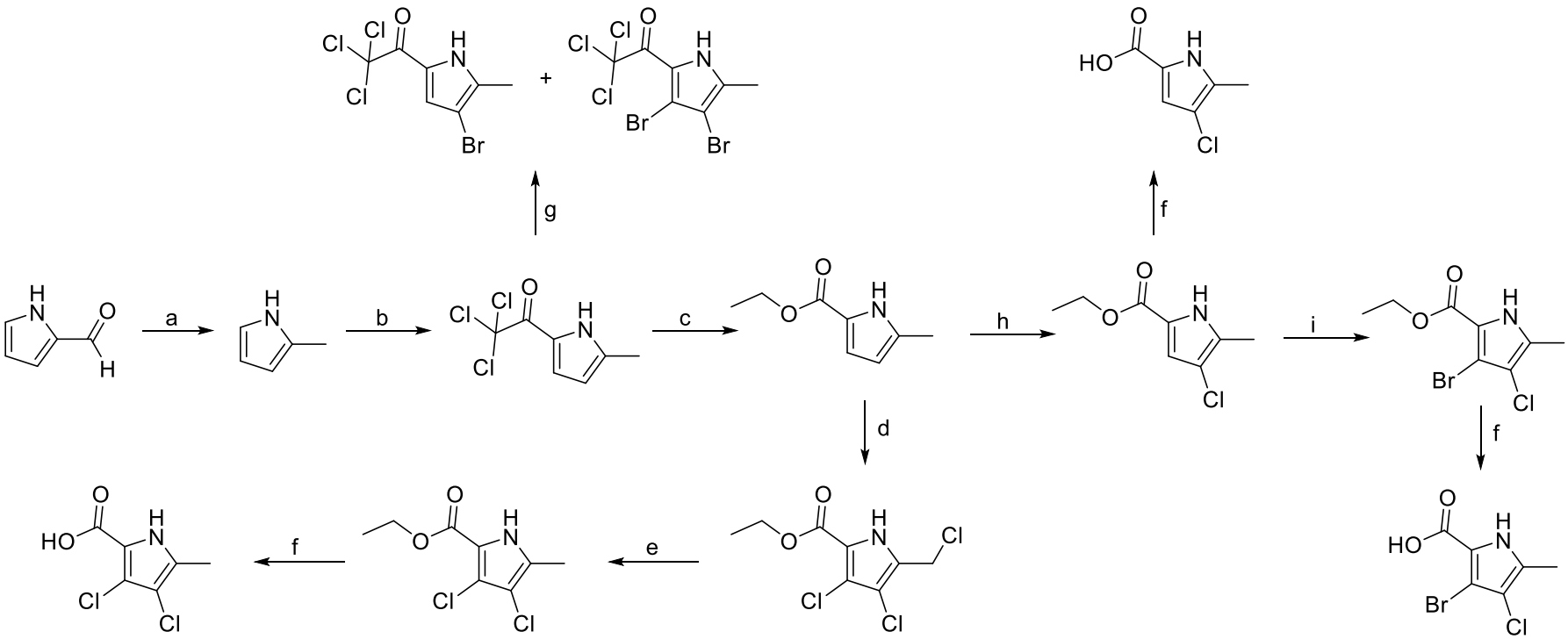


- MIC wild-type *E. coli* > 256 μg/mL
- MIC *impA E. coli* 16 μg/mL
- MIC *tolC E. coli* 32 μg/mL

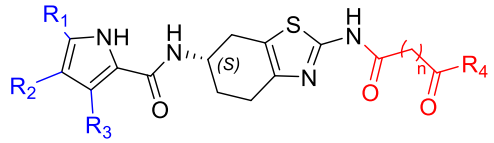


	***Ec* GyrB**	***Sa* GyrB**	***Ec* ParE**	***Sa* ParE**
pocket volume	157 Å^3	140 Å^3	149 Å^3	155 Å^3
	1*H*-indole-2-carboxamide	4,5-dibromo-1*H*-pyrrole-2-carboxamide	4,5-dichloro-1*H*-pyrrole-2-carboxamide	
volume	143 Å^3	135 Å^3	126 Å^3	

Optimisation of the pyrrole moiety

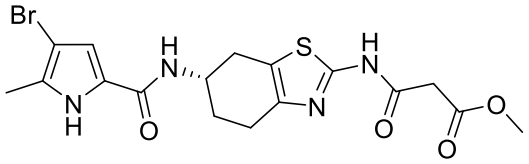


Scheme 4. Reagents and conditions. a) KOH, NH_2NH_2, ethylene glycol, reflux, 24 h; b) trichloroacetyl chloride, Et_3N, THF, 0°C, 3 h; c) Na, EtOH, r.t., 1h; d) 3 eq SO_2Cl_2, CCl_4, 0 °C - r.t., 2 h; e) H_2, Pd/C, DMF, r.t., 24 h; f) 10 M NaOH, EtOH, reflux, 4 h. 1h; g) Br_2, $CHCl_3$, 0°C, 1 h; h) 1 eq SO_2Cl_2, CCl_4, 0 °C - r.t., 2 h; i) NBS, $CHCl_3$, 40 °C, 10 h.

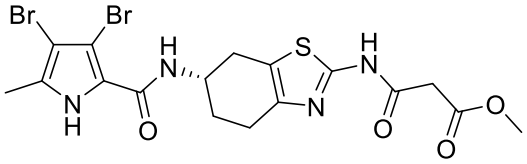


	R_1	R_2	R_3	R_4	*n*	DNA gyrase IC_{50} [μM] *E. coli*	DNA gyrase IC_{50} [μM] *S. aureus*	topoisomerase IV IC_{50} [μM] *E. coli*	topoisomerase IV IC_{50} [μM] *S. aureus*
(*S*)-42	H	H	H	OMe	1	n.a.	n.t.	n.t.	n.t.
(*S*)-43	H	H	H	OH	1	n.a.	n.a.	n.a.	n.a.
(*S*)-44	Me	H	H	OMe	1	n.a.	n.t.	n.t.	n.t.
(*S*)-45	Me	H	H	OH	1	21	n.a.	n.a.	n.a.
(*S*)-46	Me	Br	H	OMe	1	0.73	47	230	35
(*S*)-47	Me	Br	Br	OMe	1	1.0	31	n.a.	n.a.
(*S*)-48	Me	Br	H	OH	1	0.72	7.3	152	5.2
(*S*)-49	Me	Br	Br	OH	1	0.020	12	n.a.	n.a.
(*S*)-50	Me	Cl	H	OMe	1	0.32	8.4	~100	n.a.
(*S*)-51	Me	Cl	Cl	OEt	0	0.23	0.71	n.a.	n.a.
(*S*)-52	Me	Cl	Cl	OMe	1	0.29	26	n.a.	n.a.
(*S*)-53	Me	Cl	H	OH	1	0.19	2.0	n.a.	~50
(*S*)-54	Me	Cl	Cl	OH	0	0.044	0.43	209	6.2
(*S*)-55	Me	Cl	Cl	OH	1	0.022	0.56	~100	~50
(*S*)-56	Me	Cl	Br	OMe	1	0.25	1.5	n.a.	~100
(*S*)-57	Me	Cl	Br	OH	1	0.026	0.61	~100	6.6

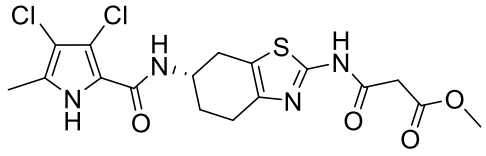
Antibacterial activity



MIC (*S.aureus*) = 125 μM
MIC (*E. faecalis*) = 125 μM

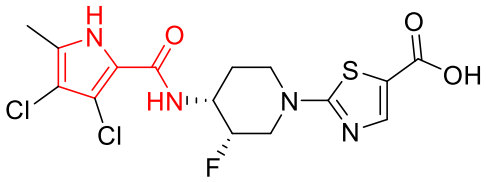


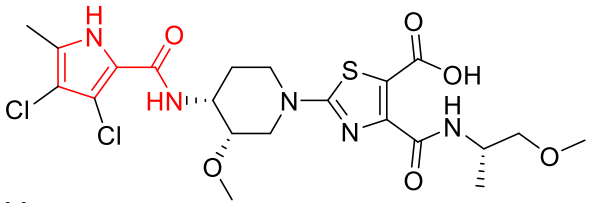
MIC (*S.aureus*) = 25 μM
MIC (*E. faecalis*) = 25 μM



MIC (*S.aureus*) = 50 μM
MIC (*E. faecalis*) = 50 μM

MIC (*E. coli* TOP10 wt) > 64 μg/mL
MIC (*E. coli* TOP10 *tolC*-) = 2 μg/mL





pyrrolamides

S. aureus GyrB IC_{50} = 4 nM
MIC (*S. aureus*) = 2 μg/mL
MIC (*E. coli*) = >64 μg/mL
MIC (*E. coli* ΔtolC) = 0.5 μg/mL

S. aureus GyrB IC_{50} <10 nM
E. coli ParE IC_{50} = 73 nM
MIC (*S. aureus*) = 0.036 μg/mL
MIC (*E. coli*) = 24 μg/mL
MIC (*E. coli* ΔtolC) = 0.94 μg/mL

Positive control information:			**Ciprofloxacin**			
			E. faecalis	*S. aureus*	*E. coli*	*P. aeruginosa*
		MIC90 (μM)	3,02	1,51	0,05	3,02
		MIC90 (μg/ml)	1	0,5	0,016	1

Conclusions

- starting from marine alkaloid oroidin, series of potent 4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazole-based *E. coli* DNA gyrase inhibitors were designed and synthesized
- optimisation of the pyrrole moiety resulted in improved *S. aureus* DNA gyrase inhibition and antibacterial activity
- more potent inhibitory activity and optimisation of physico-chemical properties to improve antibacterial activity still in progress

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INTEGRATE

MARES

SEVENTH FRAMEWORK PROGRAMME

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za raziskovalno dejavnost
Republike Slovenije