C2D2 Centre for Chronic Diseases & Disorders

Probing linker design in citric acid-ciprofloxacin conjugates as new antimicrobials

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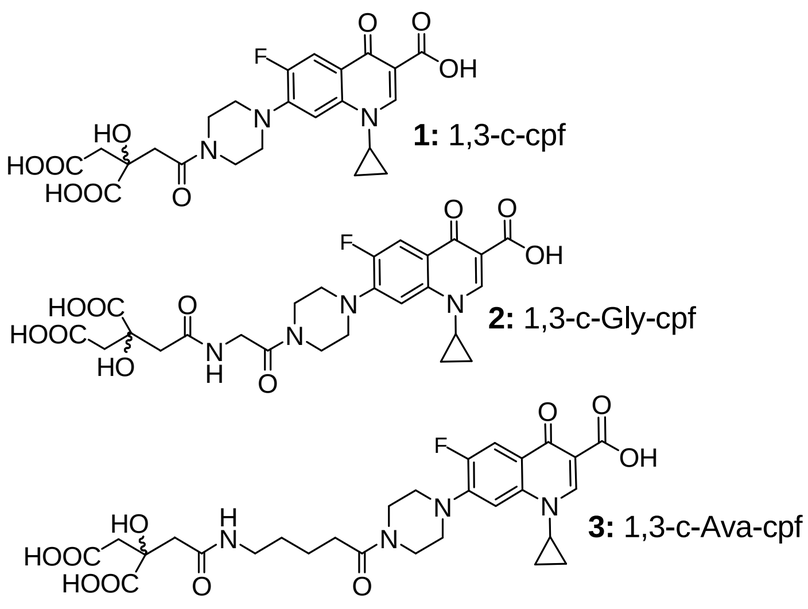
Structurally related citric acid-ciprofloxacin conjugates were synthesised to investigate the influence of the properties of the linker between the two functional units on antibacterial activities:



MICs were correlated with the DNA gyrase inhibitory activity and trends rationalised by computational modelling.

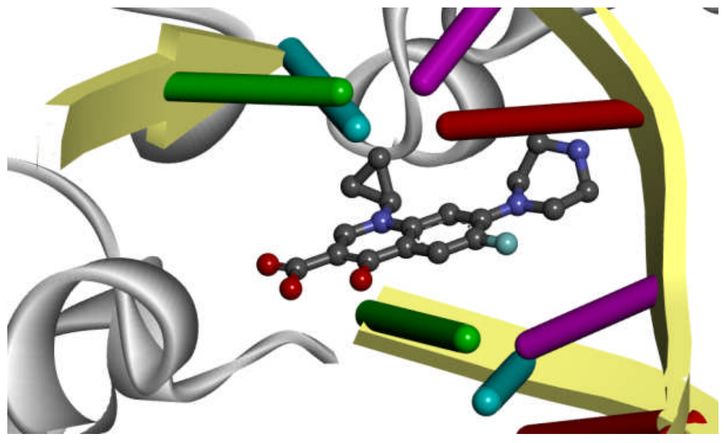


Fig. 1: Detail of the ciprofloxacin/DNA/gyrase structure (pdb code: 2xct).

Minimum inhibitory concentrations (MICs) were determined against a panel of reference strains and clinical isolates of bacteria associated with infection in humans:

bacterial strains	control	conjugates								
	ciprofloxacin	**1**			**2**			**3**		
	MIC µg/mL	MIC nmol/mL	MIC µg/mL	nmol/mL	ratio[a]	MIC µg/mL	nmol/mL	ratio[a]	MIC µg/mL	nmol/mL	ratio[a]
Staphylococcus aureus (Oxford) NCTC 6571	0.25	0.755	16	31.6	41.8	16	28.4	37.6	R	R	
Staphylococcus aureus (HG-1) [c d]	R	R	R			R	R		R	R	
Staphylococcus aureus-15 NCTC 13142 [e]	0.5	1.51	8	15.8	10.5	16	28.4	18.8	R	R	
Staphylococcus aureus-16 NCTC 13143 [c e]	R	R	R			R	R		R	R	
Staphylococcus aureus BIG 0052 [c d]	0.5	1.51	16	31.6	20.9	16	28.4	18.8	R	R	
Staphylococcus epidermidis NCTC 11047	0.125	0.377	16	31.6	83.8	16	28.4	75.3	R	R	
Staphylococcus epidermidis NCTC 2749	0.25	0.755	8	15.8	20.9	8	14.2	18.8	32	52.9	70
Staphylococcus haemolyticus NCTC 11042	0.125	0.377	16	31.6	83.8	16	28.4	75.3	R	R	
Escherichia coli NCTC 10418	0.06	0.181	0.25	0.5	2.8	0.125	0.222	1.23	1	1.6	8.8
Escherichia coli BIG 0046 [c]	R	R	R			R	R		R	R	
Coliform BIG 0051 [c]	R	R	R			R	R		R	R	
Pseudomonas aeruginosa (Environmental) BIG 0039	0.25	0.755	4	7.9	10.5	2	3.56	4.72	16	26.5	35
Pseudomonas aeruginosa (Clinical) BIG 0037	2	6.04	16	31.6	5.2	16	28.4	4.7	R	R	
Pseudomonas aeruginosa NCTC 10662	0.25	0.755	4	7.9	10.5	2	3.56	4.72	16	26.5	35
Pseudomonas aeruginosa BIG 0063	0.25	0.755	4	7.9	10.5	8	14.2	18.8	16	26.5	35
Serratia marcescens BIG 0011 = NCTC 1377	0.06	0.181	1	2	11	0.5	0.889	4.91	4	6.6	36.5
Burkholderia cepacia BIG 0009 = NCTC 10744	0.5	1.51	16	31.6	20.9	8	14.2	9.40	32	52.9	35
Burkholderia cepacia BIG 117	1	3.02	16	31.6	10.5	8	14.2	4.70	R	R	
Burkholderia cepacia BIG 118	0.25	0.755	16	31.6	41.8	8	14.2	18.8	R	R	
Burkholderia cepacia BIG 119	4	12.1	R	R		32	56.9	4.7	R	R	
Burkholderia cepacia BIG 120	2	6.04	16	31.6	5.2	32	56.9	9.4	R	R	
Burkholderia cepacia BIG 121	8	24.1	R	R		R	R		R	R	
Stenotrophomonas maltophilia N1127 [f]	2	6.04	32	63.3	10.5	32	56.9	9.4	R	R	
Stenotrophomonas maltophilia N1124 [f]	4	12.1	R	R		32	56.9	4.7	R	R	
Stenotrophomonas maltophilia N1119 [f]	1	3.02	32	63.3	20.1	R	R		R	R	

[a] ratio = MIC of conjugate (nmol/mL) / MIC of ciprofloxacin (nmol/mL)
[b] R = resistant, MIC > 36 µg/mL.
[c] ciprofloxacin resistant strain
[d] methicillin-resistant *Staphylococcus aureus* (MRSA)
[e] epidemic methicillin-resistant *Staphylococcus aureus* (EMRSA)

The ability of selected compounds to penetrate *Pseudomonas aeruginosa* colony biofilms was assessed:

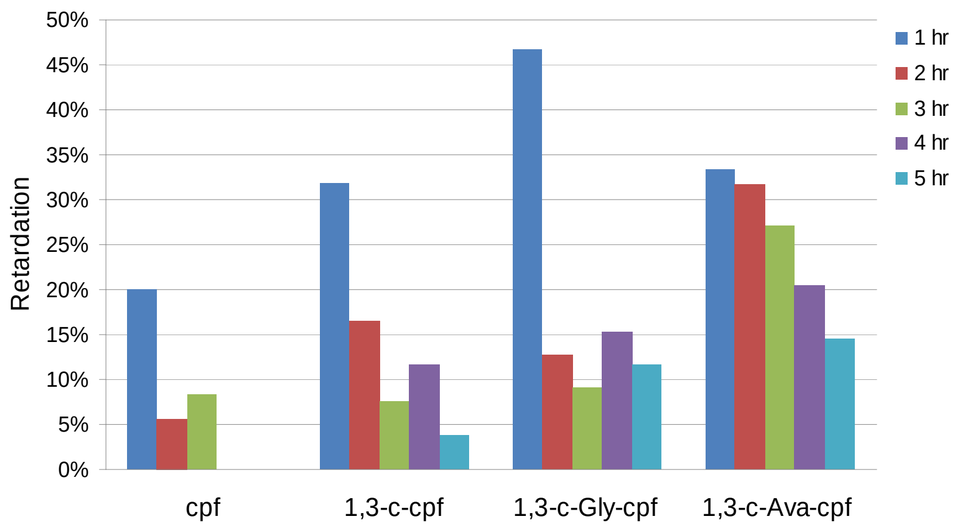


Fig. 2: % Retardation of indicated compounds by *P. aeruginosa* LESB58 colony biofilms.

Conclusions:

- long linker groups provide less stable binding to DNA gyrase
- the optimal linker group should not contain more than 1-2 atoms

Future studies will be directed towards the enhancement of the DNA inhibitory activity through introduction of biolabile linkers designed to release ciprofloxacin from the citric acid unit once transported into the cell.

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