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In vitro Activity and Pharmacodynamic Evaluation of Dual Targeting Inhibitors (DTI) of Bacterial DNA Gyrase and Topoisomerase IV against Extracellular and Intracellular Forms of Ciprofloxacin-susceptible (CIP^S) and Ciprofloxacin-resistant (CIP^R) *Pseudomonas aeruginosa* and *Staphylococcus aureus*

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Abstract

Background: Treatment of severe infections caused by PA or SA remains challenging due to (i) acquisition of resistance determinants to currently approved fluoroquinolones, and (ii) their capacity to survive intracellularly. We have examined the in vitro extracellular and intracellular activities of DTIs towards CIP^S and CIP^R PA and SA.

Methods: The Table shows the strains used. MICs were measured by microdilution (CLSI method). Extracellular and intracellular activities (24 h) were assessed in broth and in infected THP-1 cells using a PD model (1, 2) for determination of apparent static concentrations (Cs ([mg/L]) and maximal relative efficacies (Emax [delta $\log_{10}$ cfu]).

Results: Data show that, compared to CIP, DTIs display (i) similar MICs towards both CIP^S and CIP^R but are susceptible to efflux; (ii) bactericidal (or close to) effects in broth (~ 3 $\log_{10}$ cfu decrease) against both bacteria and in THP-1 cells for PA; (iii) significant effects (1.3-2.2 log cfu decrease) in THP-1 cells for SA.

Conclusions: DTIs are active *in vitro* against both CIP^S and CIP^R PA and SA (largely disregarding underlying mechanisms) and effective against their intracellular forms. This project has been funded in whole or in part with Federal funds from the National Institute of Allergy and Infectious Diseases, NIH, Department of Health and Human Services, under Contract No. HHSN272200800042C

1. Intrinsic activity (MIC; mg/L) *

	CIP	GP1	GP2	GP4	GP6
P. aeruginosa					
PAO1 (reference)	0.125	4	1	4	0.5
PA256 (MDR isolate / CIP^R)	32	2	1	4	1
PA17 (MexXY+/mutation CIP^R)	32	1	0.5	2	0.5
PA397 (deleted for all efflux)	0.008	0.125	0.125	0.125	0.125
S. aureus					
ATCC 33591 (MRSA / CIP^R)	0.25	0.06	0.03	0.06	0.03
SA1 (MSSA NorA+ / CIP^R)	4	0.25	0.062	0.125	0.125
NRS18 (MRSA / CIP^R)	16-32	0.125	0.03	0.06	0.06
ATCC 8/72 (MRSA / CIP^R)	256	0.06	0.03	0.06	0.06

2. Pharmacodynamic evaluation

P.aeruginosa	parameter	GP1	GP2	GP4	GP6
PAO1 (CIP^S)					
Broth	C_s (mg/L) [b]	2.82	1.24	2.82	0.97
	E_{max} [c]	-3.42±0.38	-2.91±0.31	-3.49±0.57	-2.90±0.35
THP-1	C_s (mg/L) [b]	2.17	0.63	2.45	1.16
	E_{max} [c]	-2.49±0.29	-2.25±0.24	-2.51±0.20	-2.22±0.18
PA256 (CIP^R)					
Broth	C_s (mg/L) [b]	2.18	0.62	4.83	1.46
	E_{max} [c]	-3.70±0.66	-4.46±0.22	-4.66±0.89	-4.23±0.50
THP-1	C_s (mg/L) [b]	13.8	1.17	3.55	0.86
	E_{max} [c]	-3.90±0.57	-3.07±0.12	-3.73±0.18	-3.44±0.06
S. aureus					
ATCC 33591 (MRSA CIP^R)					
Broth	C_s (mg/L) [b]	~ 0.04	~ 0.01	~ 0.04	~ 0.03
	E_{max} [c]	-3.4 ± 0.3	-3.0 ± 0.2	-3.8 ± 0.3	-3.2 ± 0.4
THP-1	C_s (mg/L) [b]	~ 0.01	~ 0.01	~ 0.02	~ 0.01
	E_{max} [c]	-1.2 ± 0.2	-1.3 ± 0.3	-1.6 ± 0.1	-1.3 ± 0.2
ATCC 8/72 (MRSA CIP^R)					
Broth	C_s (mg/L) [b]	~ 0.03	~ 0.02	~ 0.02	~ 0.03
	E_{max} [c]	-2.3 ± 0.2	-3.1 ± 0.4	-2.9 ± 0.3	-2.3 ± 0.2
THP-1	C_s (mg/L) [b]	~ 0.10	~ 0.02	~ 0.10	~ 0.09
	E_{max} [c]	-1.9 ± 0.2	-2.1 ± 0.2	-1.4 ± 0.2	-1.5 ± 0.2

* EUCAST breakpoints: PA, S < or = 0.5 – R > 1 mg/L; SA: S < or = 1 – R > 1 mg/L

[b] Concentration resulting in no apparent bacterial growth (number of cfu identical to the original inoculum), as determined by graphical intrapolation;

[c] Maximal relative efficacy (decrease in the numbers of CFU [in $\log_{10}$ units] at 24 h from the corresponding original inoculum), as extrapolated for an infinitely high antibiotic concentration.

Results

1. MICs (mg/L) towards *P. aeruginosa* and *S. aureus*

	CIP *	GP-1	GP-2	GP-4	GP-6
P. aeruginosa					
PAO1 (reference)	0.125	4	1	4	0.5
PA256 (MDR isolate / CIP^R)	32	2	1	4	1
PA17 (MexXY+/mutation CIP^R)	32	1	0.5	2	0.5
PA397 (deleted for all efflux)	0.008	0.125	0.125	0.125	0.125
S. aureus					
ATCC 33591 (MRSA / CIP^R)	0.25	0.06	0.03	0.06	0.03
SA1 (MSSA NorA+ / CIP^R)	4	0.25	0.062	0.125	0.125
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ATCC 8/72 (MRSA / CIP^R)	256	0.06	0.03	0.06	0.06

* EUCAST breakpoints: R > 1 mg/L

All 4 DTIs tested (GP-1, GP2, GP-4, GP-6) show:
- **activity towards *P. aeruginosa* (including ciprofloxacin-resistant isolates)**
- **lower MICs values than ciprofloxacin towards *S. aureus***

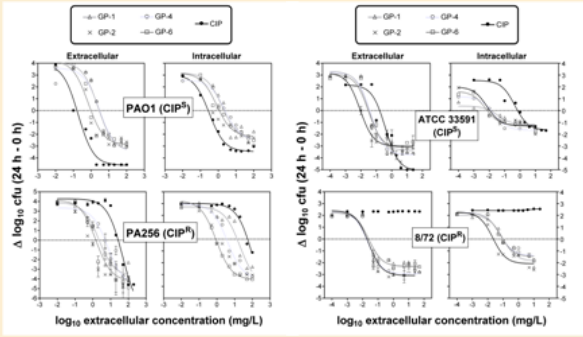
Regression parameters of dose-response curves illustrated in the figure

	parameters	GP-1	GP-2	GP-4	GP-6
P.aeruginosa					
PAO1 (CIP^S)					
Broth	C_s [1]	2.8	1.2	2.8	1.0
	E_{max} [2]	-3.4±0.4	-2.9±0.3	-3.5±0.6	-2.9±0.4
THP-1	C_s [1]	2.2	0.6	2.5	1.2
	E_{max} [2]	-2.5±49	-2.3±0.2	-2.5±0.2	-2.2±0.2
PA256 (CIP^R)					
Broth	C_s [1]	2.2	0.6	4.8	1.5
	E_{max} [2]	-3.7±0.7	-4.5±0.2	-4.7±0.9	-4.2±0.5
THP-1	C_s [1]	13.8	1.2	3.6	0.9
	E_{max} [2]	-3.9±0.6	-3.1±0.1	-3.7±0.2	-3.4±0.1
S. aureus					
ATCC 33591 (MRSA CIP^R)					
Broth	C_s [1]	~ 0.04	~ 0.01	~ 0.04	~ 0.03
	E_{max} [2]	-3.4 ± 0.3	-3.0 ± 0.2	-3.8 ± 0.3	-3.2 ± 0.4
THP-1	C_s [1]	~ 0.01	~ 0.01	~ 0.02	~ 0.01
	E_{max} [2]	-1.2 ± 0.2	-1.3 ± 0.3	-1.6 ± 0.1	-1.3 ± 0.2
ATCC 8/72 (MRSA CIP^R)					
Broth	C_s [1]	~ 0.03	~ 0.02	~ 0.02	~ 0.03
	E_{max} [2]	-2.3 ± 0.2	-3.1 ± 0.4	-2.9 ± 0.3	-2.3 ± 0.2
THP-1	C_s [1]	~ 0.10	~ 0.02	~ 0.10	~ 0.09
	E_{max} [2]	-1.9 ± 0.2	-2.1 ± 0.2	-1.4 ± 0.2	-1.5 ± 0.2

[1] Concentration (mg/L) resulting in no apparent bacterial growth (number of cfu identical to the original inoculum), as determined by graphical intrapolation;

[2] Maximal relative efficacy (decrease in the numbers of CFU [in $\log_{10}$ units] at 24 h from the corresponding original inoculum), as extrapolated for an infinitely high antibiotic concentration.

2. Extracellular and intracellular activities of antibiotics towards both *P. aeruginosa* and *S. aureus* in a pharmacodynamic model



Concentration-killing effects of DTIs and ciprofloxacin towards both the extracellular and intracellular forms of bacteria (*P. aeruginosa and S. aureus).* The ordinate shows the change in cfu per mL of broth (extracellular) or per mg of cell protein (intracellular) after 24 h, compared to the original inoculum. All values are means ± SD (n=2-3).

DTIs are :
- **bactericidal (3 $\log_{10}$ cfu decrease) against both the extracellular and the intracellular forms of *P. aeruginosa*, irrespective of its resistance phenotype to ciprofloxacin**
- **highly potent (extremely low C_s values compared to ciprofloxacin) towards both the extracellular and intracellular forms of *S. aureus* and with marked maximal activity irrespective of its resistance phenotype to ciprofloxacin (as for ciprofloxacin, however, a bactericidal effect is only observed for extracellular bacteria)**

Conclusion

The dual targeting inhibitors investigated show *in vitro* efficacy towards the extracellular and intracellular forms of both ciprofloxacin-susceptible and ciprofloxacin-resistant *P. aeruginosa* and *S. aureus*.

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Background and aim

P. aeruginosa and *S. aureus* are both the causative agents of severe nosocomial diseases. Treatment of these infections remains challenging, due to their ability (i) to resist to antibiotics and (ii) to enter and sojourn in phagocytes where they not only behave as opportunistic intracellular bacteria but also become less susceptible to many antibiotics. This may explain the recurrent and persistent character of both pseudomonal and staphylococcal infections.

In an *in vitro* models of intracellular infection (THP-1 macrophages; [1]), fluoroquinolones are amongst the most active agents, probably in relation to their bioavailability (free movement between subcellular compartments) and their intense bactericidal activity. This has triggered us to assess the extracellular and intracellular activities of the novel Dual Targeting gyrase/topoisomerase inhibitors (DTIs) against *P. aeruginosa* and *S. aureus,* in comparison with those of ciprofloxacin (CIP).

Materials & Methods

Bacterial strain and susceptibility testing.
P. aeruginosa strain ATCC PAO1 and *S.aureus strain* ATCC 33591 were used thourough as reference strains. Clinical isolates resistant to fluoroquinolones were also used for comparison (see 1). MICs were determined by the microdilution method in Mueller Hinton Broth (pH 7.4, 24 h).

Cells and cell infection.
All experiments were done using human THP-1 cells, a myelomonocytic cell line displaying macrophage-like activity. Briefly, opsonized bacteria (obtained after a 45 min incubation in the presence of 10 % human serum) were phagocytisedd by THP-1 cells (1 h for S. aureus, 2 h for P. aeruginosa), followed by elimination of non-addherent and non-internalized bacteria (45-60 min incubation in the presence of gentamicin). Cells were then transferred to fresh medium supplemented with increasing concentrations of antibiotics for 24 h

Determination of the dose-response activity of antibiotics towards extracellular and intracellular bacteria.
Extracellular (Mueller Hinton Broth) and Intracellular (infected THP-1 cells) activities of antibiotics were determined as described previously ([1-3]), using increasing concentrations of antibiotics (24 h). Results were expressed as the change in the bacterial inoculum at 24 h, compared to time 0. Data were used to fit a Hill equation, allowing determination of the values of two key pharmacological descriptors of antibiotic activity (see ref. 1 for a detailed description of these parameters): (i) Static concentration [Cstatic; no change from time 0]; (ii) Maximal relative efficacy [Emax; maximal relative efficacies of antibiotics, as determined for an infinitively large concentration of antibiotics];).

References

(1) Barcia-Macay *et al.*, Antimicrob Agents Chemother. (2007); 51:1627-32
(2) Lemaire et al, J Antimicrob Chemother. (2011); 66:596-607
(3) Buyck *et al.* 51th ICAAC, Poster A1-1753

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