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Potent *In Vitro* Antibacterial Activity of DS-8587, a Novel Broad-Spectrum Quinolone, against *Acinetobacter baumannii*

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We investigated the *in vitro* activity of DS-8587, a novel fluoroquinolone, against *Acinetobacter baumannii*. The MICs of DS-8587 against clinical isolates and its inhibitory activity against target enzymes were superior to those of ciprofloxacin and levofloxacin. Furthermore, the antibacterial activity of DS-8587 was less affected by *adeA*/*adeB*/*adeC* or *abeM* efflux pumps than was that of ciprofloxacin and the frequency of single-step mutations with DS-8587 was lower than that with ciprofloxacin. DS-8587 might be an effective agent against *A. baumannii* infection.

Acinetobacter baumannii, a Gram-negative coccobacillus, is an increasingly important nosocomial pathogen (1, 2). *A. baumannii* infection causes serious clinical problems worldwide, due to an increasing number of *A. baumannii* clinical isolates becoming resistant to the most commonly clinically utilized antibiotics (3–6). Colistin and tigecycline are the last-resort agents to treat multidrug-resistant (MDR) *A. baumannii*, but unfortunately, strains resistant to these agents have already appeared (7, 8). Therefore, development of a new treatment option for MDR *A. baumannii* is an urgent need.

DS-8587 is a novel fluoroquinolone which has potent activity against pathogens that cause community and nosocomial infections (9). In this study, we assessed the *in vitro* potency of DS-8587 against *A. baumannii*.

(Part of this work was presented at the 52nd Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA [10].)

DS-8587 (Fig. 1) and levofloxacin were synthesized at Daiichi Sankyo, Co., Ltd. Ciprofloxacin, tigecycline, and imipenem were purchased from LKT Laboratories, Inc. (St. Paul, MN). Norfloxacin, amikacin, gentamicin, and chloramphenicol were purchased from Sigma-Aldrich (St. Louis, MO). *A. baumannii* clinical isolates were collected by the LVFX Surveillance Group in 2007 in Japan (11). Isolates were confirmed to be *A. baumannii* by the presence of $bla_{\text{oxa-51-like}}$ (12) and sequencing of the 16S-23S rRNA intergenic spacer region (13). Susceptibility testing was performed by broth microdilution according to the recommended CLSI method (14).

The antibacterial activities of DS-8587 and reference compounds against clinical isolates of *A. baumannii*, including quinolone-susceptible isolates and those with mutations in the quinolone resistance-determining regions (QRDRs) of *gyrA* and *parC*, are shown in Table 1. DS-8587 possessed excellent antibacterial activity against wild-type *gyrA*/*parC* strains, with MICs of ≤0.015 to 0.06 μg/ml. These MICs were 4- to 8-fold and 8- to 16-fold lower than those of levofloxacin and ciprofloxacin, respectively. For clinical isolates with *gyrA*/*parC* mutations, the MICs of DS-8587 were 0.5 to 1 μg/ml, which were 4- to 16-fold and 64- to 128-fold lower than those of levofloxacin and ciprofloxacin, respectively. The MIC ranges of tigecycline, imipenem, amikacin, and gentamicin for these clinical isolates were 0.12 to 2, 0.12 to 64, 2 to 16, and 1 to >128 μg/ml, respectively. Compared to the reference compounds, DS-8587 exhibited the most potent antibacterial activity against *A. baumannii* clinical isolates.

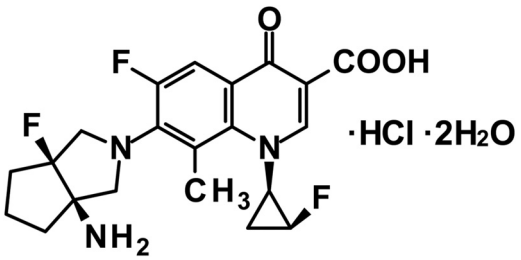

FIG 1 Chemical structure of DS-8587.

To examine the influence of mutations in *gyrA* on the antibacterial activity of DS-8587, we selected an isogenic mutant of *A. baumannii* ATCC 19606 (CIP-4) under ciprofloxacin selection pressure. CIP-4 acquired a single mutation in the *gyrA* QRDR (Ser81Leu) and no mutation in *parC*. The MIC of DS-8587 against CIP-4 was 1 μg/ml, which was 8-fold higher than that for the parent strain (ATCC 19606). In contrast, the corresponding MICs of levofloxacin and ciprofloxacin were 8 and 32 μg/ml, which were 16- and 32-fold higher than those against the parent strain, respectively.

To evaluate the inhibitory activity of DS-8587 against wild-type *A. baumannii* DNA gyrase and topoisomerase IV and altered DNA gyrase and topoisomerase IV, we purified GyrA (wild type and Ser81Leu mutant; position equivalent to Ser83 in *Escherichia coli*) and GyrB for a supercoiling assay and ParC (wild type and Ser84Leu mutant; position equivalent to Ser80 in *E. coli*) and ParE for a decatenation assay, respectively (15, 16). The 50% inhibitory concentrations (IC_{50}s) of DS-8587 for

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TABLE 1 Antibacterial activity against clinical isolates of *gyrA*/*parC* wild-type, *gyrA* mutant, and *gyrA*/*parC* mutant strains of *A. baumannii*

Strain	MIC (μg/ml)							QRDR mutation	
	DS-8587	Levofloxacin	Ciprofloxacin	Tigecycline	Imipenem	Amikacin	Gentamicin	*gyrA*	*parC*
19041	≤0.015	0.12	0.25	0.25	0.25	8	1		
19247	≤0.015	0.12	0.25	0.5	0.25	4	1		
19276	≤0.015	0.12	0.25	0.12	0.25	2	1		
19192	0.03	0.12	0.25	0.12	0.25	4	1		
19289	0.03	0.12	0.25	0.25	0.25	4	1		
19066	0.06	0.25	0.5	1	0.25	16	4		
19347	0.25	2	4	0.12	0.25	8	1	Ser81Leu	
19309	0.25	4	16	0.5	0.25	4	4	Ser81Leu	
19426	0.5	8	64	1	0.25	4	64	Ser81Leu	Ser84Leu
19477	1	8	64	2	2	8	>128	Ser81Leu	Ser84Leu
19570	1	8	64	2	0.12	4	64	Ser81Leu	Ser84Leu
19424	1	8	64	2	2	8	64	Ser81Leu	Ser84Leu
19483	1	4	64	2	64	4	4	Ser81Leu	Glu88Lys
19485	1	8	64	2	64	4	4	Ser81Leu	Glu88Lys

TABLE 2 Inhibitory activity against the wild-type and altered *A. baumannii* target enzymes

Compound	IC_{50} (μg/ml) against enzyme:			
	DNA gyrase		Topoisomerase IV	
	Wild type	Ser81Leu (GyrA)	Wild-type	Ser84Leu (ParC)
DS-8587	1.06	10.02	1.70	5.81
Levofloxacin	3.95	78.45	5.51	43.59
Ciprofloxacin	5.16	84.14	3.51	32.34

wild-type and altered (Ser81Leu in *gyrA*) DNA gyrase were 3.7- and 7.8-fold lower than those of levofloxacin and 4.9- and 8.4-fold lower than those of ciprofloxacin, respectively (Table 2). The IC_{50}s of DS-8587 for wild-type and altered (Ser84Leu in *parC*) topoisomerase IV were 3.2- and 7.5-fold lower than those of levofloxacin and 2.1- and 5.6-fold lower than those of ciprofloxacin, respectively (Table 2).

Efflux-mediated resistance has been reported in *A. baumannii* (17). Among those efflux pumps, expression levels of *adeA*/*adeB*/*adeC* and *abeM* and corresponding antimicrobial susceptibilities in clinical isolates have been examined (18–21). To assess the influence of *adeA*/*adeB*/*adeC* and *abeM* on the antibacterial activity of DS-8587, we selected two isogenic mutants which overexpressed one of these efflux pumps. The *adeA*/*adeB*/*adeC*-overexpressing strain (19483 TGC-2) was obtained under the selection pressure of tigecycline (22). The *abeM*-overexpressing strain (NFLX-16-2) was obtained under the selection pressure of norfloxacin (21). In addition, we generated an *abeM* deletion mutant (2-1-12) from NFLX-16-2 by allelic exchange (23). As shown by quantitative real-time PCR analysis (primer pairs are shown in Table 3) (22), the expression levels of *adeB* in 19483 TGC-2 and *abeM* in NFLX-16-2 were 10.2- and 3.4-fold higher than those in the parental strains,

TABLE 3 Primers used in this study

Target gene	Primer	Sequence (5′–3′)
adeB	qadeB-F	GCCTGCTTTATTGGCTGCTC
	qadeB-R	GGCAACCCTTCATTCCAAAC
abeM	qAbeM-F	TCAAGCAGGGTTCGGGTTA
	qAbeM-R	TCGGCAACTAATGGTGTGGT
rpoB	qrpoB-F	TGCGCGTTCAACTGGTTCT
	qrpoB-R	TGCCCACACTTCCATCTCAC

TABLE 4 Antibacterial activity against laboratory-selected strain of *adeA*/*adeB*/*adeC*-overexpressing *A. baumannii*

Strain	MIC (μg/ml)			
	DS-8587	Levofloxacin	Ciprofloxacin	Tigecycline
19483[a]	1	4	64	2
19483 TGC-2[b]	8	64	1,024	16

[a] *gyrA* (Ser81Leu) and *parC* (Glu88Lys) mutant strain (same strain shown in Table 2).
[b] Tigecycline-nonsusceptible strain derived from 19483, overexpressing *adeABC*.

TABLE 5 Antibacterial activity against laboratory-selected *abeM*-overexpressing strain and deletion mutant of *A. baumannii*

Strain	MIC (μg/ml)			
	DS-8587	Levofloxacin	Ciprofloxacin	Norfloxacin
ATCC 19606	0.12	0.5	1	8
NFLX-16-2[a]	0.25	1	4	64
2-1-12[b]	0.12	0.5	1	8

[a] Norfloxacin-resistant strain derived from ATCC 19606, overexpressing *abeM*. No amino acid change in the QRDRs of *gyrA* and *parC*.
[b] Derivative of NFLX-16-2 with deletion of *abeM*.

respectively. MICs of levofloxacin and ciprofloxacin for the *adeA*/*adeB*/*adeC*-overexpressing strain were both 16-fold higher (Table 4) than those for the parent strain. In contrast, DS-8587 and tigecycline MICs were 8-fold higher than those for the parental strains. MICs of norfloxacin and ciprofloxacin for the *abeM*-overexpressing mutant compared to its *abeM* deletion mutant strain (2-1-12) showed 8- and 4-fold-higher MICs, respectively (Table 5). Meanwhile, MICs of both DS-8587 and levofloxacin showed only a 2-fold increase with the mutants.

Frequencies of single-step resistance selection (16) with DS-8587 and ciprofloxacin at 4× MIC for two strains (19289 and 19347, the same strains shown in Table 1) were 4.2×10^{-8} and 7.3×10^{-8} with DS-8587 and 2.4×10^{-6} and 9.6×10^{-6} with ciprofloxacin, respectively. The mutant prevention concentration (MPC)/MIC ratios of DS-8587 and ciprofloxacin were 16 to 32 and both 32 for these two strains, respectively.

Our results demonstrate the potent *in vitro* antibacterial activity of DS-8587 against *A. baumannii* with excellent inhibitory activity against target enzymes and reduced efflux by *adeA*/*adeB*/*adeC* and *abeM* compared with other quinolones tested. These results may explain the lower frequency of single-step mutations with DS-8587 than with ciprofloxacin. These data, taken together with its reported antibacterial activity against MDR *A. baumannii* (MIC, 0.5 to 1 μg/ml) and *in vitro* bactericidal activity (24–26), support the clinical development of DS-8587 to treat infections caused by *A. baumannii*.

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