Antimicrobial activity of CHIR-090, an inhibitor of lipopolysaccharide biosynthesis, against the Burkholderia cepacia complex

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Antimicrobial Activity of CHIR-090, an Inhibitor of Lipopolysaccharide Biosynthesis, against the *Burkholderia cepacia* Complex▽

A striking characteristic of cystic fibrosis (CF) is susceptibility to life-limiting bacterial infections of the respiratory tract (13, 26). Members of the *Burkholderia cepacia* complex are a particular cause of anxiety to CF individuals (8, 14, 23) since they display high resistance to antibiotics and biocides (3, 32), possibly linked to their relatively large (~8- to 9-Mbp) genomes (16). At present, the *B. cepacia* complex consists of 17 species, the majority of which have been recovered from CF patients (4, 10, 11, 36, 37, 40). As an aid to *B. cepacia* complex studies, two panels of *B. cepacia* complex reference isolates (Table 1) have been assembled that include *B. multivorans* and *B. cenocepacia* strains, the two most prevalent species responsible for CF infections (11, 22). A promising target for the development of new antibiotics against multiresistant Gram-negative pathogens are the lipopolysaccharide (LPS) biosynthetic and modification pathways (27, 29, 30, 39). LPS (also known as endotoxin) is the major component of the bacterial outer membrane. Nine enzymes are required to form the basic core Kdo_2-lipid A, and the first six of these enzymes are essential in *Escherichia coli*. Also, we discovered that a putative locus involved in Ara4N synthesis and LPS modification was essential to *B. cenocepacia* (28). The metal-dependent UDP-[3-*O*-(*R*-3-hydroxymyristoyl)]-*N*-acetylglucosamine deacetylase (LpxC) (Fig. 1) that catalyzes the second step in lipid A biosynthesis (18, 41) has been targeted and, like many metalloenzymes, can be inhibited by hydroxamate-containing compounds (7, 9, 17). The synthetic antibiotic CHIR-090 (Fig. 1) (*N*-aroyl-L-threonine hydroxamic acid [international patent WO 2004/062601 A2]) (1) has been shown to be a slow, tight-binding inhibitor of the LpxCs from different species (5, 6, 24) and displayed good antimicrobial activity against several Gram-negative bacteria (6).

We determined the activity of CHIR-090 against the *B. cepacia* complex (Table 1) initially by disc diffusion growth inhibition assay according to published guidelines (2). Individual isolates displayed remarkable differences in susceptibility to CHIR-090, even within a single species. Interestingly, CHIR-090 was active against all representative strains of *B. multivorans*, *B. vietnamiensis*, *B. dolosa*, and *B. ambifaria*. We prepared a panel of clinically relevant *B. multivorans* strains for MIC determination and included *E. coli* and *Pseudomonas aeruginosa* (Table 2). The CHIR-090 MICs were strain dependent, and the values obtained ranged from 0.1 to >100 μg/ml.

The LPSs from a number of *Burkholderia* species display unique structural and inflammatory properties (12, 33); however, there appears to be no correlation between CHIR-090 activity and the LPS profiles of individual strains. For example, CHIR-090 is not active against smooth LPS strain *B. cenocepacia* K56-2 or its deep-rough LPS derivative SAL1 (20). A BLAST sequence analysis of the *Burkholderia* genomes (*Burkholderia* Genome Database) revealed that the LpxC genes are highly conserved and display high sequence homology to LpxCs from *P. aeruginosa* and *E. coli*; thus, the reason(s) why CHIR-090 is not active against certain members of the *B. cepacia* complex remains to be clarified. Our study reports the potential of therapeutic agents against *Burkholderia* targeted at LPS biosynthesis. Such agents may, possibly in combination

TABLE 1. Inhibition of strains of *Burkholderia* genomovars I to IX by CHIR-090

Strain	Inhibition zone diam (mm)[a]
Panel 1	
B. cepacia (I)	
ATCC 25416	—[b]
ATCC 17759	—
CEP509	—
B. multivorans (II)	
C5393	20
LMG 13010	14
C1576	15
CF-A1-1	22
JTC	23
C1962	24
ATCC 17616	17
249-2	26
B. cenocepacia (III)	
J2315	—
BC7	—
K56-2	—
C5424	19
C6433	—
PC184	18
CEP511	—
J415	21
ATCC 17765	—
SAL-1	—
B. stabilis (IV)	
LMG 14294	—
C7322	14
LMG 14086	—
LMG 18888	—
B. vietnamiensis (V)	
PC259	26
LMG 16232	23
FC441	9
LMG 10929	23
Panel 2	
B. dolosa (VI)	
AU0645	18
CEP021	24
E12	23
STM1441	21
B. ambifaria (VII)	
AMMD	23
ATCC 53266	24
CEP0996	22
B. anthina (VIII)	
W92	—
C1765	13
J2552	—
AU1293	10
B. pyrrocinia (IX)	
ATCC 15958	20
ATCC 39277	22
BC011	23
C1469	—

[a] The values shown are CHIR-090 inhibition zones in the disc diffusion assay (40 μg/disc).
[b] —, CHIR-090 gave no zone of growth inhibition with this particular strain.

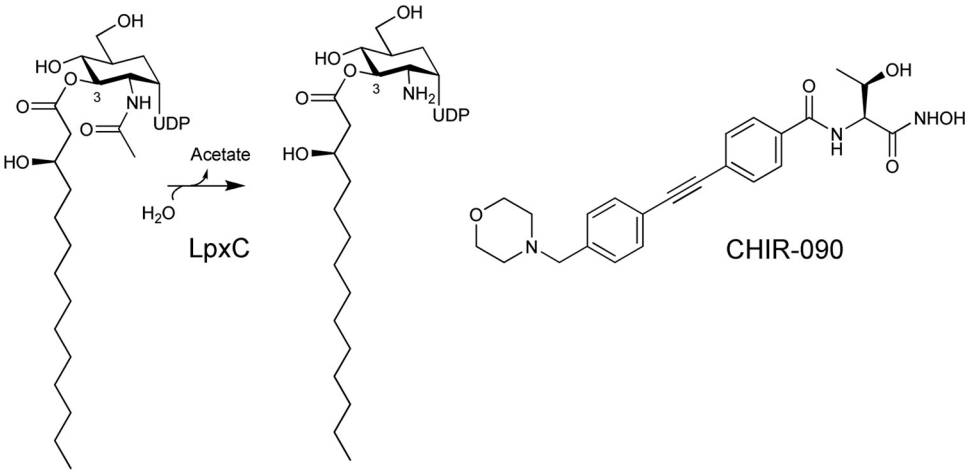

FIG. 1. Reaction catalyzed by the deacetylase LpxC and chemical structure of CHIR-090.

TABLE 2. MICs of CHIR-090 and polymyxin B against a panel of bacterial strains

Strain	Source, reference	MIC (mg/ml)[a]	
		CHIR-090	Polymyxin B
E. coli ATCC 25922	ATCC	0.05	0.78
P. aeruginosa			
ATCC 27853	ATCC	0.78	3.13
ATCC 10145	ATCC	0.78	3.13
B. multivorans (II)			
C5393	Vancouver CF clinic, 21	3.13	>100
LMG 13010	Belgian CF clinic, 31	>100	>100
C1576	Glasgow epidemic, 38	12.5	>100
CF-A1-1	Cardiff CF clinic, 25	1.56	>100
JTC	CGD[b] patient, 34	1.56	>100
C1962	Brain abscess, 15	3.13	>100
ATCC 17616	Environmental strain, 35	6.25	50
249-2	Derived from ATCC 17616	0.10	>100

[a] The antibiotic concentrations used ranged from 0 to 100 μg/ml.
[b] CGD, chronic granulomatous disease.

with nanoemulsions (19), provide a breakthrough in the treatment of CF-related infections.

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