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In vitro Activities of LB20304, a New Fluoroquinolone

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The *in vitro* activity of LB20304 was evaluated against clinical isolates and compared with those of Q-35, ciprofloxacin, sparfloxacin, lomefloxacin and ofloxacin. LB20304 demonstrated 16- to 64-fold more potent activity than ciprofloxacin against gram-positive bacteria. LB20304 inhibited 90% of the isolates of methicillin-susceptible *Staphylococcus aureus* (MSSA) at a concentration of 0.016 μg/ml (MIC_{90}). MIC_{90} values of LB20304 against methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-susceptible *Staphylococcus epidermidis* (MSSE), methicillin-resistant *S. epidermidis* (MRSE) and *Streptococcus pneumoniae* were 2 μg/ml, 0.016 μg/ml, 0.5 μg/ml and 0.031 μg/ml, respectively. LB20304 was also very active against gram-negative bacteria. Against *Escherichia coli, Klebsiella pneumoniae, Serratia marcescens, Pseudomonas aeruginosa* and *Acinetobacter calcoaceticus*, MIC_{90}s of LB20304 were 0.031 μg/ml, 0.25 μg/ml, 2 μg/ml, 8 μg/ml and 0.5 μg/ml, respectively. Its activity was comparable to that of ciprofloxacin but much better than those of Q-35, sparfloxacin, ofloxacin and lomefloxacin. LB20304 also exhibited the most potent activity among quinolones tested against laboratory standard strains, ofloxacin-resistant strains, β-lactamase-producing strains and anaerobic strains. The inhibitory effect (IC_{50}) of LB20304 on DNA gyrase from *Micrococcus luteus*, determined by the supercoiling assay, was 8-fold more potent than that of ciprofloxacin. LB20304 did not induce topoisomerase-associated DNA cleavage even at a concentration of 10 mg/ml, although ciprofloxacin induced DNA cleavage at a concentration of 1 mg/ml.

Key words : LB20304, Quinolone, MIC, DNA gyrase, Topoisomerase

INTRODUCTION

Quinolones are a class of antibiotics structually related to nalidixic acid. They exhibit bactericidal activity primarily by inhibiting bacterial DNA gyrase which controls the shape and the function of bacterial DNA through its unique supercoiling and relaxing activities (Sugino *et al*, 1977). The early quinolones had a limited spectrum of activity, low potency, high frequency of spontaneous bacterial resistance, low serum drug concentrations and short half-life, which virtually restricted their use to only urinary tract infection.

Recently, a number of new fluoroquinolone derivatives such as norfloxacin, ofloxacin, enoxacin, ciprofloxacin, lomefloxacin and fleroxacin were developed and introduced into the market. These new fluorinated quinolones differed from their predecessors in their broad antibacterial spectrum, including both gram-negative and gram-positive bacteria. They also exhibited high potency, low incidence of resistance, high oral bioavailability, extensive tissue penetration and long elimination half-life. However, these fluoroquinolone agents possessed only moderate activities against many gram-positive cocci, including *Staphylococci* and *Streptococci* which are major pathogenic strains of respiratory tract infections (Raviglione *et al*, 1990; Thys *et al*, 1989). Since it has been suggested that increasing resistance to the new fluoroquinolones is due to the moderate activity of these drugs against gram-positive bacteria (Blumberg *et al*, 1991; Jones, 1992; Kaatz *et al*, 1991), recent efforts have been directed toward the synthesis of novel quinolone compounds that can provide improved activities against gram-positive organisms while retaining the broad spectrum activity of ciprofloxacin (Fuchs *et al*, 1991; Piddock, 1994; Sato *et al*, 1992).

LB20304, [7-(3-aminomethyl-4-methoxyimino-pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-[1.8]-naphthyridine-3-carboxylic acid], is a new quinolone antibacterial agent synthesized at LG Chemical Ltd., Tae-jon, Korea (Fig. 1). This compound showed a broad-spectrum antibacterial activity. Especially, It demonstrated potent antibacterial ac-

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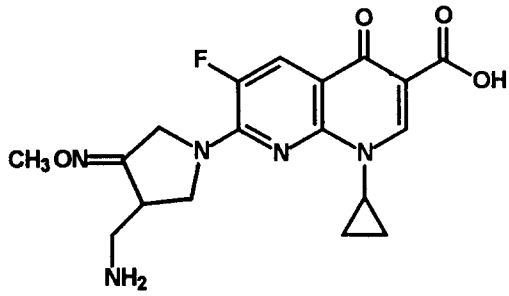

Fig. 1. Chemical structure of LB20304

tivities against gram-positive bacteria *in vitro* efficacy studies (Kim *et al*, 1995; Oh *et al*, 1995). The improved activity against gram-positive pathogens, particularly *Streptococci* and *Staphylococci*, suggests a significant therapeutic potential for this compound. In view of its strong activity against not only gram-positive strains but also the family *Enterobacteriaceae*, LB20304 could be used to treat a broad spectrum of human infections, such as respiratory tract, urinary tract, skin and skin structures, bone and gastro-intestinal tract infections.

In this study, the *in vitro* activities of LB20304 against clinical isolates, laboratory standard strains, ofloxacin-resistant strains, β-lactamase-producing strains and anaerobic strains were compared with those of Q-35, ciprofloxacin, sparfloxacin, lomefloxacin and ofloxacin. We also studied the comparative effects of LB20304 on DNA gyrase and human topoisomerase II.

MATERIALS AND METHODS

Antimicrobial agents

LB20304, Q-35 and OPC-17116 were synthesized at Biotech Research Institute, LG Chem Research Park. The comparative quinolones, such as ciprofloxacin, sparfloxacin, lomefloxacin and ofloxacin, were obtained directly from their manufacturers.

Test organisms

All strains used in this study were clinical isolates from specimens of human or laboratory standard strains obtained from American Type Culture Collection (ATCC) and Glaxo Group Research Ltd. The clinical isolates were collected in Korea from 1994 to 1995. All isolates were stored frozen at -70°C.

Susceptibility tests

The *in vitro* efficacy of LB20304 was determined by Minimal Inhibitory Concentration (MIC) values expressed in μg/ml. MICs of LB20304 against laboratory standard strains, ofloxacin-resistant strains, β-lactamase-producing resistant strains and clinical isolates were determined by the agar dilution method as described by the National Committee for Clinical Laboratory Standards M7-A3 (NCCLS, 1993). Mueller-Hinton medium (Difco Laboratories, Detroit, MI) was used for testing aerobic and facultative organisms. For *Streptococcus pneumoniae*, Mueller-Hinton Broth (MHB) was supplemented with 5% defibrinated sheep blood. Test strains were grown for 18 h in MHB. These overnight cultures were diluted with the same fresh medium to a density of approximately 10^7 CFU/ml and applied to Mueller-Hinton Agar (MHA) plates which have serially diluted antimicrobial agents by use of an automatic MIC-2000 multipin inoculator (Dynatech Laboratories, Inc., Alexandria, VA.) to yield 10^4 CFU per spot. MICs were determined after 18 h of incubation at 35°C. The concentrations of the bacterial suspensions were determined by measuring the optical density or the turbidity and were verified by determining standard colony counts on antibiotic-free agar plates. MIC was considered to be the lowest concentration that completely inhibited growth on agar plates, disregarding a single colony or a faint haze caused by the inoculum.

Against anaerobic strains, the *in vitro* activity of LB 20304 was determined by the agar dilution method as described by the National Committee for Clinical Laboratory Standards M11-A3 (NCCLS, 1993) by using Wilkins-Chalgren agar. Plates were incubated in an anaerobic chamber (Bactron Anaerobic/Environmental Chamber, Sheldon Manufacturing, Inc.) for 48 h at 37°C. MICs were determined by the same method as above.

Inhibitory activity on DNA gyrase

Bacterial DNA gyrase is the target of non-intercalative quinolone-based drugs such as ciprofloxacin and norfloxacin. To study the inhibitory activity of LB20304 on DNA gyrase, the reaction mixtures of *Micrococcus luteus* DNA gyrase (GIBCO, BRL), pBR322 DNA relaxed by topoisomerase I (GIBCO, BRL) and various concentration of quinolones were incubated at 37°C for 2 hr. The reaction was stopped by addition of proteinase K and was subjected to 1% agarose gel electrophoresis (Gellert *et al*, 1979). The inhibitory effects of quinolones on supercoiling activity of DNA gyrase were determined following staining of the gel with ethidium bromide. IC_{50} values (the drug concentration which inhibited gyrase activity by 50%) were calculated from the density of the band of supercoiled DNA.

Effects on DNA cleaving activity by human topoisomerase II

Some quinolones, at high concentration, can inhibit

the activity of eukaryotic topoisomerase II and finally stimulate DNA cleavage mediated by the eukaryotic enzyme (Barrett *et al*, 1989; Liu, 1989).

The effects of LB20304 and ciprofloxacin on DNA cleaving activity by human topoisomerase II (TopoGEN, Columbus) were estimated by the method of SDS-precipitation of topoisomerase-DNA complex (Robinson *et al*, 1991; Liu *et al*, 1983). The reaction mixtures of human topoisomerase II, ^{32}P-labeled pBR322 and various concentrations of quinolone compounds were incubated at 37°C for 6 min and then the reaction was terminated by adding SDS and salmon sperm DNA solution. The precipitates of topoisomerase-DNA complex achieved by addition of KCl solution were resuspended and transferred to a vial containing scintillation fluid, and total cpm of the precipitate was counted.

RESULTS

In vitro antibacterial activity

LB20304 showed a broad spectrum antibacterial activity against a wide range of bacteria covering gram-positive and gram-negative bacteria. Table I shows the *in vitro* MICs of LB20304, ciprofloxacin and sparfloxacin, lomefloxacin and ofloxacin against clinical isolates collected from 1994 to 1995 in Korea. LB 20304 showed the most potent antibacterial activity against gram-positive bacteria among the compounds tested. Against the methicillin-susceptible strains of *Staphylococcus aureus* (MSSA), MIC_{90} of LB20304 was 0.016 μg/ml. It was 64-fold more potent than ciprofloxacin. MIC_{90} of LB20304 against the methicillin-resistant strains of *Staphylococcus aureus* (MRSA) was 2 μg/ml. It was 16-fold more active than ciprofloxacin. Against the methicillin-susceptible strains of *Staphylococcus epidermidis* (MSSE), LB 20304 (MIC_{90}, 0.016 μg/ml) was 16-fold more active than ciprofloxacin. LB20304 with a MIC_{90} of 0.5 μg/ml exhibited 16-fold more potent activity than ciprofloxacin against the methicillin-resistant strains of *Staphylococcus epidermidis* (MRSE). Against *S. pneumoniae*, the activity of LB20304 (MIC_{90}, 0.031 μg/ml) was 64-fold more potent than that of ciprofloxacin. LB20304 was also highly active against gram-negative bacteria. Against *E. coli, K. pneumoniae* and *S. marcescens*, MIC_{90}s of LB20304 were 0.031, 0.25 and 2 μg/ml, respectively. LB20304 was as active as ciprofloxacin but more active than the other quinolones. Against *P. aeruginosa*, the activity of LB 20304 (MIC_{90}, 8 μg/ml) was similar to that of ciprofloxacin but much more potent than the other quinolones tested. LB20304 with a MIC_{90} of 0.25 μg/ml was more active against *A. calcoaceticus* than ciprofloxacin.

Table I. Comparative *in vitro* activities of LB20304 against clinical isolates

Microorganism (No. of strains)	Antimicrobial Agents	MIC (μg/ml)[a]		
		Range	50%	90%
MSSA (33)	LB20304	≤0.008-0.031	≤0.008	0.016
	Q-35	0.016-8	0.063	0.13
	Ciprofloxacin	0.13-128	0.5	1
	Sparfloxacin	0.031-16	0.063	0.13
	Lomefloxacin	0.25->128	1	2
	Ofloxacin	0.25-32	0.5	0.5
MRSA (36)	LB20304	≤0.008-128	0.13	2
	Q-35	0.016-128	0.5	2
	Ciprofloxacin	0.13->128	8	32
	Sparfloxacin	0.031-128	1	16
	Lomefloxacin	0.25->128	16	128
	Ofloxacin	0.25->128	4	8
MSSE (17)	LB20304	≤0.008-0.016	≤0.008	0.016
	Q-35	0.016-0.13	0.063	0.13
	Ciprofloxacin	0.13-0.25	0.25	0.25
	Sparfloxacin	0.063-0.25	0.13	0.25
	Lomefloxacin	0.13-0.5	0.25	0.25
	Ofloxacin	0.25-0.5	0.25	0.5
MRSE (39)	LB20304	≤0.008-1	0.063	0.5
	Q-35	0.063-4	0.5	1
	Ciprofloxacin	0.031-128	1	8
	Sparfloxacin	0.063-8	0.25	4
	Lomefloxacin	0.016-0.128	2	64
	Ofloxacin	0.13-32	1	8
Streptococcus pneumoniae (60)	LB20304	≤0.008-1	0.016	0.031
	Q-35	0.031-16	0.25	0.5
	Ciprofloxacin	0.5-32	1	2
	Sparfloxacin	0.063-16	0.25	0.5
	Lomefloxacin	4-64	8	16
	Ofloxacin	0.031-8	0.25	0.25
Escherichia coli (63)	LB20304	≤0.008-16	0.016	0.031
	Q-35	0.063-32	0.25	0.5
	Ciprofloxacin	≤0.008-8	0.016	0.031
	Sparfloxacin	≤0.008-32	0.031	0.063
	Lomefloxacin	0.063-32	0.13	0.25
	Ofloxacin	0.031-16	0.13	0.25
Klebsiella pneumoniae (45)	LB20304	0.016-1	0.063	0.25
	Q-35	0.063-16	0.25	2
	Ciprofloxacin	≤0.008-2	0.031	0.25
	Sparfloxacin	0.031-4	0.13	0.5
	Lomefloxacin	0.13-8	0.25	2
	Ofloxacin	0.063-8	0.13	1
Serratia marcescens (52)	LB20304	0.063-2	0.25	2
	Q-35	0.5-8	1	8
	Ciprofloxacin	0.031-4	0.13	2
	Sparfloxacin	0.13-4	0.5	4
	Lomefloxacin	0.13-8	0.5	4
	Ofloxacin	0.13-8	0.5	4
Acinetobacter calcoaceticus (51)	LB20304	0.031-2	0.063	0.5
	Q-35	0.13-16	0.5	2
	Ciprofloxacin	0.13-32	0.5	2
	Sparfloxacin	0.016-4	0.31	0.25
	Lomefloxacin	0.25-64	1	4
	Ofloxacin	0.13-8	0.5	2
Pseudomonas aeruginosa (42)	LB20304	0.25->128	1	8
	Q-35	2->128	8	128
	Ciprofloxacin	0.13-32	0.5	8
	Sparfloxacin	0.5-128	2	16
	Lomefloxacin	1-128	4	128
	OPC-17116	0.5-64	1	16

[a]50% and 90%-MICs at which 50% and 90% of isolates were inhibited, respectively.

Table II. *In vitro* activity of LB20304 against β-lactamase-producing resistant strains

Organism		β-lactamase	Type	MIC (μg/ml)				
				LB20304	Ceftazidime	Ceftriaxone	Cefoperazone	Cefpirome
S. aureus	MS 15009/I258		V	0.13	4	32	32	8
E. coli	ML4901/Rms 213		Va	0.13	4	4	8	0.13
E. coli	ML1410 RGN14	TEM-1	III	0.5	0.063	0.031	1	0.031
E. coli	ML1410 RGM238	OXA-1	II	0.5	0.13	0.031	0.13	0.031
E. coli	ML1410 RGN823	TEM-2	Ia	0.5	0.25	0.031	16	0.13
E. coli	1193E	TEM-1	III	≤0.008	0.25	0.063	8	0.063
E. coli	3455E	TEM-3	III	0.25	8	8	16	2
E. coli	3739E	TEM-5	III	0.13	8	2	8	0.5
E. coli	3457E	TEM-7	III	0.031	16	0.25	0.5	4
E. coli	2639E	TEM-9	III	0.031	>128	4	32	8
E. coli	3140	TEM	III	0.016	8	4	32	4
E. coli	3151	TEM	III	≤0.008	2	0.25	4	0.25
E. coli	E182	TEM	III	0.13	32	128	128	16
E. coli	E191	TEM	III	≤0.008	0.5	4	8	0.5
E. cloacae	GN 7471		Ia	4	>128	16	32	16
E. cloacae	1194E	CHR IND+VE		0.031	128	>128	32	2
E. cloacae	P99	P99	Ia	≤0.008	64	128	128	4
E. cloacae	D103	TEM	III	0.031	32	64	128	2
E. aerogenes	D108	TEM	III	0.5	2	32	>128	4
E. aerogenes	D109	TEM	III	8	2	16	128	2
E. aerogenes	D111	TEM	III	1	2	16	>128	2
K. pneumoniae	4058	TEM	III	0.063	32	8	128	8
K. pneumoniae	4059	TEM	III	0.13	0.13	0.031	0.5	0.031
K. pneumoniae	F159	TEM	III	1	128	8	64	2
K. pneumoniae	F161	TEM	III	0.25	64	32	128	16
K. pneumoniae	F162	TEM	III	0.13	8	16	32	2
K. pneumoniae	F163	TEM	III	0.25	16	32	32	2
K. pneumoniae	F169	TEM	III	0.13	64	64	64	8
K. pneumoniae	F170	TEM	III	1	128	16	64	2
K. aerogenes	1976E	SHV-1	III	0.13	0.25	0.13	32	0.25
K. aerogenes	1082E	K1+	IV	0.031	0.5	8	>128	1
M. morganii	1	TEM	III	≤0.008	32	4	64	0.5
M. morganii	2	TEM	III	≤0.008	128	8	128	1
P. vulgaris	GN 76	FEC-1	Ic	0.13	0.031	≤0.008	1	0.13
S. marcescens	1	TEM	III	0.25	32	64	>128	32
S. marcescens	3	TEM	III	2	32	32	>128	8
P. aeruginosa	GN 918		Id	2	4	64	32	8

The activity of LB20304 against β-lactamase-producing strains is shown in Table II. LB20304 exhibited potent activities against *E. coli* and *K. pneumoniae* strains harboring the TEM-1, TEM-2, TEM-3, TEM-5, TEM-7, TEM-9 or not characterized TEM β-lactamases, which make them resistant to cephalosporin antibiotics such as ceftazidime, ceftriaxone and cefoperazone. LB20304 also inhibited type 1 β-lactamase producing *Enterobacter cloacae* organisms.

LB20304 showed more potent antibacterial activities than the other reference quinolones tested against laboratory standard strains (Table III), ofloxacin-resistant strains (Table IV) and anaerobic bacteria (Table V).

Inhibitory effects on DNA gyrase

The inhibitory effects of LB20304 and ciprofloxacin on DNA gyrase from *M. luteus* are shown in Fig. 2. The 50% inhibitory concentrations (IC_{50}s) of LB20304 and ciprofloxacin on the supercoiling activities of DNA gyrase were 11 μg/ml and 84 μg/ml, respectively. LB20304 was 8-fold more potent than ciprofloxacin in inhibitory activity on DNA gyrase.

Effects on DNA cleavaging activity by human topoisomerase II

The effects of LB20304 and ciprofloxacin on DNA cleaving activity by human topoisomerase II are shown in Fig. 3. Although ciprofloxacin induced enzyme mediated-DNA cleavage at a concentration of 1 mg/ml, LB20304 did not induce DNA cleavage even at as high as concentration of 10 mg/ml. These results suggest that LB20304 is less cytotoxic than ciprofloxacin for eukaryotic cells.

DISCUSSION

Although fluoroquinolones have been recognized

Table III. Comparative *in vitro* activity of LB20304 against standard strains

Strains		MIC (μg/ml)							
		LB20304	Ciprofloxacin	Sparfloxacin	Q-35	Lomefloxacin	Ofloxacin	Norfloxacin	Enoxacin
S. aureus	6538p	≤0.008	0.13	0.063	0.063	0.5	0.5	0.25	0.5
S. aureus	giorgio	≤0.008	0.13	0.063	0.063	0.5	0.5	0.5	0.5
S. aureus	77	0.008	0.25	0.13	0.063	0.5	0.25	1	0.5
S. aureus	241	4	64	16	4	128	64	128	32
S. epidermidis	887E	≤0.008	0.13	0.063	0.063	0.5	0.25	0.25	0.25
S. epidermidis	178	4	128	16	4	128	32	>128	64
E. faecalis	29212A	0.063	1	0.5	0.5	4	2	4	2
B. subtilis	ATCC 6633	≤0.008	0.031	0.031	0.5	0.25	0.063	0.13	0.13
M. luteus	ATCC 9341	0.25	2	1	0.5	8	2	-	8
E. coli	10536	≤0.008	≤0.008	0.016	0.5	0.13	0.031	0.016	0.13
E. coli	3190Y	≤0.008	≤0.008	0.016	0.031	0.031	0.016	0.016	0.016
E. coli	851E	≤0.008	0.016	0.031	0.13	0.13	0.063	0.016	0.13
E. coli	TEM3 3455E	0.25	0.25	0.25	2	1	1	0.25	2
E. coli	TEM5 3739E	0.13	0.13	0.13	1	0.5	0.5	0.25	1
E. coli	TEM9 2639E	0.031	0.016	0.13	0.25	0.13	0.063	0.031	0.13
P. aeruginosa	1912E	0.25	0.25	1	2	1	1	1	0.5
P. aeruginosa	10145	0.5	0.25	1	4	2	2	1	1
P. aeruginosa	6065Y	8	4	16	32	32	16	8	32
A. calcoaceticus	15473A	0.063	0.25	0.031	0.25	0.5	0.25	2	2
C. diversus	2046E	0.031	0.016	0.063	0.25	0.13	0.13	0.063	0.13
E. cloacae	IND+VE 1194E	0.031	0.016	0.063	0.25	0.13	0.13	0.063	0.13
E. cloacae	P99	≤0.008	≤0.008	0.016	0.063	0.031	0.031	0.031	0.063
K. aerogenes	SHV-1 1976E	0.13	0.13	0.13	0.5	0.5	0.25	0.5	1
K. aerogenes	K1+1082E	0.031	0.016	0.063	0.25	0.13	0.13	0.063	0.13
P. vulgaris	6059A	0.5	0.031	1	1	0.25	0.13	0.063	0.25
S. marcescens	1826E	0.25	0.13	1	1	0.5	0.5	0.25	0.25
S. typhimurium	14028A	0.031	0.031	0.063	0.25	0.13	0.13	0.13	0.25

Table IV. *In vitro* activity of LB20304 against ofloxacin resistant strains

Strains		MIC (μg/ml)				
		LB20304	Ciprofloxacin	Sparfloxacin	Lomefloxacin	Ofloxacin
S. aureus	179	1	16	4	128	32
S. aureus	241	1	16	4	>128	32
S. aureus	293	1	16	8	128	32
S. aureus	303	1	16	4	128	32
S. aureus	17613	4	128	16	128	32
S. aureus	17740	1	16	4	128	32
S. aureus	17746	1	16	4	128	32
S. aureus	17845	1	16	4	128	32
S. aureus	8236	1	16	4	128	64
S. epidermidis	178	4	64	8	128	32
S. epidermidis	291	2	64	8	128	32
S. epidermidis	31989	4	64	16	128	32
S. epidermidis	32965	8	64	16	64	32
E. munchen		2	4	2	8	4
Enteroccocus	21777	1	4	1	8	4
Enteroccocus	knothe101	0.13	1	0.5	8	4
Enteroccocus	D30	0.063	1	0.5	32	16
Acinetobacter	J178	2	8	1	8	4
Acinetobacter	J180	2	8	4	32	16
Klebsiella	30-30	2	2	2	8	4
Klebsiella	30-92	1	2	2	8	4
Serratia	knothe alt2	4	2	4	8	4
Serratia	knothe alt5	4	2	8	8	4
Serratia	knothe alt9	4	2	4	8	4
Serratia	knothe alt10	4	2	8	4	2
Serratia	Y39	4	4	4	4	2
Serratia	Y42	2	2	4	4	2

Table V. *In vitro* activity of LB20304 against ofloxacin resistant strains

Strains		MIC (μg/ml)				
		LB20304	Ciprofloxacin	Lomefloxacin	Ofloxacin	Norfloxacin
Bacteroides fragilis	ATCC 25285	0.063	4	8	2	32
Bacterodies ovatus	ATCC 8483	0.031	0.063	0.25	0.25	0.25
Bacteroides thetaiotaomicron	ATCC 29741	2	16	16	8	>128
Bacteroides vulgatus	ATCC 29327	4	32	16	8	128
Clostridium histolyticum	ATCC 19401	4	32	16	8	128
Clostridium perfringens	ATCC 13124	0.031	0.25	1	0.5	1
Fusobacterium nucleatum	ATCC 25586	0.5	0.063	1	0.25	0.5
Peptostreptococcus productus	ATCC 35244	0.25	0.063	0.25	0.25	0.25
Propionibacterium acnes	ATCC 11827	4	32	16	8	>128

Method: Agar dilution method
Conditions: 1. Wilkims-Chalgren Medim, 2. H_2 5%, CO_2 5%, N_2 90%, 3. 35°C, 48 hrs, 4. 10^4 cfu/spot

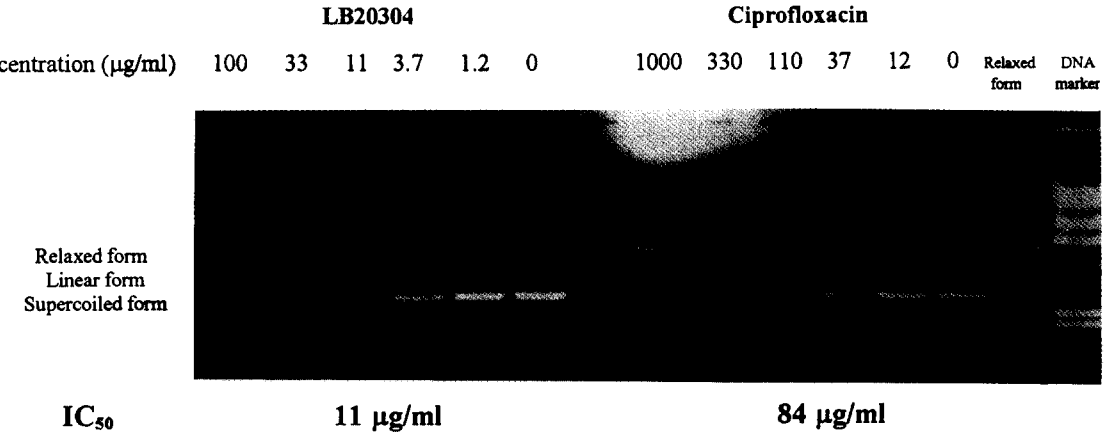

Fig. 2. Inhibitory effects of LB20304 and ciprofloxacin against *M. luteus* DNA gyrase

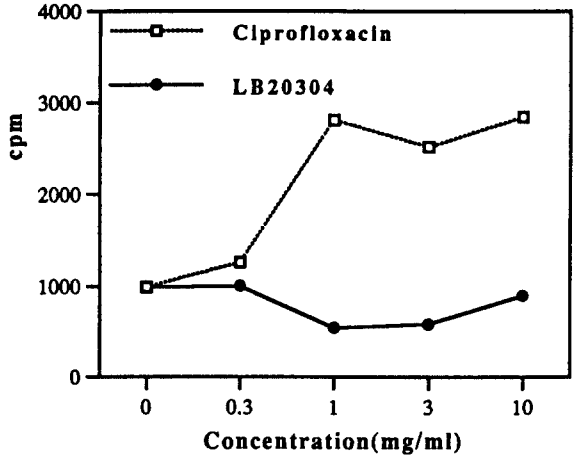

Fig. 3. Effects of LB20304 on DNA cleavaging activity by human topoisomerase II

as effective antimicrobial agents against gram-negative and gram-positive bacterial infections (Wolfson *et al*, 1992), there is still a need to develop new compounds with improved activity against *S. pneumoniae* and methicillin-resistant *Staphylococci*. And the increasing use of fluoroquinolones has apparently led to a dramatic increase in quinolone resistance among gram-positive bacteria, particularly methicillin resistant *S. aureus* and *S. epidermidis* (Blumberg *et al*, 1991; Jones, 1992; Kaatz *et al*, 1991). Therefore, the insufficient activity of current fluoroquinolones against gram-positive and anaerobes has precluded their use in infections caused by these organisms.

LB20304 exhibited a broader spectrum of antibacterial activity than Q-35, ciprofloxacin, sparfloxacin, lomefloxacin and ofloxacin. Especially, LB 20304 was more active than any other fluoroquinolones tested against gram-positive bacteria, including MRSA, MRSE and anaerobic strains. LB 20304 demonstrated an advantage in the spectrum of activity because it had improved activity against gram-positive pathogens, such as *Streptococci* and *Staphylococci*, with potent gram-negative activity still being maintained. In addition, LB20304 demonstrated good pharmacokinetic profiles after oral administration in animals (Oh *et al*, 1995). These characteristics suggest a significant therapeutic potential for this compound.

Bacterial DNA gyrase is the target for non-intercalative quinolone-based drugs such as ciprofloxacin and norfloxacin. But quinolone-based drugs, at high concentrations, can also inhibit the catalytic DNA strand passage activity of eukaryotic topoisomerase II (Robinson *et al*, 1991). It has been re-

ported that some of the potent quinolones, such as ciprofloxacin and CP-67,015, stimulate DNA cleavage mediated by the eukaryotic enzyme (Barret *et al*, 1989). These drugs stabilize covalent enzyme-cleaved DNA complexs that are normal reaction intermediates in the catalytic cycle of topoisomerase II. As a consequence of their action on enzyme-mediated DNA cleavage-religation, these drugs promote the formation of enzyme associated DNA breaks in the genomes of the treated cells and finally convert topoisomerase II into a cellular poison (Barrett *et al*, 1989; Liu, 1989; Liu *et al*, 1983). Although LB20304 strongly inhibits the supercoiling activity of DNA gyrase from *M. luteus*, it is less active than ciprofloxacin against human topoisomerase II. That is, its selectivity on human Topoisomerase II was higher than that of ciprofloxacin. This result showed that LB 20304 might be less cytotoxic for mammalian cells than ciprofloxacin.

In view of its improved activities and pharmacokinetic profiles, LB20304 might have great therapeutic potentials in the treatment of various infections in humans. Further studies would be necessary to establish the clinical usefulness of this compound.

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