Journal of Antimicrobial Chemotherapy (2001) **48**, 195–201

JAC

The inhibition and selectivity of bacterial topoisomerases by BMS-284756 and its analogues

Laura E. Lawrence[a]*, Ping Wu[a], Li Fan[a], Kristine E. Gouveia[b], Amy Card[a], Margaret Casperson[a], Kenneth Denbleyker[a] and John F. Barrett[a]

[a]Bristol-Myers Squibb Pharmaceutical Research Institute, Infectious Diseases, Department of Microbiology, 5 Research Parkway, Wallingford, CT 06492; [b]Oregon Health Science University, Portland, OR, USA

Analogues of BMS-284756, a novel des-F(6)-quinolone, were synthesized and evaluated in order to determine the effects of modification of substituents on *in vitro* target inhibition. BMS-340281 (stereoisomer of BMS-284756), BMS-340280 (C-6 fluorinated analogue of BMS-284756), BMS-340278 (C-8-H derivative), BMS-433366 (C-8 methoxy analogue) and fluoroquinolone comparators were evaluated for antibacterial activity. The MICs of BMS-284756 were generally found to be within two-fold of the MICs of BMS-284756 analogues against a panel of Gram-positive and -negative organisms. BMS-284756 had MICs of 0.03–0.125 mg/L against *Streptococcus pneumoniae* strains with GyrA and ParC mutations, and was the most active quinolone. BMS-284756 and its analogues had similar activity compared with ciprofloxacin and moxifloxacin against topoisomerase IV decatenation, but were three times more active than levofloxacin. The IC_{50} of BMS-284756 for human topoisomerase II (hTopo II) was 3000 times higher than its IC_{50} for DNA gyrase, and no whole-cell cytotoxicity was noted. Two analogues, BMS-340280 and BMS-340278, demonstrated moderate inhibition against hTopo II and cytotoxicity in the cellular assay. BMS-284756 demonstrated greater Gram-positive antibacterial activity and similar inhibition of targets compared with other fluoroquinolones, and more favourable selectivity compared with the other BMS-284756 analogues.

Introduction

The quinolone BMS-284756 (T-3811ME), identified by Toyama Chemical Co., lacks the classical C-6 fluorine of fluoroquinolones but has fluorine incorporated through a C-8 difluoromethyl ether linkage.[1] This novel des-F(6)-quinolone is now in late-stage clinical development by Bristol-Myers Squibb and Toyama Chemical Co. BMS-284756 has a broad spectrum of antibacterial activity, including good activity against anaerobes, exceptional activity against Gram-positive bacteria and the potential to cover quinolone-resistant pathogens in the clinic.[2–5] BMS-284756 has also demonstrated *in vivo* efficacy in an experimental model of systemic infection with a *Staphylococcus aureus* topoisomerase mutant strain and a model of experimental pneumonia with a penicillin-resistant *Streptococcus pneumoniae* strain.[2]

The evaluation of over 10 000 quinolone derivatives following the introduction of nalidixic acid in 1962 has resulted in thorough knowledge of the structure–activity relationship (SAR) for many quinolone substituents.[6,7] The first quinolones contained a bicyclic aromatic core in which a pyridone is essential for antibacterial activity.[6] These quinolones exhibited moderate Gram-negative activity and were used in the clinic to treat urinary tract infections.[8,9] The addition of a piperazine ring at C-7 resulted in an increase in Gram-negative activity and oral absorption.[10,11] Norfloxacin was the first of the quinolones to have the C-6 fluorine that increased quinolone activity and cell penetration.[12] Further modifications at the N-1, C-7 and C-8 positions yielded *S. aureus* activity as well as activity against *S. pneumoniae* and anaerobes.[6] In the 7-piperazine-substituted quinolones, the retention of the fluorine atom at position C-6 was found to increase dramatically both potency (DNA gyrase inhibition) and antibacterial activity.[13] Thus, most currently marketed fluoroquinolones have maintained the C-6 fluorine substituent, e.g. levofloxacin.[14,15] Certain preclinical quino-

*Corresponding author. Tel: +1-203-677-6547; Fax: +1-203-677-6771; E-mail: lawrencl@bms.com

lones without the C-6 fluorine exhibit overall antibacterial activity, but a fluorine at C-8 is required for good Gram-positive activity for these quinolones.[16] The recent innovation of an *N*-cyclopropyl-4-quinolone with a C-7 isoindolin-5-yl substituent by Toyama Chemical Co., BMS-284756, indicates that presence of the C-6 fluorine is not essential for increased potency or antibacterial activity.[1]

In order to characterize the target specificity and to determine the initial SAR of BMS-284756, analogues of BMS-284756 were synthesized by Toyama Chemical Co. BMS-340281 is the *S*-stereoisomer of BMS-284756, while BMS-340280 is the C-6 fluorinated counterpart to BMS-284756. Modifications around the C-8 position include removal of the difluoromethyl ether (BMS-340278) and replacement of this group with a methoxy group (BMS-433366). The various BMS-284756 analogues were tested for antibacterial activity and inhibition of bacterial and mammalian enzymes using an *Escherichia coli* DNA gyrase supercoiling assay, a *S. aureus* topoisomerase IV decatenation assay, a human topoisomerase II (hTopo II) relaxation assay and a cellular cytotoxicity assay.

Materials and methods

Chemicals

BMS-284756 and BMS-284756 analogues were obtained from Toyama Chemical Company (Toyama, Japan). Ciprofloxacin was obtained from Bayer Corporation (West Haven, CT, USA). Levofloxacin was obtained from Ortho-McNeil Pharmaceutical (Raritan, NJ, USA) or was extracted from tablets. Levofloxacin was extracted from 500 mg commercial tablets, purified by recrystallization, and determined to be >99.9% pure by high-pressure liquid chromatography (HPLC) analysis. Moxifloxacin was extracted from 400 mg commercial tablets, purified by recrystallization, and determined to be >99.9% pure by HPLC analysis. Compounds were solubilized in sterile water and stored at –80°C, except for the hTopo II assay and cytotoxicity assays in which compounds were solubilized in 0.1 M NaOH in order to achieve higher stock drug concentrations.

Preparation of relaxed DNA substrate for the DNA gyrase supercoiling inhibition assay

pBR322 DNA (Promega, Madison, WI, USA) was relaxed for 2 h at 37°C with 13 U of topoisomerase I (Promega, Madison, WI, USA) per microgram of DNA in a 50 mM Tris–HCl pH 7.5 buffer containing 0.1 mM EDTA, 1 mM dithiothreitol (DTT), 50% (v/v) glycerol and 50 mM NaCl. The DNA was purified from the DNA/topoisomerase I mixture using a QIAquick spin column (Qiagen Inc., Chatsworth, CA, USA) to remove the topoisomerase, and the DNA was resuspended in 10 mM Tris–HCl pH 8.0.[11]

Expression and purification of topoisomerases

The subunits of DNA gyrase, GyrA and GyrB, were purified from *E. coli* overexpression constructs obtained from M. Gellert (National Institutes of Health in Bethesda, MD, USA) according to the methods of Mizuuchi *et al.*[17] One unit of DNA supercoiling or cleavable complex activity was defined as the amount that completely converts the DNA to supercoiled DNA in 30 min at 37°C. *S. aureus* GrlA and GrlB proteins were cloned and purified as fusion proteins of maltose-binding protein (MBP) according to the method of Tanaka *et al.*[18]

E. coli DNA gyrase assay

The assay was performed according to the methods of Mizuuchi *et al.*[17] and others:[19] 0.3 μg of relaxed pBR322 were incubated with 1 U of *E. coli* DNA gyrase for 30 min at 37°C in 30 μL of 35 mM Tris–HCl pH 7.5 containing 1.4 mM ATP, 1.8 mM spermidine, 5 mM DTT, 0.14 mM EDTA, 6.5% glycerol, 24 mM KCl, 4 mM $MgCl_2$ and 0.36 mg/L of molecular grade bovine serum albumin (BSA). Serial dilutions of each quinolone were added to the above reaction mix for 50% inhibition (IC_{50}) determinations. Reactions were stopped with 15 μL of 0.5% SDS containing 6 mM EDTA, 5.35% glycerol, and 0.013% bromophenol blue. Supercoiled and relaxed forms of pBR322 were separated by agarose gel electrophoresis. The DNA was visualized following staining with 0.5 mg/L of ethidium bromide. The fluorescence was visualized and quantified for IC_{50} determinations using an AlphaImager 2200 system (AlphaInnotech Corporation, San Leandro, CA, USA).

S. aureus topoisomerase IV decatenation assay

The decatenation of kinetoplast DNA (kDNA) (Topogen Inc., Columbus, OH, USA) was measured using assay conditions as defined by Tanaka *et al.*[18] Each reaction contained 0.4 μg of kDNA in a 50 mM Tris pH 8.0 buffer containing 120 mM KCl, 10 mM $MgCl_2$, 0.5 mM ATP, 0.5 mM DTT and 30 mg/L of BSA and was incubated at 37°C for 60 min. Minimal differences in decatenation activity were noted between MBP-fusion topoisomerase IV and cleaved topoisomerase IV as measured in control experiments in the absence of quinolone. One unit of MBP-fusion topoisomerase IV activity was determined to be the amount of subunits which completely converted all catenated DNA to decatenated DNA in 30 min at 37°C.

Human topoisomerase II assay

Quinolones of various concentrations were incubated with 0.25 μg of supercoiled pBR322 DNA with 1 U of hTopo II (Topogen) for 15 min at 37°C in 40 μL of a 100 mM Tris–HCl pH 8.0 buffer containing 120 mM KCl, 10 mM $MgCl_2$, 30 mg/L BSA, 0.5 mM DTT and 5 mM ATP. The reaction was stopped by adding 5 μL of 0.5 M EDTA. Five microlitres

of gel loading buffer (Life Technologies, Rockville, MD, USA) were added to the stopped reaction mixture. Approximately 20 μL of the reaction mixture was loaded and electrophoresed onto a 1% agarose gel in 1× TBE/0.5 mg/L ethidium bromide to separate the relaxed and supercoiled forms of DNA.

Cytotoxicity assay

Cellular cytotoxicity was measured by a colorimetric assay that makes use of the tetrazolium salt, MTS.[20] A suspension of human HeLa (ATCC no. CCL2) or HEp-2 cells (ATCC no. CCL23) was prepared at a density of 10×10^3 cells/mL in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS) (Life Technologies, Gaithersburg, MD, USA) or 5×10^3 cells/mL in DMEM containing 10% FCS, respectively. Cells were incubated under 5% CO_2 at 37°C for 3–4 h before addition of quinolone to allow adherence of cells to the 96-well plate. Compounds were assayed in duplicate and prepared as a 100× stock solution at 40 mM in 0.1 M NaOH. Following the addition of serial dilutions (400–0.18 μM) of quinolone to adhered cells, the plates were incubated for 48 h at 37°C. The cells were then washed once with medium, and 100 μL of 0.6 mM MTS/0.02 mM PMS in DMEM with 10% FBS was added. Cells plated with quinolone and tetrazolium salt were incubated under 5% CO_2 at 37°C for 1 h and read in a SpectraMAX 320 plate reader (Molecular Devices, Sunnyvale, CA, USA) at 490 nm. CC_{50} values were calculated by comparing the OD values of the compound containing samples with the vehicle controls.

Antibiotic susceptibility testing

MICs were determined by the broth microdilution technique according to the NCCLS approved guidelines.[21] The MIC was defined as the lowest concentration of antimicrobial agent that prevented visible growth at 24 h.

Results

The activities of analogues were compared in order to determine the importance of the C-6 fluorine substitution, the C-8 methoxy substitution and the stereoisomerization of BMS-284756 (Figure 1). In general, the MICs of the

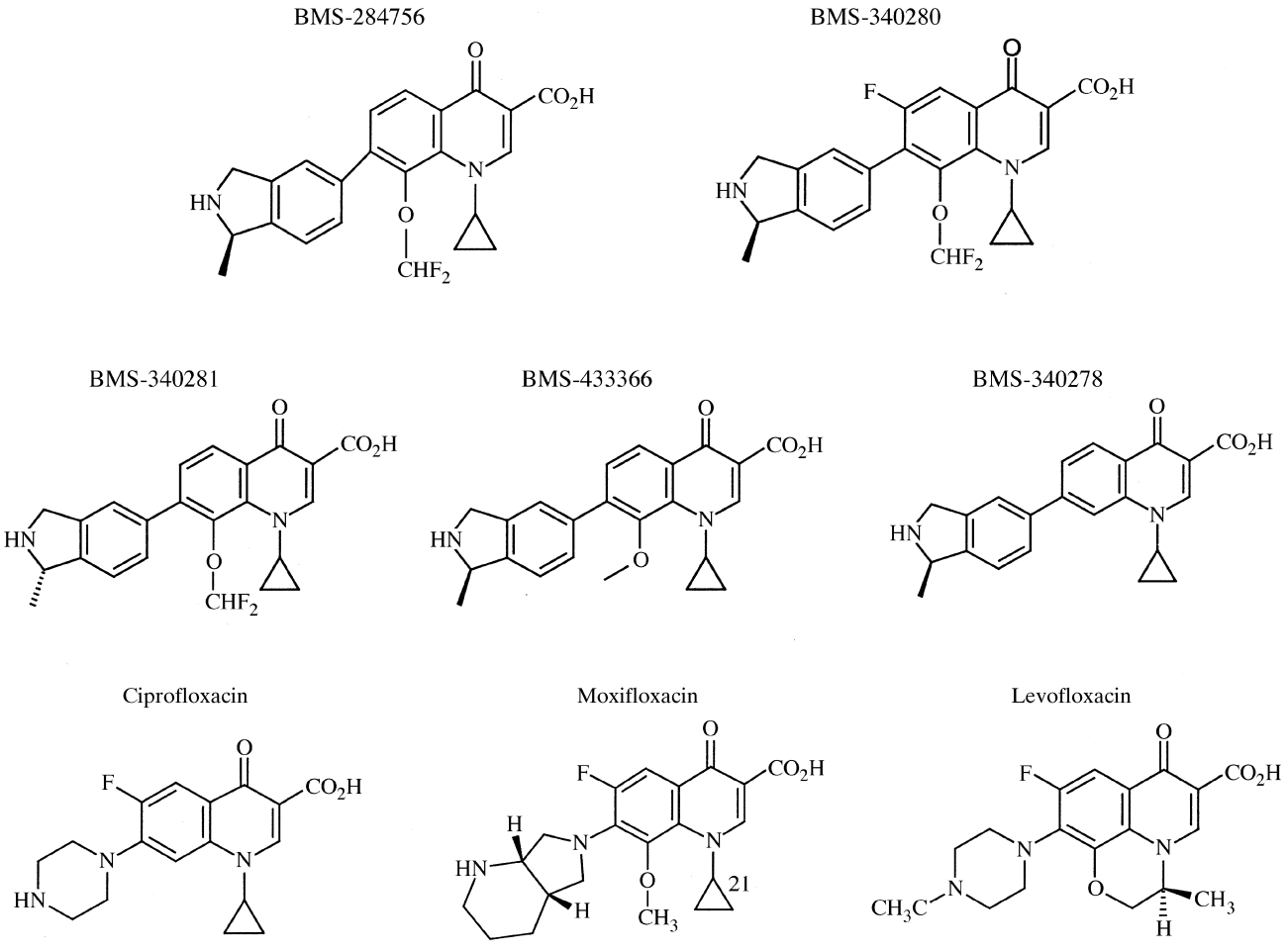

Figure 1. Chemical structures of BMS-284756, BMS-284756 analogues and comparator quinolones.

Table I. Antimicrobial activity of BMS-284756, BMS-284756 analogues and fluoroquinolone comparators against Gram-negative and Gram-positive isolates

Organism	Genotype	MIC (mg/L)							
		BMS-284756	BMS-340281	BMS-340280	BMS-340278	BMS-433366	LVX[a]	MOXI[b]	CIP[c]
E. coli A15119	WT	0.015	0.03	0.015	0.007	0.015	0.03	0.015	0.007
E. coli A29179	GyrA Ser83Leu	0.06	0.125	0.125	0.06	0.25	0.25	0.25	0.125
Enterococcus faecalis A24412	WT	0.06	0.125	0.06	0.06	0.25	0.5	0.25	0.5
E. faecalis A29451	GyrA Ser84Ile	1	2	0.5	4	2	8	8	32
S. pneumoniae A29417	WT	0.015	0.03	0.015	0.03	0.125	0.5	0.25	1
S. pneumoniae A29415/1027	ParC Ser79Phe	0.03	0.06	0.015	0.03	0.125	0.5	0.5	1
S. pneumoniae A29416/1687	ParC Asp83Tyr GyrA Ser81Phe	0.125	0.125	0.06	0.25	2	4	1	16
S. aureus A24407	WT	0.007	0.015	0.007	0.015	0.03	0.125	0.03	0.125
S. aureus MR A27223	homogeneous MR	0.007	0.015	0.007	0.003	0.03	0.125	0.03	0.06
S. aureus MR A29449/F1953	GyrA Glu88Lys ParC Ser80Tyr	0.06	0.125	0.06	0.5	1	2	1	4
S. aureus MR A29448/F2161	GyrA Ser84Leu Glu88Lys ParC Ser80Phe	0.5	2	0.25	4	4	64	2	64
S. aureus A29191	NorA overexpressor	0.06	0.06	0.06	0.06	0.06	0.25	0.03	0.5

[a]Levofloxacin; [b]moxifloxacin; [c]ciprofloxacin.

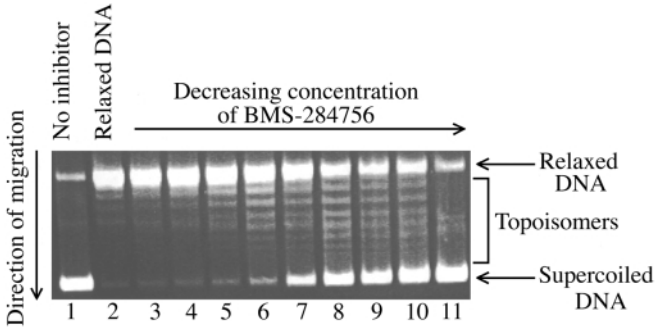

Figure 2. Concentration-dependent inhibition of *E. coli* DNA gyrase supercoiling activity by BMS-284756. Lane 1, no drug control; lane 2, relaxed pBR322 substrate; lanes 3–11, BMS-284756 at 6.25, 3.12, 1.56, 0.78, 0.39, 0.195, 0.098, 0.049 and 0.024 mg/L, respectively. IC_{50} = 0.17 mg/L.

analogues against Gram-negative and -positive wild-type and resistant bacteria were equivalent to or within two-fold of the MICs of BMS-284756 (Table I). BMS-284756 was active against all clinical strains tested including those with single or multiple mutations in topoisomerases. In particular, against resistant *S. aureus* strains A27223, A29449 and A29448, and *S. pneumoniae* strains A29415 and A29416, BMS-284756 and its analogues had the lowest MICs and were more active than the comparators (Table I).

The inhibition of DNA gyrase by the quinolones was measured using an *E. coli* DNA gyrase supercoiling inhibition assay (SCIA) (Figure 2 and Table II). The IC_{50}s of BMS-284756 and BMS-284756 analogues were indistinguishable with values ranging from 0.17 to 0.32 mg/L. The inhibition of DNA gyrase by BMS-284756 and its analogues was also comparable to ciprofloxacin, levofloxacin and moxifloxacin.

Inhibition of *S. aureus* topoisomerase IV decatenation activity by all quinolones was measured as shown in Table II. BMS-284756, BMS-340278 and ciprofloxacin had the lowest IC_{50}s while levofloxacin had the least inhibition of enzymatic activity with an IC_{50} value of 13.7 mg/L. BMS-340280 and BMS-340281 were similar to moxifloxacin with moderate topoisomerase IV inhibition.

The selectivities between bacterial and human topoisomerases of BMS-284756, BMS-284756 analogues and quinolone comparators were determined by monitoring their inhibition of relaxation activity of recombinant hTopo II (Table II). BMS-340278 and BMS-340280 had the greatest inhibition of hTopo II with IC_{50}s of 89.2 and 128.1 mg/L, respectively, distinguishing them from BMS-284756 and the other BMS-284756 analogues which had IC_{50}s of 463–1131 mg/L. BMS-340281, BMS-284756 and the comparator quinolones had selectivity ratios of human/*E. coli* topoisomerase IC_{50}s of >3000, suggesting that inhibition of human enzymes at clinically achievable concentrations would not be relevant for these compounds.

Similar to hTopo II inhibition, BMS-340278 and BMS-340280 had significant cytotoxicity as determined by the measurement of CC_{50}s in the MTS cytotoxicity assay of HEp-2 cells (Table II). BMS-433366 had a CC_{50} of 136 mg/L against HEp-2 cells, which was comparable to the CC_{50} of ciprofloxacin. The remainder of the compounds, including BMS-284756, had no inhibition at the highest soluble concentration tested. BMS-284756, BMS-340281, levofloxacin and moxifloxacin were the least cytotoxic quinolones tested. Similar cytotoxicity results were observed for all quinolones when tested against the HeLa cells. However, BMS-340280 was less cytotoxic in HeLa cells.

Discussion

In vitro testing demonstrated that BMS-284756 has excellent activity against resistant strains of bacteria with multiple topoisomerase mutations and bacterial enzymatic targets. Likewise, all analogues of BMS-284756 were almost equivalent in terms of activity, indicating little difference in intrinsic inhibition against both bacterial targets. Thus, although there is a significant change in removal of the C-6 fluorine, BMS-284756 and BMS-340280 exhibited similar target activity to other quinolones, inhibiting both DNA gyrase and topoisomerase IV from *E. coli* and *S. aureus*, respectively.

BMS-284756 is highly selective towards bacterial topoisomerases with little activity against the mammalian homologue topoisomerase. However, removal of the C-8 difluoromethyl ether linkage (BMS-340278) or replacement of the C-8 difluoromethyl ether linkage with a methoxy group (BMS-433366) decreased target selectivity, as measured by the MTS cytotoxicity assay or hTopo II assay. The methoxy derivative also demonstrated reduced antibacterial activity against *S. pneumoniae*. Although the addition of the C-6 fluorine to BMS-284756 (BMS-340280) did not increase or decrease *in vitro* inhibition of the targets, it did result in increased inhibition of hTopo II and increased mammalian cellular toxicity. BMS-284756 as a des-fluoroquinolone does not follow the traditional SAR of recent fluoroquinolones, which required the C-6 fluorine for enhanced potency and antibacterial activity. With extremely potent activity against resistant Gram-positive organisms, BMS-284756 may be a useful addition in the clinic for the treatment of respiratory diseases and complicated skin and surgical infections.

Acknowledgements

We thank Toyama Chemical Company for the kind provision of the *S. aureus* MBP–topoisomerase IV clones, synthesis of BMS-284756 analogues and for the provision of mutant *S. aureus* strains. We also thank David Hooper for providing the NorA *S. aureus* strain and Jane Thanassi for sequencing of *S. pneumoniae* mutant strains.

Table II. IC_{50}s and CC_{50}s (mg/L) of BMS-284756, analogues and comparator quinolones against bacterial topoisomerases, human topoisomerases and mammalian cell lines[a]

Quinolone	*E. coli* DNA gyrase		*S. aureus* topoisomerase IV		hTopo II		hTopo II/*E. coli* DNA gyrase	HEp-2 cytotoxicity	HeLa cytotoxicity
	IC_{50}	S.D.	IC_{50}	S.D.	IC_{50}	S.D.	IC_{50}/IC_{50}	CC_{50}	CC_{50}
BMS-284756	0.17	0.08	2.19	0.33	509.7	95.9	3034	>166	>166
BMS-340278	0.19	0.05	2.04	0.47	89.2	17.6	465	8.2	10.0
BMS-340280	0.16	0.02	4.60	0.21	128.1	21.9	816	41.1	203
BMS-340281	0.18	0.07	3.98	0.87	1130.8	49.5	6462	>211	>211
BMS-433366	0.32	0.02	2.69	0.96	462.5	54.4	1445	136	164
Ciprofloxacin	0.18	0.08	2.34	0.07	873.1	31.3	4824	115	94.2
Levofloxacin	0.32	0.04	13.74	1.76	>2000	–	>6289	>150	>150
Moxifloxacin	0.36	0.04	4.69	0.21	>2000	–	>5510	>189	>189

S.D., standard deviation.
[a]Values are the average of at least two experiments.

References

1. Hayashi, K., Todo, Y., Hamamoto, S., Ojima, K., Yamada, M., Kito, T. *et al.* (1997). T-3811, a novel des-F(6)-quinolone: synthesis and *in vitro* activity of 7-(isoindolin-5-yl) derivatives. In *Program and Abstracts of the Thirty-seventh Interscience Conference on Antimicrobial Agents and Chemotherapy*, *Toronto, Ontario, Canada, 1997.* Abstract F158, p. 173. American Society for Microbiology, Washington, DC.

2. Takahata, M., Mitsuyama, J., Yamashiro, Y., Yonezawa, M., Araki, H., Todo, Y. *et al.* (1999). *In vitro* and *in vivo* antimicrobial activities of T-3811ME, a novel des-F(6)-quinolone. *Antimicrobial Agents and Chemotherapy* **43**, 1077–84.

3. Davidson, R. J., De Azavedo, J., Bast, D., Arbique, J., Bethune, R., Duncan, C. *et al.* (2000). The activity of BMS-284756, a novel des-(6)F-quinolone, against ciprofloxacin susceptible and non-susceptible *Streptococcus pneumoniae*. In *Program and Abstracts of the Fortieth Interscience Conference on Antimicrobial Agents and Chemotherapy*, *Toronto, Ontario, Canada, 2000.* Abstract E1053, p. 175. American Society for Microbiology, Washington, DC.

4. Fung-Tomc, J. C., Minassian, B., Kolek, B., Huczko, E., Alekunes, L., Stickle, T. *et al.* (2000). Antibacterial spectrum of a novel des-fluoro(6)quinolone, BMS-284756. *Antimicrobial Agents and Chemotherapy* **44**, 3351–6.

5. Lawrence, L. E., Frosco, M., Ryan, B. M., Chaniewski, S., Yang, H., Hooper, D. C. *et al.* (2000). Bactericidal activity of BMS-284756, a novel des-F(6)-quinolone, against *Staphylococcus aureus* strains with topoisomerase mutations. In *Program and Abstracts of the Fortieth Interscience Conference on Antimicrobial Agents and Chemotherapy*, *Toronto, Ontario, Canada, 2000.* Abstract E1044, p. 174. American Society for Microbiology, Washington, DC.

6. Gootz, T. D. & Brighty, K. E. (1998). Chemistry and mechanism of action of the quinolone antibacterials. In *The Quinolones*, 2nd edn, (Andriole, V. T., Ed.), pp. 29–80. Academic Press, San Diego, CA.

7. Lesher, G. Y., Froelich, E. J., Gruett, M. D., Bailey, J. H. & Brundage, R. P. (1962). 1,8-naphthyridine derivatives: a new class of chemotherapeutic agents. *Journal of Medicinal and Pharmaceutical Chemistry* **5**, 1063–5.

8. Moellering, R. C. (1996). The place of quinolones in everyday clinical practice. *Chemotherapy (Basel)* **42**, 54–61.

9. Radl, S. (1996). From chloroquine to antineoplastic drugs? The story of antibacterial quinolones. *Archiv der Pharmazie* **329**, 115–9.

10. Koga, H., Itoh, A., Murayama, S., Suzue, S. & Inkura, T. (1980). Structure–activity relationships of antibacterial 6,7- and 7,8-disubstituted 1-alkyl-1,4-dihydro-4-oxoquinoline-3-carboxylic acids. *Journal of Medicinal Chemistry* **23**, 1358–63.

11. Hooper, D. C. & Wolfson, J. S. (1985). The fluoroquinolones: structures, mechanisms of action and resistance, and spectra of activity *in vitro*. *Antimicrobial Agents and Chemotherapy* **28**, 716–21.

12. Domagala, J. M., Hanna, L. D., Heifetz, C. L., Hutt, M. P., Mich, T. F., Sanchez, J. P. *et al.* (1986). New structure–activity relationships of the quinolone antibacterials using the target enzyme: the development and application of a DNA gyrase assay. *Journal of Medicinal Chemistry* **29**, 394–404.

13. Domagala, J. (1994). Structure–activity and structure–side-effect relationships for the quinolone antibacterials. *Journal of Antimicrobial Chemotherapy* **33**, 685–706.

14. Une, T., Fujimoto, T., Sato, K. & Osada, Y. (1988). In vitro activity of DR-3355, an optically active ofloxacin. *Antimicrobial Agents and Chemotherapy* **32**, 1336–40.

15. Fujimoto, T. & Mitsuhashi S. (1990). In vitro antibacterial activity of DR-3355, the S-(-)isomer of ofloxacin. *Chemotherapy (Japan)* **36**, 268–76.

16. Balkovec, J. M., Black, R., Hammond, M. L., Heck, J. V., Zambias, R. A., Abruzzo, G. *et al*. (1992). Potent non-6-fluoro-substituted quinolone antibacterials: synthesis and biological activity. *Journal of Medicinal Chemistry* **35**, 198–200.

17. Mizuuchi, K., Mizuuchi, M., O'Dea, M. H. & Gellert, M. (1984). cloning and simplified purification of *Escherichia coli* DNA gyrase A and B proteins. *Journal of Biological Chemistry* **258**, 9199–201.

18. Tanaka, M., Onodera, Y., Uchida, Y., Sato, K. & Hayakawa, I. (1997). Inhibitory activities of quinolones against DNA gyrase and topoisomerase IV purified from *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy* **41**, 2362–6.

19. Barrett, J. F., Bernstein, J., Krause, H. M., Hilliard, J. J. & Ohemeng, K. A. (1993). Testing potential gyrase inhibitors of bacterial DNA gyrase: a comparison of the supercoiling inhibition assay and "cleavable complex" assay. *Analytical Biochem*istry **214**, 313–7.

20. Goodwin, C. J., Holt, S. J., Downes, S. & Marshall, N. J. (1995). Microculture tetrazolium assays: a comparison between two new tetrazolium salts, XTT and MTS. *Journal of Immunological Methods* **179**, 95–103.

21. National Committee for Clinical Laboratory Standards. (1997). *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically—Fourth Edition: Approved Standard M7-A4*. NCCLS, Villanova, PA.

Received 1 February 2001; returned 2 April 2001; revised 9 May 2001; accepted 14 May 2001