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Activity of new N-acylated ciprofloxacin derivatives against faculative intracellular bacteria

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(54) **ACTIVITY OF NEW N-ACYLATED CIPROFLOXACIN DERIVATIVES AGAINST FACULATIVE INTRACELLULAR BACTERIA**

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(21) Appl. No.: **13/031,991**

(22) Filed: **Feb. 22, 2011**

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(51) **Int. Cl.**
C07D 241/04 (2006.01)

(52) **U.S. Cl.** **544/357**
(58) **Field of Classification Search** 544/362
See application file for complete search history.

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(57) **ABSTRACT**

Bartonella species are facultative intracellular pathogens responsible for a range of diseases in animals and in humans. A selection of N-acyl ciprofloxacin analogues, chemically synthesized from ciprofloxacin, have been tested in vitro for activity against *Bartonella* species as models for therapeutic development. Nine *Bartonella* strains, including five of *B. henselae*, two of *B. quintana*, and one each of *B. elizabethae* and *B. vinsonii*, have been tested for susceptibility to different N-acyl ciprofloxacin derivatives. Several techniques have been used to test the in vitro antibacterial activity of the derivatives. Seven of them, labeled RC4-125, RC4-143, RC4-147, RC5-28, RC5-29, RC5-32 and RC5-69 showed significant intracellular anti-*Bartonella* activity. These synthetically derived N-acyl ciprofloxacin derivatives may be useful in the therapeutic treatment of infections caused by *Bartonella*.

2 Claims, 5 Drawing Sheets

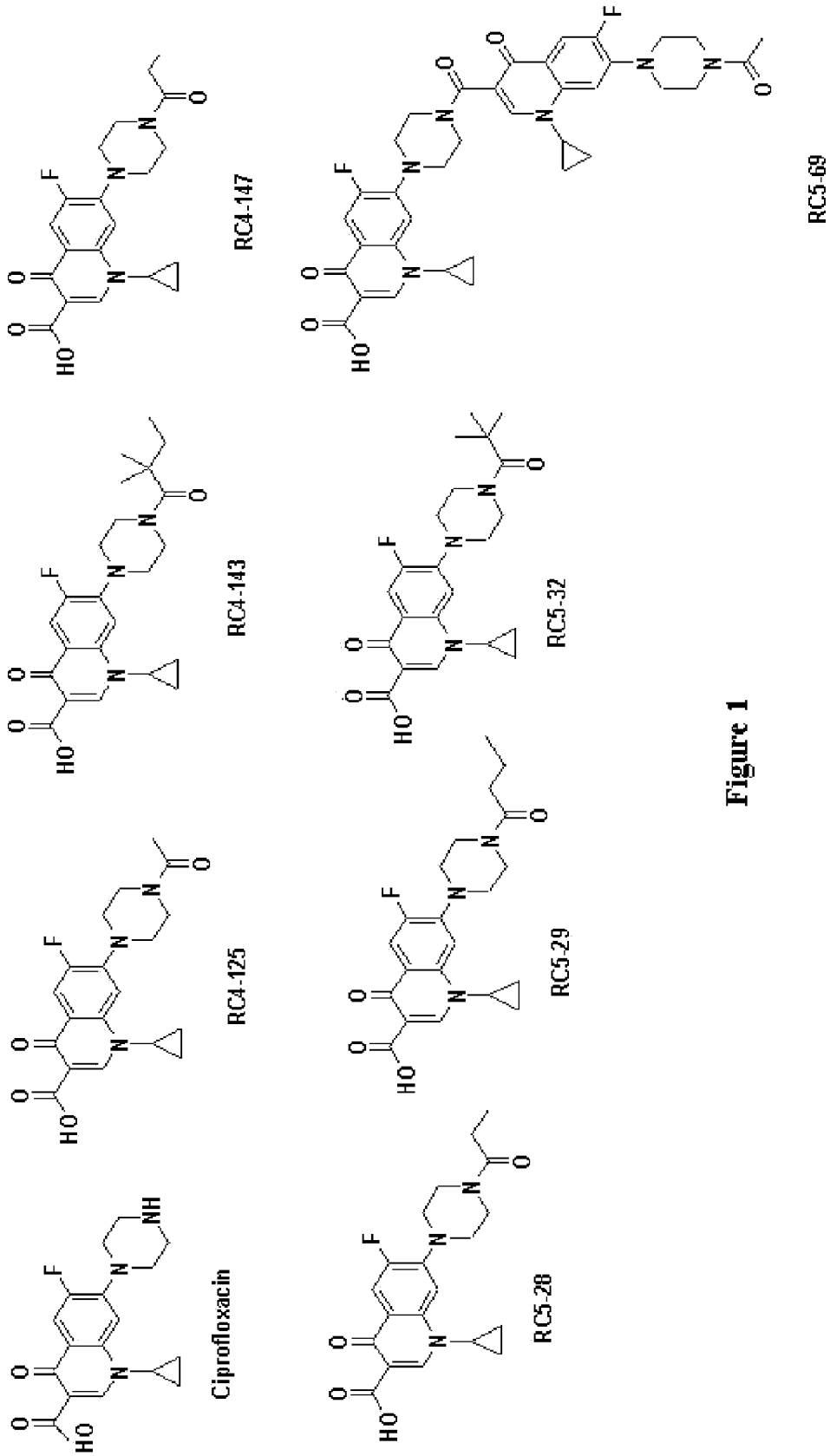

Ciprofloxacin
RC4-125
RC4-143
RC4-147
RC5-28
RC5-29
RC5-32
RC5-69

Figure 1

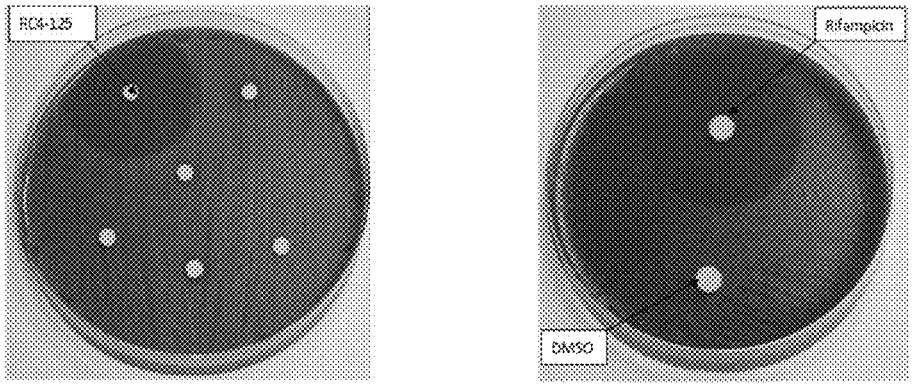


Figure 2

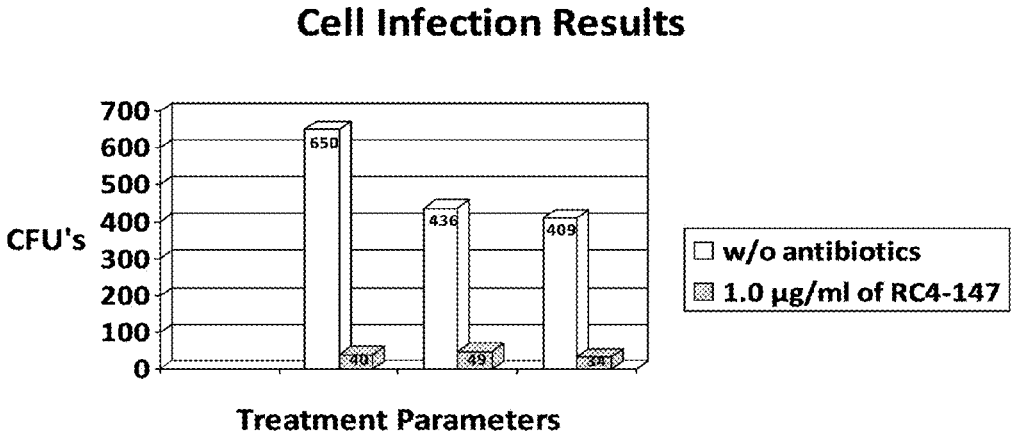


Figure 3

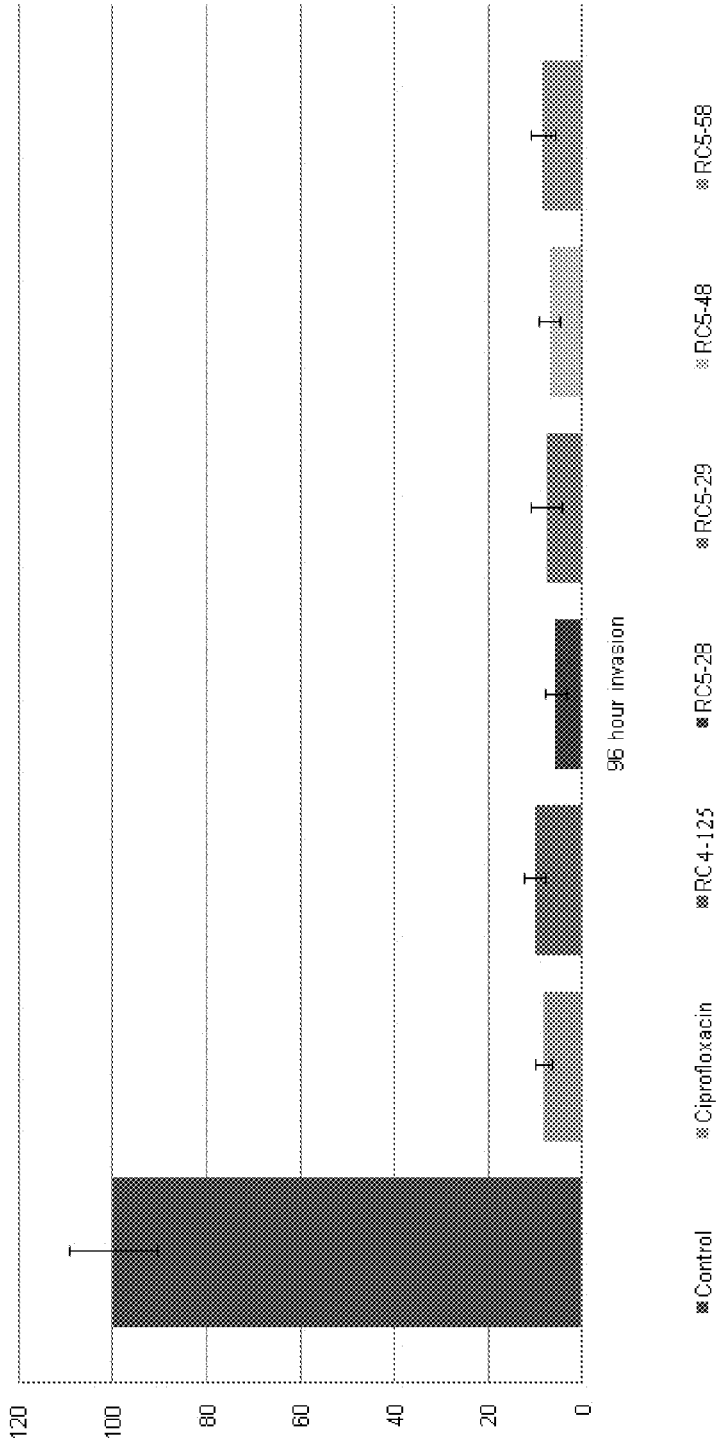

Cell Invation Results
120
100
80
60
40
20
0
96 hour invasion
Control
Ciprofloxacin
RC4-125
RC5-28
RC5-29
RC5-48
RC5-58

Figure 4

	N- acyl ciprofloxacin derivatives														Controls			
	125		129		139		141		143		145		147		Rifampin		DMSO	
Strain	Zone	MIC	Zone	MIC	Zone	MIC	Zone	MIC	Zone	MIC	Zone	MIC	Zone	MIC	Zone	MIC	Zone	MIC
B. henselae Houston-1 "smooth"	65	1.0-10	6	1.0-10	51	0.5-1.0	52	1.0-5.0	50	0.1-0.5	32	1.0-10	56	0.1-0.2	48	0.03-0.06*	6	>>>10
B. henselae Houston-1 "rough"	52	1.0-10	35	1.0-10	56	0.5-1.0	60	1.0-5.0	51	0.1-0.5	32	1.0-10	55	0.1-0.2	56	0.03-0.06*	6	>>>10
B. henselae SD-2	57	1.0-10	29	1.0-10	56	0.5-1.0	50	1.0-5.0	58	0.1-0.5	29	1.0-10	56	0.1-0.2	49	0.03-0.06*	6	>>>10
B. henselae SA-1	52	1.0-10	6	1.0-10	50	0.5-1.0	53	1.0-5.0	52	0.1-0.5	27	1.0-10	58	0.1-0.2	47	0.03-0.06*	6	>>>10
B. elizabethae	58	1.0-10	21	1.0-10	26	0.5-1.0	28	1.0-5.0	34	0.1-0.5	20	1.0-10	37	0.1-0.2	42	0.03*	6	>>>10
B. henselae Marseille	61	1.0-10	31	1.0-10	35	0.5-1.0	34	1.0-5.0	38	0.1-0.5	19	1.0-10	42	0.1-0.2	16	0.03-0.06*	6	>>>10
B. quintana Fuller	6	1.0-10	11	1.0-10	29	0.5-1.0	30	1.0-5.0	27	0.1-0.5	16	1.0-10	30	0.1-0.2	44	0.06-0.25*	6	>>>10
B. quintana D-Perm	47	1.0-10	6	1.0-10	29	0.5-1.0	28	1.0-5.0	27	0.1-0.5	16	1.0-10	32	0.1-0.2	40	0.06-0.25*	6	>>>10
B. vinsonii	6	1.0-10	20	1.0-10	39	0.5-1.0	29	1.0-5.0	38	0.1-0.5	18	1.0-10	45	0.1-0.2	39	0.12*	6	>>>10

Figure 5

	N-acyl ciprofloxacin Derivatives														Controls			
	RC5-17		RC5-28		RC5-29		RC5-32		RC5-48		RC5-58		RC5-69		Rifampin		DMSO	
Strain	Zone	MIC	Zone	MIC	Zone	MIC	Zone	MIC	Zone	MIC	Zone	MIC	Zone	MIC	Zone	MIC	Zone	MIC
B. henselae Houston-1 "smooth"	65	0.1	56	0.1	34	0.1	40	0.5	52	0.5	62	0.5	43	0.5	48	0.03-0.06*	6	>>>10
B. henselae Houston-1 "rough"	52	0.1	60	0.1	68	0.1	61	0.5	60	0.2	43	0.5	35	0.5	56	0.03-0.06*	6	>>>10
B. henselae SD-2	57	0.1	66	0.1	52	0.1	41	0.5	50	0.5	54	0.5	40	0.5	49	0.03-0.06*	6	>>>10
B. henselae SA-1	52	0.1	59	0.1	44	0.1	38	0.5	53	0.5	49	0.5	39	0.5	47	0.03-0.06*	6	>>>10
B. elizabethae	58	0.1	53	0.1	44	0.1	36	0.5	28	0.5	37	0.5	30	0.5	42	0.03*	6	>>>10
B. henselae Marseille	61	0.2	63	0.1	48	0.1	42	0.5	34	0.5	38	0.5	27	0.5	16	0.03-0.06*	6	>>>10
B. quintana Fuller	62	0.2	58	0.2	32	0.2	33	0.8	30	1.0	36	1.0	17	0.8	44	0.06-0.25*	6	>>>10
B. quintana D-Perm	47	0.1	54	0.2	42	0.2	38	0.8	28	1.0	32	1.0	16	0.8	40	0.06-0.25*	6	>>>10
B. vinsonii	68	0.1	69	0.1	50	0.1	36	0.5	29	1.0	40	0.5	29	0.5	39	0.12*	6	>>>10

Figure 6

ACTIVITY OF NEW N-ACYLATED CIPROFLOXACIN DERIVATIVES AGAINST FACULATIVE INTRACELLULAR BACTERIA

CROSS-REFERENCE TO RELATED APPLICATION

This application claims priority from U.S. Provisional Application No. 61/306,224 filed on Feb. 19, 2010, entitled "*Bartonella* as a Model Intracellular Pathogen for Developing Therapeutics".

STATEMENT OF GOVERNMENT INTEREST

This invention was made with government support under HR0011-08-0087 awarded by the Department of Defense/Defense Advanced Research Projects Agency. The government has certain rights in the invention.

FIELD OF INVENTION

This invention relates to the field of treating pathogens and infectious diseases.

BACKGROUND OF INVENTION

Bartonella is a genus of Gram-negative, facultative intracellular bacteria. *Bartonella* species are responsible for a range of diseases in animals and in humans. Some species have been recognized as emerging pathogens. The wide range of disease manifestations caused by *Bartonella* depends on the infecting species and the immune status of the patient. Most *Bartonella* species are sensitive to quinolones in vitro, though treatment failures have been reported. Quinolones vary in their antibacterial spectrum and activity. Fluoroquinolones, a type of quinolone, effectively inhibit DNA synthesis by disrupting DNA breakage-reunion reactions. Ciprofloxacin is a fluoroquinolone that inhibits the bacterial enzymes DNA gyrase and topoisomerase IV.

SUMMARY OF INVENTION

A selection of N-acyl ciprofloxacin analogues, RC4-125, RC4-143, RC4-147, RC5-28, RC5-29, RC5-32 and RC5-69 (FIG. **1**), which are chemically derived from ciprofloxacin and differ only in their side chains, are found to have significant antibacterial against *Bartonella* species.

The N-acyl ciprofloxacin analogues RC4-125, RC4-143, RC4-147, RC5-28, RC5-29, RC5-32 and RC5-69 can therefore be especially useful in the treatment of bacterial infections caused by *Bartonella* species.

BRIEF DESCRIPTION OF THE DRAWINGS

For a fuller understanding of the invention, reference should be made to the following detailed description, taken in connection with the accompanying drawings, in which:

FIG. **1** is a schematic depiction of chemical structures of ciprofloxacin and three of the N-acyl ciprofloxacin analogues: RC4-125, RC4-143, RC4-147, RC5-28, RC5-29, RC5-32 and RC5-69.

FIG. **2** is a schematic depiction of a Kirby-Bauer disk diffusion test of N-acyl ciprofloxacin analogues against *Bartonella henselae* Houston-1 rough. Each disk contained 20 μg of antibiotic, 2.0 μg of rifampicin as the control, or 20 μL of undiluted dimethylsulfoxide (DMSO). Zones of inhibition of bacterial proliferation are measured in mm.

FIG. **3** is a schematic depiction of activity of N-acyl ciprofloxacin analogue RC4-147 against intracellular *Bartonella henselae* Houston-1. Results were obtained from 24-hour human mammary epithelial cell (HMEC) culture infections and are expressed as intracellular colony forming units (CFUs).

FIG. **4** is a schematic depiction of cell invasion results for 96 hour incubation time in 1.0 μg/mL of N-acyl ciprofloxacin derivatives. Values are shown as percentage of colony forming units in comparison to control which contained cell culture media only.

FIGS. **5** and **6** shows two tables, Table 1 and Table 2. For select ciprofloxacin analogues, Kirby-Bauer disk diffusion zone sizes and minimum inhibitory concentrations (MICs) are indicated. Zones of inhibition were measured in mm MICs were determined by agar dilutions and measured in μg/mL. Values marked with * are obtained from Rolain J M, Broqui P, Koehler J E, Maguina C, Dolan M J, Raoult D: Recommendations for treatment of human infections caused by *Bartonella* species, *Antimicrob Agents Chemother,* 48(6): 1921-1933, 2004.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

In the following descriptions, reference is made to the accompanying drawings, which form a part hereof, and within which are shown by way of illustration specific embodiments by which the invention may be practiced. It is to be understood that other embodiments may be utilized and structural changes may be made without departing from the scope of the invention.

A selection of N-acyl ciprofloxacin analogues, chemically synthesized from ciprofloxacin have been tested for in vitro activity against *Bartonella* species. Nine *Bartonella* strains, including five of *B. henselae*, two of *B. quintana* and one each of *B. elizabethae* and *B. vinsonii*, have been tested for susceptibility to ten N-acyl ciprofloxacin derivatives.

The Kirby-Bauer disk diffusion assay, agar dilution testing, DNA gyrase assay, broth dilution testing, and an HMEC-1 cell assay have been done in vitro to characterize the activity each fluoroquinolone compound, using ciprofloxacin as the control.

Seven of the fluoroquinalone compounds, viz. RC4-125, RC4-143 and RC4-147, RC5-28, RC5-29, RC5-32 and RC5-69 showed significant anti-Bartonella activity. These compounds gave zones of growth inhibition greater than 30 mm in disks impregnated with 20 μg of drug, minimal inhibitory concentrations of 0.1-10.0 μg/mL and significant activity against intracellular bacteria.

Kirby-Bauer Disk Diffusion

6 mm paper disks were saturated with 20 μg of the fluoroquinalone compound to be tested or 2.0 μg of the rifampicin control in 20 μL of DMSO. The solvent control was 20 μL of undiluted DMSO. Nine strains of *Bartonella* were harvested from 4-day-old plates and resuspended in 1.0 mL sterile heart infusion broth (HIB) and adjusted to a McFarland 2.0 turbidity standard. Each suspension was spread onto a 150 mm chocolate agar plate and allowed to dry. Then, disks containing the fluoroquinalone compounds, rifampicin, or DMSO were placed on the plates in a designated pattern. Inoculated plates were incubated for 1 week, and the zones of inhibition were measured to the nearest mm. The results obtained are shown in FIGS. **2**, **5** and **6**.

Agar Dilution Testing

Agar was prepared with the N-acyl ciprofloxacin-derived compounds at concentrations of 10 μg/mL, 1.0 μg/mL and 0.1

μg/mL. An equivalent volume of DMSO was added to a control plate. Nine strains of *Bartonella* were harvested from 4-day-old plates, resuspended in 1.0 mL sterile HIB and adjusted to a McFarland 2.0 turbidity standard. Using a specific template, 12.5 μL of broth suspensions of each *Bartonella* strain was pipetted onto each of the fluoroquinalone compound-containing chocolate agar plates and the DMSO control plate. After 1 week, the growth of each *Bartonella* strain was examined and recorded.

Assay for Intracellular Activity

HMEC-1 cells were maintained in MCDB131 medium supplemented with 10% FBS, 5% L-glutamine, 10 ng/mL epidermal growth factor, 1 μg/mL hydrocortisone, penicillin/streptomycin and amphotericin B. HMEC-1 cells were infected with *Bartonella henselae* Houston-1 at a multiplicity of infection (MOI) of 100 for 4 hour in medium without ciprofloxacin-derived compounds. After infection, the cells were washed 2× with PBS and then treated with gentamicin (200 μg/mL) for 1 hour. Infected cells were washed as before and medium containing ciprofloxacin-derived compound was added at concentrations of 0.1 μg/mL, 1.0 μg/mL and 10 μg/mL. Infected cells were then incubated for 24, 48 and 96 hour. Ciprofloxacin-derived compounds were then removed, and the infected cells were washed as before and lysed with 0.1% saponin Lysates were plated on chocolate agar. After incubation for 8 days, CFU's were counted to determine the number of viable intracellular bacteria. Results are shown in FIG. **4**.

Compound RC4-147 showed greater than a tenfold decrease in CFUs when infected HMEC cells were treated with 1.0 μg/mL for 24 hours. This suggests that this synthetically-derived N-acyl ciprofloxacin derivative could be an effective therapeutic for the treatment of infections caused by intracellular bacteria such as *Bartonella*. Results are shown in FIG. **3**.

Seven compounds, RC4-125, RC4-143, RC4-147, RC5-28, RC5-29, RC5-32 and RC5-69 showed unambiguous anti-Bartonella activity. These seven compounds gave zones of growth inhibition greater than 30 mm on disks impregnated with 20 lag of drug. MICs were 1.0-10.0 μg/mL for RC4-125, 0.1-0.5 μg/mL for RC4-143 and 0.1-0.2 μg/ml for RC4-147, RC5-28, RC5-29, 0.5-0.8 μg/mL for RC5-32 and RC5-69.

DNA Gyrase Analysis

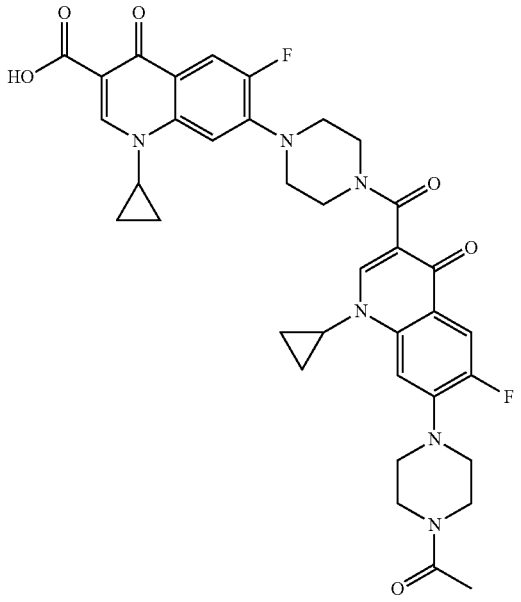
Activity of each ciprofloxacin derivative against DNA gyrase was tested using relaxed circular pUC19 DNA in the presence of *E. coli* DNA gyrase at ciprofloxacin derivative concentrations of 1.0 μg/mL, 10 μg/mL and 25 μg/mL. Samples were incubated at 37° C. for 1 hour and then analyzed by gel electrophoresis to quantify the amounts of relaxed and supercoiled DNA. The DNA gyrase assay verified DNA gyrase as the target of the synthetically-derived N-acyl ciprofloxacin derivatives.

It will be seen that the advantages set forth above, and those made apparent from the foregoing description, are efficiently attained and since certain changes may be made in the above construction without departing from the scope of the invention, it is intended that all matters contained in the foregoing description or shown in the accompanying drawings shall be interpreted as illustrative and not in a limiting sense.

It is also to be understood that the following claims are intended to cover all of the generic and specific features of the invention herein described, and all statements of the scope of the invention which, as a matter of language, might be said to fall there between.

What is claimed is:

1. A compound represented by the formula:



or a pharmaceutically acceptable salt or ester thereof.

2. A method of treating *Bartonella* infection, comprising administering the compound of claim **1** to a subject in need thereof.

* * * * *