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Monocyclic and Tricyclic Analogs of Quinolones: Mechanism of Action

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The mode of action of Ro 13-5478 and Ro 14-9578, monocyclic and tricyclic quinolone analogs, respectively, was examined for *Escherichia coli* and *Staphylococcus aureus*. The compounds showed antibacterial activity and effects on cell morphology, replicative DNA biosynthesis, and gyrase-catalyzed DNA supercoiling that were comparable to those shown by nalidixic acid and by oxolinic acid compounds. The results suggest that their site of action is DNA gyrase and that a bicyclic quinolone nucleus is not essential for activity.

Quinolone antibacterial agents, including nalidixic acid and oxolinic acid, act on susceptible bacteria by immediately, selectively, and reversibly inhibiting DNA synthesis (3, 7). The quinolones also inhibit DNA replication, but not DNA repair, in permeabilized cell systems (16, 19). The molecular target of the quinolones is DNA gyrase, a unique and essential procaryotic enzyme involved in the negative supercoiling (unwinding) of double-stranded DNA (4, 5). DNA gyrase acts by introducing a transient double-strand break in DNA (12), which is bridged via phosphodiester bonds to a tyrosine residue in each of the two A subunits of the enzyme and the 5′ ends of the two DNA strands (20). Quinolones interfere with the enzyme at this step, leading to DNA breakage (18), which may in turn trigger the SOS response (8, 9). In *Escherichia coli*, the SOS response manifests itself morphologically as extensive filamentation (10).

This study describes the antimicrobial activity and mechanism of action of Ro 13-5478 and Ro 14-9578, which are two lead compounds from a series of novel quinolone analogs. These compounds have an aromatic substituent joined to the pyridone ring at the 6 position, rather than fused across the 5 and 6 positions as is the case with most quinolones. The results of the study suggest that the two analogs act like classical quinolones, most likely by inhibiting the A subunit of DNA gyrase.

(This material was presented in part at the 25th Interscience Conference on Antimicrobial Agents and Chemotherapy, Minneapolis, Minn., 29 September to 2 October 1985 [N. H. Georgopapadakou, B. P. Dix, P. Angehrn, A. Wick, and G. L. Olson, Program Abstr. 25th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 129, 1985].)

Nalidixic acid, oxolinic acid, Trizma base, streptomycin sulfate, heparin-agarose, type II agarose (medium electroendosmosis [EEO]), sodium dodecyl sulfate, Brij 58, HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid), ATP, dithiothreitol, dATP, dTTP, dGTP, dCTP, methylene blue, and ethidium bromide were obtained from Sigma Chemical Co., St. Louis, Mo. DL-(+)-*meso*-Diamino[G-^{3}H]pimelic acid (700 μCi/mol), [U-^{14}C]uridine (479 μCi/mol), [2-^{14}C]thymidine (50 μCi/mol), and [*methyl*-^{3}H]dTTP (50 mCi/μmol) were from Amersham Corp., Arlington Heights, Ill. L-[U-^{14}C]phenylalanine (522 μCi/mol) was from ICN Pharmaceuticals, Irvine, Calif. Aquasol II was from New England Nuclear Corp., Boston, Mass. Whatman 3MM filter paper, trichloroacetic acid, and all solvents (analytical grade) were from Fisher Scientific Co., Pittsburgh, Pa.; 48-well plates were from Costar, Cambridge, Mass.; and the horizontal gel apparatus (15 by 13.3 cm) and the 20-well, 2.5-mm-thick Lucite comb were from Aquebogue Machine and Repair Shop, Aquebogue, N.Y. Redistilled phenol was obtained from International Biotechnologies, New Haven, Conn.; and the calf thymus topoisomerase I, reference pBR322 plasmid, and *Micrococcus luteus* DNA gyrase were from Bethesda Research Laboratories, Inc., Gaithersburg, Md. Antibiotic Medium 3 and Yeast Extract were from Difco Laboratories, Detroit, Mich.; and the Diagnostic Sensitivity Test Agar was from Oxoid Ltd., Basingstoke, United Kingdom.

The synthesis and biological properties of the monocyclic and tricyclic quinolone series will be described elsewhere (G. L. Olson, A. Wick, W. Hasler, G. Bernard, D. Ehrlich, P. Angehrn, N. H. Georgopapadakou, and M. Pieri, manuscript in preparation).

E. coli ATCC 25922, *Enterobacter cloacae* ATCC 13047, *Klebsiella pneumoniae* ATCC 27736 and *Staphylococcus aureus* ATCC 6538 and ATCC 25923 were from the American Type Culture Collection, Rockville, Md.; *Proteus vulgaris* NCTC 8313 was obtained from the National Collection of Type Cultures, Central Public Health Laboratory, London, United Kingdom; and *E. coli* H560 was obtained from B. Bachmann of the *E. coli* Genetic Stock Center, Yale University, New Haven, Conn. *E. coli* UB1005 and MK47 were gifts, respectively, from D. Clark of South Illinois University and M. Gellert of the National Institutes of Health. All other strains were from the Roche culture collection.

Cell morphology was determined after a 3-h incubation of bacteria (1% inoculum from an overnight culture) with the test compound in Antibiotic Medium 3 at 37°C. Cells were stained with 0.04% methylene blue. In vivo macromolecular biosynthesis was performed with growing *E. coli* ATCC 25922 cells as previously described (6).

Replicative DNA biosynthesis was measured as the ATP-dependent incorporation of [^{3}H]thymidine into trichloroacetic acid-insoluble material by toluene-treated *E. coli* H560 cells (6, 14). DNA supercoiling was performed with partially purified DNA gyrase from *E. coli* MK47 (13, 15) and with pBR322, which had been isolated from *E. coli* RR1 and relaxed with calf thymus topoisomerase I, as the substrate.

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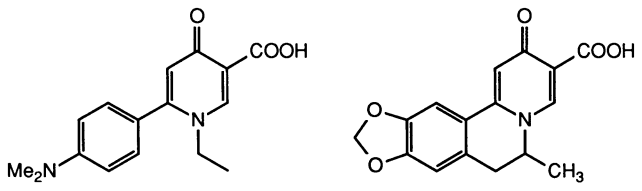

Ro 13-5478 Ro 14-9578

FIG. 1. Structures of compounds used.

Relaxed and supercoiled plasmids were separated by electrophoresis on an 0.8% agarose gel, which was then stained with 0.5 μg of ethidium bromide per ml and visualized with a Transilluminator (Ultraviolet Products, San Gabriel, Calif.).

Ro 13-5478 is representative of the monocyclic quinolone analogs, while Ro 14-9578 is representative of the tricyclic analogs (Fig. 1). Tricyclic analogs are invariably restricted conformationally, a feature designed to separate antibacterial from pharmacological effects. The two compounds were active against *E. coli* ATCC 25922 and *S. aureus* ATCC 25923, and they inhibited in vitro replicative DNA biosynthesis (Tables 1 and 2). Ro 13-5478 had activity comparable to that of nalidixic acid, while Ro 14-9578 had activity comparable to that of oxolinic acid. Both compounds had substantially reduced activity against *E. coli* UB1005 (2), a strain specifically resistant to quinolones (Table 1). Replicative DNA biosynthesis in permeabilized *E. coli* UB1005 cells is approximately 10 times more resistant to standard quinolones relative to that in *E. coli* H560 and strain ATCC 25922 (N. H. Georgopapadakou and B. A. Dix, unpublished data). Ro 13-5478 and Ro 14-9578 had, respectively, fair and good activity against several gram-negative bacteria and *S. aureus* (Table 1).

Ro 13-5478 and Ro 14-9578 inhibited DNA supercoiling catalyzed by DNA gyrase from *E. coli* MK47 (Table 2) and produced DNA cleavage at 10 μg/ml. The MK47 strain was chosen because it overproduces the limiting gyrase B protein, thus allowing activity assays to be performed with crude extracts (13). The effects of Ro 13-5478 and Ro 14-9578 on macromolecular biosynthesis are shown in Fig. 2. Similarly to norfloxacin, both compounds inhibited, preferentially, DNA biosynthesis in intact *E. coli* cells. Their effects on cell morphology were also similar to that of norfloxacin. All three compounds produced filaments in *E. coli* and enlarged cells in *S. aureus* (data not shown). The effect of quinolones on *E. coli* morphology is well documented and has been attributed to the SOS response (8, 10). The effect on *S. aureus* has so far been only descriptively studied (17,

TABLE 1. Antibacterial spectrum of Ro 13-5478 and Ro 14-9578

Organism	MIC (μg/ml)[a]			
	Ro 13-5478	Ro 14-9578	Nalidixic acid	Oxolinic acid
Escherichia coli ATCC 25922	6.3	3.1	6.3	1.6
Escherichia coli UB1005	>100	50	>100	10
Klebsiella pneumoniae ATCC 27736	25	1.6	12.5	1.6
Enterobacter cloacae ATCC 13047	50	3.1	6.3	3.1
Proteus mirabilis 2117	50	6.3	12.5	12.5
Proteus vulgaris NCTC 8313	25	3.1	6.3	0.8
Providencia rettgeri 969	25	1.6	3.1	0.8
Morganella morganii 6137	25	1.6	6.3	1.6
Serratia marcescens 803-15	25	3.1	3.1	0.8
Pseudomonas aeruginosa BA	50	>100	>100	12.5
Staphylococcus aureus ATCC 25923	50	1.6	100	6.3

[a] Determined by the broth dilution method with Antibiotic Medium 3 (for *E. coli* UB1005) or by the agar diffusion method with Diagnostic Sensitivity Test Agar (for all other organisms) (1). Inoculum size, 10^4 CFU/ml.

TABLE 2. Effects of Ro 13-5478, Ro 14-9578, and three reference compounds on *E. coli* replicative DNA biosynthesis and DNA gyrase (supercoiling assay)

Type	Compound	IC_{50}[a] (μg/ml)	
		DNA biosynthesis	DNA supercoiling
Monocyclic	Ro 13-5478	20	20
Tricyclic	Ro 14-9578	35	20
Reference	Nalidixic acid	100	50
	Oxolinic acid	7	10
	Norfloxacin	0.5	2

[a] IC_{50}, 50% inhibitory concentration.

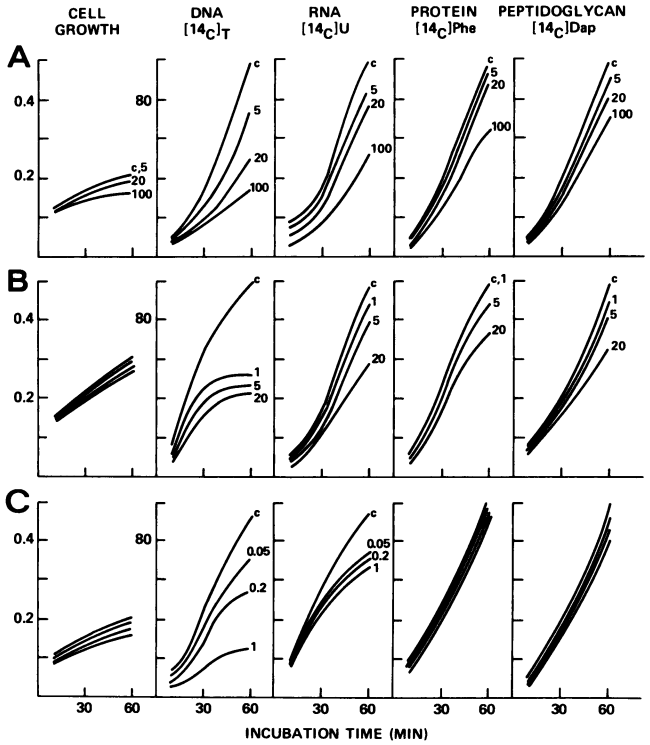

FIG. 2. Effects of Ro 13-5478 (A) and Ro 14-9578 (B) on cell growth and macromolecular biosynthesis in *E. coli* ATCC 25922. Norfloxacin (C) is included for comparison. Growth is shown as the optical density at 660 nm, and macromolecular biosynthesis is shown as the percentage of maximal incorporation of the respective radioactive precursor. Antibiotic concentrations (micrograms per milliliter) are indicated; c, untreated control. Dap, Diaminopimelic acid.

21) but may also be a manifestation of the SOS response in that organism (11).

In conclusion, structurally novel monocyclic and tricyclic analogs act like classical quinolones, which suggests that a bicyclic quinolone nucleus is not essential for activity.

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