Antimycobacterial Activities of Novel Levofloxacin Analogues

KATSUHiRO KAwAKAMI,* KENJI NAMBA, MAYUMI TANAKA, NORIKazu MAtSUHASHI, KENiJI SATO, AND MAKOTO TAKEmURA

New Product Research Laboratories I, Daiichi Pharmaceutical Co., Ltd., Edogawa-ku, Tokyo 134-8630, Japan

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In order to investigate structure-activity relationships between antimycobacterial activities and basic substituents at the C-10 position of levofloxacin (LVFX), we synthesized a series of pyridobenzoxazine derivatives by replacement of the N-methylpiperazinyl group of LVFX with various basic substituents. A compound with a 3-aminopyrrolidinyl group had one-half the activity of LVFX against Mycobacterium avium, M. intracellulare, and M. tuberculosis. Mono- and dimethylation of the 3-amino moiety of the pyrrolidinyl group increased the activities against M. avium and M. intracellulare but not those against M. tuberculosis. On the other hand, dialkylation at the C-4 position of the 3-aminopyrrolidinyl group enhanced the activities against M. avium, M. intracellulare, and M. tuberculosis. Thus, introduction of an N-alkyl or a C-alkyl group(s) into the 3-aminopyrrolidinyl group may contribute to an increase in potency against M. avium, M. intracellulare, and/or M. tuberculosis, probably through elevation of the lipophilicity. However, among the compounds synthesized, compound VII, which was a 2,8-diazabicyclo[4.3.0]nonanyl derivative with relatively low lipophilicity, showed the most potent activity against mycobacterial species: the activity was 4- to 32-fold more potent than that of LVFX and two to four times as potent as that of gatifloxacin. These results suggested that an increase in the lipophilicity of LVFX analogues in part contributed to enhancement of antimycobacterial activities but that lipophilicity of the compound was not a critical factor affecting the potency.

During the past decade, an increase in the number of patients with tuberculosis has been one of the most serious health problems in many countries (2, 27). In particular, the new pandemic combination of tuberculosis with human immunodeficiency syndrome (2, 3, 20) and the appearance of multidrug-resistant Mycobacterium tuberculosis (6, 10, 32) have aggravated attempts to treat these patients. In addition, the number of patients infected with Mycobacterium avium-M. intracellulare complex (MAC) is on the increase (5, 9). However, an effective therapy for MAC infection has not yet been established. Given these observations, the development of effective drugs for the mycobacterial infections described above has been keenly desired.

Recently, new quinolone antibacterial agents have been developed and marketed and are widely used clinically. They have potent and broad activities against both gram-negative and gram-positive pathogens. These agents also have been evaluated and shown to have potent activities against certain types of mycobacterial species in vitro tests and in experimental animals (ofloxacin [26, 29, 31], levofloxacin [LVFX] [18, 22, 33], ciprofloxacin [4, 34], sparfloxacin [12, 21, 30], gatifloxacin [GFLX, formerly AM-1155] [28], and sitafloxacin [formerly DU-6859a] [25]).

LVFX is a representative new quinolone which is characterized by its potency, safety, and good pharmacokinetic profiles in humans. This agent has a unique pyridobenzoxazine structure. In the previous paper, members of our group reported the synthesis of pyridobenzoxazines bearing a series of 3-aminopyrrolidinyl substituents at the C-10 position and evaluated their activities against gram-negative and -positive bacteria (13). In this paper, we report the in vitro activities of novel pyridobenzoxazine derivatives having various basic substituents against M. avium, M. intracellulare, and M. tuberculosis and the structure-activity relationships (SARs) between basic substituents and antimycobacterial activities.

MATERIALS AND METHODS

Organisms. M. avium (four strains), M. intracellulare (four strains), and M. tuberculosis (12 strains) were grown in MYCOBACTERIA 7H11 agar medium (Difco Laboratories, Detroit, Mich.) supplemented with 10% oleic acid-albumin-dextrose-catalase (OADC) (Difco Laboratories). Drugs. Rifampin (RFP; Sigma-Aldrich Japan, Tokyo, Japan) and isoniazide (INH; Sigma-Aldrich Japan) were obtained commercially and were used as positive drugs. LVFX was synthesized at New Product Research Laboratories I, Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan, as was GFLX. The synthesis of pyridobenzoxazine derivatives I, IV, V, and VI has been reported previously (13); other compounds were newly prepared, and brief descriptions of the synthetic methodology as well as the physicochemical properties of the compounds are given below. The structures of all the compounds synthesized are shown in Fig. 1.

All melting points (mp) were taken on a micro-mp apparatus (MP-500D; Yanagimoto Co., Kyoto, Japan) and are uncorrected. Proton nuclear magnetic resonance spectra (1H-NMR) were recorded at 400 MHz with a JNM-EX400 spectrometer (JEOL, Tokyo, Japan) in 0.1 N NaOD. Chemical shifts are expressed in ppm (δ) with sodium 2,2-dimethyl-2-silapentane-5-sulfonate as an internal standard. Elemental analyses were indicated only by the symbols of the elements; analytical results were within ±0.4% of the theoretical values unless otherwise noted. Optical rotation (αD) was measured at 589 nm with a SEPA-300 polarimeter (Horiba Co., Kyoto, Japan).

Representative procedure: 10-[(S,5)-2,8-diazabicyclo[4.3.0]nonan-8-yl]-9-fluoro-2,3-dihydro-3(5)-methyl-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid (VII). A solution of 9,10-difluoro-2,3-dihydro-3(5)-methyl-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid (VII) was added to the residue with ice-water cooling, and then the mixture was stirred at room temperature for 30 min. After evaporation of the Et3N, water was added to the residue with ice-water cooling and then was neutralized with 10% aqueous HCl to pH 7.4, and triethylamine (Et3N) (0.5 ml) in dimethyl sulfoxide (4.0 ml) was added to the mixture and stirred at room temperature for 30 min. After filtration, and then dissolved in 80% aqueous methanol (20 ml). Et3N (5.0 ml) was added to the solution, and the mixture was refluxed for 5 h. After concentration, the residue was dissolved in chloroform (CHCl3), and then the mixture was stirred at room temperature for 30 min. The precipitate was washed with water, collected by filtration, and then dissolved in 80% aqueous methanol (20 ml). Et3N (5.0 ml) was added to the solution, and the mixture was refluxed for 5 h. After concentration, the residue was dissolved in chloroform (CHCl3), which was washed with 10% aqueous citric acid and brine, dried over anhydrous sodium sulfate (Na2SO4), and then evaporated to dryness. The residue was dissolved in concentrated HCl (10 ml) with ice-water cooling and stirred for 5 min at room temperature. The mixture was adjusted to pH 11 with 20% aqueous NaOH with ice-water cooling and then was neutralized with 10% aqueous HCl to pH 7.4, which was extracted with CHCl3. The extract was dried over Na2SO4, and evaporated to dryness to yield a crude VII, which was recrystallized from ethanol-28% NH4OH to yield VII (260 mg, 67%) as slightly yellow needles. mp, 296 to 300 °C. 2.0 mmol), and triethylamine (Et3N) (0.5 ml) in dimethyl sulfoxide (4.0 ml) was added to the solution, and the mixture was refluxed for 5 h. After concentration, the residue was dissolved in chloroform (CHCl3). The extract was dried over Na2SO4, and evaporated to dryness.
299°C (decomposition). 1H-NMR δ: 8.40 to 8.41 (2H, m), 7.34 to 7.36 (1H, m), 3.76 to 3.80 (2H, m), 4.19 and 4.39 (each 1H, d, J = 11.72 Hz), 4.46 to 4.45 (1H, m), 7.39 (1H, d, J = 14.65 Hz), 8.34 (1H, s). Elemental analysis results were as follows. Calculated for C9H16FN3O4: C, 60.79; H, 5.91; N, 11.19. Found: C, 62.01; H, 5.72; N, 10.85. [11]-pyrido[1,2,3-de]7-oxo-7-aza[1,4]benzoxazine-6-carboxylic acid (II).

9-Fluoro-2,3-dihydro-3(S)-methyl-10-[3(S)-N,N-dimethylamino-1-pyrrolidinyl]-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazin-6-carboxylic acid (III). mp 220 to 224°C (decomposition). 1H-NMR δ: 1.39 to 1.40 (3H, s), 1.47 to 1.48 (3H, d, J = 6.35 Hz), 1.72 to 1.82 (1H, m), 1.26 to 2.21 (1H, m), 1.26 (6H, s), 2.86 to 2.94 (1H, m), 3.45 to 3.60 (3H, m), 3.67 to 3.73 (1H, m), 4.30 and 4.45 (each 1H, d, J = 11.23 Hz), 4.51 to 4.58 (1H, m), 7.43 (1H, d, J = 14.16 Hz), 8.30 (1H, s). Elemental analysis results were as follows. Calculated for C11H16FN3O4: C, 62.33; H, 5.79; N, 11.19. Found: C, 57.20; H, 5.88; N, 11.37. [11]-pyrido[1,2,3-de]7-oxo-7-aza[1,4]benzoxazine-6-carboxylic acid (VII).

FIG. 1. Structures of pyridobenzoxazine derivatives.
TABLE 1. MICs of pyridobenzoxazine derivatives and other drugs for MAC and M. tuberculosis strains

<table>
<thead>
<tr>
<th>Drug</th>
<th>MIC (µg/ml)</th>
<th>M. avium</th>
<th>M. intracellulare</th>
<th>RFP-s M. tuberculosis</th>
<th>RFP-r M. tuberculosis</th>
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<tr>
<td></td>
<td></td>
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<tr>
<td>F</td>
<td>0.3</td>
<td>6.25–25</td>
<td>15.6</td>
<td>6.25–50</td>
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<td>II</td>
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<td>3.13–50</td>
<td>17.2</td>
<td>6.25–25</td>
<td>41.1</td>
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<tr>
<td>III</td>
<td>17.7</td>
<td>1.56–25</td>
<td>9.0</td>
<td>3.13–12.5</td>
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<tr>
<td>IV</td>
<td>1.8</td>
<td>1.56–25</td>
<td>3.1</td>
<td>0.78–6.25</td>
<td>4.1</td>
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<td>V</td>
<td>10.7</td>
<td>1.56–12.5</td>
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<td>2.3</td>
<td>0.78–3.13</td>
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<tr>
<td>VII</td>
<td>2.7</td>
<td>0.20–1.56</td>
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<tr>
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<td>62.5</td>
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<tr>
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<td>7.8</td>
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<td>7.0</td>
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<tr>
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<td>5.1</td>
<td>1.56–50</td>
<td>16.8</td>
<td>6.25–25</td>
<td>14.1</td>
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<td>GFLX</td>
<td>5.9</td>
<td>0.78–6.25</td>
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<td>6.25–12.5</td>
<td>10.9</td>
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</table>

# NT, not tested.

## Footnotes

a. MIC, minimum inhibitory concentration.

b. The numbers of strains tested for the species listed were as follows: M. avium, 4; M. intracellulare, 4; RFP-s M. tuberculosis, 7; RFP-r M. tuberculosis, 5.

c. RFP-s, RFP-susceptible (RFP MIC, ≤12.5 µg/ml).

d. RFP-r, RFP-resistant (RFP MIC, >100 µg/ml).

# ACKNOWLEDGMENTS

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## REFERENCES


