Full Paper

Synthesis and Antibacterial Activity of Nitroaryl Thiadiazole-Levofloxacin Hybrids

Alireza Foroumadi[1], Shahla Mansouri[2], Saeed Emami[3], Javad Mirzaei[4], Maedeh Sorkhi[1], Nosratollah Saeid-Adeli[2], and Abbas Shafiee[1]

[1] Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran
[2] Department of Microbiology, Faculty of Medicine, Kerman University of Medical Sciences, Kerman, Iran
[3] Department of Medicinal Chemistry, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran
[4] School of Chemistry, University College of Science, University of Tehran, Tehran, Iran

Novel levofloxacin-containing hybrids carrying a 5-(nitroaryl)-1,3,4-thiadiazol-2-yl group were synthesized and evaluated *in vitro* against Gram-positive and Gram-negative bacteria. Preliminary data indicated that levofloxacin-nitrofuran and levofloxacin-nitroimidazole hybrids have a potent activity against Gram-positive organisms with enhanced anti-staphylococcal activity compared with the parent quinolone (*N*-desmethyl levofloxacin).

Keywords: Antibacterial activity / Levofloxacin / Quinolones / 1,3,4-Thiadiazole

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Introduction

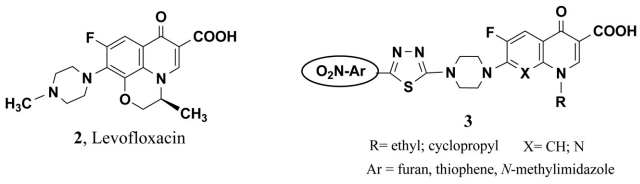
Antimicrobial resistance is now well documented for many pathogens, and studies with a variety of bacteria indicate that resistance can develop within just a few years [1]. As the prevalence of multidrug-resistant strains of *Staphylococcus aureus* and coagulase-negative staphylococci has increased worldwide, there has been an attendant need for effective new agents [2]. Fluoroquinolones, which were introduced in the 1980s, initially fulfilled this need and remain important in the treatment of a wide range of infections. However, resistance against many members of this class of agents, particularly older ones such as ciprofloxacin **1** and levofloxacin **2**, is increasing in staphylococci [3].

One strategy for slowing the development of resistance is the design of antibacterial hybrids with dual-mechanism of action. Using this approach, two pharmacophores of different drugs are combined in one molecule. These two pharmacophores, by addressing the active site of two different targets, offer the possibility to overcome the current resistance and, in addition, reduce the appearance of new resistant strains.

Because of high flexibility for structural variation at the 7-cyclic amine moiety of quinolones, this strategy was already applied to quinolone-containing hybrids via C-7 connection [4]. In addition, a position on the quinolone molecule, where substitution of bulky groups is permitted, is the C-7 position. Furthermore, it has been proposed that for Gram-positive organisms, increasing molecular mass and bulkiness of a substituent at the C-7 position is not a barrier to penetration [4]. Based on these considerations, several types of hybrids including quinolone-nitrofuran [5], quinolone-nitrothiophene [6], and quinolone-nitroimidazole [7] hybrids **3** have been synthesized and evaluated by us. Preliminary data indicated that these quinolone-nitroheterocycle hybrids **3** have a potent activity against Gram-positive organisms with enhanced anti-staphylococcal activity compared with the parent fluoroquinolones (ciprofloxacin, norfloxacin, and enoxacin).

In continuing our efforts to find new quinolone-nitroaryl hybrids, herein we report the synthesis and

Correspondence: Dr. Alireza Foroumadi, Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, 14174 Tehran, Iran.
E-mail: aforoumadi@yahoo.com
Fax: +98 21 664-61178



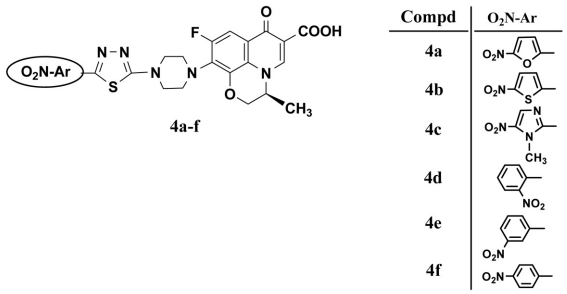
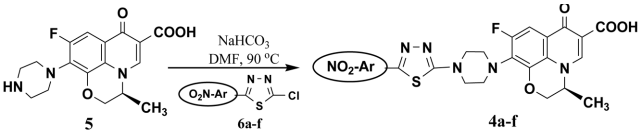


Figure 1. Chemical structure of synthesized compounds.

antibacterial activity of levofloxacin-containing hybrids **4a – f**, carrying the 5-(nitroaryl)-1,3,4-thiadiazol-2-yl group (Fig. 1).

Results and discussion

Our synthetic route to target compounds **4a-f** is diagrammed in Scheme 1. Reaction of *N*-desmethyl levofloxacin **5** with 2-chloro-5-(nitroaryl)-1,3,4-thiadiazole **6a – f** in ethanol in the presence of $NaHCO_3$ at reflux temperature gave compounds **4a – f** [5]. The intermediate *N*-desmethyl levofloxacin **5** was prepared according to the known method [8], by the reaction of piperazine with (–)-9,10-difluoro-2,3-dihydro-3-methyl-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid. The requisite 2-chloro-5-(nitroaryl)-1,3,4-thiadiazole **6a – f** was prepared according to the previously described methods [5 – 7, 9].



Scheme 1. Synthetic route to target compounds **4a – f**.

Compounds **4a – f** were tested *in vitro* by the conventional agar-dilution method [10] against Gram-positive and Gram-negative bacteria. The MIC (minimum inhibitory concentration) values were determined by comparison to the parent quinolones *N*-desmethyl levofloxacin **5** and levofloxacin **2** as reference drugs (Table 1).

Generally, the MIC values of the tested compounds indicated that 5-(nitroheteroaryl)-thiadiazole derivatives **4a – c** exhibited significant antibacterial activity, while all regio-isomers of 5-(nitrophenyl)-thiadiazole derivatives **4d – f** did not show activity against the tested strains at concentrations ≤ 4 μmg/mL.

As is evident from the data for compounds **4a – c**, higher susceptibilities (lower MICs) were observed with Gram-positive and lower susceptibilities, with Gram-negative bacteria.

The MIC values of nitrofuran **4a** and nitroimidazole **4c** against *Staphylococcus* strains indicate that these compounds possessed a comparable or better activity (MIC = 0.03 – 0.5 μg/mL) with respect to the reference drugs (MIC = 0.25 – 4 μg/mL). However, nitrothiophene derivative **4b** and *N*-desmethyl levofloxacin are statistically equivalent in antibacterial activity against *Staphylococcus* strains. Comparison between MICs of the nitrofuran **4a** and *N*-desmethyl levofloxacin against *Staphylococcus* strains revealed that incorporation of the 5-(5-

Table 1. *In vitro* antibacterial activities of compounds **4a – f** and reference drugs *N*-desmethyl levofloxacin **5** and levofloxacin **2** against selected strains (MICs in μg/mL).

Microorganisms	4a	4b	4c	4d	4e	4f	5	2
Staphylococcus aureus ATCC 25923	0.25	1	1	>4	>4	>4	4	0.5
Staphylococcus aureus ATCC 6538p	0.25	1	0.5	>4	>4	>4	2	0.25
Staphylococcus epidermidis ATCC 14940	0.06	1	0.06	>4	>4	>4	1	0.5
Staphylococcus epidermidis ATCC 12228	0.03	1	0.03	>4	>4	>4	1	0.25
Bacillus subtilis ATCC 6051	0.12	0.5	0.06	>4	>4	>4	1	0.5
Enterococcus feacalis NCTC 6013	0.25	4	4	>4	>4	>4	0.06	1
Serratia marcescens PTCC 1111	4	>4	>4	>4	>4	>4	1	0. 5
Escherichia coli ATCC 25922	1	>4	2	>4	>4	>4	0.03	0.03
Escherichia coli NCTC 12900	0.5	>4	4	>4	>4	>4	0.03	0.03
Klebsiella pneumoniae ATCC 10031	4	>4	>4	>4	>4	>4	0.25	0.25
Salmonella typhi ATCC 19430	0.5	>4	1	>4	>4	>4	0.06	0.03
Shigella flexner NCTC 8516	0.25	>4	1	>4	>4	>4	0.06	0.03
Pseudomonas aeruginosa ATCC 27853	>4	>4	>4	>4	>4	>4	>4	4

nitro-2-furan-2-yl)-1,3,4-thiadiazole moiety on to the piperazine ring of *N*-desmethyl levofloxacin increases the activity 8 to 33 times. Antibacterial screening of compounds **4a – c** against *B. subtilis* reveals that compounds **4a** and **4c** show a better activity (MIC = 0.06 – 0.12 μg/mL) with respect to the reference drugs (MIC = 0.5 – 1 μg/mL).

Generally, compounds **4a – c** are less active than the reference drugs against Gram-negative bacteria. However, compound **4a** showed moderate activity (MIC = 0.25 – 4 μg/mL) against Gram-negative bacteria, with the exception for antibacterial activity against *P. aeruginosa* (MIC >4 μg/mL).

Previously, we reported that novel nitroheteroaryl-1,3,4-thiadiazolyl quinolones differing from ciprofloxacin, norfloxacin, or enoxacin solely by the linkage of various nitroheteroaryl-1,3,4-thiadiazolyl groups to the piperazinyl residue at C-7 of the parent drug have particularly high *in vitro* activity against Gram-positive cocci such as *S. aureus* [5 – 7]. Similarly, our new series of nitroheteroaryl-1,3,4-thiadiazole derivatives **4a – c** exhibit high activity against Gram-positive and marginal activity against Gram-negative bacteria. From a structural point of view, compounds **4a – c** could be considered hybrid drugs, since they incorporate moieties of both nitroheterocycles and levofloxacin. Although the nature of the C-7 substituent is known to influence quinolone activity in bacteria [4], we identify addition of the 5-(5-nitroheteroaryl)-1,3,4-thiadiazol-2-yl groups as a particular chemical modification that allows manipulation of selectivity and potency. Indeed, the presence of the 1,3,4-thiadiazole with different nitroheteroaryl on the piperazine ring of levofloxacin shifted the activity of classic antibacterial quinolone levofloxacin from being more active against Gram-negative to Gram-positive bacteria. The low observed level of activity of compounds **4a – c** against Gram-negative bacteria may be a consequence of the interaction with their target enzymes or the result of a permeability mechanism. It has been reported that DNA gyrase is the primary target for quinolones in Gram-negative bacteria and that topoisomerase IV is the secondary target [11, 12]. The interactions of quinolone hybrids **4a – c** with these two target enzymes could lead to differences in susceptibility. On the other hand, it appears that most quinolones cross the Gram-negative outer membrane through protein channels called porins, although some may diffuse directly across the lipid bilayer [13, 14]. Thus, the outer membrane of Gram-negative bacteria is the major permeability barrier for quinolones to access their target site and to develop their antibacterial activity. It could be hypothesized that the increasing of molecular mass and bulkiness of substituent at C-7 position hinder penetration of quinolones **4a – c** into Gram-negative organisms through the porin channels. In contrast, Gram-positive bacteria do not possess an outer membrane, and so lack outer membrane proteins. Therefore, accumulation of quinolones by Gram-positive bacteria e.g. staphylococci is thought to take place by simple diffusion across the cytoplasmic membrane. Accordingly, it seems that compounds like **4a – c** with high molecular mass and bulky groups at the C-7 position of the piperazine ring, accumulated in Gram-positive bacteria more favorably than levofloxacin and *N*-desmethyl levofloxacin. Generally, our findings are in accordance with the earlier reports, where substitution at C-7 of quinolones is not only responsible for antibacterial activity but also for distinguishing between Gram-positive and Gram-negative bacteria [4 – 7, 15].

In conclusion, 5-(5-nitroheteroaryl)-1,3,4-thiadiazole groups are well tolerated in the terms of Gram-positive activity, as exemplified by the potency of 5-nitrofuran analog **4a** (MIC range of 0.03 – 0.25 μg/mL). Thus, introduction of 5-(5-nitroheteroaryl)-1,3,4-thiadiazol-2-yl groups at the N-4 position of piperazine ring in *N*-desmethyl levofloxacin molecule changes the antibacterial profile of quinolones and enhanced potency against staphylococci.

We would like to thank the Cipla Company for providing (–)-9,10-difluoro-2,3-dihydro-3-methyl-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid, and Iran National Science Foundation (INSF) for their financial support.

Experimental

Chemicals and all solvents used in this study were purchased from Merck AG and Aldrich Chemical (Darmstadt and Steinhein, resp., Germany). The 2-chloro-5-(nitroaryl)-1,3,4-thiadiazoles **6a – f** [5 – 7, 9] and *N*-desmethyl levofloxacin **5** [8] were prepared according to the literature. Melting points were determined on a Kofler hot stage apparatus (C. Reichert, Vienna, Austria) and are uncorrected. The IR spectra were obtained on a Shimadzu 470 spectrophotometer (potassium bromide disks; Shimadzu, Tokyo, Japan). ^{1}H-NMR spectra were measured using a Bruker 500 spectrometer (Bruker, Rheinstetten, Germany), and chemical shifts are expressed as δ (ppm) with tetramethylsilane as internal standard. Merck silica gel 60 F_{254} plates were used for analytical TLC. Yields are of purified product and were not optimized.

General procedure for the synthesis of compounds *4a – f*

A mixture of compound **6** (0.5 mmol), *N*-desmethyl levofloxacin **5** (174 mg, 0.5 mmol) and $NaHCO_3$ (42 mg, 0.5 mmol) in ethanol (5 mL) was refluxed for 8 h. After consumption of *N*-desmethyl levofloxacin (monitored by TLC), water (15 mL) was added and the precipitate was filtered, washed with water, and crystallized from methanol-chloroform to give compound **4**.

*2,3-Dihydro-9-fluoro-3-methyl-10-[4-[5-(5-nitro-furan-2-yl)-1,3,4-thiadiazol-2-yl]piperazin-1-yl]-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid (**4a**)*
Yield 63%, m. p. 263–265°C, IR ν_{max} (KBr) cm^{-1}: 1722 (C=O), 1620, 1531, and 1350 (NO_2); ^{1}H-NMR (500 MHz, $CDCl_3$) 1.65 (d, 3H, *J* = 6.4 Hz, CH_3), 3.54 and 3.81 (m, 8H, piperazine), 4.40 (dd, *J* = 11.2, 2.4 Hz, 1H, H-2a), 4.50 (dd, *J* = 11.2, 4.8 Hz, 1H, H-2b), 4.52 (m, 1H, H-3), 7.21 (d, 1H, *J* = 4.0 Hz, furan), 7.45 (d, 1H, *J* = 4.0 Hz, furan), 7.79 (d,1H, H-8, $J_{H,F}$ = 11.6 Hz), 8.64 (s, 1H, H-5).

*2,3-Dihydro-9-fluoro-3-methyl-10-[4-[5-(5-nitro-thiophen-2-yl)-1,3,4-thiadiazol-2-yl]piperazin-1-yl]-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid (**4b**)*
Yield 74%, m. p. 270–272°C, IR ν_{max} (KBr) cm^{-1}: 1721 (C=O), 1618, 1516, and 1337 (NO_2); ^{1}H-NMR (500 MHz, DMSO-d_6) 1.45 (d, 3H, *J* = 6.4 Hz, CH_3), 3.42 – 3.52 (m, 4H, piperazine), 3.68 – 3.77 (m, 4H, piperazine), 4.37 – 4.42 (m, 1H, H-2), 4.57 – 4.63 (m, 1H, H-2), 4.91 – 4.96 (m, 1H, H-3), 7.60 (d, 1H, *J* = 4.2 Hz, thiophene), 7.66 (d, 1H, $J_{H,F}$ = 11.8 Hz, H-8), 8.18 (d, 1H, *J* = 4.2 Hz, thiophene), 8.99 (s, 1H, H-5).

*2,3-Dihydro-9-fluoro-3-methyl-10-[4-[5-(1-methyl-5-nitro-1H-imidazol-2-yl)-1,3,4-thiadiazol-2-yl]piperazin-1-yl]-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid (**4c**)*
Yield 58%, m. p. 247 – 249°C, IR ν_{max} (KBr) cm^{-1}: 1724 (C=O), 1615, 1530, and 1365 (NO_2); ^{1}H-NMR (500 MHz, DMSO-d_6) 1.47 (d, 3H, *J* = 6.4 Hz, CH_3), 3.47-3.51 (m, 4H, piperazine), 3.74 – 3.78 (m, 4H, piperazine), 4.35 (s, 3H, N-CH_3), 4.39 – 4.43 (m, 1H, H-2), 4.60 – 4.64 (m, 1H, H-2), 4.92 – 4.97 (m, 1H, H-3), 7.63 (d, 1H, $J_{H,F}$ = 11.8 Hz, H-8), 8.24 (s, 1H, imidazole), 8.24 (s, 1H, imidazole), 9.00 (s, 1H, H-5).

*2,3-Dihydro-9-fluoro-3-methyl-10-[4-[5-(2-nitrophenyl)-1,3,4-thiadiazol-2-yl]piperazin-1-yl]-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid (**4d**)*
Yield 55%, m. p. 271–273°C, IR ν_{max} (KBr) cm^{-1}: 1726 (C=O), 1627, 1592, and 1340 (NO_2); ^{1}H-NMR (500 MHz, DMSO-d_6) 1.47 (d, 3H, *J* = 6.7 Hz, CH_3), 3.46 – 3.53 (m, 4H, piperazine), 3.71 – 3.76 (m, 4H, piperazine), 4.39 – 4.44 (m, 1H, H-2), 4.59 – 4.64 (m, 1H, H-2), 4.90 – 4.97 (m, 1H, H-3), 7.64 (d, 1H, $J_{H,F}$ = 12 Hz, H-8), 7.71 – 7.75 (m, 1H, phenyl), 7.82 – 7.86 (m, 2H, phenyl), 8.00 (d, 1H, *J* = 8.0 Hz, phenyl), 8.96 (s, 1H, H-5), 15.12 (s, 1H, COOH).

*2,3-Dihydro-9-fluoro-3-methyl-10-[4-[5-(3-nitrophenyl)-1,3,4-thiadiazol-2-yl]piperazin-1-yl]-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid (**4e**)*
Yield 82%, m. p. 304 – 306°C (dec), IR ν_{max} (KBr) cm^{-1}: 1711 (C=O), 1626, 1520, and 1370 (NO_2); ^{1}H-NMR (500 MHz, DMSO-d_6) 1.49 (d, 3H, *J* = 6.6 Hz, CH_3), 3.45 – 3.52 (m, 4H, piperazine), 3.70 – 3.77 (m, 4H, piperazine), 4.40 – 4.45 (m, 1H, H-2), 4.60 – 4.65 (m, 1H, H-2), 4.91 – 4.98 (m, 1H, H-3), 7.64 (d, 1H, $J_{H,F}$ = 12 Hz, H-8), 7.81 (t, 1H, *J* = 7.9 Hz, H-5 phenyl), 8.22 (d, 1H, *J* = 7.7 Hz, H-6 phenyl), 8.31 (d, 1H, *J* = 7.7 Hz, H-4 phenyl), 8.55 (brs, 1H, H-2 phenyl), 8.96 (s, 1H, H-5), 15.08 (s, 1H, COOH).

*2,3-Dihydro-9-fluoro-3-methyl-10-[4-[5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl]piperazin-1-yl]-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid (**4f**)*
Yield 80%, m. p. >340°C (dec), IR ν_{max} (KBr) cm^{-1}: 1714 (C=O), 1623, 1530, and 1350 (NO_2); ^{1}H-NMR (500 MHz, DMSO-d_6) 1.47 (d, 3H, *J* = 6.7 Hz, CH_3), 3.48 – 3.52 (m, 4H, piperazine), 3.72 – 3.76 (m, 4H, piperazine), 4.39 – 4.44 (m, 1H, H-2), 4.59 – 4.64 (m, 1H, H-2), 4.91 – 4.98 (m, 1H, H-3), 7.64 (d, 1H, $J_{H,F}$ = 12 Hz, H-8), 8.26 (d, 2H, *J* = 8.8 Hz, phenyl), 8.40 (d, 2H, *J* = 8.8 Hz, phenyl), 9.00 (s, 1H, H-5), 15.15 (s, 1H, COOH).

Antibacterial activity

Two-fold dilution of the test compounds **4a – f** and the standard antibacterial agents **2** and **5**, were prepared in DMSO (1 mL). Each dilute was added to molten Mueller-Hinton agar (19 mL) at 50°C to give the final concentrations ranging from 0.015 to 8 μg/mL. The plates were inoculated with $1-5 \times 10^4$ CFU of microorganisms; including a control plate (containing 1 mL DMSO without any antibacterial agent) and incubated at 35 – 37°C for 18 h. The MIC was determined as the lowest concentration of the agent that completely inhibits visible growth of the microorganisms.

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