European Journal of Medicinal Chemistry 44 (2009) 1205–1209

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and antibacterial activity of nitroaryl thiadiazole–gatifloxacin hybrids

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ARTICLE INFO

Article history:
Received 18 April 2008
Received in revised form 1 September 2008
Accepted 4 September 2008
Available online 19 September 2008

Keywords:
Quinolones
Gatifloxacin
1,3,4-Thiadiazole
Nitrothiophene
Nitrofuran
Nitroimidazole
Antibacterial activity

ABSTRACT

A number of gatifloxacin analogues containing a nitroaryl-1,3,4-thiadiazole moiety attached to the piperazine ring at C-7 position were prepared and evaluated as antibacterial agents against a panel of Gram-positive and Gram-negative bacteria. Among synthesized compounds, nitrofuran analog **6a** exhibited more potent inhibitory activity against Gram-positive bacteria including *Staphylococcus epidermidis* (MIC = 0.0078 μg/mL), *Bacillus subtilis* (MIC = 0.0039 μg/mL), *Enterococcus faecalis* (MIC = 0.125 μg/mL) and *Micrococcus luteus* (MIC = 0.125 μg/mL), with respect to other synthesized compounds and reference drug gatifloxacin. The target compounds were also assessed for their cytotoxic activity against normal mouse fibroblast (NIH/3T3) cells using MTT assay. The results indicated that these compounds exhibit antibacterial activity at non-cytotoxic concentrations.

1. Introduction

During recent years much attention has been devoted to the synthesis of new fluoroquinolones and to their antibacterial activity. Antibacterial 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. Resistance against many members of fluoroquinolones, particularly older ones, such as ciprofloxacin **1**, is increasing. Further advances in quinolone field are likely to provide better compounds capable of dealing with the resistant strains [1–3].

The inhibition of DNA gyrase or DNA topoisomerase IV and cell permeability of the quinolones are greatly influenced by the nature of the C-7 substituent on the standard structure of 4-quinolone-3-carboxylic acids. In addition, the substitution of bulky groups is permitted at the C-7 position [4–6]. Furthermore, it has been proposed that for Gram-positive organisms, increasing molecular mass and bulkiness of a substituent at the C-7 position are not barriers to penetration. With these in mind, previously several hybrids of 5-(nitroaryl)-1,3,4-thiadiazoles and different quinolones including ciprofloxacin **1**, norfloxacin **2**, enoxacin **3** and levofloxacin **4** have been synthesized with enhanced antibacterial activity against some Gram-positive organisms compared to the parent quinolones [7,8]. Gatifloxacin **5**, is a novel extended-spectrum fluoroquinolone (fourth-generation) with improved Gram-positive and anaerobe coverage compared with older agents such as ciprofloxacin **1** [9]. However, dysglycemia has been noted as the life-threatening adverse effect of gatifloxacin, which led to its withdrawal from the market in the United States in 2006 [10]. Thus, there exists continuous need for novel gatifloxacin derivatives, with better activity profile and tolerability, to overcome the limitations of gatifloxacin.

In continuing our efforts to find new quinolone–nitroarylthiadiazole hybrids, herein we report the synthesis and antibacterial activity of gatifloxacin hybrids **6** carrying a 5-(nitroaryl)-1,3,4-thiadiazol-2-yl group (Fig. 1).

2. Results and discussion

Our synthetic route to target compounds **6a–f** is presented in Fig. 2. The requisite 2-chloro-5-(nitroaryl)-1,3,4-thiadiazole **7a–f**,

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doi:10.1016/j.ejmech.2008.09.012

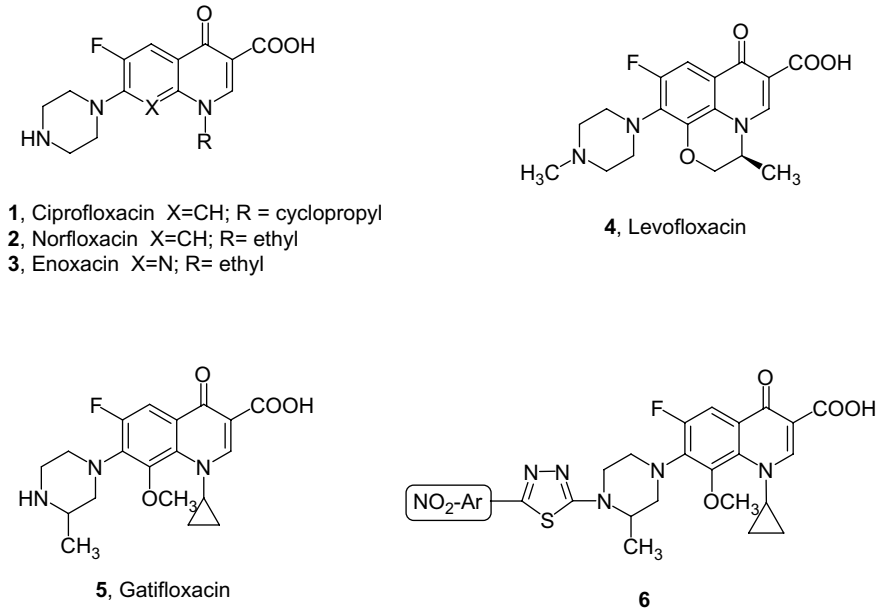


Fig. 1. Chemical structures of some piperazinyl quinolones and target compounds nitroaryl thiadiazole–gatifloxacin hybrids **6**.

was prepared according to the previously described method [7,8]. Reaction of gatifloxacin **5**, with 2-chloro-5-(nitroaryl)-1,3,4-thiadiazole **7a–f**, in DMF in the presence of $NaHCO_3$ at 85–90 °C, gave compounds **6a–f** (Table 1) [11,12].

Compounds **6a–f**, were tested *in vitro* by the conventional agar dilution method against a panel of microorganisms including *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 4940, *Streptococcus pneumonia* ATCC 1240, *Bacillus subtilis* ATCC 6051, *Enterococcus faecalis* NCTC 6013, *Micrococcus luteus* ATCC 1110, *Escherichia coli* ATCC 25922, *Salmonella typhi* ATCC 19430, *Shigella flexneri* NCTC 8516, *Klebsiella pneumonia* ATCC 10031, *Serratia marcescens* PTCC 1111 and *Pseudomonas aeruginosa* ATCC 27853 [13]. The minimum inhibitory concentration values (MICs) were determined by comparison to the parent quinolone, gatifloxacin **5** as reference drug (Table 2).

The MIC values of compounds **6a–f** against *Staphylococcus* strain indicated that some compounds possess a comparable or better activity with respect to gatifloxacin. Compound **6c**, exhibited the most potent inhibitory activity against *S. aureus* (MIC = 0.0313 μg/mL), and compound **6a** showed the most inhibitory activity against *S. epidemidis* (MIC = 0.0078 μg/mL), which were two and eightfold more potent than their parent quinolone (gatifloxacin **5**), respectively. The MIC values of compounds **6a–f** against *S. pneumonia* indicated that 4-nitrophenyl analog **6f** showed the most potent activity (MIC = 0.0625 μg/mL). Its activity was fourfold more than that of gatifloxacin. In addition, compounds **6a**, **b** and **d** exhibited potent activity (MIC = 0.25 μg/mL) comparable to gatifloxacin. Furthermore, the data obtained indicate that all compounds have more or equal inhibitory activity against *B. subtilis* (MIC = 0.0039–0.5 μg/mL), in comparison to reference drug (MIC = 0.5 μg/mL), with the exception of **6e** (MIC = 32 μg/mL). Most tested compounds had respectable *in vitro* activity against *E. faecalis*, but were less active than reference drug, with the exception of **6a** (MIC = 0.125 μg/mL), which was fourfold more potent than gatifloxacin. Compounds **6a**, **d** and **f** possessed a comparable or better activity against *M. luteus* (MIC = 0.125–1 μg/mL), with respect to gatifloxacin (MIC = 1 μg/mL).

Generally, compounds **6a–d** and **f** showed moderate to good activity against Gram-negatives including *E. coli*, *S. typhi*, *S. flexneri*, *K. pneumoniae* and *S. marcescens* but were less active than reference drug, with the exception of **6c** for *K. pneumoniae* (MIC = 0.5 μg/mL). In contrast, all the synthesized compounds did not show significant activity against another Gram-negative bacteria, *P. aeruginosa* (MICs >64).

Among synthesized compounds, nitrofuran analog **6a** exhibited the most potent inhibitory activity against Gram-positive bacteria including *S. epidermidis* (MIC = 0.0078 μg/mL), *B. subtilis* (MIC = 0.0039 μg/mL), *E. faecalis* (MIC = 0.125 μg/mL) and *M. luteus* (MIC = 0.125 μg/mL), with respect to other synthesized compounds and reference drug. Its inhibitory activity against *E. coli* (MIC = 1 μg/mL) and *S. pneumonia* (MIC = 0.25 μg/mL) was equal to reference drug gatifloxacin.

The *in vitro* cytotoxic activity of the test compounds **6a–f** against normal mouse fibroblast cell line (NIH/3T3) was investigated using

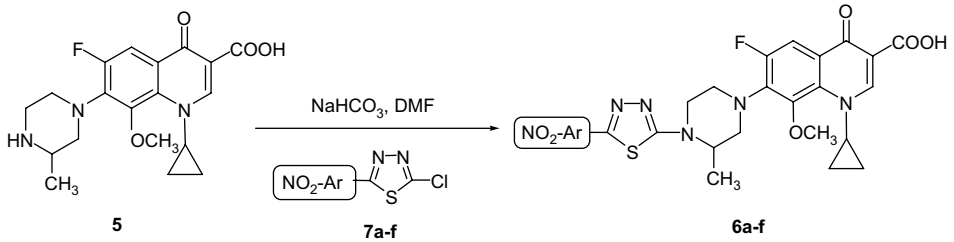
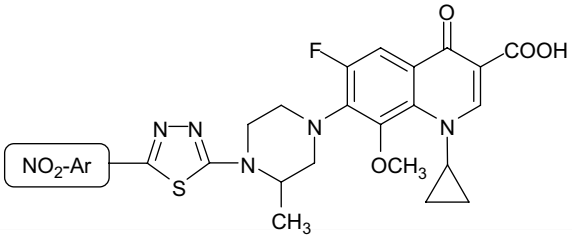
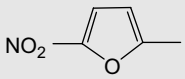
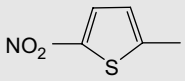
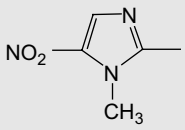
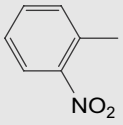
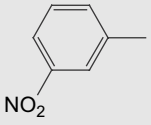
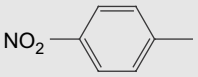


Fig. 2. Synthetic route to target compounds **6a–f**.

Table 1
Structural and physicochemical data of compounds **6a–f**



Compound	NO_2-Ar	Mp (°C)	Formula	Yield (%)	Anal. Calcd (Found) %		
					C	N	H
6a		263–265	$C_{25}H_{23}FN_6O_7S$	58	52.63 (52.88)	14.73 (14.71)	4.06 (4.03)
6b		250–252	$C_{25}H_{23}FN_6O_6S_2$	64	51.19 (51.31)	14.33 (14.35)	3.95 (4.02)
6c		272–274	$C_{25}H_{25}FN_8O_6S$	62	51.36 (51.28)	19.17 (19.17)	4.31 (4.32)
6d		270–272	$C_{27}H_{25}FN_6O_6S$	69	55.86 (55.99)	14.48 (14.33)	4.34 (4.41)
6e		168–170	$C_{27}H_{25}FN_6O_6S$	66	55.86 (55.90)	14.48 (14.45)	4.34 (4.48)
6f		267–269	$C_{27}H_{25}FN_6O_6S$	67	55.86 (55.77)	14.48 (14.53)	4.34 (4.30)

MTT colorimetric assay [14]. The IC_{50} values obtained for these compounds in comparison with reference drug gatifloxacin **5** are shown in Table 3. All compounds did not show significant activity against normal mouse fibroblast cell line at concentrations $<100\ \mu M$. Among the test compounds, 5-nitroheteroaryl derivatives **6a–c** and 4-nitrophenyl analog **6f** showed inferior toxicity with IC_{50} values of $>200\ \mu M$ and there was no significant differences between the cytotoxicity of these compounds. As can be seen from the results, these compounds display antibacterial activity at non-cytotoxic concentrations.

In the previous works, we identified that the incorporation of 5-(5-nitroheteroaryl)-1,3,4-thiadiazol-2-yl groups as a particular chemical modification at the piperazine residue of piperazinyl quinolones such as ciprofloxacin, norfloxacin, enoxacin and levofloxacin allows manipulation of selectivity and potency of these compounds [7,8]. As is evident from the data of present work, the *N*-[nitroaryl-1,3,4-thiadiazol-2-yl]gatifloxacin derivatives **6a–f** exhibit high activity against Gram-positive and less activity against Gram-negative bacteria. Similarly, *N*-[nitroaryl-1,3,4-thiadiazol-2-yl] moieties are well tolerated at the piperazine ring of gatifloxacin and the type of nitroaryl pendent on the 1,3,4-thiadiazole can modulate the antibacterial activity. Structurally, these new series of compounds distinct from ciprofloxacin derivatives solely by the introduction of methoxy group to the C-8 position of quinolone core and 3-methyl group to the piperazinyl residue at C-7 of the parent drug. Comparison between MIC values of gatifloxacin derivatives and corresponding ciprofloxacin derivatives reveal that these modifications in nitroaryl thiadiazole–quinolone hybrids can improve the antibacterial potency especially against Gram-positives.

3. Experimental

3.1. Chemistry

Chemical and all solvents used in this study were purchased from Merck AG and Aldrich chemical. Melting points were

Table 2
In vitro antibacterial activities of compounds **6a–f** and reference drug gatifloxacin against selected strains (MICs in μg/mL)

Microorganisms	6a	6b	6c	6d	6e	6f	5[a]
Staphylococcus aureus ATCC 25923	0.125	0.5	0.0313	32	>64	32	0.25
Staphylococcus epidermidis ATCC 4940	0.0078	0.125	0.5	0.0625	0.0313	0.0625	0.0625
Streptococcus pneumonia ATCC 1240	0.25	0.25	1	0.25	1	0.0625	0.25
Bacillus subtilis ATCC 6051	0.0039	0.5	0.5	0.0625	32	0.0313	0.5
Enterococcus faecalis NCTC 6013	0.125	2	4	2	>64	1	0.5
Micrococcus luteus ATCC 1110	0.125	4	2	0.5	1	0.25	1
Escherichia coli ATCC 25922	1	32	16	16	>64	16	1
Salmonella typhi ATCC 19430	1	1	0.25	16	>64	8	0.0625
Shigella flexneri NCTC 8516	0.5	1	1	16	>64	8	0.0625
Klebsiella pneumonia ATCC 10031	1	16	0.5	64	>64	64	0.5
Serratia marcescens PTCC 1111	8	8	64	16	>64	16	0.5
Pseudomonas aeruginosa ATCC 27853	>64	>64	>64	>64	>64	>64	2

[a] Gatifloxacin.

determined on a Kofler hot stage apparatus and are uncorrected. The IR spectra were obtained on a Shimadzu 470 spectrophotometer (potassium bromide disks). ^{1}H NMR spectra were recorded using a Bruker 500 spectrometer and chemical shifts are expressed as δ (ppm) with tetramethylsilane as internal standard.

3.2. General procedure for the synthesis of compounds **6a–f**

A mixture of compound **7** (0.5 mmol), gatifloxacin **5** (0.5 mmol), and $NaHCO_3$ (42 mg, 0.5 mmol) in DMF (10 mL), was heated at 85–90 °C for 12 h. After consumption of gatifloxacin (monitored by TLC), H_2O (20 mL) was added and the precipitate was filtered and washed with water to give the crude **6**. Purification was achieved by passage through a short silica gel column (chloroform–ethanol; 95:5). The product was crystallized from DMF–H_2O to give **6a–f**.

3.2.1. 1-Cyclopropyl-6-fluoro-7-[4-[5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-yl]-3-methylpiperazin-1-yl]-8-methoxy-4-oxo-quinoline-3-carboxylic acid (**6a**)

^{1}H NMR (500 MHz, DMSO-d_6) δ: 0.95–1.58 (m, 4H, cyclopropyl), 1.40 (d, 3H, CH_3-piperazine, $J = 6.5$ Hz), 3.33–3.90 (m, 8H, piperazine, and cyclopropyl), 3.72 (s, 3H, CH_3O), 7.48 (s, 1H, H_3-furan), 7.80 (d, 1H, H_5-quinolone, $J = 12$ Hz), 8.10 (s, 1H, H_4-furan), 8.73 (s, 1H, H_2-quinolone), 14.82 (s, 1H, COOH). IR (KBr, cm^{-1}): 1724 and 1619 (C=O), 1521 and 1351 (NO_2). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 8.93, 13.44, 34.98, 45.56, 49.66, 53.85, 63.79, 106.59 (d, C_8 quinolone, $^3J_{C\text{-}F} = 3.24$ Hz), 112.99, 114.21 (d, C_5 quinolone, $^2J_{C\text{-}F} = 22.35$ Hz), 121.32 (d, C_{4a} quinolone, $^3J_{C\text{-}F} = 7.45$ Hz), 122.34, 122.87, 138.43, 138.98 (d, C_7 quinolone, $^2J_{C\text{-}F} = 9.60$ Hz), 146.38 (d, C_6 quinolone, $^1J_{C\text{-}F} = 245.00$ Hz), 148.91, 149.19, 153.40, 154.83, 165.34, 165.98, 176.56 (d, C_4 quinolone, $^4J_{C\text{-}F} = 2.25$ Hz).

3.2.2. 1-Cyclopropyl-6-fluoro-7-[4-[5-(5-nitrothiophen-2-yl)-1,3,4-thiadiazol-2-yl]-3-methylpiperazin-1-yl]-8-methoxy-4-oxo-quinoline-3-carboxylic acid (**6b**)

^{1}H NMR (500 MHz, DMSO-d_6) δ: 0.90–1.54 (m, 4H, cyclopropyl), 1.41 (d, 3H, CH_3-piperazine, $J = 6.5$ Hz), 3.30–3.99 (m, 8H, piperazine and cyclopropyl), 3.72 (s, 3H, CH_3O), 7.59 (s, 1H, H_3-thiophen), 7.81 (d, 1H, H_5-quinolone, $J = 12$ Hz), 8.16 (s, 1H, H_4-thiophen), 8.71 (s, 1H, H_2-quinolone), 14.84 (s, 1H, COOH). IR (KBr, cm^{-1}): 1729 and 1618 (C=O), 1511 and 1347 (NO_2). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 8.87, 13.69, 35.12, 45.24, 49.83, 53.88 (C_5 piperazine), 63.83, 106.80 (d, C_8 quinolone, $^3J_{C\text{-}F} = 3.30$ Hz), 113.12, 114.27 (d, C_5 quinolone, $^2J_{C\text{-}F} = 22.20$ Hz), 121.14 (d, C_{4a} quinolone, $^3J_{C\text{-}F} = 7.50$ Hz), 127.98, 129.23, 137.19, 138.38, 139.28 (d, C_7 quinolone, $^2J_{C\text{-}F} = 9.75$ Hz), 146.24 (d, C_6 quinolone, $^1J_{C\text{-}F} = 245.20$ Hz), 149.11, 153.35, 154.69, 165.47, 165.85, 176.42 (d, C_4 quinolone, $^4J_{C\text{-}F} = 2.30$ Hz).

3.2.3. 1-Cyclopropyl-6-fluoro-7-[4-[5-(1-methyl-5-nitro-1H-imidazol-2-yl)-1,3,4-thiadiazol-2-yl]-3-methylpiperazin-1-yl]-8-methoxy-4-oxo-quinoline-3-carboxylic acid (**6c**)

^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.04–1.53 (m, 4H, cyclopropyl), 1.41 (d, 3H, CH_3-piperazine, $J = 6.5$ Hz), 3.30–3.98 (m, 8H, piperazine and cyclopropyl), 3.75 (s, 3H, CH_3O), 4.35 (s, 3H, CH_3-imidazole), 7.80 (d, 1H, H_5-quinolone, $J = 11.5$ Hz), 8.23 (s, 1H, imidazole), 8.73 (s, 1H, H_2-quinolone), 14.78 (s, 1H, COOH). IR (KBr, cm^{-1}): 1736 and 1623 (C=O), 1516 and 1357 (NO_2). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 8.99, 13.75, 18.65, 35.08, 45.30, 49.75, 53.90, 63.80, 106.80 (d, C_8 quinolone, $^3J_{C\text{-}F} = 3.30$ Hz), 113.00, 114.20 (d, C_5 quinolone, $^2J_{C\text{-}F} = 22.00$ Hz), 121.30 (d, C_{4a} quinolone, $^3J_{C\text{-}F} = 7.00$ Hz), 132.90, 134.10, 138.40, 139.30 (d, C_7 quinolone, $^2J_{C\text{-}F} = 9.50$ Hz), 146.20 (d, C_6 quinolone, $^1J_{C\text{-}F} = 245.10$ Hz), 149.21, 153.25, 165.70, 165.70, 171.42, 176.42 (d, C_4 quinolone, $^4J_{C\text{-}F} = 2.30$ Hz).

3.2.4. 1-Cyclopropyl-6-fluoro-7-[4-[5-(2-nitrophenyl)-1,3,4-thiadiazol-2-yl]-3-methylpiperazin-1-yl]-8-methoxy-4-oxo-quinoline-3-carboxylic acid (**6d**)

^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.04–1.50 (m, 4H, cyclopropyl), 1.42 (d, 3H, CH_3-piperazine, $J = 6.5$ Hz), 3.30–4.31 (m, 8H, piperazine and cyclopropyl), 3.76 (s, 3H, CH_3O), 7.62 (m, 2H, phenyl), 7.70 (m, 1H, phenyl), 7.95 (d, 1H, H_5-quinolone, $J = 12$ Hz), 8.05 (d, 1H, phenyl, $J = 6.5$ Hz), 8.86 (s, 1H, H_2-quinolone), 14.82 (s, 1H, COOH). IR (KBr, cm^{-1}): 1731 and 1623 (C=O), 1541 and 1372 (NO_2). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 8.54, 13.56, 34.88, 45.23, 49.77, 53.92, 63.74, 106.80 (d, C_8 quinolone, $^3J_{C\text{-}F} = 3.25$ Hz), 113.22, 114.12 (d, C_5 quinolone, $^2J_{C\text{-}F} = 22.25$ Hz), 121.00 (d, C_{4a} quinolone, $^3J_{C\text{-}F} = 7.38$ Hz), 121.58, 128.58, 129.03, 135.65, 138.43, 139.02, 139.32 (d, C_7 quinolone, $^2J_{C\text{-}F} = 9.75$ Hz), 146.24 (d, C_6 quinolone, $^1J_{C\text{-}F} = 245.20$ Hz), 149.11, 149.43, 153.50, 165.49, 165.79, 176.34 (d, C_4 quinolone, $^4J_{C\text{-}F} = 2.28$ Hz).

3.2.5. 1-Cyclopropyl-6-fluoro-7-[4-[5-(3-nitrophenyl)-1,3,4-thiadiazol-2-yl]-3-methylpiperazin-1-yl]-8-methoxy-4-oxo-quinoline-3-carboxylic acid (**6e**)

^{1}H NMR (500 MHz, DMSO-d_6) δ: 0.99–1.48 (m, 4H, cyclopropyl), 1.43 (d, 3H, CH_3-piperazine, $J = 6.5$ Hz), 3.35–4.33 (m, 8H, piperazine and cyclopropyl), 3.75 (s, 3H, CH_3O), 7.81 (d, 1H, H_5-quinolone,

Table 3
Cytotoxic activity of compounds **6a–f** in comparison with gatifloxacin **5**, against mouse fibroblast (NIH/3T3) cell line

Compound	IC_{50} (μM)[a]
6a	238 ± 27
6b	243 ± 35
6c	246 ± 37
6d	168 ± 32
6e	161 ± 4.8
6f	223 ± 31
5	596 ± 42

[a] IC_{50} is the concentration required to inhibit 50% of cell growth. The values represent mean ± SD.

$J = 12$ Hz), 7.83 (t, 1H, phenyl, $J = 8$ Hz), 8.30–8.31 (m, 1H, phenyl), 8.39–8.41 (m, 1H, phenyl), 8.43–8.54 (m, 1H, phenyl), 8.73 (s, 1H, H_2-quinolone), 14.90 (s, 1H, COOH). IR (KBr, cm^{-1}): 1738 and 1618 (C═O), 1531 and 1347 (NO_2). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 8.52, 13.66, 35.02, 45.29, 49.83, 54.08, 63.68, 106.92 (d, C_8 quinolone, $^3J_{C\text{-}F} = 3.30$ Hz), 113.22, 114.23 (d, C_5 quinolone, $^2J_{C\text{-}F} = 22.30$ Hz), 120.89 (d, C_{4a} quinolone, $^3J_{C\text{-}F} = 7.50$ Hz), 122.08, 123.07, 128.23, 129.88,138.38, 139.33 (d, C_7 quinolone, $^2J_{C\text{-}F} = 9.$ 75 Hz), 139.59, 146.29 (d, C_6 quinolone, $^1J_{C\text{-}F} = 245.15$ Hz), 149.21, 153.43, 165.50, 165.77, 176.41 (d, C_4 quinolone, $^4J_{C\text{-}F} = 2.25$ Hz).

3.2.6. 1-Cyclopropyl-6-fluoro-7-[4-[5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl]-3-methylpiperazin-1-yl]-8-methoxy-4-oxo-quinoline-3-carboxylic acid (**6f**)

^1H NMR (500 MHz, DMSO-d_6) δ: 1.00–1.59 (m, 4H, cyclopropyl), 1.52 (d, 3H, CH_3-piperazine, $J = 6.5$ Hz), 3.42–4.32 (m, 8H, piperazine and cyclopropyl), 3.79 (s, 3H, CH_3O), 7.80 (d, 1H, H_5-quinolone, $J = 11.5$ Hz), 8.07 (d, 2H, phenyl, $J = 8.5$ Hz), 8.40 (d, 2H, phenyl, $J = 8.5$ Hz), 8.72 (s, 1H, H_2-quinolone), 14.85 (s, 1H, COOH). IR (KBr, cm^{-1}): 1726 and 1623 (C═O), 1521 and 1332 (NO_2). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 8.58, 13.59, 34.92, 45.14, 49.88, 53.92, 63.78, 106.84 (d, C_8 quinolone, $^3J_{C\text{-}F} = 3.32$ Hz), 113.13, 114.32 (d, C_5 quinolone, $^2J_{C\text{-}F} = 22.25$ Hz), 121.09 (d, C_{4a} quinolone, $^3J_{C\text{-}F} = 7.45$ Hz), 122.08, 129.03, 138.38, 139.28 (d, C_7 quinolone, $^2J_{C\text{-}F} = 9.$ 75 Hz), 139.02, 146.24 (d, C_6 quinolone, $^1J_{C\text{-}F} = 245.20$ Hz), 149.11, 149.43, 153.34, 165.54, 165.84, 176.40 (d, C_4 quinolone, $^4J_{C\text{-}F} = 2.28$ Hz).

3.3. Antibacterial activity [13]

Twofold dilution of test compounds **6a–f** and the standard antibacterial agent (gatifloxacin **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 0.002 to 64 μg/mL. The bacterial inocula were prepared by suspending overnight colonies from Mueller–Hinton agar media in 0.85% saline. The inocula were adjusted photometrically at 600 nm to a cell density equivalent to approximately 0.5 McFarland standard (1.5×10^8 CFU/mL). The suspensions were then diluted in 0.85% saline to give 10^7 CFU/mL. Petri dishes were spot-inoculated with 1 μL of each prepared bacterial suspension (10^4 CFU/spot) and incubated at 35–37 °C for 18 h. The minimum inhibitory concentration (MIC) was the lowest concentration of the test compound, which resulted in no visible growth on the plate. To ensure that the solvent had no effect on bacterial growth, a control test was performed with test medium supplemented with DMSO at the same dilutions as used in the experiment.

3.4. Cytotoxic activity [14]

The *in vitro* cytotoxic activity of the test compounds against normal mouse fibroblast (NIH/3T3) cell line was assessed by MTT colorimetric assay. Briefly, cultures in the exponential growth phase were trypsinized and diluted in complete growth medium to give a total cell count of 5×10^4 cells/mL. The cell suspension (100 μL) was added to wells of sterile 96-well plates. After plating, 50 μL of a serial dilution of every agent was added. Each compound dilution was assessed in triplicate. Three wells containing only normal mouse fibroblast (NIH/3T3) cells suspended in 150 μL of complete medium were used as control for cell viability. The plates were then incubated for 72 h. After incubation, 30 μL of a 5 mg/mL solution of MTT was added to each well and the plate was incubated for another 1 h. After incubation, the culture medium was replaced with 100 μL of DMSO. Then, the absorbance of each well was measured by using a microplate reader at 492 nm wavelengths. For each compound, dose–response curve was measured with different drug concentrations, and the concentration causing 50% cell growth inhibition (IC_{50}) compared with the control was calculated.

Acknowledgments

We would like to thank the Cipla Pharmaceutical Company for providing gatifloxacin. This work was supported by grants from the Research Council of Kerman University of Medical Sciences and Iran National Science Foundation (INSF).

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