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Short communication

Synthesis and in vitro antibacterial evaluation of *N*-[5-(5-nitro-2-thienyl)-1,3,4-thiadiazole-2-yl] piperazinyl quinolones

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Abstract

A series of *N*-[5-(5-nitro-2-thienyl)-1,3,4-thiadiazole-2-yl]piperazinyl quinolones (**7a–c**) were synthesized and evaluated for in vitro antibacterial activity against some Gram-positive and Gram-negative bacteria. The antibacterial data revealed that compounds **7a–c** had strong and better activity against tested Gram-positive organisms than the reference quinolones such as ciprofloxacin, norfloxacin and enoxacin. However, all three compounds were nearly inactive against Gram-negative bacteria. Compound **7a** (ciprofloxacin analogue) was the most active compound against Gram-positive bacteria (MIC = 0.008–0.015 μg mL^{-1}).

Keywords: Quinolones; 5-Nitrothiophene; Synthesis; Antibacterial activity

1. Introduction

Because resistance to antimicrobial drugs is widespread, new antimicrobial and understanding of their mechanisms of action are vital [1]. The quinolones are compounds of intense interest because of their broad antibacterial spectrum both to Gram-positive and Gram-negative bacteria and their in vivo chemotherapeutic efficacy [2,3]. Further advances in quinolone development are likely to provide better compounds for clinical use [4]. The fluoroquinolones are the only direct inhibitors of DNA synthesis by binding to the enzyme-DNA complex; they stabilize DNA strand breaks created by DNA gyrase and topoisomerase IV. Ternary complexes of drug, enzyme, and DNA block progress of the replication fork [1]. The inhibition of DNA gyrase and cell permeability of quinolones is greatly influenced by the nature of C-7 substituent on the standard structure of 4-quinolone-3-carboxylic acid [5]. In addition, substitution of bulky functional groups is permitted at the C-7 position [6]. During recent years a number of quinolones with substitution on piperazine ring at C-7 position of the basic structure of quinolones were synthesized and evaluated for antibacterial activities [7–10].

In our previous report, we described the preparation and in vitro antibacterial activity of certain quinolones with an additional [5-(5-nitro-2-furyl)-1,3,4-thiadiazole-2-yl] moiety on the C-4 position of piperazine group [11]. All of these compounds demonstrated a potent and selective activity against Gram-positive bacteria. In continuing our efforts to enhance the antibacterial activity of the quinolones against Gram-positive bacteria, herein we report the synthesis and antibacterial activity of a new series of *N*-piperazinyl quinolones containing [5-(5-nitro-2-thienyl)-1,3,4-thiadiazole-2-yl] moiety (**7a–c**), designed by replacement of oxygen in furan ring with a sulfur, as potential new antibacterial agents.

2. Chemistry

The synthesis of *N*-substituted piperazinyl quinolones (**7a–c**) was achieved with a versatile and efficient synthetic route outline in Fig. 1. The 5-nitrothiophene-2-carboxaldehyde diacetate (**2**) [12] was prepared by nitration of 2-thiophenecarboxaldehyde (**1**). The reac-

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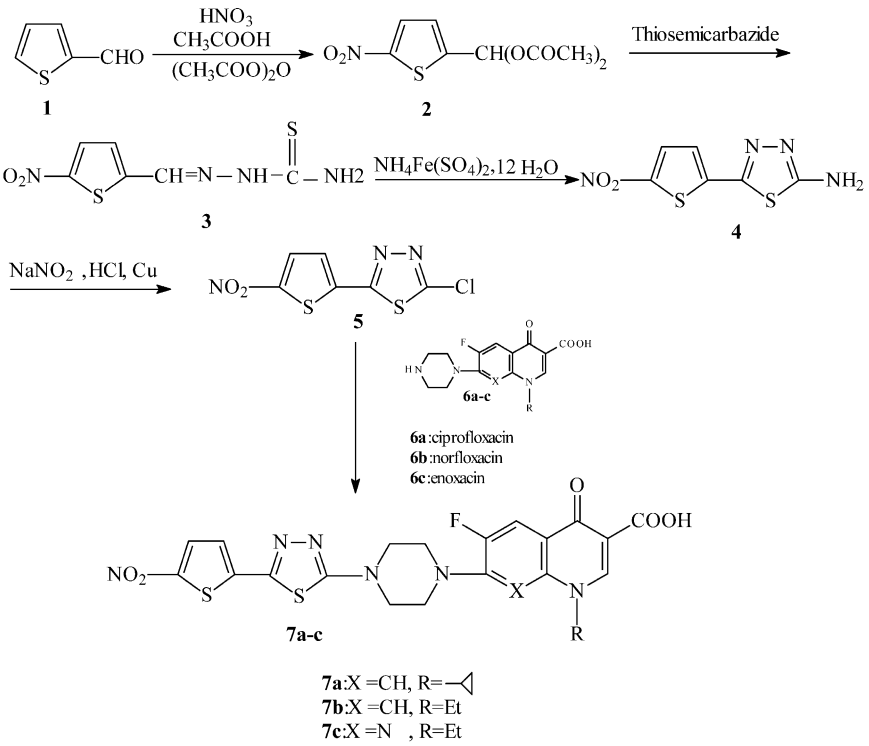

Fig. 1. Synthetic pathways to compounds **7a–c**.

tion of **2** with thiosemicarbazide in refluxing ethanol, afforded 5-nitro-2-thiophenecarboxaldehyde thiosemicarbazone (**3**) [13] in high yield. The 2-amino-5-(5-nitro-2-thienyl)-1,3,4-thiadiazole (**4**) was obtained by oxidative cyclization of **3** in the presence of ammonium ferric sulfate [14].

Diazotization of **4** in hydrochloric acid in the presence of copper powder [15] gave 2-chloro-5-(5-nitro-2-thienyl)-1,3,4-thiadiazole (**5**) in good yield. Reaction of the latter with piperazinyl quinolones (**6a–c**) in dimethylformamide (DMF) afforded compounds **7a–c** in good yields (Fig. 1).

3. Pharmacology

The in-vitro antibacterial activity of the tested compounds against Gram-positive [*Staphylococcus aureus* ATCC 6538p, *Staphylococcus epidermidis* ATCC 12228 and *Bacillus subtilis* PTCC 1023(PTCC = persian type culture collection)] and Gram-negative (*Escherichia coli* ATCC 8739, *Klebsiella pneumoniae* ATCC 10031, *Pseudomonas aeruginosa* ATCC 9027 and *Enterobacter cloacae* PTCC 1003) bacteria was tested by use of conventional agar dilution procedures [16] and compared with that of ciprofloxacin, norfloxacin and enoxacin.

Twofold serial dilutions of the compounds and reference drugs were prepared in Muller–Hinton agar. Drugs (6.4 mg) were dissolved in dimethylsulfoxide (DMSO; 1 mL) and the solution was diluted with water (9 mL). Further progressive double dilution with melted Muller–Hinton agar were performed to obtain the required concentrations of 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.13, 0.06, 0.03, 0.015, 0.0075 and 0.00375 μg mL^{-1}. Petri dishes were incubated with $1–5 \times 10^4$ colony forming units (cfu) and incubated at 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.

4. Results and discussion

The antibacterial activity of **7a–c** was assessed in side-by-side comparison with ciprofloxacin, norfloxacin and enoxacin against some Gram-positive (*S. aureus*, *S. epidermidis* and *B. subtilis*) and Gram-negative (*E. coli*, *K. peneumoniae*, *P. aeruginosa* and *E. cloacae*) bacteria using conventional agar dilution procedure and the results are summarized in Table I. The antibacterial data indicated that compounds **7a–c** had a strong and better activity against tested Gram-positive organisms than the reference quinolones. However, all three compounds were nearly inactive against tested Gram-negative bacteria. The antibacterial data revealed that the 5-nitrothiophene derivatives **7a–c** possesses similar

Table I
In vitro antibacterial activity of **7a–c** and standard quinolones (MIC μg mL^{-1})

Compound	*S. aureus*	*S. epidermidis*	*B. subtilis*	*E. coli*	*K. pneumoniae*	*E. cloacae*	*P. aeruginosa*
7a [a]	0.015	0.008	0.008	8	32	32	>64
7b [b]	0.5	0.5	0.03	64	>64	>64	>64
7c [c]	0.5	0.25	0.008	32	>64	>64	>64
Ciprofloxacin	0.5	0.25	0.015	0.125	0.03	0.06	1
Norfloxacin	1	1	0.06	0.5	0.125	0.125	4
Enoxacin	1	0.5	0.125	0.5	0.125	0.25	4

[a] Ciprofloxacin analogue.
[b] Norfloxacin analogue.
[c] Enoxacin analogue.

antibacterial profiles as compared with their 5-nitrofuran counterparts [11]. The selective antibacterial activity of *N*-[5-(5-nitro-2-thienyl)-1,3,4-thiadiazole-2-yl] piperazinyl quinolones (**7a–c**) against Gram-positive bacteria is in contrast to the good antibacterial activity of the unsubstituted piperazinyl quinolones such as ciprofloxacin against both Gram-positive and Gram negative bacteria. However, it is known that the nature of the functional group at the C-7 position of the quinolone ring system has strong influence on the spectrum and extent of antibacterial activity. Furthermore, the C-7 substituents were proposed as the domain that interacts with the enzyme for further strengthening drug binding [17].

The MIC values of compounds **7a–c** indicated that the ciprofloxacin analogue (**7a**) was the most active compound against *S. aureus* (MIC = 0.015μg mL^{-1}) and *S. epidermidis* (MIC = 0.008 μg mL^{-1}). The strong antibacterial activity of this compound against tested Gram-positive bacteria suggested further investigation on this compound.

5. Experimental

5.1. Chemistry

Melting points were 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 on a Varian Unity 400 spectrometer and chemical shifts are reported in parts per million (δ) relative to tetramethyl silane (TMS) as an internal standard. The mass spectra were run on a Finigan TSQ-70 spectrometer (Finigan, USA) at 70 eV. Elemental analyses were carried out on a CHN-O-rapid elemental analyzer (GmbH-Germany) for C, H and N, and the results are within ±0.4% of the theoretical values. Merck silica gel 60 F_{254} plates were used for analytical TLC; column chromatography was performed on Merck silica gel (70–230 mesh).

5.1.1. 5-Nitrothiophene-2-carboxadehyde diacetate (*2*)

A solution of thiophene-2-carboxaldehyde (26.9 g, 0.24 mol) in acetic anhydride (85 mL) was added to a stirring solution of conc. HNO_3 (19.2 g) and glacial acetic acid (140 mL) below 30 °C and the mixture was stirred for 0.5 h at 50–60 °C. The solution obtained was poured into crushed ice (200 g), and the resulting mixture was kept refrigerated for 1 h. The precipitate was filtered, washed with water and crystallized from ethanol to give 27.9 g of **2** in 45% yield. M.p. 73–74 °C (lit. [12] m.p. 74 °C).

5.1.2. 5-Nitrothiophene-2-carboxaldehyde thiosemicarbazone (*3*)

Conc. HCl (1 mL) was added to a mixture of compound **2** (5.2g, 20 mmol) and thiosemicarbazide (1.8 g, 20 mmol) in ethanol 95% (50 mL) and the mixture was refluxed for 3 h. After cooling the precipitate was filtered and washed with cold 90% ethanol. The product was crystallized from ethanol to give 4.1 g of **3** in 89% yield. M.p. 255–258 °C (lit. [13] m.p. 252–255 °C).

5.1.3. 2-Amino-5-(5-nitro-2-thienyl)-1,3,4-thiadiazole (*4*)

A mixture of compound **3** (4.6 g, 20 mmol) and ammonium ferric sulfate dodecahydrate (29 g, 60 mmol) in H_2O (150 mL) was refluxed for 6 h. The reaction mixture was cooled and the solids isolated by filteration were washed with water, air dried and crystallized from ethanol to give 90% (4.1 g) of **4**. M.p. 210–211 °C. IR (KBr) ν_{max}: 3100, 3075 (NH_2), 3050 (CH thienyl), 1520 and 1340 cm^{-1} (NO_2). Mass *m/z* (relative abundance %): 229(81), 228 (M^+, 99), 212 (8), 95 (26), 82 (18), 74 (100), 69 (58), 60 (73), 45 (55).

5.1.4. 2-Chloro-5-(5-nitro-2-thienyl)-1,3,4-thiadiazole (*5*)

Compound **4** (2.28 g, 10 mmol) was ground with an excess of $NaNO_2$(1.59 g, 30 mmol) and the mixture was introduced in small portions and with stirring, into a ice cooled solution of conc. HCl (30 mL) and water (13

mL), containing Cu powder (0.45 g). The reaction mixture was allowed to reach room temperature and heated to 55 °C for 20 min. The reaction mixture was cooled and extracted with $CHCl_3$ (three times). The combined extracts were washed with $NaHCO_3$ solution, dried (Na_2SO_4) and evaporated to give crude **5**. Purification was achieved by passage through a short silica gel column with chloroform as eluent. The product was crystallized from ethanol to give 63% (1.56 g) of **5**. M.p. 175–177 °C. IR (KBr) ν_{max}: 1334, 1497 (NO_2), 3090 cm^{-1} (CH thienyl) ^{1}H-NMR ($CDCl_3$, 80 MHz): 7.40 (d, 1H, $J = 4.8$ Hz) and 7.91 (d, 1H, $J = 4.8$ Hz). Mass *m/z* (relative abundance %): 249 (36), 248 (20), 247 (81), 219 (14), 217 (33), 189 (19), 138 (10), 126 (16), 111 (17), 95 (43), 93 (100), 79 (45), 69 (83), 57 (68), 45 (38).

*5.1.5. General procedure for the synthesis of N-substituted piperazinyl quinolones (**7a–c**)*

A mixture of compound **5** (0.5 mmol), piperazinyl quinolone **6** (0.5 mmol) and $NaHCO_3$ (42 mg, 0.5 mmol) in DMF (10 mL), was heated under reflux at 85–90 °C for 12 h. After cooling, water was added (10 mL) and the precipitate was filtered off, washed with water and crystallized from DMF–H_2O to give 50–60% of **7**.

*5.1.5.1. 1-Cyclopropyl-6-fluoro-7-{4-[5-(5-nitro-2-thienyl)-1,3,4-thiadiazole-2-yl]-1-piperazinyl}-4-oxo-1,4-dihydro-3-quinolone carboxylic acid (**7a**).* M.p. 305–306 °C; yield 50%; IR (KBr) ν_{max}: 1720, 1620 (C=O), 1520, 1350 cm^{-1} (NO_2). ^{1}H-NMR (DMSO-d_6, 400 MHz): 1.19–1.32 (m, 4H, cyclopropyl), 3.50 (brs, 5H, 1H cyclopropyl and 4H piperazine), 3.80 (brs, 4H, piperazine) 7.62 (d, 1H, thienyl, $J = 4.8$ Hz), 7.65 (d, 1H, H_8-quinoline, $J_{H,F} = 7.0$ Hz), 7.98 (d, 1H, H5-quinoline, $J_{H,F} = 12.8$ Hz), 8.18 (d, 1H, thienyl, $J = 4.8$ Hz), 8.69 (s, 1H, H2-quinoline). Anal. $C_{23}H_{19}FN_6O_5S_2$ (C, H, N).

*5.1.5.2. 1-Ethyl-6-fluoro-7-{4-[5-(5-nitro-2-thienyl)-1,3,4-thiadiazole-2-yl]-1-piperazinyl}-4-oxo-1,4-dihydro-3-quinolone carboxylic acid (**7b**).* M.p. 310–311 °C; yield 60%; IR (KBr) ν_{max}: 1720, 1620 (C=O), 1520, 1350 cm^{-1} (NO_2). ^{1}H-NMR (DMSO-d_6, 400 MHz): 1.42 (t, 3H, CH_3, $J = 6.4$ Hz), 4.61 (2H, CH_2, $J = 6.4$Hz), 3.50–3.60 (m, 4H, piperazine), 3.75–3.85 (m, 4H, piperazine), 7.27 (d, 1H, H_8-quinoline, $J_{H,F} = 7.2$ Hz), 7.62 (d, 1H, thienyl, $J = 4.8$ Hz), 7.99 (d, 1H, H_5-quinoline, $J_{H,F} = 13.2$ Hz), 8.18 (d, 1H, thienyl, $J = 4.8$ Hz), 8.99 (s, 1H, H_2-quinoline). Anal. $C_{22}H_{19}FN_6O_5S_2$ (C, H, N).

*5.1.5.3. 1-Ethyl-6-fluoro-7-{4-[5-(5-nitro-2-thienyl)-1,3,4-thiadiazole-2-yl]-1-piperazinyl}-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid (**7c**).* M.p. 306–307 °C; yield 57%; IR (KBr) ν_{max}: 1725, 1620 (C=O), 1515, 1350 cm^{-1} (NO_2). ^{1}H-NMR (DMSO-d_6, 400 MHz): 1.41 (t, 3H, CH_3, $J = 7.2$ Hz), 4.53 (2H, CH_2, $J = 7.2$Hz), 3.70–3.86 (m, 4H, piperazine), 3.94–4.12 (m, 4H, piperazine), 7.19 (d, 1H, thienyl, $J = 4.8$ Hz), 8.17 (d, 1H, thienyl, $J = 4.8$ Hz), 8.17 (d, 1H, H_5-naphthyridine, $J_{H,F} = 13.2$ Hz), 9.02 (s, 1H, H_2-naphthyridine). Anal. $C_{21}H_{18}FN_7O_5S_2$ (C, H, N).

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