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European Journal of Medicinal Chemistry 38 (2003) 1001–1004
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
www.elsevier.com/locate/ejmech

Laboratory note

Synthesis of 6-fluoro-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid derivatives as potential antimicrobial agents

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Received 17 March 2003; received in revised form 18 July 2003; accepted 29 July 2003

Abstract

In the present study, a series of 1-ethyl/benzyl-6-fluoro-7-(substituted piperazin-1-yl)1,4-dihydro-4-oxo-quinoline-3-carboxylic acid were synthesized and characterized by IR, ^{1}H-NMR, mass spectral and elemental analysis. The in vitro antibacterial and antifungal activities of the compounds were evaluated by paper disc diffusion method. The minimum inhibitory concentrations (MIC) of the compounds were also determined by agar streak dilution method. The in vivo antibacterial activity of the compounds against *Escherichia coli* was also evaluated by mouse protection test. All the compounds exhibited significant antibacterial and weak antifungal activities. The in vivo antibacterial activity (ED_{50}) against *E. coli* was 50–160 mg kg^{-1} in the order of **7** < **9** < **8** < **10**. 1-Ethyl-6-fluoro-7-(2,5-dioxo-piperazin-1-yl)1,4-dihydro-4-oxo-quinoline-3-carboxylic acid (**7**) was found to exhibit the most potent in vitro antimicrobial activity with MIC of 4.1, 3.1, 3.1, 2.4, 1, 1, 25 and >100 μg mL^{-1} against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Micrococcus luteus*, *Bacillus cereus*, *E. coli*, *Klebsiella pneumoniae*, *Candida albicans* and *Aspergillus niger*.

Keywords: Fluoroquinolone; Antibacterial; Antifungal

1. Introduction

Most of the quinolone antibacterial research has been focussed on the functionality at C-7 position since the introduction of norfloxacin. Piperazine substitution at C-7 position has resulted in a wide range of clinically useful fluoroquinolone antibacterial agents namely ciprofloxacin, perfloxacin, pefloxacin, ofloxacin, amifloxacin, fleroxacin, lomefloxacin, sparfloxacin, difloxacin, enoxacin, enrofloxacin, levofloxacin, marbofloxacin and orbifloxacin. Gatifloxacin and grepafloxacin exhibit potent broad-spectrum activity including *Streptococcus pneumoniae*.

Fluoroquinolones with 7-piperazinyl [1–8], 1-ethyl [9–13] and 1-benzyl [1,11,14] have been reported to possess potent antibacterial [1–14], antifungal [7], antiviral activities [2,7]. It was therefore envisaged that a new series of 2,5-dioxo/2,5-dioxo-3,6-dimethyl-piperazinyl fluoroquinolones with 1-ethyl/benzyl substitution would result in compounds with potent antibacterial and antifungal activities. In the present study, a series of 1-ethyl/benzyl-6-fluoro-7-(substituted piperazin-1-yl)1,4-dihydro-4-oxo-quinoline-3-carboxylic acid were synthesized and screened for antibacterial and antifungal activities.

2. Chemistry

In the present study, 3-chloro-4-fluoroaniline was heated with diethyl-ethoxymethylenemalonate to form diethyl-*N*-(3-chloro-4-fluoro-phenyl)aminomethylenemalonate (**1**) which was cyclized to ethyl-7-chloro-6-fluoro-4-hydroxy-quinoline-3-carboxylate (**2**). Ethylation/benzylation by treatment with ethyliodide or benzyl chloride in the presence of anhydrous potassium car-

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0223-5234/03/$ - see front matter
doi:10.1016/S0223-5234(03)00151-X

bonate formed ethyl-1-ethyl-6-fluoro-1,4-dihydro-4-oxo-quinoline-3-carboxylate (**3**) and ethyl-1-benzyl-6-fluoro-1,4-dihydro-4-oxo-quinoline-3-carboxylate (**4**), respectively. The 1-ethyl/benzyl esters were hydrolysed with aqueous sodium hydroxide to produce the corresponding carboxylic acids (**5** and **6**) [1]. 1-Ethyl/benzyl-6-fluoro-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid (**5** and **6**) were reacted with 2,5-dioxo-piperazine/2,5-dioxo-3,6-dimethyl-piperazinyl to obtain the desired products (**7**–**10**).

3. Biological investigation

The in vitro antibacterial (*Staphylococcus aureus* ATCC 9144, *Staphylococcus epidermidis* ATCC 155, *Micrococcus luteus* ATCC 4698, *Bacillus cereus* ATCC 11778, *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 11298) and antifungal (*Candida albicans* ATCC 2091 and *Aspergillus niger* ATCC 9029) activities of the compounds were evaluated by paper disc diffusion method. The minimum inhibitory concentrations (MIC) of the compounds were also determined by agar streak dilution method. The in vivo antibacterial activity of the compounds against *E. coli* was also evaluated by mouse protection test. Acute oral toxicity test was performed for all the synthesized compounds as per organization of economic co-operation and development (OECD) guidelines.

4. Results and discussion

All the compounds exhibited highly significant antibacterial and moderate antifungal activities. The compounds were active against all the tested microorganism compared to ciprofloxacin with a range of MIC values of 4.1–25, 3.1–25.4, 3.1–20.2, 2.4–25, 1–10.2, 1–10.9 and 25–50.2 μg mL^{-1} against *S. aureus*, *S. epidermidis*, *M. luteus*, *B. cereus*, *E. coli*, *K. pneumoniae* and *C. albicans*, respectively. The compounds exhibited very weak activity against *A. niger* (MIC: >100 μg mL^{-1}). 1-Ethyl-6-fluoro-7-(2,5-dioxo-piperazin-1-yl)1,4-dihydro-4-oxo-quinoline-3-carboxylic acid (**7**) was found to exhibit the most potent in vitro antimicrobial activity with MIC of 4.1, 3.1, 3.1, 2.4, 1, 1, 25 and >100 μg mL^{-1} against *S. aureus*, *S. epidermidis*, *M. luteus*, *B. cereus*, *E. coli*, *K. pneumoniae*, *C. albicans* and *A. niger*. The ED_{50} (in vivo antibacterial screening) of the compounds against *E. coli* was 50–160 mg kg^{-1} in the order of $\mathbf{7} < \mathbf{9} < \mathbf{8} < \mathbf{10}$. The compounds (**7**–**10**) did not cause mortality upto 2000 mg kg^{-1} in acute oral toxicity (OECD-423 guidelines) and were considered as safe (x-unclassified).

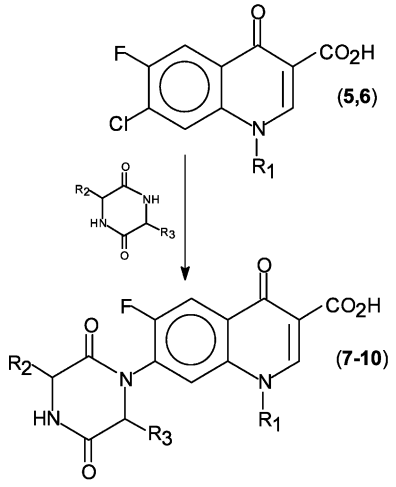
5. Experimental protocols

5.1. Chemistry

The melting points were taken in open capillary tube and are uncorrected. The IR spectra of the compounds were recorded on ABB Bomem FTIR spectrometer MB104 with KBr pellets. ^{1}H-NMR spectra were recorded on 300 MHz-Bruker DPX 200. The chemical shifts are reported as parts per million downfield from tetra methyl silane. Mass spectra were recorded on Shimadzu GC MS QP 5000. Microanalyses for C, H, N were performed in Heraeus CHN Rapid Analyzer. All the compounds gave satisfactory chemical analyses ($\pm$ 0.4%). The purity of the compounds were checked by TLC on precoated SiO_2 gel (HF_{254}, 200 mesh) aluminum plates (E Merck) using *n*-hexane–ethyl acetate (8:2) as mobile phase and visualized by iodine vapors.

5.1.1. General method of synthesis 7–10

The starting material (**5**, **6**) for the synthesis of the title compounds have been previously published [1]. A mixture of 1-ethyl/benzyl-6-fluoro-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid (0.01 mol) (**5**, **6**), 2,5-dioxo-piperazine/2,5-dioxo-3,6-dimethyl-piperazine (0.05 mol) and dimethyl formamide (20 mL) was heated to 130–140 °C for 10 h with stirring. The reaction mixture was evaporated to dryness under reduced pressure and water (20 mL) was added. The resulting precipitate was



7 – $R_1 = C_2H_5$, $R_2 = R_3 = H$

8 – $R_1 = CH_2\text{-}C_6H_5$, $R_2 = R_3 = H$

9 – $R_1 = C_2H_5$, $R_2 = R_3 = CH_3$

10 – $R_1 = CH_2\text{-}C_6H_5$, $R_2 = R_3 = CH_3$

Scheme 1.

filtered, washed with water, vacuum dried and recrystallized using dimethyl formamide (Scheme 1).

*5.1.1.1. 1-Ethyl-6-fluoro-7-(2,5-dioxo-piperazin-1-yl)1,4-dihydro-4-oxo-quinoline-3-carboxylic acid (**7**).* Yield = 98%, m.p. 236–238 °C. ^{1}H-NMR (DMSO-d_6) δ: 8.95 (s, 1H, 2H), 7.88 (d, J_{H-F} = 13.5 Hz, 1H, 5H), 7.13 (d, J_{H-F} = 7.3 Hz, 1H, 8H), 4.59 (q, 2H, J_{H-H} = 6.7 Hz, CH_2), 3.26 (s, 2H, 6′-CH_2), 2.86 (s, 2H, 3′-CH_2), 1.42 (t, 3H, J_{H-H} = 6.9 Hz, CH_3). IR (KBr) cm^{-1}: 1713 (COOH), 1615 (CO), 1339 (C–N), 1265 (C–F), 808, 764 (Ar–H). EI MS *m/z* (M^+): 347.20 (Calcd for $C_{16}H_{14}FN_3O_5$: 347.29).

*5.1.1.2. 1-Benzyl-6-fluoro-7-(2,5-dioxo-piperazin-1-yl)1,4-dihydro-4-oxo-quinoline-3-carboxylic acid (**8**).* Yield = 67%, m.p. 258–260 °C. ^{1}H-NMR (DMSO-d_6) δ: 9.21 (s, 1H, 2H), 8.17 (d, J_{H-F} = 10.3 Hz, 1H, 5H), 7.87 (d, J_{H-F} = 7.4 Hz, 1H, 8H), 6.99–7.61 (m, 5H, C_6H_5), 5.86 (s, 2H, N–CH_2), 3.15 (s, 2H, 6′-CH_2), 2.89 (s, 2H, 3′-CH_2). IR (KBr) cm^{-1}: 1717 (COOH), 1611 (CO), 1342 (C–N), 1268 (C–F), 808, 754 (Ar–H). EI MS *m/z* (M^+): 409.25 (Calcd for $C_{21}H_{16}FN_3O_5$: 409.37).

*5.1.1.3. 1-Ethyl-6-fluoro-7-(2,5-dioxo-3,6-dimethyl-piperazin-1-yl)1,4-dihydro-4-oxo-quinoline-3-carboxylic acid (**9**).* Yield = 57%, m.p. 242–244 °C. ^{1}H-NMR (DMSO-d_6) δ: 9.04 (s, 1H, 2H), 8.40 (d, J_{H-F} = 9.2 Hz, 1H, 5H), 8.08 (d, J_{H-F} = 5.9 Hz, 1H, 8H), 4.62 (q, J_{H-H} = 6.7 Hz, 2H, CH_2), 4.23 (q, J_{H-H} = 6.6 Hz, 1H, 6′-CH), 3.99 (q, J_{H-H} = 6.6 Hz, 1H, 3′-CH), 1.53 (t, J_{H-H} = 6.8 Hz, 3H, CH_2–CH_3), 1.40 (d, J_{H-H} = 6.7 Hz, 3H, 6′-CH_3), 1.33 (d, J_{H-H} = 6.8 Hz, 3H, 3′-CH_3). IR (KBr) cm^{-1}: 1717 (COOH), 1611 (CO), 1343 (C–N), 1268 (C–F), 808, 753 (Ar–H). EI MS *m/z* (M^+): 375.25 (Calcd for $C_{18}H_{18}FN_3O_5$: 375.35).

*5.1.1.4. 1-Benzyl-6-fluoro-7-(2,5-dioxo-3,6-dimethyl-piperazin-1-yl)1,4-dihydro-4-oxo-quinoline-3-carboxylic acid (**10**).* Yield = 59%, m.p. 274–275 °C. ^{1}H-NMR (DMSO-d_6) δ: 9.18 (s, 1H, 2H), 8.19 (d, J_{H-F} = 11.4 Hz, 1H, 5H), 7.87 (d, J_{H-F} = 6.6 Hz, 1H, 8H), 7.05–7.64 (m, 5H, C_6H_5), 5.87 (s, 2H, N–CH_2), 4.01 (q, J_{H-H} = 7 Hz, 1H, 6′-CH), 3.82 (q, J_{H-H} = 7.1 Hz, 1H, 3′-CH), 1.35 (d, J_{H-H} = 6.4 Hz, 3H, 6′-CH_3), 1.26 (d, J_{H-H} = 6.5 Hz, 3H, 3′-CH_3). IR (KBr) cm^{-1}: 1714 (COOH), 1615 (CO), 1331 (C–N), 1264 (C–F), 808, 764 (Ar–H). EI MS *m/z* (M^+): 437.60 (Calcd for $C_{23}H_{20}FN_3O_5$: 437.43).

5.2. In vitro antimicrobial activity

The antibacterial activity of the synthesised compounds was tested against *S. aureus*, *S. epidermidis*, *M. luteus*, *B. cereus*, *E. coli* and *K. pneumoniae* using nutrient agar medium (Hi-Media Laboratories, India). The antifungal activity of the compounds was tested against *C. albicans* and *A. niger* using sabouraud dextrose agar medium (Hi-Media Laboratories, India).

5.2.1. Paper disc diffusion method

The sterilized [15] (autoclaved at 120 °C for 30 min) medium (40–50 °C) was innoculated (1 mL/100 mL of medium) with the suspension (10^5 cfu mL^{-1}) of the microorganism (matched to McFarland barium sulphate standard) and poured into a petridish to give a depth of 3–4 mm. The paper impregnated with the test compounds (200 μg mL^{-1} in dimethyl formamide) was placed on the solidified medium. The plates were preincubated for 1 h at room temperature and incubated at 37 °C for 24 and 48 h for antibacterial and antifungal activities, respectively. Ciprofloxacin (100 μg/disc) and ketoconazole (100 μg/disc) was used as standard for antibacterial and antifungal activities, respectively. The observed zone of inhibition is presented in Table I.

5.2.2. Minimum inhibitory concentration (MIC)

MIC [16] of the test compounds were determined by agar streak dilution method. A stock solution of the synthesised compound (100 μg mL^{-1}) in dimethyl formamide was prepared and graded quantities of the test compounds were incorporated in specified quantity of molten sterile agar (nutrient agar for antibacterial activity and sabouraud dextrose agar medium for antifungal activity). A specified quantity of the medium (40–50 °C) containing the compound was poured into a petridish to give a depth of 3–4 mm and allowed to solidify. Suspension of the microorganism were prepared to contain approximately 10^5 cfu mL^{-1} and applied to plates with serially diluted compounds in dimethyl formamide to be tested and incubated at 37 °C for 24 and 48 h for bacteria and fungi, respectively. The MIC was considered to be the lowest concentration of the test substance exhibiting no visible growth of bacteria or fungi on the plate. The observed MIC is presented in Table I.

5.3. In vivo antibacterial activity

5.3.1. Animals

Inbred male swiss albino mice (20–25 g) were used for the in vivo antibacterial activity. They were kept in colony cages at 25±2 °C, relative humidity 45–55% under 12 h light and dark cycle. The animals were fed with standard animal feed and water ad libitum. All the animals were acclimatized for a week before use. The test compounds and the standard drugs were administered orally by gavage in the form of a suspension (1% carboxy methylcellulose as vehicle). Acute oral toxicity test was performed for all the synthesized compounds according to organization of economic co-operation and

Table I
Antimicrobial activity of the synthesized compounds

Compounds	In vitro activity—zone of inhibition (MIC)								In vivo activity (ED_{50})
	S. aureus	*S. epidermidis*	*M. luteus*	*B. cereus*	*E. coli*	*K. pneumoniae*	*C. albicans*	*A. niger*	*E. coli*
	ATCC 9144	ATCC 155	ATCC 4698	ATCC 11778	ATCC 25922	ATCC 11298	ATCC 2091	ATCC 9029	ATCC 25922
7	19(4.1)	20(3.1)	20(3.1)	22(2.4)	22(1)	24(1)	16(25)	13(>100)	50
8	18(5)	13(4.1)	15(5.3)	15(3.2)	17(3.1)	17(3)	13(50.1)	11(>100)	100
9	17(4.5)	18(4.2)	18(4.2)	21(3)	19(2.1)	20(2.6)	16(30.4)	12(>100)	85
10	10(25)	10(25.4)	10(20.2)	12(25)	14(10.2)	14(10.9)	11(50.2)	7(>100)	160
Ciprofloxacin (100 μg/disc)	24(0.2)	24(0.39)	23(0.1)	25(0.3)	25(0.2)	26(0.1)	–	–	–
Ketoconazole (100 μg/disc)	–	–	–	–	–	–	21(1)	19(6.1)	–

Zone of inhibition in mm, MIC in μg mL^{-1} and ED_{50} in mg kg^{-1}.

development (OECD) guidelines. All the animal experimentation was performed as per the recommendations and the protocols of the institutional animals ethics committee.

5.3.2. Acute oral toxicity

Acute oral toxicity [17] was performed as per OECD-423 guidelines (acute toxic class method). Swiss albino mice ($n = 3$) of either sex selected by random sampling technique was used for the study. The animals were kept fasting for 3–4 h providing only water ad libitum, after which the test compounds (suspended in olive oil) were administered orally at the dose level of 5 mg kg^{-1} by intragastric tube and observed for 3 days. If mortality was observed in two to three animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. In the present study, mortality was not observed and the procedure was repeated for further higher doses such as 50, 300 and 2000 mg kg^{-1}.

5.3.3. Mouse protection test

The in vivo antibacterial activity [7] of the compounds against *E. coli* was determined in male swiss albino mice ($n = 6$). The mice were infected intraperitoneally with a suspension (10^5 cfu mL^{-1}) containing an amount of *E. coli* greater than its LD_{100}. The mice were treated orally (p.o.) with a specified amount of the synthesised compound **1** and 4 h after infection. ED_{50} values were calculated by extrapolation among survival rate in each group after a week. The ED_{50} values represent the total dose of the compound (mg kg^{-1}) required to protect 50% of the mice from an experimentally induced lethal systemic infection of *E. coli*.

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