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Synthesis of Fluoroquinolones derivatives as Antimicrobial Agents

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ABSTRACT

Fluoroquinolones are well known to have an anti-infective action. In the present study we have described the synthesis of novel florouquinolones derivative as antimicrobial agent. The biological test highlighted a good inhibitory activity for the 7-Chloro-1-Alkyl-6-fluoro-8-nitro-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid derived synthons especially against pathogenic *Gram-negative* bacteria (*Pseudomonas aeruginosa*) and *Gram-positive* bacteria (*Staphylococcus aureus* and *Streptococcus agalactiae*). The binding interactions were monitored and could explain the good inhibitory activity of the synthesized derivatives of florouquinolones.

Keywords: Florouquinolones derivatives, Antibacterial activity, *Gram-negative* bacteria and *Gram-positive* bacteria.

INTRODUCTION

Anti-infective agents played a major role in saving human lives. Among these agents are the fluoroquinolones class, which had risen to be highly appreciated, especially if there was microbial resistance against penicillin and macrolide. Fluoroquinolone pharmacophore (Fig. 1) is well known to have antibacterial activity, and since 1980 there were many generation introduced to the market[1]. The fluoroquinolones were found to be effective to combat urinary tract infection[1] ideal in treating *Neisseria gonorrhea*[1] and highly effective to treat tuberculosis[2]. In addition, fluoroquinolone nucleus are presented widely in biologically active compounds such as PDE 4 inhibitors[3], PIM kinase inhibitors[4], GSK β inhibitors[5].

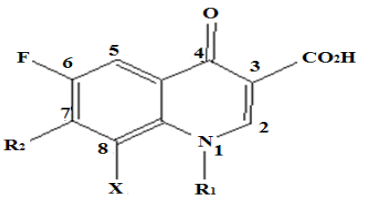


Fig. 1. Fluoroquinolone general formula

The encouraging properties of fluoroquinolones such as; broad spectrum of activity, good oral bioavailability, good tissue penetrability and low incidence of adverse effect[6] gave them high appreciation and encouraged researcher to

investigate their usefulness as a source of potent antibacterial drugs.

In our previous work, we have described various modifications to the main structure of fluoroquinolone, including the introduction of different substituent's at position 1 and 7 (Fig. 1) and in continuation for obtaining a new fluoroquinolone derivatives with excellent antibacterial activity, our team reported synthon C (1, 2, 3 and 4) as a potent antibacterial agents.

MATERIALS AND METHODS

Experimental

Molecular modelling

Computational software

The following software packages were utilized:

- CS ChemDraw Ultra 6.0, Cambridge Soft Corp. (http://www.cambridgesoft.Com), USA.2D Structure drawing was performed employing.
- Discovery Studio 4.5 (DS 4.5) Standalone Applications, including docking Biovia® (www.3ds.com), USA.
- Accelrys Enterprise Platform Server (AEP) (www.accelrys.com), USA.
- The crystal structures of gyrase enzyme were obtained from the protein data bank (http://www.rcsb.org/).

Molecular modelling studies

We docked our synthesized molecules using the Dock Ligands (LibDock) docking alogarithim implemented in the DS 4.5 into the binding pocket of the successful DNA gyraseenzyme namely : (PDB code: 5L3J, resolution 2.83 A).

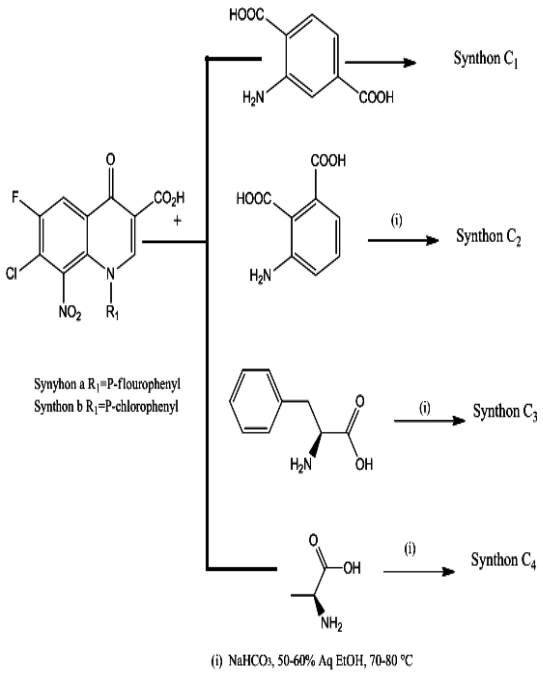
Chemistry

General

All chemicals, reagents and solvents were of analytical/synthetic grade that purchased from Sigma-Aldrich and Acros, Belgium, and used directly without further purification. Nuclear magnetic resonance spectra (NMR) were recorded on Bruker, Avance DPX-300 spectrometer. High-resolution mass spectra (HRMS) were measured in positive or negative ion mode using electrospray ion trap (ESI) technique by collision induced dissociation on a Bruker APEX-4 (7 Tesla) instrument. Melting points were determined in open capillaries on a Stuart scientific electro-thermal melting point apparatus, and are uncorrected. Infra-red (IR) spectra were recorded using Shimadzu 8400F FT-IR spectrophotometer (KBr discs). Microanalyses were performed using EuroVector Elemental Analyser, model (EA3000 A), Jordan University.

Synthesis of synthon (A)

The synthesisof 7-Chloro-1-Alkyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid was previously described by our group[4,7,8]. Then adding a substitution at position 7 of (a and b) synthons, was prepared according to reported method[4,8] provided the nitro derivatives synthon C (Scheme 1). The synthesized compounds gave satisfactory analytical and spectroscopic data in accordance with their depicted structures.



Scheme 1. Synthesis of flurouquinolone derivatives

2-[(3-carboxy-1-(4-fluorophenyl)-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinolin-7-yl)amino] terephthalic acid (C_1)

2-Aminoterphthalic acid (3.2 g, 18mmol) was reacted with synthon a (2.0 g, 5.26mmol) and dimethyl sulfoxide (DMSO) 40 ml and pyridine 10ml was heated at 70°C for 10 days under reflux conditions. The mixture was left to cool, then pH was adjusted by 3.5N HCl dried to give the title compound as dark brown solid; Yield ≈ 1.6 g (60%);

m.p. = 264°C; ^{1}H-NMR (300 MHz,DMSO-d_6): δ 7.00 (d, 2H, H-2'',, H-6''), 7.02 (d, 2H,H-3'',H-5''), 7.77-8.1 (m, 3H, ArH), 8.46 (d, 1H, H-5), 8.90 (s, 1H, H-2), 9.24 (br s, 1H, NH),13.50 – 15.40 (2 br s, $C_{(3)}$COOH and $C_{(2')}$COOH);IR (NaCl): ν 3417, 2067, 1701, 1643, 1265 cm^1; Anal.Calcd. for $C_{24}H_{13}F_2N_3O_9$ (525.06), C, 54.87; H, 2.49; F, 7.23; N, 8.0; Found: C, 54.77,H, 2.43; N, 7.03.

2-[(3-Carboxy-1-(4-fluorophenyl)-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinolin-7-yl)amino] phthalic acid (C_2)

A stirred mixture of 2-Aminophthalic acid (1.6 g, 9mmol), synthon a (1.0 g, 2.63 mmol) and DMSO 20 ml and pyridine 5 ml was heated at 70°C for 10 days under reflux conditions. The same procedure carried out as with synthon C_1 was done and yielded brownish solid compound ; Yield ≈ 0.8 g (60%); m.p.=260°C; ^{1}H-NMR (300MHz, DMSO-d_6): δ 7.04-7.11(m, 4H, H-2'', H-3'', H-5'', H-6''), 7.94-8.09 (m, 3H, ArH), 8.11 (d, 3JH-F = 9 Hz, 1H, H-5), 8.90 (m, 1H, H-2), 9.15 (br s, 1H, NH); IR (NaCl): ν 3417, 2067, 1701, 1643, 1265 cm^1; Anal. Calcd. for $C_{24}H_{13}F_2N_3O_9$ (525.06), C, 54.87; H, 2.49; F, 7.23; N, 8.0; Found: C, 54.77, H, 2.43; N, 7.03.

7-[(2-Carboxy-1-phenylethyl)amino]-1-(4-fluorophenyl)-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (C_3)

A stirred mixture of 3-phenyl β-alanine (4.06 g, 24mmol), synthon a(2.0 g, 5.2mmol) and sodium hydrogen carbonate (3.0 g, 36mmol) in 50% aqueous ethanol (280 mL) was heated at 70-80°C for 6 days under reflux condition. The mixture was worked up as described for synthon C_1. Yellow solid was collected; Yield 2.2 g (74.4%); m.p.: 280°C; ^{1}H-NMR (300MHz, DMSO-d_6): δ 2.76 (br m, 2H, CH_2-COOH, 4.31 (br s, 1H, CH-NH), 7.10-7.21 (4H, P-fluorophenyl), 7.46-7.55 (br m,5H, ArH), 7.86 (d, 3JH-F = 14.1 Hz, 1H, H-5), 8.36 (s, 1H, H-2), 15.70(br s, 2H, 2 COOH); IR (NaCl): υ 3417, 2098, 1643, 1481, 1411, 1319, 1188, 1010 cm^{-1}; Anal. Calcd. for $C_{25}H_{17}F_2N_3O_7$ (509.1): C, 58.94; H, 3.36; N, 8.25. Found: C, 58.94; H, 3.36; N, 8.25.1.H, 3.36; N, 8.25.

7-(2-Carboxy-ethylamino)-1-(4-chloro-phenyl)-6-fluoro-8-nitro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid.(C_4)

A mixture of β-alanine (1.05 g, 11.5mmol), Synthon b (1.0 g, 2.2mmol) and sodium hydrogen carbonate (3 g, 35.8mmol) in 50% aqueous ethanol (120 mL) was heated for 6 days under reflux conditions. The product wasworked up as described for synthon C_1 and yielded a yellowish color. ^{1}HNMR (300 MHz, DMSO-d_6): δ 2.28 (d, J = 8.1, 2H,CH_2-COOH), 3.66 (m, 2H, CH_2-NH), 7.51 (br t, J = 5.7 Hz, 1H, $NHCH_2$),7.51–7.54 (m, 2H, H-3', H-5'), 7.61–7.67 (m, 2H, H-2', H-6'),8.2 (H-5), 8.62 (s, 1H, H-2); HRMS (ESI,_ve): m/z [M_H]_448.04 $C_{19}H_{12}ClFN_3O_7$ requires 448.0353.

Biological Evaluation

Test microorganisms

Six pathogenic bacterial strains were used in the antimicrobial assays, four *Gram-positive* (*Staphylococcus aureus* ATCC29213, *Staphylococcus saprophyticus* ATCCRBAA 750, *Streptococcus agalactiae* ATCC13813,*Streptococcus pyogenes* ATCC19615, and two *Gram-negative* (*Pseudomonas aeruginosa* ATCC27853, *Escherichia coli* ATCC11775). Those pathogens were chosen based on their clinical and pharmacological importance. Antibacterial activities were evaluated by the agar well diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI)[9-11] and the European Committee on Antimicrobial Susceptibility Testing (EUCAST)[12-13].

Measurement of antibacterial activity of the synthetic compounds

Preparation of synthetic compounds for microbiological assay

Stock solutions of 20 and 30 mg of each synthetic compound dissolved in 1 mL of dimethyl sulfoxide (DMSO), as solvent. They were sterilized by filtration, and stored at 4°C. The antimicrobial activity of the synthesized compounds was evaluated by the agar well diffusion method[14].

Determination of antibacterial activity by agar well diffusion method

All the synthetic compounds of different concentrations were screened for their antibacterial activities against the *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Streptococcus agalactiae*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Escherichia coli* by agar well diffusion assay. Isolated pure colonies from fresh grown bacteria were transferred from the plates into sterile normal saline solution and vortexed to form bacterial homogenous suspensions. The turbidity

was then adjusted to 0.5 McFarland standard units, and a volume of the inoculum was spread on the entire surface of agar. Then, a hole with a diameter of 6-8 mm was punched aseptically using a sterile cork borer, and a volume (20-100 µl) of the synthetic compound was introduced at the desired concentration into the well. Control experiments were carried out under similar conditions using amoxicillin (20 mg), ciprofloxacin (5 mg) and gentamicin (10 mg), as positive controls, and sterile distilled water as negative control. The zones of growth inhibition were measured in millimeters (mm) after 18-24 h of incubation at 37°C. The sensitivities of the microorganisms to the synthetic compounds were determined by measuring the sizes of inhibitory zones, and values <8 mm were considered as being not active against the tested bacterial strains[15].

RESULTS AND DISCUSSION

The synthesized derivatives have been obtained in a good yield, and showed good antibacterial activity against both *Gram-positive* and *Gram-negative* bacteria (Table 1), and it's well known that position 7 modifications can bring about the major changes in potency. Attachment of aromatic rings having an amino substitution results in improved activity and it also affects the pharmacokinetics of the compound[16].

The lipophilic substitution at position 1 led to synthons with a good activity against *Gram-positive* bacteria (Fig. 2), and position 7 substitution with 2-aminophthalic acid and 3-aminoterphthalic acid imparted both *Gram-negative* and *Gram-positive* activity(Figure 2).

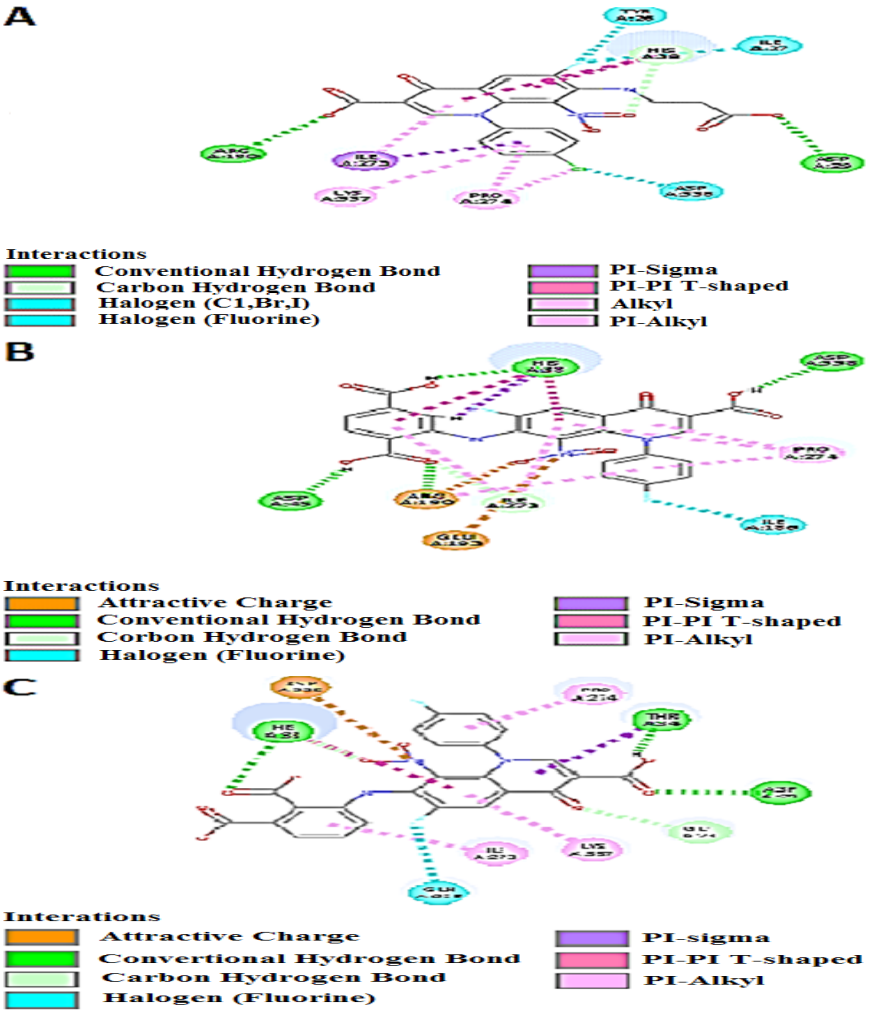


Fig. 2. A. Diagram that shows the main interactions of compound C_1 inside the bindingpocket of gyrase enzyme (PDB code: 5L3J), B. Diagram that shows the main interactions of compound C_2 inside the binding pocket of gyrase enzyme (PDB code: 5L3J), C. Diagram that shows the main interactions of compound C_3 inside the binding pocket of gyrase enzyme (PDB code: 5L3J)

Table 1: The antibacterial activity of prepared synthetic compounds and standard drugs against bacterial testing strains

		Zone of inhibition (mm)					
Compound	Concentration (mg/ml)	*E. coli*	*P. aeruginosa*	*Staph. aureus*	*Staph. saprophyticus*	*Strep. agalactiae*	*Strep. pyogenes*
C_1	20	17	22	23	12	15	23
	30	20	17	20	17	23	26
C_2	20	18	-	23	16	14	25
	30	21	-	20	17	22	21
C_3	20	20	20	19	18	20	23
	30	20	20	18	12	17	20
C_4	20	22	22	25	-	-	-
	30	-	-	13	10	19	20
Amoxacillin (20 mg) (+ve)		31	20	20	25	19	30
Ciprofloxacin (5 mg) (+ve)		20	16	20	26	25	22
Sterile D.W. (-ve)		-	-	-	-	-	-

E.: *Escherichia*, *Staph. Staphylo coccus*, P.: *Pseudomonas, Strep.*: *Streptococcus*, (-):no inhibition, (+ve): positive control, (-ve): negative control, D. W.: distilled water.

CONCLUSION

In summary, we have efficiently synthesized a novel series of fluoroquinolone-modified analogues. Biological testing showed that some of the derivatives have good antibacterial activity in a series of primary assays.

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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