# Design, Synthesis and Docking Studies of Some Novel Fluoroquinolone Compounds with Antibacterial Activity

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*A new series of fluoroquinolone compounds have been obtained by Gould-Jacobs method. The compounds have been characterized by physic-chemical methods (elemental analysis, FTIR, NMR, UV-Vis) and by antimicrobial activity against Gram-positive and Gram-negative microorganisms. For the synthesized compounds have been performed calculations of characteristics and molecular properties, using Spartan'14 Software from Wavefunction, Inc. Irvine, CA. and molecular docking studies using CLC Drug Discovery Workbench 2.4 software, to identify and visualize the most likely interaction ligand (fluoroquinolone) with the receptor protein.*

*Keywords: quinolones, fluoroquinolones, antimicrobial activity, molecular docking*

Infectious diseases are the second leading cause of death worldwide [1] Treatment of infectious diseases becomes more difficult when common pathogens such as Staphylococcus aureus, Pseudomonas aeruginosa, and Mycobacterium tuberculosis develops resistance to drug that were effective at one time. Antibiotics are a special class of therapeutic agents whose misuse affects not only the individual patient but also the entire community. This is due to the fact that, at some point after the widespread introduction and use of new antibiotics, appear almost inevitable antibiotic-resistant bacteria that occur in significant waves (both in veterinary and human populations). The apparition of antibiotic resistance is inevitable [2, 3]. The question it is not if it develop resistance to a new antibiotic, the question it is when will develop the resistance to this new antibiotic. A survey conducted in the 2011, against on 21 antibiotics released starting from 2000, highlighted that the two directions of discovery and development of new antibiotics, natural products and products obtained through chemical synthesis, are still valid. From class of natural products were highlighted two drug: daptomycin (lipopeptide) and retapamulin (Terpenoid pleuromutilin). Out of the 9 antibiotics obtained by chemical synthesis, released in the past 12 years, one belongs to the oxazolidinone class (linezolid) and the other 8 antibiotics belong to the class of fluoroquinolones.

**Experimental part**

Melting points were determined in opened capillary on Melting point apparatus OptiMelt and are uncorrected. Progress of the reaction was followed by TLG on Merck silica gel $60F_{254}$ plates eluted with the solvent system: tetrahydrofuran:dioxan: ammoniac (60:20:30) (v:v:v).$^1$H- and $^{13}$C-NMR spectra were recorded in $CDCl_3$, DMSO-$d_6$ and trifluoroacetic acid, on two instruments Varian, Varian Gemini 300 BB (operating at 300 MHz for proton and 75 MHz for carbon) and UNITY 400 Plus(operating at 400 MHz for proton and 100 MHz for carbon).Tetramerthylsilane as internal standard was the reference for the chemical shifts. All chemical shifts are given in the delta scale (ppm vs internal TMS). FT IR was recorded on an instrument Bruker Vertex 70 with diamond optic. UV-Vis was recorded on an instrument UV -Vis LAMBDA 12. Elemental analysis was performed on a Perkin Elmer CHNS/O Analyzer 2400 Series II.

Synthesis of 1-ethyl-6-fluoro-7-(piperidin-1-yl)-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid.(FPQ32) *[7].* A mixture of 1-ethyl-6-fluoro-7-chloro-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid (4) [18] (scheme 1) (2.69 g, 0.01 mol), piperidine (4.25 g, 0.05 mol) and DMF (30 mL) was stirred 5 hours at 110-120$^0$C. After 5 h was added $H_2O$ (30 mL) and acetic acid (*p*H=7) and the resulting precipitate was filtered off. The crude product was recrystallized from DMF to yield FPQ32 (mp 202.4-204.4$^0$C; yield 60%) $^1$H-NMR(dmso-d6, δ ppm, J Hz): 8.91(s, 1H, H-2); 7.83(d, 1H, H-5, $^3$J(F-H)=13.5 Hz); 7.12(d, 1H, H-8, $^4$J(F-H)=7.0 Hz); 4.57(q, 2H, H-17, 7.1); 3.28(t, 4H, 2H-20, 2H-24, 4.5); 1.71(bs, 4H, 2H-21, 2H-23); 1.63(bs, 2H, H-22); 1.42(t, 3H, H-18, 7.1).$^{13}$C-NMR(dmso-d6, δ ppm): 176.01(C-4); 166.01(C-19); 152.84(q, C-6, J(F-C$^6$)=248.2 Hz); 148.23(C-2); 146.12(d, C-7, J(F-C$^7$)=9.5 Hz); 137.17(C-9); 118.75(d, C-10, J(F-C$^7$)=8.1 Hz); 110.93(d, C-5, J(F-C$^5$)=22.7 Hz); 106.90(C-3); 105.49(C-8); 50.63(d, C-20, C-24, $^4$J(F-C$^{20}$)= $^4$J (F-C$^{24}$)=4.4 Hz); 48.91(C-17); 25.27(C-21, C-23); 23.54(C-22); 14.21(C-18).FT-IR(solid in ATR, ν cm$^{-1}$): 3050w; 3001w; 2942m; 2923sh; 2852w; 1730s; 1614s; 1550w; 1520m; 1474vs; 1442vs; 1376m; 1352m; 1299m; 1264s; 1246vs; 1218m; 1198m; 1151w; 1134w; 1114m; 1110m; 1090m; 1069w; 1040w; 974w; 950m; 921w; 887w; 863m; 835w; 823w; 806m; 750m; 712w; 698w; 665w; 636w.

Synthesis of 1-ethyl-6-fluoro-7-(piperidin-1-yl)-8-chloro-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid (FPQ-33) To a solution of FPQ-32(3.18 g; 0.01 mol) in $CHCl_3$ (50 mL) was added 2.56 mL $SO_2Cl_2$, and the mixture was stirred at room temperature. After 30 min the mixture was washed with water. The $CHCl_3$ layer was dried over $Na_2SO_4$, and evaporated to dryness. The crude quinolone was recrystallized from DMF to yield FPQ-33 (mp 187-190$^0$C, yield 60%).$^1$H-NMR(dmso-d6, δ ppm, J Hz): 8.89(s, 1H, H-2); 7.97(d, 1H, H-5, $^3$J(F-H$^5$)=12.0 Hz); 4.84(q, 2H, H-17, 7.1); 3.26(m, 4H, H-20, H-24); 1.66(m, 6H, H-21, H-22, H-23); 1.40(t, 3H, H-18, 7.1).$^{13}$C-NMR(dmso-d6, δ ppm): 175.91(C-4); 165.20(C-19); 155.90(d, C-6, J(F-C$^6$)=250.0 Hz); 152.41(C-2); 144.55(d, C-7, $^2$J(F-C$^7$)=14.3 Hz); 136.42(C-9); 123.09(d, C-10, $^3$J(F-C$^{10}$)=7.7 Hz); 118.83(C-8);110.79(d, C-5, $^2$J(F-C$^5$)=23.0 Hz); 107.73(C-3); 53.04(C-17); 52.10(d, C-20, C-24, $^4$J(F-C$^{20, 24}$)=5.1 Hz); 25.98(C-21, C-23); 23.50(C-22); 15.63(C-18).FT-IR(solid in ATR, ν cm$^{-1}$): 3057m; 2929s; 2846m; 1720vs; 1615vs; 1557sm;

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1491s; 1437vs; 1378m; 1347m; 1248m; 1214m; 1150w; 1090m; 1034w; 926m; 885w; 806m; 782w; 736w; 647w.Elemental Analyses: Calculated for: $C_{17}H_{18}ClFN_2O_3$: C, 57.88%; H, 5.14%; N, 7.94%. Found: C, 57.18%; H, 5.49%; N, 7.79%.

*Synthesis of 1-ethyl-6-fluoro-7-(4-acetyl-3-methyl-piperazin-1-yl)-1,4-dihydro-4-oxo–quinoline-3-carboxylic acid (AcFPQ27)* starting from 1-ethyl-6-chloro-7-(3-methyl-piperazin-1-yl)-1,4-dihydro -4-oxo-quinoline-3-carboxylic acid (FPQ27)[19] Acetyl chloride (30 mL) was added to a solution of FPQ 27 (3.31 g; 0.01 mol) in acetic acid(30 mL) and then the mixture was stirred 4 h at reflux temperature. At the end of the reaction, the mixture was cooled and was poured onto 200 mL of water. The precipitate formed was filtered off, washed with water, and after drying, was recrystallized from DMF to yield AcFPQ 27. (mp 247,5-248,9$^0$C, yield 54%.). $^1$H-NMR(dmso-d6, δ ppm, J Hz): 8.93(s, 1H, H-2); 7.89(d, 1H, H-5, $^3$J(F-H$^5$)=13.3 Hz); 7.17(d, 1H, H-8, $^4$J(F-H$^5$)= 3.0 Hz); 4.57(q, 2H, H-17, 7.1); 4.31(m, 1H, H-22); 3.63÷3.86(m, 6H, H-piperazine); 2.50(s, 3H, H-26); 2.07(d, 3H, H-24, 6.5); 1.42(t, 3H, H-18, 7.1). $^{13}$C-NMR(dmso-d6, δ ppm): 176.08(C-4); 168.32(C-25); 166.03(C-19); 152.73(d, C-6, J(F-C$^6$)=247.5 Hz); 148.41(C-2); 145.76(d, C-7, $^2$J(F-C$^7$)=9.9 Hz); 137.15(C-9); 121.47(d, C-10, $^3$J(F-C$^{10}$)=7.7 Hz); 111.13(d, C-5, $^2$J(F-C$^5$)=22.7 Hz); 107.07(C-8); 105.92(C-3); 49.02(C-piperazine); 42.31(C-piperazine); 52.99(C-17); 21.49(C-26); 15.21(C-24); 14.30(C-18). FT-IR(solid in ATR, ν cm$^{-1}$): 3030w; 2974m; 2835w; 1720s; 1627vs; 1520m; 1472vs; 1426vs; 1377m; 1348m; 1322s; 1281m; 1250m; 1125w; 1082m; 1040w; 994w; 967m; 807m; 754m; 595m. Elemental Analyses: Calculated for: $C_{19}H_{22}ClFN_3O_4$: C, 60.79%; H, 5.91%; N, 11.19%. Found: C, 60.31%; H, 6.20%; N, 11.08%.

*Synthesis of 1-ethyl-6,8-dichloro-7(4-acetyl-3-methyl-piperazin-1-yl)-1,4-dihydro-4-oxo-quinoline -3-carboxylic acid (AcFPQ29)* 2.56 mL $SO_2Cl_2$ was added to a solution of AcFPQ27 (3.75g; 0.01 mol) in DClE (150 mL), and the mixture was stirred at 40-50$^0$C. After 2 h the mixture was washed with water. The organic layer was dried over $Na_2SO_4$, and evaporated to dryness. The crude quinolone was recrystallized from DMF to yield AcFPQ29 (mp 262.7-264.8 $^0$C, yield 52%). $^1$H-NMR($CDCl_3$+tfa, δ ppm, J Hz): 8.97(s, 1H, H-2); 8.01(d, 1H, H-5, $^3$J(F-H$^5$)=11.8 Hz); 4.57(q, 2H, H-17, 7.1);3.80÷3.05(m, 7H, H-piperazine); 2.36(m, 1H, H-23); 2.21(s, 3H, H-27); 1.46(t, 3H, H-18, 7.1); 1.41(d, 3H, H-25, 6.4).$^{13}$C-NMR($CDCl_3$+tfa, δ ppm): 176.68(C-4); 170.55(C-4); 166.37(C-19); 153.82(d, C-6, J(F-C$^6$)=256.4 Hz); 151.78(C-2); 143.68(d, C-7, $^2$J(F-C$^7$)=14.6 Hz); 136.27(C-9); 125.45(C-8); 112.19(d, C-5, $^2$J(F-C$^5$)=23.3 Hz); 109.15(C-10); 108.43(C-3); 55.16(C-21); 53.78(C-20); 50.46(C-23); 45.41(C-24); 50.89(C-17); 20.49(C-27); 16.08(C-25); 15.29(C-18).FT-IR(solid in ATR, ν cm$^{-1}$): 3026w; 2990w; 2861w; 1724s; 1633vs; 1616vs; 1555m; 1529w; 1489m; 1427vs; 1387s; 1369s; 1336s; 1291m; 1252m; 1232s; 1212s; 1167m; 1119w; 1099m; 1042m; 1005m; 985m; 918m; 899w; 886m; 803m; 740w. Elemental Analyses: Calculated for: $C_{20}H_{22}ClFN_3O_4$: C, 56.81%; H, 5.24%; N, 9.94%. Found: C,56.90%; H, 5.18%; N, 10.04%.

*Synthesis of 1-ethyl-6-fluoro-7-(3-methyl-piperazin-1-yl)-8-chloro-1,4-dihydro-4-oxo–quinoline-3- carboxylic acid. HCl (FPQ29)*[14] A solution of AcFPQ29 (1.2 g, 0.003 mol) with HCl conc. (30 mL) was heated under reflux temperature, 6 hours and then was evaporated to dryness. The crude compound was recrystallized from DMF to yield FPQ29.HCl (mp 280-283$^0$C; yield 40%). $^1$H-NMR(dmso-d6, δ ppm, J Hz): 8.52(s, 1H, H-2); 7.90(d, 1H, H-5, $^3$J(F-H$^5$)=12.3 Hz); 4.57(q, 2H, H-17, 7.1); 4.24(m, 1H, H-22); 3.60÷3.00(m, 6H, H-piperazine); 2.07(d, 3H, H-24, 6.5); 1.42(t, 3H, H-18, 7.1).$^{13}$C-NMR(dmso-d6, δ ppm): 175.87(C-4); 168.14(C-19); 156.25(d, C-6, J(F-C$^6$)=252.2 Hz); 152.19(C-2); 143.25(d, C-7, $^2$J(F-C$^7$)=14.6 Hz); 136.37(C-9); 123.63(d, C-10, $^3$J(F-C$^{10}$)=5.6 Hz); 120.34(C-3); 110.85(d, C-5, $^2$J(F-C$^5$)=24.5 Hz); 106.56(C-8); 54.47(C-21); 53.92(C-17); 52.04(C-22); 47.17(C-piperazine); 43.89(C-piperazine); 15.70(C-24); 15.19(C-18).FT-IR(solid in ATR, ν cm$^{-1}$): 3058w; 2935w; 2891w; 2752m; 2770s; 2458s; 1723s; 1612s; 1556w; 1508s; 1494s; 1441vs; 1400m; 1376m; 1306m; 1253m; 1200w; 1088m; 1038w; 976w; 931m; 887m; 808m; 735w.Elemental Analyses: Calculated for: $C_{17}H_{20}Cl_2FN_2O_3$: C, 50.51%; H, 4.99%; N, 10.39%. Found: C,50.71%; H, 5.18%; N, 10.21%.

*Synthesis of 1-ethyl-6-fluoro-7-(4-methyl-piperazin-1-yl)-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid(Pefloxacine)(PF)* [7] A mixture of 1-ethyl-6-fluoro-7-chloro-1,4-dihydro-4-oxo-quinoline -3-carboxylic acid (4) [18] (2.7 g, 0.01 mol) and 4-methyl-piperazine (5.00 g, 0.05 mol) and pyridine (30 mL) was stirred at reflux for 8 h. The mixture was concentrated to give crude quinolone. The crude product was dissolved in acetic acid 10 % and then was precipitated to *p*H 7.2 with sodium hydroxide 2N. The resulting precipitate was filtered off. And then was recrystallized from DMF to yield PF (pefloxacine) (mp 269.2-271.8$^0$C; yield 66%). $^1$H-NMR(dmso-d6, δ ppm, J Hz, T=333K): 15.20(bs, 1H, H-9, deuterable); 8.91(s, 1H, H-2); 7.91(d, 1H, H-5, $^3$J(F-H$^5$)=13.4 Hz); 7.16(d, H-8, $^4$J(F-H$^8$)=7.3 Hz); 4.57(q, 2H, H-10, 7.1); 3.34(m, 2H, H-12, H-15, syst. $A_2B_2$); 3.18(s, 3H, H-16); 2.52(m, 2H, H-13, H-14, syst. $A_2B_2$); 1.43(t, 3H, H-11, 7.1). FT-IR(solid in ATR, ν cm$^{-1}$): 3057w; 3011w; 2966w; 2915m; 2934m; 2884m; 2844m; 2805w; 1732s; 1614s; 1520m; 1473vs; 1440vs; 1401m; 1372s; 1291s; 1249s; 1199s; 1137s; 1103m; 1079m; 1056w; 1040m; 1007m; 951m; 928m; 890m; 853w; 832m; 804w; 749m; 705w.

*Synthesis of 1-ethyl-6-fluoro-7-(4-methyl-piperazin-1-yl)-8-chloro-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid (FPQ 51)* 2.56 mL $SO_2Cl_2$ was added to a solution of PF (3.33g; 0.01 mol) in chloroform (150 mL), and the mixture was stirred at 40-50$^0$C. After 2 h the mixture was washed with water. The organic layer was dried over $Na_2SO_4$, and evaporated to dryness. The crude quinolone was recrystallized from DMF to yield FPQ51, mp 219.6-221.5$^0$C, yield 58%). $^1$H-NMR(dmso-d6, δ ppm, J Hz, T=333K): 8.90(s, 1H, H-2); 8.00(d, 1H, H-5, $^3$J(F-H$^5$)=12.1 Hz); 4.83(q, 2H, H-10, 7.1); 3.32(m, 2H, H-16, H-12); 2.53(m, 4H, H-13, H-15); 2.26(s, 3H, H-14'); 1.41(t, 3H, H-11, 7.1). $^{13}$C-NMR(dmso-d6, δ ppm): 175.88(d, C-4, $^4$J(F-C$^4$)=2.4 Hz); 165.06(C-9); 155.80(d, C-6, J(F-C$^6$)=251.3 Hz); 152.42(C-2); 143.70(d, C-7, $^2$J(F-C$^7$)=15.2 Hz); 136.48(C-1a); 123.53(d, C-4a, $^3$J(F-C$^{4a}$)=7.1 Hz); 118.93(C-8); 110.79(d, C-5, $^2$J(F-C$^5$)=23.7 Hz); 107.72(C-3); 54.90(C-14'); 52.96(C-10); 50.49(d, C-16, C-12 $^4$J(F-C$^{16}$)=4.9 Hz); 45.80(C-13, C-15); 15.54(C-11). FT-IR(solid in ATR, ν cm$^{-1}$): 3055m; 2968w; 2931m; 2860w; 2843m; 2799m; 2758m; 1716vs; 1617s; 1557s; 1531m; 1489s; 1439vs; 1371m; 1353m; 1286m; 1251m; 1210m; 1150m; 1134m; 1090m; 1075m; 1039m; 1006m; 978w; 944w; 929m; 915m; 887m; 837w; 805m; 738m. Elemental Analyses: Calculated for: $C_{17}H_{19}ClFN_3O_3$: C, 55.51%; H, 5.21%; N, 11.42%. Found: C, 55.49%; H, 5.18%; N, 11.50%.

*Biological Assays:* The quinolone derivatives were evaluated for *in vitro* activity by determining minimum inhibitory concentration against a variety of bacteria: E. coli ATCC8739, S. aureus ATCC6538 and *P. aeruginosa* ATCC 9027, by agar dilution method [21].

*Molecular mechanics calculations:* Molecular, topological, conformational characteristics on 3D quinolones optimized structure were calculated using Spartan 14 Software

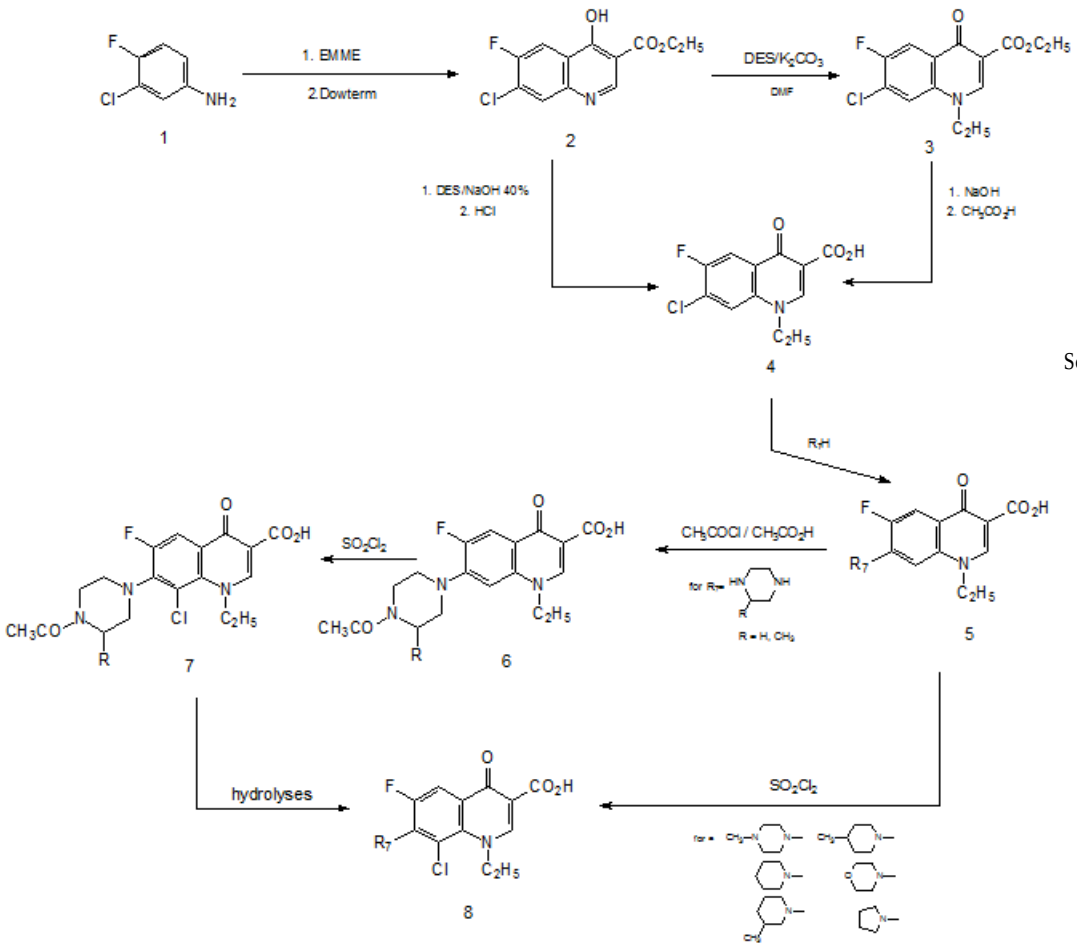
*Docking studies:* Molecular docking approach**,** using CLC Drug Discovery Workbench Software was conducted in order to achieve accurate predictions on optimized conformation for both, the quinolone (as ligand) and their target receptor protein to form a stable complex.

## Results and discussions

The synthesis of the novel quinolones (table 1) followed a Gould-Jacobs cyclization process [17-20] (scheme 1). Appropriate unsubstituted aniline (1) is reacted with diethylethoxymethylenemalonate (EMME) to produce the resultant anilinomethylenemalonate. A subsequent thermal process induces Gould-Jacobs cyclization to afford the corresponding 4-hidroxy-quinoline-3-carboxylate ester (2). The following operation is the alkylation of the quinolone which is usually accomplished by reaction with a suitable alkyl halide or dialkyl sulphates to produce the qinolone-3-carboxylate ester (3). The final manipulation is acid or basic hydrolysis to cleave the ester generating the biologically active free carboxylic acid (4).The biologically active free carboxylic acid (4) was also obtained from the corresponding 4-hidroxy-quinoline-3-carboxylate ester (2) by alkylation with dialkyl sulphates in presence of alkali.. The displacement of 7-chloro group with a heterocyclic, yielded compounds (5). 8-Chloro-quinoline-3-carboxylic acid (8) was synthesized from 8-unsubstituted quinoline-3-carboxylic acid (5) by chlorination with sulfuryl chloride (when $R_7$=4-methyl-piperazine). When, $R_7$ = 3-methyl-piperazine, or piperazine, is necessary to protect the nitrogen atom from piperazine group. After chlorination and hydrolysis is obtained the final compound (8) ($R_7$ = 3-methyl-piperazine, or piperazine).

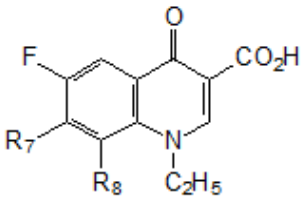
The structure of fluoroquinolone derivatives has been confirmed by physico-chemical techniques: elemental analysis, $^1$H-NMR, $^{13}$C-NMR, FT IR and UV-Vis. The introduction of chlorine atom in 8 position of the quinolone compound was proved by $^1$H-NMR spectra, by the disappearance of the proton characteristic signal of the proton from 8 position of unsubstituted compounds ($R_8$=H): δ ppm =7.12 (NF) [20], 7.16 (PF), 7.1(FPQ27) [19], 7.12 (FPQ 32), 7.13 (Q 83) [17], 7.1 (FPQ 35 [20], 7.16 (FPQ 25) [18] and by UV-Vis spectra (table 3). UV absorption spectra study was carried out using solutions with concentration of 10μg/mL in chloroform, and for FPQ 27 and FPQ 29 compounds in DMSO. Interpretation of the UV absorption spectrum of the 8 chloroquinolone compounds: FPQ 50, FPQ 51, FPQ 29, Q 85, FPQ 33, FPQ 30, FPQ 28, FPQ 36, has been made in comparison with that of the 8-unsubstitued-quinolone compounds: NF, PF, FPQ 27, Q 83, FPQ 32, FPQ 24, FPQ 25, FPQ 35 respectively, shows that: the presence of the quinolone nucleus determines in chloroform the appearance of electronic transitions in the field of 260-300 nm and 310-360 nm and the introduction of chlorine atom in 8 position produces a batocroma displacement of the entire spectrum.

The quinolone compounds were evaluated for *in vitro* activity by determining minimum inhibitory concentration against Escherichia Coli ATCC 8739, Staphylococcus Aureus ATCC 6538 and Pseudomonas aeruginosa ATCC 9027, by agar dilution method [21] (table 3). After analyzing chemical structure-biological activity relationships, it was observed that the presence of chlorine in 8 position of the quinolones compounds, leads to increased the antimicrobial activity for the compounds having piperidinyl, morpholinyl and pyrrolidinyl moiety in 7-position For 7-piperazinyl quinolones, the chlorine atom from 8-position leads to decreased the activity against all the tested strains.




Scheme 1. Preparation of fluoroquionolone compounds

**Table 1**
THE STRUCTURE OF THE FLUOROQUINOLONE COMPOUNDS




| Compound | $R_7$ | $R_8$ |
|---|---|---|
| **NF:**1-Ethyl-6-fluoro-7-(piperazin-1-yl)-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid[20] | piperazinyl | H |
| **FPQ50:**1-Ethyl-6-fluoro-7-(piperazin-1-yl)-8-chloro-1,4-dihydro-4-oxo -quinoline-3-carboxylic acid[20] | piperazinyl | Cl |
| **PF:**1-Ethyl-6-fluoro-7-(4-methyl-piperazin-1-yl)-1,4-dihydro-4-oxo -quinoline-3-carboxylic acid | 4-methyl- piperazinyl | H |
| **FPQ51:**1-Ethyl-6-fluoro-7-(4-methyl-piperazin-1-yl)-8-chloro-1,4-dihydro -4-oxo-quinoline-3-carboxylic acid | 4-methyl- piperazinyl | Cl |
| **FPQ27:**1-Ethyl-6-fluoro-7-(3-methyl-piperazin-1-yl)-1,4-dihydro-4-oxo -quinoline-3-carboxylic acid[19] | 3-methyl- piperazinyl | H |
| **FPQ29.HCl:**1-Ethyl-6-fluoro-7-(3-methyl-piperazin-1-yl)-8-chloro-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid.HCl | 3-methyl- piperazinyl | Cl |
| **FPQ35:**1-Ethyl-6-fluoro-7-(pyrrolidin-1-yl)-1,4-dihydro-4-oxo-quinoline -3-carboxylic acid[20] | pyrrolidinyl | H |
| **FPQ36:]:**1-Ethyl-6-fluoro-7-(pyrrolidin-1-yl)-8-chloro-1,4-dihydro-4-oxo -quinoline-3-carboxylic acid[20] | pyrrolidinyl | Cl |
| **FPQ32:**1-Ethyl-6-fluoro-7-(piperidin-1-yl)-1,4-dihydro-4-oxo-quinoline -3-carboxylic acid | piperidinyl | H |
| **FPQ33::**1-Ethyl-6-fluoro-7-(piperidin-1-yl)-8-chloro-1,4-dihydro-4-oxo -quinoline-3-carboxylic acid | piperidinyl | Cl |
| **Q83:**1-Ethyl-6-fluoro-7-(4-methyl-piperidin-1-yl)-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid[17] | 4-methyl-piperidinyl | H |
| **Q85:**1-Ethyl-6-fluoro-7-(4-methyl-piperidin-1-yl)-8-chloro-1,4-dihydro -4-oxo-quinoline-3-carboxylic acid[17] | 4-methyl-piperidinyl | Cl |
| **FPQ24:**1-Ethyl-6-fluoro-7-(3-methyl-piperidin-1-yl)-1,4-dihydro-4-oxo -quinoline-3-carboxylic acid[18] | 3-methyl-piperidinyl | H |
| **FPQ30:**1-Ethyl-6-fluoro-7-(3-methyl-piperidin-1-yl)-8-chloro-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid[19] | 3-methyl-piperidinyl | Cl |
| **FPQ25:**1-Ethyl-6-fluoro-7-(morpholin-1-yl)-8-chloro-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid[18] | morfolinyl | H |
| **FPQ28:**1-Ethyl-6-fluoro-7-(morpholin-1-yl)-8-chloro-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid[18, 19] | morfolinyl | Cl |

**Table2**
CHARACTERISTIC ABSORPTION BAND OF THE FLUOROQUINOLONE COMPOUNDS IN CHLOROFORM (*DMSO)

| Characteristic bands [nm] | Absorbance | Characteristic bands [nm] | Absorbance | Δλ [nm] |
|---|---|---|---|---|
| NF | | FPQ50 | | |
| 284.83<br>321.02 | 1.2290<br>0.35548 | 300.69<br>342.58 | 0.78017<br>0.32716 | 15.86<br>21.56 |
| PF | | FPQ 51 | | |
| 284.89<br>321.19 | 1.3646<br>0.38586 | 299.11<br>343.06 | 0.83119<br>0.33143 | 14.20<br>21.87 |
| FPQ 27* | | FPQ 29* | | |
| 287.62 | 0.15411 | 296.07 | 0.40968 | 8.45 |
| FPQ 35 | | FPQ 36 | | |
| 290.89<br>314.88<br>353.08 | 2.1271<br>0.34365<br>0.41815 | 301.59<br>342.35<br>364.89 | 0.75654<br>0.18883<br>0.180004 | 10.70<br>27.47<br>11.81 |
| FPQ 32 | | FPQ 33 | | |
| 288.85<br>286.65<br>315.65 | 1.4631<br>1.4715<br>0.39844 | 292.08<br>299.03<br>341.94 | 0.8547<br>0.83413<br>0.34626 | 3.92<br>12.38<br>26.29 |
| Q 83 | | Q 85 | | |
| 287.65<br>321.03 | 1.4249<br>0.38039 | 301.95<br>342.29 | 0.81140<br>.29187 | 14.30<br>21.26 |
| FPQ 24 | | FPQ 30 | | |
| 287.32<br>320.51 | 0.89341<br>0.24348 | 302.26<br>342.53 | 0.90543<br>0.32868 | 14.94<br>22.02 |
| FPQ 25 | | FPQ 28 | | |
| 283.74<br>321.02<br>334.33 | 1.3746<br>0.40963<br>0.40991 | 2989.98<br>334.89<br>343.95 | 0.78409<br>0.32626<br>0,34652 | 15.24<br>13.87<br>9.65 |

| Compound | Minimum inhibitory concentration (MIC) ( μg/ml) | | |
|---|---|---|---|
| | *Escherichia coli* ATCC 8739 | *Staphylococcus aureus* ATCC 6538 | *Pseudomonas aeruginosa* ATCC 9027 |
| NF | < 0.08 | 0.32 | 0.32 |
| FPQ 50 | 2.00 | 4.00 | 16 |
| PF | < 0.08 | 1,28 | 1,28 |
| FPQ 51 | 2.00 | 4,00 | 32 |
| FPQ27 | 2.00 | 4.00 | 1.00 |
| FPQ 29 | 0.30 | 1.21 | 4.83 |
| FPQ 35 | 31.25 | 2.00 | 128 |
| FPQ 36 | 16 | 0.25 | 16 |
| FPQ 32 | 16.00 | <0.125 | 8,00 |
| FPQ 33 | 32.00 | <0,125 | 1,28 |
| Q 83 | 32 | <0,125 | 128 |
| Q 85 | 128 | <0,125 | 128 |
| FPQ 24 | 128 | 2 | 128 |
| FPQ 30 | 128 | <0.125 | 1.28 |
| FPQ25 | 8.00 | 2.00 | 128 |
| FPQ28 | 16.00 | 0.32 | 128 |

**Table 3**
ANTIBACTERIAL ACTIVITY IN VITRO

The molecular modeling study has been performed using SPARTAN'14 software package [22]. In this study, the DFT/B3LYP/6-31 G* level of basis set has been used for the computation of molecular structure, vibrational frequencies and energies of optimized structures. In order to perform structure–activity relationship (SAR) studies, some electronic properties, such as HOMO (Highest Occupied Molecular Orbital) and LUMO (Lowest Unoccupied Molecular Orbital) energy values, HOMO and LUMO orbital coefficients distribution, molecular dipole moment, and molecular electrostatic potential (MEP), have been calculated. F*rontier molecular orbital analysis:* Molecular orbital analysis Frontier molecular orbital's (FMOs) play crucial role in the chemical stability of a molecule and in the interactions between atoms. They are considered to be effective in determining the characteristics of the molecules such as optical properties and biological activities. Among these, the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) are the most important. The HOMO represents the ability of a molecule to donate an electron, while the LUMO is an electron acceptor. [23, 24]. The HOMO and LUMO surfaces of 8-chloro-fluoroquinolone compounds, calculated at the B3LYP/6-31G* level can be seen in figure 1 for the gas phase.

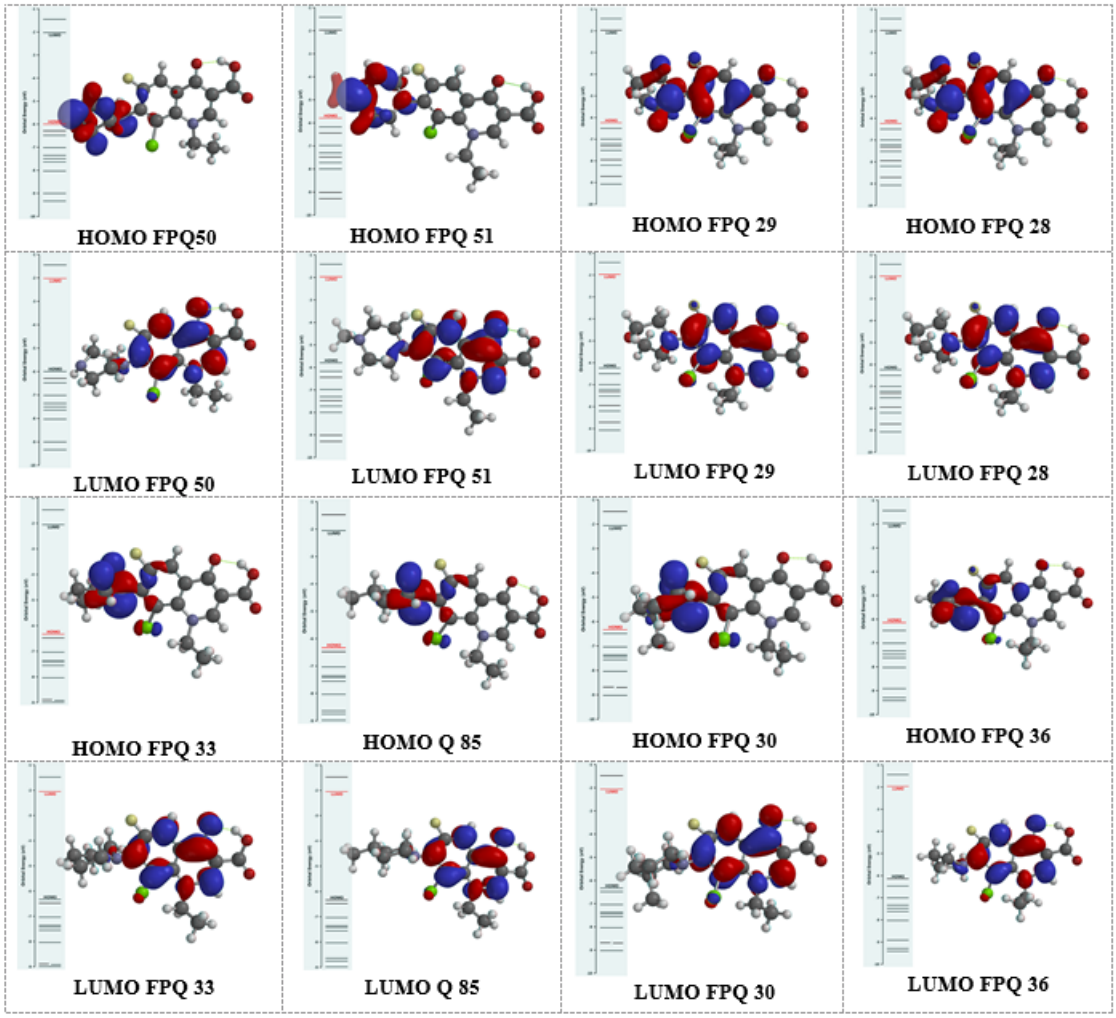



Fig. 1. HOMO, LUMO surfaces of 8-chloro-fluoroquinolone compounds

**Table 4**
FRONTIER MOLECULAR ORBITAL ENERGIES OF QUINOLONE COMPOUNDS (eV)

| Compound | $E_{HOMO}$ | $E_{LUMO}$ | $\Delta E_{LUMO-HOMO}$ |
|---|---|---|---|
| NF | -5.76 | -1.41 | 4.35 |
| FPQ 50 | -6.00 | -2.02 | 3.98 |
| PF | -5.77 | -1.43 | 4.34 |
| FPQ 51 | -5.79 | -1.97 | 3.82 |
| FPQ 27 | -5.76 | -1.40 | 4.36 |
| FPQ 29 | -6.01 | -1.96 | 4.05 |
| FPQ 32 | -6.36 | -1.82 | 4.54 |
| FPQ 33 | -6.33 | -2.05 | 4.28 |
| Q 83 | -6.36 | -1.82 | 4.54 |
| Q 85 | -6.33 | -2.05 | 4.28 |
| FPQ 24 | -6.34 | -1.82 | 4.52 |
| FPQ 30 | -6.33 | -2.06 | 4.27 |
| FPQ 25 | -6.02 | -1.58 | 4.44 |
| FPQ 28 | -6.24 | -1.97 | 4.27 |
| FPQ 35 | -5.77 | -1.39 | 4.38 |
| FPQ 36 | -6.14 | -1.97 | 4.17 |

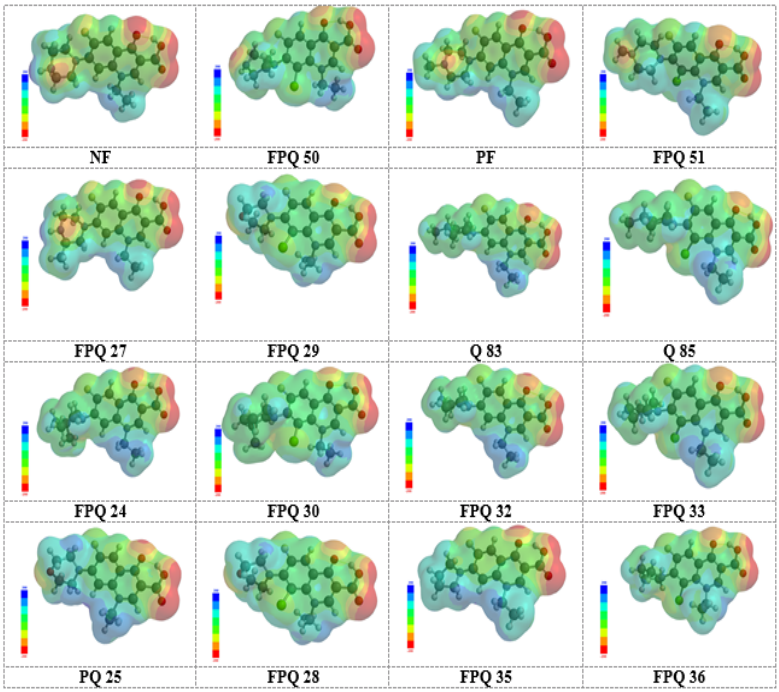



Fig. 2. Electrostatic potential MEP of fluoroquinolone compounds

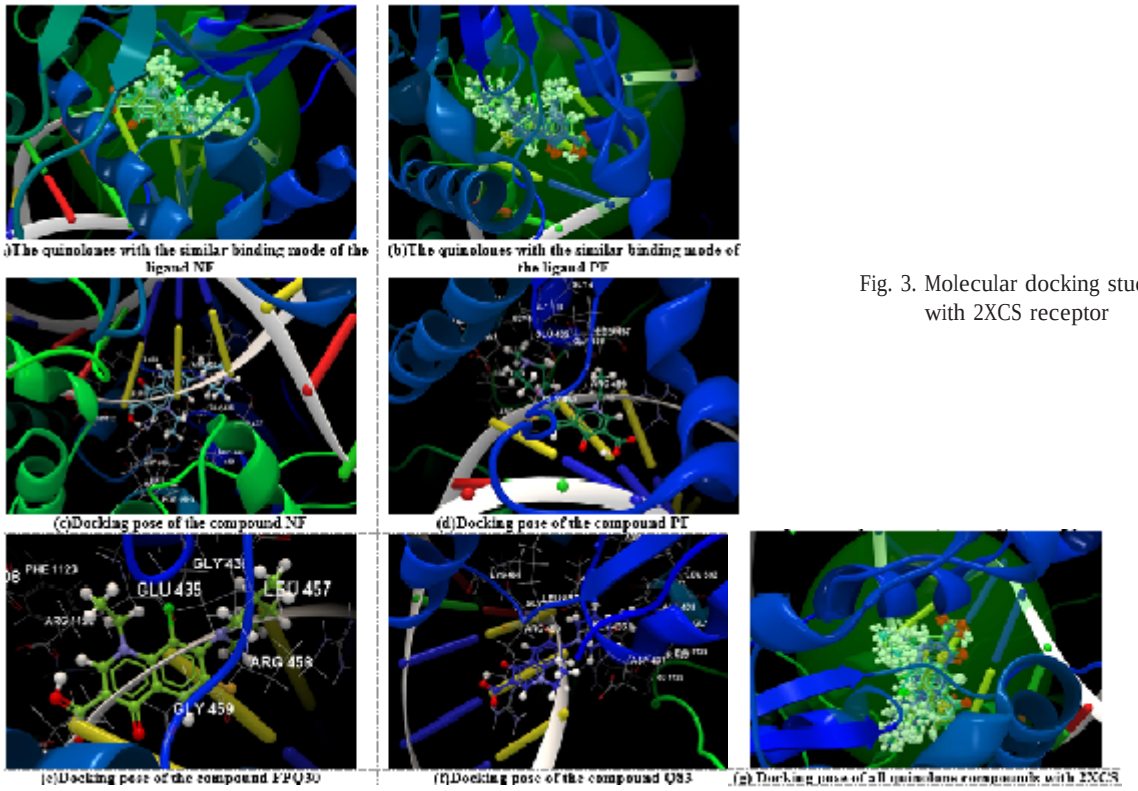



Fig. 3. Molecular docking studies with 2XCS receptor

The frontier orbital gap helps to characterize chemical reactivity of the molecule (table 4). HOMO and LUMOs determine the way in which it interacts with other species. The introduction of the electron-withdrawing substituent (chlorine) at position C 8 in quinolone compounds decreases the HOMO-LUMO gap as compared to their corresponding 8-unsubstituted quinolone compounds.

*Molecular Electrostatic Potential (MEP):* Molecular electrostatic potential (MEP) have been evaluated using B3LYP method with the basis set 6-31G* to investigate the chemical reactivity of a molecule. The MEP is especially important for the identification of the reactive sites of nucleophilic or electrophilic attack in hydrogen-bonding interactions and for the understanding of the process of biological recognition [23,24]. An electrostatic potential map for quinolone compound shows hydrophilic regions in red (negative potential) and blue (positive potential) and hydrophobic regions in green (fig. 2).

*Molecular Docking* The docking studies have been carried out using CLC Drug Discovery Workbench Software. The score and hydrogen bonds formed with the amino acids from group interaction atoms are used to predict the binding modes, the binding affinities and the orientation of the docked quinolone compound (fig.3d) in the active site of the protein-receptor (table 5). The protein-ligand complex have been realized based on the X-ray structure of S. Aureus DNA GYRASE, who was downloaded from the Protein Data Bank (PDB ID: 2XCS) [25].

The result of molecular docking study for quinolone FPQ 30, compound with a good activity *in vitro* against Staphylococcus Aureus ATCC 6538 (MIC < 0.125 μg/mL) and with a good activity against MRSA [19], reveals docking

**Table 5**

THE LIST OF INTERMOLECULAR INTERACTIONS BETWEEN THE LIGAND MOLECULES DOCKED WITH 2XCS USING CLC DRUG DISCOVERY WORKBENCH SOFTWARE

| Comp. | Score/ RMSD | Interacting group | Hydrogen bond | Bond length |
|---|---|---|---|---|
| NF | -47.02 / 0.66 | PHE1123(B), ARG1122(B), ASP508(D), ASP512(B), SER438(D), ASP437(D), GLY436(D), ALA439(D), VAL434(D), ILE516(D), LYS460(D), ARG458 (D), LEU457(D), GL 459(D), GLU435(D) | O $sp^2$ from COOH(OH)-$Osp^2$ from GLU 435<br>N $sp^3$from piperazine - $Osp^2$ from ASP 437 | 3.028 Å<br>3.244 Å |
| FPQ50 | -47.21 / 0.06 | LYS460(D), ILE516(D), ASP512(D), ASP510(D), ASP508(D), GLU435(D), LEU457(D), PRO456(D), ASP437(D), ARG458(D), GLY459(D), GLY436(D), ALA439(D), SER438(D), ARG1122(B), PHE1123(B) | O $sp^2$ from COOH(OH)- $Osp^2$ from GLU 435<br>N $sp^3$from piperazine - $Nsp^2$ from ARG 458 | 2.759 Å<br>3.054 Å |
| PF | -50.26 / 0.07 | ASP437(D), GLY440(D), ALA439(D), GLY436(D), SER438(D), GLU435(D), LEU457(D), ARG458(D), GLY459(D), PHE1123(B), ARG1122(B), GLY582(D), GLY584(D), LEU583(D), ASP508(D) | O $sp^2$ from COOH(CO)- $Nsp^2$ from ARG 458 | 2.809 Å |
| FPQ51 | -49.54 / 0.32 | ASP437(D), ALA439(D), GLY436(D), SER438(D), GLU435(D), LEU457(D), ARG458(D), GLY459(D), PHE1123(B), ARG1122(B), GLY582(D), GLY584(D), LEU583(D), ASP508(D) | O $sp^2$ from COOH(CO)- $Nsp^2$ from ARG 458 | 2.745 Å |
| FPQ27 | -50.44 / 0.68 | PHE1123(B), ARG1122(B), ASP508(D), GLY584(D), LEU583(D), ALA439(D), PRO456(D), ASP437(D), LEU457(D), GLU435(D), GLY459(D), ARG458(D), GLY436(D), GLU477(D), SER438(D) | O $sp^2$ from COOH(OH) - $Osp^2$ from GLU 435<br>N $sp^3$from piperazine - $Nsp^2$ from ARG 458<br>N $sp^3$from piperazine - $Nsp^2$ from ARG 458<br>N $sp^3$from piperazine - $Osp^2$ from ASP 437 | 2.673 Å<br>3.126 Å<br>3.352 Å<br>3.168 Å |
| FPQ29 | -46.53 / 0.05 | LYS460(D), GLY459(D), ARG458(D), LEU457(D), GLY436(D), GLU435(D), ARG1122(B), PHE1123(B), ASP508(D), PRO456(D), ASP437(D), ALA439(D), ILE516(D), ASP458(D) | O $sp^2$ from COOH(OH) - $Osp^2$ from GLU 435<br>N $sp^3$from piperazine - $Nsp^2$ from ARG 458 | 2.741 Å<br>3.119 Å |
| FPQ32 | -48.72 / 0.16 | ASP512(D), ILE516(D), LYS460(D), GLY459(D), ARG458(D), ARG1122(B), GLU435(D), LEU457(D), ASP437(D), PRO456(D), PHE1123(B), ASP508(D), GLY436(D), SER 438(D), ALA439(D) | O $sp^2$ from COOH(OH) - $Osp^2$ from GLU 435 | 2.597 Å |
| FPQ33 | -46.16 / 0.08 | PRO456(D), ALA439(D), ASP437(D), GLY436(D), SER438(D), LEU457(D), GLU435(D), ASP508(D), ARG458(D), GLY459(D), LYS460(D), ASP512(D), ASP510(D), ARG1122(B), PHE1123(B) | O $sp^2$ from COOH(OH) - $Osp^2$ from GLU 435 | 2.899 Å |
| Q83 | -51.79 / 0.71 | ARG458(D), LEU457(D), GLY459(D), LYS 460(D), ASP437(D), SER438(D), GLY436(D), ARG1122(B), ASP508(D), LEU583(D), LYS581(D), GLY 584(D), PHE1123(B), ALA439(D), GLY582(D), GLU435(D) | O $sp^2$ from COOH(CO)- $Nsp^2$ from ARG 458 | 3.034 Å |
| Q85 | -46.49 / 0.08 | ARG458(D), ALA1120(B), ARG1122(B), PHE1123(B), SER438(D), ASP437(D), GLY436(D), GLU435(D), LEU457(D), GLY459(D), LEU583(D), ASP508(D), GLY582(D) | O $sp^2$ from COOH(CO)- $Nsp^2$ from ARG 458 | 2.661 Å |
| FPQ24 | -50.01 / 0.008 | ARG1122(B), PH 1123(B), GLY436(D), PRO456(D), LEU457(D), ARG458(D), GLY459(D), GLU435(D), ALA 439(D), SER438(D), ASP508(D), LYS460(D), ASP512(D), ASP437(D) | O $sp^2$ from COOH(OH) - $Osp^2$ from GLU 435 | 2.706 Å |
| FPQ30 | -49.13 / 0.61 | LYS 460(D), GLY459(D), ILE516(D), ASP512(D), ASP508(D), ARG458(D), LEU457(D), GLU435(D), GLY436(D), PRO456(D), ASP437(D), ARG1122(B), PHE1123(B), ALA439(D), SER438(D) | O $sp^2$ from COOH(OH) - $Osp^2$ from GLU 435 | 2.706 Å |
| FPQ25 | -46.70 / 0.06 | ILE516(D), ASP508(D), ASP512(D), LYS460(D), GLU435(D), GLY459(D), PHE1123(B), ARG458(D), ALA439(D), ARG1122(B), ASP437(D), SER438(D), LEU457(D), GLY436(D) | O $sp^2$ from COOH(OH) - $Osp^2$ from GLU 435 | 2.849 Å |
| FPQ28 | -46.83 / 0.60 | LYS 460(D), ARG458(D), GLY459(D), ILE516(D), ASP512(D), ASP512(D), GLU435(D), LEU457(D), GLY436(D), ASP508(D), PHE1123(B), ARG1123(B), ALA439(D), SER438(D) | O $sp^2$ from COOH(OH) - $Osp^2$ from GLU 435<br>-O $sp^3$ from morpholine - $Nsp^2$ from ARG 458 | 2.619 Å<br>3.378 Å |
| FPQ35 | -47.27 / 0.13 | ARG1122(B), PHE1123(B), ASP437(D), GLY436(D), ARG458(D), GLY459(D), LEU 457(D), PRO456(D), GLY440(D), ALA439(D), GLY582(D), LEU583(D), SER438(D), GLY584(D), ASP508(D), VAL434(D), GLU435(D). | O $sp^2$ from COOH(CO)- $Nsp^2$ from ARG 458 | 3.083 Å |
| FPQ36 | -45.97/ 0.09 | ASP437(D), SER438(D), GLY436(D), ALA439(D), ARG458(D), GLY459(D), LYS 460(D), LEU457(D), GLU435(D), ASP508(D), PHE1123(B), ARG1122(B) | O $sp^2$ from COOH(CO)- $Nsp^2$ from ARG 458 | 2.923 Å |

score -48.72 (RMSD 0.16) and shows the occurrence of one hydrogen bonds with GLU 435 (2.597 Å) (fig.3e). The orientation of the FPQ 30 is the same of NF(Norfloxacine). Same orientation show also the compounds: FPQ 50, FPQ 32, FPQ 33, FPQ 27, FPQ 29, FPQ24, FPQ 25 and FPQ 28 (Fig.3a). Docking score of NF compound is - 47.02 (RMSD 0.66). NF shows the occurrence of two hydrogen bonds with GLU 435 (3.028 Å), and ASP 437 (3.244 Å) (fig.3c). The better score docking have been obtained from quinolone Q83: - 51.79 (RMSD 0.71). Q83 shows the occurrence of one hydrogen bonds with ARG 458 (3.034 Å) (fig.3f), and its orientation is the same of PF (Pefloxacine) (fig.3b). Compound Q83 shows also a good activity i*n vitro* against Staphylococcus Aureus ATCC 6538 (MIC < 0.125µg/mL). The same orientation show also the compounds: FPQ 51, Q 85, FPQ 35, and for FPQ 36. Docking score of PF is -50.26 (RMSD 0.07).PF shows the occurrence of one hydrogen bond with ARG 458 (2.809 Å). (fig.3d).

## Conclusions

In the present study, we have reported the synthesis of some quinolone compounds. The quinolones were evaluated for their antibacterial activity against Gram-positive and Gram-negative microorganisms. The results indicated that the synthesised compounds showed significant antimicrobial activities. In silico molecular docking simulation was performed to position all quinolone compounds into the preferred binding site of the protein receptor S. Aureus DNA GYRASE, to predict the binding modes, the binding affinities and the orientation. The docking studies revealed that the all compounds showed good docking score. The docking score is a measure of the antimicrobial activity of the studied compounds.

*Acknowledgements: This paper has been financed through the NUCLEU Program, which is implemented with the support of ANCSI, project no. PN 09-11 01 01 and PN 09-11 01 09.*

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Manuscript received: 08.08.2017