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RESEARCH ARTICLE

Novel Azole-Functionalited Flouroquinolone Hybrids: Design, Conventional and Microwave Irradiated Synthesis, Evaluation as Antibacterial and Antioxidant Agents

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Abstract: ***Background*:** The synthesis of new hybrid molecules consisting of several heterocyclic pharmacophores namely fluoroquinolone, 1,2,4-triazole, 1,3,4-oxadiazole and piperazine was carried out by conventional and successfully optimized microwave mediated techniques.

***Methods*:** The structures of new compounds were confirmed using spectroscopic techniques. These compounds were screened for their antibacterial activity against *E. coli*, *P. aeruginosa*, *Y. pseudotuberculosis*, *S. aureus, E. faecalis*, *B. cereus* and *M. smegmatis*.

***Results and Conclusion*:** Fourteen of these hybrids exhibited excellent antibacterial activities on the test microorganisms when compared with ampicillin with the mic values varying between 0.03-0.25 μg/mL.

Keywords: Fluoroquinolone, 1,2,4-triazole, 1,3,4-oxadiazole, piperazine, antibacterial activity, antioxidant activity, microwave.

1. INTRODUCTION

The increasing community- and hospital- acquired infectious diseases caused by resistant bacteria to most classes of antibacterial drugs resulted in a pressing and urgent need for designing of new antibiotic candidates. The declaration of The European Centre for Disease Prevention and Control (ECDC) reporting "Every year, the infections caused by resistant bacteria gives rise to 25,000 deaths with a cost of over 1.5 billion Eur because of healthcare spending and labor losses in the Europa" reveals that this is a public health problem with also socio-economic loses [1-5]. Mortality in these patients infected by resistant bacteria was reported as two times higher than the patients infected by nonresistant bacteria [2]. It is clear that pharmaceutical industry today is not able to meet the urgent need for discovery of new and effective antibiotics, despite much efforts aiming to deal with resistance problem by development of new class of antibacterial agents with different mode of action. Some of the main reasons of this undesirable situation are the increasing expenses of antibacterial resistant drugs, strict legal requirements and high prices of antibiotics. In order to overcome this situation, the collaboration of all sectors including regulations, society and researchers is necessary [6]. In order to overcome the drug resistance, the mostly implemented approach aiming the development of new antibacterial agents is to improve the pharmacodynamics of the existing drugs by changing their structural units or producing new derivatives. This approach has been accepted as an attractive strategy by scientific communities since this method does not require the discovery of new antibacterial scaffolds or validation of novel biological targets, which has proven to be an extremely difficult and time consuming task [7].

More recently, the concept of hybrid molecules, which contain two or more pharmacophore groups binding together covalently in one molecular framework, has been introduced in the medicinal chemistry field. These compounds that are obtained by molecular hybridization of several pharmacophore groups, act by inhibiting two or more conventional targets simultaneously, and this multiple target strategy has resulted in the development of a number of bioactive hybrid molecules [8].

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DNA gyrase and topoisomerase IV are known as essential and immanent enzymes responsible for bacterial cell growth and division [9]. Fluoroquinolones, proceeding as DNA gyrase and bacterial topoisomerase IV inhibitors, which can inhibit DNA synthesis by forming complexes with DNA gyrase or topoisomerase II enzyme, were discovered as broad-spectrum antibiotics in 1980s. Since their introduction to clinical use, they were extensively applied for the treatment of infectious diseases. But unfortunately, the wide use of fluoroquinolones in clinical settings resulted in the emergence of resistant bacterial strains. The resistance problem along with the decline of the antibiotic discovery and development studies made mandatory the design of new antibiotics [8, 10, 11].

Several successful syntheses of fluoroquinolone hybrids including arylfuran [8], benzofuroxan [12], piperazine [13, 14], thiadiazole [15], triazole [16] or quinolone [17] unit have been reported, most of which were obtained by substitution on piperazine ring at C-7 position of the basic structure of quinolones.

Since some preferable properties including flexibility, convergence and economic nature, multicomponent reactions have received considerable attention in organic, combinatorial and medicinal chemistry leading to the formation of several compounds possessing elaborate biological activities [18-20]. Moreover, the growing emphasis of "green chemistry techniques" in new drug discovery protocols has increased the popularity of the scope of microwave assisted organic synthesis due to some superior properties such as mild reaction conditions, shortened reaction times, increased reaction yields, relatively less decomposition, less formation of by-products *etc* [21-23]. Thus, the development of new strategies including a combination of multicomponent reactions and microwave irradiation techniques without any harmful organic solvent and catalyst has become one of the most important strategies of synthetic organic chemistry.

Discovery of new agents with antioxidant properties has become another extraordinary active area of preventive medicinal chemistry [24-26] and some fluoroquinolones and related compounds were reported as antioxidant capacity in addition to antimicrobial activity [27-30]. Moreover, synthesizing some fluoroquinolone hybrid compounds with their antioxidant capacity and antibacterial activity are currently continuing in our laboratory [31, 32].

In this context, we reported here the conventional and microwave irradiated green synthesis, antimicrobial and antioxidant activity screening studies of novel fluoroquinolone-azole hybrids by the molecular hybridization strategy. The rational design of the targeted compounds was depicted in Fig. (**1**).

2. EXPERIMENTEL

2.1. General

All the chemicals were purchased from Fluka Chemie AG Buchs (Switzerland) and used without further purification. Microwave-assisted syntheses were carried out using monomode CEM-Discover microwave apparatus. Reactions were monitored by thin-layer chromatography (TLC) on silica gel 60 F254 aluminium sheets. Melting points of the synthesized compounds were determined in open capillaries on a Büchi B-540 melting point apparatus and are uncorrected. The mobile phase was ethyl acetate:diethyl ether (1:1), and detection was made using UV light. FT-IR spectra were recorded using a Perkin Elmer 1600 series FTIR spectrometer. ^{1}H NMR and ^{13}C NMR spectra were registered in DMSO-d_6 on a BRUKER AVENE II 400 MHz NMR Spectrometer (400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C). The chemical shifts are given in ppm relative to Me_4Si as an internal reference, J values are given in Hz. The elemental analysis was performed on a Costech Elemental Combustion System CHNS-O elemental analyzer. All the compounds gave C, H and N analysis within ±0.4% of the theoretical values. The Mass spectra were obtained on a Quattro LC-MS (70 eV) Instrument.

2.1.1. Ethyl 2-[4-(4-nitrophenyl)piperazin-1-yl]acetate (2)

Method 1

Ethyl bromoacetate (10 mmol) was added to the solution of 1-(4-nitrophenyl) piperazine (10 mmol) in tetrahydrofuran drop wise and the mixture was stirred at room temperature in the presence of triethylamine (30 mmol) for 3 h. The solid formed was removed by filtration, and the solvent was

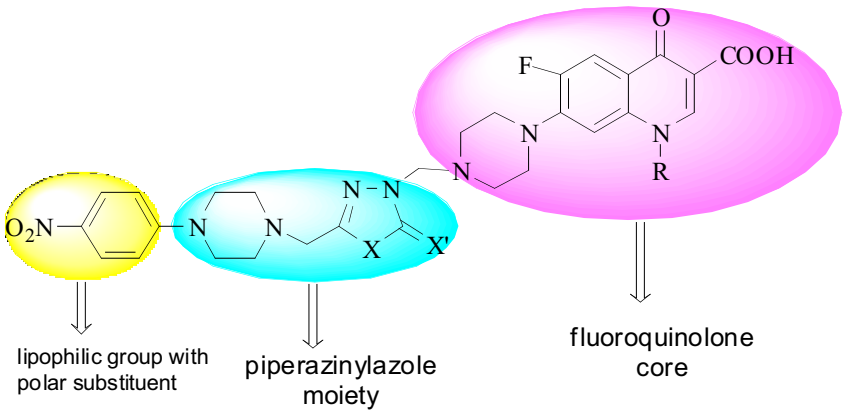


Fig. (1). The rational design of the new fluoroquinolone-azole hybrids.

evaporated under reduced pressure. The yellow solid obtained was purified by crystallization from ethyl acetate to afford the desired compound. Yield 84%.

Method 2

The mixture of ethyl bromoacetate (10 mmol), triethylamine (30 mmol) and 1-(4-nitrophenyl)piperazine (10 mmol) was irradiated in monomode microwave reactor in closed vessel with pressure control at 80 W maximum power for 8 min. The formed yellow product was washed with water and purified by crystallization from ethyl acetate. Yield 99%. mp.115-116°C.

FT-IR ($\upsilon_{max,}$ cm^{-1}): 3080 (ar-H), 1742 (C=O), 1313 and 1587 (NO_2), 1207 (C-O). ^{1}H NMR (DMSO-d_6, δ ppm): 1.24 (t, 3H, CH_3, *J*= 8.0 Hz), 2.69 (s, 4H, $2CH_2$), 3.34 (s, 2H, CH_2), 3.50 (s, 4H, $2CH_2$), 4.14 (q, 2H, CH_2, *J*= 8.0 Hz), 7.06 (d, 2H, ar-H, *J*= 8.0 Hz), 8.09 (d, 2H, ar-H, *J*=8.0 Hz). ^{13}C NMR (DMSO-d_6, δ ppm): 14.70 (CH_3), 46.90 ($2CH_2$), 52.00 ($2CH_2$), 58.64 (CH_2), 60.48 (CH_2), arC: [113.20 (CH), 113.24 (CH), 126.28 (CH), 126.30 (CH), 137.44 (C), 155.29 (C)], 170.41 (C=O). LC-MS m/z (%): 316.12 ([M+ Na]$^+$, 6), 188.16 (100). Elemental Anal. Calcd. for $C_{14}H_{19}N_3O_4$ (%):C, 57.33; H, 6.53; N, 14.33, Found (%): C, 57.34; H, 6.52; N, 14.33.

2.1.2. [4-(4-Nitrophenyl)piperazin-1-yl]acetohydrazide (3)

Method 1

Hydrazine hydrate (30 mmol) was added to the solution of compound **2** (10 mmol) in absolute ethanol and the mixture was refluxed for 5 h. On removing the solvent under reduced pressure, a solid obtained. This was recrystallized from ethanol to give the target compound. Yield 75%.

Method 2

The mixture of hydrazine hydrate (30 mmol) and compound **2** (10 mmol) was irradiated in monomode microwave reactor in closed vessel with pressure control at 120 W for 5 min. The crude product obtained was recrystallized from ethanol to give the pure compound. Yield: 97%. Mp, 163-164°C.

FT-IR ($\upsilon_{max,}$ cm^{-1}): 3328 and 3248 (NH, NH_2), 3006 (ar-H), 1631 (C=O), 1474 and 1322 (NO_2). ^{1}H NMR (DMSO-d_6, δ ppm): 2.58-2.63 (m, 4H, $2CH_2$+DMSO), 3.06 (s, 2H, CH_2), 3.55 (s, 4H, $2CH_2$), 4.32 (s, 2H, NH_2), 7.09 (s, 2H, ar-H), 8.11 (s, 2H, ar-H), 9.06 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 46.88 ($2CH_2$), 52.89 ($2CH_2$), 60.16 (CH_2), arC: [113.22 (2CH), 126.36 (2CH), 137.39 (C), 155.31 (C)], 168.65 (C=O). LC-MS m/z (%): 303.11 ([M+1+ Na]$^+$, 15), 302.11 ([M+ Na]$^+$, 100). Elemental Anal. Calcd. For $C_{12}H_{17}N_5O_3$ (%):C, 51.60; H, 6.14; N, 25.08, Found (%): C, 51.61; H, 6.13; N, 25.08.

2.1.3. 5-{[4-(4-Nitrophenyl)piperazin-1-yl]methyl}-1,3,4-oxadiazole-2-thiol (4)

Method 1

The solution of KOH (10 mmol) in water was added to the solution of compound **3** (10 mmol) in ethanol and the mixture was refluxed in the presence of CS_2 (20 mmol) for 10 hours. Then, it was cooled to room temperature and acidified to pH 6 with 37 % HCl. On cooling the mixture in cold overnight, a solid obtained. This was recrystallized from acetone to give the title compound **4** as a white solid. Yield: 76%.

Method 2

The mixture of compound **3** (10 mmol), KOH (10 mmol) and CS_2 (20 mmol) in ethanol was irradiated in the monomode microwave reactor in closed vessel at 200 W for 12 min. Then, it was acidified to pH 6 with 37 % HCl. On cooling the mixture in cold overnight, a solid obtained. This was recrystallized from acetone to give the title compound **4** as a white solid. Yield: 97%, mp. 184-186°C.

FT-IR ($\upsilon_{max,}$ cm^{-1}): 3373 (NH), 1587-1314 (NO_2). ^{1}H NMR (DMSO-d_6, δ ppm): 2.58-2.61 (m, 4H, $2CH_2$), 2.70 (t, 2H, CH_2, *J*= 8.0 Hz)3.44-3.48 (m, 4H, $2CH_2$), 7.00 (d, 2H, ar-H, *J*= 4.0 Hz), 8.02 (d, 2H, ar-H, *J*= 4.0 Hz) 8.04 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 46.40 (CH_2), 46.65 (CH_2), 51.46 (CH_2), 51.85 (CH_2), 58.37 (CH_2), arC: [113.10 (CH), 113.16 (CH), 126.14 (2CH), 137.40 (C), 155.05 (C)], 161.05 (oxadiazole C-5), 178.51 (oxadiazole C-2). LC-MS m/z (%): 322.24 ([M+1]$^+$, 10), 294.34 (100). Elemental Anal. Calcd. for $C_{13}H_{15}N_5O_3S$ (%): C, 48.59; H, 4.70; N, 21.79, Found (%): C, 48.59; H, 4.70; N, 21.78.

2.1.4. General Method for The Synthesis of Compounds 5a-b

Method 1

The solution of ciprofloxacin (for **5b**) or norfloxacin (for **5a**) (10 mmol) in dimethyl formamide was stirred at room temperature in the presence of formaldehyde (30 mmol) for 15 min. Then, compound **4** was added into it and stirred at room temperature for additional 24 h. The reaction mixture was poured into ice-water and a solid obtained. This crude product was collected by filtration and recrystallized from dimethyl sulfoxide to afford the desired product.

Method 2

The mixture of ciprofloxacin (for **5b**) or norfloxacin (for **5a**) (10 mmol), compound **4** and formaldehyde (30 mmol) was irradiated in monomode microwave reactor in closed vessel with pressure control at 80 W for 15 min. The solid obtained was recrystallized from dimethyl sulfoxide to afford the desired product.

1-Ethyl-6-fluoro-7-{4-(5-([4-(4-nitrophenyl)piperazin-1-yl]methyl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl)methyl] piperazin-1-yl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5a)

mp.: 220-221°C, Yield: 70% (Method 1), 88% (Method 2). FT-IR ($\upsilon_{max,}$ cm^{-1}): 3365 (COOH), 3071 (ar-H), 2829 (C=S), 1724 (C=O), 1626 (C=O), 1496 and 1329 (NO_2), 1224 (C-O). ^{1}H NMR (DMSO-d_6, δ ppm): 1.39- 1.43 (m, 3H, CH_3), 2.64-92 (m, 8H, $4CH_2$), 3.34-3.50 (m, 8H, $4CH_2$+ H_2O), 3.79 (s, 2H, CH_2), 4.58 (s, 2H, CH_2), 5.05 (s, 2H, CH_2), 7.01 (d, 2H, ar-H, *J*= 8.0 Hz), 7.17 (d, 1H, ar-H, *J*= 8.0 Hz), 7.90-7.97 (m, 1H, ar-H), 8.01-8.11 (m, 2H, ar-H), 8.95 (s, 1H, quinolone =CH), 15.36 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6, δ ppm): 14.79 (CH_3), 43.30 (CH_2), 45.60 (CH_2), 45.31 (CH_2), 46.68 ($2CH_2$), 48.36 (CH_2), 49.54

(CH_2), 49.79 ($2CH_2$), 51.98 (CH_2), 69.92 (CH_2), arC: [113.19 (2CH), 126.15(2CH), 137.71 (C), 154.59 (C),], 106.26 and 106.45 (d, CH, *J*= 9.0 Hz), 107.54 (C), 111.80 (CH), 119.81 (C), 137.45(C), 145.85 (C), 148.99 (CH), 152.65 (C), 155.59 (oxadiazole C-5), 166.63 (C=O), 173.99 (oxadiazole C-2), 176.86 (C=O). LC-MS m/z (%):653,22 ($[M+1]^+$, 100). Elemental Anal. Calcd. for $C_{30}H_{33}FN_8O_6S$ (%): C, 55.21; H, 5.10; N, 17.17, Found (%): C, 55.28; H, 5.17; N, 17.21.

1-Cyclopropyl-6-fluoro-7-{4-(5-[4-(4-nitrophenyl)piperazin-1-yl]methyl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl]methyl) piperazin-1-yl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5b)

mp.: 215-216°C, Yield: 75% (Method 1), 89% (Method 2). FT-IR ($\upsilon_{max,}$ cm^{-1}): 3333 (COOH), 3022 (ar-H), 1718 (C=O), 1679 (C=O), 1491 and 1356 (NO_2), 1229 (C-O). ^{1}H NMR (DMSO-d_6, δ ppm): 1.18 (s, 2H, CH_2), 1.31 (d, 2H, CH_2, *J*= 4.0 Hz), 2.65-2.73 (m, 4H, $2CH_2$), 2.89-2.94 (m, 4H, $2CH_2$), 3.32 (bs, 6H, $3CH_2$+ H_2O), 3.47 (s, 3H, CH + CH_2), 3.80 (s, 2H, CH_2), 5.06 (s, 2H, CH_2), 7.05 (d, 2H, ar-H, *J*= 12.0 Hz), 7.54-7.60 (m, 1H, ar-H), 7.87-7.95 (m, 2H, ar-H), 8.03 (d, 1H, ar-H, *J*= 8.0 Hz), 8.65 (s, 1H, quinolone =CH), 15.17 (bs, 1H, COOH). ^{13}C NMR (DMSO-d_6, δ ppm): 8.04 ($2CH_2$), 36.30 (CH), 46.68 ($2CH_2$), 48.35 (CH_2), 49.73 ($2CH_2$), 51.06 (CH_2), 51.47 (CH_2), 51.96 ($2CH_2$), 69.91 (CH_2), arC: [113.18 (2CH), 126.12 (2CH), 139.59 (C),], 107.03 (CH), 107.22 (C), 111.31 and 111.54 (d, CH, *J*= 23.0 Hz), 119.09 and 119.17 (d, C, *J*= 8.0 Hz), 137.43 (C), 145.41 (C), 148.45 (CH), 152.19 and 154.67 (d, C-F, $J_{C\text{-}F}$= 248.0 Hz), 155.06 (oxadiazole C-5), 166.37 (C=O), 176.82 (C=O), 178.53 (oxadiazole C-2). LC-MS m/z (%): 665,22 ($[M+1]^+$, 100). Elemental Anal. Calcd. for $C_{31}H_{33}FN_8O_6S$ (%):C, 56.02; H, 5.00; N, 16.86, Found (%): C, 56.08; H, 5.07; N, 16.91.

2.1.5. General Method for The Synthesis of Compounds 6a-f

Method 1

A mixture of compound **3** (10 mmol) and the corresponding iso(thio)cyanate (10 mmol) was stirred in dichloromethane at room temperature for 12 h (for **6b**), 21 h (for **6d** and **6e**) or 20 h (for **6a**, **6c** and **6f**). On evaporating the solvent under reduced pressure, a solid obtained. The crude product was recrystallized from ethanol (for **6a, 6c, 6d** and **6f**) or ethyl acetate (for **6b** and **6e**) to afford the desired product.

Method 2

A mixture of compound **3** (10 mmol) and the corresponding iso(thio)cyanate (20 mmol) was irradiated in monomode microwave reactor in closed and vessel with pressure control at 80 W for 12 min. The crude product obtained was recrystallized from ethanol to (for **6a**, **6c**, **6d** and **6f**) or ethyl acetate (for **6b** and **6e**) to give the pure compound.

N-Benzyl-2-{[4-(4-nitrophenyl)piperazin-1-yl]acetyl} hydrazincarbothioamide (6a)

mp. 209-210°C Yield: 59% (Method 1), 73% (Method 2). FT-IR ($\upsilon_{max,}$ cm^{-1}): 3285, 3268, 3231, 3119 (NH), 1743 (C=O), 1484 and 1307 (NO_2).^{1}H NMR (DMSO-d_6, δ ppm): 2.64 (s, 4H, $2CH_2$), 3.15 (s, 2H, CH_2), 3.38 (s, 2H, CH_2 + H_2O), 3.70 (s, 4H, $2CH_2$), 7.02 (d, 2H, ar-H, *J*= 8.0 Hz), 7.06-7.43 (m, 5H, ar-H), 8.05 (d, 2H, ar-H, *J*= 8.0 Hz), 9.59 (bs, 2H, 2NH), 9.94 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 46.73($2CH_2$), 51.88 (CH_2), 52.72 ($2CH_2$), 59.99 (CH_2), arC: [113.10 (4CH), 126.19 (4CH), 128.62 (CH), 137.28 (C), 139.58 (2C)], 155.15 (C=O), 181.34 (C=S).LC-MS m/z (%): 430.16 ($[M+2]^+$, 24), 429.16 ($[M+1]^+$, 94), 220.10 (100). Elemental Anal. Calcd. for$C_{20}H_{24}N_6O_3S$ (%):C, 56.06; H, 5.65; N, 19.61, Found (%): C, 56.05; H, 5.67; N, 19.61.

N-Benzyl-2-{[4-(4-nitrophenyl)piperazin-1-yl]acetyl} hydrazincarboxamide (6b)

mp. 203-204°C Yield: 78% (Method 1), 97% (Method 2). FT-IR ($\upsilon_{max,}$ cm^{-1}): 3298, 3231, 3183 (NH), 1697 (C=O).^{1}H NMR (DMSO-d_6, δ ppm): 2.62 (s, 4H, $2CH_2$), 3.09 (s, 2H, CH_2), 3.48 (s, 4H, $2CH_2$ + H_2O), 4.25 (s, 2H, CH_2), 6.97 (d, 3H, ar-H, *J*= 32.0 Hz), 7.28 (s, 4H, ar-H), 7.91 (s, 1H, NH), 8.05 (s, 2H, ar-H), 9.53 (s, 2H, 2NH).^{13}C NMR (DMSO-d_6, δ ppm): 43.13(CH_2), 50.36 ($2CH_2$), 51.54 ($2CH_2$), 57.67 (CH_2), arC: [112.41 (2CH), 126.40 (2CH), 126.92 (CH), 127.59 (2CH), 128.43 (CH), 138.88 (C), 139.85 (C), 158.23 (C)], 156.89 (C=O), 171.95 (C=O).LC-MS m/z (%): 412.19 ($[M]^+$, 100), 413.19 ($[M+1]^+$, 22.1). Elemental Anal. Calcd. For$C_{20}H_{24}N_6O_4$ (%): C, 58.24; H, 5.87; N, 20.38, Found (%): C, 57.85; H, 5.67; N, 19.44.

N-Ethyl-2-{[4-(4-nitrophenyl)piperazin-1-yl]acetyl} hydrazincarbothioamide (6c)

mp.124-125°C Yield: 75% (Method 1), 96% (Method 2). FT-IR ($\upsilon_{max,}$ cm^{-1}): 3286, 3228, 3202, 3120 (NH), 1743 (C=O), 1485 and 1319 (NO_2).^{1}H NMR (DMSO-d_6, δ ppm): 1.19 (t, 3H, CH_3, *J*= 8.0 Hz), 2.63-2.65 (m, 4H, $2CH_2$), 3.29 (s, 2H, CH_2), 3.45-3.49 (m, 4H, $2CH_2$), 4.09 (q, 2H, CH_2, *J*= 8.0 Hz), 7.01-7.04 (m, 2H, ar-H, *J*= 8.0 Hz), 7.85 (bs, 1H, NH), 8.03-8.08 (m, 2H, ar-H), 9.12 (bs, 1H, NH), 9.68 (bs, 1H, NH).^{13}C NMR (DMSO-d_6, δ ppm): 14.58 (CH_3), 46.79($2CH_2$), 51.87 ($2CH_2$), 58.54 (CH_2), 60.36 (CH_2), arC: [113.08 (CH), 113.11 (CH), 126.16 (2CH), 137.32 (2C)], 155.16 (C=O), 170.30 (C=S). LC-MS m/z (%): 367.16 ($[M+1]^+$, 100). Elemental Anal. Calcd. for $C_{15}H_{22}N_6O_3S$ (%):C, 49.17; H, 6.05; N, 22.93, Found (%): C, 49.14; H, 6.08; N, 22.96.

N-Phenyl-2-{[4-(4-nitrophenyl)piperazin-1-yl]acetyl} hydrazincarbothioamide (6d)

mp.192-194°C Yield: 73% (Method 1), 92% (Method 2). FT-IR ($\upsilon_{max,}$ cm^{-1}): 3314, 3277, 3216 (NH), 3053 (ar-H), 1744 (C=O). ^{1}H NMR (DMSO-d_6, δ ppm): 2.48 (s, 4H, $2CH_2$), 3.29 (s, 2H, CH_2), 3.44 (s, 4H, $2CH_2$), 6.92 (d, 2H, ar-H, *J*= 4.0 Hz), 7.13 (m, 1H, ar-H), 7.22 (m, 2H, ar-H), 7.34 (m, 2H, ar-H), 7.99 (d, 2H, ar-H, *J*= 4.0 Hz), 8.03 (bs, 1H, NH), 8.84 (bs, 2H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 52.00 ($2CH_2$), 54.90 ($2CH_2$), 61.84 (CH_2), arC: [112.30 (2CH), 124.80 (2CH), 126.56 (2CH),128.41 (CH), 129.35 (2CH), 137.42 (C), 138.54 (C), 155.76 (C)], 170.33 (C=O), 181.12 (C=S). LC-MS m/z (%): 415.15 ($[M+1]^+$, 100), 223.18 (32). Elemental Anal. Calcd. For $C_{19}H_{22}N_6O_3S$ (%): C, 55.06; H, 5.35; N, 20.28; Found (%): C, 54.14; H, 5.02; N, 19.98.

N-Phenyl-2-{[4-(4-nitrophenyl)piperazin-1-yl]acetyl} hydrazincarboxamide (6e)

mp. 211-212°C Yield: 76% (Method 1), 93% (Method 2). FT-IR ($\upsilon_{max,}$ cm^{-1}): 3258, 3223, 3199 (NH), 1689 (C=O), 1481 and 1332 (NO_2). ^{1}H NMR (DMSO-d_6, δ ppm): 2.62 (s, 4H, $2CH_2$), 3.13 (s, 2H, CH_2), 3.49 (s, 4H, $2CH_2$), 6.94-7.02 (m, 3H, ar-H), 7.26 (d, 2H, ar-H, *J*=4.0 Hz), 7.46-7.52 (m, 2H, ar-H), 8.05 (d, 2H, ar-H, *J*=16.0 Hz), 8.09 (s, 1H, NH), 8.82 (s,1H, NH), 9.66 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 52.16 ($2CH_2$), 53.89 ($2CH_2$), 60.80 (CH_2), arC: [113.30 (2CH), 121.60 (2CH), 124.88 (2CH), 128.00 (CH), 128.90 (2CH), 136.92 (C), 139.40 (C), 155.70 (C)], 153.80 (C=O), 170.52 (C=O). LC-MS m/z (%): 398.17 ($[M]^+$, 100), 400.19 ($[M+2]^+$, 27). Elemental Anal. Calcd. For $C_{19}H_{22}N_6O_4$ (%):C, 57.28; H, 5.57; N, 21.09, Found (%): C, 56.67; H, 5.14; N, 20.84.

N-(4-fluorophenyl)-2-{[4-(4-nitrophenyl)piperazin-1-yl] acetyl}hydrazincarbothioamide (6f)

mp.201-202°C Yield: 79% (Method 1), 94% (Method 2). FT-IR ($\upsilon_{max,}$ cm^{-1}): 3329, 3254, 3221 (NH), 3081 (ar-H), 1736 (C=O), 1488 and 1311 (NO_2).^{1}H NMR (DMSO-d_6, δ ppm): 2.51 (s, 2H, CH_2), 2.65 (s, 4H, $2CH_2$), 3.34 (s, 4H, $2CH_2$), 7.05 (d, 2H, ar-H, *J*= 8.0 Hz), 7.18 (m, 2H, ar-H), 7.42 (s, 2H, ar-H), 8.06 (d, 2H, ar-H, *J*= 8.0 Hz), 9.61 (bs, 2H, 2NH), 9.92 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 50.87 ($2CH_2$), 54.93 ($2CH_2$), 56.90 (CH_2), arC: [111.33 (2CH), 113.79 (2CH), 121.41 (2CH), 126.29 (2CH), 137.65 (C), 150.87 (C), 155.18 (C)], 168.75 (C=O), 181.10 (C=S). LC-MS m/z (%): 433.14 ($[M+1]^+$, 100), 434.15 (45), 220.18 (22). Elemental Anal. Calcd. For $C_{19}H_{21}FN_6O_3S$ (%):C, 52.77; H, 4.89; N, 19.43, Found (%): C, 51.94; H, 4.32; N, 19.01.

2.1.6. General Method For The synthesis of Compounds (7a-f)

Method 1

A solution of corresponding carbo(thio)amide **6** (10 mmol) in ethanol/water (1:1) was refluxed in the presence of 2N NaOH (20 mmol) for 7 h (for 7**a**), 8 h (for **7b**), 12 h (for **7c**) or 13 h (for **7d** and **7e**). The resulting solution was then cooled to room temperature and acidified to pH 5 with 37% HCl. The precipitate formed was collected by filtration, washed with water, and recrystallized from dimethylformamide to give the target compounds.

Method 2

A solution of corresponding carbo(thio)amide **6** (10 mmol) and 2N NaOH (20 mmol) in ethanol/water (1:1) was irradiated in monomode microwave reactor in open vessel at 150 W for 14 min. The resulting solution was then acidified to pH 5 with 37% HCl. The precipitate formed was collected by filtration, washed with water, and recrystallized from dimethylformamide to give the target compounds.

Benzyl-5-{[4-(4-nitrophenyl)piperazin-1-yl]methyl}-4H-1,2,4-triazole-3-thiol (7a)

mp.218-220 °C, Yield: 87% (Method 1), 95% (Method 2). FT-IR ($\upsilon_{max,}$ cm^{-1}): 3113 (NH), 3035 (ar-CH), 2821 (SH), 1482 and 1305 (NO_2). ^{1}H NMR (DMSO-d_6, δ ppm): 1.04-1.07 (m, 4H, $2CH_2$), 2.50-3.46 (m, 6H, $3CH_2$ + H2O), 5.34 (s, 2H, CH_2), 6.94-6.97 (m, 2H, ar-H), 6.98-7.57 (m, 5H, ar-H), 8.03 (d, 2H, ar-H, *J*= 8.0 Hz), 13.88 (bs, 1H, SH). ^{13}C NMR (DMSO-d_6, δ ppm): 46.37 (CH_2), 46.67 (CH_2), 46.77 (CH_2), 51.88 (CH_2), 52.06 (CH_2), 52.24 (CH_2), arC: [113.09 (CH), 113.14 (CH), 126.14 (CH), 127.37 (CH), 127.79 (CH), 128.75 (CH), 128.85 (CH), 129.40 (CH), 129.65 (CH), 134.50 (C), 136.69 (C), 149.57 (C)], 155.05 (triazole C-5), 181.34 (triazole C-3). LC-MS m/z (%): 433.13 ($[M+ Na]^+$, 10), 412.16 ($[M+2]^+$, 22), 411.15 ($[M+1]^+$, 100). Elemental Anal. Calcd. for$C_{20}H_{22}N_6O_2S$ (%):C, 58.52; H, 5.40; N, 20.47, Found (%): C, 58.57; H, 5.43; N, 20.49.

5-{[4-(4-Nitrophenyl)piperazin-1-yl]methyl}-4-benzyl-4H-1,2,4-triazole-5-one (7b)

mp.242-243 °C, Yield: 73% (Method 1), 85% (Method 2). FT-IR ($\upsilon_{max,}$ cm^{-1}): 3164 (NH), 3027 (ar-CH), 1689 (C=O), 1479 and 1313 (NO_2). ^{1}H NMR (DMSO-d_6, δ ppm): 2.41 (d, 2H, CH_2, *J*=4.0 Hz), 2.50 (t, 2H, CH_2, *J*=4.0 Hz), 3.24 (s, 2H, CH_2), 3.32 (s, 2H, CH_2), 3.36 (s, 4H, $2CH_2$ +H_2O), 4.89 (s, 2H, CH_2), 6.97 (d, 2H, ar-H, *J*=4.0 Hz), 7.26 (d, 3H, ar-H, *J*=8.0 Hz), 7.32 (d, 2H, CH_2, *J*=4.0 Hz), 8.03 (d, 2H, ar-H, *J*= 12.0 Hz), 11.75 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 42.43 (CH_2), 51.27 ($2CH_2$), 51.78 ($2CH_2$), 56.11 (CH_2), arC: [112.41 (2CH), 126.45 (2CH), 128.01 (CH), 128.53 (2CH), 128.76 (2CH), 137.06 (C), 138.88 (C), 146.90 (C)], 155.70 (triazole C-5), 158.23 (triazole C-3). LC-MS m/z (%): 417.18 ($[M+ Na]^+$, 43), 395.19 ($[M+1]^+$, 100). Elemental Anal. Calcd. For $C_{20}H_{22}N_6O_3$ (%): C, 60.90; H, 5.62; N, 21.31, Found (%): C, 59.97; H, 5.13; N, 20.89.

5-{[4-(4-Nitrophenyl)piperazin-1-yl]methyl}-4-ethyl-4H-1,2,4-triazole-3-thiol (7c)

mp.215-216°C, Yield: 75% (Method 1), 88% (Method 2). FT-IR ($\upsilon_{max,}$ cm^{-1}): 3088 (ar-CH), 2830 (SH), 1589 and 1336 (NO_2). ^{1}H NMR (DMSO-d_6, δ ppm): 1.27 (t, 3H, CH_3, *J*= 8.0 Hz), 2.48-3.54 (m, 2H, CH_2 + DMSO), 2.63 (d, 2H, CH_2, *J*= 8.0 Hz), 3.12 (s, 2H, CH_2), 3.43-3.46 (m, 2H, CH_2 + H2O), 3.62 (s, 2H, CH_2), 4.02 (q, 2H, CH_2, *J*= 8.0 Hz), 6.99 (d, 2H, ar-H, *J*= 12.0 Hz), 8.02 (d, 2H, ar-H, *J*= 12.0 Hz), 13.60 (bs, 1H, SH). ^{13}C NMR (DMSO-d_6, δ ppm): 13.75 (CH_3), 46.65 (CH_2), 46.73 (CH_2), 51.89 ($2CH_2$), 52.07 (CH_2), 58.68 (CH_2), arC: [113.09 (CH), 113.19 (CH), 126.14 (CH), 126.16 (CH), 137.29 (C), 149.26 (C)], 155.11 (triazole C-5), 171.78 (triazole C-3). LC-MS m/z (%): 389.43 ($[M+ K+2]^+$, 100), 375.47 ($[M+ Na+4]^+$, 85). Elemental Anal. Calcd. for$C_{15}H_{20}N_6O_2S$ (%):C, 51.71; H, 5.79; N, 24.12, Found (%): C, 51.75; H, 5.82; N, 24.10.

5-{[4-(4-Nitrophenyl)piperazin-1-yl]methyl}-4-phenyl-4H-1,2,4-triazole-3-thiol (7d)

mp.198-200 °C, Yield: 81% (Method 1), 92% (Method 2). FT-IR ($\upsilon_{max,}$ cm^{-1}): 3109 (NH), 3018 (ar-CH), 2850 (SH), 1480 and 1310 (NO_2). ^{1}H NMR (DMSO-d_6, δ ppm): 2.37 (t, 4H, $2CH_2$, *J*=12.0 Hz), 3.28 (t, 4H, $2CH_2$, *J*=12.0 Hz), 3.36 (s, 2H, CH_2), 6.97 (d, 2H, ar-H, *J*=8.0 Hz), 7.36-7.41 (m, 3H, ar-H), 7.53-7.57 (m, 2H, ar-H), 8.03 (d, 2H, ar-H, *J*= 8.0

Hz), 13.90 (s, 1H, SH). ^{13}C NMR (DMSO-d_6, δ ppm): 48.84 (CH_2), 50.50 ($2CH_2$), 52.19 ($2CH_2$), arC: [113.35 (2CH), 126.29 (2CH), 127.21 (2CH), 129.35 (2CH), 132.60 (CH), 137.65 (C), 143.18 (C), 143.18 (C)], 156.79 (triazole C-5), 174.33 (triazole C-3). LC-MS m/z (%): 419.14 ([M+ Na]$^+$, 100), 420.15 ([M+2]$^+$, 31). Elemental Anal. Calcd. For $C_{19}H_{20}N_6O_2S$ (%): C, 57.56; H, 5.08; N, 21.20, Found (%): C, 57.02; H, 4.89; N, 20.79.

5-{[4-(4-Nitrophenyl)piperazin-1-yl]methyl}-4-phenyl-4H-1,2,4-triazole-5-one (7e)

mp.242-243 °C, Yield: 68% (Method 1), 81% (Method 2). FT-IR ($\upsilon_{max,}$ cm^{-1}): 3184 (NH), 3046 (ar-CH), 1676 (C=O), 1475 and 1305 (NO_2). ^{1}H NMR (DMSO-d_6, δ ppm): 2.64 (t, 4H, $2CH_2$, *J*=12.0 Hz), 3.28 (t, 4H, $2CH_2$, *J*=12.0 Hz), 3.35 (s, 2H, CH_2), 6.92-7.06 (m, 3H, ar-H), 7.23-7.25 (m, 1H, ar-H), 7.27-7.51 (m, 3H, ar-H), 8.03 (d, 2H, ar-H, *J*= 8.0 Hz), 9.62 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 51.16 ($2CH_2$), 51.74 ($2CH_2$), 53.16 (CH_2), arC: [113.33 (2CH), 125.52 (2CH), 126.29 (2CH), 127.01 (CH), 129.21 (2CH), 133.28 (C), 137.65 (C), 151.98 (C)], 151.85 (triazole C-3), 157.15 (triazole C-5). LC-MS m/z (%): 419.16 ([M+ K]$^+$, 48), 381.17 ([M+1]$^+$, 100). Elemental Anal. Calcd. For $C_{19}H_{20}N_6O_3$ (%): C, 59.99; H, 5.30; N, 22.09, Found (%): C, 59.18; H, 5.04; N, 21.88.

5-{[4-(4-Nitrophenyl)piperazin-1-yl]methyl}-4-(4-fluorophenyl)-4H-1,2,4-triazole-3-thiol (7f)

mp.234-235 °C, Yield: 82% (Method 1), 93% (Method 2). FT-IR ($\upsilon_{max,}$ cm^{-1}): 3183 (NH), 3056 (ar-CH), 2831 (SH). ^{1}H NMR (DMSO-d_6, δ ppm): 2.37 (t, 4H, $2CH_2$, *J*=12.0 Hz), 3.28 (t, 4H, $2CH_2$, *J*=12.0 Hz), 3.42 (s, 2H, CH_2), 6.97 (d, 2H, ar-H, *J*=8.0 Hz), 7.36-7.41 (m, 2H, ar-H), 7.53-7.57 (m, 2H, ar-H), 8.03 (d, 2H, ar-H, *J*= 8.0 Hz), 13.90 (s, 1H, SH). ^{13}C NMR (DMSO-d_6, δ ppm): 50.36 ($2CH_2$), 51.54 ($2CH_2$), 56.98 (CH_2) arC: [112.41 (2CH), 116.54 (2CH), 126.40 (2CH), 129.13 (2CH), 134.49 (C), 138.88 (C), 158.23 (C), 162.88 (d, J_{C-F}=42.0 Hz], 156.82 (triazole C-5), 174.45 (triazole C-3). LC-MS m/z (%): 414.13 ([M+1]$^+$, 100), 425.15 ([M+2]$^+$, 83), 436.13 ([M+Na]$^+$, 24). Elemental Anal. Calcd. For $C_{19}H_{19}FN_6O_2S$ (%):C, 55.06; H, 4.62; F, 4.58; N, 20.28, Found (%): C, 54.72; H, 4.13; N, 19.89.

2.1.7. General Method for The synthesis of Compounds (8a-l)

Method 1

The solution of ciprofloxacin (for **8g-l**) or norfloxacin (for **8a-f**) (10 mmol) in dimethyl formamide was stirred at room temperature in the presence of formaldehyde (37 %, 30 mmol) for 15 min. Then, the corresponding compound **7** was added into it and stirred at room temperature for additional 24 h. The reaction mixture was poured to ice-water and a solid obtained. This crude product was collected by filtration and recrystallized from dimethyl sulfoxide to afford the desired product.

Method 2

The mixture of ciprofloxacin (for **8g-l**), norfloxacin (for **8a-f**) (10 mmol), the corresponding compound **7** and formaldehyde (30 mmol) was irradiated in monomode microwave reactor in closed vessel with pressure control at 80 W for 15 min. The solid obtained was recrystallized from dimethyl sulfoxide to afford the desired product.

1-Ethyl-6-fluoro-7-{4-[(3-{[4-(4-nitrophenyl)piperazin-1-yl]methyl}-4-benzyl-5-thioxo-4,5-dihydro-1H-1,2,4-triazole-1-yl)methyl]piperazin-1-yl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (8a)

mp. 216 °C (dec.), Yield: 87% (Method 1), 94% (Method 2). FT-IR ($\upsilon_{max,}$ cm^{-1}): 3060 (ar-CH), 1716 (C=O), 1630(C=O), 1447 and 1332 (NO_2), 1248 (C-O). ^{1}H NMR (DMSO-d_6, δ ppm): 1.43 (t, 3H, CH_3, *J*= 4.0 Hz) 2.44 (s, 4H, $2CH_2$), 2.95 (s, 4H, $2CH_2$), 3.21-3.24 (m, 4H, $2CH_2$+H_2O), 3.37 (s, 4H, $2CH_2$), 3.54 (s, 2H, CH_2), 4.58 (s, 2H, CH_2), 5.20 (s, 2H, CH_2), 5.42 (s, 2H, CH_2), 6.92 (d, 2H, ar-H, *J*= 8.0 Hz), 7.16-7.33 (m, 5H, ar-H), 7.91 (d, 2H, ar-H, *J*= 12.0 Hz), 8.01 (d, 2H, ar-H, *J*= 8.0 Hz), 8.91 (s, 1H, quinolone =CH), 15.24 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6, δ ppm): The spectrum could not be obtained, due to the slight solubility in any NMR solvent. LC-MS m/z (%): 764.29 ([M+Na]$^+$, 10), 744.31 ([M+3]$^+$, 15), 743.31 ([M+2]$^+$, 45), 742.30 ([M+1]$^+$, 100). Elemental Anal. Calcd. For $C_{37}H_{40}FN_9O_5S$ (%): C, 59.91; H, 5.43; N, 16.99, Found (%): C, 59.94; H, 5.47; N, 17.03.

1-Ethyl-6-fluoro-7-{4-[(3-{[4-(4-nitrophenyl)piperazin-1-yl]methyl}-4-benzyl-5-oxo-4,5-dihydro-1H-1,2,4-triazole-1-yl)methyl]piperazin-1-yl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (8b)

mp. 235 °C, Yield: 88% (Method 1), 96% (Method 2). FT-IR ($\upsilon_{max,}$ cm^{-1}): 3053 (ar-CH), 1744 (C=O), 1660(C=O), 1442 and 1328 (NO_2), 1241 (C-O). ^{1}H NMR (DMSO-d_6, δ ppm): 1.41 (t, 3H, CH_3, *J*= 12.0 Hz), 2.44 (s, 4H, $2CH_2$), 2.51 (s, 4H, $2CH_2$), 2.82 (s, 4H, $2CH_2$), 3.23 (s, 4H, $2CH_2$), 3.39 (s, 2H, CH_2), 4.58 (d, 2H, CH_2, *J*=4.0 Hz), 4.69 (s, 2H, CH_2), 4.96 (s, 2H, CH_2), 6.94 (d, 2H, ar-H, *J*= 8.0 Hz), 7.17 (d, 1H, ar-H, *J*=8.0 Hz), 7.25-7.33 (m, 5H, ar-H), 7.91 (d, 1H, ar-H, *J*= 16.0 Hz), 8.02 (d, 2H, ar-H, *J*= 8.0 Hz), 8.94 (s, 1H, quinolone =CH), 15.33 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6, δ ppm): 12.41 (CH_3), 42.69 (CH_2), 48.86 (CH_2), 50.36 ($2CH_2$), 50.76 ($2CH_2$), 51.43 ($2CH_2$), 51.54 ($2CH_2$), 56.37 (CH_2), 63.93 (CH_2), arC: [112.41 (2CH), 114.25 and 114.45 (d, CH, *J*=4.0 Hz), 126.43 (2CH), 128.01 (CH), 128.53 (2CH), 128.76 (2CH), 138.88 (C), 158.23 (C)], 102.55 (CH), 111.26 (C), 118.19 (C), 137.01 (C), 138.84 (triazole C-5), 146.55 and 146.90 (d, C, *J*= 9.0 Hz), 148.80 (CH), 150.10 and 152.20 (d, C-F, J_{C-F} = 105.0 Hz), 149.78 (C=O), 153.02 (triazole C-3), 167.02 (C=O), 176.57 (C=O). LC-MS m/z (%): 748.31 ([M+Na]$^+$, 23), 727.32 ([M+2]$^+$, 39), 726.31 ([M+1]$^+$, 100). Elemental Anal. Calcd. For $C_{37}H_{40}FN_9O_6$ (%): C, 61.23; H, 5.56; N, 17.37, Found (%): C, 60.91; H, 5.17; N, 17.02.

1-Ethyl-6-fluoro-7-{4-[(3-{[4-(4-nitrophenyl)piperazin-1-yl]methyl}-4-ethyl-5-thioxo-4,5-dihydro-1H-1,2,4-triazole-1-yl)methyl]piperazin-1-yl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (8c)

mp. 265-266 °C, Yield: 90% (Method 1), 97% (Method 2). FT-IR ($\upsilon_{max,}$ cm^{-1}): 3037 (ar-CH), 1708 (C=O), 1626

(C=O), 1463 and 1363 (NO_2). ^{1}H NMR (DMSO-d_6, δ ppm): 1.30 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 2.48-2.55 (m, 8H, $4CH_2$ + DMSO), 2.67-2.71 (s, 2H, CH_2), 2.86 (s, 2H, CH_2), 3.31-3.40 (m, 4H, $2CH_2$+H_2O), 3.68 (s, 2H, CH_2), 4.08 (bs, 2H, CH_2), 4.56 (s, 2H, CH_2), 5.09 (s, 2H, CH_2), 6.97 (d, 2H, ar-H, *J*= 8.0 Hz), 7.14 (s, 1H, ar-H), 7.89 (s, 1H, ar-H), 8.00 (d, 2H, ar-H, *J*= 8.0 Hz), 8.92 (s, 1H, quinolone =CH), 15.24 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6, δ ppm): The spectrum could not be obtained, due to the slight solubility in any NMR solvent. LC-MS m/z (%): 680.77 ([M+1]$^+$, 100). Elemental Anal. Calcd. For $C_{32}H_{38}FN_9O_5S$ (%):C, 56.54; H, 5.63; N, 18.54, Found (%): C, 56.58; H, 5.67; N, 18.53.

1-Ethyl-6-fluoro-7-{4-[(3-{[4-(4-nitrophenyl)piperazin-1-yl]methyl}-4-phenyl-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl]piperazin-1-yl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (8d)

mp. 248 °C, Yield: 84% (Method 1), 91% (Method 2). FT-IR ($\upsilon_{max,}$ cm^{-1}): 3372 (OH), 3044 (ar-H), 1716 (C=O), 1625(C=O), 1494 and 1325 (NO_2), 1226 (C-O). ^{1}H NMR (DMSO-d_6, δ ppm): 1.37 (t, 3H, CH_3), 2.77 (s, 4H, $2CH_2$), 2.93 (s, 4H, $2CH_2$), 3.29 (s, 4H, $2CH_2$), 3.36 (bs, 4H, $2CH_2$+ H_2O), 4.22 (s, 2H, CH_2), 4.56 (s, 2H, CH_2), 5.20 (s, 2H, CH_2), 5.85 (s, 1H, ar-H), 6.30-6.35 (m, 1H, ar-H), 6.73 (s, 3H, ar-H), 7.95 (s, 2H, ar-H), 7.40-7.46 (m, 3H, ar-H), 7.93 (d, 1H, ar-H, *J*= 8.0 Hz), 8.90 (s, 1H, quinolone =CH), 15.33 (bs, 1H, COOH). ^{13}C NMR (DMSO-d_6, δ ppm): 14.36 (CH_3), 48.70 ($2CH_2$), 49.56 ($2CH_2$), 49.91 ($2CH_2$), 51.53 ($2CH_2$), 51.85 ($2CH_2$), 69.74 (CH_2), arC: [101.40 and 101.64 (d, CH, *J*= 24.0 Hz), 115. 76 (2CH), 115.72 (CH), 116.05 (CH), 119.54 (CH), 129. 13 (2CH), 129.25 (C), 130.81 (2CH), 144.67 (C), 149.69 (C)], 106. 97 (CH), 108.74 (C), 111.38 and 111.62 (d, CH, *J*= 24.0 Hz), 120. 95 (C), 139.94 (C), 144.51 (C), 148.49 (CH), 151.81 (triazole C-5), 152.53 and 155.01 (d, C-F, $J_{C\text{-}F}$ = 248.0 Hz), 166.79 (C=O), 173.04 (triazole C-3), 176.79 (C=O). LC-MS m/z (%): 728,27 ([M+1] $^+$, 100). Elemental Anal. Calcd. For$C_{36}H_{38}FN_9O_5S$ (%): C, 59.41; H, 5.26; N, 17.32, Found (%):C, 59.38; H, 5.27; N, 17.31.

1-Ethyl-6-fluoro-7-{4-[(3-{[4-(4-nitrophenyl)piperazin-1-yl]methyl}-4-phenyl-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl]piperazin-1-yl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (8e)

mp. 190-192 °C, Yield: 83% (Method 1), 92% (Method 2). FT-IR ($\upsilon_{max,}$ cm^{-1}): 3046 (ar-CH), 1748 (C=O), 1679 (C=O), 1492 and 1320 (NO_2), 1251 (C-O). ^{1}H NMR (DMSO-d_6, δ ppm): 1.44 (s, 3H, CH_3), 2.44 (s, 4H, $2CH_2$), 2.52 (s, 4H, $2CH_2$), 2.72 (s, 4H, $2CH_2$), 2.89 (s, 4H, $2CH_2$), 3.26 (bs, CH_2 + H_2O), 4.61 (s, 2H, CH_2), 4.73 (s, 2H, CH_2), 6.96 (s, 2H, ar-H), 7.20 (s, 2H, ar-H), 7.55 (s, 4H, ar-H), 7.93 (s, 1H, ar-H), 8.03 (s, 2H, ar-H), 8.96 (s, 1H, quinolone =CH), 15.36 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6, δ ppm): 12.42 (CH_3), 48.86 (CH_2), 50.36 ($2CH_2$), 50.76 ($2CH_2$), 51.44 ($2CH_2$), 51.56 ($2CH_2$), 55.68 (CH_2), 63.93 (CH_2), arC: [112.41 (CH), 114.24 and 114.45 (d, CH, *J*=10.5 Hz), 118.19 (CH), 126.40 (2CH), 126.58 (2CH), 128.91 (2CH), 129.32 (CH), 134.33 (C),], 102.55 (CH), 111.26 (C), 138.74 (C), 138.89 (C), 148.43 (triazole C-5), 146.66 and 146.88 (d, C, *J*= 11.0 Hz), 150.12 and 152.21 (d, C-F, $J_{C\text{-}F}$ = 105.0 Hz), 158.28 (C), 148.86 (CH), 150.31 (triazole C-3), 167.05 (C=O), 177.18 (C=O). LC-MS m/z (%): 734.30 ([M+Na]$^+$, 28), 712.29 ([M+1] $^+$, 100), 666.28 (36). Elemental Anal. Calcd. for $C_{36}H_{38}FN_9O_6$ (%): C, 60.75; H, 5.38; N, 17.71; Found (%): C, 60.11; H, 5.05; N, 16.96.

1-Ethyl-6-fluoro-7-{4-[4-(4-fluorophenyl)]-[(3-{[4-(4-nitrophenyl)piperazin-1-yl]methyl}-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl[piperazin-1-yl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (8f)

mp. 242 °C, Yield: 83% (Method 1), 93% (Method 2). FT-IR ($\upsilon_{max,}$ cm^{-1}): 3070 (ar-H), 1728 (C=O), 1656 (C=O), 1492 and 1323 (NO_2), 1228 (C-O). ^{1}H NMR (DMSO-d_6, δ ppm): 1.42 (s, 3H, CH_3), 2.38 (s, 4H, $2CH_2$), 2.51 (s, 4H, $2CH_2$), 2.70 (s, 2H, CH_2), 2.99 (s, 2H, CH_2), 3.26 (s, 4H, $2CH_2$), 4.59 (s, 4H, $2CH_2$), 5.20 (s, 2H, CH_2), 6.95 (s, 2H, ar-H), 7.19 (s, 1H, ar-H), 7.40 (s, 2H, ar-H), 7.58 (s, 2H, ar-H), 7.92 (d, 1H, ar-H, *J*= 12.0 Hz), 8.02 (s, 2H, ar-H), 8.95 (s, 1H, quinolone =CH), 15.37 (bs, 1H, COOH). ^{13}C NMR (DMSO-d_6, δ ppm): 12.45 (CH_3), 48.05 (CH_2), 50.35 ($2CH_2$), 50.94 ($2CH_2$), 51.85 ($2CH_2$), 52.05 ($2CH_2$), 55.74 (CH_2), 63.51 (CH_2), arC: [112.41 (2CH), 114.25 and 114.48 (d, CH, *J*= 11.5 Hz), 115.85 and 116.07 (d, 2CH, *J*= 11.0 Hz), 126.40 (2CH), 129.84 (2CH), 133.36 (C), 138.75 (C), 158.23 (C), 161.32 and 163.41(d, C-F, $J_{C\text{-}F}$ = 104.5 Hz)], 102.45 (CH), 111.38 (C), 118.19 (C), 138.45 (C), 146.77 (C), 148.81 (CH), 150.22 and 152.15 (d, C-F, $J_{C\text{-}F}$ = 96.5 Hz), 154.35 (triazole C-5), 167.02 (C=O), 171.13 (triazole C-3), 178.41 (C=O). LC-MS m/z (%): 746.27 ([M+1] $^+$, 100), 747.25 ([M+2] $^+$, 37). Elemental Anal. Calcd. For $C_{36}H_{37}F_2N_9O_5S$ (%):C, 57.98; H, 5.00; N, 16.90, Found (%): C, 56.97; H, 4.83; N, 16.32.

1-Cyclopropyl-6-fluoro-7-{4-[(3-{[4-(4-nitrophenyl) piperazin-1-yl]methyl}-4-benzyl-5-thioxo-4,5-dihydro-1H-1,2,4-triazole-1-yl)methyl]piperazin-1-yl}-4-oxo-1,4-dihydroquinoline-3-carboxylicacid (8g)

mp. 225-226 °C, Yield: 91% (Method 1), 97% (Method 2). FT-IR ($\upsilon_{max,}$ cm^{-1}): 3086 (ar-CH), 1732 (C=O), 1668(C=O), 1495 and 1322 (NO_2), 1256 (C-O). ^{1}H NMR (DMSO-d_6, δ ppm): 1.16 (d, 2H, CH_2, *J*=8.0 Hz), 1.34 (d, 2H, CH_2, *J*= 8.0 Hz) 2.47 (s, 4H, $2CH_2$), 2.98 (s, 4H, $2CH_2$), 3.16 (s, 4H, $2CH_2$), 3.38 (s, 4H, $2CH_2$), 3.55 (s, 2H, CH_2), 3.81 (s, 1H, CH), 5.20 (s, 2H, CH_2), 5.43 (s, 2H, CH_2), 6.92 (d, 2H, ar-H, *J*= 8.0 Hz), 7.30 (s, 5H, ar-H), 7.55 (d, 1H, ar-H, *J*= 4.0 Hz), 7.88 (d, 1H, ar-H, *J*= 12.0 Hz), 8.01 (d, 2H, ar-H, *J*= 8.0 Hz), 8.66 (s, 1H, quinolone =CH), 15.01 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6, δ ppm): 8.04 ($2CH_2$), 36.24 (CH), 46.57 ($2CH_2$), 47.97 ($2CH_2$), 49.99 ($2CH_2$), 50.26 ($2CH_2$), 52.13 ($2CH_2$), 69.22 (CH_2), arC: [109.37 (C), 113.17 (2CH), 126.01 (2CH), 127.35 (2CH), 127.87 (2CH), 128.87 (CH), 136.48 (C), 139.68 (C), 148.49 (C)], 106. 84 (CH), 107.52 (C), 111.39 and 111.62 (d, CH, *J*= 23.0 Hz), 119.22 (C), 137.77(C), 145.64 and 145.84 (d, C, *J*= 10.0 Hz), 148.29 (CH), 149.46 (triazole C-5), 152.53 and 155.01 (d, C-F, $J_{C\text{-}F}$ = 248.0 Hz), 166.20 (C=O), 170.47 (triazole C-3), 176.87 (C=O). LC-MS m/z (%): 776.31 ([M+Na]$^+$, 10), 754.33 ([M+1] $^+$, 100). Elemental Anal. Calcd. for $C_{38}H_{40}FN_9O_5S$ (%):C, 60.54; H, 5.35; N, 16.72, Found (%): C, 60.58; H, 5.37; N, 16.69.

1-Cyclopropyl-6-fluoro-7-{4-[(3-{[4-(4-nitrophenyl) piperazin-1-yl]methyl}-4-benzyl-5-oxo-4,5-dihydro-1H-1,2,4-triazole-1-yl)methyl]piperazin-1-yl}-4-oxo-1,4-dihydroquinoline-3-carboxylicacid (8h)

mp. 151-153 °C, Yield: 88% (Method 1), 94% (Method 2). FT-IR ($\upsilon_{max,}$ cm^{-1}): 3046 (ar-CH), 1725 (C=O), 1674 (C=O), 1491 and 1319 (NO_2), 1251 (C-O). ^{1}H NMR (DMSO-d_6, δ ppm): 1.20 (s, 2H, CH_2), 1.33 (s, 2H, CH_2) 2.44 (s, 4H, $2CH_2$), 2.51 (s, 4H, $2CH_2$), 2.73 (s, 2H, CH_2), 3.25 (s, 4H, $2CH_2$), 3.39 (s, 4H, $2CH_2$), 3.81 (s, 1H, CH), 4.94 (s, 2H, CH_2), 5.04 (d, 2H, CH_2,J=8.0 Hz), 6.96-6.98 (m, 2H, ar-H), 7.28-7.35 (m, 5H, ar-H), 7.56-7.60 (m, 1H, ar-H), 7.91 (d, 1H, ar-H, *J*= 12.0 Hz), 8.04 (d, 2H, ar-H, *J*= 8.0 Hz), 8.66 (s, 1H, quinolone =CH), 15.22 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6, δ ppm): 9.15 ($2CH_2$), 37.62 (CH), 42.69 (CH_2), 50.35 ($2CH_2$), 50.78 ($2CH_2$), 51.44 ($2CH_2$), 51.58 ($2CH_2$), 57.38 (CH_2), 65.95 (CH_2), arC: [112.44 (CH), 114.67 (C), 126.43 (2CH), 128.11 (2CH), 128.53 (2CH), 128.76 (2CH), 136.30 (C), 138.85 (C), 158.23 (C)], 107.59 (CH), 109.37 (C), 113.23 and 113.33 (d, CH, *J*= 10.5 Hz), 137.06 (C), 147.79 (CH), 148.95 and 150.86 (d, C, *J*= 95.5 Hz), 150.78 (triazole C-3), 153.02 (triazole C-5), 167.05 (C=O), 178.57 (C=O). LC-MS m/z (%): 776.31 ([M+K]$^+$, 27), 738.31 ([M+1] $^+$, 100). Elemental Anal. Calcd. for $C_{38}H_{40}FN_9O_6$ (%): C, 61.86; H, 5.46; N, 17.09, Found (%): C, 61.08; H, 5.11; N, 16.73.

1-Cyclopropyl-6-fluoro-7-{4-[(3-{[4-(4-nitrophenyl) piperazin-1-yl]methyl}-4-ethyl-5-thioxo-4,5-dihydro-1H-1,2,4-triazole-1-yl)methyl]piperazin-1-yl}-4-oxo-1,4-dihydroquinoline-3-carboxylicacid (8i)

mp. 240-241 °C (dec.), Yield: 86% (Method 1), 92% (Method 2). FT-IR ($\upsilon_{max,}$ cm^{-1}): 3019 (ar-CH), 1719 (C=O), 1626 (C=O), 1464 and 1330 (NO_2). ^{1}H NMR (DMSO-d_6, δ ppm): 1.15 (d, 2H, CH_2, *J*=8.0 Hz), 1.30 (s, 5H, CH_3 +CH_2), 2.54 (s, 4H, $2CH_2$), 2.87 (s, 2H, CH_2), 3.06 (s, 2H, CH_2), 3.31-3.40 (m, 8H, $4CH_2$ + H_2O), 3.68 (s, 2H, CH_2), 3.80 (s, 1H, CH_2), 4.09 (s, 1H, CH), 5.09 (s, 2H, CH_2), 6.96-7.01 (m, 2H, ar-H), 7.51-7.56 (m, 1H, ar-H), 7.84-7.89 (m, 2H, ar-H), 8.01 (d, 1H, ar-H, *J*= 8.0 Hz), 8.64 (s, 1H, quinolone =CH), 15.11 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6, δ ppm): 8.04 ($2CH_2$), 36.24 (CH), 46.57 ($2CH_2$), 47.97 ($2CH_2$), 49.99 ($2CH_2$), 50.26 ($2CH_2$), 52.13 ($2CH_2$), 69.22 (CH_2), arC: [111.62 (C), 113.17 (2CH), 126.01 (2CH), 139.68 (C)], 106. 84 (CH), 107.52 (C), 111.39 (CH), 119.22 (C), 137.77(C), 145.64 and 145.84 (d, C, *J*= 10.0 Hz), 148.29 (CH), 152.53 and 155.01 (d, C-F, $J_{C\text{-}F}$ = 248.0 Hz) 155.21 (triazole C-5), 166.20 (C=O), 170.47 (triazole C-3), 176.87 (C=O). LC-MS m/z (%): 692.78 ([M+1] $^+$, 100). Elemental Anal. Calcd. For $C_{33}H_{38}FN_9O_5S$ (%): C, 57.30; H, 5.54; N, 18.22, Found (%): C, 57.33; H, 5.58; N, 18.26.

1-Cyclopropyl-6-fluoro-7-{4-[(3-{[4-(4-nitrophenyl) piperazin-1-yl]methyl}-4-phenyl-5-thioxo-4,5-dihydro-1H-1,2,4-triazole-1-yl)methyl]piperazin-1-yl}-4-oxo-1,4-dihydroquinoline-3-carboxylicacid (8j)

mp. 225-226 °C (dec.), Yield: 90% (Method 1), 95% (Method 2). FT-IR ($\upsilon_{max,}$ cm^{-1}): 3355 (OH), 3070 (ar-H), 1721 (C=O), 1624(C=O), 1494 and 1324 (NO_2), 1225 (C-O). ^{1}H NMR (DMSO-d_6, δ ppm): 1.04 (d, 2H, CH_2, *J*=8.0 Hz), 1.30 (d, 2H, CH_2, *J*= 8.0 Hz) 2.77 (s, 4H, $2CH_2$), 2.95 (s, 4H, $2CH_2$), 3.24 (s, 4H, $2CH_2$), 3.40 (bs, 4H, $2CH_2$+ H_2O), 3.76 (s, 1H, CH), 4.22 (s, 2H, CH_2), 5.20 (s, 2H, CH_2), 5.73 (d, 1H, ar-H, *J*= 8.0 Hz), 6.34-6.79 (m, 4H, ar-H), 7.95 (d, 2H, ar-H, *J*= 8.0 Hz), 7.40-7.47 (m, 3H, ar-H), 7.95 (d, 1H, ar-H, *J*= 8.0 Hz), 8.58 (s, 1H, quinolone =CH), 15.18 (bs, 1H, COOH). ^{13}C NMR (DMSO-d_6, δ ppm): 8.03 ($2CH_2$), 36.04 (CH), 48.77 ($2CH_2$), 49.06 ($2CH_2$), 49.96 ($2CH_2$), 51.35 ($2CH_2$), 51.58 (CH_2), 68.74 (CH_2), arC: [101.48 and 101.72 (d, CH, *J*= 24.0 Hz), 115.67 (2CH), 115.79 (CH), 116.72 (CH), 129.31 (2CH), 129.40 (C), 130.83 (2CH), 144.35 (C), 149.39 (C),], 106. 79 (CH), 108.64 (C), 111.36 and 111.60 (d, CH, *J*= 24.0 Hz), 119.45 and 120.59 (d, C, *J*=114.0 Hz), 139.49 (C), 144.46 (C), 148.24 (CH), 151.52 and 155.28 (d, C-F, $J_{C\text{-}F}$ = 376.0 Hz), 151.19 (triazole C-5), 166.43 (C=O), 169.79 (triazole C-3), 176.57 (C=O). LC-MS m/z (%): 740,27 ([M+1] $^+$, 100). Elemental Anal. Calcd. For $C_{37}H_{38}FN_9O_5S$ (%): C, 60.07; H, 5.18; N, 17.04, Found (%):C, 60.08; H, 5.17; N, 17.01.

1-Cyclopropyl-6-fluoro-7-{4-[(3-{[4-(4-nitrophenyl) piperazin-1-yl]methyl}-4-phenyl-5-oxo-4,5-dihydro-1H-1,2,4-triazole-1-yl)methyl]piperazin-1-yl}-4-oxo-1,4-dihydroquinoline-3-carboxylicacid (8k)

mp. 165-166 °C, Yield: 79% (Method 1), 88% (Method 2). FT-IR ($\upsilon_{max,}$ cm^{-1}): 3034 (ar-CH), 1728 (C=O), 1668 (C=O), 1489 and 1321 (NO_2), 1250 (C-O). ^{1}H NMR (DMSO-d_6, δ ppm): 1.18 (s, 2H, CH_2), 1.33 (s, 2H, CH_2) 2.42 (s, 4H, $2CH_2$), 2.51 (s, 4H, $2CH_2$), 2.89 (s, 4H, $2CH_2$), 3.25 (s, 4H, $2CH_2$), 3.70 (s, 2H, CH_2), 3.81 (s, 1H, CH), 4.73 (s, 2H, CH_2), 6.91-7.02 (m, 2H, ar-H), 7.23-7.27 (m, 1H, ar-H), 7.48 (s, 1H, ar-H), 7.53 (d, 4H, ar-H, *J*=4.0 Hz), 7.83 (d, 1H, ar-H, *J*= 12.0 Hz), 8.00 (t, 2H, ar-H, *J*= 16.0 Hz), 8.62 (s, 1H, quinolone =CH), 15.16 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6, δ ppm): 9.15 ($2CH_2$), 37.62 (CH), 50.24 ($2CH_2$), 50.81 ($2CH_2$), 51.45 ($2CH_2$), 51.62 ($2CH_2$), 55.68 (CH_2), 63.95 (CH_2), arC: [109.26 (C), 112.53 (2CH), 113.17 and 114.68 (C), 126.40 (2CH), 128.15 (2CH), 128.54 (2CH), 128.76 (2CH), 136.36 (C), 138.79 (C), 158.58 (C)], 107.55 (CH), 109.41 (C), 113.42 (d, CH, *J*= 12.5 Hz), 137.16 (C),147.81 (CH), 148.76 and 150.97 (d, C, *J*= 110.5 Hz), 151.78 (triazole C-5), 153.52 (triazole C-3), 167.46 (C=O), 176.77 (C=O). LC-MS m/z (%): 746.30 ([M+Na]$^+$, 31), 724.29 ([M+1] $^+$, 100). Elemental Anal. Calcd. for $C_{37}H_{38}FN_9O_6$ (%): C, 61.40; H, 5.29; N, 17.42; Found (%): C, 61.02; H, 5.08; N, 16.93.

1-Cyclopropyl-6-fluoro-7-{4-[4-(4-fluorophenyl)]-[(3-{[4-(4-nitrophenyl)piperazin-1-yl]methyl}-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl]piperazin-1-yl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (8l)

mp. 201-202 °C, Yield: 80% (Method 1), 91% (Method 2). FT-IR ($\upsilon_{max,}$ cm^{-1}): 3043 (ar-CH), 1744 (C=O), 1665 (C=O), 1491 and 1320 (NO_2), 1254 (C-O). ^{1}H NMR (DMSO-d_6, δ ppm): 1.08 (s, 2H, CH_2), 1.33 (s, 2H, CH_2), 2.54 (s, 4H, $2CH_2$), 2.72 (d, 8H, $4CH_2$, *J*=16.0 Hz), 2.82 (s, 2H, CH_2), 3.36 (s, 4H, $2CH_2$), 4.12 (s, 1H, CH), 5.23 (s, 2H, CH_2), 6.23 (s, 1H, ar-H), 6.85 (d, 2H, ar-H, *J*=8.0 Hz), 7.05 (d, 2H, ar-H, *J*=12.0 Hz), 7.37-7.45 (m, 4H, ar-H), 8.01-8.08 (m, 2H, ar-H), 8.66 (s, 1H, quinolone =CH), 15.21 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6, δ ppm): 7.78 ($2CH_2$), 36.81

(CH), 52.05 ($4CH_2$), 52.71 ($2CH_2$), 54.82 ($2CH_2$), 60.71 (CH_2), 65.77 (CH_2), arC: [112.34 (2CH), 115.53 and 116.09 (d, 2CH, *J*= 28.0 Hz), 126.42 (2CH), 129.88 (2CH), 133.54 (C), 138.75 (C), 158.35 (C), 161.42 and 163.42 (d, C-F, J_{C-F} = 100.0 Hz)], 102.73 (CH), 111.41 (C), 114.14 and 114.37 (d, CH, *J*= 11.5 Hz), 118.21 (C), 138.21 (C), 146.44 (C), 148.75 (CH), 150.16 and 152.32 (d, C-F, J_{C-F} = 108.0 Hz), 154.65 (triazole C-5), 167.74 (C=O), 173.25 (triazole C-3), 177.19 (C=O). LC-MS m/z (%):758.27 ($[M+1]^+$, 100), 712.26 (37), 680.25 (26). Elemental Anal. Calcd. for $C_{37}H_{38}FN_9O_6$ (%):C, 58.64; H, 4.92; N, 16.63; Found (%): C, 58.01; H, 4.23; N, 16.02.

2.2. Antimicrobial Activity

The test microorganisms were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and were as follows: *Escherichia coli* (*E. coli*) ATCC35218, *Yersinia pseudotuberculosis* (*Y. pseudotuberculosis*) ATCC911, *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC43288, *Enterococcus faecalis* (*E. faecalis*) ATCC29212, *Staphylococcus aureus* (*S. aureus*) ATCC25923, *Bacillus cereus* (*B. cereus*) 709 Roma, *Mycobacterium smegmatis* (*M. smegmatis*) ATCC607. All the newly synthesized compounds were weighed and dissolved in hexane to prepare extract stock solution of 20.000 microgram/milliliter (µg/mL). The antimicrobial effects of the substances were tested quantitatively in respective broth media by using double microdilution and the minimal inhibition concentration (MIC) values (±g/mL) were determined. The antibacterial and antifungal assays were performed in Mueller-Hinton broth (MH) (Difco, Detroit, MI) at pH.7.3 and buffered Yeast Nitrogen Base (Difco, Detroit, MI) at pH 7.0, respectively. The microdilution test plates were incubated for 18-24 h at 35 °C. Brain Heart Infusion broth (BHI) (Difco, Detriot, MI) wasused for *M. smegmatis*, and incubated for 48-72 h at 35 °C [39]. Ciprofloxacin, Norfloxacin, Ampicillin and Strepromicin were used as standard antibacterial and antimicobacterial drugs, respectively. Dimethylsulfoxide with dilution of 1:10 was used as solvent control. The results obtained were submitted in Table **2**.

2.3. Antioxidant Activity Studies

2.3.1. DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Activity

The scavenging activity of different chemicals was determined using the free radical DPPH (2,2-diphenyl-1-picrylhydrazyl), as described by Blois [40]. A 100-µL chemical solution was mixed with 1 ml of freshly prepared methanolic DPPH solution. The reaction mixture was incubated for 30 min at room temperature in the dark and was then measured at 520 nm. The activity was expressed as µmol Trolox equivalent.

2.3.2. FRAP (The Ferric Reducing Ability of Plasma)

FRAP was measured using the method described by Benzie & Strain [41] with some modification. To 100 µl of each sample was added 2900 µl freshly prepared FRAP reagent containing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyle-s-triazine) and 20 mM $FeCl.6H_2O$ in proportions of 10:1:1 (v/v). The mixture was incubated for 30 min at 37ºC and measured at 593 nm. The values were expressed as µmol of Trolox/g.

2.3.3. CUPRAC (Cupric Ion Reducing Antioxidant Capacity)

CUPRAC was measured following the procedure described by Apak *et al.* [42] with some modification. Briefly, 100 µL of each chemical solution was mixed with 900 µL bidistilled water, 1 ml acetate buffer solution (1 mM, pH: 7.0), 1 ml $CuCl_2$ (10 mM) and 1 ml 7.5 mM neocuproine to a final volume of 4 ml. The reaction mixture was then incubated in the dark for 30 min at room temperature, and the absorbance of the reaction mixture was measured at 450 nm against water blank. Trolox was used as the standard calibration curves, and the results were expressed as µmol Trolox equivalent per g.

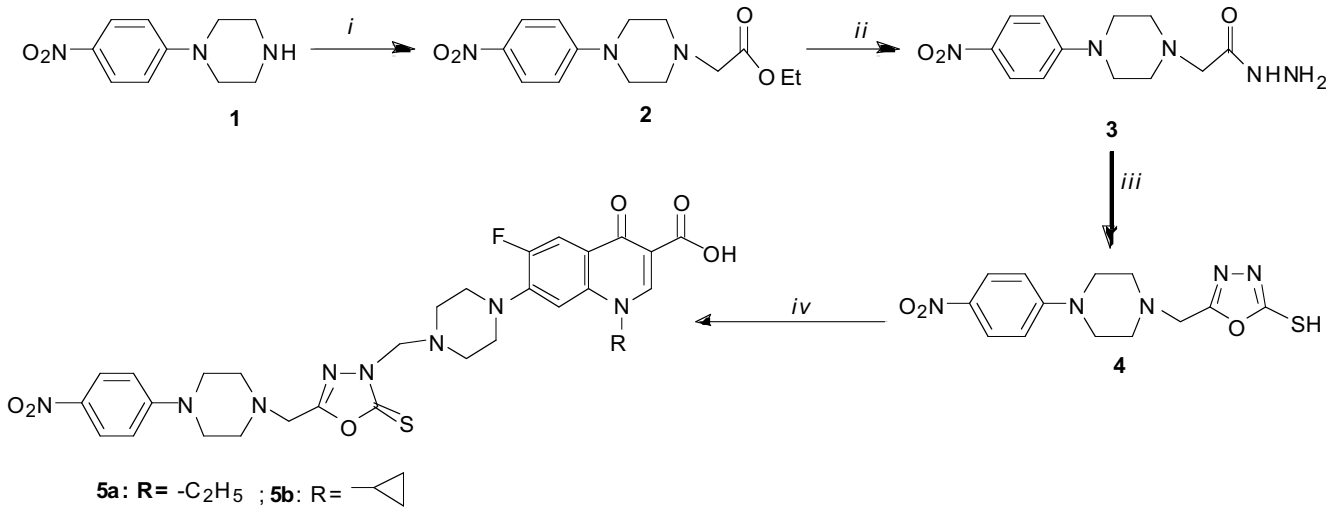
3. RESULTS AND DISCUSSION

3.1. Chemistry

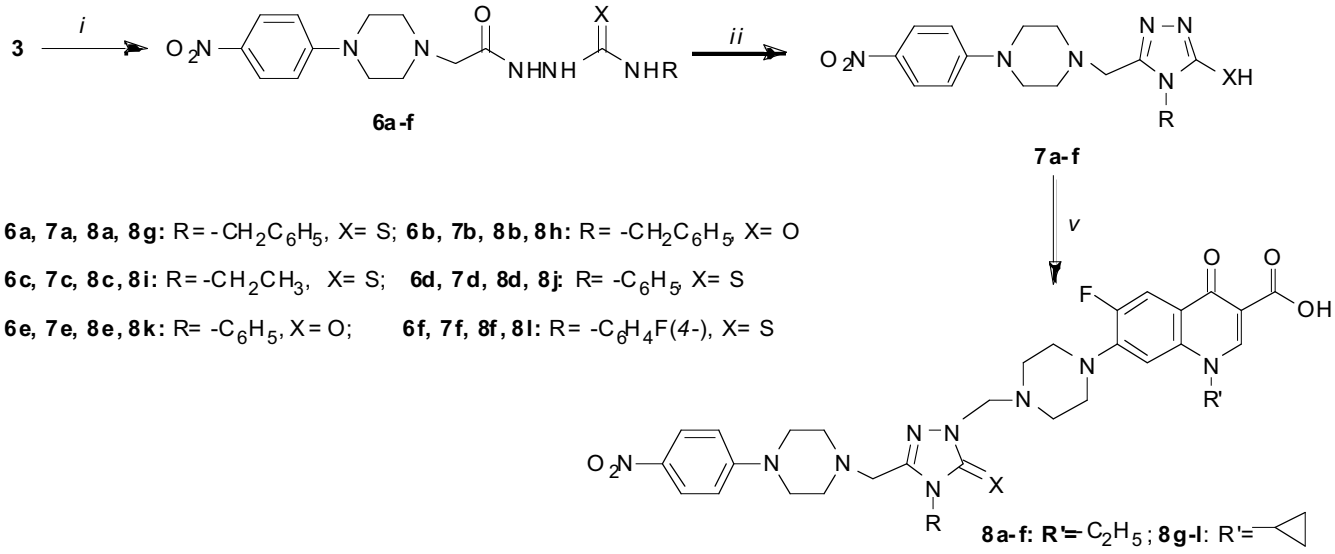
In this study, we attempted to conventional and MW mediated green synthesize of new fluoroquinolone-azole hybrids as possible antimicrobial drug candidates. On the basis of ^1H, ^{13}C NMR, FT IR and EI- MS data, the structures of the target products were established. All synthesized compounds were checked for purity and identity using elemental analysis. The MICs against clinically important pathogens were determined as well. The synthetic methodologies adopted to obtain the target compounds were depicted in Schemes **1** and **2**.

The treatment of the starting compound, 1-(4-nitrophenyl) piperazine (**1**) with ethyl bromoacetate to give ethyl 2-[4-(4-nitrophenyl)piperazin-1-yl]acetate (**2**) was carried out under conventional and also microwave (MW) irradiated conditions with a view to maximizing the yield of the product and minimizing the reaction time. With the assessing of MW irradiated method, the yield of the reaction was improved to good level (99 %) however, more significantly, the reaction time for complete consumption of starting materials was lowered from 3 h with conventional heating to a remarkable 8 min. Moreover, MW irradiated method with no solvent supplied more ecofriendly way. Compound **2** was confirmed by the disappearance of broad singlet for NH and the presence of triplet at the region 1.24 ppm and a quartet at 4.14 ppm due to the presence of ethyl group proton in the ^1H NMR spectrum of compound 2. This group appeared at 14.70 ppm (CH_3) and 60.48 ppm (CH_2) in the ^{13}C NMR spectrum. Further, in the FT IR, the appearance of the stretching band due to C=O group at the region 1741 cm^{-1} confirmed the formation of ester (**2**).

The substitution of ester group by hydrazide function generated [4-(4-nitrophenyl)piperazin-1-yl]acetohydrazide (**3**), which were confirmed by the appearance of broad signal for $-NHNH_2$ group in FT IR. Further, the protons of hydrazide function were resonated at 4.32 (NH_2) and 9.06 (NH) as D_2O exchangeable singlets confirming the formation of ace-



Scheme 1. *i*: $BrCH_2COOEt$, *ii*: NH_2NH_2, *iii*: KOH, CS_2, EtOH-H_2O, *iv*: HCHO and norfloxacin (for **5a**) or ciprofloxacin (for **5b**).



Scheme 2. *i*: suitable isocyanate or isothiocyanate, *ii*: KOH, EtOH-H_2O, *v*: HCHO and norfloxacin (for **8a-f**) or ciprofloxacin (for **8g-l**).

tohydrazide (**3**). In order to optimize microwave (MW) irradiation conditions, MW was applied at different power values of 120 and 150 W without any solvent, while the conventional synthesis of compound **3** required ethanol as reaction solvent. The complete conversion of the compound **2** in best yield was observed after microwave irradiation at 120 W maximum power for 5 min. Higher MW power or longer reaction time caused to lower yield.

Compound **3** produced 5-{[4-(4-nitrophenyl)piperazin-1-yl]methyl}-1,3,4-oxadiazole-2-thiol (**4**) on the treatment with CS_2in basic media. The ^{13}C NMR observations reveal the appearance 1,3,4-oxadiazole C-2 and C-5 carbons at the region 161.05 ppm and 178.51 ppm confirming the formation of 1,3,4-oxadiazole. On the other hand, carbo(thio)amides (**6a-f**), which were obtained from the reaction of **3** with alkyliso(thio)cyanates, were confirmed by the presence of additional signals at the related chemical shift values originated from alkyliso(thio)cyanate moiety. These compounds exhibited mass fragmental and elemental analysis data confirming the assigned structures. Two different procedures containing conventional and microwave prompted techniques were applied for the syntheses. In the conventional heating, the reaction yielding compounds **6a-f** was completed in 12-21 h with 59-79 % yield, while reaction time was 12 min with the yield 73-97 %.

The basic treatment of compounds **6a-f** yielded the corresponding 1,2,4-triazoles (**7a-f**), which can be considered as important tools for further condensation reactions leading to the formation of new bioactive molecules. The reaction was carried out in water as a none toxic ecofriendly solvent under reflux and also microwave conditions. With the use of MW, higher yield was assessed, however, the important effect of MW irradiation was on reaction time. Microwave irradiation decreased the reaction time from 7-13 h to 14 min and increased the yields from 73-87 % to 81-95%. The optimum reaction condition was assessed at 150 Watt maximum

Table 1. Optimization of the model reaction conditions for compound 8a under MW conditions.

Entry	Solvent	MW (Watt)	Time (min)	Yield (%)
1	EtOH	100	10	63
2	EtOH-H_2O (2:1)	100	10	65
3	H_2O	100	10	62
4	DMF	100	12	84
5	DMF	100	14	87
6	-	80	15	94
7	-	75	12	94
8	MeCN	100	12	74
9	THF	100	12	78
10	DCM	100	14	79

power. The FT IR spectra of compounds **7a-f** have -SH or -NH stretching bands at 2821-2850 cm^{-1} and C=O stretching band at 1676-1689 of 1,2,4-triazole nucleus. In the ^1H NMR spectra, resonances assigned to the -SH or NH protons on 1,2,4-triazole ring were detected at 13.60-13.90 ppm (SH) and 9.62-11.75 ppm (NH), respectively which are supported by the literature findings [32-38]. ^{13}C NMR spectra of these compounds have resonances of triazole C-5 and C-3 at 155.05-157.15 ppm and 158.23-181.34 ppm, respectively.

The one-pot, three-component Mannich type reaction of compounds **4** and **7a-f** with norfloxacin and ciprofloxacin yielded the corresponding fluoroquinolone-azole hybrids (**5a, b** and **8a-l**). This reaction proceeds *via* the formation of immonium salt which subsequently attacks the *N*-1 of triazole or oxadiazole *N*-3 giving rise to the corresponding Mannich bases. In the preliminary experiment, to optimize the conditions for this condensation, the synthesis of compound **8a** was selected as model reaction and some reaction parameters including time and MW power were screened on the model reaction (Table **1**). With the aim to provide further improvement for this synthetic approach, the model reaction was also performed in the presence of different organic solvents such as acetonitrile, EtOH, EtOH-H_2O, H_2O, THF, MeCN and DMF, however, the best result was assessed in solvent free media and the corresponding product was obtained in nearly quantitative yields within 15 min (Table **1**, entry 6) in model reaction under microwave irradiation. In comparison with the long refluxing time in hazardous solvent, microwave irradiation provided more efficient and green way for one pot Mannich type condensation with relatively higher product yield. The number of signals and their chemical shifts are in accordance with the assigned structures for compounds **8a-l**. In the ^1H and ^{13}C NMR spectra, additional signals corresponding tonorfloxacin or ciprofloxacin were recorded at the related chemical shift values, while the spectra of these compounds showed the disappearance of the characteristic bands of triazole (or oxadiazole)-NH or SH. Moreover, the preparation of Mannich bases (**8a-l**) was verified by registration of their mass spectrums which were in accordance with their molecular masses and the elemental analysis data (carbon, hydrogen and nitrogen) were ±0.4% of the theoretical values.

3.2. Biological Activity

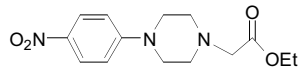
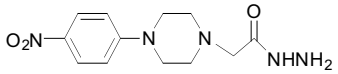
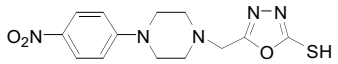
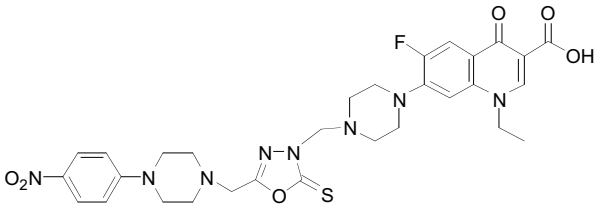
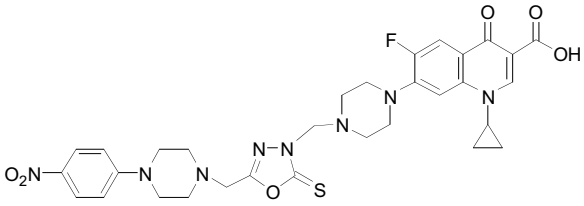
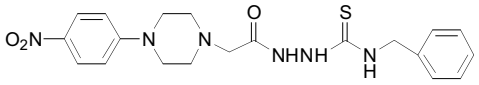
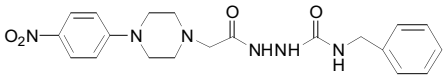
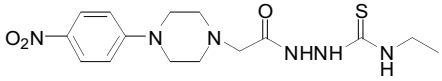
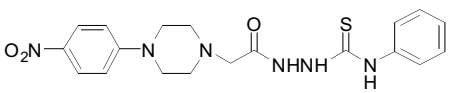
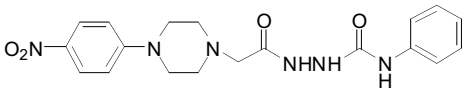
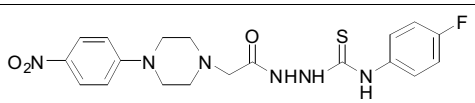
3.2.1. Antimicrobial Activity

All the compounds were tested for their antimicrobial activities and the results were presented in Table **2**. This reveals that the newly synthesized compounds, exhibited good to slight activities against some of the test microorganisms with the mic values varying between <0.03-250 μg/mL. All the newly synthesized compounds except **6a-f** exhibited activity on *Mycobacterium smegmatis* (Ms), a nonpigmented rapidly growing mycobacterium leading to mortality with the mic values varying between 0.03-62.50 μg/mL. In fact, the activity on Ms of **5a,b** and **8a-l** was better than standard drug streptomycin with mic 4 μg/mL It is evident from Table **2** that the aminoalkylation of triazoles **7a-f** and oxadiazole, **4** with fluoroquinolones namely norfloxacin and ciprofloxacin caused to excellent activity towards the test bacteria with the mic values between <0.03-0.99 μg/mL. It can be concluded that the good activity of these compounds is due to the presence of fluoroquinolone core in their structures. In fact, the mic values of compounds **5a,b** and **8a-l** are the same or very close to mic values of norfloxacin and ciprofloxacin.

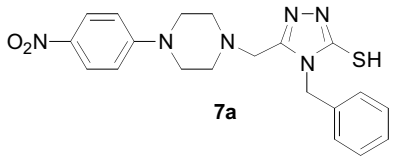
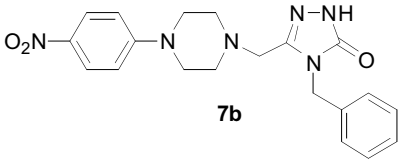
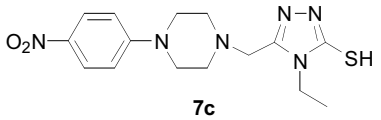
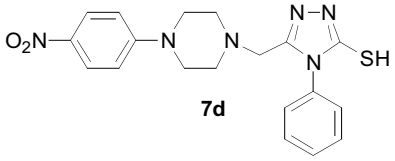
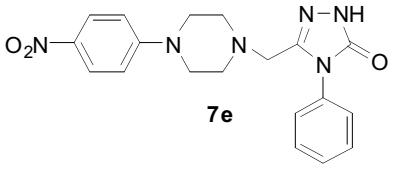
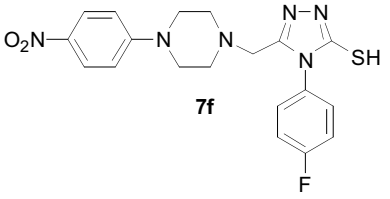
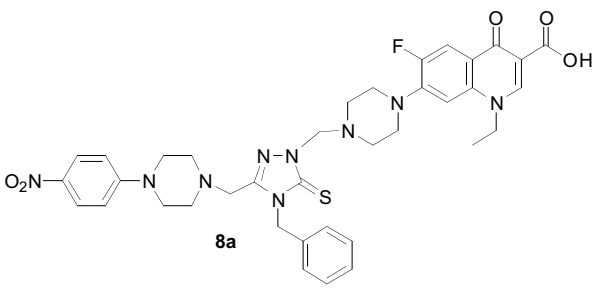
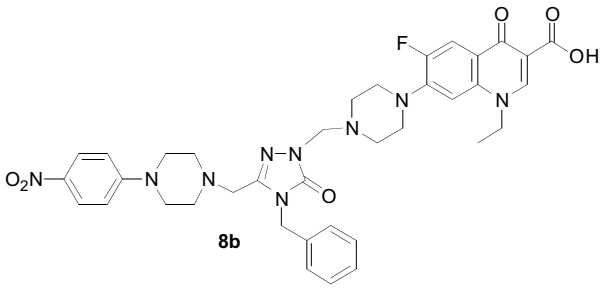
3.2.2. Antioxidant Activity

To determine antioxidant capacity (AC, μmol TE/g) value of the newly synthesized compounds, DPPH, FRAP and CUPRAC assays were assayed. The AC values of the compounds are given in Table **3** and their statisticall processing (one-way ANOVA) indicated that AC values significantly ($P<0.05$) differed with in and among measured tests. Compound **8j** (134.32μmol TE/g), followed **8d** and **8c** for DPPH, which are fluoroquinolone-triazole-piperazine hybrids, **6c** (1595.4μmol TE/g), followed **6d** (784.61μmol TE/g) and **6e** (505.17μmol TE/g), which are carbothioamides, for FRAP and **8c** (940.20μmol TE/g) followed **8j** (582.38μmol TE/g) and **8d** (442.36μmol TE/g) for CUPRAC had the highest AC values, while compounds **7b** (2.94 and 201.62μmol TE/g)

Table 2. Screening for their antimicrobial activity of the newly synthesized 29 compounds.

Compound No.	Microorganizms and Minimal Inhibition Concentrations(μg/mL)						
	Ec	Pa	Yp	Sa	Ef	Bc	Ms
 **2**	250	250	250	250	250	250	35
 **3**	250	250	250	250	250	250	35
 **4**	250	250	250	250	250	250	62.5
 5a	0,25	<0,25	<0,25	0,25	<0,25	<0,25	1.0
 **5b**	0.03	0.03	0.03	0.03	0.03	0.03	0.03
 **6a**	-	125	250	250	125	250	-
 **6b**	-	-	250	-	125	250	-
 **6c**	250	250	125	250	125	-	-
 **6d**	250	-	125	-	125	250	-
 **6e**	250	-	-	250	125	-	-
 **6f**	250	250	125	250	125	-	-

(Table 2) contd….

Compound No.	Microorganizms and Minimal Inhibition Concentrations(μg/mL)						
	Ec	Pa	Yp	Sa	Ef	Bc	Ms
 **7a**	250	250	250	250	250	250	62.5
 **7b**	-	125	250	250	250	-	62.5
 **7c**	250	250	250	125	250	250	35
 **7d**	-	250	250	-	250	-	62.5
 **7e**	125	250	250	250	250	250	35
 **7f**	125	125	250	250	250	250	62.5
 **8a**	0.03	0.48	0.06	0.24	0.488	0.48	0.244
 **8b**	0,99	0,50	0,25	0,25	0,50	0,25	0,99

(Table 2) contd….

Compound No.	Microorganizms and Minimal Inhibition Concentrations(μg/mL)						
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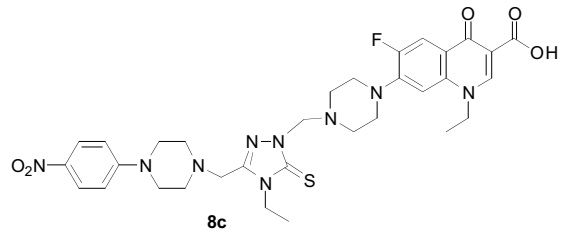
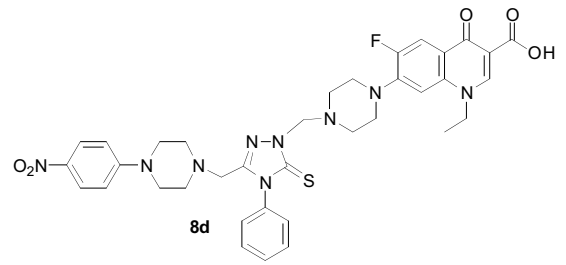
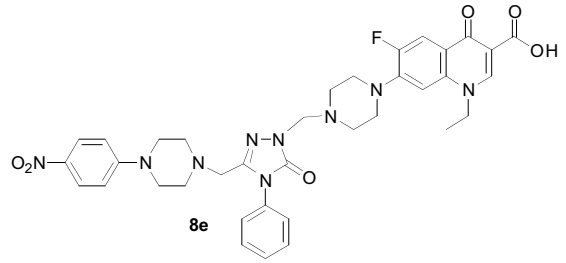
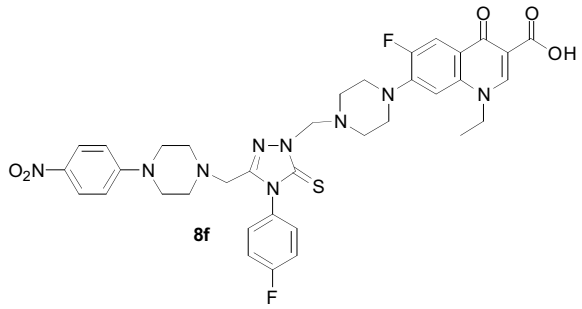
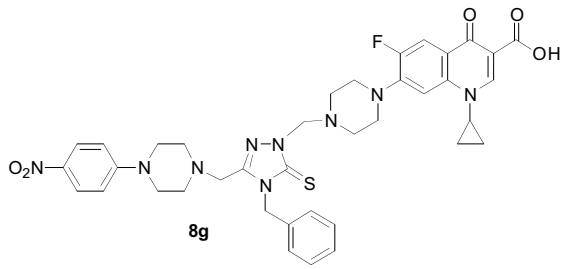
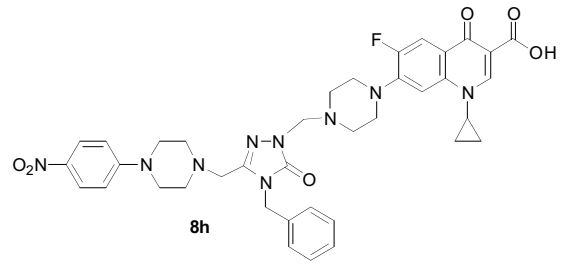
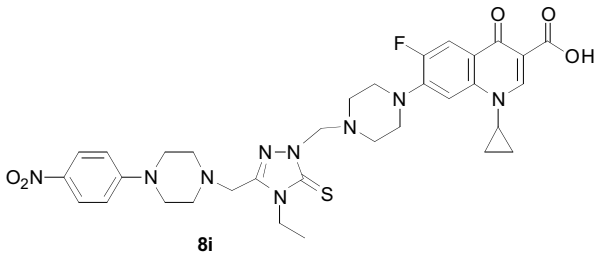
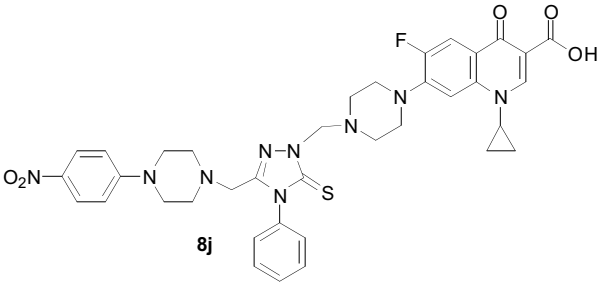
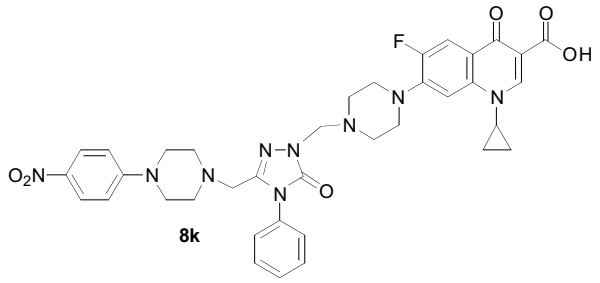
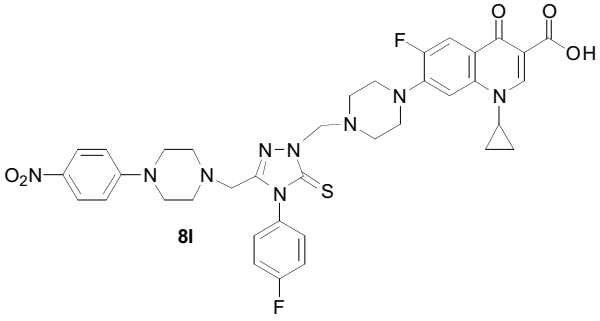
 **8c**	0,99	0,50	0,25	0,99	0.122	0,25	0.244
 **8d**	0.03	0.24	0.06	0.48	0.122	0.24	0.244
 **8e**	0.12	0.48	0,25	0.48	0.122	0,25	0.25
 **8f**	0.12	0.48	0.24	0.48	0.25	0.24	0.244
 **8g**	<0.03	0.06	<0.03	0.12	0.122	0.12	0.244
 **8h**	<0.03	0.24	<0.03	0.24	0.48	0.24	0.244

(Table 2) contd….

Compound No.	Microorganizms and Minimal Inhibition Concentrations(μg/mL)						
	Ec	Pa	Yp	Sa	Ef	Bc	Ms
 **8i**	0.25	0.25	0.25	0.25	0.122	0.122	0.244
 **8j**	<0.03	0.03	0.06	0.24	0.25	0.12	0.25
 **8k**	<0.03	0.24	0.03	0.24	0.122	0.03	0.244
 **8l**	<0.03	0.24	0.12	0.48	0.122	0.12	0.244
Cip.	<0.03	<0.03	<0.03	0.122	0.122	0.06	-
Norf.	<0.041	<0.041	<0.041	<0.041	<0.041	<0.041	-
Amp.	10	18	>128	35	10	15	-
Strep.	-	-	-	-	-	-	4

Ec: *Escherichia coli* ATCC 25922, Pa; *Pseudomonas aeruginosa* ATCC 43288, Yp; *Yersinia pseudotuberculosis* ATCC 911, Sa; *Staphylococcus aureus* ATCC 25923, Ef; *Enterococcus faecalis* ATCC 29212, Bc; *Bacillus cereus* 702 Roma, Ms; *M. smegmatis* ATCC607, Cip.; Ciprofloxacin, Norf.; Norfloxacin Amp.; Ampicillin, Strep.; Streptomycin, (—); no activity.

for DPPH and CUPRAC and **2** (37.95μmol TE/g) for FRAP had the lowest AC values among the synthesized compounds (Table **3**). In the table, for the compounds **6a**, **7a** and **7d**, no AC value for FRAP and CUPRAC was determined (Table **3**).

PCA was carried out by treating data obtained from the three different antioxidant capacity tests. This separation was mainly across PC1, with FRAP, CUPRAC and DPPH having positive scoreson PC1 explained 48.75% of the total variation (79.78%). Respectively, compounds **6c**, **6d, 8a, 8e** and **8f** at the right upper plan and compounds **5a**, **5b**, **8b**, **8c**, **8d**, **8j** and **8g** at the right lower plan were low associated and correlated with FRAP, CUPRAC and DPPH ranging from 0.097 to 0.387 (Fig. (**2**) at the left upper plan). The remaining compounds **6b, 6e, 6f, 7b, 8h, 8i, 8k** and **8l** at the left

Table 3. Antioxidant capacity (AC) values of 29 synthesized novel compounds. Values represent the mean ± SD of three determinations.

Compd. No.	DPPH (μmol TE/g)	FRAP (μmol TE/g)	CUPRAC (μmol TE/g)
2	9.48 ± 0.20^{b}	37.95 ± 2.55^{a}	269.50 ± 1.68^{c}
3	7.87 ± 0.29^{a}	82.61 ± 3.63^{c}	221.62 ± 2.54^{a}
4	3.94 ± 0.18^{a}	91.34 ± 4.52^{ab}	215.44 ± 6.51^{a}
5a	56.49 ± 2.32^{h}	182.70 ± 11.90^{e}	329.15 ± 11.35^{g}
5b	64.32 ± 5.08^{j}	258.46 ± 3.15^{d}	382.34 ± 12.42^{h}
6a	64.33 ± 0.05^{g}	n.d.**	n.d.**
6b	26.09 ± 0.33^{c}	283.65 ± 35.42^{ef}	254.30 ± 4.47^{c}
6c	63.56 ± 0.00^{g}	1595.4 ± 67.49^{i}	287.32 ± 7.95^{d}
6d	64.18 ± 0.01^{g}	784.61 ± 34.46^{h}	309.77 ± 8.79^{e}
6e	44.98 ± 0.32^{d}	505.17 ± 42.45^{g}	261.91 ± 16.99^{c}
6f	35.63 ± 0.28^{d}	408.22 ± 36.49^{g}	289.74 ± 13.43^{c}
7a	61.60 ± 0.03^{fg}	n.d.**	n.d.**
7b	2.94 ± 0.29^{a}	71.61 ± 4.63^{ab}	201.62 ± 7.54^{a}
7c	59.28 ± 0.22^{f}	116.70 ± 4.92^{bc}	299.20 ± 6.76^{de}
7d	54.97 ± 0.05^{e}	n.d**.	n.d.**
7e	53.46 ± 0.15^{e}	38.76 ± 2.74^{a}	221.33 ± 6.39^{b}
7f	36.32 ± 0.19^{f}	125.36 ± 3.88^{bc}	278.20 ± 4.65^{de}
8a	42.66 ± 4.33^{d}	325.64 ± 19.21^{f}	424.08 ± 4.89^{f}
8b	62.29 ± 1.30^{h}	118.84 ± 3.78^{c}	337.56 ± 12.17^{e}
8c	73.26 ± 3.60^{h}	245.23 ± 21.97^{de}	940.20 ± 12.42^{i}
8d	120.82 ± 2.32^{i}	272.82 ± 21.90^{e}	442.36 ± 12.75^{g}
8e	82.47 ± 2.09^{e}	137.64 ± 18.53^{c}	347.10 ± 11.18^{f}
8f	69.63 ± 3.04^{d}	225.19 ± 15.20^{e}	332.54 ± 6.79^{i}
8g	70.45 ± 0.30^{h}	126.45 ± 4.80^{c}	315.32 ± 12.40^{e}
8h	61.59 ± 2.76^{i}	172.24 ± 14.98^{e}	312.47 ± 10.57^{g}
8i	60.28 ± 2.42^{h}	218.46 ± 4.43^{c}	268.46 ± 11.67^{e}
8j	134.32 ± 5.98^{j}	204.96 ± 4.17^{d}	582.38 ± 16.33^{h}
8k	53.75 ± 2.21^{h}	235.32 ± 18.86^{e}	744.75 ± 11.89^{i}
8l	59.66 ± 4.87^{d}	195.90 ± 12.39^{f}	284.19 ± 5.67^{f}

An analysis of variance (SPSS version 11.5. one-way ANOVA) was used for comparisons among the means. Values with the same letter at superscript within a column are not significantly different at P<0.05. * Not detected.

upper and **6a**, **7a**, **7c, 7d**, **7e**, **7f**, **4, 3** and **2** at the left lower plan located on PC2 with negative loadings, accounting 31.04% of the variance, were not associate and correlate with any measuring tests (Fig. **2**).

3.2.2.1. Statistical Analysis

Triplicate measurements (n = 3) were performed during the measurements of antioxidant capacity (AC) of the compounds. The AC values were presented as mean ± pooled

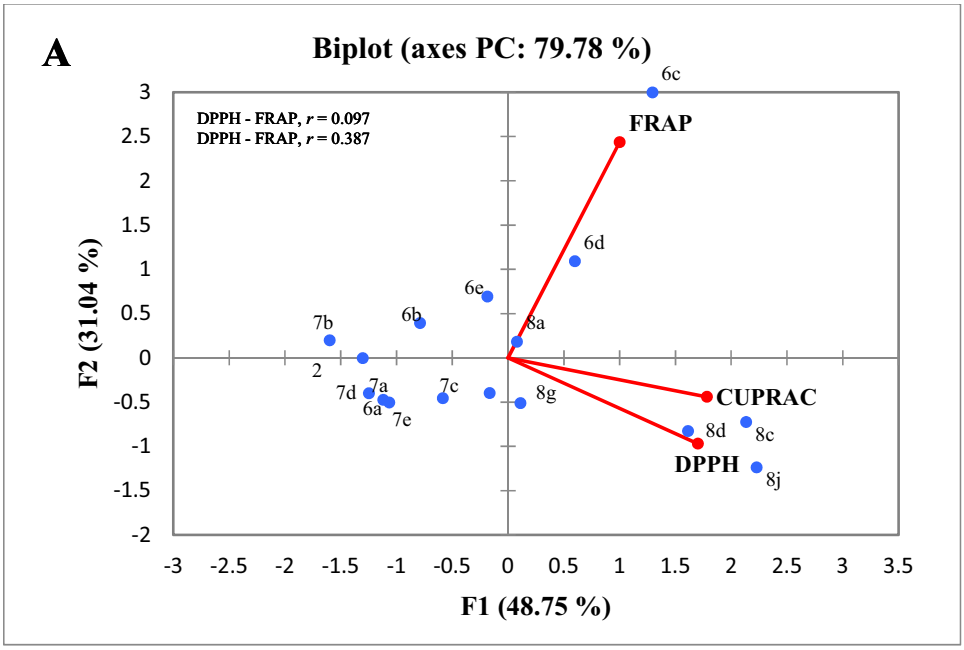


Fig. (2). Loading bi-plot of principle component analysis (PCA) of 29 synthesized novel compounds contributing to values obtained from three antioxidant capacity assays measured by FRAP, CUPRAC and DPPH.

standard deviation (mean ± SD). For statistical evaluation, one-way ANOVA and Duncan's multiple range test for comparison among the means at significance level of $P < 0.05$ (IBM SPSS Statistics V22.0) were employed. A statistical software package (XLSTAT version 2015.6) using ADDINSOFT (Damremont, Paris, France) was also employed to perform principal component analysis (PCA).

CONCLUSION

This study reports the conventional and successfully developed microwave assisted synthesis of some new Norfloxacin and Ciprofloxacin derivatives incorporating several other heterocyclic moieties namely 1,3,4-oxadiazole, 1,2,4-triazole and piperazine. Hence, we combined all these potential chemotherapeutic units by Mannich reaction, a one pot three component condensation. The structures of new compounds were confirmed by IR, ^{1}H-, ^{13}C NMR, mass spectroscopic and elemental analysis techniques. In addition, the newly synthesized compounds were screened for their antimicrobial and antioxidant activities. The antimicrobial screening suggests that among the newly synthesized compounds, the ones containing fluoroquinolone moiety in their structures exhibited excellent activities against most of the test microorganisms. Among the synthesized compounds, the fluoroquinolone derivatives **8j, 8d** and **8c** displayed antioxidant activity for DPPH, carbo(thio)amides **6c, 6d** and **6e** for FRAP and fluoroquinolones **8c, 8j** and **8d** exhibited antioxidant activity for CUPRAC, while triazole compound **7b** for DPPH and CUPRAC and ester **2** for FRAP had the lowest AC values. For the synthesis of target compounds, microwave irradiation was considered more efficient and eco-friendly.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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