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Synthesis of Novel Fluoroquinolone-Triazole Hybrid Compounds as Antimicrobial Agents

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Abstract: The hydrazide compound (**2**) was synthesized starting from 1-(2-fluorophenyl)piperazine via two steps. The reaction of compound (**2**) with different alkyl(aryl)isothiocyanates afforded the corresponding compounds (**3a-c**). 1,3-Thiazolidine derivatives (**4a-c**) were synthesized from the treatment of (**3a-c**) with ethyl bromoacetate. Mannich bases (**6a-d**) were synthesized with the treatment of (**5a-c)** with various suitable amines in the presence of formaldehyde. Compound (**3**) derivatives were converted to 1,2,4-triazole as the starting material of fluoroquinolone analogues (**11a-c**). Finally, synthesized compounds were examined their biological properties and some of these showed potent activity.

Keywords: Fluoroquinolone; 1,2,4-triazole; mannich base; biological properties.
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INTRODUCTION

Nowadays, bacterial strength towards used antibiotics has become a major worldwide problem [1]. Although new strategies have been developed for control and treatment of microbial infections, biological strength proceeds to be one of primary anxiety to the community welfare and academic society around the world. Due to the re-emergence of microbial threats with increased antibacterial resistance, high safety profile with novel and more active antibacterial agents are required [2]. 1,2,4-Triazole moiety is recognized to be a sophisticated molecule which is mainly used in the design of possible bioactive form. Its derived functions possess medicinal important activities [3-10]. Several drugs have fungicidal activity like fluconazole, consisting of 1,2,4-triazole nucleus [11].

Quinolones are mostly used in the fight against serious hospital-acquired and community-acquired infections. Therefore, quinolones became an important class of antibacterial agents [12]. These agents possess excellent safety profile, favorable pharmacokinetic characteristics, and broad antibacterial spectrum, respectively. In this way, they are well tolerated with against genitourinary infections and common respiratory tract pathogens [13].

Over the years, heterocyclic compounds have been attracted scientific attention because of their diverse biological activities [14]. Among them, 4-thiazolidinones are the derivatives of thiazole have been indicated to own a varied range of biological properties containing antitubercular, anti-inflammatory, antitumor, antihistaminic, antibacterial, and anticonvulsant activities [15]. In recent years, to overcome the drug resistance problem, 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 [1]. Mannich reaction involves condensation of a compound with active hydrogens with suitable amine and formaldehyde [16].

Mannich base functional group can increase the lipophilicity of essential amines and amides, which results in the increase of absorption through bio-membranes [17]. Lipophilic properties of fluoroquinolones affect their ability to cross bacterial membranes [18]. In light of these considerations, we reported here the synthesis and investigation of 1,2,4-triazoles fused fluoroquinolone or Mannich bases as hybrid molecules own biological properties.

MATERIALS AND METHODS

Chemistry

Used chemicals were purchased from Fluka Chemie AG Buchs (Switzerland). Melting points were measured in open capillaries on a Büchi B-540 device. Reactions were determined by thin-layer chromatography (TLC) on silica gel 60 F254 aluminum sheets. FT-IR spectra were recorded using a *Perkin Elmer* 1600 series FT IR spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO-d_6 on a *BRUKER AVENE II* 400 MHz (^{1}H) NMR Spectrometer. 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 checked on *a Costech Elemental Combustion System* CHNS-O elemental device. All the compounds displayed C, H and N analysis within ±0.4% of the technical rates. The mass spectra were registered on a *Quattro GC-MS* (70 eV) apparatus. CEM Discovery monomode synthesis reactor was used for microwave synthesis.

EXPERIMENTAL SECTION

Ethyl [4-(2-fluorophenyl)piperazin-1-yl]acetate (1)

To the mixture of 1-(2-fluorophenyl)piperazine (10 mmol) and triethylamine (15 mmol) in dry tetrahydrofuran, ethyl bromoacetate (10 mmol) was added at 0–5 °C. Then, the reaction mixture was taken to room temperature and stirred for additional 24 h. The precipitated salt was removed by filtration and the solvent was evaporated. The oily mass was purified with ethyl acetate. Yield 97%, mp 36-38°C. ^{1}H NMR (DMSO-d_6, δ ppm): 1.20 (t, 3H, CH_3, *J*= 6.8 Hz), 2.68 (brs, 4H, $2CH_2$), 3.01 (brs, 4H, $2CH_2$), 3.40 (s, 2H, CH_2), 4.10 (q, 2H, CH_2, *J*= 7.2 Hz), 6.94-7.04 (m, 2H, arH), 7.05-7.13 (m, 2H, arH). ^{13}C NMR (DMSO-d_6, δ ppm): 14.57 (CH_3), 50.44 (CH_2), 50.47 (CH_2), 52.43 ($2CH_2$), 58.79 (CH_2), 60.33 (CH_2), arC: [116.23 and 116.44 (d, CH, *J*= 21.0 Hz), 119.67 and 119.70 (d, CH, *J*= 3.0 Hz), 122.70 and 122.78 (d, CH, *J*= 8.0 Hz), 125.22 and 125.26 (d, CH, *J*= 4.0 Hz), 140.24 and 140.32 (d, C, *J*= 8.0 Hz), 154.19 and 156.62 (d, C, J_{C-F}= 243.0 Hz)], 170.28 (C=O). FT IR (v_{max}, cm^{-1}): 3066 (Aromatic CH), 2982 (Aliphatic CH), 1745 (C=O). EI MS *m/z* (%): 267.01 ($[M+1]^+$, 40), 256.98 (55), 125.82 (100). Anal. Calcd. for $C_{14}H_{19}FN_2O_2$ (266.31); C, 63.14; H, 7.19; N, 10.52%. Found: C, 63.21; H, 7.08; N, 10.55%.

2-[4-(2-Fluorophenyl)piperazin-1-yl]acetohydrazide (2)

Hydrazine hydrate (2.5 mmol) was added to the solution of compound **1** (1 mmol) in ethanol and the mixture was irradiated in monomode microwave reactor in closed vessel at 125 °C for 20 min. After evaporation, a solid was obtained. This was purified with

ethyl acetate. Yield 95%, mp 84-86°C. ^{1}H NMR (DMSO-d_6, δ ppm): 2.58 (brs, 4H, $2CH_2$), 3.01 (brs, 4H, $2CH_2$), 3.68 (brs, 2H, CH_2), 4.25 (s, 2H, NH_2), 6.94-6.96 (m, 1H, arH), 7.02 (d, 1H, arH, *J*= 8.0 Hz), 7.07-7.13 (m, 2H, arH), 8.94 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 50.40 (CH_2), 50.43 (CH_2), 53.25 ($2CH_2$), 60.26 (CH_2), arC: [116.25 and 116.46 (d, CH, *J*= 21.0 Hz), 119.62 and 119.65 (d, CH, *J*= 3.0 Hz), 122.65 and 122.73 (d, CH, *J*= 8.0 Hz), 125.24 and 125.27 (d, CH, *J*= 3.0 Hz), 140.27 and 140.35 (d, C, *J*= 8.0 Hz), 154.17 and 156.60 (d, C, J_{C-F}= 243.0 Hz)], 168.63 (C=O). FT IR (ν_{max}, cm^{-1}): 3256 and 3225 (NH_2), 3174 (NH), 3037 (Aromatic CH), 2819 (Aliphatic CH), 1692 (C=O). EI MS *m/z* (%): 252.80 ($[M]^+$, 60), 225.09 (32), 180.23 (100). Anal. Calcd for $C_{12}H_{17}FN_4O$ (252.29); C, 57.13; H, 6.79; N, 22.21%. Found: C, 57.23; H, 6.69; N, 22.09%.

General procedure for preparation of compounds 3a-c

The corresponding alkyl(aryl)isothiocyanate (1 mmol) was added to the solution of compound **2** (1 mmol) in ethanol and the reaction was irradiated in monomode microwave reactor in closed vessel at 150°C for 10 min. After evaporation, a solid appeared. This solid was purified with acetone:diethyl ether (1:2 v/v).

2-{[4-(2-Fluorophenyl)piperazin-1-yl]acetyl}-*N*-phenylhydrazine carbothioamide (3a)

Yield: 80%, mp 183-185°C. ^{1}H NMR (DMSO-d_6, δ ppm): 2.68 (brs, 4H, $2CH_2$), 3.05 (brs, 4H, $2CH_2$), 3.14 (s, 2H, CH_2), 6.95-7.11 (m, 5H, arH), 7.12-7.35 (m, 2H, arH), 7.43 (brs, 2H, arH), 9.59 (brs, 2H, 2NH), 9.90 (brs, 1H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 50.42 (CH_2), 53.28 ($2CH_2$), 60.22 ($2CH_2$), arC: [116.28 and 116.48 (d, CH, *J*= 20.0 Hz), 119.69 (2CH), 122.68 and 122.76 (d, CH, *J*= 8.0 Hz), 125.30 (2CH), 128.60 (3CH), 140.36 (C), 140.94 and 141.03 (d, C, *J*= 9.0 Hz), 154.19 and 156.62 (d, C, J_{C-F}= 243.0 Hz)], 161.21 (C=O), 181.24 (C=S). FT IR (ν_{max}, cm^{-1}): 3223 (2NH), 3120 (NH), 3065 (Aromatic CH), 2945 (Aliphatic CH), 1670 (C=O), 1237 (C=S). EI MS *m/z* (%): 410.45 ($[M+Na]^+$, 17), 401.23 (43), 321.00 (56), 126.90 (100). Anal. Calcd. for $C_{19}H_{22}FN_5OS$ (387.47); C, 58.90; H, 5.72; N, 18.07%. Found: C, 58.70; H, 5.92; N, 18.37%.

***N*-ethyl-2-{[4-(2-fluorophenyl)piperazin-1-yl]acetyl}hydrazinecarbothioamide (3b)**

Yield: 95%, mp 156-157°C. ^{1}H NMR (DMSO-d_6, δ ppm): 1.06 (t, 3H, CH_3, *J*= 8.0 Hz), 2.64 (brs, 4H, $2CH_2$), 3.03 (brs, 4H, $2CH_2$), 3.08 (s, 2H, CH_2), 3.44 (q, 2H, CH_2, *J*= 6.4 Hz), 6.93-7.08 (m, 1H, arH), 7.10-7.12 (m, 1H, arH), 7.14 (s, 2H, arH), 7.85 (s, 1H, NH), 9.13 (s, 1H, NH), 9.65 (brs, 1H, NH). ^{13} C NMR (DMSO-d_6, δ ppm): 14.91 (CH_3), 50.37 (CH_2), 50.39 (CH_2), 53.26 ($2CH_2$), 60.16 ($2CH_2$), arC: [116.26 and 116.47 (d, CH,

J= 21.0 Hz), 119.64 and 119.67 (d, CH, *J*= 3.0 Hz), 122.65 and 122.73 (d, CH, *J*= 8.0 Hz), 125.26 and 125.29 (d, CH, *J*= 3.0 Hz), 140.27 and 140.36 (d, C, *J*= 9.0 Hz), 154.19 and 156.62 (d, C, J_{C-F}= 243.0 Hz)], 169.32 (C=O), 181.75 (C=S). FT IR (ν_{max}, cm^{-1}): 3211 (2NH), 3152 (NH), 3078 (Aromatic CH), 2955 (Aliphatic CH), 1667 (C=O), 1235 (C=S). EI MS *m/z* (%): 340.34 ($[M+1]^+$, 55), 158.09 (100). Anal. Calcd. for $C_{15}H_{22}FN_5OS$ (339.43); C, 53.08; H, 6.53; N, 20.63%. Found: C, 53.23; H, 6.66; N, 20.87%.

_N_-benzyl-2-{[4-(2-fluorophenyl)piperazin-1-yl]acetyl}hydrazinecarbothioamide (3c)

Yield: 97%, mp 191-192°C. ^{1}H NMR (DMSO-d_6, δ ppm): 2.64 (brs, 4H, $2CH_2$), 3.09 (brs, 2H, CH_2), 3.36 (s, 4H, $2CH_2$), 4.73 (d, 2H, CH_2, *J*= 5.6 Hz), 6.95-7.14 (m, 4H, arH), 7.22-7.30 (m, 5H, arH), 8.40 (brs, 1H, NH), 9.33 (s, 1H, NH), 9.77 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 47.16 (CH_2), 50.35 (CH_2), 50.38 (CH_2), 53.26 ($2CH_2$), 60.17 (CH_2), arC: [116.27 and 116.48 (d, CH, *J*= 21.0 Hz), 119.64 and 119.66 (d, CH, *J*= 2.0 Hz), 122.66 and 122.74 (d, CH, *J*= 8.0 Hz), 125.26 and 125.30 (d, CH, *J*= 4.0 Hz), 127.07 (2CH), 127.47 (CH), 128.50 (2CH), 139.73 (C), 140.27 and 140.35 (d, C, *J*= 8.0 Hz), 154.18 and 156.61 (d, C, J_{C-F}= 243.0 Hz)], 168.63 (C=O), 180.21 (C=S). FT IR (ν_{max}, cm^{-1}): 3221 (2NH), 3174 (NH), 3065 (Aromatic CH), 2918 (Aliphatic CH), 1666 (C=O), 1232 (C=S). EI MS *m/z* (%): 402.43 ($[M+1]^+$, 17), 190.25 (100). Anal. Calcd. for $C_{20}H_{24}FN_5OS$ (401.50); C, 59.83; H, 6.03; N, 17.44%. Found: C, 60.00; H, 6.23; N, 18.00%.

General procedure for preparation of compounds 4a-c

Ethyl bromoacetate (1 mmol) and dried sodium acetate (5 mmol) were added to the solution of compound **3a-c** (1 mmol) in ethanol and the medium was irradiated in monomode microwave reactor in closed vessel at 150°C for 45 min. With removal of solvent with evaporation, an oily mass occurred, then it was purified with ethanol:water (1:3).

2-[4-(2-Fluorophenyl)piperazin-1-yl]-_N_'-[(4-oxo-3-phenyl-1,3-thiazolidin-2-ylidene]acetohydrazide (4a)

Yield: 60%, mp 150-151°C. ^{1}H NMR (DMSO-d_6, δ ppm): 2.38 (brs, 4H, $2CH_2$), 2.81 (brs, 4H, $2CH_2$), 3.41 (s, 4H, $2CH_2$), 6.95 (d, 2H, arH, *J*= 8.0 Hz), 7.07 (t, 2H, arH, *J*= 8.0 Hz), 7.44-7.54 (m, 5H, arH), 9.05 (brs, 1H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 33.19 (CH_2), 40.59 (CH_2), 45.92 ($2CH_2$), 50.58 (CH_2), 60.39 (CH_2), arC: [116.28 and 116.48 (d, 2CH, *J*= 20.0 Hz), 119.60 (2CH), 122.75 and 122.83 (d, 2CH, *J*= 8.0 Hz), 127.99 (CH), 128.29 (2CH), 136.30 (C), 140.21 and 140.28 (d, C, *J*= 7.0 Hz), 154.56 and 156.60 (d, C, J_{C-F}= 204.0 Hz)], 158.56 (C=N), 165.59 (C=O), 171.99 (C=O). FT IR (ν_{max}, cm^{-1}): 3277 (NH), 3071 (Aromatic CH), 2919 (Aliphatic CH), 1728 (2C=O), 1499 (C=N).

EI MS *m/z* (%): 450.01 ([M+Na]$^+$, 23), 232.21 (100). Anal. Calcd. for $C_{21}H_{22}FN_5O_2S$ (427.50); C, 59.00; H, 5.19; N, 16.38%. Found: C, 59.23; H, 5.00; N, 16.46%.

***N*'-[3-ethyl-4-oxo-1,3-thiazolidin-2-ylidene]-2-[4-(2-fluorophenyl)piperazin-1-yl]acetohydrazide (4b)**

Yield: 55%, mp 164-165°C. ^{1}H NMR (DMSO-d_6, δ ppm): 1.14 (t, 3H, CH_3, *J*= 7.2 Hz), 2.67 (brs, 2H, CH_2), 3.05 (brs, 2H, CH_2), 3.13 (s, 2H, CH_2), 3.36 (brs, 4H, $2CH_2$), 3.68 (d, 2H, CH_2, *J*=7.2 Hz), 4.04 (s, 2H, CH_2), 6.95-7.12 (m, 4H, arH), 10.05 (brs, 1H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 12.56 (CH_3), 32.21 (CH_2), 37.96 (CH_2), 50.60 (CH_2), 50.63 (CH_2), 53.19 ($2CH_2$), 60.19 (CH_2), arC: [116.28 and 116.49 (d, 2CH, *J*= 21.0 Hz), 119.67 and 119.70 (d, 2CH, *J*= 3.0 Hz), 122.74 and 122.82 (d, C, *J*= 8.0 Hz), 154.19 and 156.61 (d, C, J_{C-F}=242.0 Hz)], 158.38 (C=N), 165.47 (C=O), 171.54 (C=O). FT IR (ν_{max}, cm^{-1}): 3197 (NH), 3073 (Aromatic CH), 2979 (Aliphatic CH), 1713 (C=O), 1692 (C=O), 1497 (C=N). EI MS *m/z* (%): 379.98 ([M]$^+$, 11), 301.12 (45), 204.26 (56), 128.80 (100). Anal. Calcd. for $C_{17}H_{22}FN_5O_2S$ (379.45); C, 53.81; H, 5.84; N, 18.46%. Found: C, 54.00; H, 5.99; N, 18.59%.

***N*'-[3-benzyl-4-oxo-1,3-thiazolidin-2-ylidene]-2-[4-(2-fluorophenyl)piperazin-1-yl]acetohydrazide (4c)**

Yield: 45%, mp 198-199°C. ^{1}H NMR (DMSO-d_6, δ ppm): 2.66 (brs, 2H, CH_2), 3.04 (brs, 2H, CH_2), 3.12 (brs, 2H, CH_2), 3.39 (s, 4H, $2CH_2$), 4.14 (s, 2H, CH_2), 4.84 (s, 2H, CH_2), 6.95-7.05 (m, 2H, arH), 7.08-7.12 (m, 2H, arH), 7.32-7.36 (m, 5H, arH), 10.12 (brs, 1H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 33.17 (CH_2), 40.54 (CH_2), 45.92 (CH_2), 50.58 (CH_2), 53.17 ($2CH_2$), 60.37 (CH_2), arC: [116.28 and 116.49 (d, 2CH, *J*= 21.0 Hz), 119.66 (CH), 122.75 and 122.83 (d, 2CH, *J*= 8.0 Hz), 127.98 (CH), 128.24 (2CH), 128.85 (CH), 136.29 (C), 140.20 and 140.28 (d, C, *J*= 8.0 Hz), 154.56 and 156.60 (d, C, J_{C-F}= 204.0 Hz)], 158.46 (C=N), 165.52 (C=O), 171.90 (C=O). FT IR (ν_{max}, cm^{-1}): 3198 (NH), 3066 (Aromatic CH), 2828 (Aliphatic CH), 1720 (C=O), 1689 (C=O), 1496 (C=N). EI MS *m/z* (%): 442.09 ([M+1]$^+$, 19), 376.20 (21), 305.75 (100), 231.67 (60), 104.30 (21). Anal. Calcd. for $C_{22}H_{24}FN_5O_2S$ (441.52); C, 59.85; H, 5.48; N, 15.86%. Found: C, 60.00; H, 5.34; N, 15.89%.

General procedure for preparation of compounds 5a-c

Compounds **3a-c** (1 mmol) and 20 mL of 2 N NaOH in water was irradiated in monomode microwave reactor in closed vessel at 150 °C for 15 min. Then, the reaction mixture was cooled to room temperature and acidified to pH 4 with 37% HCl. The resulting solid was filtered off, and purified with dimethyl sulfoxide-water (1:4).

5-{[4-(2-Fluorophenyl)piperazin-1-yl]methyl}-4-phenyl-4*H*-1,2,4-triazole-3-thiol (5a)

Yield: 87%, mp 179-180°C. ^{1}H NMR (DMSO-d_6, δ ppm): 2.38 (s, 2H, CH_2), 2.81 (brs, 4H, $2CH_2$), 3.41 (brs, 4H, $2CH_2$), 6.95 (d, 2H, arH, *J*= 7.6 Hz), 7.05-7.08 (m, 2H, arH), 7.44-7.54 (m, 5H, arH), 13.90 (s, 1H, SH). ^{13}C NMR (DMSO-d_6, δ ppm): 50.33 (CH_2), 52.03 ($2CH_2$), 52.42 ($2CH_2$), arC: [116.24 and 116.45 (d, CH, *J*= 21.0 Hz), 119.60 and 119.63 (d, CH, *J*= 3.0 Hz), 122.73 and 122.81 (d, CH, *J*= 8.0 Hz), 125.21 and 125.24 (d, CH, *J*= 3.0 Hz), 128.70 (CH), 129.37 (2CH), 129.61 (2CH), 134.51 (C), 140.06 and 140.14 (d, C, *J*= 8.0 Hz), 154.10 and 156.53 (d, C, J_{C-F}= 243.0 Hz)], 149.62 (triazole C-3), 168.68 (triazole C-5). FT IR (ν_{max}, cm^{-1}): 3035 (Aromatic CH), 2942 (Aliphatic CH), 1499 (C=N). EI MS *m/z* (%): 369.56 ([M]$^+$, 23), 200.76 (100). Anal. Calcd. for $C_{19}H_{20}FN_5S$ (369.46); C, 61.77; H, 5.46; N, 18.96%. Found: C, 61.56; H, 5.66; N, 18.99%.

4-Ethyl-5-{[4-(2-fluorophenyl)piperazin-1-yl]methyl}-4*H*-1,2,4-triazole-3-thiol (5b)

Yield: 75%, mp 171-172°C. ^{1}H NMR (DMSO-d_6, δ ppm): 1.26 (brs, 3H, CH_3), 2.50 (brs, 2H, CH_2), 3.03 (brs, 4H, $2CH_2$), 3.38 (s, 2H, CH_2), 3.64 (brs, 2H, CH_2), 4.07 (brs, 2H, CH_2), 6.98-7.12 (m, 4H, arH), 13.69 (brs, 1H, SH). ^{13}C NMR (DMSO-d_6, δ ppm): 13.82 (CH_3), 52.62 ($5CH_2$), arC: [116.34 and 116.54 (d, CH, *J*= 20.0 Hz), 119.81 (CH), 123.15 (CH), 125.29 and 125.32 (d, CH, *J*= 3.0 Hz), 141.26 (C), 154.15 and 156.58 (d, C, J_{C-F}= 243.0 Hz)], 167.73 (triazole C-3 and triazole C-5). FT IR (ν_{max}, cm^{-1}): 3083 (Aromatic CH), 2927 (Aliphatic CH), 1497 (C=N). EI MS *m/z* (%): 321.48 ([M]$^+$, 47), 301.40 (31), 276.21 (100). Anal. Calcd. for $C_{15}H_{20}FN_5S$ (321.42); C, 56.05; H, 6.27; N, 21.79%. Found: C, 56.15; H, 6.35; N, 21.90%.

4-Benzyl-5-{[4-(2-fluorophenyl)piperazin-1-yl]methyl}-4*H*-1,2,4-triazole-3-thiol (5c)

Yield: 67%, mp 228-230°C. ^{1}H NMR (DMSO-d_6, δ ppm): 2.50 (brs, 4H, $2CH_2$), 2.83 (s, 2H, CH_2), 3.43 (brs, 4H, $2CH_2$), 5.41 (s, 2H, CH_2), 6.99 (s, 2H, arH), 7.09-7.12 (m, 2H, arH), 7.25-7.36 (m, 5H, arH), 13.97 (s, 1H, SH). ^{13}C NMR (DMSO-d_6, δ ppm): 47.75 ($3CH_2$), 52.30 ($3CH_2$), arC: [116.37 and 116.57 (d, CH, *J*= 20.0 Hz), 119.80 (CH), 125.35 (CH), 127.46 (CH), 128.21 (2CH), 129.02 (CH), 129.39 (2CH), 136.31 (2C), 154.11 and 156.54 (d, C, J_{C-F}= 243.0 Hz)], 157.78 (triazole C-3) 168.58 (triazole C-5). FT IR (ν_{max}, cm^{-1}): 3091 (Aromatic CH), 2925 (Aliphatic CH), 1498 (C=N). EI MS *m/z* (%): 383.50 ([M]$^+$, 21), 326.45 (36), 280.40 (78), 225.60 (21), 170.00 (100). Anal. Calcd. for $C_{20}H_{22}FN_5S$ (383.49); C, 62.64; H, 5.78; N, 18.26%. Found: C, 62.70; H, 5.88; N, 18.32%.

General procedure for preparation of compounds 6a-d

To the solution of **5a-c** (10 mmol) in dimethylformamide, a suitable amine (10 mmol) and formaldehyde (50 mmol) was added and the mixture was stirred at room temperature for 24 h. Then water was added to the reaction. The solid precipitated was filtered off and purified with dimethyl sulfoxide:water (1:3).

4-Benzyl-5-{[4-(2-fluorophenyl)piperazin-1-yl]methyl}-2-[(4-methylpiperazin-1-yl)methyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (6a)

Yield: 60%, mp 72-73°C. ^{1}H NMR (DMSO-d_6, δ ppm): 2.30 (brs, 2H, CH_2), 2.45 (brs, 7H, CH_3+2CH_2), 2.76 (brs, 8H, 4CH_2), 3.46 (brs, 2H, CH_2), 3.52 (s, 2H, CH_2), 5.35 (s, 2H, CH_2), 5.38 (s, 2H, CH_2), 6.93 (s, 2H, arH), 7.08 (d, 2H, arH, *J*=4.8 Hz), 7.26-7.32 (m, 5H, arH). ^{13}C NMR (DMSO-d_6, δ ppm): 40.90 (CH_3), 46.75 (2CH_2), 47.74 (CH_2), 47.81 (CH_2), 50.04 (CH_2), 50.23 (CH_2), 52.31 (CH_2), 52.47 (CH_2), 54.98 (CH_2), 69.11 (CH_2), 71.03 (CH_2), arC: [116.24 and 116.45 (d, CH, *J*= 21.0 Hz), 119.59 (CH), 122.75 and 122.83 (d, CH, *J*= 8.0 Hz), 125.23 (CH), 125.26 (CH), 127.42 (CH), 127.79 (CH), 127.79 (CH), 128.87 (CH), 140.10 and 140.17 (d, C, *J*= 7.0 Hz), 148.38 (C), 154.16 and 156.59 (d, C, $J_{C\text{-}F}$= 243.0 Hz)], 149.57 (triazole C-3), 168.72 (triazole C-5). FT IR (ν_{max}, cm^{-1}): 3033 (Aromatic CH), 2919 (Aliphatic CH), 1498 (C=N), 1238 (C=S). EI MS *m/z* (%): 496.43 ([M+1]$^+$, 31), 345.90 (76), 301.24 (40), 278.65 (35), 201.10 (100). Anal. Calcd. for $C_{26}H_{34}FN_7S$ (495.66); C, 63.00; H, 6.91; N, 19.78%. Found: C, 62.87; H, 7.09; N, 19.79%.

4-Benzyl-5-{[4-(2-fluorophenyl)piperazin-1-yl]methyl}-2-[(4-phenylpiperazin-1-yl)methyl]-2,4-dihydro-3H-1,2,4-triazole-3-thione (6b)

Yield: 56%, mp 65-67°C. ^{1}H NMR (DMSO-d_6, δ ppm): 2.43-2.50 (m, 6H, 3CH_2), 2.76 (brs, 6H, 3CH_2), 3.35 (brs, 6H, 3CH_2), 3.46 (s, 2H, CH_2), 5.36 (d, 2H, CH_2, *J*= 7.2 Hz), 6.90-6.95 (m, 4H, arH), 7.06-7.09 (m, 3H, arH), 7.25-7.27 (m, 4H, arH), 7.33 (d, 3H, arH, *J*= 7.2 Hz). ^{13}C NMR (DMSO-d_6, δ ppm): 46.75 (CH_2), 47.74 (CH_2), 48.67 (CH_2), 50.05 (CH_2), 50.27 (CH_2), 52.30 (CH_2), 52.46 (CH_2), 52.65 (CH_2), 52.68 (CH_2), 69.07 (CH_2), 71.02 (CH_2), arC: [116.25 and 116.45 (d, CH, *J*= 20.0 Hz), 122.83 (CH), 125.24 and 125.27 (d, CH, *J*= 3.0 Hz), 127.19 (2CH), 127.41 (2CH), 127.79 (CH), 127.79 (2CH), 128.87 (2CH), 129.34 (2CH), 140.11 and 140.19 (d, C, *J*= 8.0 Hz), 148.71 (C), 151.32 (C), 154.16 and 156.59 (d, C, $J_{C\text{-}F}$= 243.0 Hz)], 149.59 (triazole C-3), 168.54 (triazole C-5). FT IR (ν_{max}, cm^{-1}): 3092 (Aromatic CH), 2919 (Aliphatic CH), 1498 (C=N), 1238 (C=S). EI MS *m/z* (%): 557.74 ([M+1]$^+$, 34), 501.23 (43), 456.78 (35), 301.80 (75), 250.54 (100), 125.12 (51). Anal. Calcd. for $C_{31}H_{36}FN_7S$ (557.73); C, 66.76; H, 6.51; N, 17.58%. Found: C, 66.79; H, 6.65; N, 17.60%.

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

Yield: 70%, mp 213-215°C. ^{1}H NMR (DMSO-d_6, δ ppm): 1.41 (t, 3H, CH_3, J= 6.4 Hz), 2.40 (brs, 4H, $2CH_2$), 2.78 (brs, 6H, $3CH_2$), 2.99 (s, 2H, CH_2), 3.34 (brs, 6H, $3CH_2$), 4.57 (q, 2H, CH_2, J= 6.8 Hz), 5.21 (s, 2H, CH_2), 6.90-6.96 (m, 3H, arH), 7.03-7.08 (m, 2H, arH), 7.50-7.54 (m, 5H, arH), 7.87 (d, 1H, arH, J= 13.6 Hz), 8.92 (s, 1H, quinolone CH), 15.32 (s, 1H, OH). ^{13}C NMR (DMSO-d_6, δ ppm): 14.73 (CH_3), 49.23 ($2CH_2$), 49.58 ($2CH_2$), 49.99 ($2CH_2$), 50.17 (CH_2), 51.89 (CH_2), 52.38 (CH_2), 68.97 (CH_2), 70.98 (CH_2), 107.50 (C), arC: [106.46 (CH), 111.54 and 111.77 (d, CH, J= 23.0 Hz), 115.57 and 115.77 (d, 2CH, J= 20.0 Hz), 117.48 and 117.55 (d, 2CH, J= 7.0 Hz), 119.79 (C), 128.60 (2CH), 129.45 (2CH), 129.79 (CH), 134.80 and 134.95 (d, C, J= 15.0 Hz), 137.66 (C), 145.77 (C), 148.23 and 148.46 (d, C, J= 23.0 Hz), 152.05 and 154.53 (d, C, $J_{C\text{-}F}$= 248.0 Hz), 155.27 and 157.61 (d, C, $J_{C\text{-}F}$= 234.0 Hz], 148.99 (CH), 162.79 (triazole C-3), 169.75 (triazole C-5), 175.73 (C=O), 176.58 (C=O). FT IR (ν_{max}, cm^{-1}): 3450 (OH), 3068 (Aromatic CH), 2920 (Aliphatic CH), 1719 (2C=O), 1497 (C=N), 1241 (C=S). EI MS *m/z* (%): 718.25 ([M+H_2O]$^+$, 34), 521.00 (65), 431.09 (67), 340.01 (23), 309.90 (67), 295.24 (100), 123.67 (32). Anal. Calcd. for $C_{36}H_{38}F_2N_8O_3S$ (700.80); C, 61.70; H, 5.47; N, 15.99%. Found: C, 61.90; H, 5.32; N, 16.16%.

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

Yield: 79%, mp 225-226°C. ^{1}H NMR (DMSO-d_6, δ ppm): 1.17 (brs, 2H, CH_2), 1.33 (brs, 2H, CH_2), 2.40 (brs, 4H, $2CH_2$), 2.79 (brs, 4H, $2CH_2$), 3.00 (s, 2H, CH_2), 3.34 (brs, 6H, $3CH_2$), 3.47 (s, 2H, CH_2), 3.81 (brs, 1H, CH), 5.21 (s, 2H, CH_2), 6.92 (d, 2H, arH, J= 8.4 Hz), 7.04-7.07 (m, 2H, arH), 7.51-7.56 (m, 6H, arH), 7.87 (d, 1H, arH, J= 13.2 Hz), 8.64 (s, 1H, quinolone CH), 15.19 (s, 1H, OH). ^{13}C NMR (DMSO-d_6, δ ppm): 8.08 ($2CH_2$), 36.27 (CH), 49.21 ($2CH_2$), 49.80 ($2CH_2$), 50.09 ($2CH_2$), 51.83 ($2CH_2$), 52.38 (CH_2), 68.95 (CH_2), 107.01 (C), arC: [107.21 (CH), 111.30 (CH), 115.55 and 115.77 (d, 2CH, J= 22.0 Hz), 117.28 and 117.49 (d, 2CH, J= 21.0 Hz), 119.12 (C), 128.75 (CH), 129.43 (2CH), 129.78 (2CH), 134.95 and 135.11 (d, C, J= 16.0 Hz), 139.62 (2C), 148.13 and 148.35 (d, C, J= 22.0 Hz), 152.20 and 155.27 (d, c, $J_{C\text{-}F}$= 307.0 Hz), 157.61 and 162.76 (d, C, $J_{C\text{-}F}$= 514.0 Hz], 148.49 (CH), 166.39 (triazole C-3), 169.79 (triazole C-5), 173.27 (C=O), 176.88 (C=O). FT IR (ν_{max}, cm^{-1}): 3277 (OH), 3071 (Aromatic CH), 2919 (Aliphatic CH), 1728 (2C=O), 1499 (C=N), 1257 (C=S). EI MS *m/z* (%): 712.00 ([M]$^+$, 10), 651.00 (67), 550.34 (89), 210.50 (100). Anal. Calcd. for $C_{37}H_{38}F_2N_8O_3S$ (712.81); C, 62.34; H, 5.37; N, 15.72%. Found: C, 62.50; H, 5.41; N, 16.00%.

Diethyl {[(3-chloro-4-fluorophenyl)amino]methylene}malonate (7)

The synthesis of this compound was achieved according to published procedure [19]. Yield: 94%, mp 58°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.3-1.4 (m, 6H, $2CH_3$ of OCH_2CH_3), 4.2-4.3 (m, 4H, $2CH_2$ of OCH_2CH_3), 6.9-7.2 (m, 3H, ArH), 8.3 (d, 1H, Vinylic, J_{H-H}= 13 Hz), 10.9 (d, 1H, NH, J_{H-H}= 13 Hz). IR (KBr, cm^{-1}): 1722, 1658, 1622. ESI MS *m/z*: 338 $[M+Na]^+$. Anal. Calcd. for $C_{14}H_{15}ClFNO_4$: C, 53.26; H, 4.79; N, 4.44: found: C, 53.27: H, 4.74: N, 4.43%.

Ethyl 7-chloro-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (8)

The cyclization of **7** (1 mmol) that was published previously [19] was achieved by irradiation with diphenyl ether (12 mL) in monomode microwave reactor in closed vessel at 240°C for 5 min. A white solid occurred by cooling the reaction. Then this solid was washed with ethyl acetate and purified with dimethylformamide. Yield: 75%, mp>290°C. ^{1}H NMR (DMSO-d_6, δ ppm): 1.28 (brs, 3H, CH_3), 4.21 (brs, 2H, CH_2), 7.63 (s, 1H, ArH), 7.74 (s, 1H, ArH) 8.48 (s, 1H, quinolone CH), 12.37 (brs, 1H, NH). FT IR (ν_{max}, cm^{-1}): 3103 (NH), 3095 (Aromatic CH), 2987 (Aliphatic CH), 1698 (2C=O).

Ethyl 7-chloro-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (9)

To obtain compound **9** that was published previously [19], the solution of compound **8** (1 mmol) in dimethylformamide, ethyl bromide (5 mmol) and K_2CO_3 (2 mmol) was added. The reaction was irradiated in monomode microwave reactor in closed vessel at 75 °C for 20 min. A solid was appeared by evaporating the solvent. This solid was washed with water and purified with dimethylformamide:water (1:3). Yield 90%, mp 134-135°C. ^{1}H NMR (DMSO-d_6, δ ppm): 1.28 (t, 3H, CH_3, J=8.0 Hz), 1.35 (t, 3H, CH_3, J=8.0 Hz), 4.22 (q, 2H, CH_2, J=3.6 Hz), 4.42 (q, 2H, CH_2, J=7.2 Hz), 8.03 (d, 1H, ArH, J=9.6 Hz), 8.18 (d, 1H, ArH, J= 6.0 Hz), 8.70 (s, 1H, quinolone CH). ^{13}C NMR (DMSO-d_6, δ ppm): 14.74 (CH_3), 14.81 (CH_3), 48.78 (CH_2), 60.37 (CH_2), 110.45 (C), arC: [112.90 and 113.13 (d, CH, J=23.0 Hz), 120.52 (CH), 125.91 and 126.11 (d, C, J= 20.0 Hz), 129.13 and 129.19 (d, C, J= 6.0 Hz), 136.18 (C), 153.59 and 156.04 (d, C, J_{C-F}= 245.0 Hz)], 149.93 (CH), 164.75 (C=O), 171.86 (C=O). FT IR (ν_{max}, cm^{-1}): 3073 (Aromatic CH), 2918 (Aliphatic CH), 1716 (C=O), 1688 (C=O). EI MS *m/z* (%): 320.04 ($[M+Na]^+$, 25), 125.14 (100). Anal. Calcd. for $C_{14}H_{13}ClFNO_3$ (297.70); C, 56.48; H, 4.40; N, 4.70%. Found: C, 56.70; H, 4.44; N, 5.00%.

7-Chloro-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (10)

The compound **9** (10 mmol) was refluxed with 2 N NaOH (100 mL) for 2 h [19]. Then, the resulting solution was cooled to room temperature and acidified with acetic acid. The

precipitate solid was filtered off, washed with water, and purified with dimethyl sulfoxide-water (1:4). Yield: 90%, mp>300°C. ^{1}H NMR (DMSO-d_6, δ ppm): 1.39 (t, 3H, CH_3, J=7.2 Hz), 4.61 (q, 2H, CH_2, J=7.2 Hz), 8.15 (d, 1H, ArH, J=8.8 Hz), 8.42 (d, 1H, ArH, J= 6.0 Hz), 9.05 (s, 1H, quinolone CH), 14.96 (brs, 1H, OH). FT IR (ν_{max}, cm^{-1}): 3057 (Aromatic CH), 1715 (2C=O).

General procedure for the preparation of compounds 11a-c

The mixture of compound **10** (1 mmol), triethylamine (3 mmol) and compounds **5a-c** (1.1mmol) in dimethylformamide was irradiated in monomode microwave reactor in closed vessel at 80°C for 15 min. After evaporation of the solvent, a solid was formed, which was washed with water and recrystallized with dimethylsulfoxide: water (1:3).

1-Ethyl-6-fluoro-7-[(5-{[4-(2-fluorophenyl)piperazin-1-yl]methyl}-4-phenyl-4*H*-1,2,4-triazol-3-yl)thio]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (11a)

Yield 70%, mp 200-201°C. ^{1}H NMR (DMSO-d_6, δ ppm): 1.39 (brs, 3H, CH_3), 2.39 (brs, 4H, $2CH_2$), 2.80 (brs, 4H, $2CH_2$), 3.35 (s, 2H, CH_2), 4.60 (brs, 2H, CH_2), 6.92 (s, 2H, arH), 7.05 (s, 2H, arH), 7.47 (s, 3H, arH), 7.52 (s, 2H, arH), 8.13 (brs, 1H, arH), 8.41 (s, 1H, arH), 9.05 (s, 1H, quinolone CH), 14.74 (brs, 1H, OH). ^{13}C NMR (DMSO-d_6, δ ppm): 14.25 (CH_3), 50.25 (CH_2), 51.25 ($2CH_2$), 54.26 ($2CH_2$), 55.80 (CH_2), 107.56 (C), arC: [115.23 and 115.34 (d, CH, J= 11.0 Hz), 116.45 and 116.67 (d, CH, J= 22.0 Hz), 119.20 and 119.28 (d, C, J= 8.0 Hz), 121.20 and 121.38 (d, 2CH, J= 18.0 Hz), 124.26 and 124.34 (d, 2CH, J= 8.0 Hz), 128.23 (2CH), 129.90 (CH), 132.18 (2CH), 136.67 and 136.74 (d, C, J= 7.0 Hz), 138.90 (2C), 141.56 (C), 152.78 and 156.10 (d, C, J_{C-F}= 332.0 Hz), 159.12 and 162.90 (d, C, J_{C-F}= 378.0 Hz)], 148.55 (CH), 155.25 (triazole C-3), 161.67 (triazole C-5), 170.09 (C=O), 172.34 (C=O). FT IR (ν_{max}, cm^{-1}): 3456 (OH), 3057 (Aromatic CH), 2919 (Aliphatic CH), 1714 (2C=O), 1499 (C=N). EI MS *m/z* (%): 625.14 ([M+Na]$^+$, 18), 447.54 (90), 392.35 (100), 292.18 (91). Anal. Calcd. for $C_{31}H_{28}F_2N_6O_3S$ (602.65); C, 61.78; H, 4.68; N, 13.95%. Found: C, 61.65; H, 4.72; N, 14.09%.

1-Ethyl-7-[(4-ethyl-5-{[4-(2-fluorophenyl)piperazin-1-yl]methyl}-4*H*-1,2,4-triazol-3-yl)thio]-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (11b)

Yield: 65%, mp 183-185°C. ^{1}H NMR (DMSO-d_6, δ ppm): 1.30 (brs, 3H, CH_3), 1.39 (brs, 3H, CH_3), 2.58 (brs, 4H, $2CH_2$), 2.99 (brs, 4H, $2CH_2$), 3.35 (s, 2H, CH_2), 3.64 (s, 2H, CH_2), 4.03 (s, 2H, CH_2), 7.08 (s, 4H, arH), 8.16 (brs, 1H, arH), 8.42 (brs, 1H, arH), 9.06 (s, 1H, quinolone CH), 13.63 (s, 1H, OH). ^{13}C NMR (DMSO-d_6, δ ppm): 13.78 (CH_3), 14.00 (CH_3), 50.15 ($2CH_2$), 51.95 (CH_2), 53.26 ($2CH_2$), 55.81 ($2CH_2$), 107.59 (C), arC: [115.33 and 115.44 (d, CH, J= 11.0 Hz), 116.47 and 116.67 (d, CH, J= 20.0 Hz), 119.28 and 119.36 (d, C, J= 8.0 Hz), 121.20 and 121.37 (d, 2CH, J= 17.0 Hz), 124.26 and

124.39 (d, 2CH, *J*= 15.0 Hz), 136.08 and 136.14 (d, C, *J*= 6.0 Hz), 137.90 (C), 141.66 (C), 152.80 and 156.12 (d, C, J_{C-F}= 332.0 Hz), 160.12 and 162.90 (d, C, J_{C-F}= 278.0 Hz)], 148.55 (CH), 155.95 (triazole C-3), 161.60 (triazole C-5), 170.89 (C=O), 172.33 (C=O). FT IR (v_{max}, cm^{-1}): 3400 (OH), 3093 (Aromatic CH), 2917 (Aliphatic CH), 1714 (2C=O), 1499 (C=N). EI MS *m/z* (%): 591.37 (100), 577.35 ($[M+Na]^+$, 18), 563.46 (46). Anal. Calcd. for $C_{27}H_{28}F_2N_6O_3S$ (554.61); C, 58.47; H, 5.09; N, 15.15%. Found: C, 58.61; H, 5.19; N, 15.32%.

7-[(4-Benzyl-5-{[4-(2-fluorophenyl)piperazin-1-yl]methyl}-4*H*-1,2,4-triazol-3-yl)thio]-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (11c)

Yield: 57% mp 184-186°C. ^{1}H NMR (DMSO-d_6, δ ppm): 1.39 (s, 3H, CH_3), 2.44 (brs, 4H, $2CH_2$), 2.76 (brs, 4H, $2CH_2$), 3.46 (brs, 2H, CH_2), 4.61 (s, 2H, CH_2), 5.33 (s, 2H, CH_2), 6.92 (d, 2H, arH, *J*= 8.8 Hz), 7.08 (s, 2H, arH), 7.26-7.33 (m, 5H, arH), 8.21 (d, 1H, arH, *J*= 8.0 Hz), 8.44 (s, 1H, arH), 9.08 (s, 1H, quinolone CH), 13.44 (s, 1H, OH). ^{13}C NMR (DMSO-d_6, δ ppm): 14.23 (CH_3), 49.25 (CH_2), 50.35 ($2CH_2$), 51.26 ($2CH_2$), 53.80 ($2CH_2$), 107.50 (C), arC: [115.26 and 115.33 (d, CH, *J*= 7.0 Hz), 116.55 and 116.77 (d, CH, *J*= 22.0 Hz), 119.20 and 119.29 (d, C, *J*= 9.0 Hz), 121.25 and 121.38 (d, 2CH, *J*= 13.0 Hz), 124.27 and 124.34 (d, 2CH, *J*= 7.0 Hz), 128.20 (2CH), 129.99 (CH), 132.48 (2CH), 136.57 and 136.64 (d, C, *J*= 7.0 Hz), 138.75 (2C), 141.50 (C), 152.78 and 156.10 (d, C, J_{C-F}= 332.0 Hz), 158.00 and 162.20 (d, C, J_{C-F}= 420.0 Hz)], 148.78 (CH), 155.29 (triazole C-3), 161.97 (triazole C-5), 170.19 (C=O), 172.84 (C=O). FT IR (v_{max}, cm^{-1}): 3400 (OH), 3092 (Aromatic CH), 2918 (Aliphatic CH), 1718 (2C=O), 1498 (C=N). EI MS *m/z* (%): 655.39 ($[M+K]^+$, 46), 653.37 (100). Anal. Calcd. for $C_{32}H_{30}F_2N_6O_3S$ (616.68); C, 62.32; H, 4.90; N, 13.63%. Found: C, 62.40; H, 5.12; N, 13.87%.

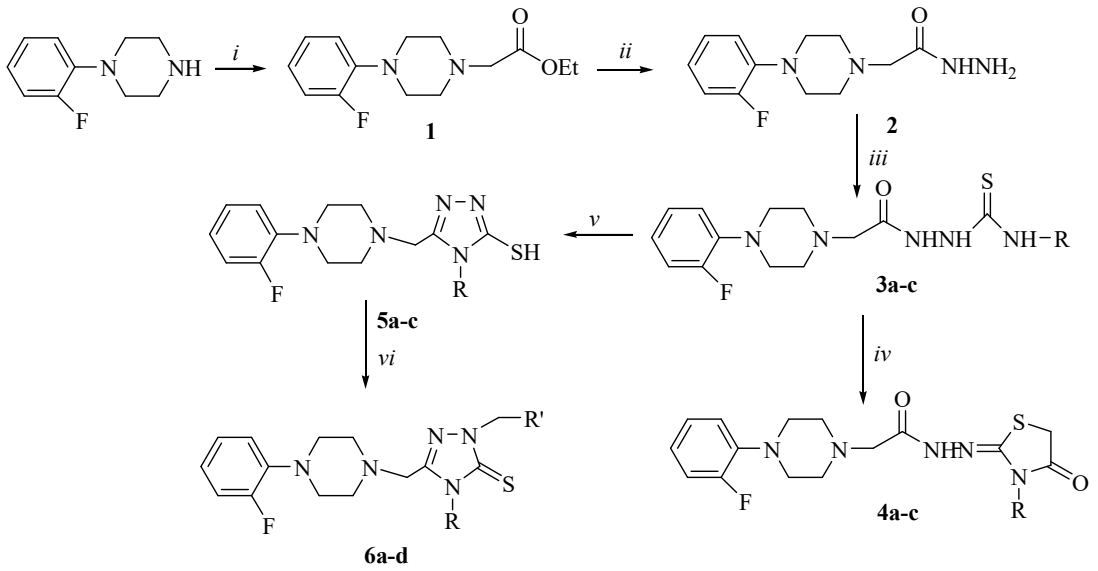
Antimicrobial Activity Assessment

The test microorganisms were purchased from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey). All the newly synthesized compounds were weighed and dissolved in hexane for preparing the 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 (µg/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 micro dilution test plates were incubated for 18-24 h at 35 °C. Brain Heart Infusion broth (BHI) (Difco, Detriot, MI) was used for *M. smegmatis*, and incubated for 48-72 h at 35 °C [22]. Ampicillin (10 µg) and fluconazole (5µg) were used as standard antibacterial and antifungal drugs, respectively. Dimethylsulfoxide with dilution of 1:10 was used as solvent control.

RESULTS AND DISCUSSION

The new Mannich base derivatives **6a-d** and novel fluoroquinolone analogues **11a-c** described herein were synthesized and examined in terms of biological activity as shown in Schemes 1 and 2, respectively. Newly compounds were obtained according to the synthetic route summarized in Scheme 1. Firstly, compound **1** was obtained from the treatment of ethyl bromoacetate with 1-(2-fluorophenyl)piperazine provided commercially. FT-IR spectrum of compound **1** indicated absorption band at 1745 cm^{-1} belonging the carbonyl function.

In ^{1}H NMR spectra, it was noticed that the protons of ethyl group peaks at 1.20 and 4.10 ppm. With the conversion of compound **1** to hydrazide (**2**), the signals of ester function were not observed in the ^{1}H and ^{13}C NMR spectrum. Additional peaks are due to the presence of $–NHNH_2$ and were indicated at 4.25 and 8.94 ppm. Moreover, in the FT-IR spectrum, absorption bands were registered at 3256 and 3225 cm^{-1} as a broad peak typical for this structure. Reaction of compound **2** with suitable isothiocyanates phenyl-, ethyl- and benzyl isothiocyanates respectively generated the corresponding carbonothioylhydrazino derivatives **3a-c**, which behave as suitable intermediates in the reactions leading to the formation of nitrogen- and/or sulfur-containing compounds having biological activity. The reaction was investigated in ethanol at microwave irradiated conditions. The FT-IR spectra of derivatives **3a-c** showed an absorption band at 1237, 1235 and 1232 cm^{-1} which can be attributed to -C=S function. The synthesis of (1,3-thiazoline-2-ylidene) acetohydrazide derivatives (**4a-c**) was carried out by the condensation of **3a-c** with ethyl bromoacetate and dried sodium acetate in ethanol by refluxing. ^{1}H NMR spectrum of **4a-c** showed signal at between 3.41-4.14 ppm which can be attributed to thiazolidinone nucleus. In addition, signals of $–CH_2$ groups resulting from thiazolidinone ring resonated at 33.19 (for **4a**), 32.21 (for **4b**), 33.17 (for **4c**) ppm in the ^{13}C NMR spectrum. Another evidence for the compounds, **4a-c** showed the molecular ion peak, suggesting the assigned structure. The basic reaction of **3a-c** yielded 1,2,4-triazole (**5a-c**) which can be considered as important tools for further condensation reactions leading to the formation of novel bioactive molecules (Scheme 1). The reaction was carried out in water, which is not a toxic solvent, under microwave conditions.



3a, **4a**, **5a**: **R**= -C_6H_5; **3b**, **4b**, **5b**: **R**= -CH_2CH_3; **3c**, **4c**, **5c**: **R**= - $CH_2C_6H_5$
6a: **R**= - $CH_2C_6H_5$; **R'**= methyl piperazine; **6c**: **R**= -C_6H_5; **R'**= norloxacin
6b: **R**= - $CH_2C_6H_5$; **R'**= phenyl piperazine; **6d**: **R**= -C_6H_5; **R'**= ciprofloxacin

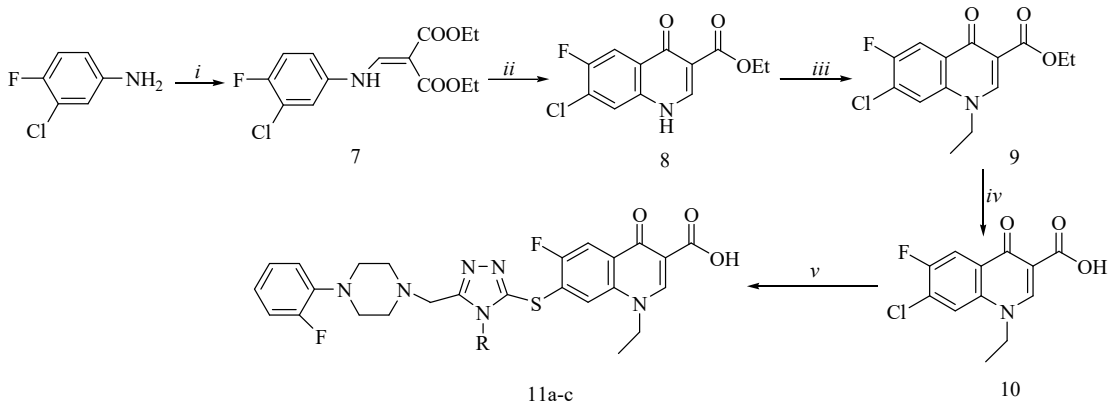
Scheme 1. Reagents and conditions: *i*: $BrCH_2COOEt$, Et_3N, THF, rt; *ii*: $H_2NNH_2.H_2O$, MW; *iii*: suitable alkyl(aryl)isothiocyanate, EtOH, MW; *iv*: $BrCH_2COOEt$, NaOAc, MW; *v*: NaOH, MW; *vi*: HCHO, suitable amine, DMF, rt.

In the ^{1}H NMR spectrum of **5a-c**, the SH signals were observed at 13.90 ppm (for **5a**), 13.69 (for **5b**) 13.97 (for **5c**) as a proof of cyclization. In the ^{13}C NMR spectrum, triazole C-3 and C-5 carbon atoms of compounds **5a-c** resonated at 149.62-157.78 ppm (triazole C-3) and 167.73-168.68 ppm (triazole C-5), respectively consistent with literature findings [20, 21]. The mass spectrum of compounds showed peaks according to their molecular formula.

The preparation of compound (**7**) was obtained with the reaction of 3-chloro-4-fluoroaniline with diethylethoxymethylene malonate according to published procedure [19]. Then compound **7** was converted to (**8**) by cyclization with diphenyl ether. The reaction was carried out by microwave irradiation. With the use of MW conditions, higher yield was assessed; however, the important effect of MW irradiation was on reaction time. The complete conversion of the compound **7** in best yield was observed after microwave irradiation at 240 °C for 5 min. Compounds **8-10** were published in the literature [19]. Subsequently, compound **8** was alkylated with ethyl bromide using K_2CO_3 in dimethylformamide to give the corresponding compound (**9**). As different from compound **8**, ^{1}H and ^{13}C NMR spectrum of **9** showed extra peaks owing to ethyl group at the belonging chemical shift values, while the signal originated from any amino group was not recorded. In addition, mass spectrum and elemental analysis results confirm with the structure. Compound **10** was synthesized by the treatment of compound **9** with NaOH under reflux conditions [19]. In the ^{1}H NMR spectrum of compound **10**, the -OH

peak was recorded 14.96 ppm while the peaks belonging to ester group were not seen in the ^{1}H NMR spectrum.

The new fluoroquinolones **11a-c** described herein were synthesized as shown in Scheme 2.



11a: R= -C_6H_5 ; **11b**: R= -CH_2CH_3; **11c**: R= -$CH_2C_6H_5$

Scheme 2: Reagents and conditions: *i*: diethyl ethoxymethylenemalonate, 100°C, reflux; *ii*: diphenyl ether, MW, 240°C for 5 min; *iii*: ethyl bromide, K_2CO_3, DMF, MW, 75°C for 20 min; *iv*: 2N NaOH, reflux; *v*: Et_3N, compounds 5a-c, DMF, MW, 80°C, 15 min.

To obtain new fluoroquinolone-triazole hybrid compounds, compound **10** was subjected to react with compounds **5a-c** in the presence of triethylamine. This reaction was achieved under microwave conditions. The best MW condition in terms of yields and product stability was assessed at 80°C, 15 min in dimethylformamide. In ^{1}H NMR spectrum of these compounds, additional signals derived from fluorophenyl ring and piperazine nucleus appeared at the related regions. Additionally, all the other aromatic and aliphatic carbons also appeared at the appropriate chemical shift regions. Mass spectrum of **11a-c** was showed $[M+Na]^+$ and $[M+K]^+$ ion peaks suitable their molecular formula.

Antimicrobial activity

Newly synthesized compounds were screened for their biological properties and the results were illustrated in Table 1. This reveals that the most compounds own well activities against to test microorganisms with the mic values varying between 0.24-500 µg/mL. Compounds **3a,** and **3b** showed medium activity towards *Enterococcus faecalis* (Ef), with the mic values 250 µg/mL while, compound **3c** showed slight antifungal activity on *Candida albicans* (Ca) and *Saccharomyces cerevisiae* (Sc). Compounds **4a-c** displayed moderate activity on *Mycobacterium smegmatis* (Ms). The compounds carrying an 1,2,4-triazole moiety (**5a-c**) showed moderate activity on Ms with the mic value 62.50-125

µg/mL and slight activity towards Ca with mics 62.5-500 µg/mL. Moreover, the conversion of compounds **5a-c** to compounds **6a-d**, which can be regarded as Mannich bases, resulted in a remarkable increase in the antimicrobial activity. Among these, the better activity was observed for compounds **6c** and **6d** carrying a fluoroquinolone unit. According to Table 1, it is more attractive to speculate the observation that the excellent antimicrobial activity results were observed for compounds **11a-c** which triazole-fluoroquinolone hybrid compounds on the test microorganisms, except Ca and Sc with the mic values between 0.24-1.9 µg/mL. In fact, the activity of these compounds was better than reference drugs, as shown table 1.

Table 1. Antimicrobial activity of the synthesized compounds (µg/mL).

	Microorganisms and Minimal Inhibition Concentration								
Comp.No	Ec	Yp	Pa	Sa	Ef	Bc	Ms	Ca	Sc
3a	-	-	-	-	250	-	62.5	500	-
3b	-	-	-	-	250	-	62.5	500	-
3c	-	-	-	-	-	-	-	125	62.5
4a	-	-	-	-	-	62.5	31.3	125	-
4b	-	-	-	-	-	-	125	500	-
4c	-	-	-	-	-	-	250	500	-
5a	-	-	-	-	-	-	62.5	500	125
5b	-	-	-	-	-	-	125	250	-
5c	-	-	-	125	-	-	62.5	62.5	-
6a	31.3	62.5	62.5	-	-	-	-	500	-
6b	31.3	-	-	-	-	-	-	125	250
6c	<3,9	<3,9	<3,9	<3,9	<3,9	<3,9	125	-	-
6d	<0,24	<0,24	<0,24	<0,24	<0,24	<0,24	<0,24	-	-
11a	<0.26	<0,26	<0.26	<0.26	<0.26	<0.26	<0.26	-	-
11b	<1,9	<1,9	<1,9	<1,9	<1,9	<1,9	<1,9	-	-
11c	<0,24	<0,24	<0,24	<0,24	<0,24	<0,24	<0,24	-	-
Amp.	10	18	>128	10	35	15			
Strep.							4		
Flu.								<8	<8

Ec: Escherichia coli ATCC 25922, Yp: Yersinia pseudotuberculosis ATCC 911, Pa: Pseudomonas aeruginosa ATCC 43288, Sa: Staphylococcus aureus ATCC 25923, Ef: Enterococcus faecalis ATCC 29212, Bc: Bacillus cereus 702 Roma, Ms: M. smegmatis ATCC607, Ca: Candida albicans ATCC 60193, Sc: Saccharomyces cerevisiae RSKK 251, Amp.: Ampicillin, Strep.: Streptomycin, Flu.: Fluconazole, (—): no activity.

CONCLUSION

This study reports the synthesis of novel hybrid compounds including fluoroquinolone and Mannich base derivatives starting from 1-(2-fluorophenyl)piperazine. The antimicrobial activity studies were examined. The results show that 1,2,4-triazole attached to

fluoroquinolones (**11a-c**) displayed excellent activity against to test microorganism except *Candida albicans* and *Saccharomyces cerevisiae*.

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Türkçe Öz ve Anahtar Kelimeler

Antimikrobiyal Araçlar Olarak Yeni Florokinolin-Triazol Hibrit Bileşiklerinin Sentezi

Serap Başoğlu Özdemir

Öz: Hidrazid bileşiği (**2**) 1-(2-florofenil)piperazin bileşiğinden iki kademede sentezlenmiştir. Bileşik (**2**)'nin farklı alkil (aril) izotiyosiyanatlarla muamele edilmesi ile (**3a-c**) bileşikleri elde edilmiştir. 1,3-Tiyazolidin türevleri (**4a-c**), (**3a-c**) bileşiklerinin etil bromoasetat ile reaksiyonundan sentezlenmiştir. Mannich bazları (**6a-d**), (**5a-c**) bileşiklerinin formaldehit varlığında çeşitli uygun aminlerle tepkimeye sokulmasıyla elde edilmiştir. Bileşik (**3**) türevleri, florokinolon analogları (**11a-c**) için başlangıç bileşiği olması amacıyla 1,2,4-triazol halkalarına dönüştürülmüştür. Son olarak, sentezlenmiş bileşiklerin biyolojik özellikleri incelenmiş ve bunların bazıları kuvvetli etki göstermiştir.

Anahtar kelimeler: Florokinolin; 1,2,4-triazol; mannich bazı; biyolojik özellikler.
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