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Synthesis and antimicrobial activity evaluation of new norfloxacine-azole hybrids

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Abstract: Norfloxacin-azole hybrids **3** and **6a,b** were synthesized starting from norfloxacin. The treatment of these compounds with amines as a one-pot three-component reaction produced the corresponding amino derivatives **4a,b**, **7a–g** and **8a,b** in good yields. The conventional and microwave-assisted methods were used with the latter method being more efficient. The structures of the synthesized compounds were characterized by elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and MS. All compounds were screened for their antimicrobial activities. Most of them exhibit excellent antibacterial activity but are not active against selected fungi.

Keywords: antimicrobial activity; microwave; norfloxacin; one-pot; 1,3,4-oxadiazole; three-component reaction; 1,2,4-triazole.

Introduction

The adaptation of microorganisms to survive in the presence of antibiotics has been described as the phenotypic expression of antibiotic resistance. Resistance is becoming increasingly serious, which causes not only great damage to human health but also economic loss, and the treatment of infectious diseases remains an important and challenging problem as antibiotics are increasingly becoming ineffective.

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Due to the frequent inadequacy of standard antibiotic therapy, efforts have been focused on addressing the problem of multidrug-resistant bacteria [1–7].

Fluoroquinolones have emerged as the dominant class of broad-spectrum antibiotics for the treatment of a wide variety of both Gram-negative and Gram-positive bacterial infections. These antibacterial agents act by inhibiting bacterial enzymes topoisomerase IV and DNA gyrase. Azoles have attracted special attention by synthetic organic chemists due to their potential applications as bioactive compounds, and an increasing effort has been directed toward their use in medicinal chemistry [8–11].

In recent years, to overcome the drug resistance problem, the concept of hybrid molecules, which contain two or more pharmacophore groups bonded together covalently in one molecular framework, has been introduced. It has been reported that the compounds 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. The heterocyclic pharmacophores are selected on the basis of their known biological activity profiles [12–14]. For the hybridization of fluoroquinolones, the most common strategy has been the introduction of new substituents in the C-7 position [15].

Multi-component reactions with at least three components in a one-pot process to give a single product represent a unique strategy [16, 17]. Moreover, improvements have been achieved applying microwave irradiation as an effective and non-polluting method for the green synthesis of bioactive molecules [18–21]. The superior properties of microwave- irradiated techniques are attributed to both thermal and specific non-thermal effects induced by these irradiations, providing rapid and convenient chemical synthesis [22]. Therefore, the combination of one-pot multicomponent reactions and microwave-irradiation techniques have been an attractive methodology for production of new bioactive compounds.

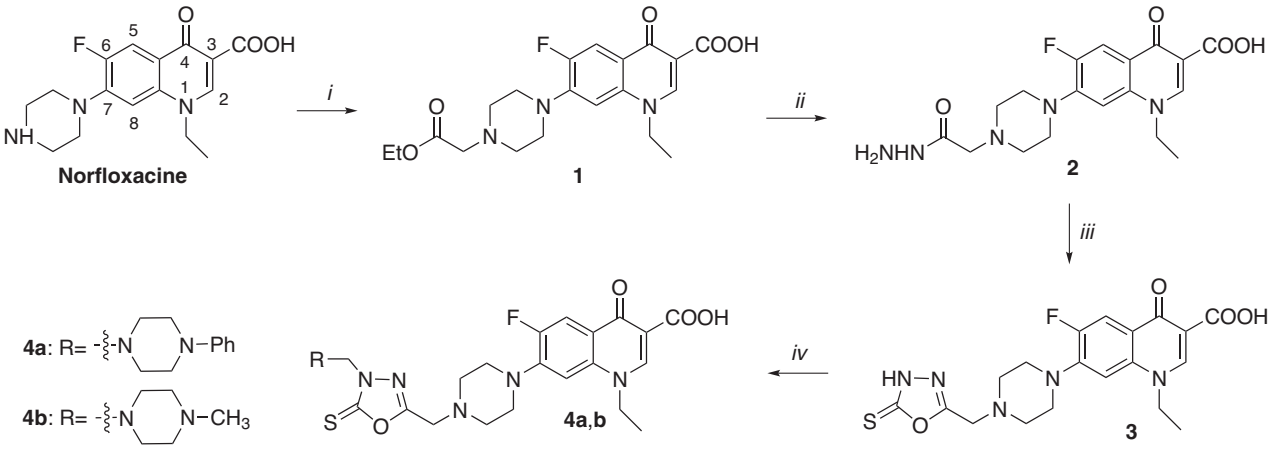
Our rationale for developing new chemotherapeutic agents involves the hybridization of two biologically active molecules into a single hybrid molecule.

Results and discussion

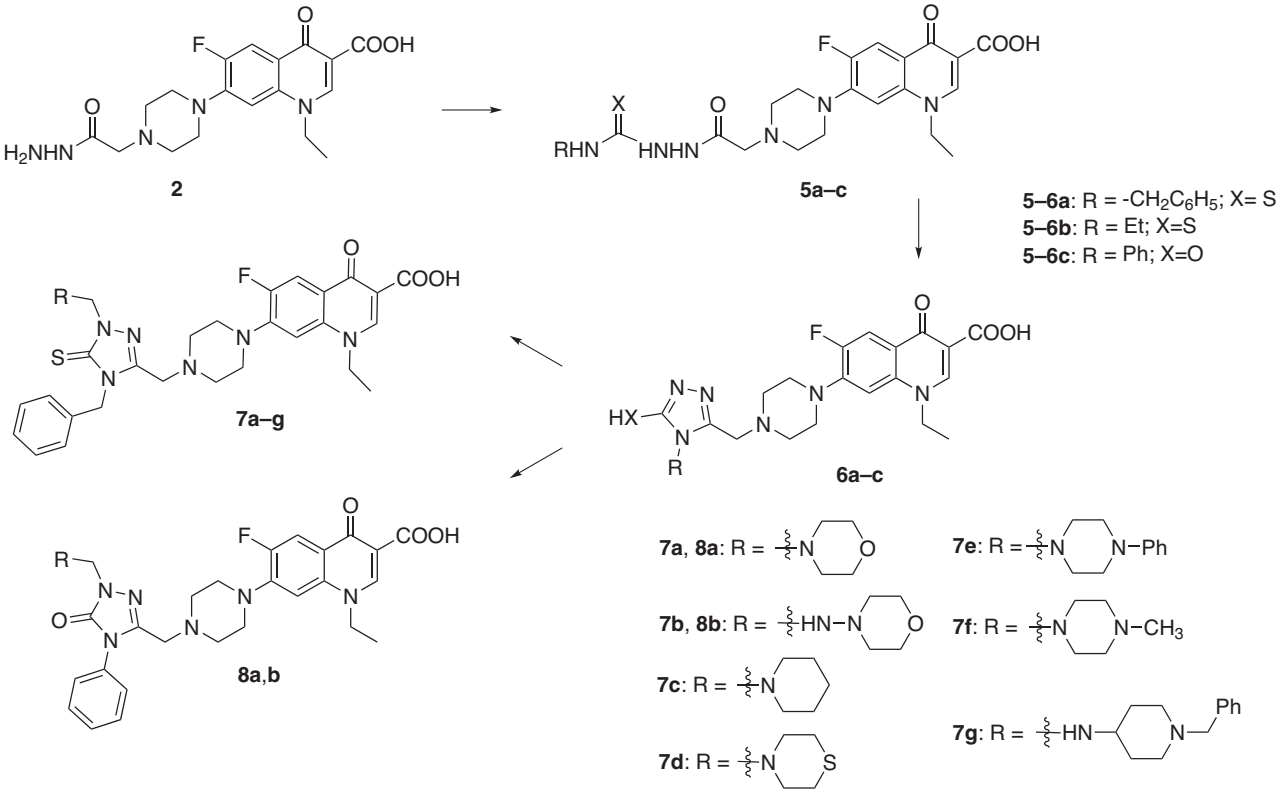
Chemistry

For the synthesis of the target hybrids, the strategy depicted in Schemes 1 and 2 was chosen.

The use of microwave irradiation provided an efficient and green synthetic approach with dramatically reduced reaction times and improved yields [23]. Norfloxacin and ethyl bromoacetate were subjected to condensation in dimethylformamide (DMF) yielding product **1**. The synthesis of compound **1** was achieved in a moderate yield (74%) in DMF with a reaction time of 24 h by the procedure reported by Zsoldos-Mádyet and co-workers. On the other hand, a similar reaction under microwave irradiation afforded compound **1** in good yield after 10 min. The



Scheme 1 Reagents and conditions: (i) $BrCH_2CO_2Et$ in DMF, TEA, 24 h, rt. (method 1) or $BrCH_2CO_2Et$, EtOH, TEA, 15 min, 200 W (method 2); (ii) H_2NNH_2, chloroform, 24 h, rt. (method 1) or 15 min 80 W MW irradiation MW (method 2); (iii) KOH, CS_2, EtOH-H_2O, 10 h, reflux (method 1) or 20 min 150 W MW irradiation MW (method 2); (iv) amine, DMF, HCHO, 24 h, rt.



Scheme 2

treatment of compound **1** with hydrazine hydrate under the conventional [23] and microwave-assisted conditions afforded hydrazide **2**. Compared to conventional thermal heating in chloroform, the microwave-irradiation technique without solvent decreased the reaction time from 24 h to 15 min, and the yield was increased from 78% to 94%. The given structure of **2** is fully consistent with the spectral data. In particular, the mass spectral fragmentation confirms the proposed structure.

The cyclization of hydrazide **2** with carbon disulfide was achieved in ethanol under conventional heating and also microwave-irradiated conditions yielding 1,3,4-oxadiazole **3**. The use of microwave irradiation resulted in an increased yield from 81% to 97%. However, the most striking effect of the irradiation was the decrease of the reaction time from 10 h to 20 min. In the ^{1}H NMR spectrum of compound **3**, the signals derived from the hydrazide function disappeared and a new signal due to -NH proton on the 1,3,4-oxadiazole ring is seen at 9.23 ppm as a D_2O-exchangeable singlet. The C=S stretching band is observed at 1256 cm^{-1} in the IR spectrum. The LC MS and elemental analysis data also support the proposed structure.

The treatment of hydrazide **2** with isothiocyanates furnished the corresponding products **5a–c** (Scheme 2). The IR spectra of derivatives **5a,b** show an absorption band at 1265 cm^{-1} (for **5a**) and 1250 cm^{-1} (for **5b**) indicating the presence of a C=S double bond. Furthermore, the IR and ^{1}H NMR spectra of **5a–c** exhibit signals for three NH protons (exchangeable with D_2O), while no signal derived from an -NH_2 group is observed. The treatment of **5a–c** with a base resulted in cyclization to 1,2,4-triazole derivatives **6a–c**. The reaction was investigated in ethanol-water under classical heating conditions and under microwave irradiation conditions. The progress of the reactions was monitored by thin layer chromatography (TLC). With the use of the microwave irradiation, the yield was improved to 94–97% and the reaction time for complete consumption of the starting material was decreased from 15–18 h to a remarkable 25 min. The optimal microwave power in terms of yield and product stability was found to be 120 W in a closed vessel. A decrease in microwave power resulted in lower yields and longer reaction times. In an attempt to optimize the reaction conditions for the irradiation, solvents were also screened. The best results were obtained in a mixture of methanol and water. In the ^{1}H NMR spectra of compounds **6a,b**, a singlet characteristic for the -SH group is recorded at 13.88 ppm, while the NH proton on a 1,2,4-triazol ring of **6c** resonates at 11.89 ppm. The IR spectra of compounds **6a,b** exhibit absorption bands originating from the -SH function at 2823–2824 cm^{-1}. In addition, compounds **6a–c** gave a mass fragmentation pattern and elemental analysis data consistent with the assigned structures. The one-pot three-component synthesis of compounds **4a,b, 7a–g** and **8a–c** was achieved by the amination of compounds **3** and **6a–c** with amines in the presence of formaldehyde in DMF (conventional method) or without solvent (irradiation method). These structures merge azine, azole and norfloxacin units into a single molecule in an attempt to obtain antimicrobial agents with improved properties (Scheme 2) [24, 25]. Initially, to optimize the conditions for this three-component reaction, compound **7a** was selected as the model product, and the model reaction was performed in a polar solvent, in the absence of solvent and in the presence of a Lewis or Bronsted acid catalyst such as *p*-TSA, $FeCl_3$, $InCl_3$ and HCl. The solvent-free reaction with HCl as a catalyst is the fastest method yielding the desired product **7a** after 18 min at 100 W with the yield of 86%.

Biological activity

All compounds were screened for their antimicrobial activities *in vitro* [26, 27] and the results are presented in Tables 1 and 2. The antimicrobial drugs norfloxacin, ampicillin, streptomycin and fluconazole were used as reference drugs. The results presented in Table 1 reveal that most of the synthesized fluoroquinolones effectively inhibit the growth of all tested microbial strains *in vitro* except *Candida albicans* (Ca) and *Saccharomyces cerevisiae* (Sc). Compound **1**, which is a norfloxacin derivative carrying an ethoxycarbonylmethyl function on the piperazine ring shows the best minimum bactericidal concentration (MBC) values with <0.041 μg/mL, exhibiting equal strong antimicrobial efficacy in comparison with norfloxacin, and stronger activity than ampicillin. This compound displays moderate antifungal activity on Ca and Sc. Compounds **2** and **3**, containing a hydrazide group or a 1,3,4-oxadiazole ring attached to the norfloxacin core with a methylene linker shows good MBC values in the range of 0.24–15.6 μg/mL. Table 2 reveals that compounds **2** and **3** inhibit the activity of *Escherichia coli* (Ec), *Staphylococcus aureus* (Sa) and *Enterococcus faecalis* (Ef) equally to norfloxacin. Compounds **2** and **3** demonstrate better antibacterial activity than the reference drug ampicillin. Moreover, with the minimum inhibitory concentration (MIC) values of 0.24 μg/mL, these compounds exhibit a better mycobacterial inhibition on *Mycobacterium smegmatis* (Ms) than the standard drug streptomycin (MBC 4 μg/mL).

On the other hand, compounds **4a** and **4b** show excellent MBC values of 0.24 μg/mL on Ec and Ms. Compound **5a** shows little activity. By contrast, the analogue **5b** is

Table 1 Screening for antimicrobial activity.

Comp.	Minimum inhibitory concentration (μg/mL)								
	Ec	Yp	Pa	Sa	Ef	Bc	Ms	Ca	Sc
1	<0.041	<0.041	<0.041	<0.041	<0.041	<0.041	<0.041	41.5	20.3
2	<0.24	15.6	15.6	<0.24	<0.24	<0.24	0.97	500	–
3	<0.24	15.6	15.6	<0.24	<0.24	<0.24	0.97	500	–
4a	<0.24	0.98	1.95	1.95	1.95	1.95	<0.24	–	500
4b	<0.24	125	–	125	125	0.49	<0.24	–	250
5a	15.6	62.5	125	125	62.5	62.5	0.97	500	–
5b	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24	0.48	500	–
5c	7.8	31.3	–	7.8	7.8	62.5	0.97	500	–
6a	1.9	1.9	–	<0.24	<0.24	<0.24	15.6	500	–
6b	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24	0.48	500	
6c	7.8	31.3	–	7.8	7.8	62.5	0.97	500	–
7a	500	125	–	62.5	31.25	31.25	–	–	–
7b	<0.24	15.65	–	<0.241	<0.24	<0.24	–	–	–
7c	<0.24	250	<0.24	<0.24	7.81	7.81	125	–	–
7d	250	250	–	31.25	250	125	–	–	–
7e	<0.24	15.65	–	<0.24	<0.24	<0.24	–	–	–
7f	<0.24	250	–	<0.24	<0.24	7.81	125	–	–
7g	62.5	250	–	<0.24	<0.24	7.81	125	–	–
8a	<0.24	31.3	–	7.8	7.8	62.5	0.97	–	–
8b	<0.24	250	–	<0.24	<0.24	<0.24	–	–	–
Norf	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24	–	7.81
Amp	10	18	>128	35	10	15			
Strep							4		
Flu								<8	<8

Ec, *Escherichia coli*; Yp, *Yersinia pseudotuberculosis*; Pa, *Pseudomonasaeruginosa*; Sa, *Staphylococcus aureus*; Ef, *Enterococcus faecalis*; Bc, *Bacillus cereus*; Ms, *Mycobacterium smegmatis*; Ca, *Candida albicans*; Sc, *Saccharomyces cerevisiae*; Norf, norfloxacin; Amp, ampicillin; Strep, streptomycin; Flu, fluconazole; (–), no activity.

highly active with the MBC values of 0.24 μg/mL that are equal to those of norfloxacin. Many compounds are more active against Ms than the standard drug streptomycin. Strong activities of **6a** and **6b** against several microbial strains should be noted. Several derivatives **7** and **8** also show respectable activities in comparison to the properties of the standard drugs.

Thus, most of the newly synthesized compounds exhibit strong antimicrobial efficacy in comparison with norfloxacin, and these compounds are more potent than the reference drug ampicillin. Activity of some compounds is stronger than activity of streptomycin against Ms. Comparison of our previous [24, 27, 28] and current data shows that when the fluorophenylene linker is present between the norfloxacin core and the azole unit, the antimicrobial activity decreases compared with the structure without the linker.

Conclusions

This study reports the conventional and microwave-mediated synthesis of some new hybrid compounds with a norfloxacin core. Microwave-irradiated method is a more efficient and eco-friendly procedure. Antimicrobial screening studies were performed. The results show that most of the newly synthesized compounds exhibit strong antibacterial activity compared with norfloxacin itself.

Experimental

Synthesis

All chemicals were purchased from Fluka Chemie AG (Buchs, Switzerland). Melting points were determined in open capillaries on a Buchi B-540 melting point apparatus and are uncorrected. Progress of the reactions was monitored by TLC on silica gel 60 F254 aluminum sheets. The mobile phase was ethyl acetate and UV light was used for the detection. IR spectra were recorded in potassium bromide pellets using a Perkin-Elmer 1600 series Fourier transform-infrared (FT-IR) spectrometer. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded in dimethyl sulfoxide (DMSO)-d_6 on a Bruker Avance II 400 spectrometer. Elemental analysis was performed on a Costech Elemental Combustion System CHNS-O elemental analyzer. The mass

Table 2 Screening for minimum bactericidal concentration (MBC).

Comp.	Minimum bactericidal concentration (μg/mL)						
	Ec	Yp	Pa	Sa	Ef	Bc	Ms
1	0.082	0.082	0.082	0.082	0.082	0.082	0.082
2	0.54	0.53	0.50	0.54	0.52	0.48	1.94
3	0.48	31.5	31.5	0.51	0.48	0.48	1.97
4a	0.50	1.96	3.90	3.92	3.91	3.90	0.49
4b	0.48	>125	–	>125	>125	0.98	0.48
5a	31.63	125	>125	>125	125	125	1.98
5b	0.48	0.48	0.48	0.48	0.48	0.48	0.99
5c	15.6	62.6	–	15.6	15.8	125	1.95
6a	3.8	3.9	–	0.48	0.48	0.48	31.2
6b	0.48	0.48	0.48	0.48	0.48	0.48	0.96
6c	15.6	62.8	–	15.6	15.6	125	1.94
7a	–	>125	–	125	62.65	62.58	–
7b	0.52	31.3	–	0.51	0.48	0.48	–
7c	0.48	–	0.48	0.50	15.77	15.71	>125
7d	–	–	–	62.5	–	>125	–
7e	0.48	31.3	–	0.48	0.48	0.52	–
7f	0.50	–	–	0.48	0.51	15.62	>125
7g	125	–	–	0.48	0.48	15.72	>125
8a	0.48	62.6	–	15.6	15.66	125	1.94
8b	0.48	–	–	0.49	0.48	0.48	–
Norf	0.48	0.48	0.48	0.50	0.49	0.48	0.48
Amp	36	>128	71	20	30	10	
Strep							9

Ec, *Escherichia coli*; Yp, *Yersinia pseudotuberculosis*; Pa, *Pseudomonas aeruginosa*; Sa, *Staphylococcus aureus*; Ef, *Enterococcus faecalis*; Bc, *Bacillus cereus*; Ms, *Mycobacteriumsmegmatis*; Norf, norfloxacin; Amp, ampicillin; Strep, streptomycin; (–), no activity.

spectra were obtained using a Quattro LC-MS (70 eV) spectrometer. Microwave-assisted reactions were performed in a CEM Discovery synthesis reactor.

7-(4-(2-Ethoxy-2-oxoethyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (1)

Method 1 [29] A mixture of compound **1** (25 mmol) and sodium bicarbonate (10 mmol) in dry DMF was treated dropwise at 0–5°C with ethyl bromoacetate (3.74 mL, 15 mmol). Then, the mixture was stirred for 24 h at room temperature. The progress of the reaction was monitored by TLC. The precipitate was removed by filtration and the solution was extracted with chloroform. The extract was dried with sodium sulfate and concentrated under reduced pressure. The residue was crystallized from acetone to give the title compound as a white solid.

Method 2 A solution of norfloxacin (10 mmol) and sodium ethoxide (10 mmol) in dry ethanol was irradiated in a microwave reactor in closed vessel at 100 W for 4 min. Then, ethyl bromoacetate (15 mmol) was added dropwise at 0–5°C, and the mixture was irradiated for an additional 15 min. The crude product was collected by filtration and crystallized from acetone.

Yield 74% (method 1), 98% (method 2); mp 215°C; IR (υ_{max}, cm^{-1}): 3055, 1735, 1625, 1614, 1254; ^1H NMR: δ 1.41 (t, *J* = 8 Hz, 3H), 1.62 (t, *J* = 8 Hz, 3H), 2.70 (s, 4H), 2.94 (s, 4H), 3.53 (s, 2H), 4.31 (q, *J* = 8 Hz, 2H), 4.79 (q, *J* = 8 Hz, 2H), 7.39 (d, *J* = 8 Hz, 1H), 8.12 (d, *J* = 12.7 Hz, 1H), 9.16 (s, 1H), 15.49 (s, 1H). LC-MS. Calcd for $C_{20}H_{24}FN_3O_5$, $[M+H+Na]^+$: *m/z* 429.17. Found: *m/z* 429.25.

7-[4-(2-Hydrazino-2-oxoethyl)piperazin-1-yl]-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (2)

Method 1 [29] A mixture of compound 1 (10 mmol), hydrazine hydrate (30 mL) and chloroform (30 mL) was stirred at room temperature for 24 h. After treatment with ethanol, the resultant white solid was filtered off and crystallized from DMSO/H_2O (1:10).

Method 2 A mixture of compound **1** (1 mmol) and hydrazine hydrate (6 mL) was irradiated in a microwave reactor in a closed vessel with pressure control at 80 W for 15 min. The solid product obtained was crystallized from DMSO/H_2O (1:10).

Yield 78% (method 1), 94% (method 2); mp 253–256°C; IR (υ_{max}, cm^{-1}): 3403, 3307, 3210, 3058, 1706, 1685, 1626, 1267; ^1H NMR: δ 1.42 (t, *J* = 8 Hz, 3H), 2.67 (s, 4H), 3.02 (s, 2H), 3.36 (s, 4H), 4.25 (bs, 2H), 4.59 (q, *J* = 8 Hz, 2H), 7.16 (d, *J* = 4 Hz, 1H), 7.90 (d, *J* = 12.2 Hz, 1H), 8.95 (s, 1H), 8.99 (s, 1H), 15.35 (s, 1H). LC-MS. Calcd for $C_{18}H_{22}FN_5O_4$, $[M+Na]^+$: *m/z* 414.17. Found: m/z 414.14.

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

Method 1 A solution of KOH (10 mmol) in water was added to a solution of compound **2** (10 mmol) in ethanol and the mixture was heated under reflux 10 h in the presence of CS_2 (20 mmol), then cooled to room temperature and acidified to pH 6 with 37% HCl. Upon cooling in the refrigerator, the resultant solid was crystallized from acetone to give a white solid.

Method 2 A mixture of compound **2** (10 mmol), KOH (10 mmol) and CS_2 (20 mmol) in ethanol was irradiated in a microwave reactor in a closed vessel with pressure control at 150 W for 20 min. After cooling to room temperature, the mixture was acidified to pH 6 with 37% HCl. Upon cooling in the refrigerator, the resultant solid was filtered off and crystallized from acetone.

Yield 81% (method 1), 97% (method 2); mp 199–200°C; IR (υ_{max}, cm^{-1}): 3198, 3036, 1694, 1656, 1256; ^1H NMR: δ 1.41 (bs, 3H), 3.16 (bs, 4H), 3.43 (m, 4H), 3.65 (s, 2H), 4.60 (s, 2H), 7.20 (s, 1H), 7.92 (d, *J* = 12 Hz, 1H), 8.96 (s, 1H), 9.23 (s, 1H), 15.30 (s, 1H); ^{13}C NMR: δ 176.7, 168.5, 166.5, 165.6, 151.9 and 154.4 (d, *J* = 248 Hz), 149.1, 144.9 (d, *J* = 10 Hz), 137.6, 120.2, 111.6 (d, *J* = 12 Hz), 107.6, 106.5 (d, *J* = 10 Hz), 61.0, 52.2, 52.0, 50.3, 49.6, 48.0, 14.9; LC MS: *m/z* 433.3, $[M]^+$. Anal. Calcd for $C_{19}H_{20}FN_5O_4S$: C, 52.65; H, 4.65; N, 16.16. Found: C, 52.69; H, 4.71; N, 16.08.

General method for the synthesis of compounds 4a,b; 7a-e and 8a,b

Method 1 A secondary amine (10 mmol) was added to a solution of compound **3** (10 mmol) (for **4a,b**), **6a** (10 mmol) (for **7a–e**) or **6c**

(10 mmol) (for **8a,b**) in DMF containing HCl (50% mmol) and the mixture was stirred at room temperature in the presence of formaldehyde (37%, 30 mmol) for 24 h. Then, the solvent was removed under reduced pressure and the residue of the product was crystallized from DMF/H_2O (1:3).

Method 2 A mixture of a secondary amine (1 mmol), compound **6a** (1 mmol) (for **7a–e**) or **6c** (1 mmol) (for **8a,b**), HCl (50% mmol) and formaldehyde (37%, 3 mmol) was irradiated in a microwave reactor in a closed vessel with pressure control at 100 W for 7 min. The solid product was crystallized from DMF/H_2O (1:3).

1-Ethyl-6-fluoro-4-oxo-7-{4-{[4-[(4-phenylpiperazin-1-yl)methyl]-5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl}methyl)piperazin-1-yl}-1,4-dihydroquinoline-3-carboxylic acid (4a) Yield 52% (method 2); mp 133–135°C; IR (υ_{max}, cm^{-1}): 3419, 3100, 1700, 1626, 1460, 1256; ^1H NMR: δ 1.41 (m, 3H), 2.80 (m, 10H), 3.17 (m, 6H), 3.82 (s, 2H), 4.58 (m, 2H), 5.01 (d, J = 8 Hz, 2H), 6.74–7.20 (m, 6H), 7.92 (m, 1H), 8.95 (s, 1H), 15.36 (s, 1H); ^{13}C NMR: δ 178.4, 176.6, 166.6, 153.3 (d, J_{CF} = 246 Hz), 148.9, 145.8, 145.8, 137.7, 129.3, 119.8, 119.4, 116.1, 111.6 (d, J = 27 Hz), 107.6, 106.5 (d, J = 15 Hz), 53.1, 52.8, 52.5, 52.1, 51.6, 50.3, 49.8, 49.5, 48.8, 46.5, 43.3, 14.8; LC MS: *m/z* 607.2, $[M]^+$. Anal. Calcd for $C_{30}H_{34}FN_7O_4S$: C, 59.29; H, 5.64; N, 16.13. Found: C, 59.26; H, 5.67; N, 16.12.

1-Ethyl-6-fluoro-7-{4-{[4-(4-methylpiperazin-1-yl)methyl]-5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl}methyl)piperazin-1-yl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (4b) Yield 49% (method 2); mp 123–125°C; IR (υ_{max}, cm^{-1}): 3412, 3100, 1700, 1625, 1469, 1256; ^1H NMR: δ 1.38–1.49 (m, 6H), 2.89 (bs, 8H), 3.03 (bs, 4H), 3.09–3.25 (m, 4H), 3.82–4.23 (m, 4H), 5.09 (d, J = 8 Hz, 2H), 6.12–7.00 (m, 1H), 8.01 (m, 1H), 8.95 (s, 1H), 15.66 (s, 1H); ^{13}C NMR: δ 178.5, 176.9, 166.8, 152.3 (d, J_{CF} = 348 Hz), 148.9, 146.0, 145.9, 137.9, 111.7 (d, J = 27Hz), 107.6, 106.5 and 106.6 (d, J = 15 Hz), 52.8, 52.5, 50.6, 50.0, 49.6, 48.9, 46.9, 43.3, 25.1, 14.8; LC MS: 545.2, $[M]^+$. Anal. Calcd for $C_{25}H_{32}FN_7O_4S$: C, 55.03; H, 5.91; N, 17.97. Found: C, 55.26; H, 6.07; N, 17.72.

7-{4-{(4-Benzyl-1-(morpholin-4-ylmethyl)-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl]methyl}piperazin-1-yl}-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylicacid (7a) Yield 80% (method 1), 97% (method 2); mp 247°C; IR (υ_{max}, cm^{-1}): 3450, 3047, 1719, 1626, 1443, 1255; ^1H NMR: δ 1.42 (s, 3H), 2.51 (s, 4H), 2.70 (s, 4H), 3.07 (s, 4H), 3.33 (m, 2H), 3.57 (s, 4H, $2CH_2$), 4.56 (s, 2H), 5.09 (s, 2H), 5.42 (s, 2H), 7.04 (s, 1H), 7.27–7.36 (m, 5H), 7.85 (s, 1H), 8.94 (s, 1H), 15.30 (s, 1H); ^{13}C NMR: δ 176.6, 169.9, 156.5, 153.5 (d, J_{CF} = 248 Hz), 148.9, 148.4, 145.8, 137.6, 127.8, 127.3, 119.8, 111.6 (d, J_{CF} = 23 Hz), 107.6, 106.2, 69.3, 66.5, 52.2, 52.1, 50.9, 49.5, 47.9, 14.8; LCMS: *m/z* 621.2, $[M]^+$. Anal. Calcd for $C_{31}H_{36}FN_7O_4S$: C, 59.89; H, 5.84; N, 15.77. Found: C, 60.14; H, 5.93; N, 15.61.

7-{4-{(4-Benzyl-1-[(morpholin-4-ylamino)methyl]-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl]methyl}piperazin-1-yl}-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (7b) Yield 85% (method 1), 97% (method 2); mp 145°C; IR (υ_{max}, cm^{-1}): 3493, 3051, 1708, 1625, 1447, 1255; ^1H NMR: δ 1.42 (s, 3H), 2.51 (s, 6H), 3.08 (s, 4H), 3.35 (bs, 6H), 3.57 (s, 2H), 4.57 (s, 2H), 5.37–5.46 (m, 4H), 6.93–7.34 (m, 7H), 7.87 (s, 1H), 8.95 (s, 1H), 15.33 (s, 1H); ^{13}C NMR: δ 176.6, 168.6, 166.5, 155.0, 151.8 (d, J_{CF} = 140 Hz), 149.0, 145.8, 137.6, 136.5, 127.9, 127.8, 127.4, 120.0, 111.6 (d, J_{CF} = 22 Hz), 107.6, 106.3, 71.0, 52.2, 49.5, 47.7, 46.8, 14.8 ; LC MS: *m/z* 637.4, $[M+H]^+$. Anal. Calcd for $C_{31}H_{37}F_2N_8O_4S$: C, 58.47; H, 5.86; N, 17.60. Found: C, 58.64; H, 5.89; N, 17.57.

7-{4-{[4-Benzyl-1-(piperidin-4-ylmethyl)-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl]methyl}piperazin-1-yl}-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (7c) Yield: 85% (method 1), 97% (method 2); mp 235–236°C; IR (υ_{max}, cm^{-1}): 3311, 3032, 1718, 1689, 1449, 1262; ^1H NMR: δ 1.33 (s, 3H), 1.43 (s, 4H), 1.49 (s, 1H), 2.51 (s, 4H), 2.68 (s, 4H), 3.09 (s, 4H), 3.30 (s, 2H), 4.57 (s, 2H), 5.06 (s, 2H), 5.42 (s, 2H), 7.06 (s, 1H, NH), 7.27 (d, J = 6 Hz, 3H), 7.35 (d, J = 6 Hz, 2H), 7.88 (m, 2H), 8.94 (s, 1H), 15.23 (s, 1H, OH); ^{13}C NMR: δ 176.6, 169.6, 166.5, 153.7 (d, J_{CF} = 235 Hz)], 149.0, 148.3, 145.7, 137.6, 136.5, 128.8, 127.9, 127.5, 127.1, 111.7 (d, J = 43 Hz), 107.6, 106.0, 72.7, 70.3, 52.3, 51.6, 49.4, 47.7, 25.9, 24.0, 14.8; LC MS: *m/z* 620.2, $[M+H]^+$. Anal. Calcd for $C_{32}H_{38}FN_7O_3S$: C, 62.02; H, 6.18; N, 15.82. Found: C, 62.04; H, 6.18; N, 15.87.

7-{4-{[4-Benzyl-1-(thiomorpholin-4-ylmethyl)-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl]methyl}-piperazin-1-yl}-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinolin-3-carboxylic acid (7d) Yield 85% (method 1), 94% (method 2); mp 176°C; IR (υ_{max}, cm^{-1}): 3359, 3051, 2956, 1719, 1629, 1495, 1257; ^1H NMR: δ 1.41 (t, J = 8 Hz, 3H), 2.28 (bs, 4H), 2.50 (bs, 4H), 2.70–2.91 (m, 8H), 4.13 (s, 2H), 4.59 (d, J = 8 Hz, 2H), 5.18 (s, 2H), 5.35 (s, 2H), 7.18–7.34 (m, 6H), 7.91 (d, J = 8 Hz, 1H), 8.95 (s, 1H); ^{13}C NMR: δ 181.1, 176.6, 166.6, 159.9, 157.5 (d, J = 518 Hz), 149.0, 148.6 and 149.0 (d, J = 38 Hz), 141.1, 137.6, 137.5, 128.9, 127.8, 127.1, 119.8 (d, J = 18 Hz), 111.6 (d, J = 23 Hz), 107.5, 106.4, 79.9, 69.0, 54.5, 53.0, 50.0, 49.5, 48.4, 47.8, 27.1, 14.8 ; LC MS: *m/z* 637.7, $[M]^+$. Anal. Calcd: C, 58.38; H, 5.69; N, 15.37. Found: C, 58.41; H, 5.69; N, 15.33.

7-{4-{[4-Benzyl-1-(4-phenylpiperazin-1-yl)methyl]-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl]methyl}piperazin-1-yl}-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (7e) Yield 91% (method 1), 97% (method 2); mp 230°C; FT IR (υ_{max}, cm^{-1}): 3067, 1492, 1724, 1237; ^1H NMR: δ 1.41 (s, 3H), 2.50 (s, 4H), 2.86 (s, 4H), 3.02 (s, 4H), 3.14 (m, 4H), 3.35 (s, 2H), 4.55 (s, 2H), 5.18 (s, 2H), 5.42 (s, 2H), 6.75 (s, 1H), 6.91 (s, 2H), 7.02 (s, 1H), 7.19–7.34 (m, 7H), 7.85 (s, 1H), 8.93 (s, 1H), 15.29 (s, 1H); ^{13}C NMR: δ 178.6, 176.5, 166.5, 154.5, 152.0, 149.9 (d, J_{CF} = 290 Hz), 148.9, 145.6, 137.5, 136.1, 129.3, 128.9, 127.8, 127.2, 119.8, 119.2, 115.8, 111.6 (d, J_{CF} = 23 Hz), 107.7, 106.1, 69.1, 52.2, 52.1, 51.5, 50.3, 49.5, 48.7, 47.9, 46.8. LC MS: *m/z* 696.92, $[M]^+$. Anal. for $C_{37}H_{41}FN_8O_3S$: C, 63.77; H, 5.93; N, 16.08. Found: C, 63.84; H, 5.93; N, 15.88.

7-{4-{[4-Benzyl-1-(4-methylpiperazin-1-yl)methyl]-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl]methyl}piperazin-1-yl}-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (7f) Yield 88% (method 1), 94% (method 2); mp 212–213°C; IR (υ_{max}, cm^{-1}): 3451, 3032, 1706, 1690, 1629, 1449, 1263; ^1H NMR: δ 1.48 (s, 3H), 2.28 (s, 3H), 2.31 (s, 4H), 2.51 (s, 4H), 2.61 (s, 4H), 3.39 (s, 4H), 3.47 (s, 2H), 4.55 (bs, 2H), 5.11 (s, 2H), 5.43 (s, 2H), 7.05 (s, 1H), 7.17 (bs, 2H), 7.36 (s, 2H), 7.58 (bs, 1H), 7.98 (s, 1H), 8.91 (s, 1H), 15.19 (s, 1H); ^{13}C NMR; δ 176.1, 169.7, 166.5, 154.8, 150.1 (d, J_{CF} = 357 Hz), 148.2, 145.5, 137.6, 136.6, 128.5, 127.5, 127.2, 119.4, 111.5 and 111.7 (d, J = 20 Hz), 107.6, 106., 68.63, 54.2, 52.5, 49.5, 47.6, 46.8, 45.6, 14.8; LC MS: *m/z* 635.31, $[M+H]^+$. Anal. for $C_{32}H_{39}FN_8O_3S$: C, 60.55; H, 6.19; N, 17.65. Found: C, 60.55; H, 6.16; N, 17.69.

7-{4-{[4-Benzyl-1-[(1-benzylpiperidin-4-yl)amino]methyl}-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl]methyl}piperazin-1-yl}-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (7g) Yield 28% (Method 1), 97% (Method 2); mp.: 177–178°C; IR (υ_{max}, cm^{-1}): 3325, 3068, 1724, 1491, 1233; ^1H NMR (DMSO-d_6, δ ppm): 1.42 (bs,

3H), 1.72–1.85 (m, 4H), 2.50 (s, 4H), 2.66–2.67 (m, 2H), 3.04 (s, 4H), 3.35–3.39 (m, 4H), 3.56 (m, 2H), 4.57–4.58 (m, 2H), 5.06 (s, 1H), 5.39 (bs, 2H), 5.55 (s, 2H), 7.05 (s, 1H), 7.25–7.28 (m, 10H), 7.89 (d, J = 12.8 Hz, 1H), 8.96 (s, 1H), 10.21 (s, 1H); ^{13}C NMR: δ 176.7, 170.0, 167.9, 156.5, 153.2 (d, J_{CF} = 248 Hz), 148.9, 148.5, 145.7, 138.9, 137.6, 136.6, 129.2, 128.9, 128.3, 128.1, 127.8, 127.2, 120.0, 111.7 (d, J_{CF} = 23 Hz), 107.6, 106.2, 62.6, 61.4, 52.1, 52.0, 49.5, 47.4, 32.4, 14.7; LC MS: *m/z* 724.89, $[M]^+$. Anal. Calcd for $C_{39}H_{45}FN_8O_3S$: C, 64.62; H, 6.26; N, 15.46. Found: C, 64.64; H, 6.23; N, 15.48.

1-Ethyl-6-fluoro-7-{4-{[1-(morpholin-4-ylmethyl)-5-oxo-4-phenyl-4,5-dihydro-1*H*-1,2,4-triazol-3-yl]methyl}piperazin-1-yl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (8a) Yield 70% (method 1), 98% (method 2); mp 237°C; IR (υ_{max}, cm^{-1}): 3451, 3037, 1719, 1443; ^{1}H NMR: δ 1.41 (s, 3H), 2.50 (s, 4H), 2.72 (s, 4H), 3.01 (s, 4H), 3.33 (m, 2H), 3.57 (s, 4H), 4.56 (s, 2H), 5.42 (s, 2H), 7.02 (s, 1H), 7.32 (m, 5H), 7.81 (s, 1H), 8.92 (s, 1H), 15.31 (s, 1H); ^{13}C NMR: δ 176.7, 176.6, 169.9, 156.5, 153.6 (d, J_{CF} = 246 Hz),148.9, 148.4, 145.8, 137.6, 136.1, 127.8, 127.5, 127.3, 119.8, 111.6 (d, J_{CF} = 24 Hz), 107.5, 106.1, 69.3, 66.5, 52.2, 52.1, 50.9, 49.5, 47.9, 14.8; LC MS: *m/z* $[M]^+$: *m/z* 591.23, $[M]^+$. Anal. Calcd for $C_{30}H_{34}FN_7O_5$: C, 60.90; H, 5.79; N, 16.57. Found: C, 60.94; H, 5.73; N, 16.51.

1-Ethyl-6-fluoro-7-{4-{[1-[(morpholin-4-ylamino)methyl]-5-oxo-4-phenyl-4,5-dihydro-1*H*-1,2,4-triazol-3-yl]methyl}piperazin-1-yl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (8b) Yield 95% (method 1), 99% (method 2); mp 178°C; IR (υ_{max}, cm^{-1}): 3492, 3357, 3051, 1718, 1625, 1447; ^{1}H NMR: δ 1.42 (s, 3H), 2.49 (s, 4H), 3.08 (s, 4H), 3.35 (bs, 4H), 3.57 (s, 4H), 4.57 (s, 2H), 5.37 (s, 2H), 5.46 (bs, 2H), 6.93–7.34 (m, 7H), 7.85 (s, 1H), 8.95 (s, 1H), 15.33 (s, 1H); ^{13}C NMR: δ 168.6, 166.5, 156.2, 155.0, 150.6 and 152.0 (d, J_{CFn} = 140.0 Hz), 149.0, 145.8, 137.6, 136.5, 127.9, 127.8, 127.4, 120.0, 111.6 (d, J_{CF} = 21 Hz), 107.54, 106.2, 71.0, 52.2, 49.5, 47.7, 46.8, 14.8; LC MS *m/z* $[M]^+$: *m/z* 607.39, $[M]^+$. Anal. Calcd for $C_{30}H_{35}FN_8O_5$: C, 59.40; H, 5.82; N, 18.47. Found: C, 59.44; H, 5.82; N, 18.47.

General methods for synthesis of compounds 5a–c

Method 1 An isothiocyanate (20 mmol) in dry DMF was added dropwise to a solution of compound **2** (10 mmol) in dry DMF. The mixture was stirred at room temperature for 24 h, then poured into ice water. The resultant precipitate was filtered off and crystallized from an appropriate solvent.

Method 2 The mixture of an isothiocyanate (20 mmol) and compound **2** (10 mmol) was irradiated in a microwave reactor in a closed vessel with pressure control at 150 W for 5 min. The resultant solid was crystallized from an appropriate solvent.

7-{4-{2-{2-[(Benzylamino)carbonothioyl]hydrazino}-2-oxoethyl}piperazin-1-yl}-1-ethyl-6-fluoro}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5a) Crystallized from DMSO/H_2O (1:10); yield 90% (method 1), 98% (method 2); mp 210–211°C; IR (υ_{max}, cm^{-1}): 3306, 3256, 3205, 3032, 1705, 1690, 1627, 1265; ^{1}H NMR: δ 1.41 (t, J = 8.0 Hz, 3H), 2.70 (s, 4H), 3.14 (s, 2H), 3.38 (m, 4H), 4.58 (q, J = 8 Hz, 2H), 4.73 (d, J = 4 Hz, 2H), 7.14–7.35 (m, 6H), 7.89 (d, J = 12 Hz, 1H), 8.41 (bs, 1H), 8.94 (s, 1H), 9.35 (bs, 1H), 9.81 (bs, 1H), 15.38 (s, 1H); ^{13}C NMR: δ 176.6, 176.6, 171.9, 166.8, 151.4 (d, J_{CF} = 37 Hz), 148.91, 145.9 and 146.0 (C, J = 9 Hz), 137.7 (d, J = 9.8 Hz), 128.7, 128.5, 127.8, 127.8, 127.5, 119.7 (d, J = 12 Hz), 111.7 (d, J = 21 Hz), 106.1 (d, J = 2.0 Hz), 60.0, 52.8, 56.5, 52.3, 49.8, 49.5, 47.2, 14.8; LC MS: *m/z* 541.19, $[M+H]^+$. Anal. Calcd for $C_{26}H_{29}FN_6O_4S$: C, 57.76; H, 5.41; N, 15.55. Found: C, 57.56; H, 5.68; N, 15.25.

1-Ethyl-7-{4-{2-{2-[(ethylamino)carbonothioyl]hydrazino}-2-oxoethyl}piperazin-1-yl}-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5b) Crystallized from acetone; yield 95% (method 1), 100% (method 2); mp 210–211°C; IR (υ_{max}, cm^{-1}): 3342, 3402, 3279, 3139, 3051, 1703, 1655, 1250; ^{1}H NMR: δ 1.07 (t, J = 4 Hz, 3H), 1.42 (t, J = 8.0 Hz, 3H), 2.71 (s, 4H), 3.13 (s, 2H), 3.38 (s, 4H), 3.45 (q, 2H), 4.58 (q, J = 4 Hz, 2H), 7.15 (d, J = 4 Hz, 1H), 7.88 (d, J = 12 Hz, 1H), 8.94 (s, 1H), 9.14 (bs, 1H), 9.69 (bs,1H), 9.98 (s, 1H), 15.36 (s, 1H); ^{13}C NMR: δ 182.0, 176.6, 166.6, 165.2, 152.3 (d, J = 379 Hz), 148.9, 145.89 and 145.98 (d, J = 9 Hz), 137.67, 119.7 (d, J = 7 Hz), 111.7 (d, J = 23 Hz), 107.5, 106.2 (d, J = 9 Hz), 59.9, 50.0, 49.8, 49.8, 49.5, 38.9, 14.9, 14.8; LC MS: *m/z* 479.19, $[M+H]^+$. Anal. Calcd for $C_{21}H_{27}FN_6O_4S$: C, 52.71; H, 5.69; N, 17.56. Found: C, 52.98; H, 5.84; N, 17.48.

7-{4-{2-[2-(Anilinocarbonyl)hydrazino]-2-oxoethyl}piperazin-1-yl}-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5c) Crystallized from DMSO/H_2O (1:10); yield 98% (method 1), 100% (method 2); mp 233–234°C; FT IR (υ_{max}, cm^{-1}): 3343, 3296, 3220, 3137, 1712, 1668, 1659, 1258; ^{1}H NMR: δ 1.42 (s, 3H), 2.74 (s, 4H), 3.15 (s, 2H), 3.39 (s, 4H), 4.59 (s, 2H), 6.95–7.50 (m, 5H), 7.88–8.05 (m, 2H), 8.79 (s, 2H), 8.94 (s, 1H), 9.14 (s, 1H), 9.64 (s, 1H), 15.39 (s, 1H); ^{13}C NMR: δ 176.6, 169.6, 166.6, 155.7, 149.2 and 154.3 (d, J = 507 Hz), 145.9, 148.9, 140.1, 137.7, 129.1, 122.3, 119.6, 118.9, 118.8, 111.6, 107.5, 106.1, 60.0, 52.8, 49.8, 49.8, 49.5, 14.8; LC MS: *m/z* 511.19, $[M+H]^+$ Anal. Calcd for $C_{21}H_{27}FN_6O_4S$: C, 58.82; H, 5.33; N, 16.46. Found (%),C, 58.89; H, 5.39; N, 16.34.

General methods for synthesis of compounds 6a–c

Method 1 A mixture of 2N NaOH (25 mL) and compound **5** in ethanol was heated under reflux for 18 h, then cooled to room temperature and neutralized to pH 7 with 37% HCl. The resultant white solid was filtered off, washed with water and crystallized from acetone.

Method 2 A mixture of compound **5** (10 mmol) and 2N NaOH (25 mL) was irradiated in a microwave reactor in a closed vessel with pressure control at 120 W for 25 min, then cooled to room temperature and neutralized to pH 7 with 37% HCl. The white solid formed was filtered off, washed with water and crystallized from acetone.

7-{4-[(4-Benzyl-5-mercapto-4*H*-1,2,4-triazol-3-yl)methyl]piperazin-1-yl}-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (6a) Yield 91% (method 1), 97% (method 2); mp 255–257°; IR (υ_{max}, cm^{-1}): 3342, 3065, 2823, 1716, 1629; ^{1}H NMR: δ 1.42 (s, 3H), 3. 07 (s, 6H), 3.50 (s, 4H), 4.52 (s, 2H), 5.36 (s, 2H), 7.06 (s, 1H), 7.26–7.35 (m, 5H), 7.87 (d, J = 12 Hz, 1H), 8.95 (s, 1H), 13.88 (s, 1H), 15.35 (s, 1H);^{13}C NMR: δ 176.6, 168.7, 166.6, 153.0 (d, J_{CF} = 248 Hz), 149.51, 148.96, 145.7 (d, J = 10 Hz), 137.6, 136.7, 128.9, 127.8, 127.4, 119.7 (d, =8 Hz), 111.6 (d, J = 22 Hz), 107.5, 106.2, 523, 522, 49.5, 46.7, 14.0; LC MS: *m/z* 523.18, $[M+H]^+$. Anal. Calcd for $C_{26}H_{27}FN_6O_3S$: C, 59.76; H, 5.21; N, 16.08. Found: C, 59.89; H, 5.28; N, 16.02.

7-{4-[(4-Ethyl-5-mercapto-4*H*-1,2,4-triazol-3-yl)methyl]piperazin-1-yl}-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (6b) Yield 80% (method 1), 94% (method 2); mp 305–306°C; IR (υ_{max}, cm^{-1}): 3450, 3052, 2824, 1727, 1629, 1257; ^{1}H NMR: δ 1.32 (s, 3H), 1.42 (s, 3H), 2.66 (s, 2H), 2.75 (s, 4H), 3.19 (s, 2H),

3.69 (s, 4H), 4.58 (s, 2H), 7.18 (s, 1H), 7.90 (d, J = 3 Hz, 1H), 8.93 (s, 1H), 13.88 (s, 1H), 15.35 (s, 1H); ^{13}C NMR: δ 176.6, 171.9, 166.5, 152.1 and 154.6 (d, J = 244 Hz), 149.3, 148.9, 145.7 and 145.9 (d, J = 11 Hz), 137.6 (d, J = 4 Hz), 119.7 (d, J = 7 Hz), 111.6 (d, J = 22 Hz), 107.6, 106.4 (d, J = 22 Hz), 59.4, 52.5, 52.1, 52.2, 49.9, 49.9, 49.5, 45.6, 14.8, 13.8; LC MS: m/z 483.31, $[M+Na]^+$. Anal. Calcd for $C_{21}H_{25}FN_6O_3S$: C, 54.77; H, 5.47; N, 18.25. Found: C, 54.89; H, 5.51; N, 18.07.

1-Ethyl-6-fluoro-4-oxo-7-{4-[(5-oxo-4-phenyl-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)methyl] piperazin-1-yl}-1,4-dihydroquinoline-3-carboxylic acid (6c) Yield 85% (method 1), 97% (method 2); mp 315–317°C; IR (υ_{max}, cm^{-1}): 3273, 3181, 3058, 1715, 1625, 1250; ^{1}H NMR: δ 1.40 (s, 3H), 3. 18 (s, 6H), 3.38 (s, 4H), 4.55 (s, 2H), 7.11 (d, J = 2 Hz, 1H), 7.43–7.52 (m, 5H), 7.89 (d, J = 4 Hz, 1H) 8.92 (s, 1H), 11.89 (s, 1H); ^{13}C NMR: δ 176.7, 167.1, 164.8, 153.6 (d, J_{CF} = 248 Hz), 149.2, 144.6, 137.8, 134.1, 129.7, 128.9, 127.8, 119.8, 112.1, 107.5, 106.6, 53.0, 52.4, 50.1, 49.7, 15.1 ; LC MS m/z: 492.19, $[M]^+$. Anal. Calcd for $C_{25}H_{25}FN_6O_4$: C, 60.97; H, 5.12; N, 17.06. Found: C, 60.93; H, 5.15; N, 17.07.

Microorganisms

The test microorganisms were obtained from the Hifzissihha Institute of RefikSaydam (Ankara, Turkey): *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, *Candida albicans* (*C. albicans*) ATCC60193 and *Saccharomyces cerevisiae* (*S. cerevisia*) RSKK 251. All newly synthesized compounds were weighed and dissolved in DMSO to prepare stock solution of 20.0 μg/mL.

Minimum inhibitory concentration (MIC) assay

The antimicrobial effects of the substances were tested quantitatively in broth media by using double microdilution to determine the MIC values (μg/mL). The antibacterial and antifungal assays were performed in Mueller-Hinton broth (MH) (Difco, Detroit, MI, USA) at pH 7.3 and buffered Yeast Nitrogen Base (Difco, Detroit, MI, USA) 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, USA) was used for Ms, and the incubation period was 48–72 h at 35°C [26]. Norfloxacin (10 μg), ampicillin (10 μg), streptomycin (10 μg) and fluconazole (5 μg) were used as reference antibacterial and antifungal drugs. DMSO with dilution of 1:10 was used as the solvent control.

Minimum bactericidal concentration (MBC) assay

The MBC assay [27] was performed in sterile 2.0-mL microfuge tubes against the test bacteria cultured overnight in MH broth. Serial dilutions of test compounds at different concentrations ranging from 0 to 125 mg/mL were prepared in the MH broth. To the test compound, 100 mL of overnight cultured bacterial suspension was added to reach a final concentration of 1.5×10^8 cfu/mL (equal to 0.5 McFarland standard) and the mixture was incubated at 37°C for 24 h. After the incubation, MBC was determined by sampling 10 mL of suspension from the tube onto the MH agar plate and the mixture was incubated for 24 h at 37°C to observe the growth of the test organism. MBC is the lowest concentration of the test compound required to kill a particular bacterial strain. All experiments were carried out in duplicates and mean values are reported.

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