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Original article

Microwave assisted synthesis of some hybrid molecules derived from norfloxacin and investigation of their biological activities

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ABSTRACT

Norfloxacin was converted to 7-(4-amino-2-fluorophenyl)piperazin derivative (**2**) via the formation of nitro compound. The synthesis of the norfloxacin derivatives containing 1,3-thiazole or 1,3-thiazolidin moiety was performed from the reaction of 4-chlorophenacylbromide or ethyl bromoacetate with compounds **4**–**7** obtained starting from **2**. 3-Fluoro-4-[4-(2-methoxyphenyl)piperazin-1-yl]aniline (**14**), 5-{[4-(2-methoxyphenyl)piperazin-1-yl]methyl}-4-phenyl-4*H*-1,2,4-triazole-3-thiol (**18**) and {[4-(2-methoxy phenyl)piperazin-1-yl]methyl}-1,3,4-oxadiazol-2-thiol (**19**) were obtained starting from 1-(2-methoxyphenyl)piperazine by several steps. The treatment of hydrazide (**16**) with several aldehydes afforded *N′*-[(2-hydroxyphenyl)methylen]- (**20**), *N′*-[(3-hydroxy-4-methoxy phenyl)methylen]- (**21**) or *N′*-[1*H*-indol-3-ylmethylene]-2-[4-(2-methoxyphenyl)piperazin-1-yl]acetohydrazide (**22**). Then, compounds **14**, **18**, **19** and **22** were condensed with 7-[4-(chloroacetyl)piperazin-1-yl]-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3**) that was obtained from norfloxacine.

All newly synthesized compounds were screened for their antimicrobial activities and some of them exhibited excellent activity. Moreover, one compound was found to have antiurease activity.

1. Introduction

Although significant progress has been made for the treatment and control of microbial infections by introducing new strategies and combinatorial therapy, antimicrobial resistance continues to be one of major concerns to the public health and scientific communities worldwide. Because, infections caused by resistant pathogens fail to response to treatment resulting in prolonged illness and greater risk of death. The alarming rates of emerging and reemerging microbial threats coupled with increasing antibacterial resistance have emphasized the urgent need for new and more effective antibacterial agents with high safety profile [1–5].

Since the introduction in use of nalidixic acid for the treatment of bacterial infections in 1962, a number of derivatives have been synthesized. Among them, fluoroquinolones such as norfloxacin, pefloxacin, enoxacin, ofloxacin, and ciprofloxacin play a major role in the treatment of bacterial community or hospital acquired diseases by displaying excellent pharmacokinetic properties, high antimicrobial activity, and low side effects [6–10].

These agents are known as specific inhibitors of the bacterial DNA gyrase, an enzyme which is responsible for negatively supercoiling covalently closed circular DNA, and also in catenation and decatenation reactions [10]. These compounds have had good success against Gram-negative bacteria, but they are resistant to Gram-positive pathogens, such as *Staphylococcus aureus*. Although they displays some adverse events such as CNS side effects, phototoxicity, and arthropathy, the more serious events are rare, and there exists a continuous need for novel quinolones to overcome the limitations of existing drugs [9,11].

It was reported that, the fluorine atom and the 1-alkyl, 1,4-dihydro-4-oxo-quinoline-3-carboxylic acid skeleton of fluoroquinolones is essential for potency represented in binding with type-II topoisomerase enzymes, DNA gyrase and topoisomerase. The 6-fluoro and 7-piperazinyl groups were reported as responsible for the broad spectrum and antipseudomonal activities of fluoroquinolones. Moreover, C-7 substituent is the most adaptable site for chemical modifications. This area also controls the pharmacokinetic properties of the drugs, with the basic nitrogen. Various substitutions of piperazine nucleus at C-7 position has resulted in a

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wide range of clinically useful fluoroquinolone antibacterials such as norfloxacin, ciprofloxacin, perfloxacin, pefloxacin, ofloxacin, amifloxacin, fleroxacin, lomefloxacin, sparfloxacin, difloxacin, enoxacin, enrofloxacin, levofloxacin, marbofloxacin, and orbifloxacin [9,10,12–16]. Furthermore, *N*-1 alkylation is essential to keep the molecule in tautomeric keto form, because keto form possess antibacterial activity, while enol form is completely inactive [17]. It is known that the molecule part linked the piperazine nucleus at C-7 is regarded as the drug–enzyme interaction domain [9,18–20]. In addition, C-7 substituent dominantly controls cell permeability and inhibition of DNA gyrase and ultimately influences the bioactivity, spectrum, solubility and pharmacokinetics [9,21]. In the 7-piperazinyl quinolone skeleton, piperazine ring displays enough structural convenience to allow several molecular modifications. Much efforts have been devoted on the structural modification at C-7 position in quinolones by incorporating several substituents containing some five- and six-membered heterocycles especially *N*-containing heterocycles such as pyridine, thiadiazole, piperidine etc [22].

1,2,4-Triazole ring is known as versatile lead molecule for designing potential bioactive structures. Its derivatives have been incorporated to yield a wide variety of therapeutically important activities including antibacterial [23–25] anti-inflammatory [26], CNS depressant [27], anti-tubercular [28] anti-HIV [29], and antiproliferative [30]. 1,2,4-Triazole system is a structural element of many drugs that have anti-fungal activity such as fluconazole, itraconazole, and voriconazole [31]. Also, there are other known drugs containing 1,2,4-triazole nucleus, e.g., triazolam, a benzodiazepine class psychotropic drug, rizatriptan (antimigraine), nefazodone (antidepressant), ribavirin (antiviral), alprazolam (analgesic) and etizolam (hypnotic and sedative). Vorozole, letrozole and anastrozole are aromatase inhibitors and used especially for the treatment of breast cancer, which interrupts synthesis of estrogen in the body [32–36].

Another new class of synthetic antimicrobial agents, oxazolidinones, have been reported to possess activity against numerous multidrug-resistant Gram-positive organisms [37]. Beside the drugs linezolid and eperezolid, a number of various oxazolidinone derivatives have been synthesized as antimicrobial compounds [38–41].

Another privileged scaffold thiazolidinone constitute a very attractive target for combinatorial synthesis due to its structure activity relationship, and it belongs to an important class of N and S containing heterocycles, which are widely used as key building blocks in the field of design, synthesis of new pharmaceutical agents [42,43]. Further, the presence of the N–C–S linkage in the thiazolidines is also responsible for nematocidal, fungicidal, antibacterial and antiviral activities [43].

It is well known that more efficacious antibacterial agents can be designed by joining two or more biologically active heterocyclic systems together in a single molecular framework [1,44–46]. These synergistic antimicrobial combinations have several major advantages, including the potential to slow down the development of drug resistance, a broader antimicrobial spectrum, and a potential reduction in the dose and undesired side effects of each drug [46]. An example of a combination treatment that has been used successfully is the administration of amphotericin B and flucytosine for the management of cryptococcal meningitis [47–49].

It has been reported that the combination of antibacterial quinolones with other pharmacophores or drug fragments might result in pharmaceutically important hybrid molecules with difunctional targets, drug synergism or new action mechanisms to overcome drug resistance. In this connection, a number of studies have been devoted for the synthesis of quinolone hybrids incorporating several bioactive moieties at C-7 position of quinolone scaffold [50].

N-Mannich base derivatives of imides, amides, amines, hydantoin and urea derivatives have been used as potentially useful prodrug candidates. The group linked to parent amine by Mannich reaction is believed to increase the lipophilicity of molecule at physiological pH values by decreasing their protonation, and this restriction of protonation results in enhancement of absorption through bio-membranes [51,52]. The lipophilicity of fluoroquinolones can influence their ability to cross bacterial membranes [10]. Furthermore, the neutral species of fluoroquinolones are more lipophilic than zwitterionic form. It was reported that lipophilicity an important factor affecting the intestinal absorption of quinolones, and steric and electronic effects or charge density, can affect lipophilicity [10,53].

Bacterial urease enzymes, which accelerate hydrolysis of urea to ammonia gas with the reaction rate at least 10^{14} over the spontaneous reaction, have been reported as important virulent factors including several important pathogenesis such as pyelonephritis, hepatic coma, peptic ulceration, injection-induced urinary stones and stomach cancer [54–56]. The detrimental impact of ureases is not only on human health. As a result of urease activity, the NH_3 lost from fertilizers is an economic impact for farmers. Moreover, the interference of NH_3 to the atmosphere from urea will subsequently be deposited to land or water. The result of this is eutrophication and acidification of natural ecosystems on a regional scale [57].

In the present study, as a part of our general program in the continued research for new hybrid molecules with antimicrobial and antiurease activity, it has been planned to introduce biologically active 1,2,4-triazole, 1,3-thiazole, 1,3-oksazole, 1,3-thiazolidinone or 1,3-oksazole nucleus at position-4 of piperazine ring of norfloxacine, that is a synthetic chemotherapeutic antibacterial drug occasionally used to treat common as well as complicated urinary tract infections [58].

2. Results and discussion

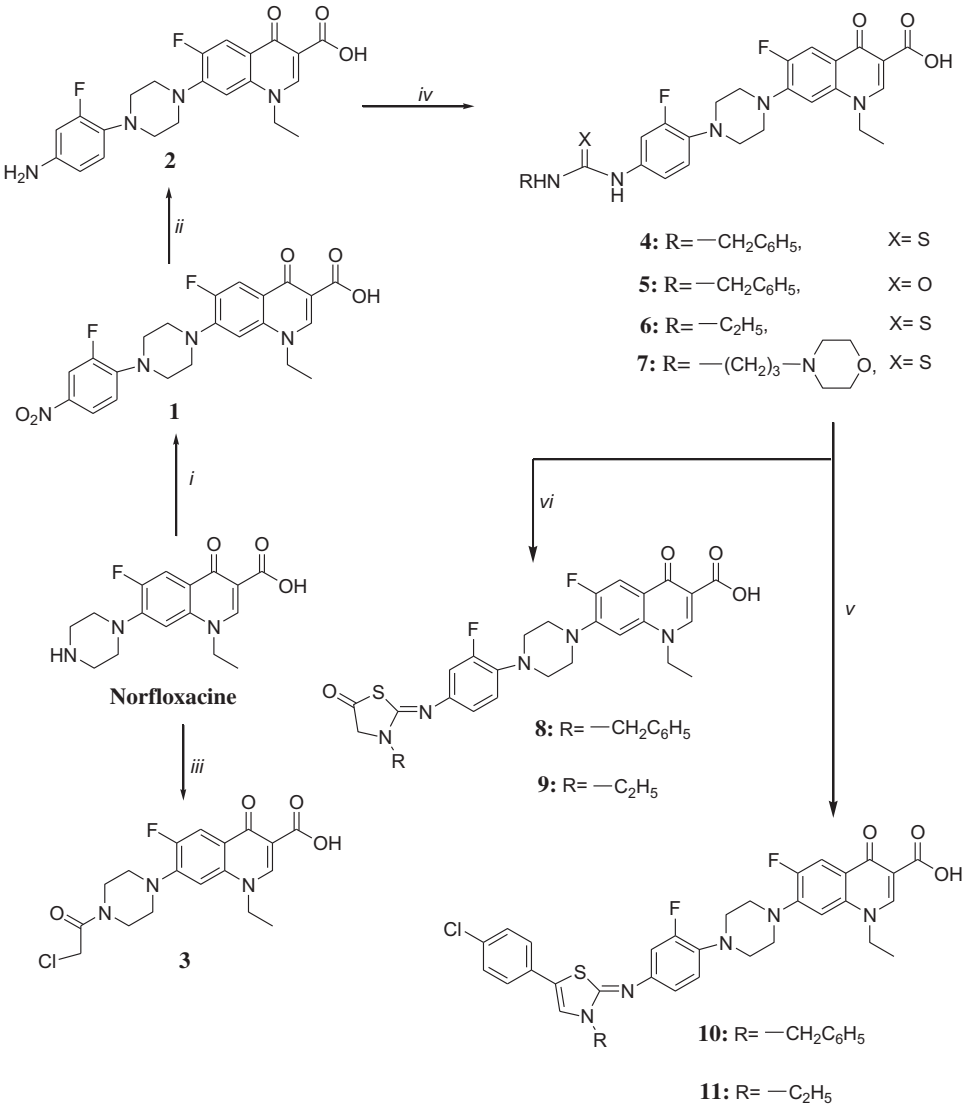
2.1. Chemistry

The synthetic strategies adopted to obtain the target compounds are depicted in Schemes 1, 2 and 3. In the present study, the synthesis of compound **1** was performed by condensation of 3,4-difluoronitrobenzene with norfloxacin and this compound was characterized by the presence of two strong bands at 1475 and 1335 cm^{-1} in the FT-IR spectrum due to nitro group. These signals disappeared, when compound **1** was converted to the corresponding amine (**2**) by the reduction of nitro group, instead, two strong absorption bands was recorded at 3433 and 3332 cm^{-1} derived from $-NH_2$ function. In the ^1H NMR spectrum, this group resonated at 5.03 ppm as D_2O exchangeable singlet.

The treatment of norfloxacin with chloroethanoylchloride yielded compound **3** [59] that was used as an intermediate for further condensations.

The treatment of norfloxacin with several iso(thio)cyanates generated the corresponding carbono(thio)ylamino derivatives (**4**–**7**). The FT-IR spectra of derivatives **4**, **6** and **7** showed an absorption band at 1245 (for **4**), 1209 (for **6**) or 1246 cm^{-1} (for **7**) indicating the presence of C=S double bond in the structure. Moreover, additional signals derived from benzyl, ethyl or 3-morpholin-4-ylpropylamino moiety at the position 7 of norfloxacin skeleton observed at the related chemical shift values in the ^1H and ^{13}C NMR spectra of compounds **4**–**7**. Furthermore, the FT-IR and ^1H NMR spectra of these compounds displayed signals derived from two NH protons (exchangeable with D_2O), while no signal derived from $-NH_2$ group was present.

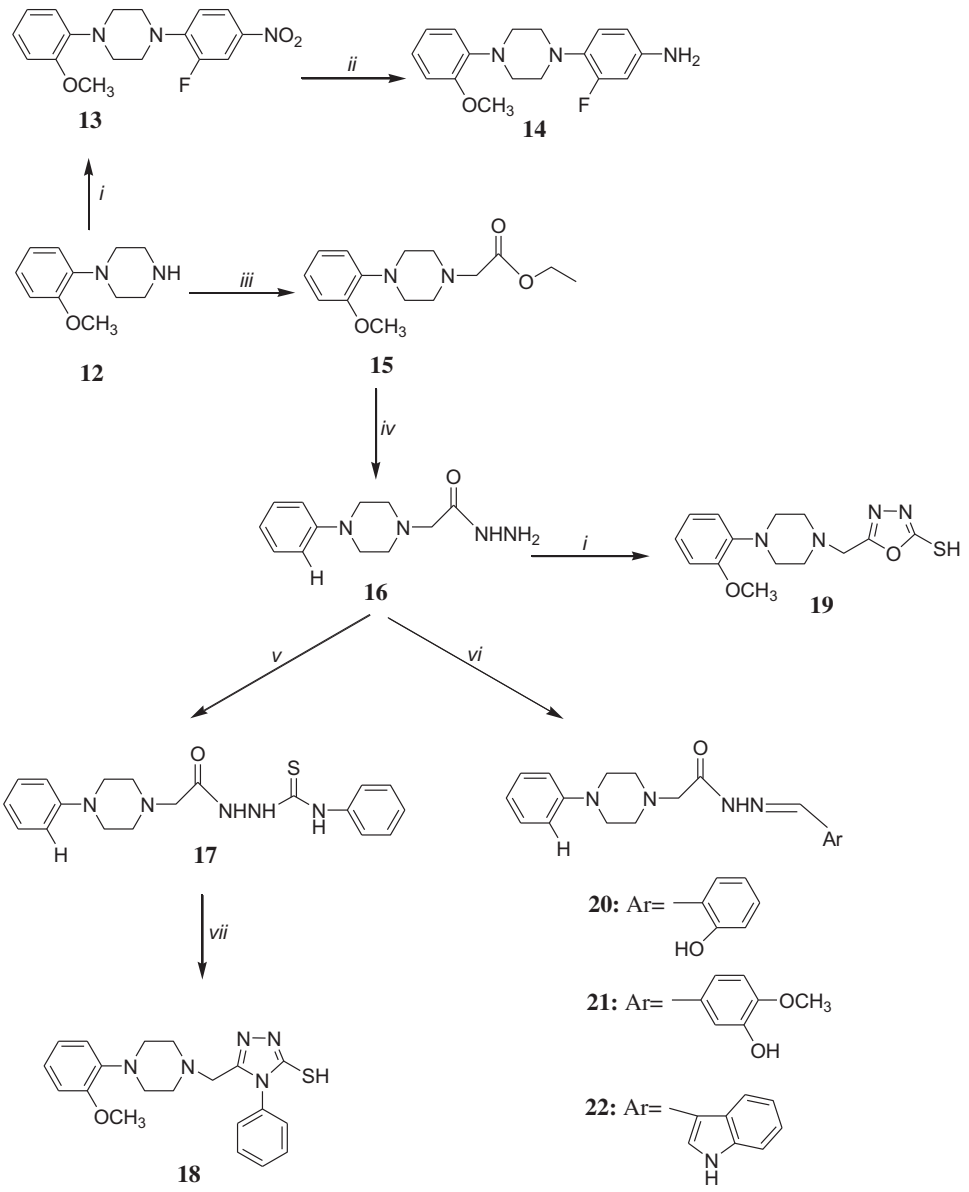
The synthesis of 7-[4-(4-{[3-alkyl-4-oxo-1,3-thiazolidin-2-ylidene]amino}-2-fluorophenyl)piperazin-1-yl]-1-ethyl-6-fluoro-



Scheme 1. *i*: 3,4-Difluoronitrobenzene in acetonitrile, $NaHCO_3$, reflux or MW irradiation; *ii*: Pd/C in *n*-butanol, hydrazine hydrate reflux or MW irradiation; *iii*: chloroethanoyl-chloride, triethylamine in THF, room temperature; *iv*: benzylisothiocyanate (for **4**), benzylisocyanate (for **5**), ethylisothiocyanate (for **6**) or 4-morpholinopropylisothiocyanate (for **7**) in ethanol, reflux or MW irradiation; *v*: ethyl bromoacetate in glacial acetic acid, dried sodium acetate, MW irradiation; *vi*: 4-chlorophenacylbromide in dry chloroform, dried sodium acetate, reflux or MW irradiation.

4-oxo-1,4-dihydroquinoline-3-carboxylic acids (**8** and **9**) was carried out by microwave irritation of the mixture of compound **4** or **6** and ethyl bromoacetate in glacial acetic acid at 150 °C, 200 W in the presence of dried sodium acetate. On the other hand, the condensation of compounds **4** and **6** with 4-chlorophenacylbromide by microwave irradiation or refluxing in chloroform produced 7-[4-(4-{[3-alkyl-5-(4-chlorophenyl)-1,3-thiazol-2(3*H*)-ylidene]amino}-2-fluoro phenyl)piperazin-1-yl]-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acids (**10** and **11**). The disappearance of NH signals in the FT-IR and ^{1}H NMR supported the condensation leading to the formation of compounds **8**–**11**. Another evidence for the cyclocondensation between compounds **4** or **6** and ethyl bromoacetate is the appearance of a signal at 4.02 ppm (for **9**) or 4.11 ppm (for **8**) in the ^{1}H NMR spectra, which correspond the C5 protons of thiazolidinone nucleus. These carbons resonated at 37.39 ppm (for **9**) or 45.37 ppm (for **8**) in the ^{13}C NMR spectra. The additional support for the formation of the targeted compounds, **8**–**11** was obtained by the appearance of [M + 1] ion peaks at corresponding *m*/*z* values confirming their molecular masses; and these compounds have given elemental analysis results consistent with the proposed structures.

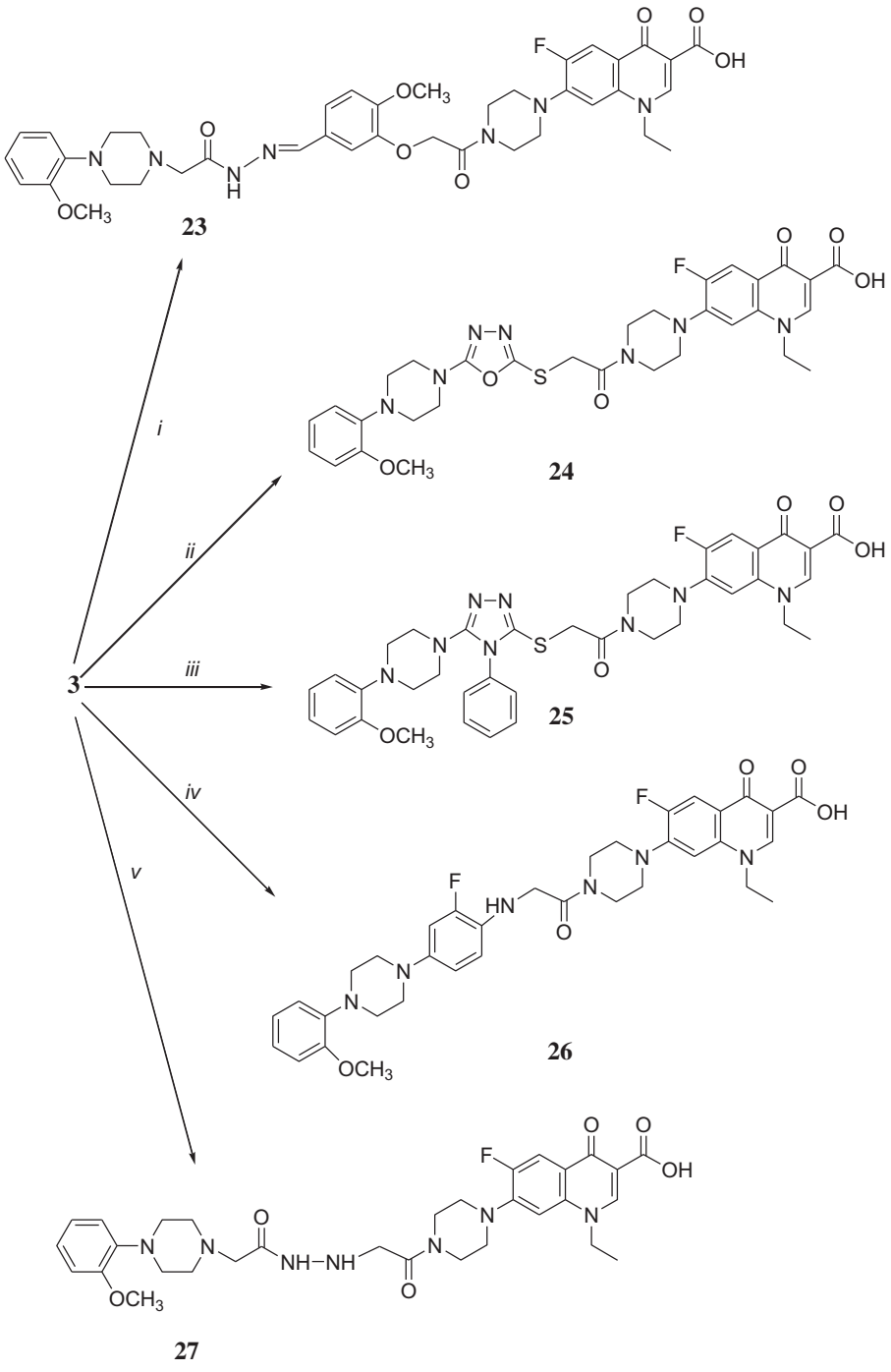
Substituted hydrazides can be considered as useful tools for the synthesis of nitrogen, sulfur or oxygen containing compounds [25]. Compound **16** that was obtained starting from 1-(2-methoxyphenyl)piperazine by two steps, was converted to 5-{[4-(2-methoxyphenyl)piperazin-1-yl]methyl}-4-phenyl-4*H*-1,2,4-triazole-3-thiol (**18**) with the aim to merge two heterocyclic nuclei responsible biological activity namely piperazine and 1,2,4-triazole in a single molecule. Similarly, the synthesis of compound **19** was performed by the reaction of the hydrazide (**16**) with CS_2 in basic media. This idea originated from the intent to introduce a 1,3,4-oxadiazole nucleus to piperazine skeleton. A singlet characteristic for the –SH group was recorded at 13.85 (for **18**) or 14.35 (for **19**) ppm in the ^{1}H NMR spectra of compounds **18** and **19**. FT-IR spectra of these compounds exhibited absorption bands originated from –SH function at 2829 and 2848 cm^{-1}. Moreover compounds **18** and **19** gave reasonable mass fragmentation and elemental analysis data consistent with the assigned structures.



Scheme 2. *i*: 3,4-Difluoronitrobenzene in tetrahydrofuran, room temperature; *ii*: Pd–C in *n*-butanol, hydrazine hydrate, reflux; *iii*: ethyl bromoacetate in tetrahydrofuran, triethylamine, room temperature; *iv*: Hydrazine hydrate in absolute ethanol, reflux; *v*: phenylisothiocyanate in absolute ethanol, reflux; *vi*: NaOH in water, reflux; *vii*: KOH in water/ethanol, CS_2, reflux; *viii*: 2-hydroxybenzaldehyde (for **20**), 3-hydroxy-4-methoxybenzaldehyde (for **21**) or ındol-3-carbaldehyde (for **22**) in ethanol, reflux.

The treatment of hydrazide, **16** with 2-hydroxybenzaldehyde, 3-hydroxy-4-methoxybenzaldehyde or indol-3-carbaldehyde in ethanolic solution generated the corresponding imine compounds, *N*′-[(substituted phenyl)methylen]-2-[4-(2-methoxy phenyl)piperazin-1-yl]acetohydrazides (**20**–**22**). The condensation of compound **21** with compound **3** resulted in the formation of 1-ethyl-6-fluoro-7-[4-({2-methoxy-4-[({[4-(2-methoxyphenyl)piperazin-1-yl]acetyl}hydrazono)methyl]phenoxy}acetyl)piperazin-1-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**23**). The structures of these compounds (**20**–**23**) were confirmed on the basis of spectroscopic methods and elemental analysis. It is interesting to note that the compounds having arylidenehydrazide structure may exist as *E*/*Z* geometrical isomers about C=N double bond and as *cis*/*trans* amide conformers [60,61]. According to the literature [61], the compounds containing imine bond are present in higher percentage in dimethyl-d_6 sulfoxide solution in the form of geometrical *E* isomer about C=N double bond. The *Z* isomer can be stabilized in less polar solvents by an intramolecular hydrogen bond. In the present study, the spectral data was obtained in dimethyl-d_6 sulfoxide solution and no signal belonging to *Z* isomer has been observed. On the other hand, the *cis*/*trans* conformers of *E* isomer were present in the dimethyl-d_6 sulfoxide solution of compounds **20**–**23**.

The synthesis of compounds **24** and **25** was carried out by the condensation of compound **3** with **18** and **19**, respectively, with the aim to introduce a 1,3,4-oxadiazole or 1,2,4-triazole nucleus to norfloxacin skeleton. These compounds exhibited FT-IR, ^{1}H NMR and ^{13}C NMR spectra consistent with the assigned structures. Moreover, [M + K] ion peaks have been included in their mass spectra, and rational elemental analysis results have been obtained for these compounds. The condensation of **14** and **16** with compound **3** yielded the corresponding norfloxacin derivatives (**26** and**27**) containing at the position 7 a 3-fluoro-4-[4-(2-methoxyphenyl)piperazin-1-yl]phenylglycyl (for **26**) or 4-[(2-{[4-(2-methoxyphenyl)



Scheme 3. *i*: NaOH and compound **21** in dimethyl sulfoxide, 80 °C; *ii*: NaOH and compound **24** in dimethyl sulfoxide, 80 °C; *iii*: NaOH and compound **18** in dimethyl sulfoxide, 80 °C; *iv*: NaOH and compound **14** in dimethyl sulfoxide, 80 °C; *v*: NaOH and compound **16** in dimethyl sulfoxide, 80 °C.

piperazin-1-yl]acetyl}hydrazino)acetyl] (for **27**) moiety, and their structures have been elucidated by spectroscopic methods and elemental analysis. ^{1}H NMR spectrum of derivative **26** displayed only one signal due to NH group at 8.69 ppm integrating one proton as a result of condensation, while ^{1}H NMR spectrum of derivative **27** showed two signals due to two NH protons at 8.52 and 11.57 ppm. The signal originated from methoxy group resonated at 3.96 (for **27**) or 3.97 (for **26**) ppm in the ^{1}H NMR spectra of these compounds. These carbons were recorded at 55.40 (for **27**) and 56.09 (for **26**) in the ^{13}C NMR spectra. Other groups resonated in the related chemical shift values in the ^{1}H NMR and ^{13}C NMR spectra. The additional support for the formation of the targeted compounds (**26** and **27**) was obtained by the appearance of molecular ion peaks at corresponding *m*/*z* values confirming their molecular masses. Moreover, compounds **26** and **27** gave reasonable elemental analysis results consistent with the proposed structures.

2.2. Biological activity

2.2.1. Antimicrobial activity

The nitro compound (**1**) exhibited good antimicrobial activity toward the test microorganisms except *Candida albicans* (Ca) and

Saccharomyces cerevisiae (Sc), which are yeast like fungi. With the reduction of nitro group to amine function, excellent antibacterial activities were observed for compound **2** with the MIC values of <1.9 or 7.8 μg/mL. Similarly, the chloroacetylation of norfloxacin gave the compound **3** having excellent antimicrobial activity on test microorganisms with the MIC values varying between 0.21 and 15.6 μg/μL, except *Ca.* and *Sc.* Even this result is better than standard drugs Ampicillin and Streptomycin. Among the carbonothioylamino derivatives (**4**, **6** and **7**), compound **4** was found to have excellent activity against the test microorganisms except *Ca.* and *Sc.* with the MIC values between 0.97 and 31.5 μg/mL. On the other hand, the carbonylamino derivative (**5**) of **4** displayed marginal activities against *Escherichia coli* (*Ec.*), enteric bacteria, *S. aureus* (*Sa.*) and *Enterococcus faecalis* (*Ef.*), which are Gram positive cocci, *Bacillus cereus* (*Bc.*) that is Gram positive spore bacillus, *C. albicans* (*Ca.*) and *S. cerevisiae* (*Sc.*). It can be speculated that this lower activity is due to the slight solubility of compound **5** even in dimethyl sulfoxide. The carbonthioylamino derivative containing a morpholin-4-ylpropyl nucleus (**7**) demonstrated good-moderate activities on the test microorganisms except *Mycobacterium smegmatis* (*Ms.*), a nonpigmented rapidly growing mycobacterium, *Ca.* and *Sc.*, while other carbonthioylamino derivative including an ethyl group instead of morpholin-4-ylpropyl nucleus (**6**) was found to have slight activity toward *Ec.*, *Ca.* and *Sc.* with the MIC values of 250–500 μg/mL.

Among the imine compounds (**20**–**22**), compounds **20** and **21**, which contain a 2-hydroxyphenylmethylene or 4-(hydroxy-3-methoxyphenyl)methylene moiety in the 4-(2-methoxyphenyl) piperazin-1-yl]acetohydrazide skeleton, displayed good-moderate activity against the test microorganisms with the MIC values between 250 and 31.5 μg/mL.

According to the results obtained, it can be concluded that the conversion of norfloxacin to the derivatives which contain a bulky group in the position 7 (compounds **23**–**27**) resulted in completely inactivation of these compounds against the test microorganisms.

2.2.2. Antiurease activity

The synthesized compounds were assayed for their in vitro inhibitory activity against Jack bean urease. Three of those compounds showed perfect urease inhibition. Thiourea with IC_{50} value 51.62 ± 7.28 μg/mL was used as standard inhibitor. Among tested compounds **25** was found to be the best inhibitory effect against urease with an IC_{50} value of 20.51 ± 9.09 μg/mL. The other compounds have moderate or no inhibitory activity (Table 2). Dose dependent inhibitory effect of compound **25** was depicted in Fig. 1. At low concentrations of compound **25** caused urease inhibitory activity than thiourea. This compound might be considered as potential antibiotics to treat infections.

3. Experimental

3.1. General

All the chemicals were purchased from Fluka Chemie AG Buchs (Switzerland) and used without further purification. Melting points of the synthesized compounds were determined in open capillaries on a Büchi B-540 melting point apparatus and are uncorrected. Reactions were monitored by thin-layer chromatography (TLC) on silica gel 60 F254 aluminum sheets. The mobile phase was ethyl acetate:ethyl 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) or Varian-Mercury 200 MHz NMR Spectrometer (200 MHz for ^{1}H and 50 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 EI-MS* (70 eV) Instrument.

3.1.1. 1-Ethyl-6-fluoro-7-[4-(2-fluoro-4-nitrophenyl)piperazin-1-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (*1*)

3.1.1.1. Method 1. The solution of norfloxacin (10 mmol) and 3,4-difluoronitrobenzene (50 mmol) in acetonitrile was refluxed in the presence of $NaHCO_3$ (30 mmol) for 5 h. Then, the mixture was poured into ice water. The yellow precipitated product was filtered off and recrystallized from dimethyl sulfoxide. Yield 86%, m.p. 314 °C.

3.1.1.2. Method 2. The mixture of norfloxacin (10 mmol), 3,4-difluoronitrobenzene (50 mmol) and $NaHCO_3$ (30 mmol) was irradiated in closed vessels with the pressure control at 100 °C for

Table 1
Screening for antimicrobial activity of the compounds (μg/mL).

Compound no	Microorganisms[a] and minimal inhibition concentration								
	Ec	*Yp*	*Pa*	*Sa*	*Ef*	*Bc*	*Ms*	*Ca*	*Sc*
1	31.5	31.5	–	–	–	–	–	–	–
2	<1.9	<1.9	7.81	<1.9	<1.9	<1.9	1.95	–	–
3	0.21	1.7	15.6	0.21	0.21	0.21	0.42	–	–
4	<1.9	<1.9	31.5	<1.9	<1.9	<1.9	0.97	–	–
5	250	–	–	500	500	500	–	500	250
6	500	–	–	–	–	–	–	250	250
7	3.4	62.5	500	15.6	31.25	125	–	–	–
8	15.6	62.5	–	125	125	125	62.5	250	250
9	125	–	–	250	250	250	–	250	250
10	–	–	–	–	250	–	–	250	250
11	–	–	–	–	–	1000	–	–	–
20	31.3	31.3	62.5	31.3	15.6	31.3	31.3	250	62.5
21	31.3	31.3	31.3	31.3	15.6	31.3	31.3	62.5	31.3
Amp.	10	18	>128	35	10	15			
Srp.							4		
Flu								<8	<8

[a] *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*: *Mycobacterium smegmatis* ATCC607, *Ca*: *Candida albicans* ATCC 60193, *Sc*: *Saccharomyces cerevisiae* RSKK 251, Amp.: Ampicillin, Strep.: Streptomycin, Flu.: Fluconazole, (–): no activity.

Table 2
Inhibitory activities and IC_{50} values of the synthesized compounds against Jack Bean urease.

Compound no	% Inhibition	IC_{50} (μg/mL)
	100 μg/mL	
25	86.2%	20.51 ± 9.09
Thiourea	92.2%	51.62 ± 7.28

4 min (hold time) at 200 W maximum power. After the completion of the reaction, (monitored by LC), the mixture was poured into ice water. The precipitate was collected by filtration and recrystallized from dimethyl sulfoxide. Yield 90%, m.p. 314 °C, FT-IR (ν_{max}, cm^{-1}): 3051 (ar–CH), 1732 and 1615 (2C=O), 1475 and 1335 (NO_2), 1237 (C–O). Elemental analysis for $C_{22}H_{20}F_2N_4O_5$, calculated (%), C, 57.64; H, 4.40; N, 12.22, found (%), C, 57.79; H, 4.42; N, 12.03. ^{1}H NMR (DMSO-d_6, δ ppm): 1.43 (t, 3H, CH_3, J = 6.0 Hz), 3.56 (s, 8H, $4CH_2$), 4.61 (q, 2H, CH_2, J = 6.0 Hz), 7.26 (bs, 2H, arH), 7.93–8.10 (m, 3H, arH), 8.97 (s, 1H, olefinic–CH), 15.29 (s, 1H, OH, exch. D_2O). ^{13}C NMR (DMSO-d_6, δ ppm): The spectrum could not be obtained, due to the slight solubility in any NMR solvent. EI MS m/z (%): 481.42 ($[M + Na]^+$, 42), 460.39 ($[M + 2]^+$, 25), 459.45 ($[M + 1]^+$, 100), 441.43 (18), 213.17 (31), 183.07 (22), 155.91 (25), 153.85 (50), 148.97 (56), 134.95 (31), 118.93 (81), 105.98 (84).

*3.1.2. 7-[4-(4-Amino-2-fluorophenyl)piperazin-1-yl]-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**2**)*

3.1.2.1. Method 1. Pd/C (5 mmol) catalyst was added to the solution of compound **1** (10 mmol) in *n*-butanol, and the mixture was refluxed in the presence of hydrazine hydrate (50 mmol) for 20 h (The progress of the reaction was monitored by TLC). Then, the catalyst was separated by filtration and washed with hot dimethylformamide. The combined filtrate poured into ice water and an orange solid was obtained. This was recrystallized from dimethylformamide:water (1:5). Yield 30%, m.p. 257 °C.

3.1.2.2. Method 2. Pd/C (5 mmol) catalyst and hydrazine hydrate were added to the solution of compound **1** (10 mmol) in *n*-butanol, and the reaction mixture was irradiated in closed vessels with the pressure control at 150 °C for 50 min (hold time) at 200 W maximum power (The progress of the reaction was monitored by TLC). Then, the catalyst was separated by filtration and washed by hot DMF. The combined filtrate poured into ice water The obtained orange solid was recrystallized from dimethylformamide:water (1:5). Yield 70%, m.p. 257 °C. FT-IR (ν_{max}, cm^{-1}): 3433 and 3332 (NH_2), 3051 (ar–CH), 2955 and 2853 (aliphatic C–H), 1702 and 1627 (2C=O), 1254 (C–O). Elemental analysis for $C_{22}H_{22}F_2N_4O_3$, calculated (%), C, 61.68; H, 5.18; N, 13.08, found (%), C, 61.58; H, 5.21; N, 13.15. ^{1}H NMR (DMSO-d_6, δ ppm): 1.41 (s, 3H, CH_3), 3.03 (s, 4H, $2CH_2$), 3.42 (s, 4H, $2CH_2$), 4.58 (d, 2H, CH_2, J = 6.0 Hz), 5.03 (s, 2H, NH_2, exch. D_2O), 6.33–6.39 (m, 2H, arH), 6.84 (t, 1H, arH), 7.21 (d, 1H, arH), 7.90 (d, 1H, arH, J = 12.0 Hz), 8.94 (s, 1H, olefinic–CH), 15.30 (s, 1H, OH, exch. D_2O). ^{13}C NMR (DMSO-d_6, δ ppm): 15.10 (CH_3), 49.79 (CH_2), 50.58 ($2CH_2$), 51.75 ($2CH_2$), arC: [106.80 (CH), 107.77 (C), 110.26 (CH), 112.02 (CH), 117.19 (CH), 119.36 (C), 119.66 (CH), 120.12 (C), 120.54 (C), 137.85 (2C), 145.78 (C), 146.35 (C), 150.51 (CH)], 166.85 and 176.90 (2C=O). EI MS m/z (%): 451.51 ($[M + Na]^+$, 15), 429.54 ($[M + 1]^+$, 28), 380.67 (56), 246.33 (43), 149.16 (100), 138.21 (98), 121.19 (68).

*3.1.3. 7-[4-(Chloroacetyl)piperazin-1-yl]-1-ethyl-6-fluoro-4-oxo-1,4-dihydro quinoline-3-carboxylic acid (**3**)*

Chloroethanoylchloride (15 mmol) was added to the mixture of compound **2** (10 mmol) and triethylamine (30 mmol) in THF cooled to −5 °C drop wise over a 2-hour period. Then, the temperature was allowed to reach to room temperature and the mixture was stirred for 4 h. The precipitate was filtered off and washed by water. The resulting white oily product was recrystallized form dimethyl sulfoxide to afford the desired compound. Yield 90%, m.p. 241–242°, FT-IR (ν_{max}, cm^{-1}): 3055 (ar–CH), 2984 (aliphatic C–H), 1713, 1656 and 1623 (C=O). ^{1}H NMR (DMSO-d_6) δ ppm: 1.42 (t, 3H, CH_3, J = 8.0 Hz), 3.35–3.38 (m, 4H, $2CH_2$), 3.58–3.70 (m, 4H, $2CH_2$), 4.48 (s, 2H, CH_2), 4.59 (q, 2H, CH_2, J = 8.0 Hz), 7.20 (d, 1H, arH, J = 8.0 Hz), 7.92 (d, 1H, arH, J = 12.0 Hz), 8.96 (s, 1H, olefinic–CH), 15.31 (s, 1H, OH, exch. D_2O). ^{13}C NMR (DMSO-d_6) δ ppm: 15.06 (CH_3), 42.03 (CH_2), 42.54 ($2CH_2$), 45.71 (CH_2), 49.76 (CH_2), 50.14 (CH_2), arC: [106.95 (d, CH, J = 2.0 Hz), 107.78 (C), 111.92 (d, CH, J = 22.0 Hz), 120.35 (d, C, J = 7.0 Hz), 137.80 (C), 145.76 (d, C, J = 9.0 Hz), 149.29 (CH), 153.49 (d, C, J = 248.0 Hz)], 165.51 (C=O), 166.85 (C=O), 176.90 (C=O). EI MS m/z (%): 418.08 ($[M]^+$, 100), 381.29 (48), 342.11 (24).

*3.1.4. 7-[4-(4-{[(Benzylamino)carbonothioyl]amino}-2-fluorophenyl)piperazin-1-yl]-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**4**)*

3.1.4.1. Method 1. The solution of compound **2** (10 mmol) in absolute ethanol was refluxed with benzylisothiocyanate (20 mmol) for 40 h. On cooling the reaction mixture to room temperature, a solid formed. This crude product was collected by filtration and recrystallized from acetone. Yield 84%, m.p. 199–200 °C.

3.1.4.2. Method 2. The mixture of compound **2** (10 mmol) and benzylisothiocyanate (20 mmol) in absolute ethanol was irradiated in open vessels under reflux condition at 78 °C for 1 h (hold time) at 200 W maximum power. The white solid formed was recrystallized from acetone. Yield 90%, m.p. 199–200 °C. FT-IR (ν_{max}, cm^{-1}): 3248 and 3186 (NH), 3045 (ar–CH), 2949 and 2847 (aliphatic C–H), 1731 and 1615 (C=O), 1245 (C–O and C=S). Elemental analysis for $C_{30}H_{29}F_2N_5O_3S$, calculated (%), C, 62.38; H, 5.06; N, 12.12, found (%), C, 62.18; H, 5.19; N, 12.12. ^{1}H NMR (DMSO-d_6, δ ppm): 1.42 (s, 3H, CH_3), 3.03 (s, 2H, CH_2), 3.20 (s, 2H, CH_2), 3.37 (s, 4H, $2CH_2 + H_2O$), 4.60–4.73 (m, 4H, $2CH_2$), 7.08 (s, 2H, CH), 7.23–7.43 (m, 7H, arH), 7.92–7.92 (d, 1H, arH, J = 7.0 Hz), 8.20 (s, H, NH, exch. D_2O), 8.96 (s, 1H, olefinic–CH), 9.61 (s, H, NH, exch. D_2O), 15.33 (s, 1H, OH, exch. D_2O). ^{13}C NMR (DMSO-d_6, δ ppm): 15.10 (CH_3), 49.79 (CH_2), 50.57

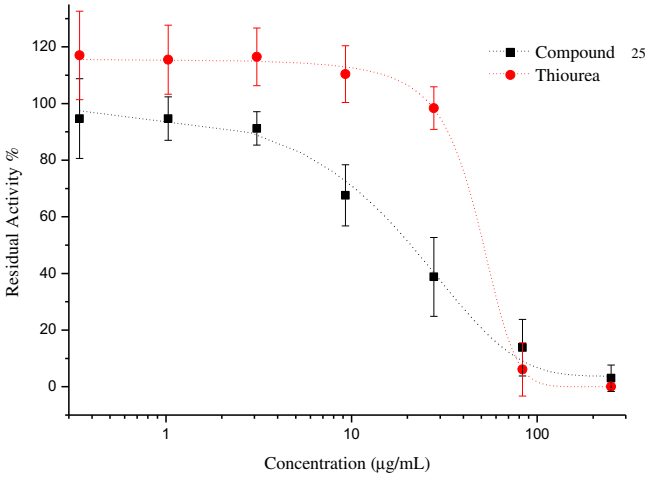


Fig. 1. Dose-dependent inhibitory effect of compound **25**. Thiourea was used as standard inhibitor. Inhibitory effect of compound **25** and Thiourea were measured at the range of 250 to 0.114 μg/mL concentrations. Residual activities of compounds are expressed as the mean ± S.D. in triplicate.

(CH_2), 50.80 ($2CH_2$), 51.70 ($2CH_2$), arC: [102.74 (CH), 106.73 (CH), 107.74 (C), 110.27 (CH), 111.84 (d, CH J = 23.5 Hz), 120.09 (C), 121.52 (CH), 127.58 (CH), 128.00 (CH), 128.09 (CH), 128.97 (2CH), 129.37 (d, C, J = 9.5 Hz), 137.85 (C), 139.66 (C), 146.08 (d, C, J = 6.0 Hz), 146.44 (d, C, J = 10.0 Hz), 149.20 (CH), 153.65 (d, C, J = 251.5 Hz), 155.85 (d, C, J = 365.0 Hz)], 166.85 and 176.86 (2C=O), 181.40 (C=S). EI MS m/z (%): 609.81 (100), 578.20 ($[M + 1]^+$, 55), 517.17 (75), 101.00 (70).

*3.1.5. 7-[4-(4-{[(Benzylamino)carbonyl]amino}-2-fluorophenyl) piperazin-1-yl]-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**5**)*

3.1.5.1. Method 1. The solution of compound (**2**) (10 mmol) in absolute ethanol was refluxed with benzylisocyanate (20 mmol) for 48 h. On cooling the reaction mixture to room temperature, a solid was formed. This crude product was collected by filtration and recrystallized from dimethyl sulfoxide:water (1:5). Yield 82%, m.p. 319–320 °C.

3.1.5.2. Method 2. The mixture of compound **2** (10 mmol) and benzylisocyanate (20 mmol) in absolute ethanol was irradiated in open vessels under reflux condition at 78 °C for 1 h (hold time) at 200 W maximum power. The yellow solid formed was recrystallized from dimethyl sulfoxide:water (1:5). Yield 95%, m.p. 319–320 °C. FT-IR (ν_{max}, cm^{-1}): 3325 and 3292 (NH), 3047 (ar–CH), 2956 and 2839 (aliphatic C–H), 1730 and 1616 (C=O), 1245 (C–O), 1223 (C–O). Elemental analysis for $C_{30}H_{29}F_2N_5O_4$, calculated (%), C, 64.16; H, 5.21; N, 12.47, found (%), C, 64.32; H, 5.01; N, 12.56. ^{1}H NMR (DMSO-d_6, δ ppm): 1.40 (t, 3H, CH_3, J = 6.0 Hz), 3.11 (s, 4H, $2CH_2$), 3.55 (s, 4H, $2CH_2$), 4.22 (d, 2H, CH_2, J = 4.0 Hz), 4.60 (q, 2H, CH_2, J = 6.0 Hz), 6.33 (s, H, NH, exch. D_2O), 6.99 (s, 2H, arH), 7.24–7.29 (m, 6H, arH), 7.44 (d, 1H, arH, J = 15.2 Hz), 7.87 (d, 1H, arH, J = 13.4 Hz), 8.63 (s, 1H, NH, exch. D_2O), 8.93 (s, 1H, olefinic–CH), 15.36 (s, 1H, OH, exch. D_2O). ^{13}C NMR (DMSO-d_6, δ ppm): 14.36 (CH_3), 42.70 (CH_2), 49.05 (CH_2), 49.68 ($2CH_2$), 50.39 ($2CH_2$), arC: [106.02 (d, CH, J = 14.6 Hz), 106.20 (CH), 107.08 (C), 111.14 (d, C, J = 23.3 Hz), 113.53 (CH), 119.37 (CH), 126.68 (CH), 127.00 (2CH), 128.34 (2CH), 133.13 (d, C, J = 9.4 Hz), 136.27 (d, C, J = 11.0 Hz), 137.15 (C), 140.26 (C), 145.42 (d, C, J = 11.1 Hz), 148.49 (CH), 152.91 (d, C, J = 248.0 Hz), 154.95 (d, C, J = 241.0 Hz)], 155.11, 166.08 and 176.12 (3C=O). EI MS m/z (%): 561.215 ($[M + 1]^+$, 100), 471.42 (50), 328.16 (75), 150.07 (33).

*3.1.6. 1-Ethyl-7-[4-(4-{[(ethylamino)carbonothioyl]amino}-2-fluorophenyl)piperazin-1-yl]-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**6**)*

3.1.6.1. Method 1. The solution of compound **2** (10 mmol) in absolute ethanol was refluxed with ethylisothiocyanate (20 mmol) for 65 h. On cooling the reaction mixture to room temperature, a solid formed. This crude product was collected by filtration and recrystallized from dimethyl sulfoxide:water (1:5). Yield 91%, m.p. 241–242 °C.

3.1.6.2. Method 2. The mixture of compound **2** (10 mmol) and ethylisothiocyanate (20 mmol) in absolute ethanol was irradiated in open vessels under reflux condition at 78 °C for 1 h (hold time) at 200 W maximum power. The yellow solid formed was recrystallized from dimethyl sulfoxide:water (1:5). Yield 95%, m.p. 241–242 °C. FT-IR (ν_{max}, cm^{-1}): 3352 and 3149 (NH), 3047 (ar–CH), 2985 and 2813 (aliphatic C–H), 1713 and 1626 (2C=O), 1263, 1248, 1228 and 1209 (C–O and C=S). Elemental analysis for $C_{25}H_{27}F_2N_5O_3S$, calculated (%), C, 58.24; H, 5.28; N, 13.58, found (%), C, 58.29; H, 5.21; N, 13.56. ^{1}H NMR (DMSO-d_6, δ ppm): 1.09 (t, 3H, CH_3, J = 7.0 Hz), 1.42 (s, 3H, CH_3), 3.19 (s, 4H, $2CH_2$), 3.36 (s, 2H, CH_2 + H_2O), 3.45 (s, 4H, $2CH_2$), 4.61 (s, 2H, CH_2), 7.04 (s, 2H, arH), 7.32–7.38 (m, 2H, arH + NH, exch. D_2O), 7.75 (s, 1H, arH), 7.92 (d, 1H, arH, J = 14.0 Hz), 8.95 (s, 1H, olefinic–CH), 9.41 (s, 1H, NH, exch. D_2O), 15.33 (s, 1H, OH, exch. D_2O). ^{13}C NMR (DMSO-d_6, δ ppm): 14.14 (CH_3), 14.38 (CH_3), 38.65–40.10 (DMSO + CH_2), 49.05 (CH_2), 50.09 (CH_2), arC: [106.09 (CH), 107.07 (C), 111.00 (CH), 111.22 (CH), 119.17 (CH), 119.38 (CH), 119.45 (C), 134.38 (C), 135.81 (d, C, J = 9.3 Hz), 137.10 (C), 145.36 (d, C, J = 10.4 Hz), 148.43 (CH), 152.88 (d, C, J = 248.0 Hz), 154.26 (d, C, J = 242.0 Hz)], 166.07 and 176.09 (2C=O), 179.97 (C=S). EI MS m/z (%): 538.10 ($[M + Na]^+$, 30), 516.12 ($[M + 1]^+$, 100), 389.16 (46), 102.10 (57).

*3.1.7. 1-Ethyl-6-fluoro-7-{4-[2-fluoro-4-({[(3-morpholin-4-ylpropyl)amino]carbonothioyl}amino)phenyl]piperazin-1-yl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**7**)*

3.1.7.1. Method 1. The solution of compound **2** (10 mmol) in absolute ethanol was refluxed with 4-morpholinopropylisothiocyanate (20 mmol) for 80 h. On cooling the reaction mixture to room temperature, a solid formed. This crude product was collected by filtration and recrystallized from acetone. Yield 70%, m.p. 215–216 °C.

3.1.7.2. Method 2. The mixture of compound **2** (10 mmol) and 4-morpholinopropylisothiocyanate (20 mmol) in absolute ethanol was irradiated in open vessels under reflux condition at 76–78 °C for 1 h (hold time) at 200 W maximum power. The yellow solid formed was recrystallized from acetone. Yield 70%, m.p. 215–216 °C. FT-IR (ν_{max}, cm^{-1}): 3342 and 3169 (NH), 3048 (ar–CH), 2936 (aliphatic C–H), 1727 and 1614 (2C=O), 1246 (C–O). Elemental analysis for $C_{30}H_{36}F_2N_6O_4S$, calculated (%), C, 58.62; H, 5.90; N, 13.67, found (%), C, 58.32; H, 5.72; N, 13.70. ^{1}H NMR (DMSO-d_6, δ ppm): 1.43 (t, 3H, CH_3, J = 8.3 Hz), 1.72 (s, 2H, CH_2), 3.21 (s, 4H, $2CH_2$), 3.21 (s, 2H, CH_2), 3.34 (s, 2H, CH_2), 3.50 (d, 12H, $6CH_2$), 4.62 (q, 2H, CH_2 J = 8.3 Hz), 7.07–7.11 (m, 1H, CH), 7.27 (s, 1H, CH), 7.36 (d, 1H, arH, J = 12.0 Hz), 7.80 (bs, 1H, NH, exch. D_2O), 7.96 (d, 1H, arH, J = 16.0 Hz), 8.04–8.09 (m, 1H, CH), 8.98 (s, 1H, olefinic–CH), 9.53 (bs, 1H, NH, exch. D_2O), 15.36 (s, 1H, OH, exch. D_2O). ^{13}C NMR (DMSO-d_6, δ ppm): 14.38 (CH_3), 24.92 (CH_2), 42.43 (CH_2), 48.87 (CH_2), 49.05 (CH_2), 49.61 (CH_2), 50.08 (CH_2), 53.08 ($2CH_2$), 55.78 (CH_2), 65.81 ($2CH_2$), arC: [106.58 (d, CH, J = 9.8 Hz), 107.06 (C), 111.11 (d, CH, J = 23.0 Hz), 112.23 (d, CH, J = 26.0 Hz), 118.68 (d, CH, J = 119.0 Hz), 119.42 (d, C, J = 8.8 Hz), 121.24 (CH), 137.10 (C), 139.67 (d, C, J = 8.6 Hz), 145.37 (d, C, J = 10.2 Hz), 148.46 (CH), 152.83 (d, C, J = 248.0 Hz), 154.36 (d, C, J = 230.0 Hz), 156.74 (C)], 166.06 and 176.08 (2C=O), 180.13 (C=S). EI MS m/z (%): 614.24 ($[M + 1]^+$, 100), 586.21 (35), 381.48 (25), 203.10 (10).

*3.1.8. 7-[4-(4-{[3-Benzyl-4-oxo-1,3-thiazolidin-2-ylidene]amino}-2-fluorophenyl)piperazin-1-yl]-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**8**)*

3.1.8.1. Method 1. The mixture of compound **3** (10 mmol) and ethyl bromoacetate (10 mmol) in glacial acetic acid was irradiated in closed vessels with the pressure control at 150 °C for 75 min (hold time) at 200 W maximum power in the presence of dried sodium acetate (10 mmol). Then, the solution was poured into ice water and a white solid formed. This was recrystallized from acetone. Yield 75%, m.p. 159–160 °C.

3.1.8.2. Method 2. The mixture of compound **3** (10 mmol) and dried sodium acetate (20 mmol) in glacial acetic acid was stirred at room temperature for 15 min. After that ethyl bromoacetate (15 mmol) was added into it and irradiated in closed vessels with the pressure control at 160 °C for 60 min (hold time) at 200 W maximum power. Then, the solution was poured into ice water and a white solid formed. This was recrystallized from acetone. Yield 90%, m.p. 159–160 °C. FT-IR (ν_{max}, cm^{-1}): 3060 (ar–CH), 2927 and 2834 (aliphatic C–H), 1720, 1624 and 1612 (3C=O), 1247 (C–O).

Elemental analysis for $C_{32}H_{29}F_2N_5O_4S$, calculated (%), C, 62.23; H, 4.73; N, 11.34, found (%), C, 62.18; H, 4.89; N, 11.21. ^{1}H NMR (DMSO-d_6, δ ppm): 1.42 (s, 3H, CH_3), 3.20 (s, 4H, $2CH_2$), 3.47 (s, 4H, $2CH_2$), 4.11 (s, 2H, CH_2), 4.62 (s, 2H, CH_2), 4.90 (s, 2H, CH_2), 6.73–6.80 (m, 2H, arH), 7.10 (t, 1H, arH), 7.20–7.34 (m, 6H, arH), 7.92 (d, 1H, arH, J = 14.0 Hz), 8.95 (s, 1H, olefinic–CH), 15.35 (s, 1H, OH, exch. D_2O). ^{13}C NMR (DMSO-d_6, δ ppm): 14.37 (CH_3), 32.54 (CH_2), 45.37 (CH_2), 49.05 (CH_2), 49.58 (CH_2), 50.12 ($2CH_2$), arC: [106.13 (CH), 107.09 (C), 109.34 (d, C, J = 22.0 Hz), 116.99 (C), 119.41 (C), 120.02 (CH), 127.42 (CH), 128.10 (2CH), 128.97 (2CH), 135.91 (C), 136.04 (d, C, J = 8.0 Hz), 137.16 (C), 142.96 (C), 148.52 (CH), 153.61 (d, C, J = 387.0 Hz), 155.12 (d, C, J = 227.0 Hz)], 166.07, 171.91 and 176.14 (3C=O). EI MS m/z (%): 640.12 ([M + Na]$^+$, 19), 618.14 ([M + 1]$^+$, 100), 389.17 (35), 212.08 (10).

3.1.9. 1-Ethyl-7-[4-(4-{3-ethyl-4-oxo-1,3-thiazolidin-2-ylidene] amino}-2-fluoro phenyl)piperazin-1-yl]-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**9**)

3.1.9.1. Method 1. The mixture of compound **5** (10 mmol) and ethyl bromoacetate (15 mmol) in glacial acetic acid was refluxed in the presence of dried sodium acetate (20 mmol) for 60 h. The solution poured into ice water. The white solid formed recrystallized from acetone. Yield 77%, m.p. 260–261 °C.

3.1.9.2. Method 2. The mixture of compound **5** (15 mmol) and dried sodium acetate (20 mmol) in glacial acetic acid was stirred at room temperature for 15 min. After that ethyl bromoacetate (15 mmol) was added and irradiated in closed vessels with the pressure control at 160 °C for 60 min (hold time) at 200 W maximum power. The solution poured into ice water. The white solid formed recrystallized from acetone. Yield 95%, m.p. 260–261 °C. FT-IR (ν_{max}, cm^{-1}): 3053 (ar–CH), 2961 and 2845 (aliphatic C–H), 1728 and 1625 (3C=O), 1249 (C–O). Elemental analysis for $C_{27}H_{27}F_2N_5O_4S$, calculated (%), C, 58.37; H, 4.90; N, 12.61, found (%), C, 58.46; H, 4.98; N, 12.55. ^{1}H NMR (DMSO-d_6, δ ppm): 1.16 (t, 3H, CH_3, J = 4.0 Hz), 1.42 (s, 3H, CH_3), 3.20 (s, 4H, $2CH_2$), 3.46 (s, 4H, $2CH_2$), 3.72 (q, 3H, CH_3, J = 4.0 Hz), 4.02 (s, 2H, CH_2), 4.62 (s, 2H, CH_2), 6.77–6.84 (m, 1H, arH), 7.11–7.26 (m, 3H, arH), 7.91 (d, 1H, arH, J = 14.0 Hz), 8.96 (s, 1H, olefinic–CH), 15.36 (s, 1H, OH, exch. D_2O). ^{13}C NMR (DMSO-d_6, δ ppm): 12.23 (CH_3), 14.38 (CH_3), 32.55 (CH_2), 37.39 (CH_2), 49.05 (CH_2), 49.59 ($2CH_2$), 50.13 ($2CH_2$), arC: [106.12 (CH), 107.08 (C), 109.39 (d, CH J = 21.6 Hz), 111.15 (d, CH, J = 22.3 Hz), 117.06 (CH), 119.43 (d, C, J = 8.2 Hz), 119.95 (CH), 135.85 (d, C, J = 8.2 Hz), 137.15 (C), 143.25 (d, C, J = 9.0 Hz), 145.37 (d, C, J = 11.5 Hz), 148.49 (CH), 153.55 (d, C, J = 379.0 Hz), 154.14 (C=N), 155.04 (d, C, J = 245.0 Hz)], 166.06, 171.62 and 176.14 (3C=O). EI MS m/z (%): 578.09 ([M + Na]$^+$, 20), 556.11 ([M + 1]$^+$, 65), 389.16 (100), 242.24 (25), 212.07 (30), 102.10 (72).

3.1.10. 7-[4-(4-{[3-Benzyl-5-(4-chlorophenyl)-1,3-thiazol-2(3H)-ylidene]amino}-2-fluorophenyl)piperazin-1-yl]-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**10**)

3.1.10.1. Method 1. The mixture of compound (**3**) (10 mmol) and 4-chlorophenacylbromide (10 mmol) in dry chloroform was refluxed in the presence of dried sodium acetate (50 mmol) for 40 h. Then, the reaction mixture was allowed to reach room temperature and the precipitated salt was separated by filtration. After removing the solvent under reduced pressure, an oily product was recrystallized from acetone. Yield 93%, m.p. 226–227 °C.

3.1.10.2. Method 2. The mixture of compound **3** (10 mmol) and dried sodium acetate (20 mmol) in glacial acetic acid was stirred at room temperature for 15 min. After that 4-chlorophenacylbromide (15 mmol) was added and irradiated in closed vessels with the pressure control at 160 °C for 60 min (hold time) at 200 W maximum power. The solution poured into ice water. The white solid formed and recrystallized from acetone. Yield 93%, m.p. 226–227 °C. FT-IR (ν_{max}, cm^{-1}): 3098 and 3060 (ar–CH), 2980 and 2830 (aliphatic C–H), 1713, 1624 and 1625 (2C=O), 1245 (C–O). Elemental analysis for $C_{38}H_{32}ClF_2N_5O_3S$, calculated (%), C, 64.08; H, 4.53; N, 9.83, found (%), C, 64.36; H, 4.63; N, 9.69. ^{1}H NMR (DMSO-d_6, δ ppm): 1.43 (s, 3H, CH_3), 3.20 (s, 4H, $2CH_2$), 3.48 (s, 4H, $2CH_2$), 4.60 (s, 2H, CH_2), 5.04 (s, 2H, CH_2), 6.37 (s, 1H, CH), 6.77–7.48 (m, 13H, arH), 7.94 (d, 1H, arH, J = 12.0 Hz), 8.94 (s, 1H, olefinic–CH), 15.34 (bs, 1H, OH). ^{13}C NMR (DMSO-d_6, δ ppm): 15.09 (CH_3), 48.43 (CH_2), 49.67 (CH_2), 50.38 ($2CH_2$), 51.01 ($2CH_2$), 98.06 (CH), arC: [106.79 (CH), 109.78 (d, CH, J = 21.0 Hz), 111.89 (d, CH, J = 22.0 Hz), 117.47 (CH), 120.38 (C), 120.95 (CH), 127.14 (2CH), 127.76 (CH), 129.12 (2CH), 129.39 (2CH), 130.42 (C), 131.16 (2CH), 134.69 (2C), 135.29 (d, C, J = 9.0 Hz), 137.77 (2C), 139.21 (C), 146.48 (d, C, J = 102.0 Hz), 147.07 (C), 149.17 (CH), 153.67 (d, C, J = 264.0 Hz), 156.13 (d, C, J = 262.0 Hz)], 159.82 (C=N), 166.86 and 176.75 (2C=O). EI MS m/z (%): 712.16 ([M + 1]$^+$, 100), 609.78 (20), 242.27 (13), 100.99 (13).

3.1.11. 7-[4-(4-{[5-(4-Chlorophenyl)-3-ethyl-1,3-thiazol-2(3H)-ylidene]amino}-2-fluorophenyl)piperazin-1-yl]-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**11**)

3.1.11.1. Method 1. The mixture of compound **5** (10 mmol) and 4-chlorophenacylbromide (10 mmol) in dry chloroform was refluxed in the presence of dried sodium acetate (50 mmol) for 58 h. Then, the reaction mixture was allowed to reach room temperature and the precipitated salt was separated by filtration. After removing the solvent under reduced pressure, an oily product was recrystallized from acetone. Yield 70%, m.p. 280–281 °C.

3.1.11.2. Method 2. The mixture of compound **5** (15 mmol) and dried sodium acetate (20 mmol) in glacial acetic acid was stirred at room temperature for 15 min. After that 4-chlorophenacylbromide (15 mmol) was added and irradiated in closed vessels with the pressure control at 160 °C for 60 min (hold time) at 200 W maximum power. The solution poured into ice water. The white solid formed and recrystallized from acetone. Yield 90%, m.p. 280–281 °C. FT-IR (ν_{max}, cm^{-1}): 3044 (ar–CH), 2921 and 2849 (aliphatic C–H), 1731 and 1614 (C=O), 1245 (C–O). Elemental analysis for $C_{33}H_{30}ClF_2N_5O_3S$, calculated (%), C, 60.96; H, 4.65; N, 10.77, found (%), C, 61.06; H, 4.78; N, 10.57. ^{1}H NMR (DMSO-d_6, δ ppm): 1.08 (s, 3H, CH_3), 1.42 (s, 3H, CH_3), 3.21 (s, 4H, $2CH_2$), 3.48 (s, 4H, $2CH_2$), 3.77 (s, 2H, CH_2), 4.63 (s, 2H, CH_2), 6.28 (s, 1H, CH), 6.70–6.86 (m, 2H, arH), 7.10 (s, 1H, arH), 7.28 (s, 1H, arH), 7.55 (s, 4H, arH), 7.95 (d, 1H, arH, J = 14.0 Hz), 8.97 (s, 1H, olefinic–CH), 15.32 (s, 1H, OH, exch. D_2O). ^{13}C NMR (DMSO-d_6, δ ppm): 13.11 (CH_3), 14.39 (CH_3), 30.66 (CH), 38.84–40.09 (DMSO + CH_2), 49.06 (CH_2), 49.65 (CH_2), 49.70 (CH_2), 50.29 ($2CH_2$), arC: [106.17 (CH), 107.07 (C), 109.16 (d, CH, J = 21.0 Hz), 111.16 (d, CH, J = 23.0 Hz), 116.85 (CH), 119.40 (C), 120.21 (CH), 128.86 (2CH), 129.94 (C), 130.62 (2 CH), 134.03 (2C), 134.41 (C), 137.16 (C), 138.35 (C), 145.44 (d, C, J = 10.0 Hz), 148.54 (CH), 154.21 (d, C, J = 262.0 Hz), 154.17 (C), 156.74 (C=N)], 166.10 (C=O), 176.16 (C=O). EI MS m/z (%): 649. 17 ([M + 1]$^+$, 100), 621.14 (60), 416.91 (44), 238.72 (25).

3.1.12. 1-(2-Fluoro-4-nitrophenyl)-4-(2-methoxyphenyl)piperazine (**13**)

The mixture of 1-(2-methoxyphenyl)piperazine (**12**) (20 mmol) and 3,4-difluoronitrobenzene (10 mmol) in tetrahydrofuran was stirred at room temperature for 1 h. Then, ice water was poured into the reaction mixture and was stirred for 10 min. The red solid formed and recrystallized from ethyl acetate. Yield 61%, m.p. 101–102 °C. FT-IR (ν_{max}, cm^{-1}): 3193 (ar–CH and NH), 1247 (O–C).

Elemental analysis for $C_{17}H_{18}N_3O_3F$, calculated (%), C, 61.62; H, 5.48; N, 12.68, found (%), C, 61.55; H, 5.52; N, 12.61. ^{1}H NMR (DMSO-d_6, δ ppm): 3.11 (s, 4H, $2CH_2$), 3.41 (s, 4H, $2CH_2$), 3.79 (s, 3H, CH_3), 6.92 (d, 4H, arH, $J = 9.0$ Hz), 7.21 (t, 1H, arH, $J = 9.0$ Hz), 8.05 (2H, d, arH, $J = 10.2$ Hz). ^{13}C NMR (DMSO-d_6, δ ppm): 38.91–40.99 (DMSO + CH_2), 41.41 (CH_2), 50.08 (CH_2), 50.17 (CH_2), 50.57 (CH_2), 56.00 (OCH_3), arC: [112.50 (CH), 113.17 (CH), 118.73 (CH), 118.82 (CH), 121.52 (CH), 121.98 (CH), 123.57 (CH), 140.01 (C), 141.34 (C), 146.15 (C), 150.34 (C–F), 155.26 (C–OCH_3)].

3.1.13. 3-Fluoro-4-[4-(2-methoxyphenyl)piperazin-1-yl]aniline (**14**)

Pd–C (20 mmol) catalyst was added to the solution of compound **13** (10 mmol) in *n*-butanol, and the mixture was allowed to reflux in the presence of hydrazine hydrate (60 mmol) for 3 h (the progress of the reaction was monitored by TLC). The catalyst was removed by filtration. After evaporating the solvent under reduced pressure, a white solid appeared. This crude product was recrystallized from ethyl acetate to afford the desired compound. Yield 65%, m.p. 78–79 °C. FT-IR (ν_{max}, cm^{-1}): 3306 and 3229 (NH and NH_2), 3066 (ar–CH), 1197 (O–C). Elemental analysis for $C_{17}H_{20}N_3OF$, calculated (%), C, 67.75; H, 6.69; N, 13.94, found (%), C, 67.77; H, 6.57; N, 13.89. ^{1}H NMR (DMSO-d_6, δ ppm): 3.05 (s, 4H, $2CH_2$), 3.36 (s, 4H, $2CH_2$), 3.78 (s, 3H, CH_3), 5.01 (s, 2H, NH_2, exch. D_2O), 6.32 (brs, 2H, arH), 6.94 (brs, 5H, arH). ^{13}C NMR (DMSO-d_6, δ ppm): 39.32–40.98 (DMSO + CH_2), 41.39 (CH_2), 50.05 (CH_2), 50.16 (CH_2), 50.56 (CH_2), 56.00 (OCH_3), arC: [112.50 (CH), 112.64 (CH), 118.72 (CH), 118.81 (CH), 121.51 (CH), 121.96 (CH), 123.56 (CH), 140.00 (C), 141.18 (C), 146.02 (C), 152.66 (C–F), 155.22 (C–OCH_3)].

3.1.14. Ethyl [4-(2-methoxyphenyl)piperazin-1-yl]acetate (**15**)

The solution of ethyl bromoacetate (10 mmol) in tetrahydrofuran was added to the solution of 1-(2-methoxyphenyl) piperazine (10 mmol) in tetrahydrofuran drop wise and the mixture was stirred at room temperature in the presence of triethylamine (10 mmol) for 2 h. The solid formed was removed by filtration, and the solvent was evaporated under reduced pressure. The oily crude product was recrystallized from ethanol to afford the desired compound. Yield 99%, m.p. 66–68 °C. FT-IR (ν_{max}, cm^{-1}): 3062 (ar–CH), 1745 (C=O), 1197 (O–C). Elemental analysis for $C_{15}H_{22}N_2O_3$, calculated (%), C, 64.73; H, 7.97; N, 10.06, found (%), C, 64.59; H, 8.17; N, 10.26. ^{1}H NMR (DMSO-d_6, δ ppm): 1.19 (t, 3H, CH_3, $J = 7.0$ Hz), 2.64 (s, 4H, $2CH_2$), 2.94 (s, 4H, $2CH_2$), 3.20 (s, 2H, CH_2), 3.75 (s, 3H, OCH_3), 4.10 (q, 2H, OCH_2, $J = 7.0$ Hz), 6.87–6.91 (m, 4H, arH). ^{13}C NMR (DMSO-d_6, δ ppm): 14.85 (CH_3), 50.67 ($2CH_2$), 52.92 ($2CH_2$), 55.95 (OCH_3), 59.20 (CH_2), 60.54 (OCH_2), arC: [112.48 (CH), 118.64 (CH), 121.48 (CH), 123.11 (CH), 141.85 (C), 152.63 (C–OCH_3)], 170.61 (C=O); EI MS *m/z* (%): 106.01 (53), 149.97 (23), 151.0 (13), 205.1 (16), 279.3 ($[M + 1]^+$, 100), 280.29 ($[M + 2]^+$, 20), 301.29 ($[M + Na]^+$, 16), 365.5 (26).

3.1.15. [4-(2-Methoxyphenyl)piperazin-1-yl]acetohydrazide (**16**)

Hydrazine hydrate (30 mmol) was added to the solution of compound **15** (10 mmol) in absolute ethanol and the mixture was refluxed for 9 h. Then, the solvent was removed under reduced pressure and the obtained oily mass was extracted with 5 mL of dichloromethane three times. The combined organic layer was dried on $MgSO_4$ and the solvent was removed under reduced pressure. The solid obtained was recrystallized from ethanol to give the target compound. Yield 77%, m.p. 97–100 °C. FT-IR (ν_{max}, cm^{-1}): 3306 and 3229 (–NH and NH_2), 3037 (ar–CH), 1645 (C=O). Elemental analysis for $C_{13}H_{20}N_4O_2$, calculated (%), C, 59.07; H, 7.63; N, 21.20, found (%), C, 59.30; H, 7.56; N, 21.15. ^{1}H NMR (DMSO-d_6, δ ppm): 2.63 (s, 4H, $2CH_2$), 2.96 (s, 4H, $2CH_2$), 3.10 (s, 2H, NH_2, exch. D_2O), 3.38 (s, 2H, CH_2), 3.75 (s, 3H, OCH_3), 6.91 (d, 4H, arH, $J = 7.5$ Hz), 10.04 (s, 1H, NH, exch. D_2O). ^{13}C NMR (DMSO-d_6, δ ppm): 50.76 (CH_2), 50.92 (CH_2), 53.69 ($2CH_2$), 55.96 (OCH_3), 58.34 (CH_2), arC: [112.47 (CH), 118.65 (CH), 121.48 (CH), 123.18 (CH), 141.85 (C), 152.62 (C–OCH_3)], 171.48 (C=O).

3.1.16. {[4-(2-Metoxyphenyl)piperazin-1-yl]acetyl}-N-phenylhydrazincarbothioamide (**17**)

Phenylisothiocyanate (10 mmol) was added to the solution of compound **16** (10 mmol) in absolute ethanol and the mixture was refluxed for 6 h. On cooling the mixture in cold overnight, a solid obtained. This was recrystallized from ethanol to afford the desired compound. Yield 51%, m.p. 177–180 °C. FT-IR (ν_{max}, cm^{-1}): 3562 and 3456 (3NH), 3158 (ar–CH), 1734 (C=O), 1227 (C=S). Elemental analysis for $C_{20}H_{25}N_5O_2S$, calculated (%), C, 60.13; H, 6.31; N, 17.53, found (%), C, 60.29; H, 6.21; N, 17.68. ^{1}H NMR (DMSO-d_6, δ ppm): 2.65 (s, 4H, $2CH_2$), 2.98 (s, 4H, $2CH_2$), 3.13 (s, 2H, CH_2), 3.75 (s, 3H, OCH_3), 6.89 (d, 4H, arH $J = 7.0$ Hz), 7.16 (bs, 1H, arH), 7.33–7.41 (m, 4H, arH), 9.62 and 9.78 (bs, 3H, 3NH, exch. D_2O). ^{13}C NMR (DMSO-d_6, δ ppm): 32.62–41.14 (DMSO + CH_2), 50.48 (CH_2), 53.69 (CH_2), 55.96 (OCH_3), 56.63 (CH_2), 60.43 (CH_2), arC: [112.43 (CH), 118.62 (CH), 121.52 (2CH), 123.22 (CH), 124.43 (CH), 128.90 (2CH), 139.53 (C), 141.75 (C), 152.58 (C–OCH_3)], 171.39 (C=O), 181.14 (C=S). EI MS *m/z* (%): 120.81 (49), 135.89 (85), 149.90 (42), 150.78 (32), 161.92 (21), 163.98 (17), 189.95 (15), 204.96 (37), 254.96 (17), 279.18 (51), 339.18 (13), 384.29 (13), 385.29 (26), 423.40 ($[M + 1 + Na]^+$, 100), 424.40 ($[M + 2 + Na]^+$, 37).

3.1.17. 5-{[4-(2-Methoxyphenyl)piperazin-1-yl]methyl}-4-phenyl-4H-1,2,4-triazole-3-thiol (**18**)

A solution of compound **17** (10 mmol) in water was refluxed in the presence of 2 N NaOH (20 mmol) for 1 h; then, the resulting solution was cooled to room temperature and acidified to pH 4 with 37% HCl. The precipitate formed was filtered off, washed with water and recrystallized from ethanol to afford the desired compound. Yield 66%, m.p. 244–246 °C. FT-IR (ν_{max}, cm^{-1}): 3054 (ar–CH), 2829 (SH), 1593 (C=N). Elemental analysis for $C_{20}H_{23}N_5OS$, calculated (%), C, 62.97; H, 6.08; N, 18.36, found (%), C, 62.81; H, 6.28; N, 18.42. ^{1}H NMR (DMSO-d_6, δ ppm): 2.37 (s, 2H, CH_2), 2.77 (s, 4H, $2CH_2$), 3.40 (s, 4H, $2CH_2$), 3.73 (s, 3H, OCH_3), 6.80–6.93 (m, 4H, arH), 7.47 (bs, 5H, arH), 13.85 (s, 1H, SH, exch. D_2O). ^{13}C NMR (DMSO-d_6, δ ppm): 49.81 ($2CH_2$), 51.63 (CH_2), 52.18 ($2CH_2$), 55.25 (CH_3), arC: [111.85 (CH), 117.86 (CH), 120.74 (C), 122.39 (CH), 128.22 (2CH), 128.86 (2CH), 129.11 (CH), 134.00 (C), 140.98 (C), 149.23 (C)], 151.86 (triazole C-3), 168.14 (C=S).

3.1.18. {[4-(2-Methoxyphenyl)piperazin-1-yl]methyl}-1,3,4-oxadiazol-2-thiol (**19**)

The solution of KOH (10 mmol) in water was added to the solution of compound **16** in ethanol and the mixture was refluxed 7 h in the presence of CS_2 (0.19 mL, 10 mmol). Then, it was cooled to room temperature and acidified to pH 4 with 37% HCl. On cooling the mixture in cold overnight, a solid obtained. This was recrystallized from ethanol to give the target compound. Yield 22%, m.p. 125 °C. FT-IR (ν_{max}, cm^{-1}): 3050 (ar–CH), 2848 (SH), 1454 (C=N). Elemental analysis for $C_{14}H_{18}N_4O_2S$, calculated (%), C, 54.88; H, 5.92; N, 18.29, found (%), C, 54.71; H, 5.86; N, 18.40. ^{1}H NMR (DMSO-d_6, δ ppm): 2.63 (s, 4H, $2CH_2$), 2.95 (s, 4H, $2CH_2$), 3.75 (s, 5H, OCH_3 + CH_2), 6.86–6.91 (m, 4H, arH), 14.35 (bs, 1H, SH, exch. D_2O). 13C NMR (DMSO-*d*6, δ ppm): 50.62 (CH_2), 51.93 (CH_2), 52.92 ($2CH_2$), 55.91 (OCH_3), 55.98 (CH_2), arC: [112.39 (CH), 118.68 (CH), 121.44 (CH), 123.26 (CH), 141.59 (C=N), 152.63 (C–OCH_3)], 161.46 (triazole C), 178.73 (triazole C–S). EI MS *m/z* (%): 205.19 (46), 278.3 (13), 279.28 (30), 305.38 ($[M - 1]^+$, 100), 306.45 ($[M]^+$, 22).

*3.1.19. N′-[(2-Hydroxyphenyl)methylen]-2-[4-(2-methoxyphenyl) piperazin-1-yl]acetohydrazide (**20**)*

2-Hydroxybenzaldehyde (10 mmol) was added to the solution of compound **16** (10 mmol) in ethanol, and the mixture was refluxed in the presence of one drop of H_2SO_4 for 3 h. On cooling the mixture in cold overnight, a solid formed. This was recrystallized from ethanol to afford the desired compound. Yield 86%, m.p. 164–165 °C. FT-IR (ν_{max}, cm^{-1}): 3169 (OH and NH), 3058 (ar–CH), 1447 (C=N), 1239 (O–C). Elemental analysis for $C_{20}H_{24}N_4O_3$, calculated (%), C, 65.20; H, 6.57; N, 15.21, found (%), C, 65.40; H, 6.47; N, 15.36. ^{1}H NMR (DMSO-d_6, δ ppm): 2.81 (s, 4H, $2CH_2$), 3.06 (s, 4H, $2CH_2$), 3.77 (s, 5H, $OCH_3 + CH_2$), 6.90–7.49 (m, 6H, arH), 7.26 (bs, 1H, arH), 7.51 and 7.71 (s, 1H, arH, *cis*/*trans* conformers), 8.36 and 8.54 (1H, N=CH, *cis*/*trans* conformers), 10.15 and 11.10 (1H, brs, OH, *cis*/*trans* conformers exch. D_2O), 11.63 and 11.85 (1H, s, NH, *cis*/*trans* conformers, exch. D_2O). ^{13}C NMR (DMSO-d6, δ ppm): 47.66 (CH2), 49.36 (CH2), 52.80 (CH2), 55.56 (OCH3), 56.21 (CH2), 59.34 (CH2), arC: [112.08 (CH), 116.50 (CH), 118.24 (CH), 118.45 (CH), 119.72 (CH), 121.06 (CH), 122.99 (CH), 129.56 (CH), 139.88 (C), 140.90 (C), 148.11 (CH), 152.11 (C–OCH3), 157.29 (C–OH)], 166.98 (C=O).

*3.1.20. N′-[(3-Hydroxy-4-methoxyphenyl)methylen]-2-[4-(2-methoxyphenyl)piperazin-1-yl]acetohydrazide (**21**)*

3-Hydroxy-4-methoxybenzaldehyde (10 mmol) was added to the solution of compound **16** (10 mmol) in ethanol, and the mixture was refluxed in the presence of one drop of H_2SO_4 for 2 h. On cooling the mixture in cold overnight, a solid formed. This was recrystallized from dimethyl sulfoxide to afford the desired compound. Yield 82%, m.p. 114–116 °C. FT-IR (ν_{max}, cm^{-1}): 3387 (OH), 3194 (ar–CH and NH), 1687 (C=O), 1247 (O–C). Elemental analysis for $C_{21}H_{26}N_4O_4$, calculated (%), C, 63.30; H, 6.58; N, 14.06, found (%), C, 63.33; H, 6.41; N, 14.26. ^{1}H NMR (DMSO-d_6, δ ppm): 3.25 (s, 3H, OCH_3), 2.78 (bs, 4H, $2CH_2$), 3.19 (bs, 4H, $2CH_2$), 3.95 (s, 5H, $OCH_3 + CH_2$), 6.89–6.96 (m, 6H, arH), 7.17–7.21 (m, 1H, arH), 7.82 and 8.12 (s, 1H, N=CH, *cis*/*trans* conformers), 9.50 (1H, s, OH, exch. D_2O), 11.20 and 11.40 (1H, s, NH, *cis*/*trans* conformers, exch. D_2O). ^{13}C NMR could not been obtained due to the reason that the solubility of this compound in any NMR solvent. EI MS *m*/*z* (%): 149.9 (14), 189.95 (38), 204.96 (100), 206.03 (23), 399.31 ($[M + 1]^+$, 94), 400.01 ($[M + 2]^+$, 41).

*3.1.21. N′-[1H-Indol-3-ylmethylene]-2-[4-(2-methoxyphenyl) piperazin-1-yl]acetohydrazide (**22**)*

Indole-3-carbaldehyde (10 mmol) was added to the solution of compound **16** (10 mmol) in ethanol, and the mixture was refluxed in the presence of one drop of H_2SO_4 for 2 h. On cooling the mixture in cold overnight, a solid formed. This was recrystallized from ethanol to afford the desired compound. Yield 82%, m.p. 114–116 °C. FT-IR (ν_{max}, cm^{-1}): 3190 (ar–CH and NH), 1689 (C=O), 1242 (O–C). Elemental analysis for $C_{22}H_{25}N_5O_2$, calculated (%), C, 67.50; H, 6.44; N, 17.89, found (%), C, 67.56; H, 6.35; N, 17.92. ^{1}H NMR (DMSO-d_6, δ ppm): 2.77 (s, 4H, $2CH_2$), 3.05 (s, 4H, $2CH_2$), 3.77 (s, 5H, $OCH_3 + CH_2$), 6.92 (d, 3H, arH, $J = 8.2$ Hz), 7.18 (brs, 3H, arH), 7.42 (brs, 2H, arH), 7.83 (s, 1H, NH = CH), 8.19 and 8.43 (1H, N=CH, *cis*/*trans* conformers), 11.00 and 11.62 (1H, s, NH, *cis*/*trans* conformers, exch. D_2O), indol–NH was not observed. ^{13}C NMR (DMSO-d_6, δ ppm): 48.59 (CH_2), 50.01 (CH_2), 53.17 (CH_2), 53.56 (CH_2), 55.99 (OCH_3), 56.66 (CH_2), arC: [112.49 (CH), 118.70 (CH), 118.88 (CH), 121.17 (CH), 121.52 (CH), 122.58 (CH), 123.39 (CH), 123.88 (CH), 124.53 (C), 124.62 (CH), 137.59 (C), 140.61 (C), 141.49 (C), 152.54 (C–OCH_3)], 148.53 (CH), 169.99 (C=O). EI MS *m*/*z* (%): 190.07 (18), 205.15 (38), 392.42 ($[M + 1]^+$, 100), 393.43 ($[M + 2]^+$, 19).

*3.1.22. General method for the synthesis of compound **23**–**27***

The mixture of compound **3** (10 mmol) and compound **14** (for **26**), **16** (for **27**), **18** (for **25**), **19** (for **24**) or **21** (for **23**) (10 mmol) in dimethyl sulfoxide was stirred at 80 °C in the presence of sodium hydroxide (50 mmol) for 12 h (for **24**), 48 h (for **23**), 52 h (for **25**), 62 h (for **26**) or 73 h (for **27**). Then, the reaction mixture was allowed to reach room temperature and neutralized with dilute HCl. The solution poured into ice water. The white solid formed was recrystallized from acetone.

*3.1.22.1. 1-Ethyl-6-fluoro-7-[4-({2-methoxy-4-[({[4-(2-methoxyphenyl)piperazin-1-yl]acetyl}hydrazono)methyl]phenoxy} acetyl)piperazin-1-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**23**).* Yield 57%, m.p. 195–197 °C. FT-IR (ν_{max}, cm^{-1}): 3426 (OH), 3056 (ar–CH), 1725 and 1699 (4C=O). Elemental analysis for $C_{39}H_{44}FN_7O_8$, calculated (%), C, 61.81; H, 5.85; N, 12.94, found (%), C, 61.68; H, 5.92; N, 12.86. ^{1}H NMR (DMSO-d_6, δ ppm): 1.42 (t, 3H, CH_3 $J = 8.0$ Hz), 3.26–3.69 (m, 8H, $4CH_2 + H_2O$), 3.76–3.83 (m, 12H, $6CH_2$), 4.43–4.46 (m, 3H, OCH_3), 4.60 (q, 2H, CH_2 $J = 8.0$ Hz), 5.01 (s, 4H, $2CH_2$), 7.21 (bs, 3H, arH), 7.23 (bs, 2H, arH), 7.93 (d, 2H, arH $J = 6.0$ Hz), 7.97 (d, 1H, arH $J = 2.0$ Hz), 8.70 (s, 1H, arH), 8.96 (s, 1H, olefinic–CH), 8.90 and 9.40 (1H, s, NH, *cis*/*trans* conformers exch. D_2O), 15.33 (1H, bs, OH, exch. D_2O). ^{13}C NMR (DMSO-d_6, δ ppm): 15.02 (CH_3), 41.75 ($2CH_2$), 42.48 (CH_2), 44.41 (CH_2), 49.76 ($2CH_2$), 50.27 ($2CH_2$), 51.34 (CH_2), 57.20 ($2OCH_3$), 61.89 ($2CH_2$), arC: [106.92 (CH), 107.84 (C), 109.43 (C), 111.82 (CH), 112.06 (CH), 112.51 (CH), 112.74 (CH), 118.90 (2CH), 121.52 (2CH), 123.54 (C), 136.83 (C), 137.84 (C), 144.65 (C), 149.24 (CH), 151.90 (C), 152.27 (C), 153.32 (d, C, $J = 284.0$ Hz), 154.36 (C)], 149.71 (CH), 164.50, 166.05, 166.73 and 172.20 (4C=O). EI MS *m*/*z* (%): 758.32 ($[M + 1]^+$, 89), 701.24 (25), 570.22 (40), 473.34 (43), 381.29 (100).

*3.1.22.2. 1-Ethyl-6-fluoro-7-{4-[({5-[4-(2-methoxyphenyl)piperazin-1-yl]-1,3,4-oxadiazol-2-yl}thio)acetyl]piperazin-1-yl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**24**).* Yield 43%, m.p. 77–79 °C. FT-IR (ν_{max}, cm^{-1}): 3054 (ar–CH), 1720 and 1623 (C=O). Elemental analysis for $C_{31}H_{34}FN_7O_6S$, calculated (%), C, 57.13; H, 5.26; N, 15.04, found (%), C, 56.95; H, 5.38; N, 15.24. ^{1}H NMR (DMSO-d_6, δ ppm): 1.41 (s, 3H, CH_3), 3.38 (bs, 16H, $2CH_2$), 3.71–3.78 (m, 6H, $8CH_2$), 4.60 (bs, 3H, OCH_3), 6.90–7.19 (m, 4H, arH), 7.91 (bs, 2H, arH), 8.96 (bs, 1H, olefinic–CH), 15.32 (bs, 1H, OH, exch. D_2O). ^{13}C NMR (DMSO-d_6, δ ppm): 14.75 (CH_3), 33.91 (CH_2), 37.19 (CH_2), 41.77 (CH_2), 42.16 (CH_2), 45.78 (CH_2), 45.96 (CH_2), 49.75 ($2CH_2$), 50.15 (CH_2), 52.62 ($2CH_2$), 56.06 (OCH_3), arC: [106.77 (CH), 107.84 (C), 111.81 (CH), 112.61 (d, CH, $J = 67.0$ Hz), 118.97 (CH), 120.16 (C), 121.51 (2CH), 137.84 (2C), 149.22 (C), 152.25 (2CH), 152.60 (oxadiazole C-2), 153.48 (d, C, $J = 247.0$ Hz), 165.47 (oxadiazole C-5)], 166.71 and 176.84 (3C=O). EI MS *m*/*z* (%): 690.22 ($[M + K]^+$, 20), 666.24 (100), 413.26 (40), 381.29 (72), 344.61 (38).

*3.1.22.3. 1-Ethyl-6-fluoro-7-(4-{[(5-{[4-(2-methoxyphenyl)piperazin-1-yl]methyl}-4-phenyl-4H-1,2,4-triazol-3-yl)thio]acetyl}piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**25**).* Yield 68%, m.p. 70–71 °C. FT-IR (ν_{max}, cm^{-1}): 3423 (OH), 3056 (ar–CH), 1715 and 1626 (C=O). Elemental analysis for $C_{38}H_{41}FN_8O_5S$, calculated (%), C, 61.61; H, 5.58; N, 15.13, found (%), C, 61.49; H, 5.66; N, 14.89. ^{1}H NMR (DMSO-d_6, δ ppm): 1.42 (t, 3H, CH_3, $J = 8.0$ Hz), 2.79 (bs, 4H, $2CH_2$), 3.40–3.55 (m, 8H, $4CH_2 + H_2O$), 3.67–3.78 (m, 7H, $OCH_3 + 2CH_2$), 4.35 (s, 2H, CH_2), 4.60 (q, 2H, CH_2, $J = 8.0$ Hz), 6.80–6.93 (m, 4H, arH), 7.20 (d, 1H, arH, $J = 8.0$ Hz), 7.52–7.58 (m, 5H, arH), 7.96 (d, 1H, arH, $J = 6.0$ Hz), 8.97 (s, 1H, olefinic–CH), 15.33 (s, 1H, OH, exch. D_2O). ^{13}C NMR (DMSO-d_6, δ ppm): 14.21 (CH_3), 36.01 ($2CH_2$), 41.28 ($2CH_2$), 45.11 ($2CH_2$), 49.05 ($2CH_2$), 49.47 (CH_2), 52.25 ($2CH_2$), 55.27 (OCH_3), arC: [106.14 (CH), 107.10 (CH), 111.36 (d, CH, $J = 23.0$ Hz), 117.88 (2CH), 119.14 (CH), 119.52 (C), 120.74 (CH), 122.45 (CH), 127.19 (2CH), 129.73 (CH), 137.13 (C), 145.12 (C), 148.58 (CH), 151.52 (2C), 152.76 (d, C, $J = 248.0$ Hz), 151.88 (C and triazole C-3)], 154.00 (C), 165.43 (C=O), 166.04 (C=O and triazole C-5), 176.16

(C=O). EI MS *m/z* (%): 765.27 ([M + K]$^+$, 28), 741.29 (100), 413.26 (28), 382.29 (63).

*3.1.22.4. 1-Ethyl-6-fluoro-7-[4-(N-{3-fluoro-4-[4-(2-methoxyphenyl) piperazin-1-yl]phenyl}glycyl)piperazin-1-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**26**).* Yield 33%, m.p. 177–178 °C. FT-IR (ν_{max}, cm^{-1}): 3100 (NH), 3057 (ar–CH), 1720 and 1623 (C=O). Elemental analysis for $C_{35}H_{39}FN_6O_5$, calculated (%), C, 65.41; H, 6.12; N, 13.08, found (%), C, 65.56; H, 6.32; N, 12.98. ^{1}H NMR (DMSO-d_6) δ ppm: 1.42 (bs, 3H, CH_3), 3.47 (bs, 8H, $4CH_2$), 3.79 (bs, 8H, $4CH_2$), 3.97 (s, 3H, OCH_3), 4.60 (bs, 2H, CH_2), 5.01 (bs, 2H, CH_2), 6.99 (bs, 3H, arH), 7.08 (bs, 2H, arH), 7.20 (bs, 2H, arH), 7.82 (bs, 1H, arH), 7.92 (bs, 1H, arH), 8.69 (s, 1H, NH, exch. D_2O), 8.96 (s, 1H, olefinic–CH), 15.33 (bs, 1H, OH, exch. D_2O). ^{13}C NMR (DMSO-d_6) δ ppm: 15.02 (CH_3), 43.17 ($2CH_2$), 44.40 ($2CH_2$), 49.76 ($2CH_2$), 50.98 ($2CH_2$), 56.09 (OCH_3), 61.89 ($2CH_2$), arC: [106.92 (2CH), 107.84 (C), 111.83 (CH), 112.94 (d, CH, J = 22.0 Hz), 112.75 (2CH), 118.87 (CH), 121.55 (2CH), 136.84 (C), 137.84 (C), 144.64 (C), 145.77 (C), 149.26 (CH), 152.27 (C), 153.33 (d, C, J = 282.0 Hz), 154.38 (C), 164.51 (C), 166.05 (C)], 166.77, 172.18 and 176.86 (3C=O).

*3.1.22.5. 1-Ethyl-6-fluoro-7-{4-[(2-{[4-(2-methoxyphenyl)piperazin-1-yl]acetyl}hydrazino)acetyl]piperazin-1-yl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**27**).* Yield 49%, m.p. 192–195 °C. FT-IR (ν_{max}, cm^{-1}): 3410 (OH), 3058 (ar–CH), 1707 (C=O). Elemental analysis for $C_{31}H_{38}FN_7O_6$, calculated (%), C, 59.70; H, 6.14; N, 15.72, found (%), C, 59.56; H, 5.94; N, 15.92. ^{1}H NMR (DMSO-d_6, δ ppm): 1.42 (s, 3H, CH_3), 3.14–3.50 (m, 8H, $4CH_2 + H_2O$), 3.68–3.96 (m, 15H, $OCH_3 + 6CH_2$), 4.58 (s, 2H, CH_2), 6.88–7.18 (m, 4H, arH), 7.85–7.91 (m, 2H, arH), 8.52 (s, 1H, NH, exch. D_2O), 8.94 (s, 1H, olefinic–CH), 11.57 (s, 1H, NH, exch. D_2O), 15.30 (bs, 1H, OH, exch. D_2O), NH was not observed. ^{13}C NMR (DMSO-d_6, δ ppm): 14.04 (CH_3), 40.85 ($2CH_2$), 49.05 ($2CH_2$), 49.45 ($2CH_2$), 52.14 (CH_2), 53.25 (CH_2), 55.40 (OCH_3), 57.93 (CH_2), 59.71 ($2CH_2$), arC: [105.94 (CH), 107.07 (C), 111.18 (d, CH, J = 23.0 Hz), 111.91 (CH), 111.95 (CH), 118.23 (CH), 119.46 (C), 120.79 (CH), 137.07 (2C), 145.08 (C), 148.50 (CH), 151.83 (C), 152.72 (d, C, J = 248.0 Hz)], 166.01 (C=O), 167.18 (C=O), 172.20 (C), 176.06 (C=O). EI MS *m/z* (%): 623.27 ([M]$^+$, 26), 503.20 (30), 381.29 (100), 353.26 (16).

3.2. Biological activity

3.2.1. 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*) ATCC 911, *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC 43288, *Enterococcus faecalis* (*E. faecalis*) ATCC 29212, *Staphylococcus aureus* (*S. aureus*) ATCC 25923, *Bacillus cereus* (*B. cereus*) 709 Roma, *Mycobacterium smegmatis* (*M. smegmatis*) ATCC607, *Candida albicans* (*C. albicans*) ATCC 60193 and *Saccharomyces cerevisiae* (*S. cerevisiae*) RSKK 251, Ar: *Arthrobacter oxydans* (laboratory strain), Ct: *Candida tropicalis*, ATCC 13803, Pv: *Proteus vulgaris* ATCC 13315, Ac: *Acinetobacter* sp. (laboratory strain), except *Serretia marcescens* (Sm), *Acinetobacter* sp. (Ac) and *Klebsiella oxitoka* (Ko) which are laboratory strains. 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 (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 microdilution 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 [62]. Ampicillin (10 μg) and fluconazole (5 μg) were used as standard antibacterial and antifungal drugs, respectively. Dimethylsulphoxide with dilution of 1:10 was used as solvent control. Only positive results were presented in Table 1.

3.2.2. Urease inhibition assay

Reaction mixtures comprising 25 μL of Jack Bean Urease, 55 μL of buffer (100 mM urea, 0.01 M K2HPO4, 1 mM EDTA and 0.01 M LiCl, pH 8.2) and 100 mM urea were incubated with 5 μL of the test compounds at room temperature for 15 min in microtiter plates. The production of ammonia was measured by indophenol method and used to determine the urease inhibitory activity. The phenol reagent (45 μL, 1% w/v phenol and 0.005% w/v sodium nitroprusside) and alkali reagent (70 μL, 0.5% w/v sodium hydroxide and 0.1% v/v NaOCl) were added to each well and the increasing absorbance at 625 nm was measured after 20 min, using a microplate reader (Molecular Device, USA). The percentage inhibition was calculated from the formula 100 − ($OD_{testwell}/OD_{control}$) × 100. Thiourea was used as the standard inhibitor. In order to calculate IC_{50} values, different concentrations of synthesized compounds and standard were assayed at the same reaction conditions [63].

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.06.045.

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