Bioorganic Chemistry 85 (2019) 308–318

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Piperazine-azole-fluoroquinolone hybrids: Conventional and microwave irradiated synthesis, biological activity screening and molecular docking studies

Arif Mermer[a], Ozlem Faiz[b], Ahmet Demirbas[a], Neslihan Demirbas[a,*], Manikandan Alagumuthu[c], Sivakumar Arumugam[c]

[a] Karadeniz Technical University, Department of Chemistry 61080 Trabzon, Turkey
[b] Recep Tayyip Erdogan University, Department of Chemistry, 53100 Rize, Turkey
[c] Dept. of Biotechnology, School of Bio-Sciences and Technology, VIT, Vellore 632014, India

ARTICLE INFO

Keywords:
Fluoroquinolone
1,2,4-triazole
Microwave irradiation
Antimicrobial activity
DNA gyrase
Topoisomerase IV
Molecular docking

ABSTRACT

A series of new 1,2,4-triazole and 1,3,4-oxadiazole derivatives was obtained via several steps sequential reactions of phenyl piperazine. Then, these compounds were converted to the corresponding fluoroquinolone hybrids via one pot three component Mannich reaction. All the reactions were examined under conventional and microwave mediated conditions, and optimum conditions were determined. The effect of different solvents and microwave power on microwave prompted reactions was investigated as well. All the newly synthesized compounds were characterized by FTIR, ^{1}H NMR, ^{13}C NMR and EI MS spectral techniques. The antimicrobial activity, DNA gyrase and Topoisomerase IV inhibition potentials were performed. The results obtained showed that fluoroquinolone hybrids possess good antimicrobial activity. Moreover, Fluoroquinolone-azole-piperazine hybrids synthesized in the present study displayed excellent DNA gyrase inhibition. To unveil the interaction mode of compounds to receptor, a molecular docking study was performed. With an average least binding energy of −9.5 kcal/mol, all compounds were found to have remarkable inhibitory potentials against DNA gyrase (*E. coli*).

1. Introduction

The increasing multidrug resistance among bacteria, viruses and fungi to currently used drugs has emerged an alarming and reemerging microbial threat and has become a major public health concern worldwide with nearly 15 million deaths every year. Several pathogens which appeared to be under control have once again been fatal since they have developed a variety of strategies to enhance their survival skills in the presence of antimicrobial agents. Estimates suggested that unless significant progress has been made, drug resistant infections may result in ten million deaths annually by 2050. In order to manage drug resistance, the development of novel chemotypes which act upon novel molecular targets, has emerged as an urgent and crucial requirement. However, there have only been prepared a few molecules with new mode of action to medical use. More recently, the molecular hybridization concept based on the combination of structural units of two or more drug fragments through the fusion in one molecular framework with improved properties has been adopted as a new and attractive strategy [1–11]. The key advantage of hybrid molecules consisting of several pharmacophore groups each with different mode of action is to inhibit bacterial targets via unique binding sites or via novel modes of action or to exhibit dual mode of action [9,12–19]. Moreover, molecular hybrids can display better pharmacokinetic profile, therapeutic index and more importantly low tendency to resistance. Quinolones have been the centre of considerable scientific and clinical interest since their discovery in the early 1960s. This is because they potentially offer many of the attributes of an ideal antibiotic, combining high potency, a broad spectrum of activity, good bioavailability, oral and intravenous formulations, high serum levels, a large volume of distribution indicating concentration in tissues and a potentially low incidence of side-effects. Much research has attempted to make these potential attributes real. The first quinolone nalidixic acid was developed, but it took more than a decade before additional compounds, such as flumequin, norfloxacin and enoxacin became available for clinical use. The main use for all these agents was the treatment of urinary tract infection [20]. Since the discovery of norfloxacin (NFLX) by Koga et al. in the early 1980s, quinolones have become one of the most attractive agents in the chemotherapy of both community-acquired and serious hospital-acquired infections [20–22].

* Corresponding author.
E-mail address: neslihan@ktu.edu.tr (N. Demirbas).

https://doi.org/10.1016/j.bioorg.2019.01.009
Received 6 August 2018; Received in revised form 1 January 2019; Accepted 6 January 2019
Available online 08 January 2019
0045-2068/

These drugs perform their activity by binding to two type II bacterial topoisomerase enzymes, DNA gyrase and topoisomerase IV. This causes to permanent double stranded DNA breaks, and results in cell death [23]. However, the rapidly increasing level of resistance towards quinolone class antibacterial drugs has limited their therapeutic efficacy and served the scientists to design either novel structural classes of inhibitors targeting gyrase and topoisomerase IV; or to discover new quinolone hybrids [24,25]. During the past 30 years, hundreds of quinolone derivatives have been discovered, and a large body of structure–activity relationship (SAR) has been accumulated. The activity of quinolone class antibacterial agents arises from bicyclic heterocyclic ring and also the structure of peripheral substituents and their spatial relationship. These groups can provide additional affinity towards bacterial enzymes, enhance the cell penetration or alter pharmacokinetic properties [26]. The in silico studies on quinolone class antibiotics have showed that the basic group at C-7 position is the most suitable site for chemical modifications. it constitutes an area with a great influence on potency, spectrum and safety and most of studies aiming the discovery of new quinolone hybrids have been focused on the functionalization at C-7 in quinolone skeleton [23,27–29]. In general, 5- and 6-membered *N*-containing rings including piperazinyl, pyrrolidinyl and piperidinyl groups have been proven as optimal substituents [23,30].

N-Containing heterocycles which have been regarded as important tools medicinally, have given a new aspect to new drug development studies. The therapeutic efficacy of 1,2,4-triazole and 1,3,4-oxadiazole derivatives have been well studied in the several pathological conditions including inflammation, cancer, pain, tuberculosis or hypertension [31–34]. The compounds including these units have been reported to have several biological activities such as antimicrobial, anticancer, antioxidant, analgesic, and antihypertensive [35–37]. Microwave irradiated syntheses have been proven as an effective technique in terms of environment, reaction time, high yields, ease of work-up and isolation of products [38]. Moreover, with the emergence of 'green chemistry concept', the scientists have shifted their focus to solvent-free methods which provide facile reactions conditions with high yields of pure products and eliminate or minimize the use of organic solvents which are often expensive, toxic and difficult to remove [39–41]. In light of these aforementioned statements, we have focused on the design, eco-friendly synthesis and antibacterial evaluation of new piperazine-azole-fluoroquinolone conjugates (Fig. 1). Phenyl piperazine scaffold has been selected as the key prototype structural unit and the combination of phenyl piperazine skeleton with azole and fluoroquinolone units has been achieved by both the conventional and microwave irradiated techniques.

2. Results and discussion

2.1. Chemistry

The designed fluoroquinolone-triazole hybrid compounds **7a, b** and **8a–j** have been prepared according to the synthetic procedure presented in Scheme 1. In order to development more efficient synthetic procedures for these reactions, microwave (MW) irradiation has also been applied in addition to conventional methods. The compounds **2** and **3** have been synthesized following the procedure reported earlier [42,43]. Initially, the carbo(thio)amide derivatives (**5a–e**) have been obtained from the nucleophilic attack of hydrazide-NH_2 to carbon atom of iso(thio)cyanate function. The evidence for the formation of compounds **5a–e** have been provided by FT IR, ^{1}H NMR, ^{13}C NMR and EI-MS. For the synthesis of **6a–e**, two methods have been used including conventional heating under reflux conditions (Method 1) or microwave mediated conditions (Method 2). The latter method has provided more efficient procedure with much shorter reaction times varying between 15 and 20 min, whereas 9–15 h have been needed for conventional method (Table 1). Moreover, MW irradiation has promoted the reaction yields from 77–84% to 84–90%. In method 2, higher microwave energy than 150 W has caused to a decrease in yields of compounds **6a–e**.

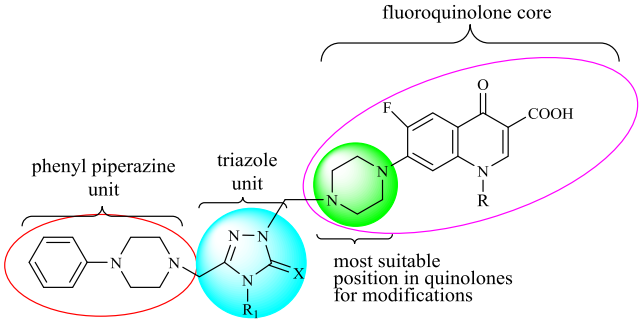


Fig. 1. General representation of the target compounds.

Our previous studies have showed that 1,2,4-triazoles containing a C═S (SH) group rather prefer the mercapto form, while the derivatives including C═O (OH) function are almost present in keto form [44–48]. The treatment of hydrazide (**3**) with carbon disulfide in the presence of KOH has yielded the corresponding 1,3,4-oxadiazole (**4**) under conventional heating and microwave mediated conditions. MW irradiation has decreased the reaction time from 10 h to 15 min and increased the reaction yield from 84% to 92%. The screening of the reaction condition has showed that the presence of catalysts has no significant impact on the yield of the desired compound. The best result has been obtained in DMF at 50 W maximum power without any catalyst (Table 2, Entry 5) at a yield of 93%. The remaining hybrid compounds, **7b, 8b–j** have been obtained through applying the optimized conditions described above (Table 3). The structures of compounds **7a,b**; **8a–j** has been identified on the basis of FT IR, ^{1}H NMR, ^{13}C NMR and mass spectrometric data.

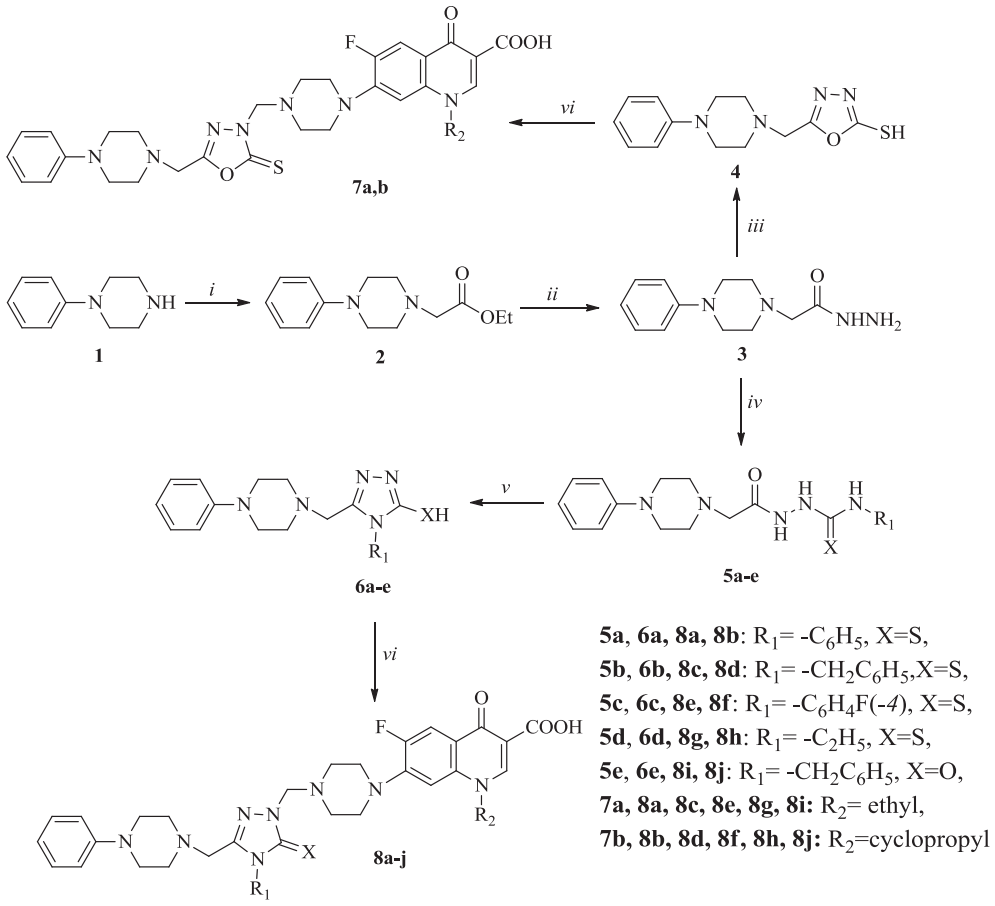
2.2. Biological activity

2.2.1. Antimicrobial activity

Antimicrobial activity of all compounds obtained were presented in Table 4 (Only positive results were presented). Among these, carbo(thio)amide and triazole derivatives did not show any activity against the test microorganism. The fluoroquinolone derivatives exhibited good, moderate and excellent activity almost all microorganism with the MIC values between 0.125 and 65 μg/mL. Compare with the standard drug Ciprofloxacin, these compounds showed good-moderate activity except for **7b**, **8b** and **8h** which showed the same MIC values for *K. pneumoniae* and only **8h** showed 0.5 μg/mL MIC value against *A. haemolyticus*. **7b** and **8b** showed excellent activity against *S. aureus* against ampicillin used as a standard drug for gram positive microorganisms. At the same time, **7b**, **8b**, **8h**, **8j** exhibited better activity against *E. faecalis* than the standard drug with the MIC value 8 μg/mL. On the other hand, Gentamicin was used as standard drug for Gram-negative microorganism. For *E. coli*, all compounds showed excellent activity against Gentamicin, also **7b**, **8b** and **8h** were found to have excellent activity for *K. pneumoniae* with the MIC value 0.25 μg/mL. On the other hand, excellent activities with the MIC values varying 0.5–2 μg/mL were observed for compounds **7b**, **8b** and **8h** for *A. haemolyticus* and *P. aeruginosa*. **7b**, **8b** and **8h** showed the best antimicrobial activity against test microorganisms when the results were evaluated collectively.

2.2.2. Results of DNA gyrase and Topo-IV inhibition studies

DNA gyrase and Topoisomerase IV are type-II topoisomerases enzymes, involving in the negative supercoiling which is a vital part of bacterial survival. Thus, we have assessed the inhibition potentials of present study compounds against these two enzymes. The results of DNA gyrase and Topo-IV inhibition study is depicted in Table 5. As per our observations, the activity has been totally a dose dependent



Scheme 1. Synthetic pathway for the preparation of new compounds. i: $BrCH_2COOEt$, TEA, THF; ii: $NH_2NH_2{\cdot}H_2O$, EtOH; iii: CS_2, KOH(aq), EtOH; iv: R_1NCX; v: NaOH(aq), EtOH; vi: HCHO, DMF, Ciprofloxacin or Norfloxacin.

Table 1
Time, power, yield data and melting point for compounds **4** and **5a–e**.

Compound	Microwave irradiation method			Conventional method		Melting point (°C)
	Time (min)	Power (W)	Yield (%)	Time (h)	Yield (%)	
4	15	150	92	10	84	201–203
5a	15	150	89	10	81	231–232
5b	15	150	86	13	77	207–208
5c	15	150	85	11	80	203–204
5d	15	150	90	9	84	223–224
5e	20	150	84	15	78	202–203

Table 2
Optimization of the model reaction conditions for compound **8a**.

Entry	Time (min)	Power (W)	Yield (%)	Solvent	Catalyst (10%)
1	20	50	86	THF	–
2	25	70	84	THF	–
3	20	50	82	EtOH	–
4	25	70	80	EtOH	–
5	**20**	**50**	**93**	**DMF**	–
6	25	70	89	DMF	–
7	20	50	93	–	*p*-TSA
8	20	50	93	–	$FeCl_3$
9	20	50	91	–	HCl
10	20	50	92	–	$InCl_3$

Table 3
The results of reaction yield and time for the synthesized compounds **7a,b** and **8a–j** in Microwave Irradiation and Conventional Method.

Compound	Microwave irradiation method		Conventional method	
	Time (min)	Yield (%)	Time (h)	Yield (%)
7a	20	91	24	86
7b	20	93	24	86
8a	20	93	24	86
8b	20	89	24	82
8c	20	91	24	85
8d	20	94	24	88
8e	20	96	24	86
8f	20	90	24	81
8g	20	95	24	89
8h	20	92	24	82
8i	20	89	24	79
8j	20	88	24	81

(Fig. 2a). Compounds **5a** to **6e** have been found totally inactive for the activity. On the other hand, fluoroquinolone-azole-piperazine hybrids have showed excellent activity with lowest IC_{50} values. Remarkably, compounds **8c**, **8f**, **8i**, **8j**, **7a** and **7b** were the excellent group compounds for DNA gyrase inhibition (Fig. 2b). At this point we have recognized the consistence between the molecular docking and the enzyme inhibition study in which the result has favored DNA gyrase. Meanwhile, the low selective TOPO-IV inhibition activity was also recognized from the obtained IC_{50} values. Among all compounds, **8j** was exhibited most potent activity with a calculated relative % activity of 85% and IC_{50} of 0.75 μM. A maximum activity was found at the

Table 4
Screening for the activity of newly synthesized compounds **7a,b** and **8a–j**.

Compound	Microorganisms and minimal inhibition concentration (μg/mL)					
	Gram-positive microorganism		Gram-negative microorganism			
	Sa	Ef	Ec	Pa	Kp	Ah
7a	16	32	0.5	8	4	16
7b	2	8	0.125	2	0.25	1
8a	16	64	2	16	4	–
8b	2	8	0.125	2	0.25	1
8c	16	32	0.5	16	2	–
8d	8	16	0.125	4	0.5	4
8e	16	32	1	16	2	16
8f	8	16	0.125	4	0.5	2
8g	16	32	0.5	8	1	8
8h	4	8	0.125	2	0.25	0.5
8i	16	32	0.5	16	2	16
8j	8	8	0.125	4	2	4
Cip.	1	0.5	0.03	0.25	0.25	0.25
Amp.	1.56	12.5	–	–	–	–
Gen.	–	–	0.78	1.56	0.39	0.78

Sa: *Staphylococcus aureus* ATCC 25923, Ef: *Enterococcus faecalis* ATCC 29212, Ec: *Escherichia coli* ATCC 25922, Pa: *Pseudomonas aeruginosa* ATCC 27853, Kp: *Klebsiella pneumoniae* ATCC 13883, Ah: *Acinetobacter haemolyticus* ATCC 19002. Cip.: Ciprofloxacin, Amp.: Amphicilin, Gen.: Gentamicin.

Table 5
Results of DNA gyrase and DNA Topo-IV inhibition studies.

Comp. no.	IC_{50} (μM) DNA gyrase	IC_{50} (μM) DNA Topo-IV
4	10.12 ± 0.14	>25.00
5a	>25.00	>25.00
5b	>25.00	>25.00
5c	>25.00	>25.00
5d	>25.00	>25.00
5e	>25.00	>25.00
6a	>25.00	>25.00
6b	>25.00	>25.00
6c	>25.00	>25.00
6d	>25.00	>25.00
6e	>25.00	>25.00
7a	2.25 ± 0.28	15.24 ± 1.05
7b	3.25 ± 0.34	12.48 ± 1.25
8a	5.24 ± 0.88	>25.00
8b	4.88 ± 0.76	>25.00
8c	1.25 ± 0.22	14.36 ± 1.45
8d	2.05 ± 0.38	15.78 ± 1.57
8e	1.84 ± 0.15	>25.00
8f	1.00 ± 0.18	>25.00
8g	0.88 ± 0.18	16.46 ± 1.74
8h	1.50 ± 0.12	18.67 ± 1.68
8i	0.95 ± 0.08	6.78 ± 1.08
8j	0.75 ± 0.07	8.24 ± 1.64

10^{-6} canceration, which indicates the necessity of low amount of target candidate drug to have a maximum activity. The activity variation and druggability suggestions against the present study compound was assessed by comparing the DNA gyrase and Topo-IV inhibition study results of the compounds with the standard drug, Novobiocin's (Novo) results. Novo exhibited a significant DNA gyrase as well as TOPO-IV activities. A relative % activity of 82.52% and an IC_{50} value of 1.0 μM was showed by Novo against DNA gyrase inhibition. In Fig. 2a and b, the results of Novo and potentially identified compound **8j** were compared. In the dose response curve, even though the curve elevation started equally and slopped at same concentrations, a slight variation could be seen between **8j** and Novo. This activity equality or slightly elevated activity of **8j** indicate its druggability potentials as a future DNA gyrase inhibitor.

2.2.3. Results of molecular docking studies

From the molecular docking study results, the attained free energy value reveals that the compounds **8a–j** having a fortunate selectivity towards DNA gyrase over TOPO-IV (ParC, PDB ID: 1ZVU) (Fig. 3). Based on the molecular mechanistic values, we have categorized the compounds as most active, intermediates and inactive groups. Mostly, the first category was used to evaluate *in vitro* experiments. The most significant binding affinity of the docked compounds was recognized through the obtained inhibitory constant (*ki*). Compounds **7a**,**b** and **8a–j** fortunately have established with a significant binding affinity (*ki* values < 1 ± 0.5 μM). The average and sum of all docking molecular mechanics values have given a clear-cut idea about the activity selectivity of compounds towards the DNA gyrase inhibition (average least binding energy value of −11.214 kcal/mol). Meanwhile, TOPO-IV activity average was also reliable (−8.24 kcal/mol). While discussing about the ligand efficiency, with a significant average value of −0.32, fluoroquinolone-1,2,4-triazole hybrids were found to have efficiency towards 2XCT (DNA gyrase). The average inhibitory constant (*ki*) indicates the possible bioactivity potentials of the compounds in low molecular concentrations towards both DNA gyrase (0.0035 μM) as well as the TOPO-IV (0.5 μM).

2.2.4. Molecular assessments of Receptor-Ligand interactions of DNA gyrase and TOPO-IV and compounds *7a, 7b* and *8a–j*

To unveil the mechanism of action and molecular interaction by means of hydrogen bonding and non-covalent bonding (π-π interaction and π-cation interactions), the established best docked poses have been analyzed. Various drug efficacy factors have been contributing in these types' communications/interactions between drug and receptor. These factors are mainly controlling the strength, duration, and different types of the drug-receptor interaction. In fact, these factors are decisively dictating the strength of the interaction established by a drug (by constituting the complex) against its receptor. This has been known as the affinity. Therefore, the binding affinity is considered as one of the

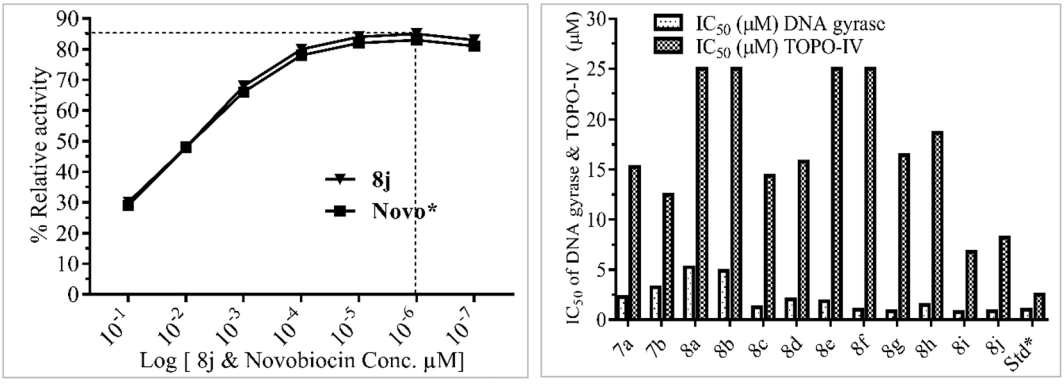


Fig. 2. a. Dose response curve of **8j** against DNA gyrase inhibition. b. DNA gyrase and TOPO-IV inhibition potential of compounds **7a**, **7b** and **8a–j**. *Novobiocin.

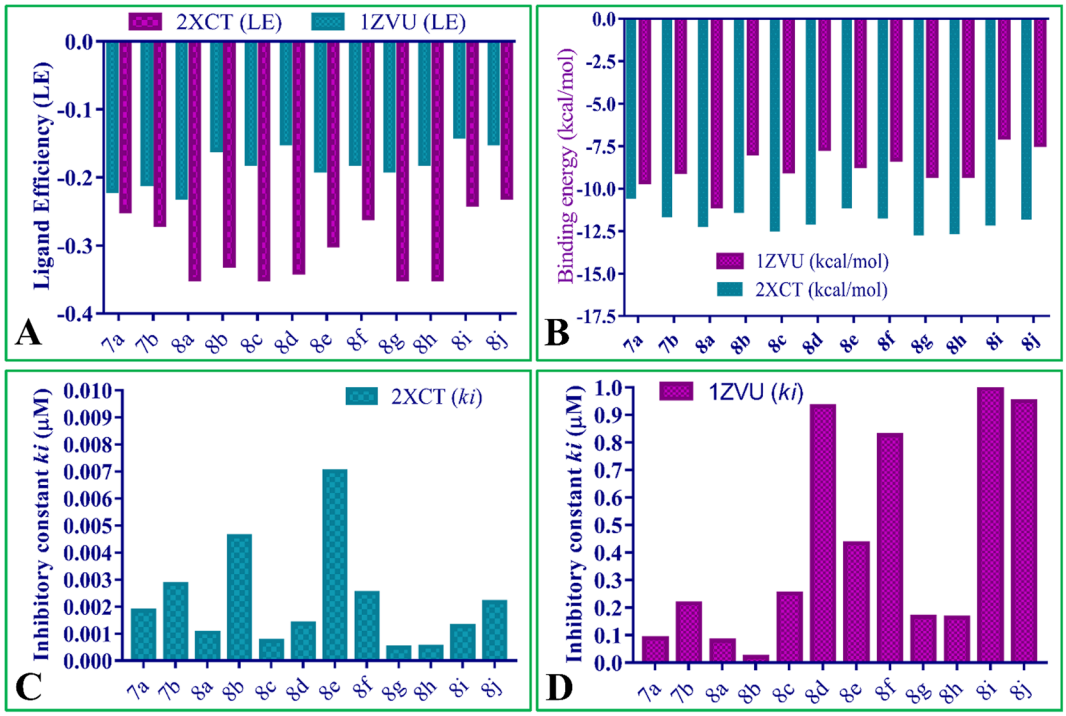


Fig. 3. Results of molecular docking studies. 3A. Ligand efficiency values; 3B. Binding energy values; 3C. Obtained inhibitory constant (*ki*) value for PDB ID: 2XCT; 3D. Obtained inhibitory constant (*ki*) value for PDB ID: 1ZVU.

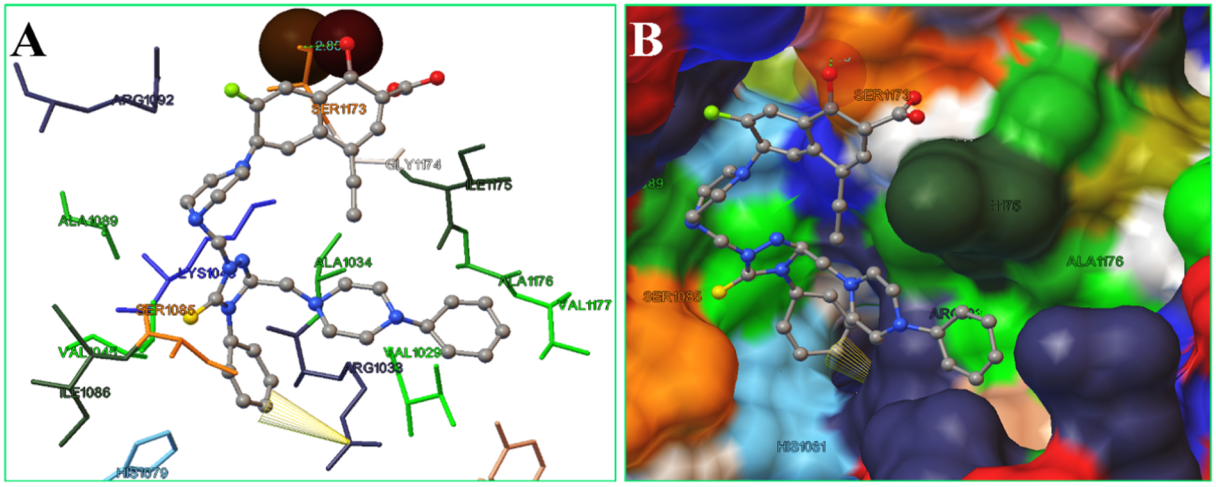


Fig. 4. A. Interaction of compound **8a** to DNA Gyrase (PDB: 2XCT). B. Interaction of compound **8a** to DNA Gyrase (PDB: 2XCT) binding pocket.

chief in silico measurement to adjudge on new chemical entity's drug efficacy.

Various intermolecular forces are involved in the interaction establishment between drug and binding sites such as Van der Waals forces (weak bonds and transient reversible effects), Hydrogen bonds (intermediate bonds and transient reversible effects), Covalent bonds (strong bonds and long-lasting or irreversible effects) and non-covalent bonding (electromagnetic interactions among molecules). Fig. 4a and b has illustrated the interaction of top scored compounds to DNA gyrase (PDB ID: 2XCT) and TOPO-IV (PDB ID: 1ZVU). Ser1173 was the main amino acid residue to establish hydrogen bond and Arg1033to establish the non-covalent cation-π interaction DNA gyrase (PDB ID: 2XCT). Docking results were also suggested to evaluate further possible *in vitro* and *in vivo* analysis to unveil the drug ability of the present study compounds.

3. Experimental

3.1. General

All the chemicals were purchased from Fluka Chemie AG Buchs and Sigma Aldrich 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 aluminium sheets. The mobile phase was ethyl acetate:diethyl ether (1:1), and detection was made using UV light. FT-IR spectra were recorded using a Perkin Elmer 1600 series FTIR spectrometer. ^{1}H NMR and ^{13}C NMR spectra were registered in DMSO-d_6 on a BRUKER AVENE II 400 MHz NMR spectrometer (400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C). The chemical shifts are given in ppm relative to Me_4Si as an internal reference, *J* values are given in Hz. The mass spectra were

obtained on a Quattro LC-MS (70 eV) Instrument. Microwave irradiated reactions were performed in a CEM Discovery monomod synthesis reactor.

*3.1.1. 5-[(4-Phenylpiperazin-1-yl)methyl]-1,3,4-oxadiazole-2-thiol **(4)***

Method 1. Compound **3** (10 mmol) and CS_2 (15 mmol) were added to a solution of KOH (10 mmol) in 10 mL H_2O and 10 mL ethanol and the reaction mixture was refluxed for 10 h. Then, the reaction content was acidified with conc. HCl to pH 6. The precipitate formed was filtered off, washed with H_2O and recrystallized from ethanol to afford the desired compound. Yield: 84%.

Method 2. The solution of compound **3** (10 mmol), CS_2 (15 mmol) and KOH (10 mmol) in H_2O + ethanol was irradiated in monomod microwave reactor in closed vessel with the pressure control at 100 °C, 150 W maximum power for 15 min (hold time). After the completion of the reaction, (monitored by TLC), the mixture was acidified with conc. HCl to pH 6. The precipitate formed was filtered off, washed with H_2O and recrystallized from ethanol to afford the desired compound. Yield: 92%.

FT-IR (υ_{max}, cm^{-1}): 3011 (*ar*—CH), 2855 (—SH), 1602 (C═N). ^{1}H NMR (DMSO-d_6, δ ppm): 2.65 (d, 4H, J = 4.0 Hz, $2CH_2$), 3.15 (s, 4H, $2CH_2$), 3.74 (s, 2H, CH_2), 6.79 (t, 1H, J = 12.0 Hz, arH), 6.93 (d, 2H, J = 8.0 Hz, arH), 7.21 (t, 2H, J = 12.0 Hz, arH), 14.42 (s, 1H, SH). ^{13}C NMR (DMSO-d_6, δ ppm): 48.52 ($2CH_2$), 51.56 (CH_2), 52.42 ($2CH_2$), arC: [116.00 (2CH), 119.45 (CH), 129.37 (2CH), 151.30 (C)], 161.09 (oxadiazole C-5), 178.49 (oxadiazole C-2). EI MS *m/z* (%): 106.04 (44), 163.08 (33), 175.16 (49), 277.23 ($[M+1]^+$, 100), 315.22 ($[M+K]^+$, 40).

*3.1.2. General method for the synthesis of compounds **5a–e***

A mixture of compound **3** (10 mmol) and the corresponding iso (thio)cyanate (15 mmol) in dichloromethane was stirred at room temperature for 24 h (monitored by TLC). After evaporating the solvent under reduced pressure, a solid obtained. The crude product was purified by crystallization from appropriate solvents.

*3.1.2.1. N-Phenyl-2-[(4-phenylpiperazin-1-yl)acetyl] hydrazincarbothioamide **(5a)***. Recrystallized from acetone. Yield: 95%, Mp. 184–185 °C. FT-IR (υ_{max}, cm^{-1}): 3306, 3273 ve 3210 (3NH), 3112 (*ar*—CH), 1665 (C═O), 1235 (C═S). ^{1}H NMR (DMSO-d_6, δ ppm): 2.69 (s, 4H, $2CH_2$), 3.18 (s, 6H, $3CH_2$), 6.78 (t, 1H, J = 16.0 Hz, arH), 7.17–7.24 (m, 3H, arH), 7.35 (t, 2H, J = 16.0 Hz, arH), 7.45 (d, 2H, J = 5.0 Hz, arH), 9.59 (bs, 2H, 2NH), 9.92 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 46.71 ($2CH_2$), 52.71 ($2CH_2$), 59.95 (CH_2), arC: [113.11 (2CH), 115.15 (CH), 124.19 (2CH), 126.19 (3CH), 129.83 (2CH), 135.90 (C), 137.31 (C)], 155.15 (C═O), 181.69 (C═S). EI MS *m/z* (%): 173.27 (96), 208.31 (52), 220.26 (49), 294.40 (49), 369.44 ($[M]^+$, 100).

*3.1.2.2. N-Benzyl-2-[(4-phenylpiperazin-1-yl)acetyl] hydrazincarbothioamide **(5b)***. Recrystallized from acetone. Yield: 90%, Mp. 193–194 °C. FT-IR (υ_{max}, cm^{-1}): 3204 (3NH), 3052 (*ar*—CH), 1671 (C═O), 1233 (C═S). ^{1}H NMR (DMSO-d_6, δ ppm): 2.64 (s, 4H, $2CH_2$), 3.15 (s, 2H, CH_2), 3.38 (s, 2H, CH_2 + su), 3.70 (s, 4H, $2CH_2$), 7.02 (d, 2H, J = 8.0 Hz, arH), 7.06–7.43 (m, 5H, arH), 8.05 (d, 2H, J = 8.0 Hz, arH), 9.59 (bs, 2H, 2NH), 9.94 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 46.69 (CH_2), 51.82 ($2CH_2$), 54.10 ($2CH_2$), 59.93 (CH_2), arC: [113.13 (d, J = 7.0 Hz, 2CH), 126.16 (d, J = 5.0 Hz, 2CH), 127.47 (CH), 129.13 (CH), 129.57 (d, J = 5.0 Hz, 2CH), 130.55 (2CH), 133.46 (C), 155.08 (C)], 169.52 (C═O), 192.95 (C═S). EI MS *m/z* (%): 106 (100), 135 (42), 320 (53), 331 (48), 384 ($[M+1]^+$, 48).

*3.1.2.3. N-(4-Fluorophenyl)-2-[(4-phenylpiperazin-1-yl)acetyl]hydrazin Carbothioamide **(5c)***. Recrystallized from acetone. Yield: 92%, Mp. 188–190 °C. FT-IR (υ_{max}, cm^{-1}): 3184 (3NH), 3032 (*ar*—CH), 1669 (C═O), 1226 (C═S). ^{1}H NMR (DMSO-d_6, δ ppm): 2.66 (s, 4H, $2CH_2$), 3.16 (d, 4H, J = 16.0 Hz, $2CH_2$), 3.36 (s, H_2O + CH_2), 6.78 (t, 1H, J = 12.0 Hz, arH), 6.94 (d, 2H, J = 8.0 Hz, arH), 7.16–7.23 (m, 4H, arH), 7.41 (s, 2H, arH), 9.63 (bs, 2H, 2NH), 9.90 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 48.48 ($2CH_2$), 53.24 ($2CH_2$), 60.28 (CH_2), arC: [115.24 (d, J = 22.0 Hz, 2CH), 115.83 (2CH), 119.23 (2CH), 123.99 (CH), 129.39 (2CH), 135.95 (C), 151.45 (C), 152.02–154.50 ($d_{C\text{-}F}$, J = 248.0 Hz, C)], 169.23 (C═O), 181.60 (C═S). EI MS *m/z* (%): 106 (93), 388 ($[M+1]^+$, 100), 389 ($[M+2]^+$, 86).

*3.1.2.4. N-(4-Ethyl)-2-[(4-phenylpiperazin-1-yl)acetyl] hydrazincarbothioamide **(5d)***. Recrystallized from acetone. Yield: 90%, Mp. 175 °C. FT-IR (υ_{max}, cm^{-1}): 3232 (3NH), 3063 (*ar*—CH), 1670 (C═O), 1234 (C═S). ^{1}H NMR (DMSO-d_6, δ ppm): 1.08 (t, 3H, J = 16.0 Hz, CH_3), 2.67 (s, 4H, $2CH_2$), 3.13 (s, 2H, $2CH_2$), 3.18 (s, 4H, $2CH_2$), 3.48 (s, 2H, CH_2), 6.78 (t, 1H, J = 16.0 Hz, arH), 6.94 (d, 2H, J = 8.0 Hz, arH), 7.22 (t, 2H, J = 16.0 Hz, arH),7.82 (bs, 1H, NH), 9.12 (bs, 1H, NH), 9.65 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 14.92 (CH_3), 38.91 (CH_2), 48.37 ($2CH_2$), 53.19 ($2CH_2$), 59.85 (CH_2), arC: [115.84 (2CH), 119.26 (CH), 129.39 (2CH), 151.40 (C)], 176.89 (C═O), 190.63 (C═S). EI MS *m/z* (%): 322 ($[M+1]^+$, 100), 323 ($[M+2]^+$, 85).

*3.1.2.5. N-Benzyl-2-[(4-phenylpiperazin-1-yl)acetyl]hydrazincarboxamide **(5e)***. Recrystallized from acetone. Yield: 86%, Mp. 211–212 °C. FT-IR (υ_{max}, cm^{-1}): 3380 ve 3304 (3NH), 3037 (*ar*—CH), 1713 (C═O), 1663 (C═O). ^{1}H NMR (DMSO-d_6, δ ppm): 2.62 (s, 4H, $2CH_2$), 3.09 (s, 4H, $2CH_2$), 3.48 (bs, H_2O + $2CH_2$), 4.25 (s, 2H, CH_2), 7.01 (s, 3H, arH), 7.28 (s, 7H, arH), 7.91 (s, 1H, NH), 8.05 (s, 1H, NH), 9.53 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 43.14 (CH_2), 46.68 ($2CH_2$), 52.64 ($2CH_2$), 59.89 (CH_2), arC: [113.05 (2CH), 126.18 (CH), 126.94 (CH), 127.02 (2CH), 127.41 (CH), 127.48 (CH), 128.53 (CH), 128.59 (CH), 137.28 (C), 155.12 (C)], 158.65 (C═O), 169.53 (C═O). EI MS *m/z* (%): 113.02 (60), 368.36 ($[M+1]^+$, 100).

*3.1.3. General method for the synthesis of compounds **6a–e***

Method 1. A solution of corresponding carbo(thio)amide **5a–e** (10 mmol) in ethanol/water (1:1) was refluxed in the presence of 2 N NaOH for 10 h (for **6a**), 13 h (for **6b**), 11 h (for **6c**), 9 h (for **6d**) and 15 h (for 6e) (the progress of the reaction was monitored by TLC). Then, the resulting solution was cooled to room temperature and acidified to pH 5 with 37% HCl. The precipitate formed was filtered off, washed with water, and recrystallized from an appropriate solvent to give the target compound.

Method 2. The mixture of the corresponding compound **5** (10 mmol) and 2 N NaOH in ethanol (10 mL) was irradiated in monomod microwave reactor in closed vessel (physical parameters, reaction time and yields were given in Table 4). Then the resulting solution was cooled to room temperature and acidified to pH 5 with 37% HCl. The precipitate formed was filtered off, wash with water, and recrystallized from an appropriate solvent to give the target compound.

*3.1.3.1. 4-Phenyl-5-[(4-phenylpiperazin-1-yl)methyl]-4H-1,2,4-triazol-3-thiol **(6a)***. Recrystallized from ethanol. FT-IR (υ_{max}, cm^{-1}): 3095 (*ar*—CH), 2759 (—SH), 1598 (C═N). ^{1}H NMR (DMSO-d_6, δ ppm): 2.50 (t, 4H, J = 5.0 Hz, $2CH_2$), 3.19 (s, 4H, $2CH_2$), 3.40 (s, 2H, CH_2), 7.04 (d, 2H, J = 8.0 Hz, arH), 7.43 (t, 4H, J = 12.0 Hz, arH), 7.66–7.70 (m, 2H, arH), 8.06 (d, 2H, J = 8.0 Hz, arH), 13.42 (s, 1H, SH). ^{13}C NMR (DMSO-d_6, δ ppm): 45.54 (CH_2), 48.59 ($2CH_2$), 51.45 (d, J = 22.0 Hz, $2CH_2$), arC: [116.76 (2CH), 126.53 (CH), 128.39 (2CH), 129.61 (CH), 130.25 (2CH), 130.87 (CH), 134.08 (CH), 148.40 (C), 151.57 (C)], 150.20 (triazol C-5), 169.61 (triazol C-3). EI MS *m/z* (%): 205.16 (1 0 0), 265.35 (42), 307.30 (35), 352.33 ($[M+1]^+$, 92), 374.28 ($[M+Na]^+$, 30).

*3.1.3.2. 4-Benzyl-5-[(4-phenylpiperazin-1-yl)methyl]-4H-1,2,4-triazol-3-thiol **(6b)***. Recrystallized from ethanol. FT-IR (υ_{max}, cm^{-1}): 3090 (*ar*—CH), 2751 (—SH), 1597 (C═N). ^{1}H NMR (DMSO-d_6, δ ppm):

2.38 (s, 4H, $2CH_2$), 3.27 (s, 4H, $2CH_2$), 3.54 (s, 2H, CH_2), 4.89 (s, 2H, CH_2), 6.96 (s, 1H, arH), 7.53 (s, 2H, arH), 7.58 (s, 4H, arH), 8.02 (s, 3H, arH), 13.89 (s, 1H, SH). ^{13}C NMR (DMSO-d_6, δ ppm): 46.67 (CH_2), 51.42 (CH_2), 51.88 (CH_2), 51.97 ($2CH_2$), arC: [117.72 (2CH), 126.13 (2CH), 127.61 (CH), 129.39 (2CH), 130.02 (CH), 130.78 (2CH), 139.14 (C), 148.40 (C), 150.79 (C)], 153.05 (triazol C-5), 174.54 (triazol C-3). EI MS *m/z* (%): 134.98 (44), 208.18 (50), 288.46 (45), 388.32 ([M + Na]$^+$, 100).

3.1.3.3. 4-(4-Fluorophenyl)-5-[(4-phenylpiperazin-1-yl)methyl]-4H-1,2,4-triazol-3-thiol ***(6c)***. Recrystallized from ethylacetate. FT-IR (υ_{max}, cm^{-1}): 3087 (*ar*—CH), 2830 (—SH), 1589 (C═N). ^{1}H NMR (DMSO-d_6, δ ppm): 2.37 (t, 4H, J = 12.0 Hz, $2CH_2$), 3.28 (t, 4H, J = 12.0 Hz, $2CH_2$), 3.42 (s, 2H, CH_2), 6.97 (d, 2H, J = 8.0 Hz, arH), 7.36 (s, 1H, arH), 7.40 (d, 2H, J = 8.0 Hz, arH), 7.55 (q, 2H, J = 16.0 Hz, arH), 8.03 (d, 2H, J = 8.0 Hz, arH), 13.90 (s, 1H, SH). ^{13}C NMR (DMSO-d_6, δ ppm): 46.66 (CH_2), 50.58 ($2CH_2$), 51.89 ($2CH_2$), arC: [113.13 (2CH), 116.28 (d, J = 23.0 Hz, 2CH), 121.22 (CH), 126.13 (2CH), 131.17 (d, J = 9.0 Hz, 2CH), 137.41 (C), 149.59 (C), 161.29–163.74 ($d_{C\text{-}F}$, J = 245.0 Hz, C)], 155.05 (triazol C-5), 168.95 (triazol C-3). EI MS *m/z* (%): 175 (100), 285 (64), 301 (63), 315 (62), 369 ([M]$^+$, 46).

3.1.3.4. 4-Ethyl-5-[(4-phenylpiperazin-1-yl)methyl]-4H-1,2,4-triazol-3-thiol ***(6d)***. Recrystallized from ethanol. FT-IR (υ_{max}, cm^{-1}): 3089 (*ar*—CH), 2762 (—SH), 1597 (C═N). ^{1}H NMR (DMSO-d_6, δ ppm): 1.30 (t, 3H, J = 16.0 Hz, CH_3), 2.58 (s, 4H, $2CH_2$), 3.12 (t, 4H, J = 12.0 Hz, $2CH_2$), 3.64 (s, 2H, CH_2), 4.04 (q, 2H, J = 8.0 Hz, CH_2), 6.78 (t, 1H, J = 16.0 Hz, arH), 6.93 (d, 2H, J = 8.0 Hz, arH), 7.21 (q, 2H, J = 8.0 Hz, arH), 13.63 (s, 1H, SH). ^{13}C NMR (DMSO-d_6, δ ppm): 13.72 (CH_3), 39.26 (CH_2), 48.69 ($2CH_2$), 52.33 (CH_2), 52.85 ($2CH_2$), arC: [115.97 (2CH), 119.44 (CH), 129.38 (2CH), 149.47 (C)], 151.36 (triazol C-5), 167.36 (triazol C-3). EI MS *m/z* (%): 106 (100), 213 (41), 304 ([M + 1]$^+$, 24).

3.1.3.5. 4-Benzyl-5-[(4-phenylpiperazin-1-yl)methyl]-1H-1,2,4-triazol-5-one ***(6e)***. Recrystallized from acetone. FT-IR (υ_{max}, cm^{-1}): 3083 (*ar*—CH), 1578 (C═N). ^{1}H NMR (DMSO-d_6, δ ppm): 2.50 (t, 4H, J = 4.0 Hz, $2CH_2$), 3.32 (s, 2H, CH_2), 3.36 (s, H_2O + CH_2), 4.89 (s, 2H, CH_2), 6.97 (d, 2H, J = 8.0 Hz, arH), 7.26 (d, 3H, J = 8.0 Hz, arH), 7.31 (s, 1H, arH), 7.34 (d, 2H, J = 8.0 Hz, arH), 8.04 (d, 2H, J = 12.0 Hz, arH), 11.75 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 44.25 (CH_2), 46.41 ($2CH_2$), 52.13 ($2CH_2$), 53.28 (CH_2), arC: [113.00 (2CH), 121.38 (CH), 126.13 (2CH), 127.48 (CH), 128.50 (2CH), 128.87 (2CH), 137.40 (C), 144.65 (C)], 154.96 (triazol C-3), 156.06 (triazol C-5). EI MS *m/z* (%): 168.93 (68), 205.22 (42), 372.33 ([M + Na]$^+$, 100).

3.1.4. General method for the synthesis of compounds ***7a,b*** *and* ***8a–j***

Method 1. The solution of ciprofloxacin (for **7b**, **8b**, **8d**, **8f**, **8h**, **8j**) or norfloxacin (for **7a**, **8a**, **8c**, **8e**, **8g**, **8i**), (10 mmol) in dimethyl formamide was stirred at room temperature in the presence of formaldehyde (37%, 30 mmol) for 15 min. Then, the corresponding compound **4** or **6** (10 mmol) was added into it and stirred for additional 24 h. The reaction mixture was poured to ice-water and a solid obtained. This crude product was recrystallized from an appropriate solvent to give the desired compound.

Method 2. A mixture of ciprofloxacin or norfloxacin (10 mmol), the corresponding compound **7** or **8** (10 mmol) and formaldehyde (37%, 30 mmol) in DMF was irradiated in a monomod microwave reactor in a closed vessel under pressure control at 50 W for 20 min. The solid obtained was purified by recrystallization from an appropriate solvent.

3.1.4.1. 1-Ethyl-6-fluoro-4-oxo-7-(4-{[5-[(4-phenylpiperazin-1-yl) methyl]-2-tioxo-1,3,4-oxadiazole-3(2H)-yl]methyl}piperazin-1-yl)-1,4-dihydroquinolin-3-carboxylic acid ***(7a)***. Recrystallized from ethyl acetate:ethanol (3:1). Mp. 153–154 °C. FT-IR (υ_{max}, cm^{-1}): 3065 (*ar*—CH), 1721 (C═O), 1520 (C═N), 1238 (C═S). ^{1}H NMR (DMSO-d_6, δ ppm): 1.41 (t, 3H, J = 12.0 Hz, CH_3), 2.66 (s, 4H, $2CH_2$), 2.74 (s, 2H, CH_2), 2.90 (s, 4H, $2CH_2$), 2.94 (s, 4H, $2CH_2$), 3.13 (s, 4H, $2CH_2$), 3.78 (s, 2H, CH_2), 4.58 (d, 2H, J = 5.0 Hz, CH_2), 5.07 (s, 2H, CH_2), 6.67 (t, 1H, J = 16.0 Hz, arH), 6.91 (d, 2H, J = 8.0 Hz, arH), 7.19 (t, 3H, J = 12.0 Hz, arH), 7.96 (s, 1H, arH), 8.95 (s, 1H, CH), 15.34 (s, 1H, OH). ^{13}C NMR (DMSO-d_6, δ ppm): 14.81 (CH_3), 48.80 ($2CH_2$), 45.92 ($2CH_2$), 49.81 ($2CH_2$), 51.66 ($2CH_2$), 52.46 ($2CH_2$), 69.93 (CH_2), arC: [106.47 (CH), 107.55 (C), 111.66 (d, J = 23.0 Hz, CH), 115.98 (2CH), 119.44 (CH), 119.71 (C), 129.36 (2CH), 137.63 (C), 145.76 (d, J = 11.0 Hz, C), 151.33 (C), 152.05–154.53 ($d_{C\text{-}F}$, J = 248.0 Hz, C)], 148.98 (CH), 159.47 (oxadiazole C-5), 166.56 (C═O), 176.63 (C═O), 178.48 (oxadiazole C-2). EI MS *m/z* (%): 205.21 (100), 361.27 (50), 517.26 (45), 630.28 ([M + Na]$^+$, 40).

3.1.4.2. 1-Cyclopropyl-6-fluoro-4-oxo-7-(4-{[5-[(4-phenylpiperazin-1-yl) methyl]-2-tioxo-1,3,4-oxadiazole-3(2H)-yl]methyl}piperazin-1-yl)-1,4-dihydroquinolin-3-carboxylic acid ***(7b)***. Recrystallized from ethyl acetate:ethanol (3:1). Mp. 176 °C. FT-IR (υ_{max}, cm^{-1}): 3014 (*ar*—CH), 1724 (C═O), 1598 (C═N), 1261 (C═S). ^{1}H NMR (DMSO-d_6, δ ppm): 1.17 (s, 2H, CH_2), 1.32 (d, 2H, J = 8.0 Hz, CH_2), 2.66 (s, 4H, $2CH_2$), 2.74 (s, 2H, CH_2), 2.90 (s, 2H, CH_2), 2.96 (s, 4H, $2CH_2$), 3.13 (s, 4H, $2CH_2$), 3.79 (s, 2H, CH_2), 3.96 (s, 1H, CH), 5.07 (s, 2H, CH_2), 6.77 (t, 1H, J = 16.0 Hz, arH), 6.90 (d, 2H, J = 8.0 Hz, arH), 7.19 (t, 2H, J = 16.0 Hz, arH), 7.55 (d, 1H, J = 8.0 Hz, arH), 7.87 (d, 1H, J = 12.0 Hz, arH), 8.66 (s, 1H, CH), 15.18 (s, 1H, OH). ^{13}C NMR (DMSO-d_6, δ ppm): 8.04 ($2CH_2$), 36.30 (CH), 48.60 ($2CH_2$), 49.74 (CH_2), 49.82 ($2CH_2$), 51.66 ($2CH_2$), 52.46 ($2CH_2$), 69.91 (CH_2), arC: [107.02 (CH), 107.20 (C), 111.42 (d, J = 23.0 Hz, CH), 115.97 (2CH), 119.04 (C), 119.28 (d, J = 32.0 Hz, CH), 129.37 (2CH), 134.69 (C), 139.58 (C), 145.44 (d, J = 10.0 Hz, C), 151.32 (C), 152.18–154.66 ($d_{C\text{-}F}$, J = 248.0 Hz, C)], 148.43 (CH), 159.46 (oxadiazole C-5), 166.38 (C═O), 176.80 (C═O), 178.49 (oxadiazole C-2). EI MS *m/z* (%): 619.02 ([M]$^+$, 100).

3.1.4.3. 1-Ethyl-6-fluoro-4-oxo-7-[4-({4-phenyl-3-[(4-phenylpiperazin-1-yl)methyl]-5-tioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl}methyl)piperazin-1-yl]-1,4-dihydroquinolin-3-carboxylic acid ***(8a)***. Recrystallized from ethyl acetate:ethanol (3:1). Mp. 203–205 °C. FT-IR (υ_{max}, cm^{-1}): 3021 (*ar*—CH), 1721 (C═O), 1548 (C═N), 1261 (C═S). ^{1}H NMR (DMSO-d_6, δ ppm): 1.44 (s, 3H, CH_3), 2.40 (s, 4H, $2CH_2$), 2.92 (s, 4H, $2CH_2$), 3.02 (s, 4H, $2CH_2$), 3.39 (s, 4H, $2CH_2$), 3.48 (s, 2H, CH_2), 4.58 (s, 2H, CH_2), 5.22 (s, 2H, CH_2), 6.75 (s, 1H, arH), 6.82 (s, 1H, arH), 7.17 (s, 3H, arH), 7.52 (d, 5H, J = 16.0 Hz, arH), 7.91 (s, 1H, arH), 8.91 (s, 1H, CH), 15.26 (bs, 1H, OH). ^{13}C NMR (DMSO-d_6, δ ppm): 14.80 (CH_3), 46.63 ($2CH_2$), 49.56 ($2CH_2$), 49.97 (CH_2), 50.10 ($2CH_2$), 51.67 (CH_2), 51.89 ($2CH_2$), 69.02 (CH_2), arC: [106.41 (CH), 107.54 (C), 113.12 (2CH), 116.35 (d, J = 23.0 Hz, 2CH), 119.76 (C), 122.45 (2CH), 124.50 (2CH), 126.11 (2CH), 131.28 (CH), 137.54 (d, J = 23.0 Hz, 2C), 139.50 (C), 142.33 (C), 148.40 (C), 152.30–155.02 ($d_{C\text{-}F}$, J = 272.0 Hz, C)], 148.94 (CH), 158.65 (triazol C-5), 166.60 (C═O), 170.01 (triazol C-3), 176.63 (C═O). EI MS *m/z* (%): 205.18 (1 0 0), 460.27 (26), 682.14 ([M]$^+$, 34).

3.1.4.4. 1-Cyclopropyl-6-fluoro-4-oxo-7-[4-({4-phenyl-3-[(4-phenylpiperazin-1-yl)methyl]-5-tioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl}methyl)piperazin-1-yl]-1,4-dihydroquinolin-3-carboxylic acid ***(8b)***. Recrystallized from ethyl acetate:ethanol (3:1). Mp. 243–244 °C. FT-IR (υ_{max}, cm^{-1}): 3018 (*ar*—CH), 1728 (C═O), 1599 (C═N), 1255 (C═S). ^{1}H NMR (DMSO-d_6, δ ppm): 1.18 (s, 2H, CH_2), 1.33 (s, 2H, CH_2), 2.40 (s, 4H, $2CH_2$), 2.92 (s, 4H, $2CH_2$), 3.03 (s, 4H, $2CH_2$), 3.27 (s, 4H, $2CH_2$), 3.48 (s, 2H, CH_2), 3.82 (s, 1H, CH), 5.22 (s, 2H, CH_2), 6.75 (d, 1H, J = 5.0 Hz, arH), 6.82 (d, 2H, J = 8.0 Hz, arH), 7.17 (d, 2H, J = 5.0 Hz, arH), 7.50 (d, 2H, J = 8.0 Hz, arH), 7.55 (d, 4H, J = 8.0 Hz, arH), 7.89 (t, 1H, J = 12.0 Hz, arH), 8.65 (s, 1H, CH), 15.13 (s, 1H, OH). ^{13}C NMR (DMSO-d_6, δ ppm): 8.07 ($2CH_2$),

36.31 (CH), 48.46 ($2CH_2$), 49.90 (CH_2), 50.09 ($2CH_2$), 51.90 ($2CH_2$), 52.42 ($2CH_2$), 68.95 (CH_2), arC: [106.93 (CH), 107.21 (C), 111.40 (d, J = 24.0 Hz, CH), 115.80 (2CH), 118.50 (CH), 119.03 (d, J = 8.0 Hz, C), 119.34 (2CH), 128.73 (CH), 129.33 (CH), 129.41 (2CH), 129.78 (CH), 134.97 (C), 139.59 (C), 145.58 (C), 148.49 (C), 152.19–154.67 (d_{C-F}, J = 248.0 Hz, C)], 148.49 (CH), 151.23 (triazol C-5), 166.37 (C═O), 169.80 (triazol C-3), 176.79 (C═O). EI MS *m/z* (%): 205.15 (100), 696.11 ($[M+2]^+$, 83).

3.1.4.5. 1-Ethyl-6-fluoro-4-oxo-7-[4-({4-benzyl-3-[(4-phenylpiperazin-1-yl)methyl]-5-tioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl}methyl)piperazin-1-yl]-1,4-dihydroquinolin-3-carboxylic acid ***(8c)***. Recrystallized from ethyl acetate:acetone (1:1). Mp. 248–249 °C. FT-IR (υ_{max}, cm^{-1}): 3063 (*ar*—CH), 1719 (C═O), 1546 (C═N), 1259 (C═S). ^{1}H NMR (DMSO-d_6, δ ppm): 1.41 (s, 3H, CH_3), 2.44 (s, 4H, $2CH_2$), 2.87 (s, 4H, $2CH_2$), 2.94 (s, 4H, $2CH_2$), 3.33 (s, 4H, $2CH_2$), 3.52 (s, 2H, CH_2), 4.58 (s, 2H, CH_2), 5.17 (s, 2H, CH_2), 5.43 (s, 2H, CH_2), 6.75 (s, 1H, arH), 6.82 (s, 2H, arH), 7.17 (s, 3H, arH), 7.27 (s, 2H, arH), 7.30 (s, 2H, arH), 7.90 (d, 1H, J = 8.0 Hz, arH), 8.94 (s, 1H, CH), 15.33 (s, 1H, OH). ^{13}C NMR (DMSO-d_6, δ ppm): 14.81 (CH_3), 46.74 (CH_2), 47.87 (CH_2), 48.20 ($2CH_2$), 49.52 (CH_2), 49.91 (CH_2), 50.16 (CH_2), 52.33 ($2CH_2$), 52.63 ($2CH_2$), 69.02 (CH_2), arC: [106.38 (CH), 107.54 (C), 115.84 (2CH), 119.33 (2CH), 119.64 (C), 127.29 (2CH), 127.43 (CH), 128.87 (2CH), 129.36 (2CH), 136.44 (C), 137.63 (C), 145.72 (C), 148.61 (C), 152.03–154.50 (d_{C-F}, J = 247.0 Hz, C)], 148.97 (CH), 151.24 (triazol C-5), 166.57 (C═O), 169.85 (triazol C-3), 176.61 (C═O). EI MS *m/z* (%): 694.29 (92), 696.23 ($[M]^+$, 100).

3.1.4.6. 1-Cyclopropyl-6-fluoro-4-oxo-7-[4-({4-benzyl-3-[(4-phenylpiperazin-1-yl)methyl]-5-tioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl}methyl)piperazin-1-yl]-1,4-dihydroquinolin-3-carboxylic acid ***(8d)***. Recrystallized from ethyl acetate:petroleum ether (3:1). Mp. 176–178 °C. FT-IR (υ_{max}, cm^{-1}): 3028 (*ar*—CH), 1728 (C═O), 1542 (C═N), 1256 (C═S). ^{1}H NMR (DMSO-d_6, δ ppm): 1.13 (s, 2H, CH_2), 1.33 (s, 2H, CH_2), 2.45 (s, 4H, $2CH_2$), 2.92 (s, 6H, $3CH_2$), 2.96 (s, 4H, $2CH_2$), 3.30 (s, 4H, $2CH_2$), 3.53 (s, 2H, CH_2), 3.81 (s, 1H, CH), 5.21 (s, 2H, CH_2), 5.41 (s, 2H, CH_2), 6.76 (s, 1H, arH), 6.83 (s, 2H, arH), 7.18 (d, 2H, J = 6.0 Hz, arH), 7.28 (s, 3H, arH), 7.30 (d, 2H, J = 5.0 Hz, arH), 7.57 (s, 1H, arH), 7.90 (d, 1H, J = 12.0 Hz, arH), 8.66 (s, 1H, CH), 15.17 (s, 1H, OH). ^{13}C NMR (DMSO-d_6, δ ppm): 8.06 ($2CH_2$), 36.30 (CH), 47.72 ($2CH_2$), 47.88 (CH_2), 48.24 ($2CH_2$), 49.83 (CH_2), 50.14 ($2CH_2$), 52.34 ($2CH_2$), 52.65 ($2CH_2$), 69.03 (CH_2), arC: [106.96 (CH), 107.21 (C), 111.43 (d, J = 23.0 Hz, CH), 115.87 (d, J = 4.0 Hz, 2CH), 119.09 (C), 119.34 (CH), 127.30 (CH), 127.43 (CH), 127.86 (CH), 128.86 (CH), 128.88 (CH), 129.36 (2CH), 136.43 (C), 139.59 (C), 148.67 (d, J = 10.0 Hz, C), 151.24 (C), 152.18–154.66 (d_{C-F}, J = 248.0 Hz, C)], 148.43 (CH), 156.07 (triazol C-5), 166.39 (C═O), 169.86 (triazol C-3), 176.81 (C═O). EI MS *m/z* (%): 205.21 (100), 644.26 (32), 710.23 ($[M+2]^+$, 73).

3.1.4.7. 1-Ethyl-6-fluoro-4-oxo-7-[4-({4-fluorophenyl-3-[(4-phenylpiperazin-1-yl)methyl]-5-tioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl}methyl)piperazin-1-yl]-1,4-dihydroquinolin-3-carboxylic acid ***(8e)***. Recrystallized from ethyl acetate:ethyl acetate (1:1). Mp. 164–165 °C. FT-IR (υ_{max}, cm^{-1}): 3063 (*ar*—CH), 1724 (C═O), 1599 (C═N), 1258 (C═S). ^{1}H NMR (DMSO-d_6, δ ppm): 1.42 (t, 3H, J = 12.0 Hz, CH_3), 2.39 (s, 8H, $4CH_2$), 2.95 (s, 4H, $2CH_2$), 3.00 (s, 4H, $2CH_2$), 3.47 (s, 2H, CH_2), 4.59 (d, 2H, J = 8.0 Hz, CH_2), 5.21 (s, 2H, CH_2), 6.76 (s, 1H, arH), 6.85 (q, 2H, J = 8.0 Hz, arH), 7.18 (d, 3H, J = 8.0 Hz, arH), 7.40 (d, 3H, J = 8.0 Hz, arH), 7.55 (d, 2H, J = 5.0 Hz, arH), 8.95 (s, 1H, CH), 15.35 (s, 1H, OH). ^{13}C NMR (DMSO-d_6, δ ppm): 14.83 (CH_3), 48.49 ($2CH_2$), 49.53 (CH_2), 49.92 (CH_2), 50.11 ($2CH_2$), 51.94 ($2CH_2$), 52.43 ($2CH_2$), 70.97 (CH_2), arC: [106.40 (CH), 107.55 (C), 111.62 (d, J = 23.0 Hz, CH), 115.70 (d, J = 6.0 Hz, CH), 115.83 (d, J = 22.0 Hz, 2CH), 119.35 (2CH), 119.66 (C), 129.35 (2CH), 131.12 (d, J = 9.0 Hz, 2CH), 137.64 (C), 145.79 (C), 148.63 (d, J = 17.0 Hz, C), 151.29 (C), 152.06–154.54 (d_{C-F}, J = 248.0 Hz, C), 163.79–166.58 (d_{C-F}, J = 279.0 Hz, C)], 148.97 (CH), 161.35 (triazol C-5), 168.62 (C═O), 169.95 (triazol C-3), 176.61 (C═O). EI MS *m/z* (%): 205.21 (100), 524.29 (27), 700.88 ($[M]^+$, 70).

3.1.4.8. 1-Cyclopropyl-6-fluoro-4-oxo-7-[4-({4-fluorophenyl-3-[(4-phenylpiperazin-1-yl)methyl]-5-tioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl}methyl)piperazin-1-yl]-1,4-dihydroquinolin-3-carboxylic acid ***(8f)***. Recrystallized from ethyl acetate:ethyl acetate (1:1). Mp. 145–146 °C. FT-IR (υ_{max}, cm^{-1}): 3070 (*ar*—CH), 1723 (C═O), 1599 (C═N), 1257 (C═S). ^{1}H NMR (DMSO-d_6, δ ppm): 1.18 (s, 2H, CH_2), 1.33 (d, 2H, J = 8.0 Hz, CH_2), 2.40 (s, 8H, $4CH_2$), 2.48 (s, 2H, CH_2), 2.94 (s, 8H, $4CH_2$), 3.82 (s, 1H, CH), 5.21 (s, 2H, CH_2), 6.76 (t, 1H, J = 12.0 Hz, arH), 6.86 (q, 2H, J = 6.0 Hz, arH), 7.18 (q, 2H, J = 8.0 Hz, arH), 7.40 (t, 2H, J = 20.0 Hz, arH), 7.53–7.59 (m, 3H, arH), 7.91 (d, 1H, J = 12.0 Hz, arH), 8.66 (s, 1H, CH), 15.20 (s, 1H, OH). ^{13}C NMR (DMSO-d_6, δ ppm): 8.07 ($2CH_2$), 36.32 (CH), 48.49 ($2CH_2$), 49.91 (CH_2), 50.06 ($2CH_2$), 51.94 ($2CH_2$), 52.43 ($2CH_2$), 70.97 (CH_2), arC: [106.99 (CH), 107.22 (C), 111.43 (d, J = 22.0 Hz, CH), 115.84 (d, J = 5.0 Hz, 2CH), 116.21 (d, J = 6.0 Hz, CH), 119.04 (C), 119.35 (CH), 129.35 (2CH), 131.12 (d, J = 9.0 Hz, 2CH), 139.62 (C), 145.56 (d, J = 9.0 Hz, C), 148.62 (d, J = 16.0 Hz, C), 151.27 (d, J = 4.0 Hz, C), 152.21–154.69 (d_{C-F}, J = 248.0 Hz, C), 166.39–168.62 (d_{C-F}, J = 223.0 Hz, C)], 148.45 (CH), 161.35 (triazol C-5), 163.79 (C═O), 169.97 (triazol C-3), 176.80 (C═O). EI MS *m/z* (%): 205.15 (48), 380.35 (67), 522.29 (44), 609.17 (49), 611.17 (50), 708.16 (82), 710.17 (85),712.12 ($[M]^+$, 100), 704.12 ($[M+2]^+$, 96).

3.1.4.9. 1-Ethyl-6-fluoro-4-oxo-7-[4-({4-ethyl-3-[(4-phenylpiperazin-1-yl)methyl]-5-tioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl}methyl)piperazin-1-yl]-1,4-dihydroquinolin-3-carboxylic acid ***(8g)***. Recrystallized from ethyl acetate:ethanol (3:1). Mp. 193–194 °C. FT-IR (υ_{max}, cm^{-1}): 3095 (*ar*—CH), 1718 (C═O), 1600 (C═N), 1243 (C═S). ^{1}H NMR (DMSO-d_6, δ ppm): 1.16 (s, 3H, CH_3), 1.33 (d, 3H, J = 8.0 Hz, CH_3), 2.60 (s, 4H, $2CH_2$), 2.74 (s, 4H, $2CH_2$), 2.92 (s, 4H, $2CH_2$), 3.27 (s, 4H, $2CH_2$), 3.70 (s, 2H, CH_2), 3.81 (s, 2H, CH_2), 4.11 (d, 2H, J = 5.0 Hz, CH_2), 5.13 (s, 2H, CH_2), 6.77 (t, 1H, J = 12.0 Hz, arH), 6.89 (d, 2H, J = 8.0 Hz, arH), 7.19 (t, 2H, J = 12.0 Hz, arH), 7.54 (d, 1H, J = 5.0 Hz, arH), 7.87 (d, 1H, J = 16.0 Hz, arH), 8.64 (s, 1H, CH), 15.13 (s, 1H, OH). ^{13}C NMR (DMSO-d_6, δ ppm): 13.50 (CH_3), 14.74 (CH_3), 48.65 ($2CH_2$), 49.51 ($2CH_2$), 49.87 (CH_2), 50.11 ($2CH_2$), 52.15 ($2CH_2$), 52.88 ($2CH_2$), 68.53 (CH_2), arC: [106.38 (CH), 107.54 (C), 111.66 (d, J = 23.0 Hz, CH), 115.93 (2CH), 119.54 (d, J = 15.0 Hz, CH), 119.68 (C), 129.43 (2CH), 137.62 (C), 145.75 (d, J = 10.0 Hz, C), 148.36 (C), 152.02–154.50 (d_{C-F}, J = 248.0 Hz, C)], 148.96 (CH), 151.28 (triazol C-5), 166.55 (C═O), 168.46 (triazol C-3), 176.59 (C═O). EI MS *m/z* (%): 205.21 (100), 517.26 (35), 658.54 ([M+1+Na]$^+$, 38).

3.1.4.10. 1-Cyclopropyl-6-fluoro-4-oxo-7-[4-({4-ethyl-3-[(4-phenylpiperazin-1-yl)methyl]-5-tioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl}methyl)piperazin-1-yl]-1,4-dihydroquinolin-3-carboxylic acid ***(8h)***. Recrystallized from ethyl acetate:ethanol (3:1). Mp. 229–230 °C. FT-IR (υ_{max}, cm^{-1}): 3073 (*ar*—CH), 1726 (C═O), 1543 (C═N), 1252 (C═S). ^{1}H NMR (DMSO-d_6, δ ppm): 1.33 (t, 4H, J = 12.0 Hz, $2CH_2$), 1.41 (t, 3H, J = 12.0 Hz, CH_2), 2.58 (s, 4H, $2CH_2$), 2.74 (s, 4H, $2CH_2$), 2.90 (s, 4H, $2CH_2$), 3.34 (s, H_2O + $2CH_2$), 3.69 (s, 1H, CH), 4.10 (d, 2H, J = 8.0 Hz, CH_2), 4.58 (d, 2H, J = 8.0 Hz, CH_2), 5.12 (s, 2H, CH_2), 6.78 (d, 1H, J = 8.0 Hz, arH), 6.88 (d, 2H, J = 8.0 Hz, arH), 7.15–7.21 (m, 3H, arH), 7.89 (d, 1H, J = 16.0 Hz arH), 8.94 (s, 1H, CH), 15.33 (s, 1H, OH). ^{13}C NMR (DMSO-d_6, δ ppm): 8.04 ($2CH_2$), 13.51 (CH_3), 36.28 (CH), 44.07 (CH_2), 48.65 ($2CH_2$), 49.80 ($2CH_2$), 50.07 ($2CH_2$), 52.16 ($2CH_2$), 52.89 ($2CH_2$), 68.53 (CH_2), arC: [106.92 (CH), 107.20 (C), 111.39 (d, J = 23.0 Hz, CH), 115.93 (2CH), 119.01 (d, J = 7.0 Hz, C), 119.46 (CH), 129.37 (2CH), 139.57 (C), 145.47 (d, J = 10.0 Hz, C), 148.38 (C), 152.16–154.64 (d_{C-F}, J = 248.0 Hz, C)], 148.40 (CH), 151.28 (triazol C-5), 166.35 (C═O), 168.47 (triazol C-3), 176.76 (C═O). EI MS *m/z* (%): 205.15 (49), 646.26 ($[M]^+$, 90), 648.27 ($[M+2]^+$, 100).

3.1.4.11. 7-[4-({4-Benzyl-5-oxo-3-[(4-phenylpiperazin-1-yl)methyl]-4,5-dihydro-1H-1,2,4-triazol-1-yl}methyl)piperazin-1-yl]-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinolin-3-carboxylic acid ***(8i)***. Recrystallized from ethyl acetate:ethanol (3:1). Mp. 253–255 °C. FT-IR (υ_{max}, cm^{-1}): 3059 (*ar*—CH), 1703 (C═O), 1677 (C═O), 1600 (C═N). ^{1}H NMR (DMSO-d_6, δ ppm): 1.41 (t, 3H, *J* = 12.0 Hz, CH_3), 2.44 (s, 4H, $2CH_2$), 2.82 (s, 4H, $2CH_2$), 3.23 (s, 4H, $2CH_2$), 3.39 (s, 6H, $3CH_2$), 4.58 (d, 2H, *J* = 5.0 Hz, CH_2), 4.96 (s, 2H, CH_2), 6.94 (d, 2H, *J* = 8.0 Hz, arH), 7.17 (d, 1H, *J* = 8.0 Hz, arH), 7.25–7.33 (m, 6H, arH), 7.91 (d, 1H, *J* = 16.0 Hz, arH), 8.02 (d, 2H, *J* = 8.0 Hz, arH), 8.94 (s, 1H, CH), 15.33 (s, 1H, OH). ^{13}C NMR (DMSO-d_6, δ ppm): 14.78 (CH_3), 44.87 (CH_2), 46.38 ($2CH_2$), 49.53 ($2CH_2$), 49.78 ($2CH_2$), 52.12 ($2CH_2$), 52.97 ($2CH_2$), 66.05 (CH_2), arC: [106.35 (CH), 107.52 (C), 112.92 (2CH), 116.04 (2CH), 119.80 (C), 126.13 (2CH), 127.41 (2CH), 127.80 (CH), 128.92 (2CH), 137.38 (C), 137.58 (d, *J* = 9.0 Hz, C), 143.57 (C), 145.82 (d, *J* = 10.0 Hz, C), 152.04–154.96 (d_{C-F}, *J* = 292.0 Hz, C)], 148.91 (CH), 155.23 (triazol C-3), 161.87 (triazol C-5), 166.59 (C═O), 176.58 (C═O). EI MS *m/z* (%): 488.32 (47), 575.20 (88), 577.15 (53), 680.16 ($[M]^+$, 100).

3.1.4.12. 7-[4-({4-Benzyl-5-oxo-3-[(4-phenylpiperazin-1-yl)methyl]-4,5-dihydro-1H-1,2,4-triazol-1-yl}methyl)piperazin-1-yl]-1-Cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinolin-3-carboxylic acid ***(8j)***. Recrystallized from ethyl acetate:ethanol (3:1). Mp. 199–200 °C. FT-IR (υ_{max}, cm^{-1}): 3059 (*ar*—CH), 1710 (C═O), 1670 (C═O), 1600 (C═N). ^{1}H NMR (DMSO-d_6, δ ppm): 1.20 (s, 2H, CH_2), 1.33 (s, 2H, CH_2), 2.44 (s, 4H, $2CH_2$), 2.73 (s, 2H, CH_2), 3.25 (s, 4H, $2CH_2$), 3.34 (s, H_2O + $4CH_2$), 3.39 (s, 2H, CH_2), 3.89 (s, 1H, CH), 5.03 (s, 2H, CH_2), 6.97 (d, 2H, *J* = 8.0 Hz, arH), 7.28–7.35 (m, 6H, arH), 7.58 (t, 1H, *J* = 16.0 Hz, arH), 7.91 (d, 1H, *J* = 12.0 Hz, arH), 8.04 (d, 2H, *J* = 8.0 Hz, arH), 8.66 (s, 1H, CH), 15.22 (s, 1H, OH). ^{13}C NMR (DMSO-d_6, δ ppm): 8.04 ($2CH_2$), 36.30 (CH), 44.81 (CH_2), 46.42 (CH_2), 48.39 (CH_2), 50.36 ($2CH_2$), 51.10 (CH_2), 52.16 ($2CH_2$), 53.04 ($2CH_2$), 67.53 (CH_2), arC: [106.92 (CH), 107.23 (C), 111.42 (d, *J* = 26.0 Hz, CH), 113.10 (2CH), 119.02 (C), 126.15 (2CH), 127.44 (CH), 127.53 (CH), 127.80 (2CH), 128.91 (2CH), 137.43 (d, *J* = 6.0 Hz, C), 139.57 (C), 143.58 (C), 152.12–154.08 (d_{C-F}, *J* = 194.0 Hz, C)], 145.63 (triazol C-3), 148.44 (CH), 155.02 (triazol C-5), 166.39 (C═O), 176.83 (C═O). EI MS *m/z* (%): 581.10 (92), 583.11 (100), 694.16 ($[M]^+$, 36).

3.2. Antimicrobial activity

The test microorganisms were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and were as follows: *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 13883, *Acinetobacter haemolyticus* ATCC 19002. All the newly synthesized compounds were weighed and dissolved in DMSO to prepare extract stock solution of 10.000 μg/mL. The antimicrobial effects of the substances were tested quantitatively in respective broth media by using double micro-dilution and the minimal inhibition concentration (MIC) values (μg/mL) were determined. The antibacterial were performed in Mueller-Hinton broth (MH) (Difco, Detroit, MI) at pH.7.3 and buffered Yeast Nitrogen Base (Difco, Detroit, MI) at pH 7.0, respectively. The micro dilution test plates were incubated for 18–24 h at 35 °C. Brain Heart Infusion broth (BHI) (Difco, Detriot, MI) was used for *M. smegmatis*, and incubated for 48–72 h at 35 °C [49]. Ampicillin (10 μg), gentamicin (10 μg) and ciprofloxacin (10 μg) were used as standard antibacterial respectively. Dimethylsulphoxide with dilution of 1:10 was used as solvent control. All results were presented in Table 3.

3.3. In vitro inhibitory activity screening and determination of IC_{50} values on E. coli gyrase

The assay for determining IC_{50} values (Inspiralis) was performed on black streptavidin-coated 96-well microtiter plates (Thermo Scientific Pierce). The plate was first rehydrated with the wash buffer supplied (20mMTris-HCl (pH 7.6),137 mM NaCl, 0.01% (w/v) BSA, 0.05% (v/v) Tween 20). Biotinylated oligonucleotide was immobilized onto the wells. The excess of oligonucleotide was then washed off and the enzyme assay carried out in the wells. The final reaction volume of 30 mL in buffer (35 mM Tris HCl pH 7.5; 24 mM KCl; 4 mM $MgCl_2$; 2 mM DTT; 1.8 mM spermidine; 1 mM ATP; 6.5% (w/v) glycerol; 0.1 mg/mL albumin) contained 1.5 U of DNA gyrase from *E. coli* 0.75 mg of relaxed pNO1 plasmid, and 3 mL of inhibitors solution in 10% DMSO and 0.008% Tween® 20. Reactions were incubated for 30 min at 37 °C and, after addition of the TF buffer (50 mM NaOAc pH 5.0, 50 mM NaCl and 50 mM MgCl2), which terminated the enzymatic reaction, for another 30 min at room temperature to allow triplex formation (biotin-oligonucleotide-plasmid). The unbound plasmid was then washed off using TF buffer, and a solution of SybrGOLD stain in T10 buffer (10 mM Tris-HCl pH 8.0 and 1 mM EDTA) was added. After mixing, the fluorescence (excitation, 485 nm; emission, 535 nm) was read using a BioTek's Synergy H4 microplate reader. Preliminary screening was performed at inhibitor concentrations of 100 mM and 10 mM. For the most potent compounds IC_{50} was determined with 7 concentrations of the inhibitors. IC_{50} values were calculated using GraphPad Prism software and represent the concentration of inhibitor where the residual activity of the enzyme is 50% in three independent measurements; the final result is given as their average value. Novobiocin (IC_{50} ¼ 0.17 mM (lit. 0.08 mM) for *E. coli* DNA gyrase was used as a positive control [50].

3.4. In vitro inhibitory activity screening and determination of IC_{50} values on E. coli topoisomerase IV

The assay for the determination of IC_{50} values (Inspiralis) was performed on the black streptavidin-coated 96-well microtiter plates (Thermo Scientific Pierce). The plate was first rehydrated with the supplied wash buffer (20 mM Tris-HCl (pH 7.6), 137 mM NaCl, 0.01% (w/v) BSA, 0.05% (v/v) Tween® 20) and biotinylated oligonucleotide was immobilized onto the wells. The excess of oligonucleotide was then washed off, and the enzyme assay carried out in the wells. The final reaction volume of 30 mL in buffer (40 mM HEPES KOH (pH 7.6), 100 mM potassium glutamate, 10 mM magnesium acetate, 10 mM DTT, 1 mM ATP, 0.05 mg/mL albumin) contained 1.5 U of topoisomerase IV from *E. coli* or *S. aureus*, 0.75 mg of supercoiled pNO1 plasmid, and 3 mL of inhibitors solution in 10% DMSO and 0.008% Tween® 20. Reactions were incubated for 30 min at 37 °C, and after addition of the TF buffer (50 mM NaOAc pH 5.0, 50 mM NaCl and 50 mM MgCl2), which terminated the enzymatic reaction, for another 30 min at room temperature to allow triplex formation (biotin-oligonucleotide-plasmid). The unbound plasmid was then washed off using TF buffer and the solution of SybrGOLD stain in T10 buffer (10 mM Tris-HCl pH 8.0 and 1 mM EDTA) was added. After mixing, fluorescence (excitation, 485 nm; emission, 535 nm) was read using a BioTek's Synergy H4 microplate reader. Preliminary screening was performed at inhibitor concentrations of 100 mM and 10 mM. For most potent compounds IC_{50} was determined with 7 concentrations of the inhibitors. IC_{50} values were calculated using GraphPad Prism software and represent the concentration of inhibitor where the residual activity of the enzyme is 50% in three independent measurements; the final result is given as their average value. Novobiocin (IC_{50} ¼ 11 mM (lit. 10 mM) for *E. coli* topoisomerase IV was used as a positive control.

3.5. Molecular docking studies

Docking studies of most active (determined through enzyme inhibition studies) new fluoroquinolone-1,2,4-triazole hybrids (only **7a**, **7b** and **8a–j**) into the binding pockets of DNA gyrase (PDB ID: 1SUU) and TOPO-IV (PDB ID: 1S16) was carried out using Autodock4.2.6, Autodock Tools 1.5.6 and the Arguslab version 4.0.1. 3D crystal structures were retrieved from the protein data bank (PDB) (Source:

www.rcsb.org/pdb/). All molecular docking tactics and parameters were referred from our previous reports [51–53]. Statistical mechanical analysis for the ligands and the lowest binding energy, ligand efficiency (it is used in drug discovery investigations to support in narrowing focus to candidate drugs with optimum amalgamations of physicochemical and pharmacological potentials [54]) and the inhibitory constant (*ki*) values were extracted. Molecular interaction (hydrogen bonding, π-π interaction, and π-cation interaction) results were validated.

4. Discussion

In our initial effort, to synthesis medicinally important small molecules, compound **8a**, has been selected as a model product to determine the optimum reaction conditions. In this trial, the model reaction has been carried out in polar solvent including ethanol, tetrahydrofuran and DMF in the presence of different Lewis and Bronsted acid catalysts, such as *p*-TSA, $FeCl_3$, $InCl_3$ and HCl under the microwave irradiated conditions. In all cases, progress of reaction has been monitored by TLC analysis. The condensation of the azoles, **4** and **6a–e** with fluoroquinolone class antibacterial drugs namely norfloxacin and ciprofloxacin has been carried out under the Mannich reaction conditions. A Mannich reaction which leads to the formation of aminoalkylated products has been descript as a one pot condensation between three components consisting of a substrate containing at least one active hydrogen, an aldehyde component and an amine reagent [55,56]. *N*-Mannich bases constitute important tools to development of new drugs containing amine or amide function [57]. It has been believed that the group linked to parent amine through a Mannich reaction cause to increase the absorption of molecules through bio-membrane as a result of their diminished protonation causing to increase the lipophilicity of molecules at physiological pH values [58]. Furthermore, the transformation into iminium salts render the Mannich bases to soluble in aqueous solvents [50].

Bringing the achieved compounds to medicinal applications, these compounds were initially tested for their antimicrobial effects and DNA gyrase and TOPO-IV inhibitions. DNA gyrase and TOPO-IV inhibition result of all compounds was compared to the standard drug, Novobiocin. The obtained potentially good DNA gyrase and TOPO-IV inhibition by the compounds was further validated through binding affinity calculations and molecular interaction validations. DNA gyrase and TOPO-IV are the enzymes that are highly involved in the synthesis of bacterial genetic material, especially in the DNA supercoiling processes [59,60]. Due to this reason these two enzymes are being the antibacterial drug targets. In the present study, compounds **7a**, **7b** and **8a–j** were showed remarkable antibacterial as well as effective DNA gyrase inhibition activities. Compound **8j** can be a future antibiotic.

5. Conclusion

We have designed the Microwave irradiated and conventional synthesis, antibacterial activity, DNA gyrase and topoisomerase IV inhibition of new piperazine functionalized fluoroquinolone hybrids. Compared to conventional method, by the microwave irradiation method, reaction yield and time was significantly decreased. At the same time, as a result of optimization studies for the synthesis of fluoroquinolone derivatives as target molecules, it was determined that Lewis acids and solvent were not affected. The antibacterial activity screening studies have revealed that new hybrid molecules consisting of fluoroquinolone-azole-piperazine conjugation (**7a,b** and **8a–j**) have good-excellent antibacterial activity against the test microorganisms. Furthermore, these hybrids have exhibited good DNA gyrase inhibition with the IC_{50} values varying between 0.134 and 1.84 μg/mL. To unveil the interaction mode of compounds to receptor, a molecular docking study has been performed. With an average least binding energy of −9.5 kcal/mol, all compounds have been found to possess remarkable inhibitory potentials against DNA gyrase (*E. coli*). Conclusively, considering their bio-activity potentials, perhaps highly substitute (**8a–j**) piperazine-azole functionalized fluoroquinolone hybrids (**7a,b** and **8a–j**) could be the future antibiotics.

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