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# Design and synthesis of ciprofloxacin-sulfonamide hybrids to manipulate ciprofloxacin pharmacological qualities: Potency and side effects

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ABSTRACT

Fluoroquinolones are a class of antibacterial agents used clinically to treat a wide array of bacterial infections. Although being potent, susceptibility to CNS side effects limits their use. It was observed that improvements in absorption, activity and side effects were achieved via modifications at the N atom of the C7 of the side chain. To meet the increasing demand for development of new antibacterial agents, nineteen novel ciprofloxacin-sulfonamide hybrid molecules were designed, synthesized and characterized by IR, $^{1}$H NMR and $^{13}$C NMR as potential antibacterial agents with dual DNA gyrase/topoisomerase IV inhibitory activity. Most of the synthesized compounds showed significant antibacterial activity that was revealed by testing their inhibitory activity against DNA gyrase, DNA topoisomerase IV as well as their minimum inhibitory concentration against *Staphylococcus aureus*. Six ciprofloxacin-sulfonamide hybrids (**3f**, **5d**, **7a**, **7d**, **7e** and **9b**) showed potent inhibitory activity against DNA topoisomerase IV, compared to ciprofloxacin (IC50: 0.55 μM), with IC50 range: 0.23−0.44 μM. DNA gyrase was also efficiently inhibited by five ciprofloxacin-sulfonamide hybrids (**3f, 5d, 5e, 7a** and **7d**) with IC50 range: 0.43−1.1 μM (IC50 of ciprofloxacin: 0.83 μM). Compounds **3a** and **3b** showed a marked improvement in the antibacterial activity over ciprofloxacin against both Gram-positive and Gram-negative pathogens, namely, *Staphylococcus aureus Newman* and *Escherichia coli ATCC8739*, with MIC = 0.324 and 0.422 μM, respectively, that is 4.2-fold and 3.2-fold lower than ciprofloxacin (MIC = 1.359 μM) against the Gram-positive *Staphylococcus aureus*, and MIC = 0.025 and 0.013 μM, respectively, that is 10.2-fold and 19.6-fold lower than ciprofloxacin (MIC = 0.255 μM) against the Gram-negative *Escherichia coli ATCC8739*. Also, the most active compounds showed lower CNS and convulsive side effects compared to ciprofloxacin with a concomitant decrease in GABA expression.



## 1. Introduction

Nowadays, the world faces a global crisis due to the emergence of multiple drug resistant bacterial strains which represent a serious threat to public health. This resistance gives rise to microbes that can overcome the prophylaxis or therapy longer than any other form. Diseases caused by infectious resistant bacteria, viruses and fungi remain a pressing problem worldwide that unless it is solved, we will reach 10 million deaths annually in 2050 [1,2].

In order to solve or slow down the emergence of resistance, combination therapy was one of the approaches that achieved some clinical success [3]. It depends on the combination of two or more antibiotics, inder the condition that they have different mechanisms of action, which will lessen the cell's ability to develop resistance [4]. However, the therapeutic effects in vivo will not necessarily correlate to in vitro results as the pharmacokinetic properties of drugs in combination are much likely to be varied [5,6].

Another winning strategy that was introduced in order to overcome the drawbacks of combination therapy is the design of

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hybrid antibacterial agents, it is based on the combination of two or more active structure units of antibiotics with different bacterial targets in a single molecular framework by one or more chemical bonds thus producing a potential weapon that will reduce their expected side effects. Also, hybrid strategies are popular for their effective role in preventing bacterial resistance with improved affinity, and efficacy compared with the parent drugs [2,7–9]. Since a single hybrid molecule targets bacterial cells through several modes of action, they are considered as new leads with complementary activities and/or multiple pharmacological targets. The newly designed hybrid molecule increases the opportunity to improve drugs' pharmacokinetic properties, toxicity profiles and to increase their retention [10,11]. Additionally, rationally designed linkers that connect two bioactive moieties may increase the chance to get better inhibition of both drug targets with decreased incidence of new resistance mutations, and may even overcome existing resistance mechanisms to individual drugs [12].

Fluoroquinolones (FQs) are one of the most prominent widely used classes of synthetic antibacterial agents in the treatment of several infectious diseases. This is because they offer many of the attributes of an ideal antibacterial combining high potency, broad spectrum of activity, good bioavailability, oral and intravenous formulations, high serum levels and a large volume of distribution indicating good concentrations in different tissues [13,14]. They play a key role in the treatment of urinary tract infections (UTI), upper and lower respiratory tract infections (RTI) and sexually transmitted diseases (STD). Beside their effects in curing skin, soft tissue, gastrointestinal, bones and joints infections [15,16]. They exert their bactericidal activity by targeting bacterial DNA gyrase and topoisomerase IV (Topo IV), where they bind to complexes that are formed between DNA and DNA gyrase or Topo IV forming DNA-enzyme-quinolone ternary complex. The produced complexes inhibit DNA replication and cell growth and are responsible for the antibacterial activity of fluoroquinolones [17].

In 1987, Ciprofloxacin which is considered one of the most popular drugs among the fluoroquinolones class was approved for clinical use. Recently, other related derivatives have been designed and synthesized. One approach, to generate new potent ciprofloxacin derivatives is by modifying N atom of the C-7 side chain through addition of a functional moiety [15]. This position acts as the most promising area for regulating drug characters and thus changes at this position can be introduced to upgrade potency, spectrum, absorption and safety of the parent drug [18,19]. Studies reported the importance of lipophilicity, which can be increased by adding substituents at this position, to ciprofloxacin antibacterial activity [20].

Unfortunately, quinolone therapy was correlated with frequent central nervous system (CNS) adverse effects including headache, dizziness, sleep disorders, agitation and scarcely convulsions. These effects are due to direct CNS interaction through binding of quinolones to γ-aminobutyric acid (GABA) receptors in the brain, hence preventing normal binding of GABA, and thus resulting in decreased GABA inhibitory activity and increased CNS stimulation. Studies revealed that the substituent at position 7 has a prominent effect on the direct CNS interaction, where it was reported that the degree of GABA inhibition was high in ciprofloxacin and norfloxacin having unsubstituted piperazine at the 7 position, whereas substituting NH of piperazine with methyl as in ofloxacin markedly decreased the degree of GABA inhibition [21,22].

In the last 20 years, literature reported the synthesis of many fluoroquinolone hybrids. Roche developed Ro 23−9424, which is a hybrid of cephalosporin and quinolone. Ro 23−9424 showed broad and potent in vitro and in vivo antibacterial activities. As a result of this, new hybrids are produced like MCB3837 (oxaquin) and CBR-2092, these hybrids have been entered in human clinical trials, and may be used in clinical practice to struggle against various upcoming diseases [23] (Fig. 1).

Sulfonamides are synthetic antimicrobial agents that are popular for their use in the treatment of bacterial infections as well as fungal ones. Their stability, bioavailability, broad spectrum of activity, beside their ease of preparation nominate them to be incorporated in massive number of drugs and encourage their continuous study. Sulfonamides inhibit the bacterial enzyme dihydropteroate synthetase (DPS) in the folic acid pathway [24]. Many hybrid drugs incorporating a sulfonamide moiety with different antibacterial agents including fluoroquinolone were reported in the literature [25−29]. In 2000, Alovero *et al.* reported the synthesis of benzylsulfonamide-ciprofloxacin hybrids through direct coupling of the benzenesulfonylamido group to the C7 piperazinyl ring. The prepared compounds were 10-fold more active than ciprofloxacin against *Staphylococcus aureus* and *Staphylococcus pneumoniae* and showed much potent inhibitory activity against gyrase enzyme [28]. In view of the aforementioned findings and in our attempt to search for effective antibacterial agents with fewer side effects, herein we report the design and synthesis of new ciprofloxacin- sulfonamide hybrids using different linkers (**3a-f**, **5a-e, 7a-e** and **9a-c**) with the prime aim of improving ciprofloxacin qualities as increasing potency and lipophilicity, as well as decreasing its CNS side effects and decreasing the incidence of new resistance mutations Fig. 2. All the synthesized compounds were tested for their inhibitory activities toward DNA gyrase, DNA topoisomerase IV, minimum inhibitory concentration against *Staphylococcus Aureus* and *Escherichia coli.* Besides, the most active compounds were tested for CNS side effects mainly convulsive activity and GABA expression and compared to the ciprofloxacin as the reference drug. Molecular docking studies were performed at DNA gyrase and DNA topoisomerase IV active sites to study the binding modes of our target compounds.

## 2. Results and discussion

### 2.1. Chemistry

In this paper, we aimed to synthesize four series of ciprofloxacin-sulfonamide hybrids through four different linkers. The synthetic pathways employed to prepare the target hybrids (**3a-g**, **5a-e, 7a-e** and **9a-c**) are depicted in Schemes 1 and 2.

The first series 1-cyclopropyl-6-fluoro-4-oxo-7-(4-((4-(un) substituted sulfamoylphenyl) diazenyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid **3a-f** involved a diazenyl

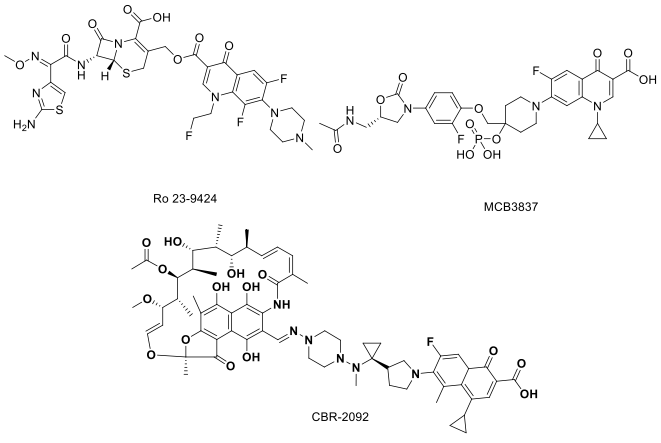



**Fig. 1.** Examples of fluoroquinolone hybrids that have entered clinical trials.

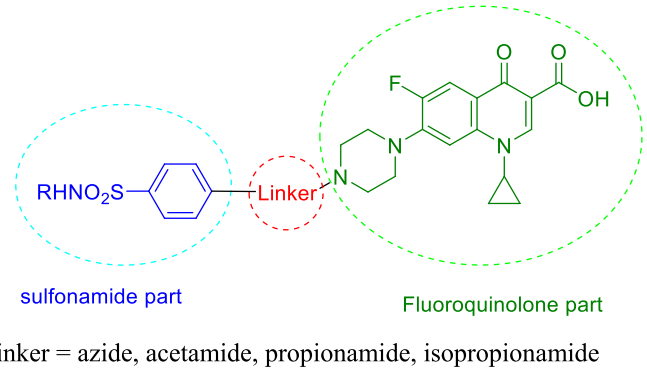
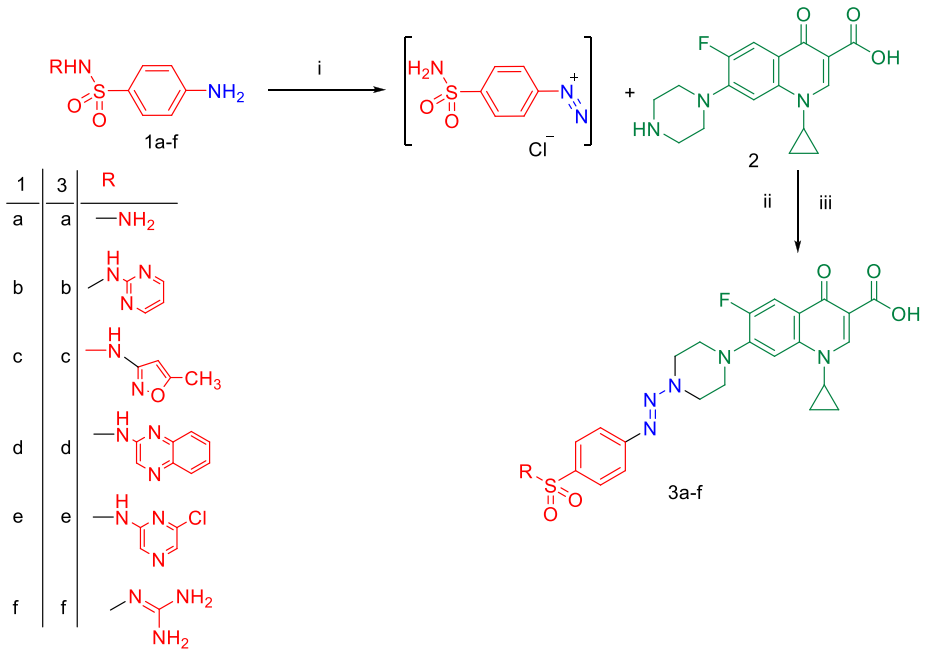



**Fig. 2.** General structure of the targeted compounds.

linker and was prepared through the reaction of ciprofloxacin with the diazonium salt of the appropriate sulfonamide **1a-f** at pH **6** (Scheme 1). The chemical structures of the new compounds (**3a-f**) were confirmed by their IR spectra which indicated the appearance of 2 bands at 1385-1307 and 1177-1149 $cm^{-1}$ corresponding to $SO_2$ group stretching, beside the appearance of two C=O stretching bands at 1740-1713 $cm^{-1}$ and 1628-1620 $cm^{-1}$, respectively. $^1H$ NMR spectra of compounds **3a-f** revealed the presence of an exchangeable signal at 14.76–15.19 ppm attributed to the acidic OH proton. The signal corresponding to $CH_2$ of piperazine was shifted downfield from 2.70 ppm (ciprofloxacin) to 3.40–4.09 ppm. This is beside the appearance of two new doublets in the aromatic region between 7.30 and 8.57 ppm corresponding to the phenyl protons of the sulfonamide moieties. $^{13}C$ NMR spectra of compounds **3a-f** showed two signals resonating between 166.4 and 176.8 ppm corresponding to the two C=O groups of the quinolone and carboxylic acid moiety. Also the aromatic region showed several signals equal to the number of carbons of the fluoroquinolone and the sulfonamide counterparts between 95 and 160 ppm. A characteristic doublet appeared at 153.1–153.4 ppm assigned to the C–F carbon with a coupling constant of 206–248 Hz, this is beside the appearance of other doublets with smaller coupling constants due to the splitting of the farther carbons by the same fluorine atom.

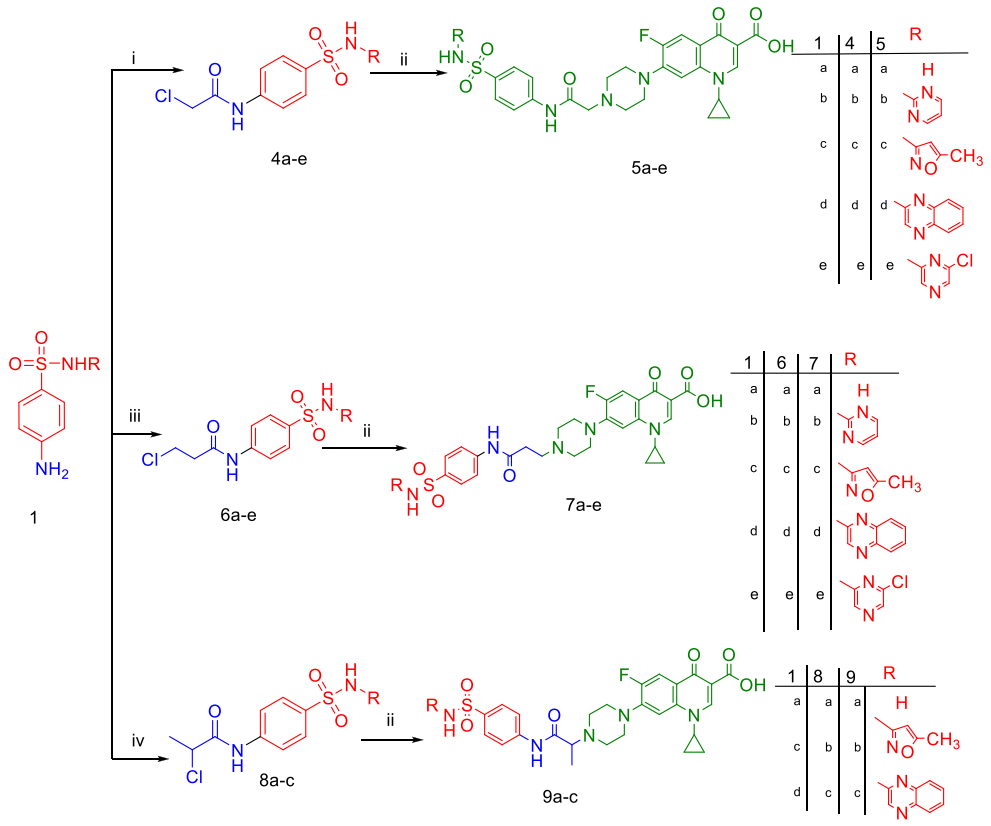
The ciprofloxacin-sulfonamide hybrids **5a-e, 7a-e** and **9a-c** involving acetamide, 3-propanamide and 2-propanamide linkers, respectively, were synthesized according to the reactions depicted in Scheme 2. These targets were synthesized first through acylation of the appropriate sulfonamide **1a-e** with the appropriate chloroacyl chloride in dry DMF. The desired acyl derivatives **4a-e, 6a-e** and **8 a-c** were obtained in good yields under mild conditions where the reaction proceeded at room temperature and without the need of a catalyst [30]. The obtained acyl derivatives were then reacted with ciprofloxacin **2** in DMF, the reaction was carried out through heating under reflux in presence of triethylamine as a basic catalyst.

The chemical structures of compounds **5a-e**, **7a-e** and **9a-c** were confirmed by their spectral data and elemental analyses. IR spectra revealed the appearance of two bands at 1357-1312 and 1192-1157 $cm^{-1}$ corresponding to the $SO_2$ group, beside the presence of a broad carboxylic OH band, two NH bands and three C=O bands at 3600-3300 $cm^{-1}$, 3440-3229 $cm^{-1}$ and 1730- 1628 $cm^{-1}$, respectively. $^1H$ NMR spectra of all compounds revealed the presence of signals corresponding to the aromatic protons of the fluoroquinolones and the sulfonamide counterparts at 6.08–8.68 ppm. Additionally, $^1H$ NMR spectra of compounds **5a-e** revealed the appearance of singlet signals at 3.29–3.35 ppm corresponding to the $CH_2$ of the acetyl moiety, these peaks have been shifted upfield compared to their corresponding starting compounds. In the $^1H$ NMR spectra of compounds **7a-e,** two triplet signals resonated at 2.57–2.65 and 2.75–2.89 ppm corresponding to $NCH_2CH_2CO$ protons. $^1H$ NMR spectra of compounds **9a-c** revealed the presence of a doublet signal at 1.23–1.27 ppm corresponding to the three methyl




Reagents and reaction conditions: (i) Sodium nitrite/ 2N HCl, ice bath 0 oC; (ii) Sodium acetate, ice bath 0 oC, 1 h: (iii) Sodium acetate, R.T, 24 h

**Scheme 1.** Synthesis of target compounds 3a-f.




Reagents and reaction conditions: (i) Chloroacetyl chloride, dry DMF, RT, 5 h; (ii) Ciprofloxacin, dry DMF/ TEA, reflux, 17 h;(iii) 3-Chloropropionyl chloride, dry DMF, RT, 5 h; (iv) 2-Chloropropionyl chloride, dry DMF, RT, 5 h.

**Scheme 2.** Synthesis of target compounds 5a-e, 7a-e and 9a-c.

protons of the propionyl group, and either a broad singlet or multiplet signals at 3.34–3.63 ppm corresponding to the CH proton adjacent to the carbonyl group. $^{13}$C NMR spectra showed three signals at 166.0–176.8 ppm corresponding to three C=O groups, beside the appearance of one or two additional signals in the aliphatic region corresponding to the added carbons of the acetyl

**Table 1**
The IC50 (μM) of the newly synthesized hybrids and the standard drug, ciprofloxacin, against topoisomerase IV enzyme and Escherichia coli DNA gyrase enzyme.

| Compound | *Staphylococcus aureus* TOPO IV aIC50 μM | *Escherichia coli* DNA Gyrase IC50 μM |
|---|---|---|
| **3 a** | 2.43 ± 0.11 | 3.56 ± 0.19 |
| **3b** | 9.5 ± 0.43 | 12.8 ± 0.7 |
| **3c** | 2.58 ± 0.12 | 1.95 ± 0.11 |
| **3d** | 12.8 ± 0.58 | 5.86 ± 0.32 |
| **3e** | 12.8 ± 0.58 | 13 ± 0.71 |
| **3f** | 0.64 ± 0.03 | 0.77 ± 0.04 |
| **5 a** | 3.77 ± 0.17 | 1.32 ± 0.07 |
| **5b** | 2.08 ± 0.09 | 2.87 ± 0.16 |
| **5c** | 4.66 ± 0.21 | 1.33 ± 0.07 |
| **5d** | 0.35 ± 0.02 | 0.55 ± 0.03 |
| **5e** | 2.29 ± 0.1 | 0.9 ± 0.05 |
| **7 a** | 0.6 ± 0.03 | 1.1 ± 0.06 |
| **7b** | 1.8 ± 0.08 | 1.99 ± 0.11 |
| **7c** | 3.91 ± 0.18 | 6.31 ± 0.34 |
| **7d** | 0.23 ± 0.01 | 0.43 ± 0.02 |
| **7e** | 0.44 ± 0.02 | 1.24 ± 0.07 |
| **9 a** | 6.66 ± 0.3 | 5.65 ± 0.31 |
| **9b** | 0.39 ± 0.02 | 1.27 ± 0.07 |
| **9c** | 1.21 ± 0.05 | 1.44 ± 0.08 |
| **Ciprofloxacin (2)** | 0.55 ± 0.02 | 0.83 ± 0.04 |

[a]IC50 value: compound concentration required to produce 50% inhibition of Topoisomerase IV enzyme and *Escherichia coli* DNA gyrase enzyme.

**Table 2**
The minimum inhibitory concentration of the tested compounds.

| Tested compound | *Staphylococcus aureus Newman* MIC (μM) | *Escherichia coli ATCC8739* MIC (μM) |
|---|---|---|
| 3a | **0.324 ± 0.14** | 0.025 ± 0.009 |
| 3b | **0.422 ± 0** | 0.013 ± 0 |
| 3c | **0.839 ± 0** | 1.119 ± 0.485 |
| 3d | 5.187 ± 1.797 | 0.389 ± 0 |
| 3e | 1.595 ± 0 | 1.329 ± 0.46 |
| 3f | 1.797 ± 0 | 2.995 ± 1.037 |
| 5a | 7.359 ± 6.373 | 12.265 ± 4.249 |
| 5b | 1.609 ± 0 | 1.341 ± 0.464 |
| 5c | 3.202 ± 0 | 7.471 ± 4.891 |
| 5d | 1.985 ± 0.86 | 0.744 ± 0 |
| 5e | 1.524 ± 0 | 2.540 ± 0.88 |
| 7a | 2.989 ± 1.035 | 0.897 ± 0 |
| 7b | 1.573 ± 0 | 2.622 ± 0.908 |
| 7c | 1.044 ± 0.452 | 5.219 ± 1.808 |
| 7d | **0.972 ± 0.421** | 0.608 ± 0.21 |
| 7e | **286.520 ± 135.067** | 286.52 ± 135.067 |
| 9a | 1.495 ± 0.518 | 0.897 ± 0 |
| 9b | **0.783 ± 0** | 1.044 ± 0.452 |
| 9c | **0.729 ± 0** | 1.944 ± 0.842 |
| Ciprofloxacin HCl | 1.359 ± 0 | 0.255 ± 0.147 |

and propionyl group, respectively. Again characteristic doublets appeared at 153.1–153.4 ppm assigned to the C–F carbon with coupling constants of 206–248 Hz, beside the appearance of other doublets with smaller coupling constants due to the splitting of the farther carbons. Additionally, all the newly synthesized compounds were further confirmed by their mass spectra which showed molecular ion peaks corresponding to the molecular weights of the investigated compounds.

## 2.2. Enzyme assay and antibacterial activity

### 2.2.1. Enzyme assessment of DNA gyrase and topoisomerase IV

All the synthesized compounds were evaluated for their inhibitory activity against the bacterial DNA Gyrase enzyme from *Escherichia coli* and DNA topoisomerase IV enzyme from *Staphylococcus aureus*, since gyrase seems to be more important to some gram negative bacteria and topoisomerase IV seems to be more important to some gram-positive bacteria [31].

*2.2.1.1. Staphylococcus aureus topoisomerase IV decatenation assay.* All the 19 synthesized compounds were screened for their ability to inhibit topo IV decatenation (using *Staphylococcus aureus* bacterial enzyme). Ciprofloxacin was used as a reference standard. The results were recorded in Table 1 and illustrated in Figs. S1 and S2 (supplementary information). From the results we can see that only few compounds, namely 5d, 7d, 7e and 9b, were better inhibitors than ciprofloxacin itself with IC50 range: 0.23–0.44 μM, IC50 of ciprofloxacin (2): 0.55 μM, and the improvement was modest in case of compounds 5d, 7e and 9b. Only compound 7d was found much potent than ciprofloxacin with 2.4-fold increased inhibitory activity. Besides, compounds 3f and 7a (IC50: 0.64 and 0.6 μM, respectively) showed comparative inhibitory activity to that of the tested standard. Looking deep in the results revealed that incorporating an amide linker was more beneficial for the activity than incorporating a triazene linker. All the hybrids incorporating a triazene linker showed decreased activity (IC50: 2.43–12.8 μM), except for the hybrid with sulfaguanidine moiety (IC50: 0.64 μM), compared to the amide containing compounds (IC50 : 0.23–6.66 μM). Also, it was observed that combining a sulfaquinoxaline and amide linkers markedly increased the inhibitory activity of the tested compounds as exemplified by the most potent derivatives 5d and 7d (IC50: 0.35 and 0.23 μM, respectively).

Amongst the compounds containing acetyl linker, compound 5d which is a sulfaquinoxaline hybrid showed the most potent activity with IC50: 0.35 μM. conserving the sulfaquinoxaline but changing the acetyl linker into a straight propionyl linker (compound 7d) increased the activity into 0.23 μM, while changing the acetyl and the straight propionyl with a branched propionyl linker (compound 9c) markedly decreased the activity to 1.21 μM.

**Table 3**
Incidence of clonic convulsion and death in mice after a single I.V. injection of Ciprofloxacin and the tested compounds (0.6 mmol/kg body weight).

| Compound | No. of animals experiencing the indicated event/total no. | |
|---|---|---|
| | convulsions | death |
| **3f** | 0/6 | 0/6 |
| **5d** | 2/6 | 0/6 |
| **7d** | 0/6 | 0/6 |
| **9b** | 1/6 | 0/6 |
| **Control** | 0/6 | 0/6 |
| **ciprofloxacin (2)** | 3/6 | 1/6 |

*Results show significant difference in incidence of convulsions between treated groups and ciprofloxacin at $P < 0.1$, ($n = 6$) using Chi square test.
• Each bar with vertical line represents mean ± SD. of 6 rats per group. * significantly different from control, # significantly different from Ciprofloxacin, @ significantly different from 3f, $ significantly different from 5d, & significantly different from 7d using one-way ANOVA followed by Tukey's multiple comparison test; $p < 0.05$.

*2.2.1.2. Escherichia coli DNA gyrase supercoiling assay.* *Escherichia coli* DNA gyrase was used as a model bacterial type-II topoisomerase to assess the activities of the 19 synthesized compounds compared to ciprofloxacin. The results were recorded in Table 1 and illustrated in Figs. S3 and S4 (supplementary information). Results revealed that only few compounds, namely 3f, 5d and 7d were better inhibitors than ciprofloxacin itself and the improvement was modest ranging from 2.0 to 1.1 fold increase in the inhibitory activity, with IC50 range: 0.43–0.77 μM compared to IC50: 0.83 μM for ciprofloxacin (2). Other two compounds, 5e and 7a (IC50 0.9 and 1.1 μM, respectively) showed similar inhibitory activity to that of ciprofloxacin (2) activity against DNA gyrase. Again, compounds 5d and 7d containing sulfaquinoxaline linked to ciprofloxacin through acetamide linker and a straight propionyl amide linker showed the most potent inhibitory activity compared to the other tested sulfonamides, with IC50: 0.55 and 0.43 μM respectively.

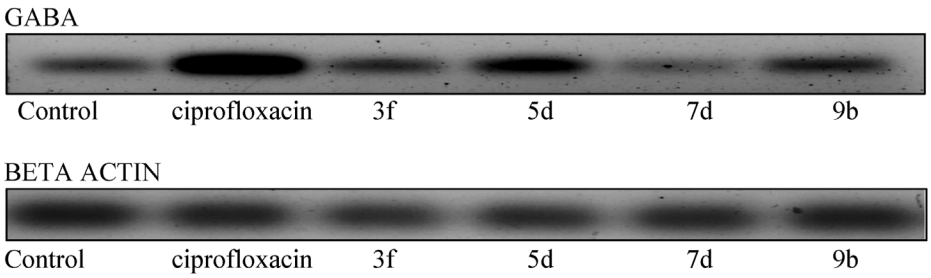



**Fig. 3.** GABA receptor expression in western *blot.*

**Table 4**
Types of interactions for the complexes formed from **Ciprofloxacin (2)** and compounds **3f, 5d, 7d** and **9b** in the active sites of DNA gyrase.

| Cpd no. | Energy score | Amino acid interaction | Interaction bonds | Bond length and interacting groups |
|---|---|---|---|---|
| 3f | −9.777 | Serine A1084 | 1 H-bond | Ser A1084 forms H bond of length 3.14 Å with oxygen of C═O |
| | | Glycine A459 | backbone acceptor | Gly A459 with the nitrogen of azide group |
| 5d | −10.811 | Serine A1084 | 1 H-bond | Ser A1084 forms H bond of length 2.88 Å with oxygen of carboxylate |
| | | Arginine A458 | hydrophobic | Arg A458 with the quinoxaline moiety |
| 7d | −9.310 | Serine A1084 | 1 H-bond | Ser A1084 forms H bond of length 3.14 Å with oxygen of carboxylate |
| | | Lysine A460 | 1 H-bond | Lys A460 forms H bond of length 2.94 Å with oxygen of sulfone |
| 9b | −10.293 | Serine A1084 | 1 H-bond | Ser A1084 forms H bond of length 3.08 Å with oxygen of carboxylate |
| 2 (ciprofloxacin) | −9.070 | Serine A1084 | 2 H- bonds | Ser A1084 forms H bond of length 2.49 Å with oxygen of C═O |

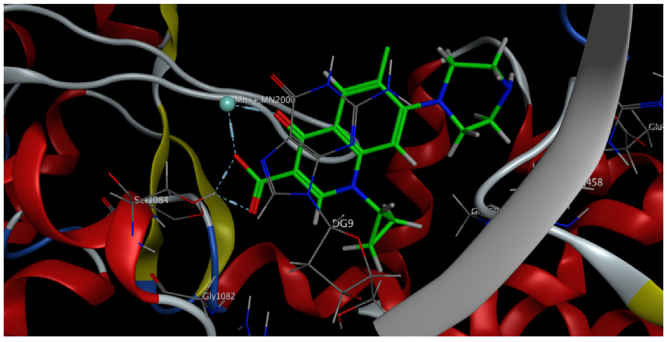



**Fig. 4.** 3D drawings of Ciprofloxacin in the active sites of DNA gyrase.

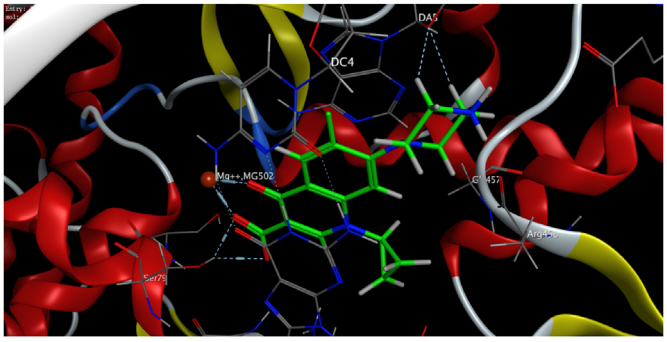



**Fig. 5.** 3D drawings of Ciprofloxacin in the active sites of DNA topoisomerase IV.

**Table 5**
Types of interactions for the complexes formed from **Ciprofloxacin (2)** and compounds **3f, 5d, 7d** and **9b** in the active sites of DNA Topoisomerase IV.

| Cpd no. | Energy score | Amino acid interaction | Interaction bonds | Bond length and interacting groups |
|---|---|---|---|---|
| 3f | −10.385 | Serine A79 | 1 H-bond | Ser A79 forms H bond of length 2.83 Å with oxygen of C═O |
| | | | Backbone acceptor | Ser A79 forms H bond with the oxygen of sulfone |
| | | Aspartate A78 | 1 H-bond | Asp A78 forms H bond of length 3.22 Å with $NH_2$ of guanidine |
| | | Lysine C48 | Backbone acceptor | Lys C48 with the fluoro of fluoroquinolone counterpart. |
| 5d | −11.217 | Serine A79 | 1 H-bond | Ser A79 forms H bond of length 3.02 Å with oxygen of carboxylate |
| | | Serine A80 | 1 H-bond | Ser A80 forms H bond of length 2.80 Å with oxygen of carbonyl of fluoroquinolone |
| | | Arg B117 | 1 H-bond | Arg B117 forms H bond of length 3.44 Å with oxygen of sulfone |
| 7d | −12.267 | Serine A79 | 1 H-bond | Ser A79 forms H bond of length 2.87 Å with oxygen of carboxylate |
| | | Aspartate A78 | 1 H-bond | Asp A78 forms H bond of length 3.51 Å with nitrogen of sulfaquinoxaline |
| | | Serine A79 | Backbone acceptor | Ser A79 with the oxygen of sulfone |
| 9b | −11.398 | Serine A79 | 1 H-bond | Ser A79 forms H bond of length 3.0 Å with oxygen of carboxylate |
| | | Aspartate A83 | 1 H-bond | Asp A83 forms H bond of length 3.07 Å with NH of isoxazole ring |
| 2 (ciprofloxacin) | −10.315 | Serine A79 | 2 H- bonds | Ser A79 forms H bond of length 3.01 and 3.04 Å with oxygen of C═O |

Analyzing the results revealed that incorporating an amide linker was more beneficial for the activity than incorporating triazene linker. All the hybrids incorporating triazene linker showed decreased activity (IC50: 1.95–13.00 μM), except for the hybrid with sulfaguanidine (IC50: 0.77 μM) moiety, compared to the amide containing compounds (IC50: 0.43–6.31 μM).

Amongst the compounds containing acetyl linker, compound 5d which is a sulfaquinoxaline hybrid showed the most potent activity with IC50: 0.55 μM, conserving the sulfaquinoxaline but replacing the acetyl linker with a straight propionyl linker (compound 7d) increased the activity into 0.43 μM, while changing the acetyl and the straight propionyl with a branched propionyl linker (compound 9c) markedly decreased the activity to 1.44 μM.

*2.2.2. Minimum inhibitory concentration determination*

All the nineteen prepared final compounds were tested for their minimum inhibitory concentrations (MIC) against the gram positive and gram negative microorganisms, *Staphylococcus aureus Newman and Escherichia coli ATCC8739*, respectively using broth microdilution method. Ciprofloxacin was used as a positive control. Test was done at the Microbiology and Immunology Department, Faculty of Pharmacy, Cairo University and results were recorded in Table 2.

Analyzing the MIC results against *Staphylococcus aureus Newman* revealed that compounds **3a** and **3b** showed a marked improvement in activity over ciprofloxacin, with MIC = 0.324 and 0.422 μM, respectively, that is 4.2-fold and 3.2-fold lower than ciprofloxacin (MIC = 1.359 μM). All other compounds -except **3d**, **5a**, **5c**, **7a** and **7e**-showed MIC values (0.729–1.985 μM) comparable to that of ciprofloxacin. Compound **7e** showed an exceptional very high MIC value (286.52 μM). Genarally compounds involving a triazene linker **3** showed better MIC range (0.324–5.187 μM) than those with amide linkers **5**, **7** and **9** (MIC: 0.729–7.359 μM, compound **7e** showed exceptional high MIC). Compounds **3a** and **3b** containing a triazene linker with sulfanilamide and sulfadiazine, respectively stood out as the most potent compounds with MIC: 0.324 and 0.422 μM, respectively. However, among the hybrids containing amide linkers, it was observed that combining a straight or branched propanamide linker with sulfamethoxazole moiety or sulfaquinoxaline moiety (**7c**, **7d**, **9b** and **9c,** MIC: 1.044, 0.972, 0.783 and 0.729 μM, respectively) was beneficial for the antibacterial activity.

Analyzing the MIC results against *Escherichia coli ATCC8739* revealed that again compounds **3a** and **3b** containing a triazene linker with sulfanilamide and sulfadiazine stood out showing a marked improvement in activity over ciprofloxacin, with MIC = 0.025 and 0.013 μM, respectively, that is 10.2-fold and 19.6-fold lower than ciprofloxacin (MIC = 0.255 μM). A single compound **3d** showed MIC value (0.389 μM) comparable to that of ciprofloxacin. Compounds involving a triazene linker **3** showed better MIC range (0.013–2.995 μM) than those with amide linkers **5**, **7** and **9** (MIC: 0.608–12.265 μM, again compound **7e** showed exceptional high MIC). It was also observed that generally, hybrid molecules containing sulfanilamide moiety namely, **3a**, **7a** and **9a** or sulfaquinoxaline moiety namely, **3d, 5d** and **7d** showed better MIC values (0.025, 0.897, 0.897, 0.389, 0.744, 0.608 μM, respectively) than the other sulfonamides.

*2.2.3. In vivo, evaluation of convulsive activity*

In our study, the convulsive activity of the four most active tested compounds **3f**, **5d**, **7d** and **9b** against bacterial topoisomerase IV and DNA gyrase were evaluated for their convulsive adverse effect and the mortality rate compared to ciprofloxacin as a reference drug, using equimolar doses to the dose of ciprofloxacin reported to cause convulsions in 50% of mice (0.6 mmol/kg body weight) [32]. The experimental tests were performed at the Pharmacology and Toxicology department in accordance with the Institutional Ethical Committee approval, Faculty of Pharmacy, Cairo University (protocol serial approval number: PC 2345). The results were recorded in Table 3. Also the separated brains were subjected to measuring GABA expression by western blot and illustrated in Fig. 3. All the tested compounds showed zero mortality rate and lower incidence or zero incidence of convulsions compared to the reference drug. Also, the tested compounds showed lower GABA expression compared to ciprofloxacin which proves that the newly designed compounds have lower or even no interaction with GABA receptors. This indicates that the tested compounds possess low or no CNS side effects.

*2.3. Molecular modeling studies*

Molecular docking is used to predict orientation of the most active compounds in each series **(3f, 5d 7d** and **9b**) inside the target protein. It predicts all possible conformations that could participate in the interpretation of the biological activity. Four compounds were evaluated using the molecular docking protocol by Molecular Operating Environment (MOE, 10.2015) software to make a computational prediction of the binding mode of these compounds with the enzyme. The X-ray crystallographic structures of DNA gyrase of *Staphylococcus aureus* co-crystallized ternary structure with ciprofloxacin as inhibitor (PDB ID: 2XCT) and DNA cleavage complex of type IV topoisomerase from *S. pneumoniae* co crystalized with levofloxacin as inhibitor (PDB ID: 3RAE) were downloaded from the protein data bank (http://www.rcsb.org/) [33–35].

For DNA gyrase: According to the docking study, all the docked compounds bind very much like ciprofloxacin with the sulfonamide moiety occupying an extended pocket for the bulky C-7 substituent. All the hybrid molecules exhibited interaction with $Mn^{2+}$ and most of them interacted with **Serine 1084** residue through one hydrogen bond. Compound **7d** formed an additional H-bond with **Lysine A460**. **Glycine A459 and Arginine A458** exhibited hydrophobic interactions in **3f** and **5d**, respectively. From the docking results depicted in Table 4 and Fig. S5 (supplementary information), it was shown that the four studied compounds showed good binding interactions to DNA gyrase which explains the potent anti-bacterial activity of these compounds, Fig. 4.

For DNA topoisomerase IV: According to the docking study, all the hybrid compounds, except **7d** and **9b**, bind very much like ciprofloxacin with the sulfonamide moiety occupying an extended pocket for the bulky C-7 substituent. All the docked compounds exhibited interaction with $Mg^{2+}$ and all the tested compounds interacted with **Serine A79** residue through one hydrogen bond. This was in addition to hydrophobic interactions that were revealed with compounds **3f** and **7d**, these two compounds **3f** and **7d** formed an additional H-bond with **Aspartate A78**. Other additional hydrogen bond interactions were revealed with the studied compounds as the hydrogen bonds between compound **5d** and **serine A80** and **arginine B117**, as well as compound **9b** with **Aspartate A83**. One more hydrophobic interaction with **Lysine C48** was observed with compound **3f**. (Table 5 and Fig. S6 (supplementary information). The aforementioned binding modes of the newly synthesized compounds and DNA topoisomerase IV explain the good antibacterial activity of these compounds, Fig. 5.

## 3. Conclusion

The current study describes the synthesis of nineteen ciprofloxacin-sulfonamide hybrid molecules **3a-f**, **5a-e, 7a-e** and **9a-c** based on six sulfonamides (sulfanilamide, sulfadiazine, sulfaquinoxaline, sulfamethoxazole, sulfaclozine and sulfaguanidine). They were evaluated for their in vitro inhibitory activity against DNA topoisomerase IV and DNA gyrase. Against DNA topoisomerase IV, six ciprofloxacin-sulfonamide hybrids (**3f**, **5d**, **7a**, **7d**, **7e** and **9b**) showed potent inhibitory activity with IC50 range: 0.23–0.44 μM. DNA gyrase was also efficiently inhibited by five ciprofloxacin-sulfonamide hybrids (**3f, 5d, 5e, 7a** and **7d**) with IC50 range: 0.43–1.1 μM. Furthermore all the synthesized hybrids were tested for their minimum inhibitory concentration against the Gram-positive and Gram-negative pathogens, namely, the standard strains: *Staphylococcus aureus Newman* and *Escherichia coli ATCC8739*, where Compounds **3a** and **3b** showed a marked improvement in the antibacterial activity over ciprofloxacin against both Gram-positive and Gram-negative pathogens, namely, *Staphylococcus aureus Newman* and *Escherichia coli ATCC8739*, with MIC = 0.324 and 0.422 μM, respectively, that is

4.2-fold and 3.2-fold lower than ciprofloxacin (MIC = 1.359 μM) against the Gram-positive *Staphylococcus aureus*, and MIC = 0.025 and 0.013 μM, respectively, that is 10.2-fold and 19.6-fold lower than ciprofloxacin (MIC = 0.255 μM) against the Gram-negative *Escherichia coli ATCC8739*. As compounds **3f**, **5d**, **7d** and **9b** emerged as the most potent inhibitors against bacterial topoisomerase IV and DNA gyrase, they were selected to be tested in *vivo* for their CNS side effects by evaluating their convulsive activity and recording mortality rates at higher doses. All the tested compounds showed zero mortality rate and lower incidence or zero incidence of convulsions compared to the reference drug. The four tested compounds showed marked lower GABA expression in the western blot test compared to ciprofloxacin. Results indicate that the tested compounds possess low or even no CNS side effects. Finally, a docking study was carried out which showed that nearly all the hybrid compounds bind very much like ciprofloxacin with the sulfonamide moiety occupying an extended pocket for the bulky C-7 substituent.

## 4. Experimental protocols

### 4.1. Chemistry

#### 4.1.1. General methods

All reagents are purchased from Sigma-Aldrich or Alfa Aesar and used without further purification. The starting material 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (Ciprofloxacin) was purchased from Memphis pharmaceuticals and used without further purification. Melting points are uncorrected and were carried out by open capillary tube method using Stuart SMP3 melting point apparatus (United Kingdom). IR spectra were recorded using Shimadzu Infrared spectrometer IR Affinity-1 (FTIR- 8400S-Kyoto-Japan) at the Micro Analytical center, Faculty of pharmacy, Cairo University. $^1$H NMR and $^{13}$C NMR spectra were recorded in $\delta$ scale given in ppm on a Bruker spectrometer (Germany) at 400 MHz and 100 MHz, respectively at the Micro Analytical center, Faculty of Pharmacy, Cairo University, and peaks were related to that of the solvents. As for the proton magnetic resonance, $D_2O$ was carried out for NH and OH exchangeable protons. Elemental analyses were carried out at the Regional Center for Mycology and Biotechnology, AL-Azhar University. Mass spectra were recorded using Shimadzu Gas chromatograph Mass spectrometer-Qp 2010 plus (Japan) at Cairo University and on Thermo Scientific ISOLT mass spectrometer at the Regional Center for Mycology and Biotechnology, AL-Azhar University. All reactions were followed by TLC using silica gel F254 plates (Merck), using chloroform: methanol (9.5: 0.5) as an eluting system and were visualized under U.V light of 254 nm. All compounds were chemically named using the chemical name facility of ChemDraw Ultra software V 14.

#### 4.1.2. Synthesis of triazenes (Scheme 1)

A suspension of the appropriate sulfonamide (**1a-f)** (2.56 mmol) in a mixture of 2 N hydrochloric acid (3 mL) and dimethylformamide (DMF) (2 mL) was cooled to 0–5 °C using an ice bath. A solution of sodium nitrite (0.18 g, 2.56 mmol) in water (3 mL) was then added dropwise with stirring and the mixture was stirred at this temperature for 1 h. The pH was adjusted to 6 by the addition of saturated solution of sodium acetate followed by the addition of an amount of 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (**2**) (0.85 g, 2.56 mmol) and, after stirring for 1 h, the mixture was neutralized using sodium acetate and left to stir for 24 h at room temperature, the formed precipitate was collected by filtration, washed with cold water and crystallized from methanol [36,37].

*4.1.2.1. 1-Cyclopropyl-6-fluoro-4-oxo-7-(4-((4–sulfamoylphenyl) diazenyl) piperazin-1-yl) –1,4-dihydroquinoline-3-carboxylic acid (3a).* Yield: (1.1 g) 83.5%. Mp:200–202 °C. IR (KBr) υmax/cm$^{-1}$: 3414, 3400 ($NH_2$, NH, OH overlapped), 3086, 3066, 3035 (CH Ar), 2843 (CH aliphatic), 1713, 1627 (2C = O), 1335, 1153 ($SO_2$). $^1$H NMR (400 MHz, DMSO) $\delta$ ppm: 1.20 (s, 2H, $CH_2$ of cyclopropyl), 1.33 (s, 2H, $CH_2$ of cyclopropyl), 3.53–3.60 (m, 4H,piperazine), 3.84–3.86 (m, 1H, CH of cyclopropyl), 4.00–4.09 (m, 4H, piperazine), 7.54 (s, 2H, $NH_2$, exch. $D_2O$), 7.61 (d, 2H, $J$ = 8.32 Hz, ArH), 7.64 (d, 1H, $J$ = 7.56 Hz, ArH), 7.83 (d, 2H, $J$ = 8.56 Hz, ArH), 7.96 (d, 1H, $J$ = 13.16 Hz, ArH), 8.68 (s, 1H,ArH), 15.19 (s, 1H, OH, exch. $D_2O$). $^{13}$C NMR (100 MHz, DMSO) $\delta$ ppm: 8.1, 36.4, 43.2, 46.9 (aliphatic carbons), 107.1 (d, $^3J_{F\text{-}c}$ = 4 Hz), 111.6 (d, $^2J_{F\text{-}c}$ = 13 Hz), 119.2 (d, $^3J_{F\text{-}c}$ = 8 Hz), 120.1, 120.9, 126.9, 127.3, 139.6, 141.3, 148.5 (d, $^2J_{F\text{-}c}$ = 27 Hz), 152.7, 153.2 (d, $^1J_{F\text{-}c}$ = 247 Hz) (Ar carbons), 166.5, 176.8 (C=O).MS (*m/z*, %): 514.94 (M+, 64.35), 513.13 ($M^+$ – 1, 25.61). Elem. anal. For $C_{23}H_{23}FN_6O_5S$ (514.53), calcd: C, 53.69; H, 4.51; N, 16.33. Found:C, 53.83; H, 4.69; N,16.50.

*4.1.2.2. 1-Cyclopropyl-6-fluoro-4-oxo-7-(4-((4-(N-(pyrimidin-2-yl) sulfamoyl) phenyl) diazenyl) piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (3b).* Yield: (1.2 g) 79.11%. Mp: 251–254 °C. IR (KBr) $\upsilon_{max}$/cm$^{-1}$: 3417, 3300 (NH + OH overlapped), 3062 (CH Ar), 2843 (CH aliphatic), 1740, 1620 (2C = O) 1307, 1149 ($SO_2$). $^1$H NMR (400 MHz, DMSO) $\delta$ *ppm*: 1.20 (s, 2H, $CH_2$ of cyclopropyl), 1.33 (d, 2H, $J$ = 6.08 Hz, $CH_2$ of cyclopropyl), 3.34 (s broad, 4H, piperazine, overlapped), 3.53 (s broad, 4H, piperazine), 3.83–3.87 (m, 1H,CH of cyclopropyl), 6.95 (t, 1H, $J$ = 4.72 Hz, ArH), 7.60 (d, 1H, $J$ = 7.16 Hz, ArH), 7.71 (d, 2H, $J$ = 8.80 Hz, ArH), 7.94–7.99 (m, 3H, ArH), 8.57 (d, 2H, $J$ = 4.72 Hz, ArH), 8.69 (s, 1H, ArH), 10.11 (s, 1H, NH, exch. $D_2O$), 15.13 (s, 1H, OH, exch. $D_2O$). $^{13}$C NMR (100 MHz, DMSO) $\delta$ *ppm*: 8.1, 36.4, 43.2, 46.9 (aliphatic carbons), 107.3, 107.3 (d, $^3J_{F\text{-}c}$ = 3 Hz), 111.6 (d, $^2J_{F\text{-}c}$ = 22 Hz), 113.9, 118.8, 119.8 (d, $^3J_{F\text{-}c}$ = 7 Hz), 124.2, 139.5, 144.5 (d, $^2J_{F\text{-}c}$ = 10 Hz),144.5, 144.8, 148.6, 153.3 (d, $^1J_{F\text{-}c}$ = 248 Hz), 158.6, 160.0 (Ar carbons), 166.4, 176.8 (C=O). MS (*m/z*, %): 593.5 ($M^+$+1,0.35), 592.5 ($M^+$, 0.35). Elem. anal. for$C_{27}H_{25}FN_8O_5S$ (592.60), calcd: C, 54.72; H, 4.25; N, 18.91. Found:C, 54.90; H, 4.37; N, 19.05.

*4.1.2.3. 1-Cyclopropyl-6-fluoro-7-(4-((4-(N-(5-methylisoxazol-3-yl) sulfamoyl) phenyl) diazenyl) piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3c).* Yield: (1.1 g) 71.90%. Mp: 237–240 °C. IR (KBr) $\upsilon_{max}$/cm$^{-1}$: 3441 (NH + OH overlapped), 3144. 3090 (CH Ar), 2974, 2932 (CH aliphatic), 1713, 1628 (C=O) 1339, 1157 ($SO_2$). $^1$H NMR (400 MHz, DMSO) $\delta$ *ppm*: 1.20 (s, 2H, $CH_2$ of cyclopropyl), 1.34 (d, 2H, $J$ = 6.36 Hz, $CH_2$ of cyclopropyl), 2.27 (s, 3H, $CH_3$), 3.60 (s broad, 4H, piperazine), 3.82–3.84 (m, 1H, CH of cyclopropyl), 4.09 (s broad, 4H, piperazine), 6.09 (s, 1H, ArH), 7.53 (d, 2H, $J$ = 8.44 Hz, ArH), 7.63 (d, 1H, $J$ = 7.32 Hz, ArH), 7.82 (d, 2H, $J$ = 8.44 Hz, ArH), 7.94 (d, 1H, $J$ = 13.12 Hz, ArH), 8.67 (s, 1H, ArH), 11.37 (s, 1H, NH, exch. $D_2O$), 15.18 (s, 1H, OH, exch. $D_2O$). $^{13}$C NMR (100 MHz, DMSO) $\delta$ *ppm*: 8.1, 12.5, 36.3, 50.2, 51.2 (aliphatic carbons), 95.9, 106.9 (d, $^3J_{F\text{-}c}$ = 2 Hz), 107.2, 111.4 (d,$^2J_{F\text{-}c}$ = 20 Hz), 119.1 (d, $^3J_{F\text{-}c}$ = 8 Hz), 121.2, 128.6, 136.4, 139.5, 144.9 (d, $^2J_{F\text{-}c}$ = 10 Hz), 148.3, 153.2 (d, $^1J_{F\text{-}c}$ = 248 Hz), 153.9, 158.1 (Ar carbons), 166.6, 170.7, 176.8 (C=O and C–O).MS (*m/z*, %): 595.39 ($M^+$, 60.35), 594.24 ($M^+$, 45.38).Elem. anal. for $C_{27}H_{26}FN_7O_6S$ (595.60), calcd: C, 54.45; H, 4.40; N, 16.46. Found: C, 54.72; H, 4.56; N, 16.29.

*4.1.2.4. 1-Cyclopropyl-6-fluoro-4-oxo-7-(4-((4-(N-(quinoxalin-2-yl) sulfamoyl)phenyl) diazenyl) piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (3d).* Yield: (1.4 g) 85.11%. Mp: charred at 204 °C. IR (KBr) $\upsilon_{max}$/cm$^{-1}$: 3421 (NH + OH overlapped), 3066 (CH Ar), 2843 (CH aliphatic), 1740, 1620 (C=O), 1385,1177 ($SO_2$). $^1$H NMR (400 MHz, DMSO) $\delta$ *ppm*: 1.21 (s, 2H, $CH_2$ of cyclopropyl), 1.35 (d,

2H, $J = 6.20$ Hz, $CH_2$ of cyclopropyl), 3.62 (s broad, 4H, piperazine), 3.84–3.86 (m, 1H, CH of cyclopropyl), 4.08 (s broad, 4H, piperazine),7.17 (t, 1H, $J = 7.46$ Hz, ArH), 7.30–7.44 (m, 3H, ArH), 7.50 (t, 1H, $J = 7.32$ Hz, ArH), 7.64–7.69 (m, 4H, 3 ArH + NH, exch. $D_2O$), 7.90 (d, 1H, $J = 10.36$ Hz, ArH), 7.98 (d, 1H, $J = 13.08$ Hz, ArH), 8.34 (s, 1H, ArH), 8.69 (s, 1H, ArH), 14.76 (s, 1H, OH, exch. $D_2O$). $^{13}$C NMR (100 MHz, DMSO) $\delta$ *ppm*: 8.1, 36.4, 43.4, 49.0 (aliphatic carbons), 107.3 (d, $^3J_{F\text{-}C} = 4$ Hz), 111.6 (d, $^2J_{F\text{-}C} = 23$ Hz), 119.5 (d, $^3J_{F\text{-}C} = 8$Hz), 120.9, 122.3, 123.5, 124.5, 127.4, 129.3, 129.8, 131.5, 132.6, 132.7, 133.0, 139.6, 139.6, 144.8 (d, $^2J_{F\text{-}C} = 11$ Hz), 148.6, 151.5, 153.3 (d, $^1J_{F\text{-}C} = 242$Hz) (Ar carbons), 166.4, 176.8 (C=O). MS (*m/z*, %): 642.62 ($M^+$, 73.30). Elem. anal. for $C_{31}H_{27}FN_8O_5S$ (642.66), calcd: C, 57.94; H, 4.23; N, 17.44. Found: C, 58.13; H, 4.45; N, 17.31.

*4.1.2.5. 7-(4-((4-(N- (6-Chloropyrazin-2-yl) sulfamoyl) phenyl) diazenyl) piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid (3e).* Yield: (1.0 g) 62.11%. Mp: 186–188 °C. IR (KBr) $\upsilon_{max}/cm^{-1}$: 3445 (NH + OH overlapped), 3089. 3067, 3013 (CH Ar), 2982, 2928 (CH aliphatic), 1724, 1628 (C=O), 1338, 1157 ($SO_2$). $^1$H NMR (400 MHz, DMSO) $\delta$ *ppm*: 1.19 (s, 2H, $CH_2$ of cyclopropyl), 1.34 (s, 2H, $CH_2$ of cyclopropyl), 3.59 (s broad, 4H, piperazine), 3.81–3.84 (m, 1H, CH of cyclopropyl), 4.08 (s broad, 4H, piperazine),7.52 (d, 2H, $J = 8.36$ Hz, ArH), 7.61 (s, 1H,ArH + NH, exch. $D_2O$), 7.90–7.95 (m, 3H, ArH), 8.16 (d, 2H, $J = 9.64$ Hz, ArH), 8.65 (s, 1H, ArH), 15.17 (s, 1H, OH, exch. $D_2O$). $^{13}$C NMR (100 MHz, DMSO) $\delta$ *ppm*: 8.1, 36.4, 49.1, 61.5 (aliphatic carbons), 107.0 (d, $^3J_{F\text{-}C} = 2$ Hz), 107.2, 111.5 (d, $^2J_{F\text{-}C} = 20$ Hz), 118.6, 119.2 (d, $^3J_{F\text{-}C} = 9$ Hz), 120.8, 129.2, 133.6, 135.3, 137.6, 139.5, 145.0 (d, $^2J_{F\text{-}C} = 10$ Hz), 145.9, 148.4, 153.2 (d, $^1J_{F\text{-}C} = 247$ Hz), 153.4 (Ar carbons), 166.5, 176.7 (C=O).MS (*m/z*, %): 627.50 ($M^+$, 0.32). Elem. anal. for $C_{27}H_{24}ClFN_8O_5S$ (627.05), calcd: C, 51.72; H, 3.86; N, 17.87. Found: C, 51.53; H, 4.04; N, 17.69.

*4.1.2.6. 1-Cyclopropyl-7-(4-((4-(N-(diaminomethylene) sulfamoyl) phenyl) diazenyl) piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3f).* Yield: (1.1 g) 77.46%. Mp: 288–290 °C. IR (KBr) $\upsilon_{max}/cm^{-1}$: 3475, 3441, 3337 ($NH_2$, NH, OH overlapped), 3066 (CH Ar), 2843 (CH aliphatic), 1713, 1627 (C=O), 1357, 1169 ($SO_2$). $^1$H NMR (400 MHz, DMSO) $\delta$ *ppm*: 1.21 (s, 2H, $CH_2$ of cyclopropyl), 1.34 (d, 2H, $J = 6.24$ Hz, $CH_2$ of cyclopropyl), 3.60 (s broad, 4H, piperazine), 3.85 (s broad, 1H, CH of cyclopropyl), 4.06 (s broad, 4H, piperazine), 6.71 (s, 4H, $2NH_2$, exch. $D_2O$),7.49 (d, 2H, $J = 8.52$ Hz, ArH), 7.64 (d, 1H, $J = 7.44$ Hz, ArH), 7.76 (d, 2H, $J = 8.56$ Hz, ArH), 7.96 (d, 1H, $J = 13.12$ Hz, ArH), 8.68 (s, 1H, ArH), 15.19 (s, 1H, OH, exch. $D_2O$). $^{13}$C NMR (100 MHz, DMSO) $\delta$ *ppm*: 8.1, 36.4, 46.6, 48.0 (aliphatic carbons), 107.1 (d, $^3J_{F\text{-}C} = 3$ Hz), 107.2, 111.5 (d, $^2J_{F\text{-}C} = 23$Hz), 119.3 (d, $^3J_{F\text{-}C} = 7$ Hz), 120.7, 127.2, 139.6, 141.8, 145.1 (d, $^2J_{F\text{-}C} = 10$ Hz), 148.6, 148.6, 153.4 (d, $^1J_{F\text{-}C} = 229$ Hz) (Ar carbons), 158.5 (C=N), 166.6, 176.8 (C=O). MS (*m/z*, %): 556.34 ($M^+$, 27.79). Elem. anal. for $C_{24}H_{25}FN_8O_5S$ (556.57), calcd: C, 51.79; H, 4.53; N, 20.13. Found: C, 51.95; H, 4.64; N, 19.87.

*4.1.3. Synthesis of acylated sulfonamides (4a-e) (Scheme 2)*

The appropriate chloroacetyl chloride (2.26 g, 20 mmol) was added dropwise with vigorous stirring to a suspension of the appropriate sulfonamide (**1a-e**) (10 mmol) in dimethylformamide (10 mL) at 0 °C and the reaction was continued at room temperature for 5 h. After completion, the mixture was poured in cold water and the precipitate was filtered off and the pure products were obtained by recrystallization from ethanol [38].

*4.1.3.1. 2-Chloro-N-(4-sulfamoylphenyl)acetamide (4a).* Yield: (2.1 g) 84.46%. Mp: 203–205 °C as reported [39].

*4.1.3.2. 2-Chloro-N-(4-(N-(pyrimidin-2-yl)sulfamoyl)phenyl)acetamide (4b).* Yield: (2.9 g) 89.06%. Mp: 220–222 °C as reported [40].

*4.1.3.3. 2-Chloro-N-(4-(N-(5-methylisoxazol-3-yl)sulfamoyl)phenyl) acetamide (4c).* Yield: (2.7 g) 81.88%. Mp: 144–146 °C as reported [41].

*4.1.3.4. 2-Chloro-N-(4-(N-(quinoxalin-2-yl)sulfamoyl)phenyl)acetamide (4d).* Yield: (3.3 g) 87.57%. Mp: 180–182 °C as reported [42].

*4.1.3.5. 2-Chloro-N-(4-(N-(6-chloropyrazin-2-yl)sulfamoyl)phenyl) acetamide (4e).* Yield: (3.30 g) 91.4%. Mp: 232–235 °C. IR (KBr) υmax/cm-1: 3622, 3475 (2NH), 3102, 3047, 3001 (CH Ar), 2940 (CH aliphatic), 1693.5 (C=O), 1331, 1165 (SO2). $^1$H NMR (400 MHz, DMSO) $\delta$ ppm: 4.30 (s, 2H, $CH_2$), 7.80 (d, 2H, $J = 8.88$ Hz, ArH), 7.95 (d, 2H, $J = 8.84$ Hz, ArH), 8.29 (s, 1H, ArH), 8.34 (s, 1H, ArH), 10.72 (s, 1H, NH, exch. $D_2O$), 11.89 (s, 1H, NH, exch. $D_2O$). $^{13}$C NMR (100 MHz, DMSO) $\delta$ ppm: 44.0 (aliphatic carbons), 119.5, 129.4, 132.7, 133.9, 137.6, 143.4, 145.9, 147.8 (Ar carbons), 165.9 (C=O). MS (*m/z*, %): 362.73 ($M^+1$, 18.18), 361.38 ($M^+$, 46.90). Elem. anal. For $C_{12}H_{10}Cl_2N_4O_3S$ (361.20), calcd: C, 39.90; H, 2.79; N, 15.51. Found:C, 40.17; H, 2.95; N, 15.73.

*4.1.4. Synthesis of ciprofloxacin-sulfonamide hybrids with amide linker (5a-e) (Scheme 2)*

A mixture of the appropriate chloroacetyl sulfonamide derivative (4a-e) (10 mmol), ciprofloxacin (2) (3.31 gm, 10 mmol) and trimethylamine (TEA) (1.01 gm, 10 mmol) in dry DMF was heated under reflux for 17 h (TLC). The reaction mixture was concentrated by evaporation under vacuum, poured on ice and the produced solid filtered and washed by water and recrystallized from ethanol [43].

*4.1.4.1. 1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(2-oxo-2-((4-sulfamoyl phenyl) amino) ethyl) piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (5a).* Yield: (3.8 g) 69.98%. Mp: 192–195 °C. IR (KBr) $\upsilon_{max}/cm^{-1}$: 3500-3300 (OH), 3337, 3225 (NH, $NH_2$), 3121. 3063 (CH Ar), 2974, 2882 (CH aliphatic), 1674 (C=O), 1312, 1157 ($SO_2$). $^1$H NMR (400 MHz, DMSO) $\delta$ *ppm*: 1.19 (s, 2H, $CH_2$ of cyclopropyl), 1.34 (d, 2H, $J = 6.36$ Hz, $CH_2$ of cyclopropyl), 2.79 (s broad, 4H, piperazine), 3.31 (s, 2H, $CH_2$), 3.42 (s, 4H, piperazine), 3.82 (broad s, 1H, CH of cyclopropyl), 7.26 (s, 2H, $NH_2$, exch. $D_2O$), 7.57 (d, 1H, $J = 6.36$ Hz, ArH), 7.78 (d, 2H, $J = 8.84$ Hz, ArH), 7.84 (d, 2H, $J = 8.80$ Hz, ArH), 7.88 (d, 1H, $J = 13.28$ Hz, ArH), 8.65 (s, 1H, ArH), 10.13 (s, 1H, NH, exch. $D_2O$), 15.20 (s, 1H, OH, exch. $D_2O$). $^{13}$C NMR (100 MHz, DMSO) $\delta$ *ppm*: 5.9, 34.2, 47.6, 50.6, 59.7 (aliphatic carbons), 104.5 (d, $^3J_{F\text{-}C} = 3$ Hz), 105.0, 109.2 (d, $^2J_{F\text{-}C} = 23$ Hz), 116.8 (d, $^3J_{F\text{-}C} = 8$ Hz), 117.5, 125.0, 136.9, 137.4, 139.9, 143.5 (d, $^2J_{F\text{-}C} = 10$ Hz), 146.1, 151.3 (d, $^1J_{F\text{-}C} = 248$ Hz) (Ar carbons), 164.4, 167.2, 174.6 (C=O). MS (*m/z*, %): 543.2 ($M^+$, 0.32), 542.1 ($M^+ - 1$, 0.41). Elem. anal. for $C_{25}H_{26}FN_5O_6S$ (543.57), calcd: C, 55.24; H, 4.82; N, 12.88. Found: C, 55.51; H, 4.93; N, 13.07.

*4.1.4.2. 1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(2-oxo-2-((4-(N-(pyrimidin-2-yl) sulfamoyl) phenyl) amino) ethyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (5b).* Yield: (4.5 g) 72.58%. Mp: 275–277 °C. IR (KBr) $\upsilon_{max}/cm^{-1}$: 3584 (OH), 3441, 3290 (NH), 3106. 3082, 3044 (CH Ar), 2924, 2851 (CH aliphatic), 1700, 1697, 1628 (C=O) 1339, 1161 ($SO_2$). $^1$H NMR (400 MHz, DMSO) $\delta$ *ppm*: 1.19 (s, 2H, $CH_2$ of cyclopropyl), 1.33 (d, 2H, $J = 6.24$ Hz, $CH_2$ of cyclopropyl), 2.77 (s broad, 4H, piperazine), 3.29 (s, 2H, $CH_2$), 3.41 (s broad, 4H, piperazine), 3.83 (broad s, 1H, CH of cyclopropyl), 7.03 (t, 1H, $J = 4.52$ Hz, ArH), 7.58 (d, 1H, $J = 7.36$ Hz, ArH), 7.83 (d, 2H, $J = 8.72$ Hz, ArH), 7.90–7.95 (m, 3H, ArH), 8.50 (d, 2H, $J = 4.76$ Hz, ArH), 8.67 (s, 1H, ArH), 10.17 (s, 1H, NH, exch. $D_2O$), 11.69 (s, 1H, NH,

exch. $D_2O$), 15.22 (s, 1H, OH, exch. $D_2O$). $^{13}C$ NMR (100 MHz, DMSO) *δ ppm*: 8.0, 36.3, 49.7, 52.7, 61.8 (aliphatic carbons), 106.7 (d, $^3J_{F\text{-}C}$ = 2 Hz), 107.1, 111.4 (d, $^2J_{F\text{-}C}$ = 23 Hz), 116.2, 118.9 (d, $^3J_{F\text{-}C}$ = 8 Hz), 119.3, 129.2, 134.8, 139.6, 142.9, 145.6 (d, $^2J_{F\text{-}C}$ = 10 Hz), 148.4, 153.4 (d, $^1J_{F\text{-}C}$ = 248 Hz), 157.4, 158.8 (Ar carbons), 166.6, 169.5, 176.8 (C═O). MS (*m/z*, %): 621.40 ($M^+$, 0.16), 620.40 ($M^+$ − 1, 0.17). Elem. anal. for $C_{29}H_{28}FN_7O_6S$ (621.64), calcd: C, 56.03; H, 4.54; N, 15.77. Found: C, 55.97; H, 4.68; N, 15.98.

*4.1.4.3. 1-Cyclopropyl -6-fluoro- 7-(4-(2-(( 4-(N-(5-methylisoxazol-3-yl) sulfamoyl) phenyl)amino)-2-oxoethyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5c).* Yield: (4.5 g) 72.0%. Mp: 260–262 °C. IR (KBr) $\upsilon_{max}/cm^{-1}$: 3641 (OH), 3464, 3286 (NH), 3086 (CH Ar), 2989, 2943, 2893 (CH aliphatic), 1732, 1697, 1628 (C═O) 1339, 1169 ($SO_2$). $^1H$ NMR (400 MHz, DMSO) *δ ppm*: 1.19 (s, 2H, $CH_2$ of cyclopropyl), 1.33 (d, 2H, *J* = 6.28 Hz, $CH_2$ of cyclopropyl), 2.30 (s, 3H, $CH_3$), 2.78 (s broad, 4H, piperazine), 3.31 (s, 2H, $CH_2$), 3.41 (s broad, 4H, piperazine), 3.82 (broad s, 1H, CH of cyclopropyl), 6.14 (s, 1H, ArH), 7.56 (d, 1H, *J* = 7.36 Hz, ArH), 7.80–7.90 (m, 5H, ArH), 8.65 (s, 1H, ArH), 10.22 (s, 1H, NH, exch. $D_2O$), 11.32 (s, 1H, NH, exch. $D_2O$), 15.20 (s, 1H, OH, exch. $D_2O$). $^{13}C$ NMR (100 MHz, DMSO) *δ ppm*: 8.0, 12.5, 36.3, 49.7, 52.7, 61.8 (aliphatic carbons), 95.8, 106.7 (d, $^3J_{F\text{-}C}$ = 3 Hz), 107.1, 111.4 (d, $^2J_{F\text{-}C}$ = 23 Hz), 118.9 (d, $^3J_{F\text{-}C}$ = 8 Hz), 119.8, 128.4, 133.8, 139.6, 143.3, 145.6 (d, $^2J_{F\text{-}C}$ = 10 Hz), 148.4, 153.4 (d, $^1J_{F\text{-}C}$ = 248 Hz), 158.0 (Ar carbons), 166.6, 169.5, 170.8, 176.7, (C═O and C–O). MS (*m/z*, %): 625.10 ($M^+$, 0.32), 624.10 ($M^+$ − 1, 0.19). Elem. anal. for $C_{29}H_{29}FN_6O_7S$ (624.64), calcd: C, 55.76; H, 4.68; N, 13.45. Found: C, 55.98; H, 4.75; N, 13.68.

*4.1.4.4. 1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(2-oxo-2-((4-(N-(quinoxalin-2-yl) sulfamoyl) phenyl) amino)ethyl) piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (5d).* Yield: (5.0 g) 74.43%. Mp: charred at 168–171 °C. IR (KBr) $\upsilon_{max}/cm^{-1}$: 3445, 3267 (NH, OH overlapped), 3098, 3063 (CH Ar), 2858 (CH aliphatic), 1701,1667, 1628 (3C = O), 1338, 1161 ($SO_2$). $^1H$ NMR (400 MHz, DMSO) *δ ppm*: 1.19 (s broad, 2H, $CH_2$ of cyclopropyl), 1.32 (s broad, 2H, $CH_2$ of cyclopropyl), 2.76 (s broad, 4H, piperazine), 3.29 (s, 2H, $CH_2$), 3.43 (s, 4H, piperazine, overlapped), 3.79–3.82 (m, 1H, CH of cyclopropyl), 7.54 (d, 1H, *J* = 7.44 Hz, ArH), 7.62 (t, 1H, *J* = 7.56 Hz, ArH), 7.75 (t, 1H, *J* = 6.92 Hz, ArH), 7.81 (d, 1H, *J* = 7.68 Hz, ArH), 7.85–7.94 (m, 4H, ArH), 8.07 (d, 2H, *J* = 8.80 Hz, ArH), 8.61 (s, 1H, ArH), 8.65 (s, 1H, ArH), 10.19 (s, 1H, NH, exch. $D_2O$), 15.21 (s, 1H, OH, exch. $D_2O$). $^{13}C$ NMR (100 MHz, DMSO) *δ ppm*: 8.0, 36.3, 49.6, 52.6, 61.7 (aliphatic carbons), 106.7 (d, $^3J_{F\text{-}C}$ = 3 Hz), 107.1, 111.4 (d, $^2J_{F\text{-}C}$ = 23 Hz), 119.0 (d, $^3J_{F\text{-}C}$ = 8 Hz), 119.4, 127.7, 129.2, 129.5, 131.4, 134.5, 138.2, 139.6, 143.2, 145.6 (d, $^2J_{F\text{-}C}$ = 10 Hz), 146.7, 148.4, 153.4 (d, $^1J_{F\text{-}C}$ = 248 Hz), 153.9, 156.2, 161.6 (Ar carbons), 166.5, 169.4, 176.8 (C═O). MS (*m/z*, %): 671.43 ($M^+$, 23.22). Elem. anal. for $C_{33}H_{30}FN_7O_6S$ (671.70), calcd: C, 59.01; H, 4.50; N, 14.60. Found: C, 58.94; H, 4.73; N, 14.69.

*4.1.4.5. 7-(4-(2-((4-(N-(6-Chloropyrazin-2-yl)sulfamoyl)phenyl) amino)-2-oxoethyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5e).* Yield: (4.8 g) 73.2%. Mp: 170–173 °C. IR (KBr) $\upsilon_{max}/cm^{-1}$: 3441, 3271 (NH, OH overlapped), 3067 (CH Ar), 2940, 2885 (CH aliphatic), 1705, 1628 (3C = O, broad), 1335, 1161 ($SO_2$). $^1H$ NMR (400 MHz, DMSO) *δ ppm*: 1.19 (s, 2H, $CH_2$ of cyclopropyl), 1.33 (d, 2H, *J* = 6.28 Hz, $CH_2$ of cyclopropyl), 2.81 (s, 4H, piperazine), 3.35 (s, 2H, $CH_2$), 3.47 (s, 4H, piperazine, overlapped), 3.81–3.83 (m, 1H, CH of cyclopropyl), 7.57 (d, 1H, *J* = 7.40 Hz, ArH), 7.86–7.95 (m, 5H, ArH), 8.25 (s, 1H, ArH), 8.28 (s, 1H, ArH), 8.66 (s, 1H, ArH), 10.25 (s, 1H, NH, exch. $D_2O$), 15.20 (s, 1H, OH, exch. $D_2O$). $^{13}C$ NMR (100 MHz, DMSO) *δ ppm*: 8.0, 36.3, 49.4, 52.6, 61.5 (aliphatic carbons), 106.7 (d, $^3J_{F\text{-}C}$ = 3Hz), 107.1, 111.4 (d, $^2J_{F\text{-}C}$ = 23 Hz), 118.9 (d, $^3J_{F\text{-}C}$ = 8 Hz), 119.5, 129.1, 133.1, 134.3, 136.4, 139.6, 143.1, 145.5 (d, $^2J_{F\text{-}C}$ = 10 Hz), 146.0, 148.4, 148.9, 153.4 (d, $^1J_{F\text{-}C}$ = 248 Hz) (Ar carbons), 166.7, 169.2, 176.8 (C═O).MS (*m/z*, %): 657.60 ($M^+$+1, 0.33), 656.60 ($M^+$, 0.42). Elem. anal. for $C_{29}H_{27}ClFN_7O_6S$ (656.09), calcd: C, 53.09; H, 4.15; N, 14.94;. Found: C, 53.31; H, 4.28; N, 15.18.

*4.1.5. Synthesis of acylated sulfonamides (6a-e) (Scheme 2)*

Compounds **6a-e** were prepared using the same procedure adopted for synthesizing compounds **4a-e** but using 3-chloropropyl chloride instead of chloroacetyl chloride.

*4.1.5.1. 3-Chloro-N-(4-sulfamoylphenyl)propanamide (6a).* Yield: (2.2 g) 83.74%. Mp: 228–230 °C as reported [44].

*4.1.5.2. 3-Chloro-N-(4-(N-(pyrimidin-2-yl)sulfamoyl)phenyl)propanamide (6b).* Yield: (2.1 g) 61.58%. Mp: 239–241 °C as reported [45].

*4.1.5.3. 3-Chloro-N-(4-(N-(5-methylisoxazol-3-yl) sulfamoyl)phenyl) propanamide (6c).* Yield: (3.0 g) 87.2%. Mp: 189–191 °C. IR (KBr) υmax/$cm^{-1}$: 3314, 3279 (2 NH), 3113 (CH Ar), 2974 (CH aliphatic), 1678 (C═O), 1312, 1092 ($SO_2$). $^1H$ NMR (400 MHz, DMSO) *δ* ppm: 2.30 (s, 3H, $CH_3$), 2.87 (t, 2H, *J* = 6.20 Hz, CO–$CH_2$), 3.88 (t, 2H, *J* = 6.20 Hz, Cl–$CH_2$), 6.12 (s, 1H, ArH), 7.77–7.82 (m, 4H, ArH), 10.49 (s, 1H, NH, exch. $D_2O$), 11.33 (s, 1H, NH, exch. $D_2O$). $^{13}C$ NMR (100 MHz, DMSO) *δ* ppm: 12.5, 41.0, 56.5 (aliphatic carbons), 95.8, 119.3, 128.6, 133.7, 143.6, 158.0 (Ar carbons), 169.3, 170.7 (C═O and C–O).

*4.1.5.4. 3-Chloro-N-(4-(N-(quinoxalin-2-yl)sulfamoyl)phenyl)propanamide (6d).* Yield: (3.2 g) 81.8%. Mp: 215–217 °C. IR (KBr) υmax/$cm^{-1}$: 3336, 3244 (2 NH), 3106, 3036 (CH Ar), 2909 (CH aliphatic), 1694 (C═O), 1358, 1092 ($SO_2$). $^1H$ NMR (400 MHz, DMSO) *δ* ppm: 2.84 (t, 2H, *J* = 6.24 Hz, CO–$CH_2$), 3.86 (t, 2H, *J* = 6.20 Hz, Cl–$CH_2$), 7.58–7.75 (m, 2H, ArH), 7.80 (d, 2H, *J* = 8.88 Hz, ArH), 7.93 (d, 2H, *J* = 7.84 Hz, ArH), 8.08 (d, 2H, *J* = 8.52 Hz, ArH), 8.58 (s, 1H, ArH), 10.45 (s, 1H, NH, exch. $D_2O$), 11.57 (s, 1H, NH, exch. $D_2O$). $^{13}C$ NMR (100 MHz, DMSO) *δ* ppm: 19.0, 56.5 (aliphatic carbons), 112.7, 119.0, 119.3, 127.8, 128.5, 129.2, 129.7, 131.4, 131.8, 139.0, 143.6, 146.5 (Ar carbons), 169.2 (C═O).

*4.1.5.5. 3-Chloro-N-(4-(N-(6-chloropyrazin-2-yl) sulfamoyl) phenyl) propanamide (6e).* Yield: (3.21 g) 85.6%. Mp: 207–209 °C. IR (KBr) υmax/$cm^{-1}$: 3314, 3267 (2NH), 3102, 3047 (CH Ar), 2993, 2924, 2901 (CH aliphatic), 1674 (C═O), 1342, 1092 (SO2). $^1H$ NMR (400 MHz, DMSO) *δ* ppm: 2.87 (t, 2H, *J* = 6.20 Hz, $CH_2CO$), 3.88 (t, 2H, *J* = 6.20 Hz, $CH_2Cl$), 7.80 (d, 2H, *J* = 8.88 Hz, ArH), 7.93 (d, 2H, *J* = 8.88 Hz, ArH), 8.29 (s, 1H, ArH), 8.34 (s, 1H, ArH), 10.51 (s, 1H, NH, exch. $D_2O$), 11.85 (s, 1H, NH, exch. $D_2O$). $^{13}C$ NMR (100 MHz, DMSO) *δ* ppm: 40.3 (overlapped with DMSO), 41.0 (aliphatic carbons), 119.1, 129.4, 132.7, 133.4, 137.5, 143.8, 145.9, 147.9 (Ar carbons), 169.3 (C═O). MS (*m/z*, %): 375.93 (M+, 28.58). Elem. anal. For $C_{13}H_{12}Cl_2N_4O_3S$ (375.23), calcd: C, 41.61; H, 3.22; N, 14.93. Found:C, 41.85; H, 3.40; N,15.12.

*4.1.6. Synthesis of ciprofloxacin-sulfonamide hybrids with amide linker (7a-e) (Scheme 2)*

Compounds **7a-e** were prepared using the same procedure adopted for synthesizing compounds **5a-e** but using 3- chloropropionyl sulfonamide derivative (**6a-e**) instead of chloroacetyl sulfonamide derivative (**4a-e**).

*4.1.6.1. 1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(3-oxo-3-((4-sulfamoylphenyl) amino) propyl) piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (7a).* Yield: (4.5 g) 80.70%. Mp: 235–237 °C. IR (KBr) $\upsilon_{max}/cm^{-1}$: 3410 (OH), 3318, 3267, 3224 ($NH_2$,

NH), 3109, 3063 (CH Ar), 2959, 2920 (CH aliphatic), 1701 broad, 1632 (3C = O), 1331, 1157 ($SO_2$). $^1$H NMR (400 MHz, DMSO) *δ ppm*: 1.19 (s, 2H, $CH_2$ of cyclopropyl), 1.32 (d, 2H, *J* = 6.20 Hz, $CH_2$ of cyclopropyl), 2.59 (t, 2H, *J* = 6.78 Hz, O=C−C$H_2$), 2.67(broad s, 4H, piperazine), 2.76 (t, 2H, *J* = 6.82 Hz, N−C$H_2$), 3.34 (s, 4H, piperazine, overlapped), 3.80−3.83 (m, 1H, CH of cyclopropyl), 7.25 (s, 2H, $NH_2$, exch. $D_2O$), 7.58 (d, 1H, *J* = 7.44 Hz, ArH), 7.76 (s, 4H, ArH),7.92 (d, 1H, *J* = 13.28 Hz, ArH), 8.67 (s, 1H, ArH), 10.37 (s, 1H, NH, exch. $D_2O$), 15.22 (s, 1H, OH, exch. $D_2O$). $^{13}$C NMR (100 MHz, DMSO) *δ ppm*: 8.1, 36.4, 40.6, 44.9, 49.6, 50.7, 51.7 (aliphatic carbons), 107.3 (d, $^3J_{F\text{-}C}$ = 3 Hz), 107.4, 111.6 (d, $^2J_{F\text{-}C}$ = 24 Hz), 119.2, 119.4 (d,$^3J_{F\text{-}C}$ = 8 Hz), 127.2, 138.9, 139.5, 142.4, 145.4 (d, $^2J_{F\text{-}C}$ = 10 Hz), 148.5, 153.4 (d, $^1J_{F\text{-}C}$ = 248 Hz) (Ar carbons), 161.5, 166.3, 176.8 (C=O). MS (*m/z*, %): 557.23 ($M^+$, 23.78). Elem. anal. for $C_{26}H_{28}FN_5O_6S$ (557.59), calcd: C, 56.00; H, 5.06; N, 12.56. Found: C, 56.23; H, 5.18; N, 12.78.

*4.1.6.2. 1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(3-oxo-3-((4-(N-(pyrimidin-2-yl)sulfamoyl) phenyl) amino) propyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (7b).* Yield: (5.3 g) 83.38%. Mp: 270−273 °C. . IR (KBr) $\upsilon_{max}/cm^{-1}$: 3500-3300 (OH), 3441, 3291, 3194(NH), 3078, 3040 (CH Ar), 2943, 2828 (CH aliphatic), 1705, 1697, 1628 (3C = O), 1339, 1161 ($SO_2$). $^1$H NMR (400 MHz, DMSO) *δ ppm*: 1.18 (s, 2H, $CH_2$ of cyclopropyl), 1.31 (d, 2H, *J* = 6.32 Hz, $CH_2$ of cyclopropyl), 2.58 (t, 2H, *J* = 6.80 Hz, O=C−C$H_2$), 2.66 (s, 4H, piperazine), 2.75 (t, 2H, *J* = 6.40 Hz, N−C$H_2$), 3.34 (s broad, 4H, piperazine, overlapped), 3.80−3.83 (m, 1H, CH of cyclopropyl), 7.03 (t, 1H, *J* = 4.84 Hz, ArH), 7.56 (d, 1H, *J* = 7.36 Hz, ArH), 7.76 (d, 2H, *J* = 8.72 Hz, ArH), 7.88−7.95 (m, 3H, ArH), 8.49 (d, 2H, *J* = 4.84 Hz, ArH), 8.67 (s, 1H, ArH), 10.42 (s, 1H, NH, exch. $D_2O$), 11.66 (s, 1H, NH, exch. $D_2O$), 15.21 (s, 1H, OH, exch. $D_2O$).$^{13}$C NMR (100 MHz, DMSO) *δ ppm*: 8.0, 34.5, 36.3, 49.8, 52.5, 53.8 (aliphatic carbons), 106.8 (d, $^3J_{F\text{-}C}$ = 3 Hz), 107.1, 111.4 (d, $^2J_{F\text{-}C}$ = 23 Hz), 116.1, 118.8, 119.0 (d, $^3J_{F\text{-}C}$ = 7 Hz), 129.3, 134.6, 139.6, 143.4, 145.6 (d, $^2J_{F\text{-}C}$ = 10 Hz), 148.4, 153.5 (d, $^1J_{F\text{-}C}$ = 249 Hz), 157.5, 158.8 (Ar carbons), 166.6, 171.4, 176.8 (C=O). MS (*m/z*, %): 635.19 ($M^+$, 43.33). Elem. anal. for $C_{30}H_{30}FN_7O_6S$ (635.67), calcd: C, 56.68; H, 4.76; N, 15.42. Found: C, 56.91; H, 4.88; N, 15.67.

*4.1.6.3. 1-Cyclopropyl-6-fluoro-7-(4-(3-((4-(N-(5-methylisoxazol-3-yl)sulfamoyl) phenyl) amino)-3-oxopropyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (7c).* Yield: (4.5 g) 70.45%. Mp: 248−250 °C. IR (KBr) $\upsilon_{max}/cm^{-1}$: 3433 (OH), 3333, 3248 (2NH), 3089, 3051, 3013 (CH Ar), 2960, 2928 (CH aliphatic), 1721, 1670, 1628 (3C = O), 1339, 1192 ($SO_2$). $^1$H NMR (400 MHz, DMSO) *δ ppm*: 1.19 (s, 2H, $CH_2$ of cyclopropyl), 1.33 (s, 2H, $CH_2$ of cyclopropyl), 2.27 (s, 3H, $CH_3$), 2.58 (t, 2H, *J* = 4.40 Hz, O=C−C$H_2$), 2.75 (t, 2H, *J* = 4.40 Hz, N−C$H_2$), 3.35 (s broad, 4H, piperazine, overlapped), 3.63 (s broad, 4H, piperazine), 3.80−3.83 (m, 1H, CH of cyclopropyl), 6.08 (s, 1H, ArH), 7.59−7.93 (m, 6H, ArH)), 8.13 (s, 1H, NH, exch. $D_2O$), 8.66 (s, 1H, ArH), 10.47 (s, 1H, NH, exch. $D_2O$), 15.15 (s, 1H, OH, exch. $D_2O$).$^{13}$C NMR (100 MHz, DMSO) *δ ppm*: 8.0, 12.5, 36.3, 45.0, 49.4, 50.6, 52.4 (aliphatic carbons), 107.1, 107.2 (d, $^3J_{F\text{-}C}$ = 2 Hz), 111.3 (d, $^2J_{F\text{-}C}$ = 23 Hz), 119.2 (d, $^3J_{F\text{-}C}$ = 8 Hz), 119.3, 128.4, 139.4, 139.5, 145.4 (d, $^2J_{F\text{-}C}$ = 9 Hz), 148.3, 148.4, 148.4, 153.4 (d, $^1J_{F\text{-}C}$ = 247 Hz), 154.6, (Ar carbons), 161.7, 166.6, 171.5, 176.7 (C=O and C−O). MS (*m/z*, %): 639.2 ($M^+$, 0.08), 638.2 ($M^+ - 1$, 0.09). Elem. anal. for $C_{30}H_{31}FN_6O_7S$ (638.67), calcd: C, 56.42; H, 4.89; N, 13.16. Found: C, 56.71; H, 4.97; N, 13.42.

*4.1.6.4. 1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(3-oxo-3-((4-(N-(quinoxalin-2-yl)sulfamoyl) phenyl) amino) propyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (7d).* Yield: (5.2 g) 75.91%. Mp: 176−179 °C. IR (KBr) $\upsilon_{max}/cm^{-1}$: 3437 (OH), 3368, 3248 (NH), 3098, 3051, 3016 (CH Ar), 2959, 2928 (CH aliphatic), 1721, 1670, 1628 (3C=O), 1339, 1157 ($SO_2$). $^1$H NMR (400 MHz, DMSO) *δ ppm*:: 1.17−1.19 (m, 2H, $CH_2$ of cyclopropyl), 1.27−1.33 (m, 2H, $CH_2$ of cyclopropyl), 2.57 (t, 2H, *J* = 6.78 Hz,O=C−$CH_2$), 2.68 (s broad, 4H, piperazine), 2.76 (t, 2H, *J* = 6.78 Hz, N−$CH_2$), 3.35 (s broad, 4H, piperazine, overlapped), 3.80−3.83 (m, 1H, CH of cyclopropyl), 7.53−7.61 (m, 2H, ArH), 7.69−7.80 (m, 4H, ArH), 7.88−7.96 (m, 3H, 2ArH + NH), 8.05 (d, 2H, *J* = 8.76 Hz, ArH), 8.58 (s, 1H, ArH), 8.66 (s, 1H, ArH), 10.42 (s, 1H, NH, exch. $D_2O$), 15.22 (s, 1H, OH, exch. $D_2O$). $^{13}$C NMR (100 MHz, DMSO) *δ ppm*: 8.0, 34.4, 36.3, 49.6, 52.5, 53.7 (aliphatic carbons), 106.8, 107.2 (d, $^3J_{F\text{-}C}$ = 8 Hz), 111.4 (d, $^2J_{F\text{-}C}$ = 23 Hz), 118.8, 119.0 (d, $^3J_{F\text{-}C}$ = 8 Hz), 127.5, 129.1, 129.6, 131.2, 134.4, 138.1, 139.6, 140.0, 143.6, 145.5 (d, $^2J_{F\text{-}C}$ = 10 Hz), 147.1, 148.4, 148.5, 153.4 (d, $^1J_{F\text{-}C}$ = 249 Hz), 161.6 (Ar carbons), 166.5, 171.2, 176.8 (C=O). MS (*m/z*, %): 685.64 ($M^+$, 42.20). Elem. anal. For$C_{34}H_{32}FN_7O_6S$ (685.72), calcd: C, 59.55; H, 4.70; N, 14.30. Found: C, 59.63; H, 4.78; N, 14.53.

*4.1.6.5. 7-(4-(3-((4-(N-(6-chloropyrazin-2-yl)sulfamoyl)phenyl) amino)-3-oxopropyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (7e).* Yield: (4.0 g) 59.69%. Mp: 173−175 °C. IR (KBr) $\upsilon_{max}/cm^{-1}$: 3441 (OH), 3337, 3271 (2NH), 3097, 3052 (CH Ar), 2882 (CH aliphatic), 1713, 1670, 1632 (3C = O), 1335, 1192 ($SO_2$). $^1$H NMR (400 MHz, DMSO) *δ ppm*: 1.19 (s, 2H, $CH_2$ of cyclopropyl), 1.31−1.34 (m, 2H, $CH_2$ of cyclopropyl), 2.65 (t, 2H, *J* = 6.40 Hz, O=C−$CH_2$), 2.89 (t, 2H, *J* = 6.00 Hz, N−$CH_2$), 3.36 (s broad, 4H, piperazine, overlapped),3.45 (s broad, 4H, piperazine), 3.81−3.84 (m, 1H, CH of cyclopropyl), 7.57 (d, 1H, *J* = 7.48 Hz, ArH), 7.78 (d, 2H, *J* = 8.84 Hz, ArH), 7.89−7.96 (m, 3H, ArH), 8.13 (s, 1H, ArH), 8.19 (s, 1H, ArH), 8.68 (s, 1H, ArH), 10.46 (s, 1H, NH, exch. $D_2O$), 11.42 (s, 1H, NH, exch. $D_2O$), 15.20 (s, 1H, OH, exch. $D_2O$). $^{13}$C NMR (100 MHz, DMSO) *δ ppm*: 8.1, 36.4, 44.9, 49.3, 52.4, 53.5 (aliphatic carbons), 107.2 (d, $^3J_{F\text{-}C}$ = 4 Hz), 107.5, 111.5 (d, $^2J_{F\text{-}C}$ = 23 Hz), 118.9, 119.2 (d, $^3J_{F\text{-}C}$ = 7 Hz), 129.2, 133.4, 135.9, 139.6, 139.6, 143.5, 145.4 (d, $^2J_{F\text{-}C}$ = 10 Hz), 145.9, 148.5, 148.6, 153.5 (d, $^1J_{F\text{-}C}$ = 245 Hz) (Ar carbons), 161.5, 166.4, 176.8 (C=O). MS (*m/z*, %): 670.50 ($M^+$, 0.01). Elem. anal. for $C_{30}H_{29}ClFN_7O_6S$ (670.11), calcd: C, 53.77; H, 4.36; N, 14.63. Found: C, 53.84; H, 4.53; N, 14.89.

### *4.1.7. Synthesis of acylated sulfonamides (8a-c) (Scheme 2)*

Compounds **8a-c** were prepared using the same procedure adopted for synthesizing compounds **4a-e** but using 2-chloropropyl chloride instead of chloroacetyl chloride.

*4.1.7.1. 2-Chloro-N-(4-sulfamoylphenyl) propanamide (8a).* Yield: (2.2 g) 83.7%. Mp: 224−226 °C. IR (KBr) υmax/cm-1: 3333, 3310, 3210 (NH, $NH_2$), 3113 (CH Ar), 2978, 2936 (CH aliphatic), 1686 (C=O), 1331, 1161 ($SO_2$). $^1$H NMR (400 MHz, DMSO) *δ* ppm: 1.64 (d, 3H, *J* = 6.68 Hz, $CH_3$), 4.71 (q, 1H, *J* = 6.68 Hz, CH), 7.29 (s, 2H, $NH_2$, exch. $D_2O$), 7.77−7.82 (m, 4H, ArH), 10.64 (s, 1H, NH, exch. $D_2O$). $^{13}$C NMR (100 MHz, DMSO) *δ* ppm: 21.3, 55.1 (aliphatic carbons), 119.6, 127.3, 139.5, 141.9 (Ar carbons), 168.3 (C=O).

*4.1.7.2. 2-Chloro-N-(4-(N-(5-methylisoxazol-3-yl)sulfamoyl)phenyl) propanamide (8b).* Yield: (2.8 g) 81.4%. Mp: 193−195 °C. IR (KBr) υmax/cm-1: 3264, 3198 (2 NH), 3121, 3075 (CH Ar), 2982 (CH aliphatic), 1678 (C=O), 1335, 1096 ($SO_2$). $^1$H NMR (400 MHz, DMSO) *δ* ppm: 1.62 (d, 3H, *J* = 6.64 Hz, Cl−CH−C$H_3$), 2.30 (s, 3H, $CH_3$), 4.70 (q, 1H, *J* = 6.64 Hz, $^{13}$C NMR (100 MHz, DMSO) *δ* ppm: 12.5, 21.3, 55.0 (aliphatic carbons), 95.8, 119.8, 128.6, 134.3, 143.2, 158.0 (Ar carbons), 168.5, 170.8 (C=O and C−O).CH), 6.13 (s, 1H, ArH), 7.79−7.85 (m, 4H, ArH), 10.73 (s, 1H, NH, exch. $D_2O$), 11.36 (s, 1H, NH, exch. $D_2O$).

*4.1.7.3. 2-Chloro-N-(4-(N-(quinoxalin-2-yl) sulfamoyl)phenyl)propanamide (8c).* Yield: (3.5 g) 89.5%. Mp:205−207 °C. IR (KBr) υmax/cm-1:3360, 3264 (2 NH), 3102, 3067 (CH Ar), 2997, 2940, 2905 (CH

aliphatic), 1697 (C=O), 1358, 1092 (SO2). 1H NMR (400 MHz, DMSO) $\delta$ ppm: 1.59 (d, 3H, $J$ = 6.64 Hz, $CH_3$), 4.66 (q, 1H, $J$ = 6.62 Hz, CH), 7.62 (t, 1H, $J$ = 7.18 Hz, ArH), 7.74 (t, 1H, $J$ = 7.12 Hz, ArH), 7.81 (d, 2H, $J$ = 8.84 Hz, ArH), 7.94 (d, 2H, $J$ = 8.16 Hz, ArH), 8.09 (d, 2H, $J$ = 7.40 Hz, ArH), 8.61 (s, 1H,ArH), 10.68 (s, 1H, NH, exch. $D_2O$), 11.92 (s, 1H, NH, exch. $D_2O$). $^{13}$C NMR (100 MHz, DMSO) $\delta$ ppm: 21.3, 55.0 (aliphatic carbons), 119.4, 127.7, 127.9, 129.2, 129.8, 129.8, 131.3, 131.4, 131.4,138.7, 143.1, 146.5 (Ar carbons), 168.4 (C=O).MS (*m/z*, %): 390.33 (M+, 40.40). Elem. anal. For $C_{17}H_{15}ClN_4O_3S$ (390.84), calcd: C, 52.24; H, 3.87; N, 14.33. Found:C, 52.51; H, 4.04; N, 14.60.

### 4.2. Synthesis of ciprofloxacin-sulfonamide hybrids with amide linker (9a-c) (Scheme 2)

Compounds **9a-c** were prepared using the same procedure adopted for synthesizing compounds **5a-e** but using 2- chloropropionyl sulfonamide derivative (**8a-c**) instead of chloroacetyl sulfonamide derivative (**4a-e**).

#### 4.2.1. 1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(1-oxo-1-((4-sulfamoylphenyl)amino)propan-2-yl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (9a)

Yield: (3.8 g) 68.15%. Mp: 237–240 °C. IR (KBr) $\upsilon_{max}$/cm$^{-1}$: 3545 (OH), 3296, 3229 ($NH_2$, NH), 3102, 3059 (CH Ar), 2982, 2936 (CH aliphatic), 1720, 1682, 1632 (C=O), 1335, 1157 ($SO_2$). $^1$H NMR (400 MHz, DMSO) $\delta$ *ppm*: 1.18 (s, 2H, $CH_2$ of cyclopropyl),1.27 (d, 3H, $J$ = 6.76 Hz, CH–C$\underline{H}_3$), 1.32 (d, 2H, $J$ = 6.64 Hz, $CH_2$ of cyclopropyl), 2.74–2.81 (m, 4H, piperazine), 3.40 (s broad, 4H, piperazine, overlapped), 3.62–3.63 (s broad, 1H, O=C–C$\underline{H}$), 3.79–3.81 (m, 1H, $\underline{CH}$ of cyclopropyl), 7.26 (s, 2H, $NH_2$, exch. $D_2O$), 7.56 (d, 1H, $J$ = 7.44 Hz, ArH), 7.77 (d, 2H, $J$ = 8.80 Hz, ArH), 7.83–7.90 (m, 3H, ArH),8.65 (s, 1H, ArH), 10.21 (s, 1H, NH, exch. $D_2O$), 15.20 (s, 1H, OH, exch. $D_2O$).$^{13}$C NMR (100 MHz, DMSO) $\delta$ *ppm*: 8.0, 12.9, 36.3, 49.2, 50.0, 63.7 (aliphatic carbons), 106.7 (d, $^3J_{F\text{-}c}$ = 3 Hz), 107.1, 111.4 (d, $^2J_{F\text{-}c}$ = 23 Hz),119.0 (d, $^3J_{F\text{-}c}$ = 7 Hz), 119.6, 127.1, 139.0, 139.6, 142.1, 145.6 (d, $^2J_{F\text{-}c}$ = 10 Hz), 148.4, 153.5 (d, $^1J_{F\text{-}c}$ = 248 Hz) (Ar carbons), 166.5, 172.2, 176.8 (C=O). MS (*m/z*, %): 557.41 (M$^+$, 10.93). Elem. anal. for $C_{26}H_{28}FN_5O_6S$ (557.59), calcd: C, 56.00; H, 5.06; N, 12.56. Found: C, 56.23; H, 5.32; N, 12.82.

#### 4.2.2. 1-Cyclopropyl-6-fluoro-7-(4-(1-((4-(N-(5-methylisoxazol-3-yl)sulfamoyl) phenyl) amino)-1-oxopropan-2-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (9b)

Yield: (4.5 g) 70.46%. Mp: charred at 245 °C. IR (KBr) $\upsilon_{max}$/cm$^{-1}$: 3441, 3306 (2NH, OH overlapped), 3098, 3052 (CH Ar), 2982, 2943 (CH aliphatic), 1713, 1670, 1628 (3C = O), 1339, 1165 ($SO_2$). $^1$H NMR (400 MHz, DMSO) $\delta$ *ppm*: 1.17–1.21 (m, 2H, $CH_2$ of cyclopropyl), 1.26 (d, 3H, $J$ = 6.68Hz, CH–C$\underline{H}_3$), 1.30–1.34 (m, 2H, $CH_2$ of cyclopropyl), 2.30 (s, 3H, $CH_3$), 3.37–3.45 (m, 5H, piperazine + O=C–C$\underline{H}$), 3.60–3.64 (m, 4H, piperazine), 3.81–3.83 (m, 1H, $\underline{CH}$ of cyclopropyl), 7.60 (d, 1H, $J$ = 7.40 Hz, ArH), 7.80–7.93 (m, 5H, ArH), 8.13 (s, 1H, ArH), 8.66 (s, 1H, ArH), 10.30 (s, 1H, NH, exch. $D_2O$), 11.33 (s, 1H, NH, exch. $D_2O$), 15.16 (s, 1H, OH, exch. $D_2O$).$^{13}$C NMR (100 MHz, DMSO) $\delta$ *ppm*: 8.0, 12.5, 12.9, 36.4, 44.9, 49.5, 50.6 (aliphatic carbons), 95.8, 107.2, 107.4 (d,$^3J_{F\text{-}c}$ = 3 Hz), 111.4 (d, $^2J_{F\text{-}c}$ = 23 Hz),119.4 (d, $^3J_{F\text{-}c}$ = 8 Hz), 119.8, 128.4, 133.7, 135.3, 136.5, 139.5, 145.4 (d, $^2J_{F\text{-}c}$ = 10 Hz), 148.5, 153.4 (d, $^1J_{F\text{-}c}$ = 248 Hz), (Ar carbons), 158.0, 161.5, 166.4, 176.8 (C=O and C–O). MS (*m/z*, %): 638.58 (M$^+$, 56.52). Elem. anal. for $C_{30}H_{31}FN_6O_7S$ (638.67), calcd: C, 56.42; H, 4.89; N, 13.16. Found: C, 56.67; H, 4.97; N, 13.34.

#### 4.2.3. 1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(1-oxo-1-((4-(N-(quinoxalin-2-yl) sulfamoyl) phenyl) amino) propan-2-yl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (9c)

Yield: (4.8 g) 70.59%. Mp: 182–185 °C. IR (KBr) $\upsilon_{max}$/cm$^{-1}$: 3441(0H), 3375, 3267 (2NH), 3098, 3063 (CH Ar), 2955, 2932 (CH aliphatic), 1713, 1670, 1628 (C=O), 1339, 1157 ($SO_2$). $^1$H NMR (400 MHz, DMSO) $\delta$ *ppm*: 1.16–1.24 (m, 5H, $CH_2$ of cyclopropyl + CH–C$\underline{H}_3$), 1.29–1.32 (m, 2H, $CH_2$ of cyclopropyl), 2.72–2.78 (m, 4$\underline{H}$, piperazine), 3.60–3.64 (m, 4H, piperazine), 3.79–3.81 (m, 2H, $\underline{CH}$ of cyclopropyl + O=C–C$\underline{H}$), 7.50 (d, 1H, $J$ = 7.40 Hz, ArH), 7.56–7.62 (m, 2H, ArH), 7.72 (t, 1H, $J$ = 7.24 Hz, ArH), 7.80–7.95 (m, 5H, ArH), 8.08 (d, 1H, $J$ = 8.84 Hz, ArH), 8.13 (s, 1H, ArH), 8.64 (s, 1H, ArH), 10.26 (s, 1H, NH, exch. $D_2O$), 15.18 (s, 1H, OH, exch. $D_2O$). $^{13}$C NMR (100 MHz, DMSO) $\delta$ *ppm*: 8.0, 12.8, 36.3, 44.9, 49.5, 50.6 (aliphatic carbons), 106.5, 107.1 (d, $^3J_{F\text{-}c}$ = 7 Hz), 111.3 (d, $^2J_{F\text{-}c}$ = 23 Hz), 119.3 (d, $^3J_{F\text{-}c}$ = 8 Hz), 119.3, 127.7, 129.1, 129.5, 131.3, 134.3, 138.2, 139.4, 139.4, 143.4, 145.3 (d, $^2J_{F\text{-}c}$ = 10 Hz), 146.6, 148.3, 153.3 (d, $^1J_{F\text{-}c}$ = 248 Hz), 161.5, 162.8 (Ar carbons), 166.5, 172.3, 176.6 (C=O). MS (*m/z*, %): 685.31 (M$^+$, 16.19). Elem. anal. for $C_{34}H_{32}FN_7O_6S$ (685.72), calcd: C, 59.55; H, 4.70; N, 14.30. Found: C, 59.79; H, 4.57; N, 14.56.

### 4.3. Pharmacology

#### 4.3.1. Staphylococcus aureus topoisomerase IV decatenation assay

The assay kit *Staphylococcus aureus* topoisomerase IV decatenation was provided by (inspiralis) and the assay was performed according to established protocols obtained from the supplier [46]. The new compounds and the standard inhibitor ciprofloxacin were dissolved in DMSO and serially diluted at concentrations of 100, 10, 1 and 0.1 μM, and then assayed in reaction then assayed in reaction mixtures in different replicate runs. the final reaction volume was 20 μL, containing 40 mM Tris pH 7.5, 10 mM DTT, 6 mM $MgCl_2$, 100 mM potassium glutamate, 1 mM ATP, 50 mg/mL acetylated BSA and 0.2 mg kDNA substrate. The reactions were initiated by addition of 2 U of *Staphylococcus aureus* topoisomerase IV (TopoGen), and 3 μL of inhibitor solution in 10% DMSO, and then were incubated with shaking for 30 min at 37 °C. All of the reactions were terminated by the addition of 10 mL of a 3X gel-loading buffer (final concentration: 6 mM EDTA, 1.2% SDS, 0.02% bromophenol blue, and 10% glycerol blue), after which 20 mL of this was loaded on a 1% agarose, TAE (0.01 M EDTA pH 8.3, 40 mM Tris-acetate) gel that was then run at 60 V for 3 h. The gel was stained by (0.5 mg/L) ethidium bromide in TAE for 30 min and then destained in water for 20 min. Fluorescent images were taken at a wavelength of 300 nm on a UV transilluminator imaging system. The fluorescence intensity of the decatenation product was quantitated using ImageQuant software (Molecular Dynamics, Sunnyvale, CA, USA). The results as IC50 values μM (concentration of the tested compound that leads to 50% inhibition of enzyme activity) for all samples were determined by nonlinear regression analysis in GraphPad Prism [47]. The average IC50 values (μM) of the experiments were calculated for the target compounds and the reference antibiotic and then listed in Table 1.

#### 4.3.2. Escherichia coli DNA gyrase supercoiling assay

Commercially available assay kit Topogen, (Port Orange, FL). The assay was carried out according to established protocols obtained from the supplier [48,49]. The new compounds and the standard inhibitor (ciprofloxacin) were dissolved in DMSO and serially diluted at concentrations of 100, 10, 1 and 0.1 μM, and then assayed in reaction mixtures in different replicate runs. The final reaction volume was 20 μL, which included 35 mM Tris pH 7, 2 mM DTT, 24 mM KCl, 4 mM $MgCl_2$, 1.8 mM spermidine, 0.1 mg/mL acetylated BSA, 6.5% (w/v) glycerol, 1 mM ATP, 0.1 mg/mL album and 0.2 mg pBR322 substrate. The reactions were initiated by addition of 2 U of *Escherichia coli* DNA gyrase (TopoGen), and 3 μL of inhibitor solution in 10% DMSO, and then were incubated with shaking for 30 min at 37 °C. All of the reactions were terminated by the addition of 10 mL of a 3X gel-loading buffer (final concentration: 6 mM

EDTA, 1.2% SDS, 0.02% bromophenol blue, and 10% glycerol blue), after which 20 mL of this was loaded on a 1% agarose, TAE (0.01 M EDTA pH 8.3, 40 mM Tris-acetate) gel that was then run at 60 V for 3 h. The gel was stained by (0.5 mg/L) ethidium bromide in TAE for 30 min and then destained in water for 20 min. Fluorescent images were taken at a wavelength of 300 nm on a UV transilluminator imaging system. The fluorescence intensity of the supercoiled plasmid reaction product was quantitated using ImageQuant software (Molecular Dynamics, Sunnyvale, CA, USA). The results as IC50 values (μM) (concentration of the tested compound that leads to 50% inhibition of enzyme activity) for all samples were determined by nonlinear regression analysis in GraphPad Prism [47]. The average IC50 values (μM) of the experiments were calculated for the target compounds and the reference antibiotic. The results were recorded in Table 1.

#### 4.3.3. MIC determination

The antibacterial activity of the synthesized compounds was tested against a model organism of Gram-positive and Gram-negative pathogens. Namely, the following standard strains: *Staphylococcus aureus Newman* and *Escherichia coli ATCC8739*. The minimum inhibitory concentration (MIC) of the tested compounds was determined by the broth microdilution method according to the guidelines of the Clinical and Laboratory Standards Institute [50]. Ciprofloxacin hydrochloride was used as a positive control. Stock solutions of the tested compounds were prepared in DMSO (Dimethyl sulfoxide). These stock solutions (960 μg/mL) were further diluted in Mueller Hinton broth (MHB) to a final concentration of (16 μg/mL). 100 μL of sterile MHB was pipetted into each well of a sterile 96-well microplate and 100 μL of the tested compound in MHB (16 μg/mL) was then added to the first well of each row. Two-fold serial dilutions were done across the microplates (8–0.0039 μg/mL). Bacterial inoculum was then added to the wells, each well was inoculated with 10 μL of the tested bacterial suspension (108 CFU/mL). One row was used as a sterility control (neither tested compound nor tested bacterial suspension was added) and another row was used as a growth control (inoculated with the tested bacterial suspension without adding the tested compound and using DMSO as negative control). The microplates were incubated aerobically at 37 °C for 24 h. After incubation, the plates were checked and the MIC was detected as the lowest concentration with no detectable bacterial growth (no turbidity). The MIC of Compound **7e** was higher than the tested concentrations so another stock solution (61440 μg/mL) was prepared in DMSO and then the same procedures described above were adopted. The experiment was performed three independent times and the MIC was recorded as mean ± SD (standard deviation). The results were recorded in Table 2.

#### 4.3.4. In vivo, evaluation of convulsive activity

Swiss male albino mice weighing 20–25 g were used. The mice were kept in the animal house under the standard conditions of light and temperature deprived of food and not water for 24 h before the experiment. The animals were randomly divided into 6 groups (control, ciprofloxacin and 4 tested compounds), each of 6 mice. Ciprofloxacin and the potassium salt of the tested compounds dissolved in distilled water for injection and were administered intravenously with an equimolar single dose (0.6 mmol/kg body weight) [32] at fixed speed of 20 mL/kg and speed of 0.5 mL/min, while the control group received only saline. The animals were observed for 6 h, the incidence of clonic convulsions will be scored and mortality rate will be determined [32].

Animals will be sacrificed by decapitation under light anesthesia at the end of the study and the brains of three mice of each group will be separated and sent to analyze GABA expression by western blot. All dead bodies will be frozen till incineration.

*4.3.4.1. Western blot analysis.* Estimation of brain GABA protein expression was carried out using western blot methodology. Brain tissues were homogenized in a lysis buffer and quantified for protein levels using a Bio-Rad Protein Assay Kit (Bio-Rad, Hercules, CA, USA). After protein solutions are extracted from tissues, an aliquot of 50 μg protein from each sample are loaded onto a sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) and transferred to a nitrocellulose membrane using a semi dry transfer apparatus (BioRad, Hercules, CA, USA). The nitrocellulose membrane was stained in a Ponceau solution (Sigma–Aldrich, St. Louis, MO, USA) for 30 s to check uniform transfer of proteins. The membrane was blocked with a 5% blocking solution containing non-fat dry milk in tris buffered saline tween (TBST) buffer for overnight at 4 °C. The membranes were then washed with TBST and incubated with a 1:1000 dilution of antibodies to GABA (Thermo Fisher Scientific, Waltham, USA) for 1 h at room temperature of 27 °C with constant shaking. The filters were washed and subsequently probed with the secondary antibody, which was Horseradish peroxidase conjugated goat anti-mouse immunoglobulin (Amersham, Life Science Inc., Arlington Heights, IL, USA). Finally, Chemiluminescence detection was performed with the Amersham detection kit according to the manufacturer's protocols and exposed to X-ray film. The amount of GABA receptor was quantified by densitometric analysis of the autoradiograms using a scanning laser densitometer (Biomed Instrument Inc., USA). Results were expressed as arbitrary units after normalization for beta-actin (β-actin) protein expression [51]. Statistical analysis was performed using GraphPad Prism software version 6 (GraphPad Software, San Diego, CA, USA). Comparisons between means were carried out using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. A probability level of less than 0.05 was accepted as statistically significant.

### 4.4. Molecular docking studies

The enzyme was prepared for docking using Protonate 3D protocol in MOE with default options. The co-crystalized ligand was used to define the active site for docking. Triangle Matcher placement method and London dG scoring function were used for the docking protocol. All minimizations were performed with MOE until the RMSD gradient of 0.05 kcal·mol−1 Å −1 with MMFF94x force field and the partial charges were automatically calculated. The site for docking was defined by selecting the co-crystallized ligand (Ciprofloxacin or levofloxacin) for pdb: 2XCT and 3RAE, respectively. Docking setup was first validated by re-docking of the co-crystallized ligand (Ciprofloxacin and levofloxacin) in the vicinity of the active site of each enzyme. Ciprofloxacin re-docking resulted in an energy score (S) = −9.07 kcal/mol and RMSD of 0.822 Å for DNA gyrase of *Staphylococcus aureus*. Levofloxacin re-docking resulted in an energy score (S) = −10.57 kcal/mol and RMSD of 0.522 Å for DNA Topoisomerase IV of S. Pneumonia. The validated setup was then used in predicting the binding mode and the binding interactions of the newly synthesized ligands at the active site of DNA gyrase [52].

In our study, we couldn't use docking energy scores to estimate and analyze the binding mode related to biological activities where there is no difference between them.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2021.114021.

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