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Synthesis, molecular docking, and evaluation of antibacterial activity of 1,2,4-triazole-norfloxacin hybrids

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ABSTRACT

A series of 1,2,4-triazole-norfloxacin hybrids was designed, synthesized, and evaluated for *in vitro* antibacterial activity against common pathogens. All the newly synthesized compounds were characterized by Fourier-transform infrared spectrophotometry, proton and carbon nuclear magnetic resonance, and electrospray ionization-mass spectrometry. Representative compounds from each step of the synthesis were further characterized by X-ray crystallography. Many of the compounds synthesized exhibited antibacterial activity superior to that of norfloxacin toward both, gram-positive and gram-negative bacteria. The toxicity of the 1,2,4-triazole-norfloxacin hybrids toward bacterial cells was 32–512 times higher than that toward mouse fibroblast cells. Moreover, hemolysis was not observed at concentrations of 64 μg/mL, suggesting good biocompatibility. Molecular docking showed a least binding energy of −9.4 to −9.7 kcal/mol, and all compounds were predicted to show remarkable affinity for the bacterial topoisomerase IV.

1. Introduction

After over 40 years of design and development, fluoroquinolones are among the most widely used first-line, broad-spectrum antibiotics that exhibit high efficacy and low toxicity [1]. However, fluoroquinolones have lost their unique advantage in anti-infective therapy owing to the emergence of drug resistance [2–4]. Therefore, it is imperative to develop new antimicrobial agents of this class that are effective against drug-resistant strains of bacteria.

Fluoroquinolones interact with DNA gyrase and topoisomerase IV in bacterial cells. They impede bacterial DNA synthesis by affecting the incision-sealing function during DNA synthesis and interfere with the normal replication and transcription of DNA, inducing oxidative damage and consequently, cell death [5]. Existing structure-activity relationship (SAR) data suggest that the C-7 position of fluoroquinolone is the most suitable site for chemical modification [6–8]. Such modifications can allow us to improve the antibacterial activity, pharmacokinetic properties, safety index, and antimicrobial spectrum of existing compounds. Substituents at the C-7 position can enhance the binding interactions, including hydrogen bonding and van der Waal's forces, between the fluoroquinolone molecules and the amino acids in the target binding site [9,10]. Moreover, introduction of large substituents at the C-7 position does not decrease their permeability [8]. At present, several fluoroquinolone hybrids have been obtained by introducing various

Abbreviations: SAR, structure-activity relationships; IR, infrared; MS, mass spectrometry; COSY, correlated spectroscopy; ^{1}H NMR, proton nuclear magnetic resonance; ^{13}C NMR, carbon-13 nuclear magnetic resonance; DEPT, distortionless enhancement by polarization transfer; MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration; TPSA, topological polar surface area; clogP, calculated logarithm of partition coefficient between *n*-octanol and water; DMSO-d_6, deuterated dimethyl sulfoxide; OD, optical density; DMSO, dimethyl sulfoxide.

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functional groups, including isatin [11], piperazine-azole [7], quinolone-based quaternary ammonium [12], bromothiophene [13], and 1,3,4-thiadiazole [14] at the C-7 position.

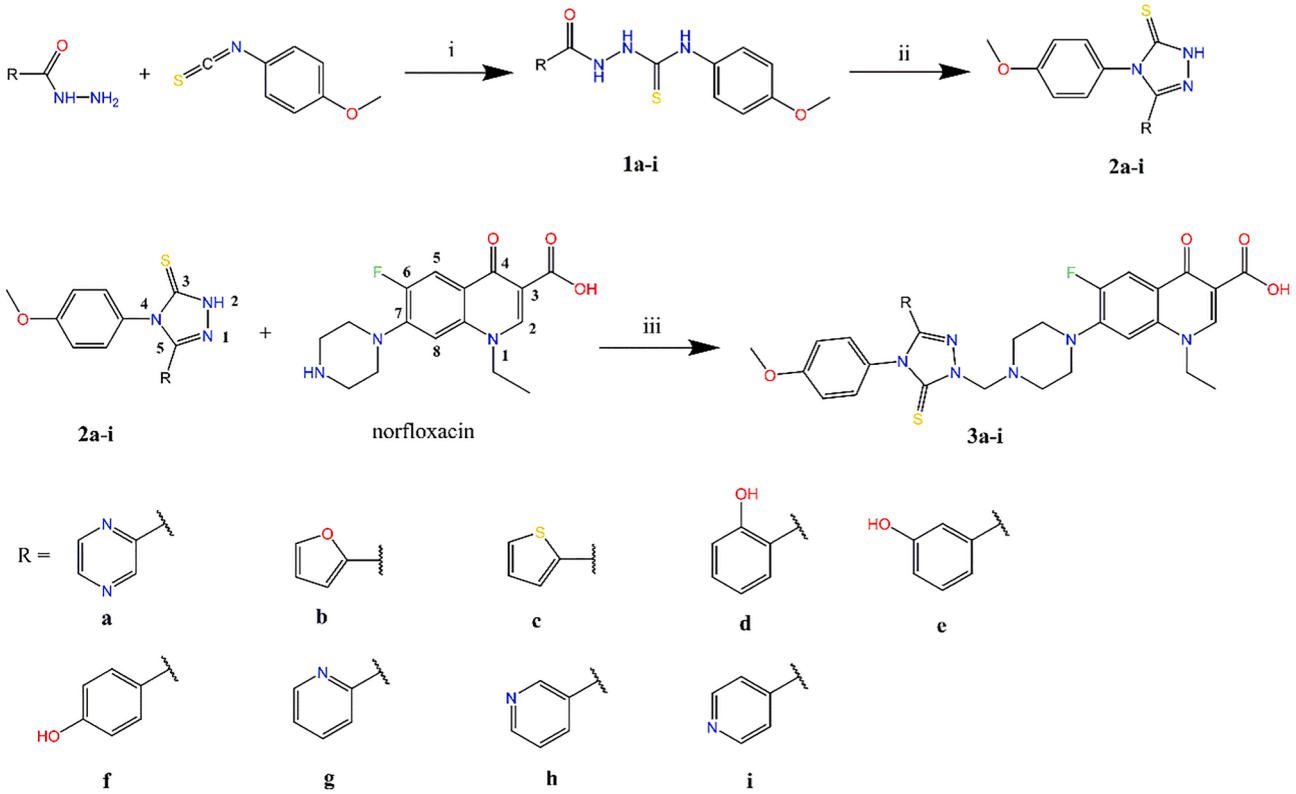
1,2,4-Triazole is a common pharmacophore found in many drug molecules, and its derivatives have been reported to exhibit varied pharmacological properties, including antibacterial activity [15]. In recent years, several series of 1,2,4-triazole-fluoroquinolone hybrids have been obtained by combining fluoroquinolones with various 1,2,4-triazole derivatives; the majority of them have been found to be far more active than the parent antibiotics [7,16], exhibiting enhanced antibacterial activity in both gram-positive and gram-negative bacteria. Prior SAR studies suggest that the C-5 and N-4 substituents of the 1,2,4-triazole-3-thione ring affect the antibacterial activity of 1,2,4-triazole-fluoroquinolone hybrids. A phenyl ring at the C-5 position has been reported to be essential for activity, and electron-donating substituents, such as hydroxyl groups, on the phenyl ring have been found to be advantageous [17]. Although the nature of N-4 substituents seems less important, *p*-methylphenyl at this position has been found to be beneficial for antibacterial activity. A change in the position of the hydroxyl group (*ortho*-, *meta*-, *para*-) on the C-5 phenyl ring was reported to slightly affect the antibacterial activity [16,18]. The replacement of the phenyl ring with a pyridyl group at C-5 position is also well tolerated [8]. It can therefore be inferred that the C-5 position substituent requires aromaticity. So far, there are no reports of 1,2,4-triazole-norfloxacin hybrids [7,19]. Therefore, we aimed to synthesize a series of novel 1,2,4-triazole-norfloxacin hybrids for development as antibacterial agents.

2. Results and discussion

2.1. Chemistry

As shown in Scheme 1, the thiosemicarbazide derivatives **1a–i** were obtained by reacting 1-isothiocyanato-4-methoxybenzene with the appropriate hydrazides in ethanol or methanol. The infrared (IR) spectra of compounds **1a–i** showed strong absorption at 1660–1694 cm^{-1} (C═O band), 1504–1514 cm^{-1} (C═S band) and 1243–1258 cm^{-1} (C—O band). The mass spectrometry (MS) data displayed molecular ion peaks corresponding to the calculated molecular weights. The proton nuclear magnetic resonance (^{1}H NMR) spectra indicated the presence of three NH groups (δ 9.50–10.87 ppm) and an —OCH_3 group (singlet at δ 3.74–3.75 ppm). The aromatic hydrogen of the representative compound **1a** was accurately identified by proton-proton correlated spectroscopy (^{1}H–^{1}H COSY) (Fig. A.2). The carbon nuclear magnetic resonance (^{13}C NMR) spectrum of compound **1a** showed the presence of 13 carbon atoms, and its distortionless enhancement by polarization transfer (DEPT) spectrum indicated the presence of one primary carbon, seven tertiary carbons, and five quaternary carbons (Fig. A.3). These data accurately correspond to the structure of compound **1a**. The IR, MS, ^{1}H NMR, and ^{13}C NMR data confirmed the successful synthesis of compounds **1a–i**. In order to further understand the spatial configuration of compounds **1a–i**, we cultured single crystals of compound **1a** (Fig. 1a). The C6—N4, C6—N5, and C6—S1 bond lengths were found to be 1.335(7) Å, 1.344(7) Å, and 1.697(4) Å, respectively, indicating that C6—N4 and C6—N5 bonds were single bonds, while C6—S1 bond was a double bond. The N4—C6—N5 bond angle was recorded as 117.0(4)°. The N3, N4, C6, and N5 atoms were nearly coplanar, showing a N3—N4—C6—N5 torsion angle of −3.9(7)°. The S1 atom was in the plane (deviation = 0.109 Å), while the O1 atom was out of the plane (deviation = 2.241 Å).

Next, compounds **1a–i** were subjected to base-promoted cyclisation in the presence of aqueous NaOH, to yield the 1,2,4-triazole derivatives **2a–i**. The structures of compounds **2a–i** were confirmed by the disappearance of the stretching vibration of the C═O bonds at 1660–1694 cm^{-1} in the IR spectra. The MS spectra of compounds **2a–i** displayed the molecular ion peaks corresponding to the desired molecular weights. The ^{1}H NMR spectra showed the presence of only one NH group (δ 13.91–14.36 ppm). The aromatic hydrogen of the representative compound **2a** was accurately identified by ^{1}H–^{1}H COSY (Fig. A.13). The ^{13}C



Scheme 1. Synthetic pathway for the target compounds **3a–i**. i: ethanol or methanol; ii: 2% NaOH, 70 °C; iii: formaldehyde, dimethylformamide.

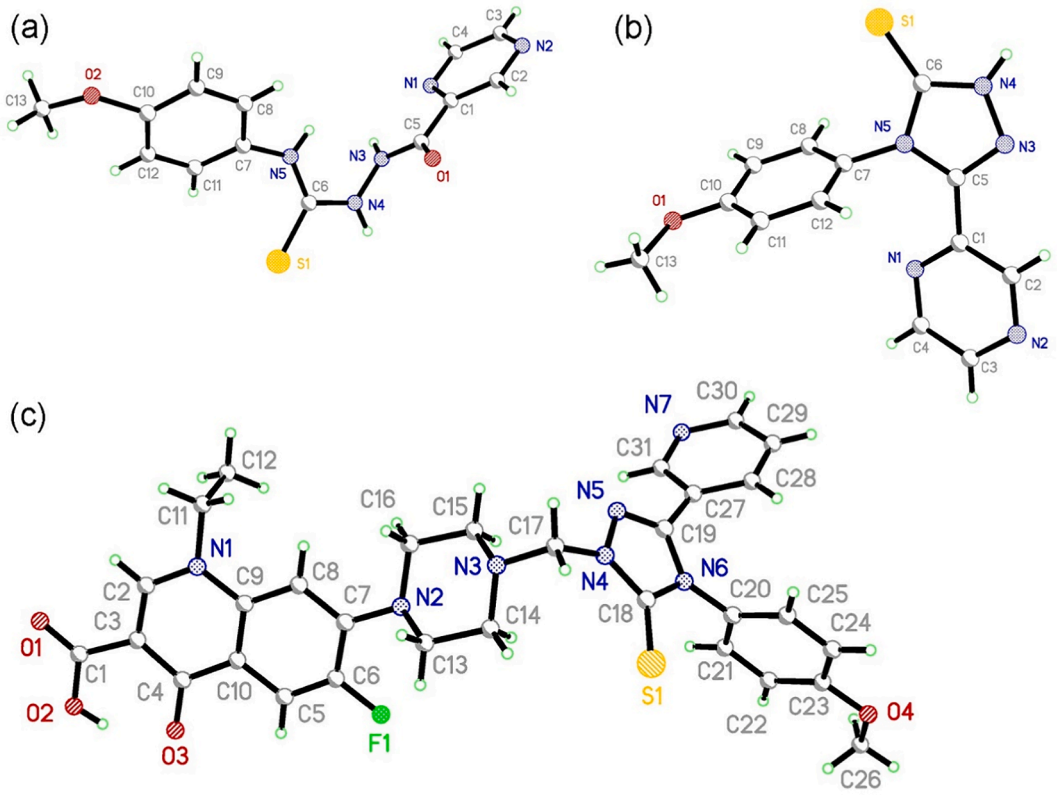


Fig. 1. The molecular structure of compounds **1a**, **2a** and **3h** with displacement ellipsoids at the 50% probability.

NMR spectrum of compound **2a** still showed the presence of 13 carbons, and the DEPT spectrum indicated the presence of one primary carbon, seven tertiary carbons, and five quaternary carbons (Fig. A.14). These data correspond to the structure of the desired compound. However, compared with **1a**, the chemical shifts of the triazole carbon and the pyrazine carbon connected with the triazole become smaller, while the chemical shifts of the three benzene carbons close to triazole become larger. All the IR, MS, ^{1}H NMR, and ^{13}C NMR data confirmed the successful synthesis of compounds **2a-i**. In order to further understand the spatial configuration of the compounds, we cultured single crystals of compound **2a** (Fig. 1b). The C5—N5, C5—N3, and C6—S1 bond lengths were found to be 1.3843(18) Å, 1.3069(19) Å, and 1.6659(15) Å, respectively, indicating that C5—N5 was a single bond, while C5—N3 and C6—S1 were double bonds. The C5—N5—C6 bond angle was recorded as 107.22(12)°, which is close to the expected value of 108° for the idealized regular pentagon. The dihedral angle between the benzene and pyrazine rings was 61.36°, in order to minimize the steric hindrance.

Following the successful synthesis of compounds **2a–i**, they were reacted with norfloxacin and formaldehyde through the Mannich reaction to yield the desired final compounds **3a–i**. The successful synthesis of compounds **3a–i** was supported by the appearance of stretching vibrations at 1627–1630 cm^{-1} (aromatic ketone) and 1720–1731 cm^{-1} (COOH) in the IR spectra. The ^{1}H NMR spectra revealed new singlet signals at δ 5.25–5.33 ppm (—CH_2), and triplet and quartet signals at δ 1.41–1.42 ppm and δ 4.58–4.61 ppm, respectively (—CH_2CH_3). The aromatic hydrogen of the representative compound **3a** was accurately identified by ^{1}H–^{1}H COSY (Fig. A.24). The ^{13}C NMR spectrum of compound **3a** showed the presence of 30 carbon atoms, and the DEPT spectrum revealed that 2 of these were primary carbons, 6 secondary, 10 tertiary, and 12 quaternary (Fig. A.25). These data correspond to the structure of the desired compound. Although MS data could not be obtained owing to the poor solubility of the compounds, the IR, ^{1}H NMR and ^{13}C NMR spectroscopic data confirmed the successful synthesis of the target hybrids **3a–i**. In order to further understand the spatial configuration of the compounds **3a–i**, we cultured single crystals of compound **3h** (Fig. 1c). The C17—N3 and C17—N4 bond lengths were found to be 1.4295(19) Å and 1.4811(18) Å, respectively, indicating that both were single bonds. The N3—C17—N4 angle was recorded as 116.32 (12)°. The maximum plane of the molecule was composed of O1, C26, and C30 (19.826 Å × 8.388 Å × 17.426 Å), and the distance from C17 to this plane was 4.232 Å.

2.2. Antibacterial activity

The antibacterial activities of the compounds were evaluated against *Escherichia coli* (gram-negative), *Pseudomonas aeruginosa* (gram-negative), and *Staphylococcus aureus* (gram-positive), by measuring the

Table 1
Minimum inhibitory concentrations and minimum bactericidal concentrations of compounds **3a–i** against common gram-positive and gram-negative bacteria.

Compound	*E. coli* (ATCC 8739)		*P. aeruginosa* (ATCC 9027)		*S. aureus* (ATCC 6538P)	
	MIC (μg/mL)	MBC/MIC	MIC (μg/mL)	MBC/MIC	MIC (μg/mL)	MBC/MIC
3a	≤0.125	1	0.5	1	2	1
3b	≤0.125	1	0.25	4	1	1
3c	≤0.125	1	0.25	1	0.5	2
3d	≤0.125	1	0.5	2	0.5	1
3e	≤0.125	1	0.5	2	1	1
3f	≤0.125	1	0.5	2	0.5	4
3g	≤0.125	1	0.5	2	0.5	1
3h	≤0.125	1	0.5	2	1	1
3i	≤0.125	1	0.25	4	0.25	4
Norfloxacin	≤0.125	1	1	4	0.5	2

Abbreviations: MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentrations.

inhibition zone diameters (see supplementary material Fig. A.47) and the minimum inhibitory concentrations (MIC). As shown in Table 1, all the hybrids and norfloxacin exhibited similar activities against *E. coli* (MIC < 0.125 μg/mL). The hybrids were observed to be more active than norfloxacin against *P. aeruginosa*, exhibiting MIC values of 0.5 μg/mL (**3a**, **3d**–**h**) or 0.25 μg/mL (**3b**, **3c**, **3i**). It is worth noting their improved activity against *P. aeruginosa*, because this pathogen is responsible for many hospital-acquired and nosocomial infections [20,21]. Compounds **3a**–**i** also exhibited activity against gram-positive bacteria, though not as strong as that against the gram-negative strains. All the hybrids exhibited potency against *S. aureus*; compound **3i** was the most active, showing an MIC of 0.25 μg/mL, which is much lower than that of norfloxacin. The hybrids **3c**, **3d**, **3f**, and **3g** also exhibited comparable activity (MIC 0.5 μg/mL). All the compounds displayed bactericidal activity against all the tested strains, and their minimum bactericidal concentrations were equal to or 1–3-fold higher than the corresponding MICs. In general, most of the synthesized hybrids demonstrated improved activity against both gram-negative and gram-positive bacteria, with compound **3i** being the most potent and exhibiting the lowest MIC value.

The SAR data revealed that the hybrids with N-4 and C-5 substituents on the 1,2,4-triazole ring exhibited better activity against *P. aeruginosa* than norfloxacin. The anti-bacterial activity of the 1,2,4-triazole-3-thione-norfloxacin analogues against the three bacterial strains was better than that reported by Mermer et al. in 2019 [7] and equivalent to that reported by Mermer et al. in 2017 [19]. This indicates that the substituents at the C-5 position of the 1,2,4-triazole-3-thiones have greater influence on the antibacterial activity of hybrids than those at the N-4 position; this is similar to the phenomenon previously reported [17]. However, there was little difference in the antibacterial activity of compounds with different types of C-5 substituents. When *p*-methoxyphenyl was retained at the N-4 position of 1,2,4-triazole, the antibacterial activity of the compounds was better than that of 1,2,4-triazole-3-thione-pipemidic acid [22], but slightly weaker than that of 1,2,4-triazole-3-thione-ciprofloxacin [16,17]. This may be because ciprofloxacin is more potent than norfloxacin. When hydroxyphenyl or pyridyl were substituted at the C-5 position of 1,2,4-triazole-3-thione, the antibacterial activity was found to be similar to that of 1,2,4-triazole-3-thione-ciprofloxacin [16,18] or 1,2,4-triazole-3-thione-1-[(*1R,2S*)-2-fluorocyclopropyl]ciprofloxacin [8]. Substitution of pyrazine, furan, and thiophene (**3a**–**c)** at the C-5 position of 1,2,4-triazole-3-thione was also well tolerated and the compounds exhibited good anti-bacterial activity. This indicates that aryl groups, rather than phenyl groups, are preferred at the C-5 position of 1,2,4-triazole-3-thione; this is consistent with our conjecture. Notably, compound **3i** exhibited promising activity against all the tested bacteria and could act as a starting compound for further optimization.

2.3. Hemolysis and toxicity

The development of a safe antibacterial agent is the top priority in any research endeavor, and the toxicity toward host tissues must be carefully assessed and minimized. Therefore, all the synthesized hybrids were thoroughly examined for cytotoxicity toward mouse fibroblast (L929) cells and hemolysis in rabbit red blood cells (Fig. 2). All the compounds displayed acceptable tolerability in L929 cells at high concentrations. Compounds **3b**, **3c,** and **3i** did not exhibit toxicity toward L929 cells, showing a cell viability of approximately 90% at a dose of 64 μg/mL. The maximal half inhibitory concentration (IC_{50}) values (concentration required to inhibit cell growth by 50%) of all the compounds were found to be greater than 64 μg/mL; this was considerably higher than the dose required to achieve antibacterial activity. These IC_{50} values were 32–512 times the MIC values of the compounds against the test pathogens, providing a broad window of safety.

The hemolytic ratios of all the hybrids are listed in Table 2. Hemolysis of rabbit red blood cells was not observed with any of the compounds tested. Concentrations of 64 μg/mL of all the compounds caused lower than 5% hemolysis. Overall, the results of the *in vitro* hemolysis and cytotoxicity assays preliminarily confirmed that our hybrids have a satisfactory toxicity profile.

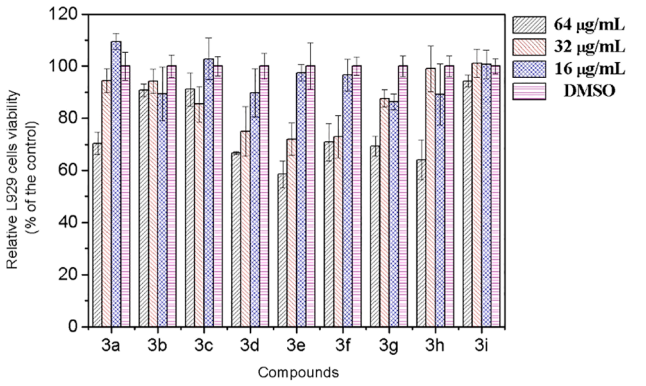


Fig. 2. Toxicity analysis of compounds **3a**–**i** conducted using the MTT assay. All compounds were tested in triplicate at concentrations of (16, 32, and 64 μg/mL) in mouse fibroblast L929 cells. Results are presented as percent viable cells relative to those treated with dimethyl sulfoxide (DMSO), which was used as a negative control to establish a baseline measure for the cytotoxic impact of each compound. The data are presented as the mean ± SD.

2.4. Physicochemical properties

Optimal physicochemical properties are essential attributes for ideal drug candidates and play an important role in the drug's ability to cross cellular barriers and reach the target successfully. Therefore, the drug-likeness of the molecules was evaluated based on Lipinski's Rule of 5 and topological polar surface area (TPSA). As shown in Table 3, all the hybrids possessed calculated logarithm of partition coefficient between *n*-octanol and water (cLogP) values of ≤ 5, as well as suitable TPSAs, indicating good solubility and permeability. Although the molecular weights and the number of hydrogen bond receptors were slighter higher than the recommended standards, they were deemed acceptable [23]. Therefore, all the hybrids were thought to possess good drug-likeness. The synthesized compounds were predicted to be able to undergo intestinal absorption in humans, and were found to be non-carcinogenic (Table A.10 in Supporting Information).

2.5. Molecular docking

Molecular docking predicted least binding energies of −9.4 to −9.7 kcal/mol with topoisomerase IV (PDB ID: 3rae), indicating that compounds **3a**–**i** are likely to exhibit good binding with the target enzyme. The least binding energy was predicted to be higher than that of norfloxacin (−7.8 kcal/mol).

To unveil the mechanism of action and molecular interactions involved, the binding affinities of the best docked poses were analyzed (Fig. A.48). Representative results are shown in Fig. 3. The *N*-terminal of the piperazine ring in norfloxacin showed hydrogen bonding with Glu474 of topoisomerase IV. However, in the case of the hybrids, the N-1 of the triazole was predicted to show hydrogen bonding with Glu474 due to the deeper insertion into the pocket. In addition, the methoxy group of the N-4 triazole substituent and the C-5 triazole substituent in the hybrids were predicted to show additional hydrogen bonding interactions with Lys415 and Pro454, respectively. The carboxyl and ketone groups of the hybrids chelated Mg^{2+}; the two coordination bond lengths were 2.20–2.28 Å and 2.28–2.49 Å, respectively. Ser79 was predicted to form a hydrogen bond with the carboxyl group of the hybrids. Meanwhile, the carboxyl group of norfloxacin only formed a weak ionic bond with Mg^{2+}. Compared with norfloxacin, the hybrids also

Table 2
Hemolytic ratios of the synthesized hybrids (**3a**–**3i**) at 64 μg/mL.

Compound	3a	3b	3c	3d	3e	3f	3g	3h	3i
Hemolytic ratio (%)	3.10	1.25	2.08	2.84	1.10	3.13	2.42	3.10	2.38

Table 3
Calculation of the physicochemical properties of hybrids **3a**–**i** as per Lipinski's Rule of 5 and the topological polar surface area.

Compound	cLogP <5	MW <500	nON <10	nOHNH <5	TPSA (Å^2) <150	nViol
3a	0.71	616	12	1	123.56	2
3b	1.68	604	11	1	110.91	2
3c	2.32	620	10	1	97.77	1
3d	2.27	630	11	2	118.00	2
3e	2.04	630	11	2	118.00	2
3f	2.06	630	11	2	118.00	2
3g	1.39	615	11	1	110.67	2
3h	1.47	615	11	1	110.67	2
3i	1.25	615	11	1	110.67	2
Norfloxacin	−0.69	319	6	2	74.57	0

Note: cLogP, Molinspiration calculated logarithm of partition coefficient between *n*-octanol and water; MW, molecular weight; nON, number of hydrogen bond acceptors; nOHNH, number of hydrogen bond donors; TPSA, topological polar surface area; nViol, number of violations. The data in the table are calculated using www.molinspiration.com.

exhibited additional aromatic-ring stacking interactions with DG1 and DA5, and the ethyl group on the aromatic ring formed a hydrogen bond with DG1. The docking results showed that the introduction of 1,2,4-triazole at the C-7 position of norfloxacin could enhance the binding affinity for topoisomerase IV. These findings were consistent with the results of the antibacterial experiment. In addition to the above interactions, the pyridine group and methoxyphenyl of compound **3i** exhibited hydrogen bonding with Thr478 and Asp435, respectively. Aromatic-ring stacking interactions of the methoxyphenyl were also predicted with Lys415. These predictions of binding interactions are consistent with the antibacterial assay results, which show that compound **3i** exhibits the lowest MIC value.

3. Conclusions

In this study, a series of substituted 1,2,4-triazole derivatives were introduced at the C-7 site of norfloxacin through Mannich reaction. Evaluation of antibacterial activity revealed that most of constructed hybrids demonstrate improved antibacterial activity against both gram-positive and gram-negative bacteria compared to the parent drug (norfloxacin). Among these, compound **3i** was found to be the most potent, with MIC values of ≤ 0.125 μg/mL, 0.25 μg/mL, and 0.25 μg/mL against

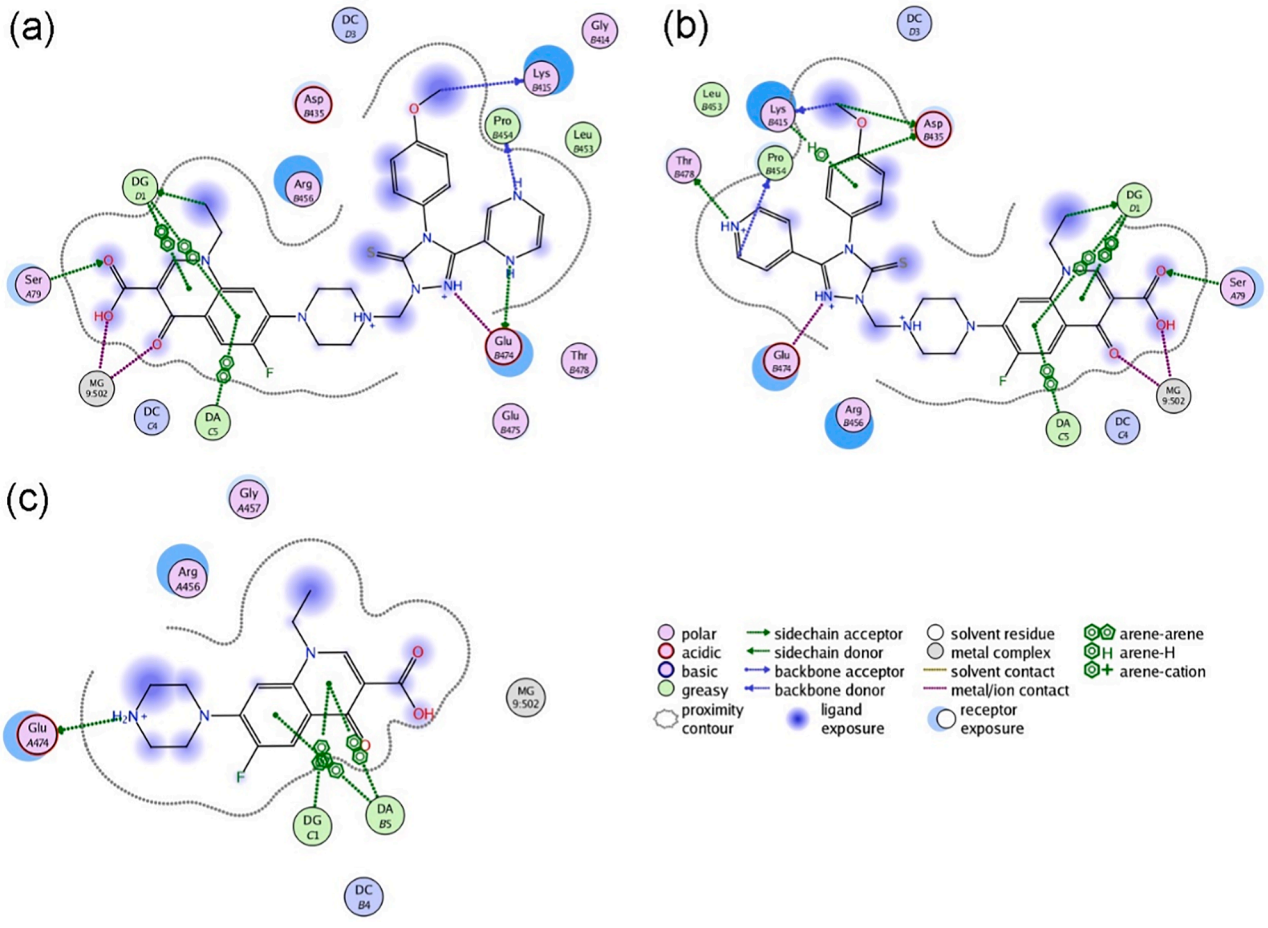


Fig. 3. Two-dimensional representation of the top ranked docking poses (that had the lowest S scores) of compound **3a** (a), compound **3i** (b) and norfloxacin (c) in the active site of *S. pneumoniae* topoisomerase IV (PDB code: 3rae).

E. coli, S. aureus, and *P. aeruginosa*, respectively, outperforming norfloxacin. It was confirmed that aromatic substituents can increase the antibacterial activity of the 1,2,4-triazole-norfloxacin hybrids, similar to the effects seen with ciprofloxacin. Molecular docking studies suggested that the modified norfloxacin analogues bound more firmly to the active site of the target, and aromatic substituents on the 1,2,4-triazole increased the binding affinity. All the hybrids synthesized exhibited a satisfactory cytotoxicity profile. Moreover, no hemolytic effects were observed when rabbit red blood cells were exposed to concentration of up to 32–512 times the MIC values of the compounds.

4. Materials and methods

4.1. General information

All chemicals were commercial products and were used without further purification. The synthesized compounds were purified by rapid purification preparative chromatography (Cheetah II, Agela Technologies, Tianjin, China). MS was conducted using a Bruker maXis impact (Bruker Corp., Billerica, MA, USA), equipped with an electrospray ionization ion source. Nuclear magnetic resonance spectra were recorded in deuterated dimethyl sulfoxide (DMSO-d_6) on a Bruker Avance (400 MHz) spectrometer, using tetramethylsilane as an internal standard (Bruker, Bremerhaven, Germany). IR spectra were recorded on a Bruker Tensor II (Bruker Corp., USA), using KBr pellets. The optical density (OD) of the bacterial suspensions was recorded using a microplate reader (Multiskan GO, Thermo Scientific, Waltham, MA, USA) at 600, 450, and 545 nm. The physicochemical properties of compounds are calculated using www.molinspiration.com [24]. Human intestinal absorption and carcinogenicity of the synthesized compounds were predicted using the online AdmetSAR server (http://lmmd.ecust.edu.cn/admetsar2) [25]. Molecular docking studies with topoisomerase IV (PDB ID: 3rae) were carried out using Molecular Operating Environment version 2014.09 (Chemical Computing Group, Montreal, Canada) [25,26].

4.2. Single-crystal X-ray diffraction

X-ray diffraction data for the compounds of **1a**, **2a**, and **3h** were recorded using a charge coupled device area detector and Oxford Gemini S Ultra (Cu-Kα, λ = 0.154178 nm) at 20 °C. The SADABS program was used for absorption correction [27]. The crystal structures were solved using direct methods [28], and then, the SHELXTL program package [29,30] was used to conduct a full-matrix least-square structure refinement based on F^2. The non-hydrogen atoms were all anisotropically refined. The hydrogen atoms were placed in geometrically idealized positions, assuming the following bond lengths: C—H = 0.93 Å (sp^2), C—H = 0.96 Å (sp^3), O—H = 0.82 Å, and N—H = 0.86 Å [31]. Further details of the X-ray structural analyses for compounds **1a**, **2a,** and **3h** are given in the Supporting Information Table A.1–A.9. Crystallographic data for the structures presented in this paper have been deposited at the Cambridge Crystallographic Data Centre (CCDC; 12 Union Road, Cambridge CB21EZ, United Kingdom).

4.3. Chemical synthesis

4.3.1. General procedure for synthesis of the thiosemicarbazide derivatives (1a–i)

According to a previously reported method [17], a mixture of the appropriate hydrazide (5 mmol) and 1-isothiocyanato-4-methoxybenzene (5 mmol) in 20 mL anhydrous ethanol or methanol was stirred at room temperature or with heat for approximately 2 h. The progress of the reaction was monitored by thin layer chromatography using silica gel plates. When the reaction was completed, the mixture was cooled and filtered. The precipitate was washed with cold ethanol and dried to obtain the desired product. For more details, please refer to **1a**[32], **1b**[33], **1c**[34], **1d**[35], **1e**[35], **1f**[35], **1g**[34], **1h**[36], and **1i**[37], respectively.

4.3.1.1. *N*-(4-methoxyphenyl)-2-(pyrazine-2-carbonyl)hydrazine-1-carbothioamide (**1a**). White solid. Yield: 90%. (methanol:dichloromethane, 1:5, Rf = 0.9). ESI-MS *m/z*: 326.0689 $[M+Na]^+$. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.87 (s, 1H, —NH—), 9.74 (s, 1H, —NH—), 9.62 (s, 1H, —NH—), 9.21 (s, 1H, pyrazinyl), 8.90 (d, J = 2.4 Hz, 1H, pyrazinyl), 8.78 (d, J = 1.4 Hz, 1H, pyrazinyl), 7.28 (d, J = 8.4 Hz, 2H, phenyl), 6.89 (d, J = 8.8 Hz, 2H, phenyl), 3.74 (s, 3H, methoxyl). ^{13}C NMR (DMSO-d_6, 400 MHz): δ 181.51 (C═S), 163.3 (C═O), 157.22 (phenyl C-methoxyl), 148.12 (pyrazinyl C), 145.17 (pyrazinyl C), 144.31 (pyrazinyl C), 143.83 (pyrazinyl C), 132.46 (phenyl C—NH), 127.85 (phenyl C), 113.70 (phenyl C), 55.68 (—CH_3). IR (KBr) (ν, cm^{-1}): 439 w, 519 w, 608 w, 744 w, 836 m, 910 w, 1020 m, 1168 m, 1249 s, 1402 w, 1465 m, 1511 s, 1694 s, 2836 w, 2957 m, 3153 m.

*4.3.1.1. 2-(Furan-2-carbonyl)-N-(4-methoxyphenyl)hydrazine-1-carbothioamide (**1b**).* White solid. Yield: 97%. (ethyl acetate, Rf = 0.74). ESI-MS *m/z*: 314.0577 $[M+Na]^+$. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.39 (s, 1H, —NH—), 9.70 (s, 1H, —NH—), 9.57 (s, 1H, —NH—), 7.91 (d, J = 0.8 Hz, 1H, furanyl), 7.29 (d, J = 8.5 Hz, 1H, phenyl), 7.24 (d, J = 3.3 Hz, 1H, furanyl), 6.89 (d, J = 8.9 Hz, 1H, phenyl), 6.67 (dd, J = 3.4, 1.7 Hz, 1H, furanyl), 3.75 (s, 3H, methoxyl). IR (KBr) (ν, cm^{-1}): 519 w, 592 w, 764 m, 804 w, 851 w, 884 w, 1027 m, 1173 s, 1251 s, 1364 w, 1467 m, 1512 s, 1588 m, 1683 s, 2969 m, 3155 m, 3226 m, 3352 w, 3460 w.

*4.3.1.2. N-(4-methoxyphenyl)-2-(thiophene-2-carbonyl)hydrazine-1-carbothioamide (**1c**).* White solid. Yield: 100%. (ethyl acetate: petroleum ether (60–90), 1:1, Rf = 0.3). ESI-MS *m/z*: 330.0342 $[M+Na]^+$. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.52 (s, 1H, —NH—), 9.75 (s, 1H, —NH—), 9.62 (s, 1H, —NH—), 7.87 (d, J = 4.1 Hz, 1H, thiophenyl), 7.86 (d, J = 5.1 Hz, 1H, thiophenyl), 7.29 (d, J = 8.3 Hz, 2H, phenyl), 7.20 (dd, J = 4.8, 3.9 Hz, 1H, thiophenyl), 6.90 (d, J = 8.9 Hz, 2H, phenyl), 3.75 (s, 3H, methoxyl). IR (KBr) (ν, cm^{-1}): 519 w, 592 w, 721 m, 848 w, 1032 w, 1142 w, 1246 s, 1355 w, 1416 m, 1512 s, 1661 s, 2839 w, 2966 m, 3155 m, 3468 w.

*4.3.1.3. 2-(2-Hydroxybenzoyl)-N-(4-methoxyphenyl)hydrazine-1-carbothioamide. (**1d**).* White solid. Yield: 86%. (ethyl acetate: petroleum ether (60–90), 1:1, Rf = 0.61). ESI-MS *m/z*: 340.0731 $[M+Na]^+$. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.91 (s, 1H, —OH), 10.74 (s, 1H, —NH—), 9.76 (s, 2H, —NH—), 7.89 (d, J = 7.6 Hz, 1H, hydroxyphenyl), 7.49–7.40 (m, 1H, hydroxyphenyl), 7.31 (d, J = 7.8 Hz, 2H, methoxyphenyl), 6.99–6.93 (m, 2H, hydroxyphenyl), 6.91 (d, J = 9.0 Hz, 2H, methoxyphenyl), 3.75 (s, 3H, methoxyl). IR (KBr) (ν, cm^{-1}): 527 w, 601 w, 697 w, 751 m, 833 w, 1036 w, 1103 w, 1178 m, 1243 s, 1302 m, 1354 m, 1477 s, 1511 s, 1605 s, 1658 s, 2837 w, 2962 m, 3172 s, 3364 w.

*4.3.1.4. 2-(3-Hydroxybenzoyl)-N-(4-methoxyphenyl)hydrazine-1-carbothioamide (**1e**).* White solid. Yield: 78%. (methanol:dichloromethane, 1:10, Rf = 0.38). ESI-MS *m/z*: *m/z*: 340.0733 $[M+Na]^+$. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.38 (s, 1H, —NH—), 9.68 (s, 1H, —OH), 9.66 (s, 1H, —NH—), 9.55 (s, 1H, —NH—), 7.39 (d, J = 7.5 Hz, 1H, hydroxyphenyl), 7.35 (s, 1H, hydroxyphenyl), 7.29 (d, J = 7.9 Hz, 2H, methoxyphenyl), 7.26 (s, 1H, hydroxyphenyl), 6.96 (dd, J = 8.0, 1.8 Hz, 1H, hydroxyphenyl), 6.89 (d, J = 8.8 Hz, 2H, methoxyphenyl), 3.75 (s, 3H, methoxyl). IR (KBr) (ν, cm^{-1}): 519 w, 617 w, 680 w, 745 w, 826 m, 886 w, 1034 m, 1213 s, 1255 s, 1362 w, 1452 s, 1514 s, 1598 s, 1675 s, 2834 w, 2967 m, 3158 s, 3235 s, 3322 m.

*4.3.1.5. 2-(4-Hydroxybenzoyl)-N-(4-methoxyphenyl)hydrazine-1-carbothioamide (**1f**).* White solid. Yield: 80%. (ethyl acetate: petroleum ether (60–90), 2:1, Rf = 0.25). ESI-MS *m/z*: 340.0744 $[M+Na]^+$. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.24 (s, 1H, —NH—), 10.08 (s, 1H, —OH), 9.63 (s, 1H, —NH—), 9.50 (s, 1H, —NH—), 7.82 (d, J = 8.6 Hz, 2H,

hydroxyphenyl), 7.29 (d, J = 8.0 Hz, 2H, methoxyphenyl), 6.89 (d, J = 8.9 Hz, 2H, methoxyphenyl), 6.83 (d, J = 8.7 Hz, 2H, hydroxyphenyl), 3.75 (s, 3H, methoxyl). IR (KBr) (ν, cm^{-1}): 517 w, 610 w, 741 w, 804 w, 834 w, 1032 m, 1106 w, 1173 s, 1256 s, 1364 w, 1486 s, 1512 s, 1610 s, 1661 s, 2834 w, 2967 m, 3008 m, 3055 m, 3229 s.

4.3.1.7. *N*-(4-methoxyphenyl)-2-picolinoylhydrazine-1-carbothioamide (**1 g**). White solid. Yield: 72%. (methanol:dichloromethane, 3:20, Rf = 0.5). ESI-MS *m/z*: 325.0742 $[M+Na]^+$. ^1H NMR (DMSO-d_6, 400 MHz): δ 10.69 (s, 1H, —NH—), 9.69 (s, 1H, —NH—), 9.62 (s, 1H, —NH—), 8.69 (d, J = 4.6 Hz, 1H, pyridyl), 8.04 (m, 2H, pyridyl), 7.68–7.62 (m, 1H, pyridyl), 7.31 (d, J = 8.4 Hz, 2H, phenyl), 6.89 (d, J = 8.9 Hz, 2H, phenyl), 3.74 (s, 3H, methoxyl). IR (KBr) (ν, cm^{-1}): 578 w, 621 w, 711 w, 745 w, 836 w, 1032 m, 1173 m, 1249 s, 1299 m, 1358 m, 1431 s, 1459 s, 1487 s, 1512 s, 1555 s, 1660 m, 3222 m, 3304 m.

*4.3.1.6. N-(4-methoxyphenyl)-2-nicotinoylhydrazine-1-carbothioamide (**1 h**).* White solid. Yield: 90%. (methanol:dichloromethane, 3:20, Rf = 0.5). ESI-MS *m/z*: 325.0741 $[M+Na]^+$. ^1H NMR (DMSO-d_6, 400 MHz): δ 10.72 (s, 1H, —NH—), 9.74 (s, 1H, —NH—), 9.67 (s, 1H, —NH—), 9.10 (d, J = 1.4 Hz, 1H, pyridyl), 8.75 (dd, J = 4.8, 1.5 Hz, 1H, pyridyl), 8.28 (d, J = 8.0 Hz, 1H, pyridyl), 7.56 (dd, J = 7.8, 4.9 Hz, 1H, pyridyl), 7.28 (d, J = 8.2 Hz, 2H, phenyl), 6.91 (d, J = 8.9 Hz, 2H, phenyl), 3.75 (s, 3H, methoxyl). IR (KBr) (ν, cm^{-1}): 521 w, 600 w, 703 w, 733 w, 804 w, 836 w, 894 w, 1027 m, 1158 m, 1248 s, 1299 m, 1372 w, 1419 w, 1512 s, 1592 m, 1683 s, 2957 m, 3178 m.

*4.3.1.7. 2-Isonicotinoyl-N-(4-methoxyphenyl)hydrazine-1-carbothioamide (**1i**).* White solid. Yield: 90%. (methanol:dichloromethane, 3:20, Rf = 0.5). ESI-MS *m/z*: 325.0733 $[M+Na]^+$. ^1H NMR (DMSO-d_6, 400 MHz): δ 10.81 (s, 1H, —NH—), 9.75 (s, 1H, —NH—), 9.70 (s, 1H, —NH—), 8.77 (d, J = 5.9 Hz, 2H, pyridyl), 7.85 (d, J = 5.8 Hz, 2H, pyridyl), 7.28 (d, J = 8.2 Hz, 2H, phenyl), 6.91 (d, J = 8.8 Hz, 2H, phenyl), 3.75 (s, 3H, methoxyl). IR (KBr) (ν, cm^{-1}): 518 w, 610 m, 690 w, 738 w, 830 m, 903 w, 1033 m, 1062 w, 1105 w, 1150 m, 1258 s, 1301 m, 1375 w, 1404 m, 1482 s, 1504 s, 1551 s, 1600 w, 1674 s, 2033 m, 2837 m, 2949 m, 3002 m, 3112 m, 3264 m.

4.3.2. General procedure for synthesis of the 1,2,4-triazole derivatives (2a–i)

According to a previously reported method [17], a solution of the corresponding thiosemicarbazide derivatives **1a–i** (2 mmol) in 8 mL or 16 mL 2% NaOH solution was stirred at 70 °C for approximately 2 h. The mixture was then cooled, and 2 M HCl was added to bring the pH down to 5. The mixture was filtered and the precipitate was collected, washed with distilled water, and dried to obtain the final compounds. For more details, please refer to **2a**[38], **2b**[33], **2c**[33], **2d**[16], **2e**[16], **2f**[16], **2g**[39], **2h**[36], and **2i**[39], respectively.

*4.3.2.1. 4-(4-Methoxyphenyl)-5-(pyrazin-2-yl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**2a**).* White solid. Yield: 86%. (methanol:dichloromethane, 1:10, Rf = 0.57). ESI-MS *m/z*: 308.0580 $[M+Na]^+$. ^1H NMR (DMSO-d_6, 400 MHz): δ 14.36 (s, 1H, triazolyl), 8.99 (d, J = 1.4 Hz, 1H, pyrazinyl), 8.67 (d, J = 2.5 Hz, 1H, pyrazinyl), 8.50 (dd, J = 2.4, 1.5 Hz, 1H, pyrazinyl), 7.27 (d, J = 8.9 Hz, 2H, phenyl), 6.99 (d, J = 8.9 Hz, 2H, phenyl), 3.80 (s, 3H, methoxyl). ^{13}C NMR (DMSO-d_6, 400 MHz): δ 170.09 (C═S), 159.74 (phenyl C-methoxyl), 148.00 (N═C—N), 146.00, 144.97, 144.55 (pyrazinyl C), 141.78 (pyrazinyl C-triazolyl), 129.98, 114.44 (phenyl C), 127.61 (phenyl C—N), 55.76 (—CH_3). IR (KBr) (ν, cm^{-1}): 416 w, 604 w, 647 w, 763 w, 800 w, 836 m, 866 w, 947 w, 974 w, 1016 m, 1136 w, 1186 m, 1253 s, 1329 m, 1379 w, 1458 m, 1496 s, 1517 s, 1558 w, 1610 w, 1647 w, 2750 w, 2917 m, 3012 m, 3116 m.

*4.3.2.2. 5-(Furan-2-yl)-4-(4-methoxyphenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**2b**).* White solid. Yield: 83%. (ethyl acetate: petroleum ether (60–90), 1:1, Rf = 0.59). ESI-MS *m/z*: 296.0472 $[M+Na]^+$, 569.1049 $[2\ M + Na]^+$. ^1H NMR (DMSO-d_6, 400 MHz): δ 14.10 (s, 1H, triazolyl), 7.82 (d, J = 1.1 Hz, 1H, furanyl), 7.35 (d, J = 8.9 Hz, 2H, phenyl), 7.12 (d, J = 8.9 Hz, 2H, phenyl), 6.51 (dd, J = 3.5, 1.8 Hz, 1H, furanyl), 5.91 (d, J = 3.3 Hz, 1H, furanyl), 3.85 (s, 3H, methoxyl). IR (KBr) (ν, cm^{-1}): 434 w, 506 w, 591 m, 625 m, 755 s, 840 s, 886 w, 977 s, 1022 s, 1076 m, 1127 m, 1182 s, 1246 s, 1275 s, 1328 s, 1372 w, 1454 s, 1519 s, 1620 m, 2565 w, 2771 s, 2923 s, 3089 s.

*4.3.2.3. 4-(4-Methoxyphenyl)-5-(thiophen-2-yl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**2c**).* White solid. Yield: 95%. (ethyl acetate: petroleum ether (60–90), 1:1, Rf = 0.67). ESI-MS *m/z*: 312.0240 $[M+Na]^+$. ^1H NMR (DMSO-d_6, 400 MHz): δ 14.05 (s, 1H, triazolyl), 7.68 (dd, J = 5.0, 0.9 Hz, 1H, thiophenyl), 7.36 (d, J = 8.9 Hz, 2H, phenyl), 7.12 (d, J = 8.9 Hz, 2H, phenyl), 7.03 (dd, J = 4.9, 3.9 Hz, 1H, thiophenyl), 6.81 (dd, J = 3.7, 0.9 Hz, 1H, thiophenyl), 3.85 (s, 3H, methoxyl). IR (KBr) (ν, cm^{-1}): 608 w, 625 w, 719 m, 735 m, 828 w, 853 w, 934 w, 1020 m, 1119 w, 1173 m, 1259 s, 1301 m, 1328 m, 1349 m, 1438 m, 1482 w, 1519 s, 1580 m, 1611 w, 2749 w, 2784 w, 2840 w, 2935 m, 2967 m, 3008 m, 3070 s, 3116 m.

*4.3.2.4. 5-(2-Hydroxyphenyl)-4-(4-methoxyphenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**2d**).* White solid. Yield: 97%. (ethyl acetate: petroleum ether (60–90), 1:1, Rf = 0.69). ESI-MS *m/z*: 340.0740 $[M+Na + H_2O]^+$. ^1H NMR (DMSO-d_6, 400 MHz): δ 13.95 (s, 1H, triazolyl), 9.89 (s, 1H, —OH), 7.31–7.28 (m, 1H, hydroxyphenyl), 7.27–7.22 (m, 1H, hydroxyphenyl), 7.17 (d, J = 8.9 Hz, 2H, methoxyphenyl), 6.90 (d, J = 8.9 Hz, 2H, methoxyphenyl), 6.81 (t, J = 7.5 Hz, 1H, hydroxyphenyl), 6.76 (d, J = 8.2 Hz, 1H, hydroxyphenyl), 3.74 (s, 3H, methoxyl). IR (KBr) (ν, cm^{-1}): 610 m, 642 m, 678 w, 708 w, 748 m, 831 m, 976 w, 1032 m, 1110 w, 1170 m, 1252 s, 1306 s, 1329 s, 1389 m, 1422 m, 1489 s, 1515 s, 1535 s, 1585 m, 1608 m, 1660 m, 2760 m, 2834 m, 2930 s, 3088 s.

*4.3.2.5. 5-(3-Hydroxyphenyl)-4-(4-methoxyphenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**2e**).* White solid. Yield: 97%. (ethyl acetate: petroleum ether (60–90), 1:1, Rf = 0.44). ESI-MS *m/z*: 322.0635 $[M+Na]^+$. ^1H NMR (DMSO-d_6, 400 MHz): δ 14.02 (s, 1H, triazolyl), 9.70 (s, 1H, hydroxyl), 7.25 (d, J = 8.9 Hz, 2H, methoxyphenyl), 7.13 (t, J = 8.0 Hz, 1H, hydroxyphenyl), 7.03 (d, J = 8.9 Hz, 2H, methoxyphenyl), 6.80 (m, J = 7.7 Hz, 2H, hydroxyphenyl), 6.69 (d, J = 7.9 Hz, 1H, hydroxyphenyl), 3.81 (s, 3H, methoxyl). IR (KBr) (ν, cm^{-1}): 612 w, 638 m, 688 w, 728 m, 763 w, 793 w, 837 w, 874 w, 989 m, 1020 w, 1034 w, 1175 m, 1209 s, 1258 s, 1305 s, 1341 s, 1408 m, 1457 m, 1486 m, 1517 s, 1555 s, 1588 m, 1608 m, 2791 m, 2840 m, 2946 s, 3109 s, 3284 s.

*4.3.2.6. 5-(4-Hydroxyphenyl)-4-(4-methoxyphenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**2f**).* White solid. Yield: 97%. (ethyl acetate: petroleum ether (60–90), 1:1, Rf = 0.44). ESI-MS *m/z*: 322.0636 $[M+Na]^+$. ^1H NMR (DMSO-d_6, 400 MHz): δ 13.91 (s, 1H, triazolyl), 9.95 (s, 1H, —OH), 7.23 (d, J = 8.9 Hz, 2H, methoxyphenyl), 7.14 (d, J = 8.7 Hz, 2H, hydroxyphenyl), 7.03 (d, J = 8.9 Hz, 2H, methoxyphenyl), 6.70 (d, J = 8.7 Hz, 2H, hydroxyphenyl), 3.81 (s, 3H, methoxyl). IR (KBr) (ν, cm^{-1}): 494 w, 529 w, 591 w, 728 w, 757 w, 828 m, 842 m, 970 w, 1023 w, 1034 w, 1109 w, 1173 m, 1213 m, 1255 s, 1279 s, 1302 m, 1336 m, 1399 w, 1432 m, 1471 w, 1512 s, 1610 s, 2764 w, 2791 w, 2837 w, 2955 m, 2990 m, 3019 m, 3050 m, 3115 s, 3235 m.

*4.3.2.7. 4-(4-Methoxyphenyl)-5-(pyridin-2-yl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**2g**).* White solid. Yield: 92%. ESI-MS *m/z*: 307.0628 $[M+Na]^+$. ^1H NMR (DMSO-d_6, 400 MHz): δ 14.18 (s, 1H, triazolyl), 8.40 (d, J = 4.8 Hz, 1H, pyridinyl), 7.90 (m, 1H, pyridinyl), 7.78 (d, J = 7.9 Hz, 1H, pyridinyl), 7.50–7.37 (m, 1H, pyridinyl), 7.21 (d, J = 8.9 Hz, 2H, phenyl), 6.97 (d, J = 8.9 Hz, 2H, phenyl), 3.79 (s, 3H, methoxyl). IR (KBr) (ν, cm^{-1}): 602 m, 638 w, 708 w, 745 m, 762 w, 796 m, 827 m, 975 w, 994 w, 1033 m, 1105 w, 1148 w, 1175 m, 1256 s, 1277 m, 1300 m,

1338 m, 1395 w, 1447 m, 1461 m, 1495 s, 1519 s, 1552 m, 1587 m, 1614 m, 2766 m, 2839 m, 2932 s, 2963 m, 3034 m, 3053 m, 3086 s, 3111 s.

*4.3.2.8. 4-(4-Methoxyphenyl)-5-(pyridin-3-yl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**2h**).* White solid. Yield: 85%. ESI-MS *m/z*: 307.0614 $[M+Na]^+$. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 14.20 (s, 1H, triazolyl), 8.60 (dd, J = 4.8, 1.3 Hz, 1H, pyridinyl), 8.53 (d, J = 1.5 Hz, 1H, pyridinyl), 7.70 (dd, J = 8.0, 1.7 Hz, 1H, pyridinyl), 7.41 (dd, J = 7.9, 4.9 Hz, 1H, pyridinyl), 7.32 (d, J = 8.8 Hz, 2H, phenyl), 7.04 (d, J = 8.9 Hz, 2H, phenyl), 3.80 (s, 3H, methoxyl). IR (KBr) (ν, cm^{-1}): 600 w, 647 m, 703 w, 744 w, 806 w, 838 w, 903 w, 967 m, 1032 m, 1105 w, 1165 w, 1256 s, 1295 s, 1326 s, 1392 w, 1445 m, 1514 s, 1552 m, 1604 m, 2557 m, 2701 m, 2820 m, 2864 m, 2959 w, 3048 w, 3083 w, 3113 w.

*4.3.2.9. 4-(4-Methoxyphenyl)-5-(pyridin-3-yl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**2i**).* White solid. Yield: 100%. ESI-MS *m/z*: 285.0792 $[M+H]^+$. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 14.30 (s, 1H, triazolyl), 8.59 (d, J = 6.0 Hz, 2H, pyridinyl), 7.33 (d, J = 8.9 Hz, 2H, pyridinyl), 7.29–7.23 (d, 2H, phenyl), 7.07 (d, J = 8.9 Hz, 2H, phenyl), 3.82 (s, 3H, methoxyl). IR (KBr) (ν, cm^{-1}): 499 w, 538 w, 607 m, 631 m, 713 w, 744 w, 833 s, 977 m, 1006 m, 1032 m, 1105 w, 1172 m, 1261 s, 1284 s, 1331 s, 1419 m, 1459 m, 1482 m, 1515 s, 1578 m, 1608 s, 2558 m, 2744 m, 2833 m, 2902 m, 2929 m, 2966 m, 3010 m, 3045 m, 3126 m, 3435 s.

4.3.3. General procedure for synthesis of the 1,2,4-triazole-norfloxacin hybrids (3a–i)

According to a previously reported method [19], 1 mmol of the corresponding 1,2,4-triazole derivatives **2a–i**, 1 mmol of norfloxacin, and 6.78 mmol formaldehyde solution (37%) were mixed in 10 mL dimethylformamide, and stirred at room temperature for 48 h. When the reaction was completed, the mixture was filtered. The precipitate was washed with ethanol and dried to yield the final compounds **3a–c**. To obtain compounds **3d–i,** the filtrate was left for the solvent to evaporate naturally for 3–7 days so that crystals or solids could form. These were washed with ethanol to yield the desired compound **3d-i**.

*4.3.3.1. 1-Ethyl-6-fluoro-7-(4-((4-(4-methoxyphenyl)-3-(pyrazin-2-yl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3a**).* White solid. Yield: 32.5%. M.p.: 255–257 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 15.34 (s, 1H, —COOH), 8.97–8.93 (m, 2H, quinoline and pyrazinyl), 8.69 (d, J = 2.4 Hz, 1H, pyrazinyl), 8.56–8.53 (m, 1H, pyrazinyl), 7.91 (d, J = 13.3 Hz, 1H, quinoline), 7.32 (d, J = 8.9 Hz, 2H, phenyl), 7.20 (d, J = 7.2 Hz, 1H, quinoline), 7.00 (d, J = 8.9 Hz, 2H, phenyl), 5.33 (s, 2H, —CH_2—), 4.59 (d, J = 7.0 Hz, 2H, —CH_2CH_3), 3.80 (s, 3H, —OCH_3), 3.38 (s, 4H, piperazinyl), 3.06 (s, 4H, piperazinyl), 1.41 (t, J = 7.1 Hz, 3H, —CH_2CH_3). ^{13}C NMR (DMSO-d_6, 600 MHz): δ 176.56 (C═O), 171.07 (C═S), 166.56 (COOH), 159.93 (phenyl), 154.09 and 152.44 (d, J = 249.8 Hz, C—F), 148.92 (quinoline), 146.77 (pyrazine), 146.28 (trizole), 145.87 and 145.81 (d, J = 10.0 Hz, quinoline), 145.17 (pyrazine), 144.58 (pyrazine), 141.56 (pyrazine), 137.59 (quinoline), 130.11 (phenyl), 128.14 (phenyl), 119.71 and 119.66 (d, J = 7.6 Hz, quinoline), 114.58 (phenyl), 111.69 and 111.53 (d, J = 23.0 Hz, quinoline), 107.53 (quinoline), 106.39 (quinoline), 69.58 (—CH_2—), 55.85 (—OCH_3), 50.07 (piperazine), 49.97 and 49.94 (piperazine), 49.52 (—CH_2CH_3), 14.79 (—CH_2CH_3). IR (KBr) (ν, cm^{-1}): 647 w, 755 w, 808 w, 987 w, 1016 m, 1166 m, 1199 m, 1255 s, 1304 m, 1326 s, 1378 m, 1408 m, 1475 s, 1517 s, 1627 s, 1723 s, 2839 w, 2937 w, 2957 w, 3050 w, 3448 w.

*4.3.3.2. 1-Ethyl-6-fluoro-7-(4-((3-(furan-2-yl)-4-(4-methoxyphenyl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3b**).* White solid. Yield: 70%. M.p.: 256–258 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 15.35 (s, 1H, —COOH), 8.95 (s, 1H, quinoline), 7.92 (d, J = 13.3 Hz, 1H, quinoline), 7.87 (d, J = 1.1 Hz, 1H, furanyl), 7.41 (d, J = 8.9 Hz, 2H, phenyl), 7.20 (d, J = 7.3 Hz, 1H, quinoline), 7.13 (d, J = 8.9 Hz, 2H, phenyl), 6.54 (dd, J = 3.5, 1.7 Hz, 1H, furanyl), 5.87 (d, J = 3.6 Hz, 1H, furanyl), 5.26 (s, 2H, —CH_2—), 4.59 (d, J = 7.1 Hz, 2H, —CH_2CH_3), 3.86 (s, 3H, —OCH_3), 3.38 (s, 4H, piperazinyl), 3.03 (s, 4H, piperazinyl), 1.41 (t, J = 7.1 Hz, 3H, —CH_2CH_3). ^{13}C NMR (DMSO-d_6, 600 MHz): δ 176.60 (C═O), 170.17 (C═S), 166.57 (COOH), 160.67 (phenyl), 154.13 and 152.48 (d, J = 249.4 Hz, C—F), 148.95 (quinoline), 146.01 (trizole), 145.91 and 145.84 (d, J = 10.0 Hz, quinoline), 142.51 (furanyl), 140.07 (furanyl), 137.62 (quinoline), 130.41 (phenyl), 127.68 (phenyl), 119.74 and 119.69 (d, J = 7.6 Hz, quinoline), 115.30 (phenyl), 113.63 (furanyl), 112.33 (furanyl), 111.70 and 111.54 (d, J = 23.2 Hz, quinoline), 107.53 (quinoline), 106.45 (quinoline), 69.42 (—CH_2—), 55.99 (—OCH_3), 50.05 (piperazine), 49.98 (piperazine), 49.53 (—CH_2CH_3), 14.79 (—CH_2CH_3). IR (KBr) (ν, cm^{-1}): 751 w, 765 w, 835 w, 987 w, 1006 w, 1112 w, 1163 w, 1196 w, 1256 s, 1322 m, 1368 w, 1406 w, 1433 m, 1459 s, 1481 s, 1514 s, 1630 s, 1683 w, 1721 m, 2836 w, 2945 w, 3069 w, 3111 w, 3448 w.

*4.3.3.3. 1-Ethyl-6-fluoro-7-(4-((4-(4-methoxyphenyl)-3-(thiophen-2-yl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3c**).* White solid. Yield: 45%. M.p.: 275–277 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 15.35 (s, 1H, —COOH), 8.95 (s, 1H, quinoline), 7.93 (d, J = 13.3 Hz, 1H, quinoline), 7.72 (d, J = 5.0 Hz, 1H, thiophenyl), 7.42 (d, J = 8.9 Hz, 2H, phenyl), 7.21 (d, J = 7.3 Hz, 1H, quinoline), 7.13 (d, J = 8.9 Hz, 2H, phenyl), 7.07–7.03 (m, 1H, thiophenyl), 6.86 (d, J = 3.8 Hz, 1H, thiophenyl), 5.25 (s, 2H, —CH_2—), 4.60 (d, J = 7.2 Hz, 2H, —CH_2CH_3), 3.86 (s, 3H, —OCH_3), 3.38 (s, 4H, piperazinyl), 3.04 (s, 4H, piperazinyl), 1.41 (t, J = 7.0 Hz, 3H, —CH_2CH_3). ^{13}C NMR (DMSO-d_6, 600 MHz): δ 176.64 (C═O), 170.37 (C═S), 166.58 (COOH), 160.79 (phenyl), 152.50 (C—F), 149.01 (quinoline), 145.94 (quinoline), 145.52 (trizole), 137.65 (quinoline), 130.88 (phenyl), 130.54 (thiophenyl), 129.51 (thiophenyl), 128.30 (phenyl), 127.67 (thiophenyl), 126.91 (thiophenyl), 119.73 (quinoline), 115.36 (phenyl), 111.73 (quinoline), 107.54 (quinoline), 106.52 (quinoline), 69.26 (—CH_2—), 56.00 (—OCH_3), 50.03 (piperazine), 49.54 (—CH_2CH_3), 14.81 (—CH_2CH_3). IR (KBr) (ν, cm^{-1}): 717 w, 754 w, 826 w, 1007 w, 1025 w, 1165 w, 1199 w, 1258 s, 1302 m, 1328 m, 1376 m, 1415 m, 1475 s, 1519 s, 1627 s, 1720 m, 2837 w, 2957 w, 3050 w, 3447 w.

*4.3.3.4. 1-Ethyl-6-fluoro-7-(4-((3-(2-hydroxyphenyl)-4-(4-methoxyphenyl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3d**).* White solid. Yield: 30%. M.p.: 203–205 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 15.35 (s, 1H, —COOH), 9.94 (s, 1H, —OH), 8.95 (s, 1H, quinoline), 7.91 (d, J = 12.8 Hz, 1H, quinoline), 7.31–7.17 (m, 5H, phenyl and quinoline), 6.93–6.76 (m, 4H, phenyl), 5.26 (s, 2H, —CH_2—), 4.59 (d, J = 6.7 Hz, 2H, —CH_2CH_3), 3.74 (s, 3H, —OCH_3), 3.39 (s, 4H, piperazinyl), 3.03 (s, 4H, piperazinyl), 1.42 (s, 3H, CH_2CH_3). ^{13}C NMR (DMSO-d_6, 600 MHz): δ 176.57 (C═O), 170.80 (C═S), 166.56 (COOH), 159.61 (phenyl), 156.36 (phenyl), 152.47 (C—F), 148.92 (quinoline and trizole), 145.93 (quinoline), 137.60 (quinoline), 132.70 (phenyl), 131.95 (phenyl), 129.65 (phenyl), 127.89 (phenyl), 119.69 (quinoline), 116.20 (phenyl), 114.19 (phenyl), 113.75 (phenyl), 113.40 (phenyl), 111.66 (quinoline), 107.53 (quinoline), 106.42 (quinoline), 69.20 (—CH_2—), 55.75 (—OCH_3), 50.23 (piperazine), 49.96 (piperazine), 49.53 (—CH_2CH_3), 14.81 (—CH_2CH_3). IR (KBr) (ν, cm^{-1}): 750 w, 807 w, 858 w, 889 w, 937 w, 969 w, 1016 m, 1041 w, 1103 w, 1145 w, 1200 w, 1261 s, 1295 m, 1369 m, 1450 s, 1477 s, 1499 s, 1515 s, 1627 s, 1723 s, 2800 w, 2846 w, 2943 w, 3435 w.

4.3.3.5. 1-Ethyl-6-fluoro-7-(4-((3-(3-hydroxyphenyl)-4-(4-methoxyphenyl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl)piperazin-1-

*yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3e**).* White solid. Yield: 40%. M.p.: 228–230 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 15.35 (s, 1H, —COOH), 9.74 (s, 1H, —OH), 8.96 (s, 1H, quinoline), 7.92 (d, J = 13.3 Hz, 1H, quinoline), 7.31 (d, J = 8.8 Hz, 2H, methoxyphenyl), 7.21 (d, J = 7.0 Hz, 1H, quinoline), 7.14 (t, J = 7.9 Hz, 1H, hydroxyphenyl), 7.04 (d, J = 8.8 Hz, 2H, methoxyphenyl), 6.85 (s, 1H, hydroxyphenyl), 6.82 (d, J = 7.8 Hz, 1H, hydroxyphenyl), 6.72 (d, J = 7.8 Hz, 1H, hydroxyphenyl), 5.27 (s, 2H, —CH_2—), 4.60 (d, J = 6.9 Hz, 2H, —CH_2CH_3), 3.82 (s, 3H, —OCH_3), 3.38 (s, 4H, piperazinyl), 3.04 (s, 4H, piperazinyl), 1.41 (t, J = 7.0 Hz, 3H, —CH_2CH_3). ^{13}C NMR (DMSO-d_6, 600 MHz): δ 176.58 (C═O), 170.37 (C═S), 166.57 (COOH), 160.06 (phenyl), 157.72 (phenyl), 154.11 and 152.46 (d, J = 249.7 Hz, C—F), 149.78 (quinoline), 148.91 (trizole), 145.90 and 145.84 (d, J = 10.1 Hz, quinoline), 137.60 (quinoline), 130.36 (phenyl), 130.13 (phenyl), 128.18 (phenyl), 126.98 (phenyl), 119.73 and 119.68 (d, J = 7.6 Hz, quinoline), 119.45 (phenyl), 118.01 (phenyl), 115.70 (phenyl), 114.94 (phenyl), 111.69 and 111.54 (d, J = 23.1 Hz, quinoline), 107.53 (quinoline), 106.40 (quinoline), 69.27 (—CH_2—), 55.89 (—OCH_3), 50.13 (piperazine), 49.99 (piperazine), 49.53 (—CH_2CH_3), 14.80 (—CH_2CH_3). IR (KBr) (ν, cm^{-1}): 690 w, 755 w, 808 w, 833 w, 893 w, 936 w, 1012 w, 1089 w, 1170 w, 1198 w, 1258 s, 1326 m, 1368 w, 1477 s, 1517 s, 1628 s, 1731 m, 2837 w, 2938 w, 3050 w, 3229 w, 3430 w.

*4.3.3.6. 1-Ethyl-6-fluoro-7-(4-((3-(4-hydroxyphenyl)-4-(4-methoxyphenyl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3f**).* White solid. Yield: 40%. M.p.: 248–250 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 15.36 (s, 1H, —COOH), 10.01 (s, 1H, —OH), 8.96 (s, 1H, quinoline), 7.95 (d, J = 6.2 Hz, 1H, quinoline), 7.29 (d, J = 8.8 Hz, 2H, methoxyphenyl), 7.20 (d, J = 7.0 Hz, 1H, quinoline), 7.18 (d, J = 8.7 Hz, 2H, hydroxyphenyl), 7.04 (d, J = 8.8 Hz, 2H, methoxyphenyl), 6.72 (d, J = 8.6 Hz, 2H, hydroxyphenyl), 5.25 (s, 2H, —CH_2—), 4.60 (d, J = 6.9 Hz, 2H, —CH_2CH_3), 3.82 (s, 3H, —OCH_3), 3.38 (s, 4H, piperazinyl), 3.04 (s, 4H, piperazinyl), 1.41 (t, J = 7.1 Hz, 3H, —CH_2CH_3). ^{13}C NMR (DMSO-d_6, 600 MHz): δ 176.59 (C═O), 170.06 (C═S), 166.57 (COOH), 160.03 (phenyl), 159.82 (phenyl), 154.11 and 152.46 (d, J = 249.7 Hz, C—F), 150.02 (quinoline), 148.92 (trizole), 145.91 and 145.84 (d, J = 10.3 Hz, quinoline), 137.60 (quinoline), 130.45 (phenyl), 128.31 (phenyl), 119.72 and 119.67 (d, J = 7.6 Hz, quinoline), 116.37 (phenyl), 115.85 (phenyl), 114.94 (phenyl), 111.69 and 111.54 (d, J = 23.0 Hz, quinoline), 107.53 (quinoline), 106.40 (quinoline), 69.16 (—CH_2—), 55.89 (—OCH_3), 50.15 (piperazine), 49.99 (piperazine), 49.52 (—CH_2CH_3), 14.79 (—CH_2CH_3). IR (KBr) (ν, cm^{-1}): 535 w, 605 w, 664 w, 703 w, 753 w, 780 w, 837 m, 897 w, 930 w, 1007 m, 1110 w, 1170 m, 1256 s, 1331 s, 1376 m, 1477 s, 1515 s, 1627 s, 1728 s, 2839 w, 2940 w, 3055 w, 3272 w, 3411 w.

*4.3.3.7. 1-Ethyl-6-fluoro-7-(4-((4-(4-methoxyphenyl)-3-(pyridin-2-yl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3g**).* White solid. Yield: 48.8%. M.p.: 272–274 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 15.35 (s, 1H, —COOH), 8.95 (s, 1H, quinoline), 8.44 (d, J = 7.9 Hz, 1H, pyridinyl), 7.91 (dd, J = 9.6, 7.2 Hz, 2H, quinoline and pyridinyl), 7.77 (d, J = 7.9 Hz, 1H, pyridinyl), 7.44 (dd, J = 7.4, 5.0 Hz, 1H, pyridinyl), 7.27 (d, J = 8.8 Hz, 2H, phenyl), 7.21 (d, J = 7.7 Hz, 1H, quinoline), 6.98 (d, J = 8.9 Hz, 2H, phenyl), 5.31 (s, 2H, —CH_2—), 4.59 (d, J = 7.1 Hz, 2H, —CH_2CH_3), 3.79 (s, 3H, —OCH_3), 3.38 (s, 4H, piperazinyl), 3.05 (s, 4H, piperazinyl), 1.41 (t, J = 7.1 Hz, 3H, —CH_2CH_3). ^{13}C NMR (DMSO-d_6, 600 MHz): δ 176.57 (C═O), 170.77 (C═S), 166.56 (COOH), 159.73 (phenyl), 154.11 and 152.45 (d, J = 249.7 Hz, C—F), 149.86 (quinoline), 148.92 (trizole), 148.88 (pyridinyl), 145.88 and 145.82 (d, J = 10.0 Hz, quinoline), 145.38 (pyridinyl), 137.73 (pyridinyl), 137.59 (quinoline), 130.10 (phenyl), 128.58 (phenyl), 125.62 (pyridinyl), 124.81 (pyridinyl), 119.72 and 119.67 (d, J = 7.6 Hz, quinoline), 114.42 (phenyl), 111.69 and 111.54 (d, J = 22.9 Hz, quinoline), 107.53 (quinoline), 106.40 (quinoline), 69.41 (—CH_2—), 55.81 (—OCH_3), 50.12 (piperazine), 49.97 and 49.95 (piperazine), 49.52 (—CH_2CH_3), 14.79 (—CH_2CH_3). IR (KBr) (ν, cm^{-1}): 614 w, 645 w, 670 w, 708 w, 754 w, 790 w, 838 w, 927 w, 994 m, 1090 w, 1163 m, 1198 m, 1253 s, 1326 m, 1376 m, 1412 m, 1475 s, 1518 s, 1627 s, 1724 m, 2837 w, 2937 w, 3050 w, 3503 w.

*4.3.3.8. 1-Ethyl-6-fluoro-7-(4-((4-(4-methoxyphenyl)-3-(pyridin-3-yl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3h**).* White solid. Yield: 81.3%. M.p.: 255–257 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 15.34 (s, 1H, —COOH), 8.95 (s, 1H, quinoline), 8.62 (dd, J = 4.8, 1.3 Hz, 1H, pyridinyl), 8.56 (d, J = 1.4 Hz, 1H, pyridinyl), 7.91 (d, J = 13.3 Hz, 1H, quinoline), 7.74 (d, J = 8.1 Hz, 1H, pyridinyl), 7.43 (dd, J = 8.0, 4.9 Hz, 1H, pyridinyl), 7.37 (d, J = 8.8 Hz, 2H, phenyl), 7.20 (d, J = 7.3 Hz, 1H, quinoline), 7.05 (d, J = 8.9 Hz, 2H, phenyl), 5.33 (s, 2H, —CH_2—), 4.59 (d, J = 7.1 Hz, 2H, —CH_2CH_3), 3.80 (s, 3H, —OCH_3), 3.38 (s, 4H, piperazinyl), 3.06 (s, 4H, piperazinyl), 1.41 (t, J = 7.1 Hz, 3H, —CH_2CH_3). ^{13}C NMR (DMSO-d_6, 600 MHz): δ 176.57 (C═O), 170.52 (C═S), 166.56 (COOH), 160.24 (phenyl), 154.09 and 152.43 (d, J = 249.7 Hz, C—F), 151.60 (pyridinyl), 149.29 (quinoline), 148.91 (trizole), 147.86 (pyridinyl), 145.90 and 145.83 (d, J = 9.9 Hz, quinoline), 137.59 (quinoline), 136.49 (pyridinyl), 130.48 (phenyl), 127.66 (phenyl), 123.96 (pyridinyl), 122.52 (pyridinyl), 119.70 and 119.65 (d, J = 7.7 Hz, quinoline), 115.02 (phenyl), 111.67 and 111.52 (d, J = 22.9 Hz, quinoline), 107.53 (quinoline), 106.37 (quinoline), 69.40 (—CH_2—), 55.91 (—OCH_3), 50.08 (piperazine), 49.98 and 49.95 (piperazine), 49.52 (—CH_2CH_3), 14.79 (—CH_2CH_3). IR (KBr) (ν, cm^{-1}): 496 w, 529 w, 554 w, 591 w, 641 w, 667 w, 707 w, 754 w, 781 w, 806 w, 840 w, 894 w, 927 w, 1009 m, 1087 w, 1170 s, 1200 m, 1252 s, 1331 s, 1385 s, 1471 s, 1517 s, 1630 s, 1673 s, 1728 s, 2764 w, 2844 w, 2950 w, 3005 w, 3050 w, 3258 w, 3367 w, 3491 w.

*4.3.3.9. 1-Ethyl-6-fluoro-7-(4-((4-(4-methoxyphenyl)-3-(pyridin-4-yl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3i**).* White solid. Yield: 65.0%. M.p.: 251–253 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 15.34 (s, 1H, —COOH), 8.96 (s, 1H, quinoline), 8.61 (d, J = 6.1 Hz, 2H, pyridinyl), 7.92 (d, J = 13.3 Hz, 1H, quinoline), 7.38 (d, J = 8.9 Hz, 2H, pyridinyl), 7.30 (dd, J = 4.6, 1.5 Hz, 2H, phenyl), 7.21 (d, J = 7.2 Hz, 1H, quinoline), 7.07 (d, J = 9.0 Hz, 2H, phenyl), 5.33 (s, 2H, —CH_2—), 4.60 (d, J = 7.2 Hz, 2H, —CH_2CH_3), 3.80 (s, 3H, —OCH_3), 3.38 (s, 4H, piperazinyl), 3.06 (s, 4H, piperazinyl), 1.41 (t, J = 7.0 Hz, 3H, —CH_2CH_3). ^{13}C NMR (DMSO-d_6, 600 MHz): δ 176.57 (C═O), 170.92 (C═S), 166.56 (COOH), 160.35 (phenyl), 154.09 and 152.44 (d, J = 249.7 Hz, C—F), 150.59 (quinoline), 148.93 (trizole), 147.74 (pyridinyl), 145.89 and 145.82 (d, J = 9.9 Hz, quinoline), 137.59 (quinoline), 133.44 (pyridinyl), 130.36 (phenyl), 127.64 (phenyl), 122.55 (pyridinyl), 119.72 and 119.67 (d, J = 9.9 Hz, quinoline), 115.12 (phenyl), 111.68 and 111.52 (d, J = 9.9 Hz, quinoline), 107.53 (quinoline), 106.38 (quinoline), 69.49 (—CH_2—), 55.94 (—OCH_3), 50.04 (piperazine), 49.97 and 49.95 (piperazine), 49.52 (—CH_2CH_3), 14.80 (—CH_2CH_3). IR (KBr) (ν, cm^{-1}): 630 w, 706 w, 754 w, 784 w, 809 w, 830 m, 926 w, 1007 m, 1089 w, 1169 m, 1198 m, 1256 s, 1302 m, 1328 s, 1376 m, 1475 s, 1518 s, 1627 s, 1723 s, 2839 w, 2936 w, 3048 w, 3448 w, 3525 w.

4.4. Molecular docking

Docking simulations were performed using Molecular Operating Environment version 2014.09. The synthesized compounds were drawn and exported to MOE. Energy minimization was done for each molecule using the MMFF94x force field. The crystal structure of *S. pneumoniae* type IV topoisomerase in complex with levofloxacin was downloaded from the protein data bank (PDB ID: 3rae) [40]. The protein obtained was prepared by adding the hydrogen atoms and computing the partial

charges. The binding pocket was identified, and flexible ligand-rigid receptor docking of the most stable conformers was performed using the alpha triangle placement method and London dG scoring function. The top-scoring pose was visually inspected.

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.

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Data statement

Crystallographic data for the structures in this paper have been deposited at the Cambridge Crystallographic Data Centre (CCDC; 12 Union Road, Cambridge CB21EZ, United Kingdom). Copies of the data can be obtained free of charge by querying the depository numbers 2009379, 2009380, and 2083295 for **1a**, **2a**, and **3h**, respectively (Fax: +44-1223-336-033; E-Mail: deposit@ccdc.cam.ac.uk, http://www.ccdc.cam.ac.uk).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2021.105270.

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