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Synthesis, Characterization, Molecular Docking, and Biological Evaluation of Novel Levofloxacin–Thiourea Derivatives as Potential Antibacterial Agents

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ABSTRACT

Fluoroquinolone resistance is a major challenge in managing bacterial infections and motivates the development of new derivatives with improved activity and pharmacokinetic properties . In this study, a series of levofloxacin–thiourea hybrids (L1–L3) were synthesized via esterification of levofloxacin, conversion to the corresponding hydrazide, and condensation with para-substituted phenyl isothiocyanates under mild conditions. The structures of the intermediates and final products were confirmed by melting point, ATR-FTIR, and ^{1}H NMR spectroscopy, which demonstrated introduction of the hydrazide and thiourea moieties while preserving the levofloxacin core. Antibacterial activity was assessed against *Escherichia coli* (G–) and *Staphylococcus aureus* (G+) using the agar well diffusion method, with levofloxacin as reference; the p-chloro derivative L2 showed inhibition zones comparable to or greater than levofloxacin at higher concentrations, whereas L1 and L3 retained substantial activity. SwissADME predictions indicated that all derivatives display high gastrointestinal absorption, acceptable polarity (TPSA 122.96 Å^2), and only one Lipinski rule violation, with moderate decreases in solubility relative to levofloxacin but overall favourable drug-likeness profiles. Molecular docking into the *Streptococcus pneumoniae* DNA gyrase–DNA complex (PDB ID: 4Z2D) showed that L1–L3 adopt poses similar to levofloxacin, preserving the key Mg^{2+}-mediated contacts and base stacking while forming additional hydrogen-bond and π–π interactions through the thiourea–aryl fragment; L2 exhibited the most favourable docking score. Together, the experimental and in silico results identify levofloxacin–thiourea derivatives, particularly the p-chloro analogue L2, as promising leads for further optimization against resistant bacterial pathogens.

1. INTRODUCTION

Fluoroquinolones are broad spectrum bactericidal agents that inhibit bacterial DNA synthesis by targeting the type II topoisomerases DNA gyrase and topoisomerase IV (Panwar et al., 2025). Levofloxacin, the S-enantiomer of ofloxacin and a third-generation fluoroquinolone, exhibits enhanced potency and a favorable pharmacokinetic profile, and it is widely used to treat respiratory, urinary, gastrointestinal, and skin infections caused by both Gram-positive and Gram-negative bacteria (Ernst et al., 1997). However, the increasing prevalence of fluoroquinolone resistance mediated by target-site mutations, decreased drug accumulation, and enzymatic modification has compromised the clinical utility of existing agents and underlines the need for novel derivatives with improved activity (Redgrave et al., 2014).

It is known that thiourea and thiosemicarbazide scaffolds are found in many bioactive agents of significant antibacterial activity by virtue of their ability to form hydrogen bonding, coordinate metal ions and interact with various enzymatic targets like DNA gyrase (Khan et al., 2025). Such substituents are common in thiourea drugs; they enhance lipophilicity and target binding, and several series using thioureas have been developed to inhibit bacterial gyrase and other enzymes19–26 (Zborovskii et al., 2025). Concurrently, structural studies have unveiled the binding mode of levofloxacin and its analogous quinolones within DNA gyrase complexes: the drug

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intercalates between DNA bases and chelates a Mg^{2+} ion disposed to cross-link the quinolone carbonyl/carboxylate to highly conserved residues of the GyrA subunit, which ultimately stabilizes the cleavage complex and thwarts DNA replication (Letuta & Berdinskiy, 2022; Spencer & Panda, 2023). In spite of several reports on levofloxacin's derivatives, including C-10 and piperazinyl modifications, the combination of levofloxacin core with thiourea linker is less investigated. In the current study, we have designed such hybrids to increase antibacterial activity and adjust pharmacokinetic properties. Herein we describe the synthesis and characterization of levofloxacin–thiourea derivatives, assessment of their antibacterial effect and SwissADME profiles, and molecular docking against

the *S. pneumoniae* DNA gyrase–DNA complex (PDB ID 4Z2D) to rationalize their binding interactions and structure–activity relationships.

2. Materials and Methods

2.1 Chemistry

The reagents and solvents adopted throughout the research investigation were obtained chiefly from HI-Media (India). Melting points were determined by the capillary method using a Stuart SMP10 electrical melting point apparatus (United Kingdom). Thin layer chromatography (TLC) was carried out on silica gel plates using ethyl acetate: methanol (8 : 2, v/v) as the developing solvent system to monitor the reactions. ATR Fourier Transform Infrared (FT-IR) spectra for compound identification were recorded on a Shimadzu IRAffinity-1S FT-IR spectrophotometer. Nuclear magnetic resonance (NMR) spectra were obtained on a Bruker NMR spectrometer.

Synthesis of levofloxacin Methyl Ester

levofloxacin (0.30 g, 0.8 mmol) was placed in a round-bottom flask and suspended in anhydrous methanol (30 mL). The mixture was cooled in an ice bath, and concentrated sulfuric acid (3–4 drops, ~0.1 mL) was added slowly with stirring. The reaction was refluxed for 6–8 h at 65 ˚C with continuous stirring. Reaction progress was monitored by TLC until complete reaction of levofloxacin.

After cooling to room temperature, the reaction mixture was neutralized by addition of saturated aqueous sodium hydrogen carbonate. Methanol was removed under reduced pressure, and the residue was diluted with distilled water (40 mL) and extracted with ethyl acetate (3 × 25 mL). The combined organic extracts were dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to afford levofloxacin methyl ester (Ibrahim et al., 2022).

Synthesis of levofloxacin Hydrazide derivative.

levofloxacin hydrazide was obtained by nucleophilic substitution of the methyl ester with hydrazine hydrate. levofloxacin methyl ester (0.37 g, 1 mmol) was dissolved in absolute ethanol (25 mL) in a round-bottom flask. Hydrazine hydrate (80% w/w, 0.25 mL, 6.5 mmol) was added dropwise under stirring. The reaction mixture was fitted with a reflux condenser and reflux for 3–4 h at 78 ˚C, while progress was monitored by TLC. After completion, the reaction mixture was cooled to room temperature and then poured into crushed ice–water (30–40 mL). The resulting precipitate was collected by filtration, washed thoroughly with cold water then dried at room temperature to give levofloxacin hydrazide (Kashid et al., 2020).

General procedure for synthesis of levofloxacin–thiourea derivatives

levofloxacin hydrazide (0.37 g, 1 mmol) was dissolved in ethanol (20 mL) in a round-bottom flask and stirred at room temperature. To this solution, the appropriate substituted phenyl isothiocyanate (1.0 mmol) was added dropwise, followed by glacial acetic acid (2–3 drops) as catalyst. The reaction mixture was stirred and heated at 40–50 **°C** for 4–6 h on a water bath, then maintaining gentle reflux at 78 ˚C, until TLC indicated complete consumption of the starting hydrazide.

After cooling to room temperature, the mixture was poured onto crushed ice with stirring. The precipitated solid was filtered under vacuum, washed with cold water and then dried at ambient temperature to furnish the corresponding levofloxacin–thiourea derivative. The crude products were purified by recrystallization from ethanol (Mahmoud et al., 2024).

2.2 In silico study

2.2.1 ADME prediction

The physicochemical properties and ADME profiles of levofloxacin and the synthesized derivatives were predicted using the Swiss ADME web server (http://www.swissadme.ch). For each compound, the 2D structures were drawn and converted to SMILES notation, which was submitted to Swiss ADME to compute molecular weight, number of hydrogen-bond donors and acceptors, topological polar surface area (TPSA), rotatable bonds, and lipophilicity (XLOGP3). Drug-likeness and oral bioavailability were assessed using Lipinski's rule of five and related filters, the bioavailability score, and the bioavailability radar plots. Predicted pharmacokinetic parameters included gastrointestinal (GI) absorption, blood–brain barrier (BBB) permeation, P-glycoprotein (P-gp) substrate status, and water solubility. In addition, the BOILED-Egg model implemented in Swiss ADME was used to visualize the likelihood of passive GI absorption and brain penetration for each compound (Daina et al., 2017).

2.2.2 Molecular docking

Molecular docking studies were carried out using MOE 2024 (Chemical Computing Group, Montreal, Canada) to investigate the binding mode of levofloxacin-based ligands within bacterial DNA gyrase. The X-ray crystal structure of the *Streptococcus pneumoniae* DNA gyrase–DNA–levofloxacin cleavage complex (PDB ID **4Z2D**) was retrieved from the Protein Data Bank and used as the receptor (figure 1).

Protein preparation

The 4Z2D structure was imported into MOE and prepared using the QuickPrep protocol (Table. 1). Non-relevant chains and crystallographic water molecules were removed, while the DNA duplex, Mg^{2+} ion, and any water molecules directly involved in ligand coordination were retained. Missing hydrogen atoms were added, partial charges were assigned, and protonation states were adjusted for pH 7.4. Local energy minimization of the protein–DNA complex was performed with the Amber10:EHT force field, restricting heavy-atom movement to relieve steric clashes while preserving the experimental geometry of the active site.

Ligand preparation

Levofloxacin and the synthesized derivatives (L1, L2, L3) were built in MOE with correct stereochemistry. The Wash module was used to assign appropriate ionization and tautomeric states at physiological pH (7.4). Each ligand was energy-minimized using the Amber10:EHT force field and a low-energy conformer ensemble was generated for docking.

Docking protocol and validation

The binding pocket was defined using the coordinates of the co-crystallized ligand levofloxacin in 4Z2D. A receptor site was generated around the ligand and used as the docking region. To validate the docking protocol, the co-crystallized ligand levofloxacin was extracted from the complex and re-docked into the prepared binding site. Docking was performed with the Dock module using the Triangle Matcher placement method and London dG scoring for initial pose generation, followed by Induced Fit refinement with GBVI/WSA dG rescoring. The best-ranked pose of levofloxacin was compared with the crystallographic pose by calculating the root-mean-square deviation (RMSD); an RMSD $\leq$ 2.0 Å was considered acceptable for protocol validation (Table. 1).

After validation, all ligands were docked into the same binding site using identical parameters. For each ligand, multiple poses were generated and ranked according to GBVI/WSA dG scores. The top-scoring pose(s) were selected for further analysis of key interactions, including hydrogen bonding, metal coordination, π–π stacking with DNA bases, and hydrophobic contacts with gyrase residues. Docking scores and interaction patterns were then correlated with the experimental antibacterial data.

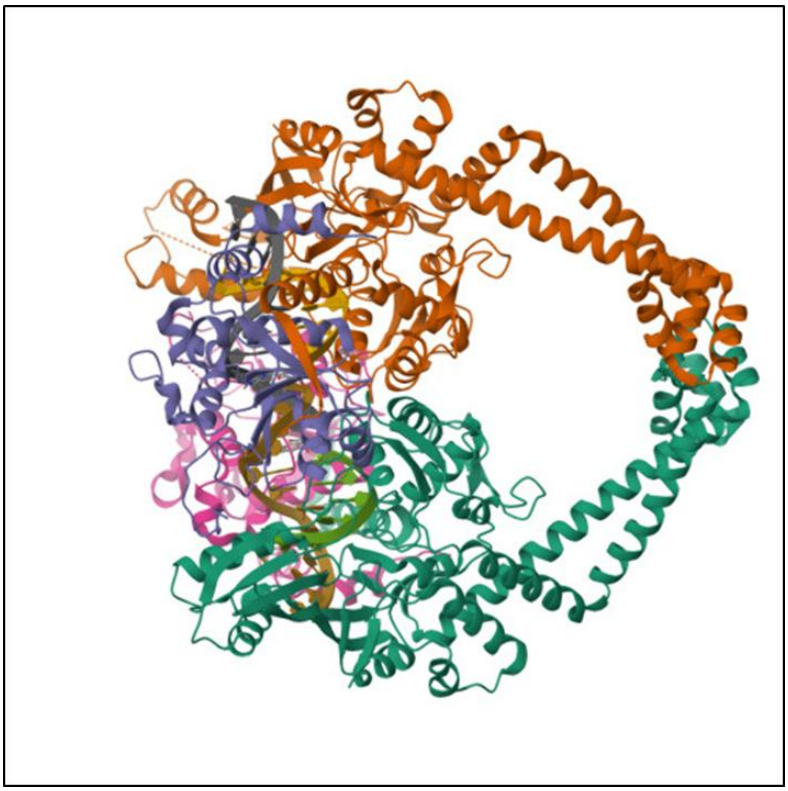


Figure 1. 3D structure of the levofloxacin–DNA cleavage complex of DNA gyrase from Streptococcus pneumoniae

Table 1. key information and characteristics of 4Z2D gyrase enzyme

Protein	method	resolution Å	Atom count	total structural weight	organism	co-crystalized ligand	docking score	RMSD
4Z2D	X-RAY DIFFRACTION	3.38	10,118	195.66 kDa	Streptococcus pneumoniae	Levofloxacin	-12.677	0.85

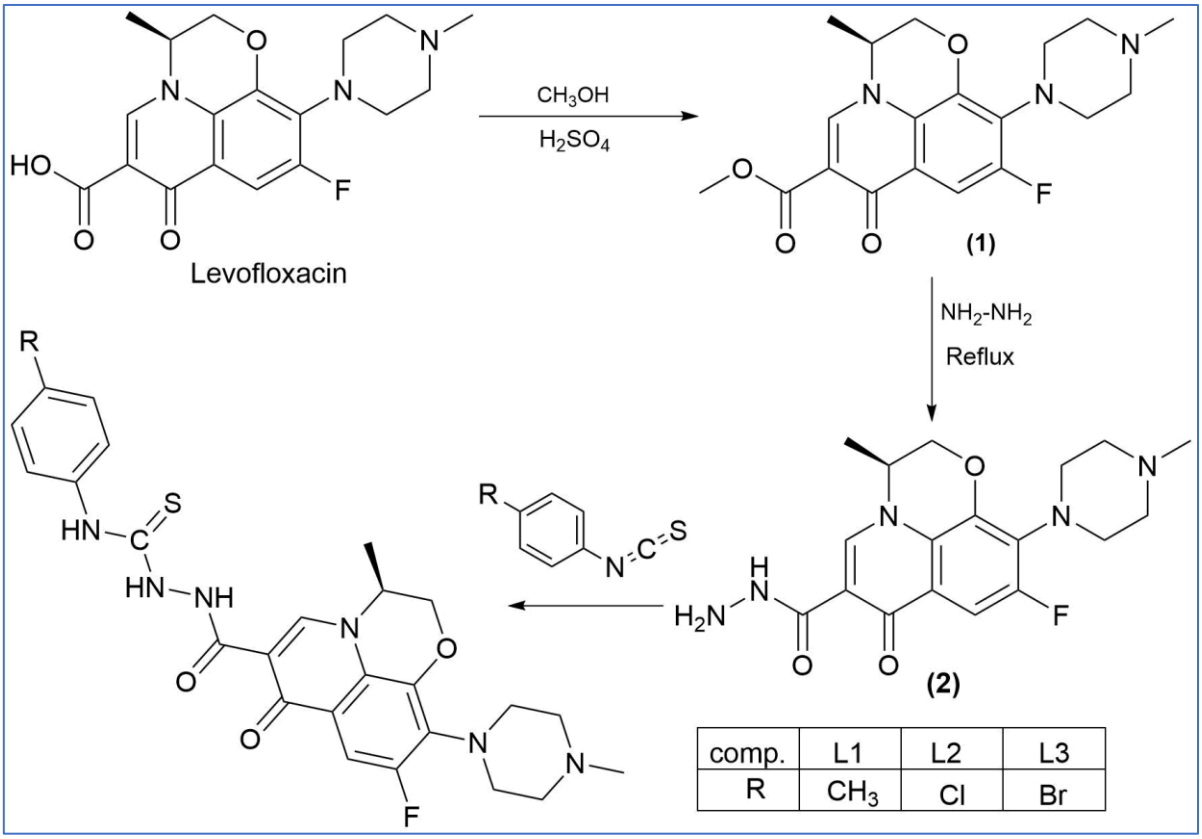
2.3 Antibacterial activity

The antibacterial activity of the synthesized levofloxacin derivatives was evaluated by the agar well diffusion method following CLSI recommendations with minor modifications. Test microorganisms are *Staphylococcus aureus (*Gram-positive bacteria) and *Escherichia coli* (Gram-negative bacteria). obtained from the microbiology laboratory culture collection and maintained on appropriate media. Bacterial inocula were prepared from overnight cultures, adjusted to 0.5 McFarland standard in sterile saline, and uniformly spread on Mueller–Hinton agar plates. After inoculation, sterile cork borers were used to punch 6-mm wells in the agar. Stock solutions of the test compounds were prepared in DMSO at 1000 µg/mL and serially diluted to obtain 500 and 250 µg/mL. Each well was filled with 50 µL of the appropriate solution. Wells containing levofloxacin solutions at the same concentrations served as positive controls, whereas wells containing DMSO alone were used as negative controls. The plates were allowed to stand at room temperature for 30 min to permit pre-diffusion of the samples, then incubated at 37 °C for 24 h. After incubation, the diameters of the inhibition zones around each well were measured in millimeters (Lin et al., 2026).

3. Results and discussion

3.1.1 chemistry

The synthetic route to the target levofloxacin derivatives involved three main steps: esterification of levofloxacin, conversion of the ester to the corresponding hydrazide, and condensation of the hydrazide with substituted phenyl isothiocyanates (Scheme 1) to afford levofloxacin–thiourea analogues (L1–L3). In each step, the structures of the intermediates and final products were confirmed by melting point, ATR-FTIR, and ^{1}H NMR spectroscopy, as summarized below.



Scheme 1. *the chemical synthesis of levofloxacin thiourea derivatives (L1-L3).*

levofloxacin methyl ester, compound (1):

Yellow viscous oil; yield 82%, m.p: not defined, ATR-FTIR (cm^{-1}) ν 3340 (broad, N–H of piperazine), 1732 (s, C=O of ester), 1685 (s, C=O of quinolone), 1254 (s, C–O–C of ester), 1193 (C–F), 1050–1020 (C–N of piperazinyl).

levofloxacin hydrazide, compound (2):

Pale yellow powder: yield 78%, m.p: 245-248 ˚C, ATR-FTIR (cm^{-1}) ν 3325, 3236 (broad, NH/NH_2), 1694 (s, C=O of hydrazide/quinolone), 1485 (C=C str), 1223 (C–F).

p-tolyl levofloxacin thiourea derivative compound (L1):

Orange powder, Yelid: 88%, m.p: 237-240 ˚C, ATR-FTIR (cm^{-1}) ν 3322, 3274 (broad, NH), 3041 (s, C–H aromatic), 2985 (C–H aliphatic), 1693, 1674 (s, C=O, quinolone/amide), 1570-1476 (C=C aromatic), 1217 (C–F). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.54 (s, 1H, N-H of amide), 10.26 (s, 1H, N-H of thiourea), 8.89 – 6.94 (m, 1H, CH of aromatic), 4.28 (d, 1H, CH of morpholine ring), 4.12 (d, 2H, CH_2 of morpholine ring), 3.49, 3.44 (m, 1H, CH2 of piperazine ring), 2.89 (s, 3H, CH_3 substitution on phenyl ring), 1.28 (s, 3H, CH3 substitution on morpholine ring).

P-chloro-levofloxacin thiourea derivative, compound (L2):

Yellow powder, yield: 86%, m.p: 223- 226 ˚C, ATR-FTIR (cm^{-1}) ν 3327, 3245 (broad, NH str.), 1696, 1674 (s, C=O, quinolone/amide), 1572, 1492 (C=C, aromatic), 1185 (C–F). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.08 (s, 1H, N-H of amide), 11.15 (s, 1H, N-H of thiourea), 8.78 – 7.28 (m, 1H, CH of aromatic), 4.13 (d, 1H, CH of morpholine ring), 4.03 (d, 2H, CH_2 of morpholine ring), 3.71, 3.48 (m, 1H, CH2 of piperazine ring), 2.65 (s, 3H, CH_3 substitution on phenyl ring), 1.21 (s, 3H, CH3 substitution on morpholine ring).

P-bromo-levofloxacin thiourea derivative, compound (L3):

Brown powder, yield: 89%, m.p: 242- 245 ˚C decompose, ATR-FTIR (cm^{-1}) ν 3300, 3290 (broad, NH str.), 1706 (s, C=O, quinolone/amide), 1584, 1491 (C=C, aromatic), 1183 (C–F). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.79 (s, 1H, N-H of amide), 11.14 (s, 1H, N-H of thiourea), 8.8 – 7.16 (m, 1H, CH of aromatic), 4.85 (d, 1H, CH of morpholine ring), 4.68 (d, 2H, CH_2 of morpholine ring), 3.52, 3.24 (m, 1H, CH2 of piperazine ring), 2.79 (s, 3H, CH_3 substitution on phenyl ring), 1.36 (s, 3H, CH3 substitution on morpholine ring).

Levofloxacin in sulfuric acid catalyzed methanol leads to levofloxacin methyl ester as a viscous yellow oil (82%). Successful esterification was confirmed by the disappearance of the carboxylic acid band and appearance of a strong ester carbonyl at 1732 cm^{-1} present in this ATR-FTIR spectrum. Refluxing treatment of the ester with excess hydrazine hydrate in ethanol afforded the related levofloxacin hydrazide (78% yield) as pale yellow solid showing a descrete melting point range (245–248 °C). The restoration of a single amide/hydrazide C=O band at 1694 cm^{-1} and broad NH/NH_2 stretches at 3325–3236 cm^{-1} signified ester conversion into the hydrazide functionality.

By performing condensation of levofloxacin hydrazide with para-substituted phenyl isothiocyanates in ethanolic solution at 40–50 °C under catalytic conditions using glacial acetic acid, the target levofloxacin–thiourea derivatives L1–L3 can be obtained at good yields (86–89%). This mild reaction conditions, and clean precipitations of product from the reaction mixtures illustrates the synthetic efficiency of this route, which should allow generation of a small library of analogues from common hydrazide intermediate.

Spectral characterization

For levofloxacin methyl ester, ATR-FTIR showed a strong ester C=O stretch at 1732 cm^{-1} and an intense C–O–C band at 1254 cm^{-1}, together with the retained quinolone carbonyl at 1685 cm^{-1} and C–F band at 1193 cm^{-1}, confirming esterification. In the hydrazide, the broad NH/NH_2 absorptions (3325, 3236 cm^{-1}) and a shifted C=O band at 1694 cm^{-1} are consistent with formation of a terminal $CONHNH_2$ group.

Introduction of the thiourea–aryl moiety in L1–L3 produced characteristic spectral changes. All three derivatives display two strong carbonyl bands in the 1706–1674 cm^{-1} region, attributable to overlapping quinolone and amide C=O stretches, along with broad NH bands around 3320–3240 cm^{-1} corresponding to the amide and thiourea NH protons. Aromatic C=C stretches appear between 1584 and 1476 cm^{-1}, while C–F bands are retained near 1183–1217 cm^{-1}, confirming that substitution occurred at the hydrazide rather than on the quinolone ring.

The ^{1}H NMR spectra provide further evidence for the proposed structures. Each thiourea derivative exhibits two downfield singlets at δ 11–12 ppm corresponding to the amide NH and thiourea NH protons, indicative of strong hydrogen bonding and conjugation with the adjacent carbonyl and C=S groups. Aromatic protons from the quinolone and para-substituted phenyl rings resonate as multiplets between δ ~8.9–6.9 ppm, while the morpholine and piperazine methine/methylene protons appear as doublets and multiplets in the δ 4.8–3.2 ppm range, preserving the characteristic pattern of the levofloxacin side chains. The para-methyl substituent on the phenyl ring in L1–L3 gives a singlet at δ ~2.6–2.9 ppm (3H), and the morpholine methyl group appears as a singlet around δ 1.2–1.4 ppm (3H), confirming the presence and environment of these groups.

Collectively, the high isolated yields, distinct changes in IR bands across each synthetic step, and diagnostic ^{1}H NMR signals for NH, aromatic, and aliphatic protons support successful synthesis of levofloxacin methyl ester, hydrazide, and the series of levofloxacin–thiourea derivatives L1–L3. The

consistency of melting points and spectral features among the thiourea analogues suggests that the synthetic route is robust and that the structural modifications at the hydrazide position do not disrupt the integrity of the levofloxacin core.

3.2 In silico study

3.2.1 Pharmacokinetic (ADME) properties

Swiss ADME calculations were performed to assess the physicochemical and pharmacokinetic behavior of levofloxacin and the new levofloxacin–thiourea derivatives (L1–L3) as summarized in table 2, 3 and figure 2.

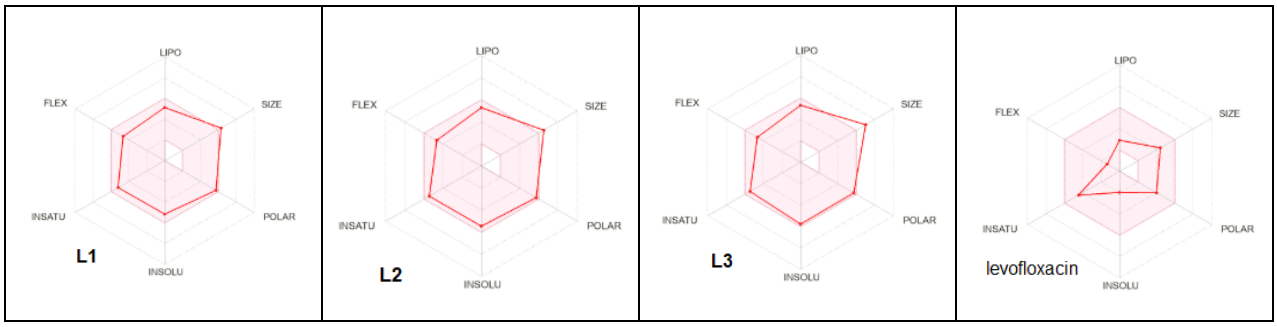


Figure 2. Radar chart showing six physicochemical properties of (L1-L3) and levofloxacin

Table 2. *Predicted physicochemical and pharmacokinetic parameters of levofloxacin and levofloxacin–thiourea derivatives (L1–L3) obtained from Swiss ADME.*

Compound	#H-bond acceptors	#H-bond donors	TPSA	solubility	GI absorption	BBB permeant	Pgp substrate	Lipinski #violations
L1	5	3	122.96	Moderately soluble	High	No	Yes	1
L2	5	3	122.96	Poorly soluble	High	No	Yes	1
L3	5	3	122.96	Poorly soluble	High	No	Yes	1
Levofloxacin	6	1	75.01	Very soluble	High	No	Yes	0

All compounds showed high predicted GI absorption, indicating that the introduction of the hydrazide–thiourea–aryl fragment did not compromise the ability of the molecules to be absorbed orally. The topological polar surface area (TPSA) of the derivatives (122.96 Å²) remained below the 140 Å² threshold generally associated with acceptable oral bioavailability, although it is higher than that of levofloxacin (75.01 Å²), reflecting the presence of additional hydrogen-bond donors and acceptors in the thiourea moiety.

In terms of solubility, levofloxacin was predicted to be very soluble, whereas L1 was moderately soluble and L2–L3 were classified as poorly soluble, consistent with their increased aromaticity and lipophilicity. Despite this reduction in solubility, all compounds complied with Lipinski's rule of five, with at most one violation for the derivatives and none for levofloxacin, suggesting a generally favourable drug-likeness profile. None of the molecules were predicted to permeate the blood–brain barrier, which is desirable for antibiotics intended to act primarily outside the central nervous system. All compounds, including levofloxacin, were identified as P-gp substrates, indicating a potential for efflux-mediated limitation of intracellular accumulation and highlighting the importance of experimental confirmation. Overall, the ADME analysis indicates that the designed levofloxacin–thiourea derivatives preserve the favourable oral absorption properties of levofloxacin, maintain acceptable polarity and molecular size, and show only moderate solubility penalties associated with increased lipophilicity. These features, together with their enhanced antibacterial activity and strong predicted binding to DNA gyrase, support their potential as promising lead compounds for further optimization.

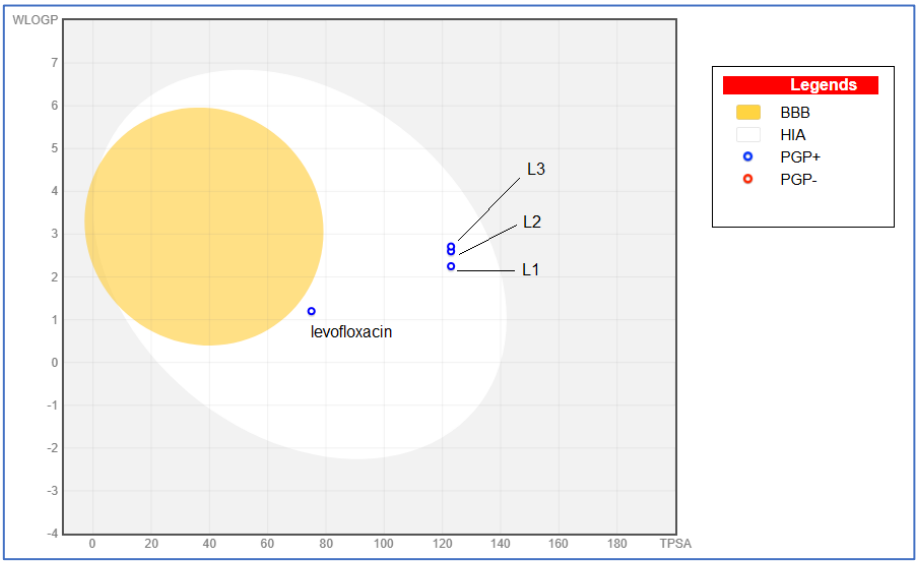


Figure 3. *BOILED-Egg diagram showing predicted gastrointestinal absorption (white region) and blood–brain barrier penetration (yellow region) for levofloxacin and levofloxacin–thiourea derivatives L1–L3, highlighting their WLOGP versus TPSA distribution.*

3.2.2 Molecular docking

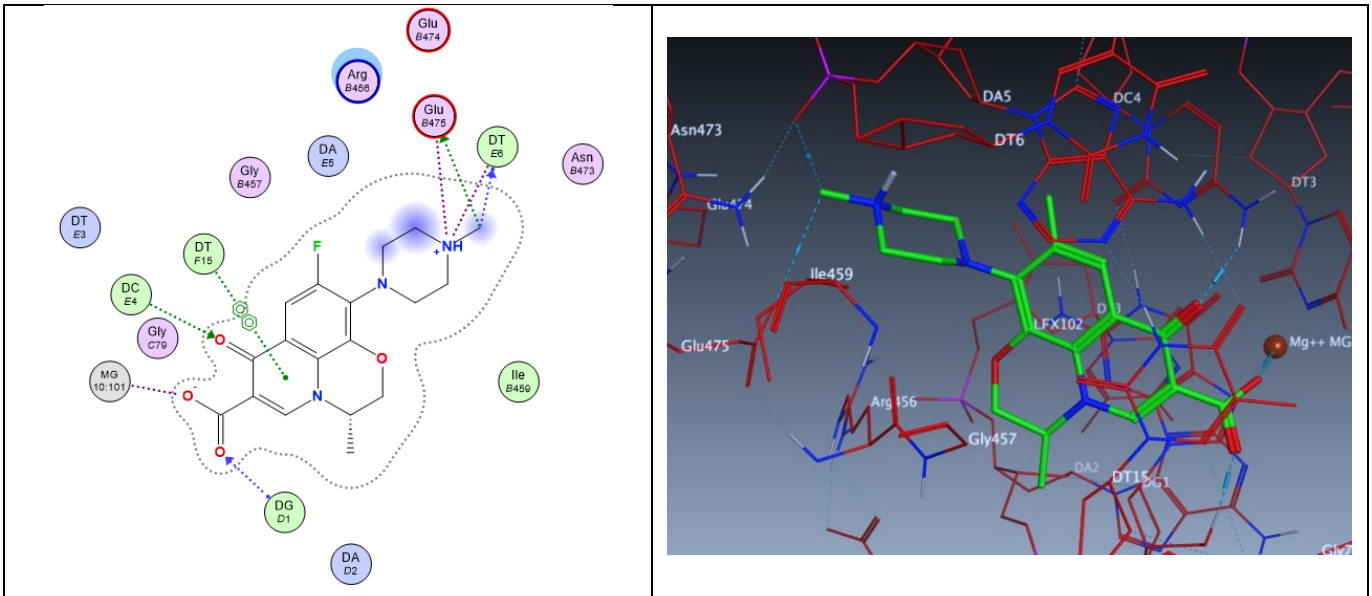
Molecular docking against the *S. pneumoniae* DNA gyrase–DNA complex (PDB ID: 4Z2D) was performed to rationalize the antibacterial activity of the synthesized levofloxacin derivatives (L1-L3) and to compare their binding with that of levofloxacin (Table 4). Re-docking of the co-crystallized levofloxacin reproduced the experimental pose with an RMSD of 0.85 Å and an S-score of −12.67 kcal/mol, confirming the reliability of the docking protocol. In the crystal pose, levofloxacin engages in a dense network of interactions, including hydrogen bonds from the quinolone carbonyl and carboxylate (C16 and O atoms) to GLU475 and the phosphate of DT6, hydrogen bonding to DNA bases DC4 and DG1, and direct coordination of the carbonyl oxygen (O5) to the catalytic Mg^{2+} ion (MG101), in addition to π–π stacking between the quinolone ring and the aromatic base of DT15. The three new ligands (L1–L3) showed S-scores in the range of −10.78 to −11.32 kcal/mol with RMSD values of 1.42–1.83 Å, indicating binding affinities comparable to the parent drug and poses that fit well within the quinolone pocket (Table 2). Compound L2 gave the best docking score (−11.32 kcal/mol), followed closely by L1 (−10.89 kcal/mol) and L3 (−10.78 kcal/mol). All derivatives preserved the key quinolone–DNA gyrase interactions, most notably a hydrogen bond from the hydrazide nitrogen (N45) to the O4 atom of thymine DT15, which anchors the ligands in the same region as levofloxacin. Additional contacts were observed for the thiourea/aryl extensions: for L1, the sulfur atom (S62) interacts with the Cα of SER82 and the carbonyl oxygen forms a hydrogen bond to SER81, while π–π stacking between the quinolone ring and DNA bases DG1 and DA5 further stabilizes the complex. L2 engages in two hydrogen bonds (N45–O4 of DT15 and N47–OG of SER81) and maintains metal coordination with MG101, together with multiple π–π stacking contacts with DG1 and DA5, which explains its slightly better docking score. For L3, the thiourea sulfur (S62) acts as a hydrogen-bond acceptor toward N4 of cytosine DC4, and the ligand retains metal interaction with MG101 and several aromatic contacts, including H–π and π–π interactions with DG1, DA5, and the side chain of GLU85. The presence of these extra interactions in L1–L3, derived from the hydrazide–thiourea–aryl moiety, compensates for the increased steric bulk and maintains high predicted affinity for the gyrase–DNA complex. the docking results confirm that the new levofloxacin–thiourea hybrids occupy the canonical quinolone binding site, preserve the essential Mg^{2+}-mediated contacts and base stacking seen for levofloxacin, and form additional hydrogen-bond and π-interactions that are consistent with their enhanced antibacterial activity observed in vitro.

Table 3. *Molecular docking scores and key interactions of levofloxacin and synthesized levofloxacin–thiourea derivatives (L1–L3) with the DNA gyrase–DNA complex (PDB ID: 4Z2D), showing S-scores, RMSD values, and main ligand–receptor contacts in the active site.*

Active site			Bonds between atoms of ligands and residues of the active site of 4Z2D.					
Comp.	**S score (kcal/mol)**	**RMSD (Å)**	**Atom of compound**	**Involved receptor atoms**	**Involved receptor residues**	**Type of interaction**	**bond Distance (Å)**	**E (kcal/mol**
Co-crystalized ligand (levofloxacin)	-12.67	0.85	C 16	OE2	GLU 475 (C)	H-donor	3.27	-1.0
			C 16	OP1	DT 6 (H)	H-donor	3.28	-0.9
			002 11	N4	DC 4 (H)	H-accepter	2.95	-2.6
			003 13	05	DG1 (F)	H-accepter	2.68	-3
			O 5	MG	MG 101 (F)	Meta1	1.97	-3.4
			N 3	OE2	GLU 475 (C)	ionic	3.84	-0.8
			N 3	OP1	DT 6 (H)	ionic	3.89	-0.9
			O 5	MG	MG 101 (F)	ionic	1.97	-16.7
			6-ring	6-ring	DT 15 (G)	Pi-Pi	3.85	
L1	-10.89	1.42	N 45	O4	DT 15 (E)	H-donor	2.9	-4.3
			O 1	OG	SER 81 (A)	H-accepter	3.27	-0.5
			S 62	CA	SER 82 (A)	H-accepter	4.02	-0.9
			O 1	MG	MG 101 (E)	Meta1	2.63	-0.9
			6-ring	6-ring	DG 1 (H)	Pi-Pi	4.04	
			6-ring	5-ring	DA 5 (F)	Pi-Pi	4.00	
L2	-11.32	1.74	N 45	O4	DT 15 (E)	H-donor	2.98	-5.6
			N 47	OG	SER 81 (A)	H-donor	2.99	-1.2
			O 1	MG	MG 101 (E)	Meta1	2.19	-3.2

			6-ring	5-ring	DG 1 (H)	Pi-Pi	4.35	
			6-ring	6-ring	DG 1 (H)	Pi-Pi	4.02	
			6-ring	5-ring	DA 5 (F)	Pi-Pi	4.03	
L3	-10.78	1.83	N 45	O4	DT 15 (E)	H-donor	3.12	-2.0
			S 62	N4	DC 4 (F)	H-accepter	3.63	-5.2
			O 1	MG	MG 101 (E)	Meta1	2.27	-2.1
			C 7	5-ring	DG 1 (H)	H-Pi	3.90	-1.4
			6-ring	CB	GLU 85 (A)	Pi-H	3.99	-0.7
			6-ring	6-ring	DG 1 (H)	Pi-Pi	4.16	
			6-ring	5-ring	DA 5 (F)	Pi-Pi	3.88	

Table 4. *2D and 3D ligands interactions with active site of 4Z2D protein.*



Co-crystalized ligand (levofloxacin)

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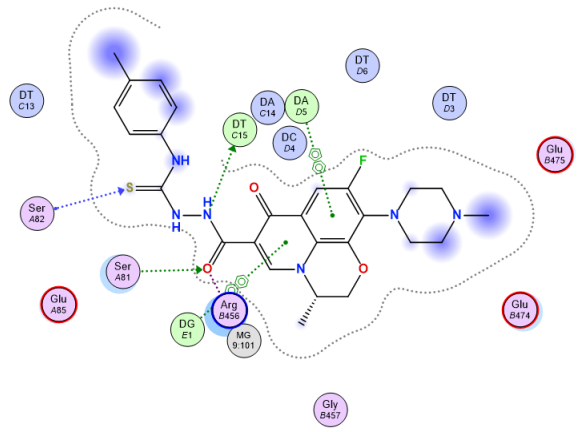
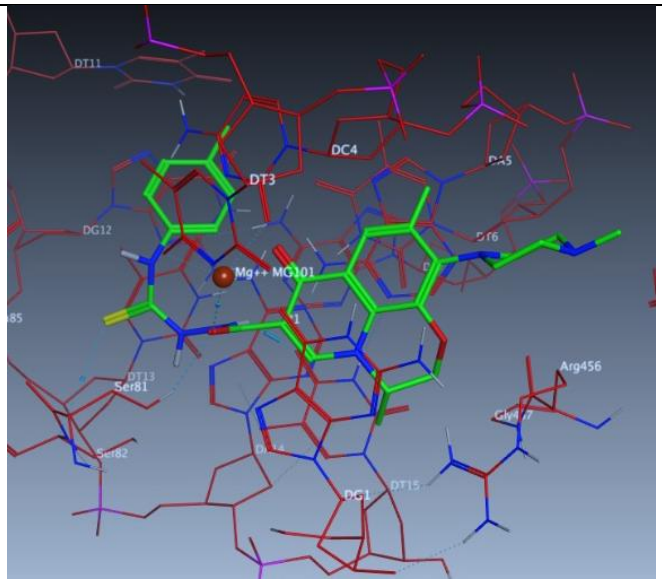
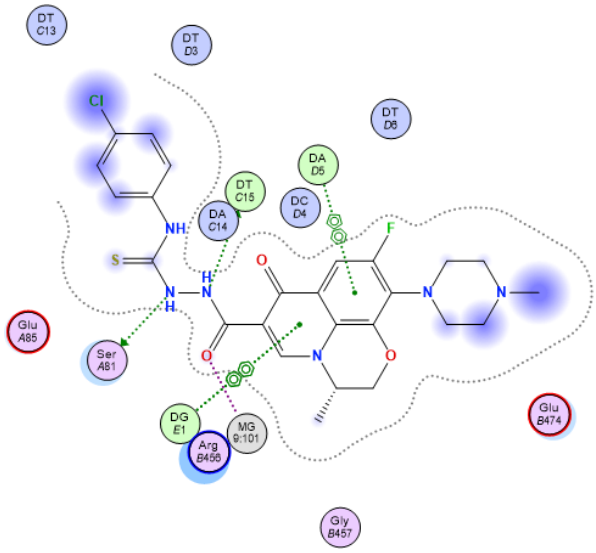
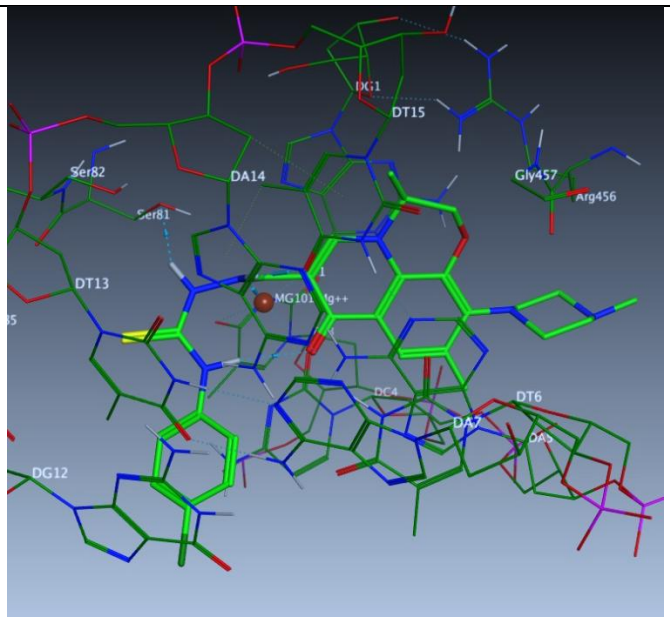


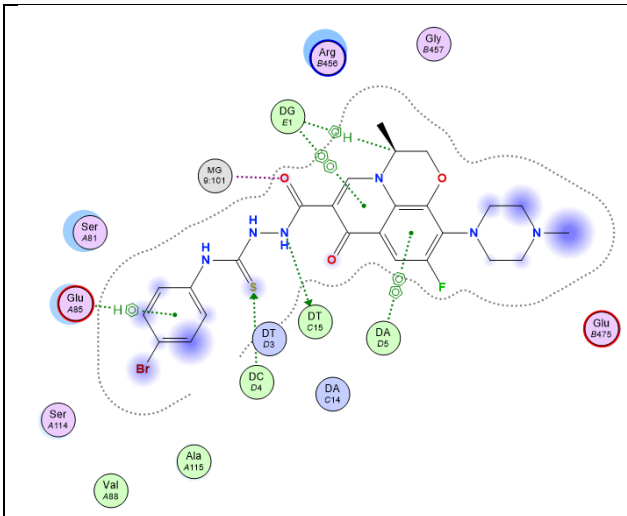


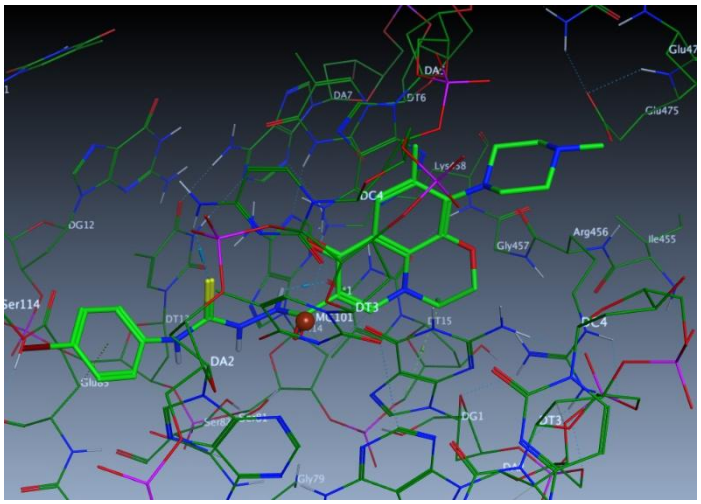
Compound L1



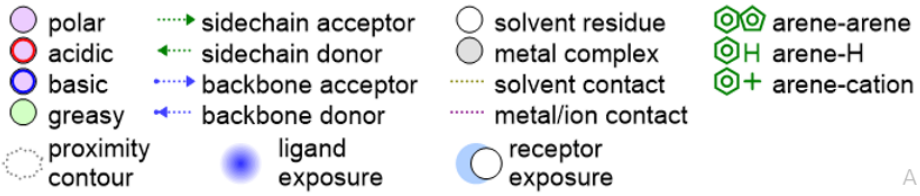


Compound L2





Compound L3



3.3 Antibacterial activity

The newly synthesized levofloxacin–thiourea derivatives were evaluated for their antibacterial activity against one Gram-negative strain (*E. coli*) and one Gram-positive strain (*S. aureus*) using the agar well diffusion method, with levofloxacin as reference, the results are summarized in table 5. Against *E. coli*, all three derivatives retained good activity; L2 (p-chloro) showed the largest inhibition zones (37, 38, and 41 mm at 250, 500, and 1000 µg/mL, respectively), slightly surpassing levofloxacin (37–40 mm) at the highest concentration, while L1 and L3 were comparable but somewhat less potent.

Table 5. *Antibacterial activity of synthesized compounds (L1-L3) using well diffusion method at different concentrations, expressed as mean inhibition zone diameters (mm, n = 3).*

Test organism	Compound	250 µg/mL	500 µg/mL	1000 µg/mL
***E. coli* (G−)**	Levofloxacin (std)	37	38	40
	L1	33	34	38
	L2	37	38	41
	L3	12	18	19
***S. aureus* (G+)**	Levofloxacin (std)	12	16	14
	L1	9	12	12
	L2	21	25	24
	L3	17	15	16

For *S. aureus*, the derivatives generally exhibited weaker activity than levofloxacin, which produced inhibition zones of 12–16 mm across the tested concentrations. L2 again emerged as the most active analogue (21–24 mm), indicating a notable enhancement in anti-staphylococcal effect relative to the parent drug, whereas L1 showed modest activity and L3 remained close to or slightly above the standard. The higher potency of the p-chloro derivative (L2) toward both Gram-negative and Gram-positive strains suggests that introduction of an electron-withdrawing chloro substituent on the thiourea phenyl ring improves antibacterial performance, in agreement with reported SAR trends for thiourea-based antibacterials and with the stronger binding predicted by docking.

4. Conclusions

In this work, levofloxacin was successfully transformed into a series of novel levofloxacin–thiourea derivatives through a concise, three-step route involving esterification, hydrazide formation, and condensation with substituted phenyl isothiocyanates. The structures of the intermediates and final products were confirmed by melting point, ATR-FTIR, and ^{1}H NMR spectroscopy, which together verified introduction of the hydrazide and thiourea pharmacophores while preserving the integrity of the levofloxacin core. The new derivatives displayed promising antibacterial activity in agar-well diffusion assays. Compound (L2) in particular produced inhibition zones that were comparable to or greater than those of levofloxacin at the same concentrations, while L1 and L3 generally retained substantial activity, indicating that modification at the hydrazide position can enhance or at least maintain the antibacterial potency of the parent drug. Swiss ADME predictions showed that all compounds retain high gastrointestinal absorption and acceptable polarity, with only minor Lipinski rule violations and moderate reductions in solubility relative to levofloxacin, suggesting that the thiourea modifications do not compromise overall drug-likeness or oral suitability.

Molecular docking into the *S. pneumoniae* DNA gyrase–DNA complex (PDB ID: 4Z2D) demonstrated that the levofloxacin–thiourea derivatives occupy the quinolone binding pocket, preserve the critical Mg^{2+}-mediated interactions and base stacking observed for levofloxacin, and establish additional hydrogen-bond and π–π contacts via the hydrazide–thiourea–aryl fragment, particularly in the case of L2. The combination of favorable in vitro antibacterial activity, acceptable in silico ADME profiles, and strong predicted target engagement supports these levofloxacin–thiourea hybrids as promising lead candidates for the development of new anti-infective agents aimed at overcoming fluoroquinolone resistance. Further work will focus on expanding the series, determining MIC values, and exploring in vivo efficacy and safety profiles.

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