Indian Journal of Chemistry
Vol. 64, November 2025, pp. 1068-1078
DOI: 10.56042/ijc.v64i11.21537

Synthesis, characterization, *in silico* and *in vitro* antimicrobial evaluation of some quinoline derivatives

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Received 12 July 2025; accepted (revised) 4 November 2025

A series of quinoline derivatives (**4a–k**) have been synthesized and tested to assess ADMET properties, docking behaviour, and antibacterial potential. A series of various quinoline derivatives have been synthesized by a simple and efficient three-component reaction using anilines, aromatic aldehydes and ethyl acetoacetate with acetonitrile as a solvent. The characterization of the synthesized compounds have been done by IR and NMR spectroscopy. Compound **4d** shows the highest binding affinity of −8.4 kcal/mol. Strong interactions that involved key amino acids have also been observed. ADMET analysis reveals outstanding intestinal absorption and CNS permeability. The antibacterial activity of synthesized quinoline derivatives have been evaluated using the agar diffusion method against two Gram-positive and two Gram-negative bacteria, with ciprofloxacin as the standard. Compounds **4c**, **4d**, and **4j** show broad-spectrum activity, with **4c** demonstrating the most consistent and potent inhibition, surpassing ciprofloxacin against *B. subtilis* and *E. coli*, making it a promising lead for further development. Two-way ANOVA analysis shows no statistically significant difference in antibacterial activity among the tested compounds ($p = 0.891$) or bacterial strains ($p = 0.431$), indicating a uniform response. However, Tukey's HSD test confirmed that no compound could significantly outperform ciprofloxacin.

Keywords: Quinoline, Molecular docking, ADMET, Antibacterial

Quinoline is a heterocyclic compound containing nitrogen and it was first synthesized by German chemist Friedlieb Ferdinand Runge in 1834 from coal tar. He called quinoline as *lekuol*[1]. It contains benzene and pyridine ring fused together to form a quinoline ring. It is one of the most significant N-based heterocyclic aromatic chemical[2]. Numerous naturally occurring chemicals (cinchona alkaloids) and pharmacologically active compounds with a wide range of biological activities include quinoline nucleus[3]. It is very important heterocyclic compound due to its broad pharmacological activities like as anti-inflammatory[4,5], antimicrobial[6,7], antimalarial[8,9], anticancer[10,11], antifungal[12], antituberculosis[13], antibacterial[14], *etc*. A wide range of variables, such as improper antibiotic administration and sales, antibiotic usage outside of the medical field, and microbial genetic factors, have contributed to the rise in antimicrobial resistance (AMR)[15]. Inadequate financial incentives for pharmaceutical companies to produce novel antimicrobial medicines have made the issue worse. Thus, it becomes a necessity to synthesize new quinoline derivatives to antagonize antimicrobial resistance[16].

Experimental Section

Materials and equipments

All chemicals and solvents utilized in this research were bought from Qualigens, Sigma-Aldrich, S. D. Fine, Himedia and Rankem Laboratories. The melting point of the synthesized compounds was calculated by using MR-VIS visual melting range instrument (Lab India). Thin layer chromatography to check reaction completion was performed in mobile phase (n-hexane:ethyl acetate::3:2). IR spectra characterization of the synthesized compounds was done on PIKE technologies. The IR graphs were drawn using Origin 2025 software. The FT-NMR Spectrometer (400 MHz, Bruker) with H^1 D_2O exchange in $CDCl_3$ was carried out and chemical shifts were denoted as δ (ppm). Every measurement was done at room temperature.

Synthesis of quinoline derivatives

In a round bottom flask anilines (**1**) (1 mmol) and aldehydes (**2**) (1 mmol) were added with 10 mL acetonitrile and then the reaction mixture was stirred for 10 minutes to completely dissolve all the reactants. Then ethyl acetoacetate (**3**) (1 mmol) was

added in the reaction mixture. Acetic acid was added in the solution to catalyse the reaction. Then the reaction mixture was refluxed with continuous stirring at 70-95 ºC for 4-6 hr. The reaction completion was noticed using TLC with mobile phase n-hexane:ethyl acetate (3:2). After reaction completion, the flask was allowed to cool at room temperature. After cooling the product (**4a-k**) was filtered and dried. The recrystallization of the products was done by using ethanol, then filtered, dried and weighed. The general scheme for the synthesis is given in Scheme 1.

The compounds were characterized by the spectral methods using IR spectroscopy and ^{1}H NMR.

In silico evaluation of quinoline derivatives

Molecular docking

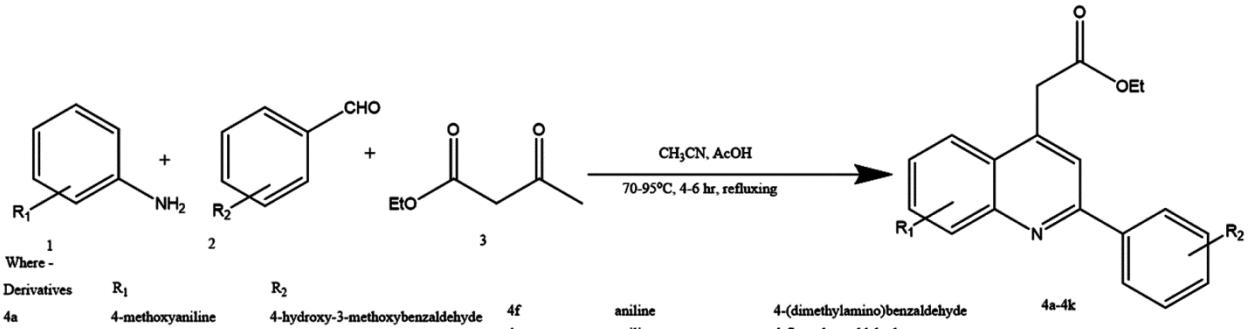
The interaction between a small molecule and a target protein at the atomic level was evaluated using a computational method known as molecular docking. It predicts the best fit conformation at the protein binding site and provides an estimate of the stability of ligand–protein complexes. Molecular docking was conducted using AutoDock software against DNA gyrase enzyme (PDB Id: 1KZN). The protein's three-dimensional structure was retrieved from the Protein Data Bank (www.rcsb.org). The protein's active site was predicted, and ligand preparation was done using Lipinski's Rule of 5. The ligand was chosen based on its hydrogen bond donors, acceptors, molecular mass, lipophilicity, and molar refractivity. The 2D structure was converted into a 3D structure, and energy was minimized to achieve the most stable form for better docking binding affinity. The docking of the ligands against the protein was analysed using a scoring function that estimates the force of non-covalent interactions between a ligand and molecular target using mathematical methods.

ADMET prediction

In terms of absorption, distribution, metabolism, and excretion studies, ADME prediction, describes the computational techniques used to forecast a chemical compound's behaviour in a biological system. pKCSM (https://biosig.lab.uq.edu.au/pkcsm/prediction) is a web server to provide an integrated freely available platform to rapidly screen multiple pharmacokinetic properties. Computational analysis of the toxicity of compound is not only faster than the determination of toxicity in animals, but also helps to reduce the amount of experimental cost. For the *in silico* prediction of chemical toxicity**,** ProTox **(**https://tox.charite.de/protox3/**)**, a freely accessible web-based platform was utilized.

In vitro Antimicrobial activity

Biological evaluation acts as the bridge between the chemical design of a drug candidate and its practical application in living organisms. The synthesized quinoline derivatives **4a-k** were evaluated for their antimicrobial activity against two Gram-positive organisms (*Streptococcus pneumoniae, Bacillus subtilis*) and two Gram-negative organisms (*Pseudomonas aeruginosa, and E. coli)* by agar well diffusion method in triplicate and results were listed as the average diameter of inhibition zones of bacterial growth in millimetres. The bacterial strain was uniformly spread using a sterile cotton swab on a sterile petri dish containing MH agar. 1 mg/mL of each test compound was added to wells (7 mm diameter holes cut in the agar gel, 20 mm apart from one another). The system was incubated for 24 h at



Where -

Derivatives	R_1	R_2
4a	4-methoxyaniline	4-hydroxy-3-methoxybenzaldehyde
4b	4-methoxyaniline	4-(dimethylamino)benzaldehyde
4c	4-methoxyaniline	3-ethoxy-4-hydroxybenzaldehyde
4d	4-methoxyaniline	3-hydroxybenzaldehyde
4e	4-methoxyaniline	4-hydroxybenzaldehyde
4f	aniline	4-(dimethylamino)benzaldehyde
4g	aniline	4-fluorobenzaldehyde
4h	aniline	3-ethoxy-4-hydroxybenzaldehyde
4i	aniline	4-hydroxy-3-methoxybenzaldehyde
4j	aniline	4-hydroxybenzaldehyde
4k	aniline	3-nitrobenzaldehyde

Scheme 1 — Synthetic route of quinoline derivatives

36±1 ºC under aerobic conditions. After incubation, confluent bacterial growth was observed. Inhibition of the bacterial growth was measured in mm and compared with that of reference compound.

Statistical analysis

For the statistical evaluation of quinoline derivatives, two-way ANOVA was performed to compare activity between multiple compounds using OriginPro learning edition with significance level of 0.05. Also, Post hoc analysis using Tukey's HSD test was applied to compare each compound with Ciprofloxacin. A Pearson's product-moment correlation was conducted to examine the relationship between molecular docking and zone of inhibition by using JASP (Version 0.19.3; JASP Team, 2025) statistical software[17,18].

Results and Discussion

Synthesis of quinoline derivatives

As shown in Scheme 1, the quinoline derivatives **4a-k** were synthesized from aryl anilines, aromatic aldehydes and ethyl acetoacetate in presence of acetic acid as catalyst and acetonitrile as solvent by stirring and refluxing at 70-95 °C for 4-6 hr. The aniline derivatives and aromatic aldehydes react together to form Schiff base (imine formation). This imine reacts with enol form of ethyl acetoacetate to form intermediates. This undergoes further cyclization to form quinoline derivatives. The compounds were characterized by IR spectroscopy and ^{1}H NMR. The characterization of the synthesized compounds is given below:

Ethyl 2-(2-(4-hydroxy-3-methoxyphenyl)-6-methoxyquinolin-4-yl) acetate, 4a: Yield 78%. IR (KBr): 3502 (-OH str), 1658 (C=N str), 1172 (C-O str), 1589 (C=C str), 1735 cm^{-1} (C=O str); ^{1}H NMR ($CDCl_3$): δ 1.25(t, 3H, CH_3 of ethyl ester), 4.15 (q, 2H, CH_2 of ethyl ester), 3.75 (s, 3H, OCH_3 on 3-position of phenyl ring), 3.90 (s, 3H, OCH_3 of quinoline ring), 3.80 (s, 2H, CH_2 between quinoline and ester group), 6.50-7.80 (m, 6H, aromatic rings of quinoline and phenyl rings), 9.80 (br s, 1H, phenolic OH).

Ethyl 2-(2-(4-(dimethylamino) phenyl)-6-methoxyquinolin-4-yl) acetate, 4b: Yield 67%. IR (KBr): 1662 (C=N str), 1026 (C-O str), 1589 (C=C str), 1650 (C=O str), 1587 cm^{-1} (N-H str); ^{1}H NMR ($CDCl_3$): δ 1.22 (t, 3H, CH_3 of ethyl ester), 4.15 (q, 2H, CH_2 of ethyl ester), 3.80 (s, 3H, OCH_3 at quinoline ring), 4.48 (s, 2H, adjacent to quinoline and ester group), 6.60-7.79 (m,6H, aromatic protons), 2.98 (dd, 6H, N-CH_3 protons).

Ethyl 2-(2-(3-ethoxy-4-hydroxyphenyl)-6-methoxyquinolin-4-yl) acetate, 4c: Yield 85%. IR (KBr): 3502 (-OH str), 1658 (C=N str), 1740 (C=O str), 1172 (C-O str), 1580 cm^{-1} (C=C str); ^{1}H NMR ($CDCl_3$): δ 1.19 (t, 3H, CH_3 of ethyl ester), 4.10 (q, 2H, CH_2 of ethyl ester), 3.81 (s, 3H, OCH_3 at quinoline ring), 4.50 (s, 2H, adjacent to quinoline and ester group), 6.60-7.79 (m,6H, aromatic protons), 9.80 (broad s, 1H, OH on phenyl ring), 1.40 (q,2H, CH_3 of ethyl ester at phenyl ring).

Ethyl 2-(2-(3-hydroxyphenyl)-6-methoxyquinolin-4-yl) acetate, 4d: Yield 74%. IR (KBr): 3325 (OH str), 1751 (C=O str), 1519 (C=C str), 1604 (C=N str), 1257 cm^{-1} (C-O str); ^{1}H NMR ($CDCl_3$): δ 9.20 (s, 1H, phenolic OH), 6.6-7.8 (m, 6H, aromatic protons), 1.19 (t, 3H, CH_3 of ethyl ester), 4.13 (q, 2H, CH_2 of ethyl ester), 4.49 (s, 2H, adjacent to quinoline and ester group), 3.81 (s, 3H, OCH_3 on quinoline).

Ethyl 2-(2-(4-hydroxyphenyl)-6-methoxyquinolin-4-yl) acetate, 4e: Yield 61%. IR (KBr): 3648 (OH str), 1751 (C=O str), 1257 (C-O str), 1604 (C=N str), 1519 cm^{-1} (C=C str); ^{1}H NMR ($CDCl_3$): δ 1.25 (t, 3H, CH_3 of ethyl ester), 4.15(q, 2H, CH_2 of ethyl ester), 4.48 (s, 2H, CH_2 between quinoline and ester group), 3.81 (s, 3H, OCH_3 on quinoline ring), 9.67 (s, 1H, phenolic OH), 6.6-7.7 (m, 2H, aromatic protons of quinoline and phenyl rings)**.**

Ethyl 2-(2-(4-(dimethylamino) phenyl) quinolin-4-yl) acetate, 4f: Yield 70%. IR (KBr): 1662 (C=N str), 1272 (C-O str), 1519 (C=C str), 1650 (C=O str), 1587 cm^{-1} (N-H str); ^{1}H NMR ($CDCl_3$): δ 3.03 (s, 6H, $N(CH_3)_2$ group), 1.25 (t, 3H, CH_3 of ethyl ester), 4.48 (s, 2H, CH_2 between quinoline and methyl ester), 4.13 (-CH_2 of ethyl ester), 6.8-8.19 (m, 2H, aromatic protons of quinoline and phenyl rings).

Ethyl 2-(2-(4-fluorophenyl) quinolin-4-yl) acetate, 4g: Yield 82%. IR (KBr): 1743 (C=O str), 1573 (C=C str), 1234 (C-O str), 1018 (C-F str), 3085 cm^{-1} (C-H str); ^{1}H NMR ($CDCl_3$): δ 1.21 (t, 3H, CH_3 of

ethyl ester), 4.45 (s, 2H, CH_2 between quinoline and ester), 4.14 (q, 2H, CH_2 of ethyl ester), 7.31 (dd, 2H, aromatic H *ortho* to F on phenyl ring), 8.25 (dd, 2H, aromatic H *meta* to F on phenyl ring), 8.19 (d, 1H, H at quinoline C-5), 7.87 (d, 1H, H at quinoline C-8), 6.80 (d, 1H, H at quinoline C-3), 7.72 (t, 1H, H at quinoline C-6) , 7.83 (t, 1H, H at quinoline C-7).

Ethyl 2-(2-(3-ethoxy-4-hydroxyphenyl) quinolin-4-yl) acetate, 4h: Yield 88%. IR (KBr): 1735 (C=O str), 1134 (C=N str), 1311 (C-O str), 3394 (OH str), 1473 cm^{-1} (C=C str); ^{1}H NMR ($CDCl_3$): δ 1.21 (t, 3H, CH_3 of ethyl ester), 4.13 (q, 2H, CH_2 of ethyl ester), 4.49 (s, 2H, adjacent to quinoline and ester group), 9.80 (broad s, 1H, OH on phenyl ring), 1.42 (q,2H, CH_3 of ethyl ester at phenyl ring), 4.13 (q, 2H, CH_2 of ethoxy on phenyl ring), 6.80 (d, 1H, H at quinoline C-3), 8.19 (d, 1H, H at quinoline C-5), 7.72 (t, 1H, H at quinoline C-6), 7.83 (t, 1H, H at quinoline C-7), 7.87 (d, 1H, H at quinoline C-8), 7.51 (d, 1H, aromatic H at C-2 of phenyl), 7.45 (d, 1H, aromatic H at C-6 of phenyl).

Ethyl 2-(2-(4-hydroxy-3-methoxyphenyl) quinolin-4-yl) acetate, 4i: Yield 59%. IR (KBr): 3332 (OH str), 3078 (C-H str), 1581 (C=C str), 1658 (C=N str), 1257 cm^{-1} (C-O str); ^{1}H NMR ($CDCl_3$): δ 1.21(t, 3H, CH_3 of ethyl ester), 4.15 (q, 2H, CH_2 of ethyl ester), 3.83 (s, 3H, OCH_3 on 3-position of phenyl ring), 4.49 (s, 2H, CH_2 between quinoline and ester group), 6.50-7.80 (m, 6H, aromatic rings of quinoline and phenyl rings), 9.80 (br s, 1H, phenolic OH), 8.19 (d, 1H, H at quinoline C-5), 7.72 (t, 1H, H at quinoline C-6), 7.83 (t, 1H, H at quinoline C-7), 7.87 (d, 1H, H at quinoline C-8), 7.51 (d, 1H, aromatic H at C-2 of phenyl), 7.45 (d, 1H, aromatic H at C-6 of phenyl).

Ethyl 2-(2-(4-hydroxyphenyl) quinolin-4-yl) acetate, 4j: Yield 49%. IR (KBr): 3425 (OH str), 1666 (C=N str), 1442 (C=C str), 1164 (C-O str), 1743 (C=O str), 3070 cm^{-1} (C-H str); ^{1}H NMR ($CDCl_3$): δ 1.21 (t, 3H, CH_3 of ethyl ester), 4.13 (q, 2H, CH_2 of ethyl ester), 4.49 (s, 2H, adjacent to quinoline and ester group), 9.67 (broad s, 1H, OH on phenyl ring), 6.80 (d, 1H, H at quinoline C-3), 8.19 (d, 1H, H at quinoline C-5), 7.72 (t, 1H, H at quinoline C-6), 7.83 (t, 1H, H at quinoline C-7), 7.87 (d, 1H, H at quinoline C-8), 6.86 (d, 1H, aromatic H *ortho* to OH of phenyl), 7.70 (d, 1H, aromatic H *meta* to phenyl).

Ethyl 2-(2-(3-nitrophenyl) quinolin-4-yl) acetate, 4k: Yield 55%. IR (KBr): 1689 (C=O str), 1342 (NO_2 str), 1095 (C-O str), 3109 cm^{-1} (C-H str); ^{1}H NMR ($CDCl_3$): δ 1.21 (t, 3H, CH_3 of ethyl ester), 4.13 (q, 2H, CH_2 of ethyl ester), 8.19 (d, 1H, H at quinoline C-5), 7.72 (t, 1H, H at quinoline C-6), 7.83 (t, 1H, H at quinoline C-7), 7.87 (d, 1H, H at quinoline C-8), 6.80 (d, 1H, H at quinoline C-3), 8.32 (dd, 1H, H *ortho* to NO_2 group), 7.84 (dd, 1H, H *meta* to NO_2 group).

In silico evaluation

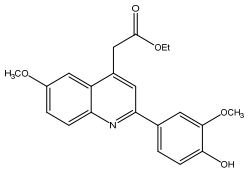
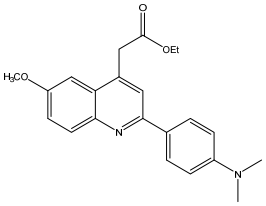
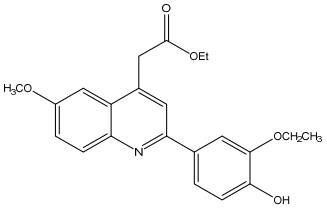
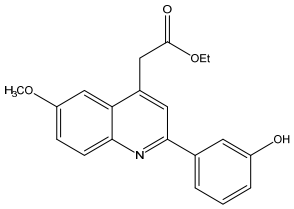
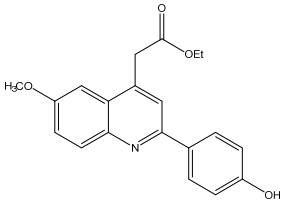
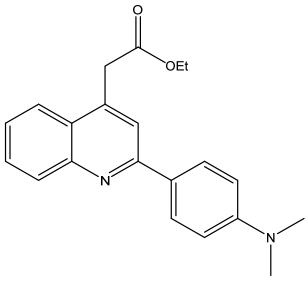
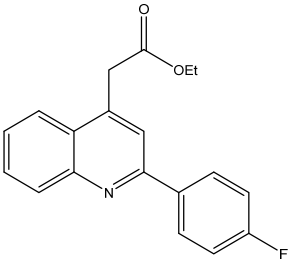
Molecular docking

The molecular docking was performed against DNA gyrase enzyme (**PDB Id**: **1KZN)** the results of the molecular docking are summarized in Table 1. Most of the synthesized compounds were deeply docked within the binding pocket region and hydrogen bonds conventional with Glu50, Ile78, Val167, Asp73, Asp49, Gly77, Pro79, Arg136, Thr165, Val120, Ile90, Asn46, Ala96, Ala47. The docking results showed that compounds **4d**, **4e**, **4g**, **4j** and **4k** (Fig. 1–5) showed much better docking score (-8.4, -8.0, -8.2, -7.8 and -7.9 kcal/mol respectively) than that of the standard drug Ciprofloxacin (-7.7 kcal/mol). The existence of electron-donating and electron-withdrawing groups has an important effect on the binding affinity of ligands to their target proteins, according to docking results in molecular modelling. Donating electrons group can increase the ligand's functional groups' nucleophilicity, increasing their reactivity with the target protein's electrophilic sites. Additionally, an electron withdrawing group might participate in hydrogen bonding, frequently forming strong bonds with amino acid residues that can greatly increase binding affinity. The docking result showed that presence of electron donating groups *viz* *m*-OH (**4d**) and *p*-OH (**4e, 4j**) and some electron withdrawing *p*-F and *m*-NO_2 (**4g** and **4k** respectively) increased the binding affinity of the compounds with protein and showed good activity.

ADMET profile of quinoline derivatives

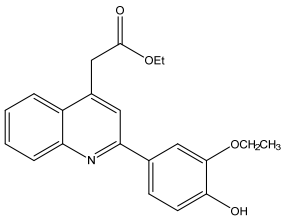
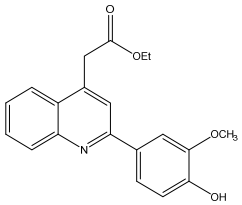
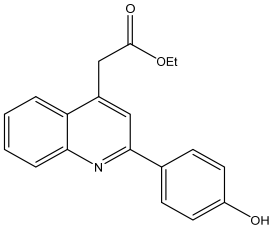
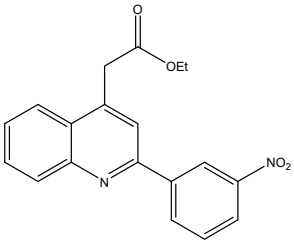
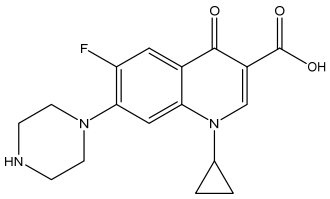
The ADMET properties of synthesized compounds were predicted by pKCSM webserver (Table 2). It is observed that these derivatives have the advantages of better intestinal absorption in human in range **96.39-**

Table 1 — Docking results of quinoline derivatives

Compd	Structure of Compd	Affinity (kcal/mol)	Amino acid interaction
4a		–7.3	Glu50, Thr165, Ile78, Val120, Ile90, Asn46, Ala96, Gly77, Arg76, Arg136, A73,
4b		–7.2	Val167, Val43, Asp73, Glu50, Arg76, Pro79, Ile90, Asn46, Ala47, Val71, Ile78, Gly77, Arg136
4c		–7.0	Asn46, Thr165, Ile78, Arg136, Pro79, Arg76, Val43, Asp49, Ile90, Gly77
4d		–8.4	Gly77, Ile75, Glu50, Asn46, Ile90, Ala47, Asp73, Pro79, Arg136, Arg76, Ile78,
4e		–8.0	Asn46, Gly77, Ile78, Glu50, Thr165, Val434, Val167, Arg76, Pro79, Ile90, Asp73, Ala47
4f		–7.1	Val167, Val43, Asn46, Arg76, Glu50, Ile78, Pro79, Arg136, Gly77, Ile90, Ala47, Val120, Pro79, Asp73,
4g		–8.2	Ile90, Asn46, Gly77, Ile78, Glu50, Asp73, Thr165, Ile90, Ala47

(*Contd.*)

Table 1 — Docking results of quinoline derivatives (*Contd.*)

Compd	Structure of Compd	Affinity (kcal/mol)	Amino acid interaction
4h		–7.3	Thr165, Ile78, Gly77, Glu50, Asn46, Ile90, Val120, Ala96, Arg136, Arg76, Pro79, Val93
4i		–7.0	Asn46, Glu50, Arg76, Arg136, Ile78, Pro79, Ile90, Asp49, Asp73, Gly77, Thr165
4j		–7.8	Gly77, Glu50, Asn46, Ile78, Val43, Thr165, Val167, Ala47, Ile90, Asp73
4k		–7.9	Val120, Asn46, Glu50, Ile78, Val167, Thr165, Ala47, Asp73, Gly77, Arg76, Ile90, Val71, Val43
Ciprofloxacin		–7.7	Pro79, Arg76, Ile78, Val43, Val120, Asn46, Val167, Ala47,, Glu50, Asp73, Thr165

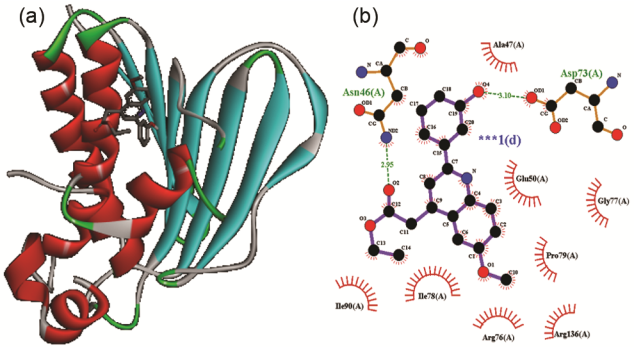


Fig. 1 — The 3D structure (a) of ethyl 2-(2-(3-hydroxyphenyl)-6-methoxyquinolin-4-yl) acetate (compound **4d**) docked with protein 1KZN, (b) compound **4d** displaying interactions with 1KZN

100. This benefit could be explained by the novel ligands' increased lipophilicity, which would facilitate their passage through the biological membranes. Consequently, upon oral administration, they could have a good bioavailability. The analysis of the BBB permeability revealed that the quinoline derivative **4g** displays the highest ability to penetrate the CNS (CNS permeability=0.251). Additionally, it is clear that all the new compounds, except **4e** and **4j**, could inhibit the main cytochrome involved in drug metabolism, cytochrome P3A. This may also attribute to the higher lipophilicity of our newly synthesized quinolines. Excretion was evaluated in the terms of total clearance, a parameter that is associated with

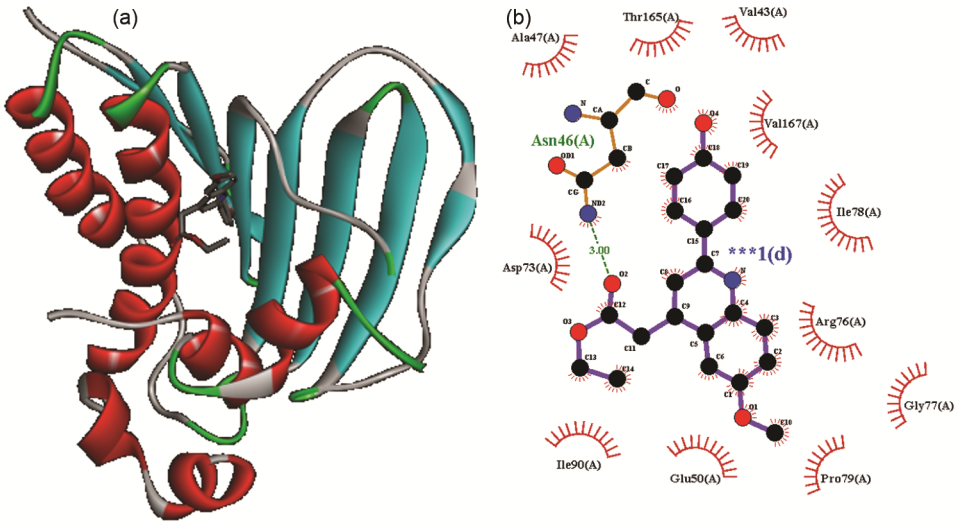


Fig. 2 — The 3D structure (a) of ethyl 2-(2-(4-hydroxyphenyl)-6-methoxyquinolin-4-yl) acetate (compound **4e**) docked with protein 1KZN, (b) compound **4e** displaying interactions with 1KZN

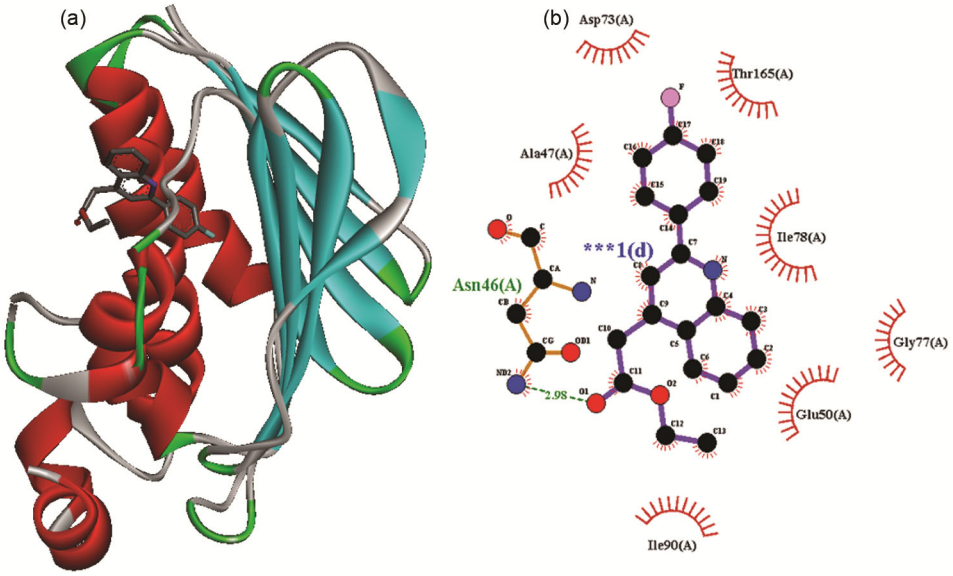


Fig. 3 — The 3D structure (a) of ethyl 2-(2-(4-fluorophenyl) quinolin-4-yl) acetate (**4g**) docked with protein 1KZN, (b) compound **4g** displaying interactions with 1KZN

bioavailability and is taken into account while deciding dose timing intervals. Observed data demonstrates that the quinoline derivatives **4b** and **4f** revealed the highest total clearance values (0.858 and 0.888, respectively). The toxicity profile of synthesized compounds was predicted by ProTox, a web-based platform (Table 3). It is observed that compounds **4c**, **4g** and **4i** showed no toxicity and are predicted to be relatively safe compounds. It is predicted that, out of all the derivatives, only **4a** showed cytotoxicity and **4d** showed mutagenicity. It is seen that neurotoxicity is more prevalent in the synthesized derivatives followed by nephrotoxicity.

In vitro antimicrobial activity evaluation

Agar diffusion method was used for the preliminary evaluation of antibacterial activity and results were listed as the average diameter of Zone of Inhibition (ZOI) of bacterial growth around the discs in millimetres. Triplicates are used in antimicrobial activity testing to ensure the reliability, accuracy, and reproducibility of the results. The antimicrobial studies were done against two gram-positive

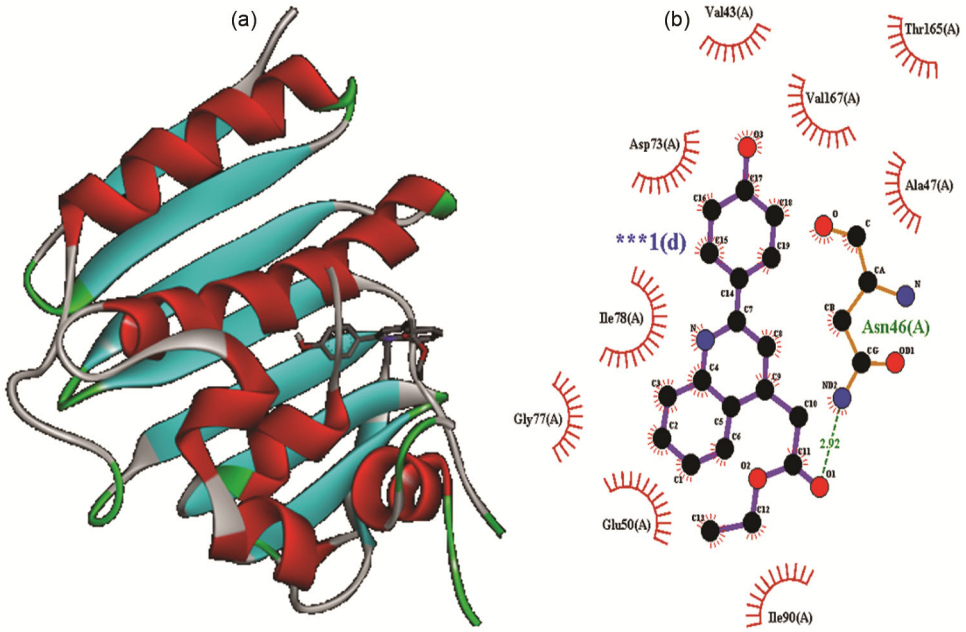


Fig. 4 — The 3D structure (a) of ethyl 2-(2-(4-hydroxyphenyl) quinolin-4-yl) acetate (**4j**) docked with protein 1KZN, (b) compound **4j** displaying interactions with 1KZN

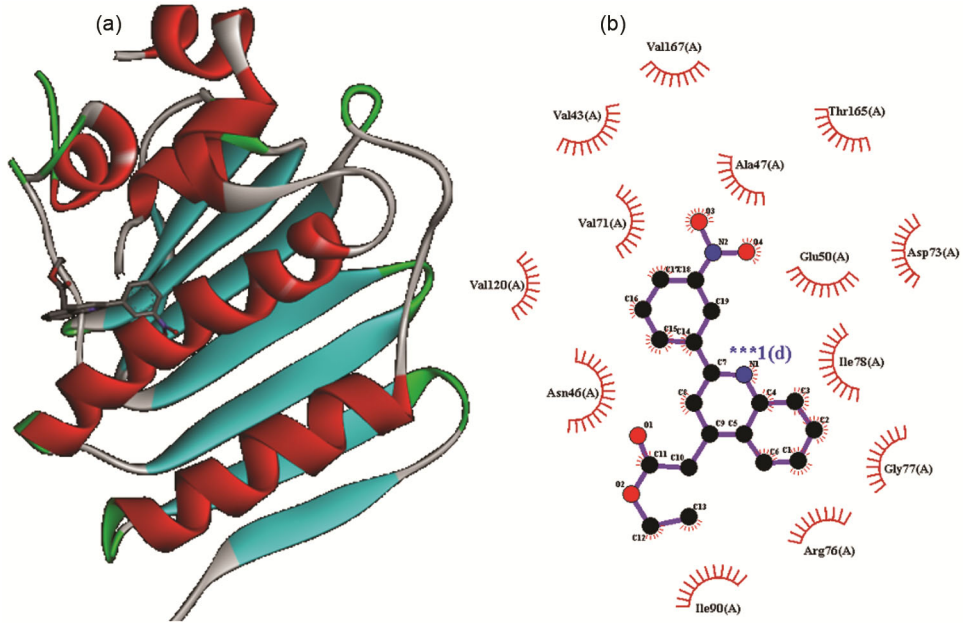


Fig. 5 — The 3D structure (a) of ethyl 2-(2-(3-nitrophenyl) quinolin-4-yl) acetate (**4k**) docked with protein 1KZN, (b) compound **4k** displaying interactions with 1KZN

(*Streptococcus pneumoniae, Bacillus subtilis*) and two Gram-negative organisms (*Pseudomonas aeruginosa, and E. coli)*. The antimicrobial activity of the synthesized compounds was compared with standard drug Ciprofloxacin (Table 4). Compound **4f** showed the highest activity against *S. pneumoniae* (20.8 ± 0.23 mm), nearly comparable to ciprofloxacin (21.0 ± 0.4 mm). Compound **4c** was the most active against *B. subtilis* (23.4 ± 0.15 mm), even surpassing ciprofloxacin (22.0 ± 0.8 mm). Compound **4j** exhibited the highest inhibition against *P. aeruginosa* (19.76 ± 0.9 mm), slightly lower than ciprofloxacin (20.0 ± 0.2 mm). Compound **4h** was the most effective against ***E. coli*** (23.9 ± 0.8 mm), higher than

Table 2 — ADME profile of synthesized compounds

	Absorption			Distribution	Metabolism		Excretion
Compd	$CaCO_2$ Permeability (log Papp in 10^{-6} cm/s)	HIA (% absorbed)	Water solubility (log m/L)	BBB (log BB)	CYP2D6	CYP3A4	Total clearance (l.og mL/min/kg)
4a	1.12	98.432	–4.803	–0.737	NO	YES	0.816
4b	1.061	100	–5.291	–0.336	NO	YES	0.858
4c	1.083	97.387	–5.127	–0.75	NO	YES	0.852
4d	1.063	98.365	–4.373	–0.416	NO	YES	0.657
4e	1.025	97.817	–4.45	–0.495	NO	NO	0.783
4f	1.055	100	–5.02	0.051	NO	YES	0.888
4g	1.834	98.799	–4.935	0.251	NO	YES	0.646
4h	1.068	96.39	–4.872	–0.494	NO	YES	0.673
4i	1.1.03	97.435	–4.597	–0.482	NO	YES	0.65
4j	1.311	96.82	–4.321	0.037	NO	NO	0.682
4k	0.782	97.923	–5.262	–0.71	NO	YES	0.769

Table 3 — Toxicity profile of the compounds

Compd	Nephrotoxicity	Neurotoxicity	Cardio-toxicity	Mutagenicity	Cytotoxicity
4a	No	Yes	No	No	Yes
4b	No	Yes	No	No	No
4c	No	No	No	No	No
4d	No	Yes	No	Yes	No
4e	Yes	No	No	No	No
4f	No	Yes	No	No	No
4g	No	No	No	No	No
4h	Yes	Yes	Yes	No	No
4i	No	No	No	No	No
4j	No	Yes	Yes	No	No
4k	Yes	No	Yes	No	No

Table 4 — Zone of inhibition of synthesized compounds against bacterial strains

	Zone of inhibition (mm)			
Compd	*Streptococcus pneumoniae*	*Bacillus subtilis*	*Pseudomonas aeruginosa*	*Escherichia coli*
4a	14.5±0.44	20.2±0.58	Nil	15.6±0.2
4b	18±0.46	Nil	17.8±0.02	17±0.6
4c	15.3±0.6	23.4±0.15	18±0.16	18.6±0.1
4d	16±0.23	17±0.2	15.3±0.98	15±0.8
4e	18.3±0.36	21.8±0.63	Nil	11.2±0.9
4f	20.8±0.23	20.4±0.63	Nil	10.8±0.4
4g	17±0.6	16.1±0.58	11.2±0.6	13±0.5
4h	Nil	17.9±0.3	18.5±0.9	23.9±0.8
4i	19±0.78	20.8±0.67	Nil	15.9±0.2
4j	18±0.93	14.9±0.31	19.76±0.9	Nil
4k	14±0.25	Nil	17.9±0.1	16.4±0.3
Ciprofloxacin	21±0.4	22±0.8	20±0.2	21±0.9

ciprofloxacin (21.0 ± 0.9 mm). Compounds **4c, 4d,** and **4g** demonstrate activity against all tested strains, indicating possible broad-spectrum potential. Some compounds like **4h** and **4e** showed no activity against certain strains (*S. pneumoniae* and *P. aeruginosa* respectively), indicating selective antibacterial behaviour. These results suggest that compound **4c** can be considered the most promising lead compound for further development due to its broad-spectrum efficacy.

Statistically evaluation

Two-way ANOVA was performed to analyse the antimicrobial activity of various compounds against

Table 5 — Post hoc analysis using Tukey's HSD test (comparison of each compound with reference compound, ciprofloxacin)

Comparison of ciprofloxacin with derivatives	Mean Difference	LCL (Lower Confidence Limit)	UCL (Upper Confidence Limit)	P-value	Statistically significant difference
4a	8.4	–10.7	27.6	0.9	No
4b	7.8	–11.3	26.9	0.9	No
4c	2.2	–16.9	21.3	1.0	No
4d	5.2	–13.9	24.3	0.9	No
4e	8.2	–10.9	27.3	0.9	No
4f	8.0	–11.1	27.1	0.9	No
4g	6.7	–12.5	25.8	0.9	No
4h	5.9	–13.2	25.1	0.9	No
4i	7.1	–12.1	26.2	0.9	No
4j	7.8	–11.3	26.9	0.9	No
4k	8.9	–10.2	28.1	0.9	No

four bacterial strains (*Streptococcus pneumoniae*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*). Two-way ANOVA analysis revealed that neither the tested compounds ($p = 0.891$) nor the bacterial strains ($p = 0.431$) had a statistically significant effect on the zone of inhibition. All test compounds performed similarly overall — statistically speaking, there's no standout compound and all bacterial strains responded similarly across treatments. High residual variability indicated experimental noise or uniform response across treatments. Post hoc analysis using Tukey's HSD test was applied to compare each compound with ciprofloxacin (Table 5). The test concludes that there is no statistically significant difference between ciprofloxacin and any of the tested compounds (p-value > 0.05). This means that the zone of inhibition for ciprofloxacin was similar to the other compounds tested, and no compound was found to be significantly better or worse than ciprofloxacin in terms of antibacterial activity. The negative lower confidence limit confirmed that the tested compounds are less effective than ciprofloxacin in inhibiting the bacteria. A Pearson's product-moment correlation (Pearson's $r = -0.041$) study concluded that there is no significant (p-value = 0.9) correlation between molecular docking score and mean ZOI values.

Conclusion

The current study highlights the successful synthesis and antimicrobial screening of quinoline derivatives using a multicomponent reaction involving aniline derivatives, aryl aldehydes, and β-keto esters under acidic conditions. The compounds showing significant antimicrobial activity, supported by favourable molecular docking results, (compounds **4d, 4e, 4g, 4j** and **4k**) showed much better binding affinity than that of the standard drug ciprofloxacin. The computational prediction of absorption, distribution, metabolism, excretion and toxicity also gave encouraging results. The toxicity profile of synthesized compounds indicated that compounds **4c, 4g** and **4i** showed no toxicity. *In vitro* antibacterial evaluation of synthesized derivatives led to the fact that there is no statistically significant difference between ciprofloxacin and any of the tested compounds. The compounds showing significant antimicrobial activity, supported by favourable molecular docking results, offer strong leads for further drug development. Future research can focus on modifying the substitution pattern on the aromatic ring and exploring electron-donating or withdrawing groups to optimize biological activity.

Supplementary Information

Supplementary information is available in the website http://nopr.niscpr.res.in/handle/123456789/58776.

Acknowledgements

The authors are thankful to Kurukshetra University, Kurukshetra, India for providing the necessary facilities to carry out this work.

References

1 Mahajan A & Chundawat T S, *Mini Rev Org Chem*, 16 (2019) 631.
2 Yadav P & Shah K, *Bioorg Chem*, 109 (2021) 104639.
3 Hariyanti H, Mauludin R, Sumirtapura Y C & Kurniati N F, *Bioint Res Appl Chem,* 13 (2022) 319.
4 Wen X, Wang S B, Liu D C, Gong G H & Quan Z S, *Med Chem Res,* 24 (2015) 2591.

5 Shaikh S F, Dhavan P P, Singh P R, Vaidya S P, Jadhav B L & Ramana M M V, *Russian J Bioorg Chem*, 47 (2021) 572.
6 Murugavel S, Sundramoorthy S, Subashini R & Pavan P, *Struct Chem*, 29 (2018) 1677.
7 Desai N C, Patel B Y & Dave BP, *Med Chem Res,* 26 (2017) 109.
8 Nqoro X, Tobeka N & Aderibigbe B, *Molecules,* 22 (2017) 2268.
9 Soares R R, Da Silva J M F, Carlos B C, Da Fonseca C C, De Souza L S A, Lopes F V, de Paula D R M, Moreira P O L, Abramo C, Viana G H R, De Pila V F, Silva A D D & Scopel K K G, *Bioorg Med Chem Lett,* 25 (2015) 2308.
10 Ibrahim D A, Abou El Ella D A, El-Motwally A M & Aly R M, *Eur J Med Chem*, 102 (2015) 115.
11 Guan Y F, Liu X J, Yuan X Y, Liu W B, Li Y R, Yu G X, Tian X Y, Zhang Y B, Song J, Li W & Zhang S Y, *Molecules*, 26 (2021) 4899.
12 El Shehry M F, Ghorab M M, Abbas S Y, Fayed E A, Shedid S A & Ammar Y A, *Eur J Med Chem*, 143 (2018) 1463.
13 Nayak N, Ramprasad J, Dalimba U, *J Fluor Chem*, 183 (2016) 59.
14 Sun N, Du R L, Zheng Y Y, Huang B H, Guo Q, Zhang R F, Wong K Y & Lu Y J, *Eur J Med Chem*, 135 (2017) 1.
15 Marston H D, Dixon D M, Knisely J M, Palmore T N & Fauci A S. *JAMA,* 316 (2016) 1193.
16 Parish T, *Expert Opin Drug Disc,* 14 (2019) 91.
17 Origin Lab Corporation, Origin Pro (Version 2025). Northampton, M A, USA: Origin Lab Corporation; 2025.
18 JASP Team, JASP, (Version 0.19.3, Computer Software) 2025,