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Synthesis and biological evaluation of novel isoxazole derivatives from acridone

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Abstract

The present study was carried out in an attempt to synthesize a new class of potential antibacterial agents. In this context, novel isoxazoles were synthesized and evaluated for their potential antibacterial behavior against four pathogenic bacterial strains. The synthesized compounds exhibited moderate-to-good antibacterial activity against these strains. The highest antibacterial activity was observed against the *Escherichia coli* strains, particularly for compounds **4a** and **4e** with phenyl and *para*-nitrophenyl groups on the isoxazole–acridone skeleton; they showed promising minimum inhibitory concentration values of 16.88 and 19.01 μg/ml, respectively, compared with the standard drug chloramphenicol (22.41 μg/ml). The synthesized compounds were subjected to in silico docking studies to understand the mode of their interactions with the DNA topoisomerase complex (PDB ID: 3FV5) of *E. coli*. The molecular docking results showed that compounds **4a–l** occupy the active site of DNA topoisomerase (PDB ID: 3FV5), stabilized via hydrogen bonding and hydrophobic interactions, which may be the reason behind their interesting in vitro antibacterial activity.

KEYWORDS
acridone, antibacterial activity, isoxazole, molecular docking

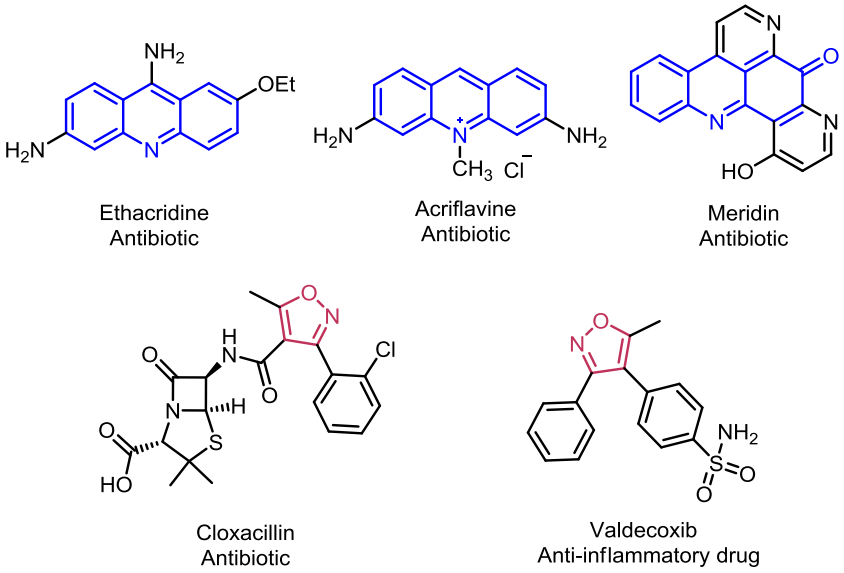
1 | INTRODUCTION

Isoxazole derivatives play an interesting role in the development of heterocyclic chemistry; they are widely used as intermediates in organic synthesis.[1] In the quest for a new antibacterial agent, we found isoxazoles core to be a privileged structure in modern medicinal chemistry, possessing a wide range of pharmacological properties like antimicrobial,[2] anticancer,[3] anti-HIV,[4] antituberculosis,[5] and anti-inflammatory[6] activities. For instance, muscimol extracted from *Amanita muscaria* is an agonist of γ-aminobutyric acid A (GABA-A) receptor, which plays a role in regulating neuronal excitability in the central nervous system.[7] Also, some isoxazole derivatives form the basis for numerous drugs, such as cloxalin and oxacillin (antibiotics), valdecoxib (COX-2 inhibitor), and zonisamide (anti-convulsant)[8] (Figure 1). Several methods have been described for the preparation of these heterocycles; the 1,3-dipolar cycloaddition of alkenes and alkynes with nitrile oxides is the most widely used method.

In contrast, acridone derivatives have attracted considerable attention from medicinal and organic chemists due to their considerable pharmacological activities.[9–13] The flatness of the polycyclic aromatic nucleus in these structures generally allows them easy intercalation between the adjacent base pairs of the double helix of DNA. This interesting property is the cornerstone of some biological activities of these molecules.[14] Many acridone derivatives have shown interesting anticancer and antitumor activities by apoptosis induction,[15] inhibition of telomerase,[16] or intercalation in DNA.[17] Moreover, research studies have led to the preparation of acridone-based molecules, which have antimicrobial potential, among these antibiotics, ethacridine, acriflavine, or meridin[18] (Figure 1).

Based on the above-mentioned findings and inspired by the potential antibacterial activity of both acridones and isoxazoles,

FIGURE 1 Acridones and isoxazoles used as pharmaceutical agents

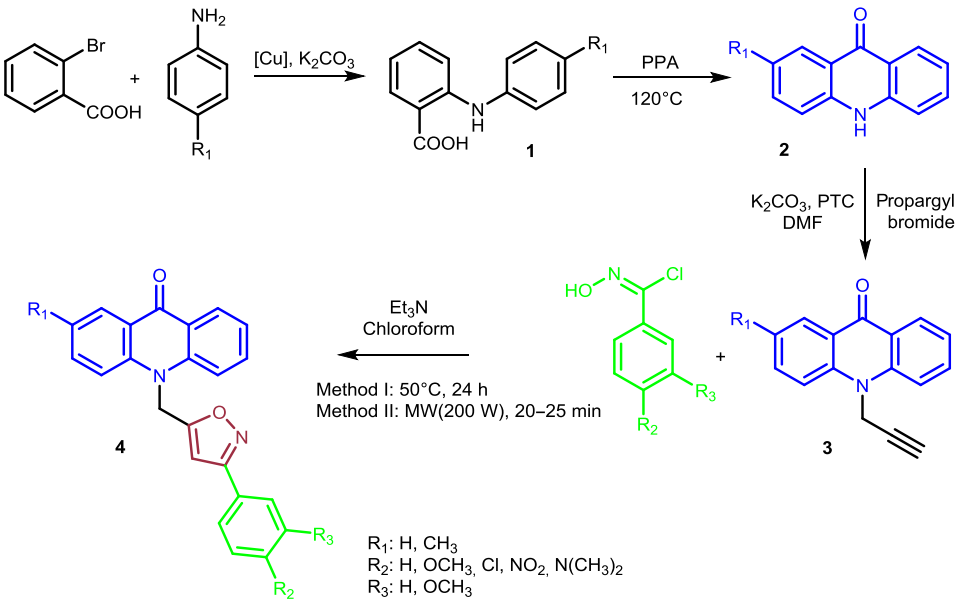


to develop novel bioactive therapeutic agents, we have focused our attention toward the preparation of novel isoxazole derivatives from acridone using friendly environmental methods. The adopted strategy for the preparation of these molecules consisted of combining these two potential pharmacophores, acridone/isoxazole, using the 1,3-dipolar cycloaddition reaction between nitrile oxides and *N*-propargyl acridone. Reactions have been conducted using microwave-assisted synthesis. The newly synthesized compounds have been then investigated for their potential antibacterial activities against four pathogenic bacteria. Finally, the molecular docking study has been applied to explain and identify the possible modes of actions between the synthesized compounds and the studied receptor.

2 | RESULTS AND DISCUSSIONS

2.1 | Chemistry

By the strategy briefly depicted in Scheme 1, we have synthesized novel isoxazoles based on acridone via 1,3-dipolar cycloaddition reaction between *N*-propargyl acridones and various nitrile oxides.



SCHEME 1 Synthesis of isoxazole derivatives from acridone

First, acridone was prepared by Ullmann condensation of *o*-bromobenzoic acid with *p*-toluidine or aniline in the presence of potassium carbonate and copper in isoamyl alcohol at reflux to produce 2-arylamino benzoic acids,[19,20] which was then cyclized with polyphosphoric acid at 120°C during 4 h to produce compounds **2a**,**b**. The propargylation of the acridone ring was attempted by refluxing excess of propargyl bromide with acridone derivatives in the presence of anhydrous potassium carbonate as base and tetra-*n*-butylammonium bromide (TBAB) as catalyst (PTC) in dimethylformamide (DMF) at 70°C.

The generation of the nitrile oxides used as dipoles was carried out "in situ" from *N*-hydroxybenzimidoyl chlorides in a basic medium. A simple synthetic strategy was used in the preparation of the *N*-hydroxybenzimidoyl chlorides. First, the aldehydes were used as precursors to generate the aldoximes,[21] then the prepared aldoximes were transformed to the corresponding *N*-hydroxybenzimidoyl chlorides with *N*-chlorosuccinimide in the presence of triethylamine in chloroform at room temperature (Scheme 2).[22]

The last step was the 1,3-dipolar cycloaddition reaction between appropriately substituted nitrile oxides and alkynes. Accordingly, different nitrile oxides and *N*-propargyl acridones were reacted in the presence of Et_3N in chloroform at 50°C for 6 h to give the 3,5-disubstituted isoxazoles (**4**) in good yields.

In the view, to obtain these isoxazoles with excellent yield and shorter reaction times, the 1,3-dipolar cycloaddition reaction was carried out under microwave irradiation. The results indicated in Table 1 show a strong acceleration of the cycloaddition reaction under microwave irradiation, which was noticed in comparison to conventional conditions that required 6–24 h of agitation rather than 20–25 min under microwave irradiation. The regioselectivity of the 1,3-dipolar cycloaddition reaction was established as no 3,4-disubstituted isoxazole regioisomers were observed. Moreover, electron-donating, as well as electron-withdrawing substituents on *N*-hydroxybenzimidoyl chlorides, gave similar results, except in the case of compounds **4e** and **4f**, we obtained mediocre yields.

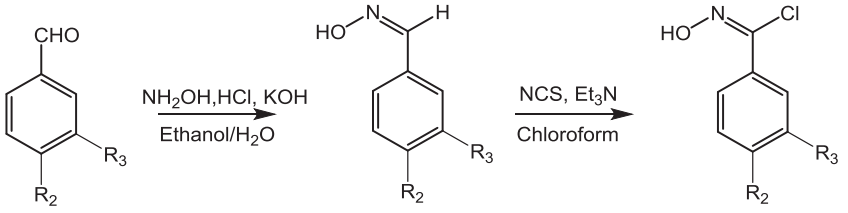
The structures of all the 3,5-disubstituted isoxazoles (**4a–l**) synthesized using the synthetic protocol described above, and were fully characterized by ^1H and ^{13}C nuclear magnetic resonance (NMR), infrared (IR), and high-resolution mass spectrometry (HRMS) analysis. The Fourier-transform infrared spectra of isoxazoles (**4a–l**) showed characteristic absorption bands in the region of 1600–1610 cm^{-1} assigned to the bond (C=N) of the isoxazole nucleus and the characteristic bands of the acridone ring were detected in the range 1640–1635 cm^{-1} corresponding to C=O bond. In contrast, the disappearance of the vibration bonds of the alkyne group in the region of 2110 and 3210 cm^{-1} confirmed the formation of the final products.

^1H NMR spectra of compounds **4a–l** were confirmed by the number of protons and the value of the chemical displacement in ppm. The analysis of the spectra of isoxazoles **4a–l** in DMSO-d_6 or $CDCl_3$ reveals the formation of 3,5-disubstituted isoxazole. In addition, ^1H NMR spectra showed a singlet at 6.08–5.65 ppm attributable to the proton of the methylene group and signals in the aromatic region 8.63–6.67 ppm relative to the aromatic protons. These structures were further supported by ^{13}C and distortionless enhancement by polarization transfer NMR spectra, the quaternary carbon of the isoxazole nucleus reveals a signal between 170 and 168 ppm, thus proving its proximity to the oxygen atom. Similarly, the isoxazolic carbon C–H showed a signal around 100 ppm; this signal would have been deshielded in the case of the 3,4-disubstituted isoxazole.

2.2 | Antibacterial activity

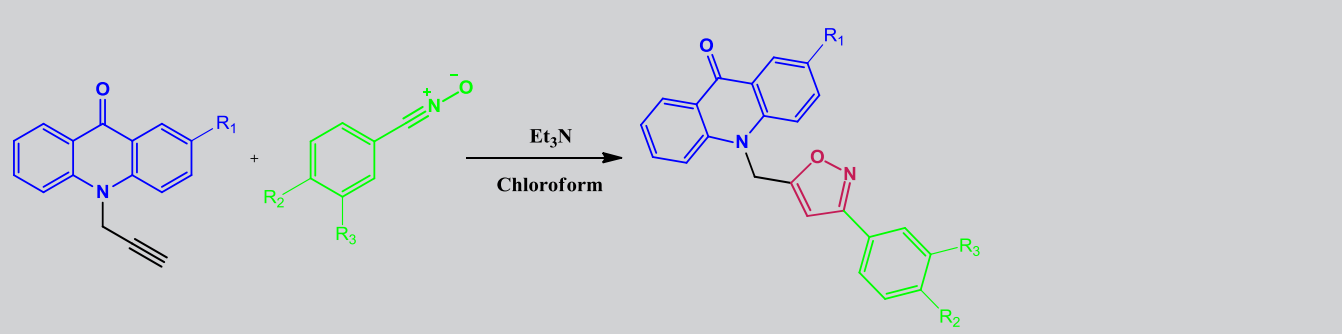
The novel isoxazole derivatives from acridone (**4a–l**) and compounds **2a**,**b** and **3a**,**b** were tested in vitro for their antibacterial activity and compared with chloramphenicol. The investigated bacterial strains involve three Gram-negative bacteria *Pseudomonas putida*, *Klebsiella pneumoniae*, *Escherichia coli*, and one Gram-positive bacterium *Staphyloccocus aureus*. The antibacterial activity has been primarily tested as the observed growth inhibition zones by the disk-diffusion method using Mueller–Hinton broth (MHB) medium. Then, minimum inhibitory concentrations (MICs) were determined for the synthesized compounds.

The test compounds exhibited a varying range of antibacterial activities against both Gram-positive as well as Gram-negative bacteria, as is obvious from the MIC values presented in Table 2. The highest antibacterial activity was obtained against *E. coli* strains. Compounds **4a** and **4e** with phenyl and *para*-nitrophenyl groups on the isoxazole–acridone skeleton showed the best antibacterial activity against *E. coli* with MIC values 16.88 and 19.01 μg/ml, respectively, compared with the standard drug chloramphenicol (22.41 μg/ml). Whereas compound **4d** with hydrogen at C-2 on the acridone ring and chloro at *para* position on the acridone–isoxazole phenyl moiety, showed very good activity against *E. coli* strains (22.39 μg/ml), which is very close to that obtained with the commercial antibiotic chloramphenicol (22.41 μg/ml). Moreover, compound **4j** with methyl at C-2 on the acridone ring and chloro at *para* position on the acridone–isoxazole phenyl moiety was found to have good



SCHEME 2 Thesis of *N*-hydroxybenzimidoyl chlorides

TABLE 1 Synthesized compounds **4a-l**



Compounds	R1	R2	R3	Yield (%)[a]	Yield (%)[b]
4a	H	H	H	76	84
4b		OMe	H	85	80
4c		OH	OMe	45	50
4d		Cl	H	69	70
4e		NO_2	H	63	60
4f		$N(CH_3)_2$	H	55	56
4g	CH_3	H	H	68	77
4h		OMe	H	80	79
4i		OH	OMe	40	45
4j		Cl	H	70	73
4k		NO_2	H	66	65
4l		$N(CH_3)_2$	H	58	63

Abbreviation: TEA, triethylamine.

[a]Reaction conditions: alkynes (1 mmol), *N*-hydroxybenzimidoyl chlorides (1.2 mmol), TEA (1.2 mmol), chloroform (5 ml) at 50°C.

[b]Reaction conditions: Reaction carried out under microwave irradiation.

antibacterial activity against *S. aureus* with MIC = 28.39 μg/ml. In addition, the compound **4l** exhibited high antibacterial potential against *P. putida* (46.85 μg/ml), which is close to that obtained with the commercial antibiotic chloramphenicol (37.03 μg/ml).

The results of the antibacterial activity expressed as MICs against the tested Gram-positive and -negative pathogens indicated that there is no significant difference in the antibacterial activity between the acridones and *N*-propargyl acridones against all bacteria. However, the introduction of the isoxazole moiety increased the antibacterial activity against these bacteria. Moreover, the results of the antibacterial activity showed that *E. coli* is more susceptible to the synthesized compounds **4a**–**l**. Concerning the effect of the substituent on the phenyl moiety of the acridone–isoxazole skeleton, the results revealed that the best MIC against *E. coli* had been achieved by the unsubstituted phenyl (**4a**) as well as those having NO_2 and Cl groups at *para* position on the phenyl group (**4d**, **4e**; Figure 2). While the presence of the methyl group on the acridone moiety and the substitution of the phenyl group decreased the antibacterial activity against *E. coli*.

2.3 | Molecular docking

In silico docking studies were performed to better understand as well as to support the in vitro antibacterial activity of the synthesized compounds for the rational design of new potent antibacterial molecules. In the current study, the most active synthesized isoxazole derivatives have been docked into the binding site of the DNA topoisomerase complex (PDB ID: 3FV5) of *E. coli*[23] to investigate the different types of interactions and clarify their probable binding modes. DNA topoisomerase has been selected for the current study because the topoisomerases are the target of many antibiotics, in addition, the acridone derivatives are known by their potential topoisomerase inhibitors.[24–26]

To validate the applied molecular docking approach, the co-crystallized ligand (1UE) was docked into the active site of the DNA topoisomerase to determine the root mean square (RMS) distance that was satisfactory with root mean square deviation (RMSD) = 0.92 Å (<2 Å). Figure S1 shows that the docked structure (red color) and the X-ray crystal structure (green color) are quite similar.

TABLE 2 Antibacterial data for the synthesized compounds

	Staphylococcus aureus	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas putida*
2a	122.83	133.41	137.93	156.31
2b	118.43	124.22	130.43	145.52
3a	97.10	80.66	97.25	100.95
3c	83.20	70.14	102.20	115.20
4a	52.13	16.88	69.77	90.91
4b	56.60	37.62	82.57	95.02
4c	58.50	38.60	80.41	90.01
4d	30.62	22.39	74.07	122.81
4e	38.46	19.01	86.76	90.91
4f	38.46	38.46	69.77	111.11
4g	47.62	31.62	78.34	90.91
4h	33.82	42.13	77.49	99.10
4i	40.62	45.32	80.66	102.15
4j	28.39	29.13	74.07	56.60
4k	43.06	29.13	90.91	74.07
4l	43.06	33.06	95.02	46.85
Chloramphenicol	11.65	22.41	15.38	37.03
DMSO	–	–	–	–

Abbreviation: DMSO, dimethyl sulfoxide.

In addition, all the 12 isoxazoles were docked into the binding pocket of the DNA topoisomerase enzyme successfully. The molecular docking representation for each synthetic compound and the superposition of all best docking poses in the enzyme-binding pocket are shown in Figures S2–S13. From the analysis of the active site of the DNA topoisomerase enzyme, it was observed that all the synthesized compounds **4a–l** fit snugly, making various close contacts with the residues lining the active site of DNA topoisomerase; the interacting amino acids of all compounds with DNA topoisomerase enzyme are shown in Table 3.

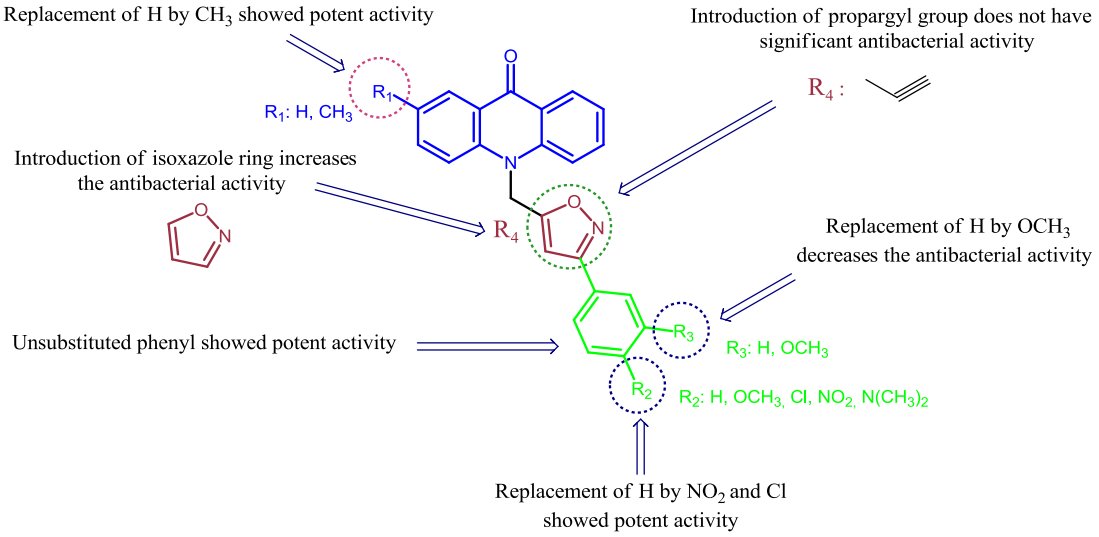


FIGURE 2 Structure–activity relationship of the novel isoxazole derivatives from acridone against *Escherichia coli*

TABLE 3 The interactions and binding affinities between the synthesized compounds **4a–l** and DNA topoisomerase enzyme of *Escherichia coli*

Compound	Interaction with nucleic acid	Hydrogen bonds	Binding affinity
4a	Arg132, Arg72, Glu46, Asn42, Gly73, Met74, Pro75, Ile90	Arg132	−6.86
4b	Gly73, Arg132, Arg72, Glu46, Asn42, Met74, Pro75, Ile90	–	−6.39
4c	Gly73, Arg72, Glu46, Asn42, Met74, Pro75, Ile90, Ile116	Gly73	−6.66
4d	Arg132, Arg72, Glu46, Asn42, Gly73, Met74, Pro75, Ile90	Arg132	−6.76
4e	Arg132, Arg72, Glu46, Asn42, Gly73, Met74, Pro75, Ile90	Arg132	−6.78
4f	Gly73, Arg132, Arg72, Glu46, Asn42, Met74, Pro75, Ile90	Gly73	−6.72
4g	Gly73, Arg72, Glu46, Asn42, Met74, Pro75, Ala49	–	−6.85
4h	Gly73, Ile116, Arg72, Glu46, Asn42, Met74, Pro75, Ile90, Arg93	Gly73, Ile116	−6.85
4i	Gly73, Arg72, Glu46, Asn42, Met74, Pro75, Ile90	Gly73	−6.81
4j	Gly73, Arg72, Glu46, Asn42, Met74, Pro75, Asp45	Gly73	−6.70
4k	Arg132, Arg72, Glu46, Asn42, Gly73, Met74, Pro75, Ala86, Thr163, Asp69	–	−6.46
4l	Gly73, Arg72, Glu46, Asn42, Met74, Pro75, Ile90, Arg93	Gly73	−6.85

The stable poses of the most active compounds **4a**, **4d**, and **4e** show one favorable hydrogen bond between the nitro group of isoxazole moiety and the hydrogen of the side chain of Arg132. The phenyl group shows hydrophobic interactions with Pro75, Arg72, and Arg132 (Figure 3), while the acridone moiety forms hydrophobic contacts with Met74, Pro75, Ile90, and Gly73. Also, compounds **4c**, **4g**, **4j**, and **4l** showed one favorable hydrogen bond between the nitro group of isoxazole moiety and the hydrogen of the side chain of Gly73, and hydrophobic interactions with the residues Pro75, Arg72 and Arg132, Met74, Pro75, and Ile90 (Figure 4). From the different interactions of the synthesized compounds depicted in Table 3 and Figures S2–S13, it can be concluded from the docking results that the most active compounds **4a**, **4d**, and **4e** form H-bond interaction with the hydrogen of the side chain of Arg132 in the active site of the DNA topoisomerase. Additionally, all synthesized compounds **4a–l** bind to the active site of the DNA topoisomerase and largely share homogeneous binding mode, especially with Pro75, Arg72, Gly73 Arg132, Met74, Pro75, and Ile90 to several DNA topoisomerase inhibitors reported in the literature.[27,28] Therefore, that can prove our docking process. The high docking scores and binding pattern reveal that these compounds are well accommodated in the active site of the enzyme and they strongly interact with the residue of the active site of the DNA topoisomerase enzyme. Hence, docking studies revealed the strong binding affinity of **4a** at the active site of DNA topoisomerase, which may be responsible for its significant in vitro antibacterial activity, especially against *E. coli*.

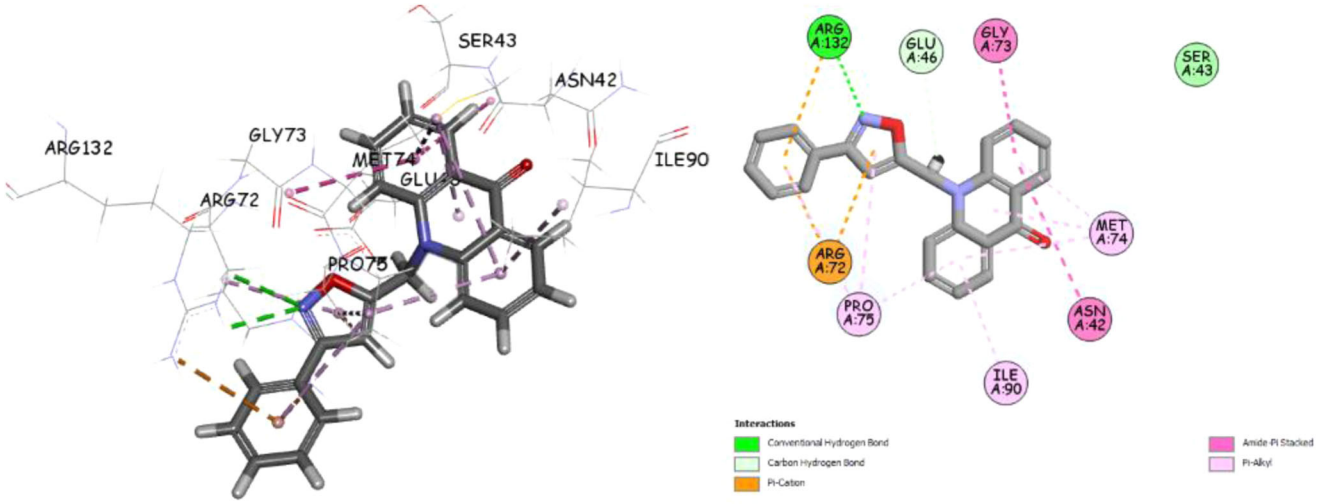


FIGURE 3 Binding mode of compound **4a** with DNA topoisomerase enzyme of *Escherichia coli*

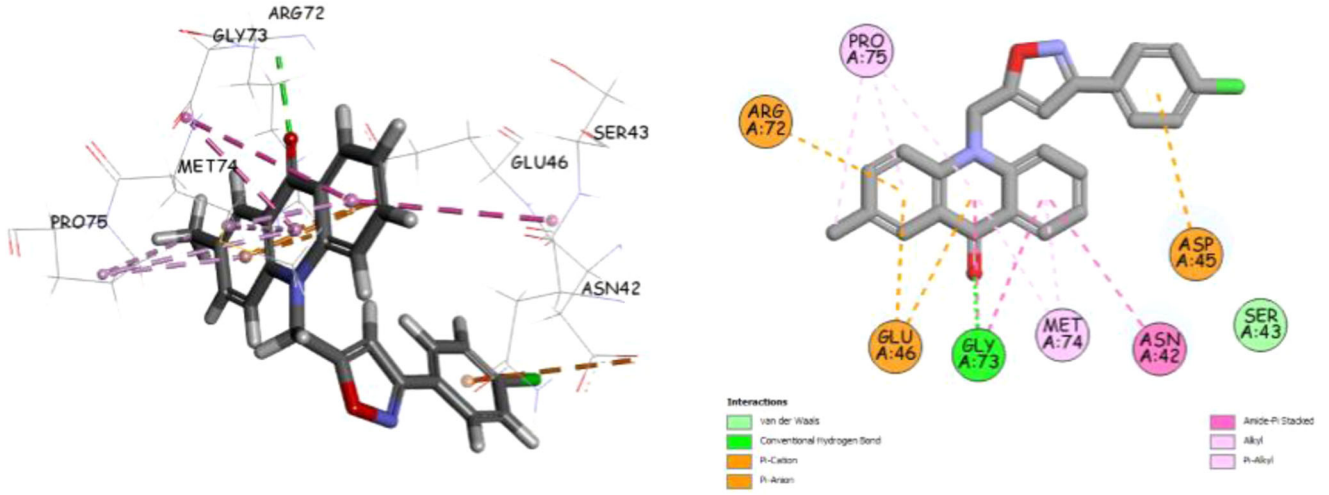


FIGURE 4 Binding mode of compound **4j** with DNA topoisomerase enzyme of *Escherichia coli*

2.4 | Prediction of toxicity

The estimation of the toxicity profile for the novel synthesized compounds **4a**–**l** is a very essential step in the design of new drug candidates. Generally, in vitro and in vivo tests are carried out to study drug safety, including a variety of adverse drug effects and toxicities. The in silico toxicity prediction is commonly applied to afford a preliminary and fast screening before further in vivo evaluation. There are numerous online tools using in silico models to access toxicity.[29,30] In this context, the toxicity profile of the synthesized compounds **4a**–**l** was evaluated using ToxPredict online tools,[31] this program predicts the probability of immunotoxicity, hepatotoxicity, carcinogenicity, and cytotoxicity, as well as the probability of binding with different biological receptors. In this study, the synthesized compounds **4a**–**l** were predicted to have moderate carcinogenicity and low hepatotoxicity and cytotoxicity profiles (Table S1). In addition, compounds **4a**–**l** show slight toxicity with LD_{50} values from 486 to 2561 mg/kg (Table S1), classifying them in categories 4 and 5 in the globally harmonized system of classification and labeling of chemicals categories.[32] These in silico studies demonstrated that compounds **4a**–**l** apparently do not have potential toxicity.

3 | CONCLUSION

In summary, novel isoxazole derivatives from acridone were synthesized, characterized, and biologically evaluated. New compounds **4a**–**l** were straightforwardly synthesized by 1,3-dipolar cycloaddition reaction between the appropriately substituted nitrile oxides and the *N*-proprayl acridones, the chemical structures of these compounds were assured by their spectral measurements (^{1}H NMR, ^{13}C NMR, IR, and HRMS). The newly synthesized compounds were screened for their antibacterial activities against four pathogenic strains, consequently, the compounds **4a**, **4d**, and **4e** were found to be the most potent agents compared with the reference drug. To predict the different modes and the stability of studied compounds into the receptor pocket responsible for antibacterial activity, molecular docking studies on DNA topoisomerase (PDB ID: 3FV5) were performed. The results showed that the occupation mode of compounds **4a**–**l** at the active site of DNA topoisomerase *E. coli* receptor (PDB ID: 3FV5), which presented hydrogen bonding and hydrophobic interactions, may be the reason behind their interesting in vitro antibacterial activity.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General remarks

All materials were purchased from commercial suppliers. The ^{1}H, ^{13}C NMR spectra were recorded with Bruker Avance 300. Mass spectrometric measurements were recorded using Exactive™ Plus Orbitrap mass spectrometer. IR spectra were recorded using JASCO FT-IR 4100 spectrophotometer. Microwave irradiation was carried out with CEM Discover™.

The InChI codes of the investigated compounds, together with some biological activity data, are provided as Supporting Information.

4.1.2 | Synthesis of 2-methyl-10-(prop-2-yn-1-yl)-acridone (3)

To a mixture of 2-methylacridone 1 (1.1 g, 5 mmol), potassium carbonate (1 g, 7.2 mmol) and TBAB (1 g, 4 mmol) in DMF (7 ml),

propargyl bromide (0.8 g, 7.5 mmol) was added and the mixture was stirred at room temperature for 6 h. After that, it was poured into water and the formed white yellow precipitate was recrystallized from methanol–DMF. White yellow solid; yield: 75%, mp = 206°C. IR (KBr): 3208 (m) (C–H), 3010 (m) (C–H), 2910 (m) (C–H), 1638 (s) (C═O), 1598 (s) (C═C) cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, 25°C, tetramethylsilane [TMS]): δ = 8.34 (d, J = 7.8 Hz, 1H, Ar–H), 8.12 (s, 1H, Ar–H), 7.87–7.86 (m, 4H, Ar–H), 7.37–7.38 (m, 2H, Ar–H), 5.32 (s, 2H, CH_2), 2.41 (s, 1H, CH), 2,38 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6, 25°C, TMS) δ 177.18, 141.78, 134.76, 134.47, 127.78, 127.07, 123.37, 122.20, 118.44, 116.35, 79.12, 76.16, 36.12, 20.18.

4.1.3 | General procedure for the synthesis of the acridon–isoxazole derivatives 4a–l

To a solution of *N*-hydroxybenzimidoyl chloride (0.5 g, 3.2 mmol) in chloroform (10 ml), alkyne (0.58 g, 2.5 mmol), and TEA (0.32 g, 3.2 mmol) were added at room temperature, the reaction mixture was stirred at 50°C for 6 h. Then, water (30 ml) was added and the mixture was extracted with chloroform, the organic layer was evaporated in high vacuum, and the obtained product was purified by flash chromatography on silica gel using hexane/diethyl ether (1:4).

Microwave-assisted procedure

A mixture of *N*-hydroxybenzimidoyl chloride (0.5 g, 3.2 mmol) and TEA (0.32 g, 3.2 mmol) was suspended in 10 ml of solvent in a glass vial equipped with a small magnetic stirring bar. To this, alkyne (0.58 g, 2.5 mmol) was added and the vial was tightly sealed. The mixture was then irradiated for 25 min at a fixed temperature (40–70°C). Microwave irradiation power was set at 200 W maximum. After completion of the reaction, water (30 ml) was added and the mixture was extracted with chloroform, the organic layer was evaporated in high vacuum, and the obtained product was purified by flash chromatography on silica gel using hexane/diethyl ether (1:4).

*10-[(3-Phenylisoxazol-5-yl)methyl)]acridin-9-one (**4a**)*

White solid; yield: 84%, mp = 220°C. IR (KBr): 3010 (m), 2974 (m) (C–H), 1640 (s) (C═O), 1617 (m) (C═N), 1596 (s) (C═C) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, 25°C, TMS): 8.62 (dd, J = 8.0, 1.7 Hz, 2H, Ar–H), 7.76 (td, J = 8.7, 7.0, 1.8 Hz, 2H, Ar–H), 7.71–7.64 (m, 2H, Ar–H), 7.47 (d, J = 8.6 Hz, 2H, Ar–H), 7.47–7.32 (m, 5H, Ar–H), 6.36 (s, 1H, CH-isoxazole), 5.71 (s, 2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$, 25°C, TMS): 177.98, 167.89, 161.97, 141.85, 136.47, 134.42, 129.23, 128.19, 128.10, 126.72, 122.85, 122.23, 114.32, 100.92, 43.56. HRMS electrospray ionization (ESI) *m/z* $[M+H]^+$ calcd. for $C_{23}H_{16}N_2O_2$: 353.1241, found: 353.1249.

*10-{[3-(4-Methoxyphenyl)-1,2-oxazol-5-yl]methyl}acridin-9-one (**4b**)*

White solid; yield: 80%, mp = 264°C. IR (KBr): 3011 (m) (C–H), 2979 (w) (C–H), 1639 (s) (C═O), 1616 (m) (C═N), 1598 (s) (C═C) cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, 25°C, TMS): 8.41 (dd, J = 8.0, 1.5 Hz, 2H, Ar–H), 7.93–7.81 (m, 4H, Ar–H), 7.80–7.72 (m, 2H, Ar–H), 7.40 (td, J = 7.9, 6.2, 1.6 Hz, 2H, Ar–H), 7.05–6.98 (m, 2H, Ar–H), 6.96 (s, 1H, CH-isoxazole), 6.03 (s, 2H, CH_2), 3.79 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6, 25°C, TMS): 176.70, 168.23, 161.61, 160.75, 141.77, 134.37, 128.13, 126.74, 121.85, 121.81, 120.45, 115.98, 114.39, 100.86, 55.25, 42.20. HRMS (ESI) *m/z* $[M+H]^+$ calcd. for $C_{24}H_{18}N_2O_3$: 383.1367, found: 383.1367.

*10-{[3-(4-Hydroxy-3-methoxyphenyl)-1,2-oxazol-5-yl]methyl}-acridin-9-one (**4c**)*

Yellow solid; yield: 45%, mp = 210°C. IR (KBr): 3410 (s) (O–H), 3010 (m) (C–H), 2974 (w) (C–H), 1640 (s) (C═O), 1616 (m) (C═N), 1598 (s) (C═C) cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, 25°C, TMS): 9.50 (s, 1H, OH), 8.41 (dd, J = 8.1, 1.7 Hz, 2H, Ar–H), 7.48–7.41 (m, 2H, Ar–H), 7.30 (dd, J = 7.9, 1.4 Hz, 2H, Ar–H), 7.22–7.12 (m, 3H, Ar–H), 6.89 (s, 1H, CH-isoxazole), 6.80 (td, J = 7.5, 1.5 Hz, 2H, Ar–H), 6.01 (s, 2H, CH_2), 3.88 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6, 25°C, TMS): 178.63, 168.83, 159.04, 148.70, 147.80, 141.33, 131.82, 126.87, 123.32, 121.86, 121.76, 121.55, 116.05, 115.83, 110.50, 100.95, 55.14, 42.78. HRMS (ESI) *m/z* $[M+H]^+$ calcd. for $C_{24}H_{18}N_2O_4$: 399.1299, found: 399.1300.

*10-{[3-(4-Chlorophenyl)-1,2-oxazol-5-yl]methyl}acridin-9-one (**4d**)*

White solid; yield: 70%, mp = 238°C. IR (KBr): 3018 (m) (C–H), 2984 (w) (C–H), 1641 (s) (C═O), 1617 (m) (C═N), 1596 (s) (C═C) cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, 25°C, TMS): 8.41 (dd, J = 8.1, 1.7 Hz, 2H, Ar–H), 7.97–7.76 (m, 6H, Ar–H), 7.59–7.49 (m, 2H, Ar–H), 7.40 (ddd, J = 7.9, 6.2, 1.6 Hz, 2H, Ar–H), 7.05 (s, 1H, CH-isoxazole), 6.06 (s, 2H, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6, 25°C, TMS): 176.70, 168.94, 161.12, 141.74, 134.99, 134.38, 129.11, 128.41, 126.95, 126.76, 121.86, 121.83, 115.94, 101.16, 42.20. HRMS (ESI) *m/z* $[M+H]^+$ calcd. for $C_{23}H_{15}ClN_2O_2$: 387.0882, found: 387.0884.

*10-{[3-(4-Nitrophenyl)-1,2-oxazol-5-yl]methyl}acridin-9-one (**4e**)*

White solid; yield: 60%, mp = 251°C. IR (KBr): 3000 (m) (C–H), 2987 (w) (C–H), 1638 (s) (C═O), 1610 (m) (C═N), 1598 (s) (C═C) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, 25°C, TMS): 8.64 (dd, J = 8.0, 1.7 Hz, 2H, Ar–H), 8.36–8.22 (m, 2H, Ar–H), 8.03–7.88 (m, 2H, Ar–H), 7.78 (td, J = 8.7, 7.0, 1.7 Hz, 2H, Ar–H), 7.56–7.35 (m, 4H, Ar–H), 6.46 (s, 1H, CH-isoxazole), 5.77 (s, 2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$, 25°C, TMS): 177.95, 168.76, 161.17, 148.92, 141.80, 134.50, 128.32, 127.77, 124.20, 122.90, 122.35, 114.17, 101.18, 43.49. HRMS (ESI) *m/z* $[M+H]^+$ calcd. for $C_{23}H_{15}N_3O_4$: 398.1062, found: 398.1062.

*10-({3-[4-(Dimethylamino)phenyl]-1,2-oxazol-5-yl}methyl)acridin-9-one (**4f**)*

White solid; yield: 56%, mp = 221°C. IR (KBr): 3013 (m) (C–H), 2977 (w) (C–H), 1640 (s) (C═O), 1615 (m) (C═N), 1591 (s) (C═C) cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, 25°C, TMS): 8.41 (dd, J = 8.0, 1.6 Hz, 2H, Ar–H), 8.04–7.82 (m, 4H, Ar–H), 7.74 (d, J = 9 Hz, 2H, Ar–H), 7.40 (td, J = 8.0, 6.2, 1.5 Hz, 2H, Ar–H), 7.07–6.99 (m, 2H, Ar–H), 6.92 (s, 1H, CH-isoxazole), 5.91 (s, 2H, $_{CH2)}$, 3.05 (s, 6H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6, 25°C, TMS): 176.13, 168.68, 161.61, 151.70, 141.76, 134.37, 128.13, 126.75, 121.86, 121.81, 120.44, 115.99,

114.38, 100.23, 42.20, 41.22. HRMS (ESI) *m/z* $[M+H]^+$ calcd. for $C_{25}H_{21}N_3O$: 396.1683, found: 396.1687.

*2-Methyl-10-[(3-phenyl-1,2-oxazol-5-yl)methyl]acridin-9-one (**4g**)*

White solid; yield: 77%, mp = 229°C. IR (KBr): 3015 (m) (C–H), 2964 (w) (C–H), 1636 (s) (C=O), 1617 (m) (C=N), 1596 (s) (C=C) cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, 25°C, TMS): 8.40 (dd, *J* = 8.0, 1.6 Hz, 1H, Ar–H), 8.19 (s, 1H, Ar–H), 7.93–7.76 (m, 5H, Ar–H), 7.67 (dd, *J* = 8.9, 2.3 Hz, 1H, Ar–H), 7.52–7.41 (m, 3H, Ar–H), 7.37 (td, *J* = 7.9, 6.5, 1.2 Hz, 1H, Ar–H), 7.01 (s, 1H, CH-isoxazole), 6.04 (s, 2H, CH_2), 2.45 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6, 25°C, TMS): 176.53, 168.69, 161.99, 141.61, 139.86, 135.62, 134.17, 131.05, 130.29, 129.01, 128.08, 126.76, 126.60, 125.99, 121.75, 121.73, 121.53, 115.97, 115.80, 101.11, 42.06, 20.16. HRMS (ESI) *m/z* $[M+H]^+$ calcd. for $C_{24}H_{18}N_2O_2$: 367.1398, found: 367.1404.

*10-{[3-(4-Methoxyphenyl)-1,2-oxazol-5-yl]methyl}-2-methylacridin-9-one (**4h**)*

White solid; yield: 79%, mp = 263°C. IR (KBr): 3011 (m) (C–H), 2987 (w) (C–H), 1637 (s) (C=O), 1616 (m) (C=N), 1598 (s) (C=C) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, 25°C, TMS): 8.63 (dd, *J* = 8.0, 1.7 Hz, 1H, Ar–H), 8.39 (s, 1H, Ar–H), 7.81–7.64 (m, 3H, Ar–H), 7.54–7.44 (m, 2H, Ar–H), 7.39 (td, *J* = 7.9, 6.9, 0.9 Hz, 2H, Ar–H), 6.93 (d, *J* = 8.9 Hz, 2H, Ar–H), 6.32 (s, 1H, CH-isoxazole), 5.70 (s, 2H, CH2), 3.85 (s, 3H, CH_3), 2.50 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$, 25°C, TMS): 178.06, 167.19, 162.52, 161.27, 141.94, 134.40, 128.26, 128.16, 122.85, 122.18, 120.69, 114.43, 114.34, 100.82, 55.34, 43.67, 20.15. HRMS (ESI) *m/z* $[M+H]^+$ calcd. for $C_{25}H_{20}N_2O_3$: 397.1473, found: 397.1507.

*10-{[3-(4-Hydroxy-3-methoxyphenyl)-1,2-oxazol-5-yl]methyl}-2-methylacridin-9-one (**4i**)*

Yellow solid; yield: 40%, mp = 214°C. IR (KBr): 3420 (s) (O–H), 3014 (m) (C–H), 2970 (m) (C–H), 1644 (s) (C=O), 1615 (m) (C=N), 1595 (s) (C=C) cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, 25°C, TMS): 9.50 (s, 1H, OH), 8.41 (dd, *J* = 8.1, 1.7 Hz, 1H, Ar–H), 7.78 (d, *J* = 1.9 Hz, 1H, Ar–H), 7.48–7.41 (m, 1H, Ar–H), 7.33–7.27 (m, 2H, Ar–H), 7.24 (d, *J* = 8.4 Hz, 1H, Ar–H), 7.22–7.12 (m, 3H, Ar–H), 6.89 (s, 1H, CH-isoxazole), 6.80 (td, *J* = 7.3, 1.3 Hz, 1H, Ar–H), 6.01 (s, 2H, CH_2), 3.88 (s, 3H, CH_3), 2.40 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6, 25°C, TMS): 178.76, 168.83, 158.04, 148.70, 147.80, 141.36, 140.06, 134.16, 132.19, 131.78, 126.88, 124.21, 123.35, 121.79, 121.64, 121.50, 116.05, 115.83, 114.86, 110.50, 100.95, 56.14, 45.78, 20.85. HRMS (ESI) *m/z* $[M+H]^+$ calcd. for $C_{25}H_{20}N_2O_4$: 399.1266, found: 399.1265.

*10-{[3-(4-Chlorophenyl)-1,2-oxazol-5-yl]methyl}-2-methylacridin-9-one (**4j**)*

Yellow solid; yield: 73%, mp = 240°C. IR (KBr): 3010 (m) (C–H), 2974 (w) (C–H), 1640 (s) (C=O), 1616 (m) (C=N), 1598 (s) (C=C) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, 25°C, TMS): 8.62 (dd, *J* = 7.9, 1.6 Hz, 1H, Ar–H), 8.39 (s, 1H, Ar–H), 7.74 (m, 3H, Ar–H), 7.57 (dd, *J* = 8.7, 2.3 Hz, 2H, Ar–H), 7.50–7.31 (m, 4H, Ar–H), 6.34 (s, 1H, CH-isoxazole), 5.69 (s, 2H, CH_2), 2.50 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$, 25°C, TMS): ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.98, 167.89, 161.97, 141.85, 136.47, 134.42, 129.23, 128.19, 128.10, 126.72, 122.85, 122.23, 114.32, 100.92, 43.56. HRMS (ESI) *m/z* $[M+H]^+$ calcd. for $C_{24}H_{17}ClN_2O_2$: 401.1040, found: 401.1050.

*2-Methyl-10-{[3-(4-nitrophenyl)-1,2-oxazol-5-yl]methyl}acridin-9-one (**4k**)*

White solid; yield: 65%, mp = 276°C. IR (KBr): 3000 (m) (C–H), 2970 (m) (C–H), 1638 (s) (C=O), 1610 (m) (C=N), 1598 (s) (C=C) cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, 25°C, TMS): 8.40 (dd, *J* = 8.0, 1.5 Hz, 1H, Ar–H), 8.33–8.25 (m, 2H, Ar–H), 8.19 (s, 1H, Ar–H), 8.15–8.07 (m, 2H, Ar–H), 7.96–7.75 (m, 3H, Ar–H), 7.68 (dd, *J* = 8.9, 2.3 Hz, 1H, Ar–H), 7.38 (td, *J* = 7.9, 6.3, 1.5 Hz, 1H, Ar–H), 7.15 (s, 1H, CH-isoxazole), 6.08 (s, 2H, CH_2), 2.46 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6, 25°C, TMS): 176.54, 169.70, 160.62, 148.35, 141.59, 139.83, 135.67, 134.23, 134.11, 131.12, 127.95, 126.79, 126.02, 124.19, 121.77, 121.59, 115.94, 115.76, 101.54, 42.09, 20.16. HRMS (ESI) *m/z* $[M+H]^+$ calcd. for $C_{24}H_{18}N_3O_4$: 412.1239, found: 412.1239.

*10-({3-[4-(Dimethylamino)phenyl]-1,2-oxazol-5-yl}methyl)-2-methylacridin-9-one (**4l**)*

Yellow solid; yield: 63%, mp = 255°C. IR (KBr): 3014 (m) (C–H), 2975 (w) (C–H), 1635 (s) (C=O), 1616 (m) (C=N), 1597 (s) (C=C) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, 25°C, TMS): 8.60 (dd, *J* = 8.0, 1.7 Hz, 1H, Ar–H), 8.38 (s, 1H, Ar–H), 7.76–7.65 (m, 2H, Ar–H), 7.55 (td, *J* = 8.5, 5.5, 3.5 Hz, 2H, Ar–H), 7.40 (m, 2H, Ar–H), 7.03 (d, *J* = 8.5 Hz, 1H, Ar–H), 6.67 (d, *J* = 8.8 Hz, 2H, Ar–H), 6.27 (s, 1H, CH-isoxazole), 5.65 (s, 2H, CH_2), 2.99 (s, 6H, CH_3), 2.49 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$, 25°C, TMS): 177.97, 167.68, 161.64, 152.01, 141.72, 139.89, 135.71, 131.91, 129.18, 128.10, 127.80, 127.43, 125.85, 122.63, 122.18, 121.15, 119.79, 115.54, 114.54, 111.87, 100.72, 43.34, 40.12, 20.59. HRMS (ESI) *m/z* $[M+H]^+$ calcd. for $C_{26}H_{23}N_3O_2$: 410.1820, found: 410.1829.

4.2 | Antibacterial activity

The novel isoxazoles (**4a–l**) were evaluated for their in vitro antibacterial activity by the disk-diffusion method. The active compounds were subjected to the determination of the MIC, using the broth microdilution method. The microorganisms used for the test were *E. coli*, *S. aureus*, *P. putida*, and *K. pneumoniae*. They were collected from clinical isolates. Bacterial inoculums were prepared by subculturing microorganisms into MHB at 37°C for 18 h and were diluted to approximately 10^6 CFU/ml. Initial solution with concentration 0.5 mg/ml of the compounds **4a–l** was prepared in DMF, further serial dilutions were made in the microplates, and 100 μl of MHB containing each test microorganism was added to the microplate,[33,34] then incubated at 36°C for 24 h. After incubation, 20 μl of tetrazolium chloride (TTC; 0.04 mg/ml) was added to each microplate. The color changes of TTC from colorless to red were accepted as microbial growth.[35]

4.3 | Molecular docking studies

Molecular docking of the compounds **4a–l** with the DNA topoisomerase complex (PDB ID: 3FV5) of *E. coli* was carried out using the AutoDock software.[36] For the preparation of protein and ligands, see Supporting Information. The Discovery Studio (version 4.5) was used for graphical visualization. Different types of interactions between protein and the docked compounds (**4a–l**) were analyzed using Discovery Studio.

CONFLICTS OF INTERESTS

The authors declare that there are no conflicts of interests.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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