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Fluorine-containing 2,3-diaryl quinolines as potent inhibitors of methicillin and vancomycin-resistant *Staphylococcus aureus*: Synthesis, antibacterial activity and molecular docking studies

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ABSTRACT

Drug resistant bacteria pose a major health concern and affect a large section of global population. Antibacterial drug discovery has stagnated owing to multiple factors including unattractive returns for major pharmaceutical companies. Thus, discovery of effective antibacterial drugs against drug-resistant bacteria is an urgent unmet need affecting healthcare systems globally. In this study, fluorine-containing 2,3-diarylquinolines (**4a-l**) and non-fluorinated analog **4m** were synthesized utilizing environmentally benign chemistry of arenediazonium salts and arynes for regioselective installation of aryl groups at C-2 and C-3 positions, respectively. *In vitro* antibacterial evaluation against various Gram-negative and Gram-positive bacteria revealed inhibitory activity of majority of these compounds against Gram-positive *S. aureus* ATCC 29213. Compounds **4e, 4i, 4j** and **4l** were most potent inhibitors with MIC values of 10.95−24.0 μM. None of the compounds inhibited Gram-negative bacteria. **4e, 4i** and **4l** also displayed low levels of cytotoxicity against Vero cells, therefore, offering high safety profiles. Importantly, **4e, 4i** and **4l** exhibited equipotent inhibition of Methicillin and Vancomycin-resistant *S. aureus*, rendering them potential hits for further development. Molecular docking studies with topoisomerase II DNA gyrase demonstrated significant interactions of these inhibitors with target protein, which provided valuable insights into their potent antibacterial activity.

1. Introduction

In recent years, frequent and widespread emergence of multidrug resistance (MDR) in deadly infectious diseases has posed challenges to mankind. The drug resistance in the bacterial infections have raised severe health concerns.[1-4] The commonly used antibiotics for treatment of bacterial infections are being increasingly rendered ineffective. World Health Organization (WHO) reports reckon the antimicrobial resistance (AMR) as one of the biggest threats to global health and economy [1,5,6]. Each year MDR bacteria kill ~25,000 in Europe, ~35,000 in the U.S., and estimated 58,000 people in India, respectively [7,8]. The severity of the matter also lies in the fact that several medical practices such as chemotherapy, surgeries and organ transplantations *etc.* that rely on the antibiotics for management of the post-treatment bacterial infections, are also at risk due to AMR [6]. The looming threat of AMR in bacterial infections demands new and effective tools and strategies to prevent and treat MDR bacterial infections. The rapid development of small-molecule based antibacterial agents offers an effective strategy to combat the re-merging resistance to existing drugs and antibiotics.

Among a variety of medicinally important heterocyclic compounds, quinoline occupies a significant position in pharmaceuticals. This privileged scaffold is a versatile pharmacophore with a broad range of therapeutic efficacy [9,10]. Several

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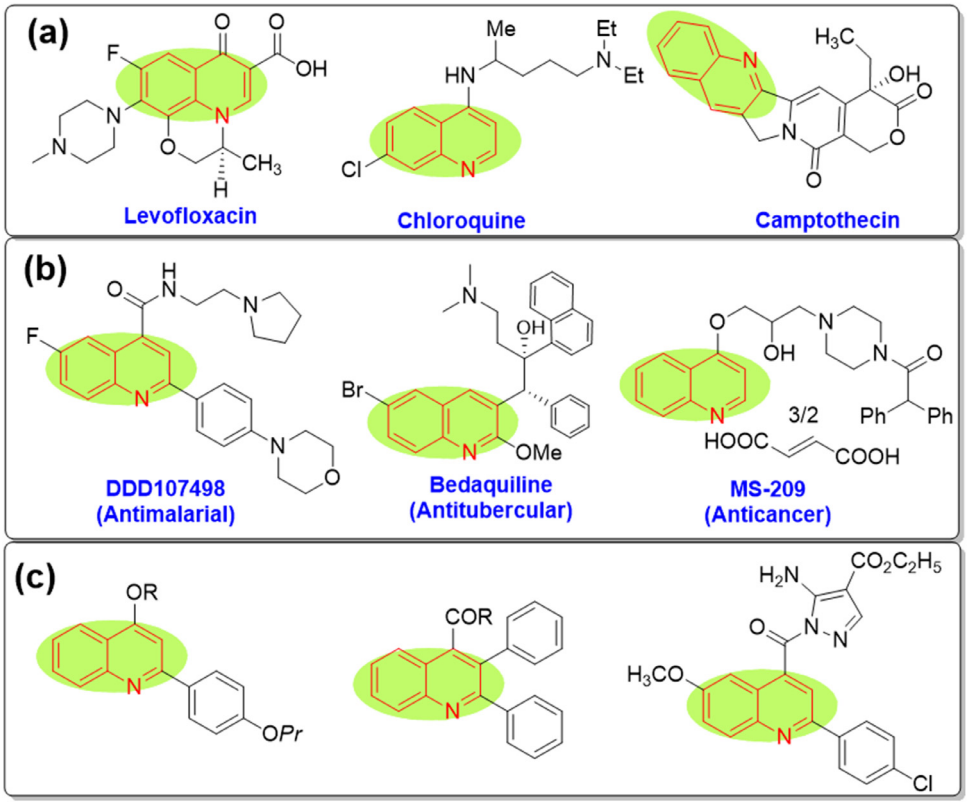


Fig. 1. Examples of (a) quinolone-derived commercial drugs; (b) drugs for drug resistant conditions; (c) antibacterial 2-arylquinolines.

quinoline-derived antibiotics and other commercial drugs are available as effective antimalarial,[11] anti-inflammatory,[12] anticancer, [13] anticonvulsant, antimicrobial [14] and anti-mycobacterial [15] (see Fig. 1a) and a plethora of biologically active candidates are under development [15,16]. Notably, several quinoline-derived drug molecules have been found effective against various drug resistance conditions Fig. 1b [17–20]. The wide therapeutic potential, generally safe pharmacological profiles and different modes of actions of quinoline scaffolds to combat drug resistance, offers their potential against the MDR bacteria. Therefore, it has consequently become vital to explore new quinoline-derived effective antibacterial agents [19,21–26].

Recently, arylquinolines were reported to display potent antibacterial activity [14,27,28] including against MDR bacteria [29–31]. Some examples of antibacterial 2-arylquinolines are shown in Fig. 1c. Lately, we prepared a library of 2,3-diarylquinolines, [32] another important class of bioactive quinolines, [27,33,34] by using transition metal-free chemistry of arynes and quinoline *N*-oxides. The antibacterial efficiency of 2-arylquinolines and therapeutic background of 2,3-dirylquinolines motivated us to investigate antibacterial activity of various 2,3-diarylquinolines towards development of antibacterial agents against MDR bacteria. Further, the pivotal role of fluorine in the enhancement of bioactivity and bioavailability of therapeutically significant molecules is well established [35–37]. Fluorinated quinolines have also been the first choice for the development of effective antibacterial drug molecules [38–40] and therefore, fluorine containing 2,3-diarylquinolines were selected as suitable candidates.

Quinoline-derived antibiotics and several antibacterial compounds work *via* inhibition of topoisomerase II DNA gyrase and this enzyme has been extensively studied as a major target for development of new antibacterial drugs [41–46]. Molecular docking studies with topoisomerase II DNA gyrase provides information about whether these compounds inhibit the enzyme or alternative mechanisms of action are operational.

2. Results and discussion

2.1. Synthesis of fluorine containing 2,3-diarylquinolines

The fluorinated 2,3-diarylquinolines **4a-m** were synthesized using our previously developed approach [32,47].

Quinoline *N*-oxide undergo regioselective C-2 arylation by radical mediated reactions of arenediazonium salts as shown in Fig. 2a [47]. The C-2 substituted quinoline *N*-oxides undergo dipolar cycloaddition reactions with benzyne and resulting cycloadduct upon rearrangement and deprotonation produces 2-substituted-3-arylquinolines (see Fig. 2b).

Various mono-, di- and trifluoroarenediazonium chlorides **2a-j** were reacted with quinolone *N*-oxide **1a** in presence of NaOAc to obtain the 2-arylquinoline *N*-oxides **3a-j**. Similarly, reactions of benzenediazonium chloride (**2k**) with 6-fluoro- (**1b**) and 7-trifluoromethyl (**1c**) quinolone *N*-oxides yielded corresponding 2-phenyl-derivatives **3k-l**, respectively. A non-fluorinated analog 2-phenylquinoline *N*-oxide **3m** was also prepared by reaction of benzenediazonium chloride (**2k**) with **1a**. [47]. 2-Arylquinoline *N*-oxides **3a-m** were further subjected to cycloaddition-rearrangement reactions with benzyne to afford desired 2,3-diarylquinolines **4a-m** as depicted in Scheme 1 [32].

All the compounds were characterized by ^{1}H, ^{13}C NMR, ^{19}F and IR spectroscopy and High-Resolution Mass Spectrometry. For example, for compound **4a**, ^{1}H NMR spectra in deuterated DMSO (*d6*) showed one singlet at δ 8.33 ppm corresponding to C-4 **H** of quinoline ring and one more singlet was observed at δ 9.33 ppm corresponding to O-**H** hydrogen at C-3 aryl substituent. In addition, signals for twelve protons were observed in aromatic region (δ 6-8 ppm), confirming total number of protons required in

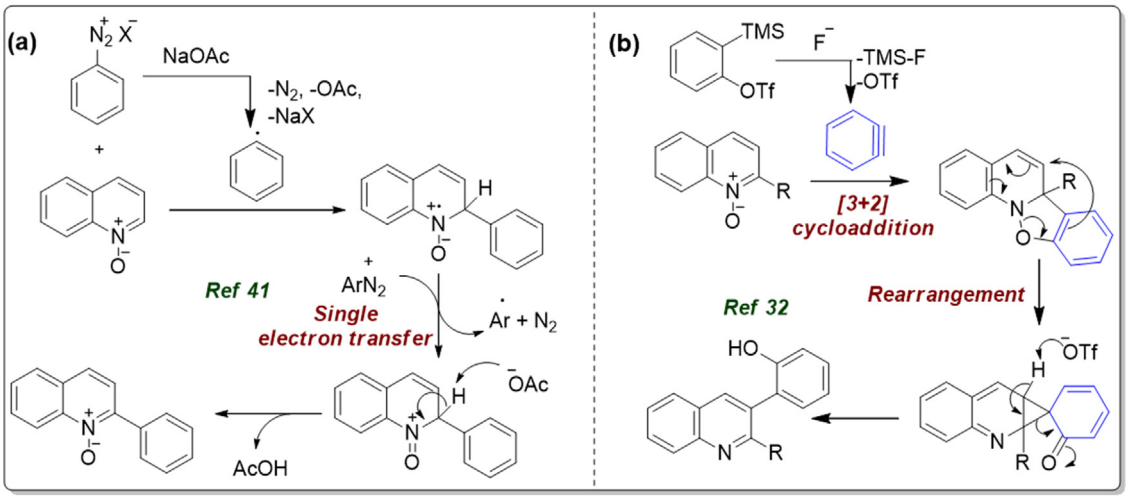


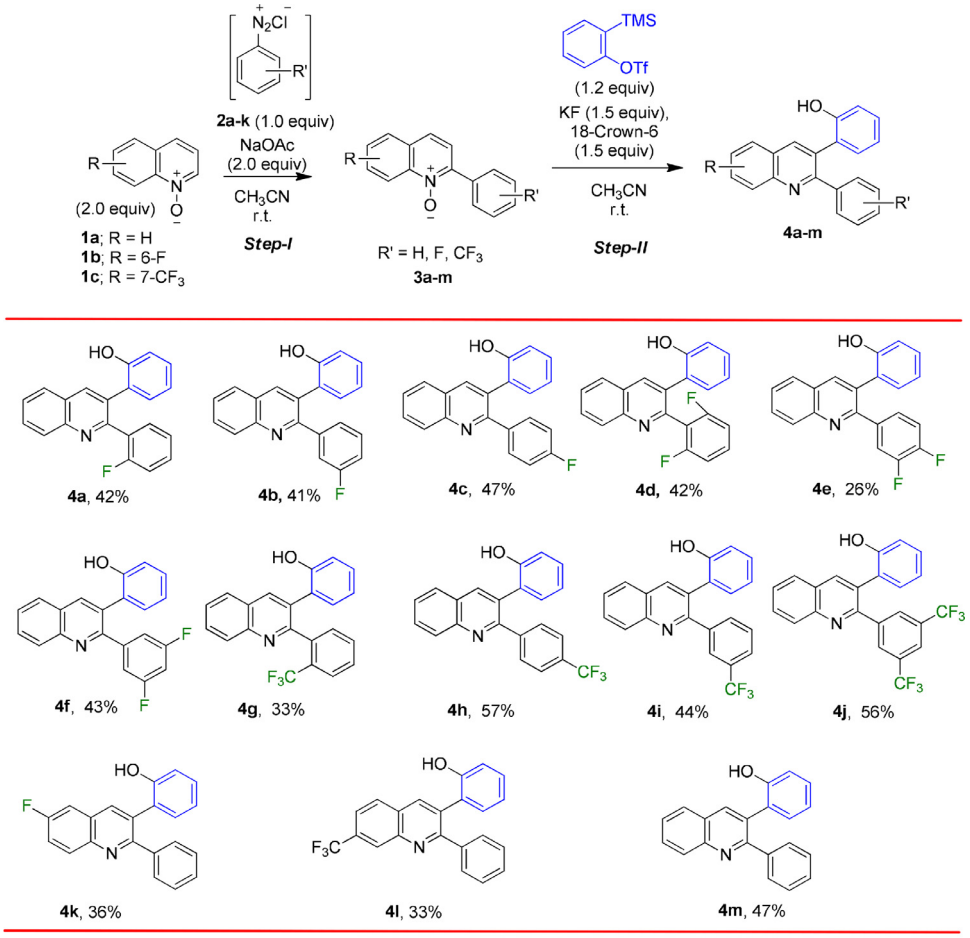
Fig. 2. Mechanisms of arylation of quinoline *N*-oxides using (a) arene diazonium salts and (b) arynes.

compound **4a** [32]. Furthermore, the ^{19}F NMR showed one doublet at −115.31 ppm, confirming presence of aryl fluorine in molecule with one-*ortho* hydrogen. In mass spectrometry, molecular ion peak at 316.1133 matched with *m/z* value of $[M + H]^+$ molecular ion of compound **4a**. ^{13}C NMR data and IR spectral data also supported the structure of the compound **4a** as reported.

2.2. Biological evaluation

2.2.1. Antibacterial activity against gram-positive and gram-negative bacteria

All synthesized compounds were screened for *in vitro* antibacterial activity against Gram-positive *S. aureus* (ATCC 29213) and Gram-negative bacteria *E. coli* (ATCC 25922), *K. pneumoniae* (BAA



*Yields are for the reactions of **3a-m**with benzyne precursor (Step-II).

Scheme 1. Synthesis of 2,3-diarylquinolines. *Yields are for the reactions of **3a-m**with benzyne precursor (Step-II).

Table 1
MIC (μM) against Gram-positive and Gram-negative bacterial pathogen panel.

S.No.	Compound	MIC (μM)				
		E. coli ATCC 25922	*S. aureus* ATCC 29213	*K. pneumoniae* BAA-1705	*baumannii* BAA-1605	*P. aeruginosa* ATCC 27853
1	**4a**	>175	101.48	>175	>175	>175
2	**4b**	>175	101.48	>175	>175	>175
3	**4c**	>175	>175	>175	>175	>175
4	**4d**	>175	96.19	>175	>175	>175
5	**4e**	>175	24.0	>175	>175	>175
6	**4f**	>175	>175	>175	>175	>175
7	**4g**	>175	>175	>175	>175	>175
8	**4h**	>175	>175	>175	>175	>175
9	**4i**	>175	21.90	>175	>175	>175
10	**4j**	>175	10.95	>175	>175	>175
11	**4k**	>175	101.47	>175	>175	>175
12	**4l**	>175	21.90	>175	>175	>175
13	**4m**	>175	>175	>175	>175	>175
14	**Levofloxacin**	0.043	0.34	>175	22.3	2.8

1705), *A. baumannii* (BAA-1605) and *P. aeruginosa* (ATCC 27853). The activity was determined as minimum inhibitory concentrations (MIC) as μM using microbroth dilution. Levofloxacin was used as a reference compound in evaluation of antibacterial activity.

As shown in Table 1, majority of compounds were active against *S. aureus* ATCC 29213 while none of the compounds was active against Gram-negative bacteria. Compounds **4e, 4i, 4j** containing fluorine atoms in C-2 phenyl ring exhibited strong antibacterial activity against *S. aureus* ATCC 29213 with MICs in 10.95−24.0 μM range. Further, trifluoromethylquinoline derived analog, **4l** was also active against *S. aureus* and displayed an activity with MIC 21.90 μM. A moderate activity with MICs closer to 100 μM was observed for C-2 fluorophenyl group containing compounds **4a, 4b, 4d** against *S. aureus* ATCC 29213. Furthermore, 6-fluoroquinoline derived, **4k** displayed some activity with MIC closer to 100 μM. Other compounds including non-fluorinated **4m** were inactive against all tested bacterial strains.

The structure activity relationship (SAR) analysis revealed that presence of at least one fluorine substituent is important for antibacterial activity. Further, among C-2 fluoroaryl substituted compounds, most active ones have fluorine at *meta*-position of aryl group (*e.g.* **4e, 4i** and **4j**). The presence of fluorine substituent in quinoline ring is also beneficial for enhancement of the activity as seen in cases of compounds **4k** (101.47 μM) and **4l** (21.90 μM) with, substitution at C-7 position has a significant effect on activity enhancement as shown in compound **4l**.

2.2.2. Cytotoxicity against Vero cells

Cytotoxicity of active compounds **4e, 4i, 4j** and **4l** was assessed against Vero cells (ATCC CCL-81), CC_{50} was determined and Selectivity index (SI = CC_{50}/MIC) was calculated. As shown in Table 2, for compounds **4e, 4i** and **4l**, CC_{50} was >100 μg/ml, resulting in a SI >12.5. Interestingly, in case of hexafluorinated **4j**, a significant inhibition of cells was observed at multiple concentrations leading to a lower selectivity index (<10). All four compounds also showed a lower toxicity as compared to doxorubicin as a reference drug [48].

Table 2
Cytotoxicity of active compounds against Vero cells (ATCC CCL-81).

Compound	MIC (μM) *S. aureus*	CC_{50} (μM) Vero cells [a]	SI (CC_{50}/MIC)
4e	24.0	>300	>12.5
4i	21.90	>270	>12.5
4j	10.95	<110	<10
4l	21.90	>270	>12.5
Doxorubicin[48]	–	18	–

[a] Maximum solubility of compounds is 100 mg/ml in DMSO, thus highest concentration tested for calculation of CC_{50} is 100 ug/ml

2.2.3. Activity of compounds against clinical strains of MDR S. aureus

Intrigued by promising antibacterial activity and high safety index exhibited by **4e, 4i** and **4l**, they were further evaluated against Methicillin-resistant (MRSA) and Vancomycin-resistant (VRSA) *S. aureus*. Gram-positive bacteria cause severe concerns to public health and the superbug MRSA is most common among the several difficult-to-treat infections, leading to high morbidity and mortality [49–53]. Vancomycin is the gold standard of treatment of severe MRSA infections, [54,55] however, Vancomycin-resistant *S. aureus* was isolated in 2002 in the U.S. continues to spread worldwide [56,57]. MRSA and VRSA infections are emerging rapidly and present a huge danger to community and hospital acquired infections [58–63]. In addition, MRSA and VRSA have been labeled as high priority pathogens by WHO, that demand the immediate search of new and effective therapeutic agents [64–68].

Compounds **4e, 4i, 4l** exhibited good inhibitory activity against several clinical MDR, MRSA and VRSA with MIC values ranging from 10.95−24.0 μM (Table 3). **4l** was an exception losing activity against VRS4 (MIC 87.59 μM) and MRSA NRS10129 (MIC >175 μM, Table 3). The results indicate that these compounds have significant potential in treating infections caused due to MDR *S. aureus*.

2.3. Molecular docking studies

Further, to get an insight into antibacterial mechanism of most active inhibitors **4e, 4i** and **4l**, molecular docking studies were performed against topoisomerase II DNA gyrase. Quinoline-derived antibiotics are shown to exert their antibacterial effects *via* inhibition of topoisomerase II DNA gyrase and this enzyme is considered as major target in antibacterial drug discovery and development [41–45]. Docking study was performed for well-known antibacterial drug Novobiocin that acts by inhibition of DNA gyrase B subunit [41]. Different poses of compounds for protein-ligand interactions were evaluated and various parameters obtained from docking were investigated. Among various parameters, binding energy (B.E.), sum of energies of van der Waals interactions, H-bonds and desolvation energy (Vdw_hb_desolv_energy), total internal energy, ligand efficiency and inhibition constant were investigated.

The most important parameter which determines stability of protein-ligand complex is B.E., indicative of amount of energy released when a drug moiety associates with the target protein. Total internal energy represents sum of all energetic changes included in scoring function of ligand binding at active site of target protein. Ligand efficiency is B.E. per atom of ligand to protein. Further, inhibition constant represents concentration of compound required

Table 3
MIC(μM) of compounds against clinical, MDR MRSA and VRSA panel

Compound/ Drug	MIC(μM)											
		MRSA							VRSA			
	ATCC 29213	NRS10100	NRS10119	NRS10129	NRS10186	NRS10191	NRS10192	NRS10193	NRS10194	NRS10198	VRS 1	VRS 4	VRS 12
4e	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0
4i	10.95	21.90	10.95	21.90	10.95	21.90	10.95	21.90	10.95	10.95	10.95	10.95	10.95
4l	10.95	21.90	10.95	>175	10.95	21.90	10.95	21.90	21.90	10.95	10.95	87.59	10.95
Levofloxacin	0.69	≤1.38	44.27	≤1.38	10.98	44.27	10.98	88.5	≤1.38	88.5	177	>177	177
Meropenem	0.65	166	>166	20.86	20.86	166	20.86	>166	1.30	166	166	83.44	41.72
Methicillin	2.63	>160	>160	160	160	>160	>160	>160	21.03	>160	>160	>160	>160
Vancomycin	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	1.38	0.69	>44	>44	>44

*ATCC 29213 is MSSA: Methicillin susceptible *S. aureus*

to produce half maximum inhibition; thus, it is indicative of potency of compound. The higher negative values of B.E. and total internal energy suggest higher stability of protein-ligand complex. However, higher values of ligand efficiency and minimum inhibition constant indicate effectiveness of drug moiety.

All the most active inhibitors **4e, 4i**, and **4l** nicely fit in catalytic core of protein, suggesting efficient inhibition of protein and therefore, their potency as antibacterials. Table 4 lists most important parameters obtained from molecular docking studies for most active compounds and Novobiocin with best docked poses against topoisomerase II DNA gyrase. Respective binding energy values for best docked pose of compounds **4e, 4i, 4l** and Novobiocin with target protein were -5.16, -4.83, -5.87 and -6.74 kcal/mol respectively.

All compounds were found to show comparable binding energies to Novobiocin, thus rendering them potent DNA gyrase inhibitors and candidates for antibacterial drug development. Binding energy values indicate comparable inhibitory activities of these compounds, which are in agreement with experimental results (Table 1). Amongst these compounds, **4l** is expected to be the most active inhibitor since it has the most negative value for binding energy and minimum value for inhibition constant as compared to other compounds.

Further, specific interactions of most active inhibitors with amino acid residues of target protein were also studied. All active inhibitors showed considerable interactions with topoisomerase II DNA gyrase (Figs. 3–5). Interestingly, OH group at C-3 aryl moiety in all inhibitors showed H-bonding with amino acids in binding pocket. Inhibitors 4e, 4i, and 4l form conventional H-bonds with the Glu1088 as depicted in Figs. 3–5. For example, inhibitors 4e and 4i form close contact with Glu1088 through π-anion interaction; π-donor H-bonds observed with G9 of DNA and Ser1084 for 4e, and G5 and C11 of DNA for 4l; π-π interactions with C11 for 4e, A7 of DNA chain with 4i have been observed. Also, these compounds have shown close proximity with different amino acids at binding sites depicted in green color in Figs. 3–5.

Close proximity is indicative of significant van der Waals interactions between amino acid and inhibitor. These multiple interactions point towards significant deformation of protein structure and compound's inhibitory potency. Notable, in case of **4i** interactions with different nucleobases of DNA chain were also observed in addition to H-bond and various electrostatic interactions namely π-π, π-anion, π-alkyl *etc*. Such interactions suggest that different residues of enzymes are present at significant distance from inhibitors, making inhibitor compounds capable of forming stable protein-ligand complex. Noteworthy interactions of inhibitors with DNA chain were observed for **4e, 4i** and **4l** which indicates that quinoline derivatives in present study possess potent antibacterial properties.

Investigation of different binding sites of protein with best pose of docked compound suggests compound **4i** and **4l** to be most potent inhibitors and higher activity of **4i** could also be explained by its different modes of interactions with various structural components of target protein.

Fig. 6 represents topoisomerase II DNA gyrase in complex with DNA and compound 4e. It is evident from Figs. 3–5 that compounds **4e, 4i** and **4l** bind to topoisomerase II DNA gyrase majorly through H-bonding with Glu1088. Furthermore, these compounds are found to have significant π-π and π-alkyl interactions with different nucleobase of DNA. These common binding modes of compounds suggest that despite having small structural dissimilarity due to different position of substituents, reported compounds are perfectly bound within catalytic cavity of target protein leading to its inactivity and consequently inhibiting bacterial growth.

Table 4
Docking parameters obtained from molecular docking study for compounds and Novobiocin

S. No.	Compound	Binding Energy (kcal/mol)	Vdw_hb_desolv_energy	Total Internal Energy	Ligand Efficiency	Inhibition Constant
1	**4e**	-5.16	-5.81	-0.78	-0.21	165.46
2	**4i**	-4.83	-5.89	-1.1	-0.18	288.22
3	**4l**	-5.87	-6.99	-1.33	-0.22	49.4
4	**Novobiocin**	-6.74	-9.85	-2.83	-0.15	11.43

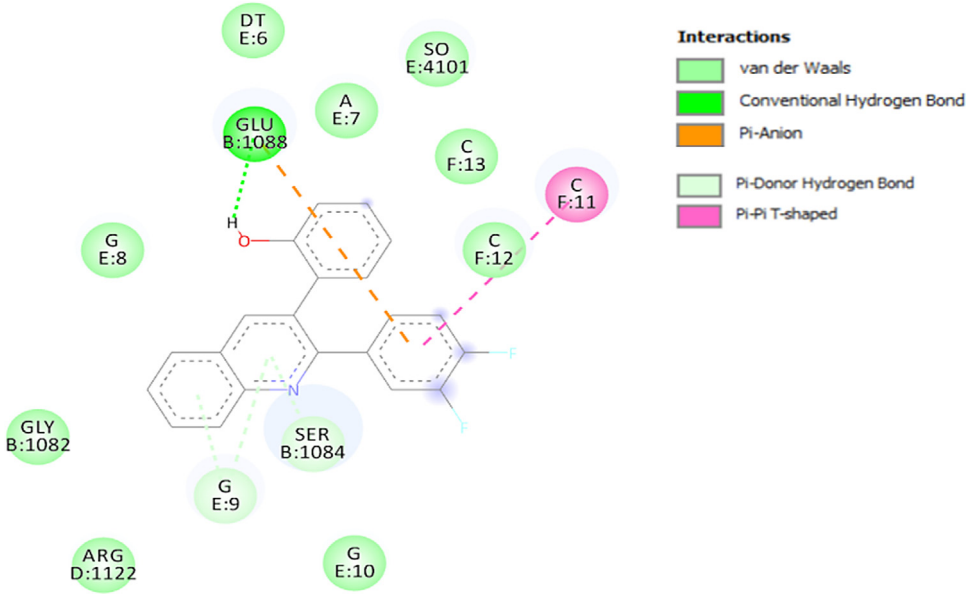


Fig. 3. 2D Protein-ligand binding interactions map for compound 4e.

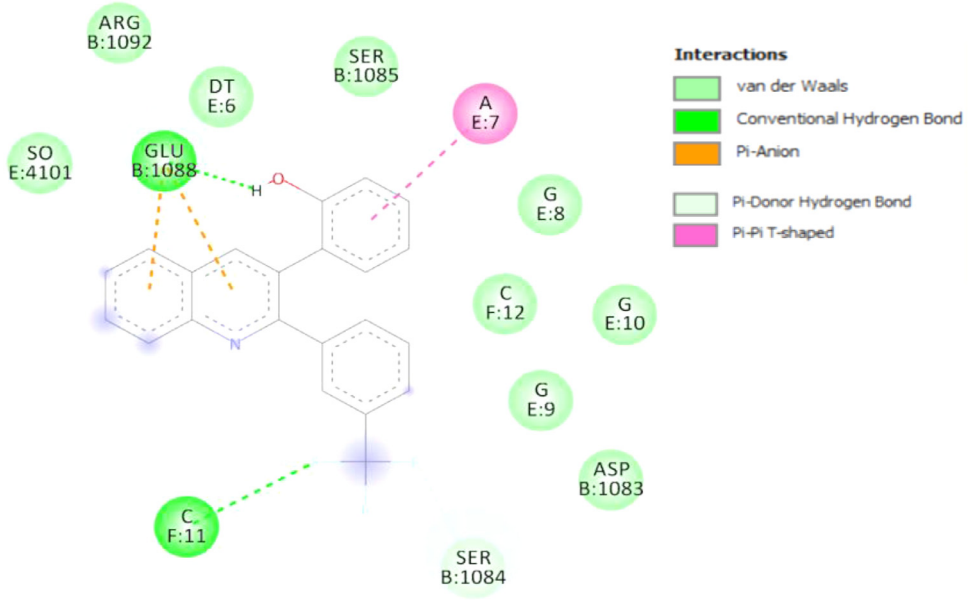


Fig. 4. 2D Protein-ligand binding interactions map for compound 4i

3. Conclusions

In the present study, antibacterial potential of various fluorine-containing 2,3-diarylquionolines and one non-fluorinated 2,3-diarylquinoline against Gram-positive and Gram-negative bacteria was demonstrated. Fluorinated compounds **4e, 4i, 4j** and **4l** exhibited good antibacterial activity against Gram-positive *S. aureus* with MIC 10.95–24.0 μM, whereas **4a, 4b, 4d** and **4k** showed moderate activity. None of the compounds was active against Gram-negative bacteria. Compounds **4e, 4i** and **4l** also exhibited lower cytotoxicity against Vero cells, however, hexafluorinated **4j** was found to be toxic. Furthermore, **4e, 4i** and **4l** demonstrated significant activity against clinical, MDR MRSA and VRSA *S. aureus.* In order to investigate their mechanism of action, molecular docking studies demonstrated significant interactions of these inhibitors with topoisomerase II DNA gyrase. The higher antibacterial activities of

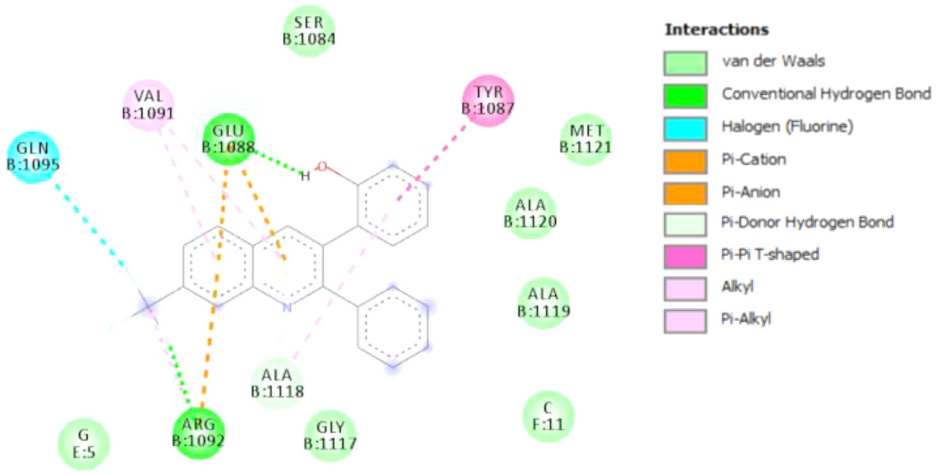


Fig. 5. 2D Protein-ligand binding interactions map for compound 4l

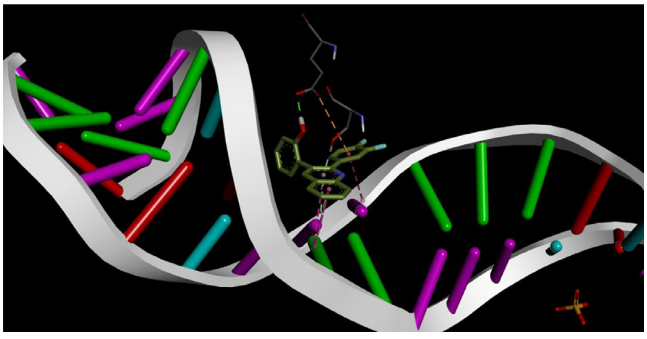


Fig. 6. Topoisomerase II DNA gyrase in complex with DNA and compound 4e.

compounds **4i** was attributed to sum of the energies *viz* binding energy, van der Waals interactions, H-bonding, desolvation energy and total internal energy. Further, specific interactions of inhibitors in binding pocket of enzyme suggested multiple modes of interactions of inhibitor **4i** with amino acid residues as well as adenine nucleobase of DNA. In all inhibitors, OH group at C-3 phenyl ring demonstrated significant H-bonding in binding pocket of protein. The binding energies of active inhibitors with topoisomerase II DNA gyrase were closer to that of a well-known antibiotic Novobiocin, indicating the promising potential of these inhibitors in antibacterial drug design. The promising antibacterial activity against MDR *S. aureus* as well as their good safety profile positions these molecules as potential candidates for effective treatment of infections caused due to MDR *S. aureus*.

4. Experimental section

4.1. Chemistry

The reactions were monitored by thin layer chromatography (TLC) on silica gel plates. The developed plates were examined under a UV lamp. ^{1}H NMR and ^{13}C NMR spectra were recorded on 500 MHzFT NMR Spectrometer model Avance Neo (Bruker) instrument.IR spectra were recorded on FTIR Spectrophotometer Model RZX (Perkin Elmer). Mass spectra were recorded on LC-MS Spectrometer Model Q-ToF Micro Waters. All chemicals and solvents used in synthesis were purchased from Sigma Aldrich, Alfa Aesar and TCI. For column chromatography, silica gel (230–400 mesh) from Merck was used. Acetonitrile was distilled over CaH_2. Reactions were carried out under ambient conditions. The detailed procedures for synthesis of compounds are described below.

4.1.1. General procedure for the synthesis of 2-arylquinoline-N-oxides (3a-m)

To an oven-dried reaction vial with magnetic stir-bar, aryldiazonium salt **2** (1.0 equiv.), quinoline N-oxide **1** (2.0 equiv.) and sodium acetate (2.0 equiv.) were added in MeCN (4ml) and allowed to stir at room temperature until the completion of reaction which was confirmed by TLC. After completion, the reaction mixture was extracted with ethyl acetate and washed with brine solution. Ethyl acetate layer was dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by column chromatography using silica gel (230-400 mesh size) and n-hexane: EtOAc as eluent.

4.1.2. General procedure for the synthesis of 2,3-diarylquinoline (4a-4m)

To an oven-dried reaction vial with magnetic stir-bar, 2-substituted quinoline-*N*-oxide **3(a-m)** (1.0 equiv.), KF (1.5 equiv.) and 18-crown-6-ether (1.5equiv.) was dissolved in MeCN, 2-trimethylsily(aryl)trifluoromethanesulfonate (1.5 equiv.) was added to the mixture and allowed to stir at room temperature until completion of reaction which was confirmed by TLC. The reaction mixture was extracted with ethyl acetate and washed with brine solution. Ethyl acetate layer was dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by column chromatography using silica gel (230-400 mesh size) and hexane: EtOAc as eluent. These compounds were characterized by ^{1}H, ^{13}C NMR, ^{19}F and IR spectroscopy, and HR-mass spectrometry.

4.1.3. 2-(2-(2-Fluorophenyl)quinolin-3-yl)phenol *(4a)*

2-(2-Fluorophenyl)quinoline 1-oxide **3a**: 100 mg (0.42 mmol); benzyne precursor : 187.1 mg (0.63 mmol); KF: 36.6 mg (0.63 mmol); 18-crown-6: 166.5 mg (0.63 mmol); CH_3CN: 4.0 mL.Column chromatography: eluting solvent 10−20% EtOAc/Hexane. Yield of **4a**:56.0 mg (42%), Yellow solid, R_f= 0.68 (35% EtOAc/Hexane).^{1}HNMR (DMSO-d_6, 500 MHz) δ 9.33 (s, 1H, OH), 8.33 (s, 1H, ArH), 8.05-8.07 (m, 2H, ArH), 7.7-7.8 (m, 1H, ArH), 7.63-7.67 (m, 1H, ArH), 7.37-7.40 (m, 1H, ArH) , 7.30-7.34 (m, 1H, ArH), 7.03-7.13 (m, 4H, ArH), 6.71-6.75 (m, 2H, ArH).^{13}C NMR (125MHz, DMSO-d_6) δ159.1 (d, J = 234.0 Hz), 155.03, 154.40, 146.30, 137.43, 132.60, 131.54, 130.96, 129.86 (d, J = 7.86 Hz), 129.62, 128.99, 128.89, 128.61, 127.79, 126.99, 126.88, 125.72, 123.45, 118.54, 115.18, 115.05(d, J = 21.3 Hz); ^{19}F NMR (DMSO-d_6, 500 MHz): δ -115.31 (d, J = 5.5 Hz). IR (KBr) υ_{max} (cm^{-1})

3060.6, 1587.1, 1489.9, 1372.0, 1156.3. HRMS (ESI-TOF) *m/z* calcd for $C_{21}H_{15}FNO$ [M + H] $^+$ 316.1138, found 316.1133.

4.1.4. 2-(2-(3-Fluorophenyl)quinolin-3-yl)phenol *(4b)*

2-(3-Fluorophenyl)quinoline 1-oxide **3b**: 48 mg (0.2 mmol); benzyne precursor : 89.5 mg (0.3 mmol); KF: 17.4 mg (0.3 mmol); 18-crown-6, 79.3 mg (0.3 mmol); CH_3CN: 2.0 mL.Column chromatography: eluting solvent 10−20% EtOAc/Hexane. Yield of **4b**: 26.0 mg (41%)Light Brown solid, R_f=0.70 (35% EtOAc/Hexane).^{1}HNMR (DMSO-d_6, 500 MHz) δ9.37 (s, 1H, OH), 8.30 (s, 1H, ArH), 8.08-8.10 (m, 1H, ArH), 8.04-8.06 (m, 1H, ArH), 7.79-7.82 (m, 1H, ArH) , 7.63-7.66 (m, 1H, ArH) , 7.25-7.30 (m, 2H, ArH) , 7.21-7.23 (m, 1H, ArH), 7.15-7.18 (m, 2H, ArH), 7.09-7.13 (m, 1H, ArH), 6.85 (t, J = 7.5 Hz, 1H, ArH), 6.77-6.79 (m, 1H, ArH).^{13}C NMR (125MHz, DMSO-d_6) δ161.45 (d, J = 237.2 Hz), 154.30, 146.45, 143.22, 138.23, 131.36, 131.03, 129.74, 129.27 (d, J = 8.75 Hz), 129.24, 128.71, 127.69, 126.86 (d, J = 4.87 Hz), 126.50, 125.02, 125.01 (d, J = 2.25 Hz), 119.09, 115.62, 115.53 (d, J = 22.5 Hz), 115.41, 114.56 (d, J = 20.62 Hz); ^{19}F NMR (DMSO-d_6, 500 MHz): δ -109.31-(-109.33) (m).IR (KBr) υ_{max} (cm^{-1}) 2922.0, 1581.3, 1483.2, 1372.7, 1177.3. HRMS (ESI-TOF) *m/z* calcd for $C_{21}H_{15}FNO$ [M+H] $^+$ 316.1138, found 316.1139.

4.1.5. 2-(2-(4-Fluorophenyl)quinolin-3-yl)phenol *(4c)*

2-(4-Fluorophenyl)quinoline 1-oxide **3c**: 100 mg (0.42 mmol); benzyne precursor: 187.1 mg (0.63 mmol); KF: 36.6 mg (0.63 mmol); 18-crown-6: 166.5 mg (0.63 mmol); CH_3CN, 4.0 mL.Column chromatography: eluting solvent 10−20% EtOAc/Hexane. Yield of **4c**: 62.0 mg (47%), brown solid, R_f = 0.70 (35% EtOAc/Hexane). ^{1}HNMR (DMSO-d_6, 500 MHz) δ 8.93 (s, 1H, OH), 8.50 (d, J = 8.5 Hz, 1H, ArH), 8.31 (d, J = 5Hz, 1H, ArH), 8.07 (t, J = 7.5 Hz, 1H, ArH), 7.88 (t, J = 7.5Hz, 1H, ArH), 7.59-7.62 (m, 2H, ArH), 7.18-7.25 (m, 5H, ArH), 6.83-6.90 (m, 2H, ArH).^{13}C NMR (125 MHz, DMSO-d_6) δ 162.91(d, J = 240.63 Hz), 155.22, 154.33, 144.62, 140.02, 132.94, 132.45, 132.14(d, J = 8.75 Hz) (2C), 131.12, 130.01, 128.72, 128.38, 127.25, 123.72, 123.04, 119.08 (2C), 115.67, 114.88 (d, J = 21.87 Hz) (2C). IR (KBr) υ_{max} (cm^{-1}) 3058.9, 1603.1, 1485.2, 1368.9, 1153.6. HRMS (ESI-TOF) *m/z* calcd for $C_{21}H_{15}FNO$ [M+H]$^+$ 316.1138, found 316.1129**.**

4.1.6. 2-(2-(2,6-Difluorophenyl)quinolin-3-yl)phenol *(4d)*

2-(2,6-difluorophenyl)quinoline 1-oxide **3d**: 50 mg (0.2 mmol); benzyne precursor: 89.5 mg (0.3 mmol); KF: 17.4 mg (0.3 mmol); 18-crown-6: 79.3 mg (0.3 mmol); CH_3CN, 2.0 mL. Column chromatography: eluting solvent 10−20% EtOAc/Hexane. Yield of **4d**: 27.0 mg (42%), orange solid, R_f=0.50 (35% EtOAc/Hexane).^{1}HNMR (DMSO-d_6, 500 MHz) δ 9.37 (s, 1H, OH), 8.37 (s, 1H, ArH), 8.06-8.09 (m, 2H, ArH), 7.80-7.84 (m, 1H, ArH), 7.67-7.70 (m, 1H, ArH), 7.34-7.40 (m, 1H, ArH), 7.07-7.10 (m, 2H, ArH), 6.99 (t, J = 10 Hz, 2H, ArH), 6.75 (td, J = 7.5, 1.0 Hz, 1H, ArH), 6.72 (d, J = 8.0 Hz, 1H, ArH).^{13}C NMR (125MHz, DMSO-d_6) δ 159.70 (d, J = 270.9 Hz), 154.61, 150.08, 146.26, 137.38 (2C), 133..52, 130.87, 130.43 (d, J = 10.1 Hz), 130.35 (d, J = 10.1 Hz), 129.77, 129.22, 128.59, 127.90 (2C),127.24 (d, J = 2.12 Hz), 124.94, 118.55 (2C), 115.04 , 111.13 (d, J = 24.6 Hz); ^{19}F NMR (DMSO-d_6, 500 MHz): δ -112.70 (d, J = 5.7 Hz). IR (KBr) υ_{max} (cm^{-1}) 3065.1, 1623.3, 1457.7, 1370.1, 1260.8. HRMS (ESI-TOF) *m/z* calcd for $C_{21}H_{14}F_2NO$ [M + H] $^+$ 334.1043, found 334.1034.

4.1.7. 2-(2-(3,4-Difluorophenyl)quinolin-3-yl)phenol *(4e)*

2-(3,4-Difluorophenyl)quinoline 1-oxide **3e**: 100 mg (0.39 mmol); benzyne precursor: 176.0 mg (0.59 mmol); KF: 34.4 mg (0.59 mmol); 18-crown-6: 155.9 mg (0.59 mmol); CH_3CN, 4.0 mL.Column chromatography: eluting solvent 10−20% EtOAc/Hexane. Yield: of **4e**: 34 mg (26%)Off white solid, R_f = 0.45 (35% EtOAc/Hexane).^{1}HNMR (DMSO-d_6, 500 MHz) δ 9.36 (s, 1H, OH) , 8.30 (s, 1H, ArH), 8.08 (d, J = 8.5Hz, 1H, ArH), 8.04 (d, J = 8.5 Hz, 1H, ArH), 7.78-7.82 (m,1H, ArH), 7.63-7.66 (m, 1H, ArH), 7.40-7.44 (m, 1H, ArH), 7.29-7.34 (m, 1H, ArH), 7.18-7.26 (m, 3H, ArH),;6.87 (t, J =7.5, 1.0 Hz, 1H, ArH), 6.77-6.79 (m, 1H, ArH).^{13}C NMR (125 MHz, DMSO-d_6) δ 154.73, 154.31, 150.90 (d, J = 11.75 Hz), 149.58 (d, J = 13.0 Hz), 148.92 (d, J = 12.25 Hz), 147.63 (d, J = 13.0 Hz), 142.21, 132.01, 131.84, 131.15, 129.92, 128.19 (2C), 127.31, 126.65, 125.39, 124.67, 119.28, 118.70 (d, J = 18.13 Hz), 116.92 (d, J = 17.13 Hz), 115.68; ^{19}F NMR (DMSO-d_6, 500 MHz): δ -139.75 (app q, J = 30 Hz). IR (KBr) υ_{max} (cm^{-1}) 2921.3, 1607.8, 1458.8, 1372.7, 1284.9. HRMS (ESI-TOF) *m/z* calcd for $C_{21}H_{14}F_2NO$ [M+H] $^+$ 334.1043, found 334.1036.

4.1.8. 2-(2-(3,5-Difluorophenyl)quinolin-3-yl)phenol *(4f)*

2-(3,5-Difluorophenyl)quinoline 1-oxide **3f**: 150 mg (0.6 mmol); benzyne precursor: 268.5 mg (0.9 mmol); KF: 52.3 mg (0.9 mmol); 18-crown-6: 237.6 mg (0.9 mmol); CH_3CN, 6.0 mL. Column chromatography: eluting solvent 10−20% EtOAc/Hexane. Yield of **4f**: 83 mg (43%), light brown solid, R_f = 0.65 (35% EtOAc/Hexane).^{1}HNMR (DMSO-d_6, 500 MHz) δ 9.46 (s, 1H, OH), 8.35 (s, 1H, ArH), 8.11 (d, J = 8.5, 1.0 Hz, ArH), 8.06 (d, J = 8Hz, 1H, ArH), 7.80-7.83 (m, 1H, ArH), 7.65-7-68 (m, 1H, ArH), 7.22-7.24 (m, 1H, ArH), 7.16-7.20 (m, 2H, ArH), 7.09-7.12 (m, 2H, ArH), 6.86-6.90 (m, 1H, ArH), 6.82 (d, J = 8Hz, 1H, ArH).^{13}C NMR (125 MHz, DMSO-d_6) δ 161.61 (d, J = 243.6 Hz), 161.50 (d, J = 243.8 Hz), 155.59, 154.27, 146.16, 144.23 (t, J = 8.9 Hz), 138.60, 131.30, 131.00. 130.02, 129.53, 128.60, 127.76, 127.21, 127.12, 126.07, 119.28, 115.49, 112.03(d, J =20.3 Hz), 111.99 (d, J = 20.3 Hz), 103.35 (t, J = 25.8 Hz); ^{19}F NMR (DMSO-d_6, 500 MHz): δ -110.93-(-110.99) (m).IR (KBr) υ_{max} (cm^{-1}) 2921.6, 1618.9, 1483.0, 1371.7, 1231.4. HRMS (ESI-TOF) *m/z*calcd for $C_{21}H_{14}F_2NO$ [M+ H] $^+$ 334.1043, found 334.1041.

*4.1.9. 4.1.9.*2-(2-(2-(Trifluoromethyl)phenyl)quinolin-3-yl)phenol *(4g)*

2-(2-(Trifluoromethyl)phenyl)quinoline 1-oxide **3g**: 100 mg (0.35 mmol); benzyne precursor: 154.8 mg (0.52 mmol); KF, 30.1 mg (0.52 mmol); 18-crown-6: 137.2 mg (0.52 mmol); CH_3CN: 4.0 mL.Column chromatography: eluting solvent 5−10% EtOAc/Hexane. Yield of **4g**: 42 mg (33%), white solid, R_f = 0.62 (20% EtOAc/Hexane).^{1}HNMR (DMSO-d_6, 500 MHz) δ 9.45 (s, 1H, OH), 8.34 (s, 1H, ArH), 8.06 (dd, J = 8.0, 1.0 Hz, 1H, ArH), 8.01(d, J = 8.5 Hz, 1H, ArH),7.78-7.81(m, 1H, ArH), 7.72-7.73 (m, 1H, ArH), 7.64-7.68 (m, 1H, ArH), 7.45-7.51(m, 2H, ArH), 7.30 (d, J = 6.5 Hz, 1H, ArH),7.04-7.07 (m, 1H, ArH), 6.93 (d, J = 6.5Hz, 1H, ArH), 6.78 (dd, J = 8.0, 0.5 Hz, 1H, ArH), 6.68 (td, J = 7.5,1.0 Hz, 1H, ArH).^{13}C NMR (125 MHz, DMSO-d_6) δ 157.47, 154.57 (2C), 145.77, 139.25 (q, J = 2.0 Hz), 137.77, 131.54, 130.80 (q, J = 5.9 Hz) (2C), 129.70, 129.03, 128.63, 128.17, 127.77, 127.50 (q, J = 29.8 Hz), 126.92, 126.81, 126.25 (q, J = 4.75Hz), 125.50, 124.23 (q, J = 272 Hz), 118.56, 115.35; ^{19}F NMR (DMSO-d_6, 500 MHz): δ -56.92 (s). IR (KBr) υ_{max} (cm^{-1}) 3053.8, 1588.69, 1486.7, 1370.7, 1262.5. HRMS (ESI-TOF) *m/z*calcd for $C_{22}H_{15}F_3NO$ [M+H] $^+$ 366.1106, found 366.1108.

4.1.10. 2-(2-(4-(Trifluoromethyl)phenyl)quinolin-3-yl)phenol *(4h)*

2-(4-(Trifluoromethyl)phenyl)quinoline 1-oxide **3h**: 50 mg (0.17 mmol); benzyne precursor: 47.6 mg (0.26 mmol); KF: 15.1 mg (0.26 mmol); 18-crown-6: 68.7 mg (0.26 mmol); CH_3CN: 2.0 mL. Column chromatography: eluting solvent 5−10% EtOAc/Hexane. Yield of **4h**: 36.0 mg (57%)off white solid, R_f = 0.50 (20% EtOAc/Hexane).^{1}HNMR (DMSO-d_6, 500 MHz) δ 9.36 (s, 1H, OH), 8.34 (s, 1H, ArH), 8.10 (d, J = 8.5 Hz, 1H, ArH), 8.06 (d, J = 7.5Hz, 1H, ArH), 7.79-7.7.82 (m, 1H, ArH), 7.62-7.67 (m, 5H, ArH), 7.23 (dd, J = 7.5, 1.5Hz, 1H, ArH), 7.18 (td, J =8.0,1.5 Hz, 1H, ArH), 6.86 (td, J = 7.5,1.0 Hz, 1H, ArH), 6.76 (dd, J = 8.0, 0.5 Hz, 1H, ArH).^{13}C NMR (125 MHz, DMSO-d_6) δ 156.97, 154.17, 146.50, 144.99, 138.31, 131.38, 131.11, 129.84, 129.57 (2C), 129.40, 128.74,

128.0 (q, J = 31.3 Hz) (2C), 127.76, 127.01, 126.27, 124.33, 124.27 (q, J = 269.7 Hz), 123.4 (q, J = 3.87 Hz), 119.22, 115.42. IR (KBr) υ_{max} (cm^{-1}) 2921.2, 1603.1, 1459.0, 1376.0, 1281.3. HRMS (ESI-TOF) m/z calcd for $C_{22}H_{15}F_3NO$ [M+H] $^+$ 366.1106, found 366.1117.

4.1.11. 2-(2-(3-(Trifluoromethyl)phenyl)quinolin-3-yl)phenol (4i)

2-(3-(Trifluoromethyl)phenyl)quinoline 1-oxide **3i**: 200 mg (0.69 mmol); benzyne precursor: 310 mg (1.04 mmol); KF, 60.3 mg (1.04 mmol); 18-crown-6, 274.4 mg (1.04 mmol); CH_3CN, 6.0 mL.Column chromatography: eluting solvent 5-10% EtOAc/Hexane. Yield of **4i**:112 mg (44%), light yellow solid, R_f = 0.45 (35% EtOAc/Hexane).^{1}HNMR (DMSO-d_6, 500 MHz) δ 9.35 (s, 1H, OH), 8.34 (s, 1H, ArH), 8.12 (d, J = 8.5 Hz, 1H, ArH), 8.07 (d, J = 7.5 Hz, 1H, ArH), 7.80-7.83 (m, 1H, ArH), 7.78 (d, J = 8.0 Hz, 1H), 7.72 (s, 1H, ArH), 7.63-7.68 (m, 2H, ArH), 7.52 (t, J = 7.5 Hz, 1H, ArH), 7.23 (dd, J = 7.5, 1.5Hz, 1H, ArH), 7.19 (td, J =8.0, 1.5 Hz, 1H, ArH), 6.87 (td, J = 7.5, 1.0 Hz, 1H, ArH), 6.76 (d, J = 8.0 Hz, 1.0H, ArH).^{13}C NMR (125 MHz, DMSO-d_6) δ 156.62, 154.22 (2C), 146.43, 141.57, 138.47, 132.78, 131.46, 131.06, 129.89, 129.38, 128.63, 128.56, 128.34 (q, J = 31.5 Hz), 127.75, 127.02 (q, J = 1.0 Hz), 126.25, 125.41 (q, J = 4.0 Hz), 124.43 (q, J =3 .4 Hz), 124.12 (q, J = 270.6 Hz), 119.23, 115.46; ^{19}F NMR (DMSO-d_6, 500 MHz): δ -61.21 (s).IR (KBr) υ_{max} (cm^{-1}) 3040.7, 1569.3, 1485.4, 1327.9, 1240.2. HRMS (ESI-TOF) m/zcalcd for $C_{22}H_{15}F_3NO$ [M+H] $^+$ 366.1106, found 366.1105.

4.1.12. 2-(2-(3,5-Bis(trifluoromethyl)phenyl)quinolin-3-yl)phenol *(4j)*

2-(3,5-Bis(trifluoromethyl)phenyl)quinoline 1-oxide **3j**: 100 mg(0.28 mmol); benzyne precursor: 25.3 mg (0.42 mmol); KF: 32.5mg (0.56 mmol); 18-crown-6: 148.02 mg (0.56 mmol); CH_3CN: 4.0mL.Column chromatography: eluting solvent 5−10% EtOAc/Hexane. Yield of **4j**: 68.0 mg (56%) off white solid, R_f=0.42 (20% EtOAc/Hexane).^{1}HNMR (DMSO-d_6, 500 MHz) δ 9.39 (s, 1H, OH), 8.40 (s, 1H, ArH), 8.17 (d, J = 8.0 Hz,1H, ArH), 8.09 (d, J = 7.5 Hz, 1H, ArH), 8.05 (s, 3H, ArH), 7.81-7.85 (m, 1H, ArH), 7.67-7.70 (m, 1H, ArH), 7.33 (dd, J = 7.5, 1.5 Hz, 1H, ArH), 7.22 (td, J = 8.0, 2.0 Hz, 1H), 6.92 (td, J = 7.5, 1.0 Hz, 1H, ArH), 6.74-6.76 (m, 1H, ArH).^{13}C NMR (125 MHz, DMSO-d_6) δ 154.93, 153.92, 146.56, 143.02, 138.45, 131.32, 131.05, 130.07, 129.71, 129.53 (q, J = 32.6 Hz) (2C), 129.17, 129.15, 128.87, 127.82, 127.37 (q, J = 5.5 Hz), 125.75, 123.20 (q, J = 271.0 Hz) (2C), 121.36 (q, J = 3.5 Hz), 119.51, 115.49 . IR (KBr) υ_{max} (cm^{-1}) 3065.9, 1589.5, 1451.3, 1366.1, 1280.8. HRMS (ESI-TOF) m/z calcd for $C_{23}H_{14}F_6NO$ [M + H] $^+$434.0980, found 434.1018.

4.1.13. 2-(6-Fluoro-2-phenylquinolin-3-yl)phenol *(4k)*

6-Fluoro-2-phenylquinoline 1-oxide **3k**: 80 mg (0.33 mmol); benzyne precursor: 149.2 mg (0.50 mmol); KF: 38.8 mg (0.68 mmol); 18-crown-6, 176.6 mg (0.68 mmol); CH_3CN:4.0 mL. Column chromatography: eluting solvent 5−10% EtOAc/Hexane. Yield of **4k**: 38 mg (36%), off white solid, R_f = 0.40 (35% EtOAc/Hexane). ^{1}HNMR (DMSO-d_6, 500 MHz) δ 9.37 (s, 1H, OH), 8.28 (s, 1H, ArH), 8.13(dd, J = 9.5, 5.5 Hz, 1H, ArH), 7.83 (dd, J = 9.5, 2.5 Hz, 1H, ArH) 7.66-7.71 (m, 1H, ArH), 7.43-7.45 (m, 2H, ArH),7.21-7.29 (m, 3H, ArH), 7.13-7.17 (m, 1H, ArH), 7.11 (dd, J = 7.5, 1.5 Hz, 1H, ArH), 6.77-6.82 (m, 2H, ArH).^{13}C NMR (125 MHz, DMSO-d_6) δ 159.77 (d, J = 243.75 Hz), 157.97 (d, J = 2.5 Hz), 154.31, 143.78, 140.61, 137.69 (d, J = 5.125 Hz), 132.29, 131.54 (d, J = 9.125 Hz), 131.06, 129.18, 128.92 (2C), 127.82, 127.44 (2C), 127.34, 126.56, 119.62 (d, J = 25.9 Hz), 118.99, 115.42, 110.63(d, J = 21.5 Hz).

4.1.14. 2-(2-Phenyl-7-(trifluoromethyl)quinolin-3-yl)phenol *(4l)*

2-Phenyl-7-(trifluoromethyl)quinoline 1-oxide **3l**: 100 mg (0.35 mmol); benzyne precursor : 154.8 mg (0.52 mmol); KF, 30.1 mg (0.52 mmol); 18-crown-6: 137.8 mg (0.52 mmol); CH_3CN: 4.0 mL. Column chromatography: eluting solvent 5−10% EtOAc/Hexane. Yield of **4l**: 42 mg (33%)white solid, R_f = 0.54 (35% EtOAc/Hexane).^{1}HNMR (DMSO-d_6, 500 MHz) δ 9.45 (s, 1H, OH), 8.46 (s, 1H, ArH), 8.41 (s, 1H, ArH), 8.29 (d, J = 10.5 Hz, 1H, ArH), 7.90 (dd, J = 10.5, 1.5 Hz, 1H, ArH),7.46-7.48 (m, 2H, ArH), 7.25-7.30 (m, 3H, ArH), 7.16-7.19 (m, 2H, ArH), 6.83 (t, J = 9.5 Hz, 1H, ArH), 6.78 (d, J = 10.5 Hz, 1H, ArH).^{13}C NMR (125 MHz, DMSO-d_6) δ 160.25, 154.29, 145.34, 139.29 (q, J = 247.9 Hz), 133.86, 131.06, 129.81, 129.62 (q, J = 40.1 Hz), 129.49, 129.00 (2C), 128.59, 128.25, 127.57 (2C), 126.31, 126.23 (q, J = 5.38 Hz), 125.53, 122.82, 121.83, 119.12, 115.47; ^{19}F NMR (DMSO-d_6, 500 MHz): δ -60.97 (s). IR (KBr) υ_{max} (cm^{-1}) 2922.5, 1593.9, 1452.5, 1322.5, 1289.3. HRMS (ESI-TOF) m/z calcd for $C_{22}H_{15}F_3NO$ [M + H]$^+$ 366.1106, found 366.1125.

4.1.15. 2-(2-Phenylquinolin-3-yl)phenol *(4m)*

2-Phenylquinoline 1-oxide **3m**: 75 mg (0.29 mmol); benzyne precursor: 130.7 mg (0.44 mmol); KF: 33.9 mg (0.58 mmol); 18-crown-6: 154.4 mg (0.58 mmol); CH_3CN: 3.5 mL. Column chromatography: eluting solvent 10−20% EtOAc/Hexane. Yield of **4m**: 47 mg (47%) brown solid, R_f = 0.66 (40% EtOAc/Hexane).^{1}HNMR (DMSO-d_6, 500 MHz) δ 9.62 (s, 1H, OH), 8.83 (s, 1H, ArH) , 8.36 (d, J = 8.5Hz, 1H, ArH), 8.25 (d, J = 8.0 Hz, 1H, ArH), 8.02 (t, J = 7.5 Hz, 1H, ArH), 7.84 (t, J = 7.5 Hz, 1H, ArH), 7.52-7.54 (m, 2H, ArH), 7.41-7.44 (m,1H, ArH), 7.35-7.38 (m, 2H, ArH), 7.17-7.20 (m, 1H, ArH), 7.14-7.16 (m, 1H, ArH), 6.80-6.85 (m, 2H, ArH).^{13}C NMR (125 MHz, DMSO-d_6) δ 156.78, 154.44 (2C), 132.31, 131.19 (2C), 129.82, 129.66, 129.51 (2C), 128.39, 128.32 (2C), 127.84 (2C), 127.18, 124.43, 119.09 (2C), 115.59 (2C).

4.2. Biology

4.2.1. Materials and methods

4.2.1.1. Growth media and Reagents. All bacterial media and supplements including Mueller-Hinton cation supplemented broth II (MHBII), Mueller-Hinton agar (MHA) and Tryptic soy broth (TSB) were purchased from Becton-Dickinson (Franklin Lakes, NJ, USA). All other chemicals and antibiotics were procured from Sigma-Aldrich (St. Louis, MO, USA). Roswell Park Memorial Institute Medium (RPMI) and Fetal Bovine Serum (FBS) were purchased from Lonza (Lonza, USA). All methods were performed in accordance with the relevant guidelines and regulations.

4.2.2. Bacterial strains

Compounds was screened against a bacterial panel consisting of ESKAPE pathogens, namely *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 29213), *Klebsiella pneumoniae* (BAA-1705), *Acinetobacter baumannii* (BAA-1605) and *Pseudomonas aeruginosa* (ATCC 27853). The panel was further expanded to include drug-resistant clinical *S. aureus* including those resistant to Vancomycin and other clinically-utilized antibiotics. These strains were procured from Biodefense and Emerging Infections Research Resources Repository/Network on Antimicrobial Resistance in *Staphylococcus aureus*/American Type Culture Collection (BEI/NARSA/ATCC, USA) and routinely cultivated on MHA and MHBII. Before starting the experiment, a single colony was picked from MHA plate, inoculated in MHBII and incubated overnight at 37 °C with shaking for 18–24 h to get the starter culture.

4.2.3. Antibiotic susceptibility testing

Antibiotic susceptibility testing of DSF was conducted according to the CLSI guidelines using the broth micro dilution assay.[69] 10 mg/mL stock solutions of test compounds were prepared in DMSO. Bacterial cultures were inoculated in MHBII and optical density (OD) was measured at 600nm, followed by dilution to achieve ~10^6 CFU/mL. The compounds were tested from 64–0.5 mg/L in two-fold serial diluted fashion with 2.5 μL of each concentration

added to well of a 96-well round bottom microtiter plate. Later, 97.5 μL of bacterial suspension was added to each well containing either test compound or appropriate controls. The plates were incubated at 37°C for 18-24 h following which the MIC was determined. The MIC is defined as the lowest concentration of the compound at which there is absence of visible growth. For each test compound, MIC determinations were carried out independently three times using duplicate samples.

4.2.4. Cell cytotoxicity of active compounds

Cell toxicity was performed against Vero cells using the MTT assay.[70] $\sim 10^3$ Cells/well were seeded in 96 well plate and incubated at 37°C in an 5% CO_2 atmosphere. After 24 h, compound was added ranging from 100-12.5 µg/mL concentration and incubated for 72 h. After the incubation was over, MTT was added in each well, incubated at 37°C for further 4 h, residual medium was discarded, 0.1 mL of DMSO was added to solubilise the formazan crystals and OD was taken at 540 nm for the calculation of CC_{50}. CC_{50} is defined as the lowest concentration of compound which leads to a 50% reduction in cell viability. Each experiment was repeated in triplicate.

4.3. Molecular docking study

Molecular docking studies were performed to investigate the various protein-ligand binding interactions of the newly synthesized antibacterial compounds in present study towards the topoisomerase II DNA gyrase. Crystal structure of topoisomerase target (PDB ID: 5BS3) obtained from the protein data bank. Autodock 4.2.6 [71], a protein-ligand docking tool have been employed for performing the molecular docking of optimized structures of compounds with protein target, whereas Biovia Discovery Studio Visualizer utilized for studying the protein-ligand interactions.[72] Optimized geometries of newly synthesized compounds have been obtained at the level of advanced PM7 [73] semi-empirical method using Gaussian 09[74] suite of quantum-chemistry software package.

Credit author statement

Shashi Janeoo Synthesis of compounds and their purification. Harminder Kaur Synthesis of compounds and their purification. Grace Kaul Biological experiments for antibacterial activity. Abdul Akhir Biological experiments cytotoxicity of the compounds. Sidharth Chopra Analysis of biological data and related intellectual inputs. Shaibal Banerjee Characterization of compounds and structure assignments. Reenu Molecular docking studies. Varinder Kumar Molecular docking studies. Rakesh Kumar Design and analysis of synthetic work, writing of manuscript.

Declaration of Competing Interest

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

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2021.130924.

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