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ARTICLE

Theoretical molecular predictions and antimicrobial activities of newly synthesized molecular hybrids of norfloxacin and ciprofloxacin

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Abstract

Antibiotics such as norfloxacin and ciprofloxacin are used to treat numerous bacterial infections. The present research work involves the molecular prediction, synthesis, characterization and in vitro antimicrobial activities of molecular hybrids of norfloxacin and ciprofloxacin. First set of compounds involve the substitutions of various amines at third position of ciprofloxacin and norfloxacin. On the other hand, second set of molecular hybrids include the substitution of different amines at seventh position along with linker (—$COCH_2$—). These synthesized compounds were identified by TLC technique and well characterized by various spectroscopic techniques such as IR, NMR, and ESI-MS. Molecular prediction of these newly synthesized compounds have been carried out using the SwissADME, ADMETLab software. Their cytotoxicity parameters have also been studied using Osiris software. It was observed that almost all these molecular hybrids suitable for their drug likeness properties. Further, these newly synthesized compounds were subjected to study their antimicrobial activities *in vitro*. The third substituted as well as most of seventh substituted molecular hybrids have shown 10-folds increase in their antibacterial activity as compared to the standard drug ciprofloxacin. Some of these compounds have also shown their potency when subjected to study their cytotoxicity test against *Escherichia coli AB 1157*, proficient to prepare damage in DNA.

1 | INTRODUCTION

Infectious diseases are generated from various pathogenic microorganisms like bacteria, virus, fungi, and so on.[1] Fluoroquinolones have been widely used to cure such infections.[2–7] Ciprofloxacin and norfloxacin, second generation fluoroquinolones, are categorized as broad spectrum antibiotics.[8,9] It is known to be the agent of choice for the treatment of various bacterial infections like shigella species which causes diarrhea and also for the treatment of tuberculosis, digestive disorder, anthrax, UTI, pneumonia, diabetic foot infection, typhoid, fever, and so on.[2–7,10,11] The presence of two carbonyl groups in these fluoroquinolones is important for binding with DNA gyrase. Some researchers have also studied the chelating process of these groups, which shows binding of these carbonyl groups with different cations.[12,13] But no clues are available which shows role of OH group of COOH at third position in these processes of chelating or binding with DNA gyrase.[14,15] Literature provides excessive data about antibiotics which mainly differ in their chemical structures having different substitution at fifth,

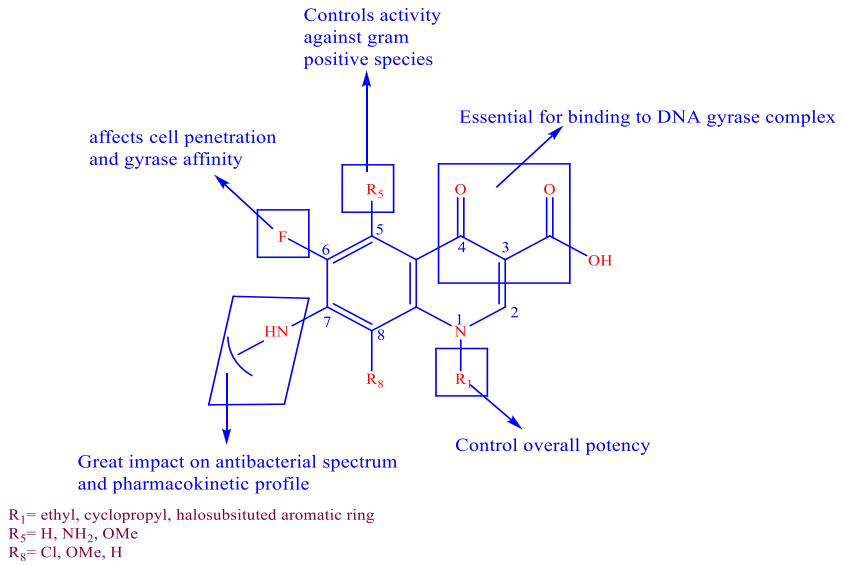


FIGURE 1 Structure of fluoroquinolones

seventh, and eighth positions of fluoroquinolones. Antibacterial activities are greatly affected by the various substituents at different positions as shown in Figure 1.[12,13] Moreover, these fluoroquinolones behave amphoterically due to the presence of basic group (secondary amine) and acidic group (carboxylic acid).[14–16]

Research has revealed some coupled reactions of ciprofloxacin with aromatic amines some of which show good antibacterial activity.[17,18] But the bacterial infections are becoming resistant to the fluoroquinolones these days. To overcome such problems, there are basically two ways to enhance the activity of drug. First, the new core molecules can be designed and synthesized which may show enhanced antibacterial activities. Second, new molecule hybrid of the existing fluoroquinolone can be formed, which may show improve biological activities with minimum side effects. In the present piece of work preclinical studies and molecular predictions of newly synthesized molecular hybrids ciprofloxacin and norfloxacin have been carried out to study various ADMET and Toxicology parameters. Further, these compounds have screened for antimicrobial activities and cytotoxicity test *in vitro*.

2 | RESULTS AND DISCUSSION

2.1 | Computational tools

An analysis of theoretical molecular predictions has provided strategy for physical properties and has been used to get computational algorithms to calculate the CNS efficacy as well as the toxicology of the newly synthesized molecules. ADMET studies and toxicological parameters are preclinical properties which help in screening the synthesis of new molecular hybrids before carrying out their biological activities. SwissADME, ADMETLab, and Osiris software are used to study and compare different physicochemical properties like binding properties, drug likeness, toxicity, and so on. Presently, we have synthesized two set of compounds of both ciprofloxacin and norfloxacin having substitutions at third position as well as seventh position. The formation, identification and characterization of the synthesized compounds have been confirmed by melting point and spectroscopic techniques. Broadly, the disappearance of stretching band (3600-3400 cm^{-1}) for OH group of carboxylic moiety in case of substitution at third position (Ia, Ib, IIa, IIb, Ic) and disappearance of NH stretching band (3400–3200 cm^{-1}) in case of substitution at seventh position (I′a, I′b, II′a, II′b), confirms the formation of coupled compounds. Moreover, number of proton count and their pattern for ^{1}H-NMR spectra as well as that of molecular ion peak in ESI-MS spectra further support the formation of all these synthesized compounds.

All the synthesized compounds have subjected to study these properties and compared with standard drug ciprofloxacin and norfloxacin. The enhancement in the binding properties has been observed for compounds II′b, I′b, and I′d. Presence of hetero atoms in the molecular system affects the hydrogen bond acceptor and hydrogen bond donor properties of the compound as shown in Table 1. The physicochemical parameter like LogP, LogD, and LogS provides data about the properties of Absorption, Distribution, and Solubility in the system as shown

TABLE 1 Parameters calculated by swissADME and ADMETlab

Compound	MW[a]	HBA[a]	HBD[a]	LogP[b]	LogD[b]	LogS[a]	TPSA[a]	%ABS[a]	BBB	GI
I	331.13	5	2	1.583	−0.705	−1.32	74.57	83.274	No	High
II	319.13	5	2	1.268	−0.757	−1.29	74.57	83.274	No	High
Ia	398.21	4	1	2.511	1.98	−3.20	57.58	89.077	Yes	High
Ib	400.19	5	1	1.357	1.491	−2.45	66.81	85.883	No	High
IIa	386.21	4	1	2.196	1.692	−3.17	57.58	89.077	Yes	High
IIb	388.19	5	1	1.042	1.369	−2.41	66.81	85.883	No	High
Ic	373.16	5	3	0.694	0.333	−2.55	109.46	71.24	No	High
Id	643.26	8	2	3.335	0.997	−4.78	120.12	67.56	No	High
II'a	456.22	6	1	2.308	0.126	−2.35	86.09	79.212	No	High
II'b	458.20	7	1	1.154	−0.187	−1.59	95.32	76.12	No	High
I'a	444.22	6	1	1.993	−0.096	−2.31	86.09	79.212	No	High
I'b	446.20	7	1	0.84	−0.242	−2.08	95.32	76.12	No	High
I'c	442.28	6	1	1.918	−0.156	−2.04	86.09	79.212	No	High
II'c	430.47	6	1	1.603	−0.246	−2.01	86.09	79.212	No	High
I'd	447.48	7	3	0.656	−0.675	−2.71	152.99	52.22	No	Low
II'd	435.47	5	3	0.341	−0.613	−2.67	152.99	56.22	No	Low

Abbreviations: %ABS = Percentage of absorption (%ABS = 109-0.345(TPSA); HBA, hydrogen bond acceptors; HBD, hydrogen bond donors; MW, molecular weight; TPSA, topological polar surface area.[21]

[a]Calculated by using SwissADME.

[b]Calculated by using ADMETlab.

in Table 1. Further the TPSA and solubility parameters inversely proportional to LogP.[19] Also low TPSA value leads to the good absorption in gastrointestinal (GI) track. Therefore, TPSA can be used as predefined tool to predict the transport path way of the particular type of drug. It was seen that compound Ia and Ic which were having TPSA value less than 70, have the tendency to penetrate Blood brain barrier (BBB) and also increase in hydrogen bonding causes the decrease in the BBB penetration as shown in Table 1. Broadly, all the compounds are having their TPSA value ranging from 57 to 120 which justify the penetration of the drug in GI.[19,20]

A uniform trend has been observed of the %ABS parameter for both set of compounds having substitutions at third position as well as seventh position. For the first set of compounds which show 89% value for compounds Ia and IIa having piperidine substitution at third position. Similar trend we have seen from other molecules having same substitution as shown in Table 1.

The studies have been extended by comparing the toxicities of all molecules using orisis software as shown in Table 2. According to that most of the compounds are showing very low toxic effects against (mutagenecity/carcinogenic) except Ic.[22]

2.2 | Antimicrobial activities

All these coupled compounds have been subjected for antimicrobial activities at six different concentration (2.5, 5, 10, 25, 50, 100 μg). Most of the synthesized compounds have shown MIC value (2.5 μg), which shows 10-folds more potency against gram positive and gram-negative strains used, except (I'c and II'c), when compared with ciprofloxacin. The zone of inhibition at minimum concentration 2.5 μg is greater for the synthesized compounds with substitution toward 3rd position (Ia, Ib, IIa, IIb, Ic, and Id) as shown in Tables 3 and 4, also represented graphically as shown in Figure 2 than for compound with substitution at 7th position (I′a, I′b, II′a, II′b, I′c, II′c, I′d, II′d) as shown in Table 5 and Table 6. Exceptionally, **IIb** compound has greater zone of inhibition even at minimum concentration for both gram positive and gram negative strains as shown in Figure 3 graphically. Further, it has been observed that these compounds are inactive against fungi *Candida albicans*. In the cytotoxicity analysis third substituted derivatives of ciprofloxacin have significance minimum inhibitory concentration and zone of inhibition as compared to standard drug ciprofloxacin. Zone of inhibition increased with the increase in concentration of the compound. These synthesized

TABLE 2 Predicted toxicity effects using Orisis software

Compound	Mutagenic	Tumorigenic	Reproductive effective	Irritant
I	None	None	None	None
II	None	None	None	None
Ia	None	None	High	None
Ib	None	None	High	None
IIa	None	None	High	None
IIb	None	None	High	None
Ic	Low	Low	Low	None
Id	None	None	High	None
II'a	None	None	None	Low
II'b	None	None	None	None
I'a	None	None	None	Low
I'b	None	None	None	None
I'c	None	None	None	High
II'c	None	None	None	High
I'd	None	None	None	None
II'd	None	None	None	None

compounds have MIC value 25 μg which is fourfold more potent than ciprofloxacin (100 μg) except II'c (250 μg) as shown in Table 7.

3 | EXPERIMENTAL

3.1 | Materials and methods

The 250 mg tablets of Norfloxacin were purchased from the market under the brand Noxitef. Ciprofloxacin was bought from Sigma-Aldrich. The melting points were recorded by open capillary method. Methanol, acetone, piperidine, and morpholine were used of Lobachem. Sodium Hydroxide and Urea was used from Merck chemicals. The IR spectra were obtained by Bruker alpha-E and RC FT-IR KBr pellets on a Perkin Elmer RXIFT Infrared spectrophotometer. NMR spectra were recorded on Bruker Avance 400 MHz spectrophotometer with TMS as an internal standard. The mass spectra were recorded on the Waters Micromass Q-T of Micro (ESI) spectrometer. All the synthesized compounds have been evaluated for their antibacterial and antifungal activity in vitro by using agar diffusion method. Four bacterial strains have been used namely, *Bacillus subtilis, Staphylococcus aureus* (Gram positive) and *Escherichia coli, Pseudomonas aeruginosa* (Gram negative) and *Candida albicans* strain were used for antifungal activity. Ciprofloxacin, norfloxacin, and fluconazole have been used as standard drug for antibacterial and antifungal activity. Cytotoxicity test has also been carried out by using agar diffusion method against strain *E. coli AB 1157*.

3.2 | Synthesis of 1-alkyl-6-fluoro-7-(piperazin-1-yl)-3-(substituted amine) quinolin-4(1*H*)-one

(2.5 mmol, 0.827 g) of ciprofloxacin or (2.5 mmol, 0.80 g) of norfloxacin was refluxed after the addition of 15 mL of methanol and 2 to 3 drops of concentrated sulphuric acid for 8 to 10 hour at 35°C, respectively. After the formation of ester derivatives of ciprofloxacin or norfloxacin, (2.5 mmol) different aliphatic amines were added in the reaction mixture and refluxed for 7 to 8 hours as shown in Scheme 1. The product formation, as monitored by thin layer chromatographic technique, was extracted after the removal of solvent by vacuum rotary evaporator. The solid product was extracted and washed with the mixture of methanol-chloroform (2:8).[17] The pure samples were filtered, dried, and identified by using different spectroscopic techniques.

3.3 | Synthesis of 1-alkyl-6-fluoro-4-oxo-7-(4-(2-(substituted amine)acetyl) piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid

(9 mmol, 2.98 g) of ciprofloxacin or (9 mmol, 2.87 g) of norfloxacin was stirred with 40 mL dichloromethane and trimethylamine in equimolar ratio (9 mmol), respectively, for 20 minutes at 0 to 5 °C followed by drop wise addition

TABLE 3 Antibacterial activities against gram positive strains of 3rd-sustituted molecular hybrids (zone of inhibition in mm)

Bacterial strains →	*B. subtilis*							*S. aureus*						
Concentration →	2.5 μg	5 μg	10 μg	25 μg	50 μg	100 μg	MIC μg	2.5 μg	5 μg	10 μg	25 μg	50 μg	100 μg	MIC μg
Compound														
Ia	10	12	14	17	20	23	2.5	15	18	21	25	28	*	2.5
Ib	12	14	16	20	23	25	2.5	14	17	20	26	30	*	2.5
IIa	15	16	24	25	28	*	2.5	16	18	20	21	25	*	2.5
IIb	18	21	23	25	27	*	2.5	8	14	20	25	30	*	2.5
Ic	10	15	18	20	21	24	2.5	16	20	22	26	30	*	2.5
Id	11	13	15	18	22	25	2.5	15	19	21	26	28	*	2.5
Ciprofloxacin	-	-	-	8	10	15	25	-	-	-	13	18	21	25

TABLE 4 Antibacterial activities against gram negative strains of 3rd-sustituted molecular hybrids (zone of inhibition in mm)

Bacterial strains →	*E. coli*							*P. aeruginosa*						
Concentration →	2.5 μg	5 μg	10 μg	25 μg	50 μg	100 μg	MIC μg	2.5 μg	5 μg	10 μg	25 μg	50 μg	100 μg	MIC μg
Compound														
Ia	15	19	20	22	25	27	2.5	11	13	15	17	19	21	2.5
Ib	12	14	16	20	23	25	2.5	14	17	20	26	30	*	2.5
IIa	15	18	22	23	28	*	2.5	18	20	25	27	30	*	2.5
IIb	16	20	24	27	30	*	2.5	20	21	23	25	30	*	2.5
Ic	11	15	20	24	26	30	2.5	10	15	18	20	21	24	2.5
Id	15	17	20	23	26	28	2.5	7	10	13	15	18	23	2.5
Ciprofloxacin	-	-	-	18	20	23	25	-	-	-	0	0	1	100

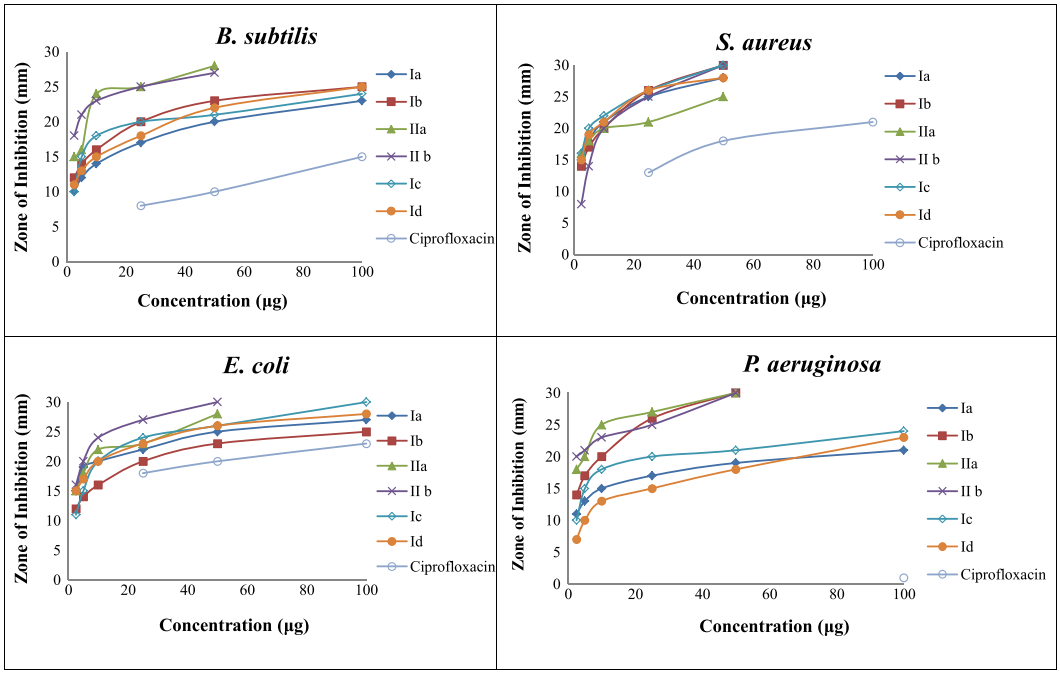


FIGURE 2 Graphical representation of 3rd-substituted molecular hybrids

of 1.1 mL (14 mmol) of α-chloroacetyl chloride. After the complete addition of the α-chloroacetyl chloride the mixture was initially stirred at 0 to 5°C for 15 to 20 minutes followed by stirring at room temperature for 36 hours. The formation of the product has been monitored by single spot on thin layer chromatography which differs from that of reactant. The reaction mixture was then filtered and the resulting solid was dissolved in dichloromethane. The crude sample was given the washings of saturated solution of sodium chloride (3 × 30 mL). Organic layer was dried over anhydrous sodium sulphate and after the removal of solvent pure sample was obtained as shown in Scheme 2.[8]

Thirty five micro liter of acetonitrile was added to 1.2 mmol of chloroacetyl derivatives of Ciprofloxacin or norfloxacin. The reaction mixture was stirred after the addition of 2.4 mmol triethylamine, 1.2 mmol of sodium iodide, and 2.4 mmol of secondary aliphatic amines for 48 hours, respectively. The reaction was filtered and resulting solid was dissolved in dichloromethane, which was given the washings of saturated solution of sodium chloride (3 × 30 mL). The organic layer collected and dried over anhydrous sodium sulphate, pure sample was obtained after the removal of solvent as shown in Scheme 2.[23]

3.4 | Characterization data

3.4.1 | 1-cyclopropyl-6-flouro-7-(piperazin-1-yl)-3-(piperidine-1-carbonyl)quinolin-4-(1*H*)-one (Ia)

Pure creamish solid; yield 62% to 63%; mp 240 to 242°C decompose; IR (KBr) cm^{-1}: ν_{max} 3317.67 (N-H str.), 3045.45, 3014.52 (Ar C-H str.), 2961.58, 2909.60, 2846.53 (C-H str.), 1617.28, (C=O; keto), 1590.32 (C=O), 1331.46 (C-N); ^{1}H-NMR (400 MHz, DMSO) δ (ppm): 8.66 (s, 1H, H-2), 7.87 (d, 1H, H-5, $J_{H\text{-}F}$ = 13.44 Hz), 7.53 (d, 1H, H-8, J = 7.56 Hz), 3.82 (d, 2H, H-4′, H-1 of cyclopropyl, J = 3.6 Hz), 3.55 (s, 4H, H-2′, 4′), 3.37 to 3.16 (m, 6H, H-3′, 5′, 3″), 2.90 (s, 6H, H-2″, 6″, 5″), 1.58 (s, 2H, H-4″), 1.30 (d, 2H, cyclopropyl, J = 6.12 Hz), 1.18 (m, 2H, cyclopropyl); ESI-MS: Molecular ion peak = 398 (M^+ − NH) m/z = 382.

3.4.2 | 1-cyclopropyl-6-flouro-3-(morpholine-4-carbonyl)-7-(piperazin-1-yl)quinoline-4-(1*H*)-one (Ib)

Pure creamish solid; yield 58 to 59%; mp 246 to 248°C decompose; IR (KBr) cm^{-1}: ν_{max} 3355.67 (N-H str.), 3045.70 (Ar C-H str.), 2921.72, 2850.71 (C-H str.), 1621.55 (C=O;

TABLE 5 Antibacterial activities against gram positive strains of 7th-sustituted molecular hybrids (zone of inhibition in mm)

Bacterial strains →	*B. subtilis*							*S. aureus*						
Concentration →	2.5 μg	5 μg	10 μg	25 μg	50 μg	100 μg	MIC μg	2.5 μg	5 μg	10 μg	25 μg	50 μg	100 μg	MIC μg
Compound														
I′a	0	6	9	12	13	19	5	13	15	17	19	21	23	2.5
I′b	8	11	13	15	17	20	2.5	13	15	18	20	28	*	2.5
II′a	7	12	15	17	19	25	2.5	15	19	20	23	27	*	2.5
II′b	0	0	5	8	10	15	10	5	6	9	12	15	18	2.5
I′c	0	0	0	3	5	7	25	0	0	0	3	5	10	25
II′c	0	0	0	0	3	5	50	0	0	0	0	3	7	50
I′d	3	6	6	10	12	14	2.5	5	8	8	11	15	19	2.5
II′d	0	6	9	11	17	21	5	5	10	10	14	16	18	2.5
Ciprofloxacin	-	-	-	8	10	15	25	-	-	-	13	18	21	25

TABLE 6 Antibacterial activities against gram negative strains of seven-sustituted molecular hybrids (zone of inhibition in mm)

Bacterial strains →	*E. coli*							*P. aeruginosa*						
Concentration →	2.5 μg	5 μg	10 μg	25 μg	50 μg	100 μg	MIC μg	2.5 μg	5 μg	10 μg	25 μg	50 μg	100 μg	MIC μg
Compound														
I′a	4	5	8	10	13	17	2.5	11	13	15	17	19	21	2.5
I′b	5	7	9	11	14	16	2.5	12	13	15	18	20	25	2.5
II′a	0	0	5	7	9	13	10	15	16	18	21	23	*	2.5
II′b	0	0	4	6	8	9	10	0	9	10	12	14	16	5
I′c	0	0	0	0	0	10	100	0	0	0	0	5	10	50
II′c	0	0	0	3	5	8	25	0	0	0	5	6	8	25
I′d	3	3	0	5	5	11	2.5	5	6	10	16	18	21	2.5
II′d	3	6	5	10	12	14	2.5	0	10	16	18	21	22	2.5
Ciprofloxacin	-	-	-	18	20	23	25	-	-	-	0	0	1	100

keto), 1581.63 (C=O), 1334.70 (C-N), 1293.60 (C-O); ^{1}H-NMR (400 MHz, DMSO) δ (ppm): 8.71 (s, 1H, H-2), 7.87 (d, 1H, H-5, J_{H-F} = 13.28 Hz), 7.50 (d, 1H, H-8, J = 7.36 Hz), 3.78 (d, 1H, H-4′), 3.64 (d, 2H, H-3″, J = 4.28 Hz), 3.62 (s, 4H, H-2′, 6′), 3.30 (t, 4H, H-3′, 5′), 3.01 (t, 4H, H-2″, 6″), 2.84 (t, 1H, H-1 cyclopropyl), 1.38 (d, 2H, H-5″, J = 6.6 Hz), 1.24 (s, 2H, cyclopropyl), 1.20 (s, 2H, cyclopropyl); ^{13}C-NMR (400 MHz, DMSO) δ (ppm): 176.26, 165.96, 154.21, 147.86, 145.54, 145.44, 139.15, 118.41, 110.95, 110.73, 106.64, 106.10, 50.09, 50.05, 44.94, 35.80, 7.51; ESI-MS: Molecular ion peak (M^+- Δ) m/z = 360.

3.4.3 | 1-ethyl-6-flouro-7-(piperazin-1-yl)-3-(piperidine-1-carbonyl)quinolin-4(1*H*)-one (IIa)

Pure creamish solid; yield 62 to 63%; mp 266 to 268°C decompose; IR (KBr) cm^{-1}: ν_{max} 3392.94 (N-H str.), 2996.74 (Ar C-H str.), 2892.29, 2825.27 (C-H str.), 1678.12 (C=O; keto), 1629.81 (C=O; amine), 1389.58 (C-N), 1360.02 (C-O); ^{1}H-NMR (400 MHz, DMSO) δ (ppm): 8.96 (s, 1H, H-2), 7.93 (d, 1H, H-5, J_{H-F} = 12.84 Hz), 7.25 (d, 1H, H-8,), 4.61 (s, 2H, -CH_2CH_3), 3.51 (11H, H-2′, 3′, 4′, 5′, 6′) 3.06 (8H, H-2″, 3″, 4″, 5″, 6″), 1.42 (s, 3H, -CH_2CH_3); ^{13}C-NMR (400 MHz, DMSO) δ (ppm): 176.07, 166.06, 148.57, 144.38, 137.04, 119.92, 111.44, 111.22, 107.08, 106.41, 52.93,49.14, 46.51, 42.75, 14.38; ESI-MS: m/z = 342.

3.4.4 | 1-ethyl-6-flouro-3-(morpholine-4-carbonyl)-7-(piperazin-1-yl)quinoline-4-(1*H*)-one (IIb)

Pure creamish solid; yield 58 to 59%; mp 260 to 262°C decompose; IR (KBr) cm^{-1}: ν_{max} 3405.76 (N-H str.), 2988.59 (Ar C-H str.), 2827.11 (C-H str.), 1710.02 (C=O; keto), 1628.92 (C=O; amine), 1389.80 (C-N), 1368.98 (C-O); ^{1}H-NMR (400 MHz, DMSO) δ (ppm): 8.96 (s, 1H, H-2),7.92 (d, 1H, H-5), 7.25 (s, 1H, H-8, J_{H-F} = 13.04 Hz), 4.61 (s, 2H, CH_2CH_3, J = 7.36 Hz), 3.43 (8H, H-2′,3′,4′, 5′, 6′), 3.10 (9H, H-2″, 3″, 4″, 5″, 6″, J = 4.28 Hz), 1.42 (s, 3H, s, 1H); ^{13}C-NMR (400 MHz, DMSO) δ (ppm): 176.11, 166.00, 153.92, 151.44, 148.63, 144.43, 144.33, 137.06, 119.90, 111.44, 111.22, 107.12, 106.42, 52.85, 49.08, 46.72, 42.83, 14.42; ESI-MS: m/z = 334.

3.4.5 | N-carbamoyl-1-cyclopropyl-6-flouro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxamide (Ic)

Pure light brown solid, yield 69% to 70%, mp 276 to 278°C decompose; IR (KBr) cm^{-1}: N_{max} 3394.60 (N-H str.), 3063.49 (Ar C-H str.), 2807.52 (C-H str.), 1717.51 (C=O; keto), 1690 53 (C=O; amide), 1629.42 (C=O; amide), 1494.42 (1^0 N-H), 1459.43 (2^0 N-H), 1381.59 (C-N). ^{1}H-NMR (400 MHz, DMSO) δ (ppm): 14.88 (s, 1H, N-H, D_2O exchangable proton), 8.89 (s, 2H, NH_2, D_2O exchangeable proton), 8.65 (s, 1H, H-2), 7.88 (d, 1H, H-5, J_{H-F} = 13.02 Hz), 7.59 (d, 1H, H-8, J = 7.2 HZ), 3.84 (s, 1H, H-4′, D_2O exchangeable proton), 3.72 (q, 1H, H-1 of cyclopropyl), 3.65, 3.55 (d, 4H, H-2′, 6′), 3.35 (dd, 4H, H-3′, 5′), 1.33 (d, 2H, cyclopropyl, J = 6.2 Hz), 1.18 (s, 2H, cyclopropyl); ^{13}C-NMR (400 MHz, DMSO) δ (ppm): 176.29, 165.79, 154.05, 151.58, 148.09, 144.08, 143.98, 139.00, 119.30, 119.22, 111.22, 110.99, 106.88,106.76, 46.38, 46.33, 42.69, 35.94, 7.58; ESI-MS: Molecular ion peak = 373 (M^+ − CH_2 = CH_2) m/z = 346, base peak = 332

3.4.6 | 1-cyclopropyl-7-(4-(1-cyclopropyl-6-flouro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carbonyl)piperazin-1-yl)-6-flouro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (Id)

Creamish yellow solid, yield 78 to 79% mp 298 to 300°C decompose; IR (KBr) cm^{-1}: ν_{max} 3418.30 (O-H str.), 3022.26 (Ar C-H str.), 2826.28 (C-H str.), 1720.25 (C=O; keto), 1629.23 (C=O; amine), 1340.35 (C-N); ^{1}H-NMR (400 MHz, DMSO) δ (ppm):14.94 (1brs, 1H, OH), 8.92 (s, 1H, H-2), 8.69 (s, 1H, H-2″), 7.91 (d, 1H, H-5, J_{H-F} = 13 Hz), 7.58 (d, 1H, H-8, J = 6.96 Hz), 3.83 to 3.35 (m, 10H, H-2′, 3′, 4′, 5′, 6′, H-1 of cyclopropyl), 1.35 (d, 2H, cyclopropyl, J = 6.4 Hz), 1.17 (s, 2H, cyclopropyl); ESI-MS: Molecular ion peak (M^+ + K) m/z = 684.

3.4.7 | 1-cyclopropyl-6-flouro-4-oxo-7-(4-(piperidine-1-carbnyl)piperazin-1-yl)-1,4- dihydroquinoline-3-carboxylic acid (I′a)

Pure yellow solid; yield 60 to 61%; mp 248 to 250°C decompose; IR (KBr) cm^{-1}: 3094 (Ar C-H str.), 1723 (C=O; acid), 1645 (C=O; amide), 1630 (C=O; keto), 1253 (C-N-C stretch of piperidine ring); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 14.96 (brs, 1H, COOH), 8.75 (s, 1H, H-2), 8.01 (d, 1H, H-5), 7.37 (s, 1H, H-8), 5.3 (s, 1H), 3.88 (d, 4H, H-3, 5′), 3.57 to 3.29 (7H, $COCH_2$, H-cyclopropyl, H-2′, H-6′), 2.48 (s, 4H, H-2″, 6″), 1.86 (d, 2H), 1.60 (s, 4H, cyclopropyl), 1.43 (d, 4H), 1.23 (d, 2H, H-4″); ESI-MS: Molecular ion peak (M + 1) m/z = 457.

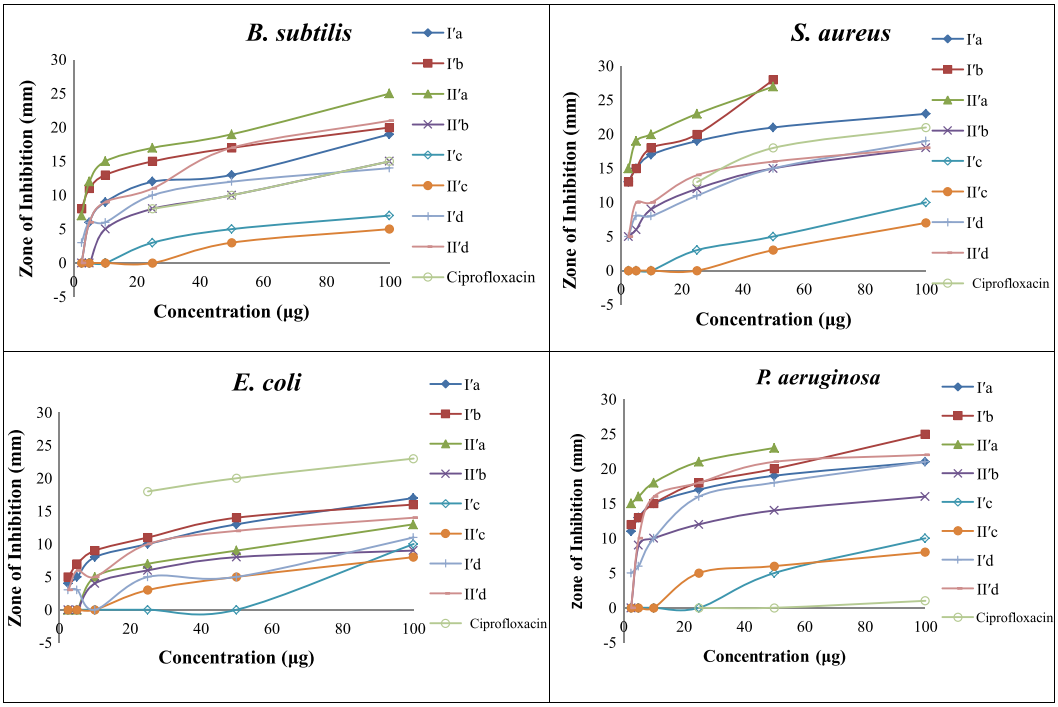
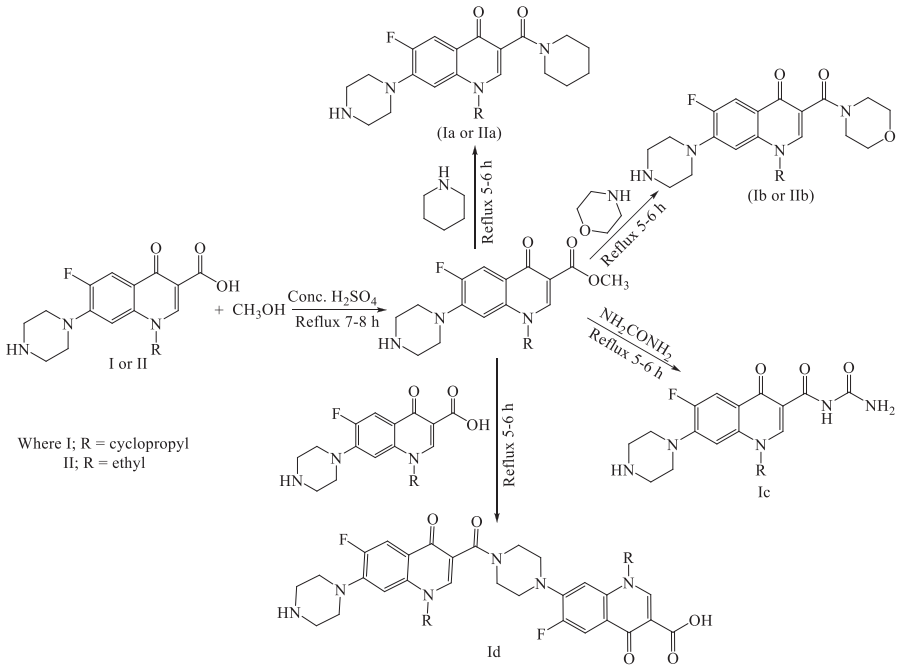


FIGURE 3 Graphical representation of 7th-substituted molecular hybrids

TABLE 7 Cytotoxicity test (zone of inhibition in mm)

Strain →	*E. coli AB 1157*						
Conc. →	25 µg	50 µg	100 µg	250 µg	500 µg	1000 µg	MIC µg
Compound							
Ia	21	23	25	29	31	*	25
Ib	25	27	28	31	34	*	25
Ic	20	22	24	28	30	*	25
Id	20	23	25	27	30	40	25
IIb	*	*	*	*	*	*	25
I'c	8	10	8	12	14	16	25
II'c	0	0	0	8	10	12	250
I'd	12	16	16	18	20	21	25
II'd	12	18	20	21	21	23	25
Ciprofloxacin	-	-	1	3	8	*	100

Note. *Zones could not measure due to merging.



SCHEME 1 1-alkyl-6-fluoro-7-(piperazin-1-yl)-3-(substituted amine) quinolin-4(1*H*)-one

3.4.8 | 1-cyclopropyl-6-flouro-7-(morpholine-4-carbonyl)-7-(piperazin-1-yl)quinoline-4-oxo-1,4-dihydroquninoline-3-carboxylic acid (I′b)

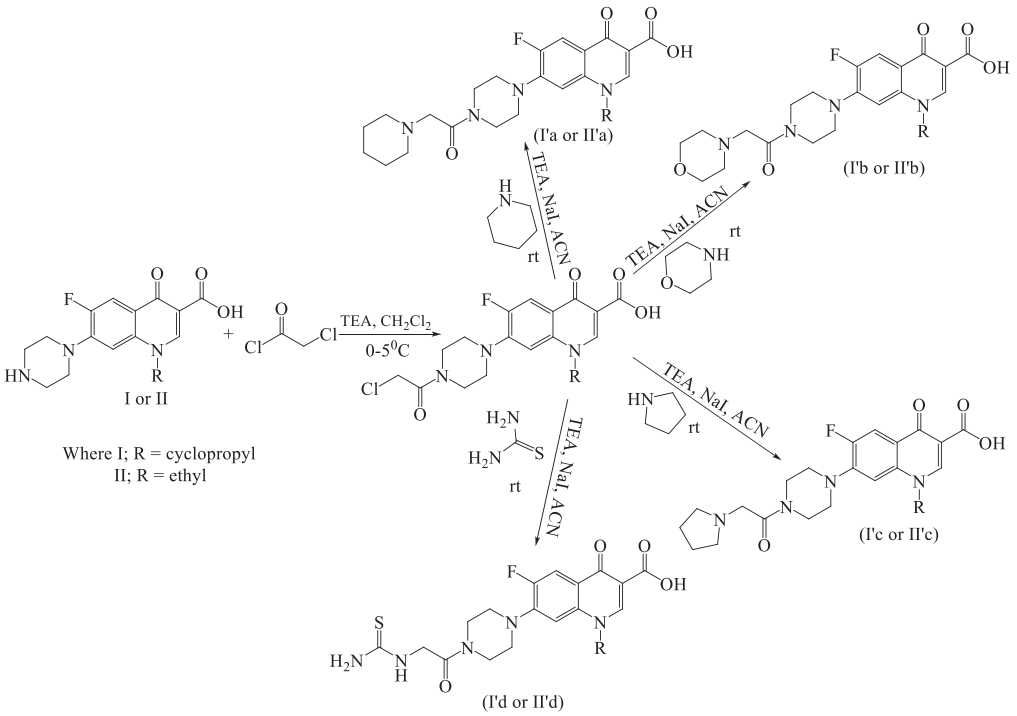
Pure creamish solid; yield 58 to 59%; mp 252 to 254°C decompose; IR (KBr) cm^{-1}: ν_{max} 3049.3 (Ar C-H str.), 2907.9, 2851.0 (C-H str.), 1737.6 (C=O; acid), 1643.6 (C=O; amide), 1626.5 (C=O; keto), 1385.5 (C-N), 1297.7 (C-O), 1117.6 (C-O-C); ^{1}H-NMR (400 MHz, DMSO) δ (ppm): 15.24 (brs, 1H, COOH), 8.67 (s, 1H, H-2), 7.93 (d, 1H, H-5, $J_{H\text{-}F}$ = 13.28 Hz), 7.58 (d, 1H, H-8, J = 7.4 Hz), 5.74 to 3.33 (18H, $COCH_2$, H-3′, 5′, 3″, 5″, H-2′, 6′, 6″, H-cyclopropyl), 1.31 (d, 2H, H-cyclopropyl), 1.19 (t, 2H); ESI-MS: Molecular ion peak (M + Na) m/z = 481, (M + 1) = 459, (M + 2) m/z = 460.

3.4.9 | 1-ethyl-6-flouro-4-oxo-7-(4-(piperidine-1-carbnyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (II′a)

Dark brown solid; yield 61 to 62%; mp 262 to 264°C decompose; IR (KBr) cm^{-1}: ν_{max} 3069 (Ar C-H str.), 1625 (C=O; acid), 1657 (C=O; amide), 1628 (C=O; keto), 1292 (C-N-C) stretch of piperidine ring); ^{1}H-NMR (400 MHz, DMSO) δ (ppm): 15.11 (brs, 1H, COOH), 8.94 (s, 1H, H-2), 8.26 to 7.78 (1H, H-5), 7.13 to 7.09 (1H, H-8, $J_{H\text{-}F}$ = 7.28 Hz), 4.59 (s, 2H, CH_2CH_3), 3.78 to 3.14 (10H, $COCH_2$, H-3′, 5′, H-2′, 6′), 2.68 to 2.36 (4H, H-2″, 6″), 1.49 (s, 2H), 1.40 (d, 4H, H-3″, 4″, 5″), 1.23 (s, 3H, CH_2CH_3); ESI-MS: Molecular ion peak (M + 2) m/z = 446, (M + 1) m/z = 445.

3.4.10 | 1-ethyl-6-flouro-3-(morpholine-4-carbonyl)-7-(piperazin-1-yl)quinoline-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (II′b)

Mustard brown solid; yield 66 to 67%; mp 260 to 262°C decompose; IR (KBr) cm^{-1}: ν_{max} 3069 (Ar C-H str.), 1626 (C=O), 1628.92 (C=O), 1292 (C-N-C), 1127 (C-O-C); ^{1}H-NMR (400 MHz, DMSO) δ (ppm): 15.30 (brs, 1H, COOH), 8.96 (s, 1H, H-2), 7.93 (d, 1H, H-5, $J_{H\text{-}F}$ = 12 Hz), 7.07 (d, 1H, H-8, J = 5.3 Hz), 4.59 (2H, CH_2CH_3), 3.88 to 3.21 (17H, H-3″, $COCH_3$, H-3″, 5″, H-3′, 5′, H-2′, 6′), 2.44 to 1.41 (7H, H-2″, 6″, H-5″, CH_2CH_3); ESI-MS: Molecular ion peak (M + Na) m/z = 469, (M + Na + 1) m/z = 470, (M + 1) m/z = 447.



SCHEME 2 Synthesis of 1-alkyl-6-fluoro-4-oxo-7-(4-(2-(substituted amine)acetyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid

3.4.11 | 1-cyclopropyl-6-flouro-4-oxo-7-(4-(2-(pyrrolidin-1-yl)acetyl)piperazin-1-yl)-1,4-dihydroquninoline-3-carboxylic acid (I′c)

Light yellow solid; yield 52 to 53%; mp 264 to 266°C decompose; IR (KBr) cm^{-1}: ν_{max} 3050, 2957.52 (Ar C-H str.), 2849.3 (C-H str.), 2723.4 (O-H str.),1739.4 (C=O; carboxylic acid) 1720.38 (C=O; keto), 1643.45 (C=O; amide), 1384.68 (C-N), 1248.66 (C-O); ^{1}H-NMR (400 MHz, DMSO) δ (ppm): 8.96 (s, 1H, H-2),7.92 (d, 1H, H-5), 7.25 (s, 1H, H-8, J_{H-F} = 13.04 Hz), 4.61 (s, 1H, 3" CH_2 and 2H, 8′,), 3.51 (4H, H-,3′, 5′,), 3.39 (4H, H-2′, 6′, J = 4.28 Hz), 3.25(s, 4H, 10′, 13′) 1.69 (s, 4H, 10′, 13′); ESI-MS: Molecular ion peak m/z = (M + 1) = 443.

3.4.12 | 1-ethyl-6-flouro-4-oxo-7-(4-(2-(pyrrolidin-1-yl)acetyl)piperazin-1-yl)-1,4-dihydroquninoline-3-carboxylic acid (II′c)

Creamish solid; yield 50 to 51%; mp 252 to 254°C decompose; IR (KBr) cm^{-1}: ν_{max} 2956.69 (Ar C-H str.), 2848.92 (C-H str.), 2727.6 (O-H str.), 1723.80 (C=O; keto), 1624.55 (C=O; amine), 1378.22 (C-N), 1258.78 (C-O); ^{1}H-NMR (400 MHz, DMSO) δ (ppm): 15.52 (s, 1H, 2" OH), 8.92 (s, 1H, H-2),7.92 (d, 1H, H-5, J_{H-F} = 12.72 Hz), 7.18 (s, 1H, H-8,), 4.57 (s, 2H, 3"CH_2CH_3), 4.39 (2H, H-8' $COCH_2$), 3.75 (t, H-2′, 3′, 5′, 6′), 3.41 (4H, 10′, 13′), 1.69 (s, 4H, 11′, 12′), 1.29 (t, 3H, H-4" CH_2CH_3); ESI-MS: Molecular ion peak m/z = (M + 1) = 431.

3.4.13 | 3.4.13 7-(4-(carbamothioylglycyl) piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (I′d)

Light green solid; yield: 46 to 47%; mp 274 to 276°C decompose; IR (KBr) cm^{-1}: ν_{max} 3393.5 (N-H str.), 2917.35 (Ar C-H str.), 2850.6 (C-H str.), 2617.3 (O-H str.), 1714 (C=O; carboxylic acid), 1615.36 (C=O; ester), 1577.97 (C=O; amine), 1377.11(C=S), 1291.88 (C-N), 1255.81 (C-O);^{1}H-NMR (400 MHz, DMSO) δ (ppm):8.67 (s, 1H, H-2), 7.92 (d, 1H, H-5, J_{H-F} = 13.2 Hz), 7.55 (d, 1H, H-8, J_{H-F} = 6.8 Hz), 3.84 (d, 2H, H-8′), 3.36 to 2.33 (m, 8H, 2′, 3′, 5′, 6′,), 1.25 to 1.92 (3H, -NH, -NH_2), 0.94 to 0.93 (4H, 5″, 6″); ESI-MS: Molecular ion peak m/z = (M + K) = 486.

3.4.14 | 3.4.14 7-(4-(carbamothioylglycyl) piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (II′d)

Light brown solid; yield: 45 to 46%; mp 268 to 270°C decompose; IR (KBr) cm^{-1}: ν_{max} 3401.59 (N-H str.), 2918.09 (Ar C-H str.), 2849.98 (C-H str.), 1707.39 (C=O; carboxylic acid), 1615.36 (C=O; ester), 1577.97 (C=O; amine), 1377.11(C=S), 1291.88 (C-N), 1255.81 (C-O); ^{1}H-NMR (400 MHz, DMSO) δ (ppm):15.31 (s, 1H, OH), 8.96 (s, 1H, H-2), 7.94 (s, 1H, H-5), 7.21 (s, 1H, H-8,), 4.60 (2H, $-CH_2CH_3$), 3.69 (2H, 8′), 3.69 to 1.42 (8H, 2′, 3′, 5′, 6′,), 1.42 (3H, -NH, $-NH_2$), 1.12 (3H, $-CH_2CH_3$); ESI-MS: Molecular ion peak m/z = (M + K) = 474.

3.5 | Antibacterial assay

Antibacterial activity was studied against two gram-positive strains *B. subtilis* and *S. aureus* and two gram-negative strains *E. coli* and *P. aeruginosa*. For the analysis, agar diffusion method was carried out. For the solid cultural media 10 g peptone, 10 g sodium chloride, 5 g yeast extract and 20 g agar were dissolved in 1000 ml of distilled water. The stock cultures were inoculating in broth media and grown at 37°C for 18 hours and also revived. The above stock solution poured in the petri plates and wells were made in the plate. For 18 hours old cultures (100 μL 10^{-4} cfu) inoculated for each plate and spread consistently on the plates. The wells were filled after 20 minutes containing different concentrations of samples and antibiotic. After that at 37°C all the plates incubated and zone of inhibition in diameter (mm) noted after 24 hours.[24,25]

3.6 | Antifungal assay

These coupled compounds were also subjected to study antifungal activity against fluconazole as standard. The Agar diffusion method was used for studying the results against the fungi *Candida albicans*. The solid culture media Czapek-Dox Agar prepared which is the composition of 30 g sucrose, 2 g sodium nitrite, 1 g K_2HPO_4, 0.5 g $MgSO_4.7H_2O$, 0.5 g KCl, 0.01 g $FeSO_4$, and 20 g agar dissolved in 1000 mL of distilled water. This stock culture is inoculated in broth media and grown at 27°C for 48 hours and also revived. The above stock solution poured in the petri plate and wells were made in each petri plate. For 48 hours old cultures (100 μL 10^4 CFU) inoculated for each plate and spread consistently on the plate. The wells were filled after 20 minutes containing different concentrations of samples and antibiotic. After that at 27°C all the plates incubated and zone of inhibition noted in diameter (mm) after 96 hours.[24,25]

3.7 | Cytotoxic assay

The wild-type strain *E. coli AB 1157* was used for the cytotoxicity studies. For the solid cultural media 10 g tryptone, 10 g sodium chloride, 5 g yeast extract and 20 g agar were dissolved in 1000 mL distilled water. Initially, the stock culture of bacteria was revived by inoculating in broth medium and grown at 37°C for 18 hours. All the compounds were dissolved in DMSO. The LB agar plates were prepared and wells were made in the solidified LB agar plate. Each plate was inoculated with 18 hours old cultures and spread evenly on the plates. After 20 minutes, the wells were filled with compounds at different concentration. All the plates were incubated at 37°C to 24 hours and zone of inhibition measured in diameter mm.[24,25]

4 | CONCLUSIONS

The molecular predictions for almost all the synthesized compounds were evaluated and have shown highly efficient results except Ia and IIa, which entered in the blood brain barrier due to their low TPSA value. The molecular hybrids at third position of ciprofloxacin and norfloxacin are showing promising antibacterial activity as compared to seventh substituted molecular hybrids. But all the molecular hybrids are inactive against fungal strain *Candida albicans*. Moreover, IIb compound have shown highest antibacterial activity. Enhanced cytotoxicity results of all the synthesized compounds have been observed as compared to ciprofloxacin. It is further long way to study and synthesized more molecular hybrid with substitution toward third position and to find their efficiency for binding with DNA gyrase.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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