

Ciprofloxacin-Tethered 1,2,3-Triazole Conjugates: New Quinolone Family Compounds to Upgrade Our Antiquated Approach against Bacterial Infections

Alka Agarwal,* Preeti Singh, Anand Maurya, Upendra Kumar Patel, Alka Singh, and Gopal Nath



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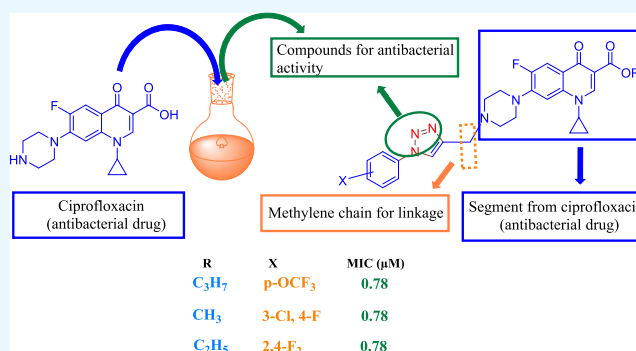


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ABSTRACT: A newer ciprofloxacin series containing 1,2,3-triazole conjugates of ciprofloxacin was designed, synthesized, and well characterized using modern analytical techniques by reacting diversified anilines with ciprofloxacin obtained from ciprofloxacin hydrochloride. The newer conjugates were evaluated for their antimicrobial activity against various strains, viz. *Staphylococcus aureus* (ATCC25923), *Enterococcus faecalis* (clinical isolate), *Staphylococcus epidermidis* (ATCC3594), *Escherichia coli* (ATCC25922), *Pseudomonas aeruginosa* (ATCC27853), *Salmonella typhi* (clinical isolate), *Salmonella typhimurium* (clinical isolate), *Acinetobacter baumannii* (ATCC19606), *Aeromonas hydrophila* (ATCC7966), *Plesiomonas shigelloides* (ATCC14029), and *Sphingobium paucimobilis* (MTCC6362) *in vitro*. Interestingly, some of the conjugates showed superior antimicrobial activity as compared to the control drug ciprofloxacin. The three compounds **4i**, **4j**, and **4n** showed strong activity with minimum inhibitory concentration (MIC) 0.78 μM , while the compound **4g** showed MIC 1.56 μM against *S. typhi* (clinical). The compound **4a** showed good efficacy against *S. aureus* (ATCC25923) and *S. typhi* (clinical) with MIC 3.12 μM , while the compound **4b** exhibited efficacy with MIC 3.12 μM against *S. aureus* (ATCC25923) and the control drug ciprofloxacin showed MIC 6.25 μM . Among all of the synthesized compounds, **4e**, **4f**, **4g**, **4h**, **4p**, **4q**, **4t**, and **4u** displayed less than 20% hemolysis, while the rest of the compounds showed hemolysis in the range of 21–48%. Moreover, the structure of compound **4b** was also established by single-crystal X-ray diffraction studies.



1. INTRODUCTION

Quinolones are synthetic antibacterial agents that inhibit DNA gyrase and topoisomerase enzymes in bacteria.¹ Since their discovery back in 1970, quinolones and their various synthetic analogs continue to be the most effective and widely used common antibiotics.² Ciprofloxacin, a second-generation fluoroquinolone, displays broad-spectrum antibiotic activity. Acute uncomplicated infections such as urinary tract infections, cystitis, shigellosis, acute sinusitis disorder, and chronic bacterial prostatitis are generally treated by ciprofloxacin and its analogs.^{3,4} Ciprofloxacin functions by expressing ternary complexes with enzyme-DNA, thus restricting the repair of bacterial DNA and transcription of RNA.^{1,2} The remarkable antibacterial activity, efficient pharmacokinetics, and insignificant side effects of ciprofloxacin have led to its extended use as a fluoroquinolone antibiotic. However, excessive use of ciprofloxacin in recent times has led to emergence of bacterial resistance,⁵ and thus, it is no longer useful to treat various bacterial infections. Therefore, there is an urgent need to find an alternative drug with high efficacy, negligible toxicity, and cost effectiveness. Further, the complexation of quinolone antibiotics with metals is also reported to enhance their

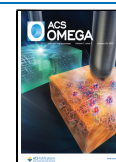
biological activity by decreasing the bacterial resistance of the drug due to increase of liposolubility, thereby leading to greater intracellular accumulation. This is another approach that would add to the already existing batteries of antibiotic arsenal in derivatization of the previously effective existing drugs.

The most common and rational strategy for the modification of ciprofloxacin is the introduction of a substituent at position C-7.⁶ Previous studies have demonstrated that a large group at this position does not affect the drug permeability across the bacterial membrane and concurrently modifies its strength and spectrum.^{7,8} This strategy has delivered a wide variety of newer conjugates containing 1,2,3-triazole,^{9–11} carboxamide,¹² poly-(2-oxazoline) and poly(ethylene glycol),¹³ amino-glycoside,¹⁴ nitrofurans,¹⁵ nitroimidazole,¹⁶ antitumor,¹⁷ 1,3,4-thiadiazole,¹⁸ isatin,¹⁹ bromothiophene,²⁰ acyl group²¹ etc.

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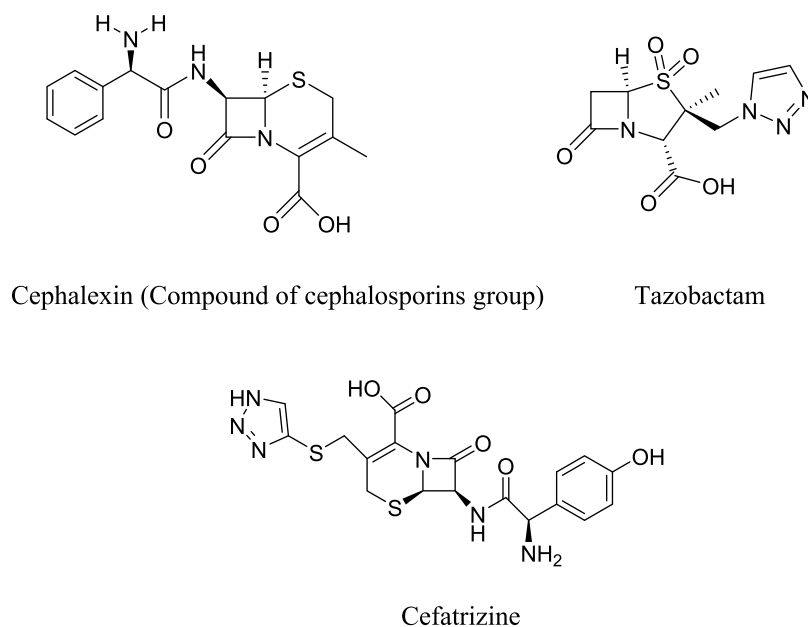


Figure 1. Marketed antibacterial drugs.

Huisgen 1,3-dipolar cycloaddition reactions (1,3-DCRs) serve as an extremely versatile and significant approach for the production of pharmacologically active compounds with one or more five-membered heterocyclic rings. The synthesis approach chosen for 1,3-DCRs might fulfill the stringent criteria that define click chemistry reactions: the reaction must be modular, render good yields, produce only harmless byproducts that can be separated by non-chromatographic processes, and be stereospecific but not necessarily enantioselective in nature.^{22–24} Click chemistry is capable of generating novel pharmacophores that represent a wide range of functional organic molecules.¹⁰ The advantages of click chemistry include simple and efficient reaction conditions, readily obtainable starting materials and reagents, reaction under solvent-free conditions, including use of benign solvents such as water or alcohol, and easy and simple workup procedure for isolation of the final title compound.²² Further, 1,3-DCRs have emerged as an alternative method to obtain 1,2,3-triazole compounds for screening of their broad-spectrum biological activity, including antibacterial,²⁴ antitubercular,²⁵ antitumor,²⁶ anti-HIV,²⁷ antifungal, and antiphytopathogenic activities. Moreover, the 1,2,3-triazole scaffold is also an integral component of the current extensively used clinical antibacterial drugs, such as cephalexin, tazobactam, and cefatrizine, as shown in Figure 1.

Encouraged by our previous studies,²⁸ we aimed to design and synthesize a newer efficacious series of ciprofloxacin conjugates containing 1,2,3-triazole for antimicrobial studies (Scheme 1).

These ciprofloxacin analogues were screened against Gram-positive and Gram-negative bacterial strains, and their toxicity was also evaluated against human red blood cells (hRBCs).

There are a few reports in literature on ciprofloxacin–triazole hybrids exhibiting synergistic biological activities.²⁸ Keeping these facts in mind, we have synthesized a series of ciprofloxacin-tethered triazoles with ester on one side and triazole ring on the other side of ciprofloxacin and evaluated their antibacterial and hemolytic activities.

2. RESULTS AND DISCUSSION

2.1. Chemistry. New ciprofloxacin–triazole compounds (4a–u) were synthesized in three simple steps starting from ciprofloxacin hydrochloride as summarized in Scheme 1. Briefly, the free ciprofloxacin (1) was achieved by dissolving ciprofloxacin hydrochloride in a surplus of 5% aqueous solution of sodium bicarbonate after the usual workup procedure as reported elsewhere.²⁹ Further, free ciprofloxacin was treated with propargyl bromide and NaHCO₃ at 100 °C to obtain a propargylated ciprofloxacin (2).³⁰ Furthermore, propargylated ciprofloxacin (2) was dissolved in three different solvents separately, viz. methanol, ethanol, and propanol, for esterification of the COOH group. Subsequently, concentrated H₂SO₄ was added to this mixture and the reaction mixture was refluxed for 7 h to obtain the corresponding esters (3). The compounds (3a–c) containing the propargyl group were used as substrate to further generate the 1,4-disubstituted 1,2,3-triazole library of 21 compounds (4a–u) by reacting various substituted aromatic azides by click chemistry. The structure of compound 4b was also confirmed by X-ray crystallographic studies to validate the design criteria, and the crystal data of compound 4b is presented in Figure 2.

2.2. Biological Activity. **2.2.1. Antibacterial Activity.** A study on 21 compounds (4a–u) containing ciprofloxacin with 1,2,3-triazole on one side and ester with varying alkyl groups on the other side was done via propargylation, followed by esterification and copper-catalyzed azide–alkyne (3 + 2) cycloaddition. All of the synthesized chemical compounds (4a–u) were evaluated for their antibacterial activity and minimum inhibitory concentration (MIC) against a broad spectrum of bacterial strains. The antibacterial activity of the compounds was examined against three strains of Gram-positive bacteria, i.e., *Staphylococcus aureus* (ATCC25923), *Enterococcus faecalis* (clinical isolate), *Staphylococcus epidermidis* (ATCC3594), and eight strains of Gram-negative bacteria, i.e., *Escherichia coli* (ATCC25922), *Pseudomonas aeruginosa* (ATCC27853), *Salmonella typhi* (clinical isolate), *Salmonella typhimurium* (clinical isolate), *Acinetobacter baumannii* (ATCC19606), *Aeromonas hydrophila* (ATCC7966), *Plesio-*

Scheme 1. Synthesis of Desired Ciprofloxacin–Triazole Conjugates and Their Design Strategy

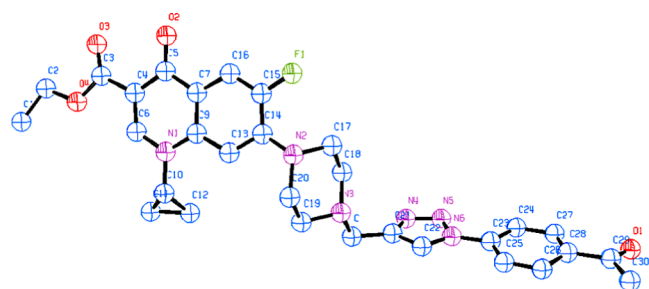
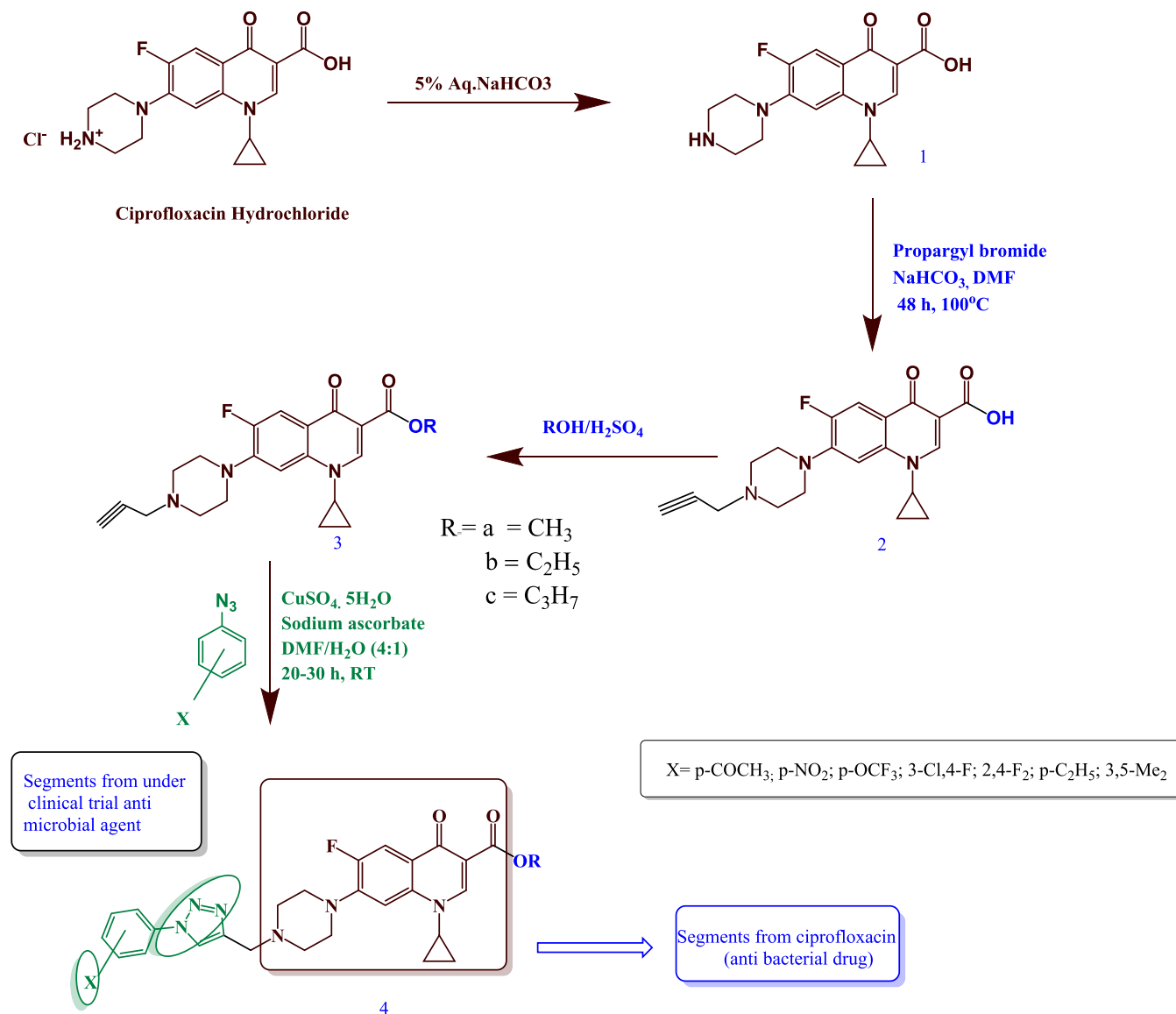


Figure 2. X-ray structure of 1,2,3-triazole-linked ciprofloxacin conjugate (4b).

monas shigelloides (ATCC14029), and *Sphingo biumpaucimobilis* (MTCC6362). The MIC results support the fact that the nature and place of the substituent as well as their isomeric consequences on phenyl rings have a dramatic effect on the antibacterial activity of the moiety (Table 1).

It is evident from Table 1 that the synthesized compounds, viz. 4a, 4b, 4g, 4h, 4i, 4j, 4k, 4m, 4n, and 4p were more potent,

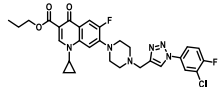
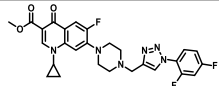
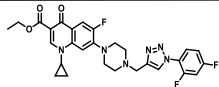
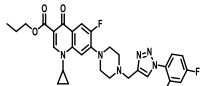
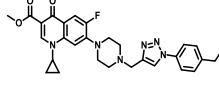
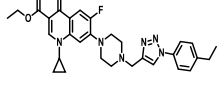
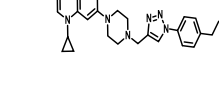
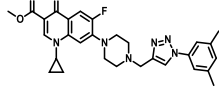
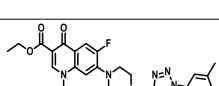
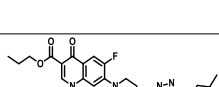
with either less or equal MIC as compared to the standard drug ciprofloxacin.

Compound 4a, with a methyl ester and *p*-acetyl substituent on triazole, showed very good efficacy against *S. aureus* (ATCC25923) and *S. typhi* (clinical) with MIC 3.12 μM, and also against *E. coli* (ATCC25922) and *A. hydrophila* (ATCC7966) with MIC 6.25 μM, while the control drug showed MIC 6.25 μM. Compound 4b with ethyl ester and *p*-COCH₃-substituted benzene showed the potent activity against *S. aureus* (ATCC25923) with MIC 3.12 μM, while this compound was also found active against *S. typhi* (clinical) and *S. paucimobilis* (MTCC6362) with MIC 6.25 μM similar to the control drug. Compound 4c with propyl ester and *p*-acetyl group on triazole was found active only against two strains, viz. *S. typhi* (clinical) and *S. paucimobilis* (MTCC6362), with MIC 6.25 μM, while it showed moderate to weak activity against the rest of the strains. Compound 4d with methyl ester and 4e with ethyl ester and *p*-NO₂ group on a benzene ring in both compounds showed weak activity against all tested strains with the MIC in the range from 12.5 to 25 μM. Compound 4f with the propyl ester group and *p*-

Table 1. Antibacterial Activity and % Hemolysis of Compounds (4a–u)

S. No.	Compounds	Gram +ve strains			Gram –ve strains								% hemolysis
		<i>S. epidermidis</i> (ATCC 3594)	<i>S. aureus</i> (ATCC 25923)	<i>E. faecalis</i> (clinical)	<i>E. coli</i> (ATCC C 25922)	<i>P. aeruginosa</i> (ATCC 27853)	<i>S. typhi</i> (clinical)	<i>S. typhimurium</i> (clinical)	<i>Acinetobacter baumannii</i> (ATCC 19606)	<i>A. hydrophila</i> (ATCC 7966)	<i>P. shigelloides</i> (ATCC 14029)	<i>S. paucimobilis</i> (MTCC 6362)	
4a		6.25	3.12	12.5	6.25	25	3.12	25	12.5	6.25	12.5	12.5	25.47
4b		12.5	3.12	12.5	12.5	25	6.25	12.5	12.5	12.5	12.5	6.25	38.78
4c		12.5	100	25	25	25	6.25	25	12.5	12.5	25	6.25	23.57
4d		12.5	25	25	25	25	12.5	25	25	25	25	12.5	36.50
4e		12.5	25	12.5	25	25	12.5	25	25	25	25	12.5	14.44
4f		12.5	12.5	6.25	6.25	25	6.25	12.5	12.5	6.25	12.5	6.25	19.77
4g		6.25	6.25	6.25	3.12	12.5	1.56	12.5	6.25	3.12	6.25	3.12	17.87
4h		6.25	6.25	100	50	25	12.5	6.25	3.12	12.5	12.5	1.56	10.27
4i		12.5	100	50	100	12.5	0.78	100	6.25	12.5	25	3.12	34.98
4j		6.25	6.25	12.5	1.56	12.5	0.78	6.25	12.5	1.56	25	1.56	39.54
4k		12.5	3.12	12.5	3.12	25	3.12	25	12.5	3.12	25	100	44.11

Table 1. continued

S. No.	Compounds	Gram +ve strains					Gram -ve strains						% hemolysis
		<i>S. epidermidis</i> (ATCC 3594)	<i>S. aureus</i> (ATCC 25923)	<i>E. faecalis</i> (clinical)	<i>E. coli</i> (ATCC 25922)	<i>P. aeruginosa</i> (ATCC 27853)	<i>S. typhi</i> (clinical)	<i>S. typhimurium</i> (clinical)	<i>Acinetobacter baumannii</i> (ATCC 19606)	<i>A. hydrophila</i> (ATCC 7966)	<i>P. shigelloides</i> (ATCC 14029)	<i>S. paucimobilis</i> (MTCC 6362)	
4l		100	100	100	100	25	6.25	100	100	6.25	100	50	31.17
4m		12.5	6.25	12.5	12.5	12.5	6.25	6.25	12.5	3.12	6.25	6.25	40.30
4n		12.5	6.25	12.5	6.25	25	0.78	12.5	12.5	3.12	6.25	6.25	33.84
4o		25	100	50	25	25	100	50	25	25	50	100	47.90
4p		12.5	6.25	50	100	25	3.12	25	6.25	6.25	12.5	100	18.63
4q		25	100	100	100	12.5	100	25	100	6.25	100	100	12.16
4r		-	100	100	100	25	100	100	100	100	100	-	21.29
4s		12.5	100	100	100	25	25	50	25	25	25	6.25	21.67
4t		12.5	100	100	100	25	100	100	100	25	25	50	15.97
4u		50	1000	100	100	25	100	100	-	12.5	-	100	18.63
Standard	Ciprofloxacin	3.12	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	3.12	12.5	5.23

nitrobenzene on triazole was found active against five strains, viz. *E. coli* (clinical), *E. faecalis* (clinical), *S. typhi* (clinical), *A. hydrophila* (ATCC7966), and *S. paucimobilis* (MTCC6362) with MIC 6.25 μM , which is comparable to the control drug. Compound **4g** with methyl ester and the *p*-trifluoromethoxy group on benzene was found most potent against *S. typhi* (clinical) with MIC 1.56 μM and against *A. hydrophila* (ATCC7966), *S. paucimobilis* (MTCC6362), and *E. coli* (clinical) with MIC 3.12 μM . Further, the compound **4g** also showed promising activity against five strains, including *S. epidermidis* (ATCC3594), *S. aureus* (ATCC25923), *A. baumannii* (ATCC19606), *P. shigelloides* (ATCC14029), and *E. faecalis* (clinical), with MIC 6.25 μM similar to the standard drug ciprofloxacin. Compound **4h** with ethyl ester having *p*-OCF₃ substituted on the benzene ring showed excellent activity against strains *S. paucimobilis* (MTCC6362) and *A. baumannii* (ATCC19606), having MIC values of 1.56 and 3.12 μM , respectively, while it showed moderate potency against *S. aureus* (clinical), *S. epidermidis* (ATCC3594), and *S. typhimurium* (clinical) with MIC values comparable to that of ciprofloxacin (6.25 μM). Compound **4i** with propyl ester and the *p*-OCF₃ group on benzene showed excellent potency against *S. typhi* (clinical) with MIC 0.78 μM , which indicates approximately 8-fold higher activity than that of the control drug ciprofloxacin with MIC 6.25 μM , while the same compound exhibited potent activity against *S. paucimobilis* (MTCC6362) and *A. baumannii* (ATCC19606) with MIC values of 3.12 and 6.25 μM , respectively. Compound **4j** having 3-chloro, 4-fluoro substitution on the benzene in triazole along with methyl ester showed excellent activity against *S. typhi* (clinical) with MIC 0.78 μM , while *E. coli* (clinical), *S. paucimobilis* (MTCC6362), and *A. hydrophila* (ATCC7966) showed MIC 1.56 μM . The same compound showed good activity against three strains, namely *S. epidermidis* (ATCC3594), *S. aureus* (ATCC25923), and *S. typhimurium* (clinical), with MIC 6.25 μM , which is comparable to the control drug. Further, compound **4k** with ethyl ester and 3-chloro, 4-fluoro substitution on benzene showed potent activity against *S. aureus* (clinical), *A. hydrophila* (ATCC7966), *E. coli* (clinical), and *S. typhi* (clinical) with MIC 3.12 μM each. Compound **4l** having propyl ester and 3-chloro, 4-fluoro group substitution on benzene showed activity against *S. typhi* (clinical) and *A. hydrophila* (ATCC7966) with MIC 6.25 μM , whereas compound **4m** with methyl ester and 2,4-difluoro on triazole showed activity against *A. hydrophila* (ATCC7966) with MIC 3.12 μM , while in *S. aureus* (clinical), *S. typhi* (clinical), *S. typhimurium* (clinical), and *P. shigelloides* (ATCC14029), and *S. paucimobilis* (MTCC6362) it showed MIC 6.25 μM , which is similar to that of the reference drug ciprofloxacin. Compound **4n** with 2,4-difluoro substitution on the benzene ring and ethyl ester showed excellent activity against *S. typhi* (Clinical) and *A. hydrophila* (ATCC7966) with MIC values of 0.78 and 3.12 μM , respectively, while the same compound showed good activity against *S. aureus* (ATCC25923), *S. paucimobilis* (MTCC6362), *P. shigelloides* (ATCC14029), and *E. coli* (clinical) with MIC 6.25 μM each, comparable to the control drug. Compound **4o** with propyl ester and a 2,4-difluoro-substituted benzene ring did not show any reasonable activity against any of the strains under the study. Compound **4p** having an ethyl group on the benzene ring and methyl ester was found most potent against *S. typhi* (clinical) with MIC 3.12 μM , while it showed good activity against three strains, viz. *S. aureus* (ATCC25923), *A.*

baumannii (ATCC19606), and *A. hydrophila* (ATCC7966), with MIC 6.25 μM , again comparable to the control drug. Compound **4q** having an ethyl group on benzene and ethyl ester showed MIC 6.25 μM against the Gram-negative bacteria *A. hydrophila* (ATCC7966), which is similar to the MIC of the control drug. No activity was seen in other strains. Compound **4r** having an ethyl group on benzene and propyl ester did not show any activity against any strain selected for study. Compound **4s** having methyl ester and a 3,5-dimethyl-substituted benzene ring exhibited potent activity against *S. paucimobilis* (MTCC6362) with MIC 6.25 μM , which is just half of the MIC of the standard drug, while no activity was seen against any of the strains. Again, compounds **4t** and **4u** having a 3,5-dimethyl benzene ring and ethyl and propyl esters, respectively, did not show any activity against any of the tested Gram strains.

Our findings indicate that the nature of the groups present in the substituent has a strong role in antimicrobial activity. Therefore, functional groups such as strong electron-withdrawing groups, e.g. trifluoromethoxy, nitro, and acetyl groups having electron-withdrawing nature at position 4 in the benzene ring, strongly affect the antibacterial activity. This hypothesis is supported by the higher activity of compounds **4f**, **4g**, **4j**, **4k**, **4m**, and **4n** against all of the Gram-positive and Gram-negative bacteria screened in the present study. Further, the ester group on the opposite side of the phenyl ring also plays a critical role in governing the activity. Thus, we can conclude from this study that position 4 is critical for determining the antibacterial activity against both Gram-positive and Gram-negative bacteria. The presence of strong electron-withdrawing groups has a major effect on the potency of the compounds. The sensitivity of the bacterial strain is another noteworthy observation. For example, *S. typhi* (clinical) showed a higher sensitivity to most of the synthesized compounds than the parent drug ciprofloxacin, except the compounds bearing electron-donating groups. Interestingly, compounds **4i**, **4j**, and **4n** were found to be most active against *S. typhi* (clinical). Thus, it is clear from this study that there is a need of second-generation synthesis based on activity data, and structure–activity relationship (SAR) should be done on lead molecules to get meaningful results.

2.2.2. Hemolytic Activity. The hemolytic activity of drugs is executed by several mechanisms, including increase in the permeability of cell membranes to absolute cell lysis. It is observed that latent damage is caused by the drugs present in oils to the membranes of erythrocytes, which undergo lysis and subsequently liberate hemoglobin. The cause underlying this hemolytic movement is to optimize the % hemolysis of the compounds against red blood cells, which fills in as an extra criterion to analyze the significance of the incorporated compounds and may likewise guide the development of such compounds as medications. While determining the toxicity profile of whole synthesized compounds, their hemolytic activities were evaluated by following the route originated by Nielson et al.³¹ at a stable concentration of 100 μM on human RBCs. The results showed that these compounds caused 10–48% hemolysis. Interestingly, most of the synthesized compounds (**4e**, **4f**, **4g**, **4h**, **4p**, **4q**, **4t**, and **4u**) displayed less than 20% hemolysis, while the standard drug ciprofloxacin showed 5.23% hemolysis. The outcomes of the hemolytic experiment of the tested compounds are represented in Table 1 in conjunction with the antibacterial activity results.

3. CONCLUSIONS

Briefly, twenty-one newer synthetic ciprofloxacin analogues were screened against various antibacterial strains. From a small library pool, three compounds, viz. **4i**, **4j**, and **4n**, showed excellent activity against *S. typhi* (clinical) strains with MIC 0.78 μ M, while two compounds, viz. **4h** and **4j**, showed excellent activity against *S. paucimobilis* (MTCC6362) and *A. hydrophila* (ATCC7966) with MIC 1.56 μ M, respectively. Moreover, nine compounds, viz. **4a**, **4b**, **4g**, **4h**, **4i**, **4k**, **4m**, **4n**, and **4p**, showed very good activity with MIC 3.12 μ M against various strains, whereas the control drug ciprofloxacin showed MIC 6.25 μ M. Moreover, the electron-withdrawing groups such as $-\text{OCF}_3$, $-\text{NO}_2$, and $-\text{COCH}_3$, at *para*-position in the benzene ring, have a strong influence on determining the antibacterial activity. The study of hemotoxicity revealed a negligible toxicity profile for all of the compounds. Thus, there is need to study the structure–activity relationship (SAR) of lead molecules to generate second-generation compounds for antimicrobial studies. Moreover, the lead compounds from second generation may be combined with existing clinical drugs to generate multifunctional hybrids in order to establish meaningful structure–activity relationship (SAR), aimed toward lowering their further effective concentrations and higher antimicrobial activity. This idea may solve to some extent the drug resistance problem in bacteria, and work in this direction is progressing well. Moreover, single-crystal X-rays of the compound validate the structure of our designed compound.

4. EXPERIMENTAL SECTION

All chemicals and solvents used in this study were purchased from Sigma-Aldrich and E. Merck (India). The reactions during synthesis were monitored by thin-layer chromatography (TLC) on precoated silica gel 60 F254 (mesh), and spots were visualized with UV light. Silica gel (60–120 mesh) was used for column chromatography. The melting points of all synthesized compounds were determined using the open capillary method and may be uncorrected. The structural assignments of the synthesized products were based on ^1H , ^{13}C NMR, MS, and single-crystal X-ray diffraction (XRD). NMR data were collected using a 400 MHz, JEOL JNM-ECS spectrometer in CDCl_3 with TMS as internal standard, and data were processed with its Delta software. The following abbreviations were used in reporting the spectra: s = singlet, bs = broad singlet, d = doublet, dd = doublet of doublets, t = triplet, m = multiple. Mass data were generated using Bruker Compass spectrometer. X-ray analysis was performed using an Oxford Diffraction Xcalibur four-circle diffractometer with an Eos CCD detector using graphite monochromatized Mo K α radiation ($\lambda = 0.71073 \text{ \AA}$).

4.1. Regeneration of Free 1-Cyclopropyl-6-fluoro-4-oxo-7-piperazin-1-ylquinoline-3-carboxylic Acid (Ciprofloxacin 1). Ciprofloxacin hydrochloride (5.0 g, 13.59 mmol) was dissolved in water (30 mL) to get a clear solution. This solution was treated with an excess of 5% aqueous sodium bicarbonate solution, resulting in the formation of a white precipitate. The precipitate was filtered off and left to dry as a free 1-cyclopropyl-6-fluoro-4-oxo-7-piperazin-1-ylquinoline-3-carboxylic acid (ciprofloxacin 1, 4.3 g, 12.98 mmol). The free ciprofloxacin was pure enough and used as starting material for propargylation reaction without purification. White solid, yield 4.3 g (96%), MP 253–255 $^\circ\text{C}$. ^1H NMR

(DMSO- d_6) δ : 8.63 (s, 1H, Ar-H), 7.87–7.84 (d, $J = 13.72 \text{ Hz}$, 1H, Ar-H), 7.51–7.50 (d, $J = 4.56 \text{ Hz}$, 1H, Ar-H), 3.79 (bs, 1H, $-\text{CH}$), 3.21 (bs, 4H, 2- CH_2), 2.90 (bs, 4H, 2- CH_2), 1.29–1.27 (m, 2H, $-\text{CH}_2$), 1.15 (bs, 2H, $-\text{CH}_2$).

4.2. Propargylation of 1-Cyclopropyl-6-fluoro-4-oxo-7-piperazin-1-ylquinoline-3-carboxylic Acid (2). To the solution of 1-cyclopropyl-6-fluoro-4-oxo-7-piperazin-1-ylquinoline-3-carboxylic acid (1 mmol) in dimethylformamide (15 mL), 25 mL of NaHCO_3 solution (1.2 mmol) and 1.2 mmol of propargyl bromide were added under vigorous stirring. The mixture was allowed to react at 100 $^\circ\text{C}$ for 48 h. The completion of the reaction was monitored by TLC. After evaporating the solvent, the residue was purified by column chromatography using a $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (98:2) mixture as eluent. Light yellow solid, yield 1.9 g (73%), MP 225–227 $^\circ\text{C}$. ^1H NMR (CDCl_3) δ : 8.71 (s, 1H, Ar-H), 7.97 (d, $J = 13.08 \text{ Hz}$, 1H, Ar-H), 7.33 (d, $J = 7.42 \text{ Hz}$, 1H, Ar-H), 7.25 (s, 1H, Ar-H), 3.53 (m, 1H), 3.37 (t, 4H), 2.92 (s, 1H), 2.78 (t, 4H), 2.29 (s, 2H), 1.36 (d, $J = 6.91 \text{ Hz}$, 2H), 1.18 (d, $J = 6.2 \text{ Hz}$, 2H); ^{13}C NMR (CDCl_3) δ : 173.2 ($-\text{CO}$), 166.5 ($-\text{CO}$), 154.6, 148.3, 144.5, 138.0, 113.4, 110.4, 104.7, 73.6, 52.1, 51.5, 49.8, 46.8, 34.4, 8.1. Chemical formula: $\text{C}_{20}\text{H}_{20}\text{FN}_3\text{O}_3$; MS m/z (ES, +ve, CH_3OH) 370.1579 [$\text{M} + \text{H}$] $^+$.

4.3. General Procedure for Synthesis of 1-Cyclopropyl-6-fluoro-4-oxo-7-(4-prop-2-ynyl-piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic Acid Methyl/Ethyl/Propyl Ester (3a–c). The compound 2 was dissolved separately in methanol, ethanol, and propanol (25 mL each). Concentrated H_2SO_4 (3 mL) was added to this mixture and the mixture was refluxed for 7 h. The completion of the reaction was monitored by TLC. After completion of the reaction as seen by TLC, the reaction mixture was cooled at room temperature; ice was added to the mixture and neutralized by aqueous sodium bicarbonate solution. The reaction mixture was extracted with chloroform. The organic layer was washed with water and dried over anhydrous sodium sulfate to get the corresponding ester.

4.3.1. 1-Cyclopropyl-6-fluoro-4-oxo-7-(4-prop-2-ynyl-piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic Acid Methyl Ester (3a). Yield 0.9 g (88.5%), MP 208–210 $^\circ\text{C}$. ^1H NMR (CDCl_3) δ : 8.51 (s, 1H, Ar-H), 8.01 (s, 1H, Ar-H), 7.25 (s, 1H, Ar-H), 3.8 (s, 2H), 3.77–3.29 (m, 8H), 2.77 (t, 4H), 2.28 (s, 1H), 1.32–1.09 (m, 4H); ^{13}C NMR (CDCl_3) δ : 177.2 ($-\text{CO}$), 167.0 ($-\text{CO}$), 147.4, 145.6, 139.3, 119.9, 112.9, 108.0, 104.8, 73.7, 51.4, 49.6, 46.8, 35.2, 8.2. Chemical formula: $\text{C}_{21}\text{H}_{22}\text{FN}_3\text{O}_3$; MS m/z (ES, +ve, CH_3OH) 384.1736 [$\text{M} + \text{H}$] $^+$.

4.3.2. 1-Cyclopropyl-6-fluoro-4-oxo-7-(4-prop-2-ynyl-piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic Acid Ethyl Ester (3b). Yield 1 g (96%), MP 194–196 $^\circ\text{C}$. ^1H NMR (CDCl_3) δ : 8.73 (s, 1H, Ar-H), 7.99 (s, 1H, Ar-H), 7.33 (s, 1H, Ar-H), 3.54 (m, 1H), 3.39–3.35 (m, 7H), 2.80–2.77 (m, 7H), 2.29 (s, 1H), 1.68 (s, 1H), 1.39 (d, $J = 7.65 \text{ Hz}$, 2H), 1.17 (t, 2H); ^{13}C NMR (CDCl_3) δ : 173.1 ($-\text{CO}$), 166.0 ($-\text{CO}$), 148.1, 144.4, 137.8, 113.6, 110.4, 104.3, 73.6, 51.5, 49.8, 46.8, 34.4, 29.3, 14.4, 8.1. Chemical formula: $\text{C}_{22}\text{H}_{24}\text{FN}_3\text{O}_3$; MS m/z (ES, +ve, CH_3OH) 398.1856 [$\text{M} + \text{H}$] $^+$.

4.3.3. 1-Cyclopropyl-6-fluoro-4-oxo-7-(4-prop-2-ynyl-piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic Acid Propyl Ester (3c). Yield 1 g (98.2%), MP 176–178 $^\circ\text{C}$. ^1H NMR (CDCl_3) δ : 8.45 (s, 1H, Ar-H), 7.96 (d, 1H, $J = 14.11 \text{ Hz}$), 7.19 (s, 1H, Ar-H), 4.20 (t, 2H), 3.34–3.24 (m, 7H), 2.74 (t, 4H), 2.25 (s, 1H), 1.74–1.73 (m, 2H), 1.26–0.94 (m, 7H);

^{13}C NMR (CDCl_3) δ : 173.2 (–CO), 165.6 (–CO), 148.0, 144.1, 138.0, 113.3, 110.8, 104.7, 66.4, 51.5, 49.8, 46.8, 34.4, 22.1, 10.5, 8.1. Chemical formula: $\text{C}_{23}\text{H}_{26}\text{FN}_3\text{O}_3$; MS m/z (ES, +ve, CH_3OH) 412.2041 $[\text{M} + \text{H}]^+$.

4.4. General Procedure for the Synthesis of Azides.

The synthesis of azides was carried out according to the published procedure.³² Briefly, aniline (1 equiv) was dissolved in 6 N HCl solution (10 mL/mmol of aniline) at room temperature and cooled upto 0 °C, followed by addition of aqueous NaNO_2 (1 equiv) solution under stirring. After 10 min of stirring at the same temperature, sodium azide (1.2 equiv) was added to the reaction mixture. This mixture was further stirred at room temperature for 2–3 h. After the completion of the reaction as seen by TLC, the reaction was worked up by extraction with chloroform. The organic layer was washed with brine solution and dried over anhydrous Na_2SO_4 . After evaporation of the solvent, the crude product was pure enough for further reactions. All of the synthesized azides were stored at –20 °C.

4.5. General Procedure for the Synthesis of 1,2,3-Triazole Scaffolds (4a–u). The synthesis of triazoles was carried out according to the published procedure.³³ Briefly, 1-cyclopropyl-6-fluoro-4-oxo-7-(4-prop-2-ynyl-piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid methyl/ethyl/propyl ester (3.1 equiv) and substituted aromatic azide (1.2 equiv) were suspended in *N,N*-dimethylformamide (25 mL/mmol of alkyne). The solution of sodium ascorbate (0.4 equiv in minimum water) was added, followed by copper(II) sulfate pentahydrate solution (0.2 equiv in minimum water). The heterogeneous mixture was stirred vigorously at room temperature until consumption of alkyne as monitored by TLC. After completion of the reaction, the mixture was poured onto ice water and the precipitate was collected by filtration. The desired products were purified by column chromatography using an eluent of 3% methanol in chloroform.

4.5.1. Methyl-7-(4-((1-(4-acetylphenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (4a). Light yellow solid, yield 0.11 g (77.5%), MP 216–218 °C. ^1H NMR (CDCl_3) δ : 8.50 (s, 1H, Ar-H), 8.12–7.86 (m, 6H, Ar-H), 3.87–3.85 (m, 3H), 3.28 (t, 3H), 2.79 (t, 4H), 2.60 (s, 3H, –COCH₃), 2.64 (s, 3H, –OCH₃), 1.98 (m, 2H), 1.29 (t, 2H), 1.10 (t, 2H); ^{13}C NMR (CDCl_3) δ : 196.6 (–CO), 172.8 (–CO), 166.7 (–CO), 154.4, 148.3, 139.9, 137.9, 136.7, 130.0, 120.7, 119.8, 113.4, 109.8, 104.8, 52.9, 52.0, 49.7, 34.4, 26.6, 8.0. Chemical formula: $\text{C}_{29}\text{H}_{29}\text{FN}_6\text{O}_4$; MS m/z (ES, +ve, CH_3OH) 545.2302 $[\text{M} + \text{H}]^+$.

4.5.2. Ethyl-7-(4-((1-(4-acetylphenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (4b). Light yellow compound, yield 0.11 g (78%), MP 208–210 °C. ^1H NMR (CDCl_3) δ : 8.48 (s, 1H, Ar-H), 8.12–8.07 (m, 5H), 7.99 (d, 2H, $J = 13.80$ Hz), 7.89 (d, 2H, $J = 9.10$ Hz), 4.35 (q, 2H, –CH₂CH₃), 3.85 (s, 2H), 3.39 (m, 1H), 3.28 (t, 4H), 2.78 (t, 4H), 2.64 (s, 3H, –OCH₃), 1.37 (t, 2H), 1.22 (m, 1H), 1.10 (t, 2H); ^{13}C NMR (CDCl_3) δ : 196.5 (–CO), 173.0 (–CO), 165.7 (–CO), 154.3, 152.1, 148.0, 145.5, 144.5, 140.1, 137.8, 136.7, 130.0, 120.7, 119.8, 113.3, 110.0, 104.7, 60.8, 52.9, 52.6, 49.7, 34.4, 26.6, 14.3, 8.0. Chemical formula: $\text{C}_{30}\text{H}_{31}\text{FN}_6\text{O}_4$; MS m/z (ES, +ve, CH_3OH) 559.2459 $[\text{M} + \text{H}]^+$.

4.5.3. Propyl-7-(4-((1-(4-acetylphenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (4c). Light yellow solid, yield

0.13 g (93%), MP 198–200 °C. ^1H NMR (CDCl_3) δ : 8.47 (s, 2H, Ar-H), 8.10–7.87 (m, 5H), 7.25 (s, 1H), 4.25 (t, 2H), 3.85 (s, 2H), 3.46 (s, 1H), 3.28 (t, 4H), 2.79 (s, 4H), 2.64 (s, 3H), 1.78 (m, 2H), 1.28 (d, $J = 7.34$ Hz, 2H), 0.98 (m, 5H); ^{13}C NMR (CDCl_3) δ : 196.5 (–CO), 173.2 (–CO), 165.8 (–CO), 152.1, 148.0, 144.4, 139.7, 138.4, 136.7, 130.0, 120.6, 119.8, 113.3, 110.0, 104.7, 66.3, 52.6, 49.7, 34.4, 26.6, 22.0, 10.5, 8.0. Chemical formula: $\text{C}_{31}\text{H}_{33}\text{FN}_6\text{O}_4$; MS m/z (ES, +ve, CH_3OH) 573.2643 $[\text{M} + \text{H}]^+$.

4.5.4. Methyl-1-cyclopropyl-6-fluoro-7-(4-((1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (4d). Light pink solid, yield 0.11 g (77%), MP 211–213 °C. ^1H NMR (CDCl_3) δ : 8.53 (s, 1H, Ar-H), 8.42 (d, 2H, $J = 9.30$), 8.12–7.98 (m, 5H), 7.25 (s, 2H), 3.89 (m, 4H), 3.31 (m, 1H), 3.29 (m, 3H), 2.81 (m, 2H), 1.74 (t, 2H), 1.29 (t, 2H), 1.10 (t, 2H); ^{13}C NMR (CDCl_3) δ : 173.0 (–CO), 166.4 (–CO), 148.3, 146.9, 145.6, 140.8, 138.2, 125.5, 120.7, 120.3, 113.2, 109.4, 104.1, 52.9, 52.6, 52.0, 49.7, 34.4, 8.1. Chemical formula: $\text{C}_{27}\text{H}_{26}\text{FN}_7\text{O}_5$; MS m/z (ES, +ve, CH_3OH) 548.2041 $[\text{M} + \text{H}]^+$.

4.5.5. Ethyl-1-cyclopropyl-6-fluoro-7-(4-((1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (4e). Light pink solid, yield 0.12 g (85%), MP 205–207 °C. ^1H NMR (CDCl_3) δ : 8.44 (s, 1H, Ar-H), 8.34 (d, 2H, $J = 8.46$ Hz), 8.09 (s, 1H), 7.99 (d, 2H, $J = 8.45$ Hz), 7.19 (s, 1H), 4.32 (q, 2H, –CH₂CH₃), 3.81 (s, 2H), 3.36 (m, 1H), 3.23 (t, 4H), 2.74 (t, 4H), 1.34–1.22 (m, 6H), 1.06 (t, 2H); ^{13}C NMR (CDCl_3) δ : 173.11 (–CO), 165.61 (–CO), 148.12, 147.03, 141.10, 137.93, 125.5, 120.35, 113.14, 110.20, 104.79, 60.78, 52.60, 49.66, 34.46, 29.35, 14.36, 8.08. Chemical formula: $\text{C}_{28}\text{H}_{28}\text{FN}_7\text{O}_5$; MS m/z (ES, +ve, CH_3OH) 562.2207 $[\text{M} + \text{H}]^+$.

4.5.6. Propyl-1-cyclopropyl-6-fluoro-7-(4-((1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (4f). Pink solid, yield 0.13 g (93%), MP 181–183 °C. ^1H NMR (CDCl_3) δ : 8.43 (s, 1H, Ar-H), 8.37 (d, 3H, $J = 9.4$ Hz), 8.16 (s, 1H), 7.98 (d, 2H, $J = 8.59$ Hz), 7.24 (s, 1H), 4.21 (t, 2H, –CH₂CH₂CH₃), 3.83 (s, 2H), 3.39–3.38 (m, 1H), 3.25 (t, 4H), 2.75 (t, 4H), 1.76–1.72 (m, 2H, –CH₂CH₂CH₃), 1.27–1.20 (m, 2H), 0.97 (t, 5H, –CH₂CH₂CH₃); ^{13}C NMR (CDCl_3) δ : 173.0 (–CO), 165.4 (–CO), 147.9, 147.0, 144.2, 141.0, 137.8, 125.4, 120.9, 120.3, 113.2, 110.1, 104.7, 52.8, 52.5, 49.7, 34.4, 22.0, 10.4, 8.0. Chemical formula: $\text{C}_{29}\text{H}_{30}\text{FN}_7\text{O}_5$; MS m/z (ES, +ve, CH_3OH) 576.2342 $[\text{M} + \text{H}]^+$.

4.5.7. Methyl-1-cyclopropyl-6-fluoro-4-oxo-7-(4-((1-(4-(trifluoromethoxy)phenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate (4g). Light yellow solid, yield 0.14 g (92%), MP 196–198 °C. ^1H NMR (CDCl_3) δ : 8.52 (s, 1H, Ar-H), 8.01 (m, 2H), 7.81 (d, 2H, $J = 8.60$ Hz), 7.39 (d, 2H, $J = 9.13$ Hz), 7.25 (s, 1H), 3.92 (m, 4H), 3.41 (m, 1H), 3.32 (t, 4H), 2.87 (t, 3H), 1.32 (t, 3H), 1.09 (t, 3H); ^{13}C NMR (CDCl_3) δ : 173.2 (–CO), 166.5 (–CO), 149.2, 148.2, 143.9, 137.6, 122.3, 121.8, 113.1, 110.0, 104.7, 52.5, 52.0, 49.4, 34.5, 8.1. Chemical formula: $\text{C}_{28}\text{H}_{26}\text{F}_4\text{N}_6\text{O}_4$; MS m/z (ES, +ve, CH_3OH) 587.2032 $[\text{M} + \text{H}]^+$.

4.5.8. Ethyl-1-cyclopropyl-6-fluoro-4-oxo-7-(4-((1-(4-(trifluoromethoxy)phenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate (4h). White solid, yield 0.11 g (72%), MP 166–168 °C. ^1H NMR (CDCl_3) δ : 8.48 (s, 1H, Ar-H), 7.97 (m, 2H), 7.80 (d, 2H, $J = 8.83$ Hz), 7.38 (d, 2H, $J = 8.83$ Hz), 7.24 (s, 1H), 4.35 (q, 2H,

–CH₂CH₃), 3.84 (s, 2H), 3.39 (m, 3H), 3.28 (t, 4H), 2.78 (t, 4H), 1.37 (3H, –CH₂CH₃), 1.09 (t, 2H); ¹³C NMR (CDCl₃) δ: 173.3 (–CO), 165.8 (–CO), 154.5, 152.2, 148.9, 148.1, 145.2, 144.3, 138.1, 135.3, 122.3, 121.8, 113.3, 110.5, 104.7, 60.8, 53.0, 52.6, 49.7, 34.4, 14.3, 8.0. Chemical formula: C₂₉H₂₈F₄N₆O₄; MS *m/z* (ES, +ve, CH₃OH) 601.2188 [M + H]⁺.

4.5.9. Propyl-1-cyclopropyl-6-fluoro-4-oxo-7-(4-((1-(4-(trifluoromethoxyphenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate (4i). Brown solid, yield 0.13 g (87%), MP 161–163 °C. ¹H NMR (CDCl₃) δ: 8.47 (s, 1H, Ar-H), 7.98–7.96 (m, 2H), 7.80 (d, 2H, *J* = 8.54 Hz), 7.38 (d, 2H, *J* = 8.63 Hz), 7.25 (s, 1H, Ar-H), 4.24 (t, 2H, –CH₂CH₂CH₃), 3.84 (s, 2H), 3.39 (m, 6H), 2.79 (t, 4H), 1.76 (m, 2H, –CH₂CH₂CH₃), 1.09 (t, 3H), 0.98 (t, 3H); ¹³C NMR (CDCl₃) δ: 173.2 (–CO), 165.8 (–CO), 154.8, 152.1, 148.0, 144.5, 137.8, 135.5, 122.2, 121.8, 113.2, 110.4, 104.7, 66.3, 52.6, 49.7, 34.4, 22.0, 10.5, 8.0. Chemical formula: C₃₀H₃₀F₄N₆O₄; MS *m/z* (ES, +ve, CH₃OH) 615.2359 [M + H]⁺.

4.5.10. Methyl-7-(4-((1-(3-chloro-4-fluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (4j). Light yellow solid, yield 0.14 g (97%), MP 190–192 °C. ¹H NMR (CDCl₃) δ: 8.52 (s, 1H, Ar-H), 7.85 (s, 1H), 7.33 (m, 3H), 3.41 (m, 5H), 3.30 (m, 5H), 2.79 (t, 4H), 1.32–1.24 (m, 3H), 1.13–1.09 (m, 2H); ¹³C NMR (CDCl₃) δ: 173.2 (–CO), 165.4 (–CO), 154, 148.1, 144.5, 137.8, 135.5, 122.9, 121.8, 113.2, 110.4, 104.7, 52.6, 49.7, 34.4, 8.1. Chemical formula: C₂₇H₂₅ClF₂N₆O₃; MS *m/z* (ES, +ve, CH₃OH) 555.1691 [M + H]⁺.

4.5.11. Ethyl-7-(4-((1-(3-chloro-4-fluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (4k). Light yellow solid, yield 0.13 g (91%), MP 115–117 °C. ¹H NMR (CDCl₃) δ: 8.48 (s, 1H, Ar-H), 7.95–7.83 (m, 3H), 7.77 (m, 1H), 7.32–7.22 (m, 1H), 4.37–4.32 (q, 2H, –CH₂CH₃), 3.83 (s, 2H), 3.393.28 (m, 5H), 2.77 (t, 4H), 1.92 (t, 2H), 1.37 (t, 5H), 1.10 (t, 2H); ¹³C NMR (CDCl₃) δ: 172.8 (–CO), 166.0 (–CO), 148.1, 145.2, 144.3, 138.2, 133.5, 122.9, 120.9, 120.1, 117.7, 109.5, 104.9, 60.8, 52.9, 52.6, 49.7, 34.4, 14.3, 8.1. Chemical formula: C₂₈H₂₇Cl F₂N₆O₃; MS *m/z* (ES, +ve, CH₃OH) 569.1845 [M + H]⁺.

4.5.12. Propyl-7-(4-((1-(3-chloro-4-fluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (4l). Brown solid, yield 0.13 g (92%), MP 103–105 °C. ¹H NMR (CDCl₃) δ: 8.47 (s, 1H), 7.96–7.84 (m, 3H), 7.66 (m, 1H), 7.32–7.21 (m, 2H), 4.26 (m, 2H), 3.79 (s, 2H), 3.40 (m, 1H), 3.28 (t, 4H), 2.79 (t, 4H), 1.80–1.78 (m, 2H), 1.29 (d, *J* = 6.41 Hz, 2H), 1.10–0.98 (m, 5H); ¹³C NMR (CDCl₃) δ: 173.0 (–CO), 165.6 (–CO), 156.4, 151.7, 148.0, 144.2, 137.9, 133.6, 122.8, 120.1, 120.0, 117.7, 112.7, 104.7, 66.3, 52.9, 52.4, 49.7, 34.4, 22.0, 10.4, 8.0. Chemical formula: C₂₉H₂₉ClF₂N₆O₃; MS *m/z* (ES, +ve, CH₃OH) 583.2034 [M + H]⁺.

4.5.13. Methyl-1-cyclopropyl-7-(4-((1-(2,4-difluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (4m). Light yellow solid, yield 0.07 g (50%), MP 172–174 °C. ¹H NMR (CDCl₃) δ: 8.49 (s, 1H, Ar-H), 7.99–7.94 (m, 3H), 7.25–7.22 (m, 1H), 7.08–7.03 (m, 2H), 3.87 (s, 4H), 3.40 (m, 1H), 3.28 (t, 4H), 2.77 (t, 4H), 2.00 (s, 1H), 1.29 (d, *J* = 6.92 Hz, 2H), 1.09 (t, 2H); ¹³C NMR (CDCl₃) δ: 173.2 (–CO), 166.3 (–CO),

154.9, 152.3, 148.3, 144.5, 137.9, 126.1, 126.0, 113.2, 113.0, 109.9, 104.7, 52.9, 52.5, 52.0, 49.7, 34.4, 8.0. Chemical formula: C₂₇H₂₅F₃N₆O₃; MS *m/z* (ES, +ve, CH₃OH) 539.2016 [M + H]⁺.

4.5.14. Ethyl-1-cyclopropyl-7-(4-((1-(2,4-difluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (4n). Light yellow solid, yield 0.05 g (36%), MP 161–163 °C. ¹H NMR (CDCl₃) δ: 8.50 (s, 1H), 8.03–7.95 (m, 3H), 7.25 (d, *J* = 6.55 Hz, 1H), 7.09–7.05 (m, 2H), 4.37 (q, 2H, –CH₂CH₃), 3.86 (s, 2H), 3.30 (s, 4H), 2.79 (t, 4H), 1.78 (s, 1H), 1.40–1.26 (m, 5H), 1.13 (t, 2H); ¹³C NMR (CDCl₃) δ: 165.89 (–CO), 148.1, 144.5, 137.8, 125.8, 113.4, 110.8, 105.1, 60.8, 52.6, 49.7, 34.4, 14.4, 8.1. Chemical formula: C₂₈H₂₇F₃N₆O₃; MS *m/z* (ES, +ve, CH₃OH) 553.2148 [M + H]⁺.

4.5.15. Propyl-1-cyclopropyl-7-(4-((1-(2,4-difluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (4o). Brown solid, yield 0.09 g (65%), MP 153–155 °C. ¹H NMR (CDCl₃) δ: 8.47 (s, 1H), 8.00–7.90 (m, 3H), 7.24 (s, 1H), 7.07–7.02 (m, 2H), 4.24 (t, 2H), 3.85 (s, 2H), 3.39 (m, 1H), 3.28 (t, 4H), 2.78 (t, 4H), 1.79 (q, 2H), 1.28–0.98 (m, 7H); ¹³C NMR (CDCl₃) δ: 173.2 (–CO), 165.8 (–CO), 154.8, 148.0, 144.9, 137.4, 125.8, 110.2, 105.2, 66.4, 52.9, 52.5, 49.7, 34.4, 22.0, 10.5, 8.1. Chemical formula: C₂₉H₂₉F₃N₆O₃; MS *m/z* (ES, +ve, CH₃OH) 567.2311 [M + H]⁺.

4.5.16. Methyl-1-cyclopropyl-7-(4-((1-(4-ethylphenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (4p). Dark brown solid, yield 0.12 g (87%) MP 172–174 °C. ¹H NMR (CDCl₃) δ: 8.52 (s, 1H, Ar-H), 8.01–7.96 (d, *J* = 13.32 Hz, 2H), 7.64 (d, *J* = 8.69 Hz, 2H), 7.34 (d, *J* = 8.93 Hz, 2H), 7.26 (s, 1H), 3.82 (s, 2H), 3.41 (m, 1H), 3.31 (t, 4H), 2.82 (s, 3H), 2.70 (q, 2H), 1.30–1.12 (m, 11H); ¹³C NMR (CDCl₃) δ: 178.39 (–CO), 159.92, 148.50, 145.08, 137.46, 129.08, 120.14, 113.28, 109.85, 52.74, 49.67, 34.07, 29.91, 15.41, 8.08. Chemical formula: C₂₉H₃₁FN₆O₃; MS *m/z* (ES, +ve, CH₃OH) 531.2499 [M + H]⁺.

4.5.17. Ethyl-1-cyclopropyl-7-(4-((1-(4-ethylphenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (4q). Dark brown solid, yield 0.11 g (80%), MP 103–105 °C. ¹H NMR (CDCl₃) δ: 8.50 (s, 1H), 7.98 (s, 2H), 7.63 (m, 3H), 7.33 (m, 4H), 4.35 (q, 2H), 3.87 (d, 4H), 3.39 (m, 2H), 3.87 (m, 3H), 2.80 (s, 2H), 1.38–1.23 (m, 10H); ¹³C NMR (CDCl₃) δ: 173.2 (–CO), 165.9 (–CO), 148.0, 145.6, 138.0, 129.0, 120.4, 113.4, 104.1, 61.1, 52.5, 49.6, 34.4, 28.4, 15.4, 14.3, 8.0. Chemical formula: C₃₀H₃₃FN₆O₃; MS *m/z* (ES, +ve, CH₃OH) 545.2658 [M + H]⁺.

4.5.18. Propyl-1-cyclopropyl-7-(4-((1-(4-ethylphenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (4r). Dark brown solid, yield 0.11 g (81%), MP 88–90 °C. ¹H NMR (CDCl₃) δ: 8.49 (s, 1H), 8.03–8.00 (m, 2H), 7.64 (d, 2H, *J* = 8.8 Hz), 7.34 (d, 3H, *J* = 8.6 Hz), 4.27 (t, 2H, –CH₂CH₂CH₃), 3.40 (m, 1H), 3.31 (m, 4H), 2.82–2.68 (m, 5H), 1.81 (q, 2H), 1.29–1.01 (m, 13H); ¹³C NMR (CDCl₃) δ: 165.8 (–CO), 147.9, 144.8, 137.6, 129.1, 120.2, 104.3, 52.1, 48.9, 34.1, 28.4, 22.0, 15.4, 10.5, 8.0. Chemical formula: C₃₁H₃₅FN₆O₃; MS *m/z* (ES, +ve, CH₃OH) 559.2832 [M + H]⁺.

4.5.19. Methyl-1-cyclopropyl-7-(4-((1-(3,5-dimethylphenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (4s). Dark brown

solid, yield 0.11 g (83%), MP 108–110 °C. ^1H NMR (CDCl_3) δ : 8.52 (s, 1H), 7.99 (s, 2H), 7.25 (d, 2H, $J = 8.68$ Hz), 7.05 (s, 1H), 6.50 (s, 1H), 3.8 (t, 3H), 3.46 (m, 7H), 2.38 (s, 10H), 1.26 (m, 4H); ^{13}C NMR (CDCl_3) δ : 166.6 (–CO), 148.3, 139.5, 138.0, 130.4, 122.0, 118.2, 113.0, 104.7, 52.5, 50.7, 33.7, 21.2, 8.0. Chemical formula: $\text{C}_{29}\text{H}_{31}\text{FN}_6\text{O}_3$; MS m/z (ES, +ve, CH_3OH) 531.2510 [$\text{M} + \text{H}$] $^+$.

4.5.20. Ethyl-1-cyclopropyl-7-(4-((1-(3,5-dimethylphenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (4t). Dark brown solid, yield 0.12 g (88%), MP 96–98 °C. ^1H NMR (CDCl_3) δ : 8.50 (s, 1H, Ar-H), 7.99 (s, 2H), 7.33–7.22 (m, 3H), 7.05 (s, 1H), 4.35 (q, 2H, – CH_2CH_3), 3.87 (d, $J = 14.7$ Hz, 4H), 3.40 (m, 1H), 3.29 (t, 4H), 2.79 (s, 2H), 2.37 (m, 6H), 1.37–1.22 (m, 7H); ^{13}C NMR (CDCl_3) δ : 166.0 (–CO), 148.3, 139.6, 138.0, 136.9, 130.3, 118.2, 113.0, 105.4, 61.5, 52.5, 49.7, 33.1, 29.3, 14.3, 8.0. Chemical formula: $\text{C}_{30}\text{H}_{33}\text{FN}_6\text{O}_3$; MS m/z (ES, +ve, CH_3OH) 545.2655 [$\text{M} + \text{H}$] $^+$.

4.5.21. Propyl-1-cyclopropyl-7-(4-((1-(3,5-dimethylphenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-6-fluoro-oxo-1,4-dihydroquinoline-3-carboxylate (4u). Dark brown solid, yield 0.11 g (82%), MP 90–92 °C. ^1H NMR (CDCl_3) δ : 8.49 (s, 1H, Ar-H), 8.02–7.96 (m, 2H), 7.34–7.24 (m, 3H), 7.06 (s, 1H), 4.27 (t, 2H, – $\text{CH}_2\text{CH}_2\text{CH}_3$), 3.89 (d, $J = 13.09$ Hz, 2H), 3.38 (m, 1H), 3.31 (t, 4H), 2.81 (t, 4H), 2.39 (s, 6H), 1.79 (q, 2H), 1.32–1.00 (m, 7H); ^{13}C NMR (CDCl_3) δ : 165.8 (–CO), 148.3, 139.7, 138.0, 130.3, 118.2, 113.1, 104.9, 66.4, 52.5, 49.9, 34.3, 22.0, 21.2, 10.5, 8.1. Chemical formula: $\text{C}_{31}\text{H}_{35}\text{FN}_6\text{O}_3$; MS m/z (ES, +ve, CH_3OH) 559.4488 [$\text{M} + \text{H}$] $^+$.

4.6. X-ray Crystallographic Analysis. The structure of the compound (**4b**) was determined by X-ray analysis. A good-quality single crystal of (**4b**) was obtained by slow evaporation at room temperature. The selected crystal was mounted on a glass fiber and used for data collection. The data was gathered at 20 °C by the X-ray scan technique using an Oxford Diffraction Xcalibur four-circle diffractometer with graphite monochromatized Mo $K\alpha$ radiation ($\lambda = 0.71073$ Å). The crystal structure was solved by direct methods using the program SHELXS-86 and refined by the full-matrix least-squares technique on F^2 by SHELXL-86. The thermal ellipsoid plot was prepared using ORTEP-3 as shown in Figure 2.

The crystallographic data, data collection, and structure refinement details are given in Table 2.

4.7. Biological Assays. **4.7.1. Determination of Anti-bacterial Activity.** The *in vitro* antibacterial studies employed the bacterial strains *S. aureus* (ATCC25923), *S. epidermidis* (ATCC35984), *E. faecalis* (clinical isolate), *E. coli* (ATCC25922), *P. aeruginosa* (ATCC27853), *P. aeruginosa* (clinical isolate), *A. hydrophila* (ATCC7966), *S. typhi* (clinical isolate), *S. typhimurium* (clinical isolate), *S. paucimobilis* (MTCC6362), and *P. shigelloides* (ATCC14029). The cultures used in the study were preserved at the Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India. All cultures were obtained from American Type Culture Collection (ATCC) or Microbial Type Culture Collection (MTCC), or were clinical strains. The fresh microbial broth cultures were prepared in normal saline before the screening procedure. Ciprofloxacin was used as the reference drug for antibacterial evaluation. Minimum inhibitory concentration (MIC) was determined using the micro-dilution method using a series of dilutions (10-fold) of all compounds. The various concentrations of the compounds

Table 2. Crystal Data, Data Collection, and Structure Refinement Details for (4b)

CCDC	1885454
molecular formula	$\text{C}_{30}\text{H}_{31}\text{FN}_6\text{O}_4$
molecular weight	558.24
temperature	293 K
wavelength	0.71073
crystal system	triclinic
space group	$P\bar{1}$
unit cell dimensions	$a = 14.5024(7)$ Å; $\alpha = 87.207(5)^\circ$ $b = 14.5614(11)$ Å; $\beta = 89.583(4)^\circ$ $c = 24.1403(12)$ Å; $\gamma = 85.244(5)^\circ$
volume	5074.2(5)
Z	14; $Z' = 7$
density	0.33 g/cm 3
absorption coefficient	0.024 mm $^{-1}$
$F(000)$	522.3
theta range for data collection	6.08–58.7
R_{int}	0.0709
index ranges	$-19 \leq h \leq 19$, $-18 \leq k \leq 19$, $-32 \leq l \leq 33$
reflections collected	72 381
completeness to theta	100%
absorption correction	multi-scan
refinement method	full-matrix least-squares on F_2
goodness-of-fit on F_2	4.323
final R indices	$R_1 = 0.9813$ $[I > 2\sigma(I)]$
	$R_2 = 0.9996$

were serially diluted in a microtiter plate. Specifically, 10 mL of standardized inoculum ($1-2 \times 10^7$ cfu/mL) was added in each tube of the microtiter plate. The plates were incubated aerobically at 37 °C for 18–24 h. The lowest concentration of the compounds at which they exhibited no visible bacterial growth and no turbidity in the solution when compared with the wells containing the control was regarded as the MIC.

4.7.2. Determination of the Hemolytic Activity of the Compounds on hRBC. The hemolytic activity was investigated by following the process published by Nielson et al.³¹ Briefly, fresh human blood was collected from the hospital and washed thrice in sterile phosphate-buffered saline (PBS) solution. After each washing, the cells were centrifuged at 3000 rpm for 7 min at room temperature and the supernatant was discarded. The RBCs were resuspended in PBS and adjusted to a final concentration of 5×10^8 RBCs/mL. An aliquot (10 mL) of the cell suspension was added to 100 mL of buffer solution containing 100 mM of the test compounds in 1% v/v dimethyl sulfoxide (DMSO) in PBS. Further, controls were also taken as 1% v/v DMSO in PBS and sterile water. The cell suspensions were incubated at 37 °C for 1 h with constant shaking. After 1 h, the solution was centrifuged at 1300 rpm for 5 min at room temperature and absorbance was recorded at 540 nm. The UV–vis absorbance values of the test compounds were expressed as % of the absorbance of sterile water (equivalent to 100% hemolysis) to obtain the % hemolysis results.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.1c05303>.

Spectra of synthesized compounds; CCDC No. 1885454 for compound (**4b**) contain the supplementary crystallographic data (PDF)

AUTHOR INFORMATION

Corresponding Author

Alka Agarwal – Department of Medicinal Chemistry, Institute of Medical Sciences, Banaras Hindu University, Varanasi UP-221005, India; orcid.org/0000-0002-8136-6543; Email: agarwal.dralka@gmail.com

Authors

Preeti Singh – Chemical Biology Laboratory, University of Delhi, Delhi 110007, India; orcid.org/0000-0002-9927-0427

Anand Maurya – Department of Medicinal Chemistry, Institute of Medical Sciences, Banaras Hindu University, Varanasi UP-221005, India

Uendra Kumar Patel – Department of Medicinal Chemistry, Institute of Medical Sciences, Banaras Hindu University, Varanasi UP-221005, India

Alka Singh – Department of Medicinal Chemistry, Institute of Medical Sciences, Banaras Hindu University, Varanasi UP-221005, India

Gopal Nath – Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi UP-221005, India

Complete contact information is available at: <https://pubs.acs.org/10.1021/acsomega.1c05303>

Notes

The authors declare no competing financial interest.

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