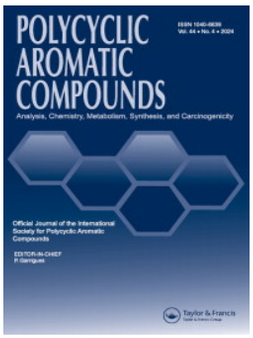


Polycyclic Aromatic Compounds

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/gpol20

Antibacterial Activity of Novel 1-Cyclopropyl-6,7-Difluoro-8-Methoxy-4-Oxo-1,4-Dihydroquinoline-3-Carbohydrazide Derivatives

Zaki Ahmed B. Munshi, Mubarak H. Shaikh, Pravinsing S. Girase, Iqrar Ahmad, Harun Patel & Bhata R. Chaudhari

To cite this article: Zaki Ahmed B. Munshi, Mubarak H. Shaikh, Pravinsing S. Girase, Iqrar Ahmad, Harun Patel & Bhata R. Chaudhari (2024) Antibacterial Activity of Novel 1-Cyclopropyl-6,7-Difluoro-8-Methoxy-4-Oxo-1,4-Dihydroquinoline-3-Carbohydrazide Derivatives, Polycyclic Aromatic Compounds, 44:4, 2703-2714, DOI: 10.1080/10406638.2023.2220866

To link to this article: https://doi.org/10.1080/10406638.2023.2220866

Published online: 09 Jun 2023.

POLYCYCLIC AROMATIC COMPOUNDS
2024, VOL. 44, NO. 4, 2703–2714
https://doi.org/10.1080/10406638.2023.2220866

Antibacterial Activity of Novel 1-Cyclopropyl-6,7-Difluoro-8-Methoxy-4-Oxo-1,4-Dihydroquinoline-3-Carbohydrazide Derivatives

Zaki Ahmed B. Munshi[a], Mubarak H. Shaikh[b], Pravinsing S. Girase[a], Iqrar Ahmad[d], Harun Patel[d], and Bhata R. Chaudhari[a,c]

[a]Department of Chemistry, JET, Z. B. Patil College, Dhule, India; [b]Department of Chemistry, Radhabai Kale Mahila Mahavidyalaya, Ahmednagar, India; [c]Ex-Principal, SSVPS's ACS College, Shindkheda, Dhule, India; [d]Division of Computer Aided Drug Design, Department of Pharmaceutical Chemistry, R.C. Patel Institute of Pharmaceutical Education and Research, Shirpur, India

ABSTRACT

We have synthesized and characterized *N*-substituted-1-cyclopropyl-6,7-difluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carbohydrazide derivatives and were evaluated for their antibacterial activity against *Staphylococcus Aureus*, *Micrococcus Luteus*, *Bacillus subtilis* and Gram-negative *Escherichia Coli*, *Pseudomonas aeruginosa* and *Flavobacterium Devorans* pathogens and found that any modification is done at C-3 position then the activity of quinolone scaffold is decreased. This shows that presence of carboxylic acid group at C-3 position is very important for antibacterial activities. To gain more molecular insight into the binding interaction of the synthesized compounds, docking studies with to *S. aureus* DNA gyrase (PDB: 2XCT) were conducted. Based on its potential anti-bacterial properties, the most active molecule, **5a**, was submitted to molecular docking simulations using Schrödinger Glide software. The fluoroquinolones mechanism of action is entirely compatible with the binding interaction of the compound **5a**. Further, all the synthesized compounds tested for *In Silico* ADME prediction and observed that all the compounds followed the criteria for orally active drug and therefore, these compounds can be further developed an oral drug candidate.

ARTICLE HISTORY
Received 1 December 2022
Accepted 29 May 2023

KEYWORDS
Antibacterial; quinoline-3-carbohydrazide; gatifloxacin; ciprofloxacin; ADME prediction

Introduction

Nalidixic acid was the first quinolone drug developed in 1960[1–3] and then pipermidic acid, piromidic acid and cinoxacin. But the clinical application of these drug was very narrow due to its narrow band of antimicrobial activity.[4–5] They were only used for the treatment of Gram negative urinary tract infection caused due to enteric bacteria and to treat bacterial enteritis.[6] After the introduction for clinical use found number of organisms quickly developed resistance to it.[7–8] After large number of structural modifications on 1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carboxylic acid core found that the presence of fluorine atom at C-6 position increased DNA gyrase inhibitory activity, it also facilitated penetration into the bacterial cell, absence of nitrogen at C-8 position enhances the biological activity spectrum.[9] The quinolones with these modifications are known as fluoroquinolones which have broad spectrum

CONTACT Zaki Ahmed B. Munshi zbmunshi@yahoo.com Department of Chemistry, JET, S Z. B. Patil College, Dhule; Mubarak H. Shaikh mubbarakshaikh01@gmail.com Department of Chemistry, Radhabai Kale Mahila Mahavidyalaya, Ahemadnagar, India

antimicrobial activity and shows good active against aerobic Gram-negative microorganisms and less active against Gram-positive microorganisms. So they are extremely useful in treating variety of infectious diseases.

A number of infectious diseases such as community-acquired pneumonia, respiratory tract infections, including acute bacterial exacerbations of chronic bronchitis, respiratory tract infections, nosocomial pneumonia, uncomplicated and complicated urinary tract infections, bacterial prostatitis, skin and other soft tissue infections, joint and bone infections, gastrointestinal infections caused due to toxigenic E. coli or Salmonella species and bacterial sinusitis are successfully treated with fluoroquinolones.[10] Due to rapid scientific progress in the last few decades had made the treatment of infectious diseases possible to some extend but still remains a very serious and challenging health problems due to several factors, which have led to the reemergence of these diseases.[11] Now a day's antibiotic resistance problem has increased considerably. Millions of people are dying worldwide because of antibacterial resistance problems.[12] So, for keeping control on the microorganism's resistance, the existing antimicrobial drugs should be used very carefully and novel drugs should be design with different modes of action. Increase in antimicrobial agents use leads to resistance against microbes, despite having the remarkable features of new antimicrobial agents and it is unpreventable. So new clinical strategies are required to delay or minimize the risk of development of antibiotic resistance and the fluoroquinolones antimicrobial agents are not exception to this. There must be clinical guidelines for the use of antimicrobial agents such as appropriate dose, period of dose and combination with other agents. The use of antimicrobial agents will continue to remain a difficult challenge worldwide. So, the introduction of the new fluoroquinolones has created a new hope and exciting era for treatment of antimicrobial infections. To delay the development of resistance to the 'newer' fluoroquinolones will also need to be constantly monitoring the changing therapeutic environment if these agents are to comprehend their full therapeutic potential.

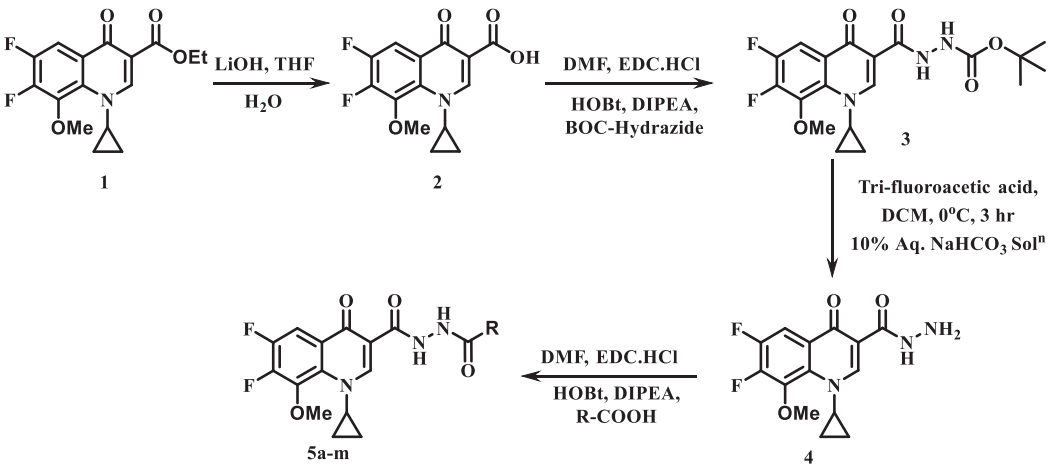
In Continuation to our earlier work,[13] herein, we have reported the synthesis of *N*-substituted 1-cyclopropyl-6,7-difluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carbohydrazide derivatives and their antibacterial evaluation, molecular docking study and *In Silico* ADME prediction.

Results and discussions

Chemistry

The achieve the synthesis of target compounds, initially hydrolysis of compound ethyl-1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4oxoquinoline-3-carboxylate **(1)** with lithium hydroxide was carried out in THF and water as a solvent (Scheme 1).

Then, the intermediate 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic acid (**2**) was further reacted with Boc hydrazide in presence of the coupling reagent 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC.HCl) and 1-Hydroxybenzotriazole (HOBt). This reaction carried out in aprotic *N,N*-Dimethylformamide (DMF) solvent and *N,N*-Diisopropylethylamine (DIPEA) as base. The *tert*-butyl *N*-[(1-cyclopropyl-6,7-difluoro-8-methoxy-4-oxo-quinoline-3-carbonyl)amino] carbamate **(3)** was isolated and subsequently Boc group deprotected in presence of trifluoroacetic acid to give 1-cyclopropyl-6,7-difluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carbohydrazide **(4)**. Finally, compound **(4)** was coupled with different acids using coupling reagent EDC.HCl and HOBt. This reaction carried out in aprotic DMF solvent and DIPEA as base (Scheme 1). Thus, the structures of different novel derivatives of *N*'-substituted-1-cyclopropyl-6,7-difluoro-8-methoxy-4-oxo-quinoline-3-carbohydrazide were shown in Figure 1.



Scheme 1. Synthesis of fluoroquinolones **5a–m**.

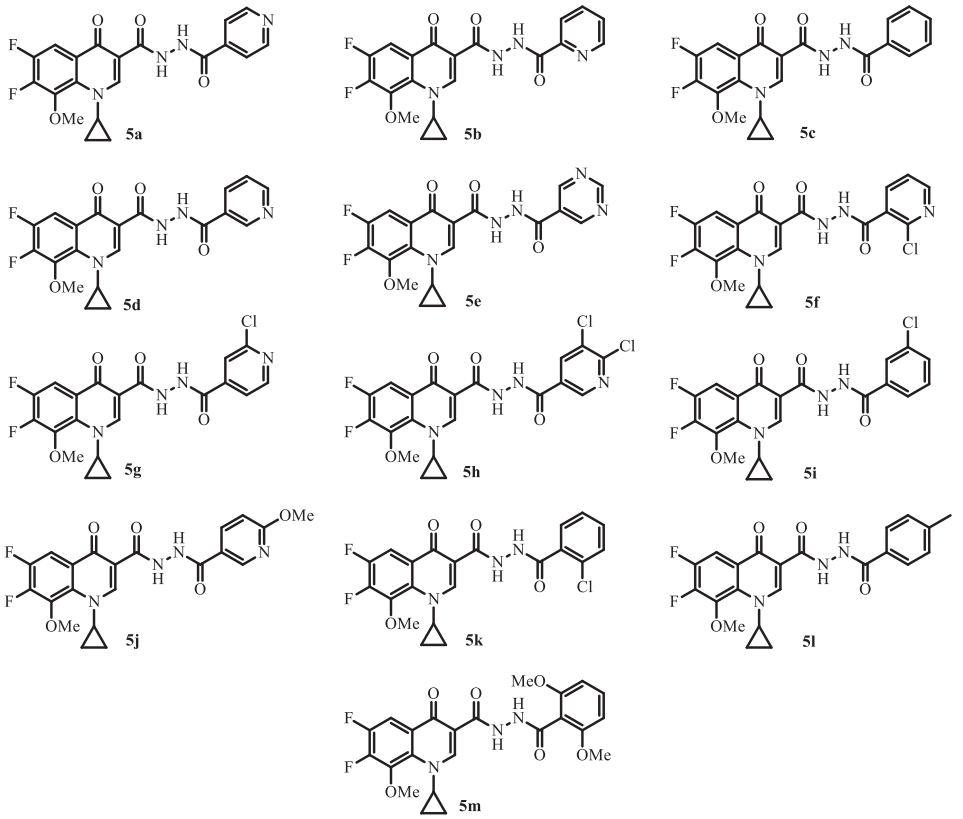


Figure 1. Structures of synthesized fluoroquinolones **5a–m**.

Biological activity

Antibacterial activity

Antibacterial activity of newly synthesized *N*-substituted-1-cyclopropyl-6,7-difluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carbohydrazide derivatives were screened by measuring the zone of growth

Table 1. *In vitro* antibacterial evaluation of compounds **2**, **3**, **4** and **5a-m** MIC values (μg/mL).

	Gram + ve bacteria			*Gram –ve bacteria*		
Entry	*SA*	*ML*	*BS*	*EC*	*PA*	*FD*
2	16.23	12.56	14.56	21	18.18	19.22
3	8.04	6.55	7.12	10.57	9.56	9.88
4	–	–	–	–	–	–
5a	9.91	8.5	8.69	14.26	11.23	10.2
5b	–	–	–	7.77	–	–
5c	–	–	–	–	–	–
5d	–	–	–	–	–	–
5e	–	–	–	–	–	–
5f	–	–	–	–	–	–
5g	–	–	–	–	–	–
5h	–	–	–	–	–	–
5i	–	–	–	–	–	–
5j	–	–	–	–	–	–
5k	–	–	–	–	–	–
5l	–	–	–	–	–	–
5m	-	-	-	-	-	-
Ciprofloxacin	26.94	24.26	22.30	32.70	27.88	23.42

SA: Staphylococcus aureus; ML: Micrococcus luteus; BS: Bacillus subtilis; EC: Escherichia coli; PA: Pseudomonas aeruginosa; FD: Flavobacterium devorans; '–': no zone of inhibition.

inhibition against the tested bacteria. These compounds were screened for antibacterial activity and were compared with ciprofloxacin as standard. The results were summarized in Table 1.

Computational study

Molecular docking

To gain more molecular insight into the binding interaction of the synthesized compounds, docking studies with to *S. aureus* DNA gyrase (PDB: 2XCT) were conducted. Fluoroquinolones interact with DNA gyrase with particular domains and conformations, preventing catalysis of DNA strand passage and stabilizing DNA-enzyme complexes that impede the DNA replication process and cause double breaks in the DNA, resulting in their bactericidal effect. Based on its potential anti-mycobacterial properties, the most active molecule, **5a**, was submitted to molecular docking simulations using Schrodinger Glide software. The fluoroquinolones mechanism of action is entirely compatible with the binding interaction of the compound **5a**. The fluoroquinolone scaffold of compound **5a** binds between the two central base pairs of the stretched DNA, in the midst of the two active sites, which is comparable to that of the reference ciprofloxacin.[14] Compound **5a**, had a Glide docking score of −7.443 kcal/mol, whereas the co-crystalized ligand (Ciprofloxacin) had a value of −8.495 kcal/mol. The 2D and 3D graphical representations of the ligand-protein interactions illustrated in Figure 2 were created using Maestro's ligand-interaction tool. The 2D diagram showed π-π interaction with Phe1123 through the terminal pyridine ring of in *S. aureus* DNA gyrase target. The quinolone scaffold further generates Van der Waals contacts with DNA nucleotide bases DT E8 and DG G9, specifically π-π interactions, with binding energies of −10.424 kcal/mol and −8.225 kcal/mol, respectively.

In Silico ADME prediction

The sensation of a drug is determined by a suitable ADME (absorption, distribution, metabolism and excretion) profile and its efficacy. In the current study, we have calculated molecular volume (MV), molecular weight (MW), logarithm of partition coefficient (miLog *P*), number of hydrogen bond acceptors (n-ON), number of hydrogen bonds donors (n-OHNH), topological polar surface

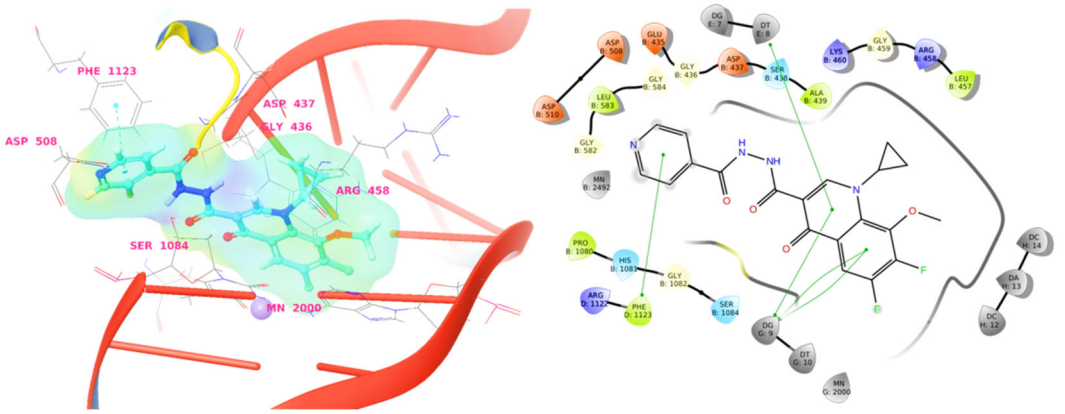


Figure 2. 3D and 2D Diagrams illustrate the binding mode of compounds **5a** interacted with the active site of *S. aureus* DNA gyrase (PDB: 2XCT) enzyme.

Table 2. Pharmacokinetic parameters important for good oral bioavailability.

Entry	% ABS	TPSA (A^2)	n-ROTB	MV	MW	miLog *P*	n-ON acceptors	n-OHNH donors	Lipinski violation	Drug likeness score
Rule	–	–	–	–	<500	≤5	<10	<5	≤1	–
5a	73.48	102.33	5	337.41	414.37	−0.83	8	2	0	1.28
5b	73.48	102.33	5	337.41	414.37	−0.71	8	2	0	0.99
5c	78.14	89.44	5	341.56	413.38	0.46	7	2	0	0.85
5d	73.48	102.33	5	337.41	414.37	−0.77	8	2	0	1.55
5e	69.24	115.22	5	333.25	415.36	−1.65	9	2	0	1.00
5f	73.48	102.33	5	350.94	448.81	0.19	8	2	0	1.14
5g	73.48	102.33	5	350.94	448.81	0.19	8	2	0	0.88
5h	73.48	102.33	5	364.48	483.26	0.85	8	2	0	0.83
5i	78.14	89.44	5	355.10	447.82	1.12	7	2	0	0.91
5j	74.95	98.67	6	367.11	443.41	0.52	8	2	0	0.82
5k	78.14	89.44	5	355.10	447.82	1.09	7	2	0	1.22
5l	78.14	89.44	5	358.12	427.41	0.91	7	2	0	0.88
5m	71.77	107.90	7	392.66	473.43	0.48	9	2	0	0.85

% ABS: percentage absorption, TPSA: topological polar surface area, n-ROTB: number of rotatable bonds, MV: molecular volume, MW: molecular weight, milogP: logarithm of partition coefficient of compound between n-octanol and water, n-ON acceptors: number of hydrogen bond acceptors, n-OHNH donors: number of hydrogen bonds donors.

area (TPSA), number of rotatable bonds (n-ROTB) and Lipinski's rule of five[15] using Molinspiration online property calculation toolkit.[16] Absorption (% ABS) was calculated by: % ABS = 109-(0.345 × TPSA)[17] Drug-likeness model score (a collective property of physic-chemical properties, pharmacokinetics and pharmacodynamics of a compound is represented by a numerical value) was computed by MolSoft[18] software.

The pharmacokinetic data of all the synthesized compounds **5a-m** was within the range of accepted values. It is detected that all the synthesized compounds exhibited a good % ABS ranging from 69.24 to 78.14% (Table 2). Also, the synthesized compounds do not violated Lipinski's rule of five, thus showing potential utility of series for developing the compound with good drug like properties. A molecule can be developed for an orally active drug candidate and must not show more than one violation of the following four criteria: miLog *P* (octanol-water partition coefficient) ≤5, molecular weight ≤500, number of hydrogen bond acceptors ≤10 and number of hydrogen bond donors ≤5.[19] The greater the value of the drug likeness model score, the greater is the possibility that the particular molecule will be active. All the synthesized compounds followed the essential criteria for orally active drug and therefore, these compounds can be further taken for the development as oral drug candidates.

Experimental

Chemistry

All the raw materials used for synthesis are obtained from commercial suppliers and purified as per requirement. The intermediate ethyl 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxo-quinoline-3-carboxylate is commercially available and synthesis of this intermediate is also reported in literature. Mass spectra were recorded on 'LCMS-Qp2010s' instrument by direct injection method. Nuclear Magnetic Resonance spectra (^{1}HNMR & ^{13}C NMR) were recorded on Bruker advance spectrometer (400 MHz) using DMSO-d_6 or $CDCl_3$ solvents. Tetramethylsilane was used as internal standard. Chemical shift (δ) are reported in parts per million. Reactions were monitored and its purity was checked by Merck pre-coated plate (silica gel 60 F_{254}) Thin Layer Chromatography was visualized with UV light. Melting points were determined in open capillary tube and are uncorrected. The FT-IR instrument used for analysis of samples was IR Affinity 1, Shimazu. The diffuse reflectance scan (DRS) method of sample preparation was used to scan samples. The samples were scanned in the range of 4000 to 400 cm^{-1}.

Synthesis of 1-Cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic acid (2)

To the suspension of ethyl-1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylate **(1)** (15.4 mmol) in THF (50 ml) was added the lithium hydroxide (16.94 mmol) solution (25 ml water) drop wise at 0 °C in 30 min. Reaction mixture was stirred at room temperature for 4 hr. Completion of reaction checked with TLC. After completion of reaction solvent was removed under the reduced pressure at 35 °C. Cool the reaction mass to 0 °C and pH was adjusted (between 5 to 6) using 2 N aq. HCl solution. Reaction mass was filtered and cake obtained was washed with water (3 × 20 mL) and dried under reduced pressure at 35 °C. Compound **(2)** obtained as white solid; yield 85%; mp 188–190 °C; ^{1}H NMR ($CDCl_3$) (400 MHz) δ: 14.39 (s,1H), 8.875 (s, 1H), 8.075–8.029 (t, 1H, $J = 8.4$ Hz), 4.151 (s, 3H), 4-145–4.127 (m, 1H), 1.303–1.284 (m, 2H), 1.144–1.129 (m, 2H); ^{13}C NMR (400 MHz, DMSO-D_6, ppm) δ: 176.13, 165.12, 150.94, 150.13- 149.51, 147.63–147, 141.13–141.01, 132.89, 122.7–122.70, 106.90, 106.75–106.56, 63.38–63.31, 41.01, 8.63; MS (ESI) m/z 296.2 $[M + H]^+$.

Synthesis of Tert-butyl-N-[(1-cyclopropyl-6,7-difluoro-8-methoxy-4-oxo-quinoline-3-carbonyl)amino]carbamate (3)

To the suspension of 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic acid **(2)** (16.9 mmol) in DMF (40 ml) were added 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC.HCl) (25.4 mmol) and 1-Hydroxybenzotriazole (HOBt) (20.3 mmol) at 0 °C and stirred for 1 hr. Boc-Hydrazide (20.3 mmol) and *N,N*-Diisopropylethylamine (50.8 mmol) were added. After stirring the reaction mixture for 1h at 0 °C, Allowed the mixture to stir at room temperature for 16 hr. Reaction was monitored by TLC. The reaction was quenched to 500 mL chilled water, filtered and washed the cake with 50 mL water. Dry the solid mass under reduced pressure at 40 °C. The crude product obtained was purified by passing through silica gel (60–120 mesh). Chloroform and methanol was used to elute the compound. Off white solid; Yield 75%; mp 180–182 °C; IR (KBr) (ν_{max}, cm^{-1})1069, 1287, 1323, 1657, 1769, 3094, 3215; ^{1}H NMR (DMSO-D_6) (400 MHz) δ: 10.750 (1H, s), 9.032(1H, s), 8.695(1H, s), 7.982–7.935 (1H, m), 4.154–4.131 (1H, m), 4.058 (3H, s), 1.403 (9H, s), 1.169–1.081 (4H, m). ^{13}C NMR (500 MHz, DMSO-D_6, ppm) δ: 173.42, 163.611, 155.004, 149.905, 149.51–149, 147.63–147, 140.69, 132.33, 124.198, 109.105, 107.025, 79.31, 63.22, 40.162, 28.013, 8.578; ES-MS: m/z 410.4 $[M + H]^+$.

Synthesis of 1-Cyclopropyl-6,7-difluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carbohydrazide (4)

Tert-butyl-*N*-[(1-cyclopropyl-6,7-difluoro-8-methoxy-4-oxo-quinoline-3-carbonyl) amino]carbamate **(3)** (9.78 mmol) was taken in a DCM (10 ml) and allowed to cooled to 0 °C. Then Trifluroacetic acid (TFA) (15 mL) was added to the reaction mass and stirred at 0 °C for 3 h. TLC run in 10% methanol and 90% chloroform system show absence of starting material. The solvent was distilled under reduced pressure at 40 °C. Reaction mass was dissolved in DCM (50 mL) and washed with 50 mL 10% $NaHCO_3$ solution. Wash DCM layer with 2×10 mL times with water and DCM layer was dried with anhydrous Na_2SO_4. Distilled out the whole solvent under reduced pressure at 40 °C. The crude product obtained was purified by crystallization with ethyl acetate (20 mL). Off white solid; Yield 76%; mp 194–196 °C; IR (KBr) (ν_{max}, cm^{-1}) 1061, 1285, 1321, 1659, 1746, 3040, 3281, 3495; ^{1}H NMR (DMSO-D_6) (400 MHz) δ: 10.33(1H, s), 8.683(1H, s), 7.962–7.814 (1H, t, $J = 8.8$ Hz), 4.588 (2H, s), 4.148–4.113 (1H, m), 4.048 (3H, s), 1.168–1.056 (4H, m); ^{13}C NMR (400 MHz, DMSO-D_6, ppm) δ: 173.28, 162.881, 149.461, 149.10, 148.99, 140.609, 132.304, 124.114, 109.604, 106.970, 63.250, 40.126, 8.658; ES-MS: m/z 310.1 $[M + H]^+$.

Synthesis of N′-substituted-1-cyclopropyl-6,7-difluoro-8-methoxy-4-oxo-1,4-dihydro quinoline-3-carbohydrazide (5a-m)

To the suspension of carboxylic acid (0.97 mmol) in DMF (5 ml) were added EDC.HCl (1.451 mmol) and HOBt (1.02 mmol) at 0 °C and stirred for 1 h. 1-Cyclopropyl-6,7-difluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carbohydrazide **(4)** (0.97 mmol) and *N*,*N*-Diisopropylethylamine (2.91 mmol) were added, After stirring the reaction mixture for 1 h at 0 °C, allow the mixture to stirred at room temperature for another 16 h. The progress of reaction was monitored by TLC. 10% Methanol and 90% chloroform solvent system was used as mobile phase. The reaction mass was quenched with 50 mL chilled water, filter and wash the cake with 5 mL water. Obtained solid mass was dried the under reduced pressure at 40 °C. Crude mass was purified by crystallization in methanol solvent.

Synthesis of 1-Cyclopropyl-6,7-difluoro-N′-isonicotinoyl-8-methoxy-4-oxo-1,4-dihydro quinoline-3-carbohydrazide (5a)

A off white color solid; Yield 47%; mp = 212–218 °C; IR (KBr) (ν_{max}, cm^{-1}): 1180, 1317, 1065, 1638, 3026, 318, 3385; ^{1}H NMR (DMSO-D_6) (400 MHz) δ: 11.457 (s, 1H), 11.192 (s, 1H), 8.766 − 8.732 (m, 3H), 8.021–7974 (m, 1H), 7.802- 7.786 (d, 2H, $J = 6.4$ Hz), 4.176–4.158 (m, 1H), 4.071 (s, 3H), 1.183–1.094 (m, 4H); ^{13}C NMR (400 MHz, DMSO-D_6, ppm) δ: 173.472, 163.142, 162.36, 150.356, 149.960, 149.67, 147.198, 140.764, 139.329, 132.399, 124.209, 121.440, 108.959, 107.132, 63.290, 40.293, 8.650; ES-MS: m/z 415.2 $[M + H]^+$.

1-Cyclopropyl-6,7-difluoro-8-methoxy-4-oxo-N′-(pyridin-2-ylcarbonyl)-1,4-dihydro-quinoline-3-carbohydrazide (5b)

A off white color solid; Yield 50%; mp = >230 °C; IR (KBr) (ν_{max}, cm^{-1}): 1057, 1285, 1319, 1651, 1719, 2318, 2980, 3267.1; ^{1}H NMR (DMSO-D_6) (400 MHz) δ: 11.509 (s, 1H), 10.786 (s, 1H), 8.725(s, 1H), 8.699 − 8.686 (d, 1H, $J = 5.2$ Hz), 8.040–7.979 (m, 3H), 7.671–7.632 (m, 1H), 4.186–4.151 (m, 1H), 4.071 (s, 3H), 1.184–1.094 (m, 4H); ^{13}C NMR (500 MHz, DMSO-D_6, ppm) δ: δ 173.50, 161.88, 161.54, 149.88, 148.75, 148.72, 147.43, 140.75, 140.66, 137.93, 132.44, 127.07, 124.18, 122.33, 108.92, 107.12, 63.28, 40.26, 8.63; ES-MS: m/z 415.3 $[M + H]^+$.

N′-Benzoyl-1-cyclopropyl-6,7-difluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carbohydrazide (5c)

A off white color solid; Yield 62.5%; mp = 226–228 °C; IR (ν_{max}, cm^{-1}): 1103, 1280, 1311, 1458, 1604, 1627, 1674, 2924, 3178; ^{1}H NMR ($CDCl_3$) (400 MHz) δ: 12.34 (s, 1H), 9.078 (s, 1H), 8.817 (s, 1H), 8.096–8.049 (m, 1H), 7.896–7.877 (d, 2H, J = 7.6 Hz), 7.538–7.442 (m, 3H), 4.117 (s, 3H), 4.089–4.054 (m, 1H), 1.256–1.095 (m, 4H); ^{13}C NMR (400 MHz, DMSO-D_6, ppm) δ: 173.52, 164.78, 162.55, 149.92, 149.61, 147.26, 140.65, 132.43, 132.31, 131.85, 128.45, 127.56, 124.18, 109.16, 107.15, 63.30, 40.26, 8.66; ES-MS: m/z 414.2 $[M+H]^+$.

1-Cyclopropyl-6,7-difluoro-8-methoxy-4-oxo-N′-(pyridin-3-ylcarbonyl)-1,4-dihydro quinoline-3-carbohydrazide (5d)

A off white color solid; Yield 87%; mp = 220–222 °C; IR (KBr) (ν_{max}, cm^{-1}): 1095, 1288, 1319, 1465, 1604, 1674, 2993, 3170; ^{1}H NMR (DMSO-D_6) (400 MHz) δ: 11.444 (s, 1H), 1.096 (s, 1H), 9.0345 (s, 1H), 8.75–8.736 (m, 2H), 8.244–8.214 (m, 1H), 8.025–7.979 (m, 1H), 7.561–7.527 (m, 1H), 4.187–4.152 (m, 1H), 4.072 (s, 3H), 1.184–1.154 (m, 2H), 1.117–1.096 (m, 2H); ^{13}C NMR (400 MHz, DMSO-D_6, ppm) δ: δ 173.511, 163.304, 162.405, 152.428, 149.960, 149.90, 148.541, 147.92, 140.775, 135.319, 132.454, 128.060, 124.217, 123.603, 109.006, 107.155, 63.297, 40.272, 8.650; ES-MS: m/z 415.3 $[M+H]^+$.

1-Cyclopropyl-6,7-difluoro-8-methoxy-4-oxo-N′-(pyrimidin-5-yl-carbonyl)-1,4-dihydro quinoline-3-carbohydrazide (5e)

A off white color solid; Yield 62%; mp = 230–232 °C; IR (KBr) (ν_{max}, cm^{-1}): 1095, 1188, 1319, 1465, 1612, 1643, 1681, 3047, 3170; ^{1}H NMR (DMSO-D_6) (400 MHz) δ: 11.570 (s, 1H), 11.376 (s, 1H), 9.355 (s, 1H), 9.195 (s, 2H), 8.737 (s, 1H), 8.025 − 7.979 (t, 1H, J = 9.2 Hz), 4.188- 4.153 (m, 1H), 4.072 (s, 3H), 1.186–1.168 (m, 2H), 1.119–1.098 (m, 2H); ^{13}C NMR (400 MHz, DMSO-D_6, ppm) δ: 173.484, 162.049, 161.272, 160.448, 156.106, 149.975, 149.694, 147.214, 140.783, 132.419, 126.242, 124.197, 108.885, 107.151, 63.317, 40.324, 8.665; m/z 416.3 $[M+H]^+$.

N′-[(2-Chloropyridin-3-yl)carbonyl]-1-cyclo-propyl-6,7-difluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carbohydrazide (5f)

A off white color solid; Yield 61%; mp = 218–220 °C; IR (KBr) (ν_{max}, cm^{-1}): 756, 794, 1072, 1180, 1327, 1465, 1604, 1635, 3047, 3163; ^{1}H NMR (DMSO-D_6) (400 MHz) δ: 11.645 (s, 1H), 11.218 (s, 1H), 8.736 (s, 1H), 8.520–8.504 (m, 1H), 8.000–7.976 (m, 2H), 7.546–7.515 (m, 1H), 4.187- 4.152 (m, 1H), 4.074 (s, 3H), 1.184–1.153 (m, 2H), 1.117 (m, 2H); ^{13}C NMR (400 MHz, DMSO-D_6, ppm) δ: 175.456, 162.386, 161.419, 150.895, 149.944, 149.744, 147.055, 140.811, 138.802, 132.407, 130.775, 124.158, 123.008, 108.820, 107.112, 63.329, 40.356, 8.662; ES-MS: m/z 449.2 $[M+H]^+$.

N′-(2-Chloroisonicotinoyl)-1-cyclopropyl-6,7-difluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carbohydrazide (5 g)

A off white color solid; Yield 57.4%; mp = 222–224 °C; IR (KBr) (ν_{max}, cm^{-1}): 763, 794, 1103, 1319, 1465, 1604, 1643, 1681, 2993, 3170; ^{1}H NMR ($CDCl_3$) (400 MHz) δ: 12.441 (s, 1H), 10.012 (s, 1H), 8.742 (s, 1H), 8.501–8.488 (d, 1H, J = 5.2 Hz), 8.073–8.028 (m, 1H), 7.796 (s, 1H), 7.683–7.263 (m, 1H), 4.133 (s, 3H), 4.127–4.069 (m, 1H), 1.634–1.226 (m, 2H), 1.103–1.061 (m, 2H); ^{13}C NMR (400 MHz, DMSO-D_6, ppm) δ: 173.47, 162.135, 161.609, 150.879, 149.983, 149.783, 147.219, 142.895, 140.783, 132.422, 123.201, 122.228, 121.086, 108.867, 107.139, 63.309, 40.320, 8.661; ES-MS: m/z 449.3 $[M+H]^+$.

1-Cyclopropyl-N′-[(5,6-dichloropyridin-3-yl)carbonyl]-6,7-difluoro-8-methoxy-4-oxo-1,4-dihydro-quinoline-3-carbohydrazide (5h)

A off white color solid; Yield 74.6%; mp = 220–222 °C; IR (KBr) (ν_{max}, cm^{-1}): 794, 941, 1180, 1319, 1465, 1550, 1597, 1620, 3001, 3147; ^{1}H NMR ($CDCl_3$) (400 MHz): δ 11.59 (s, 1H), 11.35 (s, 1H), 8.84 (s, 1H), 8.75 (s, 1H), 8.54 (s, 1H), 8.038–7.992 (m, 1H), 4.187–4.178 (m, 1H), 4.092 (s, 3H), 1.204–1.116 (m, 4H); ^{13}C NMR (400 MHz, DMSO-D_6, ppm) δ: 173.460, 162.050, 160.781, 150.317, 149.957, 149.675, 147.076, 146.521, 140.772, 138.359, 132.399, 129.392, 128.715, 124.182, 108.840, 107.36, 63.302, 40.305, 8.650; ES-MS: m/z 485.2 $[M+H]^+$.

N′-(3-Chlorobenzoyl)-1-cyclopropyl-6,7-difluoro-8-methoxy-4-oxo-1,4-dihydro quinoline-3-carbohydrazide (5i)

A off white color solid; Yield 75.9%; mp = 196–198 °C; IR (KBr) (ν_{max}, cm^{-1}): 740, 763, 794, 941, 1072, 1103, 1319, 1458, 1550, 1604, 1627, 1674, 3194; ^{1}H NMR (DMSO-D_6) (400 MHz) δ: 11.401(s, 1H), 10.999 (s, 1H), 8.730(s, 1H), 8.022–7.975 (m, 1H), 7.932 (s, 1H), 7.862–7.844 (d, 1H, J = 7.2 Hz), 7.669–7.646 (m, 1H), 7.565–7.526 (m, 1H), 4.176 4–166 (m, 1H), 4.072 (s, 3H), 1.183–1.093 (m, 4H); ^{13}C NMR (400 MHz, DMSO-D_6, ppm) δ: 173.500, 163.360, 162.453, 149.972, 149.566, 147.523, 140.722, 134.269, 133.263, 132.512, 131.702, 130.537, 127.351, 126.321, 124.231, 109.038, 107.172, 63.313, 40.273, 8.658; ES-MS: m/z 448.3 $[M+H]^+$.

1-Cyclopropyl-7-fluoro-N′-(4-methoxy-benzoyl)-4-oxo-1,4-dihydroquinoline-3-carbo hydrazide (5j)

A off white color solid; Yield 62.7%; mp = 218–220 °C; IR (KBr) (ν_{max}, cm^{-1}): 794, 848, 941, 1026, 1103, 1180, 1280, 1319, 1458, 1597, 1627, 1674, 3001, 3163; ^{1}H NMR (DMSO-D_6) (400 MHz) δ: 11.312 (s, 1H), 10.665 (s, 1H), 8.727 (s, 1H), 8.022–7.975 (m, 1H), 7.891–7.870 (d, 2H, J = 8.4 Hz), 7.039–7.016 (d, 2H, J = 9.2 Hz), 4.182–4.156 (m, 1H), 4.07 (s, 3H), 3.815 (s, 3H), 1.167 (m, 2H), 1.091 (m, 2H); ^{13}C NMR (400 MHz, DMSO-D_6, ppm) δ: 173.515, 164.275, 162.556, 162.017, 149.884, 149.576, 147.132, 140.755, 132.419, 129.451, 124.407, 124.256, 113.673, 109.212, 107.163, 63.301, 55.392, 40.233, 8.661; ES-MS: m/z 444.3 $[M+H]^+$.

N′-(2-Chlorobenzoyl)-1-cyclopropyl-6,7-difluoro-8-methoxy-4-oxo-1,4-dihydro quinoline-3-carbo- hydrazide (5k)

A off white color solid; Yield 69%; mp = 244–246 °C; IR (KBr) (ν_{max}, cm^{-1}): 771, 840, 941, 1103, 1172, 1311, 1458, 1496, 1597, 1627, 1681, 2993, 3155; ^{1}H NMR (DMSO-D_6) (400 MHz) δ: 11.490 (s, 1H), 10.933(s, 1H), 8.737(s, 1H), 8.021–7.974 (m, 1H), 7.544–7.426 (m, 4H), 4.180–4.161 (m, 1H), 4.073 (s, 3H), 1.184–1.153 (m, 2H), 1.117–1.107 (m, 2H); ^{13}C NMR (400 MHz, DMSO-D_6, ppm) δ: δ 173.480, 163.998, 161.834, 149.928, 149.490, 147.213, 140.791, 134.352, 132.399, 131.456, 130.529, 129.740, 129.491, 127.066, 124.173, 108.970, 107.104, 63.301, 63.301, 40.292, 8.360; ES-MS: m/z 448.3 $[M+H]^+$.

1-Cyclopropyl-6,7-difluoro-8-methoxy-N′-(4-methylbenzoyl)-4-oxo-1,4-dihydroquinoline-3-carbohydrazide (5 l)

A off white color solid; Yield 78.42%; mp = 240–242 °C; IR (KBr) (ν_{max}, cm^{-1}): 732, 802, 933, 1056, 1103, 1180, 1242, 1319, 1465, 1558, 1604, 1666, 1689, 2947, 3086, 3317; ^{1}H NMR ($CDCl_3$) (400 MHz) δ: 12.337 (s, 1H), 8.95 (s, 1H), 8.822 (s, 1H), 8.082 (m, 1H), 7.788–7.768 (d, 2H, J = 8 Hz), 7.274–7.262 (d, 2H, J = 4.8 Hz), 4.119 (s, 3H), 4.114–4.072 (m, 1H), 2.411 (s, 3H), 1.255–1.240 (m, 2H), 1.101–1.095 (m, 2H); ^{13}C NMR (400 MHz, DMSO-D_6, ppm) δ: δ 173.519, 164.673, 162.564, 149.930, 149.622, 147.287, 141.853, 140.776, 132.332, 129.483, 128.983, 127.573, 125.153, 109.176, 107.195, 63.321, 40.245, 21.040, 8.669; ES-MS: m/z 428.4 $[M+H]^+$.

1-Cyclopropyl-6,7-difluoro-N′-(2,6-methoxybenzoyl)-8-methoxy-4-oxo-1,4-dihydroquinolone-3-carbohydrazide (5 m)

A off white color solid; Yield 71%; mp = 240–242 °C; IR (KBr) (ν_{max}, cm^{-1}): 756, 794,941, 1111, 1180, 1257, 1311, 1465, 1589, 1635, 1689, 3016, 3217, 3371, 3556; ^{1}H NMR (DMSO-D_6) (400 MHz) δ: 11.690 (s, 1H), 10.819 (s, 1H), 8.719 (s, 1H), 8.024–7.976 (m, 1H), 7.7.324 (t, 1H, J = 8.8 Hz), 6.686–6.665 (d, 2H, J = 8.4 Hz), 4.176–4.166 (m, 1H), 4.075 (s, 3H), 3.738 (s, 6H), 1.183–1.167 (m, 2H), 1.118–1.087 (m, 2H); ^{13}C NMR (400 MHz, DMSO-D_6, ppm) δ: 173.404, 161.268, 160.249, 157.290, 157.290, 149.566, 147.223, 140.499, 132.438, 130.782, 124.133, 113.879, 109.148, 107.100, 104.144, 63.321, 55.761, 30.252, 8.660; ES-MS: m/z 474.4 $[M+H]^+$.

Biology

Antibacterial activity (Disk diffusion assay):

Various bacterial strains - Gram-positive *Staphylococcus Aureus* (NCIM No. 2079), *Micrococcus Luteus* (ATCC No. 398), *Bacillus subtilis* (NCIM No. 2250) and Gram-negative *Escherichia Coli* (NCIM No. 2109), *Pseudomonas aeruginosa* (NCIM No. 2036) and *Flavobacterium Devorans* (ATCC No. 10829) were used as test microorganism to evaluate the antimicrobial testing of newly synthesized compounds (Table 1) **2**, **3**, **4** and **5(a-m)**. Pure culture of test bacterial strain was picked with a loop and the growth was transferred into a tube containing 5 mL of a nutrient broth medium, while pure culture of test fungal strain was transferred into a tube containing 5 mL of a MGYP medium. The broth culture was incubated at 37 °C until it achieves or exceeds the turbidity of the 0.5 McFarland standards (usually to 6 h). The turbidity of the actively growing broth culture is adjusted with sterile saline or broth to obtain turbidity optically comparable to that of the 0.5 McFarland standards. This result in a suspension contains 2 x 10^8 CFU/mL of microbial cells.

Within 15 min after adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the adjusted suspension. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level. The surface of a nutrient agar plate was inoculated by streaking the swab over the entire sterile agar surface. This procedure is repeated by streaking several times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum.

Stock solution [1000 microgram per mL] of each newly synthesized compound was prepared in dimethyl sulfoxide (DMSO). The sterile disks of 6 mm diameter were used in this assay. The disk diffusion assay was carried out by taking concentration 100 microorganism per disk. The disks immersed with compounds were dispensed onto the surface of the inoculated agar plate. Also, Ciprofloxacin (10 µg/disk, Amphotericin-B (100 units/disk) [Hi-media, Mumbai, disk diameter 6 mm] moistened with DMSO were placed on agar plate as standard. Each disk was pressed down to ensure complete contact with the agar surface. The plates were placed in a refrigerator at to 8 °C for 30 min after the disks were applied. Then, the plates were incubated in incubator at 37 °C for 24 h.

Molecular docking

Molecular docking studies were performed on synthesized compounds against the crystal structure of DNA gyrase or type IIA topoisomerase from *S. aureus* (PDB ID: 2XCT) as a target enzyme. *LigPrep* was used to model the synthesized compounds by creating potential enantiomers, ionization, and tautomeric states at pH = 7.0 ± 2, and then ligands were energy minimized using default OPLS3e force field parameters.[20] The Protein Preparation Wizard was used to prepare the protein; during this procedure, crystallographic water molecules were eliminated, bond ordering and partial charges were assigned, ionization and tautomeric states of the residues were

established, and H bonds were assigned.[21] The Receptor Grid Generation tool was started by clicking on the co-crystalized ligand (Ciprofloxacin), and the default grid box was created. The docking was done in the active site of a receptor protein, utilizing the XP (extra precision) Glide simulation-based docking methodology.[22]

Conclusion

We have synthesized and characterized *N*-substituted-1-cyclopropyl-6,7-difluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carbohydrazide derivatives and were evaluated for their antibacterial activity against *Staphylococcus Aureus*, *Micrococcus Luteus*, *Bacillus subtilis* and Gram-negative *Escherichia Coli*, *Pseudomonas aeruginosa* and *Flavobacterium Devorans* pathogens and once again confirmed that at **C-3** position carboxylic acid group, Carbonyl group at **C-4** position and some heterocyclic amine or substituted heterocyclic amine etc at **C-7** position is necessary for antibacterial activity. So when compound **2** modified to compounds **5(b-m)** then these compounds shows no activity against these four stains. This shows that carboxylic acid group at **C-3** position is very important for antibacterial activities. To gain more molecular insight into the binding interaction of the synthesized compounds, docking studies with to *S. aureus* DNA gyrase (PDB: 2XCT) were conducted. Based on its potential anti-mycobacterial properties, the most active molecule, **5a**, was submitted to molecular docking simulations using Schrödinger Glide software. The fluoroquinolones mechanism of action is entirely compatible with the binding interaction of the compound **5a**. Further, all the synthesized compounds tested for *In Silico* ADME prediction and observed that all the compounds followed the criteria for orally active drug and therefore, these compounds can be further developed an oral drug candidate.

Acknowledgements

The authors are very thankful to the Management, Principal and Head of Dept. of Chemistry, JET's Z. B. Patil College, Dhule for providing the lab facilities and for encouragement. The authors are also thankful to Dr. Ulhas Patil, Head and Assistant Professor of R. C. Patel A. S. C. College, Shirpur (Dist Dhule) for testing antimicrobial activity of synthesized compounds.

Disclosure statement

No potential conflict of interest was reported by the author(s).

ORCID

Mubarak H. Shaikh http://orcid.org/0000-0002-1190-2371
Iqrar Ahmad http://orcid.org/0000-0002-7697-9572
Harun Patel http://orcid.org/0000-0003-0920-1266

References

1. M. I. Andersson, and A. P. Macgowan, "Development of the Quinolones," *Journal of Antimicrobial Chemotherapy* 51, no. 90001 (2003): 1–11. doi:10.1093/jac/dkg212
2. G. G. Zhanel, A. Walkty, L. Vercaigne, J. A. Karlowsky, J. Embil, A. S. Gin, and D. J. Hoban, "The New Fluoroquinolones: A Critical Review," *The Canadian Journal of Infectious Diseases = Journal Canadien Des Maladies Infectieuses* 10, no. 3 (1999): 207–238. doi:10.1155/1999/378394
3. R. Schaumann, and A. C. Rodloff, "Activities of Quinolones against Obligately Anaerobic Bacteria," *Anti-Infective Agents in Medicinal Chemistry* 6, no. 1 (2007): 49–56. doi:10.2174/187152107779314179

4. M. A. Cohen, M. D. Huband, G. B. Mailloux, S. L. Yoder, G. E. Roland, and C. L. Heifetz, "*In Vitro* Antibacterial Activities of the Fluoroquinolones PD 117596, PD 124816, and PD 127391," *Diagnostic Microbiology and Infectious Disease* 14, no. 3 (1991): 245–258. doi:10.1016/0732–8893(91)90039-i
5. V. Uivarosi, "Metal Complexes of Quinolone Antibiotics and Their Applications: An Update," *Molecules (Basel, Switzerland)* 18, no. 9 (2013): 11153–11197. doi:10.3390/molecules180911153
6. P. M. Shah, "Ciprofloxacin," *International Journal of Antimicrobial Agents* 1, no. 2–3 (1991): 75–96. doi:10.1016/0924-8579(91)90002-u
7. P. C. Appelbaum, and P. A. Hunter, "The Fluoroquinolone Antibacterials: Past, Present and Future Perspectives," *International Journal of Antimicrobial Agents* 16, no. 1 (2000): 5–15. doi:10.1016/s0924-8579(00)00192-8
8. P. Ball, "Quinolone Generations: Natural History or Natural Selection?," *Journal of Antimicrobial Chemotherapy* 46, no. suppl_3 (2000): 17–24. doi:10.1093/oxfordjournals.jac.a020889
9. R. Singh, and A. Debnath, "Synthesis and Biological Activities of Selected Quinolone-Metal Complexes," *Research Journal of Chemical Sciences* 3, no. 6 (2013): 83–94.
10. V. T. Andriole, "The Quinolones: Past, Present and Future," *Clinical Infectious Diseases* 41, no. 2 (2005): 113–119.
11. Y. D. Mane, S. M. Surwase, D. O. Biradar, Y. P. Sarnikar, B. H. Jawle, V. S. Shinde, and B. C. Khade, "Design and Synthesis of Diverse Pyrrole-2-Carboxamide Derivatives as a Potent Antibacterial Agents," *Journal of Heterocyclic Chemistry* 54, no. 5 (2017): 2627–2634. doi:10.1002/jhet.2859
12. M. Mentese, F. S. Beris, and N. Demirbas, "Synthesis of Some New Ciprofloxacin Hybrids as Potential Antimicrobial Agents," *Journal of Heterocyclic Chemistry* 54, no. 6 (2017): 2996–3007.
13. (a) S. V. Akolkar, M. H. Shaikh, M. K. Bhalmode, P. U. Pawar, J. N. Sangshetti, M. G. Damale, and B. B. Shingate, "Click Chemistry Inspired Syntheses of New Amide Linked 1, 2, 3-Triazoles from Naphthols: Biological Evaluation and *in Silico* Computational Study," *Research on Chemical Intermediates* 49, no. 6 (2023): 2725–2753. doi:10.1007/s11164-023-05008-4. (b) M. H. Shaikh, D. D. Subhedar, S. V. Akolkar, A. A. Nagargoje, A. Asrondkar, V. M. Khedkar, and B. B. Shingate, "New 1,2,3-Triazole-Tethered Thiazolidinedione Derivatives: Synthesis, Bioevaluation and Molecular Docking Study," *Polycyclic Aromatic Compounds* 43, no. 4 (2023): 3353–3379. (c) M. H. Shaikh, D. D. Subhedar, V. M. Khedkar, and B. B. Shingate, "[Et 3 NH][HSO 4]-Catalyzed One-Pot Solvent Free Syntheses of Functionalized [1,6]-Naphthyridines and Biological Evaluation," *Polycyclic Aromatic Compounds* 42, no. 9 (2022): 6043–6059.
14. B. D. Bax, P. F. Chan, D. S. Eggleston, A. Fosberry, D. R. Gentry, F. Gorrec, I. Giordano, M. M. Hann, A. Hennessy, M. Hibbs, et al. "Type IIA Topoisomerase Inhibition by a New Class of Antibacterial Agents," *Nature* 466, no. 7309 (2010): 935–940. doi:10.1038/nature09197
15. C. A. Lipinski, L. Lombardo, B. W. Dominy, and P. J. Feeney, "Experimental and Computational Approaches to Estimate Solubility and Permeability in Drug Discovery and Development Settings," *Advanced Drug Delivery Reviews* 46, no. 1-3 (2001): 3–26. doi:10.1016/s0169-409x(00)00129-0
16. *Molinspiration Chemoinformatics Brastislava*. Slovak Republic. 2014. http://www.molinspiration.com/cgi-bin/properties
17. Yuan H. Zhao, Michael H. Abraham, Joelle Le, Anne Hersey, Chris N. Luscombe, Gordon Beck, Brad Sherborne, and Ian Cooper, "Rate-Limited Steps of Human Oral Absorption and QSAR Studies," *Pharmaceutical Research* 19, no. 10 (2002): 1446–1457. doi:10.1023/a:1020444330011
18. Drug-likeness and molecular property prediction. http://www.molsoft.com/mprop/
19. P. Ertl, B. Rohde, and P. Selzer, "Fast Calculation of Molecular Polar Surface Area as a Sum of Fragment-Based Contributions and Its Application to the Prediction of Drug Transport Properties," *Journal of Medicinal Chemistry* 43, no. 20 (2000): 3714–3717. doi:10.1021/jm000942e
20. (a) B. Chaudhari, H. Patel, S. Thakar, I. Ahmad, and D. Bansode, "Optimizing the Sunitinib for Cardio-Toxicity and Thyro-Toxicity by Scaffold Hopping Approach," *In Silico Pharmacology* 10, no. 1 (2022): 10. doi:10.1007/s40203-022-00125-1(b) I. Ahmad, R. H. Pawara, R. T. Girase, A. Y. Pathan, V. R. Jagatap, N. Desai, Y. O. Ayipo, S. J. Surana, and H. Patel, "Synthesis, Molecular Modeling Study, and Quantum-Chemical-Based Investigations of Isoindoline-1, 3-Diones as Antimycobacterial Agents," *ACS Omega* 7, no. 25 (2022): 21820–21844. doi:10.1021/acsomega.2c01981
21. Y. O. Ayipo, W. A. Alananzeh, I. Ahmad, H. Patel, and M. N. Mordi, "Structural Modelling and in Silico Pharmacology of β-Carboline Alkaloids as Potent 5-HT1A Receptor Antagonists and Reuptake Inhibitors," *Journal of Biomolecular Structure & Dynamics* 26 (2022): 1–17.
22. (a) M. M. Farhan, M. A. Guma, M. A. Rabeea, I. Ahmad, and H. Patel, "Synthesizes, Characterization, Molecular Docking and in Vitro Bioactivity Study of New Compounds Containing Triple Beta Lactam Rings," *Journal of Molecular Structure* 1269, no. 5 (2022): 133781. doi:10.1016/j.molstruc.2022.133781.(b) M. A. Abdelgawad, J. M. Oh, D. G. T. Parambi, S. Kumar, A. Musa, M. M. Ghoneim, A. A. Nayl, A. H. El-Ghorab, I. Ahmad, H. Patel, H. Kim, and B. Mathew, "Development of Bromo- and Fluoro-Based α, β-Unsaturated Ketones as Highly Potent MAO-B Inhibitors for the Treatment of Parkinson's Disease," *Journal of Molecular Structure* 1266 (2022): 133545.