New Hydroxamic Acid Derivatives of Fluoroquinolones: Synthesis and Evaluation of Antibacterial and Anticancer Properties

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A series of new hydroxamic acid derivatives (6a–f) at C-3 position of fluoroquinolones were designed and synthesized through multistep synthesis. The design concept involved replacement of the 3-carboxylic acid in fluoquinolones with hydroxamic acid as an acid mimicking group. The synthetic work employed in this work provides a good example for the synthesis of pure hydroxamic acid based fluoroquinolones. The synthesized compounds were characterized by ^{1}H-NMR, electrospray ionization (ESI)-MS and IR. The new compounds were tested for their *in vitro* antimicrobial and anti-proliferative activity. Out of the six derivatives, compound 6e exhibited moderate antibacterial activity by inhibiting the growth of *Escherichia coli* and *Klebsiella pneumoniae* (MIC: 4.00–8.00 μg/mL). Compounds 6b and 6f displayed good growth inhibition against A549 Lung adenocarcinoma and HCT-116 Colon carcinoma cell lines.

Key words fluoroquinolone; hydroxamic acid; [1,2,3]triazolyl derivative; antibacterial activity; anticancer activity

Fluoroquinolones are a class of quinolone compounds and known as first made broad spectrum antibiotics. The fluoroquinoles such as Ciprofloxacin and Norfloxacin are extremely successful antibiotics that have potent, broad spectrum antibacterial activity, and relatively few side effects.[1)] These antibiotics exert their antimicrobial activity by binding to 2 type II bacterial topoisomerase enzymes, DNA gyrase (subunits encoded by gyrA and gyrB) and topoisomerase IV (subunits encoded by grlA and grlB for *Staphylococcus aureus*). This binding induces permanent double-stranded DNA breaks, which results in cell death.[2,3)]

Structure–activity relationship (SAR) studies of quinolone antibacterial agents showed that the basic group at the C-7 position is the most adaptable site for chemical change and an area that greatly influences their potency, spectrum and safety.[4,5)] In general, 5- and 6-membered nitrogen heterocycles have been proven to be the optimal substituents.[6)]

However, the development of resistance to this class of drugs, and the resulting loss of their effectiveness as antimicrobial therapies, poses a serious global health threat. Particularly, most clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) have become resistant to fluoroquinolone antibiotics within five years of their introduction.[7)] Also, the proportion of clinical isolates of *Pseudomonas aeruginosa* that are resistant to fluoroquinolone antibiotics has increased by 30% over the past 20 years.[8)] Furthermore, similar increases in resistance are now being observed with *Escherichia coli* on global fluoroquinolone resistance,[9)] which is a concern as *E. coli* causes more nosocomial blood stream infections than *S. aureus*.[10)] Therefore, there is a need to identify new fluoroquinolone derivatives with alternate structural features. To date all of the marketed fluoroquinolone antibiotics contain the C-3 carboxylic acid group.

In our continuous efforts to identify new antibacterial and anticancer agents,[11–14)] we have introduced hydroxamic acid at C-3 position of fluoroquinolones and hydroxamic acids are known to be bioisosteres of carboxylic acids.[15)] Also the hydroxamic acid derivatives are known to be good building blocks in anticancer and anti bacterial drug discovery.[16,17)] The design strategy of hydroxamic acid derivatives with a representative example (compound **6b**) is shown in Fig. 1.

Results and Discussion

Chemistry In the present work, the target hydroxamic acid based fuloroquinolones were synthesized in continuation to recently published[14)] carboxylic acid based fluoroquinolone derivatives. The present work also provides large scale synthesis of *O*-(tetrahydro-pyran-2-yl)hydroxylamine **3** in Chart 1 and its application in synthesis of chemically pure hydroxamic acid derivatives as shown in Chart 2.

The quinolone ester **1** was synthesized from the commercially available 2,4,5-trifluoro-benzoic acid in seven steps as per our previously published procedure.[14)] After having synthesized known compound **1**, base hydrolysis of compound **1** with lithium hydroxide at 60°C yielded carboxylic acid derivate **2** in good yield. Then compound **3** was conveniently synthesized from commercially available *N*-hydroxy phthalimide **1′** in two steps as per the literature procedure[18)] in excellent yields.

Then, our attention was turned towards synthesis of key intermediate compound **4**. Our initial efforts to convert compound **2** to compound **4** by reacting the compound **2** with compound **3** in the presence of various coupling conditions such as 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC)·HCl/*N*-hydroxybenzotriazole (HOBt)/*N*,*N*-dimethylformamide (DMF), EDC·HCl/HOBt/dimethyl sulfoxide (DMSO), EDC/HOBt/4-dimethylaminopyridine (DMAP)/DMF, EDC/HOBt/DMAP/DMSO did not result the desired compound **3**. We believe that this could be because of very low solubility of compound **2** in DMF and DMSO. Even attempts prepare

The authors declare no conflict of interest.

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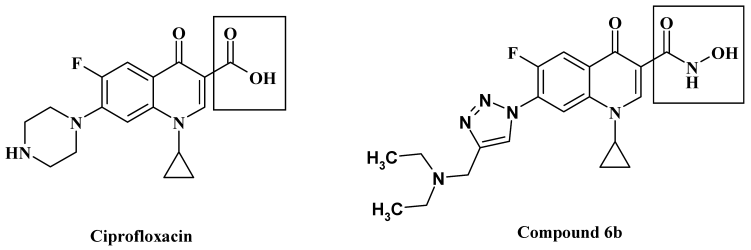


Fig. 1. Chemical Structure of Ciprofloxacin and Compound **6b**

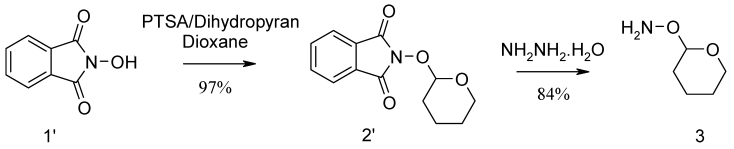


Chart 1. Scalable Synthetic Route for *O*-(Tetrahydro-pyran-2-yl)hydroxylamine (**3**)

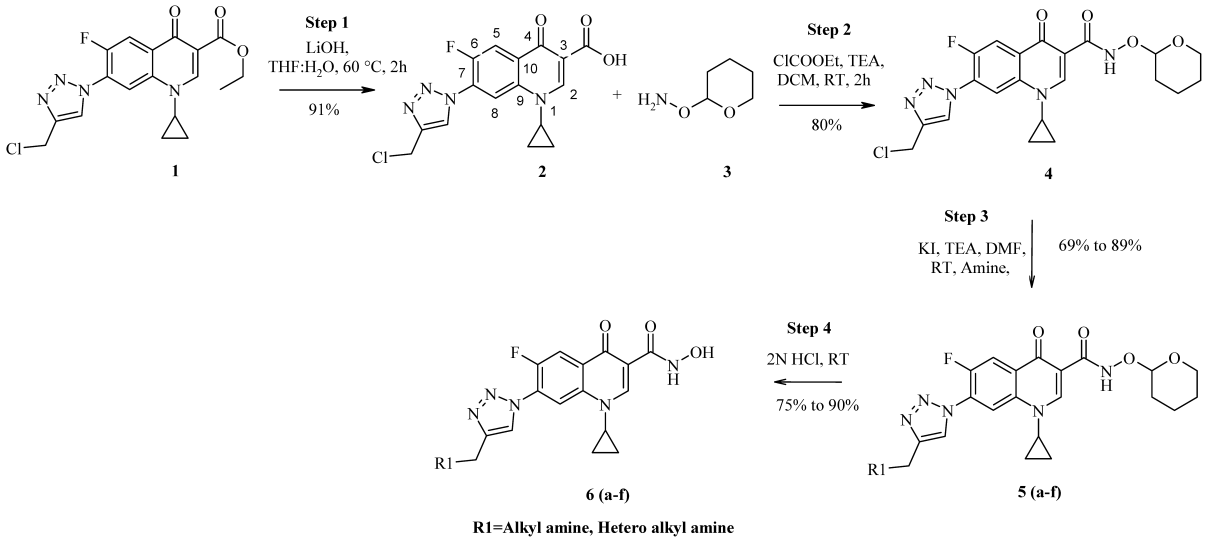


Chart 2. Synthetic Scheme for Novel Hydroxamic Acid Based Fluoroquinolones

acid chloride of compound **2** with thionyl chloride and oxalyl chloride were not successful. Fortunately, when we attempted classical mixed anhydride reaction by reacting the compound **2** with ethyl chloroformate in the presence of triethyl amine and compound **3** conveniently resulted desired compound **4** in good yield. The alkyl amino derivatives **5a**–**f** were smoothly obtained by reacting compound **4** with corresponding alkyl amines in the presence of triethyl amine and potassium iodide at room temperature.

After having synthesized pre-final compounds **5a**–**f**, our initial attempts to de-protect tetrahydropyranyl group of compound **5a** with pyridinium *p*-toluenesulfonate (PPTS) or *p*-toluenesulfonic acid (PTSA) in the presence of methanol yielded compound **6a** along with 5–10% of corresponding methoxy hydroxamic acid as an impurity. Similar results were observed when we attempted same reaction with ethanol. Attempts to remove the impurity were not successful by normal phase silica gel column chromatography as the product was highly polar in nature.

At this juncture, we have opted water as protic solvent to avoid the formation of methoxy hydroxamic acid and hydrochloric acid as an acid for the de-protection of tetrahydropyranyl group. Then reaction of the compound **5a** with 2M hydrochloric acid in water at room temperature smoothly resulted desired product **6a** with >98% HPLC purity. Similar experimental conditions were applied for the synthesis of remaining compounds **6b**–**f** in good yields.

The structures of most of the synthesized compounds were confirmed by IR, ^{1}H- and ^{13}C-NMR and LC mass spectral studies except final compounds (**6a**–**f**). We could not characterize the final compounds by ^{13}C-NMR as the solubility of these compounds are very low in DMSO, CD_3OD and D_2O. The structure of compound **2** was confirmed by their ^{1}H-NMR analyses. The ^{1}H-NMR spectrum of compound **2** confirms hydrolysis of ester group due to broad singlet at δ 14.59 corresponds to –COOH group. Further, the LC-MS showed its molecular ion peaks at 363.3 (M+H) which is in accordance with its molecular formula $C_{16}H_{12}ClFN_4O_3$.

The structure of compound **3** was interpreted by its IR, ^{1}H- and ^{13}C-NMR analyses. The IR spectrum of **3** revealed the presence of $-NH_2$ group due to the appearance of strong band at 3302 cm^{-1}. In its ^{1}H-NMR spectrum the appearance of

a singlet at δ 5.99 confirmed the presence of newly introduced $-NH_2$ group. The ^{13}C-NMR signal at 102.00 corresponds –CH– flanked by two oxygen atoms. The structure of compound **4** was confirmed by IR, ^{1}H-NMR and LC-MS analyses. In the IR spectrum strong absorbance peak at 1668 cm^{-1} indicates newly introduced amide functional group. In the ^{1}H-NMR spectrum peak δ 12.03 confirmed the newly introduced amide group. Further, the LC-MS showed its molecular ion peaks at 462.5 (M+H), which is in accordance with its molecular formula $C_{21}H_{21}ClFN_5O_4$.

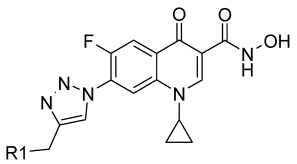
The structures of compounds **5a**–**f** were interpreted by their IR, ^{1}H- and ^{13}C-NMR and LC-MS analyses. The IR spectrum of **5a** revealed the presence of amide –C=O group due to the appearance of strong band at 1666 cm^{-1}, while that of –C=C of aryl was observed at 1619 cm^{-1}. In its ^{1}H-NMR spectrum the appearance of a singlet at δ 2.25 confirmed the presence of newly introduced dimethyl amine functional group. Further, the LC-MS showed its molecular ion peaks at 471.5 (M+H), which is in accordance with its molecular formula $C_{23}H_{27}FN_6O_4$.

The structures of compounds **6a**–**f** were elucidated by their IR, ^{1}H-NMR and LC-MS analyses. The IR spectrum of **6a** revealed the presence of hydroxamic group –C=O due to strong absorbance was observed at 1628 cm^{-1}. The ^{1}H-NMR spectrum of **6a** showed singlet at δ 4.57, for two protons which correspond to $-NCH_2Ar$ and appearance of singlet δ 10.69 (which disappeared on D_2O exchange) that corresponds to –OH proton of hydroxamic acid. The structure of **6a** was further confirmed by LC-MS. It showed the molecular ion peak at *m/z* 387.5 (M+H), which conforms to its molecular formula $C_{18}H_{19}FN_6O_3$.

The physicochemical characteristics of the newly synthesized compounds are presented in Table 1.

Antibacterial Evaluation All the newly synthesized compounds (**6a**–**f**) were evaluated for their *in vitro* antibacterial activities against human pathogens by means of standard twofold serial dilution method using agar media. The *in vitro* antibacterial activity was performed against three Gram positive bacterial strains including methicillin-sensitive *S. aureus*, *Enterococcus faecalis*, methicillin-resistant *S. aureus*, and

Table 1. Physicochemical Characteristics of Hydroxamic Acid Derivatives at C-3 Position of Fluoroquinolones (**6a**–**f**)



Compound	R1	MF/MW	Yield (%)	mp[a] (°C)
6a		$C_{18}H_{19}FN_6O_3$/386.39	84	188.1–182.3
6b		$C_{20}H_{23}FN_6O_3$/414.44	87	215.4–217.6
6c		$C_{22}H_{27}FN_6O_3$/442.50	90	184.2–185.1
6d		$C_{21}H_{23}FN_6O_3$/426.45	86	192.1–193.4
6e		$C_{20}H_{21}FN_6O_4$/428.43	88	250.5–255.7
6f		$C_{19}H_{21}FN_6O_3$/400.42	75	221.4–222.6

a) Melting point of compounds at their decomposition.

Table 2. Antibacterial Activity of New Fluoroquinolones **6a**–**f**

Compound	Gram-positive panel MIC (μg/mL)			Gram-negative panel MIC (μg/mL)		
	ATCC 29213 *Staphylococcus aureus* (MSSA)	ATCC 29212 *Enterococcus faecalis*	ATCC 33591 *Staphylococcus aureus* (MRSA)	ATCC 35218 *Escherichia coli*	ATCC 35657 *Klebsiella pneumoniae*	ATCC 25238 *Moraxella catarrhalis*
6a	>64	>64	>64	>64	>64	>64
6b	64	64	>64	16	8	16
6c	64	64	>64	64	32	64
6d	>64	>64	>64	64	64	64
6e	>64	>64	>64	8	4	8
6f	>64	>64	>64	>64	>64	>64
Linezolid	4	16	2	>64	>64	8
Ciprofloxacin	0.5	1	0.5	0.015	0.007	0.03

three Gram-negative strains including *Klebsiella pneumoniae*. Ciprofloxacin and linezolid were used as reference standards.

The data generated from this study (Table 2) showed that none of the target compounds exhibited potency in inhibiting the growth of Gram-positive bacteria. However, the *in vitro* activity of compound **6e** against Gram-negative bacteria such as *E. coli*, *K. pneumonia* and *Moraxella catarrhalis* are comparable to linezolid and less active when compared to ciprofloxacin.

The antibacterial activity of compounds (**6a**–**f**) suggested that replacement of carboxylic acid with hydroxamic acid group at C-3 position of the fluoroquinolone completely reduced the antibacterial activity against Gram-positive strains. However, few of these compounds are (**6c**, **d**) are moderately active against Gram-negative strains.

Anti Cancer Evaluation All compounds were screened for their *in vitro* anticancer activity against representative human cancer cell lines (A549: Lung adenocarcinoma; HeLa: Cervical adenocarcinoma; HCT-116: Colon carcinoma; PANC1: Pancreatic carninoma) obtained from American Type Culture Collection (ATCC). Suberoylanilide hydroxamic acid (SAHA) was used as reference standards.

The data generated from this study (Fig. 2) showed that among the six compounds, two compounds **6b** and **6f** are found to have better anticancer activity against A549, HeLa, HCT-116 and PANC1 cell lines. However, all the synthesized compounds are less potent when compared to marketed anticancer drug SAHA.

The anticancer activity of compounds (**6a**–**f**) suggested that introduction of bulky alkyl amines reduced the anticancer activity when compared to less hindered alkyl amines *i.e.*, isopropyl amine in compound **6f**. Also diethyl amine in compound **6b** is optimum to have good anticancer activity against A549 cell line.

Conclusion

A series of new hydroxamic acid derivatives (**6a**–**f**) at C-3 position of fluoroquinolones were designed and synthesized through multistep synthesis. The design concept involved replacement of the 3-carboxylic acid in fluoquinolones with hydroxamic acid as an acid mimicking group. The synthetic work employed in this work provides a good example for the synthesis of pure hydroxamic acid based fluoroquinoles. The new compounds were tested for their *in vitro* antimicrobial and anti-proliferative activity. Out of the six derivatives, compound **6e** exhibited moderate antibacterial activity by inhibiting the growth of *E. coli* and *K. pneumoniae* (minimum inhibitory concentration (MIC): 4.00–8.00 μg/mL). The cancer activity of compounds **6b** and **6f** displayed good growth inhibition (30–40%) against A549 lung adenocarcinoma and HCT-116 colon carcinoma cell lines.

Experimental

Chemistry Chemicals were obtained from Sigma-Aldrich Co. Final purifications were carried out using Merck silica gel 230–400 mesh. TLC experiments were performed on alumina-backed silica gel 40 F254 plates (Merck, Darmstadt, Germany). The plates were illuminated under UV (254 nm) and $KMnO_4$. Melting points were determined using Buchi B-540 and are uncorrected. All ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AM-300 (300 MHz for ^{1}H-NMR and 75 MHz for ^{13}C-NMR), Bruker BioSpin Corp., Germany. Molecular weights of unknown compounds were checked by LC-MS 6200 series Agilent Technology. Chemical shifts are reported in ppm (δ) with reference to internal standard tetramethylsilane (TMS). The signals are designated as follows: s,

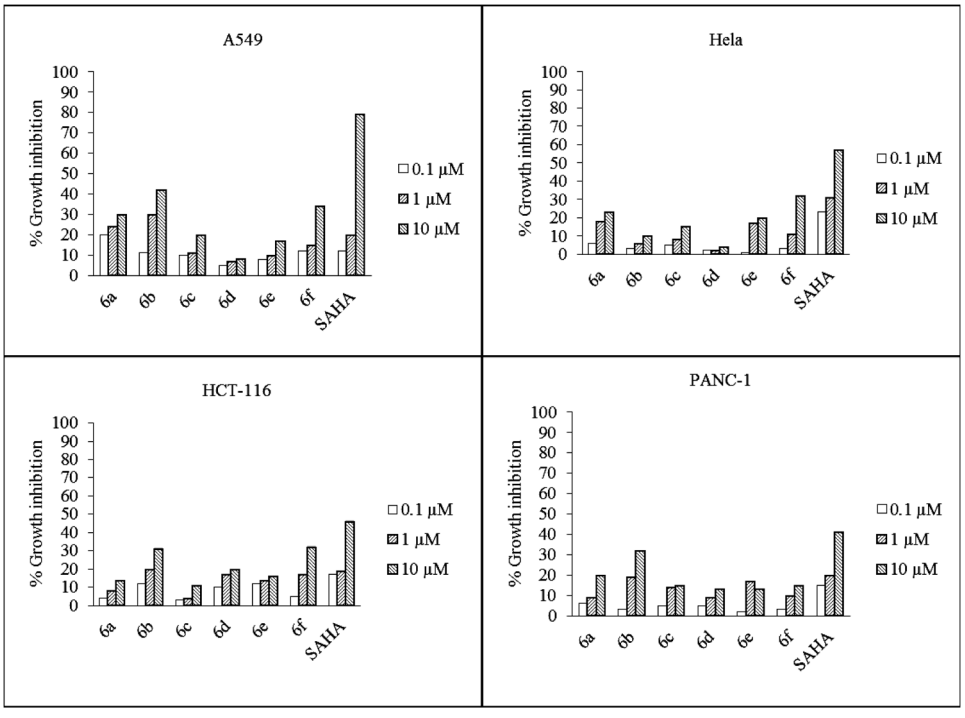


Fig. 2. Anticancer Activity of New Fluoroquinolones **6a**–**f**

Percentage growth inhibition in different cell lines. A549: Lung adenocarcinoma; HeLa: Cervical adenocarcinoma; HCT-116: Colon carcinoma; PANC1: Pancreatic carcinoma.

singlet; d, doublet; t, triplet; m, multiplet; br s, broad singlet. IR Spectra were recorded using a Bruker Alpha Fourier transform (FT)-IR spectrometer using a diamond attenuated total reflectance (ATR) single reflectance module (24 scans).

Procedure for the Synthesis of 2-(Tetrahydro-pyran-2-yloxy)isoindole-1,3-dione (2′) To a solution of *N*-hydroxy phthalimide **1′** (150 g, 0.92 mol) in dichloromethane (3.0 L) were added 1,4-dioxane (2.0 L), *p*-toluenesulfonic acid (3.16 g, 0.02 mol) and dihydropyran (116 g, 1.36 mol) at 25–30°C. Reaction mass was stirred at 25–30°C over a period of 3 h. Completion of the reaction was monitored by TLC. Then reaction mass was quenched with 10% $NaHCO_3$ solution (1.5 L) and layers were separated. Aqueous layer was further extracted with dichloromethane (3.0 L). Combined organic layer was washed with water (1.5 L) and brine (1.5 L). Organic layer was dried with sodium sulfate and volatiles were evaporated under reduced pressure to obtain compound **2′** as a white solid (220 g, 97%). mp 121–123°C; IR (ATR, cm^{-1}) *v*: 2937 (–C–H), 1733 (amide –C=O), 1376 (alkane –C–H), 693 (alkene =C–H); ^{1}H-NMR (DMSO-d_6 300 MHz) δ (ppm): 1.62–1.64 (3H, m, tetrahydropyran $-CH_2$), 1.77–1.81 (2H, m, tetrahydropyran $-CH_2$), 1.91–1.93 (1H, m, tetrahydropyran $-CH_2$), 3.58 (1H, d, *J*=10.2 Hz, tetrahydropyran $-OCH_2$), 4.28–4.33 (1H, m, tetrahydropyran $-OCH_2$), 5.32 (1H, s, tetrahydropyran –OCH), 7.88 (4H, ArH); ^{13}C-NMR (DMSO-d_6 75 MHz) δ: 17.79, 24.79, 27.63, 61.81, 102.86, 123.72, 128.91, 135.31, 163.80; LC-MS (electrospray ionization (ESI), *m/z*): 248.1 (M+H).

Procedure for the Synthesis of *O*-(Tetrahydro-pyran-2-yl)hydroxylamine (3) To a solution of compound **2′** (200 g, 0.80 mol) in methyl *tert*-butyl ether (1.8 L), hydrazine hydrate (48.6 g, 0.97 mol) was added drop wise at 25–30°C. The resulting reaction mass refluxed at 80°C over a period of 1 h. Reaction completion was monitored by TLC. The resulting reaction mass was cooled to 10°C and reaction mass was filtered through celite pad to remove phthalimide impurities. Then filtrate was dried with sodium sulfate and concentrated. The product was further purified by high vacuum distillation (120°C, 1 mmHg) to obtain compound **3** (80 g, 84%) as colorless oil which was solidified upon storage at 4–8°C as a white solid. bp 307°C; IR (ATR, cm^{-1}) *v*: 3302 (–NH); 2939 (–C–H), 1595 (–C–H bend), 1032 (ether–C–O); ^{1}H-NMR (DMSO-d_6 300 MHz) δ (ppm): 1.43–1.44 (4H, m, tetrahydropyran $-CH_2$), 1.54–1.65 (2H, m, tetrahydropyran $-CH_2$), 3.40–3.46 (1H, m, tetrahydropyran $-OCH_2$), 3.73–3.80 (1H, m, tetrahydropyran $-OCH_2$), 4.56–4.57 (1H, m, tetrahydropyran –OCH), 5.99 (2H, s, $-NH_2$); ^{13}C-NMR (DMSO-d_6 75 MHz) δ: 19.90, 25.48, 29.20, 61.76, 102.00. LC-MS (ESI, *m/z*): 118.2 (M+H).

Procedure for the Synthesis of 7-(4-Chloromethyl-[1,2,3]-triazol-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (2) To a solution of compound **1** (5.0 g, 12.8 mmol) in tetrahydrofuran (50.0 mL) was added lithium hydroxide (1.6 g, 38.4 mmol) in water (20.0 mL) at room temperature. Then the reaction mixture was allowed to stir at 60°C over a period of 2h. Completion of the reaction was monitored by TLC. Then volatiles were evaporated under reduced pressure, the crude mass obtained was diluted with water (50.0 mL), acidified with 1.0 N HCl (pH 3.0). Then acidic suspension was extracted with dichloromethane (2×100 mL), dried over sodium sulphate and concentrated under reduced pressure to obtain compounds **2** (4.2 g, 91%) as off-white solid. mp 219.7–224.8°C. IR (ATR, cm^{-1}) *v*: 3078 (–C–H), 1716 (acid –C=O), 1620 (–C=C), 1490 (alkane –C–H). ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 1.26–1.34 (4H, m, cyclopropyl $-CH_2$), 3.94 (1H, br s, cyclopropyl –CH), 5.01 (2H, s, $-CH_2Cl$), 8.38 (1H, d, *J*=10.5 Hz, ArH), 8.78 (1H, d, *J*=6.0 Hz, ArH), 8.83 (1H, s, triazole H), 8.93 (1H, s, ArH), 14.59 (1H, br s, –COOH); ^{13}C-NMR (DMSO-d_6 75 MHz) δ: 8.19, 36.35, 36.80, 108.21, 113.39 (d, *J*=21.75 Hz, C5-F coupling), 116.08, 126.44, 126.53, 126.58, 126.67, 129.70 (d, *J*=13.5 Hz, C7-F coupling), 138.50, 145.23, 149.96 and 153.31 (d, *J*=251.25 Hz, C6-F coupling), 150.22, 165.65, 176.95. LC-MS (ESI, *m/z*): 363.3 (M+H). *Anal.* Calcd for $C_{16}H_{12}ClFN_4O_3$: C, 52.98; H, 3.33; N, 15.45. Found: C, 52.95; H, 3.29; N, 15.44.

Preparation of 7-(4-Chloromethyl-[1,2,3]triazol-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (Tetrahydro-pyran-2-yloxy)amide (4) To a suspension of compound **2** (2.5 g, 6.9 mmol) in dichloromethane (50 mL) were added triethyl amine (4.0 mL, 27.5 mmol) and ethyl chloroformate (0.64 mL, 8.2 mmol) at 0°C under nitrogen atmosphere. Then reaction mixture stirred at 0°C over a period of 30 min and compound **3** was added to the reaction mixture at same temperature. The reaction mixture was slowly allowed to reach room temperature (25°C) and continued stirring for a period of 1 h. Completion of the reaction was monitored by TLC. The resulting reaction mixture was diluted with dichloromethane (250 mL), washed with water (2×100 mL) and dried over sodium sulphate. The crude product obtained upon evaporation of the solvent was purified by silica gel column chromatography (5% methanol in dichloromethane) to obtain product **4** as pale yellow solid (2.6 g, 80%). mp: 188.2–191.1°C; IR (ATR, cm^{-1}) *v*: 2937 (–C–H), 1668 (amide –C=O), 1610 (–C=C), 1020 (alkane –C–F); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 1.19–1.23 (2H, m, cyclopropyl $-CH_2$), 1.31–1.33 (2H, m, cyclopropyl $-CH_2$), 1.56 (3H, br s, tetrahydropyran $-CH_2$), 1.73 (3H, br s, tetrahydropyran $-CH_2$), 3.58–3.61 (1H, m, tetrahydropyran $-OCH_2$), 3.86 (1H, br s, cyclopropyl –CH), 3.99–4.01 (1H, m, tetrahydropyran $-OCH_2$), 5.00 (3H, br s, $-CH_2Cl$, tetrahydropyran –O–CH–O), 8.30 (1H, d, *J*=10.8 Hz, ArH), 8.67 (1H, d, *J*=6.0 Hz, ArH), 8.73 (1H, s, triazole H), 8.90 (1H, s, ArH), 12.03 (1H, s, –CONH); ^{13}C-NMR (DMSO-d_6 75 MHz) δ: 8.43, 18.36, 25.07, 27.97, 35.32, 35.65, 102.12, 111.63, 112.76, 114.81 (d, *J*=22.5 Hz, C5-F coupling), 124.12 (d, *J*=11.2 Hz, C10-F coupling), 127.84, 128.78 (d, *J*=12.75 Hz, C7-F coupling), 137.69, 146.06, 148.11, 148.46, 151.79 (d, *J*=249.75 Hz, C6-F coupling), 161.92, 174.21; LC-MS (ESI, *m/z*): 378.9 (M-dihydropyran+H), 462.5 (M+H). *Anal.* Calcd for $C_{21}H_{21}ClFN_5O_4$: C, 54.61; H, 4.58; N, 15.16. Found: C, 54.53; H, 4.59; N, 15.16.

General Procedure for the Preparation of 1-Cyclopropyl-6-fluoro-7-(4-alkylaminomethyl-[1,2,3]triazol-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (Tetrahydro-pyran-2-yloxy)amides (5a–f) To a solution of compound **4** (200 mg, 0.43 mmol) in *N*,*N*-dimethylformamide (5.0 mL) was added triethylamine (156 μL, 1.08 mmol), potassium iodide (29 mg, 0.17 mmol) and the corresponding amine (1.08 mmol) at ice bath temperature. After stirring at ice bath temperature for 10 min, the reaction mass was allowed to stir at room temperature over a period 4–6 h. After completion of reaction, reaction mass was diluted with ethyl acetate (300 mL), washed with water (3×25 mL) and dried over sodium sulphate. The residue obtained upon evaporation of the

solvent was further purified by silica gel column chromatography using methanol–chloroform) as the eluent.

1-Cyclopropyl-7-(4-dimethylaminomethyl-[1,2,3]triazol-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (Tetrahydro-pyran-2-yloxy)amide (**5a**): This compound was prepared by reaction of compound **4** with dimethyl amine in the presence of triethylamine and potassium iodide. It was obtained as a pale yellow solid (176 mg, 87%), mp: 198.5–204.1°C; IR (ATR, cm^{-1}) *v*: 2937 (ArH), 1666 (amide –C=O), 1610 (–C=C), 1480 (–C–O), 1020 (–C–F); ^{1}H-NMR (DMSO-d_6 300 MHz) δ (ppm): 1.19–1.22 (2H, m, cyclopropyl $-CH_2$), 1.31–1.33 (2H, m, cyclopropyl $-CH_2$), 1.56 (3H, brs, tetrahydropyran $-CH_2$), 1.73 (3H, brs, tetrahydropyran $-CH_2$), 2.25 (6H, s, $-N(CH_3)_2$), 3.58–3.61 (1H, m, tetrahydropyran $-OCH_2$), 3.69 (2H, s, $-CH_2-N$), 3.87 (1H, brs, cyclopropyl –CH), 3.99 (1H, brs, tetrahydropyran –O–CH_2), 4.99 (1H, s, tetrahydropyran –O–CH–O), 8.28 (1H, d, *J*=10.8 Hz, ArH), 8.64–8.68 (2H, m, ArH), 8.73 (1H, s, triazole H), 12.02 (1H, s, –CONH); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 8.40, 18.36, 25.07, 27.97, 35.30, 45.08, 54.08, 60.42, 102.09, 111.51, 112.59, 114.69 (d, *J*=21.75 Hz, C5-F coupling), 124.08 (d, *J*=12.0 Hz, C10-F coupling), 127.51 (d, *J*=6.75 Hz, C7-F coupling), 129.20, 137.69, 146.21, 148.01, 148.54 & 151.80 (d, *J*=244.5 Hz, C6-F coupling), 161.97, 174.22; LC-MS (ESI, *m/z*): 471.5 (M+H). *Anal*. Calcd for $C_{23}H_{27}FN_6O_4$: C, 58.71; H, 5.78; N, 17.86. Found: C, 58.68; H, 5.80; N, 17.84.

1-Cyclopropyl-7-(4-diethylaminomethyl-[1,2,3]triazol-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (Tetrahydro-pyran-2-yloxy)amide (**5b**): This compound was prepared by reaction of compound **4** with diethyl amine in the presence of triethylamine and potassium iodide. It was obtained as a pale yellow solid (170 mg, 79%), mp 201.8–203.4°C; IR (ATR, cm^{-1}) *v*: 3010 (ArH), 1671 (amide –C=O), 1614 (–C=C), 1481 (–C–O), 1034 (–C–F); ^{1}H-NMR (DMSO-d_6 300 MHz) δ (ppm): 1.07 (6H, t, *J*=6.9 Hz, diethylamine $-CH_3$), 1.19–1.23 (2H, m, cyclopropyl $-CH_2$), 1.31–1.33 (2H, m, cyclopropyl $-CH_2$), 1.56 (3H, brs, tetrahydropyran $-CH_2$), 1.74 (3H, brs, tetrahydropyran $-CH_2$), 2.50 (4H, q, *J*=6.9 Hz, diethylamine $-NCH_2$), 3.58–3.61 (1H, m, tetrahydropyran $-OCH_2$), 3.87–3.99 (4H, m, cyclopropyl –CH, $-CH_2-N$, tetrahydropyran –O–CH_2), 4.99 (1H, s, tetrahydropyran –O–CH–O), 8.28 (1H, d, *J*=10.8 Hz, ArH), 8.64–8.66 (2H, m, ArH), 8.73 (1H, s, triazole H), 12.02 (1H, s, –CONH); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 8.41, 14.54, 18.37, 25.07, 27.96, 35.32, 46.23, 55.12, 62.21, 102.07, 111.46, 112.51, 114.69 (d, *J*=21.75 Hz, C5-F coupling), 123.72 (d, *J*=11.20 Hz, C10-F coupling), 127.43, 129.21 (d, *J*=12.70 Hz, C7-F coupling), 137.69, 147.34, 148.51 & 151.82 (d, *J*=248.25 Hz, C6-F coupling), 162.32, 174.24; LC-MS (ESI, *m/z*): 499.6 (M+H). *Anal.* Calcd for $C_{25}H_{31}FN_6O_4$: C, 60.23; H, 6.27; N, 16.86. Found: C, 60.25; H, 6.31; N, 16.83.

1-Cyclopropyl-7-(4-dipropylaminomethyl-[1,2,3]triazol-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (Tetrahydro-pyran-2-yloxy)amide (**5c**): This compound was prepared by reaction of compound **4** with dipropyl amine in the presence of triethylamine and potassium iodide. It was obtained as an off-white solid (175 mg, 77%), mp 183.2–187.8°C; IR (ATR, cm^{-1}) *v*: 2957 (ArH), 1668 (amide –C=O), 1609 (–C=C), 1476 (–C–O), 1018 (–C–F); ^{1}H-NMR (DMSO-d_6 300 MHz) δ (ppm): 0.86 (6H, t, *J*=7.2 Hz, dipropylamine $-CH_3$), 1.20 (2H, brs, cyclopropyl $-CH_2$), 1.30–1.33 (2H, m, cyclopropyl $-CH_2$), 1.51–1.57 (7H, brs, dipropylamine $-CH_2$, tetrahydropyran $-CH_2$), 1.74 (3H, brs, tetrahydropyran $-CH_2$), 2.38–2.40 (4H, m, dipropylamine $-NCH_2$), 3.58–3.61 (1H, m, tetrahydropyran $-OCH_2$), 3.82–3.99 (4H, m, cyclopropyl –CH, $-CH_2-N$, tetrahydropyran –O–CH_2), 4.99 (1H, s, tetrahydropyran –O–CH–O), 8.30 (1H, d, *J*=10.8 Hz, ArH), 8.64–8.66 (2H, m, ArH), 8.74 (1H, s, triazole H), 12.04 (1H, s, –CONH); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 8.44, 11.83, 18.35, 20.31, 25.06, 27.96, 35.30, 48.76, 55.85, 62.15, 102.06, 111.46, 112.51, 114.62 (d, *J*=21.75 Hz, C5-F coupling), 123.72 (d, *J*=11.25 Hz, C10-F coupling), 127.43, 129.23 (d, *J*=12.75 Hz, C7-F coupling), 137.69, 147.34, 147.99, 148.52 and 151.83 (d, *J*=248.25 Hz, C6-F coupling), 161.97, 174.25; LC-MS (ESI, *m/z*): 527.3 (M+H). *Anal.* Calcd for $C_{27}H_{35}FN_6O_4$: C, 61.58; H, 6.70; N, 15.96. Found: C, 61.61; H, 6.74; N, 15.94.

1-Cyclopropyl-6-fluoro-4-oxo-7-(4-piperidin-1-ylmethyl-[1,2,3]-triazol-1-yl)-1,4-dihydroquinoline-3-carboxylic Acid (Tetrahydro-pyran-2-yloxy)amide (**5d**): This compound was prepared by reaction of compound **4** with piperidine in the presence of triethylamine and potassium iodide. It was obtained as a off-white solid (160 mg, 73%), mp 195.2–196.5°C; IR (ATR, cm^{-1}) *v*: 2959 (ArH), 1668 (acid –C=O), 1614 (–C=C), 1477 (–C–O), 1025 (–C–F); ^{1}H-NMR (DMSO-d_6 300 MHz) δ (ppm): 1.19–1.23 (2H, m, cyclopropyl $-CH_2$), 1.31–1.33 (2H, m, cyclopropyl $-CH_2$), 1.35–1.55 (9H, m, piperidine $-CH_2$, tetrahydropyran $-CH_2$), 1.73 (3H, brs, tetrahydropyran $-CH_2$), 2.50 (4H, brs, piperidine $-NCH_2$), 3.58–3.68 (3H, m, tetrahydropyran $-OCH_2$, $-CH_2-N$), 3.87 (1H, brs, cyclopropyl –CH), 3.99 (1H, brs, tetrahydropyran –O–CH_2), 4.99 (1H, s, tetrahydropyran –O–CH–O), 8.29 (1H, d, *J*=10.8 Hz, ArH), 8.64–8.66 (2H, m, ArH), 8.73 (1H, s, triazole H), 12.04 (1H, s, –CONH); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 8.44, 15.54, 18.36, 21.28, 25.08, 27.96, 35.31, 46.68, 54.12, 62.16, 102.08, 111.46, 112.50, 114.69 (d, *J*=21.75 Hz, C5-F coupling), 123.72 (d, *J*=11.25 Hz, C10-F coupling), 127.45, 129.24 (d, *J*=6.75 Hz, C7-F coupling), 137.70, 147.35, 148.52 and 151.83 (d, *J*=248.25 Hz, C6-F coupling), 162.22, 174.24; LC-MS (ESI, *m/z*): 511.7 (M+H). *Anal.* Calcd for $C_{26}H_{31}FN_6O_4$: C, 61.16; H, 6.12; N, 16.46; Found: C, 61.20; H, 6.13; N, 16.44.

1-Cyclopropyl-6-fluoro-7-(4-morpholin-4-ylmethyl-[1,2,3]-triazol-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (Tetrahydro-pyran-2-yloxy)amide (**5e**): This compound was prepared by reaction of compound **4** with morpholine in the presence of triethylamine and potassium iodide. It was obtained as pale yellow solid (196 mg, 89%), mp 192.6–193.5°C; IR (ATR, cm^{-1}) *v*: 2939 (ArH), 1670 (amide –C=O), 1612 (–C=C), 1478 (–C–O), 1024 (–C–F); 1.19–1.23 (2H, m, cyclopropyl $-CH_2$), 1.31–1.33 (2H, m, cyclopropyl $-CH_2$), 1.56 (3H, brs, tetrahydropyran $-CH_2$), 1.73 (3H, brs, tetrahydropyran $-CH_2$), 3.33 (4H, brs, morpholine $-NCH_2$), 3.59 (5H, brs, tetrahydropyran $-OCH_2$, morpholine $-OCH_2$), 3.71 (2H, s, $-CH_2-N$), 3.86 (1H, brs, cyclopropyl –CH), 3.99 (1H, brs, tetrahydropyran –O–CH_2), 4.99 (1H, s, tetrahydropyran –O–CH–O), 8.28 (1H, d, *J*=10.8 Hz, ArH), 8.64–8.69 (2H, m, ArH), 8.73 (1H, s, triazole H), 12.03 (1H, s, –CONH); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 8.40, 18.37, 25.07, 27.97, 35.31, 48.76, 55.12, 62.14, 62.43, 73.11, 102.09, 111.47, 112.50, 114.64 (d, *J*=21.75 Hz, C5-F coupling), 123.70, (d, *J*=13.50 Hz, C10-F coupling), 127.42, 129.22 (d, *J*=12.75 Hz, C7-F coupling), 137.70, 147.32, 148.53 and 151.84 (d, *J*=248.25 Hz, C6-F coupling), 162.42, 174.26; LC-MS (ESI, *m/z*): 513.2 (M+

H). *Anal.* Calcd for $C_{25}H_{29}FN_6O_5$: C, 58.58; H, 5.70; N, 16.40. Found: C, 58.60; H, 5.74; N, 16.38.

1-Cyclopropyl-6-fluoro-7-[4-(isopropylamino-methyl)-[1,2,3]triazol-1-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (Tetrahydro-pyran-2-yloxy)amide (**5f**): This compound was prepared by reaction of compound **4** with isopropyl amine in the presence of triethylamine and potassium iodide. It was obtained as pale yellow solid (145 mg, 69%), mp 186.5–187.8°C; IR (ATR, cm^{-1}) ν: 2964 (ArH), 1665 (amide –C=O), 1615 (–C=C), 1478 (acid –C–O), 1022 (–C–F); ^{1}H-NMR (DMSO-d_6 300 MHz) δ (ppm): 1.19 (6H, d, J=6.3 Hz, isopropyl amine $-CH_3$), 1.19–1.23 (2H, m, cyclopropyl $-CH_2$), 1.31–1.32 (2H, m, cyclopropyl $-CH_2$), 1.56 (3H, brs, tetrahydropyran $-CH_2$), 1.73 (3H, brs, tetrahydropyran $-CH_2$), 2.89–2.91 (1H, m, isopropyl amine-NCH), 3.34–3.35 (1H, brs, –NH), 3.58–3.61 (1H, m, tetrahydropyran $-OCH_2$), 3.87–3.99 (4H, m, cyclopropyl –CH, $-CH_2$–N, tetrahydropyran $-O-CH_2$), 4.99 (1H, s, tetrahydropyran –O–CH–O), 8.29 (1H, d, J=10.8 Hz, ArH), 8.63–8.68 (2H, m, ArH), 8.73 (1H, s, triazole H), 12.03 (1H, s, –CONH). ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 8.40, 18.34, 18.95, 25.06, 27.97, 35.30, 38.64, 55.87, 62.20, 102.09, 111.51, 112.52, 114.62 (d, J=21.75 Hz, C5-F coupling), 123.72 (d, J=11.25 Hz, C10-F coupling), 127.42, 129.28 (d, J=12.75 Hz, C7-F coupling), 137.70, 147.35, 148.51 and 151.82 (d, J=248.25 Hz, C6-F coupling), 162.15, 174.22; LC-MS (ESI, *m/z*): 485.4 (M+H). *Anal.* Calcd for $C_{24}H_{29}FN_6O_4$: C, 59.49; H, 6.03; N, 17.34. Found: C, 59.52; H, 6.11; N, 17.31.

General Procedure for the Synthesis of 1-Cyclopropyl-7-(4-alkylaminomethyl-[1,2,3]triazol-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid Hydroxyamides (6a–f) To a solution of 2 N hydrochloric acid (10.0 mL) was added compounds **5a–f** (0.5 mmol) at room temperature. The resulting suspension was allowed to stir at 25°C over a period of 12 h. The completion of the reaction was monitored by TLC. The solids obtained upon evaporation of the volatiles under reduced pressure were washed with diethyl ether (2×10 mL).

1-Cyclopropyl-7-(4-dimethylaminomethyl-[1,2,3]triazol-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid Hydroxyamide (**6a**): This compound was prepared by de-protection of tetrahydropyranyl group of compound **5a** with 2 M hydrochloric acid. It was obtained as a yellow solid, IR (ATR, cm^{-1}) ν: 2968 (ArH), 2492 (–OH), 1628 (–C=O), 1611 (–C=C), 1452 (–C–O), 1043 (–C–F); ^{1}H-NMR (DMSO-d_6 300 MHz) δ (ppm): 1.18–1.23 (2H, m, cyclopropyl $-CH_2$), 1.32–1.33 (2H, m, cyclopropyl $-CH_2$), 2.82 (6H, s, $N(CH_3)_2$), 3.87 (1H, brs, cyclopropyl –CH), 4.57 (2H, s, $-NCH_2$), 8.33 (1H, d, J=10.8 Hz, ArH), 8.69 (1H, d, J=6.3 Hz, ArH), 8.76 (1H, s, triazole H), 9.01 (1H, s, ArH), 10.69 (1H, brs, –NHOH), 11.50 (1H, s, –CONH). LC-MS (ESI, *m/z*): 387.5 (M+H). *Anal.* Calcd for $C_{18}H_{19}FN_6O_3$: C, 55.95; H, 4.96; N, 21.75. Found: C, 55.98; H, 5.11; N, 21.73.

1-Cyclopropyl-7-(4-diethylaminomethyl-[1,2,3]triazol-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid Hydroxyamide (**6b**): This compound was prepared by de-protection of tetrahydropyranyl group of compound **5b** with 2 M hydrochloric acid. It was obtained as a pale yellow solid, IR (ATR, cm^{-1}) ν: 2942 (ArH), 2538 (–OH), 1627 (–C=O), 1617 (–C=C), 1479 (–C–O), 1030 (–C–F); ^{1}H-NMR (DMSO-d_6 300 MHz) δ (ppm): 1.19 (2H, brs, cyclopropyl $-CH_2$), 1.31 (2H, brs, cyclopropyl $-CH_2$), 1.34 (6H, t, J=6.9 Hz, diethylamine $-CH_3$), 3.13 (4H, q, J=6.9 Hz, diethylamine $-NCH_2$), 3.86 (1H, brs, cyclopropyl –CH), 4.58 (2H, s, CH_2–N), 8.32 (1H, d, J=11.1 Hz, ArH), 8.69 (1H, d, J=11.1 Hz, ArH), 8.75 (1H, s, triazole H), 9.08 (1H, s, ArH), 10.86 (1H, brs, –NHOH), 11.49 (1H, s, –CONH). LC-MS (ESI, *m/z*): 499.6 (M+H). *Anal.* Calcd for $C_{20}H_{23}FN_6O_3$: C, 57.96; H, 5.59; N, 20.28. Found: C, 57.98; H, 5.60; N, 20.25.

1-Cyclopropyl-7-(4-dipropylaminomethyl-[1,2,3]triazol-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid Hydroxyamide (**6c**): This compound was prepared by de-protection of tetrahydropyranyl group of compound **5c** with 2 M hydrochloric acid. It was obtained as a pale yellow solid. IR (ATR, cm^{-1}) ν: 2968 (ArH), 2492 (–OH), 1628 (–C=O), 1611 (–C=C), 1452 (–C–O), 1043 (–C–F); ^{1}H-NMR (DMSO-d_6 300 MHz) δ (ppm): 0.95 (6H, t, J=6.9 Hz, dipropylamine $-CH_3$), 1.20–1.23 (2H, m, cyclopropyl $-CH_2$), 1.31–1.33 (2H, m, cyclopropyl $-CH_2$), 1.79–1.83 (4H, m, dipropylamine $-CH_3$), 3.02–3.04 (4H, m, –dipropylamine $-NCH_2$), 3.87 (1H, m, cyclopropyl –CH), 4.59 (2H, s, $-CH_2$–N), 8.33 (1H, d, J=11.7 Hz, ArH), 8.70 (1H, d, J=6.0 Hz, ArH), 8.76 (1H, s, triazole H), 9.08 (1H, s, ArH), 10.54 (1H, brs, –NHOH), 11.50 (1H, s, –CONH). LC-MS (ESI, *m/z*): 499.6 (M+H). *Anal.* Calcd for $C_{22}H_{27}FN_6O_3$: C, 59.72; H, 6.15; N, 18.99. Found: C, 59.75; H, 6.17; N, 18.98.

1-Cyclopropyl-6-fluoro-4-oxo-7-(4-piperidin-1-ylmethyl-[1,2,3]triazol-1-yl)-1,4-dihydroquinoline-3-carboxylic Acid Hydroxyamide (**6d**): This compound was prepared by de-protection of tetrahydropyranyl group of compound **5d** with 2 M hydrochloric acid. It was obtained as a off-white solid. IR (ATR, cm^{-1}) ν: 2942 (ArH), 2541 (–OH), 1628 (–C=O), 1611 (–C=C), 1494 (acid –C–O), 1033 (–C–F); ^{1}H-NMR (DMSO-d_6 300 MHz) δ (ppm): 1.19 (2H, brs, cyclopropyl $-CH_2$), 1.32–1.34 (2H, m, cyclopropyl $-CH_2$), 1.68–1.80 (6H, m, piperidine $-CH_2$), 2.95–2.98 (2H, m, piperidine $-CH_2$), 3.41–3.45 (2H, m, piperidine $-CH_2$), 3.87 (1H, brs, cyclopropyl –CH), 4.53 (2H, s, $-CH_2$–N), 8.32 (1H, d, J=10.8 Hz, ArH), 8.70 (1H, d, J=4.8 Hz, ArH), 8.75 (1H, s, triazole H), 9.05 (1H, s, ArH), 10.90 (1H, brs, –NHOH), 11.49 (1H, s, –CONH). LC-MS (ESI, *m/z*): 427.5 (M+H). *Anal.* Calcd for $C_{21}H_{23}FN_6O_3$: C, 59.15; H, 5.44; N, 19.71. Found: C, 59.15; H, 5.45; N, 19.70.

1-Cyclopropyl-6-fluoro-7-(4-morpholin-4-ylmethyl-[1,2,3]-triazol-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid Hydroxyamide (**6e**): This compound was prepared by de-protection of tetrahydropyranyl group of compound **5e** with 2 M hydrochloric acid. It was obtained as a yellow solid. IR (ATR, cm^{-1}) ν: 2958 (ArH), 2541 (–OH), 1628 (–C=O), 1610 (–C=C), 1489 (–C–O), 1007 (–C–F); ^{1}H-NMR (DMSO-d_6 300 MHz) δ (ppm): 1.19 (2H, brs, cyclopropyl $-CH_2$), 1.33 (2H, brs, cyclopropyl $-CH_2$), 3.21 (2H, brs, morpholine $-NCH_2$), 3.39 (2H, brs, morpholine $-NCH_2$), 3.78 (2H, brs, morpholine $-OCH_2$), 3.97 (3H, brs, morpholine $-OCH_2$, cyclopropyl –CH), 4.63 (2H, s, $-NCH_2$), 8.32 (1H, d, J=9.6 Hz, ArH), 8.69 (1H, brs, ArH), 8.75 (1H, s, triazole H), 9.05 (1H, s, ArH), 11.49 (1H, s, –CONH), 11.66 (1H, brs, –NHOH). LC-MS (ESI, *m/z*): 513.2 (M+H). *Anal.* Calcd for $C_{20}H_{21}FN_6O_4$: C, 56.07; H, 4.94; N, 19.62. Found: C, 56.11; H, 4.99; N, 19.60.

1-Cyclopropyl-6-fluoro-7-[4-(isopropylamino-methyl)-[1,2,3]triazol-1-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid Hydroxyamide (**6f**): This compound was prepared by de-protection of tetrahydropyranyl group of compound **5f** with 2 M hydrochloric acid. IR (ATR, cm^{-1}) ν: 2967 (ArH),

2509 (–OH), 1622 (–C=O), 1610 (–C=C), 1452 (–C–O), 1007 (–C–F); ^{1}H-NMR (DMSO-d_6 300 MHz) δ (ppm): 1.19–1.23 (2H, m, cyclopropyl $-CH_2$), 1.31–1.32 (2H, m, cyclopropyl $-CH_2$), 1.33 (6H, d, J=6.3 Hz, isopropyl amine $-CH_3$), 3.35–3.36 (1H, m, isopropyl amine –NCH), 3.87 (1H, brs, cyclopropyl –CH), 4.42 (2H, s, $-CH_2$–N), 8.33 (1H, d, J=10.8 Hz, ArH), 8.64 (1H, d, J=6.0 Hz, ArH), 8.76 (1H, s, triazole H), 8.98 (s, ArH), 9.35 (3H, brs,–NHOH, isopropyl $-NH_2^+$), 11.49 (1H, s, –CONH); LC-MS (ESI, m/z): 401.0 (M+H). *Anal.* Calcd for $C_{19}H_{21}FN_6O_3$: C, 56.99; H, 5.29; N, 20.99. Found: C, 56.97; H, 5.60; N, 20.95.

Antibacterial Assay All compounds were screened for their *in vitro* antibacterial activity against representative Gram-positive and Gram-negative strains, by means of standard twofold serial dilution method using agar media. MIC is defined as the minimum concentration of the compound required to give complete inhibition of bacterial growth after incubation at 35°C for 24 h.

Results Interpretation After incubation, readings are taken manually and the optical density is measured at 600 nm. The lowest concentration of the test item/s which prevents visible growth of a microorganism is considered as minimal inhibitory concentration.

Cell Proliferation Assay Human cancer cell lines obtained from American Type Culture Collection (ATCC) were grown in appropriate medium supplemented with 10% fetal calf serum (FCS) and 100 U/mL penicillin, 100 μg/mL streptomycin. Cells were incubated in 5% CO_2 atmosphere at 37°C. Cells were seeded in 96-well plates at a density of 5×10^3 cells per well in 100 μL and were allowed to attach for 24 h. Stock concentrations of the compounds were made in DMSO. One hundred microliters of media containing various concentrations of compounds (0.1, 1, 10 μM) were added to the cells and were incubated for 48 h. SAHA was tested as a reference compound in the assay. On the day of termination, 40 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) (Sigma, St. Louis, MO, U.S.A.) solution (5 mg/mL) was added to the medium and the cells were incubated for 3 h. The medium was then aspirated and 100% DMSO was added to solubilize the violet MTT-formazan product. The absorbance at 570 nm was measured by spectrophotometry (Biotek, Synergy HT 96-well plate reader). The assay was carried out in triplicates for each concentration. Results are expressed as percentage of growth inhibition with respect to the vehicle-treated control wells.

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References

1) Mitscher L. A., *Chem. Rev.*, **105**, 559–592 (2005).
2) De Souza M. V. N., *Mini Rev. Med. Chem.*, **5**, 1009–1017 (2005).
3) Hooper D. C., *Clin. Infect. Dis.*, **32**, S9–S15 (2001).
4) Bryskier A., Chantot J. F., *Drugs*, **49** (Suppl. 2), 16–28 (1995).
5) Koga H., Itoh A., Murayama S., Suzue S., Irikura T., *J. Med. Chem.*, **23**, 1358–1363 (1980).
6) Domagala J. M., *J. Antimicrob. Chemother.*, **33**, 685–706 (1994).
7) Acar J. F., Goldstein F. W., *Clin. Infect. Dis.*, **24** (Suppl. 1), S67–S73 (1997).
8) Cooper M. A., Shlaes D., **472**, 32 (2011).
9) Dalhoff A., *Interdiscip. Perspect. Infect. Dis.*, **2012**, 976273 (2012).
10) de Kraker M. E., Davey P. G., Grundmann H., BURDEN Study Group, *PLoS Med.*, **8**, e1001104 (2011).
11) Selvakumar N., Rajulu G. G., Reddy K. C., Chary B. C., Kumar P. K., Madhavi T., Praveena K., Reddy K. H., Takhi M., Mallick A., Amarnath P. V., Kandepu S., Iqbal J., *Bioorg. Med. Chem. Lett.*, **18**, 856–860 (2008).
12) Takhi M., Selvakumar N., Kandepu S., Rajulu Gavara G., Iqbal J., WO Patent 073056 A1 (2009).
13) Selvakumar N., Rajulu Gavara G., Pazhanimuthu A., Ganesh S., WO Patent 021209 A1 (2011).
14) Rajulu G. G., Naik H. S. B., Viswanathan A., Agarwal D. S., Sambasivam G., Koppolu K. P., *Med. Chem. Res.*, **22**, 3843–3856 (2013).
15) Burger A., "A Guide to the Chemical Basis of Drug Design," EUA, Wiley, New York, 1983, pp. 24–29
16) Hyun-Jung K., Suk-Chul B., *Am. J. Transl. Res.*, **3**, 166–179 (2011).
17) Liang X., Lee C. J., Chen X., Chung H. S., Zeng D., Raetz C. R., Li Y., Zhou P., Toone E. J., *Bioorg. Med. Chem.*, **19**, 852–860 (2011).
18) Martin N. I., Woodward J. J., Marletta M. A., *Org. Lett.*, **8**, 4035–4038 (2006).