European Journal of Medicinal Chemistry 51 (2012) 52–59

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and in vitro antiplasmodial activities of fluoroquinolone analogs

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ARTICLE INFO

Article history:
Received 17 October 2011
Received in revised form
4 January 2012
Accepted 3 February 2012
Available online 21 February 2012

Keywords:
Fluoroquinolones
Antimalarial activity
Plasmodium falciparum
In vitro

ABSTRACT

Fluoroquinolone analogs were synthesized by simple alkylation followed by click chemistry and evaluated for their antimalarial in vitro against chloroquine sensitive strain of *Plasmodium falciparum* while ciprofloxacin was used as standard. Our results showed that the compound **12** was found most active with IC_{50} value of 1.33 μg/mL while ciprofloxacin showed IC_{50} = 8.81 μg/mL. Therefore, screening of either known or unknown quinolone/fluoroquinolone analogs are worthwhile to find more potent antimalarial drugs which might prove useful in the treatment of mild or severe malaria in human either alone or in combination with existing antimalarial drugs.

1. Introduction

Malaria remains one of the forefront disease affecting approximately 400–500 million people worldwide that results in nearly one million deaths each year more especially young children and woman [1,2]. The widely used first and second generation antimalarial drugs such as chloroquine, quinine, pyrimethamine, etc are losing their effectiveness due to the development of drug resistance in malaria parasite [3]. In order to overcome drug resistance, constant efforts are being made by chemist to find newer and cheaper drugs to cure malaria. At present, only artemisinin, an extract isolated from Chinese worm wood *Artemisia annua* and its semi-synthetic derivatives such as artemether, arteether, and artesunic acid, successfully treat chloroquine sensitive and chloroquine resistant malaria and are the only available drugs to treat multi-drug resistant malaria [4]. Till now, artemisinin and its derivatives have not shown development of resistance in parasite so far [5]. However, the paucity of natural artemisinin and complex synthesis of its semi-synthetic derivatives have forced chemists worldwide to find simple, cheap and efficient lead molecules so as to check malaria infection, as this disease is common in mainly poor countries (Fig. 1). Moreover, WHO has advocated combination therapy with artemisinin to further delay the drug resistance in the parasites [6]. Therefore, there is an urgent need to find new drugs that can have different mode of action against parasites *Plasmodium falciparum*.

It is well established that certain antibiotics such as tetracycline [7], rifampin [8] clindamycin [9] erythromycin [10], chloramphenicol [11] show antimalarial activity in vivo either alone or in combination with other more commonly used antimalarial drugs [12] (Fig. 1). Despite of slow antimalarial activity against malaria parasite, antibiotic such as doxycycline are frequently used for antimalarial prophylaxis along with more efficient antimalarial drugs [13]. Fluoroquinolone derivatives are best known drugs to treat various infectious diseases. Some of them such as enoxacin, grepafloxacin, clinafloxacin and ciprofloxacin were also found to be active against chloroquine resistant strain of *P. falciparum* [14]. However, the exact mode of action of these antibiotics on malarial parasite is still elusive [15]. Recent research on *P. falciparum* revealed that parasite mitochondria is the main target of these antibiotics [16–20]. Further, recent research findings suggest that these antibiotics affect the apicoplast of *Plasmodium* which in turn affects the parasite viability at the second or later generation [21–24]. However, the main draw back in use is the relatively slow antimalarial action of fluoroquinolones and the enhanced activity after prolonged contact limits their clinical application in treating malarial parasite. Thus, more efficient compounds are warranted. In fact, quinolones as part of combination therapy could be used when

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doi:10.1016/j.ejmech.2012.02.006

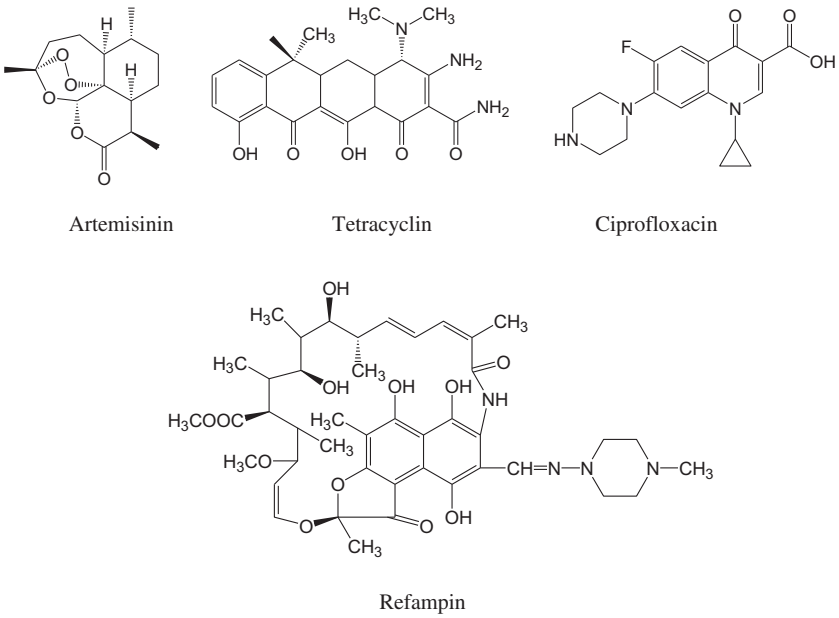


Fig. 1. Chemical structure of artemisinin, tetracycline, ciprofloxacin and refampin.

administered in conjunction with a rapidly acting antimalarial drug [25]. However, studies of antimalarial activity of quinolones are limited to some of the well known antibiotic such as ciprofloxacin, nalidixic acid and pefloxacin, etc [26–29]. Therefore, there is still scope to screen fluoroquinolones against malaria parasite irrespective of their activity against bacteria to find an agent with promising antimalarial activity. Recently, we have reported several synthetic molecules which showed antimalarial activities in vitro [30,31] as well as combination approach. To further extend our work, we synthesized fluoroquinolones having various alkyl (**1**–**11**) and substituted triazolyl (**12**–**21**) groups at position-1.

2. Chemistry

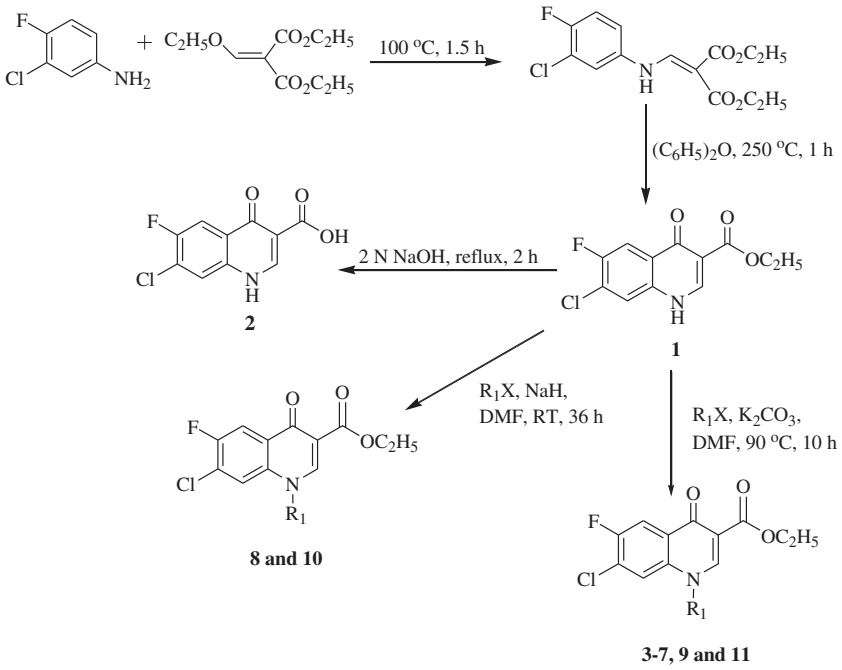
The fluoroquinolones (**1**–**11**) were synthesized by reported procedure [32] and is outlined in Scheme 1. Briefly, the condensation of 3-chloro-4-fluoroaniline with diethylethoxymethylene malonate ester at 100 °C followed by cyclization in diphenyl ether at 250 °C yielded 7-chloro-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester (**1**) in good yield. Subsequently, compound **1** was alkylated with various alkyl halides using either K_2CO_3 or NaH in DMF to gave the corresponding *N*-alkylated products **3**–**11**. The compound 7-chloro-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester (**1**) was hydrolyzed into corresponding acid 7-chloro-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (**2**) using 2 N NaOH solution. Further, 1,2,3-triazole compounds (**12**–**21**) were synthesized by click chemistry [33–35] as shown in Scheme 2. In a general approach, the compound 7-chloro-6-fluoro-4-oxo-1-(2-propynyl)-1,4-dihydroquinoline-3-carboxylic acid ethyl ester (**8**) was treated with various alkyl or aryl azides in DMF/H_2O (1:1, v/v) using copper sulfate pentahydrate ($CuSO_4.5H_2O$) and sodium ascorbate to yielded 7-chloro-6-fluoro-4-oxo-1-(1-alkyl/aryl [1,2,3] triazol-4-ylmethyl)-quinoline-3-carboxylic acid ethyl ester (**12**–**21**). All synthesized compounds were characterized by FT-IR, ESI-MS, ^{1}H and ^{13}C NMR.

2.1. The X-ray crystal structure

The molecular structure of compound **8** was also confirmed by X-ray crystallography (Fig. 2). X-ray intensity data were collected on CrysAlis PRO (Oxford Diffraction, 2009) with graphite monochromated Mo Kα radiation (λ = 0.71073 Å) at 293 (2) K. The structure was solved by direct method using SHELXL-97 and refined by full matrix least-squares method on F^2 (SHELXL-97). The unit cell parameters obtained for the single crystal; a = 4.6888(5) Å, α = 90°; b = 19.2740 (19) Å, β = 93.960 (10)°; c = 15.5464 (17) Å, γ = 90° and volume = 1401.6 (3) (Å^3), which clearly indicates that it exhibits monoclinic crystal system with the space group of $P2_1/n_1$. The detailed structural data have been deposited with CCDC-834819. Crystallographic data collection, crystal data and the refinement details are summarized in Table 1.

3. Biological results and discussion

Quinolone scaffolds possess wide range of biological properties ranging from cytotoxic [26], antibacterial [27] to anti-HIV [28]. However, their inhibitory role against the malaria parasites has not yet been explored thoroughly except few known drugs. Keeping these facts in mind, we designed and synthesized two series of fluoroquinolone analogs. In the first series, substituted fluoroquinolone **1** was alkylated with normal and branched chain alkyl groups as well as some polar groups such as −OH, −CN and −C≡CH etc. at position-1 and generated **3**–**11** compounds. Again, the compound **8** containing propargyl group was used as a substrate to further generate small quinolone libraries of compounds **12**–**21** by exploiting click chemistry. Various substituted fluoroquinolones were tested in vitro against chloroquine sensitive 3D7 strain of *P. falciparum* using ciprofloxacin as a standard. The compound **2** showed IC_{50} value 4.32 μg/mL while compounds **3** and **4** in which position-1 was substituted by methyl and ethyl groups respectively showed very weak activity with 11.8 μg/mL and 15.2 μg/mL respectively. Further, increase in carbon length by one more carbon such as propyl group suddenly enhanced antimalarial activity with IC_{50} 4.86 μg/mL as seen in compound **5**. Interestingly, further increase in carbon length by one carbon atom such as in n-butyl as in compound **6** or branching of alkyl chain such as in case of isopropyl had decreased activity slightly with IC_{50} 6.96 μg/mL as seen in compound **7**. Similar decrease in activity was also observed when benzyl group was introduced at position-1 (compound **11** with IC_{50} 6.8 μg/mL). Interestingly, the insertion of an alkyl chain, having

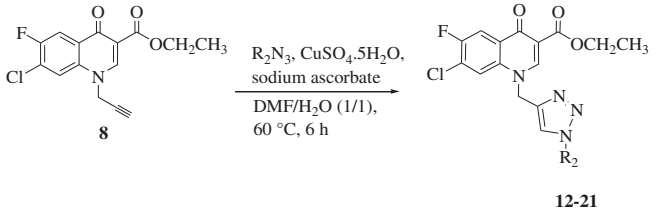


Scheme 1. Schematic representation of synthesis of *N*-1 alkylated fluoroquinolone analogs.

polar group such as 2-hydroxyethyl (compound **9**, IC_{50} = 4.26 μg/mL), 2-cyanoethyl (compound **10**, IC_{50} = 2.56 μg/mL) and −C≡CH (compound **8**, IC_{50} = 3.46 μg/mL) showed very good and enhanced activity. These results clearly indicate that the substitution at position-1 of fluoroquinolone scaffold with medium size alkyl chain such as propyl or polar group may exhibit promising antimalarial activity. Further, we also noticed that methyl and ethyl substituted fluoroquinolone were found least active in this series. We presume that small carbon chain alkyl groups are not suitable for activity. The overall activities data are tabulated in Table 2.

We further extended our work and synthesized a second series of fluoroquinolones by exploiting click chemistry on propargyl group present at position-1 in compound **8** and generated ten analogs. The activity data are given in Table 3. We observed that compounds with different substituent's on position-1 of 1,2,3-triazole ring showed different activities. The compounds **13**, **15**, **18**, **19**, **20** and **21** which bears propyl, methyl acetate, 3-chloropheyl, 4-fluorophenyl, 3-chloro-4-fluorophenyl and 7-chloroquinolinyl groups showed very good activity. On the other hand, the compound **14**, **16** and **17** having 2-hydroxyethyl, benzyl, 4-chlorophenyl groups respectively found to be least active. It is interesting to note that the presence of Cl at the position-3 of the phenyl ring appears to be significant. Thus, we can speculate that compounds having electron withdrawing substituents such as cyano (compound **11**) and chloro (mainly at position-3 of phenyl ring in triazolyl series) were crucial for inhibitory activity. Further, the substitution on position-1 of the fluoroquionolone with various groups markedly affects the antimalarial activity suggests that this position makes a specific contribution to the binding with targeted site. Interestingly, the compound **12** in which the position-1 of triazole ring was left unsubstituted found the most active (IC_{50} = 1.33 μg/mL) antimalarial agent among both series, suggesting that hydrogen of triazole ring might be forming hydrogen bond with the target site, thus interfering with the normal function of parasite life.

Fluoroquinolones inhibit the A subunit of DNA gyrase, an enzyme responsible for various reactions including the production of negative superhelical twists within circular double-stranded DNA. This enzyme is commonly found in bacteria. *P. falciparum* contains a functional mitochondrion which is sensitive to a wide



Scheme 2. Schematic representation of synthesis of *N*-1 triazolyl substituted fluoroquinolone analogs.

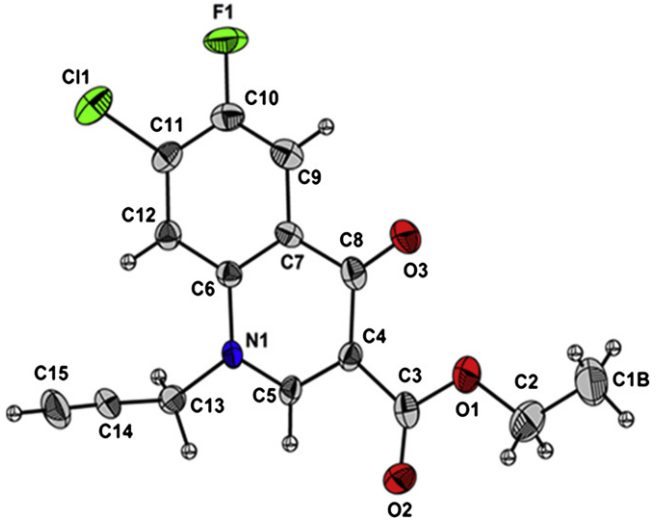


Fig. 2. Ortep diagram of the compound **8** drawn in 30% thermal probability ellipsoids showing atomic numbering schemes. Only one part of the disordered component (C1B) has been shown.

Table 1
Summary of crystal data of compound **8**.

CCDC deposit No.	834819
Identification code	Shelxl
Empirical formula	C_{15} H_{11} Cl F N O_3
Formula weight	307.70
Temperature (K)	293(2)
Crystal system, space group	Monoclinic, $P2_1/n_1$
Unit cell dimensions	$a = 4.6888(5)$ Å, $\alpha = 90°$.
	$b = 19.2740(19)$ Å, $\beta = 93.960(10)°$
	$c = 15.5464(17)$ Å, $\gamma = 90°$
Volume (Å^3)	1401.6(3)
Z, calculated density (Mg/m^3)	4, 1.458
Absorption coefficient (mm^{-1})	0.293
F(000)	632
Theta range for data collection	3.37–29.11°
h, k, l	5, 23, 19
Data completeness	0.998
Goodness-of-fit on F^2	0.799
$WR2$	0.1978 (2750)
Extinction coefficient	0.0000(17)

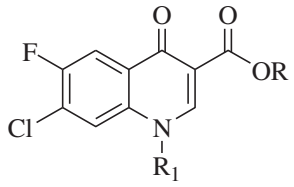
range of inhibitors [36]. Mitochondrial inhibitors, including the antibiotics which are specific for 70S ribosome are also lethal to malaria parasite [37]. This is most satisfactory explanation for the inhibitory activity of fluoroquinolones against parasite. However, the exact mode of action of fluoroquinolones against malaria parasites are still elusive.

Our results are in good agreement with previously published data for trovafloxacin, ciprofloxacin, pefloxacin and temafloxacin tested against chloroquine sensitive and resistant strains of *P. falciparum* [26–29]. Moreover, these in vitro results are encouraging and therefore, these results could be taken as a starting point for further design of antimalarial compounds and also for drug combinations. In light of the above results, it is worthwhile to further screening of large number of quinolones or fluoroquinolones from known or unknown libraries for antimalarial activity to find more potent and lead analogs.

3.1. In vitro cytotoxicity

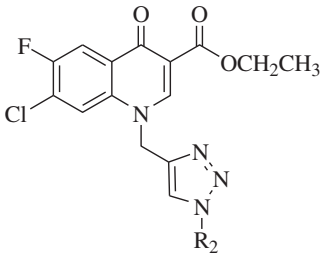
The cytotoxicity of some of these compounds were evaluated in human embryonic kidney cells (HEK-293) using MTT assay and IC_{50} values were calculated. In alkyl series, compound **2** (antimalarial activity, 4.32 μg/mL) was found non-toxic while in triazole series, compounds **19** and **20** (antimalarial activities, 4.3 μg/mL and 3.4 μg/mL respectively) exhibited IC_{50} value of 63.79 μM and 41.87 μM respectively. The compound **21**(antimalarial activity 4.06 μg/mL) was found non-toxic. The most potent compound **12** with IC_{50} = 1.33 μg/mL was found to be least toxic among screened compounds. In general, the compounds with better antimalarial activity were showed negligible or very low toxicity profile. Thus, these results are further support the significance of this study.

Table 2
Comparison of IC_{50} values of *N*-1 alkylated fluoroquinolones **1**–**11** with ciprofloxacin.



S. No.	R	R_1	IC_{50} concentration (μg/mL ± SE)
1	$-CH_2CH_3$	−H	5.81 ± 0.07
2	−H	−H	4.32 ± 0.44
3	$-CH_2CH_3$	$-CH_3$	11.83 ± 0.67
4	$-CH_2CH_3$	$-CH_2CH_3$	15.20 ± 0.12
5	$-CH_2CH_3$	$-(CH_2)_2CH_3$	4.86 ± 0.05
6	$-CH_2CH_3$	$-(CH_2)_3CH_3$	6.96 ± 0.15
7	$-CH_2CH_3$	$-CH(CH_3)_2$	6.966 ± 1.097
8	$-CH_2CH_3$	$-CH_2C{\equiv}CH$	3.46 ± 0.60
9	$-CH_2CH_3$	$-(CH_2)_2OH$	4.26 ± 0.4096
10	$-CH_2CH_3$	$-CH_2CN$	2.56 ± 0.30
11	$-CH_2CH_3$	$-CH_2Ph$	6.83 ± 0.20

Table 3
Comparison of IC_{50} values of *N*-1 (1-alkyl/aryl [1,2,3] triazol-4-yl-methyl) fluoroquinolones **12**–**21** with ciprofloxacin.



S. No.	R_2	IC_{50} concentration (μg/mL ± SE)
12	−H	1.33 ± 0.67
13	$-(CH_2)_2CH_3$	5.4 ± 0.92
14	$-(CH_2)_2OH$	14.33 ± 0.73
15	$-CH_2COOCH_3$	5.26 ± 0.87
16	$-CH_2Ph$	12.2 ± 0.40
17	4-Chlorophenyl	13.0 ± 2.05
18	3-Chlorophenyl	2.73 ± 0.23
19	4-Fluorophenyl	4.3 ± 0.54
20	3-Chloro-4-fluorophenyl	3.4 ± 0.72
21	7-Chloroquinolinyl	4.06 ± 0.19
Ciprofloxacin		8.82 ± 0.06

4. Conclusion

We successfully synthesized small fluoroquinolone libraries by alkylation at position-1 by simple alkylation as well as by click chemistry and screened against malaria parasites. The majority of compounds showed very good activities with IC_{50} ranging from 1.33 μg/mL to 6.96 μg/ml as compared to ciprofloxacin (IC_{50} 8.82 μg/mL). Out of twenty one compounds, sixteen compounds showed better activity than ciprofloxacin while the compound **12** showed highest activity among all studied compounds with IC_{50} 1.33 μg/mL. This study provides new window and to rethink about screening of either existing or new libraries of quinolone derivatives to find more potent as well as synergistic partner in the combination therapy along with existing drug to cure malaria. Further, structure–activity relationship (SAR) and mechanistic approach should be taken into account while considering designing and screening of compounds. More research in this direction is under progress and results will be published in due course of times.

5. Experimental

Various chemicals and solvents used in this study were purchased from E. Merck (India) and Sigma–Aldrich chemicals. Melting points were determined by using open capillary method and are uncorrected. ^{1}H NMR spectral data were recorded on Brucker Avance spectrometer at 300 MHz and Jeol JNM ECX spectrometer at 400 MHz, respectively, using TMS as an internal standard. The chemical shifts values were recorded on δ scale and the

coupling constants (*J*) in hertz. The following abbreviations were used in reporting spectra: s = singlet, d = doublet, t = triplet, q = quartet, m = multiple. IR spectra were obtained on a Perkin Elmer Fourier-transform infrared (FT-IR) Spectrophotometer (Spectrum 2000) in potassium bromide disk. ESI-MS spectra were obtained on a Waters micromass LCT Mass spectrometer. Elemental analysis was done on Elementar GmbH VarioEl analyzer.

5.1. General procedure for the synthesis of 7-chloro-6-fluoro-4-oxo-1,4-dihydroquinoline 3-carboxylic acid ethyl ester (**1**)

Step (1): The synthesis of 7-chloro-6-fluoro-4-oxo-1,4-dihydroquinoline 3-carboxylic acid ethyl ester was achieved according to published procedure [32]. Briefly, 3-chloro-4-fluoroaniline (50.0 mmol, 7.2 g) and diethyl ethoxymethylenemalonate (55.0 mmol, 11.1 mL) were heated at 100 °C for 1.5 h. After this period, the reaction mixture was cooled at room temperature and ethanol formed during reaction was removed under vacuo to yield crude product and was purified by recrystallization with n-hexane to give corresponding malonate ester (17.2 g). Yield: 94%; m.p. 58 °C; ESI-MS (*m*/*z*): 338 $[M + Na]^+$; ^1H NMR (300 MHz, $CDCl_3$): δ 1.3–1.4 (m, 6H, $2CH_3$ of OCH_2CH_3), 4.2–4.3 (m, 4H, $2CH_2$ of OCH_2CH_3), 6.9–7.2 (m, 3H, ArH), 8.3 (d, 1H, Vinylic, J_{H-H} = 13 Hz), 10.9 (d, 1H, NH, J_{H-H} = 13 Hz); IR (KBr): 1722, 1658, 1622 cm^{-1}. Elemental analysis: calcd. For $C_{14}H_{15}ClFNO_4$: C, 53.26; H, 4.79; N, 4.44: found: C, 53.27: H, 4.74: N, 4.43%.

Step (2): The cyclization of malonate ester was achieved by heating diphenyl ether (50 mL) in an oil bath at 250 °C and the above malonate ester (20 mmol, 6.3 g) was added slowly. The reaction mixture was refluxed for 1 h with stirring, a white solid was formed on cooling. Solid was filtered, washed with hexane and purified through recrystallization from DMF to give **1** (4.4 g). Yield: 70%; m.p.: 300 °C; ESI-MS (*m*/*z*): 292 $[M + Na]^+$; ^1H NMR (300 MHz CF_3COOD): δ 1.01 (t, 3H, CH_3 of OCH_2CH_3, J_{H-H} = 7.2 Hz), 4.1 (q, 2H, CH_2 of OCH_2CH_3, J_{H-H} = 7.5 Hz), 7.4 (d, 1H, ArH, J_{H-F} = 9 Hz), 7.8 (d, 1H, ArH, J_{H-F} = 6 Hz), 8.8 (s, 1H, 2-H); IR (KBr): 3103, 1699, 1618 cm^{-1}; Elemental analysis calcd. for $C_{12}H_9ClFNO_3$: C, 53.45; H, 3.36; N, 5.19%; found: C, 53.29; H, 3.54; N, 5.08%.

5.2. 7-Chloro-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**2**)

The compound **1** (2.7 g, 10.0 mmol) was refluxed with 2 N NaOH (25.0 mL) for 2 h. The mixture was allowed to cool at room temperature and acidified with acetic acid. Solid was filtered, washed with water and dried under vacuum. The solid was recrystallized with DMF to give **2** (2.30 g).

Yield: 85%; m.p. 285 °C; ESI-MS (*m*/*z*): 283 $[M + K]^+$; ^1H NMR (300 MHz, DMSO-D_6): δ 8.03 (d, 1H, ArH, J_{H-F} = 6.6 Hz), 8.09 (d, 1H, ArH, J_{H-F} = 9.1 Hz), 8.8 (s, 1H, 2-H), 13.5 (s, 1H, NH)), 14.8 (s, 1H, COOH); IR (KBr): 3071, 2918, 1698, 1625, 1474 cm^{-1}; Elemental analysis calcd. for $C_{10}H_5ClFNO_3$: C, 49.71; H, 2.09; N, 5.80%; found: C, 48.15; H, 2.65; N, 5.62%.

5.3. General procedure for the synthesis various N-1 substituted quinolones (**3**–**9**)

A mixture of compound **1** (1.0 mmol, 0.27 g), K_2CO_3 (5.0 mmol, 0.69 g), alkyl halide (5.0 mmol, 0.810 g), in 20 mL DMF was heated at 90 °C. After 10–15 h, the mixture was evaporated to dryness, dissolved in water and extracted with chloroform. The combined organic layer was dried over Na_2SO_4 and evaporated to dryness. The crude products were purified by column chromatography using $CHCl_3$/MeOH (95/5) as eluent to afford corresponding *N*-1 substituted quinolones (**3**–**9**).

5.3.1. 7-Chloro-6-fluoro-1-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester (**3**)

Yield: 70%; mp. 218 °C; ESI-MS (*m*/*z*): 306 $[M + Na]^+$; ^1H NMR (300 MHz, $CDCl_3$): δ 1.37 (t, 3H, CH_3 of OCH_2CH_3, J_{H-H} = 6.9 Hz), 3.8 (s, 3H, CH_3 of $-NCH_3$), 4.3 (q, 2H, CH_2 of OCH_2CH_3, J_{H-H} = 6.9 Hz), 7.5 (d, 1H, ArH, J_{H-F} = 5.4 Hz), 8.1 (1H, d, ArH, J_{H-F} = 9 Hz), 8.41 (s, 1H, 2-H); IR (KBr): 3069, 1721, 1677 cm^{-1}; Elemental analysis: calcd. for $C_{13}H_{11}ClFNO_3$: C, 55.04; H, 3.91; N, 4.94%; found: C, 54.70; H, 3.93; N, 4.88%.

5.3.2. 7-Chloro-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester (**4**)

Yield: 90%; m.p. 135 °C; ESI-MS (*m*/*z*): 320 $[M + Na]^+$; ^1H NMR (300 MHz, $CDCl_3$): δ 1.3 (t, 3H, CH_3 of NCH_2CH_3, J_{H-H} = 7.2 Hz), 1.5 (t, 3H, CH_3 of OCH_2CH_3, J_{H-H} = 7.2 Hz), 4.2 (q, 2H, CH_2 of NCH_2CH_3, J_{H-H} = 7.2 Hz), 4.4 (q, 2H, CH_2 of OCH_2CH_3, J_{H-H} = 7.2 Hz), 7.54 (d, 1H, ArH, J_{H-F} = 5.7 Hz), 8.2 (d, 1H, J_{H-F} = 9 Hz), 8.4 (s, 1H, 2-H); IR (KBr): 3422, 2684, 1719, 1615 cm^{-1}; Elemental analysis: calcd. for $C_{14}H_{13}ClFNO_3$: C, 56.48; H, 4.40; N, 4.70%; found: C, 53.44; H, 3.7; N, 4.5%.

5.3.3. 7-Chloro-6-fluoro-4-oxo-1-propyl-1,4-dihydroquinoline-3-carboxylic acid ethyl ester (**5**)

Yield: 60%; m.p. 156 °C; ESI-MS (*m*/*z*): 334 $[M + Na]^+$; ^1H NMR (300 MHz, CDCl3): δ 1.03 (t, 3H, CH_3 of $NCH_2CH_2CH_3$, J_{H-H} = 7.3 Hz), 1.3 (t, 3H, CH_3 of $CH_2CH_2CH_3$, J_{H-H} = 7 Hz), 1.8 (m, 2H, CH_2 of $NCH_2CH_2CH_3$), 4.1 (t, 2H, CH_2 of $NCH_2CH_2CH_3$, J_{H-H} = 7.3 Hz), 4.5 (q, 2H, CH_2 of OCH_2CH_3, J_{H-H} = 7 Hz), 7.5 (d, 1H, ArH, J_{H-F} = 5.6 Hz), 8.2 (d, 1H, ArH, J_{H-F} = 9 Hz), 8.4 (s, 1H, 2-H); ^{13}C NMR (300 MHz, $CDCl_3$ + DMSO-d_6): δ 10.47, 13.93, 21.36, 55.28, 60.31, 109.90, 113.24, 118.11, 126.34, 128.78, 135.12, 148.90, 153.15, 164.43, 172.20; IR (KBr): 3045, 1728, 1613 cm^{-1}; Elemental analysis: calcd. for $C_{15}H_{15}ClFNO_3$: C, 57.79; H, 4.85; N, 4.49%; found: C, 57.39; H, 4.83; N, 4.43%.

5.3.4. 1-Butyl-7-chloro-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester (**6**)

Yield: 75%; m.p. 140 °C; ESI-MS (*m*/*z*): 348 $[M + Na]^+$; ^1H NMR (300 MHz, $CDCl_3$): δ 0.9 (t, 3H, CH_3 of $CH_2CH_2CH_2CH_3$, J_{H-H} = 7.2 Hz), 1.3 (t, 3H, CH_3 of OCH_2CH_3, J_{H-H} = 7.2 Hz), 1.8 (m, 4H, 2 CH_2 of $CH_2CH_2CH_2CH_3$), 4.1 (t, 2H, CH_2 of $CH_2CH_2CH_2CH_3$, J_{H-H} = 7.2 Hz), 4.3 (q, CH_2 of OCH_2CH_3, J_{H-H} = 7.2 Hz), 7.52 (d, 1H, ArH, J_{H-F} = 5.4 Hz), 8.2 (d, 1H, ArH, J_{H-F} = 9.3 Hz), 8.4 (s, 1H, 2-H); IR (KBr): 2957, 1721, 1612 cm^{-1}; Elemental analysis: calcd. for $C_{16}H_{17}ClFNO_3$: C, 58.99; H, 5.26; N, 4.30%; found: C, 45.14; H, 3.68; N, 3.52%.

5.3.5. 7-Chloro-6-fluoro-1-isopropyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester (**7**)

Yield: 50%; m.p. 128–130 °C; ESI-MS (*m*/*z*): 334 $[M + Na]^+$; ^1H NMR (300 MHz, $CDCl_3$): δ 1.3 (t, 3H, CH_3 of OCH_2CH_3, J_{H-H} = 7.2 Hz), 1.5–1.6 (d, 6H, 2 CH_3 of $(CH_3)_2CH$, J_{H-H} = 7.2 Hz), 4.3 (q, 2H, CH_2 of OCH_2CH_3, J_{H-H} = 6.9 Hz), 4.7 (m, 1H, CH of $(CH_3)_2CH$), 7.6 (d, 1H, ArH, J_{H-F} = 5.7 Hz), δ 8.2 (d, 1H, ArH, J_{H-F} = 9 Hz), 8.6 (s, 1H, 2-H); IR (KBr): 2365, 1672, 1574 cm^{-1}; Elemental analysis calcd. for $C_{15}H_{15}ClFNO_3$: C, 57.79; H, 4.85; N, 4.95%; found: C, 62.15; H, 4.1; N, 4.90%.

5.3.6. 7-Chloro-6-fluoro-4-oxo-1-(-2propynyl)-1,4-dihydroquinoline-3-carboxylic acid ethyl ester (**8**)

Sodium hydride (0.2 g, 60% oil suspension) was washed with n-hexane and added to a stirred suspension of **1** (1.35 g, 5.0 mmol) in DMF and stirred at room temperature for 50 min to dissolve all solid subsequently propargyl bromide (0.53 mL, 6.0 mmol) was added to it portion wise and reaction mixture was stirred for 36 h. The solution was concentrated in vacuo and, dissolved in water,

extracted with chloroform (3 × 25 mL) and the combined organic layer was dried over sodium sulfate, evaporated in vacuo to afford crude compound (**8**). The crude product was purified by column chromatography using $CHCl_3$/MeOH (93/7) as eluent.

Yield: 75%; m.p. 216–218 °C; ESI-MS (*m/z*): 307 $[M]^+$; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.3 (t, 3H, CH_3 of OCH_2CH_3, J_{H-H} = 7.2 Hz), 2.7 (s, 1H, CH of $CH_2C{\equiv}CH$), 4.3 (q, 2H, CH_2, of OCH_2CH, J_{H-H} = 7.2 Hz), 4.9 (s, 2H, CH_2 of $NCH_2C{\equiv}CH$), 7.7 (d, 1H, ArH, J_{H-F} = 5.7 Hz), 8.1 (d, 1H, ArH, J_{H-F} = 9 Hz), 8.6 (s, 1H, 2-H); ^{13}C NMR (300 MHz, 300 MHz, $CDCl_3$ + DMSO-d_6): δ 13.75, 42.98, 60.10, 74.67, 110.33, 112.85, 118.50, 126.11, 128.32, 134.74, 148.42, 153.16, 163.87, 172.12; IR (KBr): 3065, 2987, 2928, 1719, 1613 cm^{-1}; Elemental analysis calcd. for $C_{14}H_{11}ClFNO_3$: C, 58.55; H, 3.60; N, 4.55%; found: C, 58.50; H, 3.55; N, 4.50%.

5.3.7. 7-Chloro-6-fluoro-1-(2-hydroxy-ethyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester (**9**)

Yield: 78%; m.p. 210 °C; ESI-MS (*m/z*): 335 $[M + Na]^+$; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.3 (t, 3H, CH_3 of OCH_2CH_3, J_{H-H} = 7.2 Hz), 2.0 (s, 1H, −OH) 4.1–4.2 (m, 6H, $3CH_2$, two CH_2 of CH_2CH_2OH and one CH_2 of OCH_2CH_3), 7.3 (d, 1H, ArH, J_{H-F} = 9.3 Hz), 7.4 (d, 1H, ArH, J_{H-F} = 5.7 Hz), 8.5 (s, 1H, 2-H); IR (KBr): 2928, 1721, 1614 cm^{-1}; Elemental analysis: calcd. for $C_{14}H_{13}ClFNO_4$: C, 53.60; H, 4.18; N, 4.46%; found: C, 51.40; H, 4.14; N, 4.35%.

5.4. 7-Chloro-1-cyanomethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester (**10**)

Sodium hydride (0.2 g, 60% oil suspension) was added to a stirred suspension of **1** (1.35 g, 5.0 mmol) in DMF (30 mL) and stirred at room temperature for 50 min to dissolve all solid followed by addition of bromo acetonitrile (0.42 mL, 6.0 mmol) into it and continued stirring for additional for 36 h. After this period, the reaction mixture was concentrated in vacuo, redissolved into water and extracted with chloroform (3 × 25 mL). The combined organic layer was dried on anhydrous sodium sulfate and evaporated in vacuum to give titled compound (1.1 g). The crude product was purified by column chromatography using $CHCl_3$/MeOH (94/6) as eluent.

Yield: 88%; m.p. 222–224 °C; ESI-MS (*m/z*): 308 $[M]^+$; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.0 (t, 3H, CH_3 of OCH_2CH_3, J_{H-H} = 3 Hz), 4.0 (q, 2H, CH_2 of OCH_2CH_3 J_{H-H} = 7 Hz), 5.2 (s, 2H, CH_2 of NCH_2CN), 7.6 (d, 1H, ArH, J_{H-F} = 4 Hz), 7.7 (d, 1H, ArH, J_{H-F} = 4 Hz), 8.4 (s, 1H, 2-H); IR (KBr): 3048, 2117, 1672, 1654 cm^{-1}; Elemental analysis calcd. for $C_{14}H_{10}ClFN_2O_3$: C, 54.47; H, 3.27; N, 9.07%.; found: C, 54.40; H, 3.2; N, 8.9%.

5.5. 1-Benzyl-7-chloro-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester (**11**)

Yield: 55%, m.p. 142 °C; ESI-MS (*m/z*): 384 $[M + Na]^+$; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.3 (t, 3H, CH_3 of OCH_2CH_3, J_{H-H} = 7.2 Hz), 4.3 (d, 2H, CH_2 of NCH_2Ar, J_{H-H} = 6.9 Hz), 5.3 (s, 2H, CH_2 of NCH_2Ar), 7.1–7.4 (m, 6H, ArH), 8.1 (d, 1H, ArH, J_{H-F} = 9 Hz), 8.5 (s, 1H, 2-H); IR (KBr): 3057, 2370, 1720, 1615 cm^{-1}; Elemental analysis calcd. for $C_{19}H_{15}ClFNO_3$: C, 6343; H, 4.20; N, 3.89%; found: C, 61.48; H, 4.07; N, 3.85%.

5.6. 7-Chloro-6-fluoro-4-oxo-1-(1H-[1,2,3]triazol-4-ylmethyl)-1,4-dihydroquinoline-3-carboxylic acid ethyl ester (**12**)

The compound 7-chloro-6-fluoro-4-oxo-1-(2-propynyl)-1,4-dihydroquinoline-3-carboxylic acid ethyl ester **11** (0.153 g, 0.5 mmol) and sodium azide (0.065 g, 1.0 mmol) was dissolved in a mixture of DMF and water (1:1, 10 mL). Sodium ascorbate (0.04 g. 0.2 mmol, in 500 μl of water) was added to reaction mixture followed by the addition of $CuSO_4 \cdot 5H_2O$ (0.02 g, 0.09 mmol in 200 μL of water). The reaction mixture was stirred vigorously for 6 h at 60 °C. After completion of the reaction, ice-cold water was added in to the reaction mixture. The precipitate thus obtained was collected by filtration and washed with water. Again this precipitate was dissolved in 3 N HCl (10 mL) and stirred for 30 min at room temperature, and extracted with ethyl acetate (25 mL × 3). Combined organic layer was dried over sodium sulfate and solvent was evaporated in vacuum to give compound (**12**).

Yield: 50%; m.p. >300 °C, ESI-MS (*m/z*): 350 $[M]^+$; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.3 (t, 3H, CH_3 of OCH_2CH_3, J_{H-H} = 7.2 Hz), 4.2 (q, 2H, CH_2 of OCH_2CH_3, J_{H-H} = 6.9 Hz), 5.8 (s, 2H, CH_2 of NCH_2-triazole), 8.0 (m, 2H, ArH), 8.2 (d, 1H, ArH, J_{H-F} = 6.6 Hz), 8.9 (s, 1H, 2-H); ^{13}C NMR (300 MHz, 300 MHz, DMSO-d_6): δ 14.27, 47.88, 60.03, 110.12, 112.34, 120.49, 125.11, 128.53, 135.99, 140.94, 150.28, 152.78, 156.06, 164.20, 171.51; IR (KBr): 2982, 1718, 1612 cm^{-1}; Elemental analysis calcd. for $C_{15}H_{12}ClFN_4O_3$: C, 51.37; H, 3.45; N, 15.97%.; found: C, 51.32; H, 3.43; N, 15.90%.

5.7. General procedure for the synthesis of various N-(1-substituted-[1,2,3]triazol-4-ylmethyl)-1,4 substituted quinolones (**13**–**21**)

An equimolar ratio of alkyne, 7-chloro-6-fluoro-4-oxo-1-(2-propynyl)-1,4-dihydro-quinoline-3-carboxylic acid ethyl ester **11** (0.153 g, 0.5 mmol) and azide, **13**–**21** (0.5 mmol) was dissolved in a mixture of DMF and water (1:1, 10 mL). Sodium ascorbate (0.04 g. 0.2 mmol, in 500 μl of water) was added to above suspension followed by the addition of $CuSO_4 \cdot 5H_2O$ (0.02 g, 0.09 mmol in 200 μL of water). The reaction mixture was stirred vigorously for 6 h at 60 °C. After the completion of reaction, reaction mixture was diluted with ice-cold water added and precipitate thus obtained was collected by filtration, washed with water and dried under vacuum. The crude product was purified by column chromatography using chloroform and methanol as eluent.

5.7.1. 7-Chloro-6-fluoro-4-oxo-1-(1-propyl-1H-[1,2,3]triazol-4-ylmethyl)-1,4-dihydroquinoline-3-carboxylic acid ethyl ester (**13**)

Yield: 60%; m.p. >300 °C; ESI-MS (*m/z*): 392 $[M]^+$; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.6–0.8 (m, 6H, $2CH_3$), 2.0 (m, 2H, CH_2 of $NCH_2CH_2CH_3$), 3.7 (t, 2H, CH_2 of $CH_2CH_2CH_3$, J_{H-H} = 6 Hz), 4.6 (q, 2H, CH_2 of OCH_2CH_3, J_{H-H} = 6 Hz), 4.7 (s, 2H, CH_2 of NCH_2), 7.2 (s, 1H, C=CH of triazole), 7.3 (d, 1H, ArH, J_{H-F} = 6 Hz), 7.5 (d, 1H, ArH, J_{H-F} = 9 Hz), 8.1 (s, 1H, 2-H); IR (KBr): 3047, 2928, 1695, 1614 cm^{-1}; Elemental analysis: calcd. for $C_{18}H_{18}ClFN_4O_3$: C, 55.04; H, 4.62; N, 14.26%; found: 55.01; H, 4.60; N, 14.22%.

5.7.2. 7-Chloro-6-fluoro-1-[1-(2-hydroxy-ethyl)-1H-[1,2,3]triazol-4-ylmethyl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester (**14**)

Yield: 70%; m.p. 222–224 °C; ESI-MS (*m/z*): 394 $[M]^+$; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.2 (t, 3H, CH_3 of OCH_2CH_3, J_{H-H} = 6.7 Hz), 2.5 (s, 1H, −OH), 3.7 (t, 2H, CH_2, of NCH_2CH_2OH, J_{H-H} = 5.3 Hz), 4.2 (q, 2H, CH_2, of OCH_2CH_3, J_{H-H} = 7 Hz), 4.3 (t, 2H, CH_2 of NCH_2CH_2OH, J_{H-H} = 5 Hz), 5.7 (s, 2H, CH_2 of NCH_2-triazole), 8.0 (d, 1H, ArH, J_{H-F} = 9.4 Hz), 8.2 (s, 1H, C=CH of triazole), 8.3 (d, 1H, ArH, J_{H-F} = 6 Hz), 8.9 (s, 1H, 2-H); IR (KBr): 3047, 2924, 1678, 1610 cm^{-1}; Elemental analysis calcd. for $C_{14}H_{11}ClFNO_3$: C, 51.72; H, 4.09; N, 14.19%; found: C, 51.70; H, 4.06; N, 14.16%.

5.7.3. 7-Chloro-6-fluoro-1-(1-methoxycarbonylmethyl-1H-[1,2,3]triazol-4-ylmethyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester (**15**)

Yield: 65%; m.p 222 °C; ESI-MS (*m/z*): 422 $[M]^+$; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.2 (t, 3H, CH_3 of OCH_2CH_3, J_{H-H} = 6 Hz), 3.9 (s, 3H, CH_3 of OCH_3), 4.2 (q, 2H, CH_2 of OCH_2CH, J_{H-H} = 6 Hz), 5.1 (s,

2H, CH_2 of NCH_2-triazole), 5.4 (s, 2H, CH_2 of NCH_2COOCH_3), 7.7 (s, 1H, C═CH of triazole), 7.8 (d, 1H, ArH, $J_{H-F} = 6$ Hz), 8.2 (d, 1H, ArH, $J_{H-F} = 9$ Hz), 8.6 (s, 1H, 2-H); IR (KBr): 3421, 2084, 1710, 1615 cm^{-1}; Elemental analysis: calcd. for $C_{18}H_{61}ClFN_4O_5$: C, 51.13; H, 3.81; N, 13.25%; found: C, 51.11; H, 3.80; N, 13.23%.

*5.7.4. 1-(1-Benzyl-1H-[1,2,3]triazol-4-ylmethyl)-7-chloro-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester (**16**)*

Yield: 70%; m.p. 232–234 °C; ESI-MS (*m*/*z*): 440 $[M]^+$; ^1H NMR (400 MHz, $CDCl_3$): δ 1.2 (t, 3H, CH_3 of OCH_2CH_3, $J_{H-H} = 7.3$ Hz), 4.3 (q, 2H, CH_2, of OCH_2CH_3, $J_{H-H} = 6.8$ Hz), 5.2 (s, 2H, CH_2 of NCH_2-triazole), 5.4 (s, 2H, CH_2 of NCH_2Ar), 7.2–7.4 (m, 6H, aromatic), 7.7 (1H, d, ArH, $J_{H-F} = 5.9$ Hz), 8.1 (d, 1H, ArH, $J_{H-F} = 9.1$ Hz), 8.5 (s, 1H, 2-H); IR (KBr): 2989, 1715, 1611 cm^{-1}; Elemental analysis calcd. for $C_{22}H_{18}ClFN_4O_3$: C, 59.94; H, 4.12; N, 12.71%.; found: C, 59.9.; H, 4.1; N, 12.69%.

*5.7.5. 7-Chloro-1-[1-(4-chloro-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester (**17**)*

Yield: 70%: m.p. 274–276 °C; ESI-MS (*m*/*z*): 460 $[M]^+$; ^1H NMR (300 MHz, DMSO-d_6): δ 1.2 (t, 3H, CH_3 of OCH_2CH_3, $J_{H-H} = 6.9$ Hz), 4.2 (q, 2H, CH_2 of OCH_2CH_3, $J_{H-H} = 7.2$ Hz), 5.8 (s, 2H, CH_2 of NCH_2-triazole), 7.6 (d, 2H, ArH, $J_{H-H} = 8.7$ Hz), 7.8 (d, 2H, ArH, $J_{H-H} = 8.7$ Hz), 8.0 (d, 1H, ArH, $J_{H-F} = 9.3$ Hz), 8.2 (d, 1H, ArH, $J_{H-H} = 5.7$ Hz), 8.9 (d, 2H, ArH, $J_{H-F} = 15.9$ Hz); IR (KBr): 3073, 2926, 1725, 1615 cm^{-1}; Elemental analysis calcd. for $C_{21}H_{15}Cl_2FN_4O_3$: C, 54.68; H, 3.28; N, 12.15%; found: 54.62; H, 3.250; N, 12.1%.

*5.7.6. 7-Chloro-1-[1-(3-chlorophenyl)-1H-[1,2,3]triazol-4-ylmethyl]-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester (**18**)*

Yield: 60%; m.p. >206–208 °C; ESI-MS (*m*/*z*): 460 $[M]^+$; ^1H NMR (400 MHz, $CDCl_3$): δ 1.2 (t, 3H, CH_3 of OCH_2CH_3, $J_{H-H} = 6.9$ Hz), 4.2 (q, 2H, CH_2 of OCH_2CH_3, $J_{H-H} = 6.9$ Hz), 5.8 (s, 2H, CH_2 of NCH_2-triazole), 7.2 (m, 2H, ArH), 7.8 (d, 1H, ArH, $J_{H-F} = 8.1$ Hz), 7.9 (m, 2H, ArH), 8.2 (d, 1H, ArH, $J_{H-F} = 5.7$ Hz), 8.93 (s, 1H, ArH), 8.95 (s, 1H, 2-H); ^{13}C NMR (300 MHz, 300 MHz, DMSO-d_6): δ 14.29, 48.01, 60.04, 110.33, 112.39, 112.68, 118.76, 119.95, 120.45, 122.30, 125.19 128.68, 131.59, 134.15, 136.02, 137.39, 142.97, 150.39, 152.83, 164.22, 171.57; IR (KBr): 3196, 2925, 1672, 1616 cm^{-1}; Elemental analysis: calcd. for $C_{21}H_{15}Cl_2FN_4O_3$: C, 54.68; H, 3.28; N, 12.15%; found: C, 54.65; H, 3.20; N, 12.10%.

*5.7.7. 7-Chloro-6-fluoro-1-[1-(4-fluoro-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester (**19**)*

Yield: 78%; m.p. 236–238 °C; ESI-MS (*m*/*z*): 444 $[M]^+$; ^1H NMR (400 MHz, $CDCl_3$): δ 1.3 (t, 3H, CH_3 of OCH_2CH_3, $J_{H-H} = 6.9$ Hz), 4.2 (q, 2H, CH_2 of OCH_2CH_3, $J_{H-H} = 7.2$ Hz), 5.4 (s, 2H, CH_2 of NCH_2-triazole), 7.4 (m, 2H, ArH), 7.7 (d, 1H, ArH, $J_{H-F} = 7.2$ Hz), 7.8 (m, 2H, ArH), 8.0 (d, 1H, ArH, $J_{H-F} = 8.7$ Hz), 8.4 (s, 1H, C═CH of triazole), 8.6 (s, 1H, 2-H); IR (KBr): 3080, 2928, 1726, 1612 cm^{-1}; Elemental analysis: calcd. for $C_{21}H_{15}ClF_2N_4O_3$: C, 56.7.; H, 3.40; N, 12.6%; found: 56.68; H, 3.350; N, 12.52%.

*5.7.8. 7-Chloro-1-[1-(3-chloro-4-fluorophenyl)-1H-[1,2,3]triazol-4-ylmethyl]-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester (**20**)*

Yield: 80%; m.p. 238–240 °C; ESI-MS (*m*/*z*): 478 $[M]^+$; ^1H NMR (400 MHz, $CDCl_3$): δ 1.2 (t, 3H, CH_3 of OCH_2CH_3, $J_{H-H} = 6$ Hz), 4.1 (q, 2H, CH_2 of OCH_2CH_3, $J_{H-H} = 7$ Hz), 5.2 (s, 2H, CH_2 of NCH_2-triazole), 7.2 (m, 3H, ArH), 7.4 (d, 1H, ArH, $J_{H-H} = 5$ Hz), 8.2 (d, 1H, ArH, $J_{H-F} = 9$ Hz), 8.3 (s, 1H, C═CH of triazole), 8.5 (s, 1H, 2-H); IR (KBr): 3080, 2926, 1725, 1613 cm^{-1}; Elemental analysis: calcd. for $C_{21}H_{14}Cl_2F_2N_4O_3$: C, 52.63; H, 2.94; N, 11.69%; found: 52.50; H, 2.90; N, 11.65%.

*5.7.9. 7-Chloro-6-fluoro-4-oxo-1-(1-quinolin-4-yl-1H-[1,2,3]triazol-4-ylmethyl)-1,4-dihydro-quinoline-3-carboxylic acid ethyl ester (**21**)*

Yield: 75%; m.p. 238–240 °C; ESI-MS (*m*/*z*): 511 $[M]^+$; ^1H NMR (300 MHz, DMSO-d_6): δ 1.2 (t, 3H, CH_3 of OCH_2CH_3, $J_{H-H} = 6.9$ Hz), 4.2 (q, 2H, CH_2 of OCH_2CH_3, $J_{H-H} = 6.9$ Hz), 5.9 (s, 2H, CH_2 of NCH_2-triazole), 7.7–7.8 (m, 2H, ArH), 7.9 (d, 1H, ArH, $J_{H-H} = 9.3$ Hz), 8.0 (d, 1H, ArH, $J_{H-H} = 9.6$ Hz), 8.2 (s, 1H, 2-H), 8.3 (d, 1H, ArH, $J_{H-H} = 5.7$ Hz), 8.9 (d, 2H, ArH, $J_{H-F} = 15.6$ Hz), 9.1 (d, 1H, ArH, $J_{H-H} = 4.5$ Hz); IR (KBr): 3048, 2129, 1667, 1613 cm^{-1}; Elemental analysis calcd. for $C_{24}H_{16}Cl_2FN_5O_3$: C, 56.26; H, 3.15; N, 13.67%; found: C, 56.24; H, 3.13; N, 13.60%.

6. MTT assay for cell viability

Various human embryonic kidney cells (HEK-293) were maintained as monolayer at 37 °C in 5% CO_2 using DMEM medium. Approximately, 4000 cells/well were seeded in 96-well plate containing 200 mL of medium and incubated for 24 h. The culture medium was replaced by fresh medium containing 1, 10, 20, 30, 50 and 100 μM concentration of compounds 2, 12, 19, 20 and 21 respectively and incubated for 24, 48 and 72 h. The cell viability was determined by the MTT assay following the procedure described by Price and McMillan [38]. The light absorbance was measured at 570 nm wave length using a microplate reader (Infinite M200; Tecan Group Ltd., Männedorf, Switzerland).

Acknowledgement

SKA is thankful to Department of Science and Technology (DST), New Delhi, India (scheme no. SR/S0/BB-65//2003) and University of Delhi, Delhi, India for financial support. This work was partly supported to VKB by the Department of Biotechnology, Ministry of Science and Technology, Government of India. SKA is extremely thankful to Dr Vibha Tandon, Department of Chemistry, University of Delhi, Delhi, India for providing cytotoxicity data.

Appendix. Supplementary material

Supplementary data related to this article can be found online at doi:10.1016/j.ejmech.2012.02.006.

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