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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

New azetidine-3-carbonyl-*N*-methyl-hydrazino derivatives of fluoroquinolones: synthesis and evaluation of antibacterial and anticancer properties

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Abstract A series of nine new *N*-(substituted azetidine-3-carbonyl)-*N*-methyl-hydrazino derivatives at C-7 position of fluoroquinolones were designed and synthesized through multistep synthesis. The synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR, ESI–MS and IR. The new compounds were tested for their in vitro antimicrobial and anti-proliferative activity. Out of the nine derivatives, compound **6i** exhibited good antibacterial activity by inhibiting the growth of Methicillin-sensitive *Staphylococcus aureus*, Methicillin-resistant *S. aureus* and ATCC 35218 *Escherichia coli* (MIC: 0.25–16.00 μg/mL). Compounds **6c**, **6g–h** are displayed good growth inhibition against MCF-7 Breast carcinoma, HCT-116 Colon carcinoma and A549 Lung adenocarcinoma cell lines. Compound **6h** is the most active against MCF-7 cell line with superior growth inhibition compared to ciprofloxacin and reference anticancer drug SAHA.

Keywords Synthesis · Reductive amination · Fluoroquinolones · Antibacterial activity · Anticancer activity

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Introduction

Fluoroquinolones are a class of quinolone compounds and known as the 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 (Mitscher, 2005). These antibiotics exert their antimicrobial activity by binding to 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 (De Souza, 2005; Hooper, 2001).

Most of the quinolones currently in the market or under development are generally characterized by a broad antibacterial spectrum, but their activity against clinically important Gram-positive *cocci*, including *Staphylococci*, *Streptococci*, and *Enterococci*, is relatively moderate. This insufficient activity has not only limited their use in infections caused by these organisms, but has also contributed to the rapidly developing quinolone resistance. Thus, recent efforts have been directed towards the synthesis of new quinolones that can provide improved Gram-positive antibacterial activity, while retaining the Gram-negative activity of early fluoroquinolones, such as ciprofloxacin (Piddock *et al.,* 1994).

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 (Bryskier and Chantot, 1995; Koga *et al.,* 1980). In general, 5- and 6-membered nitrogen heterocycles have been proven to be the optimal substituents (Domagala, 1994).

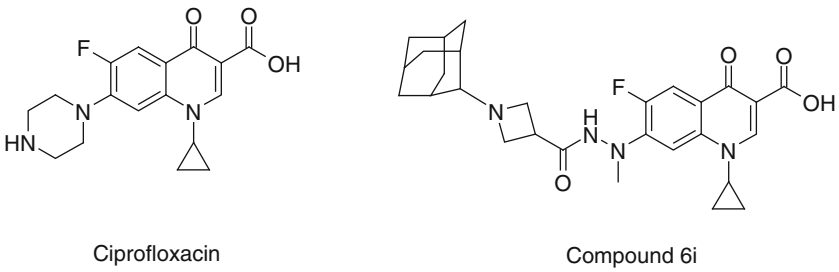


Fig. 1 Chemical structure of ciprofloxacin and compound **6i**

In our continuous efforts to identify new antibacterial and anticancer agents (Selvakumar *et al.,* 2008; Takhi *et al.,* 2009; Selvakumar *et al.,* 2011), we have introduced basic 4-membered nitrogen-containing heterocycle such as *N*-(substituted azetidine-3-carbonyl) group via *N*-methyl-hydrazine linker at C-7 position of fluoroquinolones. Also the azetidine-based derivatives are known to be good building blocks in drug discovery (Ferraris *et al.,* 2007; Ikee *et al.,* 2008). To the best of our knowledge, there is no literature available to reveal the effect of *N*-(substituted azetidine-3-carbonyl)-*N*-methyl-hydrazino group at C-7 position of fluoroquinolones for their antibacterial and anticancer activity. The design strategy of *N*-(substituted azetidine-3-carbonyl)-*N*-methyl-hydrazino derivatives with a representative example (compound **6i**) is shown in Fig. 1.

Experimental

Chemistry

Chemicals were obtained from Sigma-Aldrich Co. The 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 ^1H and ^{13}C NMR spectra were recorded on a Bruker AM-300 (300 MHz for ^1H 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 TMS. The signals are designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; brs, broad singlet. IR Spectra were recorded using a Bruker Alpha FTIR spectrometer using a diamond ATR single reflectance module (24 scans).

*General procedure for the synthesis of N-alkyl substituted azetidine 3-carboxylic acid derivatives (**2a–i**)*

To a suspension of 3-azetidine carboxylic acid **1** (1.0 g, 9.9 mmol) in methanol (20 mL) corresponding aldehyde/ketone (10.9 mmol) and 10 % Pd/C (0.2 g) at room temperature were added. The reaction mixture was allowed to stir at room temperature under hydrogen pressure (20 psi) over a period of 8 h. Completion of the reaction was monitored by TLC. The resulting reaction mixture was filtered through Celite pad and filtrate was concentrated under reduced pressure. The residue obtained upon evaporation of the volatiles was washed with diethyl ether (2 × 10 mL).

*1-(4-Trifluoromethyl-benzyl)-azetidine-3-carboxylic acid (**2a**)* This compound was prepared by reductive amination of 4-trifluoromethyl-benzaldehyde with 3-azetidine carboxylic acid. It was obtained as a off-white solid (2.0 g, 80 %), MP: 79.6–81.7 °C. IR (ATR, cm^{-1}) υ: 3466 (–OH); 3288 (ArH), 1569 (acid –C=O), 1325 (–C=C). ^1H NMR (DMSO-d_6-D_2O 300 MHz) δ (ppm): 3.14–3.22 (1H, m, azetidine–CH), 3.59 (2H, t, $J = 8.5$ Hz, azetidine–CH_2), 3.71 (2H, t, $J = 8.7$ Hz, azetidine–CH_2), 3.97 (2H, s, –NCH_2Ar), 7.54 (2H, d, $J = 8.1$ Hz, ArH), 7.70 (2H, d, $J = 8.1$ Hz, ArH). ^{13}C NMR (DMSO-d_6 75 MHz) δ: 34.14 (C-16), 56.83 (C-17 & C-17′), 61.66 (CH_2, –CH_2Ph), 122.94 & 126.54 (d, $J = 270$ Hz, –CF_3), 125.48 & 125.53 (d, $J = 3.75$ Hz, –ArC), 128.01 & 128.43 (d, $J = 31.5$ Hz, C, –$ArCCF_3$), 129.42 (ArC), 142.80 (ArC), 174.72 (C-15). LC–MS (ESI, *m/z*): 260.1 (M + H).

*1-(4-Methoxy-benzyl)-azetidine-3-carboxylic acid (**2b**)* This compound was prepared by reductive amination of 4-methoxy-benzaldehyde with 3-azetidine carboxylic acid. It was obtained as a off-white solid (1.85 g, 85 %). MP: 149.4–152.5 °C. IR (ATR, cm^{-1}) υ: 3497 (–OH); 3021 (ArH), 1592 (acid –C=O), 1514 (acid –C–O), 1172 (ether –C–O). ^1H NMR (CD_3OD 300 MHz) δ (ppm): 3.32–3.45 (1H, m, azetidine–CH), 3.79 (3H, s, –OMe), 4.16 (4H, d, $J = 8.4$ Hz, azetidine–CH_2), 4.28 (2H, s, –NCH_2Ar), 7.00 (2H, d, $J = 8.7$ Hz, ArH), 7.39 (2H, d, $J = 8.4$ Hz, ArH). ^{13}C NMR (DMSO-d_6 75 MHz) δ: 34.03 (C-16), 55.46 (C-17 & C-17′), 56.46 (–OMe), 63.01 (CH_2, –CH_2Ph), 114.14 (ArC), 128.37 (ArC), 130.24 (ArC), 158.93 (ArC), 174.62 (C-15). LC–MS (ESI, *m/z*): 222.1 (M + H).

*1-(4-Fluoro-benzyl)-azetidine-3-carboxylic acid (**2c**)* This compound was prepared by reductive amination of

4-fluoro-benzaldehyde with 3-azetidine carboxylic acid. It was obtained as a pale yellow solid (1.6 g, 81 %), MP: 129.5–130.2 °C. IR (ATR, cm^{-1}) υ: 3450 (–OH); 3042 (ArH), 1591 (acid –C=O), 1510 (acid –C–O), 1026 (–C–F). ^{1}H NMR (CD_3OD 300 MHz) δ (ppm): 3.37–3.48 (1H, m, azetidine–CH), 4.19 (4H, d, $J = 8.1$ Hz, azetidine–CH_2), 4.36 (2H, s, –NCH_2Ar), 7.21 (2H, t, $J = 6.6$ Hz, ArH), 7.50–7.55 (2H, m, ArH). ^{13}C NMR (DMSO-d_6 75 MHz) δ: 34.14 (C-16), 56.17 (C-17 & C-17′), 59.53 (CH_2, –CH_2Ph), 115.79 (ArC), 115.96 (ArC), 131.51 (ArC), 160.60 &163.83 (d, $J = 242.25$ Hz, C–F), 174.62 (C-15). LC–MS (ESI, *m/z*): 210.1 (M + H).

1-Thiazol-4-ylmethyl-azetidine-3-carboxylic acid ***(2d)*** This compound was prepared by reductive amination of 2-thiazole-4-carbaldehyde with 3-azetidine carboxylic acid. It was obtained as a white solid (1.7 g, 74 %), MP: 180.2–181.3 °C. IR (ATR, cm^{-1}) υ: 3443 (–OH); 1572 (acid –C=O), 1522 (acid –C–O). ^{1}H NMR (DMSO-d_6-D_2O 300 MHz) δ (ppm): 3.07–3.17 (1H, m, azetidine–CH), 3.24 (2H, t, $J = 6.6$ Hz, azetidine–CH_2), 3.43 (2H, t, $J = 7.8$ Hz, azetidine–CH_2), 3.62 (2H, s, –NCH_2thiazole), 7.43 (1H, s, thiazole H), 9.02 (1H, s, thiazole H). ^{13}C NMR (DMSO-d_6 75 MHz) δ: 34.00 (C-16), 56.44 (C-17 & C-17′), 57.08 (CH_2, –CH_2Ph), 117.15 (thiazole C), 152.95 (thiazole C), 154.60 (thiazole C), 174.38 (C-15). LC–MS (ESI, *m/z*): 199.0 (M + H).

1-Isopropyl-azetidine-3-carboxylic acid ***(2e)*** This compound was prepared by reductive amination of acetone with 3-azetidine carboxylic acid. It was obtained as a pale yellow solid (1.2 g, 85 %), MP: 131.1–132.6 °C. IR (ATR, cm^{-1}) υ: 3349 (–OH); 2904 (ArH), 1595 (acid –C=O), 1512 (acid –C–O). ^{1}H NMR (DMSO-d_6 300 MHz) δ (ppm): 0.89 (6H, d, $J = 6.6$ Hz, $(CH_3)_2$), 2.47–2.50 (1H, m, isopropyl–CH), 3.00–3.05 (1H, m, azetidine–CH), 3.30 (2H, t, $J = 6.9$ Hz, azetidine–CH_2), 3.49 (2H, t, $J = 8.1$ Hz, azetidine–CH_2). ^{13}C NMR (DMSO-d_6 75 MHz) δ: 17.73 (–$(CH_3)_2$), 33.36 (C-16), 54.76 (C-17 & C-17′), 56.56 (isopropyl–CH), 174.89 (C-15). LC–MS (ESI, *m/z*): 144.1 (M + H).

1-Cyclopentyl-azetidine-3-carboxylic acid ***(2f)*** This compound was prepared by reductive amination of cyclopentanone with 3-azetidine carboxylic acid. It was obtained as a off-white solid (1.25 g, 74 %), MP: 132.4–133.1 °C. IR (ATR, cm^{-1}) υ: 3278 (–OH); 1576 (acid –C=O), 1510 (acid –C–O). ^{1}H NMR (DMSO-d_6-D_2O 300 MHz) δ (ppm): 1.40–1.61 (6H, m, cyclopentyl–CH_2), 1.76–1.82 (2H, m, cyclopentyl–CH_2), 3.08 (1H, t, $J = 8.4$ Hz, cyclopentyl–CH), 3.53–3.55 (1H, m, azetidine–CH), 3.80–3.99 (4H, m, azetidine–CH_2). ^{13}C NMR (DMSO-d_6 75 MHz) δ: 24.45 (cyclopentyl–CH_2), 28.95 (cyclopentyl–CH_2), 33.61 (C-16), 55.57 (C-17 & C-17′), 67.22 (cyclopentyl–CH), 174.89 (C-15). LC–MS (ESI, *m/z*): 170.1 (M + H).

1-Cyclohexyl-azetidine-3-carboxylic acid ***(2g)*** This compound was prepared by reductive amination of cyclohexanone with 3-azetidine carboxylic acid. It was obtained as a pale yellow solid (1.4 g, 78 %), MP: 149.3–152.7 °C. IR (ATR, cm^{-1}) υ: 3499 (–OH); 1592 (acid –C=O), 1514 (acid –C–O). ^{1}H NMR (DMSO-d_6-D_2O 300 MHz) δ (ppm): 1.19–1.23 (5H, m, cyclohexyl–CH_2), 1.52–1.56 (1H, m, cyclohexyl–CH_2), 1.67–1.71 (2H, m, cyclohexyl–CH_2), 2.91 (1H, t, $J = 10.8$ Hz, cyclohexyl–CH), 3.02–3.14 (1H, m, azetidine–CH), 3.90–4.03 (4H, m, azetidine–CH_2). ^{13}C NMR (DMSO-d_6 75 MHz) δ: 23.84 (cyclohexyl–CH_2), 27.91 (cyclohexyl–CH_2), 28.64 (cyclohexyl–CH_2), 32.75 (C-16), 54.89 (C-17 & C-17′), 65.89 (cyclohexyl–CH), 173.88 (C-15). LC–MS (ESI, *m/z*): 184.1 (M + H).

1-Cycloheptyl-azetidine-3-carboxylic acid ***(2h)*** This compound was prepared by reductive amination of cycloheptanone with 3-azetidine carboxylic acid. It was obtained as a pale yellow solid (1.56 g, 80 %), MP: 154.1–155.6 °C. IR (ATR, cm^{-1}) υ: 3499 (–OH); 1595 (acid –C=O), 1520 (acid –C–O). ^{1}H NMR (DMSO-d_6-D_2O 300 MHz) δ (ppm): 1.42–1.52 (6H, m, cycloheptyl–CH_2), 1.59–1.72 (4H, m, cycloheptyl–CH_2), 2.49–2.53 (1H, m, cycloheptyl–CH), 3.03–3.11 (1H, m, azetidine–CH), 3.40 (2H, t, $J = 7.2$ Hz, azetidine–CH_2), 3.59 (2H, t, $J = 8.4$ Hz, azetidine–CH_2). ^{13}C NMR (DMSO-d_6 75 MHz) δ: 23.92 (cycloheptyl–CH_2), 28.03 (cycloheptyl–CH_2), 29.00 (cycloheptyl–CH_2), 32.94 (C-16), 54.99 (C-17 & C-17′), 66.20 (cycloheptyl–CH), 173.64 (C-15). LC–MS (ESI, *m/z*): 198.1 (M + H).

1-Adamantan-2-yl-azetidine-3-carboxylic acid ***(2i)*** This compound was prepared by reductive amination of 2-adamantanone with 3-azetidine carboxylic acid. It was obtained as a white solid (1.7 g, 74 %), MP: 160.1–162.1 °C. IR (ATR, cm^{-1}) υ: 3450 (–OH); 1559 (acid –C=O), 1521 (acid –C–O). ^{1}H NMR (DMSO-d_6-D_2O 300 MHz) δ (ppm): 1.23 (1H, s, adamantyl–CH), 1.33 (2H, d, $J = 11.7$ Hz, adamantyl–CH_2), 1.58–1.78 (9H, m, adamantyl–CH), 1.97 (2H, d, $J = 11.7$ Hz, adamantyl–CH_2), 2.20 (1H, s, adamantyl–CH), 3.06–3.10 (2H, m, azetidine–CH_2), 3.12–3.19 (1H, m, azetidine –CH), 3.39 (2H, t, $J = 6.9$ Hz, azetidine–CH_2). ^{13}C NMR (DMSO-d_6 75 MHz) δ: 27.14 (adamantyl–CH), 27.32 (adamantyl–CH_2), 28.05 (adamantyl–CH), 31.01 (adamantyl–CH), 32.98 (C16), 36.02 (adamantyl–CH_2), 36.50 (adamantyl–CH_2), 37.61 (adamantyl–CH), 40.81 (adamantyl–CH_2), 54.89 (C-17 & C-17′), 70.72 (adamantyl–NCH), 173.65 (C-15). LC–MS (ESI, *m/z*): 236.2 (M + H).

*Procedure for the synthesis of 1-cyclopropyl-6-fluoro-7-(N -methyl-hydrazino)-4-oxo-1,4-dihydro-quinoline -3-carboxylic acid ethyl ester (**4**)*

To a solution of compound **3** (1.0 g, 3.4 mmol) in pyridine (20.0 mL) methyl hydrazine (173 mg, 3.7 mmol) at 25 °C was added. Then the reaction mixture was allowed to stir at 90 °C over a period of 5 h. After completion of the reaction, volatiles were evaporated under reduced pressure to obtain crude product as yellow colour solid. The crude product was purified by silica gel column chromatography (5 % methanol in chloroform) to obtain product **4** (750 mg, 68 %) as a pale yellow solid. MP: 199.3 °C-201.4 °C. IR (ATR, cm^{-1}) υ: 3337 (ArH), 1702 (ester –C=O), 1620 (quinolone–C=O), 1476 (ester –C–O), 1195 (–C–F). ^{1}H NMR (DMSO-d_6 300 MHz) δ (ppm): 1.08 (2H, bs, cyclopropyl–CH_2), 1.22–1.29 (5H, m, cyclopropyl–CH_2 & ethyl–CH_3), 3.22 (3H, s, –NCH_3), 3.56 (1H, bs, cyclopropy–CH), 4.20 (2H, q, $J = 6.6$ Hz, ethyl–OCH_2), 4.72 (2H, s, –NH_2), 7.67 (1H, d, $J = 14.1$ Hz, ArH), 7.87 (1H, d, $J = 7.5$ Hz, ArH), 8.39 (1H, s, ArH). ^{13}C NMR (DMSO-d_6 75 MHz) δ: 8.03 (C-12 & C-12′), 14.76 (C-19), 35.17 (C-11), 46.65 (d, $J = 9.75$ Hz, C-14), 60.09 (C-18), 105.30 (C-3), 109.28 (C-8), 111.92 (d, $J = 23.25$ Hz, C-5), 120.75 (d, $J = 6.75$ Hz, C-10), 138.39 (C-9), 146.07 (d, $J = 10.5$ Hz, C-7), 148.37 (C-2), 148.64 & 151.88 (d, $J = 243.0$ Hz, C-6), 165.01 (C-13), 172.01 (C-4). LC–MS (ESI, *m/z*): 306.2 (M–CH_2 + H).

*General procedure for the synthesis of 1-cyclopropyl-6 -fluoro-7-(1-alkylazetidine-3-carbonyl-N-methyl -hydrazino)-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ethyl esters (**5a–i**)*

To a solution of compounds **2a–i** (2.15 mmol) in *N*,*N* -dimethylformamide (2.5 mL) *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide HCl (EDC.HCl) (558 mg, 3.0 mmol), 4-dimethylaminopyridine (DMAP) (38 mg, 0.3 mmol) at 0 °C under nitrogen atmosphere were added. After 5 min, compound **4** (500 mg, 1.5 mmol) was added to the reaction mixture at 0 °C and reaction mixture was slowly allowed to reach room temperature (25 °C) and continued stirring at room temperature over a period of 6 h. The resulting reaction mass was poured into crushed ice to precipitate the product. The precipitated solid was filtered and dried under reduced pressure to yield the title compounds **5a–i**.

*1-Cyclopropyl-6-fluoro-7-{N-methyl-N′-[1-(4-trifluoro-methyl-benzyl)-azetidine-3-carbonyl]-hydrazino}-4-oxo-1,4 -dihydro-quinoline-3-carboxylic acid ethyl ester (**5a**)* This compound was prepared by coupling of compound **4** with compound **2a** in the presence of EDC.HCl. It was obtained as a off-white solid (650 mg, 74 %), MP: 147.7–150.3 °C. IR (ATR, cm^{-1}) υ: 3246 (ArH), 1728 (ester –C=O), 1659 (quinolone –C=O), 1619 (amide –C=O), 1581 (–C=C), 1064 (–C–F). ^{1}H NMR (DMSO-d_6 300 MHz) δ (ppm): 1.05 (2H, bs, cyclopropyl–CH_2–), 1.18–1.29 (5H, m, cyclopropyl–CH_2– & –OCH_2CH_3), 3.26–3.33 (6H, m, –NCH_3, –NCH_2Ph, azetidine –CH), 3.48 (2H, bs, azetidine –CH_2), 3.57 (1H, bs, cyclopropyl–CH), 3.67 (2H, bs, azetidine –CH_2), 4.20 (4H, q, $J = 6.9$ Hz, 2H. –OCH_2), 7.34 (1H, d, $J = 7.8$ Hz, ArH), 7.49 (1H, d, $J = 7.8$ Hz, ArH), 7.66–7.74 (3H, m, ArH), 8.41 (1H, s, ArH), 10.47 (1H, s, –CO–NH–). ^{13}C NMR (DMSO-d_6 75 MHz) δ: 7.99 (C-12 & C-12′), 14.73 (C-19), 33.15 (C-16), 35.09 (C-11), 42.57 (C-17), 42.63 (C-17′), 56.54 (C-14), 60.97 (C-19), 62.25 (CH_2, –NCH_2Ph), 104.00 (C-3), 109.55 (C-8), 112.5 (d, $J = 22.5$ Hz, C-5), 119.44, 123.01, 126.62 & 130.23 (q, $J = 270.75$ Hz, –CF_3), 121.59 (d, $J = 6.0$ Hz, C-10), 125. 51 (d, $J = 3.75$ Hz, –ArC), 127.39, 127.81, 128.23 & 128. 65 (q, $J = 32.25$ Hz, ArC–CF_3), 129.16 (ArC), 138.29 (C-9), 142.62 (d, $J = 9.0$ Hz, C-7), 148.67 (C-2), 148.67 & 151.93 (d, $J = 244.5$ Hz, C-6), 166.89 (C-13), 170.85 (C-15), 171.95 (C-4). LC–MS (ESI, *m/z*): 561.1 (M + H).

*1-Cyclopropyl-6-fluoro-7-{N′-[1-(4-methoxy-benzyl)-azetidine-3-carbonyl]-N-methyl-hydrazino}-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ethyl ester (**5b**)* This compound was prepared by coupling of compound **4** with compound **2b** in the presence of EDC.HCl. It was obtained as a off-white solid (640 mg, 81 %), MP: 151.2–152.1 °C. IR (ATR, cm^{-1}) υ: 3240 (ArH), 1729 (ester –C=O), 1662 (quinolone –C=O), 1618 (amide –C=O), 1581 (–C=C), 1065 (–C–F). ^{1}H NMR (DMSO-d_6 300 MHz) δ (ppm): 1.13 1.33 (m, 7H, cyclopropyl–CH_2– & –OCH_2CH_3), 3.26 (s, 3H, –NCH_3), 3.56 (1H, bs, cyclopropyl–CH), 3.35–3.73 (m, 7H, azetidine –CH_2, CH & N–CH_2Ph), 4.20 (q, $J = 7.2$ Hz, 2H. –OCH_2), 6.89 (d, $J = 8.7$ Hz, 2H), 7.28 (d, $J = 7.8$ Hz, 2H), 7.35 (d, $J = 7.8$ Hz, 1H), 7.72 (d, $J = 13.8$ Hz, 1H), 8.41 (s, 1H), 10.45 (s, 1H, –CO–NH–). ^{13}C NMR (DMSO-d_6 75 MHz) δ: 8.03 (C-12 & C-12′), 14.76 (C-19), 32.00 (C-16), 35.15 (C-11), 42.68 (C-17), 42.75 (C-17′), 55.51 (C-14), 56.14 (–OCH_3), 60.20 (–NCH_2), 103.84 (C-3), 109.57 (C-8), 111.96 (d, $J = 22.2$ Hz, C-5), 114.25 (ArC), 118.28 (d, $J = 7.5$ Hz, C-10), 130.00 (ArC), 130.10 (ArC), 138.32 (C-9), 144.02 (d, $J = 9.0$ Hz, C-7), 148.71 (C-2), 148.97 & 152.27 (d, $J = 247.5$ Hz, C-6), 163.92 (ArC), 166.93 (C-13), 170.85 (C-15), 171.97 (C-4). LC–MS (ESI, *m/z*): 523.1 (M + H).

*1-Cyclopropyl-6-fluoro-7-{N′-[1-(4-fluoro-benzyl)-azetidine-3-carbonyl]-N-methyl-hydrazino}-4-oxo-1,4-dih ydro-quinoline-3-carboxylic acid ethyl ester (**5c**)* This compound was prepared by coupling of compound **4** with compound **2c** in the presence of EDC.HCl. It was obtained as a pale yellow solid (620 mg, 79 %), MP: 142.5–143.8 °C. IR

(ATR, cm^{-1}) υ: 3245 (ArH), 1728 (ester –C=O), 1665 (quinolone –C=O), 1619 (amide –C=O), 1585 (–C=C), 1074 (–C–F). ^{1}H NMR (DMSO-d_6 300 MHz) δ (ppm): 1.06 1.20 (4H, m, cyclopropyl–CH_2–), 1.27 (3H, t, $J = 6.9$ Hz, –OCH_2CH_3), 3.26 (3H, s, –NCH_3), 3.15–3.26 (2H, m, azetidine–CH & cyclopropyl–CH), 3.35–3.54 (6H, m, azetidine –CH_2 and–NCH_2Ph), 4.20 (2H, q, $J = 6.9$ Hz, –OCH_2), 7.13 (2H, t, $J = 8.1$ Hz, ArH), 7.30–7.35 (3H, m, ArH), 7.72 (1H, d, $J = 13.8$ Hz, ArH), 8.41 (1H, s, ArH), 10.45 (1H, s, –CO–NH–). ^{13}C NMR (DMSO-d_6 75 MHz) δ: 8.02 (C-12 & C-12′), 14.76 (C-19), 33.10 (C-16), 36.21 (C-11), 42.69 (C-17), 42.76 (C-17′), 56.28 (C-14), 60.10 (C-18), 62.13 (–NCH_2), 103.88 (C-3), 107.03 (C-8), 111.97 (d, $J = 23.25$ Hz, C-5), 115.38 (d, $J = 21.0$ Hz, ArC), 118.28 (d, $J = 7.5$ Hz, C-10), 120.70 (ArC), 139.49 (C-9), 144.02 (d, $J = 8.25$ Hz, C-7), 148.58 (C-2), 149.04 & 152.34 (d, $J = 247.5$ Hz, C-6), 130.4 (d, $J = 8.25$ Hz, ArC), 160.22 & 163.24 (d, $J = 163.24$ Hz, ArC–F), 166.44 (C-13), 170. 84 (C-15), 171.96 (C-4). LC–MS (ESI, *m/z*): 511.5 (M + H).

*1-Cyclopropyl-6-fluoro-7-[N-methyl-N'-(1-thiazol-4-ylmethyl-azetidine-3-carbonyl)-hydrazino]-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ethyl ester (**5d**)* This compound was prepared by coupling of compound **4** with compound **2d** in the presence of EDC.HCl. It was obtained as a yellow solid (410 mg, 54 %), MP: 161.2–163.1 °C. IR (ATR, cm^{-1}) υ: 3244 (ArH), 1728 (ester –C=O), 1665 (quinolone –C=O), 1620 (amide –C=O), 1584 (–C=C), 1076 (–C–F). ^{1}H NMR (DMSO-d_6 300 MHz) δ (ppm): 1.06–1.20 (4H, m, cyclopropyl–CH_2–), 1.27 (3H, t, $J = 6.9$ Hz, –OCH_2CH_3), 3.25 (3H, s, –NCH_3), 3.15–3.26 (2H, m, azetidine–CH & cyclopropyl–CH), 3.35–3.54 (4H, m, azetidine–CH_2), 3.57 (2H, s, –NCH_2 thiazole), 4.19 (2H, q, $J = 6.9$ Hz, –OCH_2), 7.30–7.31 (1H, m, ArH), 7.43 (1H, s, thiazole H), 7.72 (1H, d, $J = 13.8$ Hz, ArH), 8.41 (1H, s, ArH), 9.02 (1H, s, thiazole H), 10.45 (1H, s, –CO–NH–). ^{13}C NMR (DMSO-d_6 75 MHz) δ: 8.02 (C-12 & C-12′), 14.74 (C-19), 33.11 (C-16), 36.22 (C-11), 42.70 (C-17), 42.75 (C-17′), 55.54 (C-14), 57.14 (–NCH_2), 60.21 (C-18), 103.85 (C-3), 107.06 (C-8), 111.94 (d, $J = 22.2$ Hz, C-5), 117.26 (thiazole C), 118.30 (d, $J = 7.5$ Hz, C-5), 139.45 (C-9), 144.02 (d, $J = 9.0$, C-7), 148.64 (C-2), 149.81 & 152.21 (d, $J = 247.5$ Hz, C6), 152.95 (thiazole C), 154.62 (thiazole C), 165.02 (C-13), 170.90 (C-15), 171.92 (C-4). LC–MS (ESI, *m/z*): 500.1 (M + H).

*1-Cyclopropyl-6-fluoro-7-[N'-(1-isopropyl-azetidine-3-carbonyl)-N-methyl-hydrazino]-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ethyl ester (**5e**)* This compound was prepared by coupling of compound **4** with compound **2e** in the presence of EDC.HCl. It was obtained as a off-white solid (530 mg, 79 %), MP: 124.3–125.2 °C. IR (ATR, cm^{-1}) υ: 3225 (ArH), 1728 (ester –C=O), 1664 (quinolone –C=O), 1619 (amide –C=O), 1586 (–C=C), 1077 (–C–F); ^{1}H NMR (DMSO-d_6 300 MHz) δ (ppm): 0.88 (6H, d, $J = 6.6$ Hz, $(CH_3)_2$), 1.05–1.22 (7H, m, cyclopropyl–CH_2, –OCH_2CH_3), 2.46 (1H, m, isopropyl–CH), 3.06–3.15 (4H, m, azetidine–CH_2), 3.25 (3H, s, –NCH_3), 3.32 (1H, bs, azetidine–CH), 3.57 (1H, m, cyclopropyl–CH), 4.21 (2H, q, $J = 7.1$ Hz, –OCH_2), 7.32 (1H, bs, ArH), 7.71 (1H, d, $J = 13.2$ Hz, ArH), 8.41 (1H, s, ArH), 10.35 (1H, s, –CO–NH–). ^{13}C NMR (DMSO-d_6 75 MHz) δ: 8.02 (C-12 & C-12′), 14.76 (C-19), 19.54 (–$(CH_3)_2$), 31.72 (C-16), 36.22 (C-11), 42.68 (C-17), 42.75 (C-17′), 54.84 (C-14), 57.86 (CH, –$NCH(CH_3)_2$)), 60.22 (C-18), 103.88 (C-3), 107.06 (C-8), 111.97 (d, $J = 22.5$ Hz, C-5), 118.34 (C-10), 139.49 (C-9), 144.02 (d, $J = 9.0$ Hz, C-7), 148.66 (C-2), 152.29 & 148.99 (d, $J = 247.5$ Hz, C-6), 166.44 (C-13), 170.94 (C-15), 172.11 (C-4). LC–MS (ESI, *m/z*): 445.2 (M + H).

*7-[N'-(1-Cyclopentyl-azetidine-3-carbonyl)-N-methyl-hydrazino]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ethyl ester (**5f**)* This compound was prepared by coupling of compound **4** with compound **2f** in the presence of EDC.HCl. It was obtained as a pale yellow solid (550 mg, 78 %), MP: 133.2–135.1 °C. IR (ATR, cm^{-1}) υ: 3226 (ArH), 1729 (ester –C=O), 1664 (quinolone –C=O), 1620 (amide –C=O), 1585 (–C=C), 1070 (–C–F). ^{1}H NMR (DMSO-d_6 300 MHz) δ (ppm): 1.06–1.45 (15H, m, cyclopropyl–CH_2, –OCH_2CH_3 & cyclopentyl–CH_2–), 2.63 (1H, bs, cyclopentyl–CH–), 3.06–3.15 (4H, m, azetidine–CH_2), 3.26 (3H, s, –NCH_3), 3.31 (1H, bs, cyclopropyl–CH), 3.57 (1H, m, azetidine–CH), 4.20 (2H, q, $J = 6.9$ Hz, –OCH_2), 7.34 (1H, bs, ArH), 7.72 (1H, d, $J = 13.2$ Hz, ArH), 8.41 (1H, s, ArH), 10.38 (1H, s, –CO–NH–). ^{13}C NMR (DMSO-d_6 75 MHz) δ: 8.03 (C-12 & C-12′), 14.76 (C-19), 24.36 (cyclopentyl–CH_2), 29.80 (cyclopentyl–CH_2), 32.19 (C-16), 36.21 (C-11), 42.68 (C-17), 42.75 (C-17′), 55. 02 (C-14), 60.09 (C-18), 68.88 (cyclopentyl-NCH), 103.81 (C-3), 107.08 (C-8), 111.99 (d, $J = 23.25$ Hz, C-5), 118.27 (d, $J = 6.7$ Hz, C-10), 139.48 (C-9), 143.02 (d, $J = 9.0$ Hz, C-7), 148.64 (C-2), 152.28 & 148.98 (d, $J = 247.5$ Hz, C-6), 166.43 (C-13), 171.03 (C-15), 172.21 (C-4). LC–MS (ESI, *m/z*): 471.2 (M + H).

*7-[N'-(1-Cyclohexyl-azetidine-3-carbonyl)-N-methyl-hydrazino]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ethyl ester (**5g**)* This compound was prepared by coupling of compound **4** with compound **2g** in the presence of EDC.HCl. It was obtained as a pale yellow solid (550 mg, 75 %), MP: 135.4–137.2 °C. IR (ATR, cm^{-1}) υ: 3231 (ArH), 1729 (ester –C=O), 1664 (quinolone –C=O), 1617 (amide –C=O), 1588 (–C=C), 1061 (–C–F). ^{1}H NMR (DMSO-d_6 300 MHz) δ (ppm): 1.24–1.35 (13H, m, cyclopropyl–CH_2, –OCH_2CH_3 & cyclohexyl–CH_2–),

1.53–1.71 (5H, m, cyclohexyl–CH & CH_2–), 3.18 (3H, s, –NCH_3), 4.17–4.24 (6H, m, azetidine –CH, –CH_2 and cyclopropyl–CH), 4.20 (2H, q, $J = 6.9$ Hz, –OCH_2), 7.37 (1H, d, $J = 2.5$ Hz, ArH), 7.73 (1H, d, $J = 13.2$ Hz, ArH), 8.42 (1H, s, ArH), 10.63 (1H, s, –CO–NH–). ^{13}C NMR (DMSO-d_6 75 MHz) δ: 8.03 (C-12 & C-12′), 14.76 (C-19) 24.38 (cyclohexyl–CH_2), 30.22 (cyclohexyl–CH_2), 31.35 (C-16), 36.21 (C-11), 42.68 (C-17), 42.78 (C-17′), 54.86 (C-14), 60.10 (C-18), 68.52 (cyclohexyl–NCH), 103.84 (C-3), 107.08 (C-8), 111.97 (d, $J = 23.25$ Hz, C-5), 118.31 (d, $J = 7.5$ Hz, C-10), 139.48 (C-9), 143.98 (d, $J = 9.0$ Hz, C-7), 148.59 (C-2), 148.88 & 152.18 (d, $J = 247.5$ Hz, C-6), 166.42 (C-13), 170.84 (C-15), 171.95 (C-4). LC–MS (ESI, *m/z*): 485.2 (M + H).

*7-[N′-(1-Cycloheptyl-azetidine-3-carbonyl)-N-methyl-hydrazino]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ethyl ester (**5h**)* This compound was prepared by coupling of compound **4** with compound **2h** in the presence of EDC.HCl. It was obtained as a off-white solid (540 mg, 72 %), MP: 141.5–138.1 °C. IR (ATR, cm^{-1}) υ: 3226 (ArH), 1725 (ester –C=O), 1666 (quinolone –C=O), 1618 (amide –C=O), 1550 (–C=C), 1051 (–C–F). ^{1}H NMR (DMSO-d_6 300 MHz) δ (ppm): 1.07–1.80 (20H, m, cyclopropyl–CH_2, –OCH_2CH_3 & cycloheptyl–CH_2–), 3.18 (3H, s, –NCH_3), 3.62 (1H, bs, cyclopropyl–CH), 4.17–4.24 (5H, m, azetidine–CH and –CH_2), 4.20 (2H, q, $J = 6.9$ Hz, –OCH_2), 7.39 (1H, d, $J = 6.9$ Hz, ArH), 7.74 (1H, d, $J = 13.8$ Hz, ArH), 8.43 (1H, s, ArH), 10.77 (1H, s, –CO–NH–). ^{13}C NMR (DMSO-d_6 75 MHz) δ: 8.04 (C-12 & C-12′), 14.76 (C-19), 24.08 (cycloheptyl–CH_2), 28.30 (cycloheptyl–CH_2), 30.32 (cycloheptyl–CH_2), 31.57 (C-16), 36.24 (C-11), 42.68 (C-17), 42.76 (C-17′), 54.74 (C-14), 60.10 (C-18), 67.95 (cycloheptyl–NCH), 103.87 (C-3), 107.06 (C-8), 111.96 (d, $J = 23.25$ Hz, C-5), 118.31 (d, $J = 7.5$ Hz, C-10), 139.46 (C-9), 143.98 (d, $J = 9.0$ Hz, C-7), 148.63 (C-2), 148.99 & 152.29 (d, $J = 247.5$ Hz, C-6), 166.42 (C-13), 170.70 (C-15), 172.24 (C-4). LC–MS (ESI, *m/z*): 499.5 (M + H).

*7-[N′-(1-Adamantan-2-yl-azetidine-3-carbonyl)-N-methyl-hydrazino]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ethyl ester (**5i**)* This compound was prepared by coupling of compound **4** with compound **2i** in the presence of EDC.HCl. It was obtained as a off-white solid (650 mg, 80 %), MP: 162.2–164.5 °C. IR (ATR, cm^{-1}) υ: 3222 (ArH), 1720 (ester –C=O), 1662 (quinolone –C=O), 1609 (amide –C=O), 1547 (–C=C), 1042 (–C–F). ^{1}H NMR (DMSO-d_6 300 MHz) δ (ppm): 1.07–2.00 (22H, m, cyclopropyl–CH_2, –OCH_2CH_3 & adamantyl–CH_2–), 3.18 (3H, s, –NCH_3), 3.60 (1H, bs, cyclopropyl–CH), 4.17–4.24 (5H, m, azetidine–CH and –CH_2), 4.21 (2H, q, $J = 6.9$ Hz, –OCH_2), 7.36 (1H, d, $J = 6.9$ Hz, ArH), 7.74 (1H, d, $J = 13.8$ Hz, ArH), 8.42 (1H, s, ArH), 10.85 (1H, s, –CO–NH–). ^{13}C NMR (DMSO-d_6 75 MHz) δ: 8.04 (C-12 & C-12′), 14.76 (C-19), 27.16 (adamantyl –C), 27.50 (adamantyl–C), 28.75 (adamantyl–C), 31.60 (C-16), 32.48 (adamantyl–C), 36.14 (C-11), 36.60 (adamantyl–CH), 42.59 (C-17), 42.66 (C-17′), 54.43 (C-14), 60.20 (C-18), 71.66 (adamantyl–NCH), 104.71 (C-3), 109.58 (C-8), 111.95 (d, $J = 23.25$ Hz, C-5), 118.32 (d, $J = 7.5$ Hz, C-10), 138.30 (C-9), 142.56 (d, $J = 9.0$ Hz, C-7), 148.65 (C-2), 148.67 & 151.97 (d, $J = 246.75$ Hz, C-6), 164.91 (C-13), 170.85 (C-15), 171.94 (C-4). LC–MS (ESI, *m/z*): 537.5 (M + H).

*General procedure for the synthesis of 1-cyclopropyl-6-fluoro-7-(1-alkylazetidine-3-carbonyl-N-methyl-hydrazino)-4-oxo-1,4-dihydro-quinoline-3-carboxylic acids (**6a–i**)*

To a solution of compounds **5a–i** (0.9 mmol) in tetrahydrofuran (18.0 mL) was added lithium hydroxide (2.67 mmol) in water (12.0 mL) at room temperature. Then the reaction mixture was allowed to stir at 60 °C over a period of 4 h. Completion of the reaction was monitored by TLC.

The crude mass obtained upon evaporation of volatiles was diluted with water (5.0 mL) and acidified with formic acid (pH: 3.0). Then acidic solution was extracted with dichloromethane (2 × 25 mL), dried over sodium sulphate and concentrated under reduced pressure to obtain compounds **6a–i**.

*1-Cyclopropyl-6-fluoro-7-{N-methyl-N′-[1-(4-trifluoromethyl-benzyl)-azetidine-3-carbonyl]-hydrazino}-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (**6a**)* This compound was prepared by base hydrolysis of compound **5a** with lithium hydroxide. It was obtained as a pale yellow solid (415 mg, 88 %), MP: 134.1–136.3 °C. IR (ATR, cm^{-1}) υ: 3281 (–OH), 2837 (ArH), 1731 (acid –C=O), 1616 (amide–C=O), 1504 (–C=C), 1324 (–C–F). ^{1}H NMR (DMSO-d_6 300 MHz) δ (ppm): 1.15 (bs, 2H, cyclopropyl–CH_2–), 1.24–1.26 (m, 2H, cyclopropyl–CH_2), 3.24–3.29 (6H, bs, –NCH_3, –NCH_2Ph, azetidine–CH), 3.45 (2H, bs, azetidine–CH_2), 3.64 (2H, bs, azetidine –CH_2), 3.75 (1H, bs, cyclopropyl–CH), 7.44–7.50 (3H, m, ArH), 7.66–7.68 (2H, m, ArH), 7.88 (1H, d, $J = 13.8$ Hz, ArH), 8.64 (1H, s, ArH), 10.58 (1H, s, –CO–NH–), 15.24 (1H, s, –COOH). ^{13}C NMR (DMSO-d_6 75 MHz) δ: 8.01 (C-12 & C-12′), 33.15 (C-16), 36.21 (C-11), 42.67 (C-17), 42.74 (C-17′), 56.50 (C-14), 62.18 (-NCH_2Ph), 103.81 (C-3), 107.04 (C-8), 111.96 (d, $J = 23.25$ Hz, C-5), 118.28 (d, $J = 6.7$ Hz, C-10), 123.00 & 126.61 (d, $J = 270.75$ Hz, –CF_3), 125.53 (d, $J = 3.75$ Hz, -ArC), 127.81 & 128.23 (d, $J = 31.5$ Hz, ArC–CF_3), 129.29 (ArC), 139.47 (C-9), 143.57 (ArC), 144.02 (d, $J = 9.0$ Hz, C-7), 148.65 (C-2), 149.01 & 152.31 (d,

$J = 247.5$ Hz, C-6), 166.43 (C-13), 170.88 (C-15), 176.71 (d, $J = 2.25$ Hz, C-4). LC–MS (ESI, *m/z*): 533.1 (M + H).

*1-Cyclopropyl-6-fluoro-7-{N'-[1-(4-methoxy-benzyl)-azetidine-3-carbonyl]-N-methyl-hydrazino}-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (**6b**)* This compound was prepared by base hydrolysis of compound **5b** with lithium hydroxide. It was obtained as a off-white solid (380 mg, 86 %), MP: 131.4–132.6 °C. IR (ATR, cm^{-1}) υ: 3330 (–OH), 2933 (ArH), 1732 (acid –C=O), 1662 (amide –C=O), 1620 (quinolone –C=O), 1478 (–C=C), 1329 (–C–F). ^{1}H NMR (DMSO-d_6 300 MHz) δ (ppm): 1.15–1.26 (4H, m, cyclopropyl–CH_2–), 3.19 (1H, bs, azetidine–CH), 3.21 (3H, s, –NCH_3), 3.37–3.47 (6H, m, azetidine–CH_2, –CH_2Ph), 3.72 (3H, s, –OMe), 3.73 (1H, bs, cyclopropyl–CH), 6.86 (2H, d, $J = 8.4$ Hz, ArH), 7.17 (2H, d, $J = 8.4$ Hz, ArH), 7.44 (1H, d, $J = 7.8$ Hz, ArH), 7.88 (1H, d, $J = 13.8$ Hz, ArH), 8.64 (1H, s, ArH), 10.57 (1H, s, –CO–NH–), 15.24 (1H, bs, –COOH). ^{13}C NMR (DMSO-d_6 75 MHz) δ: 8.03 (C-12 & C-12′), 33.07 (C-16), 36.20 (C-11), 42.70 (C-17), 42.77 (C-17′), 55.45 (C-14), 56.13 (–OCH_3), 62.14 (-NCH_2), 103.84 (C-3), 107.06 (C-8), 111.96 (d, $J = 22.2$ Hz, C-5), 114.09 (ArC), 118.28 (d, $J = 7.5$ Hz, C-10), 130.00 (ArC), 130.10 (ArC), 139.49 (C-9), 144.02 (d, $J = 9.0$ Hz, C-7), 148.64 (C-2), 148.97 & 152.27 (d, $J = 247.5$ Hz, C-6), 163.92 (ArC), 166.44 (C-13), 170.94 (C-15), 176.71 (d, $J = 3.0$ Hz, C-4). LC–MS (ESI, *m/z*): 495.1 (M + H).

*1-Cyclopropyl-6-fluoro-7-{N'-[1-(4-fluoro-benzyl)-azetidine-3-carbonyl]-N-methyl-hydrazino}-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (**6c**)* This compound was prepared by base hydrolysis of compound **5c** with lithium hydroxide. It was obtained as a yellow solid (375 mg, 87 %), MP: 125.9–127.0 °C. IR (ATR, cm^{-1}) υ: 3258 (–OH), 2978 (ArH), 1734 (acid –C=O), 1663 (amide –C=O), 1623 (quinolone –C=O), 1510 (–C=C), 1329 (–C–F). ^{1}H NMR (DMSO-d_6 300 MHz) δ (ppm): 1.15–1.26 (4H, m, cyclopropyl–CH_2–), 3.18 (1H, bs, azetidine–CH), 3.20 (3H, s, –NCH_3), 3.25–3.46 (4H, m, azetidine–CH_2), 3.51 (2H, s, –CH_2Ph), 3.75 (1H, bs, cyclopropyl–CH), 7.12 (2H, t, $J = 9.0$ Hz, ArH), 7.28 (2H, t, $J = 7.5$ Hz, ArH), 7.44 (1H, d, $J = 7.5$ Hz, ArH), 7.88 (1H, d, $J = 13.5$ Hz, ArH), 8.65 (1H, s, ArH), 10.57 (1H, s, –CO–NH–), 15.25 (1H, s, –COOH). ^{13}C NMR (DMSO-d_6 75 MHz) δ: 8.02 (C-12 & C-12′), 33.10 (C-16), 36.21 (C-11), 42.69 (C-17), 42.76 (C-17′), 56.28 (C-14), 62.00 (-NCH_2), 103.85 (C-3), 107.05 (C-8), 111.97 (d, $J = 23.25$ Hz, C-5), 115.38 (d, $J = 21.0$ Hz, ArC), 118.28 (d, $J = 7.5$ Hz, C-10), 120.69 (ArC), 139.48 (C-9), 144.02 (d, $J = 8.25$ Hz, C-7), 148.68 (C-2), 149.04 & 152.34 (d, $J = 247.5$ Hz, C-6), 130.5 (d, $J = 8.25$ Hz, ArC), 160.22 & 163.24 (d, $J = 163.24$ Hz, ArC-F), 166.43 (C-13), 170.94 (C-15), 176.74 (C-4). LC–MS (ESI, *m/z*): 483.5 (M + H).

*1-Cyclopropyl-6-fluoro-7-[N-methyl-N'-(1-thiazol-4-ylmethyl-azetidine-3-carbonyl)-hydrazino]-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (**6d**)* This compound was prepared by base hydrolysis of compound **5d** with lithium hydroxide. It was obtained as a off-white solid (270 mg, 64 %), MP: 186.2–189.1 °C. IR (ATR, cm^{-1}) υ: 3258 (–OH), 2977 (ArH), 1732 (acid –C=O), 1665 (amide –C=O), 1623 (quinolone –C=O), 1511 (–C=C), 1329 (–C–F). ^{1}H NMR (DMSO-d_6 300 MHz) δ (ppm): 1.12–1.24 (4H, m, cyclopropyl–CH_2–), 3.18 (1H, bs, azetidine–CH), 3.20 (3H, s, –NCH_3), 3.25–3.46 (4H, m, azetidine–CH_2), 3.52 (2H, s, –NCH_2thiazole), 3.75 (1H, bs, cyclopropyl–CH), 7.42 (1H, s, thiazole H), 7.44 (1H, d, $J = 7.5$ Hz, ArH), 7.88 (1H, d, $J = 13.5$ Hz, ArH), 8.64 (1H, s, ArH), 9.02 (1H, s, thiazole H), 10.55 (1H, s, –CO–NH–), 15.24 (1H, s, –COOH). ^{13}C NMR (DMSO-d_6 75 MHz) δ: 8.03 (C-12 & C-12′), 33.10 (C-16), 36.22 (C-11), 42.70 (C-17), 42.75 (C-17′), 55.55 (C-14), 57.12 (-NCH_2), 103.86 (C-3), 107.06 (C-8), 111.94 (d, $J = 22.2$ Hz, C-5), 117.24 (thiazole C), 118.30 (d, $J = 7.5$ Hz, C-5), 139.46 (C-9), 144.02 (d, $J = 9.0$, C-7), 148.65 (C-2), 149.81 & 152.21 (d, $J = 247.5$ Hz, C-6), 153.00 (thiazole C), 154.62 (thiazole C), 166.41 (C-13), 170.92 (C-15), 176.71 (d, $J = 3.0$, C-4). LC–MS (ESI, *m/z*): 472.4 (M + H).

*1-Cyclopropyl-6-fluoro-7-[N'-(1-isopropyl-azetidine-3-carbonyl)-N-methyl-hydrazino]-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (**6e**)* This compound was prepared by base hydrolysis of compound **5e** with lithium hydroxide. It was obtained as a yellow solid (255 mg, 68 %), MP: 120.1–122.2 °C. IR (ATR, cm^{-1}) υ: 3235 (–OH), 2975 (ArH), 1728 (acid –C=O), 1659 (amide –C=O), 1620 (quinolone –C=O), 1522 (–C=C), 1324 (–C–F). ^{1}H NMR (DMSO-d_6 300 MHz) δ (ppm): 0.87 (6H, d, $J = 6.6$ Hz, $(CH_3)_2$), 1.15–1.26 (4H, m, cyclopropyl–CH_2), 2.46–2.48 (1H, m, isopropyl–CH), 3.12 (1H, bs, azetidine–CH), 3.19 (3H, s, -NCH_3), 3.24–3.43 (4H, m, azetidine–CH_2), 3.76 (1H, bs, cyclopropyl–CH), 7.46 (1H, d, $J = 7.2$ Hz, ArH), 7.86 (1H, d, $J = 13.5$ Hz, ArH), 8.64 (1H, s, ArH), 10.60 (1H, s, –CO–NH–), 15.25 (1H, bs, –COOH). ^{13}C NMR (DMSO-d_6 75 MHz) δ: 8.02 (C-12 & C-12′), 19.54 (–$(CH_3)_2$), 31.72 (C-16), 36.22 (C-11), 42.68 (C-17), 42.75 (C-17′), 54.84 (C-14), 57.82 (isopropyl-NCH), 103.87 (C-3), 107.06 (C-8), 111.97 (d, $J = 22.5$ Hz, C-5), 118.34 (C-10), 139.49 (C-9), 144.02 (d, $J = 9.0$ Hz, C-7), 148.65 (C-2), 152.29 & 148.99 (d, $J = 247.5$ Hz, C-6), 166.44 (C-13), 170.94 (C-15), 176.71 (C-4). LC–MS (ESI, *m/z*): 417.5 (M + H).

*7-[N'-(1-Cyclopentyl-azetidine-3-carbonyl)-N-methyl-hydrazino]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (**6f**)* This compound was prepared by base hydrolysis of compound **5f** with lithium hydroxide. It was obtained as a pale yellow solid (294 mg, 74 %), MP: 146.1–148.6 °C. IR (ATR, cm^{-1}) υ: 3230 (–OH), 2975 (ArH), 1732 (acid –C=O), 1659 (amide –C=O), 1620 (quinolone –C=O), 1500 (–C=C), 1322 (–C–F). ^{1}H NMR (DMSO-d_6 300 MHz) δ (ppm): 1.47–1.50 (12H, m, cyclopropyl–CH_2– & cyclopentyl–CH_2), 2.73 (1H, bs, cyclopentyl–CH), 3.12 (1H, bs, azetidine–CH), 3.19 (3H, s, –NCH_3), 3.24–3.43 (4H, m, azetidine–CH_2), 3.76 (1H, bs, cyclopropyl–CH), 7.45 (1H, d, $J = 7.5$ Hz, ArH), 7.88 (1H, d, $J = 13.8$ Hz, ArH), 8.17 (1H, s, ArH), 8.65 (1H, s, ArH), 10.59 (1H, s, –CO–NH–), 15.23 (1H, bs, –COOH). ^{13}C NMR (DMSO-d_6 75 MHz) δ: 8.03 (C-12 & C-12′), 24.37 (cyclopentyl–CH_2), 29.75 (cyclopentyl–CH_2), 32.20 (C-16), 36.21 (C-11), 42.68 (C-17), 42.75 (C-17′), 55.02 (C-14), 68.88 (cyclopentyl–NCH), 103.81 (C-3), 107.08 (C-8), 111.99 (d, $J = 23.25$ Hz, C-5), 118.27 (d, $J = 6.7$ Hz, C-10), 139.48 (C-9), 143.02 (d, $J = 9.0$ Hz, C-7), 148.64 (C-2), 152.28 & 148.98 (d, $J = 247.5$ Hz, C-6), 166.43 (C-13), 171.03 (C-15), 176.70 (C-4). LC–MS (ESI, *m/z*): 443.1 (M + H).

*7-[N'-(1-Cyclohexyl-azetidine-3-carbonyl)-N-methyl-hydrazino]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (**6g**)* This compound was prepared by base hydrolysis of compound **5g** with lithium hydroxide. It was obtained as a pale yellow solid (315 mg, 77 %), MP: 153.2–154.6 °C. IR (ATR, cm^{-1}) υ: 3210 (–OH), 2927 (ArH), 1692 (amide –C=O), 1619 (quinolone –C=O), 1468 (–C=C), 1331 (–C–F). ^{1}H NMR (DMSO-d_6 300 MHz) δ (ppm): 1.22–1.66 (14H, m, cyclopropyl–CH_2– & cyclohexyl –CH_2), 2.11 (1H, bs, cyclohexyl–CH), 3.24 (3H, s, –NCH_3), 3.32–3.51 (5H, m, azetidine–CH & –CH_2), 3.76 (1H, bs, cyclopropyl–CH), 7.46 (1H, d, $J = 7.8$ Hz, ArH), 7.88 (1H, d, $J = 13.8$ Hz, ArH), 8.65 (1H, s, ArH), 10.62 (1H, s, –CO–NH–), 15.22 (1H, bs, –COOH). ^{13}C NMR (DMSO-d_6 75 MHz) δ: 8.03 (C-12 & C-12′), 24.35 (cyclohexyl–CH_2), 30.21 (cyclohexyl–CH_2), 31.32 (C-16), 36.21 (C-11), 42.68 (C-17), 42.75 (C-17′), 54.86 (C-14), 68.54 (cyclohexyl–NCH), 103.85 (C-3), 107.08 (C-8), 111.97 (d, $J = 23.25$ Hz, C-5), 118.31 (d, $J = 7.5$ Hz, C-10), 139.48 (C-9), 143.98 (d, $J = 9.0$ Hz, C-7), 148.64 (C-2), 148.88 & 152.18 (d, $J = 247.5$ Hz, C-6), 166.42 (C-13), 170.85 (C-15), 176.69 (C-4). LC–MS (ESI, *m/z*): 457.6 (M + H).

*7-[N'-(1-Cycloheptyl-azetidine-3-carbonyl)-N-methyl-hydrazino]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (**6h**)* This compound was prepared by base hydrolysis of compound **5h** with lithium hydroxide. It was obtained as a pale yellow solid (300 mg, 71 %), MP: 148.2–151.3 °C. IR (ATR, cm^{-1}) υ: 2922 (ArH), 1730 (acid –C=O), 1694 (amide –C=O), 1623 (quinolone –C=O), 1489 (–C=C), 1333 (–C–F). ^{1}H NMR (DMSO-d_6 300 MHz) δ (ppm): 1.12–1.33 (16H, m, cyclopropyl–CH_2– & cycloheptyl –CH_2), 1.47 (1H, bs, cycloheptyl–CH), 3.24 (3H, s, –NCH_3), 3.25–3.50 (5H, m, azetidine–CH & –CH_2), 3.75 (1H, bs, cyclopropyl–CH), 7.45 (1H, d, $J = 7.5$ Hz, ArH), 7.88 (1H, d, $J = 13.8$ Hz, ArH), 8.64 (1H, s, ArH), 10.58 (1H, s, –CO–NH–), 15.23 (1H, bs, –COOH). ^{13}C NMR (DMSO-d_6 75 MHz) δ: 8.04 (C-12 & C-12′), 24.06 (cycloheptyl–CH_2), 28.25 (cycloheptyl–CH_2), 30.29 (cycloheptyl–CH_2), 31.57 (C-16), 36.22 (C-11), 42.68 (C-17), 42.74 (C-17′), 54.74 (C-14), 67.92 (cycloheptyl–NCH), 103.87 (C-3), 107.06 (C-8), 111.96 (d, $J = 23.25$ Hz, C-5), 118.31 (d, $J = 7.5$ Hz, C-10), 139.46 (C-9), 143.98 (d, $J = 9.0$ Hz, C-7), 148.63 (C-2), 148.99 & 152.29 (d, $J = 247.5$ Hz, C-6), 166.42 (C-13), 170.70 (C-15), 176.69 (C-4). LC–MS (ESI, *m/z*): 471.3 (M + H).

*7-[N'-(1-Adamantan-2-yl-azetidine-3-carbonyl)-N-methyl-hydrazino]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (**6i**)* This compound was prepared by base hydrolysis of compound **5i** with lithium hydroxide. It was obtained as a off-white solid (380 mg, 83 %), MP: 158.2–159.3 °C. IR (ATR, cm^{-1}) υ: 3221 (–OH), 2926 (ArH), 1724 (acid –C=O), 1690 (amide –C=O), 1622 (quinolone –C=O), 1481 (–C=C), 1344 (–C–F). ^{1}H NMR (DMSO-d_6 300 MHz) δ (ppm): 1.12–1.99 (18H, m, cyclopropyl–CH_2– & adamantyl–CH & CH_2), 2.12 (1H, bs, adamantyl–CH), 3.06–3.43 (5H, m, azetidine–CH & –CH_2), 3.24 (3H, s, –NCH_3), 3.76 (1H, bs, cyclopropyl–CH), 7.45 (1H, d, $J = 7.8$ Hz, ArH), 7.85 (1H, d, $J = 13.8$ Hz, ArH), 8.65 (1H, s, ArH), 10.58 (1H, s, –CO–NH–), 15.24 (1H, bs, –COOH). ^{13}C NMR (DMSO-d_6 75 MHz) δ: 8.04 (C-12 & C-12′), 27.40 (adamantyl–C), 27.54 (adamantyl–C), 28.75 (adamantyl–C), 31.59 (C-16), 32.48 (adamantyl–C), 36.13 (C-11), 36.60 (adamantyl–CH), 42.70 (C-17), 42.77 (C-17′), 54.48 (C-14), 71.63 (adamantyl–NCH), 103.80 (C-3), 107.05 (C-8), 111.95 (d, $J = 23.25$ Hz, C-5), 118.32 (d, $J = 7.5$ Hz, C-10), 139.43 (C-9), 143.99 (d, $J = 9.7$ Hz, C-7), 148.65 (C-2), 148.96 & 152.25 (d, $J = 246.75$ Hz, C-6), 166.51 (C-13), 171.11 (C-15), 176.63 (C-4). LC–MS (ESI, *m/z*): 509.3 (M + H).

Antibacterial activity

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. Minimum inhibitory concentration (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.

Anticancer activity

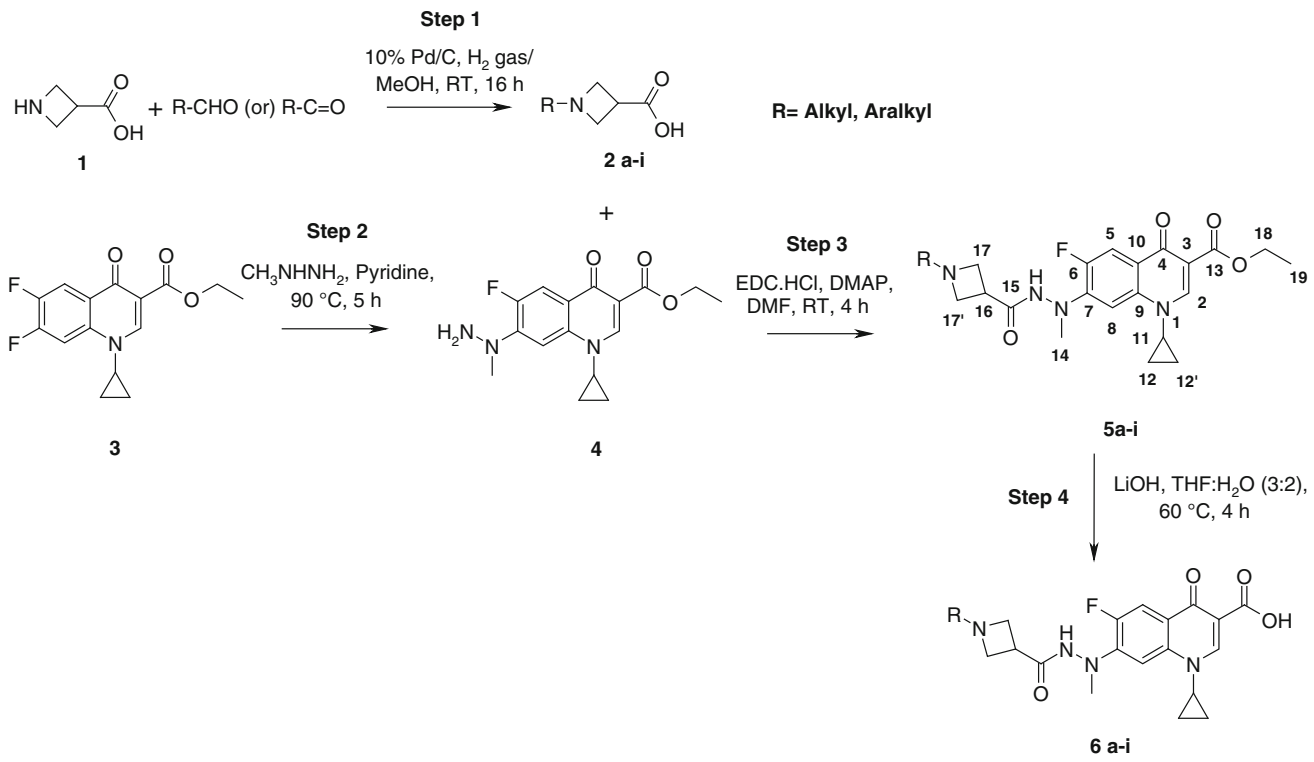
All compounds were screened for their in vitro anticancer activity against representative human cancer cell lines obtained from American Type Culture Collection (ATCC). These cell lines were grown in appropriate medium supplemented with 10 % FCS and 100 U/mL Penicillin, 100 μg/mL Streptomycin. Cells 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. 100 μL of media containing various concentrations of compounds (0.1, 1, 10 and 50 μM) were added to the cells and were incubated for 48 h. The marketed anticancer drug—Vorinostat (SAHA) was tested as a reference compound in the assay. On the day of termination, 50 μL of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) (Sigma, St Louis, MO, USA) 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.

Results and discussion

Chemistry

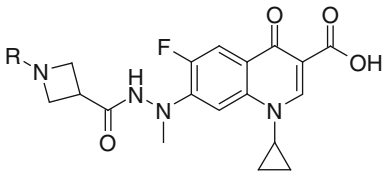
The new fluoroquinolone derivatives described herein were synthesized as shown in Scheme 1. The quinolone ester **3** was conveniently synthesized from the commercially available 2,4,5-trifluoro-benzoic acid in four steps as per the reported procedure (Ledoussal *et al.,* 1992) and the spectral data of the compound were in agreement with the reported data (Dubar *et al.,* 2009). After having synthesized known compound **3,** reaction of compound **3** with methylhydrazine at 90 °C yielded selectively 7-*N*-methylhydrazino derivate **4** in good yield.

Then, our attention was turned towards synthesis of key azetidine-based building blocks (**2a–i**) via Pd/C-catalyzed reductive amination. Our initial efforts to convert compound **1** to compound **2a** by reacting the compound **1** with 4-trifluoromethyl-benzaldehyde in the presence 10 % Pd/C and hydrogen gas pressure (60 psi) did not result the desired compound **2a**. Moreover, we have observed presence of 1-methyl-4-trifluoromethyl-benzene and compound **1** in the reaction mixture. We believe that this could be because compound **2a** formed further underwent debenzylation to form 1-methyl-4-trifluoromethyl-benzene. Hence, when we attempted the same reaction under reduced hydrogen gas pressure

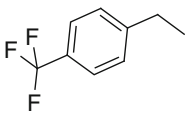
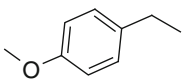
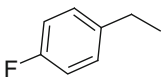


Scheme 1 Synthetic route to synthesize compounds **6a–i**

Table 1 Physicochemical characteristics of *N*-(substituted azetidine-3-carbonyl)-*N*-methyl-hydrazino derivatives of fluoroquinolones (**6a–i**)



6 a-i

Compound	R	MF/M. wt.	Yield (%)	Mp[a] (°C)
6a		$C_{26}H_{24}F_4N_4O_4$/532.50	88	134.1–136.3
6b		C26H27FN4O5/494.53	86	131.4–132.6
6c		$C_{25}H_{24}F_2N_4O_4$/482.49	87	125.9–127.0
6d		$C_{22}H_{22}FN_5O_4S$/471.51	64	186.2–189.1
6e		$C_{21}H_{25}FN_4O_4$/416.46	68	120.1–122.2
6f		$C_{23}H_{27}FN_4O_4$/442.49	74	146.1–148.6
6g		$C_{24}H_{29}FN_4O_4$/456.52	77	153.2–154.6
6h		$C_{25}H_{31}FN_4O_4$/470.55	71	148.2–151.3
6i		$C_{30}H_{37}FN_4O_4$/536.65	83	158.2–159.3

[a] Melting point of compounds at their decomposition

(20 psi), compound **2a** resulted in good yield. Similar reaction conditions were applied to synthesize remaining compounds **2b–i**.

The alkyl azetidino derivatives **5a–i** were smoothly obtained by reacting compound **4** with corresponding acids (**2a–i**) in the presence of *N*-ethyl-*N'*

-(3-dimethylaminopropyl)carbodiimide HCl (EDC.HCl) and 4-dimethylaminopyridine (DMAP) at room temperature. Finally, the target compounds **6a–i** were synthesized in good yields by base hydrolysis of compounds **5a–i** with lithium hydroxide at 60 °C.

The structures of all the newly synthesized compounds were confirmed by IR, ^{1}H and ^{13}C NMR and LC mass spectral studies. The structure of compound **4** was confirmed by their ^{1}H NMR analyses. The ^{1}H NMR spectrum of compound **4** confirms introduction of methyl hydrazine due to sharp singlet at δ 3.22 corresponds to $-NCH_3$ and singlet at 4.72 corresponds to NH_2 group.

The structures of compounds **2a-i** were interpreted by their IR, ^{1}HNMR and LCMS analyses. The IR spectrum of **2a** revealed the presence of acid –OH group due to the appearance of strong band at 3,466 cm^{-1}, while that of acid –C=O of azetidine was observed at 1,569 cm^{-1}. In its ^{1}H NMR spectrum the appearance of a singlet at δ 3.97, doublet at δ 7.54 and double at δ 7.70 confirmed the presence of newly introduced $-NCH_2Ar$ group. Further, the LCMS showed its molecular ion peaks at 260.1 (M + H) which is in accordance with its molecular formula $C_{12}H_{12}F_3NO_2$.

The structures of compounds **5a-i** were interpreted by their IR, ^{1}HNMR and LCMS analyses. The IR spectrum of **5a** revealed the presence of ester –C=O group due to the appearance of strong band at 1728 cm^{-1}, while that of –C=O of amide was observed at 1,619 cm^{-1}. In its ^{1}H NMR spectrum the appearance of a singlet at δ 10.47 confirmed the presence of newly introduced amide functional group. Further, the LCMS showed its molecular ion peaks at 561.1 (M + H), which is in accordance with its molecular formula $C_{28}H_{28}F_4N_4O_4$.

The structures of compounds **6a–i** were elucidated by their IR, ^{1}H and ^{13}C NMR and LCMS analyses. The IR spectrum of **6a** revealed the presence of acid –OH group due to the appearance of absorbance bands at 3,281 cm^{-1}, while that of –C=O of amide was observed at 1,616 cm^{-1}. The ^{1}H NMR spectrum of **6a** showed singlet at δ 3.64, for two protons which correspond to $-NCH_2Ar$ and appearance of singlet δ 15.24 (which disappeared on D_2O exchange) that corresponds to –OH proton of acid. The ^{13}C NMR signals at 166.43, 170.83 and 176.71 corresponds to the presence of three types of carbonyl functional groups in the molecule and also typical signal at 8.01 confirms the presence of cyclopropyl group. The structure of **6a** was further confirmed by LCMS. It showed the molecular ion peak at m/z 533.1 (M + H), which conforms to its molecular formula $C_{26}H_{24}F_4N_4O_4$.

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

Antibacterial evaluation

All the newly synthesized nine compounds (**6a–i**) 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 *Staphylococcus aureus,* Vancomycin-Resistant *Enterococcus faecalis,* Methicillin-resistant *Staphylococcus aureus*, and three Gram-negative strains including *Klebsiella pneumoniae* (clinical). Ciprofloxacin and Linezolid were used as reference standards.

The data generated from this study (Table 2) showed that some of the target compounds exhibit good potency in inhibiting the growth of Gram-positive bacteria such as *Staphylococci aureus* including MRSA and VRE. *faecalis* (0.25–8.00 μg/mL). The in vitro activity of compound **6i**

Table 2 Antibacterial activity of new fluoroquinolones **6a–i**

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	16	32	32	4	4	2
6b	32	64	>64	4	2	1
6c	34	64	>64	4	2	2
6d	16	64	64	4	4	2
6e	16	32	32	8	8	2
6f	16	32	32	4	4	2
6g	16	32	64	2	1	4
6h	16	32	64	2	2	0.5
6i	4	16	4	0.25	0.5	0.25
Linezolid	4	16	2	>64	>64	8
Ciprofloxacin	0.5	1	0.5	0.015	0.007	0.03

Table 3 Anticancer activity of new fluoroquinolones **6a–i**

Percentage growth inhibition in different cell lines				
Compound	Concn. (μM)	A549	HCT-116	MCF-7
6a	1	31	9	16
	10	32	18	21
	50	37	34	23
6b	1	16	5	10
	10	20	15	13
	50	22	16	15
6c	1	11	19	26
	10	21	24	38
	50	35	31	52
6d	1	5	16	16
	10	20	20	17
	50	24	22	20
6e	1	15	18	17
	10	17	20	24
	50	21	20	26
6f	1	24	20	22
	10	24	22	25
	50	25	22	26
6g	1	28	5	25
	10	28	16	32
	50	40	17	45
6h	1	19	14	23
	10	19	20	50
	50	21	25	56
6i	1	7	29	23
	10	8	30	29
	50	8	30	30
Ciprofloxacin	1	6	7	24
	10	8	7	29
	50	9	8	41
SAHA	1	10	19	19
	10	79	46	24
	50	87	60	33

A549: Lung adenocarcinoma; HCT-116: Colon carcinoma; MCF-7: Breast carcinoma

against Gram-positive bacteria such as Methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecalis* are comparable to the marketed drugs Linezolid and Ciprofloxacin.

The in vitro activity of compound **6i** against Gram-negative bacteria such as *Klebsiella pneumonia, Acinetobacter baumannii* and *Moraxella catarrhalis* are superior to the marketed drug Linezolid and slightly less active when compared to Ciprofloxacin.

The antibacterial activity of compounds (**6e–i**) suggested that introduction of *N*-(lipophilic alkyl-substituted azetidine-3-carbonyl)-*N*-methyl-hydrazino derivatives at C-7 position of the fluoroquinolone improved the antibacterial activity against Gram-positive strains with retention of activity against Gram-negative strains. However, *N*-(aryl-substituted azetidine-3-carbonyl)-*N*-methyl-hydrazino derivatives (**6a–c**) are less potent than the reference compounds Linezolid and Ciprofloxacin.

Anticancer evaluation

All compounds were screened for their in vitro anticancer activity against representative human cancer cell lines (MCF-7 Breast carcinoma, HCT-116 Colon carcinoma and A549 Lung adenocarcinoma) obtained from American Type Culture Collection (ATCC). Ciprofloxacin and SAHA were used as reference standards.

The data generated from this study (Table 3) showed that some of the target compounds exhibit good potency in inhibiting the growth of MCF-7, A549 and HCT-116 cell lines. The in vitro anticancer activity of compound **6h** against MCF-7 cell line is superior to the marketed drugs Ciprofloxacin and SAHA. However, all the synthesized compounds are less potent when compared to SAHA against A549 and HCT-116 cell lines.

The anticancer activity of compounds (**6a–i**) suggested that introduction of bulky functional groups ($-CF_3$ in compound **6a** and –OMe in compound **6b**) on para position of the aromatic ring reduced the anticancer activity when compared to less hindered fluorine group in compound **6c**. Also *N*-cycloheptyl group in compound **6h** is optimum to have good anticancer activity against MCF-7 cell line.

Conclusion

A series of nine new *N*-(substituted azetidine-3-carbonyl)-*N*-methyl-hydrazino derivatives at C-7 position of fluoroquinolones were designed and synthesized through multi-step synthesis. The synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR, ESI–MS and IR. The new compounds were tested for their in vitro antimicrobial and anti-proliferative activity. Out of the nine derivatives, compound **6i** exhibited good antibacterial activity by inhibiting the growth of Methicillin-sensitive *Staphylococcus aureus* (MSSA), Methicillin-resistant *Staphylococcus aureus* (MRSA) and ATCC 35218 *Escherichia coli* (MIC: 0.25–16.00 μg/mL).

The anticancer activity of compound **6h** against MCF-7 cell line is superior with growth inhibition (56 %) when compared to the reference compounds ciprofloxacin (41 %) and marketed anticancer drug SAHA (33 %).

The results from in vitro anticancer studies suggested that *N*-(substituted azetidine-3-carbonyl)-*N*-methyl-hydrazino derivatives at C-7 position of fluoroquinolones are

very potent against MCF-7 breast cancer cell line. Hence, further structure–activity relationship studies on compounds **6c** and **6h** are in progress in our laboratory.

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