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Synthesis, characterization and *in vitro* antimicrobial and antimycobacterial activity of novel quinazolinone–flouroquinolone hybrids

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ABSTRACT

Novel quinazoline-flouroquinolone hybrids are synthesized using ciprofloxacin/gatifloxacin and 2-chloromethyl-3-(*N*-isonicotinamide-yl)–substituted-4H-quinazolinone (**3a-j**). The synthesized compounds were characterized on the basis of their elemental and spectral analysis (IR, ^{1}H-NMR, ^{13}C-NMR and mass spectrometry). All newly synthesized compounds were evaluated for their *in vitro* antimycobacterial activity against *Mycobacterium tuberculosis* $H_{37}Rv$, *in vitro* antibacterial activity against selected human pathogens *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella typhi, Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis* and antifungal activity against *Candida albicans, Aspergillus niger, A. clavatus* strains. The *in vitro* antimicrobial data suggest that 6-bromo and 8-bromo compounds are more active and also ciprofloxacin derivatives are more potent than gatifloxacin.

Keywords: *flouroquinolones, gatifloxacin, ciprofloxacin, antimicrobial activity, antimycobacterial activity, quinazolinone*

1. INTRODUCTION

Tuberculosis (TB), caused predominantly by *Mycobacterium tuberculosis* (MTB) and spred *via* the aerosol route is obstinate and remarkably flourishing pathogen that has latently infected a third of the world population [1, 2]. There were an estimated 8.8 million incident cases of TB (range, 8.5 million–9.2 million) globally in 2010, 1.1 million deaths (range, 0.9 million–1.2 million) among HIV-negative cases of TB and an additional 0.35 million deaths (range, 0.32 million–0.39 million) among people who were HIV-positive [3]. Despite the availability of four drug regimen to treat tuberculosis, loss of human lives is essentially unabated due to poverty, emergence of MDR, XDR and TDR strains of the bacterium and its synergy with HIV and other immune-compromised diseases [4]. Since no effective vaccine is available, the major strategy to conflict the scattering of TB is chemotherapy and the ever-increasing drug resistance, toxicity, side effects of currently used antituberculosis drugs and the absence of their bactericidal activity [5]. In order to tackle these new situations, it is crucial to develop new treatment guidelines and promising results have been obtained with fluoroquinolones [6-8].

The excellent oral bioavailability, convenient dosing schedules, favorable adverse incident profiles, and broad spectrum of activity of the newer flouroquinolone makes them attractive alternatives for treatment of many types of infections particularly when caused by organisms resistant to other available antimicrobial agents [9]. Number of modifications to the quinolone molecule, primarily at the N-1 position and the C-6, C-7, and C-8 positions has led to some significant changes in the

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antimicrobial activity, pharmacokinetic profiles, and metabolic properties of the newer fluoroquinolones [10]. Structure-activity relationship (SAR) studies of flouroquinolones indicated that modification at the C-7 position is the most adaptable site for chemical change and has a great impact modulating potency, spectrum, biopharmaceutics and pharmacokinetics and also the substitution of bulky functional group is acceptable at the C-7 position [11]. During recent years a number of quinolones with substitution on piperazine ring at C-7 position of the basic structure of quinolones were synthesized and evaluated for antibacterial activities [12-15]. On the other hand, it was suggested that the lipophilicity of the flouroquinolones plays an important role in the penetration of these compounds into bacterial cells, and simply increasing the lipophilic temperament at C-7 position could also increase the anti-TB activity [16, 17]. Therefore, reasonable modification at C-7 position is likely to produce more effective anti-TB agents.

Further molecular modification can enhance pharmacokinetics or pharmacodynamics aspects of the drugs. There are many strategy used to design drugs using these strategies such as molecular hybridization, prodrug and bioisosterism [18]. In view of the above mentioned facts and our interest in the synthesis of compounds with antimicrobial activity [19-22], here we report quinazolinone-fluoroquinolone hybrids by attachment of 2-chloromethyl-3-(N-isonicotinamide-yl) – substituted-4H-quinazolinone(3a-j) at 4-position of piperazine ring of fluoroquinolones. Thus, by making change in substituents present on C-7 position, we studied the effect on *in vitro* antimycobacterial and antimicrobial activity.

2. EXPERIMENTAL SECTION

Chemical and all solvents used in this study were purchased from Sigma aldrich. Melting points were determined in one end open capillaries and are uncorrected. The purity of the compounds was monitored by ascending thin layer chromatography (TLC) on silica gel-G 60 F_{254} (Merck) coated aluminium plates, visualized by iodine vapor. Spectra were obtained as follows: The IR spectra were obtained on a Perkin-Elmer 1720 FT-IR spectrometer (KBr pellets). ^{1}H-NMR spectra were recorded on a Bruker AC 400 MHz spectrometer, using DMSO-d_6 as solvent and ^{13}C- NMR spectra were recorded on a Bruker AC 100 MHz spectrometer, and their chemical shifts are reported in δ ppm units with respect to TMS as internal standard. Mass spectra were recorded on Shimadzu LCMS 2010 spectrometer. Elemental analyses (C, H, and N) were conducted using a Carlo Erba analyzer model 1106.

2.1. General procedure for the synthesis of Substituted *N*-chloroacetyl-substituted-anthranilic acid (2a-j). *N*-chloroacetyl-substituted-anthranilic acid was synthesized using substituted anthranilic acid **1a-j** (0.01 mol) and chloroacetylchloride (0.02 mol) according to reported procedure [19]. The solid thus obtained was recrystallized from ethanol to give the title compound.

2.2. General procedure for the synthesis of 2-chloromethyl-3-(*N*-isonicotinamide-yl) – substituted-4*H*-quinazolinone (3a-j). *N*-chloroacetyl-substituted-anthranilic acid **2a-j** (0.01 mol) was refluxed for 3-4 h with isonicotinic acid hydrazide (0.01 mol) following the reported procedure [19].

2.3. General procedure for the synthesis of 1-cyclopropyl-6-fluoro-7-(4-{substituted-4-oxo-3-[*N*-isonicotinamide-yl]-3,4-dihydro-quinazoline-2-ylmethyl}-piperazine-1-yl)-4-oxo,1,4-dihydro-quinoline-3-carboxylic acid (4a-j). A mixture of compounds **3a-j** (0.5 mmol), ciprofloxacin (0.5 mmol) and $NaHCO_3$ (0.5 mmol) in DMF (10 ml), was heated at 85–90 ºC for 9-10 h. After consumption of ciprofloxacin (monitored by TLC), H_2O (20 ml) was added and the

precipitate was filtered and washed with water to give the desired compounds **4a-j**. The product was crystallized from DMF–H_2O to give **4a-j**.

2.3.1. 1-cyclopropyl-6-fluoro-7-(4-{4-oxo-3-[*N*-isonicotinamide-yl]-3,4-dihydro-quinazoline-2-ylmethyl}-piperazine-1-yl)-4-oxo,1,4-dihydro-quinoline-3-carboxylic acid 4a. Yield, 67%; m.p. 213-215 ºC; FTIR (KBr, *v* cm^{-1}): 3340 (NH aromatic), 1236 (NH aliphatic), 3045 (C-H aromatic), 2946 (OH), 2874 (C-H aliphatic), 1864 (CH_2 cyclic), 1734 (C=O of carboxylic acid), 1671 (C=O of amide), 1646 (C=O of quinazolinone), 1321 (C=N), 1265 (N-N), 1153 (C-O-C), 1034 (C-F), 813 (C-C aliphatic); ^{1}H-NMR (400MHz, DMSO-d_6) δ ppm: 0.72-1.25 (m, 4H, cyclopropyl-H), 2.13 (s, 2H, CH_2), 3.17-3.80 (m, 9H, piperazine-H and cyclopropyl-H), 6.86-8.13 (m, 11H, Ar-H), 8.46 (s, 1H, NH-CO), 14.37 (s, 1H, COOH); ^{13}C-NMR (100MHz, DMSO-d_6) δ ppm: 8.17 (C_{30},C_{31}), 36.58 (C_{29}), 42.37-44.58 (C_{16}-C_{19}), 50.53 (C_{15}), 107.15 (C_{22}), 107.48 (C_{27}), 111.41 (C_{25}), 119.60 (C_{23}), 122.34-148.49 (C_1-C_6,C_{10}-C_{11},C_{14},C_{20},C_{24},C_{28}), 150.37 (C_{12}-C_{13}), 153.52 (C_{21}), 164.14 (C_8), 166.27 (C_{32}), 167.10 (C_7), 168.43 (C_9), 176.68 (C_{26}); MS: m/z $[609.21]^+$; Analysis calculated for $C_{34}H_{28}FN_7O_5$: C, 63.05; H, 4.63; N, 16.08. Found: C, 63.13; H, 4.75; N, 16.20%.

2.3.2. 1-cyclopropyl-6-fluoro-7-(4-{6-bromo-4-oxo-3-[*N*-isonicotinamide-yl]-3,4-dihydro-quinazoline-2-ylmethyl}-piperazine-1-yl)-4-oxo,1,4-dihydro-quinoline-3-carboxylic acid 4b. Yield, 71%; m.p. 251-253 ºC; FTIR (KBr, *v* cm^{-1}): 3341 (NH aromatic), 1234 (NH aliphatic), 3046 (C-H aromatic), 2947 (OH), 2873 (C-H aliphatic), 1865 (CH_2 cyclic), 1733 (C=O of carboxylic acid), 1670 (C=O of amide), 1645 (C=O of quinazolinone), 1324 (C=N), 1268 (N-N), 1155 (C-O-C), 1031 (C-F), 821 (C-C aliphatic); ^{1}H-NMR (400MHz, DMSO-d_6) δ ppm: 0.68-1.34 (m, 4H, cyclopropyl-H), 2.20 (s, 2H, CH_2), 3.25-3.73 (m, 9H, piperazine-H and cyclopropyl-H), 6.79-8.12 (m, 10H, Ar-H), 8.54 (s, 1H, NH-CO), 14.32 (s, 1H, COOH); ^{13}C-NMR (100MHz, DMSO-d_6) δ ppm: 8.14 (C_{30},C_{31}), 36.62 (C_{29}), 42.36-44.68 (C_{16}-C_{19}), 50.58 (C_{15}), 107.18 (C_{22}), 107.42 (C_{27}), 111.46 (C_{25}), 119. 62 (C_{23}), 121.52 (C_1), 122.40-148.53 (C_2-C_6,C_{10}-C_{11},C_{14},C_{20},C_{24},C_{28}), 150.34 (C_{12}-C_{13}), 153.56 (C_{21}), 164.12 (C_8), 166.21 (C_{32}), 167.15 (C_7), 168.39 (C_9), 176.73 (C_{26}); MS: m/z $[687.12]^+$; Analysis calculated for $C_{32}H_{27}BrFN_7O_5$: C, 55.82; H, 3.95; N, 14.24. Found: C, 55.89; H, 3.77; N, 14.34%.

2.3.3. 1-cyclopropyl-6-fluoro-7-(4-{6-iodo-4-oxo-3-[*N*-isonicotinamide-yl]-3,4-dihydro-quinazoline-2-ylmethyl}-piperazine-1-yl)-4-oxo,1,4-dihydro-quinoline-3-carboxylic acid 4c. Yield, 65%; m.p. 239-241 ºC; FTIR (KBr, *v* cm^{-1}): 3343 (NH aromatic), 1236 (NH aliphatic), 3048 (C-H aromatic), 2947 (OH), 2876 (C-H aliphatic), 1867 (CH_2 cyclic), 1735 (C=O of carboxylic acid), 1673 (C=O of amide), 1646 (C=O of quinazolinone), 1321 (C=N), 1271 (N-N), 1152 (C-O-C), 1034 (C-F), 828 (C-C aliphatic); ^{1}H-NMR (400MHz, DMSO-d_6) δ ppm: 0.71-1.23 (m, 4H, cyclopropyl-H), 2.27 (s, 2H, CH_2), 3.20-3.68 (m, 9H, piperazine-H and cyclopropyl-H), 6.74-8.15 (m, 10H, Ar-H), 8.62 (s, 1H, NH-CO), 14.39 (s, 1H, COOH); ^{13}C-NMR (100MHz, DMSO-d_6) δ ppm: 8.21 (C_{30},C_{31}), 36.58 (C_{29}), 42.31-44.63 (C_{16}-C_{19}), 50.60 (C_{15}), 95.81 (C_1), 107.20 (C_{22}), 107.45 (C_{27}), 111.43 (C_{25}), 119.64 (C_{23}), 122.51-148.43 (C_2-C_6,C_{10}-C_{11},C_{14},C_{20},C_{24},C_{28}), 150.31 (C_{12}-C_{13}), 153.60 (C_{21}), 164.18 (C_8), 166.17 (C_{32}), 167.21 (C_7), 168.32 (C_9), 176.60 (C_{26}); MS: m/z $[735.11]^+$; Analysis calculated for $C_{32}H_{27}IFN_7O_5$: C, 52.26; H, 3.70; N, 13.33. Found: C, 52.43; H, 3.81; N, 13.22%.

2.3.4. 1-cyclopropyl-6-fluoro-7-(4-{6-chloro-4-oxo-3-[*N*-isonicotinamide-yl]-3,4-dihydro-quinazoline-2-ylmethyl}-piperazine-1-yl)-4-oxo,1,4-dihydro-quinoline-3-carboxylic acid 4d. Yield, 59%; m.p. 262-264 ºC; FTIR (KBr, *v* cm^{-1}): 3338 (NH aromatic), 1235 (NH aliphatic), 3052 (C-H aromatic), 2937 (OH), 2883 (C-H aliphatic), 1864 (CH_2 cyclic), 1741 (C=O of carboxylic acid), 1672 (C=O of amide), 1641 (C=O of quinazolinone), 1328 (C=N), 1264 (N-N), 1153 (C-O-C),

1038 (C-F), 831 (C-C aliphatic); ^{1}H-NMR (400MHz, DMSO-d_6) δ ppm: 0.57-1.30 (m, 4H, cyclopropyl-H), 2.31 (s, 2H, CH_2), 3.18-3.71 (m, 9H, piperazine-H and cyclopropyl-H), 6.86-8.10 (m, 10H, Ar-H), 8.59 (s, 1H, NH-CO), 14.43 (s, 1H, COOH); ^{13}C-NMR (100MHz, DMSO-d_6) δ ppm: 8.16 (C_{30},C_{31}), 36.61 (C_{29}), 42.27-44.68 (C_{16}-C_{19}), 50.51 (C_{15}), 107.16 (C_{22}), 107.38 (C_{27}), 111.48 (C_{25}), 119.53 (C_{23}), 122.49-148.54 (C_1-C_6,C_{10}-C_{11},C_{14},C_{20},C_{24},C_{28}), 150.34 (C_{12}-C_{13}), 153.64 (C_{21}), 164.21 (C_8), 166.22 (C_{32}), 167.15 (C_7), 168.32 (C_9), 176.56 (C_{26}); MS: m/z $[643.17]^+$; Analysis calculated for $C_{32}H_{27}ClFN_7O_5$: C, 59.68; H, 4.23; N, 15.22. Found: C, 59.72; H, 4.13; N, 15.34%.

2.3.5. 1-cyclopropyl-6-fluoro-7-(4-{8-bromo-4-oxo-3-[*N*-isonicotinamide-yl]-3,4-dihydro-quinazoline-2-ylmethyl}-piperazine-1-yl)-4-oxo,1,4-dihydro-quinoline-3-carboxylic acid 4e. Yield, 63%; m.p. 254-256 ºC; FTIR (KBr, *v* cm^{-1}): 3342 (NH aromatic), 1238 (NH aliphatic), 3038 (C-H aromatic), 2936 (OH), 2871 (C-H aliphatic), 1863 (CH_2 cyclic), 1740 (C=O of carboxylic acid), 1683 (C=O of amide), 1641 (C=O of quinazolinone), 1331 (C=N), 1258 (N-N), 1143 (C-O-C), 1042 (C-F), 826 (C-C aliphatic); ^{1}H-NMR (400MHz, DMSO-d_6) δ ppm: 0.64-1.23 (m, 4H, cyclopropyl-H), 2.36 (s, 2H, CH_2), 3.24-3.70 (m, 9H, piperazine-H and cyclopropyl-H), 6.93-8.16 (m, 10H, Ar-H), 8.65 (s, 1H, NH-CO), 14.52 (s, 1H, COOH); ^{13}C-NMR (100MHz, DMSO-d_6) δ ppm: 8.20 (C_{30},C_{31}), 36.57 (C_{29}), 42.23-44.54 (C_{16}-C_{19}), 50.53 (C_{15}), 107.14 (C_{22}), 107.36 (C_{27}), 111.45 (C_{25}), 116.83 (C_4), 119.56 (C_{23}), 122.43-148.58 (C_1-C_3,C_5,C_{10}-C_{11},C_{14},C_{20},C_{24},C_{28}), 150.37 (C_{12}-C_{13}), 151.24 (C_6), 153.60 (C_{21}), 164.14 (C_8), 166.26 (C_{32}), 167.13 (C_7), 168.35 (C_9), 176.58 (C_{26}); MS: m/z $[687.12]^+$; Analysis calculated for $C_{32}H_{27}BrFN_7O_5$: C, 55.82; H, 3.95; N, 14.24. Found: C, 55.93; H, 3.82; N, 14.30%.

2.3.6. 1-cyclopropyl-6-fluoro-7-(4-{8-iodo-4-oxo-3-[*N*-isonicotinamide-yl]-3,4-dihydro-quinazoline-2-ylmethyl}-piperazine-1-yl)-4-oxo,1,4-dihydro-quinoline-3-carboxylic acid 4f. Yield, 72%; m.p. 246-248 ºC; FTIR (KBr, *v* cm^{-1}): 3347 (NH aromatic), 1243 (NH aliphatic), 3042 (C-H aromatic), 2938 (OH), 2874 (C-H aliphatic), 1866 (CH_2 cyclic), 1745 (C=O of carboxylic acid), 1680 (C=O of amide), 1638 (C=O of quinazolinone), 1335 (C=N), 1260 (N-N), 1143 (C-O-C), 1040 (C-F), 825 (C-C aliphatic); ^{1}H-NMR (400MHz, DMSO-d_6) δ ppm: 0.52-1.28 (m, 4H, cyclopropyl-H), 2.29 (s, 2H, CH_2), 3.16-3.66 (m, 9H, piperazine-H and cyclopropyl-H), 6.78-8.14 (m, 10H, Ar-H), 8.63 (s, 1H, NH-CO), 14.38 (s, 1H, COOH); ^{13}C-NMR (100MHz, DMSO-d_6) δ ppm: 8.23 (C_{30},C_{31}), 36.63 (C_{29}), 42.24-44.56 (C_{16}-C_{19}), 50.54 (C_{15}), 90.23 (C_4), 107.22 (C_{22}), 107.31 (C_{27}), 111.50 (C_{25}), 119.51 (C_{23}), 122.36-148.45 (C_1-C_3,C_5,C_{10}-C_{11},C_{14},C_{20},C_{24},C_{28}), 150.43 (C_{12}-C_{13}), 153.68 (C_{21}), 156.53 (C_6), 164.23 (C_8), 166.18 (C_{32}), 167.19 (C_7), 168.37 (C_9), 176.61 (C_{26}); MS: m/z $[735.11]^+$; Analysis calculated for $C_{32}H_{27}IFN_7O_5$: C, 52.26; H, 3.70; N, 13.33. Found: C, 52.31; H, 3.62; N, 13.40%.

2.3.7. 1-cyclopropyl-6-fluoro-7-(4-{8-chloro-4-oxo-3-[*N*-isonicotinamide-yl]-3,4-dihydro-quinazoline-2-ylmethyl}-piperazine-1-yl)-4-oxo,1,4-dihydro-quinoline-3-carboxylic acid 4g. Yield, 61%; m.p. 261-263 ºC; FTIR (KBr, *v* cm^{-1}): 3349 (NH aromatic), 1240 (NH aliphatic), 3042 (C-H aromatic), 2941 (OH), 2869 (C-H aliphatic), 1862 (CH_2 cyclic), 1743 (C=O of carboxylic acid), 1685 (C=O of amide), 1644 (C=O of quinazolinone), 1329 (C=N), 1253 (N-N), 1147 (C-O-C), 1039 (C-F), 826 (C-C aliphatic); ^{1}H-NMR (400MHz, DMSO-d_6) δ ppm: 0.48-1.29 (m, 4H, cyclopropyl-H), 2.33 (s, 2H, CH_2), 3.20-3.60 (m, 9H, piperazine-H and cyclopropyl-H), 6.88-8.03 (m, 10H, Ar-H), 8.74 (s, 1H, NH-CO), 14.46 (s, 1H, COOH); ^{13}C-NMR (100MHz, DMSO-d_6) δ ppm: 8.12 (C_{30},C_{31}), 36.56 (C_{29}), 42.18-44.50 (C_{16}-C_{19}), 50.49 (C_{15}), 107.12 (C_{22}), 107.43 (C_{27}), 111.54 (C_{25}), 119.58 (C_{23}), 122.40-148.64 (C_1-C_6,C_{10}-C_{11},C_{14},C_{20},C_{24},C_{28}), 150.52 (C_{12}-C_{13}), 153.70

(C_{21}), 164.21 (C_8), 166.13 (C_{32}), 167.16 (C_7), 168.32 (C_9), 176.66 (C_{26}); MS: m/z $[643.17]^+$; Analysis calculated for $C_{32}H_{27}ClFN_7O_5$: C, 59.68; H, 4.23; N, 15.22. Found: C, 59.51; H, 4.37; N, 15.14%.

2.3.8. 1-cyclopropyl-6-fluoro-7-(4-{6,8-dibromo-4-oxo-3-[*N*-isonicotinamide-yl]-3,4-dihydro-quinazoline-2-ylmethyl}-piperazine-1-yl)-4-oxo,1,4-dihydro-quinoline-3-carboxylic acid 4h. Yield, 58%; m.p. 267-269 ºC; FTIR (KBr, *v* cm^{-1}): 3348 (NH aromatic), 1241 (NH aliphatic), 3043 (C-H aromatic), 2935 (OH), 2874 (C-H aliphatic), 1867 (CH_2 cyclic), 1742 (C=O of carboxylic acid), 1680 (C=O of amide), 1641 (C=O of quinazolinone), 1334 (C=N), 1253 (N-N), 1148 (C-O-C), 1045 (C-F), 823 (C-C aliphatic); ^{1}H-NMR (400MHz, DMSO-d_6) δ ppm: 0.53-1.17 (m, 4H, cyclopropyl-H), 2.25 (s, 2H, CH_2), 3.18-3.56 (m, 9H, piperazine-H and cyclopropyl-H), 6.84-8.11 (m, 9H, Ar-H), 8.70 (s, 1H, NH-CO), 14.35 (s, 1H, COOH); ^{13}C-NMR (100MHz, DMSO-d_6) δ ppm: 8.17 (C_{30},C_{31}), 36.61 (C_{29}), 42.20-44.48 (C_{16}-C_{19}), 50.51 (C_{15}), 107.16 (C_{22}), 107.51 (C_{27}), 111.62 (C_{25}), 118.74 (C_4), 119.56 (C_{23}), 122.37-148.53 (C_1-C_3,C_5,C_{10}-C_{11},C_{14},C_{20},C_{24},C_{28}), 150.23 (C_6), 150.63 (C_{12}-C_{13}), 153.61 (C_{21}), 164.23 (C_8), 166.14 (C_{32}), 167.20 (C_7), 168.33 (C_9), 176.71 (C_{26}); MS: m/z $[765.03]^+$; Analysis calculated for $C_{32}H_{26}Br_2FN_7O_5$: C, 50.08; H, 3.41; N, 12.78. Found: C, 50.14; H, 3.57; N, 12.83%.

2.3.9. 1-cyclopropyl-6-fluoro-7-(4-{6,8-diiodo-4-oxo-3-[*N*-isonicotinamide-yl]-3,4-dihydro-quinazoline-2-ylmethyl}-piperazine-1-yl)-4-oxo,1,4-dihydro-quinoline-3-carboxylic acid 4i. Yield, 69%; m.p. 253-255 ºC; 3349 (NH aromatic), 1246 (NH aliphatic), 3049 (C-H aromatic), 2938 (OH), 2876 (C-H aliphatic), 1862 (CH_2 cyclic), 1746 (C=O of carboxylic acid), 1682 (C=O of amide), 1648 (C=O of quinazolinone), 1345 (C=N), 1248 (N-N), 1150 (C-O-C), 1043 (C-F), 834 (C-C aliphatic); ^{1}H-NMR (400MHz, DMSO-d_6) δ ppm: 0.61-1.28 (m, 4H, cyclopropyl-H), 2.32 (s, 2H, CH_2), 3.21-3.49 (m, 9H, piperazine-H and cyclopropyl-H), 6.90-8.16 (m, 9H, Ar-H), 8.75 (s, 1H, NH-CO), 14.41 (s, 1H, COOH); ^{13}C-NMR (100MHz, DMSO-d_6) δ ppm: 8.24 (C_{30},C_{31}), 36.57 (C_{29}), 42.17-44.38 (C_{16}-C_{19}), 50.56 (C_{15}), 92.63 (C_4), 97.42 (C_1), 107.13 (C_{22}), 107.50 (C_{27}), 111.58 (C_{25}), 119.53 (C_{23}), 122.41-148.47 (C_3,C_5,C_{10}-C_{11},C_{14},C_{20},C_{24},C_{28}), 150.52 (C_{12}-C_{13}), 151.19 (C_2), 153.64 (C_{21}), 155.74 (C_6), 164.19 (C_8), 166.20 (C_{32}), 167.16 (C_7), 168.39 (C_9), 176.75 (C_{26}); MS: m/z $[861.01]^+$; Analysis calculated for $C_{32}H_{26}I_2FN_7O_5$: C, 44.62; H, 3.04; N, 11.38. Found: C, 44.51; H, 3.15; N, 11.47%.

2.3.10. 1-cyclopropyl-6-fluoro-7-(4-{6,8-dichloro-4-oxo-3-[*N*-isonicotinamide-yl]-3,4-dihydro-quinazoline-2-ylmethyl}-piperazine-1-yl)-4-oxo,1,4-dihydro-quinoline-3-carboxylic acid 4j. Yield, 62%; m.p. 269-271 ºC; 3342 (NH aromatic), 1246 (NH aliphatic), 3045 (C-H aromatic), 2938 (OH), 2870 (C-H aliphatic), 1864 (CH_2 cyclic), 1748 (C=O of carboxylic acid), 1675 (C=O of amide), 1638 (C=O of quinazolinone), 1340 (C=N), 1256 (N-N), 1144 (C-O-C), 1042 (C-F), 830 (C-C aliphatic); ^{1}H-NMR (400MHz, DMSO-d_6) δ ppm: 0.57-1.24 (m, 4H, cyclopropyl-H), 2.35 (s, 2H, CH_2), 3.24-3.54 (m, 9H, piperazine-H and cyclopropyl-H), 6.90-8.18 (m, 9H, Ar-H), 8.64 (s, 1H, NH-CO), 14.40 (s, 1H, COOH); ^{13}C-NMR (100MHz, DMSO-d_6) δ ppm: 8.27 (C_{30},C_{31}), 36.51 (C_{29}), 42.21-44.42 (C_{16}-C_{19}), 50.63 (C_{15}), 107.18 (C_{22}), 107.53 (C_{27}), 111.60 (C_{25}), 119.54 (C_{23}), 122.44-148.51 (C_1-C_6,C_{10}-C_{11},C_{14},C_{20},C_{24},C_{28}), 150.56 (C_{12}-C_{13}), 153.68 (C_{21}), 164.16 (C_8), 166.17 (C_{32}), 167.22 (C_7), 168.41 (C_9), 176.67 (C_{26}); MS: m/z $[677.14]^+$; Analysis calculated for $C_{32}H_{26}Cl_2FN_7O_5$: C, 56.65; H, 3.86; N, 14.45. Found: C, 56.69; H, 3.72; N, 14.32%.

2.4. General procedure for the synthesis of 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl-4-{substituted-4-oxo-3-[*N*-isonicotinamide-yl]-3,4-dihydro-quinazoline-2-ylmethyl}-piperazine-1-yl)-4-oxo,1,4-dihydro-quinoline-3-carboxylic acid (5a-j). A mixture of compounds **3a-j** (0.5 mmol), gatifloxacin and $NaHCO_3$ (0.5 mmol) in DMF (10 ml), was heated at 85–90 ºC for 12-13 h.

After consumption of gatifloxacin (monitored by TLC), H_2O (20 ml) was added and the precipitate was filtered and washed with water to give the desired compounds **5a-j**. The product was crystallized from DMF–H_2O.

2.4.1. 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl-4-{4-oxo-3-[*N*-isonicotinamide-yl]-3,4-dihydro-quinazoline-2-ylmethyl}-piperazine-1-yl)-4-oxo,1,4-dihydro-quinoline-3-carboxylic acid 5a. Yield, 73%; m.p. 226-228 ºC; FTIR (KBr, *v* cm^{-1}): 3342 (NH aromatic), 1235 (NH aliphatic), 3042 (C-H aromatic), 2949 (OH), 2876 (C-H aliphatic), 2738 (CH_3), 1869 (CH_2 cyclic), 1734 (C=O of carboxylic acid), 1674 (C=O of amide), 1643 (C=O of quinazolinone), 1321 (C=N), 1264 (N-N), 1157 (C-O-C), 1034 (C-F), 826 (C-C aliphatic); ^{1}H-NMR (400MHz, DMSO-d_6) δ ppm: 0.37-1.28 (m, 4H, cyclopropyl-H), 1.46 (d, 3H, CH_3 of piperazine), 2.32 (s, 2H, CH_2), 3.18-3.56 (m, 8H, piperazine-H and cyclopropyl-H), 3.83 (s, 3H, OCH_3), 6.84-8.12 (m, 10H, Ar-H), 8.71 (s, 1H, NH-CO), 14.50 (s, 1H, COOH); ^{13}C-NMR (100MHz, DMSO-d_6) δ ppm: 8.14 (C_{30},C_{31}), 16.82 (CH_3), 36.60 (C_{29}), 42.35-44.51 (C_{16}-C_{19}), 50.52 (C_{15}), 56.13 (OCH_3), 107.18 (C_{22}), 107.24 (C_{27}), 111.43 (C_{25}), 119.57 (C_{23}), 122.38-148.52 (C_1-C_6,C_{10}-C_{11},C_{14},C_{20},C_{24},C_{28}), 150.34 (C_{12}-C_{13}), 153.50 (C_{21}), 164.16 (C_8), 166.28 (C_{32}), 167.14 (C_7), 168.49 (C_9), 176.66 (C_{26}); MS: m/z $[653.24]^+$; Analysis calculated for $C_{34}H_{32}FN_7O_6$: C, 62.47; H, 4.93; N, 15.00. Found: C, 62.58; H, 4.84; N, 15.17%.

2.4.2. 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl-4-{6-bromo-4-oxo-3-[*N*-isonicotinamide-yl]-3,4-dihydro-quinazoline-2-ylmethyl}-piperazine-1-yl)-4-oxo,1,4-dihydro-quinoline-3-carboxylic acid 5b. Yield, 64%; m.p. 247-249 ºC; FTIR (KBr, *v* cm^{-1}): 3344 (NH aromatic), 1238 (NH aliphatic), 3047 (C-H aromatic), 2943 (OH), 2872 (C-H aliphatic), 2731 (CH_3), 1863 (CH_2 cyclic), 1736 (C=O of carboxylic acid), 1672 (C=O of amide), 1644 (C=O of quinazolinone), 1329 (C=N), 1269 (N-N), 1150 (C-O-C), 1036 (C-F), 822 (C-C aliphatic); ^{1}H-NMR (400MHz, DMSO-d_6) δ ppm: 0.32-1.18 (m, 4H, cyclopropyl-H), 1.48 (d, 3H, CH_3 of piperazine), 2.41 (s, 2H, CH_2), 3.20-3.49 (m, 8H, piperazine-H and cyclopropyl-H), 3.86 (s, 3H, OCH_3), 6.89-8.17 (m, 9H, Ar-H), 8.64 (s, 1H, NH-CO), 14.46 (s, 1H, COOH); ^{13}C-NMR (100MHz, DMSO-d_6) δ ppm: 8.20 (C_{30},C_{31}), 16.83 (CH_3), 36.64 (C_{29}), 42.27-44.54 (C_{16}-C_{19}), 50.56 (C_{15}), 56.19 (OCH_3), 107.07 (C_{22}), 107.28 (C_{27}), 111.41 (C_{25}), 119.60 (C_{23}), 121.73-148.35 (C_1-C_6,C_{10}-C_{11},C_{14},C_{20},C_{24},C_{28}), 150.40 (C_{12}-C_{13}), 153.56 (C_{21}), 164.12 (C_8), 166.23 (C_{32}), 167.15 (C_7), 168.52 (C_9), 176.70 (C_{26}); MS: m/z $[732.15]^+$; Analysis calculated for $C_{34}H_{31}BrFN_7O_6$: C, 55.75; H, 4.27; N, 13.38. Found: C, 55.86; H, 4.38; N, 13.24%.

2.4.3. 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl-4-{6-iodo-4-oxo-3-[*N*-isonicotinamide-yl]-3,4-dihydro-quinazoline-2-ylmethyl}-piperazine-1-yl)-4-oxo,1,4-dihydro-quinoline-3-carboxylic acid 5c. Yield, 68%; m.p. 232-234 ºC; FTIR (KBr, *v* cm^{-1}): 3345 (NH aromatic), 1238 (NH aliphatic), 3049 (C-H aromatic), 2946 (OH), 2877 (C-H aliphatic), 2734 (CH_3), 1860 (CH_2 cyclic), 1735 (C=O of carboxylic acid), 1687 (C=O of amide), 1646 (C=O of quinazolinone), 1324 (C=N), 1268 (N-N), 1154 (C-O-C), 1033 (C-F), 821 (C-C aliphatic); ^{1}H-NMR (400MHz, DMSO-d_6) δ ppm: 0.28-1.15 (m, 4H, cyclopropyl-H), 1.53 (d, 3H, CH_3 of piperazine), 2.43 (s, 2H, CH_2), 3.16-3.43 (m, 8H, piperazine-H and cyclopropyl-H), 3.91 (s, 3H, OCH_3), 6.78-8.07 (m, 9H, Ar-H), 8.59 (s, 1H, NH-CO), 14.52 (s, 1H, COOH); ^{13}C-NMR (100MHz, DMSO-d_6) δ ppm: 8.18 (C_{30},C_{31}), 16.78 (CH_3), 36.54 (C_{29}), 42.41-44.52 (C_{16}-C_{19}), 50.53 (C_{15}), 56.18 (OCH_3), 95.81 (C_1), 107.13 (C_{22}), 107.30 (C_{27}), 111.45 (C_{25}), 119.58 (C_{23}), 122.36-148.41 (C_2-C_6,C_{10}-C_{11},C_{14},C_{20},C_{24},C_{28}), 150.33 (C_{12}-C_{13}), 153.57 (C_{21}), 164.20 (C_8), 166.32 (C_{32}), 167.19 (C_7), 168.51 (C_9), 176.62 (C_{26}); MS: m/z

$[779.14]^+$; Analysis calculated for $C_{34}H_{31}IFN_7O_6$: C, 52.38; H, 4.01; N, 12.58. Found: C, 52.24; H, 4.10; N, 12.67%.

2.4.4. 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl-4-{6-chloro-4-oxo-3-[*N*-isonicotinamide-yl]-3,4-dihydro-quinazoline-2-ylmethyl}-piperazine-1-yl)-4-oxo,1,4-dihydro-quinoline-3-carboxylic acid 5d. Yield, 61%; m.p. 258-260° C; FTIR (KBr, ν cm^{-1}): 3348.31 (NH aromatic), 1238 (NH aliphatic), 3045 (C-H aromatic), 2945 (OH), 2879 (C-H aliphatic), 2732 (CH_3), 1865 (CH_2 cyclic), 1737 (C=O of carboxylic acid), 1678 (C=O of amide), 1641 (C=O of quinazolinone), 1325 (C=N), 1268 (N-N), 1159 (C-O-C), 1040 (C-F), 830 (C-C aliphatic); ^{1}H-NMR (400MHz, DMSO-d_6) δ ppm: 0.23-1.20 (m, 4H, cyclopropyl-H), 1.60 (d, 3H, CH_3 of piperazine), 2.40 (s, 2H, CH_2), 3.19-3.48 (m, 8H, piperazine-H and cyclopropyl-H), 3.87 (s, 3H, OCH_3), 6.93-8.13 (m, 9H, Ar-H), 8.62 (s, 1H, NH-CO), 14.44 (s, 1H, COOH); ^{13}C-NMR (100MHz, DMSO-d_6) δ ppm: 8.24 (C_{30},C_{31}), 16.80 (CH_3), 36.63 (C_{29}), 42.27-44.60 (C_{16}-C_{19}), 50.57 (C_{15}), 56.20 (OCH_3), 107.08 (C_{22}), 107.25 (C_{27}), 111.46 (C_{25}), 119.60 (C_{23}), 122.53-148.65 (C_1-C_6,C_{10}-C_{11},C_{14},C_{20},C_{24},C_{28}), 150.36 (C_{12}-C_{13}), 153.58 (C_{21}), 164.15 (C_8), 166.22 (C_{32}), 167.21 (C_7), 168.57 (C_9), 176.74 (C_{26}); MS: m/z $[687.20]^+$; Analysis calculated for $C_{34}H_{31}ClFN_7O_6$: C, 59.35; H, 4.54; N, 14.25. Found: C, 59.44; H, 4.46; N, 14.31%.

2.4.5. 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl-4-{8-bromo-4-oxo-3-[*N*-isonicotinamide-yl]-3,4-dihydro-quinazoline-2-ylmethyl}-piperazine-1-yl)-4-oxo,1,4-dihydro-quinoline-3-carboxylic acid 5e. Yield, 56%; m.p. 261-263 ºC; FTIR (KBr, ν cm^{-1}): 3347 (NH aromatic), 1238 (NH aliphatic), 3050 (C-H aromatic), 2942 (OH), 2883 (C-H aliphatic), 2734 (CH_3), 1865 (CH_2 cyclic), 1730 (C=O of carboxylic acid), 1671 (C=O of amide), 1646 (C=O of quinazolinone), 1327 (C=N), 1269 (N-N), 1152 (C-O-C), 1038 (C-F), 822 (C-C aliphatic); ^{1}H-NMR (400MHz, DMSO-d_6) δ ppm: 0.25-1.16 (m, 4H, cyclopropyl-H), 1.54 (d, 3H, CH_3 of piperazine), 2.46 (s, 2H, CH_2), 3.12-3.38 (m, 8H, piperazine-H and cyclopropyl-H), 3.84 (s, 3H, OCH_3), 6.82-8.15 (m, 9H, Ar-H), 8.60 (s, 1H, NH-CO), 14.51 (s, 1H, COOH); ^{13}C-NMR (100MHz, DMSO-d_6) δ ppm: 8.23 (C_{30},C_{31}), 16.81 (CH_3), 36.56 (C_{29}), 42.23-44.61 (C_{16}-C_{19}), 50.52 (C_{15}), 56.28 (OCH_3), 107.13 (C_{22}), 107.23 (C_{27}), 111.48 (C_{25}), 119.61 (C_{23}), 122.52-148.64 (C_1-C_6,C_{10}-C_{11},C_{14},C_{20},C_{24},C_{28}), 150.33 (C_{12}-C_{13}), 153.60 (C_{21}), 164.16 (C_8), 166.29 (C_{32}), 167.22 (C_7), 168.56 (C_9), 176.73 (C_{26}); MS: m/z $[732.15]^+$; Analysis calculated for $C_{34}H_{31}BrFN_7O_6$: C, 55.75; H, 4.27; N, 13.38. Found: C, 55.68; H, 4.34; N, 13.41%.

2.4.6. 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl-4-{8-iodo-4-oxo-3-[*N*-isonicotinamide-yl]-3,4-dihydro-quinazoline-2-ylmethyl}-piperazine-1-yl)-4-oxo,1,4-dihydro-quinoline-3-carboxylic acid 5f. Yield, 65%; m.p. 255-253 ºC; FTIR (KBr, ν cm^{-1}): 3346 (NH aromatic), 1240 (NH aliphatic), 3043 (C-H aromatic), 2946 (OH), 2890 (C-H aliphatic), 2732 (CH_3), 1867 (CH_2 cyclic), 1732 (C=O of carboxylic acid), 1675 (C=O of amide), 1645 (C=O of quinazolinone), 1329 (C=N), 1270 (N-N), 1154 (C-O-C), 1032 (C-F), 826 (C-C aliphatic); ^{1}H-NMR (400MHz, DMSO-d_6) δ ppm: 0.22-1.20 (m, 4H, cyclopropyl-H), 1.57 (d, 3H, CH_3 of piperazine), 2.38 (s, 2H, CH_2), 3.15-3.42 (m, 8H, piperazine-H and cyclopropyl-H), 3.89 (s, 3H, OCH_3), 6.75-8.08 (m, 9H, Ar-H), 8.56 (s, 1H, NH-CO), 14.53 (s, 1H, COOH); ^{13}C-NMR (100MHz, DMSO-d_6) δ ppm: 8.28 (C_{30},C_{31}), 16.78 (CH_3), 36.67 (C_{29}), 42.24-44.51 (C_{16}-C_{19}), 50.55 (C_{15}), 56.18 (OCH_3), 107.13 (C_{22}), 107.30 (C_{27}), 111.42 (C_{25}), 119.58 (C_{23}), 122.51-148.67 (C_1-C_6,C_{10}-C_{11},C_{14},C_{20},C_{24},C_{28}), 150.34 (C_{12}-C_{13}), 153.59 (C_{21}), 164.16 (C_8), 166.23 (C_{32}), 167.26 (C_7), 168.54 (C_9), 176.71 (C_{26}); MS: m/z $[779.14]^+$; Analysis calculated for $C_{34}H_{31}IFN_7O_6$: C, 52.38; H, 4.01; N, 12.58. Found: C, 52.46; H, 4.13; N, 12.40%.

2.4.7. 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl-4-{8-chloro-4-oxo-3-[*N*-isonicotinamide-yl]-3,4-dihydro-quinazoline-2-ylmethyl}-piperazine-1-yl)-4-oxo,1,4-dihydro-quinoline-3-carboxylic acid 5g. Yield, 60%; m.p. 275-273 ºC; FTIR (KBr, *v* cm^{-1}): 3342 (NH aromatic), 1233 (NH aliphatic), 3042 (C-H aromatic), 2947 (OH), 2876 (C-H aliphatic), 2736 (CH_3), 1860 (CH_2 cyclic), 1740 (C=O of carboxylic acid), 1673 (C=O of amide), 1643 (C=O of quinazolinone), 1331 (C=N), 1269 (N-N), 1154 (C-O-C), 1036 (C-F), 827 (C-C aliphatic); ^{1}H-NMR (400MHz, DMSO-d_6) δ ppm: 0.26-1.16 (m, 4H, cyclopropyl-H), 1.49 (d, 3H, CH_3 of piperazine), 2.40 (s, 2H, CH_2), 3.18-3.45 (m, 8H, piperazine-H and cyclopropyl-H), 3.86 (s, 3H, OCH_3), 6.83-8.14 (m, 9H, Ar-H), 8.53 (s, 1H, NH-CO), 14.60 (s, 1H, COOH); ^{13}C-NMR (100MHz, DMSO-d_6) δ ppm: 8.31 (C_{30},C_{31}), 16.82 (CH_3), 36.56 (C_{29}), 42.21-44.63 (C_{16}-C_{19}), 50.54 (C_{15}), 56.24 (OCH_3), 107.11 (C_{22}), 107.23 (C_{27}), 111.47 (C_{25}), 119.48 (C_{23}), 122.51-148.71 (C_1-C_6,C_{10}-C_{11},C_{14},C_{20},C_{24},C_{28}), 150.34 (C_{12}-C_{13}), 153.52 (C_{21}), 164.21 (C_8), 166.17 (C_{32}), 167.25 (C_7), 168.51 (C_9), 176.75 (C_{26}); MS: m/z $[687.20]^+$; Analysis calculated for $C_{34}H_{31}ClFN_7O_6$: C, 59.35; H, 4.54; N, 14.25. Found: C, 59.43; H, 4.60; N, 14.16%.

2.4.8. 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl-4-{6,8-dibromo-4-oxo-3-[*N*-isonicotinamide-yl]-3,4-dihydro-quinazoline-2-ylmethyl}-piperazine-1-yl)-4-oxo,1,4-dihydro-quinoline-3-carboxylic acid 5h. Yield, 67%; m.p. 264-266 ºC; FTIR (KBr, *v* cm^{-1}): 3343 (NH aromatic), 1230 (NH aliphatic), 3049 (C-H aromatic), 2938 (OH), 2871 (C-H aliphatic), 2730 (CH_3), 1863 (CH_2 cyclic), 1722 (C=O of carboxylic acid), 1670 (C=O of amide), 1648 (C=O of quinazolinone), 1326 (C=N), 1267 (N-N), 1154 (C-O-C), 1051 (C-F), 828 (C-C aliphatic); ^{1}H-NMR (400MHz, DMSO-d_6) δ ppm: 0.23-1.26 (m, 4H, cyclopropyl-H), 1.53 (d, 3H, CH_3 of piperazine), 2.34 (s, 2H, CH_2), 3.16-3.40 (m, 8H, piperazine-H and cyclopropyl-H), 3.92 (s, 3H, OCH_3), 6.77-8.16 (m, 8H, Ar-H), 8.58 (s, 1H, NH-CO), 14.47 (s, 1H, COOH); ^{13}C-NMR (100MHz, DMSO-d_6) δ ppm: 8.20 (C_{30},C_{31}), 16.80 (CH_3), 36.67 (C_{29}), 42.24-44.56 (C_{16}-C_{19}), 50.58 (C_{15}), 56.17 (OCH_3), 107.02 (C_{22}), 107.32 (C_{27}), 111.44 (C_{25}), 119.62 (C_{23}), 122.75-148.86 (C_1-C_6,C_{10}-C_{11},C_{14},C_{20},C_{24},C_{28}), 150.33 (C_{12}-C_{13}), 153.59 (C_{21}), 164.31 (C_8), 166.20 (C_{32}), 167.29 (C_7), 168.65 (C_9), 176.73 (C_{26}); MS: m/z $[809.06]^+$; Analysis calculated for $C_{34}H_{30}Br_2FN_7O_6$: C, 50.33; H, 3.73; N, 12.08. Found: C, 50.48; H, 3.80; N, 12.16%.

2.4.9. 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl-4-{6,8-diiodo-4-oxo-3-[*N*-isonicotinamide-yl]-3,4-dihydro-quinazoline-2-ylmethyl}-piperazine-1-yl)-4-oxo,1,4-dihydro-quinoline-3-carboxylic acid 5i. Yield, 57%; m.p. 259-261 ºC; FTIR (KBr, *v* cm^{-1}): 3348 (NH aromatic), 1232 (NH aliphatic), 3046 (C-H aromatic), 2946 (OH), 2879 (C-H aliphatic), 2736 (CH_3), 1864 (CH_2 cyclic), 1723 (C=O of carboxylic acid), 1674 (C=O of amide), 1643 (C=O of quinazolinone), 1324 (C=N), 1264 (N-N), 1153 (C-O-C), 1047 (C-F), 824 (C-C aliphatic); ^{1}H-NMR (400MHz, DMSO-d_6) δ ppm: 0.31-1.24 (m, 4H, cyclopropyl-H), 1.58 (d, 3H, CH_3 of piperazine), 2.35 (s, 2H, CH_2), 3.20-3.43 (m, 8H, piperazine-H and cyclopropyl-H), 3.96 (s, 3H, OCH_3), 6.82-8.11 (m, 8H, Ar-H), 8.52 (s, 1H, NH-CO), 14.50 (s, 1H, COOH); ^{13}C-NMR (100MHz, DMSO-d_6) δ ppm: 8.33 (C_{30},C_{31}), 16.78 (CH_3), 36.62 (C_{29}), 42.30-44.61 (C_{16}-C_{19}), 50.45 (C_{15}), 56.32 (OCH_3), 92.73 (C_4), 97.41 (C_1), 107.18 (C_{22}), 107.38 (C_{27}), 111.51 (C_{25}), 119.46 (C_{23}), 122.45-148.69 (C_3,C_5,C_{10}-C_{11},C_{14},C_{20},C_{24},C_{28}), 150.38 (C_{12}-C_{13}), 151.36 (C_2), 153.52 (C_{21}), 155.78 (C_6), 164.18 (C_8), 166.30 (C_{32}), 167.24 (C_7), 168.58 (C_9), 176.70 (C_{26}); MS: m/z $[905.03]^+$; Analysis calculated for $C_{34}H_{30}I_2FN_7O_6$: C, 45.10; H, 3.34; N, 10.83. Found: C, 45.18; H, 3.42; N, 10.72%.

2.4.10. 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl-4-{6,8-dichloro-4-oxo-3-[*N*-isonicotinamide-yl]-3,4-dihydro-quinazoline-2-ylmethyl}-piperazine-1-yl)-4-oxo,1,4-dihydro-

quinoline-3-carboxylic acid 5j. Yield, 65%; m.p. 279-281 ºC; FTIR (KBr, *v* cm^{-1}): 3342 (NH aromatic), 1240 (NH aliphatic), 3044 (C-H aromatic), 2950 (OH), 2885 (C-H aliphatic), 2736 (CH_3), 1860 (CH_2 cyclic), 1733 (C=O of carboxylic acid), 1667 (C=O of amide), 1649 (C=O of quinazolinone), 1325 (C=N), 1268 (N-N), 1158 (C-O-C), 1034 (C-F), 820 (C-C aliphatic); ^{1}H-NMR (400MHz, DMSO-d_6) δ ppm: 0.22-1.25 (m, 4H, cyclopropyl-H), 1.55 (d, 3H, CH_3 of piperazine), 2.32 (s, 2H, CH_2), 3.13-3.38 (m, 8H, piperazine-H and cyclopropyl-H), 3.86 (s, 3H, OCH_3), 6.84-8.13 (m, 8H, Ar-H), 8.62 (s, 1H, NH-CO), 14.59 (s, 1H, COOH); ^{13}C-NMR (100MHz, DMSO-d_6) δ ppm: 8.22 (C_{30},C_{31}), 16.79 (CH_3), 36.65 (C_{29}), 42.20-44.48 (C_{16}-C_{19}), 50.53 (C_{15}), 56.21 (OCH_3), 107.13 (C_{22}), 107.43 (C_{27}), 111.49 (C_{25}), 119.61 (C_{23}), 122.37-148.74 (C_1-C_6,C_{10}-C_{11},C_{14},C_{20},C_{24},C_{28}), 150.28 (C_{12}-C_{13}), 153.62 (C_{21}), 164.35 (C_8), 166.28 (C_{32}), 167.30 (C_7), 168.61 (C_9), 176.68 (C_{26}); MS: m/z $[721.16]^+$; Analysis calculated for $C_{34}H_{30}Cl_2FN_7O_6$: C, 56.52; H, 4.18; N, 13.57. Found: C, 56.61; H, 4.27; N, 13.43%.

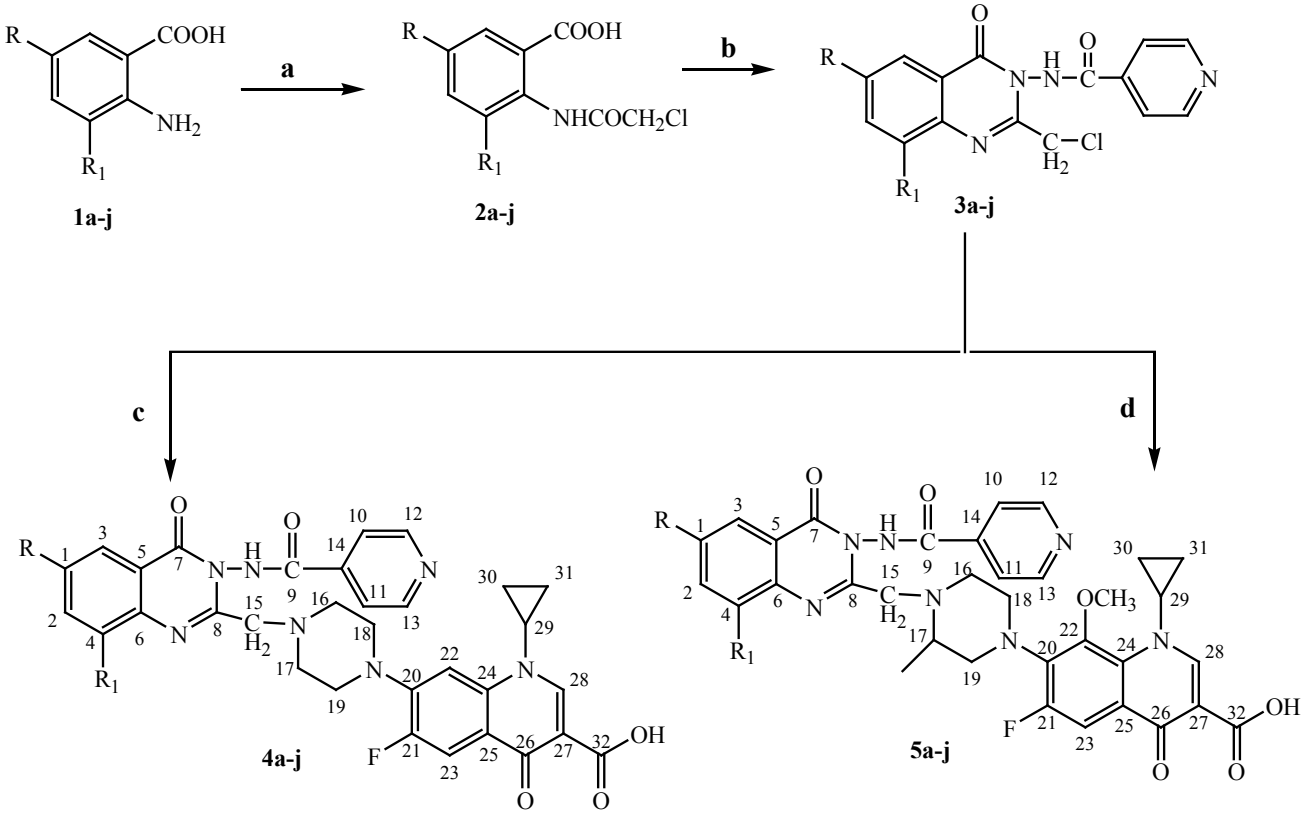
2.5. *In vitro* antimicrobial activity: The MICs of synthesized compounds were carried out by broth micro dilution method as described by Rattan [23]. Antibacterial activity was screened against *Escherichia coli* (MTCC-443), *Pseudomonas aeruginosa* (MTCC-1688), *Klebsiella pneumoniae* (MTCC-109), *Salmonella typhi* (MTCC-98), *Staphylococcus aureus* (MTCC-96), *Streptococcus pyogenes* (MTCC-442) and *Bacillus subtilis* (MTCC-441). Gentamycin, Ampicillin, Chloramphenicol, Ciprofloxacin, Norfloxacin was used as a standard antibacterial agent. Antifungal activity was screened against three fungal species *Candida albicans* (MTCC 227), *Aspergillus niger* (MTCC 282) and *A. clavatus* (MTCC 1323). Nystatin and Greseofulvin was used as a standard antifungal agent. The antimicrobial screening data are shown in **Table 1**.

2.6. *In vitro* antimycobacterial activity: *In vitro* antimycobacterial activity of all the newly synthesized compounds against *Mycobacterium tuberculosis H37Rv* strain was determined by using Lowenstein-Jensen medium [24,25] and the observed MIC of compounds are presented in **Table 2**.

3. RESULTS SECTION

The synthetic protocol used to synthesize the title compounds; **4a–j** and **5a-j** is outlined in **Scheme 1**. *N*-chloroacetyl-substituted-anthranilic acid **2a-j** was synthesized by the chloroacetylation of substituted-anthranilic acid **1a-j** with chloroacetylchloride using dry benzene as solvent. Further the heterocyclisation of **2a-j** is carried using isonicotinic acid hydrazide in presence of dry K_2CO_3, to give 2-chloromethyl-3-[*N*-isonicotinamide-yl]-substituted-quinazolin-4-one **3a-j**. Finally the condensation of compounds **3a-j** with ciprofloxacin and gatifloxacin in presence of sodium bicarbonate gives the desired compounds **4a-j** and **5a-j** respectively. The structure of all the newly synthesized compounds was established by IR, elemental analysis, (^{1}H & ^{13}C)-NMR and Mass spectral data. The structures assigned to **4a-j** were supported by IR spectra showing absorption bands at 3038-3052 cm^{-1} for (C-H aromatic), 1862-1864 cm^{-1} for (CH_2 Cyclic), 1733-1748 cm^{-1} for (C=O of carboxylic acid) and 813-834 cm^{-1} for (C-C aliphatic). ^{1}H-NMR of these compounds revealed the presence of multiplate at δ 0.48-1.34 for (cyclopropyl ring), singlet at δ 2.13-2.36 (CH_2-aliphatic). Further the proton of amide appears as singlet at δ 8.46-8.75 and singlet for COOH appear at δ 14.32-14.52. ^{13}C-NMR spectra of these compounds show the peak at δ 8.12-8.27 for (cyclopropyl ring), δ 50.49-50.63 for (CH_2), δ 153.52-153.70 for (C-F). Carbon of carboxylic acid appear at δ 166.13-166.27 and (C=O) of quinolone ring appear at δ 176.56-176.75. The structure of compound **5a-j** was confirmed by elemental analysis and IR spectra showing absorption band at 3042-3050 cm^{-1} for (C-H aromatic), 2938-2950 cm^{-1} for (OH), 2871-2890 cm^{-1} for (C-H aliphatic), 1722-1740 cm^{-1} for (C=O of carboxylic acid), 1032-1051 cm^{-1} for (C-F) and 821-830 cm^{-1} for (C-C aliphatic).

Further the presence of multiplate at δ 0.22-1.28 for proton of cyclopropyl ring, doublet at δ 1.46-1.60 for (CH_3), singlet at δ 2.32-2.46 for (CH_2-aliphatic), singlet at δ 3.83-3.96 for methoxy proton, singlet at δ 8.52-8.71 for (NH-CO) and singlet at δ 14.47-14.60 (COOH) in ^{1}H-NMR spectra confirmed the assigned structure **5a-j**. ^{13}C-NMR of compound **5a-j** shows the presence of the cyclopropyl ring at δ 8.14-8.33, δ 16.78-16.83 for (CH_3), δ 56.13-56.32 for (OCH_3), δ 153.50-153.62 for (C-F), δ 166.17-166.32 for (COOH) and carbonyl carbon of quinolone ring appear at δ 176.62-176.75 confirmed the structure.



Here,
R = H, Br, I, Cl
R_1 = H, Br, I, Cl

Scheme - 1. Reagents and Condition: (a) $ClCH_2COCl$, pyridine, dry benzene,reflux, 5-6 h; (b) isonicotinic acid hydrazide, dry K_2CO_3, benzene, reflux, 3-4 h; (c) $NaHCO_3$, ciprofloxacine, DMF, reflux, 9-10 h; (d) $NaHCO_3$, gatifloxacine, DMF, reflux, 12-13 h

***In vitro* antimicrobial activity:** The results of *in vitro* antimicrobial activities (MIC) of compounds **4a-j** and **5a-j** against various bacterial and fungal strains are summarized in **Table 1**. From *in vitro* antibacterial activity data, it is confirmed that compound **4b** and **4e** with bromo substituent showed excellent antibacterial activity against almost all tested bacterial strains while compound **5b** (6-bromo) displayed excellent antibacterial activity against all tested Gram negative strains and compound **5e** (8-bromo) exhibited the highest activity against all tested Gram positive strains. Compound **4d** with 6-chloro substituent displayed highest activity against *Kl. pneumoniae*. Other compounds are found to have good to moderate activity against all tested antibacterial strains. The *in vitro* antifungal activity data suggested that compound **4b** (6-bromo) and **4e** (8-bromo) substituent exhibited excellent antifungal activity while compounds **5b** (6-bromo), **5e** (6-bromo), **4d** (6-chloro)

and **4g** (8-chloro) showed good antifungal activity and others are exhibiting good to moderate antifungal activity.

The result indicates that the *in vitro* antimicrobial activity was more enhanced by bromo substitution; the reason behind it may be due optimal lipophilicity [24, 26-28] than by iodine and chlorine substitution. The order of *in vitro* antimicrobial activity of compound is 4b=4e>5e=5b>4d=4g>5d=5g>4f=4c>5f=5c>4j>4i>4h>4a>5i>5j>5h>5d. It was also observed that compounds with ciprofloxacin moiety are more active against *M. tuberculosis* than corresponding gatifloxacin derivatives.

Table 1. *In vitro* antimicrobial activity (MIC µg/ml) of the new compounds **4a-j** and **5a-j**

Entry	R	R_1	R_2	R_3	*E.c.*	*P.a.*	*Kl.p*	*S.t.*	*S.a.*	*Str.p.*	*B.s.*	*C.a.*	*A.n.*	*A.c.*
4a	H	H	H	H	250	200	250	250	200	250	200	500	1000	>1000
4b	Br	H	H	H	**50**	62.5	100	**50**	62.5	**50**	**50**	**200**	**200**	**250**
4c	I	H	H	H	150	125	100	100	125	100	125	250	500	500
4d	Cl	H	H	H	62.5	100	**50**	62.5	62.5	100	62.5	250	250	250
4e	H	Br	H	H	62.5	**50**	100	**50**	**50**	62.5	**50**	**200**	**250**	**200**
4f	H	I	H	H	125	100	100	125	125	150	100	500	250	500
4g	H	Cl	H	H	**50**	62.5	100	62.5	100	62.5	62.5	250	250	250
4h	Br	Br	H	H	250	200	250	250	200	200	150	1000	500	1000
4i	I	I	H	H	200	250	150	200	200	150	200	500	500	1000
4j	Cl	Cl	H	H	200	150	200	200	150	250	200	500	1000	500
5a	H	H	CH_3	OCH_3	500	250	500	500	500	250	250	>1000	>1000	1000
5b	Br	H	CH_3	OCH_3	**50**	100	62.5	100	**50**	**62.5**	**62.5**	**200**	250	250
5c	I	H	CH_3	OCH_3	150	100	125	150	150	125	125	500	1000	250
5d	Cl	H	CH_3	OCH_3	125	100	100	125	125	150	100	250	250	500
5e	H	Br	CH_3	OCH_3	62.5	100	**50**	100	**62.5**	**50**	**62.5**	250	200	250
5f	H	I	CH_3	OCH_3	150	150	125	100	150	125	100	250	500	1000
5g	H	Cl	CH_3	OCH_3	100	125	100	100	125	125	150	500	250	250
5h	Br	Br	CH_3	OCH_3	250	500	250	500	250	250	200	>1000	1000	>1000
5i	I	I	CH_3	OCH_3	200	250	250	500	250	500	200	1000	1000	1000
5j	Cl	Cl	CH_3	OCH_3	250	250	250	500	250	500	250	1000	1000	>1000
Gentamycin					0.05	1	0.05	1	0.25	0.5	-	-	-	-
Ampicilin					100	100	100	100	250	100	-	-	-	-
Chloramphenicol					50	50	50	50	50	50	-	-	-	-
Ciprofloxacin					25	25	25	25	50	50	-	-	-	-
Norfloxacin					10	10	10	10	10	10	-	-	-	-
Nystatin					-	-	-	-	-	-	-	100	100	100
Greseofulvin					-	-	-	-	-	-	-	500	100	100

E.c., *E. coli* (MTCC 443); *P.a.*, *P. aeruginosa* (MTCC 1688); *Kl.p.*, *Kl. pneumoniae* (MTCC109); *S.t., S. typhi* (MTCC98); *S.a.*, *S. aureus* (MTCC 96); *S.p., Str. pyogenes* (MTCC 442); *B.s.*, *B. subtilis* (MTCC 441); *C.A.*, *C. albicans* (MTCC 227); *A.N.*, *A. niger* (MTCC 282); *A.C.*, *A. clavatus* (MTCC 1323).

***In vitro* antimycobacterial activity:** The preliminary screening of the synthesized compounds against *Mycobacterium tuberculosis $H_{37}Rv$* is summarized in **Table 2**. Compound **4b** and **4e** containing 6-bromo and 8-bromo substituent showed excellent activity (50 µg/ml) and compounds **5b** and **5e** showed good activity (62.5 µg/ml), where as other compounds displayed moderate to good activity with 60-99% inhibition at the concentration of (100-500 µg/ml).

Table 2. *In vitro* antitubercular activity of the compounds **4a-j** and **5a-j**

Entry	*M. tuberculosis $H_{37}Rv$* (MIC µg/ml) *(MTCC 200)*	% Inhibition	clogP*
4a	250	90	-1.61
4b	**50**	**96**	-0.68
4c	150	91	-0.42
4d	100	93	-0.83
4e	**50**	**94**	-0.68
4f	150	90	-0.42
4g	100	93	-0.83
4h	200	75	0.19
4i	200	80	0.71
4j	200	84	-0.10
5a	500	68	-1.15
5b	**62.5**	**93**	-0.22
5c	200	90	0.03
5d	100	89	-0.37
5e	**62.5**	**92**	-0.22
5f	200	91	0.03
5g	100	88	-0.37
5h	500	82	0.65
5i	250	89	1.17
5j	250	71	0.35
Rifampicin	40	98	6.04
Isoniazid	0.20	99	-0.60

*Theoretical values of log P were calculated using commercially available ChemDraw program.

4. CONCLUSIONS

Quinazolinone-fluoroquinolone hybrids were synthesized and characterized for their structure elucidation. Various chemical and spectral data supported the structures thought of. Antimicrobial studies of these compounds indicated that compounds were found to exhibit good to moderate activity against some bacteria compared to standard antibiotic drugs. Among all the newly synthesized compounds, compound **4b** and **4e** is showing excellent antituberculosis effect. It was also concluded that ciprofloxacin derivatives **(4a-j)** are more potent than corresponding gatifloxacin derivatives **(5a-j).** The importance of such work lies in the possibility that the new compounds might be a more efficient drug against bacteria, mycobacteria and fungi, for which a thorough exploration

regarding the structure activity relationship, toxicity and its biological effects is essential, which is underway in our lab.

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