Synthesis and *in vitro* Antibacterial, Antitubercular and Cytotoxicity Evaluation of Lomefloxacin Derivatives

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ABSTRACT

Introduction: The fluoroquinolones antibacterial agents are one of the fastest growing groups of drugs in recent years. The various side chains on it can be altered and the resulting analogues are evaluated for their anti-microbial and antitubercular properties. Most of these agents are substituted at the 7 positions by nitrogen heterocycles. Lomefloxacin at C-7, which represents a site amenable to significant modification.

Objective: Based on evidence of research results and in search of new bioactive molecules in the fluoroquinolones, a of N-substituted piperazinyl quinolones have been designed, synthesized, characterized and evaluated for their antibacterial activity and antitubercular activity.

Method: A series of 2-((5-chloro-1, 3, 4-thiadiazol-2-yl) thio)-1-(4-subst.) ethanone (4a-4j) were prepared by diazotization of amines (3a-3j) in concentrated HCl in the presence of Cu-powder. The reaction of (4a-4j) with piperazinyl quinolone (lomefloxacin) in DMF yield (5a-5j). The synthesized compounds were evaluated against some Gram-positive and Gram-negative bacteria and antitubercular activity against Mtb WT H37Rv.

Result: The structure of the synthesized compound was confirmed by their IR, ¹H NMR, data. The antibacterial data revealed that all substituted derivatives (5a to 5j), are found to be least active against Gram-positive and Gram-negative organisms. Among all of the tested compounds, 5b (Lomefloxacin derivative) exhibited excellent antitubercular activity against Mtb WT H37Rv (MIC 0.8 µg/ml) which is comparable to that of standard. (MIC 0.8 µg/ml)

Conclusion: Although the nature of the C-7 substituent is known to enhance quinolone activity in bacteria but results of the present study reveal that the synthesized derivative shows significant antitubercular property but poor antibacterial activity.

Key Words: Antibacterial activity, Antitubercular activity, Fluoroquinolone, Lomefloxacin, N-piperazinyl quinolone, Synthesis

INTRODUCTION

Fluoroquinolones, a major class of antibiotics, are under clinical development. The antibacterial activity of Fluoroquinolones is due to the inhibition of bacterial enzymes; DNA-gyrase and topoisomerase IV. They have potent activity, rapid bactericidal effects, and a low prevalence of resistance development.¹ The fluoroquinolones exert certain adverse effects, have restricted activity against Gram-positive pathogens and methicillin-resistant *Staphylococcus aureus* (MRSA).² Therefore, there is a need of synthesizing novel quinolones with better activity profile, pharmacokinetics, and acceptability, to overcome the limitations

of existing drugs.³ Most of the quinolone antibacterial research has been focused on substitution at the C-7 as it is the most adaptable site for chemical change. C-7 position is an area that determines potency and target preference and also controls the pharmacokinetic properties of the drugs, with basic nitrogen.⁴⁻⁶ The most commonly found substitution at the C-7 position is a five- or six-membered ring. For example, aminopyrrolidine substituent at C-7 in trovafloxacin and gemifloxacin and Piperazine substitution at the C-7 position in norfloxacin, ciprofloxacin, pefloxacin, pefloxacin, ofloxacin, amifloxacin, fleroxacin, lomefloxacin, sparfloxacin, difloxacin, enoxacin, enrofloxacin, levofloxacin, marbofloxacin, and orbifloxacin which has triggered a

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wide range of clinically useful fluoroquinolone antibacterial agents.⁷⁻¹⁷ (Figure 1) The site near the C-7 substituent is regarded as the domain for drug–enzyme interaction and the cell permeability.¹⁸⁻²¹ The piperazine moiety of 7-piperazinyl quinolones possesses enough structural flexibility to allow product optimization. In the present study, we have aimed to achieve a better antimicrobial profile at a lower concentration, by preparing [(7-(4--(5-substituted-benzoylthio)-1,3,4-thiadiazol-2-yl)-3-methylpiperazine-1-yl)-1-ethyl-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid](5a to 5j) derivatives and have been evaluated for its *in vitro* antibacterial and anti-tubercular effect. (Figure 2).

MATERIALS AND METHODS

Materials

All the chemicals, reagents, and solvents used in this research were bought from E Merck Ltd, Loba chemicals Ltd, Sigma-Aldrich Ltd., Spectrochem Ltd., Hi-media, and Rankem Chemicals Ltd. Mumbai, India. Solvents used were dried and purified as and when required. The melting points reported were uncorrected and were determined in open capillaries using Thiele's melting point apparatus and measured in (°C). The yields of synthesized compounds were mentioned in tables along with respective physical constants. The FT-IR spectra were obtained Shimadzu FTIR spectrophotometer and values were measured in cm^{-1} (potassium bromide disks). ¹H NMR and ¹³C NMR were recorded at 400 MHz and 100 MHz respectively on a Bruker AM spectrometer, IISc Bangalore, and chemical shifts are expressed as δ (ppm) with tetramethylsilane as an internal standard. The FAB / EIMS mass spectra were recorded on Autospec Mass spectrometer, ICT, Hyderabad.

Methods

General Procedure for Synthesis of 2(a–j) (Figure 3)

Synthesis of substituted/unsubstituted phenacyl bromide 2(a–j)

0.1 mol of substituted/un-substituted acetophenones 1(a–j) were taken in the two-necked round bottom flask, suitable anhydrous solvents (ether, acetone, methanol, chloroform) was added with anhydrous AlCl_3 . The reaction condition was kept up either in cold or at room temperature and bromine (0.09 mol) was added with stirring. Mixtures 2(a–j) were acquired as colourless to brown to shining crystals. The product was washed twice with appropriate solvents and recrystallized from methanol to get lachrymatory crystals.²²⁻²³ Liquefying point ranges of 2a–j; R = H, Cl, Br, F, NO_2 , CH_3 , OCH_3 , NH_2 , OH, C_6H_5 ; 48–50°, 90–92°, 110–112°, 46–48, 96–98°, 52–54°, 72–74°, 80–84°, 102–104, 98–102 respectively (50–74 %).

General Procedure for Synthesis of 3(a–j). (Figure 3)

Synthesis of 2-((amino-1,3,4-thiadiazol-2-yl)thio)-1-(4-subst.) ethanone 3 (a–j).

The 2-amino-5-mercapto-1,3,4-thiadiazole (0.1 mol) was suspended in 15 ml of water and 80% potassium hydroxide (0.1 mol) was added. This solution was de-colored with activated charcoal, followed by the addition of 32 mL of ethanol and stirred rapidly with 2 (a–j) (0.1 mol). The reaction mixture was cooled for 40 minutes and it added 200 mL of cold water. It is then filtered to obtain the solid product and washed with ether and water. The 3(a–j) were obtained (Scheme 1), with 54–68% yield and melting point (80–108°C).²⁴⁻²⁵

General Procedure for Synthesis of 4(a–j). (Figure 3)

Procedure for Synthesis of 2-((5-chloro-1, 3, 4-thiadiazol-2-yl) thio)-1-(4-subst.)ethanone 4 (a–j)

Triturated 2-((amino-1,3,4-thiadiazol-2-yl) thio)-1-(4-subst.) ethanone 3(a–j) (30 mmol) with sodium nitrite (60 mmol). The triturate was introduced in the ice-cooled (0–5°C) mixture of 15 ml water and 30 ml concentrated HCl with stirring in the presence of copper powder. The product was refluxed for 1 hour at 75°C and cool. Then the mixture was extracted thrice with dry chloroform (75 ml). The combined extracts of chloroform were washed with a sodium bicarbonate solution. Then the solution was dried over sodium sulphate followed by evaporation under reduced pressure. Finally, recrystallization of the product was done using ethanol to yield 2-((5-chloro-1, 3, 4-thiadiazol-2-yl)thio)-1-(4-subst) ethanone 4 (a–j) (Scheme 1). The compound was purified by column chromatography with methanol: chloroform (1:9) as mobile phase²⁶, m.p. 85–110°C (48–60%).

General Procedure for Synthesis of 5(a–j). (Figure 3)

Synthesis of 1-subst.-6-fluoro-8-subst.-7-(3-subst.-4-(5-subst.((2-oxo-2-(p-subst.)ethyl)thio)-1,3,4-thiadiazol-2-yl) piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 5(a–j).

A combination of equimolar quantities of compound 2-((5-chloro-1, 3, 4-thiadiazol-2-yl)thio)-1-(4-subst.) ethanone 4(a–j) and piperazinyl fluoroquinolone (sparfloxacin), along with sodium-bicarbonate in 10 ml dimethyl-formamide was refluxed on an oil bath at 140–160°C for hrs. After cooling the reaction mixture, 10 ml of cold water was added to it. The precipitated product was filtered and washed with water. The product was then subjected to recrystallization using a blend of dimethylformamide and water to yield (5a–j) compounds.²⁷⁻²⁸ (Scheme 1). The Physicochemical results are shown in (Table 1)

Antibacterial Activity

Preliminary *in vitro* antibacterial activity was employed by the broth micro-dilution technique. Antibacterial Activity

was examined against two Gram-negative microorganisms, *Pseudomonas aeruginosa* and *Escherichia coli*, and two Gram-positive microorganisms, *Staphylococcus aureus* and *Bacillus subtilis*. The test compounds and reference drugs (Sparfloxacin and Rifampicin) were prepared in Mueller-Hinton agar medium by two-fold serial dilutions. The required concentrations of 0.5, 1.0, 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5, and 20.0 µg/ml was obtained by Progressive double dilutions with agar. The Petri plates were inoculated with $1-5 \times 10^4$ colonies forming units (CFU/ml) and incubated at 37°C for 18 hours.²⁹ The results are presented in (Table 2).

Anti-tubercular Activity

In vitro screening for anti-mycobacterial was performed by utilizing *M. tuberculosis* virulent H37Rv strain. The broth dilution assay for each drug for determination of MIC was determined by using the frozen culture of Middlebrook 7H9 broth supplemented with 10% ADC (albumin dextrose catalase) and 0.2% glycerol. It is used as inoculum with dilution in broth to 2×10^5 CFU/ml. In the assay, for the accommodation of compounds U-tubes were used in 0.1, 0.5, 1.5, 2.5, 05, 7.5, 10, 12.5, 15, 17.5 and 20 µg/ml dilutions.³⁰⁻³¹ The results are presented in (Table 2).

In-vitro cytotoxic study

Estimation of cell viability

Conversion of MTT [(3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrasodium bromide)] to dark blue formazan crystals due to the presence of living cells, was employed for estimation of cell viability. Colourimetric analysis was used for the estimation of MTT cleaved to the viable cells. The solution of compounds under investigation in DMSO was diluted to achieve test concentrations. The DMSO content was maintained below 0.1% in all the aliquots under investigation. The cultured Hep-G2 normal liver-cell lines were added in plates with 96 wells and then preserved with variable dilutions of investigational compounds in DMSO, at 37°C in a carbon dioxide incubator for four days. Further, the MTT reagent was instilled into the wells and incubated for four hours, and then the dark blue formazan developed was allowed to dissolve in DMSO and the colourimetric absorbance was read at 550 nm. The IC_{50} value was estimated by graph plotted between percentage cells inhibited versus concentrations.³² The findings are provided in (Table 2).

RESULTS

Spectral Data of synthesized compound

1-ethyl-6,8-difluoro-7-(3-methyl-4-(5-((2-oxo-2-phenylethyl)thio)-1,3,4-thiadiazol-2-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5a)

IR (KBr) cm^{-1} : 3422(carboxylic, O-H *str.*), 2943(Ar. C-H *str.*), 2856(Alk. CH₂ C-H *str.*), 1716(carboxylic, C=O *str.*), 1642(ketonic, C=O *str.*), 1588(Imine, C=N *str.*), 1320(ethylylic, C-H *str.*); ¹H-NMR (DMSO-*d*₆) δ ppm: 12.52(s, carboxylic, 1H, OH), 9.02(s, 1H, H2-quinoline), 7.56-7.94(m, 5H, Ar.), 7.58(s, 1H, H5-quinoline), 4.92(s, 2H, CH₂), 4.64(q, 2H, NCH₂CH₃), 2.92-3.50(m, 7H, piperazinyl), 1.38(t, 3H, NCH₂CH₃), 1.29 (s, 3H, piperazinyl CH₃).

7-(4-(5-((2-(4-chlorophenyl)-2-oxoethyl)thio)-1,3,4-thiadiazol-2-yl)-3-methylpiperazin-1-yl)-1-ethyl-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5b)

IR (KBr) cm^{-1} : 3445(carboxylic, O-H *str.*), 3005(Ar. C-H *str.*), 2852(Alk. CH₂ C-H *str.*), 1725(carboxylic, C=O *str.*), 1656(ketonic, C=O *str.*), 1583(Imine, C=N *str.*), 1307(ethylylic, C-H *str.*); ¹H-NMR (DMSO-*d*₆) δ ppm: 12.55(s, carboxylic, 1H, OH), 8.91(s, 1H, H2-quinoline), 7.60-7.92(m, 4H, Ar.), 7.42(s, 1H, H5-quinoline), 4.84(s, 2H, CH₂), 4.57(q, 2H, NCH₂CH₃), 2.94-3.47(m, 7H, piperazinyl), 1.46(t, 3H, NCH₂CH₃), 1.33(s, 3H, piperazinyl CH₃); ¹³C-NMR (DMSO-*d*₆) δ ppm: 198, 180, 161, 158, 148, 144, 130, 118, 110, 68, 40, 18; MS: *m/z* = 619 [M⁺]; CHN calcd; C₂₇H₂₄ClF₂N₅O₄S₂; C, 52.30; H, 3.90; N, 11.29; Found C, 52.34; H, 3.90; N, 11.30.

7-(4-(5-((2-(4-bromophenyl)-2-oxoethyl)thio)-1,3,4-thiadiazol-2-yl)-3-methylpiperazin-1-yl)-1-ethyl-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5c)

IR (KBr) cm^{-1} : 3444(carboxylic, O-H *str.*), 3009(Ar. C-H *str.*), 2850(Alk. CH₂ C-H *str.*), 1728(carboxylic, C=O *str.*), 1625(ketonic, C=O *str.*), 1554(Imine, C=N *str.*), 1310(ethylylic, C-H *str.*); ¹H-NMR (DMSO-*d*₆) δ ppm: 12.58(s, carboxylic, 1H, OH), 8.93(s, 1H, H2-quinoline), 7.61-7.94(m, 4H, Ar.), 7.44(s, 1H, H5-quinoline), 4.80(s, 2H, CH₂), 4.59(q, 2H, NCH₂CH₃), 2.93-3.48(m, 7H, piperazinyl), 1.48(t, 3H, NCH₂CH₃), 1.31(s, 3H, piperazinyl, CH₃); ¹³C-NMR (DMSO-*d*₆) δ ppm: 199, 181, 165, 160, 149, 128, 120, 108, 70, 42, 17.

5.4 7-(4-(5-((2-(4-fluorophenyl)-2-oxoethyl)thio)-1,3,4-thiadiazol-2-yl)-3-methylpiperazin-1-yl)-1-ethyl-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5d)

IR (KBr) cm^{-1} : 3454(carboxylic, O-H *str.*), 2926(Ar. C-H *str.*), 2853(Alk. CH₂ C-H *str.*), 1726(carboxylic, C=O *str.*), 1658(ketonic, C=O *str.*), 1584(Imine, C=N *str.*), 1327(ethylylic, C-H *str.*); ¹H-NMR (DMSO-*d*₆) δ ppm: 12.68(s, carboxylic, 1H, OH), 8.94(s, 1H, H2-quinoline), 7.98-8.02(m, 4H, Ar.), 7.78(s, 1H, H5-quinoline), 4.59(s, 2H, CH₂), 4.42(q, 2H, NCH₂CH₃), 2.50-3.50(m, 7H, piperazinyl), 1.48(t, 3H, NCH₂CH₃), 1.25(s, 3H, piperazinyl CH₃); ¹³C-NMR(DMSO-*d*₆) δ ppm: 197, 173, 148, 133, 117,

107, 98, 74, 48, 38, 37; MS: $m/z = 602$ [M^+]; CHN calcd; $C_{27}H_{24}F_3N_5O_4S_2$; C, 53.72; H, 4.01; N, 11.60; Found C, 54.12; H, 3.98; N, 11.94.

7-(4-(5-((2-(4-nitrophenyl)-2-oxoethyl)thio)-1,3,4-thiadiazol-2-yl)-3-methylpiperazin-1-yl)-1-ethyl-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5e)

IR (KBr) cm^{-1} : 3441(s, carboxylic, O-H *str.*), 3006(Ar. C-H *str.*), 1374(Ali. CH_2 C-H *str.*), 1728(carboxylic, C=O *str.*), 1624(ketonic, C=O *str.*), 1547(Imine, C=N *str.*), 1311(ethylic, C-H *str.*); 1H -NMR (DMSO- d_6) δppm : 12.56(s, 1H, carboxylic, OH), 8.92(s, 1H, H2-quinoline), 7.74-7.92(m, 4H, Ar.), 7.47(s, 1H, H5-quinoline), 4.83(s, 2H, CH_2), 4.65(q, 2H, NCH_2CH_3), 2.92-3.47(m, 7H, piperazinyl), 1.47(t, 3H, NCH_2CH_3), 1.38(s, 3H, piperazinyl CH_3); ^{13}C -NMR (DMSO- d_6) δppm : 192, 183, 165, 155, 147, 138, 129, 113, 74, 47, 11; MS : $m/z = 630$ [M^+]; CHN calcd; $C_{27}H_{24}F_2N_6O_5S_2$; C, 51.42; H, 3.84; N, 13.33; Found C, 51.40; H, 3.85; N, 13.30.

1-ethyl-6,8-difluoro-7-(3-methyl-4-(5-((2-oxo-2-(p-tolyl)ethyl)thio)-1,3,4-thiadiazol-2-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5f)

IR (KBr) cm^{-1} : 3427(s, carboxylic, O-H *str.*), 2934(Ar. C-H *str.*), 2854(Ali. CH_2 C-H *str.*), 1710(carboxylic, C=O *str.*), 1623(ketonic, C=O *str.*), 1576(Imine, C=N *str.*), 1298(ethylic, C-H *str.*); 1H -NMR (DMSO- d_6) δppm : 12.06(s, 1H, carboxylic, OH), 8.84(s, 1H, H2-quinoline), 7.54-7.68(m, 4H, Ar.), 7.38(s, 1H, H5-quinoline), 4.64(s, 2H, CH_2), 4.32(q, 2H, NCH_2CH_3), 2.83-3.27(m, 7H, piperazinyl), 2.37(s, 3H, tolyl), 1.32(t, 3H, NCH_2CH_3), 1.19(s, 3H, piperazinyl CH_3); ^{13}C -NMR (DMSO- d_6) δppm : 188, 179, 158, 147, 135, 128, 116, 108, 68, 35, 16, 7.

7-(4-(5-((2-(4-methoxyphenyl)-2-oxoethyl)thio)-1,3,4-thiadiazol-2-yl)-3-methylpiperazin-1-yl)-1-ethyl-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5g)

IR (KBr) cm^{-1} : 3445(carboxylic, O-H *str.*), 3011(Ar. C-H *str.*), 2851(Ali. CH_2 C-H *str.*), 1728(carboxylic, C=O *str.*), 1634(ketonic, C=O *str.*), 1581(Imine, C=N *str.*), 1310(ethylic, C-H *str.*); 1H -NMR (DMSO- d_6) δppm : 12.83(s, carboxylic, 1H, OH), 9.04(s, 1H, H2-quinoline), 7.14-7.98(m, 4H, Ar.), 7.84(s, 1H, H5-quinoline), 4.71(s, 2H, CH_2), 4.37(q, 2H, NCH_2CH_3), 3.83(s, 3H, methoxyl), 2.92-3.67(m, 7H, piperazinyl), 1.31(t, 3H, NCH_2CH_3), 1.11(s, 3H, piperazinyl CH_3); ^{13}C -NMR (DMSO- d_6) δppm : 191, 184, 168, 156, 138, 129, 112, 108, 60, 38, 18, 11; MS: $m/z = 617$ [$M+1$]; CHN calcd; $C_{28}H_{27}F_2N_5O_5S_2$; C, 54.62; H, 4.42; N, 11.38; Found C, 54.64; H, 4.40; N, 11.37.

7-(4-(5-((2-(4-aminophenyl)-2-oxoethyl)thio)-1,3,4-thiadiazol-2-yl)-3-methylpiperazin-1-yl)-1-ethyl-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5h)

IR (KBr) cm^{-1} : 3452(carboxylic, O-H *str.*), 3367(Ar. NH_2 , N-H *str.*), 3027(Ar. C-H *str.*), 2851(Ali. CH_2 C-H *str.*), 1736(carboxylic, C=O *str.*), 1628(ketonic, C=O *str.*), 1580(Imine, C=N *str.*), 1327(ethylic, C-H *str.*); 1H -NMR (DMSO- d_6) δppm : 12.88(s, carboxylic, 1H, OH), 9.12(s, 1H, H2-quinoline), 7.86(s, 1H, H5-quinoline), 6.83-7.72(m, 4H, Ar.), 6.31(s, 2H, Ar. NH_2), 4.72(s, 2H, CH_2), 4.32(q, 2H, NCH_2CH_3), 3.02-3.62(m, 7H, piperazinyl), 1.29(t, 3H, NCH_2CH_3), 1.14(s, 3H, piperazinyl CH_3); ^{13}C -NMR (DMSO- d_6) δppm : 197, 187, 172, 151, 133, 123, 117, 107, 67, 35, 16; MS: $m/z = 601$ [$M+1$]; CHN calcd; $C_{27}H_{26}F_2N_6O_4S_2$; C, 53.99; H, 4.36; N, 13.99; Found C, 54.02; H, 4.37; N, 13.98.

7-(4-(5-((2-(4-hydroxyphenyl)-2-oxoethyl)thio)-1,3,4-thiadiazol-2-yl)-3-methylpiperazin-1-yl)-1-ethyl-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5i)

IR (KBr) cm^{-1} : 3544(phenolic, O-H *str.*), 3410(carboxylic, O-H *str.*), 2973(Ar. C-H *str.*), 2850(Ali. CH_2 C-H *str.*), 1723(carboxylic, C=O *str.*), 1662(ketonic, C=O *str.*), 1575(Imine, C=N *str.*), 1322(ethylic, C-H *str.*); 1H -NMR (DMSO- d_6) δppm : 12.62(s, carboxylic, 1H, OH), 8.99(s, 1H, H2-quinoline), 7.79(s, 1H, H5-quinoline), 6.81-7.77(m, 4H, Ar.), 5.35(s, 1H, phenolic), 4.73(s, 2H, CH_2), 4.37(q, 2H, NCH_2CH_3), 2.94-3.37(m, 7H, piperazinyl), 1.23(t, 3H, NCH_2CH_3), 1.08(s, 3H, piperazinyl CH_3); ^{13}C -NMR (DMSO- d_6) δppm : 193, 182, 175, 154, 130, 121, 115, 110, 71, 37, 18.

7-(4-(5-((2-([1,1'-biphenyl]-4-yl)-2-oxoethyl)thio)-1,3,4-thiadiazol-2-yl)-3-methylpiperazin-1-yl)-1-ethyl-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5j)

IR (KBr) cm^{-1} : 3462(carboxylic, O-H *str.*), 3084(Ar. C-H *str.*), 2852(Ali. CH_2 C-H *str.*), 1733(carboxylic, C=O *str.*), 1656(ketonic, C=O *str.*), 1566(Imine, C=N *str.*), 1337(ethylic, C-H *str.*); 1H -NMR (DMSO- d_6) δppm : 13.04(s, carboxylic, 1H, OH), 9.10(s, 1H, H2-quinoline), 7.88(s, 1H, H5-quinoline), 7.75-8.00(m, 4H, Ar.), 7.41-7.52(m, 5H, Ar.), 4.74(s, 2H, CH_2), 4.53(q, 2H, NCH_2CH_3), 2.92-3.46(m, 7H, piperazinyl), 1.27(t, 3H, NCH_2CH_3), 1.11(s, 3H, piperazinyl CH_3); ^{13}C -NMR (DMSO- d_6) δppm : 197, 184, 179, 158, 133, 127, 121, 106, 65, 39, 21, 9; MS: $m/z = 661$ [M^+]; CHN calcd; $C_{33}H_{29}F_2N_5O_4S_2$; C, 59.90; H, 4.42; N, 10.58; Found C, 59.92; H, 4.41; N, 10.60.

DISCUSSION

Chemistry

The synthesis of p-substituted phenacyl bromide 2(a-j) formation mechanism carried out by substitution acetophenone, acetic acid, and liquid bromine, reaction mechanism followed via acetic acid-mediated H^+ ions rather than lewis acid. Probably this reaction mechanism is followed by the bromination of the methyl group and it can be restricted to mono substitution, when the reaction is carried out in acidic media in presence of anhydrous $AlCl_3$.³³ The synthesis of 2-amino-5-benzoylmethylenethio-1,3,4-thiadiazole 3(a-j) was carried out by reacting 2(a-j) and 2-amino-5-mercapto-1,3,4-thiadiazole via de-hydro-bromination mechanisms. In the reaction alkali hydroxyl ion abstract the mercapto proton, not the amino proton, because mercapto group having strong electron density centre on sulfur rather than amino nitrogen atom although nitrogen has strong electronegative element than sulfur electron density more on sulfur hence, the probability to abstract the proton of the mercapto group by hydroxyl ion is more where finding more electron density centre. Compounds 3(a-j) were directly converted to 2-chloro-5-benzoylmethylenethio-1,3,4-thiadiazole 4(a-i) by diazotization of amines followed by chlorination, which obeyed Sandmeyer reaction. This reaction is considered to be a more convenient method for introducing a halogen substituent at the desired position of an aromatic ring. The synthesis of [(7-(4--(5-sustituted-benzoylthio)-1,3,4-thiadiazol-2-yl)-3-methyl piperazine-1-yl)-1-ethyl-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid] 5(a-j) was based on aromatic nucleophilic substitution reaction mechanism involving substrate as 2-chloro-5-benzoylmethylenethio-1,3,4-thiadiazole 4(a-j) and fluoroquinolones with sodium bicarbonate in presence of N, N-dimethylformamide, (DMF) which seemed to be a convenient route to execute the synthesis of title compounds.³⁴

Microbiological assay 5(a-j)

Antibacterial activity

The synthesized compounds 5 (a-j) explored *In-vitro* antibacterial activity against two Gram-positive and two Gram-negative strains i.e. *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*, *Pseudomonas aeruginosa* respectively to determine the MICs, which gave profound conclusion about the most active analogues. As per results depicted in compounds, classified the active and non-active analogues based on screened standard drug's MICs. According to that, 1.33 to 2.50 categorized highly active; 2.50-10.0 considered to be moderately active and >10.0 deemed to be least active when compared with that of standards gatifloxacin.

Largely, most of the synthesized derivatives were found progressive to moderate activity against the Gram-positive bacterial strains of the lomefloxacin series. However, methyl

and methoxy substituted (9f & 9g) of lomefloxacin derivatives were found to be the least active (15.33 to 17.00 $\mu g/ml$) against gram-positive bacterial strains of *Staphylococcus aureus* and *Bacillus subtilis*. Apart from that, none of the derivatives was found highly active against *Escherichia coli* and *Pseudomonas aeruginosa* gram-negative strains.

Antitubercular activity

The derivatives were screened for *In-vitro* antitubercular against *H₃₇Rv* strain, using micro Alamar blue reagent, in this assay, blue colour converted into red colour indicates the test compound would be non-active up to that particular concentration. This assay has conducted based on BACTEC radiometric method. Microplate Alamar blue assay (MABA) has certain advantages over the conventional disc diffusion method³⁵. In this assay, eight double-fold serially diluted concentrations were taken between 0.8 to 100 $\mu g/ml$. In which (-Cl) substituted of lomefloxacin 9b derivatives were found to be an excellent activity (0.8 $\mu g/ml$) which is comparable to that of standards (0.8 $\mu g/ml$).

In-vitro toxicity study

The potent antitubercular agents were subjected to toxicity study with the help of MTT assay on Hep-G2 normal liver cell line. The value of PI is unitless and a higher value is considered to be the most efficient agent. The compound 5b (-Cl substituted derivative) shown PI value 68.75, indicated more toxic as compared to reference rifampicin as its PI value was 81.25. Hence these derivatives have not secured the safe candidate, as the PI value is very low as compared to reference Rifampicin. The present nucleus permits various modifications at the site of the desire position to obtain more derivatives with improve pharmacokinetics and pharmacodynamics profiles.

CONCLUSIONS

C-7 position of fluoroquinolone nucleus permits the bulkier substitutions as Benzoylmethylenethio-1, 3, 4-thiadiazole (5 a-j) provide satisfactory yields by aromatic nucleophilic substitution reaction (SN^2). The substituted derivatives of fluoroquinolone were moderate to least active against Gram-positive bacteria and *M. tuberculosis* pathogen. However, all the synthesized derivatives did not respond to the gram-negative strains. So that, these practical results offering numerous modifications in the same scaffold, to develop the new chemical entity.

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Table 1: Physicochemical data of synthesized compound (5a-5j)

Compound No	R	Nature	Molecular formula	Molecular mass	Melting point (°C)	Yield (%)
5a	-H	Amorphous	C ₂₇ H ₂₅ F ₂ N ₅ O ₄ S ₂	585	215	54
5b	-Cl	Amorphous	C ₂₇ H ₂₄ F ₂ ClN ₅ O ₄ S ₂	619	245	50
5c	-Br	Amorphous	C ₂₇ H ₂₄ F ₂ BrN ₅ O ₄ S ₂	664	250	52
5d	-F	Amorphous	C ₂₇ H ₂₄ F ₃ N ₅ O ₄ S ₂	603	185	55
5e	-NO ₂	Amorphous	C ₂₇ H ₂₄ F ₂ N ₆ O ₆ S ₂	630	240	48
5f	-CH ₃	Amorphous	C ₂₈ H ₂₇ F ₂ N ₅ O ₄ S ₂	599	228	46
5g	-OCH ₃	Amorphous	C ₂₈ H ₂₇ F ₂ N ₅ O ₅ S ₂	615	230	52
5h	-NH ₂	Amorphous	C ₂₇ H ₂₆ F ₂ N ₆ O ₄ S ₂	600	220	58
5i	-OH	Amorphous	C ₂₇ H ₂₅ F ₂ N ₅ O ₅ S ₂	601	240	52
5j	-C ₆ H ₅	Amorphous	C ₃₃ H ₂₉ F ₂ N ₅ O ₄ S ₂	661	248	54

Table 2: Antibacterial and antimycobacterial activity against the screened compounds along with In-vitro toxicity studies 5(a-j).

Compound Number	^b S. A. G(+) ^a MIC	^b B. S. G(+) ^a MIC	^b E. C. G(-) ^a MIC	^b P. A. G(-) ^a MIC	^c M. TB ^a MIC	^d IC ₅₀	^e SI
5a	15.67	17.00	31.33	65.33	Resist	--	--
5b	4.33	4.33	15.33	31.67	0.8	55	68.75
5c	8.33	7.67	16.33	17.00	3.12	--	--
5d	8.32	5.94	5.33	4.45	1.60	--	--
5e	4.00	7.00	15.67	16.00	3.12	--	--
5f	15.67	17.00	31.00	64.33	Resist	--	--
5g	15.67	15.33	66.00	64.67	12.5	--	--
5h	4.67	4.67	16.33	33.00	12.5	--	--
5i	4.67	6.67	31.67	32.67	Resist	--	--
5j	3.00	2.33	8.67	8.67	3.12	--	--
GATI*	1.33	2.00	7.67	3.67	--	--	--
RIP*	--	--	--	--	0.8	65	81.25

^a :-MIC: Minimum inhibitory concentration (in µg/ml) required to inhibit 90% inhibition against M. TB and antibacterial.

^b :-As a bacteria;SA:- *S. aureus*(Gram positive), BS:- *B. subtilis*(Gram positive); EC:- *E. Coli* (Gram negative), PA:-*P. aeruginosa*(Gram negative)

^cM. TB:- Mycobacterium Tuberculosis

^d :- IC₅₀ inhibition concentration (inhibited 50 % of total cells in LM and converted to µg/mL for SI calculation)

*:- As reference pure drug :- GATI: Gatifloxacin; RIP: rifampicin.

^e :- SI Index selectivity index (ratio between IC₅₀ and M. tuberculosis MIC value)

Figure 1

Figure 2: Targeted Derivative.

Compound	5	a	b	c	d	e	f	g	h	i	j
R	H	Cl	Br	F	NO ₂	CH ₃	OCH ₃	NH ₂	OH	C ₆ H ₅	

Figure 3: Synthetic Scheme.