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Synthesis, Characterization, Antimicrobial Activity of Novel N-Substituted β-Hydroxy Amines and βHydroxy Ethers Contained Chiral Benzoxazine Fluoroquinolones

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Abstract: Synthesis of novel N-substituted β-hydroxy amines **4(a-j)** and β-hydroxyethers **5(a-c)** with chiral benzoxazine fluoroquinolones has been described. Benzoxazinefluoroquinolone carboxylic acid **1**, on reaction with piperizine in acetonitrile in presence of triethylamine under reflux gives 7- piperazinyl benzoxazine fluoroquinolone **2**. The latter is reacted with epichlrohydrine in presence of NaOH in acetone to yield respective N-substituted epoxide **3** with retained chirality**,** the **3** on treatment with different amines gives respective β-hydroxy amines **4(a-j)**. On other hand, **3** on treatment with alcohols in presence of NaOH afforded the corresponding β-hydroxy ethers **5(a-c).** The structures of the synthesized compounds have been established on the basis of its spectral and analytical data. The antimicrobial activity of newly synthesized compounds ware evaluated against different microorganisms comparing with levofloxacin and found all the compounds exhibited remarkable activity.

Keywords: Antimicrobial activity; Chiral benzoxazine fluoroquinolones; Epichlorohydrine; 7-Piperazinyl chiral benzoxazine fluoroquinolones.

INTRODUCTION

Fluoroquinolones are well established class of board spectrum antibiotics, therapeutically useful in the treatment of various pathogenic infections. When compared to previou sly synthesized antibacterial drugs, the fluoroquinolones are having extensive potent activity against various microorganis ms [1]. These classes of antibiotics were also used in the treatment of many sexually transmitted diseases (STDs), complicated urinary tract and respiratory infections [2]. Fluoroquinolones (nalidixic acid) were discovered in 1962 as a byproduct during the synthesis of anti-malarial drug chloroquine [3]. In 1963 it was approved by Food and Drug Administration (FDA) for the treatment of urinary tract infection. After the discovery of norfloxacin, structure–activity relationships (SAR) analysis of the fluoroquinolone pharmacophore led to synthesize a new class of compounds with good solubility and enhanced biological activity [4]. The bactericidal activity generated by fluoroquinolones is caused by the inhibition of two bacterial enzymes: DNA gyrase and topoisomerase IV [5]. These derivatives act against Gram-positive and Gram-negative bacteria due to the presence of fluorine atom at 6 th position and piperazinyl group at 7th position. In the past few years, research for new quinolone antibacterial agents has focused on numerous structural patterns at C-7 and C-8 position [6-9] of quinolone carboxylic acid. Besides these studies, simple modifications were done on 7-piperazinyl fluroquinolones, and substitutions with bulky groups on N-4 of piperazine ring, resulted in increase in biological activity by inhibiting DNA gyrase [10, 11]. These kind of modifications leads to synthesize modern fluoroquinolone antibacterials [12, 13] with enhanced antimicrobial activity. The evolution as well as the high potency in quinolones is mainly because of modification at 7th position. Introduction of these new quinolones has created an exciting era in antimicrobial chemotherapy. Literature survey reveals that researchers hav e attempted to make the various substituted fluoroquinolones, which posses many desirable microbiologi cal and pharmacokinetic properties [14-16].

In view of these reports, it was thought worthwhile to synthesize and investigate the compounds containing N-substituted piperazinyl fluoroquinolones. Present work is concerned with the synthesis of N-substituted β-hydroxy amines and β-hydroxy ethers contained fluoroquinolone derivatives with the objective of discovering novel antimicrobial agents.

EXPERIMENTAL

Materials and Methods

All the reagents and solvents used analytical grade and without further purification unless otherwise mentioned. Thin layer chromatography (TLC) was carried out on aluminium sheets coated with silica gel 60 F254 (Merck). TLC plates were inspected under UV light. Elemental analyses data were obtained by employing a Perkin-Elmer 240c analyzer. IR spectra (KBr pellets) were recorded with a Perkin-Elmer-1700 spectro photometer. ^{1}H NMR and ^{13}C NMR spectra were measured on avance BRUKER-300 MHz spectrometer. Mass was recorded on Varian 300-MS spectrometer and melting points were recorded on a Polmon MP 96. Specific optical rotation performed on Rudolf model number 420766APR/6W polarimeter.

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(S)-9-fluoro-3,7-dihydro-3-methyl-7-oxo-10-(piperazin-1-yl)-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid (2).

To a solution of **1** (100g, 356 mmol) in acetonitrile (500 mL) and piperazine (45.92 g, 534mmol) was stirred for 30 min at 25-30 °C, added triethylamine (108g, 1068 mmol) and heated the reaction mass to reflux, maintained for 12h at same temperature, progress of the reaction was monitored by TLC. The reaction mass was cooled to 25-30 °C, stirred for 1h, filtered the isolated compound and washed with acetonitrile. The crude solid was recrystallized from methanol to obtain pure **2.**

Pale yellow solid, yield: 106g (86%), mp 263-265 °C; IR (KBr) ν_{max} (cm^{-1}): 3412, 2814, 1728 and 1617. ^{1}H-NMR (DMSO d_6): δ 1.22-1.24 (d, 3H, *J*=6.0 Hz), 2.48-2.55 (m, 4H), 2.27 (s,1H, D_2O exchangeable), 3.56-3.62 (m, 4H), 3.97-4.00 (m, 1H), 4.54-4.57 (m, 2H), 7.48-7.52 (d, 1H, *J* =12.0 Hz), 8.89 (s, 1H) and 15.17 (s, 1H, D_2O exchangeable, COOH). ^{13}C-NMR (DMSO d_6): δ 18.39, 51.46, 54.32, 58.71, 60.31, 107.62, 120.34, 125.41, 131.08, 140.36, 146.54, 154.31, 157.29, 166.82, 177.09. Mass (ES): *m/z* 348.07 $[M+1]^+$; Anal. Calcd. for ($C_{17}H_{18}FN_3O_4$): C, 58.78; H, 5.22; N, 12.10. Found: C, 58.64; H, 5.09; N, 12.01 %. Specific optical rotation: $[\alpha]_D^{25}$ -69.4° (c=1.0 in Methanol: DCM 1: 1)

(S)-9-fluoro-3,7-dihydro-3-methyl-10-(4-((oxiran-2-yl)methyl)piperazin-1-yl)-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid (3)

To a solution of **2** (50g, 123 mmol) in acetone (250 mL), add powdered NaOH (12g, 307 mmol) followed by epichlorohydrine (23g, 0.246 mmol) and the reaction mixture was maintained for 4h at 40-45 °C. At the end of this period, the reaction mixture was cooled to 25-30 °C and solvent was distilled off under vacuum. The residue was dissolved in water (50 mL) and adjusted the pH 6.0-7.0 with dil. HCl. The separated solid was filtered, washed with water and dried. The crude solid was recrystallized from ethyl acetate to obtain pure **3.** Off-white solid, yield: 47g (82%), mp 210-213°C; IR (KBr, cm^{-1}): 3370, 3042, 2985, 1708 and 1624. ^{1}H-NMR (DMSO d_6): δ 1.42-1.44 (d, 3H, *J*=6.0 Hz), 2.34-2.41 (m, 4H), 2.49-2.51 (d, 2H, *J*=6.0Hz), 3.23-3.32 (m, 4H), 3.51-3.53(d, 2H, *J*=6.2 Hz), 4.33-4.38 (m, 1H), 4.55-4.58 (m, 1H), 4.89-4.91 (d, 2H, *J* = 6.0 Hz), 7.52-7.56 (d, 1H, *J* =12.0 Hz), 8.94 (s, 1H) and 15.14 (s, 1H, D_2O exchangeable, COOH). ^{13}C-NMR (DMSO d_6): δ 18.34, 45.29, 49.23, 50.72, 54.56, 55.25, 58.35, 61.61, 68.49, 107.32, 120.13, 125.19, 130.44, 132.56, 140.68, 146.79, 154.28, 166.45, 176.77. Mass (ES): *m/z* 404.02 $[M+1]^+$; Anal. Calcd. for ($C_{20}H_{22}FN_3O_5$): C, 59.55; H, 5.50; N, 10.42. Found: C, 59.49; H, 5.40; N, 10.33 %. Specific optical rotation: $[\alpha]_D^{25}$ -73.06° (c=1.0 in Methanol: DCM 1: 1).

General Procedure for the Synthesis of Compounds 4(a-j).

To a solution of **3** (5g, 12.3 mmol), in methanol (50 mL) were added various amines in excess amount (aliphatic, aromatic and cyclic). The reaction mass was heated to reflux and maintained for 4h. The completion of the reaction was confirmed by TLC and the excess amount of amine as well as solvent was distilled off under vacuum, the residue was dissolved in methanol (10 mL) followed by addition of ethyl acetate (40 mL) with constant stirring at room temperature. The separated solid was filtered, washed with ethyl acetate (5 mL) to afford pure title compounds.

(S)-9-fluoro-3,7-dihydro-10-(4-(2-hydroxy-3-(methylamino)propyl)piperazin-1-yl)-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid (4a)

Off-white solid, yield: 4.5g (83%), mp 229-233 °C; IR (KBr, cm^{-1}): 3430, 2985, 1707, 1625. ^{1}H-NMR (DMSO d_6): δ 1.41-1.43(d, 3H, *J*=6.2 Hz) 2.26 (s, 1H, D_2O exchangeable), 2.40-2.49 (m, 4H), 2.57 (s, 3H), 2.91-2.95 (m, 2H), 3.09-3.12 (m, 2H), 3.26-3.36 (m, 4H), 3.66-3.71 (m,1H), 4.36-4.38 (d, 1H, *J*= 6.0 Hz),4.45-4.58 (d, 2H, *J*=6.0 Hz), 4.89 (s, 1H, D_2O exchangeable), 7.52-7.56 (d, 1H, *J* =12.0 Hz), 8.96 (s, 1H) and 15.10 (s, 1H, D_2O exchangeable, COOH). ^{13}C-NMR (DMSO d_6): δ 18.31, 33.07, 51.43, 53.64, 55.34, 59.24, 61.38, 68.77, 107.07, 120.94, 125.12, 130.69, 140.89, 146.69, 154.95, 157.32, 166.40, 176.75. Mass (ES): *m/z* 435.1 $[M+1]^+$; Anal. Calcd. for ($C_{21}H_{27}FN_4O_5$): C, 58.05; H, 6.26; N, 12.90. Found: C, 57.91; H, 6.13; N, 12.81 %. Specific optical rotation: $[\alpha]_D^{25}$ -37.84° (c=1.0 in Methanol: DCM 1: 1).

(S)-10-(4-(3-(ethylamino)-2-hydroxypropyl)piperazin-1-yl)-9-fluoro-3,7-dihydro-3-methyl-7-oxo-2H-[1,4]oxazino-[2,3,4-ij]quinoline-6-carboxylic acid (4b)

Off-white solid, Yield: 4.5g (82%); mp: 218-219 °C; IR (KBr, cm^{-1}): 3409, 2985,1707 and 1619. ^{1}H-NMR (DMSO d_6): δ 1.19-1.21 (t, 3H), 1.37-139 (d, 3H, *J*= 6.0 Hz), 2.29 (s, 1H, D_2O exchangeable), 2.48-2.52 (m, 4H), 2.61 (m, 2H), 2.91-2.95 (m, 2H), 3.11-3.14 (m, 2H), 3.31-3.38 (m, 4H), 3.58-3.63 (m,1H), 4.34-4.36 (d, 1H, *J*= 6.0 Hz),4.52-4.54 (d, 2H, *J*=6.0 Hz), 5.18 (s, 1H, D_2O exchangeable), 7.45-7.49 (d, 1H, *J* =12.0 Hz), 8.75 (s, 1H) and 15.12 (s, 1H, D_2O exchangeable, COOH). ^{13}C-NMR (DMSO d_6): δ 18.31, 21.2, 46.81, 51.26, 54.13, 55.67, 59.42, 61.23, 68.85, 107.12, 120.75, 125.31, 130.43, 140.76, 146.27, 154.63, 157.11, 166.65, 176.62. Mass (ES): *m/z* 449.2 $[M+1]^+$; Anal. Calcd. for ($C_{22}H_{29}FN_4O_5$): C, 58.92; H, 6.52, N, 12.49. Found: C, 58.76; H, 6.41; N, 12.32 %. Specific optical rotation: $[\alpha]_D^{20}$ -44.84° (c=1.0 in Methanol: DCM 1: 1).

(S)-10-(4-(3-(dipropylamino)-2-hydroxypropyl) piperazin-1-yl)-9-fluoro-3,7-dihydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid (4c)

Off-white solid, Yield: 5.2g (84%); mp: 247-250 °C; IR (KBr, cm^{-1}): 3425, 2928, 1707 and 1621cm^{-1}. ^{1}H-NMR (DMSO d_6): δ 1.07-1.12 (m, 6H), 1.34-1.41 (m, 7H), 2.25-2.32 (m, 4H), 2.48-2.52 (m, 4H), 2.77-2.79 (d, 1H, *J*=6.1 Hz), 3.06-3.13 (m, 2H), 3.51-3.54 (m, 4H), 3.66-3.72 (m, 1H), 4.28-4.33 (m, 1H),4.49- 4.51 (d, 2H, *J*=6.0 Hz), 5.06 (s, 1H, D_2O exchangeable), 7.52-7.56 (d, 1H, *J* = 12.0 Hz), 8.96 (s, 1H) and 15.14 (s, 1H, D_2O exchangeable, COOH). ^{13}C-NMR (DMSO d_6): δ 14.26, 18.42, 24.16, 51.26, 54.13, 55.67, 59.02, 59.70, 61.23, 68.92, 107.34, 120.49, 125.37, 130.58, 141.23, 146.41, 154.51, 157.34, 166.72,

176.81.Mass (ES): *m/z* 505.2 $[M+1]^+$; Anal. Calcd. for ($C_{26}H_{37}FN_4O_4$): C, 61.89; H, 7.39; N, 11.10. Found: C, 61.74; H, 7.28; N, 10.96%. Specific optical rotation: $[\alpha]_D^{25}$ -14.60° (c=1.0 in Methanol : Water 1: 1).

(S)-10-(4-(3-(tert-butylamino)-2-hydroxypropyl) piperazin-1-yl)-9-fluoro-3,7-dihydro-3-methyl-7-oxo-2H-[1,4]-oxazino[2,3,4-ij]quinoline-6-carboxylic acid (4d)

Off-white solid, Yield: 5.0g (84%); mp: 221-224 °C; IR (KBr, cm^{-1}): 3400, 2964, 1707 and 1620. ^{1}H-NMR (DMSO d_6): δ 1.13-1.21 (m, 9H) 1.36-1.38 (d, 3H, *J*=6.2 Hz), 2.27 (s, 1H, D_2O exchangeable), 2.46-2.57 (m, 4H), 2.70-2.72 (d, 2H, *J*=6.3 Hz), 3.11-3.13 (d, 2H, *J*=6.1 Hz), 3.53-3.58 (m, 4H), 3.81-3.86 (m, 1H), 4.16-4.22 (m, 1H), 4.52-4.54 (d, 2H, *J*=6.0), 4.71 (s, 1H, D_2O exchangeable), 7.43-7.47 (d, 1H, *J* = 12.0 Hz), 8.62 (s, 1H) and 15.09 (s, 1H, D_2O exchangeable, COOH). ^{13}C-NMR (DMSO d_6): δ 18.31, 34.07, 50.73, 54.14, 56.02, 59.38, 61.51, 69.27, 107.12, 121.14, 125.36, 130.71, 140.77, 146.58, 155.07, 157.41, 166.53, 176.67. Mass (ES): *m/z* 477.2 $[M+1]^+$; Anal. Calcd. for ($C_{24}H_{33}FN_4O_5$): C, 60.49; H, 6.98; N, 11.76. Found: C, 60.34; H, 6.82; N, 11.66%. Specific optical rotation: $[\alpha]_D^{25}$ -18.96° (c=1.0 in Methanol : Water 1: 1).

(S)-10-(4-(3-(benzylamino)-2-hydroxypropyl) piperazin-1-yl)-9-fluoro-3,7-dihydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid (4e)

Off-white solid, Yield: 5.0g (79%); mp: 234-236 °C; IR (KBr, cm^{-1}): 3391, 2935,1717 and 1619. ^{1}H-NMR (DMSO d_6): δ 1.36-1.38 (d, 3H, *J*=6.0 Hz), 2.29 (s, 1H, D_2O exchangeable), 2.38-2.43 (m, 4H), 2.72-2.74 (d, 2H, *J*=6.0 Hz), 3.14-3.16 (d, 2H, *J*=6.2 Hz), 3.69-3.72 (m, 4H), 3.75-3.78 (m, 1H), 3.92 (s, 2H), 4.29-4.35 (m, 1H), 4.42-4.44 (d, 2H, *J*=6.0 Hz), 4.96 (s, 1H, D_2O exchangeable), 7.18-7.31 (m, 5H), 7.42-7.46 (d, 1H, *J* =12.0 Hz), 8.96 (s, 1H) and 15.16 (s, 1H, D_2O exchangeable, COOH). ^{13}C-NMR (DMSO d_6): δ 18.46, 50.64, 54.23, 55.61, 56.37, 57.12, 59.24, 61.38, 68.77, 107.07, 109.30, 120.94, 125.12, 127.36, 128.36,129.65, 130.69, 137.28, 140.89, 146.69, 154.95, 157.32, 166.40, 177.75. Mass (ES): *m/z* 511.5 $[M+1]^+$; Anal. Calcd. for ($C_{27}H_{31}FN_4O_5$): C, 63.52; H, 6.12; N, 10.97. Found: C, 63.41; H, 6.02; N, 10.83%. Specific optical rotation: $[\alpha]_D^{25}$ -26.48° (c=1.0 in Methanol: DCM 1: 1)

(S)-9-fluoro-3, 7-dihydro-10-(4-(2-hydroxy-3-(phenylamino)propyl) piperazin-1-yl)-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid (4f)

Off-white solid Yield: 4.9g (81%); mp: 216-218 °C; IR (KBr, cm^{-1}): 3393, 2847,1718 and 1620. ^{1}H-NMR (DMSO d_6): δ 1.42-1.44 (d, 3H, *J*=6.0 Hz), 2.49-2.56 (m, 4H), 2.78-2.79 (d, 2H, *J*=6.0 Hz), 3.29-3.31 (d, 2H, *J*=6.2 Hz), 3.57-3.66 (m, 4H), 3.66-3.69 (m, 1H), 3.84 (s, 1H, D_2O exchangeable) 4.34-4.36 (m, 1H), 4.55-4.59 (m, 2H), 4.92 (s, 1H, D_2O exchangeable), 6.51-6.61 (m, 3H), 7.03-7.08 (m, 2H), 7.56-7.60 (d, 1H, *J* =12.0 Hz), 8.96 (s, 1H) and 15.16 (s, 1H, D_2O exchangeable, COOH). ^{13}C-NMR (DMSO d_6): δ 18.46, 50.28, 53.96, 55.20, 57.34, 59.34, 61.07, 68.68, 108.32, 109.73, 118.61, 119.24, 130.51, 131.26, 134.01, 144.35, 150.33, 154.95, 157.61, 166.38, 177.82. Mass (ES): *m/z* 497.2 $[M+1]^+$; Anal. Calcd. for ($C_{26}H_{29}FN_4O_5$): C, 62.89; H, 5.89; N, 11.28. Found: C, 62.73; H, 5.81; N, 11.14%. Specific optical rotation: $[\alpha]_D^{25}$ -32.04° (c=1.0 in Methanol: DCM 1: 1).

(S)-9-fluoro-2,3-dihydro-10-(4-(2-hydroxy-3-(piperidin-1-yl) propyl)piperazin-1-yl)-6-(hydroxymethyl)-3- methyl -[1,4]oxazino[2,3,4-ij]quinolin-7-one (4g)

Off-white solid Yield: 5.3g (87%); mp: 189-191 °C; IR (KBr, cm^{-1}): 3420, 2933, 1720 and 1619. ^{1}H-NMR (DMSO d_6): δ 1.43-1.45 (d, 3H, *J*=6.0 Hz), 1.65-1.78 (m, 4H), 1.85-1.89 (m, 2H), 2.51-2.56 (m, 4H), 2.71-2.73 (d, 2H, *J*=6.0 Hz), (m, 4H), 3.26-3.28 (d, 2H, *J*=6.2 Hz), 3.53-3.64 (m, 4H), 3.62-3.67 (m, 1H), 4.38-4.40 (m, 1H), 4.58-4.60 (m, 2H), 4.95 (s, 1H, D_2O exchangeable), 7.60-7.64 (d, 1H, *J* =12.0 Hz), 8.98 (s, 1H) and 15.09 (s, 1H, D_2O exchangeable, COOH). ^{13}C-NMR (DMSO d_6): δ 18.39, 24.56, 26.67, 50.72, 54.23, 55.28, 58.24, 58.93, 60.73, 65.41, 68.26, 107.07, 109.23, 120.56, 125.21, 130.51, 140.36, 146.27, 154.59, 157.23, 166.71, 177.05.Mass (ES): *m/z* 489.2 $[M+1]^+$; Anal. Calcd. for ($C_{25}H_{33}FN_4O_5$): C, 61.46; H, 6.81; N, 11.47. Found: C, 61.33; H, 6.74; N, 11.39%. Specific optical rotation: $[\alpha]_D^{25}$ -28.32° (c=1.0 in Methanol : Water 1: 1) 2.98-3.00.

(S)-9-fluoro-2,3-dihydro-10-(4-(2-hydroxy-3-(piperazin-1-yl) propyl)piperazin-1-yl)-6-(hydroxymethyl)-3-methyl-[1,4]oxazino[2,3,4-ij]quinolin-7-one(4h)

Off-white solid, Yield: 5.1g (84%); mp: 229-230 °C; IR (KBr, cm^{-1}): 3420, 2933, 1720 and 1619. ^{1}H-NMR (DMSO d_6): δ 1.43-1.44 (d, 3H, *J*=6.0 Hz), 2.03 (s, 1H, D_2O exchangeable), 2.48-2.53 (m, 4H), 2.81-2.83 (d, 2H, *J*=6.0 Hz), 3.14-3.24 (m, 8H), 3.28-3.30 (d, 2H, *J*=6.0 Hz) 3.56-3.61 (m, 4H), 3.71-3.75 (m, 1H), 4.38-4.41(m, 1H), 4.62-4.64 (d, 2H, *J*=6.0 Hz), 4.95 (s, 1H, D_2O exchangeable), 7.60-7.64 (d, 1H, *J* = 12.0 Hz), 8.98 (s, 1H) and 15.12 (s, 1H, D_2O exchangeable, COOH). ^{13}C-NMR (DMSO d_6): δ 18.31, 44.24, 50.29, 54.23, 55.34, 56.58, 59.24, 61.38, 62.34, 68.77, 108.23, 109.09, 120.69, 125.26, 130.54, 140.68, 146.43, 154.74, 157.11, 166.63, 176.84. Mass (ES): *m/z* 490.5 $[M+1]^+$; Anal. Calcd. for ($C_{24}H_{32}FN_5O_5$): C, 58.88; H, 6.51; N, 14.31. Found: C, 58.74; H, 6.38; N, 14.22%. Specific optical rotation: $[\alpha]_D^{25}$ -29.34° (c=1.0 in Methanol : Water 1: 1).

(S)-9-fluoro-2,3-dihydro-10-(4-(2-hydroxy-3-(4-methylpiperazin-1-yl)propyl) piperazin-1-yl)-6-(hydroxymethyl)-3-methyl-[1,4]oxazino[2,3,4-ij]quinolin-7-one (4i)

Off-white solid, Yield: 5.2g (83%); mp: 234-237 °C; IR (KBr, cm^{-1}): 3412, 2937, 1720 and 1620.^{1}H-NMR (DMSO d_6): δ 1.42-1.44 (d, 3H, *J*=6.0 Hz), 2.36 (s, 3H), 2.34-2.38 (m, 4H), 2.49-2.53 (m, 4H), 2.78-2.80 (d, 2H, *J*=6.1 Hz), 2.84-2.89 (m, 4H), 3.18-3.20 (d, 2H, *J*=6.0), 3.66-3.71 (m, 4H), 3.74-3.79 (m, 1H), 3.93-3.98 (m, 1H), 4.56-4.58 (d, 2H, *J*= 6.0), 5.08 (s, 1H, D_2O exchangeable), 7.56-7.60 (d, 1H, *J* =12.0 Hz), 8.90 (s, 1H) and 15.10 (s, 1H, D_2O exchangeable, COOH). ^{13}C-NMR (DMSO d_6): δ 18.35, 42.05,47.18,49.41, 53.04, 55.32, 58.71, 59.32, 60.68, 68.78, 107.10, 120.91, 125.12, 130.70, 140.96, 146.72, 154.04, 157.31, 166.35, 176.74. Mass (ES): *m/z* 504.2 $[M+1]^+$; Anal.

Calcd. for ($C_{25}H_{34}$ FN_5O_5): C, 59.63; H, 6.81; N, 13.91.Found: C, 59.52; H, 6.75 ; N, 13.83%. Specific optical rotation: $[\alpha]_D^{25}$ -21.37° (c=1.0 in Methanol : Water 1: 1).

(S)-9-fluoro-3,7-dihydro-10-(4-(2-hydroxy-3-morpholino-propyl) piperazin-1-yl)-3-methyl-7-oxo-2H-[1,4]oxazino[-2,3,4-ij]quinoline-6-carboxylic acid (4j)

Off-white solid, Yield: 5.2g (86%); mp: 235-237 °C; IR (KBr, cm^{-1}): 3420, 2933, 1720 and 1619 cm^{-1}. ^{1}H-NMR (DMSO d_6) δ: 1.36-1.38 (d, 3H, *J*=6.0 Hz), 2.52-2.56 (m, 4H), 2.72-2.74 (d, 2H, *J*=6.1 Hz), 3.01-3.08 (m, 4H), 3.12-3.17 (d, 2H, *J*=6.0), 3.54-3.59 (m, 4H), 3.64-3.69 (m, 4H), 3.82-3.86 (m, 1H), 4.08-4.18 (m, 1H), 4.43-4.45 (d, 2H, *J*= 6.0), 4.98 (s, 1H, D_2O exchangeable), 7.43-7.47 (d, 1H, *J* =12.0 Hz), 8.62 (s, 1H) and 15.08 (s, 1H, D_2O exchangeable, COOH). ^{13}C-NMR (DMSO d_6): δ 18.38, 50.23, 54.18, 55.56, 58.21, 58.71, 59.32, 60.68, 66.14, 68.68, 108.23, 109.46, 119.87, 126.31, 131.28, 141.08, 146.62, 154.31, 156.65, 166.82, 177.34. Mass (ES): *m/z* 491.2 $[M+1]^+$; Anal. Calcd. for ($C_{24}H_{31}FN_4O_6$): C, 58.77; H, 6.37; N, 11.42. Found: C, 58.63; H, 6.28; N, 11.31%. Specific optical rotation: $[\alpha]_D^{25}$ -33.80° (c=1.0 in Methanol : Water 1: 1).

General Procedure for the Synthesis of Compounds 5(a-c).

To a solution of **3** (5g , 12.39 mmol) in corresponding alcoholic NaOH (1g, 23.9 mmol in 50 mL) was refluxed for 4h. On completion of the reaction as shown by TLC, solvent was distilled off under vacuum. The residue was dissolved in water (50 mL) and adjusted pH 6.0-7.0 with dil. HCl. The separated solid was filtered, washed with water (5 mL) to give corresponding crude compounds. Latter on recrystallization from methanol afforded pure title compounds.

9-fluoro-3,7-dihydro-10-(4-(2-hydroxy-3-methoxypropyl-)piperazin-1-yl)-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]-quinoline-6-carboxylic acid (5a).

Off-white solid Yield: 4.0 g (73%); mp 237-240 °C; IR (KBr, cm^{-1}): 3415, 2939, 1715 and 1620. ^{1}H-NMR (DMSO d_6): δ 1.43-1.45 (d, 3H, *J*=6.0 Hz), 2.49-2.53 (m, 4H), 3.12-3.16 (m, 2H), 3.25 (s, 3H), 3.26-3.30 (m, 4H), 3.54-3.61 (m, 1H), 3.65-3.71(m, 2H), 4.37-4.39 (d, 1H, *J*=6.0 Hz), 4.58-4.60 (d, 1H, *J*=6.0 Hz), 4.92-4.94 (d, 1H, *J*=6.0 Hz), 5.74 (s, 1H, D_2O exchangeable, OH), 7.59-7.63 (d, 1H, *J* =12.0 Hz), 8.99 (s, 1H) and 15.12 (s, 1H, D_2O exchangeable, COOH). ^{13}C-NMR (DMSO d_6): δ 18.39, 52.38, 53.58, 55.30, 58.99, 59.68, 64.34, 74.89, 107.25, 120.78, 125.18, 130.80, 140.91, 146.75, 154.01, 157.31, 166.36, 176.79. Mass (ES): *m/z* 436.6 $[M+1]^+$; Anal. Calcd. for ($C_{21}H_{26}FN_3O_6$): C, 57.92; H, 6.02; N, 9.65. Found: C, 57.84; H, 5.91; N, 9.53%. Specific optical rotation: $[\alpha]_D^{25}$ -68.0° (c=1.0 in Methanol : DCM 1: 1).

10-(4-(3-ethoxy-2-hydroxypropyl)piperazin-1-yl)-9-fluo-ro-3,7-dihydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]-quinoline-6-carboxylic acid 5(b)

Off-white solid, Yield: 4.0 g (73%); mp 227-229 °C; IR (KBr, cm^{-1}): 3429, 2939, 1715 and 1620. ^{1}H-NMR (DMSO d_6): 1.08-1.13 (m, 2H), 1.43-1.45 (d, 3H, *J*=6.0 Hz), 2.48-2.52 (m, 4H), 3.10-3.15 (m, 2H), 3.35 (s, 3H), 3.37-3.39 (d, 2H, J=6.0 Hz), 3.58-3.63 (m, 4H), 3.62-3.68 (m, 1H), 4.35-4.37 (m, 1H), 4.55-4.57 (d, 2H, *J*=6.0 Hz), 5.21 (s, 1H, D_2O exchangeable), 7.59-7.63 (d, 1H, *J* =12.0 Hz), 8.98 (s, 1H) and 15.11 (s, 1H, D_2O exchangeable, COOH). ^{13}C-NMR (DMSO d_6): δ 18.39, 20.24, 52.12, 53.41, 55.06, 58.75 , 59.57, 64.52, 74.66, 107.08, 120.23, 125.27, 130.71, 141.13, 146.55, 154.34, 157.06, 166.42, 176.58. Mass (ES): *m/z* 450.2 $[M+1]^+$; Anal. Calcd. for ($C_{22}H_{28}FN_3O_6$): C, 58.79; H, 6.28; N, 9.35. Found: C, 58.63; H, 6.14; N, 9.22%. Specific optical rotation: $[\alpha]_D^{25}$ -58.44° (c=1.0 in Methanol : DCM 1: 1).

9-fluoro-3,7-dihydro-10-(4-(2-hydroxy-3-isopropoxypro-pyl)piperazin-1-yl)-3-methyl-7-oxo-2H-[1,4]oxazino[2,3, 4-ij]quinoline-6-carboxylic acid (5c)

Off-white solid, Yield: 4.4g (74%); mp 218-221 °C; IR (KBr, cm^{-1}): 3412, 2937, 1720 and 1620 cm^{-1}. ^{1}H-NMR (DMSO d_6): δ 1.07-1.09 (m, 6H), 1.42-1.44 (d, 3H, *J*=6.0 Hz), 2.52-2.56 (m, 4H), 3.14-3.18 (m, 2H), 3.21-3.25 (m, 1H), 3.41-3.46 (m, 4H), 3.60-3.64 (m, 1H), 3.68-3.73 (m, 2H), 4.33-4.35 (m, 1H), 4.57-4.59 (d, 2H, *J*=6.0 Hz), 5.21 (s, 1H, D_2O exchangeable), 7.56-7.60 (d, 1H, *J* =12.0 Hz), 8.99 (s, 1H) and 15.13 (s, 1H, D_2O exchangeable, COOH). ^{13}C-NMR (DMSO d_6): δ 18.34, 26.52, 51.73, 54.12, 55.61, 59.21 , 60.27, 65.02, 74.51, 76.39, 107.33, 120.61, 125.31, 130.46, 141.37, 146.74 , 154.69, 156.79, 166.51, 176.67. Mass (ES): *m/z* 464.4 $[M+1]^+$; Anal. Calcd. for($C_{23}H_{30}FN_3O_6$) : C, 59.60; H, 6.52; N, 9.07. Found: C, 59.51; H, 6.44; N, 8.96%. Specific optical rotation: $[\alpha]_D^{25}$ -47.64° (c=1.0 in Methanol: DCM 1: 1).

BIOLOGICAL EVALUATION

Antibacterial Activity

The compounds **2, 3, 4(a-j)** and **5(a-c)** were assayed for antibacterial activity against three representative Gram-positive organisms *viz*. *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 96), *Staphylococcus epidermidis* and Gram-negative organisms *viz Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 741) and *Klebsiella pneumoniae* (MTCC 618) by broth dilution method recommended by National Committee for Clinical Laboratory (NCCL) standards [17]. Levofloxacin was used as reference standard**.** The minimum inhibitory concentration (MIC) values are presented in Table **1**.

Anti-fungal Activity

In vitro antifungal activity of the newly synthesized compounds was studied against the fungal strains *viz Candida albicans* (MTCC 227), *Candida rugosa* (NCIM 3467), *Aspergillus flavus* (MTCC 277), and *Saccharomyces cervisiae* (MTCC 36) of yeasts by Agar Well Diffusion Method [18] in 100 and 150 ug/ml concentrations. The Potato Dextrose Agar (PDA) medium was suspended in distilled water (39g in 1000ml) and heated to boiling until it dissolved completely, the medium and Petri dishes were

Table 1. Minimum inhibitory concentration (MIC) values of compounds 2, 3, 4(a-j) and 5(a-c).

S. No	Test compounds	Micro organisms and minimum inhibitory concentration (MIC) µg /mL					
		B.subtilis	*S.aureus*	*S.epidermidis*	*E.coli*	*P.aeruginosa*	*K.pneumoniae*
1	**2**	6.9	0.75	1.20	0.75	2.20	2.20
2	**3**	7.5	6.17	7.37	7.37	6.68	3.75
3	**4a**	0.75	0.79	1.51	1.80	2.25	1.23
4	**4b**	1.52	0.93	0.93	0.93	0.46	7.53
5	**4c**	0.98	0.46	0.93	0.46	0.46	3.75
6	**4d**	0.61	0.38	0.24	0.24	0.34	0.91
7	**4e**	0.46	1.17	0.68	2.34	2.34	0.37
8	**4f**	0.37	1.17	0.46	1.17	1.17	8.75
9	**4g**	0.68	1.34	0.68	0.68	0.68	0.37
10	**4h**	0.92	1.64	1.34	1.37	1.37	0.75
11	**4i**	0.56	0.21	0.21	0.68	0.22	0.78
12	**4j**	0.41	0.41	0.36	0.22	0.46	1.34
13	**5a**	0.32	0.32	0.93	0.46	0.51	0.55
14	**5b**	0.75	1.17	1.68	1.34	2.34	0.79
15	**5c**	0.98	1.12	1.39	0.76	0.54	1.34
16	**Levofloxacin Ref std.**	0.78	0.19	0.19	0.19	0.19	1.56

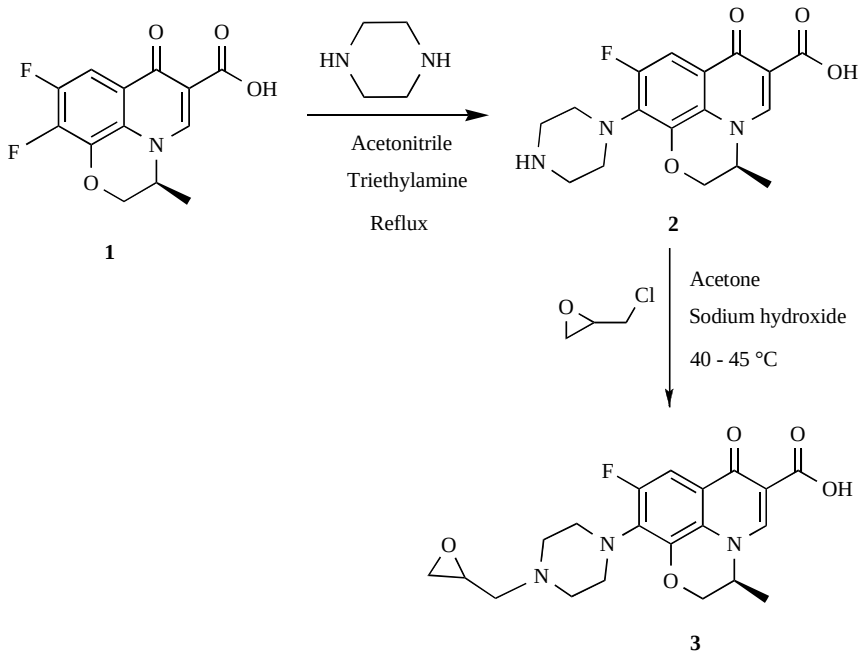
autoclaved at pressure of 15 lb/inc^2 for 20 min. Agar well bioassay was employed for testing antifungal activity**.** The medium was poured into sterile Petri dishes under aseptic conditions in a laminar air flow chamber. When the medium in the plates solidified, 0.5 ml of (week old) culture of test organism was inoculated and uniformly spread over the agar surface with a sterile L-shaped rod. Solutions were prepared by dissolving the compound in DMSO at different concentrations. After inoculation, wells were scooped out with 6 mm sterile cork borer and the lids of the dishes were replaced. To each well different concentrations of test solutions were added. Controls were maintained. The treated and the controls were kept at 27 °C for 48 h. Inhibition zones were measured, the diameter calculated in millimetre.

Some of newly synthesized compounds shown excellent to good activity against *Candida albicans* (MTCC 227) only at 150µg/mL concentrations and inactive against100µg/mL concentration, these compounds didn't shown any appreciable activity against the other fungal strains. The results of the activity against *Candida albicans* are tabulated in Table **2**.

RESULTS AND DISCUSSION

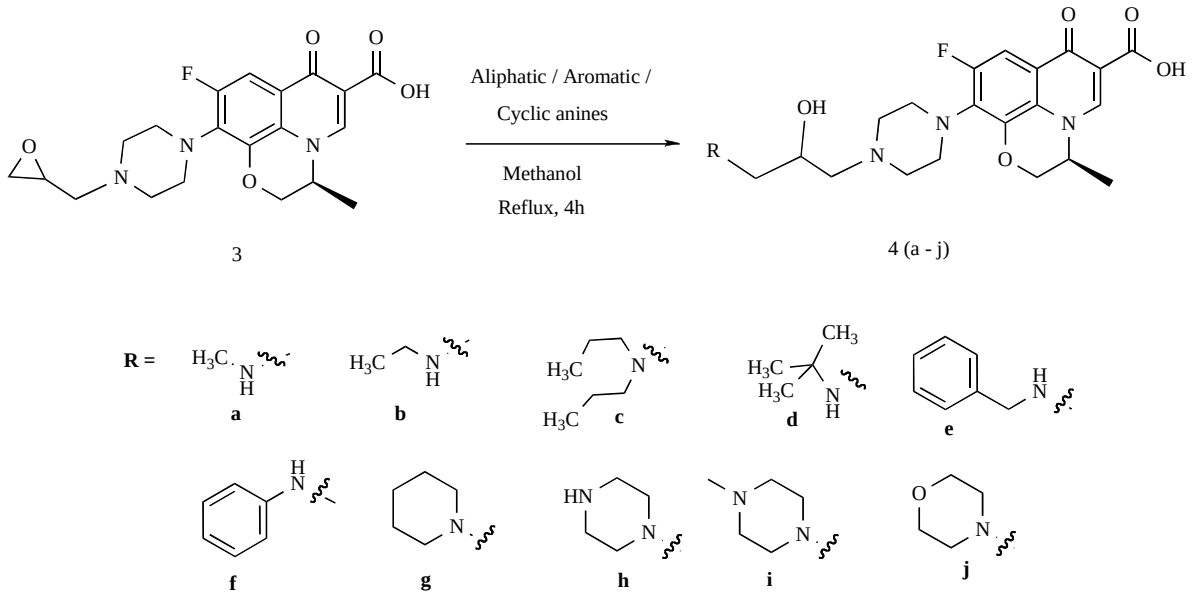
(3S)-9,10-Difluoro-3-methyl-7-oxo-3,7-dihydro-2*H*-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxylic acid **1** reacted with piperizine in presence of triethylamine in acetonitrile under reflux given the previously reported [19], (S)-9-fluoro-3,7-dihydro-3-methyl-7-oxo-10-(piperazin-1-yl)-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid **2** on treatment with epichlrohydrine in presence of NaOH in acetone at 40-45 °C yielded the corresponding 9-fluoro-3,7-dihydro-3-methyl-10-(4-((oxiran-2-yl)methyl)piperazin-1-yl)-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid **3** as shown in Scheme **1**.

The structure **3** was determined on the basis of its spectral data. The IR (KBr) spectrum of compound **3** has shown absorptions at 1708 & 1624 cm^{-1} assignable to two carbonyl groups as diagnostic absorptions. Typical aliphatic



1 **2** **3**

Scheme 1. Synthesis of Compound **3** from **1.**

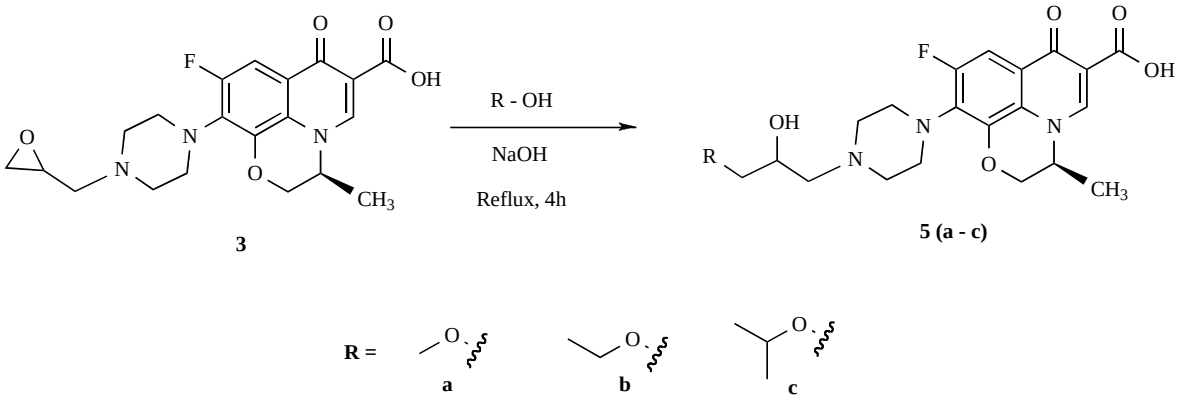


3 4 (a - j)

Scheme 2. Synthesis of Compounds **4 (a-j)** from **3**.

shift alignment in ^{1}H NMR spectrum at 3.51-3.53 (d, 2H), 4.33-4.38 (m, 1H) confirms the epoxide ring protons. However the chemical shift at 7.52-7.56 (d, 1H) with coupling constant 12.0 Hz indicates proton coupled with adjacent fluorine and broad singlet at 15.17 ppm exchangeable with D_2O confirmed the presence of carboxylic acid. In ^{13}C NMR chemical shift at 18.34 indicated the methyl group and signal at 146.51 shown the carbon attached to fluorine. Enone carbon resonated downfield than all other carbons at 176.77 ppm, where as carboxylic acid carbon resonated at 166.45.

Further, the nucleophilic opening of epoxide **3** was carried out with various amines like aliphatic amines (20% methyl amine in methanol, ethyl amine, isopropyl amine and t-butyl amine), aromatic amines (benzyl amine, aniline) and cyclic amine (piperidine, piperzine, n-methyl piperizine and morphiline) in excess amount in methanol under reflux for



Scheme 3. Synthesis of Compounds **5 (a-c)** from **3**.

Table 2. Inhibition activity of compounds 2, 3, 4(a-j) and 5(a-c) in mm at 100 & 150 µg/mL concentrations.

S. No	Test compounds	*C. albicans*	
		100 µg	**150 µg**
1	**2**	0	6
2	**3**	0	7
3	**4a**	0	3
4	**4b**	0	5
5	**4c**	0	5
6	**4d**	0	9
7	**4e**	0	6
8	**4f**	0	7
9	**4g**	0	9
10	**4h**	0	10
11	**4i**	0	8
12	**4j**	0	10
13	**5a**	0	5
14	**5b**	0	6
15	**5c**	0	6
16	**Levofloxacin Ref std.**	0	4

4h by classical approach [20-23] given the corresponding β-hydroxy amines **4(a-j),** the reaction shown in Scheme **2**.

The structure **4a** was confirmed by its spectral analysis. IR (KBr) spectrum of compound **4a** shown an absorption at 3430 cm^{-1} indicated the presence of hydroxy group and absorption at 1707 and 1625 cm^{-1} shown the presence of carbonyl groups. Chemical shift in ^{1}H NMR spectrum at 4.89 as broad singlet, exchangeable to D_2O proven the presence of –OH group. The chemical shift at 33.07 in ^{13}C NMR shown the methyl carbon and chemical shift at 68.24 is due to presence of CH_2 group adjacent to asymmetric carbon of oxazin ring. On the other hand, **3** on treatment with various alcohols in the presence of NaOH under reflux for 4h afforded the corresponding β- hydroxy ethers **5(a-c)** reactions were shown in Scheme **3**.

The structure of **5(a-c)** have confirmed based on its spectral and analytical data.

All newly synthesized compounds **3**, **4(a-j) and 5(a-c)** shown in table **1** were tested *invitro* against Gram-positive organisms viz. *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 96), *Staphylococcus epidermidis* and Gram-negative organisms viz *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 741), and *Klebsiella pneumoniae* (MTCC 618) and also synthesized compounds were screened *invitro* for antifungal activity aganist *Candida albicans* (MTCC 227), *Candida rugosa* (NCIM 3467), *Aspergillus flavus* (MTCC 277), and *Aspergillus niger* (MTCC 282). *Saccharomyces cervisiae* (MTCC 36) of yeasts. Most of the evaluated compounds exhibited remarkable antimicrobial activity.

CONCLUSION

In conclusion, we have synthesized novel N-substituted β-hydroxy amines **4(a-j)** and β-hydroxy ethers **5(a-c)** through epoxide ring opening by classical approach. All the newly synthesized compounds have been evaluated for their antibacterial activity against bacteria and fungi. The synthesized compounds were found potent against bacteria and fungi strains. Compounds **4d**, **4i** and **4j** are exhibited excellent activity against all Gram-positive and Gram-negative organisms. Compounds **4g and 5a** shown good activity and remaining compounds shown good to moderate activity compared with levofloxacin used as reference standard. In the same way compounds **4d, 4g, 4h** and **4j** were shown good anti fungal activity in 150 µg/mL concentration.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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