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ARTICLE

# Synthesis of Fluorinated Piperazinyl Substituted Quinazolines as Potential Antibacterial Agents

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## ABSTRACT

In present study, fluorinated piperazine and benzonitrile/nicotinonitrile fused quinazoline derivatives have synthesized, characterized using FT-IR, $^{1}H$ & $^{13}C$ NMR, $^{19}F$ NMR and MS analysis and evaluated as potential antibacterial agents. They were also tested against the multi-drug resistant strains. The antibacterial activity results revealed that the majority of synthesized compounds exhibited potential antibacterial with the extraordinary level of minimum inhibitory concentrations comparable to the control drugs. Moreover, the influence of presence or absence of fluoro and trifluoromethyl functional groups on the piperazine ring systems towards different biological species is elaborated. The synthesized compounds were also found non-toxic on the human cervical (HeLa) cells at their minimum inhibitory concentrations.

## KEYWORDS

Quinazoline, Fluorinated piperazine, Multidrug–resistant antibacterial activity.

## INTRODUCTION

The multidrug resistance of various microorganisms towards modern antibiotics is a biggest threat in all over the world. Quinazoline derivatives have fascinated significant attention due to their assorted pharmacological activities such as antimicrobial [1-3], anti-inflammatory [4-6], anticonvulsant [7,8], antihypertensive [9,10], antidiabetic [11,12], anticancer [13,14] and antimalarial [15,16] activities. Similarly, piperazine substituted derivatives also show wide range of biological activities [17-24]. Highly electronegative fluorine and trifluoromethyl substitution can enhance electrophilicity, lipophilicity and metabolism. Fluorinated quinolone and piperazine substituted antibiotics (Fig. 1) such as norfloxacin (I), ofloxacin (II), ciprofloxacin (III) *etc.* are widely used worldwide.

In view of that, we have combined different moieties such as benzonitrile, nicotinonitrile, fluorinated piperazine and quinazoline ring in a single molecule using a simple and mild synthetic protocol. The compounds were investigated *in vitro* against various normal antibacterial as well as multidrug resistant clinical isolates such as methicillin-resistant and quinolone-resistant *Staphylococcus aureus* using the minimum inhibitory concentration (MIC). Oxacillin and norfloxacin were used as

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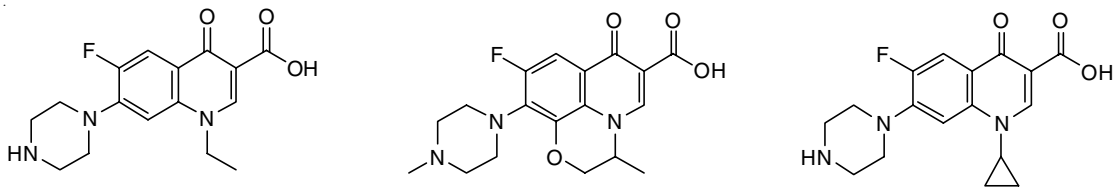


(I) (II) (III)

Fig. 1. Fluorinated quinolone and piperazine substituted antibiotics

positive controls. Synthesized analogs were also tested for their cytotoxic assay against Human cervical (HeLa) cells at their MICs. The influence of fluoro and trifluoromethyl group functionalized phenyl ring attached to the piperazine ring towards the bioassay is elaborated.

## EXPERIMENTAL

The chemicals have been purchased from, Merck and Sigma Aldrich. Analytical grade solvents were used. All reactions were routinely checked by thin layer chromatography (TLC). TLC was performed on aluminum-backed silica gel plates (silica gel 60 F254 grade, Merck DC) with spots visualized by UV light. Column chromatography was performed on silica gel LC 60A (70-200 μ). Melting points were determined in open capillaries on a Veego electronic apparatus VMP-D (Veego Instrument Corporation, Mumbai, India) and are uncorrected. Column chromatography was performed on 1½ feet (2.5 cm diameter) glass column using silica gel LC 60A (70-200 μ). The mass spectra were measured with Waters Micromass Q-Tof Micro instrument (time of flight (TOF) mass spectrometer) and GC-MS Shimadzu QP 2010. The $^{1}$H NMR and $^{13}$C NMR spectra were recorded on a Bruker and Varian spectrometers using DMSO-$d_6$ as a solvent and TMS as an internal standard with $^{1}$H resonant frequency of 400 MHz and $^{13}$C resonant frequency of 100 MHz. The $^{1}$H NMR, $^{13}$C NMR chemical shifts were reported as parts per million (ppm) downfield from TMS ($Me_4Si$). $^{19}$F NMR spectra were obtained on the same spectrometer using $CDCl_3$ as a solvent and $CFCl_3$ as an external standard with $^{19}$F resonant frequency of 400 MHz.

**Synthetic procedure for quinazoline-2,4-diol (2):** A mixture of urea (3.0 g, 50 mmol) and anthranilic acid (**1**) (0.69 g, 5 mmol) was heated at 180 ºC in flat bottom flask on magnetic stirrer. The reaction mixture was stirred for 3 h and then cooled to 100 ºC. Add equal volume of water in the reaction mixture and obtained suspension was stirred for 10 min, after which it was cooled to room temperature. The precipitate was filtered off and recrystallized from DMF to obtain 2,4-dihydroxyquinazoline (**2**) [25]. Light pink solid, yield: 80%, m.p. 298-300 °C.

**General synthetic procedure for 2,4-dichloroquinazoline (3):** A flask containing $POCl_3$ (16 mL), *N,N*-dimethylaniline (5 mL) and 2,4-dihydroxyquinazoline (**2**) (4.8 g, 30 mmol) was refluxed for 2 h. Distilled out the unreacted $POCl_3$, cool the reaction mixture to room temperature and pour it into 250 mL ice–water with constant stirring. The resulting precipitate was filtered off and washed with 50 mL water and dried to obtain 2,4-dichloroquinazoline (**3**) [26]. Yellow solid, yield: 85%, m.p. 117-119 °C. IR (KBr, $\nu_{max}$, cm$^{-1}$): 3064 (Ar–CH), 1610 (CN), 735(C–Cl). $^{1}$H NMR (400 MHz, $Me_2SO$-$d_6$): δ 8.12 (dd, $J$ = 7.2, 1.8 Hz, 1H), 7.97-7.92 (m, 2H, Ar–H), 7.77-7.73 (m, 1H, Ar–H). $^{13}$C NMR (100 MHz, $Me_2SO$-$d_6$): δ 162.1, 154.2, 153.9, 148.0, 132.7, 127.5, 126.2, 123.0.

**Synthetic procedure for 4-((2-chloroquinazolin-4-yl)-oxy)benzonitrile (4a)/6-((2-chloroquinazolin-4-yl)-oxy)-nicotinonitrile (4b):** The mixture of compound **3** (1.0 g, 5 mmol), 4-hydroxybenzonitrile/6-hydroxynicotinonitrile (0.6 g, 5 mmol), potassium carbonate (1.1 g, 8 mmol) and dried acetone (20 mL) is stirred vigorously at 30 ºC for 12 h. After the completion of the reaction, the reaction mass was treated with 100 mL ice cold water and the resulting precipitates were filtered off to obtain crude products (**4a/4b**) [27].

**Compound 4a:** White solid, recrystallization from MeCN; yield: 89%; m.p. 130-132 °C; IR (KBr, $\nu_{max}$, cm$^{-1}$): 2255 (C≡N), 1193 (C–O–C), 741 (C–Cl); $^{1}$H NMR (400 MHz, $Me_2SO$-$d_6$): δ 8.01 (dd, $J$ = 6.8, 1.8 Hz, 1H, quinazoline), 7.92-7.43 (m, 3H, quinazoline), 7.05-6.19 (m, 4H, Ar–H); $^{13}$C NMR (100 MHz, $Me_2SO$-$d_6$): δ 164.02 (1C, C–O–C, quinazoline to benzonitrile linkage), 162.43, 154.37 (1C, C–Cl), 151.25, 146.97, 141.66, 140.05, 139.24, 130.52, 129.47, 127.20, 125.43, 124.08 (10C, Ar–C), 103.71 (1C, C≡N), 95.27 (1C, C–C≡N); MS, *m/z* 282.8 [M+1]$^{+}$.

**Compound 4b:** White solid, recrystallization from MeCN; yield: 81%; m.p. 192-195 °C; IR (KBr, $\nu_{max}$, cm$^{-1}$): 2252 (C≡N), 1204 (C–O–C), 744 (C–Cl); $^{1}$H NMR (400 MHz, $Me_2SO$-$d_6$): δ 8.21 (s, 1H, CH–N of nicotinonitrile), 7.90 (dd, $J$ = 7.2, 2.0 Hz, 1H, quinazoline), 7.67-7.41 (m, 3H, quinazoline), 7.15-6.47 (m, 3H, Ar–H); $^{13}$C NMR (100 MHz, $Me_2SO$-$d_6$): δ 163.68 (1C, C–O–C, quinazoline to benzonitrile linkage), 157.26 (1C, N–C–O, nicotinonitrile), 154.19 (1C, C–Cl), 148.35, 143.08, 141.47, 137.50, 132.19, 128.26, 128.02, 127.15, 125.63 (9C, Ar–C) 104.29 (1C, C≡N), 97.45 (1C, C–C≡N); MS, *m/z* 283.4 [M+1]$^{+}$.

**General synthetic procedure for analogs 5a-5e & 6a-6e:** The oven dried flat bottom flask was charged with compound **4a/4b** (10 mmol), various 1-(4-substituted phenyl)piperazines (11 mmol), potassium carbonate (15 mmol) and ethanol (50 mL) and stirred vigorously at 30 °C for 12 h. The reaction mass was treated with 100 mL ice cold water after the completion of the reaction. The resulting crude analogs **5a-5e** and **6a-6e** were filtered off, dried and purified by column chromatography (20% ethyl acetate in hexane) [28].

**4-((2-(4-Phenylpiperazin-1-yl)quinazolin-4-yl)oxy)-benzonitrile (5a)** White solid; recrystallization from MeCN; yield: 83%; m.p. 188-189 °C; IR (KBr, $\nu_{max}$, cm$^{-1}$): 2248 (C≡N), 1203 (C–O–C), 746 (C–F); $^{1}$H NMR (400 MHz, $Me_2SO$-$d_6$): δ 8.32 (dd, $J$ = 7.2, 2.4 Hz, 1H, quinazoline), 8.02-7.68 (m, 3H, quinazoline), 7.29-6.33 (m, 9H, Ar–H), 3.68 (br s, 4H, piperazine), 3.32 (br s, 4H, piperazine); $^{13}$C NMR (100 MHz,

$Me_2SO$-$d_6$): δ 170.81 (1C, C–C, quinazoline to piperazine linkage), 162.39 (1C, C–O–C, quinazoline to benzonitrile linkage), 161.11, 156.58, 152.03, 146.76, 142.19, 140.55, 138.12, 134.31, 130.07, 129.15, 129.01, 127.93, 126.14, 125.38, 122.38, 115.21, 114.56 (17C, Ar–C), 104.47 (1C, C≡N), 98.29 (1C, C–C≡N), 49.81, 45.49 (4C, piperazine); MS, *m/z* 408.2 $[M+1]^+$.

**4-((2-(4-(2-Fluorophenyl)piperazin-1-yl)quinazolin-4-yl)oxy)benzonitrile (5b):** Light brown solid; recrystallization from MeCN; yield: 79%; m.p. 195-198 °C; IR (KBr, $\nu_{max}$, $cm^{-1}$): 2309 (C≡N), 1129 (C–O–C), 749 (C–F); $^1$H NMR (400 MHz, $Me_2SO$-$d_6$): δ 8.44 (dd, *J* = 6.9, 1.8 Hz, 1H, quinazoline), 8.18-7.83 (m, 3H, quinazoline), 7.59-6.36 (m, 8H, Ar–H), 3.85 (br s, 4H, piperazine), 3.44 (br s, 4H, piperazine); $^{13}$C NMR (100 MHz, $Me_2SO$-$d_6$): δ 172.20 (1C, C–C, quinazoline to piperazine linkage), 168.48 (1C, C–O–C, quinazoline to benzonitrile linkage), 167.23, 157.30, 150.20, 147.35, 143.35, 139.77, 137.01, 135.09, 133.44, 131.01, 129.40, 128.28, 126.21, 123.62, 120.41, 119.20, 116.24 (17C, Ar–C), 105.21 (1C, C≡N), 97.80 (1C, C–C≡N), 50.48, 47.67 (4C, piperazine); $^{19}$F NMR (400 MHz, $CDCl_3$): δ 117.02 (1F, s, F); MS, *m/z* 426.3 $[M+1]^+$.

**4-((2-(4-(4-Fluorophenyl)piperazin-1-yl)quinazolin-4-yl)oxy)benzonitrile (5c):** White solid; recrystallization from MeCN; yield: 68%; m.p. 226-228 °C; IR (KBr, $\nu_{max}$, $cm^{-1}$): 2268 (C≡N), 1198 (C–O–C), 752 (C–F); $^1$H NMR (400 MHz, $Me_2SO$-$d_6$): δ 8.14 (dd, *J* = 7.0, 2.2 Hz, 1H, quinazoline), 7.93-7.74 (m, 3H, quinazoline), 7.38-6.14 (m, 9H, Ar–H), 3.54 (br s, 4H, piperazine), 3.27 (br s, 4H, piperazine); $^{13}$C NMR (100 MHz, $Me_2SO$-$d_6$): δ 169.48 (1C, C–C, quinazoline to piperazine linkage), 161.91 (1C, C–O–C, quinazoline to benzonitrile linkage), 159.37, 157.70, 154.23, 147.97, 143.44, 141.65, 139.46, 135.03, 132.19, 130.27, 129.43, 128.37, 127.89, 126.41, 122.08, 115.14, 115.02 (17C, Ar–C), 108.79 (1C, C≡N), 96.32 (1C, C–C≡N), 50.07, 47.55 (4C, piperazine); $^{19}$F NMR (400 MHz, $CDCl_3$): δ 121.32 (1F, s, F); MS, *m/z* 426.5 $[M+1]^+$.

**4-((2-(4-(2–(Trifluoromethyl)phenyl)piperazin-1-yl)-quinazolin-4-yl)oxy)benzonitrile (5d):** Light yellow solid; recrystallization from MeCN; yield: 84%; m.p. 169-170 °C; IR (KBr, $\nu_{max}$, $cm^{-1}$): 2244 (C≡N), 1194 (C–O–C), 738 (C–F); $^1$H NMR (400 MHz, $Me_2SO$-$d_6$): δ 8.19 (dd, *J* = 8.4, 2.2 Hz, 1H, quinazoline), 7.95-7.57 (m, 3H, quinazoline), 7.32-6.48 (m, 8H, Ar–H), 3.73 (br s, 4H, piperazine), 3.57 (br s, 4H, piperazine); $^{13}$C NMR (100 MHz, $Me_2SO$-$d_6$): δ 179.32 (1C, C–C, quinazoline to piperazine linkage), 169.25 (1C, C–O–C, quinazoline to benzonitrile linkage), 165.47, 158.98, 152.21, 149.05, 145.51, 141.37, 139.12, 134.08, 131.24, 130.15, 129.49, 129.17, 128.32 (13C, Ar–C), 126.59 (1C, $CF_3$), 122.79, 121.20, 120.34 (3C, Ar–C), 115.41 (1C, C–$CF_3$), 104.93 (1C, C≡N), 98.17 (1C, C–C≡N), 48.16, 42.33 (4C, piperazine); $^{19}$F NMR (400 MHz, $CDCl_3$): δ 62.39 (3F, s, $CF_3$); MS, *m/z* 476.8 $[M+1]^+$.

**4-((2-(4-(4–(Trifluoromethyl)phenyl)piperazin-1-yl)-quinazolin-4-yl)oxy)benzonitrile (5e):** White solid; recrystallization from MeCN; yield: 78%; m.p. 183-185 °C; IR (KBr, $\nu_{max}$, $cm^{-1}$): 2258 (C≡N), 1228 (C–O–C), 741 (C–F); $^1$H NMR (400 MHz, $Me_2SO$-$d_6$): δ 8.41 (dd, *J* = 7.2, 2.4 Hz, 1H, quinazoline), 8.02-7.63 (m, 3H, quinazoline), 7.58-6.72 (m, 8H, Ar–H), 3.80 (br s, 4H, piperazine), 3.48 (br s, 4H, piperazine); $^{13}$C NMR (100 MHz, $Me_2SO$–$d_6$): δ 181.03 (1C, C–C, quinazoline to piperazine linkage), 171.12 (1C, C–O–C, quinazoline to benzonitrile linkage), 167.53, 162.19, 157.31, 149.28, 146.89, 143.15, 140.21, 136.96, 132.45, 130.52, 130.01, 129.87, 129.04 (13C, Ar–C), 127.34 (1C, $CF_3$), 123.66, 121.25, 119.98 (3C, Ar–C), 116.10 (1C, C–$CF_3$), 103.07 (1C, C≡N), 97.68 (1C, C–C≡N), 50.35, 43.96 (4C, piperazine); $^{19}$F NMR (400 MHz, $CDCl_3$): δ 63.03 (3F, s, $CF_3$); MS, *m/z* 476.5 $[M+1]^+$.

**6-((2-(4-Phenylpiperazin-1-yl)quinazolin-4-yl)oxy)-nicotinonitrile (6a):** White solid; recrystallization from MeCN; yield: 83%; m.p. 167-169 ºC; IR (KBr, $\nu_{max}$, $cm^{-1}$): 2267 (C≡N), 1222 (C–O–C), 735 (C–F); $^1$H NMR (400 MHz, $Me_2SO$-$d_6$): δ 8.59 (s, 1H, CH–N of benzonitrile), 8.17 (dd, *J* = 6.8, 2.0 Hz, 1H, quinazoline), 7.86–7.52 (m, 3H, quinazoline), 7.15–6.47 (m, 7H, Ar–H), 3.51 (br s, 4H, piperazine), 3.28 (br s, 4H, piperazine); $^{13}$C NMR (100 MHz, $Me_2SO$-$d_6$): δ 172.44 (1C, C–C, quinazoline to piperazine linkage), 164.37 (1C, C–O–C, quinazoline to benzonitrile linkage), 159.02 (1C, N–C–O, nicotinonitrile), 154.61, 154.19, 147.23, 141.58, 140.34, 138.59, 133.01, 129.43, 128.72, 127.85, 126.66, 124.32, 121.14, 113.29, 113.07 (15C, Ar–C), 106.34 (1C, C≡N), 99.05 (1C, C–C≡N), 50.28, 47.06 (4C, piperazine); MS, *m/z* 409.9 $[M+1]^+$.

**6-((2-(4-(2-Fluorophenyl)piperazin-1-yl)quinazolin-4-yl)oxy)nicotinonitrile (6b):** White solid; recrystallization from MeCN; yield: 79%; m.p. 232-236 °C; IR (KBr, $\nu_{max}$, $cm^{-1}$): 2283 (C≡N), 1230 (C–O–C), 737 (C–F); $^1$H NMR (400 MHz, $Me_2SO$-$d_6$): δ 8.41 (s, 1H, CH–N of benzonitrile), 8.23 (dd, *J* = 6.9, 1.8 Hz, 1H, quinazoline), 7.98-7.69 (m, 3H, quinazoline), 7.34-6.42 (m, 6H, Ar–H), 3.57 (br s, 4H, piperazine), 3.42 (br s, 4H, piperazine); $^{13}$C NMR (100 MHz, $Me_2SO$-$d_6$): δ 171.89 (1C, C–C, quinazoline to piperazine linkage), 166.01 (1C, C–O–C, quinazoline to benzonitrile linkage), 163.45 (1C, N–C–O, nicotinonitrile), 155.19, 152.98, 148.19, 143.28, 139.15, 134.33, 132.25, 131.48, 128.73, 127.81, 126.00, 124.29, 121.65, 119.36, 117.54 (16C, Ar–C), 104.45 (1C, C≡N), 98.80 (1C, C–C≡N), 52.96, 48.02 (4C, piperazine); $^{19}$F NMR (400 MHz, $CDCl_3$): δ 119.35 (1F, s, F); MS, *m/z* 427.9 $[M+1]^+$.

**6-((2-(4-(4-Fluorophenyl)piperazin-1-yl)quinazolin-4-yl)oxy)nicotinonitrile (6c):** Light brown solid; recrystallization from MeCN; yield: 83%; m.p. 219-221 °C; IR (KBr, $\nu_{max}$, $cm^{-1}$): 2245 (C≡N), 1196 (C–O–C), 743 (C–F); $^1$H NMR (400 MHz, $Me_2SO$–$d_6$): δ 8.32 (s, 1H, CH–N of benzonitrile), 8.26 (dd, *J* = 6.8, 4.2 Hz, 1H, quinazoline), 7.88-7.59 (m, 3H, quinazoline), 7.21-6.27 (m, 6H, Ar–H), 3.52 (br s, 4H, piperazine), 3.30 (br s, 4H, piperazine); $^{13}$C NMR (100 MHz, $Me_2SO$-$d_6$): δ 170.11 (1C, C–C, quinazoline to piperazine linkage), 163.45 (1C, C–O–C, quinazoline to benzonitrile linkage), 158.86 (1C, N–C–O, nicotinonitrile), 157.02, 154.37, 146.25, 142.38, 140.13, 136.63, 133.27, 131.78, 129.64, 127.73, 126.42, 126.02, 121.87, 116.33, 114.39 (16C, Ar–C), 107.42 (1C, C≡N), 98.46 (1C, C–C≡N), 52.01, 49.31 (4C, piperazine); $^{19}$F NMR (400 MHz, $CDCl_3$): δ 120.02 (1F, s, F); MS, *m/z* 427.5 $[M+1]^+$.

**6-((2-(4-(2–(Trifluoromethyl)phenyl)piperazin-1-yl)-quinazolin-4-yl)oxy)nicotinonitrile (6d):** White solid; recrystallization from MeCN; yield: 70%; m.p. 257-261 °C; IR (KBr, $\nu_{max}$, $cm^{-1}$): 2262 (C≡N), 1223 (C–O–C), 749 (C–F); $^1$H NMR (400 MHz, $Me_2SO$-$d_6$): δ 8.57 (s, 1H, CH–N of benzonitrile),

8.31 (dd, *J* = 7.2, 2.6 Hz, 1H, quinazoline), 8.09-7.76 (m, 3H, quinazoline), 7.49-6.14 (m, 6H, Ar–H), 3.62 (br s, 4H, piperazine), 3.44 (br s, 4H, piperazine); $^{13}$C NMR (100 MHz, $Me_2SO$-$d_6$): δ 179.18 (1C, C–C, quinazoline to piperazine linkage), 172.87 (1C, C–O–C, quinazoline to benzonitrile linkage), 166.64 (1C, N–C–O, nicotinonitrile), 161.31, 158.34, 151.57, 148.95, 142.77, 139.19, 134.62, 132.73, 129.47, 129.03, 127.84 (11C, Ar–C), 125.78 (1C, $CF_3$), 121.97, 121.26, 120.48 (3C, Ar–C), 116.25 (1C, C–$CF_3$), 108.33 (1C, C≡N), 96.11 (1C, C–C≡N), 49.64, 43.17 (4C, piperazine); $^{19}$F NMR (400 MHz, $CDCl_3$): δ 63.42 (3F, s, $CF_3$); MS, *m/z* 477.4 $[M+1]^+$.

**6-((2-(4-(4-(Trifluoromethyl)phenyl)piperazin-1-yl)-quinazolin-4-yl)oxy)nicotinonitrile (6e):** White solid; recrystallization from MeCN; yield: 74%; m.p. 208-212 °C; IR (KBr, $\nu_{max}$, $cm^{-1}$): 2270 (C≡N), 1215 (C–O–C), 744 (C–F); $^1$H NMR (400 MHz, $Me_2SO$-$d_6$): δ 8.51 (s, 1H, CH–N of benzonitrile), 8.20 (dd, *J* = 6.8, 2.2 Hz, 1H, quinazoline), 7.80-7.54 (m, 3H, quinazoline), 7.48-7.25 (m, 6H, Ar–H), 3.89 (br s, 4H, piperazine), 3.51 (br s, 4H, piperazine); $^{13}$C NMR (100 MHz, $Me_2SO$-$d_6$): δ 172.65 (1C, C–C, quinazoline to piperazine linkage), 168.59 (1C, C–O–C, quinazoline to benzonitrile linkage), 166.82 (1C, N–C–O, nicotinonitrile), 158.91, 157.29, 156.57, 151.09, 148.72, 145.51, 143.16, 139.73, 136.20, 134.27, 133.41, 133.18 (11C, Ar–C), 130.40 (1C, $CF_3$), 125.57, 124.90, 122.04 (3C, Ar–C), 120.61 (1C, C–$CF_3$), 105.23 (1C, C≡N), 96.93 (1C, C–C≡N), 49.78, 46.65 (4C, piperazine); $^{19}$F NMR (400 MHz, $CDCl_3$): δ –62.80 (3F, s, $CF_3$); MS, *m/z* 476.4 $[M+1]^+$.

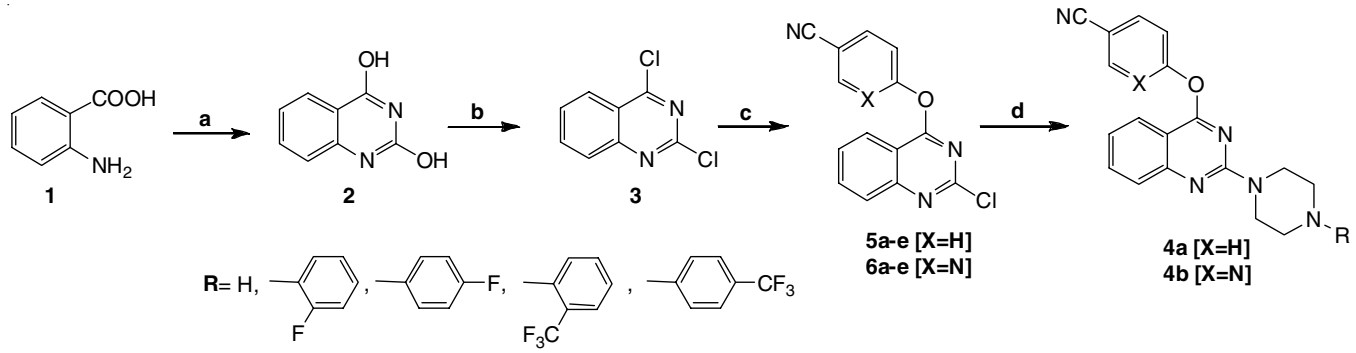
**Antibacterial activity:** The *in vitro* antibacterial activity was carried out using the minimum inhibitory concentration (MIC) and broth microdilution method with multidrug-resistant clinical isolates. The compounds were tested against Gram-positive bacteria *S. aureus* (RN4220, KCTC 503 and KCTC 209) and a Gram-negative bacterium *E. coli* 1356. The strains of multidrug-resistant clinical isolates were methicillin-resistant *S. aureus* (MRSA CCARM 3167 and MRSA CCARM 3506) and quinolone-resistant *Staphylococcus aureus* (QRSA CCARM 3505 and QRSA CCARM 3519). The microbacteria growth was measured by the absorption at 630 nm using a microtiter enzyme-linked immunosorbent assay (ELISA) reader. Test susceptible microbacteria were grown to mid-log phase in Mueller–Hinton broth (MHB) and diluted 1000-fold in the same medium. The stock solutions of the compounds in DMSO were poured into 96-well plates and used to achieve final concentrations of 64-0.5 μg/mL [29]. Oxacillin and norflo-xacin were used as positive controls for bacteria. Suspension of microbacteria was prepared to contain approximate $10^5$ CFU/mL and applied to 96-well plates with serially diluted compounds to be tested and incubated at 37 ºC for 24 h. The MIC (expressed in μg/mL) was the lowest concentration of the test substance that completely inhibited growth of the microbacteria. All experiments were carried out three times.

**Cytotoxicity:** Human cervical cell mono layers were used as an *in vitro* model of cervicovaginal epithelium for testing the cytotoxicity of the synthesized compounds. The cells were developed in Dulbecco modified Eagle medium with fetal bovine serum (FBS, 10%) and penicillin-streptomycin mixture (100 U/mL). The cells split by trypsin (0.25% in PBS; pH 7.4) and the medium was changed at 24 h intervals. They were cultured at 37 ºC in a 5% $CO_2$ incubator. The cells were grown to 3 passages and approximately 1 × $10^4$ cells were seeded into each well of a 96-well plate for 24 h. Then the medium was restored with DMEM supplemented with FBS (10%) containing various concentrations of test compounds and incubated for 48 h. Then 10 μL of MTT (5 mg/mL in PBS) was added to each well. The medium was removed and the resulting formazan crystals were dissolved with 100 μL DMSO after incubation for 4 h. The optical density was measured at 570 nm using a microtiter ELISA reader. The assay was conducted four times [30].

## RESULTS AND DISCUSSION

The synthetic route for the titled compounds **5a-6e** is sketched in Fig. 2. The initial compound 2,4-dichloroquinazoline (**3**) was synthesized from anthranilic acid according to the reported literature [31-35]. The condensation reaction of 4-hydroxybenzonitrile/6-hydroxynicotinonitrile with 2,4-dichloroquinzoline (**3**) in presence of base yields the intermediate analogs 4-((2-chloroquinazolin-4-yl)oxy)benzonitrile (**4a**)/6-((2-chloroquinazolin-4-yl)oxy)nicotinonitrile (**4b**). The reaction of this intermediates **4a/4b** with various fluorinated phenyl piperazine derivatives yields desired analogs **5a-6e**. The syntheses of titled analogs were confirmed by various analytical techniques.

In the FT-IR spectrum, presence of characteristic absorption bands at 2309 $cm^{-1}$ for C≡N group, confirms the formation of compound **5b**. Moreover, the FT-IR spectrum also revealed another two absorption bands at 1129 $cm^{-1}$ for the C–O–C




(a) Urea, 180 °C; (b) $POCl_3$, DMA, reflux; (c) 4-hydroxybenzonitrile/6-hydroxynicotinonitrile, $K_2CO_3$, acetone (d) 1-(4-substituted phenyl)piperazine, $K_2CO_3$, EtOH, Reflux

Fig. 2. Synthetic protocol for the analogs **5a–6e**

linkage. The $^{1}$H NMR spectra of compound **5b** shows two doublets (d, 3.41 and 3.85), which confirms the presence of piperazine ring. Moreover, the multiplet between 6.36-6.67 ppm indicates the presence of aromatic ring. The presence of multiplet between 7.02–8.44 ppm proves the presence of quinazoline ring. The piperazine ring gave signals at 47.6 ppm and 50.4 ppm in the $^{13}$C NMR spectra of compound **5b**. Finally the appearance of peak at 117.02 ppm in $^{19}$F NMR spectrum of compound **5b** shows the presence of fluorine atom on phenyl ring attached to the piperazine moiety. Similarly the FT–IR spectrum of most active compound **6e** shows absorption at 2270 cm$^{-1}$ for C≡N and 1215 cm$^{-1}$for the C–O–C linkage. The presence of piperazine ring in compound **6e** is confirmed by appearance of two doublets at 3.51 and 3.89 ppm in $^{1}$H NMR spectrum of compound **6e**. The appearance of peaks at 46.6 and 49.7 ppm in $^{13}$C NMR spectrum of compound **6e** also confirms the presence of piperazine ring. The $^{19}$F NMR spectrum of compound **6e** shows the peak at 62.8 ppm, which proves the presence of trifluoromethyl group in it. Moreover the mass spectrum with *m/z* 276.4 $[M+1]^{+}$ also proves the formation of compound **6e**.

**Antibacterial activity:** The *in vitro* antimicrobial activities (Table-1) of the newly synthesized compounds were evaluated against three Gram–positive bacteria (*Staphylococcus aureus* RN4220, KCTC 503 and KCTC 209) and Gram-negative bacteria (*Escherichia coli* CCARM1356). It was observed that many of the synthesized analogs displayed excellent efficacy against all the three Gram-positive bacteria specifically *Staphylococcus aureus* RN4220 and *Staphylococcus aureus* 503 (MIC values of d" 64 µg/mL) as compared to the control drugs. The introduction of fluoro and trifluoromethyl functional groups on *ortho* or *para* the phenyl ring attached to the piperazine moiety increased the antibacterial activity, relative to the non–substituted example. Moreover, trifluoromethyl piperazine fused nicotinonitrile derivatives (**6d** & **6e**) show more promising activity (MIC values between 4-8 µg/mL) as compared to benzonitrile derivatives (**5d** & **5e**, MIC values between 8-16 µg/mL). *p*-Trifluoromethyl piperazine fused nicotinonitrile **6e** has shown highest antibacterial activity (4 µg/mL) against *Staphylococcus aureus* RN4220. Similar *ortho* analog was found active against *Staphylococcus aureus* 503 at MIC level of 4 µg/mL. However, all the synthesized analogs were found inactive (MIC values of >64 µg/mL) against Gram-negative bacteria *Escherichia coli* 1356.

Synthesized analogs were further evaluated for their inhibitory activity against selected methicillin-resistant *S. aureus* (MRSA) and quinolone-resistant *S. aureus* (QRSA) (Table-2). It is clear from the data that all of the compounds exhibited higher levels of inhibitory activity than oxacillin against MRSA 3167 (methicillin-resistant *S. aureus* CCARM 3167) and MRSA 3506 (methicillin-resistant *S. aureus* CCARM 3506). Out of all the synthesized analogs, compound **6e** has shown the most potent levels of antibacterial activity (4 µg/mL) against all of the multidrug-resistant clinical isolates MRSA 3167, 3506 and QRSA 3519. However, analog **6d** is half fold less active than compound **6e**. Analog compounds **6d** and **6e** is also found to be potent against quinolone-resistant *S. aureus* CCARM 3505 at MIC level of 8 µg/mL.

TABLE-1
INHIBITORY ACTIVITIES (MIC, µg/mL) OF COMPOUNDS **5a–6e** AGAINST BACTERIA

| Compound | *S. aureus* | | | *E. coli* |
|---|---|---|---|---|
| | RN4220 | 503 | 209 | CCARM 1356 |
| **5a** | 64 | 64 | > 64 | > 64 |
| **5b** | 32 | 32 | 32 | > 64 |
| **5c** | 32 | 16 | 32 | > 64 |
| **5d** | 8 | 32 | 16 | > 64 |
| **5e** | 8 | 16 | 16 | > 64 |
| **6a** | 64 | 64 | 64 | > 64 |
| **6b** | 16 | 16 | 32 | > 64 |
| **6c** | 16 | 8 | 32 | > 64 |
| **6d** | 8 | 4 | 16 | > 64 |
| **6e** | 4 | 8 | 8 | > 64 |
| Oxacillin | 1 | 1 | 1 | > 64 |
| Norfloxacin | 2 | 2 | 4 | 16 |

TABLE 2
INHIBITORY ACTIVITIES (MIC, µg/mL) OF COMPOUNDS **5a–6e** AGAINST CLINICAL ISOLATES OF MULTIDRUG-RESISTANT GRAM-POSITIVE BACTERIAL STRAINS

| Compound | Multidrug-resistant Gram-positive strains | | | |
|---|---|---|---|---|
| | MRSA | | QRSA | |
| | 3167[a] | 3506[b] | 3505[c] | 3519[d] |
| **5a** | 32 | 64 | 64 | 64 |
| **5b** | 16 | 16 | 16 | 32 |
| **5c** | 16 | 16 | 16 | 32 |
| **5d** | 16 | 32 | 16 | 16 |
| **5e** | 8 | 16 | 16 | 16 |
| **6a** | 32 | 32 | 32 | 64 |
| **6b** | 16 | 16 | 16 | 16 |
| **6c** | 8 | 16 | 16 | 16 |
| **6d** | 8 | 8 | 8 | 16 |
| **6e** | 4 | 4 | 8 | 4 |
| Oxacillin | > 64 | > 64 | 1 | 1 |
| Norfloxacin | 4 | 4 | > 64 | > 64 |

[a]Methicillin-resistant *S. aureus* CCARM 3167; [b]Methicillin-resistant *S. aureus* CCARM 3506; [c]Quinolone-resistant *S. aureus* CCARM 3505; [d]Quinolone-resistant *S. aureus* CCARM 3519

It is known that trifluoromethyl group possesses greater inductive effect than direct fluorine substitution on aromatic compounds. In view of the electrostatic properties of fluoroalkyl groups are very likely to be an outcome of high electronegativity of fluorine atoms. Hydrogen bonds between the trifluoromethyl substituent and the enzyme may account for a better binding of the substrate. Biological results also support that trifluoromethyl group substituted derivatives are more active as compared to normal fluorinated analogs.

The cytotoxic properties of compounds **6d** and **6e** were also investigated using MTT colorimetric assay to determine if their observed antibacterial activity was caused by selective toxicity towards the bacterial cells and the results are shown in Table-3.

These compounds did not affect cell viability on the Human cervical (HeLa) cells at their MICs but showed cytotoxicity at much higher concentrations. Hence, the inconsistency between the antibacterial activity and cytotoxicity of compounds **6d** and **6e** suggests that there may be an antibacterial mechanism different from cytotoxicity.

TABLE-3
CYTOTOXIC ACTIVITY OF COMPOUNDS **6d** AND **6e** AGAINST HeLa CELL

| Compound | $IC_{50}$ (µg/mL) |
|---|---|
| **6d** | 18.32 |
| **6e** | 21.14 |

[a]Hela cell (Human cervical) monolayers were used as an *in vitro* model of cervicovaginal epithelium for testing the cytotoxicity of the new compounds. [b]$IC_{50}$ is defined as the concentration at which 50% growth is observed.

## Conclusion

The results demonstrated that most of the synthesized analogs have good levels of antibacterial activity against Gram-positive bacteria including multidrug-resistant strains of clinical isolates. It was observed that trifluoromethyl substituted derivatives exhibit good interactions and tight binding with active sites of respective receptor. Trifluoromethyl phenyl piperazine and nicotinonitrile ring substituted quinazoline derivatives **6d** and **6e** show high antibacterial activity in the range of 4-8 µg/mL MIC levels against *Staphylococcus aureus*. *p*-Trifluoromethyl substituted nicotinonitrile quinazoline analog (**6e**) has shown highest antibacterial activity (4 µg/mL) against MRSA CCARM 3167, CCARM 3506 and QRSA CCARM 3519. Compounds **6d** and **6e** were evaluated for their cytotoxicity and exhibited no significant influence on cell viability in the HeLa cells at their MIC (1 µg/mL). These derivatives may be extremely helpful for further drug discovery and design.

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