DESIGN, SYNTHESIS AND ANTIMICROBIAL ACTIVITIES OF 1-(4-OXO-3-(4-FLUOROPHENYL)-3*H*-QUINAZOLIN-2-YL)-4-(SUBSTITUTED) THIOSEMICARBAZIDE DERIVATIVES

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ABSTRACT

A new series of 1-(4-oxo-3-(4-fluorophenyl)-3*H*-quinazolin-2-yl)-4-(substituted) thiosemicarbazides (**AR1-AR10**) were obtained by the reaction of 2-hydrazino-3-(4-fluorophenyl) quinazolin-4(3*H)*-one (**6**) with different dithiocarbamic acid methyl ester derivatives. The key intermediate 3-(4-fluorophenyl)-2-thioxo-2,3-dihydro-1*H*-quinazolin-4-one **(4)** was obtained by reacting 4-fluoroaniline (**1**) with carbon disulphide and sodium hydroxide in dimethyl sulphoxide to give sodium dithiocarbamate, which was methylated with dimethyl sulfate to yield the dithiocarbamic acid methyl ester **(2)** and condensed with methyl anthranilate **(3)** in ethanol yielded the desired compound **(4)** via the thiourea intermediate. The SH group of compound (**4**) was methylated for the favorable nucleophilic displacement reaction with hydrazine hydrate, which afford 2-hydrazino-3-(4-fluorophenyl)-3*H*-quinazolin-4-one **(6)**. All synthesized compounds (**AR1-AR10**) were also screened for their antimicrobial activity against selective gram positive and gram negative by agar dilution method. In the present study compounds **AR8** and **AR9** were emerged as the most active compounds of the series.

Keywords: Quinazolinone, Substituted thiosemicarbazide, Anti-bacterial, Antitubercular activity.

INTRODUCTION

Tuberculosis (TB) is one of the leading causes of death all over the world. Tuberculosis (TB) is an infection, primarily in the lungs (a pneumonia), caused by bacteria called *Mycobacterium tuberculosis*. Emergence of multi drug resistant tuberculosis (MDR-TB) makes the conditions most alarming.[1,2] Some of the MDR isolates are resistant to as many as seven of the commonly employed antimycobacterial drugs.[3] Quinazolines and condensed quinazolines received the attention of medicinal chemists due to their potential biological activities. Among the biological activities exhibited by quinazolines the antimicrobial activities of 2,3-disubstituted quinazolines are promising.[4] Literature survey indicates that the quinazolines nucleus substituted at 2,3-position (Fig 1, **I, II**) showed significant antitubercular activity.[5,6] The pharmacophore like thiosemicarbazides and thiosemicarbazones groups (Fig 1, **III-IV**) in different heterocyclic moieties were also found to exhibit the antitubercular activity.[7-15] The present work is an extension of our ongoing efforts towards developing effective antitubercular and antimicrobial agents by a hybrid approach using the quinazoline scaffold (Fig 1). In this approach two or more pharmacophores are merged into a single molecule. Therefore, a single molecule containing more than one pharmacophore, each pharmacophores may be addressing the active site of targets and offer the possibility to selectivity, further it can also reduce unwanted side effects.[16] In the present study, we have placed the substituted thiosemicarbazide moiety at the C-2 position and 4-fluorophenyl ring at N-3 position of quinazoline ring[17,18] and studied their antitubercular and antibacterial activities against selected gram positive and negative bacteria.

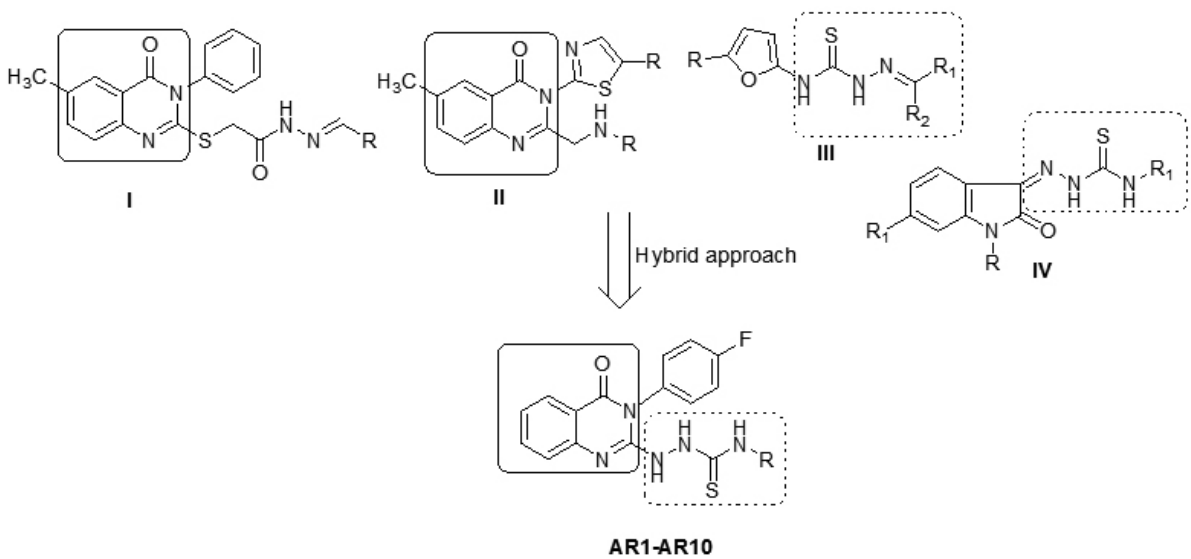

Fig. 1. Hybrid approach design of 1-(4-oxo-3-(4-fluorophenyl)-3*H*-quinazolin-2-yl)-4-(substituted)thiosemicarbazide analogs.

Methods Chemistry

Melting points (mp) were taken in open capillaries on Thomas Hoover melting point apparatus (Thomas Hoover, USA) and are uncorrected. The IR spectra were recorded in film or in potassium bromide disks on a Perkin-Elmer 398 spectrometer (Perkin-Elmer, USA). The ^{1}H spectra were recorded on a DPX-300 MHz Bruker FT-NMR spectrometer (Bruker, USA). The chemical shifts were reported as parts per million (δ ppm) tetramethylsilane (TMS) as an internal standard. Mass spectra were obtained on a JEOL-SX-102 instrument (JEOL, Japan) using fast atom bombardment (FAB positive). Elemental analysis was performed on a Perkin-Elmer 2400 CHN analyzer (Perkin-Elmer, USA) and values were within the acceptable limits of the calculated values (± 0.4%). The progress of the reaction was monitored on readymade silica gel plates (Merck, Norway) using chloroform/methanol (9:1) as a solvent system. Iodine was used as a developing agent. All chemicals and reagents used in the synthesis were obtained from Aldrich (USA), Lancaster (USA) or Spectrochem (India) and were used without further purification.

Synthesis of 3-(4-fluorophenyl)-2-thioxo-2,3-dihydro-1*H*-quinazolin-4-one (4)

A solution of 4-fluoroaniline **1** (0.02 mol) in dimethyl sulphoxide (10 mL) was stirred vigorously. To this was added carbon disulphide (1.6 mL, 0.026

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mol) and aqueous sodium hydroxide 1.2 mL (20 molar solution) drop wise during 30 min with stirring. Dimethyl sulphate (0.02 mol) was added gradually keeping the reaction mixture stirring in freezing mixture for 2 h. The reaction mixture was then poured into ice water. The solid obtained was filtered, washed with water, dried and recrystallized from ethanol. Methyl anthranilate (0.01 mol) and the above prepared *N*-(4-fluorophenyl)-methyl dithiocarbamic acid (0.01 mol), were dissolved in ethanol (20 mL). To this anhydrous potassium carbonate (100 mg) was added and refluxed for 23 h. The reaction mixture was cooled in ice and the solid separated was filtered and purified by dissolving in 10% alcoholic sodium hydroxide solution and re-precipitated by treating with dilute hydrochloric acid. The solid obtained was filtered, washed with water, dried and recrystallized from ethanol. Yield = 83 %, mp 253-255 °C. IR: 3242 (NH), 1666 (C=O), 1218 (C=S) cm^{-1}. ^1H NMR ($CDCl_3$) δ ppm: 6.45 (br s, 1H, NH), 6.75-6.77 (m, 2H, ArH), 6.97-6.99 (d, J = 7.5 Hz, 2H, Ar-H), 7.26-7.28 (m, 1H, ArH), 7.65-7.67 (d, J = 7.5 Hz, 2H, Ar-H), 7.54-7.56 (m, 1H, ArH); ^{13}C NMR ($CDCl_3$) δ ppm: 120.61, 123.45, 123.78, 125.32, 127.72, 128.45, 130.79, 131.54, 138.75, 158.69, 160.18, 178.85; MS (m/z): 273 ([M+H]$^+$, 100), 178 (58), 144 (56), 134 (40); Anal. Calcd. for $C_{14}H_9FN_2OS$: C 61.75, H 3.33, N 10.29; Found C 61.65, H 3.38, N 10.17.

Synthesis of 3-(4-fluorophenyl)-2-(methylthio)quinazolin-4(3H)-one (5)

The 3-(4-fluorophenyl)-2-thioxo-2,3-dihydro-1*H*-quinazolin-4-one **4** (0.01 mol) was dissolved in 40 mL of 2% alcoholic sodium hydroxide solution. To this dimethyl sulphate (0.01 mol) was added drop wise with stirring. The stirring was continued for 1 h, the reaction mixture was then poured into ice water. The solid obtained was filtered, washed with water, dried and recrystallized from ethanol-chloroform (75:25) mixture. Yield = 81%, mp 153-155 °C; IR: 1682 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ ppm: 2.01 (s, 3H, SCH_3), 6.65-6.67 (m, 2H, ArH), 6.95-6.97 (d, J = 7.5 Hz, 2H, Ar-H), 7.21-7.23 (m, 1H, ArH), 7.63-7.65 (d, J = 7.5 Hz, 2H, Ar-H), 7.64-7.66 (m, 1H, ArH); ^{13}C NMR ($CDCl_3$) δ ppm: 12.58, 115.57, 120.56, 123.35, 125.28, 127.78, 127.85, 128.61, 130.81, 131.86, 138.75, 158.69, 162.15; MS (*m/z*): 287 ([M+H$^+$], 100), 254 (48), 178 (45), 144 (52), 134 (33); Anal. Calcd. for $C_{15}H_{11}FN_2OS$: C 62.92, H 3.87, N 9.78; Found C 62.95, H 3.92, N 9.82.

Synthesis of 2-hydrazino-3-(4-fluorophenyl)-3*H*-quinazolin-4-one (6)

The 3-(4-fluoro phenyl)-2-methylsulfanyl-3*H*-quinazolin-4-one **5** (0.01 mol) was dissolved in ethanol (25 mL). To this hydrazine hydrate (99%) (0.1 mol) and anhydrous potassium carbonate (100 mg) was added and refluxed for 35 h. The reaction mixture was cooled and poured into ice water. The solid so obtained was filtered, washed with water, dried and recrystallized from chloroform-benzene (25:75) mixture. Yield = 72%, mp 174-176 °C. IR: 3310, 3226 ($NHNH_2$), 1680 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ ppm: 4.50 (s, 2H, NH_2), 6.68-6.70 (m, 2H, ArH), 6.97-6.99 (d, J = 7.0 Hz, 2H, Ar-H), 7.31-7.33 (m, 1H, ArH), 7.73-7.75 (d, J = 7.0 Hz, 2H, Ar-H), 7.84-7.86 (m, 1H, ArH), 8.62 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ ppm: 115.47, 120.45, 123.54, 125.47, 127.76, 128.75, 130.65, 131.76, 138.75, 158.69, 160.53, 162.42; MS (m/z): 271 ([M+H]$^+$, 100), 223 (55), 179 (48), 144 (57); Anal. Calcd. for $C_{14}H_{11}FN_4O$: C 62.22, H 4.10, N 20.73; Found C 62.62, H 4.17, N 20.67.

General procedure for synthesis of 1-(4-oxo-3-(4-fluorophenyl)-3*H*-dihydroquinazolin-2-yl)-4-(substituted)thiosemicarbazides (AR1-AR10)

A solution of primary alkyl/aryl amine (0.02 mol) in dimethyl sulfoxide (10 mL) was stirred vigorously. To this simultaneously carbon disulphide (1.6 mL) and aqueous sodium hydroxide 1.2 mL (20 molar solution) was added drop wise during 30 min with stirring. Dimethyl sulphate (0.02 mol) was added gradually by keeping the reaction mixture stirring in a freezing mixture and continued for further 2 h. The reaction mixture was then poured into ice water and the solid obtained was filtered washed with water, dried and recrystallized from ethanol.

2-hydrazino-3-(4-fluoro phenyl)-3*H*-quinazolin-4-one (**6**) (2.32 g, 0.01 mol) and methyl-*N*-(substituted)dithiocarbamate **(7)** (0.01 mol) was dissolved in ethanol and refluxed for 22-30 h (until the methyl mercapton evolution ceases). After completion of the reaction the reaction mixture cooled to room temperature. The solid obtained was filtered, dried and recrystallized from ethanol. By adapting the above procedure the compounds **AR1-AR10** were prepared.

1-[4-oxo-3-(4-fluorophenyl)-3*H*-quinazolin-2-yl]-4-[cyclohexyl] thiosemicarbazide (AR1)

Yield = 83 %; mp 192-193 °C; IR (KBr) cm^{-1}: 3356 (NH), 3283 (NH), 3243 (NH), 1660 (C=O), 1605 (C=N), 1210 (C=S); ^1H NMR ($CDCl_3$) δ ppm: 1.40-1.62 (m, 6H, CH_2), 1.82-1.84 (m, 2H, CH_2), 1.91-1.93 (m, 2H, CH_2), 2.84 (s, 1H, CH), 6.79-6.82 (m, 2H, ArH), 7.11-7.14 (m, 1H, ArH), 7.39-7.41 (d, J = 7.0 Hz, 2H, Ar-H), 7.45-7.48 (d, J = 7.0 Hz, 2H, Ar-H), 7.91-7.93 (m, 1H, ArH), 8.42 (s, 1H, NH), 8.98 (s, 1H, NH), 10.53 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ ppm: 22.68, 25.79, 32.54, 52.75, 115.47, 120.56, 123.35, 123.68, 125.28, 127.85, 128.61, 130.81, 131.86, 138.75, 158.69, 162.45, 183.74; MS (*m/z*): 412 ([M+H]$^+$, 100); Anal. Calcd. for $C_{21}H_{22}FN_5OS$: C 61.29, H 5.39, N 17.02; Found C 61.32, H 5.31, N, 17.08.

1-[4-oxo-3-(4-fluorophenyl)-3*H*-quinazolin-2-yl]-4-[benzyl] thiosemicarbazide (AR2)

Yield = 79 %; mp 232-234 °C; IR (KBr) cm^{-1}: 3321 (NH), 3295 (NH), 3264 (NH), 1692 (C=O), 1616 (C=N), 1216 (C=S); ^1H NMR ($CDCl_3$) δ ppm : 1.51 (s, 1H, NH), 3.25 (s, 1H, NH), 4.56 (s, 2H, CH_2), 6.61-6.64 (m, 2H, ArH), 6.73-6.77 (m, 2H, ArH), 7.21-7.24 (d, J = 8.0 Hz, 2H, Ar-H), 7.41-7.44 (d, J = 7.5 Hz, 2H, Ar-H), 7.56-7.59 (d, J = 8.0 Hz, 2H, Ar-H), 7.92-7.94 (d, J = 8.0 Hz, 2H, Ar-H), 7.95-7.98 (m, 1H, ArH); ^{13}C NMR ($CDCl_3$) δ ppm: 49.68, 115.65, 120.68, 123.61, 123.74, 125.32, 125.72, 126.82, 127.42, 127.75, 128.41, 130.51, 131.45, 138.56, 140.35, 158.69, 161.87, 183.12; MS (m/z): 420 ([M+H$^+$], 100); Anal. Calcd. for $C_{22}H_{18}FN_5OS$: C 62.99, H 4.33, N 16.70; Found C 65.85, H 4.74, N, 17.42.

1-[4-oxo-3-(4-fluorophenyl)-3*H*-quinazolin-2-yl]-4-[phenyl] thiosemicarbazide (AR3)

Yield = 92 %; mp 276 - 278 °C; IR (KBr) cm^{-1}: 3338 (NH), 3286 (NH), 3210 (NH), 1666 (C=O), 1600 (C=N), 1256 (C=S), 1136 (C-F); ^1H NMR ($CDCl_3$) δ ppm: 6.51-6.54 (m, 2H, ArH), 6.82-6.85 (m, 2H, ArH), 7.12-7.15 (d, J = 8.0 Hz, 2H, Ar-H), 7.32-7.41 (d, J = 7.5 Hz, 2H, Ar-H), 7.60-7.63 (d, J = 8.0 Hz, 2H, Ar-H), 7.85-7.88 (d, J = 8.0 Hz, 2H, Ar-H), 7.91-7.93 (m, 1H, ArH), 8.72 (s, 1H, NH), 8.89 (s, 1H, NH), 10.53 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ ppm: 115.64, 120.54, 123.53, 123.68, 125.22, 125.39, 126.82, 127.35, 127.82, 128.72, 130.81, 131.86, 138.75, 140.35, 158.69, 162.75, 181.55; MS (m/z): 405 ([M+H$^+$], 100); Anal. Calcd. for $C_{21}H_{16}FN_5OS$: C 62.21, H 3.98, N 17.27; Found C 62.18, H 3.95, N 17.19.

1-[4-oxo-3-(4-fluorophenyl)-3*H*-quinazolin-2-yl]-4-[2-methylphenyl] thiosemicarbazide (AR4)

Yield = 86 %; mp 240-241 °C; IR (KBr) cm^{-1}: 3338 (NH), 3270 (NH), 3242 (NH), 1655 (C=O), 1610 (C=N), 1250 (C=S), 1135 (C-F); ^1H NMR ($CDCl_3$) δ ppm: 2.31 (s, 3H, CH_3), 6.49-6.51 (m, 2H, ArH), 6.79 (br s, 1H, NH), 6.82-6.85 (m, 2H, ArH), 7.12-7.15 (d, J = 8.0 Hz, 2H, Ar-H), 7.32-7.36 (d, J = 7.5 Hz, 2H, Ar-H), 7.59-7.62 (d, J = 8.0 Hz, 2H, Ar-H), 7.75-7.78 (d, J = 8.0 Hz, 2H, Ar-H), 8.42 (br s, 1H, NH), 10.29 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ ppm: 13.58, 115.64, 120.54, 123.54, 123.63, 125.22, 125.42, 126.68, 127.82, 127.85, 128.61, 130.79, 131.86, 138.75, 138.85, 140.35, 158.69, 162.75, 182.63; MS (m/z): 419 ([M+H$^+$], 100); Anal. Calcd. for $C_{22}H_{18}FN_5OS$: C 62.99, H 4.33, N 16.70; Found C 63.01, H 4.28, N 16.57.

1-[4-oxo-3-(4-fluorophenyl)-3*H*-quinazolin-2-yl]-4-[4-methylphenyl] thiosemicarbazide (AR5)

Yield = 75 %; mp 225 - 226 °C; IR (KBr) cm^{-1}: 3342 (NH), 3278 (NH), 3218 (NH), 1662 (C=O), 1262 (C=S), 1132 (C-F); ^1H NMR ($CDCl_3$) δ ppm: 2.31 (s, 3H, CH_3), 6.51-6.53 (m, 2H, ArH), 6.75 (br s, 1H, NH), 6.72-6.75 (m, 2H, ArH), 7.17-7.20 (d, J = 7.5 Hz, 2H, Ar-H), 7.29-7.32 (d, J = 7.5 Hz, 2H, Ar-H), 7.59-7.62 (d, J = 7.5 Hz, 2H, Ar-H), 7.69-7.71 (d, J = 7.5 Hz, 2H, Ar-H), 8.45 (br s, 1H, NH), 10.15 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ ppm: 13.58, 115.64, 120.43, 123.56, 123.62, 125.75, 125.85, 126.68, 127.82, 127.93, 128.61, 130.79, 131.86, 138.69, 138.91, 140.35, 158.69, 162.75, 182.35; MS (m/z): 419 ([M+H$^+$], 100); Anal. Calcd. for $C_{22}H_{18}FN_5OS$: C 62.99, H 4.33, N 16.70; Found C 62.89, H 4.31, N 16.62.

1-(3-(4-fluorophenyl)-4-oxo-3,4-dihydroquinazolin-2-yl)-4-(3-methoxyphenyl)thiosemicarbazide (AR6)

Yield = 80 %; mp 190-192 °C; IR (KBr) cm^{-1}: 3340 (NH), 3280 (NH), 3223 (NH), 1660 (C=O), 1605 (C=N), 1270 (OCH_3), 1260 (C=S), 1130 (C-F); ^{1}HNMR ($CDCl_3$) δ ppm : 3.71 (s, 3H, OCH_3), 6.61-6.63 (m, 2H, ArH), 6.79 (br s, 1H, NH), 6.82-6.85 (m, 2H, ArH), 7.21-7.24 (d, J = 7.0Hz, 2H, Ar-H), 7.32-7.35 (d, J = 7.0 Hz, 2H, Ar-H), 7.62-7.66 (d, J = 8.0 Hz, 2H, Ar-H), 7.71-7.74 (d, J = 7.0 Hz, 2H, Ar-H), 8.45 (br s, 1H, NH), 10.25 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ ppm: 54.75, 109.87, 115.47, 117.85, 120.48, 123.48, 123.75, 125.53, 127.21, 127.75, 128.75, 129.57, 129.89, 130.81, 131.46, 137.57, 138.75, 158.69, 162.42, 181.53; MS (*m/z*): 435 ([M+H$^+$], 100); Anal. Calcd. for $C_{22}H_{18}FN_5O_2S$: C 60.68, H 4.17, N 16.08; Found C 60.62, H 4.18, N, 16.04.

1-(3-(4-fluorophenyl)-4-oxo-3,4-dihydroquinazolin-2-yl)-4-(4-methoxyphenyl)thiosemicarbazide (AR7)

Yield = 84 %; mp 220- 222 °C; IR (KBr) cm^{-1}: 3319 (NH), 3227 (NH), 3266 (NH), 1684 (C=O), 1608 (C=N), 1218 (C=S), 1100 (C-F); ^{1}HNMR ($CDCl_3$) δ ppm : 3.75 (s, 3H, OCH_3), 6.59-6.61 (m, 2H, ArH), 6.69 (br s, 1H, NH), 6.81-6.83 (m, 2H, ArH), 7.23-7.26 (d, J = 7.5 Hz, 2H, Ar-H), 7.31-7.34 (d, J = 8.0 Hz, 2H, Ar-H), 7.61-7.64 (d, J = 7.5 Hz, 2H, Ar-H), 7.73-7.76 (d, J = 7.0 Hz, 2H, Ar-H), 8.55 (br s, 1H, NH), 10.31 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ

ppm: 54.89, 108.97, 115.23, 117.71, 120.43, 123.18, 123.68, 124.53, 126.33, 126.84, 127.85, 129.57, 128.99, 130.81, 131.46, 137.57, 138.75, 158.69, 162.42, 181.25; MS (*m/z*): 435 ([M+H$^+$], 100); Anal. Calcd. for $C_{22}H_{18}FN_5O_2S$: C 60.68, H 4.17, N 16.08; Found C 60.72, H 4.08, N, 16.11.

1-[4-oxo-3-(4-fluorophenyl)-3*H*-quinazolin-2-yl]-4-[4-chlorophenyl] thiosemicarbazide (AR8)

Yield = 88 %; mp 262-264 °C; IR (KBr) cm^{-1}: 3288 (NH), 3269 (NH), 3203 (NH), 1682 (C=O), 1610 (C=N), 1258 (C=S), 1122 (C-F); ^{1}H NMR ($CDCl_3$) δ ppm: 6.42-6.45 (m, 2H, ArH), 6.53-6.55 (m, 1H, ArH), 7.22-7.24 (d, J = 8.0 Hz, 2H, Ar-H), 7.42-7.46 (d, J = 8.0 Hz, 2H, Ar-H), 7.73-7.77 (d, J = 7.5 Hz, 2H, Ar-H), 7.80-7.83 (d, J = 7.5 Hz, 2H, Ar-H), 7.92-7.94 (m, 1H, ArH), 8.63 (br s, 1H, NH), 8.92 (br s, 1H, NH), 10.63 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ ppm: 115.34, 121.39, 122.37, 123.44, 124.82, 125.87, 126.74, 127.22, 127.85, 128.61, 130.81, 131.86, 138.75, 139.45, 158.69, 161.79, 181.61; MS (*m/z*): 440 ([M+H$^+$], 100); Anal. Calcd. for $C_{21}H_{15}ClFN_5OS$: C 57.34, H 3.44, N 15.92; Found C 57.35, H 3.34, N 15.88.

4-(4-chlorophenyl)-1-(3-(4-fluorophenyl)-4-oxo-3,4-dihydroquinazolin-2-yl)thiosemicarbazide (AR9)

Yield = 82 %; mp 242-245 °C; IR (KBr) cm^{-1}: 3352 (NH), 3310 (NH), 3210 (NH), 1691 (C=O), 1615 (C=N), 1220 (C=S); ^{1}H NMR ($CDCl_3$) δ ppm: 6.72-6.75 (m, 2H, ArH), 6.92-6.95 (d, J = 7.5 Hz, 2H, Ar-H), 7.32-7.36 (d, J = 8.0 Hz, 2H, Ar-H), 7.63-7.67 (d, J = 7.5 Hz, 2H, Ar-H), 7.75-7.78 (d, J = 7.5 Hz, 2H, Ar-H), 7.92-7.94 (m, 2H, ArH), 8.45 (br s, 1H, NH), 8.75 (br s, 1H, NH), 10.35 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ ppm: 115.25, 121.89, 122.57, 123.11, 124.73, 125.87, 126.74, 127.22, 127.85, 128.61, 130.75, 131.86, 138.75, 139.45, 158.69, 161.79, 182.61; MS (*m/z*): 451 ([M+H$^+$], 100); Anal. Calcd. for $C_{21}H_{15}FN_6O_3S$: C 55.99, H, 3.36, N 18.66; Found C 57.35, H 3.34, N 15.88.

1-[4-oxo-3-(4-fluorophenyl)-3*H*-quinazolin-2-yl]-4-[pyridin-2-yl] thiosemicarbazide (AR10)

Yield = 78 %; mp 180-182 °C; IR (KBr) cm^{-1}: 3350 (NH), 3310 (NH), 3223 (NH), 1680 (C=O), 1614 (C=N), 1200 (C=S); ^{1}H NMR ($CDCl_3$) δ ppm: 6.41-6.44 (m, 1H, ArH), 6.83-6.85 (m, 2H, ArH), 7.21-7.24 (d, J = 7.5 Hz, 2H, Ar-H), 7.40-7.43 (d, J = 8.0 Hz, 2H, Ar-H), 7.61-7.65 (d, J = 7.5 Hz, 2H, Ar-H), 7.80-7.83 (d, J = 7.5 Hz, 2H, Ar-H), 7.90-7.93 (m, 1H, ArH), 8.83 (br s, 1H, NH), 9.03 (br s, 1H, NH), 10.32 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ ppm: 108.75, 112.89, 115.25, 120.48, 123.51, 123.25, 125.42, 127.53, 128.61, 130.81, 131.86, 137.89, 138.75, 147.75, 157.85, 158.69, 161.78, 181.25; MS (*m/z*): 407 ([M+H$^+$], 100); Anal. Calcd. for $C_{20}H_{15}FN_6OS$: C 59.10, H 3.72, N 20.68, Found C 59.15, H 3.75, N 20.72.

Pharmacology

Antibacterial activity

Evaluation of antibacterial activity determined by agar dilution method.[10,11]. The standard strains were procured from the American Type Culture Collection (ATCC), Rockville, USA, and the pathological strains were procured from the Department of Microbiology, MNR Medical College, Sangareddy, India. The antibacterial activity of the synthesized compounds was screened against the following bacterial strains: *P. vulgaris ATCC 9484, S. typhimurium ATCC 33068, K. pneumoniae ATCC 13883, E. tarda, P. aeruginosa ATCC 2853, B. subtilis ATCC 6051, and S. paratyphi*. All bacteria were grown on Muller–Hinton Agar (Hi-media) plates (37 °C, 24 h) and the minimum inhibitory concentration (MIC) was considered to be the lowest concentration that completely inhibited the growth on agar plates, disregarding a single colony or faint haze caused by the inoculums.[19, 20] The MIC of the test compounds was compared with the reference drug ciprofloxacin. The values are mentioned in Table 1 calculated from at least three different experiments in duplicate.

Antitubercular activity

10 fold serial dilutions of each test compound/drug were incorporated into Middle brook 7H11 agar slants with OADC Growth Supplement. Inoculums of *M. tuberculosis* H37R$_v$ were prepared from fresh Middle brook 7H11 agar slants with OADC Growth Supplement adjusted to 1 mg/mL in Tween 80 (0.05% W/V) saline diluted to 10^{-2} to give a concentrate of approximately 107 cfu/mL. A 5µL amount of bacterial suspension was spotted into 7H11 agar tubes containing 10-fold serial dilutions of drug per mL. The tubes were incubated at 37 ºC, and final readings were recorded after 28 days. Tubes having the compounds were compared with control tubes where medium alone was incubated with H37R$_v$. The concentration at which complete inhibition of colonies occurred was taken as active concentration of test compound. The MIC is defined as the minimum concentration of compound required to give complete inhibition of bacterial growth.[21-23] The MIC of the test compounds was compared with the reference drug gatifloxacin.

Cytotoxicity profile of the tested compounds

For cytotoxic assay with HeLa, approximately 10,000 cells were seeded with 0.1 mL RPMI 1640 culture medium per well of the 96-well micro plates. HeLa cells were preincubated for 48 h without the test substances. The solutions of the compounds of the corresponding concentrations were applied carefully on the monolayers of HeLa cells after the preincubation time. The monolayers of the adherent HeLa cells were fixed by glutaraldehyde and stained with a 0.05% solution of methylene blue for 15 min. After gently washing, the stain was eluted by 0.2 mL of 0.33N HCl in the wells. The optical densities were measured at 630 nm in a micro plate reader. In general, compounds showed no significant cytotoxic effect at tested concentration.[24]

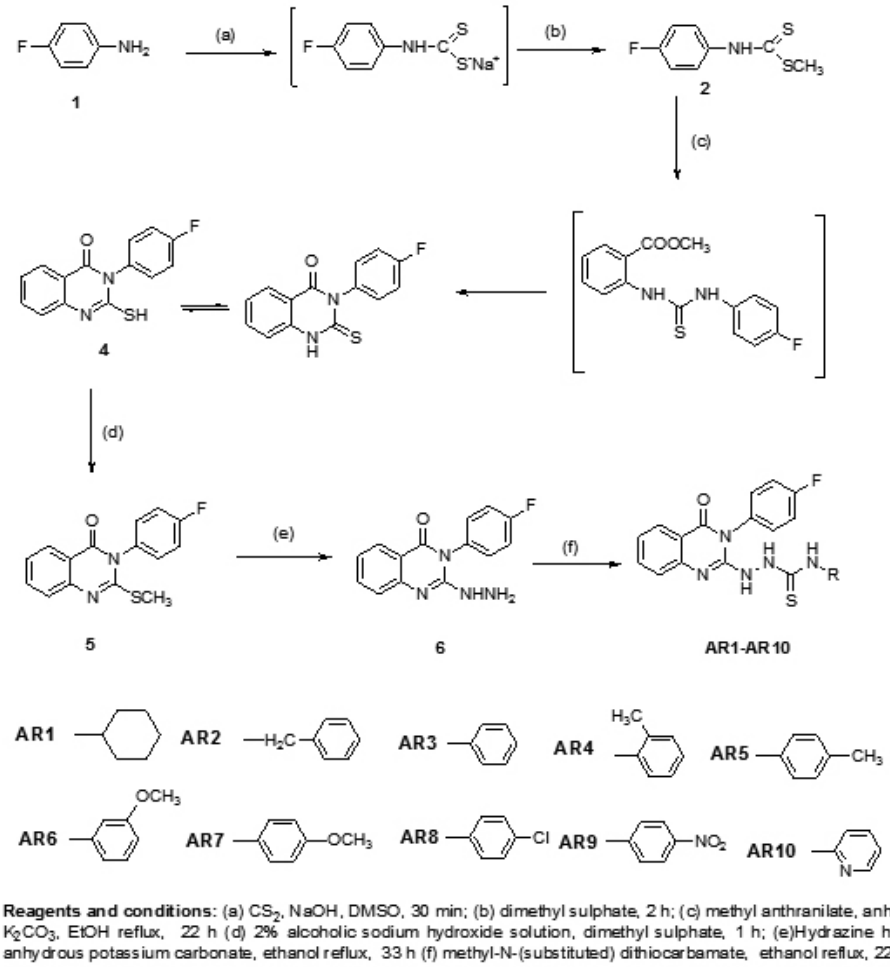
RESULTS AND DISCUSSION

Chemistry

Synthetic route depicted in Scheme 1 outline the chemistry part of the present work. The key intermediate 3-(4-fluorophenyl)-2-thioxo-2,3-dihydro-1*H*-quinazolin-4-one **(4)** was obtained by reacting 4-fluoroaniline (**1**) with carbon disulphide and sodium hydroxide in dimethyl sulphoxide to give sodium dithiocarbamate, which was methylated with dimethyl sulfate to afford the dithiocarbamic acid methyl ester **(2)**. Compound **2** on reflux with methyl anthranilate **(3)** in ethanol yielded the desired 3-(4-fluorophenyl)-2-thioxo-2,3-dihydro-1*H*-quinazolin-4-one **(4)** via the thiourea intermediate in good yield (83%). The product obtained was cyclic and not an open chain thiourea **3a**. The 3-(4-fluorophenyl)-2-methylsulfanyl-3*H*-quinazolin-4-one **5** was obtained by dissolving **4** in 2% alcoholic sodium hydroxide solution and methylating with dimethyl sulphate with stirring at room temperature. Nucleophilic displacement of methylthio group of **5** with hydrazine hydrate was carried out using ethanol as solvent to afford 2-hydrazino-3-(4-fluorophenyl)-3*H*-quinazolin-4-one **6**. The long duration of reaction (35 h) required might be due to the presence of bulky aromatic ring at position **3**, which might have reduced the reactivity of quinazoline ring system at C-2 position. The title compounds 1-(4-oxo-3-(4-fluorophenyl)-3*H*-dihydroquinazolin-2-yl)-4-(substituted) thiosemicarbazides (**AR1-AR10**) were obtained by the condensation of amino group of 2-hydrazino-3-(4-fluorophenyl)-3*H*-quinazolin-4-one **(6)** with a variety of methyl ester of dithiocarbamic esters. The formation of title product is indicated by the disappearance of peak due to NH, NH_2 of the starting material in IR and ^{1}H NMR spectrum of all the compounds **AR1-AR10**. The IR and ^{1}H NMR spectrum of these compounds showed the presence of peaks due to thiosemicarbazides, carbonyl (C=O), NH and aryl groups. The mass spectra of the title compounds showed molecular ion peaks corresponding to their molecular formulae. In mass spectrum of compounds **AR1-AR10** a common peak at *m/z* 144 corresponding to quinazolin-4-one moiety appeared. Elemental (C, H, N) analysis satisfactorily confirmed elemental composition and purity of the synthesized compounds.

Antitubercular activity

The synthesized compounds were screened for their *in vitro* antimycobacterial activity against *M. tuberculosis* strain H37R$_v$. The results are expressed in terms of Minimum Inhibitory Concentration (MIC). The results of antimycobacterial activity depicted in Table 1, indicates that the test compounds inhibited the growth of *Mycobacterium* in varying degree. Compounds with aliphatic substituents showed lesser antitubercular activity over the aryl and heteroaryl substituents. The compounds with electron withdrawing substituent on the aryl ring showed better activity over the unsubstituted or electron donating substituent on the aryl ring. Among the test compounds, 1-(4-oxo-3-(4-fluorophenyl)-3*H*-quinazolin-2-yl)-4-(4-chlorophenyl) thiosemicarbazides (**AR8**) and as well as 1-(4-oxo-3-(4-fluorophenyl)-3*H*-quinazolin-2-yl)-4-(4-nitrophenyl)thiosemicarbazides (**AR9**) exhibited the antitubercular activity at the minimum microgram (3 µg/mL) concentration.



Scheme 1. Synthesis of 1-(4-oxo-3-(4-fluorophenyl)-3*H*-quinazolin-2-yl)-4-(substituted) thiosemicarbazides.

Table 1: Antitubercular and antibacterial activity of synthesized compounds (**A1-A10**).

Microorganisms	Test Compounds (MIC in µg/ml)										Standard*
	AR1	AR2	AR3	AR4	AR5	AR6	AR7	AR8	AR9	AR10	
M. tuberculosis	63	26	26	6	13	6	13	3	3	6	1
S. typhi	63	125	63	125	125	125	63	8	8	32	4
E. coli	63	32	63	63	63	125	32	8	8	32	2
S. flexneri	63	63	63	63	125	32	125	16	16	63	1
P. vulgaris	63	63	63	125	63	32	63	16	16	32	1
Enterobacter spp.	125	125	63	63	63	16	32	16	16	16	1
K. pneumonia	125	63	63	125	63	63	32	32	16	16	1
S. enteritidis	125	63	125	63	125	125	63	32	16	16	1
B. subtilis	63	125	63	125	32	63	32	8	16	16	1
S. flexneri	125	63	63	32	125	63	32	16	16	32	1
P. aeruginosa	32	63	63	32	32	32	63	32	8	16	1

*Gatifloxacin used as a reference standard against *M. tuberculosis* whereas Ciprofloxacin used as a reference standard for other bacteria.

Antibacterial activity

Among the different substituents at C-2 position of the quinazolin-2-yl, aryl and heteroaryl substitutents exhibited better activity over the aliphatic cyclic substituents. Compounds with electron withdrawing substituents like –Cl and $–NO_2$ showed better activity over the unsubstituted and electron donating substituents. Compounds **AR8** and **AR9** were emerged as the most active compounds of the series. Compound **AR8** shown most potent activity against *S. typhi, E.coli* and *B. subtilis*. While the compound **AR9** showed most potent activity against *S. typhi, E.coli* and *P. aeruginosa.*

CONCLUSION

In summary, synthesis of new series of 1-(4-oxo-3-(4-fluorophenyl)-3*H*-dihydroquinazolin-2-yl)-4-(substituted) thiosemicarbazides have been described. These derivatives have exhibited significant antibacterial activity against the various gram positive and gram negative bacteria including *M. tuberculosis.* Among the series, compound **AR8** shown most potent activity against *S. typhi, E.coli* and *B. subtilis*. While the compound **AR9** showed most potent activity against *S. typhi, E.coli* and *P. aeruginosa.* The test compounds, **AR8** and **AR9** exhibited the antitubercular activity at the minimum microgram (3 µg/mL) concentration and offers potential for further optimization and development to new antitubercular agents.

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Conflict of Interest Statement

The authors report no conflicts of interest to declare in connection with the contents of this manuscript.

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