4. EXPERIMENTAL

4.1. Chemistry

Melting Points (mp) were determined in open capillary tubes on Thomas Hoover melting point apparatus and are uncorrected. The IR spectra were recorded in film or in potassium bromide disks using a Perkin-Elmer 398 spectrometer. The ^{1}H NMR spectra were recorded on DPX-500 MHz Bruker FT-NMR spectrometer. The chemical shifts were reported in parts per million (δ ppm) relative to TMS as an internal reference. Mass spectra were recorded on a JEOL-SX-102 instrument using fast atom bombardment (FAB positive). Elemental analysis (C, H and N) was performed on a Perkin-Elmer 2400 analyzer and values were within the acceptable limits of the calculated values. The progress of all the reactions were monitored by readymade silica gel plates (Merck) and a solvent system of chloroform-methanol (9:1). The spots were developed in iodine chamber. Spectral data (IR, NMR and mass spectra) were confirmed the structures of the synthesized compounds and the purity of these compounds were ascertained by microanalysis. Elemental (C, H and N) analysis indicated that the calculated and observed values were within the acceptable limits. All chemicals and reagents were procured from Aldrich (USA), Lancaster (UK) or Spectrochem Pvt.Ltd (India) and were used without further purification.

4.1.1. 3-^{n}Butyl-2-thioxo quinazolin-4(3*H*)-one (4)

To a vigorously stirred solution of $_{n}$Butylamine (1.46 gm; 0.02 mol) in dimethyl sulfoxide (10 mL), carbon disulphide (1.6 mL) and aqueous sodium hydroxide (1.2 mL; 20 molar solution) added dropwise. Then stirring was continued for further 30 min. Dimethyl sulphate (2.5 g; 0.02 mol) was then added gradually keeping the reaction mixture in freezing mixture with stirring and the stirring was continued for an additional 2 hrs. The reaction mixture was then poured into ice cold water. The separated solid obtained was filtered, washed with water, dried and recrystallized from ethanol to give *N*-(butyl) methyl dithiocarbamic acid. Then Methyl anthranilate (1.5 g; 0.01 mol) was added to the above prepared product in ethanol (20 mL). To this anhydrous potassium carbonate (100 mg) was also added and refluxed (conventional heating 22 h; microwave heating 25 min). The reaction mixture was cooled in crushed ice and the solid separated was filtered off and purified by dissolving in 10% alcoholic sodium hydroxide solution and reprecipitated by treating with dilute hydrocholoric acid. The solid obtained was filtered, washed with water and finally dried. Then it was recrystallized from ethanol to give (**4**).

Yield	: 2.01 gm; 86 %
Melting Point	: 240-242 ^{0}C
Rf Value	: 0.30 [Chloroform-Methanol (9:1)]

Molecular Formula	: $C_{12}H_{14}N_2OS$
Molecular Weight	: 234 [M^+]
IR (KBr) cm^{-1}	: 3250 (NH), 1669 (C=O), 1217 (C=S)
^1H NMR ($CDCl_3$) δ ppm	: 0.97-1.02 (t, 3H, CH_3), 1.40-1.52 (m, 2H, CH_2), 1.74-1.80 (m, 2H, CH_2), 4.50-4.55 (m, 2H, CH_2), 7.26-7.63 (m, 4H, Ar-H), 10.5 (s, 1H, NH)
Elemental Analysis	
Calculated	: C, 61.51; H, 6.02; N, 11.96
Found	: C, 61.55; H, 6.05; N, 11.99

4.1.2. 3-*n*Butyl-2-methylsulfanyl quinazolin-4(3*H*)-one (5)

The 3-*n*butyl-2-thioxo quinazolin-4(3*H*)-one **(4)** (2.34 gm; 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	: 2.18 gm; 88 %
Melting Point	: 170-172 °C
Rf Value	: 0.63 [Chloroform-Methanol (9:1)]

Molecular Formula	: $C_{13}H_{16}N_2OS$
Molecular Weight	: 248 [M^+]
IR (KBr) cm^{-1}	: 1680 (C=O)
^1H NMR ($CDCl_3$) δ ppm	: 0.96-1.01 (t, 3H, CH_3), 1.39-1.51 (m, 2H, CH_2), 1.73-1.77 (m, 2H, CH_2), 2.65 (t, 3H, SCH_3), 4.10-4.15 (m, 2H, CH_2), 7.33-7.69 (m, 4H, Ar-H)
Elemental Analysis	
Calculated	: C, 62.87; H, 6.49; N, 11.28
Found	: C, 62.85; H, 6.52; N, 11.25

4.1.3. 3-*n*Butyl-2-hydrazino quinazolin-4(3*H*)-one (6)

The 3-*n*butyl-2-methylsulfanyl quinazolin-4(3*H*)-one **(5)** (2.48 gm; 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 (conventional heating 30 h; microwave heating 35 min). 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	: 1.87 gm; 81%
Melting Point	: 180-182 °C
Rf Value	: 0.41 [Chloroform-Methanol (9:1)]

Molecular Formula	: $C_{12}H_{16}N_4O$
Molecular Weight	: 232 [M^+]
IR (KBr) cm^{-1}	: 3434 & 3200 ($NHNH_2$), 1656 (C=O)
^1H NMR ($CDCl_3$) δ ppm	: 0.96-1.01 (t, 3H, CH_3), 1.37-1.50 (m, 2H, CH_2), 1.66-1.74 (m, 2H, CH_2), 4.04-4.13 (m, 2H, CH_2), 4.61 (s, 2H, NH_2), 7.10-7.23 (m, 4H, Ar-H), 10.24 (s, 1H, NH).
Elemental Analysis	
Calculated	: C, 62.05; H, 6.94; N, 24.12
Found	: C, 62.10; H, 6.96; N, 24.10

4.1.4. General procedure for the synthesis of 1-(4-oxo-3-^{n}butyl-3*H*-quinazolin-2-yl)-4-(substituted) thiosemicarbazides (AS1-AS10)

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 mol) and aqueous sodium hydroxide 1.2 mL (20 molar solution) was added drop wise during 30 min with stirring. Dimethyl sulphate (1.84 gm; 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.

3-Butyl-2-hydrazino quinazolin-4(3*H*)-one (**6**) (2.32 gm; 0.01 mol) Methyl-*N*-(substituted) dithiocarbamate **(7)** (0.01 mol) was dissolved in ethanol and refluxed (conventional heating 24-30 h; microwave heating 20-25 min) 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 **AS1-AS10** were prepared.

4.1.4.1. 1-(4-oxo-3-*n*butyl-3*H*-quinazolin-2-yl)-4-(phenyl) thiosemicarbazide (AS1)

Yield	: 3.22 gm; 88 %
Melting Point	: 115-117 ˚C
Rf Value	: 0.49 [Chloroform-Methanol (9:1)]
Molecular Formula	: $C_{19}H_{21}N_5OS$
Molecular Weight	: 367 [M^+]
IR (KBr) cm^{-1}	: 3300 (NH), 3240 (NH), 1674 (C=O), 1252 (C=S)
^1H NMR ($CDCl_3$) δ ppm	: 1.04-1.09 (t, 3H, CH_3), 1.40-1.47 (m, 2H, CH_2), 1.55-1.77 (m, 2H, CH_2), 4.14-4.19 (m, 2H, CH_2), 6.84-6.87 (d, 2H, Ar-H), 7.08 (br s, 1H, NH), 7.36 (br s, 1H, NH), 7.43-7.53 (m, 4H, Ar-H), 8.02-8.08 (m, 2H, Ar-H), 8.15-8.19 (d, 1H, Ar-H), 8.77 (br s, 1H, NH)
Elemental Analysis	
Calculated	: C, C, 62.10; H, 5.76; N, 19.06
Found	: C, 62.13; H, 5.74; N, 19.01

4.1.4.2. 1-(4-oxo-3-^{n}butyl-3*H*-quinazolin-2-yl)-4-(2-methylphenyl) thiosemicarbazide (AS2)

Yield	: 2.78 gm; 73 %
Melting Point	: 100-102 ˚C
Rf Value	: 0.47 [Chloroform-Methanol (9:1)]
Molecular Formula	: $C_{20}H_{23}N_5OS$
Molecular Weight	: 381 [M^+]
IR (KBr) cm^{-1}	: 3300 (NH), 3220 (NH), 1673 (C=O), 1254 (C=S)
^1H NMR ($CDCl_3$) δ ppm	: 1.05-1.09 (t, 3H, CH_3), 1.42-1.55 (m, 2H, CH_2), 1.67-1.82 (m, 2H, CH_2), 2.46 (s, 3H, CH_3), 4.14-4.19 (m, 2H, CH_2), 6.84-6.86 (d, 1H, Ar-H), 7.06-7.34 (m, 6H, Ar-H), 7.53 (br s, 1H, NH), 8.03-8.08 (d, 1H, Ar-H), 8.77 (br s, 1H, NH), 11.78 (br s, 1H, NH)
Elemental Analysis	
Calculated	: C, 62.97; H, 6.08; N, 18.36
Found	: C, 62.96; H, 6.11; N, 18.38

4.1.4.3. 1-(4-oxo-3-^{n}butyl-3*H*-quinazolin-2-yl)-4-(3-methylphenyl) thiosemicarbazide (AS3)

Yield	: 3.08 gm; 81 %
Melting Point	: 195-197˚ C
Rf Value	: 0.52 [Chloroform-Methanol (9:1)]
Molecular Formula	: $C_{20}H_{23}N_5OS$
Molecular Weight	: 381 [M^+]
IR (KBr) cm^{-1}	: 3383 (NH), 3240 (NH), 1673 (C=O), 1233 (C=S)
^1H NMR ($CDCl_3$) δ ppm	: 0.92-0.99 (t, 3H, CH_3), 1.31-1.40 (m, 2H, CH_2), 1.83-1.89 (m, 2H, CH_2), 2.13 (s, 3H, CH_3), 4.02-4.09 (m, 2H, CH_2), 7.15-7.20 (d, 1H, Ar-H), 7.35-7.42 (m, 6H, Ar-H), 7.92-7.98 (d, 1H, Ar-H), 8.05 (br s, 1H, NH), 8.49 (br s, 1H, NH), 10.11 (br s, 1H, NH).
Elemental Analysis	
Calculated	: C, 62.97; H, 6.08; N, 18.36
Found	: C, 62.99; H, 6.05; N, 18.34

4.1.4.4. 1-(4-oxo-3-^{n}butyl-3*H*-quinazolin-2-yl)-4-(4-methylphenyl) thiosemicarbazide (AS4)

Yield	: 2.81 gm; 74%
Melting point	: 110-112 ˚C
Rf Value	: 0.47 [Chloroform-Methanol (9:1)]
Molecular Formulae	: $C_{20}H_{23}N_5OS$
Molecular Weight	: 381 [M^+]
IR (KBr) cm^{-1}	: 3405 (NH), 3238 (NH), 1673 (C=O), 1253 (C=S)
^1H NMR ($CDCl_3$) δ ppm	: 1.13-1.17 (t, 3H, CH_3), 1.53-1.57 (m, 2H, CH_2), 1.72-1.78 (m, 2H, CH_2), 2.71 (s, 3H, CH_3), 4.17-4.21 (m, 2H, CH_2), 7.03-7.06 (d, 1H, Ar-H), 7.72-7.83 (m, 6H, Ar-H), 7.93-7.99 (d, 1H, Ar-H), 8.29 (br s, 1H, NH), 9.12 (br s, 1H, NH), 10.48 (br s, 1H, NH).
Elemental Analysis	
Calculated	: C, 62.97; H, 6.08; N, 18.36
Found	: C, 62.99; H, 6.06; N, 18.30

4.1.4.5. 1-(4-oxo-3-^{n}butyl-3*H*-quinazolin-2-yl)-4-(3-methoxyphenyl) thiosemicarbazide (AS5)

Yield	: 3.17 gm; 80 %
Melting Point	: 120-122 ˚C
Rf Value	: 0.52 [Chloroform-Methanol (9:1)]
Molecular Formula	: $C_{20}H_{23}N_5O_2S$
Molecular Weight	: 397 [M^+]
IR (KBr) cm^{-1}	: 3365 (NH), 3221 (NH), 1634 (C=O), 1254 (C=S)
^1H NMR ($CDCl_3$) δ ppm	: 1.05-1.11 (t, 3H, CH_3), 1.55-1.61 (m, 2H, CH_2), 1.59-1.67 (m, 2H, CH_2), 2.32 (s, 3H, OCH_3), 3.72-3.76 (m, 2H, CH_2), 7.22-7.23 (d, 1H, Ar-H), 7.48-7.55 (m, 6H, Ar-H), 7.73-7.74 (d, 1H, Ar-H), 8.20 (br s, 1H, NH), 8.25 (br s, 1H, NH), 10.13 (br s, 1H, NH).
Elemental Analysis	
Calculated	: C, 60.43; H, 5.83; N, 17.67
Found	: C, 60.46; H, 5.87; N, 17.67

4.1.4.6. 1-(4-oxo-3-^{n}butyl-3*H*-quinazolin-2-yl)-4-(2-nitrophenyl) thiosemicarbazide (AS6)

Yield	: 2.88 gm; 70 %
Melting Point	: 115-117 ˚C
Rf Value	: 0.47 [Chloroform-Methanol (9:1)]
Molecular Formula	: $C_{19}H_{20}N_6O_3S$
Molecular Weight	: 412 [M^+]
IR (KBr) cm^{-1}	: 3340 (NH), 3239 (NH), 1651 (C=O), 1233 (C=S)
^1H NMR ($CDCl_3$) δ ppm	: 0.90-0.96 (t, 3H, CH_3), 1.53-1.61 (m, 2H, CH_2), 1.86-1.91 (m, 2H, CH_2), 3.63-3.68 (m, 2H, CH_2), 7.19-7.21 (d, 1H, Ar-H), 7.31-7.45 (m, 6H, Ar-H), 7.72-7.73 (d, 1H, Ar-H), 8.05 (br s, 1H, NH), 8.26 (br s, 1H, NH), 10.43 (br s, 1H, NH)
Elemental Analysis	
Calculated	: C, 55.33; H, 4.89; N, 20.38
Found	: C, 55.31; H, 4.92; N, 20.41

4.1.4.7. 1-(4-oxo-3-^{n}butyl-3*H*-quinazolin-2-yl)-4-(4-chlorophenyl) thiosemicarbazide (AS7)

Yield	: 3.08 gm; 77 %
Melting Point	: 130-132 ˚C
Rf Value	: 0.46 [Chloroform-Methanol (9:1)]
Molecular Formula	: $C_{19}H_{20}N_5OSCl$
Molecular Weight	: 402 [M^+], 404 [M^++2]
IR (KBr) cm^{-1}	: 3380 (NH), 3221 (NH), 1651(C=O), 1272 (C=S)
^{1}H NMR ($CDCl_3$) δ ppm	: 1.04-1.09 (t, 3H, CH_3), 1.49-1.52 (m, 2H, CH_2), 1.74-1.79 (m, 2H, CH_2), 4.14-4.19 (m, 2H, CH_2), 6.99-7.02 (d, 1H, Ar-H), 7.05-7.10 (m, 2H, Ar-H), 7.26-7.38 (m, 2H, Ar-H), 7.51-7.54 (m, 2H, Ar-H), 7.56-7.59 (d, 1H, Ar-H), 7.99 (br s, 1H, NH), 8.77 (br s, 1H, NH), 11.49 (br s, 1H, NH)
Elemental Analysis	
Calculated	: C, 56.78; H, 5.02; Cl, 8.82
Found	: C, 56.83; H, 5.03; Cl, 8.86

4.1.4.8. 1-(4-oxo-3-^{n}butyl-3*H* - quinazolin-2-yl)-4-(2-aminopyridyl) thiosemicarbazide (AS8)

Yield	: 2.94 gm; 80 %
Melting Point	: 145-147 ˚C
Rf Value	: 0.44 [Chloroform-Methanol (9:1)]
Molecular Formula	: $C_{18}H_{20}N_6OS$
Molecular Weight	: 368 [M^+]
IR (KBr) cm^{-1}	: 3522 (NH), 3365 (NH), 1632 (C=O), 1223 (C=S)
^{1}H NMR ($CDCl_3$) δ ppm	: 1.12-1.17 (t, 3H, CH_3), 1.68-1.72 (m, 2H, CH_2), 2.08-2.15 (m, 2H, CH_2), 3.76-3.79 (m, 2H, CH_2), 7.21-7.23 (d, 1H, Ar-H), 7.30-7.39 (m, 6H, Ar-H), 7.93-7.97 (d, 1H, Ar-H), 8.12 (br s, 1H, NH), 8.21 (br s, 1H, NH), 10.36 (br s, 1H, NH)
Elemental Analysis	
Calculated	: C, 58.68; H, 5.47; N, 22.81
Found	: C, 58.66; H, 5.45; N, 22.86

4.1.4.9. 1-(4-oxo-3-*n*butyl-3*H*-quinazolin-2-yl)-4-(benzyl) thiosemicarbazide (AS9)

Yield	: 3.04 gm; 80 %
Melting Point	: 125-127 ˚C
Rf Value	: 0.44 [Chloroform-Methanol (9:1)]
Molecular Formula	: $C_{20}H_{23}N_5OS$
Molecular Weight	: 381 [M^+]
IR (KBr) cm^{-1}	: 3380 (NH), 3239 (NH), 1676 (C=O), 1214 (C=S)
^1H NMR ($CDCl_3$) δ ppm	: 0.93-0.96 (t, 3H, CH_3), 1.52 (s, 2H, CH_2), 1.76-1.78 (m, 2H, CH_2), 2.17-2.20 (m, 2H, CH_2), 3.86-3.90 (m, 2H, CH_2), 7.15-7.18 (d, 1H, Ar-H), 7.37-7.46 (m, 6H, Ar-H), 7.73-7.76 (d, 1H, Ar-H), 8.04 (br s, 1H, NH), 8.27 (br s, 1H, NH), 10.48 (br s, 1H, NH)
Elemental Analysis	
Calculated	: C, 62.97; H, 6.08; N, 18.36
Found	: C, 62.98; H, 6.06; N, 18.45

4.1.4.10. 1-(4-oxo-3-^{n}butyl-3*H*-quinazolin-2-yl)-4-(cyclohexyl) thiosemicarbazide (AS10)

Yield	: 2.98 gm; 80 %
Melting Point	: 130-132 ˚C
Rf Value	: 0.43 [Chloroform-Methanol (9:1)]
Molecular Formula	: $C_{19}H_{27}N_5OS$
Molecular Weight	: 373 [M^+]
IR (KBr) cm^{-1}	: 3383 (NH), 3299 (NH), 1653 (C=O), 1227 (C=S)
^1H NMR ($CDCl_3$) δ ppm	: 1.02-1.05 (t, 3H, CH_3), 1.55-1.59 (m, 2H, CH_2), 1.96-2.01 (m, 2H, CH_2), 2.27-2.32 (m, 6H, CH_2), 2.31-2.38 (m, 4H, CH_2), 2.91-2.94 (m, 1H, CH), 3.81-3.85 (m, 6H, CH_2), 7.31-7.42 (m, 4H, Ar-H), 8.05 (br s, 1H, NH), 8.49 (br s, 1H, NH), 10.32 (br s, 1H, NH)
Elemental Analysis	
Calculated	: C, 61.10; H, 7.29; N, 18.75
Found	: C, 61.16; H, 7.35; N, 18.71

4.2. Pharmacology

4.2.1 *In vitro* antibacterial activity[121-124]

The agar dilution method was used for the evaluation of antibacterial activity. All the microorganisms used for the evalution were procured from Department of Microbiology, MNR Medical College, Sangareddy. Muller-Hinton agar plates (Hi-media) were used for the growth of the bacteria at 37 ^{o}C for 24 h. The concentration (MIC) that completely inhibited the growth of bacteria was considered to be the minimum inhibitory concentration.

4.2.2 *In vitro M. Tuberculosis* activity[125-129] (Agar Dilution Method)

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* $H_{37}RV$ were prepared from fresh Middle brook 7H11 agar slants with OADC Growth Supplement adjusted to 1mg/mL (wet weight) in Tween 80 (0.05%) 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 $H_{37}RV$. The concentration at which complete inhibition of colonies occurred was taken as active concentration of test compound. The minimum inhibitory concentration (MIC) is defined as the minimum concentration of compound required to give complete inhibition of bacterial growth.