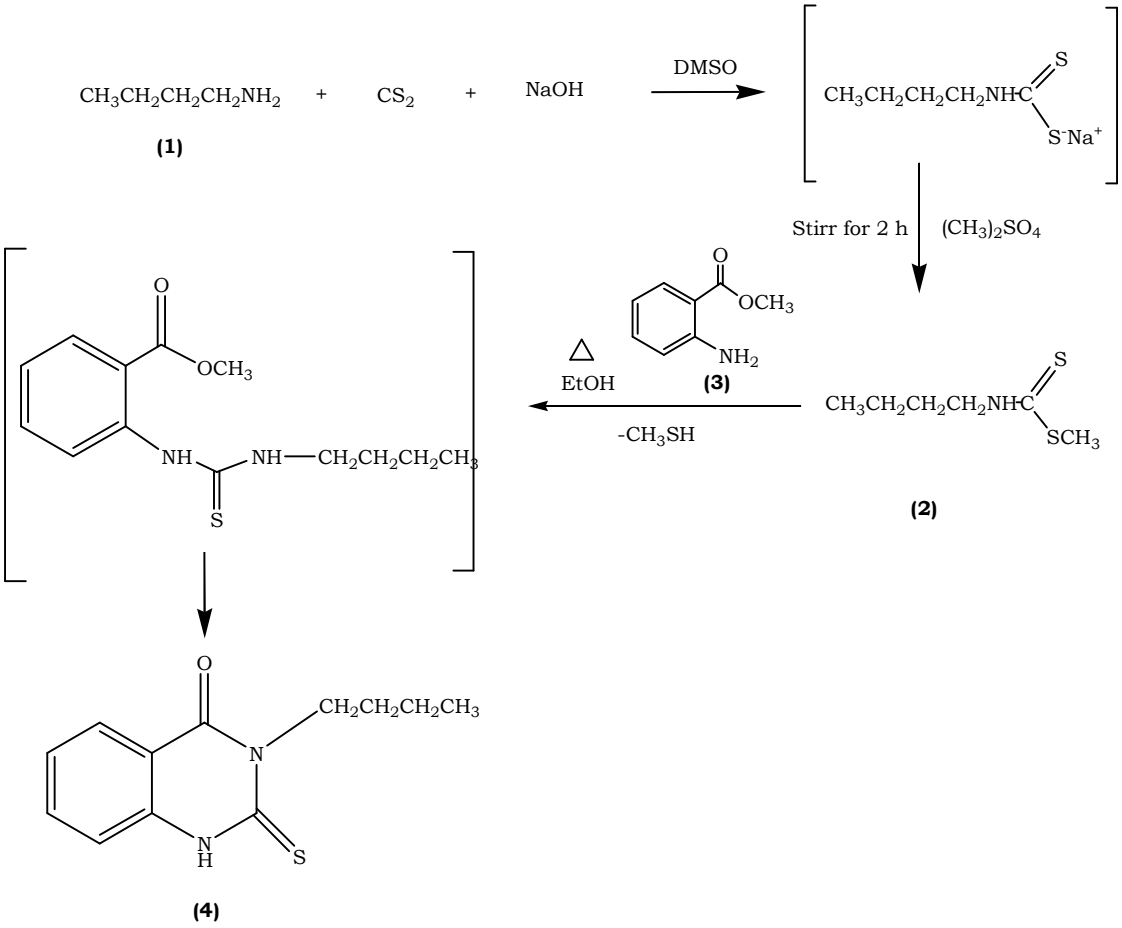
5. RESULTS AND DISCUSSION

5.1 Chemistry

5.1.1. Synthesis of 3-butyl-2-thioxo quinazolin-4(3*H*)-one (4)

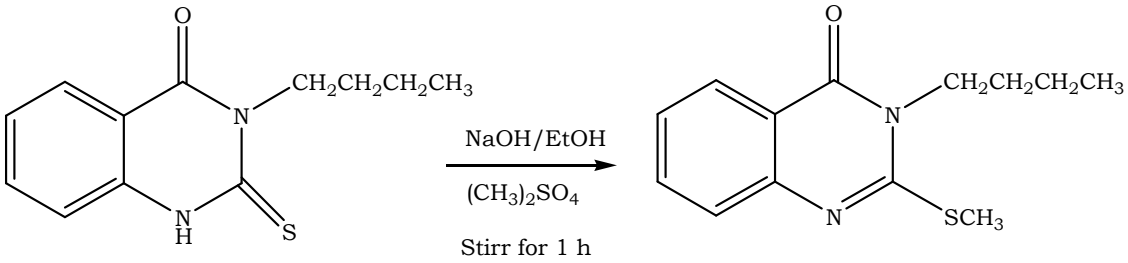
The key intermediate 3-butyl-2-thioxo quinazolin-4(3*H*)-one **(4)** was obtained by reacting butylamin**e** (**1)** with carbon disulphide and sodium hydroxide in dimethyl sulphoxide to give sodium dithiocarbamate, which was methylated with dimethyl sulphate to afford the dithiocarbamic acid methyl ester **(2)**. Compound **2** on reflux with methyl anthranilate **(3)** in ethanol at reflux (conventional heating 22 h; microwave heating 35 min) yielded the desired 3-butyl-2-thioxo quinazolin-4(3*H*)-one **(4)** via the thiourea intermediate in good yield (86%).



The IR spectra of **4** showed intense peaks at 3250 cm^{-1} for cyclic thiourea (NH), 1669 cm^{-1} for carbonyl (C=O) and 1217 cm^{-1} for thioxo (C=S) stretching. ^1H NMR spectra of compound **4** showed multiplet around δ 0.97-4.55 ppm due to butyl group, a multiplet around δ 7.26 ppm for aromatic (4H) protons and a singlet at δ 10.5 ppm indicating the presence of NH. Data from the elemental analyses have been found to be in conformity with the assigned structure. Furthermore the molecular ion recorded in the mass spectra is also in agreement with the molecular weight of the compound.

5.1.2. Synthesis of 3-Butyl-2-methylsulfanyl quinazolin-4(3*H*)-one (5)

The 3-butyl-2-methysulfanyl quinazolin-4(3*H*)-one **(5)** was obtained by dissolving **4** in 2% alcoholic sodium hydroxide solution and methylating with dimethyl sulphate with stirring at room temperature.

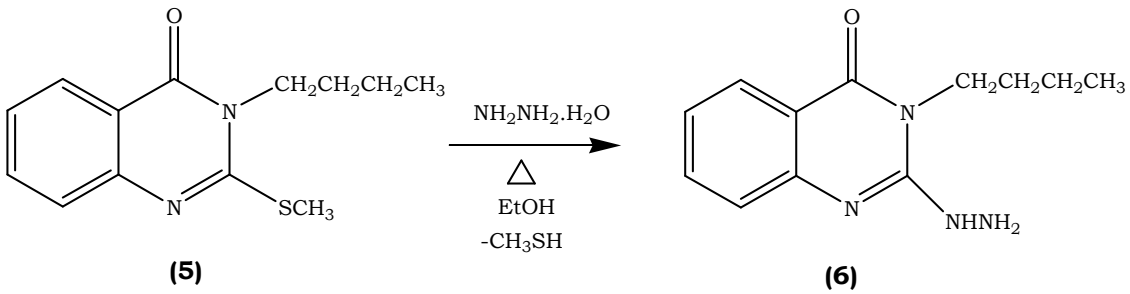


(4) **(5)**

The IR spectra of **5** showed disappearance of NH and C=S stretching signals of cyclic thiourea. It showed a peak for carbonyl (C=O) stretching at 1680 cm^{-1}. The ^{1}H NMR spectra of compound **5** showed singlet at δ 2.65 due to SCH_3 ppm; and multiplet around δ 1.39-4.15 ppm and δ 7.33-7.69 ppm was observed for butyl group and aromatic (4H) protons respectively. Data from the elemental analysis and molecular ion recorded in the mass spectra further confirmed the assigned structure.

5.1.3. Synthesis of 3-Butyl-2-hydrazino quinazolin-4(3*H*)-one (6)

Nucleophilic displacement of the methylthio group of **5** with hydrazine hydrate was carried out using ethanol as solvent to afford 3-butyl-2-hydrazino quinazolin-4(3*H*)-one **(6)**. The long duration of reaction (conventional heating 30 h; microwave heating 35 min) required might be due to the presence of bulky aromatic ring at position **3**, which might have reduced the reactivity of quinazolines ring system at C-2 position.

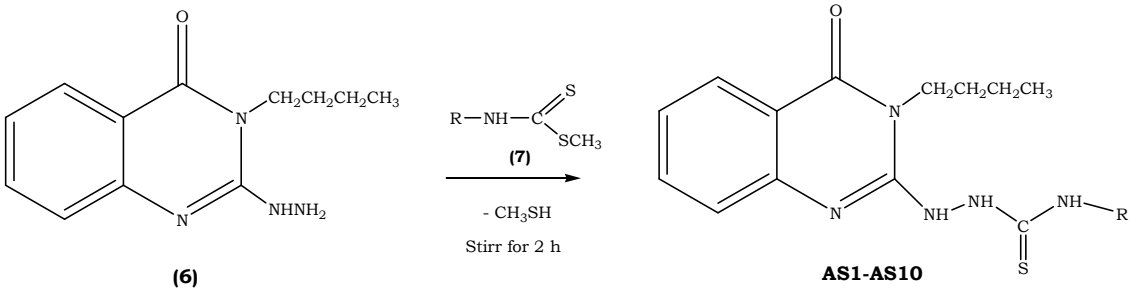


(5) **(6)**

The formation of compound **6** was confirmed by the presence of NH and NH_2 signals at 3434 and 3200 cm^{-1} in the IR spectra. It also showed a peak for carbonyl (C=O) at 1656 cm^{-1}. The ^1H NMR spectra of the compound **6** showed multiplet at δ 1.37-4.13 for butyl and δ 4.61 ppm & δ 10.24 ppm due to NH_2 and NH respectively, a multiplet at δ 7.10-7.23 ppm was observed for aromatic (4H) protons. Elemental analyses data and mass spectral data is also in agreement with the assigned structure of the compound.

5.1.4. Synthesis of 1-(4-oxo-3-*n*butyl-3*H*-quinazolin-2-yl)-4-(substituted) thiosemicarbazides (AS1-AS10)

The title compounds 1-(4-oxo-3-*n*butyl-3*H*-quinazolin-2-yl)-4-(substituted) thiosemicarbazides **(AS1-AS10)** were obtained by the condensation of amino group of 3-butyl-2-hydrazino quinazolin-4(3*H*)-one **(6)** with a variety of methyl ester of dithiocarbamic esters at (conventional heating 24-30 h; microwave heating 20-25 min).



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 ^{1}H NMR spectra showed peaks for NH around δ 8.0-10.0 ppm, a multiplet around δ 0.9-4.0 ppm for –$CH_2CH_2CH_2CH_3$, a multiplet around δ 6.84-8.77 ppm was observed for aromatic protons. The mass spectra of the title compounds showed molecular ion peaks corresponding to their molecular formula. In mass spectrum of compounds **AS1-AS10** a common peak at m/z 144 corresponding to quinazolin-4-one moiety appeared. Elemental (C, H and N) analysis satisfactorily

confirmed elemental composition and purity of the synthesized compounds.

5.2 Pharmacology

5.2.1. Antitubercular activity

The synthesized compounds were screened for their *in vitro* antimycobacterial activity against *Mycobacterium tuberculosis* strain $H_{37}Rv$ at the Tuberculosis Antimicrobial screening centre, Birla Institute of Technology & Sciences, Hyderabad campus, Hyderabad. The results are expressed in terms of Minimum Inhibitory Concentration (MIC).

The results of antimycobacterial activity depicted in **Table 4** indicate that the test compounds inhibited the growth of *mycobacterium* in varying degree. 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-butyl quinazolin-2-yl)-4-(2-nitrophenyl) thiosemicarbazide (**AS6**), 1-(4-oxo-3-^{n}butyl quinazolin-2-yl)-4-(4-chlorophenyl) thiosemicarbazide (**AS7**) and 1-(4-oxo-3-butyl quinazolin-2-yl)-4-(2-pyridyl) thiosemicarbazide (**AS8**) exhibited the antitubercular activity at the minimum micro gram concentration (6µg/ml).

5.2.2. Antibacterial activity

The title compounds are screened for their antibacterial activity against different gram positive and gram negative bacteria by agar dilution method, the results are depicted in **Table-4**. Among the different substituents on the aryl ring with electron withdrawing substituent showed better activity over the unsubstituted and electron donating substituents. Compounds **AS6** and **AS7** emerged as the most active compounds of the series. Compound **AS6** shown most potent activity against *E. coli* and *K. pneumoniae* while the compound **AS7** showed most potent activity against *S. typhi, E. coli, K. pneumoniae, S. enteritidis and B. subtilis.*

Table 4: Antibacterial activity (MIC in µg/mL) of compounds AS1-AS10

Microorganisms	AS1	AS2	AS3	AS4	AS5	AS6	AS7	AS8	AS9	AS10	Standard*
M. tuberculosis	25	13	13	13	25	6	6	6	25	25	0.4
S.typhi	63	125	63	125	63	63	32	63	125	63	4
E.coli	63	63	63	63	63	32	32	63	63	63	2
S.flexneri	125	125	63	125	63	63	63	125	125	125	1
P.vulgaris	63	63	125	125	125	63	63	63	125	63	1
Enterobacter spp.	125	125	125	125	125	63	63	63	125	125	1
K.pneumoniae	63	63	63	125	63	32	32	63	125	63	1
S.enteritidis	125	63	63	63	63	63	32	63	63	63	1
B.subtilis	125	63	63	125	63	63	32	63	63	63	1
S.flexneri	125	125	125	63	125	63	63	125	125	125	1
P.aeruginosa	63	125	125	63	125	63	63	125	63	125	1

*INH used as a reference standard against *M. Tuberculosis* whereas Ciprofloxacin used as a reference standard for other bacteria.

Figure 14: Antitubercular activity of synthesized compounds (AS1-AS10)

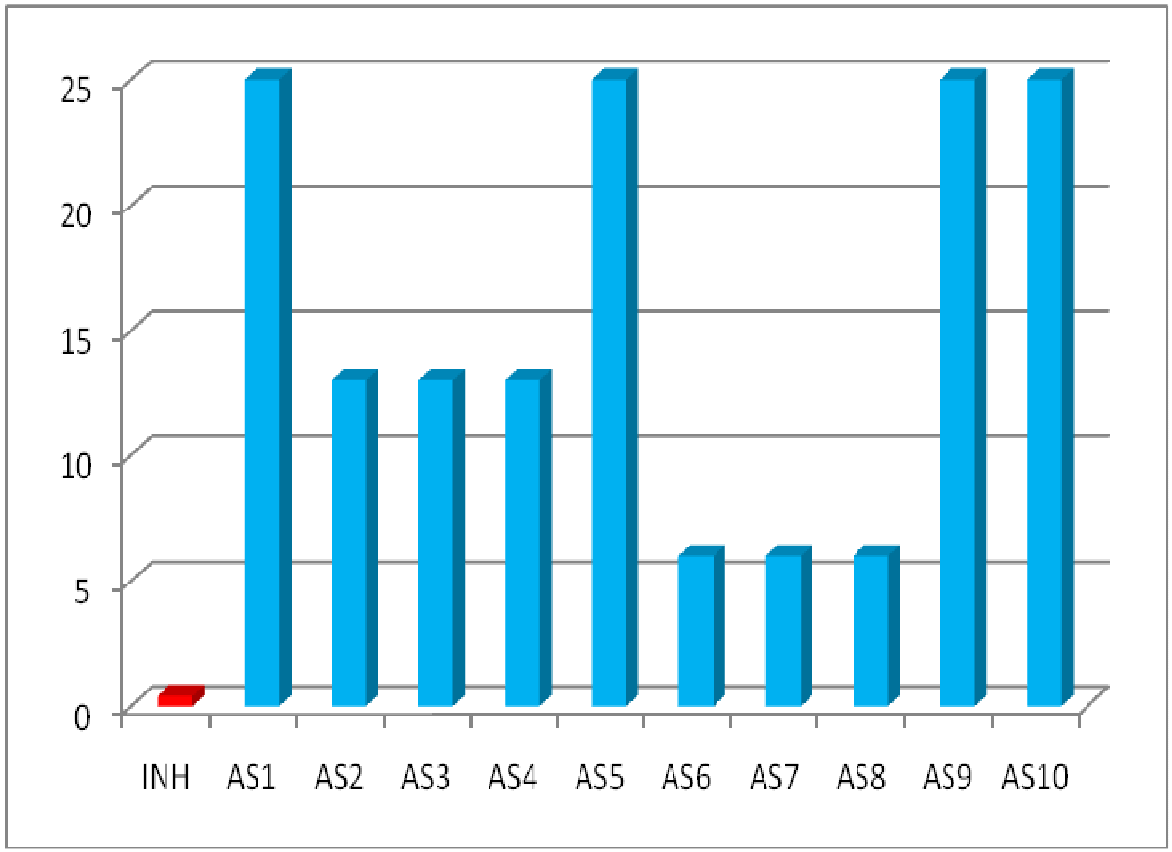


Figure 15: Antibacterial activity of synthesized compounds (AS1-AS10)