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Antimicrobial Activities of Some Synthesized 1-(3-(2-Methylphenyl)-4-Oxo-3*H*-Quinazolin-2-yl-4-(Substituted)Thiosemicarbazide Derivatives[1]

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Abstract—The substituted thiosemicarbazide moiety was placed at the C-2 position and 2-methylphenyl group at N-3 position of quinazoline ring and obtained compounds were tested for their antitubercular activities and antibacterial activities against selected gram-positive and gram-negative bacteria. The target compounds 1-(3-(2-methylphenyl)-4-oxo-3H-quinazolin-2-yl)-4-(substituted) thiosemicarbazides were obtained by the reaction of 2-hydrazino-3-(2-methylphenyl) quinazolin-4(3*H*)-one with different dithiocarbamic acid methyl ester derivatives. All synthesized compounds were also screened for their antimicrobial activity against selective gram-positive and gram-negative bacteria by agar dilution method. Among the series, 1-[3-(2-methylphenyl)-4-oxo-3*H*-quinazolin-2-yl]-4-[4-chlorophenyl]-thiosemicarbazide exhibited the most potent activity against *S. typhi, E. coli*, and *B. subtilis*, while 1-[3-(2-methylphenyl)-4-oxo-3*H*-quinazolin-2-yl]-4-[4-nitrophenyl]-thiosemicarbazide was the most potent against *E. coli, B. subtilis, P. aeruginosa, S. typhi*, and *S. flexneri*. These two compounds exhibited the antitubercular activity at the minimum concentration (3 μg/mL) that offered potential for further optimization and development of new antitubercular agents. The obtained results demonstrated promising antimicrobial and antitubercular activities of the synthesized quinazoline compounds which could be used as new scaffolds for improving their antimicrobial activity.

Keywords: quinazolinone, substituted thiosemicarbazide, anti-bacterial activity, antitubercular activity

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INTRODUCTION

Tuberculosis (TB) is one of the leading causes of death all over the world. This infection is caused primarily in the lungs (a pneumonia) by bacteria *Mycobacterium tuberculosis*. Emergence of multi drug resistant tuberculosis (MDR-TB) makes the situation the most alarming [1, 2]. Some of the MDR isolates are resistant to as many as seven of the commonly employed anti-mycobacterial 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 indicated that the quinazoline nucleus substituted at positions 2 and 3 (compounds (**I**) and (**II**) in the figure) showed significant antitubercular activity [5]. The thiosemicarbazide and thiosemicarbazone pharmacophore groups in different heterocyclic moieties (compounds (**III**) and (**IV**) in the figure) were also found to exhibit the antitubercular activity [6–14]. 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 (figure). In this approach, two or more pharmacophores are merged into a single molecule. Therefore, each of the pharmacophores in a single molecule may be addressing the active site of targets and offers the selectivity; furthermore, this may also reduce unwanted side effects [15]. In the present study, we placed the substituted thiosemicarbazide moiety at the C-2 position and 2-methylphenyl group at the N-3 position of quinazoline ring [16, 17] and studied their antitubercular and antibacterial activities against selected gram-positive and gram-negative bacteria.

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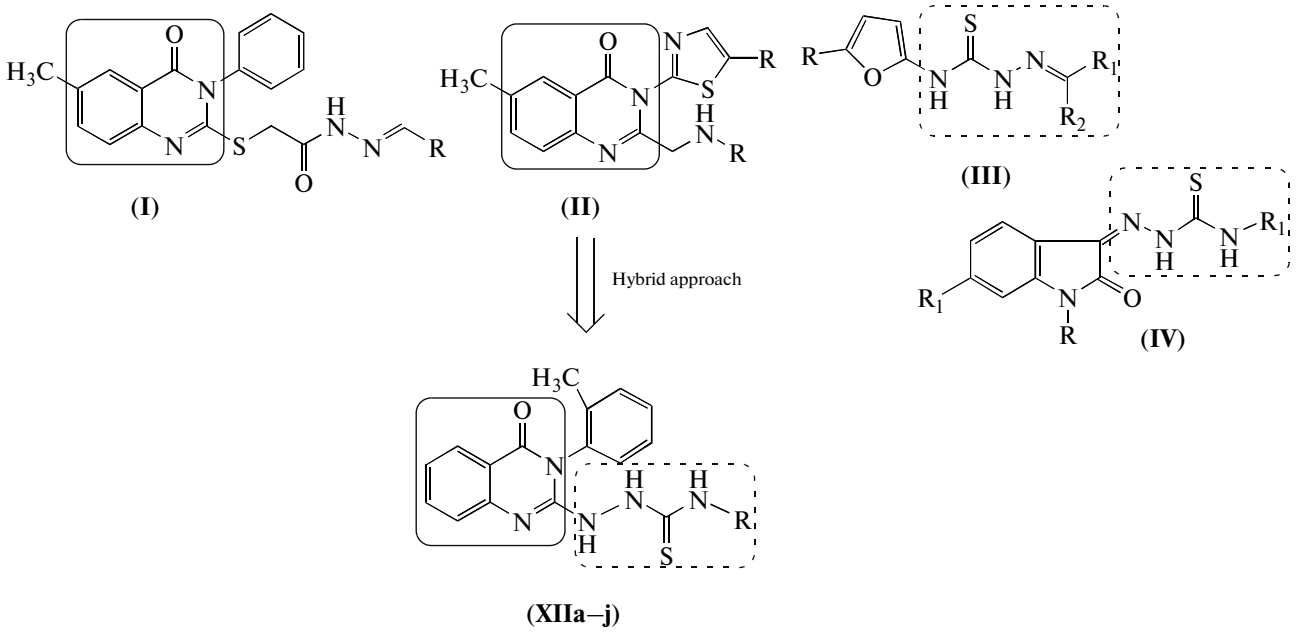


Hybrid approach to design of 1-[3-(2-methylphenyl)-4-oxo-3*H*-quinazolin-2-yl]-4-[substituted]-thiosemicarbazide analogues.

RESULTS AND DISCUSSION

Chemistry

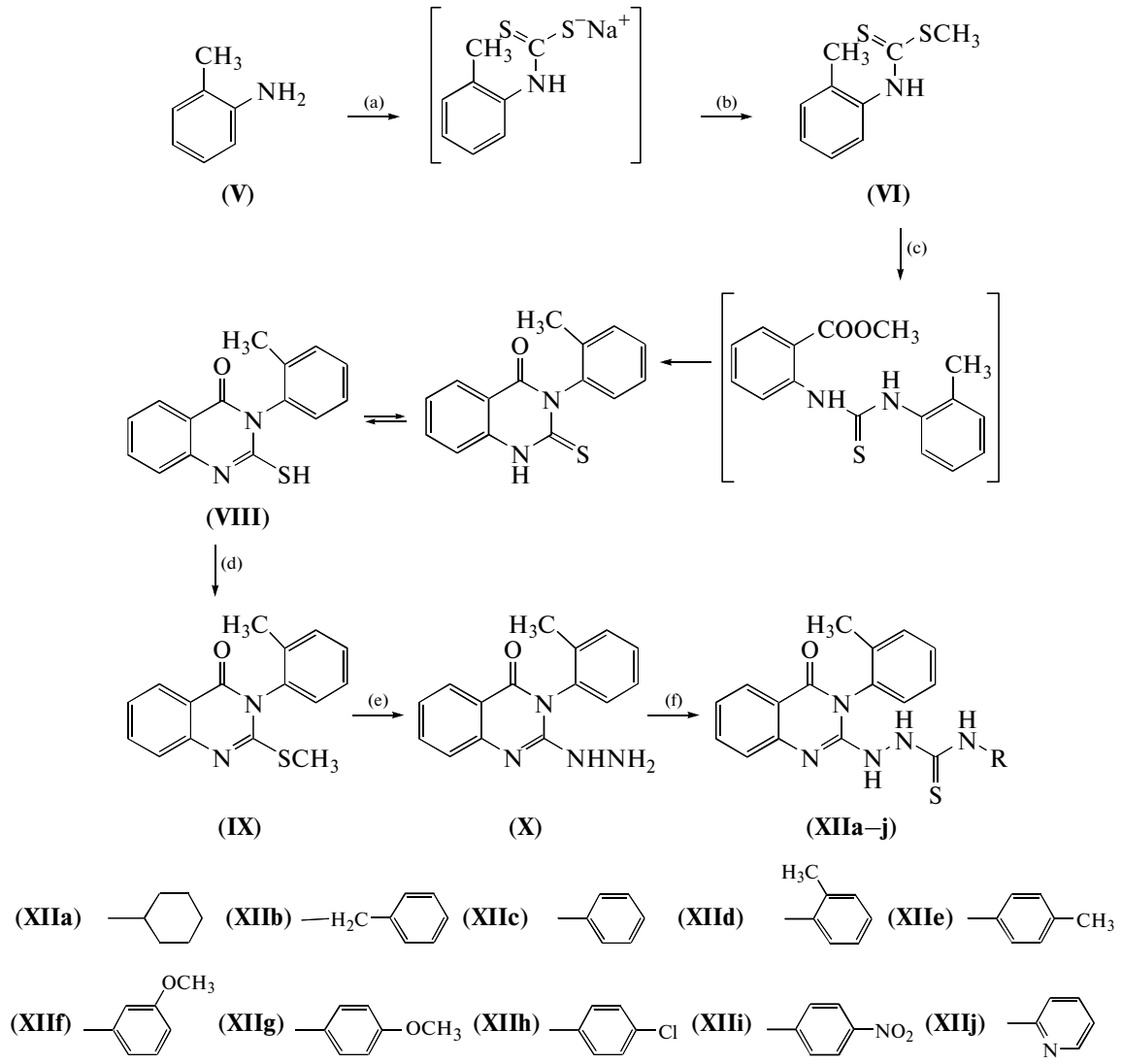
Synthetic route depicted in the scheme outlines the chemical part of the present work. The key intermediate 3-(2-methylphenyl)-2-thioxo-2,3-dihydro-1*H*-quinazolin-4-one (**VIII**) was obtained by reacting aniline (**V**) with carbon disulphide and sodium hydroxide in dimethyl sulphoxide to give sodium dithiocarbamate, which was methylated with dimethyl sulfate to afford the dithiocarbamic acid methyl ester (**VI**). Compound (**VI**) on reflux with methyl anthranilate (**VII**) in ethanol yielded the desired 3-(2-methylphenyl)-2-thioxo-2,3-dihydro-1*H*-quinazolin-4-one (**VIII**) via the thiourea intermediate in good yield (86%). The 3-(2-methylphenyl)-2-methylsulfanyl-3*H*-quinazolin-4-one (**IX**) was obtained by dissolving compound (**VIII**) in 2% alcoholic sodium hydroxide solution and methylating with dimethyl sulphate with stirring at room temperature. Nucleophilic displacement of methylthio group in compound (**IX**) with hydrazine hydrate was carried out using ethanol as a solvent to afford 3-(2-methylphenyl)-2-hydrazino-

Antitubercular and antibacterial activity of synthesized compounds (**XIIa–j**)

Microorganism	Test compound (MIC in μg/mL)										Standard*
	(XIIa)	**(XIIb)**	**(XIIc)**	**(XIId)**	**(XIIe)**	**(XIIf)**	**(XIIg)**	**(XIIh)**	**(XIIi)**	**(XIIj)**	
M. tuberculosis	63	125	63	6	6	13	13	3	3	6	1
S. typhi	66	63	16	32	63	63	63	8	8	32	4
E. coli	125	32	63	32	63	63	63	8	8	63	2
S. flexneri	125	63	63	32	63	63	63	16	16	32	1
P. vulgaris	125	63	125	63	63	125	125	8	16	63	1
Enterobacter spp.	63	63	63	63	125	63	32	16	16	32	1
K. pneumonia	125	63	63	63	63	63	125	16	16	32	1
S. enteritidis	63	63	32	32	63	32	63	16	32	32	1
B. subtilis	63	63	32	63	32	63	63	8	8	32	1
S. flexneri	63	32	63	32	125	63	125	16	8	8	1
P. aeruginosa	63	63	63	32	125	63	125	16	8	32	1

* Gatifloxacin was used as a reference standard against *M. tuberculosis,* whereas ciprofloxacin was used as a reference standard for other bacteria.

3*H*-quinazolin-4-one (**X**). The long duration of reaction (33 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-(3-(2-methylphenyl)-4-oxo-3*H*-dihydroquinazolin-2-yl)-4-(substituted)thiosemicarbazides (**XIIa–j**) were obtained by condensation of the amino group of 3-(2-methylphenyl)-2-hydrazino-3*H*-quinazolin-4-one (**X**) with a variety of methyl esters of dithiocarbamic acid (**XIa–j**). The formation of title product is indicated by the disappearance of the peak due to NH and NH_2 of the starting material in IR and ^1H NMR spectra of all the compounds (**XIIa–j**). The IR and ^1H NMR spectra 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 the mass spectra of compounds (**XIIa–j**), 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.



Reagents and conditions: (a) CS_2, NaOH, DMSO, 30 min; (b) Dimethyl sulphate, 2 h; (c) Methyl anthranilate, Anhydrous K_2CO_3, EtOH reflux, 22 h; (d) 2% alcoholic sodium hydroxide solution. Dimethyl sulphate, 1 h; (e) Hydrazine hydrate, Anhydrous potassium carbonate, Ethanol reflux, 33 h; (f) methyl-N-(substituted) dithiocarbamate, Ethanol reflux, 22–30 h.

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

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 the Table indicate that the test compounds inhibited the growth of *Mycobacterium* in varying degree. Compounds with aliphatic substituents showed lower antitubercular activity over the aryl and heteroaryl substituents. The compounds with an electron withdrawing substituent on the aryl ring showed better activity over the unsubstituted or an electron donating substituent on the aryl ring. Among the tested compounds,1-(3-(2-methylphenyl)-oxo-3*H*-quinazolin-2-yl)-4-(4-chlorophenyl)thiosemicarbazide (**XIIh**), as well as 1-(3-(2-methylphenyl)-4-oxo-3*H*-quinazolin-2-yl)-4-(4-nitrophenyl)-thiosemicarbazide (**XIIi**) exhibited the antitubercular activity at the minimum concentration (3 μg/mL).

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 (**XIIh**) and (**XIIi**) emerged as the most active compounds of the series. Compound (**XIIh**) showed the most potent activity against *S. typhi, E. coli,* and *B. subtilis,* while the compound (**XIIi**) showed the most potent activity against *E. coli, B. subtilis, P. aeruginosa, S. typhi,* and *S. flexneri.*

Cytotoxicity

Cytotoxicity studies conducted on HeLa cells suggest that the studied compounds are devoid of any signicant toxicity. The drugs at the concentration of 200 μg/mL were diluted to 3.25 μg/mL by serial two-fold dilutions. Following the incubation for 48 h in a drug, the cells were treated with methylene blue to measure their growth/viability using a spectrophotometer as described previously [18]. The most potent compounds (**XIIh**) and (**XIIi**) did not show any toxicity against HeLa cells even at concentrations greater than 100 μg/mL. Altogether, the results of SAR studies establish the 1-(3-(2-methylphenyl)-4-oxo-3*H*-quinazolin-2-yl)-4-(substituted)thiosemicarbazides nucleus as a chemical structure endowed with antimicrobial properties.

CONCLUSIONS

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

EXPERIMENTAL

Chemistry

Melting points (mp) were taken in open capillaries on a Thomas Hoover melting point apparatus (Thomas Hoover, USA) and are uncorrected. The IR spectra (ν, cm^{-1}) were recorded in potassium bromide disks on a Perkin-Elmer 398 spectrometer (Perkin-Elmer, USA). The ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a DPX-300 MHz Bruker FT-NMR spectrometer (Bruker, USA). The chemical shifts are reported as parts per million (δ, ppm) with tetramethylsilane (TMS) used 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.

3-(2-Methylphenyl)-2-thioxo-2,3-dihydro-1*H*-quinazolin-4-one (VIII). A solution of *o*-toluidine (**V**) (0.02 mol) in dimethyl sulphoxide (10 mL) was stirred vigorously. To this mixture carbon disulphide (1.6 mL) and 20 M aqueous sodium hydroxide (1.2 mL) were added dropwise 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 (**VI**) obtained was filtered, washed with water, dried, and recrystallized from ethanol. Methyl anthranilate (**VII**) (0.01 mol) and the above prepared *N*-(2-methylphenyl)-methyl dithiocarbamic acid (**VI**) (0.01 mol) were dissolved in ethanol (20 mL). Anhydrous potassium carbonate (100 mg) was added to this mixture and refluxed for 22 h. The reaction mixture was cooled in ice and the solid separated was filtered, 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

86%, mp 240–242°C; IR: 3211 (NH), 1686 (C=O), 1213 (C=S); ^{1}H NMR: 2.34 (s, 3H, CH_3), 7.11–7.13 m, 2H, Ar-H), 7.39–7.41 (m, 2H, Ar-H), 7.55–7.81 (m, 4H, ArH), 10.3 (br s, 1H, NH); ^{13}C NMR: 12.67, 120.11, 121.79, 125.69, 126.25, 126.88, 127.23, 127.95, 132.89, 133.75, 134.98, 140.75, 145.89, 160.25, 162.89; MS (*m/z*): 268 [M^+]. Anal. Calcd for $C_{15}H_{12}N_2OS$: C, 67.14; H, 4.51; N, 10.44. Found: C, 67.19; H, 4.57; N, 10.38.

3-(2-Methylphenyl)-2-methylsulfanyl-3*H*-quinazolin-4-one (IX). The 3-(2-methylphenyl)-2-thioxo-2,3-dihydro-1*H*-quinazolin-4-one (**VIII**) (0.01 mol) was dissolved in 40 mL of 2% alcoholic sodium hydroxide solution. To this mixture, dimethyl sulphate (0.01 mol) was added dropwise 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 88%, mp 130–132°C; IR: 1685 (C=O), 1610 (C=C); ^{1}H NMR: 2.01 (s, 3H, SCH_3), 2.28 (s, 3H,CH_3), 6.91–6.93 (m, 1H, Ar-H), 7.22–7.24 (m, 2H, Ar-H), 7.41–7.46 (m, 4H, Ar-H), 7.71–7.73 (d, J = 7.0 Hz, 1H, Ar-H); ^{13}C NMR: 9.85, 12.73, 120.22, 121.65, 125.73, 126.55, 126.87, 127.42, 127.85, 132.65, 133.75, 134.98, 140.75, 145.89, 160.35, 162.75; MS (*m/z*): 282 [M^+]; Anal. Calcd for $C_{16}H_{14}N_2OS$: C, 68.06; H, 5.00; N, 9.92. Found: C, 68.24; H, 5.03; N, 9.97.

3-(2-Methylphenyl)-2-hydrazino-3*H*-quinazolin-4-one (X). The 3-(2-methylphenyl)-2-methylsulfanyl-3*H*-quinazolin-4-one (**IX**) (0.01 mol) was dissolved in ethanol (25 mL). To this mixture, hydrazine hydrate (99%) (0.1 mol) and anhydrous potassium carbonate (100 mg) were added and refluxed for 33 h. The reaction mixture was cooled and poured into ice-water. The solid obtained was filtered, washed with water, dried, and recrystallized from chloroform–benzene (25 : 75) mixture. Yield 81%, mp 200–202°C; IR: 3356, 3293 ($NHNH_2$), 1672 (C=O); ^{1}H NMR: 2.31 (s, 3H, CH_3), 5.01 (s, 2H, NH_2), 6.89–6.91 (m, 1H, Ar-H), 7.21–7.23 (m, 2H, Ar-H), 7.39–7.41 (m, 4H, Ar-H), 7.68–7.70 (d, J = 6.5 Hz, 1H, Ar-H), 9.63 (s, 1H, NH); ^{13}C NMR: 12.69, 120.35, 121.77, 125.65, 126.55, 126.88, 127.23, 127.95, 132.73, 133.55, 134.15, 140.85, 145.91, 160.25, 162.93; MS (*m/z*): 266 (M^+); Anal. Calcd for $C_{15}H_{14}N_4O$: C, 67.65; H, 5.29; N, 21.04. Found: C, 67.58; H, 5.28; N, 21.02.

General Procedure for Synthesis of 1-(3-(2-Methylphenyl)-4-Oxo-3-Phenyl-3H-Dihydroquinazolin-2-yl)-4-(substituted)thiosemicarbazides (XIIa–j)

A solution of primary alkyl/aryl amine (0.02 mol) in dimethyl sulfoxide (10 mL) was stirred vigorously. To this mixture, carbon disulphide (1.6 mL) and aqueous sodium hydroxide 1.2 mL (20 M solution) were added simultaneously 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; the stirring in a freezing mixture 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 to afford methyl-*N*-(substituted) dithiocarbamate (**XIa–j**).

3-(2-Methylphenyl)-2-hydrazino-3*H*-quinazolin-4-one (VI) (2.32 g, 0.01 mol) and appropriate methyl-*N*-(substituted) dithiocarbamate (**XIa–j**) (0.01 mol) were dissolved in ethanol and refluxed for 22–30 h (until the methyl mercaptan evolution ceases). After completion of the reaction the reaction mixture was cooled to room temperature. The solid obtained was filtered, dried, and recrystallized from ethanol. By adapting the above procedure the compounds (**XIIa–j**) were prepared.

1-[3-(2-Methylphenyl)-4-oxo-3*H*-quinazolin-2-yl]-4-[cyclohexyl] thiosemicarbazide (XIIa). Yield 77%; mp 250–252°C; IR: 3290 (NH), 3272 (NH), 3223 (NH), 1656 (C=O), 1610 (C=N), 1210 (C=S); ^{1}H NMR: 1.53–1.71 (m, 6H, CH_2), 1.83 (s, 2H, CH_2), 1.92 (s, 2H, CH_2), 2.31 (s, 2H, CH_3), 2.54 (m, 1H, CH), 7.22–7.23 (m, 1H, ArH), 7.65–7.67 (m, 1H, ArH), 8.43–8.48 (d, J = 7.1 Hz, 2H, Ar-H), 8.61–8.64 (m, 1H, ArH), 8.93–8.96 (d, J = 8.0 Hz, 2H, Ar-H), 9.12–9.23 (m, 1H, ArH), 9.33 (s, 1H, NH), 9.56 (s, 1H, NH), 11.23 (s, 1H, NH); ^{13}C NMR: 12.75, 22.14, 25.89, 39.89, 52.75, 120.56, 123.35, 123.68, 125.28, 127.78, 127.85, 128.61, 130.81, 131.86, 133.52, 134.12, 138.75, 158.69, 162.45, 183.74; MS (*m/z*): 407 (M^+, 100); Anal. Calcd. for $C_{22}H_{25}N_5OS$: C, 64.84; H, 6.18; N, 17.18; Found: C, 64.83; H, 6.21; N, 17.23.

1-[3-(2-Methylphenyl)-4-oxo-3*H*-quinazolin-2-yl]-4-[benzyl] thiosemicarbazide (XIIb). Yield 70%; mp 190–192°C; IR: 3327 (NH), 3310 (NH), 3237 (NH), 1672 (C=O), 1610 (C=N), 1212 (C=S); ^{1}H NMR: 2.32 (s, 3H, CH_3), 4.62 (s, 2H, CH_2), 6.12–6.15 (m, 2H, ArH), 6.53–6.55 (m, 1H, ArH), 7.21–7.32 (d, J = 7.5 Hz, 2H, Ar-H), 7.42–7.45 (d, J = 7.0 Hz, 2H, Ar-H), 7.73–7.75 (d, J = 7.5 Hz, 2H, Ar-H), 8.00–8.01 (m, 1H, ArH), 8.05–8.07 (d, J = 7.5 Hz, 2H, Ar-H), 8.42–8.44 (m, 1H, ArH), 8.53 (s, 1H, NH), 8.85 (s, 1H, NH), 9.26 (s, 1H, NH); ^{13}C NMR: 12.95, 49.68, 120.71, 123.48, 123.68, 125.28, 125.68, 126.74, 127.22, 127.78, 127.85, 128.61, 130.81, 131.86, 133.57, 134.85, 138.75, 140.35, 158.69, 162.35, 183.58; MS (*m/z*): 415 (M^+, 100); Anal. Calcd. for $C_{23}H_{21}N_5OS$: C, 66.41; H, 5.07; N, 16.83; Found: C, 66.36; H, 4.94; N, 16.81.

1-[3-(2-Methylphenyl)-4-oxo-3*H*-quinazolin-2-yl]-4-[phenyl] thiosemicarbazide (XIIc). Yield 73%; mp 245–247°C; IR: 3395 (NH), 3183 (NH), 3270 (NH), 1676 (C=O), 1610 (C=N), 1256 (C=S); ^{1}H NMR: 2.41 (s, 3H, CH_3), 6.59–6.60 (d, J = 7.0 Hz, 1H, Ar-H) 7.02–7.05 (m, 3H, Ar-H), 7.45–7.48 (m, 3H, Ar-H), 7.55–7.58 (m, 3H, Ar-H), 7.67–7.70 (m, 3H, Ar-H),

8.01 (br s, 1H, NH), 8.7 (br s, 1H, NH), 10.22 (br s, 1H, NH); ^{13}C NMR: 12.77, 120.68, 123.55, 123.74 125.18, 125.36, 126.74, 127.22, 127.78, 127.85, 128.61, 130.81, 131.86, 133.75, 134.25, 138.75, 140.35, 158.69, 162.75, 183.75; MS (*m/z*): 401 (M^+, 100); Anal. Calcd. for $C_{22}H_{19}N_5OS$:C, 65.81; H, 4.77; N, 17.44; Found: C, 65.82; H, 4.73; N, 17.38.

1-[3-(2-Methylphenyl)-4-oxo-3*H*-quinazolin-2-yl]-4-[2-methylphenyl] thiosemicarbazide (XIId). Yield 81%; mp 245–247°C; IR: 3407 (NH), 3381 (NH), 3265 (NH), 1676 (C=O), 1650 (C=N), 1257 (C=S); ^{1}H NMR: 2.33 (s, 6H, CH_3), 6.85 (br s, 1H, NH), 6.82–6.84 (m, 1H, ArH), 7.04–7.06 (m, 1H, ArH), 7.27–7.31 (d, J = 7.0 Hz, 2H, Ar-H), 7.62–7.66 (d, J = 7.2 Hz, 2H, Ar-H), 7.83–7.87 (d, J = 7.0 Hz, 2H, Ar-H), 7.91–7.94 (m, 1H, ArH), 7.99–8.01 (d, J = 7.0 Hz, 2H, Ar-H), 8.51–8.53 (m, 1H, ArH), 8.72 (br s, 1H, NH), 10.21 (br s, 1H, NH); ^{13}C NMR: 12.58, 12.61, 120.68, 123.55, 123.74, 125.18, 125.36, 126.74, 127.22, 127.78, 127.85, 128.61, 128.95, 130.81, 131.86, 133.54, 134.25, 138.75, 138.85, 140.35, 158.69, 162.75, 181.75;MS (*m/z*): 415 (M^+, 100); Anal. Calcd. for $C_{23}H_{21}N_5OS$:C, 66.48; H, 5.09; N, 16.85; Found: C, 66.53; H, 5.12; N, 16.86.

1-[3-(2-Methylphenyl)-4-oxo-3*H*-quinazolin-2-yl]-4-[4-methylphenyl] thiosemicarbazide (XIIe). Yield 80%; mp 240–242°C; IR: 3407 (NH), 3243 (NH), 3200 (NH), 1676 (C=O), 1180 (C=N), 1256 (C=S); ^{1}H NMR: 2.27 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 5.95 (br s, 1H, NH), 6.96–6.98 (d, J = 7.0 Hz, 1H, Ar-H), 7.08–7.12 (m, 3H, Ar-H), 7.32–7.36 (m, 4H, Ar-H), 7.45–7.47 (m, 4H, Ar-H), 8.06 (br s, 1H, NH), 10.53 (br s, 1H, NH); ^{13}C NMR: 12.98, 23.85, 120.52, 123.71, 123.84, 125.42, 125.87, 126.74, 127.22, 127.78, 127.85, 128.61, 130.81, 131.86, 133.25, 134.11, 138.75, 140.45, 158.69, 162.59, 183.61; MS (*m/z*): 415 ($[M + H]^+$, 100); Anal. Calcd. for $C_{23}H_{21}N_5OS$: C, 66.48; H, 5.09; N, 16.85; Found: C, 66.52; H, 5.10; N, 16.89.

1-[3-(2-Methylphenyl)-4-oxo-3*H*-quinazolin-2-yl]-4-(3-methoxyphenyl) thiosemicarbazide (XIIf). Yield 80%; mp 210–212°C; IR: 3407 (NH), 3280 (NH), 3205 (NH), 1677 (C=O), 1620 (C=N), 1280 (OCH_3), 1260 (C=S); ^{1}H NMR: 2.32 (s, 3H, CH_3), 3.69 (s, 3H, OCH_3), 6.72 (br s, 1H, NH), 7.10–7.12 (m, 1H, Ar-H), 7.32–7.35 (m, 1H, Ar-H), 7.39–7.42 (d, J = 7.5 Hz, 2H, Ar-H), 7.49–7.54 (d, J = 7.80 Hz, 2H, Ar-H), 7.63–7.69 (d, J = 7.2 Hz, 2H, Ar-H), 7.71–7.73 (m, 1H, Ar-H), 7.82–7.84 (d, J = 7.0 Hz, 2H, Ar-H), 8.03–8.05 (m, 1H, ArH), 8.72 (br s, 1H, NH), 10.21 (br s, 1H, NH); ^{13}C NMR: 12.39, 53.85, 108.75, 109.87, 117.85, 120.48, 123.51, 123.25, 125.42, 127.53, 127.85, 128.61, 129.57, 129.99, 130.81, 131.86, 134.35, 137.57, 138.75, 158.69, 160.15, 162.42, 179.53; MS (*m/z*): 431 (M^+, 100); Anal. Calcd. for $C_{23}H_{21}N_5O_2S$: C, 64.02; H, 4.91; N, 16.23; Found: C, 64.03; H, 4.87; N, 16.33.

1-[3-(2-Methylphenyl)-4-oxo-3*H*-quinazolin-2-yl]-4-(4-methoxyphenyl) thiosemicarbazide (XIIg). Yield 81%; mp 232–233°C; IR: 3323 (NH), 3310 (NH), 3242 (NH), 1691 (C=O), 1605 (C=N), 1286 (OCH3), 1210 (C=S); ^{1}HNMR : 2.32 (s, 3H, CH_3), 3.69 (s, 3H, OCH_3), 6.51 (br s, 1H, NH), 6.55–6.57 (m, 1H, Ar-H), 6.86–6.88 (m, 1H, Ar-H), 7.24–7.29 (d, J = 7.3 Hz, 2H, Ar-H), 7.32–7.36 (d, J = 7.5 Hz, 2H, Ar-H), 7.42–7.46 (d, J = 7.2 Hz, 2H, Ar-H), 7.88–7.91 (m, 1H, Ar-H), 8.14–8.17 (d, J = 7.5 Hz, 2H, Ar-H), 8.24–8.27 (m, 1H, Ar-H), 8.84 (br s, 1H, NH), 10.82 (br s, 1H, NH); ^{13}C NMR: 12.75, 53.75, 120.61, 123.85, 123.98, 125.42, 125.87, 126.74, 127.22, 127.78, 127.85, 128.35, 128.61, 130.81, 131.86, 133.58, 138.75, 140.45, 156.79, 162.59, 181.61; MS (*m/z*): 431 (M^+, 100); Anal. Calcd. for $C_{23}H_{21}N_5O_2S$: C, 64.02; H, 4.91; N, 16.23; Found: C, 64.01; H, 4.94; N, 16.28.

1-[3-(2-Methylphenyl)-4-oxo-3*H*-quinazolin-2-yl]-4-[4-chlorophenyl] thiosemicarbazide (XIIh). Yield 80%; mp 245–247°C; IR: 3330 (NH), 3281 (NH), 3218 (NH), 1675 (C=O), 1615 (C=N), 1210 (C=S); ^{1}H NMR: 2.31 (s, 3H, CH_3), 6.61–6.64 (m, 1H, Ar-H), 7.04–7.07 (d, J = 7.5 Hz, 2H, Ar-H), 7.10–7.13 (d, J = 7.5 Hz, 2H, Ar-H), 7.90–7.93 (d, J = 7.2 Hz, 2H, Ar-H), 7.94–7.97 (m, 1H, Ar-H), 7.91–7.96 (d, J = 7.0 Hz, 2H, Ar-H), 7.97–7.99 (m, 1H, Ar-H), 9.42 (br s, 1H, NH), 10.33 (br s, 1H, NH); ^{13}C NMR: 12.75, 120.45, 123.77, 123.89, 125.23, 125.87, 126.81, 127.09, 127.58, 128.34, 127.99, 128.61, 130.81, 133.57, 131.86, 138.75, 140.45, 156.79, 162.59, 181.61; MS (*m/z*): 435 (M^+, 100); Anal. Calcd. for $C_{22}H_{18}ClN_5OS$: C, 60.47; H, 4.12; N, 16.01; Found: C, 60.48; H, 4.16; N, 16.08.

1-[3-(2-Methylphenyl)-4-oxo-3*H*-quinazolin-2-yl]-4-[4-nitrophenyl]thiosemicarbazide (XIIi). Yield 74%; mp 233–235°C; IR: 3321 (NH), 3310 (NH), 3226 (NH), 1688 (C=O), 1604 (C=N), 1208 (C=S); ^{1}H NMR: 2.11 (s, 3H, CH_3), 6.53–6.56 (m, 1H, ArH), 6.70–6.72 (m, 1H, ArH), 7.05–7.09 (d, J = 8.1 Hz, 2H, Ar-H), 7.10–7.13 (d, J = 8.1 Hz, 2H, Ar-H), 7.32–7.36 (d, J = 7.3 Hz, 2H, Ar-H), 7.63–7.66 (m, 1H, Ar-H), 7.88–7.93 (d, J = 7.3 Hz, 2H, Ar-H), 7.95–7.98 (m, 1H, Ar-H), 8.81 (br s, 1H, NH), 8.96 (br s, 1H, NH), 10.21 (br s, 1H, NH); ^{13}C NMR: 12.83, 120.39, 123.47, 123.84, 125.42, 125.87, 126.74, 127.22, 127.78, 127.85, 128.35, 128.61, 130.81, 131.66, 133.87, 138.75, 139.45, 158.69, 161.79, 181.61; MS (*m/z*): 446 ($[M+H]^+$,100); Anal. Calcd. for $C_{22}H_{18}N_6O_3S$: C, 59.02; H, 4.02; N, 18.83; Found: C, 59.00; H, 4.04; N, 18.84.

1-[3-(2-Methylphenyl)-4-oxo-3*H*-quinazolin-2-yl]-4-[pyridine-2-yl]thiosemicarbazide (XIIj). Yield 80%; mp 210–212°C; IR: 3387 (NH), 3342 (NH), 3203 (NH), 1665 (C=O), 1610 (C=N), 1254 (C=S); ^{1}H NMR: 2.28 (s, 3H, CH_3), 6.54–6.58 (m, 1H, ArH), 7.02–7.06 (m, 1H, ArH), 7.20–7.23 (d, J = 7.5 Hz, 2H, Ar-H), 7.35–7.38 (d, J = 8.1 Hz, 2H, Ar-H), 7.41–7.45

(d, J = 7.3 Hz, 2H, Ar-H), 7.53–7.58 (m, 1H, ArH), 7.71–7.74 (d, J = 7.3 Hz, 2H, Ar-H), 7.92–7.96 (m, 1H, ArH), 8.72 (s, 1H, NH), 8.92 (br s, 1H, NH), 10.51 (br s, 1H, NH); ^{13}C NMR: 12.83, 108.75, 112.89, 120.48, 123.51, 123.25, 125.42, 127.53, 127.85, 128.35, 128.61, 130.81, 131.86, 134.25, 137.89, 138.75, 147.75, 157.85, 158.69, 161.78, 181.25; MS (m/z): 402 ([M + H]$^+$, 100); Anal. Calcd. for $C_{21}H_{18}N_6OS$:C, 62.53; H, 4.45; N, 21.03; Found: C, 62.50; H, 4.43; N, 21.06.

Pharmacology

Antibacterial activity. Antibacterial activity was evaluated by agar dilution method [9, 10]. 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 (Himedia) 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 calculated from at least three different experiments in duplicateare presentedin the table.

Antitubercular activity. Serial 10-fold 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 10^7 CFU/mL. A 5-μL 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°C, 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 [21–24]. 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 10000 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 0.33 N 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 [18].

COMPETING INTERESTS

The authors declare that they have no competing interests.

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