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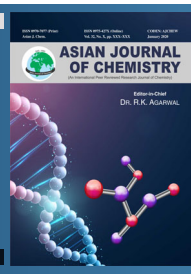

Anti-HIV, Antitubercular and Antibacterial Activities of Novel 3-(Substituted Quinazolinylamino)-2-phenyl quinazolin-4(3*H*)ones

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In the present study, we have synthesized a series of novel 2-phenyl-3-(substituted quinazolinylamino)quinazolin-4(3*H*)-ones by the reaction of 3-(substituted)-2-hydrazinoquinazoline-4(3*H*)-ones with 2-phenyl-3,1-benzoxazin-4-one. The starting material 3-(substituted)-2-hydrazino-quinazolin-4(3*H*)-ones were synthesized from various primary amines. All the synthesized compounds were screened for their antitubercular, anti-HIV and antibacterial activity against different Gram-positive and Gram-negative strains by agar dilution method. Among the test compounds, 3-(4-nitrophenyl)-2-(4-oxo-2-phenylquinazolin-3(4*H*)-ylamino)quinazolin-4(3*H*)-one (**BQZ6**) and 3-(4-chlorophenyl)-2-(4-oxo-2-phenylquinazolin-3(4*H*)-ylamino)quinazolin-4(3*H*)-one (**BQZ7**) shown most potent antibacterial activity against *E. coli*, *P. aeruginosa* and *S. aureus* with the MIC of 3 µg/mL. The compound **BQZ7** exhibited the antitubercular activity with the MIC of 25 µg/mL and anti-HIV activity with the MIC of 35.4 µg/mL against HIV1 and HIV2 and offers potential lead for further optimization and development to new antitubercular and anti-HIV agents. The results from this study confirm that the synthesized and biologically evaluated quinazolines showed promising antimicrobial, antitubercular and anti-HIV activities and are new scaffolds for antimicrobial activity.

Keywords: Quinazolines, Antibacterial activity, Antitubercular activity, Anti-HIV activity.

INTRODUCTION

Tuberculosis (TB) is an opportunistic infection that occurs more often or more severe in people with weakened immune systems than in people with healthy immune systems. Human immunodeficiency virus (HIV) weakens the immune system, increasing the risk of tuberculosis in people with HIV. Co-infection with tuberculosis and HIV poses a tremendous challenge to tuberculosis control, particularly in resource-limited treatment option. In 2015, it was estimated 10.4 million cases of tuberculosis disease, among that 1.2 million (11 %) people living with HIV, there were an estimated 456,000 deaths from HIV-associated tuberculosis [1-4]. Although exhaustive efforts to prevent and treat tuberculosis is taken the problem still continues due to multi-drug-resistant (MDR-TB) to isoniazid, rifampicin, quinolones and aminoglycosides. Recently tuberculosis threat has an additional challenge with the emergence of both multi-drug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) strains. It clearly highlights the urgent need to develop novel "druggable" molecules for the co-infection treatment and strains of MDR-TB and XDR-TB.

Recent year's quinazolines and condensed quinazolines gained much attention to medicinal chemists and pharmacologist due to their potential druggable behaviour [5]. Among that antimicrobial activity of 2,3-disubstituted quinazolines are encouraging for further development. From recent literature it is revealed that the 2,3-disubstituted quinazoline nucleus showed significant antimicrobial activity [5,6].

Quinazolines and bisquinazolines have been explored for various medicinal chemistry properties due to their widespread pharmacological activities including antineoplastic, antimycobacterial, antibacterial, antifungal, antiviral and antimalarial. In recent literature, many quinazoline derived compounds were prepared and screened for its antimicrobial activity. The current use of these agents in bacterial and viral infections have led to

the development of novel anti-HIV, antibacterial and antitubercular agents [7-15]. In present study, using the quinazoline scaffold we have developed 3-(substituted)-2-(4-oxo-2-phenylquinazolin-3(4*H*)-ylamino)quinazolin-4(3*H*)-ones by incorporating the phenyl group at the C-2 position and quinazoline moiety with desired substituents at N-3 position of quinazoline as potent anti-HIV, antitubercular and antibacterial agents based on pharmacophore approach. In this approach a molecule was created by merging two quinazolines called as bisquinazolines. Active site of targets may be addressed by this pharmacophore, which increase the opportunity for selectivity. In addition it also expected to reduce the undesirable side effects. The title compounds were synthesized by a facile synthetic methods with the good yields and studied for their anti-HIV activity in MT-4 cells against replication of HIV-1 (III B) and HIV-2 (ROD); antitubercular activity against *M. tuberculosis* $H37R_V$ by 10 fold serial dilutions of each test compound/drug and the antibacterial activity against selected Gram-positive and negative bacteria by Agar dilution method.

EXPERIMENTAL

In open capillaries melting points were measured on a Thomas Hoover melting point apparatus (Thomas Hoover, USA) and are uncorrected. Using potassium bromide disks the IR spectra (ν, cm^{-1}) were recorded on Bruker FT-IR spectrometer (Bruker, USA). Using Bruker FT NMR spectrometer (Bruker, USA) the ^1H NMR spectra were recorded in $CDCl_3$ at 300 MHz. The chemical shifts are reported (δ, ppm) using tetramethylsilane (TMS) as an internal standard. Using fast atom bombardment (FAB positive) mass spectra were obtained on a JEOL-SX-102 instrument (JEOL, Japan). PerkinElmer 2400 CHN analyzer (Perkin Elmer, USA) was used to perform elemental analysis and values were within the acceptable limits of the calculated values (± 0.4 %). Readymade silica gel plates (Merck, Norway) are used to monitor the progress of the reaction. All chemicals and reagents used in the synthesis were obtained from Merck, SD fine chemicals, Aldrich (USA), Lancaster (USA), or Spectrochem (India) and were used without further purification.

Synthesis of 3-(4-nitrophenyl)-2-thioxo-2,3-dihydro quinazolin-4-one (3): A solution of 4-nitro aniline (0.02 mol) in dimethyl sulfoxide (10 mL) was stirred vigorously. To this carbon disulphide (1.6 mL) and aqueous 20 M sodium hydroxide (1.2 mL) was added drop wise and stirring was continued for 0.5 h. Dimethyl sulphate (0.02 mol) was added to the reaction mixture and the reaction condition was kept in freezing mixture for 2 h. The resultant reaction mixture was transferred into ice cold water and stirred well. The product obtained was filtered, washed with water, dried and recrystallized from ethanol.

To the above prepared *N*-(4-nitrophenyl)-methyl dithiocarbamic acid (0.02 mol), methyl anthranilate (0.02 mol) solution in ethanol (20 mL) was added. To the above reaction mixture anhydrous potassium carbonate (100 mg) was added and refluxed for 23 h. The reaction mixture was further cooled by using ice. The resulting solid separated by filtration. The product **3** obtained was 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 %; m.p.: 281-282 °C; IR (KBr, ν_{max}, cm^{-1}): 3355 (NH), 3062 (Ar-CH), 1729 (C=O), 1607 (C=C), 1520 & 1346 (NO_2), 1251 (C=S); ^1H NMR ($CDCl_3$) δ: 6.73-6.75 (m, 2H, Ar-H), 7.26 (d, *J* = 7.5 Hz, 1H, Ar-H), 7.52-7.54 (m, 2H, Ar-H), 7.71 (d, *J* = 2.0 Hz, 1H, Ar-H),7.95 (d, *J* = 7.5 Hz, 1H, Ar-H), 8.01 (d, *J* = 2.0 Hz, 1H, Ar-H), 8.09 (m, 1H, Ar-H),9.16 (brs, 1H, CSNH); MS (*m/z*): 299 [M^+]; Anal. calcd. (%) for $C_{14}H_9N_3O_3S$: C, 56.18; H, 3.03; N, 14.04. Found (%): C, 56.31; H, 3.02; N, 14.00. (Adopting this procedure other 3-substituted-2-thioxo-2,3-dihydro-quinazolin-4-ones are prepared).

Synthesis of 2-methylthio-3-(4-nitrophenyl)quinazolin-4-one (4): The solution of compound **3** (0.01 mol) in alcoholic sodium hydroxide solution (25 mL) transferred in to conical flask. To this mixture dimethyl sulphate (0.01 mol) was added drop wise with stirring and stirring was continued for 1 h after complete addition of dimethyl sulphate. The reaction mixture was then poured into ice cold water and mixed well. The resultant solid obtained was filtered, washed with water and dried. The crude solid obtained was recrystallized from ethanol. Yield: 91 %; m.p.: 181-182 °C; IR (KBr, ν_{max}, cm^{-1}): 3059 (Ar-CH), 2957 (CH_3-CH), 1715 (C=O), 1668 (C=N), 1612 (C=C), 1534 & 1326 (NO_2), 672 (C-S-C); ^1H NMR ($CDCl_3$) δ: 2.20 (s, 3H, SCH_3), 7.08-7.10 (m, 2H, Ar-H), 7.26 (d, *J* = 7.5 Hz, 1H, Ar-H), 7.52-7.54 (m, 2H, Ar-H), 7.71 (d, *J* = 2.0 Hz, 1H, Ar-H), 7.90 (d, *J* = 7.5 Hz, 1H, Ar-H), 7.92 (d, *J* = 2.0 Hz, 1H, Ar-H); MS (*m/z*): 313 [M^+]; Anal. calcd. (%) for $C_{15}H_{11}N_3O_3S$: C, 57.50; H, 3.54; N, 13.41. Found (%): C, 57.67; H, 3.55; N, 13.38. (Adopting this procedure other 3-substituted-2-methylsulfanyl-quinazolin-4-ones are prepared).

Synthesis of 2-hydrazino-3-(4-nitrophenyl)quinazolin-4-one (5): The compound **4** (0.01 mol) was dissolved in ethanol (25 mL). To this mixture 99 % hydrazine hydrate (0.1 mol) and anhydrous potassium carbonate (100 mg) was added and refluxed for 36 h. The reaction mixture was cooled and poured into ice cold water. The solid so obtained was filtered, washed with water, dried and recrystallized using ethanol to afford the compound **5**. Yield: 83 %; m.p.: 200-201 °C; IR (KBr, ν_{max}, cm^{-1}): 3371 & 3286 (NH), 3035 (Ar-CH), 1748 (C=O), 1650 (C=N), 1621 (C=C), 1519 & 1353 (NO_2); ^1H NMR ($CDCl_3$) δ: 5.72 (brs, 1H, Ar-NH), 6.17 (brs, 2H, NH_2), 6.73-6.75 (m, 2H, Ar-H), 6.94 (d, *J* = 7.5 Hz, 1H, Ar-H), 7.42-7.44 (m, 2H, Ar-H), 7.61 (d, *J* = 2.0 Hz, 1H, Ar-H), 7.95 (d, *J* = 7.5 Hz, 1H, Ar-H), 8.18 (d, *J* = 2.0 Hz, 1H, Ar-H); MS (*m/z*): 297 [M^+]; Anal. calcd. (%) for $C_{14}H_{11}N_5O_3$: C, 56.56; H, 3.73; N, 23.56. Found (%): C, 56.45; H, 3.75; N, 23.64. (Adopting this procedure other 2-hydrazino-3-substituted-quinazolin-4-ones are prepared).

Synthesis of 2-phenyl-1, 3-benzoxazin-4-one: 14.05 g of benzoyl chloride (0.2 mol) was added to a solution of 13.7 g of anthranilic acid (0.1 mol) dissolved in 60 mL pyridine. For a period of 30 min the reaction mixture was stirred and treated with 15 mL of $NaHCO_3$ (5 %). The products separated were crystallized from alcohol. Yield: 81 %; m.p.: 120-121 °C; IR (KBr, ν_{max}, cm^{-1}): 3025 (Ar-CH), 1685 (C=O), 1620 (C=N), 1627 (C=C), 1029 (Cyclic C-O-C); ^1H NMR ($CDCl_3$) δ: 6.81-6.84 (m, 3H, Ar-H), 6.95 (d, *J* = 7.5 Hz, 2H), 7.02-7.04 (m,

3H, Ar-H), 7.51 (d, *J* = 2.0 Hz, 1H); MS (*m/z*): 223 [M$^+$]; Anal. calcd. (%) for $C_{14}H_9NO_2$: C, 75.33; H, 4.06; N, 6.27. Found (%): C, 75.11; H, 4.07; N, 6.29.

Synthesis of 3-(substituted quinazolinyl amino)-2-phenyl quinazolin-4(3*H*)ones (BQZ1-BQZ10): A mixture of 2-hydrazino-3-substituted-3*H*-quinazolin-4-one **(5)** (0.01 mol) and 2-phenyl-3,1-benzoxazin-4-one (0.01 mol) were fused at 150 °C and the solid obtained and recrystallized to afford the title compounds (**BQZ1-BQZ10**).

3-Ethyl-2-(4-oxo-2-phenylquinazolin-3(4*H*)-ylamino)-quinazolin-4(3*H*)-one (BQZ1): Yield: 86 %; m.p.: 228-229 °C; IR (KBr, ν_{max}, cm^{-1}): 3372 (NH), 3057 (Ar-CH), 2989 (CH_3-CH), 1703 (C=O), 1655 (C=N), 1610 (C=C); ^{1}H NMR ($CDCl_3$) δ: 1.52-1.83 (t, 3H, CH_3), 2.46-2.69 (q, 2H, CH_2), 5.64 (s, 1H, Ar-NH), 7.10-7.95 (m, 13H, Ar-H); MS (*m/z*): 409 [M$^+$]; Anal. calcd. For $C_{24}H_{19}N_5O_2$: C, 70.40; H, 4.68; N, 17.1043. Found (%): C, 70.19; H, 4.70; N, 17.16.

3-Allyl-2-(4-oxo-2-phenylquinazolin-3(4*H*)-ylamino)-quinazolin-4(3*H*)-one (BQZ2): Yield: 80 %; m.p.: 225-226 °C; IR (KBr, ν_{max}, cm^{-1}): 3304 (NH), 3028 (Ar-CH), 2963 (CH_2-CH), 1702 (C=O), 1649 (C=N), 1607 (C=C); ^{1}H NMR ($CDCl_3$) δ: 1.64-1.88 (d, 2H, $\underline{CH_2}CH=CH_2$), 4.72-4.95 (d, 2H, $CH_2CH=\underline{CH_2}$), 5.37-5.51 (m, 1H, $CH_2\underline{CH}=CH_2$), 5.62 (s, 1H, Ar-NH), 7.04-8.16 (m, 13H, Ar-H); MS (*m/z*): 421 [M$^+$]; Anal. calcd. (%) for $C_{25}H_{19}N_5O_2$: C, 71.25; H, 4.54; N, 16.62. Found (%): C, 71.50; H, 4.52; N, 16.57.

2-(4-Oxo-2-phenylquinazolin-3(4*H*)-ylamino)-3-phenylquinazolin-4(3*H*)-one (BQZ3): Yield: 81 %; m.p.: 240-241 °C; IR (KBr, ν_{max}, cm^{-1}): 3258 (NH), 3045 (Ar-CH), 1727 (C=O), 1662 (C=N), 1609 (C=C); ^{1}H NMR ($CDCl_3$) δ: 5.53 (s, 1H, Ar-NH), 7.27-8.10 (m, 18H, Ar-H); MS (*m/z*): 457 [M$^+$]; Anal. calcd. (%) for $C_{28}H_{19}N_5O_2$: C, 73.51; H, 4.19; N, 15.31. Found (%): C, 73.74; H, 4.17; N, 15.26.

2-(4-Oxo-2-phenylquinazolin-3(4*H*)-ylamino)-3-(pyridin-4-yl)quinazolin-4(3*H*)-one (BQZ4): Yield: 80 %; m.p.: 251-252 °C; IR (KBr, ν_{max}, cm^{-1}): 3349 (NH), 3034 (Ar-CH), 1730 (C=O), 1653 (C=N), 1616 (C=C); ^{1}H NMR ($CDCl_3$) δ: 5.60 (s, 1H, Ar-NH), 6.91-8.24 (m, 17H, Ar-H); MS (*m/z*): 458 [M$^+$]; Anal. calcd. (%) for $C_{27}H_{18}N_6O_2$: C, 70.73; H, 3.96; N, 18.33. Found (%): C, 70.45; H, 3.97; N, 18.39.

2-(4-Oxo-2-phenylquinazolin-3(4*H*)-ylamino)-3-*p*-tolylquinazolin-4(3*H*)-one (BQZ5): Yield: 83 %; m.p.: 232-233 °C; IR (KBr, ν_{max}, cm^{-1}): 3394 (NH), 3051 (Ar-CH), 2952 (CH_3-CH), 1735 (C=O), 1650 (C=N), 1623 (C=C); ^{1}H NMR ($CDCl_3$) δ: 2.98 (s, 3H, CH_3), 5.46 (s, 1H, Ar-NH), 7.13-8.25 (m, 17H, Ar-H); MS (*m/z*): 471 [M$^+$]; Anal. calcd. (%) for $C_{29}H_{21}N_5O_2$: C, 73.87; H, 4.49; N, 14.85. Found (%): C, 73.60; H, 4.48; N, 14.91.

3-(4-Nitrophenyl)-2-(4-oxo-2-phenylquinazolin-3(4*H*)-ylamino)quinazolin-4(3*H*)-one (BQZ6): Yield: 80 %; m.p.: 241-242 °C; IR (KBr, ν_{max}, cm^{-1}): 3271 (NH), 3046 (Ar-CH), 1728 (C=O), 1664 (C=N), 1602 (C=C), 1540 & 1317 (NO_2); ^{1}H NMR ($CDCl_3$) δ: 5.52 (s, 1H, Ar-NH), 7.15-8.08 (m, 17H, Ar-H); MS (*m/z*): 502 [M$^+$]; Anal. calcd. (%) for $C_{28}H_{18}N_6O_4$: C, 66.93; H, 3.61; N, 16.73. Found (%): C, 67.12; H, 3.60; N, 16.75.

3-(4-Chlorophenyl)-2-(4-oxo-2-phenylquinazolin-3(4*H*)-ylamino)quinazolin-4(3*H*)-one (BQZ7): Yield: 81 %; m.p.: 230-231 °C; IR (KBr, ν_{max}, cm^{-1}): 3343 (NH), 3039 (Ar-CH), 1704 (C=O), 1646 (C=N), 1611 (C=C), 786 (C-Cl); ^{1}H NMR ($CDCl_3$) δ: 5.61 (s, 1H, Ar-NH), 7.26-8.14 (m, 17H, Ar-H); MS (*m/z*): 493 [M^{+2}], 491 [M$^+$]; Anal. calcd. (%) for $C_{28}H_{18}ClN_5O_2$: C, 68.36; H, 3.69; N, 14.24. Found (%): C, 68.13; H, 3.70; N, 14.29.

3-(2-Chlorophenyl)-2-(4-oxo-2-phenylquinazolin-3(4*H*)-ylamino)quinazolin-4(3*H*)-one (BQZ8): Yield: 78 %; m.p.: 238-239 °C; IR (KBr, ν_{max}, cm^{-1}): 3286 (NH), 3053 (Ar-CH), 1731 (C=O), 1668 (C=N), 1605 (C=C), 764 (C-Cl); ^{1}H NMR ($CDCl_3$) δ: 5.42 (s, 1H, Ar-NH), 7.34-8.01 (m, 17H, Ar-H); MS (*m/z*): 493 [M^{+2}], 491 [M$^+$]; Anal. calcd. For $C_{28}H_{18}ClN_5O_2$: C, 68.36; H, 3.69; N, 14.24. Found (%): C, 68.19; H, 3.68; N, 14.28.

3-(2-Methoxyphenyl)-2-(4-oxo-2-phenylquinazolin-3(4*H*)ylamino)quinazolin-4(3*H*)-one (BQZ9): Yield: 78 %; m.p.: 243-244 °C; IR (KBr, ν_{max}, cm^{-1}): 3360 (NH), 3042 (Ar-CH), 2943 (CH_3-CH), 1719 (C=O), 1654 (C=N), 1628 (C=C), 1061 (C-O-C); ^{1}H NMR ($CDCl_3$) δ: 3.05 (s, 3H, OCH_3), 5.58 (s, 1H, Ar-NH), 7.02-8.37 (m, 17H, Ar-H); MS (*m/z*): 487 [M$^+$]; Anal. calcd. (%) for $C_{29}H_{21}N_5O_3$: C, 71.45; H, 4.34; N, 14.37. Found (%): C, 71.19; H, 4.36; N, 14.42.

3-(4-Methoxyphenyl)-2-(4-oxo-2-phenylquinazolin-3(4*H*)-ylamino)quinazolin-4(3*H*)-one (BQZ10): Yield: 81 %; m.p.: 215-216 °C; IR (KBr, ν_{max}, cm^{-1}): 3337 (NH), 3020 (Ar-CH), 2979 (CH_3-CH), 1706 (C=O), 1651 (C=N), 1613 (C=C), 1051 (C-O-C); ^{1}H NMR ($CDCl_3$) δ: 3.40 (s, 3H, OCH_3), 5.57 (s, 1H, Ar-NH), 7.29-8.13 (m, 17H, Ar-H); MS (*m/z*): 487 [M$^+$]; Anal. calcd. (%) for $C_{29}H_{21}N_5O_3$: C, 71.45; H, 4.34; N, 14.37. Found (%): C, 71.70; H, 4.33; N, 14.32.

Antitubercular activity: Into Middle brook 7H11 agar slants 10 fold serial dilutions of each test compound/drug were incorporated with OADC growth supplement. Fresh Middle brook 7H11 agar slants with OADC growth supplement was used to prepare inoculums of *M. tuberculosis* $H37R_V$ and adjusted to 1 mg/mL in Tween 80 (0.05 % W/V) saline diluted to 10^{-2} to give a 10^7 cfu/mL concentrate approximately. Into 7H11 agar tubes containing 10 fold serial dilutions of drug per mL a 5 µL amount of bacterial suspension was spotted. At 37 °C the tubes were incubated and after 28 days the final readings were recorded. Control tubes with medium alone were incubated with $H37R_V$ were used to compare tubes having the compounds. Active concentration of test compound was taken as the concentration at which complete inhibition of colonies occurred. The minimum concentration of compound required to give complete inhibition of bacterial growth was taken as MIC [16-18]. Against reference drug isoniazid, rifampicin and ethambutol the MIC of the test compounds was compared.

Anti-HIV activity: In MT-4 cells anti-HIV activity of the compounds (**BQZ1-BQZ10**) were tested against replication of HIV-1 (III B) and HIV-2 (ROD) [19]. The MT-4 cells were grown in RPMI-1640 DM (Dutch modification) medium (Flow laboratories, Irvine, Scotland), supplemented with 10 % (v/v) heat inactivated fetal calf serum and 20 µg/mL gentamicin (E. Merck, Darmstadt, Germany). From the culture supernatant of HIV-1 infected MT-4 cell lines HIV-1 (III B) and HIV-2 (ROD) were obtained and the virus stocks were stored at -70 °C until used. Microtiter plates was used to perform anti-HIV

assay by filled with 100 µL of medium and 25 µL volumes of compounds in triplicate so as to allow simultaneous evaluation of their effects on HIV and mock infected cells. 50 µL of HIV at 100 $CCID_{50}$ medium was added to either infected or mock infected part of microtiter tray. At 37°C the cell cultures were incubated in a humidified atmosphere of 5 % CO_2 in air. By the MTT method after 5 days of infection spectrophotometrically examined the viability of mock and HIV infected cells. The effective dose of compound achieving 50 % protection of MT-4 cells against the cytopathic effect (Virus cause cell degeneration or cell death, which can be seen by microscopical examination of cultures. Cell degeneration is manifested by certain pathological changes) of HIV (EC_{50}) and the cytotoxic dose of compound, required to reduce the viability of normal uninfected MT-4 cells by 50 % (CC_{50}) were calculated.

Antibacterial activity: Agar dilution method was used to evaluate antibacterial activity of compounds [20,21]. From the American type culture collection (ATCC), Rockville, USA the standard strains were procured and the pathological strains were procured from the department of microbiology, MNR medical college, Sangareddy, India. The antibacterial activity of the test analogs was screened against the following bacterial strains: *E. coli* ATCC 25922, *P. vulgaris* ATCC 9484, *S. typhimurium* ATCC 33068, *K. pneumoniae* ATCC 13883, *P. aeruginosa* ATCC 2853, *B. subtilis* ATCC 6051, *S. aureus* ATCC 25923, *M. luteus* ATCC 10240, *S. epidermidis* ATCC 35984, *S. albus* ATCC 17900. Muller-Hinton Agar (Hi-media) plates (37 °C, 24 h) were used for bacterial growth 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. Ciprofloxacin was used as reference drug for comparison of MIC of the test compounds.

RESULTS AND DISCUSSION

Synthetic route shown in **Scheme-I** outline the chemistry part of the present work. The key intermediate 3-substituted-2-thioxo-2,3-dihydro-1*H*-quinazolin-4-one **(3)** was obtained by reacting primary amine (**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, which on reflux with methyl anthranilate **(2)** in ethanol yielded the desired 3-substituted-2-thioxo-2,3-dihydro-1*H*-quinazolin-4-one **(3)**in a good yield. The 3-substituted-2-methylsulfanyl-3*H*-quinazolin-4-one **(4)** was obtained by dissolving compound **(3)** in 2 % alcoholic sodium hydroxide solution and methylating with dimethyl sulphate by stirring at room temperature. With hydrazine hydrate nucleophilic displacement of methylthio group of **(4)** was carried out in ethanol to afford 2-hydrazino-3-substituted-3*H*-quinazolin-4-one **(5)**. The title compounds 3-(substituted quinazolinylamino)-2-phenyl quinazolin-4(3*H*)ones (**BQZ1-BQZ10**) were obtained by the condensation of amino group of 2-hydrazino-3-phenyl-3*H*-quinazolin-4-one **(6)** with 2-phenyl-1,3-benzoxazin-4-one. The formation of title product is indicated by the disappearance of peak due to NH_2 of the starting material in the IR and ^{1}H NMR spectrum of the compounds **BQZ1-BQZ10**. In the IR and ^{1}H NMR spectrum of these peaks due to thiosemicarbazides, carbonyl (C=O), NH and aryl groups presence were confirmed. The mass spectra of test compounds molecular ion peaks corresponding to their molecular formulae were observed. 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 is depicted in Table-1, indicates that the test compounds inhibited the growth of *Mycobacterium* at the minimum microgram of 25 µg/mL concentration.

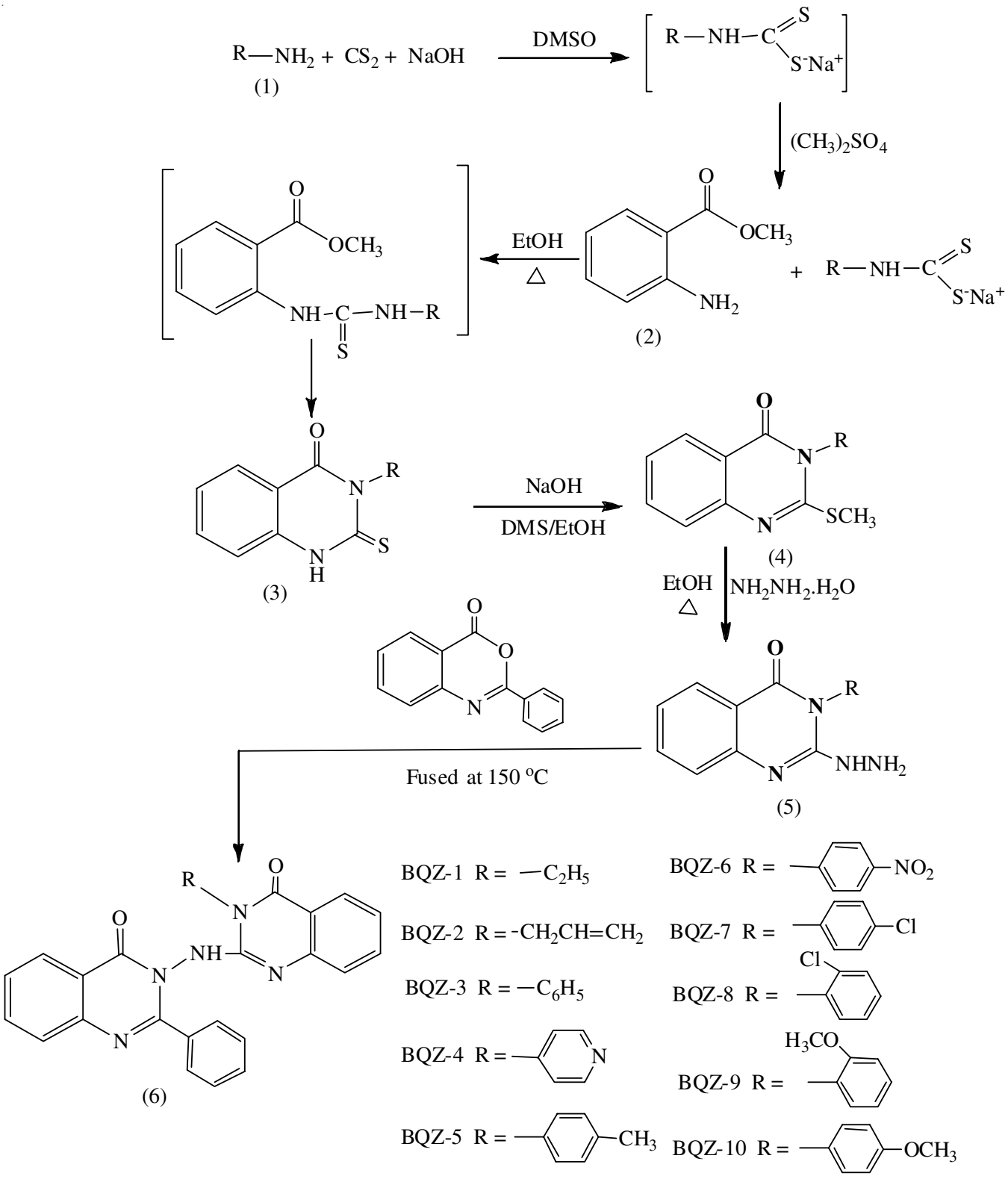
Anti-HIV activity: The results of anti-HIV activity (Table-1) indicate that all compounds exhibited mild to moderate anti-HIV activity; whereas compounds **BQZ7** containing aryl ring with electron withdrawing group exhibited anti-HIV activity at 35.4 µg/mL concentration against HIV1 and HIV2. While the test compounds with other substituent's showed mild to moderate anti-HIV activity against HIV1 and HIV2.

Antibacterial activity: Among the different substituent at N-3 position of the quinazoline, aryl and heteroaryl substituents

TABLE-1
ANTITUBERCULAR, ANTI-HIV AND ANTIBACTERIAL ACTIVITY OF TITLE COMPOUNDS (**BQZ1- BQZ10**)

Microorganism	Test compounds and standard												
	BQZ1	**BQZ2**	**BQZ3**	**BQZ4**	**BQZ5**	**BQZ6**	**BQZ7**	**BQZ8**	**BQZ9**	**BQZ10**	**STD1**	**STD2**	**STD3**
M. tuberculosis	25	25	25	25	25	25	25	25	25	25	0.05	0.1	1.56
HIV-1	100	39.6	49.5	47.6	100	35.9	35.4	39.1	54.9	91.4	0.0012	–	–
HIV-2	100	39.6	49.5	47.6	100	35.9	35.4	39.1	54.9	91.4	0.000642	–	–
S. typhi	100	25	25	25	100	25	25	25	50	50	4	–	–
E. coli	50	13	25	25	50	3	3	6	50	100	1	–	–
B. subtilis	100	25	25	25	100	25	25	25	25	50	1	–	–
K. pneumonia	50	13	13	25	50	13	13	25	25	50	1	–	–
P. vulgaris	100	13	25	13	100	13	13	50	25	50	1	–	–
P. aeruginosa	100	6	25	13	50	3	3	6	13	25	1	–	–
S. aureus	100	6	25	6	50	3	3	13	13	25	1	–	–
M. luteus	25	13	13	25	25	13	6	25	25	13	1	–	–
S. epidermidis	50	13	25	25	50	13	6	13	50	100	1	–	–
S. albus	100	25	25	25	100	25	13	25	50	50	1	–	–

Antitubercular standard: **STD1** - Isoniazid, **STD2** - Rifampicin, **STD3** - Ethambutol; Anti-HIV standard: **STD1**-AZT; Antibacterial standard: **STD1** - Ciprofloxacin.



Scheme-I: Synthesis of quinazolinyl quinazolines (**BQZ1-BQZ10**)

exhibited better activity over the aliphatic and cyclic substituent. Compounds with electron withdrawing substituents like chloro and nitro showed better activity over the unsubstituted and electron donating substituents. Among the test compounds 3-(4-nitrophenyl)-2-(4-oxo-2-phenylquinazolin-3(4*H*)-yl-amino)-quinazolin-4(3*H*)-one (**BQZ6**) and 3-(4-chlorophenyl)-2-(4-oxo-2-phenylquinazolin-3(4*H*)-ylamino)quinazolin-4(3*H*)-one (**BQZ7**) shown most potent activity against *E. coli*, *P. aeruginosa* and *S. aureus* with the MIC of 3 µg/mL. Compound **BQZ7** and **BQZ6** emerged as the most active compounds of the series.

Conclusion

Synthesis of new series of 3-(substituted)-2-(4-oxo-2-phenyl-quinazolin-3(4*H*)-ylamino)quinazolin-4(3*H*)-ones have been

described. The title compounds exhibited significant antibacterial activity against the different Gram-positive and Gram-negative bacteria including *M. tuberculosis;* and they also exhibited significant activity against HIV1 and HIV2 strains. The substituents at 3-aminoquinazoline shown varied antimicrobial activity, aryl substituents with electron withdrawing group showed most potent and the aryl/allyl substituents showed moderate activity and the alkyl, aryl substituents with electron donating groups showed the least activity. Among the test compounds, compounds 3-(4-nitrophenyl)-2-(4-oxo-2-phenylquinazolin-3(4H)-ylamino)quinazolin-4(3*H*)-one (**BQZ6**) and 3-(4-chlorophenyl)-2-(4-oxo-2-phenylquinazolin-3(4*H*)-ylamino)quinazolin-4(3*H*)-one (**BQZ7**) shown most potent activity against *E. coli*, *P. aeruginosa* and *S. aureus* with the MIC of 3 µg/mL. The compound **BQZ7** exhibited the antitubercular activity at the minimum microgram of 25 µg/mL and anti-HIV activity at 35.4 µg/mL against HIV1 and HIV2 and offers potential lead for further optimization and development to new antitubercular and anti-HIV agents.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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