ORIGINAL ARTICLE

Design and synthesis of Quinazolinone, Benzothiazole derivatives bearing guanidinopropanoic acid moiety and their Schiff bases as cytotoxic and antimicrobial agents

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Abstract   Two series of Benzothiazole, Quinazolinone derivatives bearing guanidinopropanoic acid (38 compounds including 27 intermediates) and one series of Schiff base derivatives (14 compounds) were synthesized, characterized then evaluated for their cytotoxicity against human cervix cell line (HeLa) by MTT assay; antimicrobial activity against 11 pathogenic bacteria, 10 pathogenic fungus using standard of ciprofloxacin and Clotrimazole respectively. Compounds **13–18** showed significant activity against HeLa with IC_{50} range of 2–550 μM. Compound 3-(3-(6-hydroxybenzo[*d*]thiazol-2-yl)guanidino)propanoic acid (**18**) showed potent activity against human HeLa cell line with the half maximal inhibitory concentration (IC_{50}) values of 1.8 μM which was close to the value of the positive control, doxorubicin. Antimicrobial result indicated that, compounds showed differential activity against the tested fungus and bacteria. Compounds **11**, **14**, **38** and **49** exhibited potent antibacterial and antifungal activity.

1. Introduction

Cancer is continuing to be a major health problem in developing as well as underdeveloped countries. Surpassing heart diseases, it now become the number one killer disease in the world (Alaa and Abdel-Aziz, 2007; Medina et al., 1998, 1999; Hwang et al., 1999). Guanidine-containing molecules display a wide range of biologically important roles like influenza inhibitor zanamivir, antimicrobials, anticancer, antiviral, etc. (Sarah et al., 2006; Jiawang Liu et al., 2007; Nagwa et al., 2010). Mortimer et al. (2006) had reported Benzothiazole derivatives (Fig. 1) having significant anticancer activity and was selected for preclinical development then became the foundation for a second phase of analogue work. Abdulrahman et al. (2009) reported antitumor activity of novel derivatives of Quinazolinone (Fig. 2) against three (tumour cell lines human liver cell

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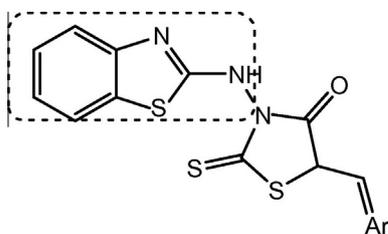 E-mail address: venkateshom2001@gmail.com (P. Venkatesh).

Figure 1 Benzo[*d*]thiazol-2-ylamino-2-thioxothiazolidin-4-one derivative.
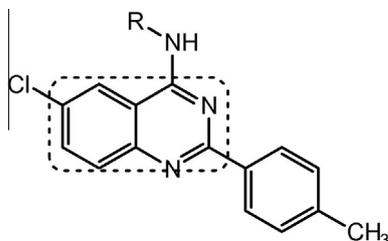
Figure 2 6-Chloro-2-p-tolylquinazolin-4-amine derivative.

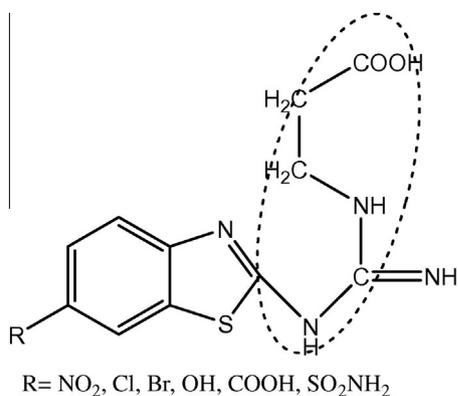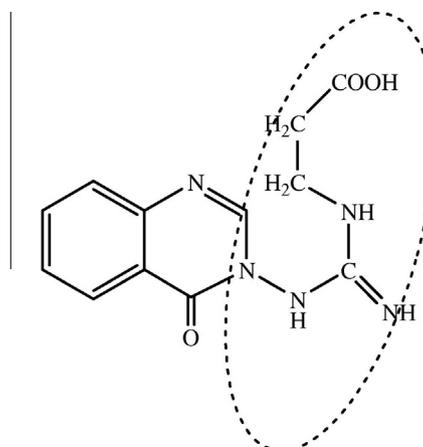R= NO₂, Cl, Br, OH, COOH, SO₂NH₂

Figure 3 3-(3-(6-Substituted Benzo[*d*]thiazol-2-yl) guanidino)propanoicacid derivative.

R=CH₃,C₆H₅, C₆H₄(OH)(OCH₃), C₆H₄(N(CH₃)₂), C₆H₄(OCH₃)

Figure 4 3-(3-(2-Substituted-4-oxoquinazolin-3(4H)-yl) guanidino)propanoicacid derivatives.

R= NO₂, OH, COOH, Cl, Br, SO₂NH₂   R₁= CH₃, C₆H₅
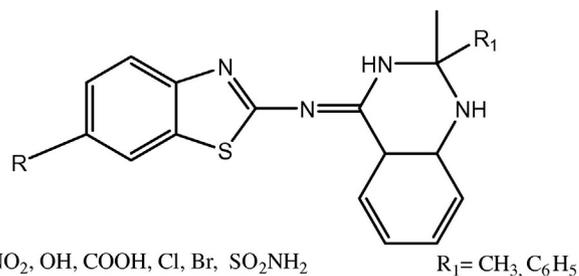
Figure 5 2-(2,2-Disubstituted-1,2,3,8a-dihydroquinazolin-4(4aH)-ylideneamino)benzo[*d*]thiazole derivative.
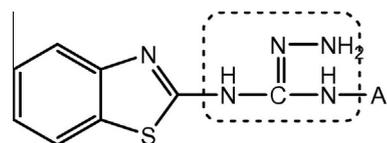
Figure 6 (Benzo[*d*]thiazol-2-yl)guanidine derivative.
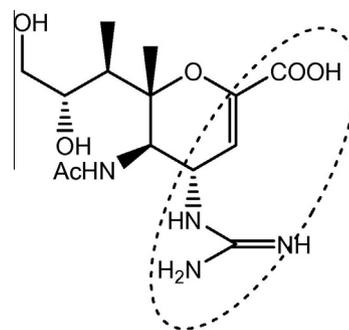
Figure 7 5-Acetamido-4-guanidino-6-methyl-4H-pyran-2-carboxylicacid derivative (influenza inhibitor zanamivir).

line (HEPG2), human breast cell line (MCF-7) and human cervix cell line (HeLa).

Consequently, both Benzothiazole and Quinazolinone moiety separately substituted with Guanidine moiety (Fig. 3 and 4) and the combination of Benzothiazole and Quinazolinone in one molecule through imine linkage (Fig. 5), can be considered as promising approach in drug-like molecules design.

Synthesised compounds (Figs. 3–5) were found in structural similarity with the earlier reported antimicrobial, anticancer (Fig. 6), and antiviral (influenza inhibitor zanamivir) (Fig. 7) compounds. So, we could expect that these compounds may also exhibit the above activities.

To confirm that, various Quinazolinones, Benzothiazole-2-amine derivatives and Schiff base derivatives (synthesized by condensation of Quinazolinone and Benzothiazole-2-amine derivatives) bearing guanidinopropanoic moiety were synthesised; evaluated for their cytotoxicity against human

cervix cell line (HeLa) by MTT assay, antimicrobial activity against 11 pathogenic bacteria and 10 pathogenic fungus.

2. Experimental

2.1. Materials and methods

All the starting materials and solvents were purchased from commercially available sources. The melting points were determined on THERMONIC MODEL-C-LMP-1, Campbell melting point apparatus (uncorrected). ^{1}H NMR were recorded on Brucker NRC-IISC 400/DRX-500/AV-III 500(S)/(L) AV-700 NMR spectrometer, ESI-MS were recorded with a Mariner System 5304 mass spectrometer and Elementary analysis was performed on a CHN-O-Rapid instrument within ±0.4% of

the theoretical values at IIT Madras, Chennai. All the reactions were monitored using thin layer chromatography (TLC) using glass plate coated with silica gel G. TLC plates were developed in iodine and UV and chloroform: methanol (1:1) was taken as mobile phase.

2.2. Synthesis

2.2.1. Series I
Compounds **1–6**, (6-substituted Benzo[d]thiazol-2-amine derivatives) and compounds **7–12**, (1-(6-substituted Benzo[d]thiazol-2-yl)thiourea were prepared from the corresponding substituted anilines and from the compounds **1–6** respectively by using earlier reported methods (Pankaj et al., 2010) (Scheme 1).
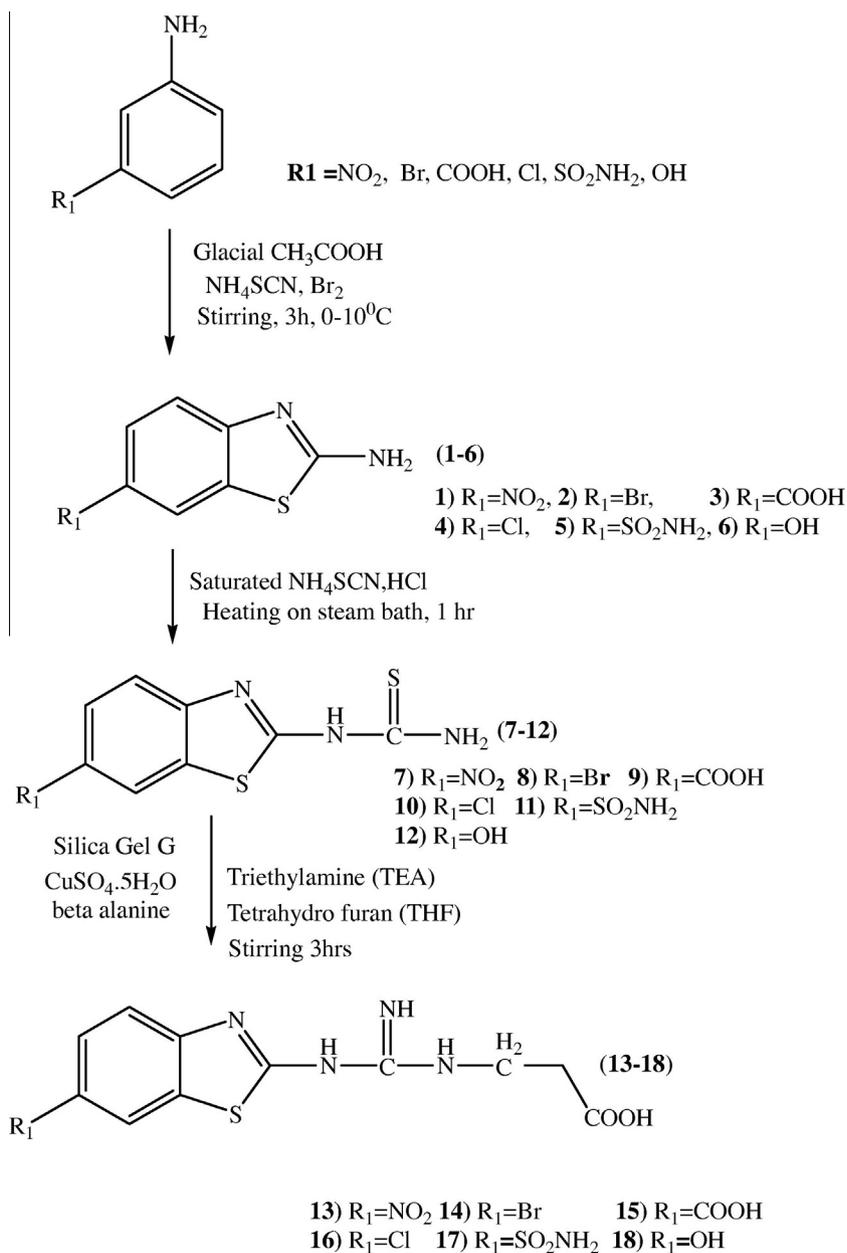
Scheme 1   Synthesis of BTGP (**13–18**) from aryl amines (**13–18**).

2.2.1.1. Synthesis of 3-(3-(6-substituted Benzo[d]thiazol-2-yl)guanidino) propanoic acid derivatives (BTGP) (Scott et al., 2001) from 1-(6-substituted Benzo[d]thiazol-2-yl)thioureas (compounds 13–18, Scheme 1). To the stirring mixture of compounds **7–12** containing Silica gel G (0.02 mol, 0.01g), Cupric sulphate pentahydrate (0.01 g), and Triethylamine (2 mL) in Tetrahydrofuran (25 mL), 1.78 g (0.02 mol) of β-alanine were added and stirring continued for 5–6 h, then kept at room temperature for 24 h. The crude product formed was recrystallised from methanol.

2.2.2. Series II

2.2.2.1. Synthesis of 2-substituted Quinazolin-4(3H)-one derivative from Anthranilamide (Anette Witt and Jan Bergman, 2000) (compounds 19–23, Scheme 2). Anthranilamide (0.05 mol, 6.8 g) and aryl/heteroaryl aldehydes (0.05 mol) were refluxed in ethanol for 3 h at 70 °C, excess solvent was removed by rotary vacuum evaporator and solid residue (Schiff base) formed was refluxed with CuCl₂ (0.05 mol, 6.72 g) in ethanol for further 3 h at 70 °C. Two-substituted Quinazolinones formed was recrystallised from ethyl acetate.

2.2.2.2. Synthesis of 3-amino-2-substituted Quinazolin-4(3H)-one derivatives from 2-substituted Quinazolin-4(3H)-one derivatives (Priyanka et al., 2010) (compounds 24–28, Scheme 2). To the compounds **19–23** (0.2 mol), equal mixture of Conc. HCl and Hydrazine hydrate (6 mL) was added drop wise with stirring at 5–10 °C. Ethylene glycol (24 mL) and substituted Quinazolinones (**19–23**, 0.03 mol) were added to the above reaction mixture and refluxed for 3 h. On cooling solid was separated, filtered, washed with water and recrystallized from ethanol.

2.2.2.3. Synthesis of 1-(2-substituted-4-oxoquinazolin-3(4H)-yl) thioureas from 3-amino-2-substituted Quinazolin-4(3H)-one derivatives (compounds 29–33, scheme 2). Thiourea analogues **29–33** were prepared from the compounds **24–28** by using earlier method (Scott et al., 2001).

2.2.2.4. Synthesis of 3-(3-(2-substituted-4-oxoquinazolin-3(4H)-yl) guanidino) propanoicacid derivatives (QGP) from 1-(2-substituted-4-Oxoquinazolin-3(4H)-yl) thioureas (Compounds 34–38, scheme 2). Guanidino propanoic acid derivatives **34–38** were prepared from the compounds **29–33**, method used was similar to the preparation of compounds **13–18**.

2.2.3. Series III

2.2.3.1. Step I: Synthesis of 2-methyl-2-substituted-2,3-dihydroquinazolin-4(1H)-ones (compounds 39–40) from Anthranilamide (Al-Obaid et al., 2010). To a stirring solution of Anthranilamide (110 mmol, 15 g) in Acetone (150 mL), *p*-Toluenesulfonic acid monohydrate (0.65 mmol, 0.125 g) was and the resulting homogeneous mixture was refluxed for 1 h. The solvent was subsequently cooled to ambient temperature and removed under reduced pressure. The resulting solid was partitioned between Ethyl acetate (250 mL) and saturated aqueous NaHCO₃ (250 mL). The layers were shaken, and organic layer was separated and washed additionally with water (2 portions, 100 mL). The organic layer was dried over Na₂SO₄ and solvent evaporated to approximately one-half its volume,

cooled and the solid obtained was recrystallized from the organic layer.

2.2.3.2. Step III: synthesis of Schiff base derivatives (compounds 41–54) from substituted 6-substituted benzo[d]thiazol-2-amine and 2-Methyl-2-substituted-2,3-dihydroquinazolin-4(1H)-ones (Jain et al., 2008). Substituted Benzothiazole s (compounds **1–6**, 0.02 mol) and Tetrahydroqunazolinones (compounds **39–40**, 0.02 mol) were dissolved in 50 mL of ethanol and refluxed for 10–16 h. The reaction mixture was concentrated to approximately to 10 mL and allowed to cool. The resultant product was filtered out and recrystallized from ethanol.

2.2.4. Characterization

2.2.4.1. 3-(3-(6-Nitrobenzo[d]thiazol-2-yl) guanidino) propanoic acid (Compound 13). Pale yellow solid, Yield, 78%, m.p., 102–104 °C, (IR) v_{max} (KBr/cm⁻¹), 3300–3235 (OH) 3128 (NH), 2354.66 (CH₂), 1692 (C=O), 1640 (C=N), 1537.95 (NO₂), 1339 (C–N), 1234 (C–S), ¹H NMR (DMSO, δ ppm): 9.964 (s, br, 1H, COOH of CH₂COOH), 7.66–7.65 (m, 2H, $C_{5,7}$-H of Benzothiazole), 7.64–7.69 (s, br),1H, –C=NH of Guanidine), 7.23–7.11 (m, 1H, C_4–H of Benzothiazole), 3.52 (s, 1H, NH) 3.23–3.31 (t, $J = 7$ Hz, 2H, CH₂), 2.76–2.87 (t, $J = 7$ Hz, 2H, CH₂). ESI–MS: 309.91 [M⁺]. Anal. Calcd for $C_{11}H_{11}N_5O_4S$ (309.3): C, 62.20; H, 5.22; N, 20.72. Found: C, 62.22; H, 5.21; N, 20.61.

2.2.4.2. 3-(3-(6-bromobenzo[d]thiazol-2-yl) guanidino) propanoic acid (Compound 14). Greenish yellow solid, Yield, 89%, m.p., 152–154 °C, (IR) v_{max} (KBr/cm⁻¹), 3288–3216 (OH) 3078 (NH), 2910 (CH₂), 1611 (C=N), 1680 (C=O), 1304 (C–N), 735 (C–Br), 1265 (C–S), ¹H NMR (DMSO, δ ppm): 9.87 (s, 1H, COOH of CH₂COOH), 7.66–7.68 (m, 2H, $C_{5,7}$-H of Benzothiazole), 7.73–7.86 (s, 1H, –C=NH of Guanidine), 7.63–7.71 (m, 1H C_4-H of Benzothiazole), 3.72 (s, 1H, –NH), 3.33–3.55 (t, $J = 9$ Hz, 2H, CH₂), 2.66–2.78 (t, $J = 9$ Hz, 2H, CH₂). ESI–MS: 346 [M⁺]. Anal. Calcd for $C_{11}H_{11}BrN_4O_2S$ (343.2): C, 38.50; H, 3.23; N, 16.32. Found: C, 38.27; H, 3.24; N, 16.58.

2.2.4.3. 2-(3-(2-carboxyethyl) guanidino) benzo[d]thiazole-6-carboxylic acid (Compound 15). Dark yellow solid, Yield, 78%, m.p., 147 °C, (IR) v_{max} (KBr/cm⁻¹), 3286–3289 (OH) 3260 (NH), 2727 (CH₂), 1678(C=O), 1474 (C=N), 1234 (C–S), ¹H NMR (DMSO, δ ppm): 10.95 (s, 1H, (br), Ar-COOH), 9.88 (s, 1H, COOH of CH₂COOH), 7.46–7.56 (m, 2H, $C_{5,7}$–H of Benzothiazole), 7.87 (s, br, 1H, –C=NH of Guanidine), 7.53–7.423 (m, 1H, C_4–H of Benzothiazole), 3.76 (s, br, 1H, –NH) 3.56–3.60 (t, $J = 8$ Hz, 2H, CH₂), 2.56–2.48 (t, $J = 8$ Hz, 2H, CH₂). ESI-MS: 308.98 [M⁺] Anal. Calcd for $C_{12}H_{12}N_4O_4S$ (308.31):C, 46.75; H, 3.92; N, 18.17. Found: C, 46.64; H, 4.02; N, 18.47.

2.2.4.4. 3-(3-(6-chlorobenzo[d]thiazol-2-yl) guanidino) propanoic acid (Compound 16). Brown solid, Yield, 69%, m.p., 115–117 °C, (IR) v_{max} (KBr/cm⁻¹), 3326–3287 (OH), 3126 (N–H), 2696 (CH₂), 1624 (C=N), 1681(C=O), 1313 (C–N), 999 (C–Cl), 1245 (C–S), ¹H NMR (DMSO, δ ppm): 9.99 (s, 1H, COOH of CH₂COOH), 7.96–7.98 (m, 2H, $C_{5,7}$-H of Benzothiazole), 7.47 (s, 1H, –C=NH of Guanidine), 7.53–7.41 (m, 1H C_4-H of Benzothiazole), 3.62 (s, 1H, NH) 3.63–3.15

(t, J = 7 Hz, 2H, CH$_2$), 2.31–2.24 (t, J = 7 Hz, 2H, CH$_2$). ESI-MS: 300.78 [M$^+$]. Anal. Calcd for C$_{11}$H$_{11}$ClN$_4$O$_2$S (298.75): C, 44.22; H, 3.71; Cl, 11.87; N, 18.75. Found: C, 44.11; H, 3.96; N, 18.45.

2.2.4.5. 3-(3-(6-sulphanilamidobenzo[d]thiazol-2-yl)guanidino) propanoic acid (Compound 17). Light green solid, Yield, 72%, m.p., 116–118 °C, (IR) v_{max} (KBr/cm^{-1}), 3311–3286 (OH), 3132 (N–H str), 2926 (CH$_2$), 1684 (C=O), 1434 (C=N), 1490 (SO$_2$NH$_2$), 1404, 641 (CH=CH aromatic), 1324 (C–N), 623 (C–S), ^{1}H NMR (DMSO, δ ppm): 9.99 (s, 1H, COOH of CH$_2$COOH), 7.96–7.99 (m, 2H, C$_{5,7}$-H of Benzothiazole), 7.38 (s, 1H 1H, –C=NH of Guanidine), 7.43–7.11 (m, 1H C$_4$-H of Benzothiazole), 3.77 (s (br), 1H, NH), 3.63–3.31 (t, J = 7 Hz, 2H, CH$_2$), 2.42–2.12 (S (br), 2H, -SO$_2$NH$_2$), 2.86–2.68 (t, J = 7 Hz, 2H, CH$_2$). ESI-MS: 343.35 [M$^+$]. Anal. Calcd for C$_{11}$H$_{13}$N$_5$O$_4$S$_2$ (343.38):C, 38.48; H, 3.82; N, 20.40. Found: C, 38.62; H, 3.89; N, 20.01.

2.2.4.6. 3-(3-(6-hydroxybenzo[d]thiazol-2-yl) guanidino) propanoic acid (Compound 18). Black solid, Yield, 59%, m.p., 146 °C, (IR) v_{max} (KBr/cm^{-1}), 3392 (OH), 3130 (N–H str), 2359 (CH$_2$), 1683 (C=O), 1548 (C=N), 1512, 1401 (C–N), 669 (C–S). ^{1}H NMR (DMSO, δ ppm): 11.24 (s, 1H, Ar-OH), 9.89 (s, 1H, COOH of CH$_2$COOH), 7.56–7.32 (m, 2H, C$_{5,7}$-H of Benzothiazole), 7.17 (s, (br), –C=NH of Guanidine), 7.63–7.51 (m, 1H C$_4$-H of Benzothiazole), 3.82 (s (br), 1H, NH) 3.33–3.51 (t, J = 7 Hz, 2H, CH$_2$), 2.76–2.56 (t, J = 7 Hz, 2H, CH$_2$). ESI-MS: 280.96 [M$^+$]. Anal. Calcd for C$_{11}$H$_{12}$N$_4$O$_3$S (280.3): C, 47.13; H, 4.32; N, 19.99. Found: C, 47.18; H, 4.68; N, 19.89.

2.2.4.7. 3-(3-(2-methyl-4-oxoquinazolin-3(4H)-yl)guanidino) propanoic acid (Compound 34). Light brown solid, Yield, 63%, m.p., 133 °C, (IR) v_{max} (KBr/cm^{-1}), 3319 (OH), 3129 (N–H str aliphatic), 2857 (CH$_2$), 1679 (C=O), 1448 (C=N), 1360 (C–N), 1265 (C–CH$_3$), ^{1}H NMR (DMSO, δ ppm): 11.24 (s, (1H, COOH), 7.68–8.09 (m, 4H of, C$_{5,6,7,8}$-H of Quinazolinone ring), 7.78 (s,(br),1H, s, 1H, –C=NH of Guanidine), 2.64–2.75 (s,1H, NH), 2.59–2.67 (t, J = 7.4 Hz, 2H, CH$_2$), 1.85 (s, 3H, –CH$_3$ attached C-2 of Quinazolinone ring), 1.35–1.75 (t, J = 7.4 Hz, 2H, CH$_2$). ESI-MS: 289.89 [M$^+$]. Anal. Calcd for C$_{13}$H$_{15}$N$_5$O$_3$ (289.29):C, 53.97; H, 5.23; N, 24.21. Found; C, 53.14; H, 5.38; N, 24.37.

2.2.4.8. 3-(3-(4-oxo-2-phenylquinazolin-3(4H)-yl)guanidino) propanoic acid (Compound 35). Pale green solid, Yield, 69%, m.p.,140 °C, (IR) v_{max} (KBr/cm^{-1}), 3331 (OH), 3133 (N–H stretch, aliphatic), 2351 (CH$_2$), 1684 (C=O), 1430 (C=N), 1492 (monsubstituted Benzene), 1393 (C–N). ^{1}H NMR (DMSO, δ ppm): 11.48 (s, 1H, COOH), 7.59–8.10 (m, 4H of, C$_{5,6,7,8}$-H of Quinazolinone ring), 7.83 (s, (br), 1H, –C=NH of Guanidine), 7.65–7.81 (d, J = 6 Hz, 2H, C$_2$-phenyl-C$_{2,6}$-H), 6.85–6.94 (m, 3H, C$_2$-phenyl-C$_{3,4,5}$-H), 2.68–2.71 (s, 1H, (br), NH), 2.31–2.39 (t, J = 7.4 Hz, 2H, CH$_2$), 1.75–1.82 (t, J = 7.4 Hz, 2H, CH$_2$). ESI-MS: 351.41 [M$^+$]. Anal. Calcd for C$_{18}$H$_{17}$N$_5$O$_3$ (351.36):C, 61.53; H, 4.88; N, 19.93. Found: C, 61.65; H, 4.97; N, 19.34.

2.2.4.9. 3-(3-(2-(4-hydroxy-3-methoxyphenyl)-4-oxoquinazolin-3(4H)-yl)guanidino) propanoic acid (Compound 36). Yellowish brown solid, Yield, 66%, m.p., 215 °C, (IR)

v_{max} (KBr/cm^{-1}), 3367 (OH), 3127 (N–H str aliphatic), 3026 (CH$_2$), 1676 (C=O), 1447 (C=N), 1360 (C–N), 1140 (O-CH$_3$). ^{1}H NMR (DMSO, δ ppm): 11.46 (s, 1H, Ar-OH), 9.91 (s, 1H, COOH of CH$_2$COOH), 7.38–7.98 (m, 4H of, C$_{5,6,7,8}$-H of Quinazolinone ring), 7.98 (s, (br), 1H, –C=NH of Guanidine), 7.75–7.85 (d, J = 6 Hz, 2H, C$_2$-phenyl-C$_{2,6}$-H), 6.57–6.52 (s, 1H, C$_2$-phenyl-C$_3$-H), 3.67 (s, 3H of –OCH$_3$), 2.85–2.90 (s,1H,(br), NH), 2.59–2.60 (t, J = 7.4 Hz, 2H, CH$_2$), 1.37–1.42 (t, J = 7.4 Hz, 2H, CH$_2$). ESI-MS: 397.47 [M$^+$]. Anal. Calcd for C$_{18}$H$_{17}$N$_5$O$_3$ (397.38): C, 61.53; H, 4.88; N, 19.93. Found; C, 61.62; H, 4.93; N, 19.73.

2.2.4.10. 3-(3-(2-(4-(dimethylamino) phenyl)-4-oxoquinazolin-3(4H)yl) guanidino) propanoic acid (Compound 37). Brown solid, Yield, 73%, m.p., 154 °C, (IR) v_{max} (KBr/cm^{-1}), 3336 (OH), 3108 (N–H str), 2678.64 (CH$_2$), 1687 (C=O), 1432 (C=N), 1187.04 (C–N), 813.813. ^{1}H NMR (DMSO, δ ppm): 11.18 (s, (1H, COOH), 7.78–8.04 (m, 4H of, C$_{5,6,7,8}$-H of Quinazolinone ring), 2.98–2.94 (s, 6H, N(CH$_3$)$_2$, 7.78 (s, (br), 1H, –C=NH of Guanidine), 7.55–7.55 (d, J = 6 Hz, 2H, C$_2$-phenyl-C$_{2,6}$-H), 6.67–6.67 (d, J = 2.4 Hz, 2H, C$_2$-phenyl-C$_{3,5}$-H), 2.94–2.98 (s,1H, NH), 2.495–2.485 (t, J = 7.4 Hz, 2H, CH$_2$), 1.15–1.12 (t, J = 7.4 Hz, 2H, CH$_2$). ESI-MS: 394.58 [M$^+$]. Anal. Calcd for C$_{20}$H$_{22}$N$_6$O$_3$ (394.43):C, 60.90; H, 5.62; N, 21.31. Found: C, 60.56; H, 5.89; N, 21.29.

2.2.4.11. 3-(3-(2-(4-Methoxyphenyl)-4-oxoquinazolin 3(4H) yl)guanidino) propanoic acid (Compound 38). Yellowish green solid, Yield, 76%, m.p., 126 °C, (IR) v_{max} (KBr/cm^{-1}), 3379 (OH), 3165 (N–H str), 1685 (C=O), 1493 (C=N), 1395 (C–N), 1253 (O-CH$_3$). ^{1}H NMR (DMSO, δ ppm): 11.56 (s, (1H, COOH), 7.85–8.10 (m, 4H of, C$_{5,6,7,8}$-H of Quinazolinone ring), 7.78 (s,(br),1H, imine proton (C=NH)), 7.85–7.97 (d, J = 6 Hz, 2H, C$_2$-phenyl-C$_{2,6}$-H), 6.57–6.68 (d, J = 2.4 Hz, 2H, C$_2$-phenyl-C$_{3,5}$-H), 3.58–3.60 (s, 1H, -OCH$_3$), 2.84–2.08 (s,1H,(br), NH), 2.53–2.73 (t, J = 7.4 Hz, 2H, CH$_2$), 1.25–1.27 (t, J = 7.4 Hz, 2H). ESI-MS: 381.79 [M$^+$]. Anal. Calcd for C$_{19}$H$_{19}$N$_5$O$_4$ (381.39); C, 59.84; H, 5.02; N, 18.36. Found; C, 59.34; H, 5.46; N, 18.15.

2.3. STEP III: 6-Chloro-N-(2,2-dimethyl-1,2,3,8a-dihydroquinazolin-4(4aH)-ylidene)benzo[d] thiazol-2-amine (Compound-41)

Black solid, Yield, 79%, m.p.,121 °C, (IR) v_{max}(KBr/cm^{-1}), 3169 (N–H str), 1633 (C=N), 1519, 1399 (C–CH$_3$), 1333 (C–N), 810 (C–Cl), 1298 (C–S), ^{1}H NMR (δ-ppm): 7.87 (m, 2H, C$_{6-7}$-H of Dihydroquinazolinone ring), 7.74–7.75 (m, 2H, C$_{5,7}$-H of Benzothiazole ring), 7.55–7.57 (m, 1H, C$_4$-H of Benzothiazole ring), 6.58–6.63 (m, 2H, C$_{6-7}$-H of Dihydroquinazolinone ring), 2.48–2.49 (s, 6H, dimethyl). ESI-MS: 346.88 [M$^+$].

Anal. Calcd for C$_{17}$H$_{17}$ClN$_4$S (344.86); C, 59.21; H, 4.97; N, 16.25. Found: C, 59.65; H, 4.63; N, 16.35.

2.3.1. N-(2,2-Dimethyl-1,2,3,8a-dihydroquinazolin-4(4aH)-ylidene)-4-nitrobenzo[d] thiazol-2-amine (Compound-42)
Yellow solid, Yield, 80%, m.p., 116 °C, (IR) v_{max} (KBr/cm^{-1}), 3161 (N–H stretch), 1617 (C=N), 1573 (NO$_2$), 1509, 807 (CH=CH aromatic), 2845 (C–CH$_3$), 1282 (C–S), ^{1}H NMR (δ-ppm): 7.87 (s, (br), 1H, –NH of Dihydroquinazolinone ring), 7.74–7.75 (m, 3H, C$_{5,6,7}$-H of Benzothiazole ring),

6.58–6.64 (m, 4H, $C_{5,6,7,8}$-H of Dihydroquinazolinone ring), 2.48–2.49 (s, 6H, dimethyl). ESI-MS: 355.91 [M^+]. Anal. Calcd for $C_{17}H_{17}N_5O_2S$ (355.41); C, 57.45; H, 4.82; N, 19.70. Found: C, 57.33; H, 4.96; N, 19.40

2.3.2. 6-Bromo-N-(2,2-dimethyl-1,2,3,8a-dihydroquinazolin-4(4aH)-ylidene)benzo[d] thiazol-2-amine (Compound-43)

Black solid, Yield, 83%, m.p., 121 °C, (IR) v_{max} (KBr/cm^{-1}), 3172 (N–H str), 1632 (C=N), 1485, 809 (CH=CH aromatic), 1332 (C–N), 751 (C–Br), 1276 (C–S), ^{1}H NMR (δ-ppm): 7.85 (s, (br), 1H, –NH of Dihydroquinazolinone ring), 7.74–7.75 (m, 2H, C_{5-7}-H of Benzothiazole ring), 7.55–7.57 (m, 1H, C_4-H of Benzothiazole ring), 6.58–6.63 (m, 4H, $C_{5,6,7,8}$-H of Dihydroquinazolinone ring), 2.47–2.48 (s, 6H, dimethyl). ESI-MS: 391.33 [M^+] Anal. Calcd for $C_{17}H_{17}BrN_4S$ (389.31); C, 52.45; H, 4.40; N, 14.39. Found: C, 52.76; H, 4.62; N, 14.46.

2.3.3. N-(2,2-Dimethyl-1,2,3,8a-dihydroquinazolin-4(4aH)-ylidene)-6-nitrobenzo[d] thiazol-2-amine (Compound-44)

Pale yellow solid, Yield, 76%, m.p., 120 °C, (IR) v_{max} (KBr/cm^{-1}), 3168 (N–H), 1632 (C=N), 1560, 1482 (NO$_2$), 1399 (C–N), 2177 (C–S), ^{1}H NMR (δ-ppm): 7.87 (s, (br), 1H, –NH of Dihydroquinazolinone ring), 7.74–7.75 (m, 2H, $C_{5,7}$-H of Benzothiazole ring), 7.54–7.57 (m, 1H, C_4-H of Benzothiazole ring), 6.58–6.63 (m,2H, $C_{5,6-7,8}$-H of Dihydroquinazolinone ring), 2.47–2.49 (s, 6H, dimethyl). ESI-MS: 355.82 [M^+]. Anal. Calcd for $C_{17}H_{17}N_5O_2S$ (355.41); C, 57.45; H, 4.82; N, 19.70. Found: C, 57.24;, H, 4.63; N, 19.75.

2.3.4. 2-(2,2-Dimethyl-1,2,3,8a-dihydroquinazolin-4(4aH)ylideneamino)benzo[d] thiazole-6-carboxylic acid (Compound-45)

Light brown solid, Yield, 89%, m.p., 135 °C, (IR) v_{max} (KBr/cm^{-1}), 3197–3135 (OH), 3173 (N–H str), 1692 (C=O), 1632 (C=N), 1484, 854 (CH=CH aromatic), 1332 (C–N), 1278 (C–S), ^{1}H NMR (δ ppm): 11.15 (s, (br), Ar-COOH), 7.87 (s, (br), 1H, –NH of Dihydroquinazolinone ring), 7.74–7.75 (m, 2H, $C_{5,7}$-H of Benzothiazole ring), 7.56–7.59 (m, 1H, C_4-H of Benzothiazole ring), 6.57–6.63 (m, 4H, $C_{5,6-7,8}$-H of Dihydroquinazolinone ring), 2.46–2.44 (s, 6H, dimethyl). ESI-MS: 354.93 [M^+]. Anal. Calcd for $C_{18}H_{18}N_4O_2S$ (354.43); C, 61.00; H, 5.12; N, 15.81. Found: C, 61.27; H, 5.37; N, 15.56

2.3.5. 2-(2,2-dimethyl-1,2,3,8a-dihydroquinazolin-4(4aH)-ylideneamino)benzo[d] thiazole-6-sulfonamide (Compound-46)

Cream colour solid, Yield, 91%, m.p., 178 °C, (IR) v_{max} (KBr/cm^{-1}), 3424 (N–H str), 1639 (C=N), 1484, 1396 (C–N), 1278 (SO$_2$NH$_2$), 1276 (C–S), ^{1}H NMR (δ-ppm): 7.78 (s, (br), 1H, –NH of Dihydroquinazolinone ring), 7.73–7.74 (m, 2H, $C_{5,7}$-H of Benzothiazole ring), 7.52–7.57 (m, 1H, C_4-H of Benzothiazole ring), 6.58–6.63 (m, 2H, $C_{5,6,7,8}$-H of Dihydroquinazolinone ring), 2.846–2.821 (S, (br), 2H, –SO$_2$NH$_2$) 2.479–2.499 (s, 6H, dimethyl). ESI-MS: 390.71 [M^+]. Anal. Calcd for $C_{17}H_{19}N_5O_2S_2$ (389.5); C, 52.42; H, 4.92; N, 17.98. Found: C, 52.12; H, 4.99; N, 17.72.

2.3.6. 2-(2,2-dimethyl-1,2,3,8a-dihydroquinazolin-4(4aH)-ylideneamino)benzo[d] thiazol-6-ol (Compound-47)

Brown solid, Yield, 93%, m.p., 180 °C, (IR) v_{max} (KBr/cm^{-1}), 3855 (OH), 3225 (NH), 1640 (C=N), 1334 (C–N), 1271 (C–S),

^{1}H NMR (δ-ppm): 10.24 (s, 1H, Ar-OH), 7.89 (s, (br), 1H, –NH of Dihydroquinazolinone ring), 7.74–7.75 (m, 2H, C_{5-7}-H of Benzothiazole ring), 7.55–7.57 (m, 1H, C_4-H of Benzothiazole ring), 6.57–6.613 (m, 2H, $C_{5,6,7,8}$-H of Dihydroquinazolinone ring), 2.481–2.490 (s, 6H, dimethyl). ESI-MS: 326.98 [M^+].

Anal. Calcd for $C_{17}H_{18}N_4OS$ (326.42); C, 62.55; H, 5.56; N, 17.16. Found: C, 62.67; H, 5.87; N, 17.11.

2.3.7. 6-chloro-N-(2-methyl-2-phenyl-1,2,3,8a-dihydroquinazolin-4(4aH)-ylidene) benzo [d] thiazol-2-amine (Compound-48)

Black solid, Yield, 69%, m.p., 123 °C, (IR) v_{max} (KBr/cm^{-1}), 3405 (N–H str), 1666 (C=N), 1274 (C–S), 775 (C–Cl), ^{1}H NMR (δ-ppm): 7.48–7.45 (m, 2H, C_{5-7}-H of Benzothiazole ring), 7.29–7.12 (m, 1H, C_4-H of Benzothiazole ring), 6.76–6.74 (m, 4H, $C_{5,6,7,8}$-H of Dihydroquinazolinone ring), 6.54–6.58 (m, 5H, C_2-phenyl of Dihydroquinazolinone ring), 3.30 (s, (br), 1H, –NH of Dihydroquinazolinone ring), 2.31 (s, 3H, C_2-CH$_3$ of dihydro Quinazolinone). ESI-MS: 408.91 [M^+]. Anal. Calcd for $C_{22}H_{19}ClN_4S$ (406.93); C, 64.93; H, 4.71; N, 13.77. Found: C, 64.81; H, 4.86; N, 13.45.

2.3.8. 6-bromo-N-(2-methyl-2-phenyl-1,2,3,8a-dihydroquinazolin-4(4aH)-ylidene)benzo [d] thiazol-2-amine (Compound-49)

Dark brown solid, Yield, 74%, m.p., 148 °C, (IR) v_{max} (KBr/cm^{-1}), 3126 (N–H str), 1723, 1666 (C=N), 1272 (C–S), 745 (C–Br). ^{1}H NMR (δ-ppm): 7.49–7.47 (m, 2H, C_{5-7}-H of Benzothiazole ring), 7.29–7.13 (m, 1H, C_4-H of Benzothiazole ring), 6.81–6.74 (m, 4H, $C_{5,6,7,8}$-H of Dihydroquinazolinone ring), 6.61–6.48 (m, 5H, C_2-phenyl of Dihydroquinazolinone ring), 3.318 (s, (br), 1H, –NH of Dihydroquinazolinone ring), 2.34 (s, 3H, C_2-CH$_3$ of Dihydroquinazolinone). ESI-MS: 453.40 [M^+] Anal. Calcd for $C_{22}H_{19}BrN_4S$ (451.38); C, 58.54; H, 4.24; N, 12.41. Found: C, 58.39; H, 4.38; N, 12.60.

2.3.9. N-(2-methyl-2-phenyl-1,2,3,8a-dihydroquinazolin-4(4aH)-ylidene)-6-nitrobenzo[d] thiazol-2-amine (Compound-50)

Yellowish brown solid, Yield, 62%, m.p., 118 °C, (IR) v_{max} (KBr/cm^{-1}), 3127 (N–H str), 1599 (C=N), 1481, 839 (CH=CH aromatic), 1443 (NO$_2$), 1298 (C–N), 1276 (C–S), ^{1}H NMR (δ-ppm): 7.49–7.46 (m, 2H, C_{5-7}-H of Benzothiazole ring), 7.28–7.14 (m, 1H, C_4-H of Benzothiazole ring), 6.73–6.76 (m, 4H, $C_{5,6,7,8}$-H of Dihydroquinazolinone ring), 6.54–6.58 (m, 5H, C_2-phenyl of Dihydroquinazolinone ring), 3.31 (s, (br), 1H, –NH of Dihydroquinazolinone ring), 2.312 (s, 3H, C_2-CH$_3$ of Dihydroquinazolinone). ESI-MS: 418.50 [M^+]. Anal. Calcd for $C_{22}H_{19}N_5O_2S$ (417.48); C, 63.29; H, 4.59; N, 16.78. Found: C, 63.12; H, 4.76; N, 16.25.

2.3.9.1. N-(2-methyl-2-phenyl-1,2,3,8a-dihydroquinazolin-4(4aH)-ylidene)-4-nitrobenzo [d] thiazol-2-amine (Compound-51).

Yellow solid, Yield, 78%, m.p185 °C, (IR) v_{max} (KBr/cm^{-1}), 3133 (N–H str), 1670 (C=N), 1576, 1558 (NO$_2$), 1281 (C–S), ^{1}H NMR (δ ppm): 7.49–7.46 (m, 3H, $C_{5,6,7}$-H of Benzothiazole ring), 6.74–6.74 (m, 3H, $C_{5,6,8}$-H of Dihydroquinazolinone ring), 6.72–6.78 (m, 5H, C_2-phenyl of Dihydroquinazolinone ring), 6.54–6.58 (t, $J = 3$ Hz, 1H, C_7-H of Dihydroquinazolinone ring), 3.32 (s, (br), 1H, –NH

of Dihydroquinazolinone ring), 2.32 (s, 3H, C_2-CH_3 of dihydro Quinazolinone). ESI-MS: 417.91 [M^+]. Anal. Calcd for $C_{22}H_{19}N_5O_2S$ (417.48); C, 63.29; H, 4.59; N, 16.78. Found: C, 63.20; H, 4.91; N, 16.55.

2.3.9.2.   2-(2-methyl-2-phenyl-1,2,3,8a-dihydroquinazolin-4(4aH)ylideneamino)benzo [d]thiazole-6-sulfonamide (Compound-52). Cream colour solid, Yield, 69%, m.p., 125 °C, (IR) v_{max} (KBr/cm^{-1}), 3121 (N–H str), 1645 (C=N), 1290 (SO_2NH_2), 1274 (C–S), ^{1}H NMR (δ-ppm): 7.49–7.46 (m, 2H, C_{5-7}-H of Benzothiazole ring), 7.29 –7.13 (m, 1H, C_4-H of Benzothiazole ring), 6.76–6.74 (m, 3H, $C_{5,6,8}$-H of Dihydroquinazolinone ring), 6.72–6.78 (t, $J = 3$ Hz, 1H, C_7-H of Dihydroquinazolinone ring), 6.54–6.58 (m, 5H, C_2-phenyl of Dihydroquinazolinone ring), 3.30 (s, (br), 1H, –NH of Dihydroquinazolinone ring), 2.34 (s, 3H, C_2-CH_3 of dihydro Quinazolinone), 2.86–2.82 (s (br), 2H, 2H, –SO_2NH_2). ESI-MS: 451.58 [M^+]. Anal. Calcd for $C_{22}H_{21}N_5O_2S_2$ (451.56); C, 58.52; H, 4.69; N, 15.51. Found: C, 58.29; H, 6.21; N, 15.82.

2.3.9.3.   2-(2-methyl-2-phenyl-1,2,3,8a-dihydroquinazolin-4(4aH)-ylideneamino)benzo[d] thiazole-6-carboxylic acid (Compound-53). Brown solid, Yield, 68%, m.p., 123 °C, (IR) v_{max} (KBr/cm^{-1}), 3414 (N–H str), 3367 (OH), 1628 (C=O), 1491 (C=N), 1276 (C–S). ^{1}H NMR (δ-ppm): 11.71 (s, (br), Ar-COOH), 7.49–7.46 (m, 2H, $C_{5,7}$-H of Benzothiazole ring), 7.29 –7.13 (m, 1H, C_4-H of Benzothiazole ring), 6.76–6.74 (m, 3H, $C_{5-6,8}$-H of Dihydroquinazolinone ring), 6.54–6.58 (t, $J = 3$ Hz, 1H, C_7-H of Dihydroquinazolinone ring), 6.32–6.31(m, 5H, C_2-phenyl of Dihydroquinazolinone ring), 3.30 (s, (br), 1H, –NH of Dihydroquinazolinone ring), 2.38 (s, 3H, C_2-CH_3 of dihydro Quinazolinone). ESI-MS: 416.92 [M^+]. Anal. Calcd for $C_{23}H_{20}N_4O_2S$ (416.5); C, 66.33; H, 4.84; N, 13.45. Found: C, 66.01; H, 4.89; N, 13.40.

2.3.9.4.   2-(2-methyl-2-phenyl-1,2,3,8a-dihydroquinazolin-4(4aH)-ylideneamino)benzo [d]thiazol-6-ol (compound 54). Black solid, Yield, 89%, m.p., 174 °C, (IR) v_{max} (KBr/cm^{-1}), 3408 (NH str), 3132 (OH), 1657 (C=N), 1214 (C–S), ^{1}H NMR (δ-ppm): 9.48 (s, (br), Ar -OH), 7.49–7.46 (m, 2H, $C_{5,7}$-H of Benzothiazole ring), 7.29–7.13 (m, 1H, C_4-H of Benzothiazole ring), 6.76–6.74 (m, 3H, $C_{5,6,8}$-H of Dihydroquinazolinone ring), 6.54–6.58 (t, $J = 3$ Hz, 1H, C_7-H of Dihydroquinazolinone ring), 3.30 (s, (br), 1H, NH of Dihydroquinazolinone ring), 6.34–6.35 (m, 5H, C_2-phenyl of Dihydroquinazolinone ring), 2.49 (s, 3H, C_2-CH_3 of dihydro Quinazolinone). ESI-MS: 388.96 [M^+]. Anal. Calcd for $C_{22}H_{20}N_4OS$ (388.49); C, 68.02; H, 5.19; N, 14.42. Found: C, 68.99; H, 5.27; N, 14.30.

3. Pharmacological evaluation

3.1. In vitro cytotoxicity

3.1.1. MTT assay method (Mosmann and Tim, 1983)
3.1.1.1. Procedure. The human cervical cancer cell line (HeLa), was obtained from National Centre for Cell Science (NCCS), Pune. The HeLa was grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were seeded into 96-well plates in 100 μl of respective medium containing 10% FBS, at plating density of 10,000 cells/well

and incubated at 37 °C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of compounds **13–18** and **34–38**. The compounds were solubilised in Dimethylsulfoxide and diluted in respective serum free medium. The cells were exposed to various concentrations of the test drugs and incubated at 37 °C, 5% CO_2, 95% air and 100% relative humidity for 48 h. Triplicate was maintained and the medium containing without drugs were served as a control. After 48 h, 15 μl of MTT (5 mg/mL) in phosphate buffered saline (PBS) was added to each well and incubated at 37 °C for 4 h. The medium with MTT was then flicked off and the formed formazan crystals were solubilised in 100 μl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The IC_{50} value resulting from 50% inhibition of cell growth was calculated graphically as a comparison with the control doxorubicin.
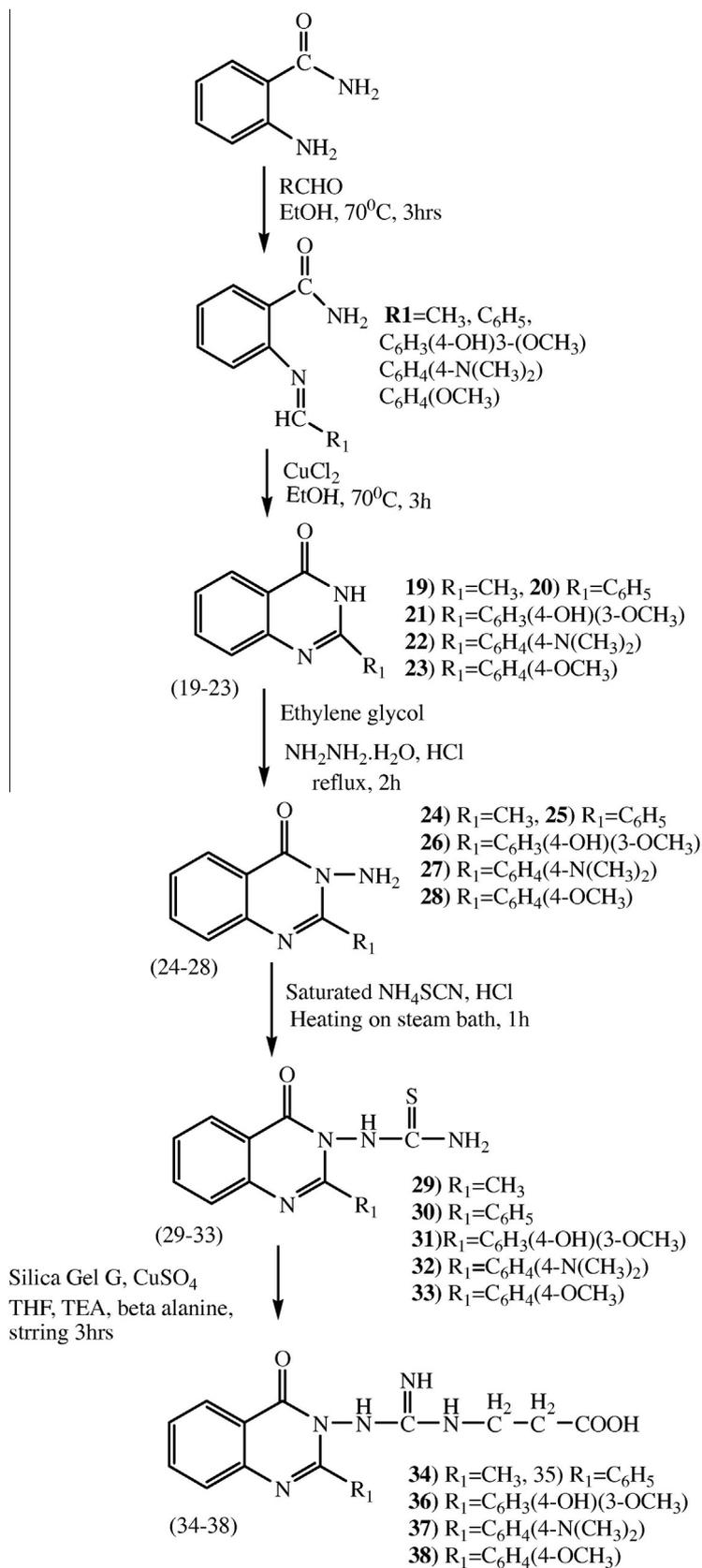
3.2. Antimicrobial evaluation

3.2.1. In vitro antimicrobial activity (Sohail Saeed et al., 2010)
The micro dilution susceptibility test in Muller-Hinton Broth and Sabouraud Liquid Medium was used for the determination of antibacterial and antifungal activity, respectively. Standard sterilized filter paper disks (5 mm diameter) impregnated with a solution of the test compound in DMF (1 mg/mL) was placed on an agar plate seeded with the appropriate test organism showed in Tables 2 and 3 in triplicates. Agar-diffusion method was used for the determination of the preliminary antibacterial and antifungal activity. Ciprofloxacin (cp) and Clotrimazole were used as reference drugs antibacterial and antifungal agents, respectively. DMSO alone was used as control at the same above-mentioned concentration. The plates were incubated at 37 °C for 24 h for bacteria and for 48 days for fungi. Compounds that showed significant growth inhibition zones (> 09 mm) using the twofold serial dilution technique, were further evaluated for their minimal inhibitory concentrations (MICs). The plates were incubated at 37 °C for 24 h. Compounds that showed significant growth inhibition zones (14 mm) using the twofold serial dilution technique, were further evaluated for their minimal inhibitory concentrations (MICs).

4. Results and discussion

4.1. Chemistry

Series 1 compounds 3-(3-(6-substitutedbenzo[d]thiazol-2-yl) guanidino) propanoic derivatives (**13–18**) were synthesized according to earlier reported method in the literature, shown in scheme 1. Compounds **13–18** prepared in 3 steps, the first step involved reaction of various substituted aromatic amines, ammoniumthiocyanate and bromine in glacial acetic acid under cold condition at 0–10 °C afforded 6-substituted benzo[d]thiazol-2-amine derivatives (**1–6**) which further treated with saturated ammoniumthiocynate and Conc. Hydrochloric acid (second step) to yield the corresponding thioureas (**7–12**). When the compounds **7–12**, in third step treated with β-alanine dissolved in tetrahydro furan containing silica gel G, $CuSO_4$. $5H_2O$ and triethyl amine (accelerates desulphuration) afforded the guanidinopropanoic acid derivatives **13–18** (Scheme 1) as a final compound of series one.

R1=CH$_3$, C$_6$H$_5$,
C$_6$H$_3$(4-OH)3-(OCH$_3$)
C$_6$H$_4$(4-N(CH$_3$)$_2$)
C$_6$H$_4$(OCH$_3$)

19) R$_1$=CH$_3$, **20)** R$_1$=C$_6$H$_5$
21) R$_1$=C$_6$H$_3$(4-OH)(3-OCH$_3$)
22) R$_1$=C$_6$H$_4$(4-N(CH$_3$)$_2$)
23) R$_1$=C$_6$H$_4$(4-OCH$_3$)

24) R$_1$=CH$_3$, **25)** R$_1$=C$_6$H$_5$
26) R$_1$=C$_6$H$_3$(4-OH)(3-OCH$_3$)
27) R$_1$=C$_6$H$_4$(4-N(CH$_3$)$_2$)
28) R$_1$=C$_6$H$_4$(4-OCH$_3$)

29) R$_1$=CH$_3$
30) R$_1$=C$_6$H$_5$
31) R$_1$=C$_6$H$_3$(4-OH)(3-OCH$_3$)
32) R$_1$=C$_6$H$_4$(4-N(CH$_3$)$_2$)
33) R$_1$=C$_6$H$_4$(4-OCH$_3$)

34) R$_1$=CH$_3$, **35)** R$_1$=C$_6$H$_5$
36) R$_1$=C$_6$H$_3$(4-OH)(3-OCH$_3$)
37) R$_1$=C$_6$H$_4$(4-N(CH$_3$)$_2$)
38) R$_1$=C$_6$H$_4$(4-OCH$_3$)

**Scheme 2** Synthesis of QGP derivatives (**34–38**) from anthranilamide.

Series 2 Compounds 3-(3-(2-substituted-4-oxoquinazolin-3(4H)-yl) guanidino) propanoic acid derivatives (**34–38**) were prepared in 4 steps as shown in scheme 2. Initially, intermediate compounds 2-substituted quinazolin-4(3H)-one derivative

(19–23) were prepared by refluxing with substituted aldehyde in ethanol for 3 h at 70 °C, when the formed non isolable intermediate Schiff bases were refluxed with $CuCl_2$ in ethanol for 3 h at 70 °C produced the compounds 19–23. In compounds 19–23, amino group was introduced at N-3 of Quinazolinone by treating with hydrazine hydrate, ethylene glycol in acidic medium, refluxing the reaction mixture for 3 h to afford 3-amino-2-substituted quinazolin-4(3H)-one derivative (24–28). Compounds 19–23 do not form hydrazone when they are treated with hydrazine hydrate since the Quinazolinones 19–23 are cyclic amides (–NH–C=O), (Scheme 2). The method adopted for the prearation of Quinazolinone thioureas 29–33 and Quinazolinone guanidino propanoic acid derivatives 34–38

(scheme 2) were similar to the preparation 7–12 and 13–18 respectively.

Series 3 compounds Schiff bases (41–54) were prepared from substituted benzo[d]thiazol-2-amines (1–7) and 2-methyl-2-substituted-2,3-dihydroquinazolin-4(1H)-ones (39–40), shown in scheme 3. Intermediate compounds 39–40 were synthesised by treating anthranilamide with acetone and acetophenone (refluxed for 90 minutes) in presence of p-toluenesulfonic acid monohydrate as cyclising agent, Dihyhydroquinazolinones (39–40) formed (via non isolable imine formation and cyclization). Finally, Schiff base derivatives (41–54) were prepared by reacting the 2-substituted-2,3-dihydroquinazolin-4(1H)-ones (39–40) and substituted benzo[d]thiazol-2-amine(1–7)
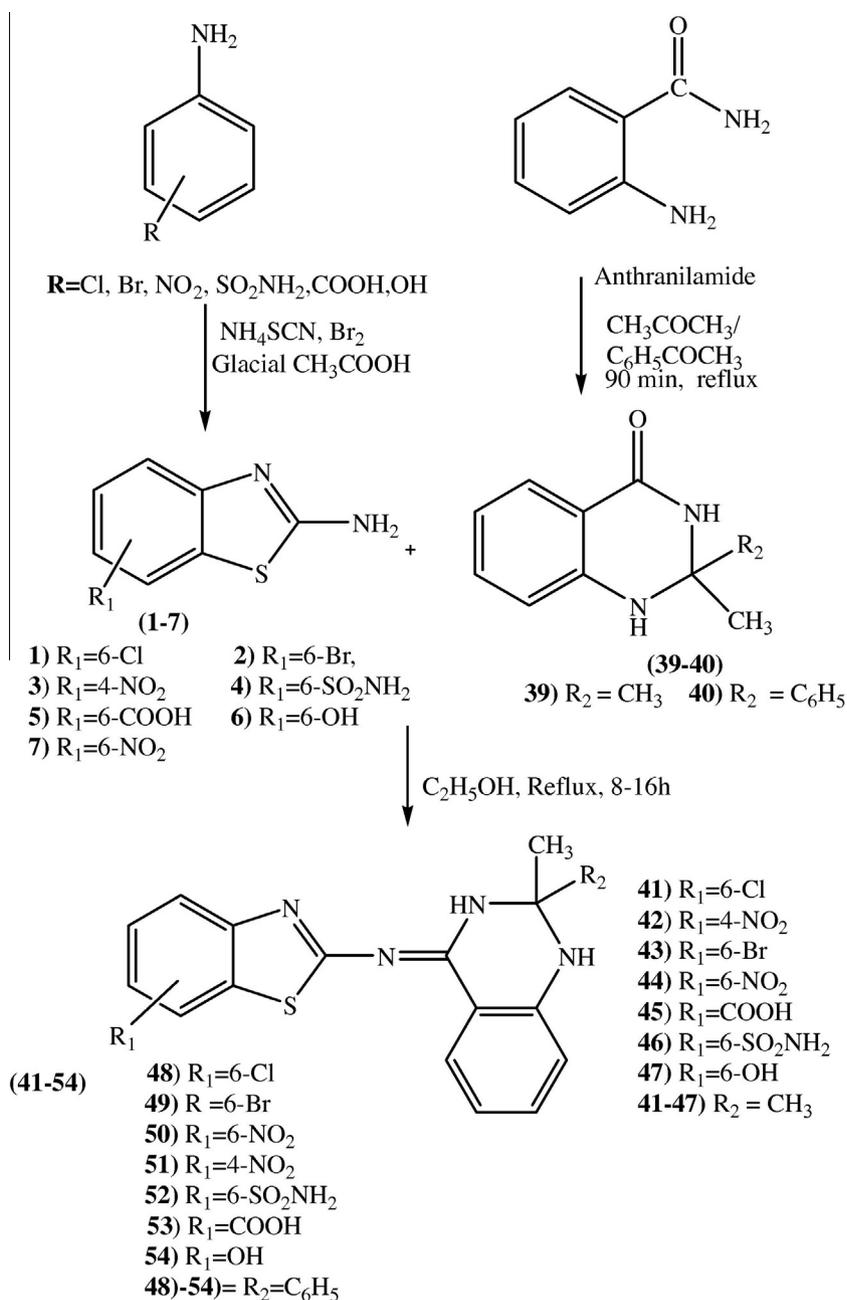
Scheme 3 Synthesis of Schiff base derivatives (41–54) from Benzothiazole-2-amines (1–7) and 2,3-dihydroquinazolin-4(1H)-ones (39–40).

Table 1 In vitro cytotoxicity of compounds **13–18**, **13–38**, **43–45**, **48–53** (20 compounds) on HeLa cell line.

S. no.	Compound no.	In vitro cytotoxicity (IC_{50} μm)	S. no.	Compound no.	In vitro cytotoxicity (IC_{50} μm)
(1)	**13**	67	(12)	**43**	865
(2)	**14**	64	(13)	**44**	743
(3)	**15**	37	(14)	**45**	867
(4)	**16**	32	(15)	**48**	750
(5)	**17**	59	(16)	**49**	782
(6)	**18**	2	(17)	**50**	875
(7)	**34**	527	(18)	**51**	855
(8)	**35**	443	(19)	**52**	795
(9)	**36**	550	(20)	**53**	810
(10)	**37**	524			
(11)	**38**	534			

In vitro cytotoxicity IC_{50} of doxorubicin is 1.8 μm.

(Scheme 1, first step compounds) then refluxed for 10–16 h, the amino group of compounds **1–7** reacted with keto group of compounds **39–40** in ethanol to afford the Schiff base derivatives (**41–54**) (scheme 3).

Infrared (IR) spectroscopy of the compounds **7–12** and **29–33**, showed characteristic bands at 1051–1196 by the thiocarbonyl moiety C=S. Carboxylic acid group of the compounds **13–18**, **34–38** showed characteristic broad bands at the range of about 3379–3216 cm^{-1} by the -OH stretching of the carboxylic acid, bands at about 1692–1679 cm^{-1} by the carboxylic C=O stretching. C=N absorption bands appeared in the region of at 1493–1430 cm^{-1}.

Compounds **41–54** showed strong absorption bands in the region of 1390–1210 cm^{-1} by C=N, while the absorption bands for –NH_2 and C=O were absent in compounds **41–54**. The ^{1}H NMR spectrum of the compounds **13–18** and **34–38** exhibited three broad singlets, one at about 9.575–11.187 ppm by the carboxylic acid proton, second at 7.585–7.788 by imine proton and third at 2.985–3.376 by –NH proton. Adjacent two methylene protons of the compounds **13–18** shown two triplets at 3.137–3.315 (J = 7.4 Hz) and 2.560–2.481 (J = 7.4 Hz). The compounds **34–38** were also shown two similar triplets at 2.495–2.485 (J = 7 Hz) and 1.156–1.120 (J = 7). Presence of two triplets with the same coupling constant in the compounds **13–18** and **34–38** confirmed presence of two adjacent methylene protons. In addition, methyl proton of compound **34** exhibited singlet at 1.452, phenolic OH proton and methoxy proton of compound **36** exhibited singlets at 6.387 and 3.946 respectively. Methoxy protons of compound **38** exhibited singlet at 3.489–3.498. Compound **41–54** were shown singlet at 7.859–7.888 ppm, which were assigned to third position –NH of dihydro Quinazolinone. Sulphanilamide group of compounds **17**, **46** and **52** exhibited broad singlet at 2.121- 2.864 while carboxylic acid protons attached to Benzothiazole ring of compounds **15**, **45** and **53** exhibited one broad singlet at 10.955–11.718. The phenolic -OH group of compounds **45** and **54** were also shown broad singlet at about 9.486–11.243 ppm.

In mass spectrum, thiourea intermediates showed base peak due to cleavage of $[C_7H_8N_2]^{+\bullet}$ at m/z = 138 and another intense peak at about m/z 108 due to deamination from base peak with relative intensity of 60%. The obtained molecular ion m/z values of the final guanidinopropanoic acid derivatives were consistent with the molecular weight of compounds and the base peak was formed after the successive removal of prop-

anoic acid moiety with one intense peak due to desulphuration from base peak with relative intensity of 62%.

4.2. In vitro cytotoxicity

The synthesized BTGP derivatives **13–18**, QGP **34–38** and Schiff base derivatives **43–45**, **48–53** (20 compounds) were evaluated for antiproliferative activities against human cancer cell line HeLa. The results were summarized in Table 1. All the compounds showed antiproliferative activities against the Hela cell line with the half maximal inhibitory concentration (IC_{50}) values of 2–550 μM (except Schiff base derivatives IC_{50} values of 743–875 μm) and compound **18** showed the most potent activity with a mean IC_{50} of 2 μM which was close to the value of the positive control, doxorubicin. Structure–activity relationship (SAR) analysis indicated that Benzothiazole bearing guanidinopropanoic acid **13–18** showed stronger activities than the Quinazolinone bearing guanidinopropanoic acid **34–38**, with all the IC_{50} values below 100 μM. In the SAR further study of **13–18**, the –OH, –COOH, and –SO_2NH_2 substituted at sixth position of Benzothiazole ring played an important role for the activity. Compounds **18** with –OH substitution at sixth showed the most potent activity against the HeLa cell lines, with the mean IC_{50} of 2 μM. From these results it is evident that hydrogen bonding substituent is necessary for the potent activity at the sixth position of Benzothiazole ring, further size of these groups also play important role in the activity, activity order is OH > COOH > SO_2NH_2. From the results it is clear that smaller hydrogen bonding group (donor Hydrogen containing) at sixth position of Benzothiazole ring necessary for the potent activity.

4.3. In vitro antibacterial activity

Twenty-three compounds (**7–17, 34–38, 44–46, 49–52** and **54**) were evaluated for in vitro antimicrobial activity against 11 pathogenic bacteria and 10 pathogenic fungus. The results were recorded for each tested compound as the average diameter of inhibition zones (IZ) of bacterial growth around the disks in millimetre. The minimum inhibitory concentration (MIC) measurement was determined for all the compounds using the twofold serial dilution method. The MIC (μg/mL) values were recorded. Most of the tested exhibited differential activity against tested bacteria and fungi.

Table 2 Antibacterial activity of the compounds **7–17**, **34–38**, **44–46**, **49–52** and **54**, MIC (μg/ml).

Organism	Compounds MIC (μg/ml)																						
	7	8	10	11	12	13	14	15	16	17	34	35	36	37	38	44	45	46	49	51	52	54	Std[a]
Micrococcus luteus	25	12.5	6.25	12.5	6.25	12.5	12.5	25	25	12.5	25	12.5	6.25	50	12.5	12.5	12.5	25	12.5	12.5	12.5	25	<0.019
Escherichia coli	50	12.5	12.5	12.5	25	50	25	6.25	50	12.5	25	25	50	50	12.5	25	–	12.5	12.5	12.5	12.5	25	1.24
Pseudomonas aeruginosa	12.5	25	12.5	6.25	25	25	3.12	25	25	25	12.5	25	25	25	12.5	12.5	–	12.5	6.25	25	6.25	12.5	19.5
Bacillus subtilis	25	6.25	25	6.25	25	12.5	12.5	6.25	25	50	12.5	12.5	25	12.5	6.25	12.5	–	–	6.25	25	6.25	25	0.5
Klebsiella pneumonia	12.5	12.5	25	6.25	12.5	12.5	25	6.25	25	12.5	25	25	12.5	12.5	6.25	12.5	–	–	25	6.25	12.5	6.25	<0.019
Salmonella paratyphi	25	50	12.5	6.25	12.5	25	25	12.5	12.5	25	25	6.25	6.25	12.5	6.25	12.5	–	–	3.125	12.5	12.5	12.5	43.4
Vibrio cholera	25	25	6.25	25	6.25	25	12.5	25	6.25	25	12.5	12.5	25	12.5	25	25	–	–	6.25	25	25	25	256
Bacillus lentus	12.5	12.5	12.5	12.5	12.5	25	12.5	6.25	50	6.25	50	25	50	25	12.5	6.25	12.5	25	6.25	50	25	12.5	0.781
Rhodosporium rubrum	50	12.5	12.5	12.5	25	12.5	12.5	50	50	12.5	6.25	50	50	12.5	6.25	6.25	–	25	12.5	12.5	25	12.5	32
Staphylococcus albus	12.5	6.25	35	25	12.5	25	25	50	6.25	12.5	6.25	50	6.25	12.5	6.25	12.5	6.25	50	25	12.5	12.5	12.5	0.5
Staphylococcus aureus	6.25	25	25	25	12.5	12.5	25	50	25	12.5	6.25	50	12.5	25	12.5	12.5	–	–	6.25	12.5	12.5	–	25

^a Ciprofloxacin, (–) activity not found.

Table 3 Antifungal activity of the compounds **7–17**, **34–38**, **44–46**, **49–52** and **54**, MIC (μg/ml).

Organism	Compounds MIC (μg/ml)																						
	7	8	10	11	12	13	14	15	16	17	34	35	36	37	38	44	45	46	49	51	52	54	Std[a]
Monascus purpureus	25	25	12.5	50	12.5	12.5	12.5	12.5	12.5	25	3.12	12.5	12.5	50	12.5	12.5	–	–	6.25	12.5	6.25	12.5	30
Aspergillus niger	–	–	–	–	–	–	–	–	–	–	12.5	–	25	25	–	6.25	12.5	12.5	12.5	12.5	12.5	25	0.24–0.34
Candida albicans	12.5	12.5	50	–	6.25	12.5	6.25	12.5	25	25	25	12.5	6.25	12.5	25	–	–	–	6.25	–	–	12.5	0.25–2.10
Aspergillus fumigatus	25	–	–	–	–	25	25	25	12.5	12.5	12.5	25	12.5	25	25	12.5	25	12.5	6.25	6.25	12.5	25	12–5–25
Aspergillus parasiticus	–	–	–	–	–	–	–	–	–	–	12.5	–	–	25	25	12.5	6.25	12.5	12.5	6.25	12.5	25	>8
Monascus ruber	–	–	–	–	–	–	12.5	–	–	12.5	25	12.5	25	12.5	12.5	25	12.5	12.5	–	25	12.5	12.5	10
Streptomyces griseus	–	–	25	6.25	25	25	12.5	25	12.5	12.5	25	12.5	–	12.5	12.5	6.25	–	12.5	3.125	6.25	–	12.5	0.05
Microsporum gypseum	–	–	–	25	12.5	25	25	12.5	25	25	12.5	12.5	25	6.25	25	–	–	–	–	–	–	–	19.53
Trichophyton rubrum	–	–	–	25	12.5	25	25	12.5	12.5	12.5	12.5	25	25	12.5	12.5	–	–	–	–	–	–	–	0.4
Trichophyton mentagrophytes	–	–	–	50	25	50	12.5	12.5	12.5	25	–	3.12	12.5	25	6.25	12.5	25	6.25	25	12.5	12.5	12.5	0.0075

^a Clotrimazole, (–) activity not found.

From the results of antibacterial activity it is evident that 6 compounds (**11**, **14**, **34**, **38**, **44** and **46**), 11 compounds (**7**, **10**, **11**, **12**, **13**, **14**, **15**, **11**, **35**, **36**, **38 and 49**), 4 compounds (**10**, **12**, **17** and **49**), 2 compounds (**15** and **17**), 3 compounds (**34**, **38**, **44**), 5 compounds (**8**, **16**, **34**, **36**, **38**) and 3 compounds (**34**, **38** and **49**) were more active against *Pseudomonas aeruginosa*, *Salmonella paratyphyi*, *Vibrio cholera*, *Bacillus lentus*, *Rhodosporium rubrum*, *Staphylococcus albus* and *Staphylococcus aureus*, respectively than the reference drug ciprofloxacin and compounds **11**, **14**, **34**, **38** and **49** showed the broad spectrum of activity (Table 2).

The results of antifungal activity indicated that, Compounds **34**, **51**, **45** and **37** were more potent against *Monascus purpureus*, *Aspergillus fumigates*, *Aspergillus parasiticus* and *Microsporum gypseum* than the reference drug Clotrimazole (Table 3).

Collectively, compounds **11**, **14**, **38** and **49** are considered to be the most active antimicrobial members identified in this study with a broad spectrum of antibacterial activity against both Gram positive and Gram negative bacteria. Particularly, compounds **14** was superior over the ciprofloxacin against *Pseudomonas aeruginosa* (**14**, MIC 3.125 and Cp MIC 19.5 µg/mL) and compound 49 highly active against *Salmonella paratyphyi* (**49**, MIC 3.125 and Cp MIC 43.4 µg/mL). Compound **34** was more active against *Monascus purpureus* (**34**, MIC 3.125 and Clotrimazole MIC 30 µg/mL).

5. Conclusion

Compound **18** exhibited potent cytotoxicity against the human cervical cancer cell line (**HeLa**). Compounds **13–17** also showed the promising cytotoxicity. Compounds **11**, **14**, **38** and **49** are considered to be the most active antimicrobial members identified in this study with a broad spectrum of antibacterial activity against both Gram positive and Gram negative bacteria. Among the three series tested for cytotoxicity and antimicrobial activity, 3-(3-(6-substitutedbenzo[*d*]thiazol-2-yl) guanidino) propanoic derivatives (**13–18**) exhibited the promising cytotoxicity and antimicrobial activity.

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