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Synthesis, antibacterial and free radical scavenging activity of some newer *N*-((10-nitro-1*H*-indolo [1, 2-c]quinazolin-12-yl)methylene)benzenamines

Original Paper

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Abstract Present research is oriented on the synthesis of some novel 12-(*N*-arylmethaniminyl)indolo[1,2-*c*]quinazoline analogs (4b1-4b11) and their characterization by ^{1}H NMR, ^{13}C NMR, FTIR and mass spectrophotometry. Their free radical scavenging activity and antibacterial potential were also evaluated. Many derivatives have shown a marked free radical scavenging capacity in all the concentrations but specifically compounds 4b7, 4b8 and 4b11 have shown good antioxidant potential with an IC_{50} value of 25.18 µmol/L, 28.09 µmol/L & 44.22 µmol/L, respectively (DPPH method) and 39.46 µmol/L, 44.47 µmol/L & 35.61 µmol/L, respectively (H_2O_2 method). The antibacterial evaluation was carried out against B. *subtilis* and *E. coli* by agar well diffusion method and it revealed that all the compounds in the series were having marked antibacterial activity but compounds 4b9 and 4b11 have shown best antibacterial potential. Then, it was concluded that the derivatives which were containing substituted anilines (4-Nitro, 4-Fluoro, 4-Bromo & 4-Chloro-2-nitro) on the carbon attached on the 12th position of indoloquinazoline moiety were having marked potential as an antibacterial and free radical scavenger.

Keywords *free radical scavenger – antibacterial – DPPH – indoloquinazoline – IC_{50}*

INTRODUCTION

Indole is considered as the chief constituent of the alkaloids present in plants (Somei & Yamada, 2003; Gupta et al., 2007). It contains versatile biological activities such as antiviral potential (Cihan-Üstündağ et al., 2019; Xu & Lv, 2009; Ran et al., 2010; Ghosh et al., 2008; Williams et al., 2004), anticancer activity (Gaikwad et al., 2019; El Sayed et al., 2018; Sreenivasulu et al., 2019; Andreani et al., 2008; Slater et al., 2001), antimicrobial study (Chodvadiya et al., 2019; Shirinzadeh et al., 2018; Mathada & Mathada, 2009; Gurkok et al., 2009), antimycobacterial activity (Cihan-Üstündağ et al.; Karah et al., 2007), free radical scavenging activity and antifungal potential (Demurtas et al., 2019; Dekker et al., 1975). The most important pharmacological activities of drugs containing indole moiety are antimicrobial activity and free radical scavenging activity. There are several indole containing structures which are reported by researchers as a good antimicrobial and antifungal agents. Among them, ethyl-3-Indolylacrylate, 5-Bromo-3-(2-Cyanovinyl) indole and 3-(2-Nitrovinyl)indole were those compounds that were active against microbes (Whitehead & Whitesitt, 1974). Haloindoles were found to be effective in between the concentration of 10-100 µg/ml. In one research study, 3-Acyl-4,7-dihydroxy indoles were reported to be active highly potent against *Escherichia coli* and *Streptococcus pyogenes* (Malesani et al., 1975). 1-Morpholino-3-Carbethoxy-5-hydroxy- 2-methylindole was reported as the most active agent against *Escherichia coli* and *Bacillus cirroflagellosus* (Donawade & Gadaginamath, 2005). 1-(4-Phenyl) and (1-Naphthyl-4*H*-1,2,4-triazole-5-thion-3-yl)indoles were found to be the most potent antimicrobial and antifungal agents (Tsotinis et al., 1997). Some thiosemicarbazide derivatives having indole nucleus and their cyclic 1,2,4-triazole and 1,3,4-thiadiazole analogs were found to have selective action against many microbes and fungi (Varvaresou et al., 2000). Many analogs containing indole moiety fused with various heterocycles were found to be potent antimicrobial agents. Among the reported compounds, 4*H*-pyrano[2,3-*f*] indole, benzotetrahydrocyclohept[1,2-*b*]indole, and 1-triazolylethylbenz[*g*]indole derivatives were reported as some of the most potent classes of antimicrobial compounds (Macchia et al., 1996; Gadaginamath & Kavali 1999; Bhovi & Gadaginamath, 2005). If we talk about another activity, indole

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has a good candidature for free radical scavenging activity. Literature tells about the involvement of free radicals in various diseases and pathophysiological events including inflammation, cancer, myocardial infarction, arthritis and neurodegenerative disorders (Bast et al., 1991; Bulkley, 1993; Halliwell & Gutteridge, 1998). This involvement of action of free radicals on crucial systems is multiple complex aspects of their involvement in a series of inflammatory disorders (Sreejayan & Rao, 1996) and those disorders which are related to nutrition (McCord, 1993; Cross et al., 1994). Various anti-inflammatory agents are there which act by the free oxygen radicals scavenging action (Santrucek & Krepelka, 1988; Santrunek & Krepelka, 1988). A huge amount of free radicals produced during the inflammation process and out of those wide variety of free radicals there are reactive oxygen species (ROS) such as, superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), singlet oxygen and peroxyl radicals, as well as reactive nitrogen species (RNS), like nitric oxide (NO) and peroxynitrite anion ($ONOO^-$). Actually, ROS and RNS are generated by the endothelial cells, Kupffer cells, neutrophils and macrophages in defense mechanism in the response of infections caused by foreign pathogenic invaders (Nikolic & Breemen, 2001; Vapaatalo, 1986; Halliwell et al., 1988; Mouithys-Mickalad et al., 2000). On the other hand, ROS are also produced by the COX enzyme processes in response to infection and mitochondria are also considered as a source of ROS (Turrens, 2003). These ROS and RNS are involved in a wide variety of diseases and disorders such as cancer, rheumatoid arthritis, and atherosclerosis, Alzheimer and Parkinson's disease, among others (Dedon & Tannenbaum, 2004). In the efforts of finding some more potential of indole nucleus, synthesis of some newer *N*-((10-nitro-1*H*-indolo[1,2-*c*]quinazolin-12-yl)methylene)benzenamine derivatives (4b1-4b11) was done and their antibacterial and antioxidant potential was reported.

MATERIALS AND METHODS

Chemistry

The purity of all the synthesized derivatives was determined by thin-layer chromatography on pre-coated silica gel aluminum sheets (Type 60 GF254, Merck) and detection of the spots was done by iodine vapors and UV-Lamp. The melting point was determined by the melting point apparatus and all the melting points were uncorrected. The FTIR spectra were recorded on 470- Shimadzu FTIR spectrophotometer and wavenumber values were expressed in cm^{-1}. NMR spectra were recorded in DMSO-*d*6 as a solvent at 300 MHz (^{1}H NMR) and 75 MHz (^{13}C NMR) on a BRUKER ADVANCE-300 spectrometer. Tetramethylsilane (TMS) was used as an internal standard. Chemical shifts (δ) are shown in parts per million (ppm). Spin multiplicities are given as s (singlet), d (doublet), dd (double doublet), t (triplet) and m (multiplet). Mass spectra were recorded on Shimadzu 2010A LC-MS spectrometer. Elemental analysis was carried out on Elemental Vario EL III Carlo Erba 1108 and the values were within ±0.04% of the theoretical values.

Experimental procedure for the synthesis of 2-(5-nitro-1H-indol-2-yl)benzenamine ***(1b)***

The mixture of 4-Nitrophenylhydrazine (15 g, 98.03 mmol) and 2-Aminoacetophenone (13.23 g, 98.03 mmol) was refluxed in acetic acid/ethanol mixture for 3 h to give 4-Nitro substituted phenyl hydrazone of 2-Aminoacetophenone. Methanesulfonic acid (220 ml) was heated to 80 °C and phosphorus pentoxide (30 g) was added very slowly with stirring till its complete dissolution (mixture A). The 4-Nitro substituted phenylhydrazone of 2-Aminoacetophenone (20 g) was added slowly to mixture A**.** The temperature of the reaction was maintained between 80 and 100 °C. Then the solution was heated further at 80 °C for half an hour. Then the reaction mixture cooled to room temperature and then it was poured over crushed ice already containing sodium hydroxide. Then the solid precipitate was filtered, it was washed thoroughly with water, and it was dried to give the crude product which was recrystallized from ethanol.
(Yield: 16.20 g, 90 %, M.P. 140-142 °C)

Experimental procedure for the synthesis of 10-nitro-1H-indolo[1,2-c]quinazoline ***(2b)***

Compound **1b**, 2-(5-nitro-1*H*-indol-2-yl)benzenamine (16 g, 63.24 mmol) was mixed with formic acid (88%, 118 ml) and the solution was heated at 90 °C for 1 h. The reaction mixture was cooled to room temperature and then poured into crushed ice. The solid precipitate was filtered, it was washed thoroughly with water, and it was dried to give the crude product which was recrystallized from ethyl alcohol.
(Yield: 12.47 g, 75 %, M.P. 200-202 °C)

Procedure for the synthesis of 10-nitro-1H-indolo[1,2-c] quinazoline-12-carbaldehyde ***(3b)***

Vilsmeyer–Haack Formylation

Phosphorous oxychloride (9.72 g, 63.52 mmol) was added slowly to *N, N*-Dimethylformamide (374 ml) at 0 °C and then the solution was stirred for 15 minutes and then it was added dropwise to 10-nitro-*H*-indolo[1,2-*c*]quinazoline (12.47 g, 47.41 mmol). Then, the mixture was stirred for a further 15 minutes and then it was refluxed for 30 minutes. It was then cooled to room temperature and then poured over the crushed ice. The precipitate was then filtered, washed with water (3 x 100 ml) and then it was boiled with aqueous sodium hydroxide (5%, 100 ml). The solid precipitate was then filtered, washed thoroughly with water till it was free from alkali, and then it was dried. The crude material was then recrystallized from ethyl alcohol to give compound 3b (Billimoria & Cava, 1994).
(11.44 g, 83 %, M.P. 233-235 °C)

General procedure for the synthesis of substituted N-((10-nitro-1H-indolo[1,2-c]quinazolin-12-yl)methylene) benzenamines ***(4b1–4b11)***

An equimolar mixture of 10-nitro-*H*-indolo[1,2-*c*]quinazoline-12-carbaldehyde and substituted anilines in methanol was refluxed for 1 h. It was poured on ice to give the precipitate. The precipitate was then filtered, washed thoroughly with cold water and then it was recrystallized with ethyl alcohol. The percentage yield was obtained in between 80–90 %.

N-((10-nitro-1H-indolo[1,2-c]quinazolin-12-yl) methylene)benzenamine ***(4b1)***

Compound 3b (1 g) was refluxed with Aniline (0.31 g). (Yield: 1.05 g, 84 %); M.P. 251-252 ˚C; Brown color FTIR (ν_{max}, cm^{-1}) 3107 (Aromatic C-H str.), 2938 (Aliphatic C-H str.), 1600 (C=N str.), 1540, 1515 (Aromatic C=C ring str.), 1493, 1350 (N=O str.), 1329 (Aromatic C-N str.); ^{1}H NMR (300 MHz, DMSO-*d6*) *δ ppm*: 7.30 (s, 5H), 7.50 (m, 2H), 7.70 (d, 1H), 7.80 (m, 2H), 8.00 (dd, *J* = 8.42, 2.45 Hz, 2H,), 8.50 (s, 1H), 9.23 (s, 1H); ^{13}C NMR (75 MHz, DMSO-*d6*) *δ ppm*: 102.02, 112.23, 115.35, 116.21, 122.32, 122.41, 125.36, 127.22, 127.18, 127.45, 127.91, 128.65, 130.87, 130.33, 134.77, 136.08, 142.12, 149.21, 149.81, 150.63, 155.74, 160.80; EIMS (m/z): $[M]^+$ 366.3689, $[M+1]^+$ 367.3719; Fragments: 262.1336, 217.2027, 189.4612, 163.8462, 108.2225, 104.0144, 45.2915, 27.1215; Anal. calcd. for $C_{22}H_{14}N_4O_2$; C, 72.12; H, 3.85; O, 8.74; N, 15.29. Found: C, 72.18; H, 3.87; O, 8.70; N, 15.30.

2-chloro-N-((10-nitro-1H-indolo[1,2-c]quinazolin-12-yl) methylene)benzenamine ***(4b2)***

Compound 3b (1 g) was refluxed with 2-Chloroaniline (0.43 g). (Yield: 1.13 g, 82%); M.P. 256-257 ˚C; Reddish brown color FTIR (ν_{max}, cm^{-1}) 3050 (Aromatic C-H str.), 2800 (Aliphatic C-H str.), 1600 (C=N) str.), 1544,1492 (Aromatic C=C ring str.), 1451, 1364 (N=O str.), 1330 (Aromatic C-N str.); 750 (C-Cl str.); ^{1}H NMR (300 MHz, DMSO-*d6*) *δ ppm*: 7.10 (d, 1H), 7.10 (m, 2H), 7.30 (d, 1H), 7.50 (s, 1H), 7.58 (t, *J* = 7.12 Hz, 1H), 7.70 (d, 1H), 7.80 (dd, *J* = 8.29, 2.43 Hz, 2H), 8.00 (dd, *J* = 8.64, 2.43 Hz, 2H), 8.50 (s, 1H), 9.23 (s, 1H); ^{13}C NMR (75 MHz, DMSO-*d6*) *δ ppm*: 102.10, 112.33, 115.20, 116.16, 123.42, 125.66, 127.34, 127.01, 127.55, 127.32, 128.09, 128.67, 128.24, 130.11, 134.55, 136.04, 139.21, 142.78, 149.90, 150.10, 155.55, 160.22; EIMS (m/z): $[M]^+$ 400.4150, $[M+1]^+$ 401.2635, $[M+2]^+$ 402.1035; Fragments: 262.3250, 217.7151, 189.1423, 163.5502, 138.0576, 108.8115, 45.2900, 27.5636; Anal. calcd. for $C_{22}H_{13}ClN_4O_2$; C, 66.24; H, 3.25; O, 8.31; N, 14.50. Found: C, 66.22; H, 3.93; O, 8.33; N, 9.48.

3-chloro-N-((10-nitro-1H-indolo[1,2-c]quinazolin-12-yl) methylene)benzenamine ***(4b3)***

Compound 3b (1 g) was refluxed with 3-Chloroaniline (0.43 g). (Yield: 1.16 g, 85 %); M.P. 254-255 ˚C; Dark brown color FTIR (ν_{max}, cm^{-1}) 3029 (Aromatic C-H str.), 2929 (Aliphatic C-H str.), 1590 (C=N str.), 1500, 1458 (Aromatic C=C ring str.), 1554, 1326 (N=O str.), 1238 (Aromatic C-N str.), 752 (C-Cl str.); ^{1}H NMR (300 MHz, DMSO-*d6*) *δ ppm*: 7.11 (d, 1H), 7.23 (t, *J* = 7.44 Hz, 1H), 7.35 (t, *J* = 7.41 Hz, 2H), 7.50 (t, *J* =7.39 Hz, 2H), 7.71 (d, 1H), 7.86 (dd, J = 8.43, 2.41 Hz, 7. 2H), 8.01 (dd, *J* = 8.28, 2.42 Hz, 2H), 8.54 (s, 1H), 9.23 (s, 1H); ^{13}C NMR (75 MHz, DMSO-*d6*) *δ ppm*: 102.21, 112.36, 115.09, 116.33, 120.40, 122.06, 125.65, 127.56, 127.37, 127.90, 127.24, 128.26, 131.82, 134.18, 135.38, 136.03, 142.19, 149.22, 150.36, 150.43, 155.74, 160.08; EIMS (m/z): $[M]^+$ 400.4533, $[M+1]^+$ 401.2345, $[M+2]^+$ 402.2030; Fragments: 262.4333, 217.5050, 189.1723, 163.5210, 138.1035, 108.2465, 45.3200, 27.8715; Anal. calcd. for $C_{22}H_{13}ClN_4O_2$; C, 66.24; H, 3.25; O, 8.31; N, 14.50. Found: C, 66.27; H, 3.22; O, 8.33; N, 14.47.

4-chloro-N-((10-nitro-1H-indolo[1,2-c]quinazolin-12-yl) methylene)benzenamine ***(4b4)***

Compound 3b (1 g) was refluxed with 4-Chloroaniline (0.43 g). (Yield: 1.13 g, 83 %); M.P. 253-254 ˚C; Reddish brown color FTIR (ν_{max}, cm^{-1}) 3046 (Aromatic C-H str.), 2928 (Aliphatic C-H str.), 1624 (C=N str.), 1590, 1500 (Aromatic C=C ring str.), 1550, 1350 (N=O str.), 1283 (Aromatic C-N str.), 750 (C-Cl str.); ^{1}H NMR (300 MHz, DMSO-*d6*) *δ ppm*: 7.22 (dd, *J* = 8.40, 2.41 Hz, 2H), 7.35 (dd, *J* = 8.43, 2.38 Hz, 2H), 7.51 (t, 7.45 Hz, 2H), 7.72 (s, 1H), 7.85 (dd, 8.46, 2.43 Hz, 2H), 8.01 (dd, 8.40, 2.42 Hz, 2H), 8.53 (s, 1H), 9.23 (s, 1H); ^{13}C NMR (75 MHz, DMSO-*d6*) *δ ppm*: 102.24, 112.09, 115.13, 116.21, 123.14, 123.33, 125.42, 127.35, 127.29, 127.98, 128.37, 130.27, 130.16, 132.22, 134.81, 136.92, 142.33, 147.01, 149.17, 150.29, 155.45, 160.38; EIMS (m/z): $[M]^+$ 400.4904, $[M+1]^+$ 401.1802, $[M+2]^+$ 402.2760; Fragments: 262.4035, 217.2651, 189.1423, 163.5662, 138.0826, 108.1540, 45.6302, 27.3016; Anal. calcd. for $C_{22}H_{13}ClN_4O_2$; C, 66.24; H, 3.25; O, 8.31; N, 14.50. Found: C, 66.26; H, 3.26; O, 8.35; N, 14.52.

2-nitro-N-((10-nitro-1H-indolo[1,2-c]quinazolin-12-yl) methylene)benzenamine ***(4b5)***

Compound 3b (1 g) was refluxed with 2-Nitroaniline (0.47 g). (Yield: 1.12 g, 80 %); M.P. 245-246 ˚C; Grey color FTIR (ν_{max}, cm^{-1}) 3020 (Aromatic C-H str.), 2954 (Aliphatic C-H str.), 1620 (C=N str.), 1585,1450 (Aromatic C=C ring str.), 1520, 1322 (N=O str.), 1220 (C-N str.); ^{1}H NMR (300 MHz, DMSO-*d6*) *δ ppm*: 7.50 (m, 4H), 7.72 (dd, *J* = 8.43, 2.44 Hz, 2H), 7.81 (dd, *J* = 8.41, 2.42 Hz, 2H), 8.01 (dd, *J* = 8.44, 2.44 Hz, 2H), 8.25 (d, 1H), 8.50 (s, 1H), 9.23 (s, 1H); ^{13}C NMR (75 MHz, DMSO-*d6*) *δ ppm*: 102.11, 112.22, 115.36, 116.49, 122.32, 123.09, 125.02, 127.00, 127.15, 127.28, 128.82, 128.53, 134.37, 136.19, 136.18, 141.26, 142.31, 144.42, 149.46, 150.50, 155.63, 160.88; EIMS (m/z): $[M]^+$ 411.7609, $[M+1]^+$ 412.3619; Fragments: 262.1356, 217.4400, 189.2350, 163.4902, 149.2949, 108.3225, 45.6415, 27.2712; Anal. calcd. for $C_{22}H_{13}N_5O_4$; C, 64.25; H, 3.62; O, 15.60; N, 17.35. Found: C, 64.27; H, 3.22; O, 15.57; N, 17.38.

3-nitro-N-((10-nitro-1H-indolo[1,2-c]quinazolin-12-yl) methylene)benzenamine ***(4b6)***

Compound 3b (1 g) was refluxed with 3-Nitroaniline (0.47 g). (Yield: 1.18 g, 84 %); M.P. 250-252 ˚C; Greyish green color FTIR (ν_{max}, cm^{-1}) 3046 (Aromatic C-H str.), 2958 (Aliphatic C-H str.), 1651 (C=N str.), 1574, 1419 (Aromatic C=C ring str.), 1508, 1360 (N=O str.), 1174 (C-N str.); ^{1}H NMR (300 MHz, DMSO-*d*6) *δ ppm*: 7.58 (m, 3H), 7.72 (dd, *J* = 8.40, 2.45 Hz, 2H), 7.83 (dd, *J* = 8.42, 2.45 Hz, 1H), 7.84 (dd, *J* = 8.39, 2.41 Hz, 1H), 8.01 (dd, *J* = 8.34, 2.29 Hz, 2H), 8.22 (t, *J* = 7.35 Hz, 2H), 8.54 (s, 1H), 9.23 (s, 1H); ^{13}C NMR (75 MHz, DMSO-*d*6) *δ ppm*: 102.25, 112.36, 115.12, 116.57, 117.31, 119.09, 125.26, 127.52, 127.78, 127.91, 128.17, 128.18, 131.31, 134.06, 136.14, 142.77, 149.81, 149.15, 149.48, 150.80, 155.36, 160.66; EIMS (m/z): $[M]^+$ 411.7618, $[M+1]^+$ 412.3629; Fragments: 262.2045, 217.3640, 189.2750, 163.5292, 149.2509, 108.3914, 45.6121, 27.2022; Anal. calcd. for $C_{22}H_{13}N_5O_4$; C, 64.30; H, 3.50; O, 15.56; N, 17.41. Found: C, 64.32; H, 3.47; O, 15.54; N, 17.44.

4-nitro-N-((10-nitro-1H-indolo[1,2-c]quinazolin-12-yl) methylene)benzenamine ***(4b7)***

Compound 3b (1 g) was refluxed with 4-Nitroaniline (0.47 g). (Yield: 1.22 g, 87 %); M.P. 252-253 ˚C; Reddish brown color FTIR (ν_{max}, cm^{-1}) 3085 (Aromatic C-H str.), 2937 (Aliphatic C-H str.), 1670 (C=N str.), 1610,1421 (Aromatic C=C ring str.), 1554, 1326 (N=O str.), 1292 (C-N str.); ^{1}H NMR (300 MHz, DMSO-*d*6) *δ ppm*: 7.51 (m, 4H), 7.73 (d, 1H), 7.83 (dd, *J* = 8.42, 2.40 Hz, 1H), 7.84 (dd, *J* = 8.39, 2.44 Hz, 1H), 8.01 (m, 2H), 8.29 (dd, *J* = 8.26, 2.29 Hz, 2H), 8.53 (s, 1H), 9.23 (s, 1H); ^{13}C NMR (75 MHz, DMSO-*d*6) *δ ppm*: 102.65, 112.33, 115.18, 116.44, 122.92, 122.29, 123.64, 123.75, 125.19, 127.05, 127.06, 127.20, 128.13, 134.45, 136.61, 142.77, 146.32, 149.48, 150.37, 155.19, 155.29, 160.04; EIMS (m/z): $[M]^+$ 411.3303, $[M+1]^+$ 412.2959; Fragments: 262.1136, 217.5940, 189.2050, 163.5450, 149.4862, 108.0545, 45.7124, 27.2821; Anal. calcd. for $C_{22}H_{13}N_5O_4$; C, 64.30; H, 3.50; O, 15.45; N, 17.41. Found: C, 64.27; H, 3.52; O, 15.42; N, 17.40.

4-fluoro-N-((10-nitro-1H-indolo[1,2-c]quinazolin-12-yl) methylene)benzenamine ***(4b8)***

Compound 3b (1 g) was refluxed with 4-Fluoroaniline (0.37 g). (Yield: 1.06 g, 81 %); M.P. 258-259 ˚C; Grey color FTIR (ν_{max}, cm^{-1}) 3080 (Aromatic C-H str.), 2920 (Aliphatic C-H str.), 1606 (C=N str.), 1558, 1325, (N=O str.), 1433, 1421 (Aromatic C=C ring str.), 1292 (Aromatic C-N str.), 1180 (C-F str.); ^{1}H NMR (300 MHz, DMSO-*d*6) *δ ppm*: 7.01 (dd, *J* = 8.29, 2.44 Hz, 2H), 7.24 (dd, *J* = 8.35, 2.43 Hz, 2H), 7.50 (s, 1H), 7.58 (t, *J* = 7.40 Hz, 1H), 7.70 (d, 1H), 7.83 (dd, *J* = 8.22, 2.46 Hz, 1H), 7.84 (d, 1H), 8.01 (dd, *J* = 8.18, 2.40 Hz, 2H), 8.53 (s, 1H), 9.23 (s, 1H); ^{13}C NMR (75 MHz, DMSO-*d*6) *δ ppm*: 102.05, 112.55, 115.69, 116.41, 116.25, 116.32, 123.28, 123.16, 125.07, 127.18, 127.00, 127.30, 128.48, 134.22, 136.31, 142.83, 144.76, 149.51, 150.19, 155.82, 160.20, 161.35; EIMS (m/z): $[M]^+$ 384.1269, $[M+1]^+$ 385.3126; Fragments: 262.9936, 217.3023, 189.4712, 163.2262, 122.5622, 108.4945, 45.3815 27.3115; Anal. calcd. for $C_{22}H_{13}FN_4O_2$; C, 68.75; H, 3.41; O, 8.33; N, 14.58. Found: C, 68.72; H, 3.39; O, 8.30; N, 14.55.

4-bromo-N-((10-nitro-1H-indolo[1,2-c]quinazolin-12-yl) methylene)benzenamine ***(4b9)***

Compound 3b (1 g) was refluxed with 4-Bromoaniline (0.59 g). (Yield: 1.30 g, 85 %); M.P. 240-241 ˚C; Dark brown color FTIR (ν_{max}, cm^{-1}) 3073 (Aromatic C-H str.), 2927 (Aliphatic C-H str.), 1613 (C=N str.), 1588,1458 (Aromatic C=C ring str.), 1518, 1323, (N=O str.) 1232 (Aromatic C-N str.), 609 (C-Br str.); ^{1}H NMR (300 MHz, DMSO-*d*6) *δ ppm:* 7.21 (dd, *J* = 8.44, 2.42 Hz, 2H), 7.42 (dd, *J* = 8.40, 2.38 Hz, 2H) 7.50 (s, 1H), 7.58 (t, J = 7.50 Hz, 1H), 7.71 (d, 1H), 7.83 (dd, *J* = 8.56, 2.39 Hz, 1H), 7.84 (dd, *J* = 8.50, 2.63 Hz, 1H), 8.01 (dd, *J* = 8.40, 2.41 Hz, 2H), 8.52 (s, 1H), 9.23 (s, 1H); ^{13}C NMR (75 MHz, DMSO-*d*6) *δ ppm*: 102.03, 112.26, 115.15, 116.33, 121.27, 124.41, 124.35, 125.83, 127.42, 127.28, 127.06, 128.30, 133.22, 133.12, 134.19, 136.90, 142.39, 148.44, 149.56, 150.07, 155.82, 160.08; EIMS (m/z): $[M]^+$ 445.1316, $[M+1]^+$ 446.2617, $[M+2]^+$ 447.5860; Fragments: 262.2627, 217.3373, 189.0577, 181.4457, 163.3219, 108.2079, 45.0195, 27.1422; Anal. calcd. for $C_{22}H_{13}BrN_4O_2$; C, 59.34; H, 2.94; O, 7.19; N, 12.58. Found: C, 59.32; H, 2.91; O, 7.16; N, 12.59.

2-chloro-4-nitro-N-((10-nitro-1H-indolo[1,2-c] quinazolin-12-yl)methylene)benzenamines ***(4b10)***

3b (1 g) was refluxed with 2-Chloro-4-nitroaniline (0.58 g). (Yield: 1.33 g, 88 %); M.P. 256-257 ˚C; Reddish brown color FTIR (ν_{max}, cm^{-1}) 3028 (Aromatic C-H str.), 2853 (Aliphatic C-H str.), 1642 (C=N str.), 1563, 1430 (Aromatic C=C ring str.), 1523,1308 (N=O str.), 1283 (C-N str.), 752 (C-Cl str.); ^{1}H NMR (300 MHz, DMSO-*d*6) *δ ppm*: 7.51 (t, *J* = 7.56 Hz, 2H), 7.58 (t, *J* = 7.39 Hz, 1H), 7.70 (d, 1H), 7.82 (m, 2H), 8.01 (dd, *J* = 8.30, 2.45 Hz, 2H), 8.12 (d, 1H), 8.26 (s, 1H), 8.51 (s, 1H), 9.23 (s, 1H); ^{13}C NMR (75 MHz, DMSO-*d*6) *δ ppm*: 102.11, 112.22, 115.47, 116.66, 120.74, 124.57, 125.33, 125.09, 127.01, 127.18, 127.77, 128.91, 128.28, 134.44, 136.56, 142.00, 145.88, 148.20, 149.01, 150.43, 155.17, 160.84; EIMS (m/z): $[M]^+$ 445.5030, $[M+1]^+$ 446.1413, $[M+2]^+$ 447.2303; Fragments: 262.3925, 217.6756, 189.1023, 182.5222, 163.8866, 108.4325, 45.4560, 27.2202; Anal. calcd. for $C_{22}H_{12}N_5O_4Cl$; C, 59.27; H, 2.71; O, 14.38; N, 15.71. Found: C, 59.28; H, 2.70; O, 14.36; N, 15.73.

4-chloro-2-nitro-N-((10-nitro-1H-indolo[1,2-c] quinazolin-12-yl)methylene)benzenamine ***(4b11)***

Compound 3b (1 g) was refluxed with 4-Chloro-2-nitroaniline (0.58 g).
(Yield: 1.29 g, 85 %; M.P. 255-256 ˚C; Light brown color

FTIR (ν_{max}, cm^{-1}) 3023 (Aromatic C-H str.), 2910 (Aliphatic C-H str.), 1612 (C=N str.), 1553, 1423 (Aromatic C=C ring str.), 1513, 1330 (N=O str.), 1285 (C-N str.), 685 (C-Cl str.); ^{1}H NMR (300 MHz, DMSO-*d6*) *δ ppm*: 7.50 (t, *J* = 7.47 Hz, 2H), 7.52 (t, *J* = 7.36 Hz, 1H), 7.71 (d, 1H), 7.87 (m, 2H), 8.01 (dd, *J* = 8.42, 2.38 Hz, 2H), 8.15 (d, 1H), 8.28 (s, 1H), 8.54 (s, 1H), 9.23 (s, 1H); ^{13}C NMR (75 MHz, DMSO-*d6*) *δ ppm*: 102.05, 112.14, 115.23, 116.14, 124.25, 125.66, 125.25, 127.36, 127.17, 127.46, 128.81, 133.62, 134.77, 136.01, 136.34, 142.71, 142.29, 143.37, 149.45, 150.40, 155.92, 160.14; EIMS (m/z): $[M]^+$ 445.5237, $[M+1]^+$ 446.2513, $[M+2]^+$ 447.4463; Fragments: 262.6625, 217.1725, 189.1882, 182.3421, 163.6131, 108.7235, 45.4560, 27.1925; Anal. calcd. for $C_{22}H_{12}N_5O_4Cl$; C, 59.27; H, 2.71; O, 14.38; N, 15.71. Found: C, 59.29; H, 2.69; O, 14.37; N, 15.74.

BIOLOGICAL ACTIVITIES

Antibacterial activity

Antibacterial activity for all derivatives was done by agar well diffusion method. Ciprofloxacin was used as the reference compound. One gram-negative bacterium *(Escherichia coli)* and a gram-positive bacterium *(Bacillus subtilis)* were taken for activity. These bacteria were obtained from Microbiology Laboratory, National JALMA Institute for Leprosy & Other Mycobacterial Diseases, Agra, India. Solutions of all derivatives having a concentration of 100 µg/ml and 150 µg/ml were prepared in Dimethylsulfoxide (DMSO). Sterilized materials were used. The bacteria were grown in nutrient broth at 37 °C for 24 h. On a water bath, nutrient agar was melted and then cooled to 45 °C. It was then shaken gently. Inoculation of each culture (1.0 ml) was done aseptically and then mixing was done by gentle shaking and then the mixture was poured into the sterilized Petri plates. The material was then allowed to settle for 1–2 h, and then it was cut to make wells of 7 mm. Each compound in the form of the solution was added to each of these wells. Then all plates were incubated at 37 °C for 24 h. After incubation time, the zone of inhibition around each well was measured in millimeters. DMSO was used as a control (Ugur et al., 2000).

Antioxidant activities

DPPH radical scavenging activity

All the synthesized derivatives were evaluated for their free radical scavenging potential using 0.1 mmol solution of DPPH in methanol. Solutions were kept in darkness for half an hour to form free radicals. Solution of different concentrations were prepared (10, 20, 30, 40 and 50 µg/ml) for test compounds as well as standard. Then, 1 ml of each test compound solution was added with the same volume of DPPH solution, then the mixture was mixed vigorously and kept for half an hour in the darkroom. The absorbance of all solutions was measured at a wavelength of 517 nm. The same procedure was performed in the triplicate manner (n = 3) and the average of the three readings was shown in the results with standard deviation. The same procedure was carried out with the solutions of ascorbic acid. Percent inhibition was calculated using equation 1 (Kaushik et al., 2016; Malviya et al., 2017).

$$\%\ inhibition = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{absorbance\ of\ control} \times 100 \quad (1)$$

H_2O_2 radical scavenging activity

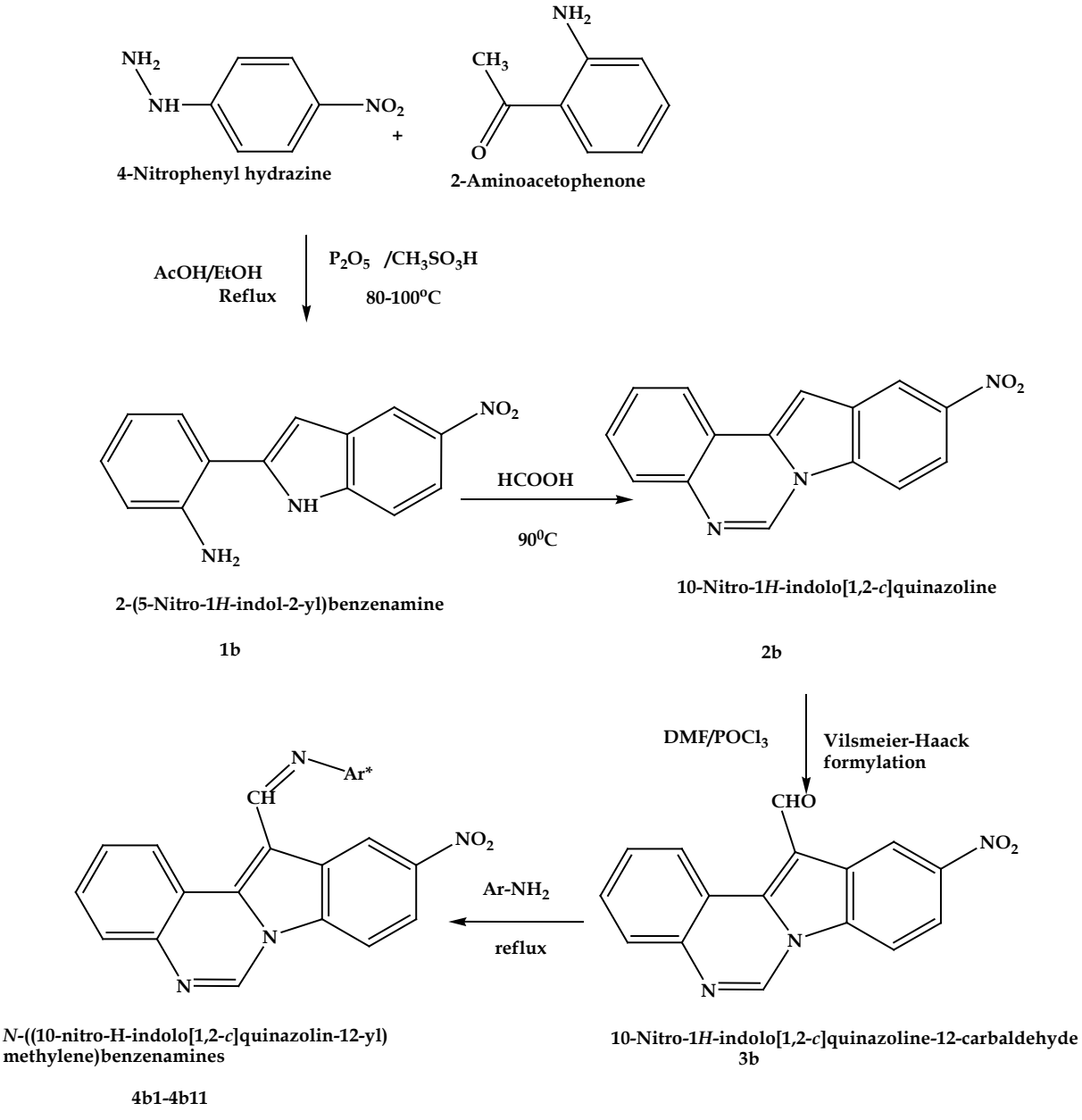
Hydrogen peroxide was used to produce hydroxyl radicals. All the solutions of test and standard compounds were prepared as above and concentrations of hydroxyl radicals were observed at 230 nm (Ningsih et al., 2016). The procedure was performed in a triplicate manner and the average of the three readings was shown in the results with standard deviation. Percent inhibition was calculated using **equation 1**.

RESULTS AND DISCUSSION

Chemistry

In the first step, phenyl hydrazone of 2-Aminoacetophenone was synthesized by the reflux of equimolar quantities of 4-Nitrophenyl hydrazine and 2-Aminoacetophenone in acetic acid/ethanol mixture for 3 h. Then, 2-(5-nitro-1*H*-indol-2-yl)benzenamine **(1b)** was synthesized after the cyclization reaction of phenylhydrazone of 2-Aminoacetophenone by phosphorous pentoxide and methanesulphonic acid. Then, cyclization of **1b** was carried out by 90% Formic acid, which produced 10-Nitro-1*H*-Indolo[1,2-*c*]quinazoline **(2b)**. In the next step, compound **2b** was allowed to undergo formylation by Vilsmeyer–Haack Formylation reaction to give 10-Nitro-12-formylindolo[1,2-*c*]quinazoline **(3b)**. Compound **3b** was then reacted with various substituted anilines to give *N*-((10-nitro-1*H*-indolo [1, 2-*c*]quinazolin-12-yl)methylene)benzenamines **(4b1–4b11)**. The synthetic **scheme** is given below.

The characterization of all derivatives was done by IR, ^{1}H NMR, ^{13}C NMR, mass spectral data, and elemental analysis. In the IR spectra of compounds **4b1–4b11,** the C-H stretching (aromatic) vibrations gave rise to a band at 3107–3020 cm^{-1}. Stretching bands for C-H (aliphatic) were observed in between 2958–2800 cm^{-1}. The stretching bands for C-Cl were observed between 752–685 cm^{-1}. The stretching vibrations of the nitro group for symmetric and asymmetric vibrations gave rise to the bands between 1558–1451 cm^{-1} and 1364–1308 cm^{-1}. Stretching of C-F and C-Br bonds were observed at 1292 cm^{-1} and 551 cm^{-1} respectively. In the ^{1}H NMR spectra of compounds **4b1–4b11**, the signals of protons of the aromatic rings appeared in the region of 7.00 and 9.23 ppm in the form of singlet, doublet, double doublet, and multiplet. The singlet due to the proton of C-H of methaniminyl group was observed at 7.50 ppm. Spectra of compounds showed the singlet due to the proton of quinazoline ring at 9.23 ppm.



***Ar:** –*Phenyl, 2-Chlorophenyl, 3-Chlorophenyl, 4-Chlorophenyl, 2-Nitrophenyl, 3-Nitrophenyl, 4-Nitrophenyl, 4-Fluorophenyl, 4-Bromophenyl, 2-Chloro-4-nitrophenyl, 4-Chloro-2-nitrophenyl*

In the ^{13}C NMR spectra of all compounds, carbons present in aromatic rings gave signals in the region of 102–160 ppm. The signal of N-C=N carbon atom of the quinazoline ring was observed at 136 ppm. The signal due to the C-Cl carbon was observed at 127–135 ppm. The signal due to the C-F carbon was observed at 161.40 ppm. The signal due to the C-Br carbon was observed at 121.63 ppm. Mass spectral data and elemental analysis were complying with the structures of all compounds. [M+ 2] peaks were found for compounds having Cl group.

Biological activities

All indoloquinazoline derivatives **(4b1–4b11)** were tested for their potential as a better antioxidant and antibacterial agent.
Antibacterial activity
The result for antibacterial activity was shown (Table 1), compound **4b9** showed 13 mm and 18 mm zone of inhibition at 100 µg/ml and150 µg/ml concentrations, respectively against *B. subtilis* and it also showed 12 mm and 17 mm zone of inhibition at 100 µg/ml and 150 µg/ml concentrations, respectively against *E. coli*. Compound **4b10** showed

Table 1: Zone of inhibition values for derivatives (4b1-4b11) at 100 µg/ml and 150 µg/ml

Compounds	Zone of inhibition (mm)			
	B. subtilis		E.coli	
	100 µg/ml	150 µg/ml	100 µg/ml	150 µg/ml
4b1	2	7	3	5
4b2	2	8	4	9
4b3	4	8	6	10
4b4	5	10	4	9
4b5	3	7	4	9
4b6	6	10	7	10
4b7	6	12	7	12
4b8	5	10	7	13
4b9	13	18	12	17
4b10	12	15	13	17
4b11	6	14	8	14
Ciprofloxacin (25 µg/ml)	20		18	

good antibacterial activity against *B. subtilis* with a zone of inhibition of 12 mm and 15 mm at 100 µg/ml and150 µg/ml concentrations, respectively and a zone of inhibition of 13 mm and 17 mm against *E. coli* at 100 µg/ml and 150 µg/ml concentrations, respectively. Ciprofloxacin showed better antimicrobial activity against *B. subtilis and E. coli* at lower concentration (25 µg/ml) with a zone of inhibition of 20 mm and 18 mm against *B. subtilis* and *E. coli* respectively.

Antioxidant activities

DPPH radical scavenging activity

All the newly synthesized indoloquinazoline derivatives were screened for free radical scavenging activity by DPPH method. Sample solutions were prepared to have concentrations of 10, 20, 30, 40 and 50 µg/ml. Ascorbic acid was taken as a standard antioxidant. Compounds **4b7, 4b8** and **4b11** were found to be good free radical scavengers with good percentage inhibition in all the concentrations and an IC_{50} value of 25.18 µmol/L, 28.09 µmol/L and 44.22 µmol/L, respectively. The percentage inhibition for all the synthesized compounds is shown (Table 2).

H_2O_2 radical scavenging activity

All the derivatives were also screened for antioxidant activity by the H_2O_2 method. Sample solutions were prepared to have concentrations of 10, 20, 30, 40 and 50 µg/ml. Ascorbic acid was taken as a standard antioxidant. Compounds **4b7, 4b8** and **4b11** were reported as good free radical scavengers with an IC_{50} value of 39.46 µmol/L, 44.47 µmol/L and 35.61 µmol/L, respectively. The percentage inhibition for all the synthesized compounds is shown in the tabular form (Table 3).

Structure-Activity Relationship

The present study suggests that all the indole derivatives that were containing substituted anilines having electron-withdrawing groups at para positions were reported to have good antioxidant and antibacterial activities. Compounds **4b9** (4-Bromophenyl) and **4b10** (4-Chloro-2-nitrophenyl) were found to contain good antibacterial activity against gram-positive and gram-negative bacteria. Derivatives **4b7** (4-Nitrophenyl), **4b8** (4-Fluorophenyl) and **4b11** (4-Chloro-2-nitrophenyl) were having good potential as an antioxidant. It is also observed that the nitro group attached at 2nd position can also enhance the antioxidant activity and chloro group attached at 2nd position along with a nitro group at 4th position can enhance the antibacterial activity.

CONCLUSION

Synthesis of some newer *N*-((10-nitro-1*H*-indolo [1, 2-*c*] quinazolin-12-yl)methylene)benzenamines **(4b1–4b11)** was done and the characterization of all the derivatives was done by the ^{1}H NMR, ^{13}C NMR, FTIR and mass spectrophotometry. Then their potential as an antioxidant and as an antibacterial was evaluated. Most of the derivatives have shown good antioxidant activity in all the concentrations. Compounds **4b7, 4b8** and **4b11** were found to be the best free radical scavengers. Antibacterial studies showed that the compounds **4b9** and **4b10** have shown the best antibacterial action. By looking at these results, the conclusion can be made that

Table 2: DPPH free radical scavenging activity of compounds 4b1–4b11

Compounds	% Inhibition at µg/ml*					
	10	20	30	40	50	IC*50* value (µmol/L)
Ascorbic acid	45.8 ± 1.21	54.01 ± 1.09	61.60 ±0.98	66.89 ± 1.23	78.01 ± 2.31	87.67
4b1	17.26 ± 4.36	20.98 ± 0.43	26.20 ± 0.69	36.84 ± 2.86	41.26 ± 1.50	189.43
4b2	4.01 ± 2.43	14.75 ± 0.79	24.29 ± 1.71	35.24 ± 0.89	37.94 ± 1.67	150.70
4b3	14.75 ± 0.42	19.67 ± 0.91	27.60 ± 0.46	32.92 ± 0.75	36.64 ± 0.45	178.80
4b4	7.42 ± 0.75	15.66 ± 0.30	20.88 ± 0.62	30.82 ± 0.45	35.13 ± 0.75	174.22
4b5	16.06 ± 0.45	21.68 ± 0.30	28.01 ± 0.30	34.93 ± 1.08	45.98 ± 0.75	141.77
4b6	4.31 ± 0.75	13.65 ± 0.62	23.18 ± 1.50	26.70 ± 0.75	32.92 ± 0.62	176.32
4b7	50.19 ± 1.05	54.71 ± 0.45	65.45 ± 0.75	73.49 ± 0.90	76.70± 0.75	25.18
4b8	50.59 ± 1.17	53.61 ± 0.79	57.02 ± 0.62	60.94 ± 0.45	67.66 ± 0.75	28.09
4b9	12.74 ± 1.83	20.57 ± 0.96	28.41 ± 0.75	32.92 ± 0.92	36.04 ± 0.74	158.38
4b10	27.40 ± 0.21	30.41 ± 0.60	33.12 ± 0.79	35.63 ± 0.62	38.75 ± 0.45	203.73
4b11	44.67 ± 0.96	50.00 ± 0.62	56.62 ± 0.82	59.83 ± 0.45	67.46 ± 0.60	44.22

**n = 3 (results are average of triplicate readings with standard deviation)*

Table 3: H2O2 free radical scavenging activity of compounds 4b1–4b11

Compounds	% Inhibition at µg/ml*					
	10	20	30	40	50	IC*50* value (µmol/L)
Ascorbic acid	44.35 ± 1.21	55.32 ± 1.34	62.09 ± 1.51	66.72 ± 1.10	77.02 ± 1.33	88.23
4b1	4.02 ± 0.57	10.18 ± 0.75	18.11 ± 0.75	24.52 ± 0.37	32.32 ± 0.57	224.22
4b2	1.88 ± 0.37	7.67 ± 1.32	16.98 ± 0.37	20.37 ± 0.75	26.03 ± 0.75	220.12
4b3	9.68 ± 0.57	20.25 ± 0.57	24.02 ± 1.32	28.80 ± 0.57	34.08 ± 0.94	191.10
4b4	2.26 ± 0.37	8.55 ± 0.94	15.34 ± 0.78	20.00 ± 0.75	28.17 ± 0.94	213.82
4b5	1.25 ± 0.57	7.92 ± 1.13	12.20 ± 1.15	19.24 ± 0.37	23.52 ± 0.78	234.89
4b6	4.90 ± 0.99	10.06 ± 0.57	19.37 ± 0.57	23.64 ± 0.94	28.17 ± 0.57	205.62
4b7	44.90 ± 0.37	54.08 ± 0.57	60.37 ± 0.37	65.15 ± 0.94	76.10 ± 0.94	39.46
4b8	43.52 ± 0.57	54.00 ± 1.15	59.11 ± 0.57	66.03 ± 0.75	72.83 ± 1.13	44.47
4b9	8.30 ± 0.75	13.96 ± 0.37	20.12 ± 0.57	28.80 ± 0.57	30.81 ± 0.57	178.53
4b10	4.90 ± 0.75	8.30 ± 0.37	13.20 ± 0.37	20.75 ± 0.37	24.90 ± 0.75	219.88
4b11	47.04 ± 0.57	53.58 ± 1.13	57.10 ± 0.57	63.39 ± 0.75	74.08 ± 0.57	35.61

**n = 3 (results are average of triplicate readings with standard deviation)*

all those compounds having 4 substituted anilines (4-Nitro, 4-Fluoro, 4-Bromo & 4-Chloro-2-nitro) on the methaniminyl group of 12th position of indolo[1,2-*c*]quinazoline moiety possess good antioxidant and antibacterial activities.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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