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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of some new glutamine linked 2,3-disubstituted quinazolinone derivatives as potent antimicrobial and antioxidant agents

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Abstract A series of novel glutamine linked 2,3-disubstituted quinazolinone conjugates was synthesized from methyl anthranilate and different substituted acids and acid chlorides. The compounds **5a**–**l** were prepared in good yields. All compounds were screened for their antibacterial activity against Gram-positive and Gram-negative bacteria and for antifungal activity against *Candida albicans* and *Aspergillus flavus* using paper disk diffusion technique. The minimum inhibitory concentrations of the compounds were also determined by agar streak dilution method. The compound **5b** was found to exhibit the most potent in vitro anti-microbial activity. When tested for their antioxidant activity, compounds **5i** and **5l** showed potent radical scavenging activity, while compound **5g** had moderate effect against 2,2-diphenyl-1-picrylhydrazyl, hydroxyl, nitric oxide, and superoxide radical scavenging assays. These results suggest that, the three quinazolinone analogs (**5g**, **5i**, and **5l**) could be considered as useful templates for future development to obtain more potent antioxidant agents.

Keywords Glutamine · Quinazolinone · Antimicrobial · Antioxidant

Introduction

Quinazolinone is a building block for naturally occurring alkaloids isolated from a number of families of plant kingdom and its derivatives are versatile lead molecules for designing potential bioactive agents (Mhaske and Argade, 2006). Quinazolinone derivatives are found to possess a number of therapeutic activities including antimicrobial, antioxidant, anti-inflammatory, and anti-cancer activities (Maggio *et al.*, 2001; Grover and Kini, 2006; Mani Chandrika *et al.*, 2008). 4(3H)-quinazolinone is one of the most frequently encountered heterocycles in medicinal chemistry with wide applications. Among the various classes of quinazolinones, 2,3-disubstituted-4(3H)-quinazolinone has been reported to be associated with anti-microbial properties. Examples of such disubstituted derivatives include substituted phenyl ring moieties, and bridged phenyl rings and aliphatic systems (Mosaad *et al.*, 2010). The 2,3-disubstituted quinazolinones have been predicted to possess antiviral and antihypertensive activities (Pandey *et al.*, 2004) and exhibited good analgesic and anti-inflammatory activities (Alagarsamy *et al.*, 2007). The presence of fluorine atom at appropriate position in the molecule alters the properties of molecule by promoting activity due to high lipid solubility and enhancement of transport mechanism (Furuyal and Ritter, 2011).

Free radicals and oxygen derivatives are constantly generated in vitro by specific metabolic processes (Suthakaran *et al.*, 2008). These radicals can easily react with most biological molecules including proteins, lipids, lipoproteins, and DNA. These can be responsible for a wide range of human conditions such as arthritis, hemorrhagic shock, coronary artery diseases, cataract, cancer, AIDS as well as age-related degenerative brain diseases (Parr and Bolwell, 2000). Thus, there is a constant need for searching new and effective therapeutic agents.

General investigation revealed that the components with antimicrobial activity have gained increasing importance due to growing worldwide concern about the increase in the rate of infection by pathogenic microbes (Davies, 1994).

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Antibacterial is a general term for drug, chemical, or other substance that either kills or slows the growth of microbe. Thus, the development of new and different antimicrobial drugs is a very important objective and much of the research program efforts are directed toward the design of new antibacterial agents.

Currently, antioxidants that exhibit DPPH radical scavenging activity are receiving increasing attention. Antioxidants act as a major defense against radical mediated toxicity by protecting the damages caused by free radicals and they have been reported to have interesting anticancer, anti-aging, and anti-inflammatory activities (Finkel and Holbrook, 2000). Literature is scanty on the study of antioxidant activity of substituted quinazolinone derivatives. This prompted the authors to synthesize a new series of biologically active quinazolinones and evaluate their antimicrobial activity by disk diffusion method and antioxidant activity by DPPH free radical scavenging, hydroxyl radical scavenging, superoxide radical scavenging, and nitric oxide (NO) radical scavenging assays. Thus, the present work is aimed at designing new and more active and less toxic synthetic antimicrobial and antioxidant agents.

Experimental

All chemicals and solvents were of AR grade. Solvents were used as received without further purification. Elemental analysis (C, H, N) was performed using a Carlo-Erba 1160 elemental analyzer. IR spectra were recorded on a JASCO FTIR-8400 spectrophotometer using KBr method. The ^{1}H-NMR spectra were recorded on a Varian AC 400 spectrometer using TMS as the internal standard and $CDCl_3$ as a solvent. Mass spectra were obtained on a Varian 1200L model mass spectrometer (solvent: CH_3OH). Melting points were determined with a Buchi 530 melting point apparatus in open capillaries and are uncorrected. Compound purity was checked by thin layer chromatography (TLC) on precoated silica gel plates (Merck, Kieselgel 60 F254, layer thickness 0.25 mm).

Synthesis

*General procedure for compounds (**2a–f**)/(**2g–l**)*

Compounds **2a–f** were prepared by the reaction of methyl anthranilate (0.01 mol) with corresponding substituted benzoyl chlorides (0.01 mol) in CH_2Cl_2, and the reaction mixture was stirred at room temperature for 20 h and the progress of the reaction was monitored by TLC. The reaction mixture was then diluted with CH_2Cl_2 and washed with water (50 ml) and brine solution (100 ml), and the combined organic portions were dried over anhydrous Na_2SO_4. The crude product was obtained after evaporation of the solvent, and recrystallization from absolute ethanol gave the desired compounds **2a–f**.

To the suspension of different substituted aromatic acids (0.01 mol) in CH_2Cl_2 an excess of thionyl chloride was added and then the resulting mixture was stirred at room temperature for 2 h. The solvent was evaporated under reduced pressure giving the acid chlorides. This acid chloride was added to a solution containing methyl anthranilate in CH_2Cl_2 and stirring was continued at room temperature for 20 h, and the reaction mixture was diluted with CH_2Cl_2 and washed with water (50 ml) and brine solution (100 ml). The combined organic portions were dried over Na_2SO_4. Evaporation of the solvent gave the crude product, and recrystallization from absolute ethanol gave desired compounds **2g–l**.

*Methyl 2-(3,5-difluorobenzamido)benzoate (**2a**)*

Yield: 72 %. IR (KBr, cm^{-1}): 3190 (NH), 1687, 1630 (2C=O). ^{1}H NMR ($CDCl_3$, δ ppm): 8.74 (s, 1H, NH), 8.21–7.20 (m, 7H, Ar–H), 3.80 (s, 3H, OCH_3).

*Methyl 2-(2,3,4-trifluorobenzamido)benzoate (**2b**)*

Yield: 70 %. IR (KBr, cm^{-1}): 3248 (NH), 1695, 1637 (2C=O). ^{1}H NMR ($CDCl_3$, δ ppm): 8.41 (s, 1H, NH), 8.19–7.12 (m, 6H, Ar–H), 3.87 (s, 3H, OCH_3).

*Methyl 2-(2,5-difluorobenzamido)benzoate (**2c**)*

Yield: 78 %. IR (KBr, cm^{-1}): 3196 (NH), 1681, 1640 (2C=O). ^{1}H NMR ($CDCl_3$, δ ppm): 8.65 (s, 1H, NH), 8.23–7.22 (m, 7H, Ar–H), 3.79 (s, 3H, OCH_3).

*Methyl 2-(4-chlorobenzamido)benzoate (**2d**)*

Yield: 72 %. IR (KBr, cm^{-1}): 3219 (NH), 1690, 1624 (2C=O). ^{1}H NMR ($CDCl_3$, δ ppm): 8.33 (s, 1H, NH), 8.21–7.15 (m, 8H, Ar–H), 3.80 (s, 3H, OCH_3).

*Methyl 2-(2-phenylacetamido)benzoate (**2e**)*

Yield: 77 %. IR (KBr, cm^{-1}): 3211 (NH), 1695, 1639 (2C=O). ^{1}H NMR ($CDCl_3$, δ ppm): 8.60(s, 1H, NH), 8.22–7.26 (m, 8H, Ar–H), 3.80 (s, 3H, OCH_3).

*Methyl 2-(2-chlorobenzyloxycarbonylamino)benzoate (**2f**)*

Yield: 80 %. IR (KBr, cm^{-1}): 3150 (NH), 1673, 1621 (2C=O). ^{1}H NMR ($CDCl_3$, δ ppm): 9.27 (s, 1H, NH), 8.21–7.04 (m, 8H, Ar–H), 3.80 (s, 3H, OCH_3).

*Methyl 2-(4-methoxybenzamido)benzoate (**2g**)*

Yield: 74 %. IR (KBr, cm^{-1}): 3271 (NH), 1654, 1620 (2C=O). ^1H NMR ($CDCl_3$, δ ppm): 8.64 (s, 1H, NH), 8.23–7.01 (m, 8H, Ar–H), 3.80, 3.89 (s, 6H, $2OCH_3$).

*Methyl 2-(4-nitrobenzamido)benzoate (**2h**)*

Yield: 70 %. IR (KBr, cm^{-1}): 3280 (NH), 1692, 1644 (2C=O). ^1H NMR ($CDCl_3$, δ ppm): 8.92 (s, 1H, NH), 8.27–7.12 (m, 8H, Ar–H), 3.80 (s, 3H, OCH_3).

*Methyl 2-(4-aminobenzamido)benzoate (**2i**)*

Yield: 76 %. IR (KBr, cm^{-1}): 3255 (NH), 1703, 1633 (2C=O). ^1H NMR ($CDCl_3$, δ ppm): 9.03 (s, 1H, NH), 8.21–7.19 (m, 8H, Ar–H), 5.72 (s, 2H, NH_2), 3.80 (s, 3H, OCH_3).

*Methyl 2-(2-methylbenzamido)benzoate (**2j**)*

Yield: 81 %. IR (KBr, cm^{-1}): 3214 (NH), 1652, 1621 (2C=O). ^1H NMR ($CDCl_3$, δ ppm): 8.52 (s, 1H, NH), 8.24–7.33 (m, 8H, Ar–H), 3.80 (s, 3H, OCH_3), 2.41 (s, 3H, CH_3).

*Methyl 2-benzamidobenzoate (**2k**)*

Yield: 70 %. IR (KBr, cm^{-1}): 3207 (NH), 1697, 1630 (2C=O). ^1H NMR ($CDCl_3$, δ ppm): 8.36 (s, 1H, NH), 8.22–7.24 (m, 8H, Ar–H), 3.80 (s, 3H, OCH_3).

*Methyl 2-(4-hydroxybenzamido)benzoate (**2l**)*

Yield: 78 %. IR (KBr, cm^{-1}): 3258 (NH), 1701, 1649 (2C=O). ^1H NMR ($CDCl_3$, δ ppm): 10.04 (s, 1H, OH), 9.27 (s, 1H, NH), 8.24–7.12 (m, 8H, Ar–H), 3.80 (s, 3H, OCH_3).

*General procedure for compounds (**3a–l**)*

Compounds **2a–l** (1.0 mmol) were dissolved separately in 1:1 mixture of methanol and CH_2Cl_2, then hydrolyzed by aqueous NaOH (2 M, 5 ml), and then each mixture was refluxed for 1–2 h. Afterward, the reaction mixture was poured into water and acidification to pH 2 with 2 M HCl gave a precipitate, which was collected by filtration, giving **3a–l** as an amorphous powder.

*2-(3,5-Difluorobenzamido)benzoic acid (**3a**)*

Yield: 98 %. IR (KBr, cm^{-1}): 3201 (NH), 2735 (COOH), 1685 (C=O). ^1H NMR ($CDCl_3$, δ ppm): 12.01 (s, 1H, COOH), 8.94 (s, 1H, NH), 8.22–7.19 (m, 7H, Ar–H).

*2-(2,3,4-Trifluorobenzamido)benzoic acid (**3b**)*

Yield: 92 %. IR (KBr, cm^{-1}): 3248 (NH), 2700 (COOH), 1647 (C=O). ^1H NMR ($CDCl_3$, δ ppm): 12.33 (s, 1H, COOH), 8.63 (s, 1H, NH), 8.22–7.14 (m, 6H, Ar–H).

*2-(2,5-Difluorobenzamido)benzoic acid (**3c**)*

Yield: 92 %. IR (KBr, cm^{-1}): 3199 (NH), 2699 (COOH), 1675 (C=O). ^1H NMR ($CDCl_3$, δ ppm): 11.96 (s, 1H, COOH), 8.69 (s, 1H, NH), 8.23–7.15 (m, 7H, Ar–H).

*2-(4-Chlorobenzamido)benzoic acid (**3d**)*

Yield: 96 %. IR (KBr, cm^{-1}): 3221 (NH), 2784 (COOH), 1678 (C=O). ^1H NMR ($CDCl_3$, δ ppm): 12.11 (s, 1H, COOH), 8.47 (s, 1H, NH), 8.21–7.21 (m, 8H, Ar–H).

*2-(2-Phenylacetamido)benzoic acid (**3e**)*

Yield: 92 %. IR (KBr, cm^{-1}): 3219 (NH), 2690 (COOH), 1680 (C=O). ^1H NMR ($CDCl_3$, δ ppm): 12.07 (s, 1H, COOH), 8.69 (s, 1H, NH), 8.21–7.26 (m, 8H, Ar–H), 3.80 (s, 3H, OCH_3).

*2-((2-Chlorobenzyloxy)carbonylamino)benzoic acid (**3f**)*

Yield: 95 %. IR (KBr, cm^{-1}): 3150 (NH), 2751 (COOH), 1672 (C=O). ^1H NMR ($CDCl_3$, δ ppm): 12.64 (s, 1H, COOH), 9.61 (s, 1H, NH), 8.23–7.14 (m, 8H, Ar–H).

*2-(4-Methoxybenzamido)benzoic acid (**3g**)*

Yield: 98 %. IR (KBr, cm^{-1}): 3290 (NH), 2739 (COOH), 1670 (C=O). ^1H NMR ($CDCl_3$, δ ppm): 12.06 (s, 1H, COOH), 9.11 (s, 1H, NH), 8.23–7.04 (m, 8H, Ar–H), 3.59 (s, 3H, OCH_3).

*2-(4-Nitrobenzamido)benzoic acid (**3h**)*

Yield: 94 %. IR (KBr, cm^{-1}): 3302 (NH), 2697 (COOH), 1700 (C=O). ^1H NMR ($CDCl_3$, δ ppm): 12.71 (s, 1H, COOH), 8.97 (s, 1H, NH), 8.24–7.12 (m, 8H, Ar–H).

*2-(4-Aminobenzamido)benzoic acid (**3i**)*

Yield: 96 %. IR (KBr, cm^{-1}): 3255 (NH), 2700 (COOH), 1701 (C=O). ^1H NMR ($CDCl_3$, δ ppm): 12.94 (s, 1H, COOH), 9.33 (s, 1H, NH), 8.21–7.17 (m, 8H, Ar–H), 5.27 (s, 2H, NH_2).

*2-(2-Methylbenzamido)benzoic acid (**3j**)*

Yield: 91 %. IR (KBr, cm^{-1}): 3216 (NH), 2730 (COOH), 1695 (C=O). ^1H NMR ($CDCl_3$, δ ppm): 12.64 (s, 1H, COOH), 8.90 (s, 1H, NH), 8.23–7.21 (m, 8H, Ar–H), 2.46 (s, 3H, CH_3).

*2-Benzamidobenzoic acid (**3k**)*

Yield: 90 %. IR (KBr, cm^{-1}): 3248 (NH), 2714 (COOH), 1695 (C=O). ^1H NMR ($CDCl_3$, δ ppm): 12.37 (s, 1H, COOH), 8.61 (s, 1H, NH), 8.22–7.17 (m, 8H, Ar–H).

*2-(4-Hydroxybenzamido)benzoic acid (**3l**)*

Yield: 97 %. IR (KBr, cm^{-1}): 3279 (NH), 2690 (COOH), 1684 (C=O). ^1H NMR ($CDCl_3$, δ ppm): 13.16 (s, 1H, COOH), 10.01 (s, 1H, OH), 9.47 (s, 1H, NH), 8.23–7.11 (m, 8H, Ar–H).

*General procedure for compounds (**4a–l**)*

Compounds **3a–l** (0.1 mmol) were refluxed in thionyl chloride (0.1 mmol) for 2 h. The solvent was evaporated under reduced pressure and recrystallization of the residue from acetonitrile gave the products **4a–l**.

*2-(3,5-Difluorophenyl)-4H-benzo[d][1,3]oxazin-4-one (**4a**)*

Yield: 70 %. IR (KBr, cm^{-1}): 1685 (C=O), 1597 (C=N). ^1H NMR ($CDCl_3$, δ ppm): 8.23–7.19 (m, 7H, Ar–H).

*2-(2,3,4-Trifluorophenyl)-4H-benzo[d][1,3]oxazin-4-one (**4b**)*

Yield: 79 %. IR (KBr, cm^{-1}): 1695 (C=O), 1587 (C=N). ^1H NMR ($CDCl_3$, δ ppm): 8.21–7.12 (m, 6H, Ar–H).

*2-(2,5-Difluorophenyl)-4H-benzo[d][1,3]oxazin-4-one (**4c**)*

Yield: 82 %. IR (KBr, cm^{-1}): 1700 (C=O), 1580 (C=N). ^1H NMR ($CDCl_3$, δ ppm): 8.26–7.10 (m, 7H, Ar–H).

*2-(4-Chlorophenyl)-4H-benzo[d][1,3]oxazin-4-one (**4d**)*

Yield: 72 %. IR (KBr, cm^{-1}): 1697 (C=O), 1600 (C=N). ^1H NMR ($CDCl_3$, δ ppm): 8.23–7.20 (m, 8H, Ar–H).

*2-Benzyl-4H-benzo[d][1,3]oxazin-4-one (**4e**)*

Yield: 72 %. IR (KBr, cm^{-1}): 1678 (C=O), 1593 (C=N). ^1H NMR ($CDCl_3$, δ ppm): 8.24–7.16 (m, 8H, Ar–H), 2.36 (s, 2H, CH_2).

*2-(2-Chlorobenzyloxy)-4H-benzo[d][1,3]oxazin-4-one (**4f**)*

Yield: 74 %. IR (KBr, cm^{-1}): 1665 (C=O), 1583 (C=N). ^1H NMR ($CDCl_3$, δ ppm): 8.27–7.14 (m, 8H, Ar–H), 2.47 (s, 2H, CH_2).

*2-(4-Methoxyphenyl)-4H-benzo[d][1,3]oxazin-4-one (**4g**)*

Yield: 77 %. IR (KBr, cm^{-1}): 1695 (C=O), 1587 (C=N). ^1H NMR ($CDCl_3$, δ ppm): 8.23–7.11 (m, 8H, Ar–H), 3.66 (s, 3H, OCH_3).

*2-(4-Nitrophenyl)-4H-benzo[d][1,3]oxazin-4-one (**4h**)*

Yield: 73 %. IR (KBr, cm^{-1}): 1700 (C=O), 1589 (C=N). ^1H NMR ($CDCl_3$, δ ppm): 8.21–7.20 (m, 8H, Ar–H).

*2-(4-Aminophenyl)-4H-benzo[d][1,3]oxazin-4-one (**4i**)*

Yield: 80 %. IR (KBr, cm^{-1}): 3390 (NH_2), 1695 (C=O), 1582 (C=N). ^1H NMR ($CDCl_3$, δ ppm): 8.22–7.15 (m, 8H, Ar–H), 5.49 (s, 2H, NH_2).

*2-o-tolyl-4H-benzo[d][1,3]oxazin-4-one (**4j**)*

Yield: 81 %. IR (KBr, cm^{-1}): 2732 (C–CH3), 1699 (C=O), 1601 (C=N). ^1H NMR ($CDCl_3$, δ ppm): 8.23–7.21 (m, 8H, Ar–H), 2.46 (s, 3H, CH_3).

*2-Phenyl-4H-benzo[d][1,3]oxazin-4-one (**4k**)*

Yield: 74 %. IR (KBr, cm^{-1}): 1703 (C=O), 1597 (C=N). ^1H NMR ($CDCl_3$, δ ppm): 8.27–7.20 (m, 9H, Ar–H).

*2-(4-Hydroxyphenyl)-4H-benzo[d][1,3]oxazin-4-one (**4l**)*

Yield: 79 %. IR (KBr, cm^{-1}): 3184 (OH), 1695 (C=O), 1592 (C=N). ^1H NMR ($CDCl_3$, δ ppm): 10.07 (s, 1H, OH), 8.23–7.11 (m, 8H, Ar–H).

*General procedure for compounds (**5a–l**)*

Equimolar quantities (0.06 mmol) of 2-substituted-3,1-benzoxazin-4-one and the primary amine (glutamine) in glacial acetic acid (5 ml) were refluxed for 6 h. The resulting reaction mixture was cooled to room temperature and poured onto crushed ice. The separated out solid was filtered, washed thoroughly with cold distilled water, vacuum dried and recrystallized from ethanol to obtain the products **5a–l**.

*2-Amino-5-(2-(3,5-difluorophenyl)-4-oxoquinazolin-3(4H)-yl)-5-oxopentanoic acid (**5a**)*

IR (KBr, cm^{-1}): 3341 (NH_2), 2660 (COOH), 1673, 1591 (C=O), 1539 (C=N). 1H NMR ($CDCl_3$, δ ppm): 12.10 (s, 1H, COOH), 8.26–7.22 (m, 7H, Ar–H), 5.47 (s, 2H, NH_2), 3.54–3.51 (t, 1H, CH), 2.53–2.50 (t, 2H, CH_2), 2.29–2.26 (q, 2H, CH_2). MS *m/z*: 387 (M^+). Analysis Calcd. for $C_{19}H_{15}F_2N_3O_4$ (%): C 58.92, H 3.90, N 10.85. Found (%): C 58.87, H 3.93, N 10.93.

*2-Amino-5-oxo-5-(4-oxo-2-(2,3,4-trifluorophenyl) quinazolin-3(4H)-yl)pentanoic acid (**5b**)*

IR (KBr, cm^{-1}): 3361 (NH_2), 2670 (COOH), 1681, 1609 (C=O), 1562 (C=N). ^{1}H NMR ($CDCl_3$, δ ppm): 12.01 (s, 1H, COOH), 8.28–7.19 (m, 8H, Ar–H), 5.37 (s, 2H, NH_2), 3.53–3.50 (t, 1H, CH), 2.51–2.48 (t, 2H, CH_2), 2.24–2.21 (q, 2H, CH_2). MS *m/z*: 405 (M^+). Analysis Calcd. for $C_{19}H_{14}F_3N_3O_4$ (%): C 56.30, H 3.48, N 10.37. Found (%): C 56.58, H 3.11, N 10.04.

*2-Amino-5-(2-(2,5-difluorophenyl)-4-oxoquinazolin-3(4H)-yl)-5-oxopentanoic (**5c**)*

IR (KBr, cm^{-1}): 3370 (NH_2), 2668 (COOH), 1694, 1628 (C=O), 1569 (C=N). ^{1}H NMR ($CDCl_3$, δ ppm): 12.11 (s, 1H, COOH), 8.29–7.19 (m, 7H, Ar–H), 5.40 (s, 2H, NH_2), 3.56–3.52 (t, 1H, CH), 2.51–2.48 (t, 2H, CH_2), 2.29–2.26 (q, 2H, CH_2). MS *m/z*: 387 (M^+). Analysis Calcd. for $C_{19}H_{15}F_2N_3O_4$ (%): C 58.92, H 3.90, N 10.85. Found (%): C 58.50, H 3.73, N 10.49.

*2-Amino-5-(2-(4-chlorophenyl)-4-oxoquinazolin-3(4H)-yl)-5-oxopentanoic acid (**5d**)*

IR (KBr, cm^{-1}): 3374 (NH_2), 2669 (COOH), 1693, 1630 (C=O), 1587 (C=N). ^{1}H NMR ($CDCl_3$, δ ppm): 12.01 (s, 1H, COOH), 8.23–7.20 (m, 8H, Ar–H), 5.35 (s, 2H, NH_2), 3.52–3.49 (t, 1H, CH), 2.52–2.50 (t, 2H, CH_2), 2.29–2.26 (q, 2H, CH_2). MS *m/z*: 384 (M^+), 386 (M^{+2}). Analysis Calcd. for $C_{19}H_{16}ClN_3O_4$ (%): C 59.15, H 4.18, N 10.89. Found (%): C 59.11, H 4.09, N 10.94.

*2-Amino-5-(2-benzyl-4-oxoquinazolin-3(4H)-yl)-5-oxopentanoic acid (**5e**)*

IR (KBr, cm^{-1}): 3354 (NH_2), 2667 (COOH), 1675, 1627 (C=O), 1589 (C=N). ^{1}H NMR ($CDCl_3$, δ ppm): 11.96 (s, 1H, COOH), 8.27–7.19 (m, 9H, Ar–H), 5.62 (s, 2H, NH_2), 4.03 (s, 2H, Ph–CH_2), 3.62–3.58 (t, 1H, CH), 2.47–2.44 (t, 2H, CH_2), 2.19–2.15 (q, 2H, CH_2). MS *m/z*: 365 (M^+). Analysis Calcd. for $C_{20}H_{19}N_3O_4$, (%): C 65.74, H 5.24, N 11.50. Found (%): C 65.50, H 5.20, N 11.26.

*2-Amino-5-(2-(2-chlorobenzyloxy)-4-oxoquinazolin-3(4H)-yl)-5-oxopentanoic acid (**5f**)*

IR (KBr, cm^{-1}): 3369 (NH_2), 2671 (COOH), 1662, 1611 (C=O), 1564 (C=N). ^{1}H NMR ($CDCl_3$, δ ppm): 12.16 (s, 1H, COOH), 8.29–7.19 (m, 8H, Ar–H), 5.40 (s, 2H, NH_2), 4.36 (s, 2H, Ph–CH_2), 3.53–3.50 (t, 1H, CH), 2.57–2.54 (t, 2H, CH_2), 2.29–2.25 (q, 2H, CH_2). MS, *m/z*: 415 (M^+), 417 (M^{+2}). Analysis Calcd. for $C_{20}H_{18}ClN_3O_5$ (%): C 57.77, H 4.36, N 10.11. Found (%): C 57.63, H 4.31, N 10.18.

*2-Amino-5-(2-(4-methoxyphenyl)-4-oxoquinazolin-3(4H)-yl)-5-oxopentanoic acid (**5g**)*

IR (KBr, cm^{-1}): 3370 (NH_2), 2669 (COOH), 1682, 1602 (C=O), 1574 (C=N). ^{1}H NMR ($CDCl_3$, δ ppm): 12.16 (s, 1H, COOH), 8.17–7.02 (m, 8H, Ar–H), 5.43 (s, 2H, NH_2), 3.67 (s, 3H, OCH_3), 3.64–3.61 (t, 1H, CH), 2.58–2.54 (t, 2H, CH_2), 2.26–2.21 (q, 2H, CH_2). MS *m/z*: 381 (M^+). Analysis Calcd. for $C_{20}H_{19}N_3O_5$ (%): C 62.99, H 5.02, N 11.02. Found (%): C 62.93, H 5.11, N 11.07.

*2-Amino-5-(2-(4-nitrophenyl)-4-oxoquinazolin-3(4H)-yl)-5-oxopentanoic acid (**5h**)*

IR (KBr, cm^{-1}): 3367 (NH_2), 2669 (COOH), 1693, 1632 (C=O), 1579 (C=N). ^{1}H NMR ($CDCl_3$, δ ppm): 12.04 (s, 1H, COOH), 8.27–7.11 (m, 8H, Ar–H), 5.47 (s, 2H, NH_2), 3.68–3.63 (t, 1H, CH), 2.47–2.44 (t, 2H, CH_2), 2.24–2.20 (q, 2H, CH_2). MS, *m/z*: 396 (M^+). Analysis Calcd. for $C_{19}H_{16}N_4O_6$ (%): C 57.58, H 4.07, N 14.14. Found (%): C 57.41, H 4.11, N 14.06.

*2-Amino-5-(2-(4-aminophenyl)-4-oxoquinazolin-3(4H)-yl)-5-oxopentanoic acid (**5i**)*

IR (KBr, cm^{-1}): 3477, 3362 (2 NH_2), 2669 (COOH), 1698, 1626 (C=O), 1555 (C=N). ^{1}H NMR ($CDCl_3$, δ ppm): 12.01(s, 1H, COOH), 8.28–7.19 (m, 8H, Ar–H), 5.39 (s, 2H, $2NH_2$), 3.64–3.60 (t, 1H, CH), 2.54–2.50 (t, 2H, CH_2), 2.24–2.21 (q, 2H, CH_2). MS, *m/z*: 366 (M^+). Analysis Calcd. for $C_{19}H_{18}N_4O_4$ (%): C 62.29, H 4.95, N 15.29. Found (%): C 62.21, H 4.99, N 15.17.

*2-Amino-5-oxo-5-(4-oxo-2-o-tolylquinazolin-3(4H)-yl)pentanoic acid (**5j**)*

IR (KBr, cm^{-1}): 3362 (NH_2), 2669 (COOH), 1697, 1634 (C=O), 1582 (C=N). ^{1}H NMR ($CDCl_3$, δ ppm): 11.61 (s, 1H, COOH), 8.23–7.19 (m, 8H, Ar–H), 5.42 (s, 2H, NH_2),

3.64–3.61 (t, 1H, CH), 2.54–2.50 (t, 2H, CH_2), 2.47 (s, 3H, CH_3), 2.26–2.23 (q, 2H, CH_2). MS *m/z*: 365 (M^+). Analysis Calcd. for $C_{20}H_{19}N_3O_4$ (%): C 65.74, H 5.24, N 11.50. Found (%): C 65.46, H 5.27, N 11.59.

*2-Amino-5-oxo-5-(4-oxo-2-phenylquinazolin-3(4H)-yl)pentanoic acid (**5k**)*

IR (KBr, cm^{-1}): 3367 (NH_2), 2674 (COOH), 1699, 1617 (C=O), 1572 (C=N). ^1H NMR ($CDCl_3$, δ ppm): 12.11 (s, 1H, COOH), 8.28–7.25 (m, 9H, Ar–H), 5.64 (s, 2H, NH_2), 3.54–3.51 (t, 1H, CH), 2.50–2.47 (t, 2H, CH_2), 2.14–2.12 (q, 2H, CH_2). MS *m/z*: 351 (M^+). Analysis Calcd. for $C_{19}H_{17}N_3O_4$ (%): C 64.95, H 4.88, N 11.96. Found (%): C 64.75, H 4.72, N 11.99.

*2-Amino-5-(2-(4-hydroxyphenyl)-4-oxoquinazolin-3(4H)-yl)-5-oxopentanoic acid (**5l**)*

IR (KBr, cm^{-1}): 3361 (NH_2), 3176 (OH), 2670 (COOH), 1691, 1635 (C=O), 1583 (C=N). ^1H NMR ($CDCl_3$, δ ppm): 11.21 (s, 1H, COOH), 10.03 (s, 1H, OH), 8.28–7.06 (m, 8H, Ar–H), 5.48 (s, 2H, NH_2), 3.58–3.55 (t, 1H, CH), 2.52–2.50 (t, 2H, CH_2), 2.01–1.99 (q, 2H, CH_2). MS, *m/z*: 367 (M^+). Analysis Calcd. for $C_{19}H_{17}N_3O_5$ (%): C 62.12, H 4.66, N 11.44. Found (%): C 62.01, H 4.53, N 11.49.

Antimicrobial activity

Microbial strains

The in vitro antimicrobial screening of the quinazolinone compounds was individually performed against a panel of bacteria and fungi including *Staphylococcus aureus* (NCIM 5021), *Pseudomonas aeruginosa* (NCIM 5029), *E. coli* (NCIM 2574), *Bacillus subtilis* (NCIM 2999), *Candida albicans* (NCIM 3102), and *Aspergilus flavus* (NCIM 524). Microbial strains were cultured overnight at 37 °C in nutrient and potato dextrose agar medium. All the pure microbial strains were obtained from National Chemical Laboratory (NCL), Pune, India.

Antimicrobial screening

The antibacterial activity of compounds was determined by agar disk diffusion method (Gillespie, 1994). Briefly, a suspension of tested bacterial strains was spread on the nutrient agar medium and potato dextrose agar for fungi. The disks (6 mm in diameter) impregnated with test chemicals, each 100 μg/ml in DMSO, were placed on the inoculated agar. These plates were kept at 4 °C for 2 h. Each plate was then incubated at 37 °C for 24 h in the case of bacteria and 48 h at 28 °C in the case of fungi. Streptomycin (10 μg/disk) and fluconazole (10 μg/disk) were used as standards for antibacterial and antifungal activity, respectively. Each sample was assayed in triplicate and the zone of inhibition was measured in millimeters.

Minimum inhibitory concentration (MIC)

The MIC of the synthesized compounds was determined by dilution method (Jones *et al.*, 1984). The compounds were dissolved in DMSO; two-fold serial concentrations of the compounds were employed to determine the MIC. In this method, the test concentrations of chemically synthesized compounds were made from 5 to 125 μg/ml. The MIC-value was determined as the lowest concentration of the compound that completely inhibited macroscopic growth of microorganism.

Antioxidant activity

DPPH free radical scavenging activity

The capacity to scavenge the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was monitored according to the Blois method (Blois, 1958). The test samples (10–100 μl) were mixed with 1 ml of DPPH solution (0.1 mM) and filled up with methanol to a final volume of 4 ml. The absorbance of the resulting solution was measured at 517 nm on a visible spectrophotometer (Model 166, Systronics, India). The free radical scavenging rate of the reaction solution was calculated as a percentage (%) of DPPH discoloration using the equation

$$I\,(\%) = \left(A_{\text{blank}} - A_{\text{sample}}/A_{\text{blank}}\right) \times 100$$

where A_{blank} is the absorbance of the control reaction mixture excluding the test compounds, and A_{sample} is the absorbance of the test compounds. Radical scavenging potential was expressed as IC_{50} value, which represents the sample concentration at which 50 % of the DPPH radicals scavenged. Tests were carried out in triplicate and the results were expressed as mean values ± standard deviations.

Hydroxyl radical scavenging assay

This assay was performed by a standard method (Jayabharathi *et al.*, 2012), with a slight modification. Hydroxyl radical scavenging of quinazolinone derivatives was carried out by measuring the competition between 2-deoxyribose and the synthesized compounds for hydroxyl radicals. The assay is based on quantification of the degradation product of 2-deoxyribose by condensation with thiobarbituric acid. The hydroxyl radicals ($^{\bullet}OH$) in aqueous media were generated

through the Fenton system. The assay was performed by mixing 0.36 ml of 2-deoxyribose (2.8 mM), 0.33 ml of phosphate buffer (20 mM, pH-7.4), 1 ml of test solution (10–100 µl), 0.1 ml of hydrogen peroxide (1 mM), 0.1 ml of ascorbic acid (100 mM), 0.1 ml of EDTA (100 mM), and 0.01 ml of $FeCl_3$ (100 mM) and this mixture was incubated at 37 °C for 1 h. Thereafter, 1 ml of cold 2.8 % trichloroacetic acid was added and reactivity was developed by adding 1 ml of thiobarbituric acid (1 % w/v) followed by heating at 100 °C for 15 min, the absorbance of the cooled mixture was measured at 532 nm. Butylated hydroxyanisole (BHA) was used as a positive control. All samples and the control were made in triplicate and the results were expressed as mean values ± standard deviations.

NO radical scavenging assay

NO radical scavenging capacity is based on the method of Padmaja *et al.*, (2011). The assay is based on generation of NO from sodium nitroprusside (SNP) and it was measured by the Griess reagent. SNP in aqueous solution at physiological pH spontaneously generates NO which interacts with oxygen to produce nitrite ions, which can be quantified by the Griess reagent. The reaction mixture containing 1 ml of SNP (10 mM), 1.5 ml of phosphate buffer (pH 7.4), and test solution (10–100 µl) was incubated for 150 min at 25 °C. Then, 1 ml of Griess reagent (1 % sulfanilamide in 3 % phosphoric acid and 0.2 % *N*-(1-naphthyl)ethylenediamine dihydrochloride) was added to the reaction mixture and allowed to stand for 3 min, the absorbance of this solution was measured at 546 nm against the reagent blank. All samples and controls were made in triplicate. IC_{50} values were determined and the results were expressed as mean values ± standard deviations.

Superoxide radical scavenging assay

Superoxide radical scavenging activity was measured as described by Kovala-Demertzi *et al.*, (2004). The assay is based on the reduction of nitroblue tetrazolium (NBT) by superoxide ions generated by the xanthine/xanthine oxidase (X–XO) system. The reaction mixture contained test samples (10–100 µl), 0.2 mM xanthine, and 0.6 mM NBT in 0.1 M phosphate buffer, pH 7.8. The reaction was started by the addition of xanthine oxidase (0.07 U ml^{-1}) to the reaction mixture, an activity which allowed yielding the absorbance change between 0.03 and 0.04 per minute, at 560 nm. The extent of NBT reduction was followed spectrophotometrically, by measuring the increase of the absorbance at 560 nm. All the experiments were replicated three times. The IC_{50} of each compound was defined as the concentration which inhibited 50 % of the NBT reduction by superoxide ions produced by the X–XO system.

Results and discussion

Chemistry

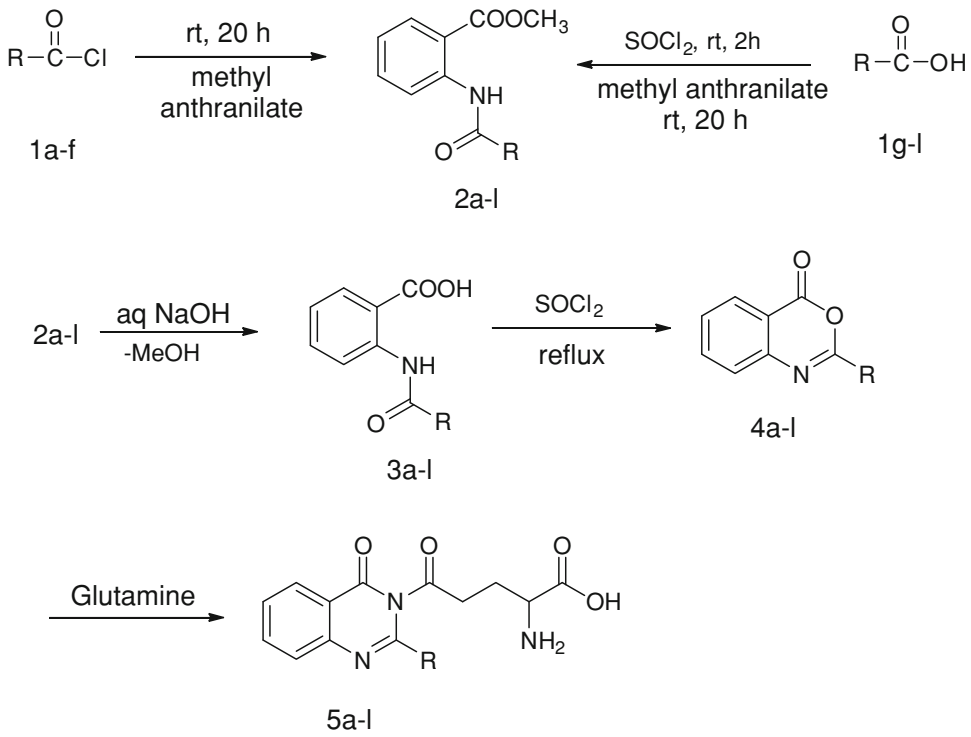
The strategy to synthesize the target quinazolinone derivatives is depicted in Scheme 1. The first synthetic step involves the amidation of methyl anthranilate with different substituted acid chlorides (**1a–f**) in dichloromethane giving rise to the respective diaryl amide intermediates. On the other hand, different substituted aromatic acids (**1g–l**) were converted to their corresponding acid chlorides by reaction with an excess of thionyl chloride in THF and allowed to react with methyl anthranilate to yield the respective diaryl amide intermediates. The products (**2a–l**) were subjected to hydrolysis using aqueous sodium hydroxide in methanol and subsequent acidification, to afford the deprotected carboxylic acid (Jeffrey *et al.*, 2008) followed by cyclization of **3a–l** using thionyl chloride to afford the desired benzoxazinones **4a–l**. Finally, benzoxazinones were coupled with glutamine to give the respective 2,3-disubstituted quinazolinone derivatives **5a–l** in high yields. TLC was run throughout the reaction to optimize the reaction for purity and completion. The physicochemical data for the newly synthesized compounds are presented. All the synthesized compounds were characterized by their physical, analytical, and spectral data. The chemical structures and physical data of all the synthesized compounds are given in Table 1. Compounds gave agreeing values in elemental analysis.

IR spectrum of compound **5e** revealed the presence of two carbonyl groups at 1,675 and 1,627 cm^{-1}. It also showed $–NH_2$ and –COOH stretching bands at 3,354 and 2,667 cm^{-1}, respectively, and these bands were observed for other compounds at expected regions. A band in the range of 2,660–2,674 cm^{-1} was obtained due to COOH stretching. In general, the IR spectra of compounds **5a–l** exhibited C=N stretching absorption bands in the range of 1,539–1,589 cm^{-1}. The IR spectra of all the compounds showed characteristic band in the region 3,341–3,374 cm^{-1} indicating the presence of ($–NH_2$) primary amine group.

The proton magnetic resonance spectra of synthesized compounds were recorded in $CDCl_3$. The chemical shift and multiplicity patterns correlated well with the proposed structures. ^1H NMR spectra of all the synthesized quinazolinone analogs showed NH_2 proton as singlet at 5.35–5.64 ppm. The signal due to acidic OH in all the analogs appeared as singlet at 11.21–12.16 ppm. In addition to acidic OH, $–OCH_3$ protons present in the compound **5g** resonated as a singlet at 3.67 ppm. Other aromatic protons were observed at the expected regions.

The mass spectra of compounds are in agreement with their structures. All the spectra exhibit parent peaks due to molecular ions (M^+) and the chlorine substituted compounds give (M^{+2}) peak. The proposed molecular formula

Scheme 1 synthesis of glutamine linked 2,3-disubstituted quinazolinone derivatives (**5a–l**)



of each compound was confirmed by its molecular formula weight with *m/z* values. The mass spectra of **5b** and **5g** showed molecular ion peak M^+ at 405 and 381, respectively, corresponding to their molecular formula.

Antimicrobial activity

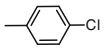
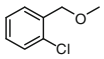
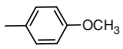
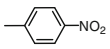
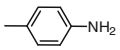
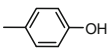
All the newly synthesized compounds were evaluated for their in vitro antibacterial activity by disk diffusion method and MIC against an assortment of two Gram-positive bacteria (*B. subtilis* and *S. aureus*), two Gram-negative bacteria (*P. aeruginosa* and *E. coli*), and antifungal activity (against *C. albicans* and *A. flavus*) was evaluated. Standard antibiotic, streptomycin and antifungal drug fluconazole were used to compare the antibacterial and antifungal activities shown by compounds. Screening results are summarized in Table 2. The newly synthesized compounds **5a–l** exerted significant inhibitory activity against the growth of tested bacterial strains. The antibacterial data revealed that all tested compounds of this investigation were found to exhibit moderate to good activity against all the tested pathogenic bacteria as compared to the standard drug streptomycin.

The most potent antibacterial activity was exhibited by compound **5b** (MIC 8.6 μg/ml) with halogen substituted ring in the respective series compared to the compounds bearing other electron donating or withdrawing groups. The inclusion of nitro group to the phenyl ring (**5h**) produced moderate activity against *S. aureus* with a MIC of 9.6 μg/ml. A close analysis of the screening results and structures of active compounds reveals that the halogen substitution of phenyl ring increased antimicrobial activity. The compound **5d** possesses active inhibition against all the tested strains and this may be attributed to the presence of chlorine in the compound. It is interesting to note that the electron withdrawing property of the phenyl ring is important, which is corroborated by the eminent activity of compounds with halogen group and decreased activity of compounds with either methyl or methoxy group in the phenyl ring. Hence, the replacement of methyl or methoxy group in place of halogens shows decrease in antibacterial activity. Other compounds exhibited moderate to good antibacterial activity against all organisms. Similarly, compound **5e** exhibited poor antibacterial activity against *S. aureus*, *E. coli*, and *P. aeruginosa* as compared to the standard drug (streptomycin).

A notable observation being that all the compounds containing COOH group showed better activity. This is due to the increase in the polarity of these compounds (Suresha *et al.*, 2009), which would penetrate the molecules through the lipid membrane and thus, they could inhibit the growth of the microorganisms. It can be concluded that the antimicrobial activity of such compounds may change by the introduction or elimination of a specific group.

The screening data of antifungal activity of these series of compounds showed wide range of antifungal activity. Compounds **5b** and **5h** exhibited good antifungal activity against *C. albicans* and *A. flavus* compared to the standard drug fluconazole. The remaining compounds were moderately

Table 1 Chemical structure, yield, and physical characterization of synthesized compounds

Compound	*R*	Yield (%)	m. p.(°C)
5a		85	194–197
5b		78	196–198
5c		80	216–218
5d		76	190–193
5e		73	214–216
5f		84	210–213
5g		82	212–215
5h		76	198–200
5i		80	211–213
5j		82	214–216
5k		79	210–212
5l		84	205–208

active against these two microorganisms. In the whole series, compound **5h** with MIC 10 μg/ml showed the highest percentage inhibition against both fungal strains, whereas none of the tested compounds restricted the fungal growth. The compounds **5a** and **5c** displayed differences in activity due to the presence of fluoro group at different positions. Instead of methyl or methoxy groups, halogen substituted compounds **5a**, **5b**, and **5c** seemed better in displaying pronounced inhibitory power against *C. albicans* and *A. flavus*. Compound **5e** showed very less potency in comparison with reference fluconazole, while all other compounds exhibited MIC between 10 and 100 μg/ml.

Antioxidant activity

Since the synthesized compounds exhibited good antimicrobial activity, it was considered worthwhile to study other potential aspects of these compounds, such as antioxidant and antiradical activity. The role of free radicals and reactive oxygen species is becoming increasingly recognized in the pathogenesis of many human diseases, including cancer, aging, and atherosclerosis (Halliwell and Whiteman, 2004). Antioxidants are playing a key role in offering cure to various life-style related diseases (Wua *et al.*, 2011). So, their antioxidant capacity cannot be fully described using a single method because antioxidant capacity is influenced by many factors. Therefore, various assays were performed to assess the radical scavenging activity of the compounds in cell free system. Accordingly, we have performed the following in vitro assays namely, inhibition of DPPH radical, superoxide radical scavenging, hydroxyl radical scavenging, and NO radical scavenging methods to assess the antioxidant properties of synthesized compounds, in comparison with the standards, such as ascorbic acid and BHA.

A freshly prepared DPPH solution exhibited a deep purple color with an absorption maximum at 517 nm. The absorbance decreased when the antioxidant molecule quenched DPPH free radical through donation of hydrogen atom or electron to form a stable DPPH molecule, resulting in a color change from purple to yellow. Hence, rapidity of the absorbance decrease was linked the more potent antioxidant activity of such compound.

The scavenging effect of the synthesized compounds is given in Table 3. All the compounds (**5a–l**) showed comparable or slightly less activity to the standards (ascorbic acid and BHA). The antioxidant activity was expressed as the 50 % inhibitory concentration (IC_{50}) based on the amount of compound required for a 50 % decrease in the initial DPPH radical concentration. It was observed that all the compounds notably reduced the concentration of DPPH free radical. It was clearly demonstrated that free radical scavenging increased with increasing concentration.

The difference in radical scavenging activity of the synthesized quinazolinone compounds (**5a–l**) were due to the difference in the stability of the oxygen centered radical formed in these compounds. The better activity of compound **5l** (IC_{50} 14.3 μg/ml) having hydroxyl group at *p*-position in the aromatic ring is due to high electron-releasing properties (positive mesomeric effect is higher than negative inductive effect) and this activates the aromatic ring. The compound **5g** (IC_{50} 18.4 μg/ml) bearing an electron donating methoxy group at para position showed better DPPH radical scavenging activity compared to **5k** (IC_{50} 29.7 μg/ml). The compound **5h** (IC_{50} 36.4 μg/ml) exhibited less activity compared to compound **5g** due to the presence of electron withdrawing nitro group instead of a methoxy group in the same position.

Observing the overall data for antioxidant activity, it is clear that compounds **5g**, **5i**, and **5l** were found to be the most efficacious antioxidants among all the compounds, obviously this confers great influence on radical scavenging

Table 2 Antimicrobial activity of the synthesized compounds

Compounds	In vitro activity zone of inhibition in mm (MIC in μg/ml)					
	Gram positive		Gram negative		Fungi	
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. flavus*
5a	11 (13)	16 (15)	12 (50)	14 (25)	11 (15)	10.4 (25)
5b	13.2 (08)	13.5 (08)	12.7 (12)	14.8 (10)	13.6 (10)	14.4 (15)
5c	14 (12.5)	12 (12.5)	11 (30)	17 (35)	11.4 (10)	10.9 (15)
5d	13 (10)	12.7 (11.2)	11.5 (15)	12 (15)	13.6 (12)	12.1 (15)
5e	10 (50)	07 (50)	11 (>100)	11 (>100)	14 (100)	10.2 (75)
5f	12 (30)	14 (50)	10 (50)	13 (50)	13.7 (25)	11 (40)
5g	14 (30)	10 (50)	11 (20)	13 (25)	10 (25)	14 (15)
5h	14 (9.6)	16 (12.5)	09 (25)	11 (25)	14.1 (10)	13.6 (12.5)
5i	10 (25)	13 (30)	15 (50)	16 (50)	12.5 (50)	11 (50)
5j	11 (50)	12 (30)	10 (50)	11 (30)	12 (25)	15 (25)
5k	11 (45)	11 (50)	15 (50)	12 (75)	14 (30)	10 (25)
5l	13 (25)	09 (25)	15 (50)	13 (50)	09 (40)	15 (40)
Streptomycin	14.1 (05)	13.4 (05)	16.5 (05)	14 (05)	–	–
Flucanazole	–	–	–	–	16 (05)	19 (05)

activity due to the presence of electron donating OCH_3, NH_2, and OH groups on the phenyl ring and their ability to scavenge DPPH.

Hydroxyl radical scavenging capacity is directly related to its antioxidant activity and hydroxyl radicals are the major active oxygen species and they mediate various types of reactions including enormous biological damage and lipid oxidation (Boguslaw, 2011). Biologically, the hydroxyl radical is widely believed to be generated when hydrogen peroxide reacts with Fe(II) (Fenton reaction). This assay showed the abilities of the compounds and standard to inhibit hydroxyl radical mediated deoxyribose degradation in an Fe^{3+}-EDTA-ascorbic acid and H_2O_2 reaction mixture. The values of IC_{50} of compounds for hydroxyl radical ranged from 17.8 to 55.1 μg/ml. The compound **5l** showed higher abilities of scavenging for hydroxyl radical, possibly due to the key role of functional group –OH, which can react with hydroxyl radical to form stable macromolecular radicals by the typical H-abstraction reaction (Ueda *et al.*, 1996). The significant increase in the scavenging activity, observed in compounds **5g** and **5i** compared to that of compound **5k** can be attributed to the presence of methoxy and amine groups and for increased protection against H_2O_2 rather than an increase in reactive oxygen species concentrations. The scavenging of hydroxyl radical by the compounds increased in dose-dependent manner.

In addition to reactive oxygen species, NO radical is implicated in pathogenesis of several diseases and a potent pleiotropic inhibitor of physiological processes. NO is a very unstable species under aerobic condition. It reacts with O_2 to produce stable products nitrate and nitrite through intermediates. Among the tested compounds, compounds **5i** and **5l** showed the most potent activity. The IC_{50} values of the compounds **5i** and **5l** were found to be 17.5 and 15.2 μg/ml, respectively. The NO generated from SNP at physiological pH and its metabolic product peroxynitrite (ONOO–) was formed after reacting with oxygen. The nitrite formation was suppressed by compounds which directly compete with oxygen in the reaction with NO. The scavenging of NO was found to increase in dose-dependent manner.

Superoxide is biologically quite toxic and is deployed by the immune system to kill invading microorganisms. It is

Table 3 Antioxidant activity of the synthesized compounds

Compounds	IC_{50} (μg/ml)			
	DPPH	$^{\bullet}OH$	$O_2^{\bullet}$	NO
5a	27.4 ± 0.11	38.1 ± 0.04	45.7 ± 0.17	40.1 ± 0.22
5b	29.7 ± 0.02	55.1 ± 0.15	56.0 ± 0.08	48.2 ± 0.13
5c	34.9 ± 0.03	41.8 ± 0.17	50.8 ± 0.13	46.1 ± 0.02
5d	37.1 ± 0.07	49.0 ± 0.09	41.8 ± 0.04	37.2 ± 0.17
5e	26.6 ± 0.16	29.8 ± 0.11	31.6 ± 0.16	36.2 ± 0.04
5f	26.1 ± 0.11	27.7 ± 0.06	31.4 ± 0.19	38.7 ± 0.12
5g	18.4 ± 0.17	23.4 ± 0.20	24.7 ± 0.17	20.1 ± 0.08
5h	36.4 ± 0.08	39.0 ± 0.16	43.2 ± 0.26	49.0 ± 0.19
5i	17.5 ± 0.05	18.0 ± 0.03	19.4 ± 0.17	17.5 ± 0.27
5j	24.9 ± 0.12	30.4 ± 0.10	25.4 ± 0.04	29.1 ± 0.14
5k	29.7 ± 0.22	31.2 ± 0.03	32.0 ± 0.16	36.0 ± 0.17
5l	14.3 ± 0.14	17.8 ± 0.19	18.3 ± 0.07	15.2 ± 0.05
AA[a]	12.6 ± 0.43	–	–	–
BHA[b]	–	15.3 ± 0.76	13.4 ± 0.29	14.6 ± 0.11

[a] Ascorbic acid

[b] Butylated hydroxyanisole

an oxygen centered radical with selective reactivity. Superoxide anion is a precursor to active free radicals. Superoxide is very harmful to cellular components (Liang *et al.*, 2010) and it has been observed to directly initiate lipid peroxidation (Wickens, 2001). The superoxide radical generated in vitro by X–XO can be measured by its ability to reduce NBT. The synthesized compounds have strong scavenging effect on superoxide radicals. The inhibitory effects of the tested compounds on superoxide radical were concentration related and the suppression ratio increased with the increasing sample concentration. The compounds clearly showed significant superoxide radical scavenging activity compared with that of standard antioxidants like BHA (IC_{50} 10.19 μM). In the present study, the compounds **5i** and **5l** showed the reduction of NBT and the IC_{50} values were found to be 19.4 and 18.3 μg/ml, respectively. These values are higher than the value observed for other synthesized compounds due to the presence of amine and hydroxyl groups. Among others, the compounds **5a**, **5b**, and **5c** showed the least scavenging activity as shown by the high value of IC_{50}.

Compounds **5i** and **5l** showed the higher scavenging effect than the other compounds. The compounds **5a** and **5c** showed difference in activity due to the presence of fluoro group at different positions. Compound **5h** showed the lowest radical scavenging activity due to the presence of strong electron withdrawing nitro group situated at para position, which would destabilize the ring.

Conclusion

In summary, we have synthesized a small library of novel 2,3-disubstituted quinazolinone analogs and assessed their antimicrobial and antioxidant activities. Most of the new compounds exhibited substantial antimicrobial and antioxidant activity, while four analogs showed diverse antimicrobial activity against selected kinds of bacteria and fungi. In particular, three compounds (**5g**, **5i**, and **5l**) showed higher potency of radical scavenging activity than other analogs with IC_{50} values of 14.3–24.7 μg/ml.

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