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Quinazolinones linked amino acids derivatives as a new class of promising antimicrobial, antioxidant and anti-inflammatory agents

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ABSTRACT

Two series of amino acids conjugated quinazolinones (1a-h and 2a-h) were synthesized by acid-amine coupling and the structures of all the compounds were confirmed through spectroscopic techniques such as IR, NMR and HRMS. The synthesized compounds were evaluated for their antimicrobial, antioxidant and anti-inflammatory activities. Biological evaluation study revealed that, the compounds 1f, 2f, 2g and 1g showed good antioxidant activity with 50% of the inhibition concentration (IC_{50}) values 35, 20, 30 and 40 µg/mL, respectively, much better than the standard BHT (IC_{50} = 45 µg/mL). The compounds 1g, 2e and 2g found to have promising anti-inflammatory activity and almost all the synthesized compounds exhibited good antimicrobial activities (antibacterial and antifungal) against all the selected pathogenic bacteria and fungi. Conjugates containing Trp, Tyr and Pro have shown better activity than the rest of the analogues in the series. The structure-activity relationship was established for these compounds.

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1. Introduction

Among the different nitrogen containing heterocycles, quinazolinone plays an important role in medicinal chemistry and subsequently have emerged as a pharmacophore. The quinazolin-4(3*H*)-one structural motifs have attracted a great deal of interest due to their ready accessibility, diverse chemical reactivity and wide gamut of biological activities like antibacterial [1], antifungal [2], antitumour [3], hypotensive [4], anti-HIV [5], anti-inflammatory [6], *etc*. Quinazolinones are excellent reservoir of bioactive substances.

Since the last half of the 20th century, bacteria have developed resistance against currently available drugs and therefore it is an ongoing effort for medicinal chemists to synthesize new chemotherapeutic agents. Infectious diseases caused by bacteria and fungi affect millions of people worldwide. Concerted and systematic progresses to discover and develop new antibiotics are always done due to the development of resistance by the microorganisms to the drugs commonly used against them. The rapid rise in bacterial resistance to the traditional antibiotics such as penicillins [7] and tetracyclines [8] had encouraged a continuing search for new classes of compounds with novel modes of antibacterial activity.

Reactive oxygen species (ROS) in the forms of superoxide anion ($^{\bullet}O_2^-$), hydroxyl radical ($^{\bullet}OH$) and hydrogen peroxide (H_2O_2) are generated from metabolism or environmental sources interact continuously with biological systems and their uncontrolled generation correlate directly with molecular level of many diseases [9]. Although a living system possesses several natural defense mechanisms, such as enzymes and antioxidant nutrients, which arrest the chain reaction of ROS initiation and production, as these substances are unable to prevent the damage completely [10]. Therefore, there has been a considerable interest to develop non-toxic and highly active antioxidant compounds that demonstrate measurable health benefits.

Inflammation is a multi-factorial process. It reflects the response of the organism to various stimuli and is related to many disorders such as arthritis, asthma and psoriasis which require prolonged or repeated treatment [11]. It is well known that non-steroidal anti-inflammatory drugs (NSAIDs) are associated with several side effects such as gastrointestinal mucosal damage, bleeding, intolerance and renal toxicity.

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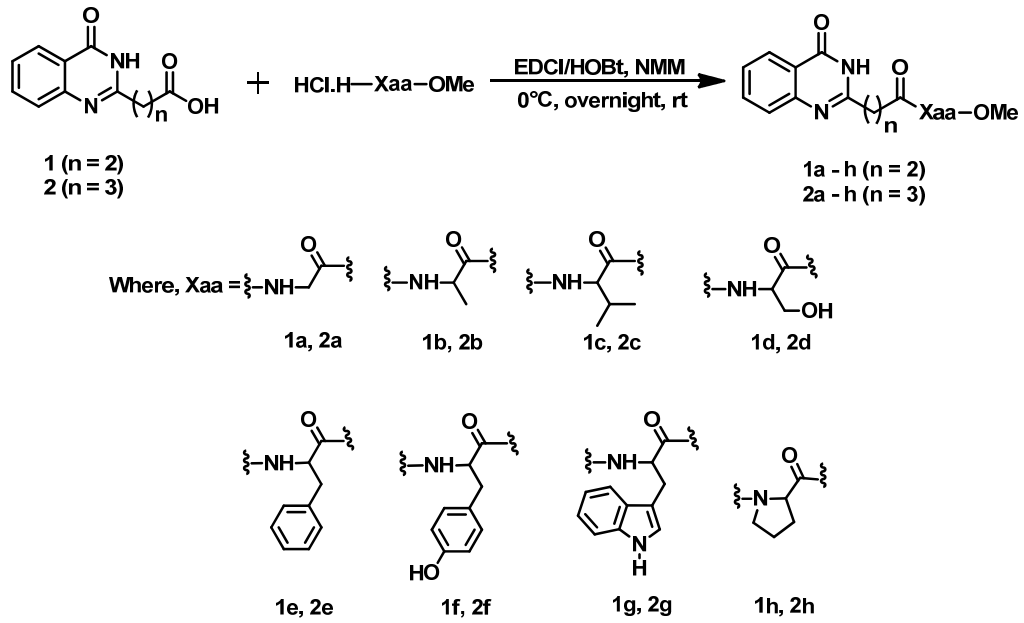


Reagents: 1-(3-Dimethylaminopropyl)-3-ethyl-carbodiimide (EDCI), 1-Hydroxy benzotriazole (HOBt), *N*-Methylmorpholine (NMM).

Scheme 1

If inflammation is not combated in right time, it leads to multiple deadly diseases. This has led intense efforts to search potent anti-inflammatory agents with fewer risks.

Moreover, microbes are the root cause of most of the diseases especially for the formation of ROS and inflammatory lesions which progresses towards the plethora of problems. Hence, the synthesis of compounds which can act against all these propaganda of disorders would be of prime interest. On the other hand, earlier reports have shown that conjugation of different amino acids/peptides to various biologically active scaffolds has fetched remarkable results, which are very promising and even enthusiastic [12-14]. Further, amino acid/ peptide based drugs have low toxicity, ample bioavailability and permeability, modest potency and good metabolic and pharmacokinetic properties [15]. Prompted by all these observations and with a further interest to develop more biologically active compounds, the present work encompasses the synthesis of novel quinazolinone-based amino acids analogues as promising antimicrobial/antioxidant/anti-inflammatory agents.

2. Experimental

2.1 Instrumentation

All the amino acids used except glycine were of *L*-configuration unless otherwise mentioned. All methyl ester amino acids, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide.HCl (EDCI), 1-hydroxybenzotriazole (HOBt) and trifluoroacetic acid (TFA) were purchased from Advanced Chem. Tech. (Louisville, Kentucky, USA). *N*-Methylmorpholine (NMM) was purchased from Sigma Aldrich (India). All other chemicals and reagents obtained from Spectrochem Pvt. Ltd. (India) and Rankem Pvt. Ltd. (India) were used without further purification. Melting points were determined on a Superfit melting point apparatus (India) and are uncorrected. FT-IR was performed using a Jasco spectrometer (Japan) using nujol media. Elemental analysis was performed by using VARIO EL III CHNS Elementar. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker multinuclear FT-NMR spectrometer (Japan) using DMSO-d_6 as solvent. The mass spectra were recorded on Waters-Synapt G2 Q-ToF in ESI mode spectrometer (USA). Progress of the reaction was monitored by TLC using silica gel coated on glass plates with the solvent system comprising chloroform/ methanol/acetic acid in the ratio 98:02:03 (R_f^a) and 95:05:03 (R_f^b) and the compounds on the TLC plates were detected by iodine vapors.

2.2. General procedure for the conjugation of methyl esters of amino acids to 3-(4-oxo-3,4-dihydroquinazolin-2-yl) propanoic acid (QZN 1) and 4-(4-oxo-3,4-dihydro quinazolin-2-yl)butanoic acid (QZN 2)

3-(4-Oxo-3,4-dihydroquinazolin-2-yl)propanoic acid (**QZN 1**) and 4-(4-oxo-3,4-dihydroquinazolin-2-yl)butanoic acid (**QZN 2**) (1 mmol) dissolved in dimethyl formamide (DMF) separately (10 mL/g of compound) and cooled to 0 °C was added NMM (1 mmol). EDCI (1 mmol) was added under stirring while maintaining the temperature at 0 °C and stirred for 15 min. HOBt (1 mmol) in DMF (2 mL) was added. The reaction mixture was stirred for an additional 10 min and a pre-cooled solution of methyl ester of amino acid (1 mmol) and NMM (1 mmol) in DMF was added slowly. After 20 min, pH of the solution was adjusted to 8 by the addition of NMM and the reaction mixture was stirred overnight at room temperature. DMF was removed under reduced pressure and the residue was poured into about 100 mL ice-cold 90% saturated $KHCO_3$ solution and stirred for 30 min. The precipitated product was taken into $CHCl_3$ and washed sequentially with 5% $NaHCO_3$ solution (2×20 mL), water (2×20 mL), 0.1 N cold HCl solution (2×20 mL) and finally brine (2×20 mL). The $CHCl_3$ layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The products so obtained were recrystallized from ether/ petroleum ether to get desired products **1a-h** and **2a-h** (Scheme 1 and 2).

Methyl 2-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)propan amido) acetate (**1a**): Color: White. Yield: 81%. R_f^a = 0.40. R_f^b = 0.44. M.p.: 195-196 °C. FT-IR (KBr, ν, cm^{-1}): 1630 (C=O) (amide), 1675 (C=O) (amide), 1690 (C=O) (ester), 3345 (NH).

^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 12.10 (1H, s, QZN-NH), 8.10 (1H, dd, *J* = 6.8 Hz, 1.4 Hz, H-5′′), 7.74 (1H, m, H-7′′), 7.60 (1H, d, *J* = 7.6 Hz, H-8′′), 7.44 (1H, m, H-6′′), 6.65 (1H, d, *J* = 7.0 Hz, NH), 3.90 (2H, d, *J* = 7.0 Hz, H-2), 3.69 (3H, s, -OCH_3), 2.81 (2H, t, *J* = 6.8 Hz, H-2′), 2.62 (2H, t, *J* = 6.8 Hz, H-3′). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 172.7 (C, C-1′), 170.2 (C, C-1), 161.2 (C, C-4′′), 156.4 (C, C-2′′), 148.5 (C, C-9′′), 133.8 (CH, C-7′′), 127.2 (CH, C-6′′), 126.2 (CH, C-8′′), 125.9 (CH, C-5′′), 120.4 (C, C-10′′), 51.9 (C, -OCH_3), 45.8 (CH_2, C-2), 30.2 (CH_2, C-2′), 28.8 (CH_2, C-1′). HRMS (ESI, *m/z*): 290.1236 [M+1]. Anal. calcd. for $C_{14}H_{15}N_3O_4$: C, 58.13, H, 5.23; N, 14.53. Found: C, 58.09; H, 5.12; N, 14.46%.

Methyl 2-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)propan amido)propanoate (**1b**): Color: White solid. Yield: 71%. R_f^a = 0.36. R_f^b = 0.42. M.p.: 138-140 °C. FT-IR (KBr, ν, cm^{-1}): 1638 (C=O) (amide), 1671 (C=O) (amide), 1682 (C=O) (ester), 3370 (NH). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 11.30 (1H, s, QZN-NH), 8.23 (1H, dd, *J* = 6.8, 1.2 Hz, H-5′), 7.72 (1H, m, H-7′′), 7.64 (1H, d, *J* = 7.2 Hz, H-8′′), 7.45 (1H, m, H-6′′), 6.68 (1H, d, *J* = 6.8 Hz, NH), 4.58 (1H, q, *J* = 6.8 Hz, H-2), 3.71 (3H, s, -OCH_3), 2.84 (2H, t, *J* = 7.2 Hz, H-2′), 2.41 (2H, t, *J* = 7.2 Hz, H-3′), 1.40 (3H, d, *J* = 6.8 Hz, CH_3-3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 172.2 (C, C-1′), 170.8 (C, C-1), 161.2 (C, C-4′′), 156.7 (C, C-2′′), 148.7 (C, C-9′′), 134.1 (CH, C-7′′), 127.7 (CH, C-6′′), 126.6 (CH, C-8′′), 126.1 (CH, C-5′′), 120.7 (C, C-10′′), 51.6 (C, -OCH_3), 48.9 (CH, C-2), 33.7 (CH_2, C-2′), 26.8 (CH_2, C-1′), 16.8 (CH_3, C-3). HRMS (ESI, *m/z*): 304.2145 [M+1]. Anal. calcd. for $C_{15}H_{17}N_3O_4$: C, 59.40, H, 5.65; N, 13.85. Found: C, 59.31; H, 5.54; N, 13.73%.

Methyl 3-methyl-2-(3-(4-oxo-3,4-dihydroquinazolin-2-yl) propanamido)butanoate (**1c**): Color: White solid. Yield: 86%. R_f^a = 0.43. R_f^b = 0.47. M.p.: 180-181 °C. FT-IR (KBr, ν, cm^{-1}): 1633 (C=O) (amide), 1685 (C=O) (amide), 1649 (C=O) (ester), 3320 (NH). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 10.92 (1H, s, QZN-NH), 8.26 (1H, dd, *J* = 6.8, 1.2 Hz, H-5′), 7.73 (1H, m, H-7′′), 7.64 (1H, d, *J* = 8.0 Hz, H-8′′), 7.44 (1H, m, H-6′′), 6.54 (1H, d, *J* = 8.4 Hz, NH), 4.60 (1H, q, *J* = 8.4 Hz, H-2), 3.72 (3H, s, -OCH_3), 3.07 (2H, t, *J* = 6.8 Hz, H-2′), 2.84 (2H, t, *J* = 6.8 Hz, H-3′), 2.20 (1H, m, H-3), 0.90 (6H, d, *J* = 6.8 Hz, $(CH_3)_2$). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 172.2 (C, C-1′), 172.0 (C, C-1), 161.8 (C, C-4′′), 156.1 (C, C-2′′), 148.4 (C, C-9′′), 134.1 (CH, C-7′′), 126.8 (CH, C-6′′), 126.1 (CH, C-8′′), 125.7 (CH, C-5′′), 120.7 (C, C-10′′), 57.4 (CH, C-2), 51.8 (C, -OCH_3), 33.7 (CH, C-3), 33.2 (CH_2, C-2′), 29.7 (CH_2, C-1′), 18.7 (CH_3, C-4), 18.4 (CH_3, C-5). HRMS (ESI, *m/z*): 332.0829 [M+1]. Anal. calcd. for $C_{17}H_{21}N_3O_4$: C, 61.62, H, 6.39; N, 12.68. Found: C, 61.49; H, 6.26; N, 12.56.

Methyl 3-hydroxy-2-(3-(4-oxo-3,4-dihydroquinazolin-2-yl) propanamido)propanoate (**1d**): Color: Brown gummy. Yield: 71%. R_f^a = 0.44. R_f^b = 0.49. FT-IR (KBr, ν, cm^{-1}): 1627 (C=O) (amide), 1638 (C=O) (amide), 1671 (C=O) (ester), 3318 (NH). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 11.09 (1H, s, QZN-NH), 8.25 (1H, dd, *J* = 6.8, 1.4 Hz, H-5′′), 7.71 (1H, m, H-7′′), 7.63 (1H, d, *J* = 8.0 Hz, H-8′′), 7.43 (1H, m, H-6′′), 6.67 (1H, d, *J* = 7.2 Hz, NH), 5.78 (1H, s, OH), 4.52 (1H, q, *J* = 7.2 Hz, H-2), 4.10 (2H, d, *J* = 7.2 Hz, H-3), 3.71 (3H, s, -OCH_3), 3.14 (2H, t, *J* = 7.2 Hz, H-2′), 2.84 (2H, t, *J* = 7.2 Hz, H-3′). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 171.7 (C, C-1′), 170.7 (C, C-1), 161.7 (C, C-4′′), 156.7 (C, C-2′′), 148.1 (C, C-9′′), 133.8 (CH, C-7′′), 128.0 (CH, C-6′′), 126.2 (CH, C-8′′), 125.9 (CH, C-5′′), 120.8 (C, C-10′′), 64.1 (CH_2, C-3), 57.2 (CH, C-2), 51.7 (C, -OCH_3), 30.6 (CH_2, C-1′), 29.4 (CH_2, C-2′). HRMS (ESI, *m/z*): 320.1053 [M+1]. Anal. calcd. for $C_{15}H_{17}N_3O_5$: C, 56.42, H, 5.37; N, 13.16. Found: C, 56.35; H, 5.26; N, 13.06%.

Methyl 2-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)propan amido)-3-phenylpropanoate (**1e**): Color: White solid. Yield: 72%. R_f^a = 0.36. R_f^b = 0.41. M.p.: 174-176 °C. FT-IR (KBr, ν, cm^{-1}): 1639 (C=O) (amide), 1652 (C=O) (amide), 1684 (C=O) (ester), 3302 (NH). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 11.30 (1H, s, QZN-NH), 8.24 (1H, d, *J* = 8.0, 1.4 Hz, H-5′′), 7.73 (1H, m, H-7′′), 7.61 (1H, d, *J* = 7.8 Hz, H-8′′), 7.46 (1H, m, H-6′′), 7.12 (3H, m, H-6, H-6′ & H-7) 7.05 (2H, m, H-5, H-5′), 6.78 (1H, d, *J* = 6.2 Hz, NH), 4.90 (1H, q, *J* = 6.2 Hz, H-2), 3.69 (3H, s, -OCH_3), 3.22 (2H, d, *J* = 6.2 Hz, H-3), 2.81 (2H, t, *J* = 7.2 Hz, H-2′), 2.44 (2H, t, *J* = 7.2 Hz, H-3′). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 172.0 (C, C-1′), 171.0 (C, C-1), 161.5 (C, C-4′′), 156.4 (C, C-2′′), 148.7 (C, C-9′′), 137.1 (C, C-4), 134.1 (CH, C-7′′), 128.9 (CH, C-6, C-6′), 128.1 (CH, C-5, C-5′), 126.7 (CH, C-6′′), 126.4 (CH, C-8′′), 125.8 (CH, C-5′′), 125.6 (CH, C-5), 120.8 (C, C-10′′), 53.5 (CH, C-2), 51.7 (C, -OCH_3), 36.7 (CH_2, C-3), 31.0 (CH_2, C-1′), 29.4 (CH_2, C-2′). HRMS (ESI, *m/z*): 380.0851 [M+1]. Anal. calcd. for $C_{21}H_{21}N_3O_4$: C, 66.48, H, 5.58; N, 11.08. Found: C, 66.40; H, 5.43; N, 11.01%.

Methyl 3-(4-hydroxyphenyl)-2-(3-(4-oxo-3,4-dihydroquina zolin-2-yl)propanamido) propanoate (**1f**): Color: Brown solid. Yield: 75%. R_f^a = 0.37. R_f^b = 0.42. M.p.: 178-179 °C. FT-IR (KBr, ν, cm^{-1}): 1632 (C=O) (amide), 1650 (C=O) (amide), 1670 (C=O) (ester), 3310 (NH). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 11.12 (1H, s, QZN-NH), 8.24 (1H, dd, *J* = 6.8, 1.4 Hz, H-5′′), 7.73 (1H, m, H-7′′), 7.65 (1H, d, *J* = 8.0 Hz, H-8′′), 7.44 (1H, m, H-6′′), 7.14 (2H, m, H-5, H-5′), 6.92 (2H, m, H-6, H-6′), 6.65 (1H, d, *J* = 6.8 Hz, NH), 5.88 (1H, s, OH), 4.54 (1H, q, *J* = 6.8 Hz, H-2), 3.68 (3H, s, -OCH_3), 3.21 (2H, d, *J* = 6.8 Hz, H-3), 2.81 (2H, t, *J* = 6.8 Hz, H-2′), 2.71 (2H, t, *J* = 6.8 Hz, H-3′). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 171.7 (C, C-1′), 170.8 (C, C-1), 161.7 (C, C-4′′), 156.7 (C, C-2′′), 153.8 (C, C-7), 148.8 (C, C-9′′), 134.7 (CH, C-7′′), 130.1 (C, C-4), 129.8 (CH, C-5, C-5′), 127.2 (CH, C-6′), 126.7 (CH, C-8′′), 125.9 (CH, C-5′′), 120.7 (C, C-10′′), 115.9 (C, C-6, C-6′), 58.2 (CH, C-2), 51.7 (C, -OCH_3), 37.8 (CH_2, C-3), 30.3 (CH_2, C-1′), 29.8 (CH_2, C-2′). HRMS (ESI, *m/z*): 396.1351 [M+1]. Anal. calcd. for $C_{21}H_{21}N_3O_5$: C, 63.79, H, 5.35; N, 10.63. Found: C, 63.68; H, 5.22; N, 10.54%.

Methyl 3-(1H-indol-3-yl)-2-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)propanamido) propanoate (**1g**): Color: Brown solid. Yield: 79%. R_f^a = 0.52. R_f^b = 0.59. M.p.: 109-110 °C. FT-IR (KBr, ν, cm^{-1}): 1641 (C=O) (amide), 1678 (C=O) (amide), 1690 (C=O) (ester), 3310 (NH). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 12.15 (1H, s, QZN-NH), 10.87 (1H, s, Indole-NH), 8.43 (1H, dd, *J* = 6.8, 1.2 Hz, H-5′′), 8.08 (1H, d, *J* = 6.8 Hz, H-6), 7.95 (1H, m, H-7′′), 7.74 (1H, d, *J* = 6.4 Hz, H-8′′), 7.53 (1H, m, H-6′′), 7.47 (1H, t, *J* = 8.4 Hz, H-7), 7.53 (1H, d, *J* = 7.6 Hz, H-9), 7.18 (1H, s, H-5), 7.07 (1H, m, H-8), 7.00 (1H, d, *J* = 7.2 Hz, NH), 4.53 (1H, q, *J* = 7.2 Hz, H-2), 3.61 (3H, s, -OCH_3), 3.18 (2H, d, *J* = 7.2 Hz, H-3), 2.82 (2H, t, *J* = 6.8 Hz, H-2′), 2.70 (2H, t, *J* = 6.8 Hz, H-3′). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 172.3 (C, C-1′), 169.4 (C, C-1), 161.7 (C, C-4′′), 156.2 (C, C-2′′), 148.2 (C, C-9′′), 137.3 (C, C-10), 133.2 (CH, C-7′′), 131.0 (CH, C-6′′), 128.8 (C, C-11), 127.1 (CH, C-8′′), 126.1 (CH, C-5′′), 123.5 (CH, C-5), 121.2 (CH, C-8), 120.9 (C, C-10′′), 116.6 (CH, C-6), 114.6 (CH, C-7), 110.7 (CH, C-9), 108.9 (C, C-4), 56.8 (CH, C-2), 51.8 (C, -OCH_3), 36.3 (CH_2, C-3), 34.7 (CH_2, C-2′), 32.3 (CH_2, C-1′). HRMS (ESI, *m/z*): 419.2134 [M+1]. Anal. calcd. for $C_{23}H_{22}N_4O_4$: C, 66.02, H, 5.30; N, 13.39. Found: C, 66.01; H, 5.20; N, 13.29%.

Methyl 1-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)propanoyl) pyrrolidine-2-carboxylate (**1h**): Color: White gummy. Yield: 83%. R_f^a = 0.41. R_f^b = 0.45. FT-IR (KBr, ν, cm^{-1}): 1634 (C=O) (amide), 1657 (C=O) (amide), 1680 (C=O) (ester). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 12.02 (1H, s, QZN-NH), 8.27 (1H, dd, *J* = 6.8, 1.2 Hz, H-5′′), 7.72 (1H, m, H-7′′), 7.70 (1H, d, *J* = 7.0 Hz, H-8′′), 7.45 (1H, m, H-6′′), 4.58 (1H, t, *J* = 6.8 Hz, $^{\alpha}$CH), 3.71 (3H, s, -OCH_3), 3.53 (2H, t, *J* = 6.8 Hz, $^{\delta}CH_2$), 2.78 (2H, t, *J* = 7.2 Hz, H-2′), 2.48 (2H, t, *J* = 7.2 Hz, H-3′), 2.18 (2H, m, $^{\beta}CH_2$), 2.01 (2H, m, $^{\gamma}CH_2$). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 171.8 (C, C-1′), 170.0 (C, C-1), 161.6 (C, C-4′′), 156.6 (C, C-2′′), 148.7 (C, C-9′′), 134.1 (CH, C-7′′), 127.5 (CH, C-6′′), 126.7 (CH, C-8′′), 125.8 (CH, C-5′′), 120.4 (C, C-10′′), 66.4 (C, $^{\alpha}$CH), 51.8 (C, -OCH_3), 46.5 (C, $^{\delta}CH_2$), 30.7 (CH_2, C-1′), 29.9 (C, $^{\beta}CH_2$), 28.3 (CH_2, C-2′), 24.3 (C, $^{\gamma}CH_2$). HRMS (ESI, *m/z*): 330.2631 [M+1]. Anal. calcd. for $C_{17}H_{19}N_3O_4$: C, 62.00, H, 5.81; N, 12.76. Found: C, 61.95; H, 5.70; N, 12.68%.

Methyl 2-(4-(4-oxo-3,4-dihydroquinazolin-2-yl)butanamido) acetate (**2a**): Color: White solid. Yield: 77%. R_f^a = 0.37. R_f^b =

0.41. M.p.: 184-185 °C. FT-IR (KBr, ν, cm^{-1}): 1627 (C=O) (amide), 1641 (C=O) (amide), 1660 (CO) (ester), 3327 (NH). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 12.16 (1H, s, QZN-NH), 8.09 (1H, dd, *J* = 6.8, 1.4 Hz, H-5′′), 7.76 (1H, m, H-7′′), 7.61 (1H, d, *J* = 7.6 Hz, H-8′′), 7.46 (1H, m, H-6′′), 6.67 (1H, d, *J* = 6.8 Hz, NH), 3.89 (2H, d, *J* = 6.8 Hz, H-2), 3.71 (3H, s, -OCH_3), 2.83 (2H, t, *J* = 6.8 Hz, H-2′), 2.62 (2H, t, *J* = 6.8 Hz, H-4′), 2.24 (2H, t, *J* = 6.8 Hz, H-3′). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 172.7 (C, C-1′), 170.2 (C, C-1), 161.2 (C, C-4′′), 156.2 (C, C-2′′), 148.4 (C, C-9′′), 133.2 (CH, C-7′′), 127.2 (CH, C-6′′), 126.8 (CH, C-8′′), 126.1 (CH, C-5′′), 120.2 (C, C-10′′), 51.8 (C, -OCH_3), 44.7 (CH_2, C-2), 33.7 (CH_2, C-3′), 31.9 (CH_2, C-1′), 25.3 (CH_2, C-2′). HRMS (ESI, *m/z*): 304.1065 [M+1]. Anal. calcd. for $C_{15}H_{17}N_3O_4$: C, 59.40, H, 5.65; N, 13.85. Found: C, 59.32; H, 5.54; N, 13.77%.

Methyl 2-(4-(4-oxo-3, 4-dihydroquinazolin-2-yl)butan amido)propanoate (**2b**): Color: White solid. Yield: 85%. R_f^a = 0.35. R_f^b = 0.41. M.p.: 187-188 °C. FT-IR (KBr, ν, cm^{-1}): 1626 (C=O) (amide), 1635 (C=O) (amide), 1668 (C=O) (ester), 3333 (NH). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 11.32 (1H, s, QZN-NH), 8.25 (1H, dd, *J* = 6.8, 1.2 Hz, H-5′), 7.74 (1H, m, H-7′′), 7.65 (1H, d, *J* = 7.6 Hz, H-8′′), 7.44 (1H, m, H-6′′), 6.66 (1H, d, *J* = 7.6 Hz, NH), 4.60 (1H, q, *J* = 7.6 Hz, H-2), 3.75 (3H, s, -OCH_3), 2.86 (2H, t, *J* = 7.0 Hz, H-2′), 2.38 (2H, t, *J* = 7.0 Hz, H-4′),2.22 (2H, q, *J* = 7.0 Hz, H-3′), 1.41 (3H, d, *J* = 7.6 Hz, CH_3-3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 173.2 (C, C-1′), 171.5 (C, C-1), 161.7 (C, C-4′′), 156.9 (C, C-2′′), 148.8 (C, C-9′′), 134.1 (CH, C-7′′), 126.7 (CH, C-6′′), 125.8 (CH, C-8′′), 125.6 (CH, C-5′′), 120.8 (C, C-10′′), 51.7 (C, -OCH_3), 47.4 (CH, C-2), 34.0 (CH_2, C-3′), 33.6 (CH_2, C-1′), 22.5 (CH_2, C-2′), 16.8 (CH_3, C-3). HRMS (ESI, *m/z*): 318.0734[M+1]. Anal. calcd. for $C_{16}H_{19}N_3O_4$: C, 60.56, H, 6.03; N, 13.24. Found: C, 60.47; H, 5.90; N, 13.18%.

Methyl 3-methyl-2-(4-(4-oxo-3, 4-dihydroquinazolin-2-yl) butanamido)butanoate (**2c**): Color: White solid. Yield: 79%. R_f^a = 0.38. R_f^b = 0.43. M.p.: 194-195 °C. FT-IR (KBr, ν, cm^{-1}): 1637 (C=O) (amide), 1670 (C=O) (amide), 1667 (C=O) (ester), 3340 (NH). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 10.94 (1H, s, QZN-NH), 8.26 (1H, dd, *J* = 6.8, 1.0 Hz, H-5′), 7.75 (1H, m, H-7′′), 7.68 (1H, d, *J* = 8.0 Hz, H-8′′), 7.45 (1H, m, H-6′′), 6.46 (1H, d, *J* = 8.4 Hz, NH), 4.59 (1H, q, *J* = 8.4 Hz, H-2), 3.76 (3H, s, -OCH_3), 2.84 (2H, t, *J* = 7.0 Hz, H-2′), 2.42 (2H, t, *J* = 7.0 Hz, H-4′), 2.21 (3H, m, H-3′, H-3), 0.95 (6H, d, *J* = 6.8 Hz, $(CH_3)_2$). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 172.1 (C, C-1′), 172.0 (C, C-1), 161.7 (C, C-4′′), 156.8 (C, C-2′′), 148.6 (C, C-9′′), 134.2 (CH, C-7′′), 126.6 (CH, C-6′′), 125.9 (CH, C-8′′), 125.6 (CH, C-5′′), 120.8 (C, C-10′′), 57.3 (CH, C-2), 51.5 (C, -OCH_3), 33.9 (CH_2, C-3′), 33.7 (CH, C-3), 29.7 (CH_2, C-1′), 22.7 (CH_2, C-2′), 18.9 (CH_3, C-4), 18.2 (CH_3, C-5). HRMS (ESI, *m/z*): 346.1044 [M+1]. Anal. calcd. for $C_{18}H_{23}N_3O_4$: C, 62.60, H, 6.66; N, 12.17. Found: C, 62.48; H, 6.57; N, 12.08%.

Methyl 3-hydroxy-2-(4-(4-oxo-3,4-dihydroquinazolin-2-yl)butanamido)propanoate (**2d**): Color: Brown gummy. Yield: 70%. R_f^a = 0.40. R_f^b = 0.46. FT-IR (KBr, ν, cm^{-1}): 1630 (C=O) (amide), 1641 (C=O) (amide), 1677 (C=O) (ester), 3340 (NH). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 11.12 (1H, s, QZN-NH), 8.23 (1H, dd, *J* = 6.8, 1.4 Hz, H-5′′), 7.70 (1H, m, H-7′′), 7.61 (1H, d, *J* = 7.8 Hz, H-8′′), 7.44 (1H, m, H-6′′), 6.65 (1H, d, *J* = 7.2 Hz, NH), 5.42 (1H, s, OH), 4.54 (1H, q, *J* = 7.2 Hz, H-2), 4.13 (2H, d, *J* = 7.2 Hz, H-3), 3.69 (3H, s, -OCH_3), 3.17 (2H, t, *J* = 7.4 Hz, H-2′), 2.83 (2H, t, *J* = 7.4 Hz, H-4′), 2.23 (2H, q, *J* = 7.4 Hz, H-3′). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 171.7 (C, C-1′), 170.3 (C, C-1), 161.8 (C, C-4′′), 156.2 (C, C-2′′), 148.7 (C, C-9′′), 134.1 (CH, C-7′′), 127.2 (CH, C-6′′), 126.9 (CH, C-8′′), 126.2 (CH, C-5′′), 120.4 (C, C-10′′), 66.2 (CH_2, C-3), 57.3 (CH, C-2), 51.9 (C, -OCH_3), 33.6 (CH_2, C-3′), 31.8 (CH_2, C-1′), 26.1 (CH_2, C-2′). HRMS (ESI, *m/z*): 334.1642 [M+1]. Anal. calcd. for $C_{16}H_{19}N_3O_5$: C, 57.65, H, 5.70; N, 12.61. Found: C, 57.52; H, 5.63; N, 12.56%.

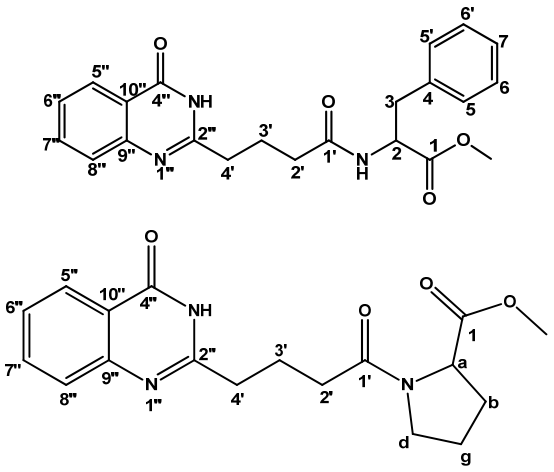
Methyl 2-(4-(4-oxo-3,4-dihydroquinazolin-2-yl)butan amido)-3-phenylpropanoate (**2e**): Color: White solid. Yield: 85%. R_f^a = 0.39. R_f^b = 0.45. M.p.: 182-183 °C. FT-IR (KBr, ν, cm^{-1}): 1631 (C=O) (amide), 1667 (C=O) (amide), 1678 (C=O) (ester), 3320 (NH). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 11.35 (1H, s, QZN-NH), 8.26 (1H, d, *J* = 8.0, 1.4 Hz, H-5′′), 7.72 (1H, m, H-7′′), 7.58 (1H, d, *J* = 8.0 Hz, H-8′′), 7.45 (1H, m, H-6′′), 7.17 (3H, m, H-6, H-6′ & H-7) 7.04 (2H, m, H-5, H-5′), 6.80 (1H, d, *J* = 7.2 Hz, NH), 4.94 (1H, q, *J* = 7.2 Hz, H-2), 3.68 (3H, s, -OCH_3), 3.17 (2H, d, *J* = 7.2 Hz, H-3), 3.04 (2H, t, *J* = 6.8 Hz, H-2′), 2.78 (2H, t, *J* = 6.8 Hz, H-4′), 2.18 (2H, t, *J* = 6.8 Hz, H-3′). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 172.0 (C, C-1′), 171.8 (C, C-1), 161.9 (C, C-4′′), 156.6 (C, C-2′′), 148.7 (C, C-9′′), 135.6 (C, C-4), 134.6 (CH, C-7′′), 129.1 (CH, C-5, C-5′), 128.5 (CH, C-6, C-6′), 127.1 (CH, C-6′′), 126.9 (CH, C-8′′), 126.6 (CH, C-5′′), 125.9 (CH, C-7), 120.9 (C, C-10′′), 53.2 (CH, C-2), 51.8 (C, -OCH_3), 37.8 (CH_2, C-3), 32.5 (CH_2, C-3′), 30.5 (CH_2, C-1′), 27.2 (CH_2, C-2′). HRMS (ESI, *m/z*): 394.1044 [M+1]. Anal. calcd. for $C_{22}H_{23}N_3O_4$: C, 67.16, H, 5.89; N, 10.68. Found: C, 67.08; H, 5.76; N, 10.57%.

Methyl-3-(4-hydroxyphenyl)-2-(4-(4-oxo-3, 4-dihydroquina zolin-2-yl)butanamido)-propanoate (**2f**): Color: Brown solid. Yield: 77%. R_f^a = 0.41. R_f^b = 0.46. M.p.: 176-177 °C. FT-IR (KBr, ν, cm^{-1}): 1637 (C=O) (amide), 1669 (C=O) (amide), 1679 (C=O) (ester), 3326 (NH). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 11.09 (1H, s, QZN-NH), 8.26 (1H, dd, *J* = 6.8, 1.4 Hz, H-5′′), 7.74 (1H, m, H-7′′), 7.64 (1H, d, *J* = 8.0 Hz, H-8′′), 7.43 (1H, m, H-6′′), 7.12 (2H, m, H-5, H-5′), 6.92 (2H, m, H-6, H-6′), 6.67 (1H, d, *J* = 6.8 Hz, NH), 5.70 (1H, s, OH), 4.53 (1H, q, *J* = 6.8 Hz, H-2), 3.69 (3H, s, -OCH_3), 3.24 (2H, d, *J* = 6.8 Hz, H-3), 2.84 (2H, t, *J* = 7.4 Hz, H-2′), 2.41 (2H, t, *J* = 7.4 Hz, H-4′), 2.20 (2H, t, *J* = 7.4 Hz, H-3′). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 172.1 (C, C-1′), 170.2 (C, C-1), 161.8 (C, C-4′′), 156.7 (C, C-2′′), 153.6 (C, C-7), 148.3 (C, C-9′′), 133.8 (CH, C-7′′), 130.7 (C, C-4), 130.2 (CH, C-5, C-5′), 126.9 (CH, C-6′′), 126.6 (CH, C-8′′), 125.9 (CH, C-5′′), 120.8 (C, C-10′′), 116.9 (C, C-6, C-6′), 57.3 (CH, C-2), 51.9 (C, -OCH_3), 37.3 (CH_2, C-3), 34.4 (CH_2, C-3′), 29.8 (CH_2, C-1′), 22.7 (CH_2, C-2′). HRMS (ESI, *m/z*): 410.0965 [M+1]. Anal. calcd. for $C_{22}H_{23}N_3O_5$: C, 64.54, H, 5.66; N, 10.26. Found: C, 64.42; H, 5.51; N, 10.13%.

Methyl-3-(1H-indol-3-yl)-2-(4-(4-oxo-3, 4-dihydroquinazo lin-2-yl)butanamido) propanoate (**2g**): Color: Reddish solid. Yield: 71%. R_f^a = 0.53. R_f^b = 0.60. M.p.: 135-136 °C. FT-IR (KBr, ν, cm^{-1}): 1640 (C=O) (amide), 1672 (C=O) (amide), 1680 (C=O) (ester), 3338 (NH). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 12.10 (1H, s, QZN-NH), 10.92 (1H, s, Indole-NH), 8.40 (1H, dd, *J* = 6.8, 1.2 Hz, H-5′′), 8.12 (1H, d, *J* = 7.6 Hz, H-6), 7.90 (1H, m, H-7′′), 7.70 (1H, d, *J* = 8.0 Hz, H-8′′), 7.51 (1H, m, H-6′′), 7.42 (1H, m, H-7), 7.30 (1H, d, *J* = 6.8 Hz, H-9), 7.19 (1H, s, H-5), 7.07 (1H, t, *J* = 6.0 Hz, H-8), 7.02 (1H, d, *J* = 6,8 Hz, NH), 4.55 (1H, q, *J* = 6.8 Hz, H-2), 3.68 (3H, s, -OCH_3), 3.20 (2H, d, *J* = 6.8 Hz, H-3), 2.88 (2H, t, *J* = 6.2 Hz, H-2′), 2.40 (2H, t, *J* = 6.2 Hz, H-4′), 2.21 (2H, q, *J* = 6.2 Hz, H-3′). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 172.9 (C, C-1′), 170.2 (C, C-1), 161.2 (C, C-4′′), 156.2 (C, C-2′′), 148.2 (C, C-9′′), 138.2 (C, C-10), 134.1 (CH, C-7′′), 130.2 (CH, C-6′′), 128.2 (C, C-11), 127.2 (CH, C-8′′), 126.9 (CH, C-5′′), 123.2 (CH, C-5), 121.3 (CH, C-8), 120.9 (C, C-10′′), 118.1 (CH, C-6), 117.1 (CH, C-7), 110.8 (CH, C-9), 108.7 (C, C-4), 57.4 (CH, C-2), 51.7 (C, -OCH_3), 36.7 (CH_2, C-3), 34.9 (CH_2, C-3′), 31.9 (CH_2, C-2′), 26.9 (CH_2, C-1′). HRMS (ESI, *m/z*): 433.1092 [M+1]. Anal. calcd. for $C_{24}H_{24}N_4O_4$: C, 66.65, H, 5.59; N, 12.96. Found: C, 66.57; H, 5.47; N, 12.88%.

Methyl 1-(4-(4-oxo-3,4-dihydroquinazolin-2-yl)butanoyl) pyrrolidine-2-carboxylate (**2h**): Color: White gummy. Yield: 87%. R_f^a = 0.34. R_f^b = 0.40. FT-IR (KBr, ν, cm^{-1}): 1640 (C=O) (amide), 1660 (C=O) (amide), 1685 (C=O) (ester). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 11.10 (1H, s, QZN-NH), 8.24 (1H, dd, *J* = 6.8, 1.2 Hz, H-5′′), 7.68 (1H, m, H-7′′), 7.62 (1H, d, *J* = 7.0 Hz, H-8′′), 7.42 (1H, m, H-6′′), 4.61 (1H, q, *J* = 5.6 Hz, $^{\alpha}$CH), 3.73 (3H, s, -OCH_3), 3.58 (2H, t, *J* = 8.6 Hz, $^{\delta}CH_2$), 3.16 (2H, t, *J* = 7.2 Hz, H-2′), 2.87 (2H, t, *J* = 7.2 Hz, H-4′), 2.18 (2H, m, $^{\beta}CH_2$), 2.06 (2H, m, $^{\gamma}CH_2$), 2.03 (2H, q, *J* = 7.2 Hz, H-3′). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 171.2 (C, C-1′), 170.2 (C, C-1), 161.7 (C, C-4′′), 156.2 (C, C-2′′), 148.3 (C, C-9′′), 133.7 (CH, C-7′′), 127.2 (CH, C-6′′), 126.6 (CH, C-8′′), 125.9 (CH, C-5′′), 120.7 (C, C-10′′), 65.9 (C, $^{\alpha}$CH), 51.7 (C, -OCH_3), 46.1 (C, $^{\delta}CH_2$), 33.7 (CH_2, C-3′), 31.8 (CH_2, C-1′), 29.7 (C, $^{\beta}CH_2$), 26.7 (CH_2, C-2′), 24.7 (C,

γCH$_2$). HRMS (ESI, *m/z*): 344.1342 [M+1]. Anal. calcd. for $C_{18}H_{21}N_3O_4$: C, 62.96, H, 6.16; N, 12.24. Found: C, 62.81; H, 6.08; N, 12.16%.



Scheme 2

2.3. Biological evaluation

2.3.1. Antimicrobial activity

2.3.1.1. Antibacterial activity

In vitro antibacterial activity was evaluated against human pathogens of Gram negative organisms namely *S. typhimurium*, *E. aerogenes*, *E. coli* and *K. pneumoniae* by agar well diffusion method [16]. The microorganisms were inoculated in to the sterilized nutrient broth and maintained at 37 °C for 24 hours. On the day of testing, bacteria were subcultured separately into 25 mL of sterilized nutrient broth. Inoculated subcultured broths were kept at room temperature for the growth of inoculums. Each test compounds (**1a-h**, **2a-h**) and standard drug (Streptomycin) of 10 mg was dissolved in 10 mL of DMSO to get a concentration of 1 mg/mL and further diluted to get a final concentration of 30 µg/mL. About 15-20 mL of molten nutrient agar was poured into each of the sterile plates. With the help of cork borer of 6 mm diameter, the cups were punched and scooped out of the set agar and the plates were inoculated with the suspension of particular organism by spread plate technique. The cups of inoculated plates were then filled with 0.1 mL of the test solution, streptomycin solution and DMSO (negative control). The plates were allowed to stay for 24 hours at 37 °C and zone of inhibition (mm) was then measured.

2.3.1.2 Antifungal activity

In vitro antifungal activity was evaluated against three fungal species namely *A. niger, F. monoliforme* and *C. albicans* by agar well diffusion method [17]. The fungal strains were subcultured separately into 25 mL of sterilized nutrient broth and incubated for one day to obtain the inoculums. Each test compounds (**1a-h**, **2a-h**) and standard drug (Bavistin) of 10 mg was dissolved in 10 mL of DMSO to get a concentration of 1 mg/mL and further diluted to get a final concentration of 30 µg/mL. Molten media of Sabouraud agar of 10-15 mL was poured into the petriplates and allowed to solidify. Fungal subculture was inoculated on the solidified media. With the help of 6 mm cork borer, the cups were punched and scooped out of the set agar. The cups of inoculated plates were then filled with 0.1 mL of the test solution, bavistin solution and DMSO (negative control). The plates were allowed to stay for 3 days at room temperature and zone of inhibition (mm) was then measured.

2.3.2 Antioxidant activity

The scavenging activity of DPPH free radicals by synthesized compounds was determined according to the reported method [18]. Briefly, 50 µL of test compounds was mixed at different concentrations (25, 50, 100, 200 and 300 µg/mL) with 1 mL of 0.1 mM DPPH in methanol solution and 450 µL of 50 mM Tris HCl buffer (pH = 7.4). Methanol (50 µL) only was used as the experimental control. After 30 min of incubation at room temperature, the reduction in the number of DPPH free radicals was measured by reading the absorbance at 517 nm. BHT (Butylatedhydroxyltoluene) was used as control similar to test concentrations. Percent inhibition was calculated from the equation (1).

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control - Absorbance of test sample}}{\text{Absorbance of control}} \times 100 \quad (1)$$

2.3.3. Anti-inflammatory activity

2.3.3.1. Human erythrocyte suspension

The whole blood was collected from a healthy volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment and collected in heparinized vacationer. The blood was washed three times with 0.9% saline and centrifuged simultaneously for 10 minutes at 3000 rpm. The packed cells were washed with 0.9% saline and 40% (*v*:*v*) suspension made using isotonic phosphate buffer which was composed of 154 mM NaCl in 10 mM Sodium Phosphate Buffer at pH = 7.4 used as stock erythrocyte or RBC suspension.

2.3.3.2. Hypotonic solution-induced haemolysis

The membrane stabilizing activity of the compounds was assessed according to the method [19] with slight modification. The test sample consisted of stock erythrocyte (RBC) suspension 0.5 mL mixed with 5 mL of hypotonic solution (50 mM NaCl in 10 mM Sodium Phosphate Buffered saline at pH = 7.4) containing different concentrations of sample (25, 50, 100, 200 and 300 µg/mL). The control consisted of 0.5 mL RBC suspension mixed with 5 mL of hypotonic buffered solution alone. The standard drug acetylsalicylic was treated similar to test concentration. The experiment was carried out in triplicate. The mixtures were incubated for 10 minutes at room temperature, centrifuged for 10 minutes at 3000rpm and absorbance of the supernatant was measured spectrophotometrically at 540 nm. The percentage inhibition of haemolysis or membrane stabilization was calculated by following equation.

$$\% \text{ Inhibition of haemolysis} = \frac{A_1 - A_2}{A_1} \times 100 \quad (2)$$

where, A_1 = Absorbance of hypotonic buffered solution alone, A_2 = Absorbance of test/standard sample in hypotonic solution.

3. Results and discussion

3.1. Chemistry

QZN 1 and **QZN 2** were synthesized following the procedure described earlier method [20-22] starting from anthranilamide and succinic anhydride or maleic anhydride to obtain propanoic acid analogue or butanoic acid analogue.

Table 1. Antibacterial and antifungal activities of the compounds.

Entry	Antibacterial activity [a,b,c]				Antifungal activity [a,b,d]		
	S. typhimurium	*E. aerogenes*	*K. pneumonia*	*E. coli*	*C. albicans*	*F. moniliforme*	*A. niger*
1	4±0.3	4±0.1	5±0.1	4±0.2	3±0.3	3±0.1	4±0.1
1a	14±0.2	16±0.7	10±0.6	10±0.8	15±0.9	11±0.4	11±0.7
1b	10±0.3	11±0.5	8±0.2	7±0.1	11±0.3	8±0.1	9±0.2
1c	12±0.4	14±0.3	10±0.1	9±0.4	13±0.1	9±0.4	10±0.6
1d	14±0.6	18±0.1	14±0.1	13±0.4	17±0.2	13±0.3	14±0.2
1e	16±0.2	20±0.2	16±0.4	17±0.1	19±0.5	17±0.1	18±0.3
1f	18±0.1	21±0.4	20±0.8	20±0.4	21±0.2	20±0.3	21±0.4
1g	21±0.6	25±0.2	21±0.7	22±0.2	25±0.8	21±0.4	23±0.2
1h	20±0.5	23±0.7	18±0.4	19±0.4	23±0.2	19±0.1	20±0.3
2	4±0.2	3±0.2	3±0.1	2±0.1	2±0.2	3±0.2	4±0.2
2a	13±0.3	14±0.3	9±0.4	8±0.40	13±0.8	9±0.3	9±0.8
2b	8±0.4	9±0.1	7±0.2	6±0.1	9±0.2	6±0.1	7±0.1
2c	10±0.4	13±0.5	9±0.1	9±0.3	11±0.1	7±0.2	8±0.3
2d	13±0.3	16±0.3	13±0.3	12±0.2	15±0.4	11±0.4	12±0.2
2e	15±0.4	18±0.2	14±0.4	15±0.4	17±0.1	15±0.1	16±0.4
2f	17±0.6	20±0.4	19±0.1	18±0.2	20±0.3	19±0.4	20±0.4
2g	20±0.4	23±0.7	20±0.3	20±0.7	24±0.7	19±0.2	21±0.3
2h	18±0.3	21±0.3	16±0.4	17±0.1	21±0.1	17±0.3	18±0.2
Std	11±0.4	13±0.6	12±0.4	13±0.2	14±0.5	11±0.3	13±0.2

[a] Values are mean of three determinations, the ranges of which are <5% of the mean in all cases.
[b] Zone of inhibition in mm.
[c] Std: Streptomycin.
[d] Std: Bavistin.

These quinazolinones (**1** and **2**) were conjugated to amino acid methyl esters using EDCI/HOBt as coupling agent and NMM as base in solution (Scheme 1). Completion of the reaction was confirmed by TLC and later by spectroscopic techniques like IR, NMR and MS. The stretching frequencies appeared at 1630-1644 (C=O) and 3300 cm^{-1} (NH) in IR spectra and in NMR spectra the peak appeared at δ ~6.65 ppm as a doublet (NH) and the absence of COOH peak confirms the conjugation. Further, the mass values obtained were in good agreement with the structures assigned. The presence of all requisite peaks and absence of extraneous peaks confirms the synthesis.

3.2. Antimicrobial studies

The efficiency of synthesized quinazolinones as antimicrobials were evaluated against various strains of Gram negative bacteria like *S. typhimurium*, *E. aerogenes*, *E. coli* and *K. pneumoniae* followed by antifungal studies against *A. niger*, *F. moniliforme* and *C. albicans* following agar dilution method. Zone of inhibition (mm) values are presented in Table 1. The standard drugs streptomycin and bavistin were used as standards for antibacterial and antifungal activities, respectively.

All the synthesized conjugates (**1a**-**h** and **2a**-**h**) showed good antimicrobial activity whereas the corresponding starting materials did not show any activity. This clearly testifies that conjugation definitely improves the activities of parent molecules (**1** and **2**). Compounds **1e**-**h** and **2e**-**h** showed excellent antimicrobial activities much better than the standards used. Whereas the compounds **1a**, **1c**, **1d**, **2a**, **2c** and **2d** exhibited comparable activities to that of the standards used.

Structure-activity relationship suggested that the antimicrobial activity of a particular molecule is apparently governed by the side chain of amino acids. The most active analogues **1g** and **2g** consist of tryptophan which has indole ring as its side chain. The activity may be due to its hydrophobic nature and the acid-base character of indole nucleus. The compounds **1h** and **2h** got second position among the series which have pyrrolidine ring of proline. The five membered ring of proline facilitates in improving the activity which could be due to steric conformation of this ring. Compounds with phenylalanine and tyrosine are also having good activity which may be attributed to the presence of aromatic phenyl ring and phenolic side chain, respectively. This confirms our earlier observations that aromatic amino acids play an important role in improving the activity [23]. Whereas the other amino acids derivatives like glycine (**1a**/**2a**) and serine (**1d**/**2d**) were also active which may be due to their simple side chain functionalities. The conjugation of alanine and valine were less active compared to reference standards may be due to steric hindrance of aliphatic side chains.

3.3. In-vitro antioxidant activity

Evaluation of antioxidant activity of the newly synthesized analogues was done by 2,2-diphenyl-1-picryl-hydroxyl (DPPH) radical scavenging activity. The antioxidant properties were expressed as 50% inhibitory concentration IC_{50} values (Table 2).

Quinazolinones scaffolds **1** and **2** exhibited poor activity and upon conjugation with amino acids improved their free radical scavenging ability. Compounds **1f**, **2f**, **1g** and **2g** showed excellent free radical scavenging property with IC_{50} values 35, 20, 40 and 30 µg/mL respectively, much better than the standard BHT (IC_{50} 45 µg/mL). This may be explained on the basis that electron donating indole of tryptophan possesses good antioxidant and free radical scavenging properties which is in great agreement with earlier results [24]. In addition, indole skeleton has been reported to act as a protective agent in different models of oxidative stress both *in vivo* and *in vitro* [25]. Further, the presence of phenolic -OH of tyrosine may be responsible for high activity of tyrosine containing analogues which is also in good agreement with earlier report [26].

3.4. In vitro anti-inflammatory activity

All the synthesized compounds were evaluated for their anti-inflammatory activity using human erythrocytes. A substantial number of compounds have been identified exhibiting good to moderate inhibitory activity compared to standard drug aspirin. IC_{50} was determined for the compounds showing more than 50% inhibition concentration (Table 2). Among the conjugates, phenylalanine and tryptophan conjugated heterocycles exhibited good activity compared to standard drug. This may be due to the presence of aromatic and hydrophobic indole group in tryptophan. The tyrosine and proline analogues exhibited moderate activity which may be due to the presence of phenolic group and five membered pyrrolidine ring system, respectively.

Table 2. Antioxidant and Anti-inflammatory of the conjugates.

Entry	Antioxidant activity [a,b], IC_{50} (µg/mL)	Anti- inflammatory activity [a,c], IC_{50} (µg/mL)
1	>300	>300
1a	>300	>300
1b	230±2.0	>300
1c	270±1.2	>300
1d	175±1.1	>300
1e	170±1.3	52±0.6
1f	35±0.9	86±0.9
1g	40±1.0	40±0.4
1h	75±1.2	88±0.9
2	285±1.2	>300
2a	>300	>300
2b	230±1.4	>300
2c	245±1.9	>300
2d	160±1.1	>300
2e	290±1.8	44±0.9
2f	20±0.5	84±0.8
2g	30±0.9	38±0.5
2h	95±1.0	88±0.3
Std	45±1.2	10±0.8

[a] Values are mean of three determinations, the ranges of which are <5% of the mean in all cases.
[b] Std: Butylated hydroxyl toluene.
[c] Std: Aspirin.

Compounds with tryptophan, tyrosine and proline showed excellent antimicrobial, antifungal, antioxidant and anti-inflammatory activities. Whereas compounds with phenyl alanine exhibited good antibacterial, antifungal and anti-inflammatory activities. Further compounds with **QZN 1** are more active than **QZN 2** with respect to antibacterial and antifungal side chains. On the other hand, **QZN 2** linked conjugates exhibited superior antioxidant and anti-inflammatory activities over **QZN 1** analogues. This small but definite difference in activity clearly indicates the importance of length of side chain in quinazolinones.

4. Conclusion

The methyl ester amino acids conjugated quinazolinones were synthesized and appraised for their efficacy as antibacterial, antifungal, antioxidant and anti-inflammatory activities. From the results, it may be summarized that some of the analogues are highly active than the standards particularly tryptophan, tyrosine and proline. This study is crystallizing in that the presence of side chain functionalities in the amino acids of the conjugates renders the moieties more potent. Further, compounds with **QZN 1** are more active than **QZN 2** with respect to antibacterial and antifungal studies. On the other hand, **QZN 2** linked conjugates exhibited superior antioxidant and anti-inflammatory activities over **QZN 1** compounds.

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