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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF NOVEL QUINAZOLINONE DERIVATIVES AS ANTI-INFLAMMATORY AGENTS

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Keywords:

5-chloro anthranilic acid, Quinazolinone, Anti-inflammatory activity, Diclofenac sodium

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ABSTRACT: A series of quinazolinone derivatives were synthesized and screened for anti-inflammatory activity. 5-chloro anthranilic acid undergoes acetylation in the presence of acetic anhydride and anhydrous sodium acetate to give 5-chloro-N-acetyl anthranilic acid as intermediate-I which upon cyclization in the presence of phosphorous pentaoxide, glacial acetic acid, and para aminobenzoic acid to yield 4-[6-chloro-2- methyl-4-oxoquinazolin -3(4H)-yl] benzoic acid as intermediate-II. This resulted in intermediate-II undergo mannich base reaction to produce novel quinazolinone derivatives (Q1 - Q16) on the reaction of formaldehyde with different aromatic amines. Sixteen different quinazolinone derivatives were synthesized. Structural assignments of these compounds have been made by elemental analysis, FTIR, 1H NMR, and mass spectral data. Among the synthesized compounds Q3, Q8, and Q 15 showed high anti-inflammatory activity against standard drug Diclofenac sodium. A majority of the tested compounds had shown good consequence to moderate anti-inflammatory activity.

INTRODUCTION: It is evident from the literature that, Quinazolinone is a heterocyclic compound play a vital role in synthetic, medicinal chemistry. The synthetic derivatives of quinazolinone are utilized as a therapeutic agent for combating against different pathological conditions. 5-chloro anthranilic acid is mainly employed for the synthesis of quinazolinone compounds as starting materials [1].

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Quinazolinone and its derivatives possess a major class of biologically active compounds which exhibited a large spectrum of therapeutic activities, including; anti-malaria [l2], analgesic [3], antioxidant [4], anticancer [5], antiviral [l6], anti-feedant [7], sedative-hypnotic [8], anticonvulsant [9], antimicrobial 10, antialgal [11], hypotensive [12] and anti-inflammatory [13].

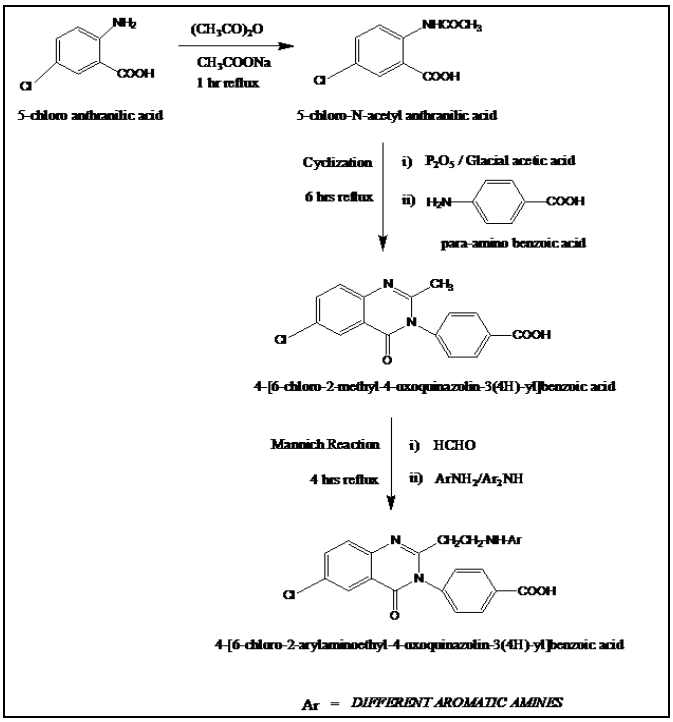
Recently quinazolinone derivatives seek the great attention of researchers in organic and medicinal chemistry due to their prompt biological activities. Encouraged by the therapeutic diversity of quinazolinone containing moiety and the comparative ease of convertibility of anthranilic acid to quinazolinone, we took up the synthesis of certain novel quinazolinone from 5-chloro

anthranilic acid and evaluated their anti-inflammatory activity [14].

EXPERIMENTAL:

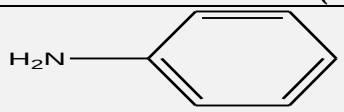
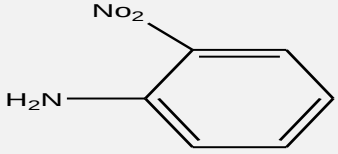
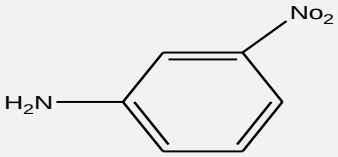
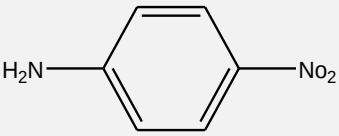
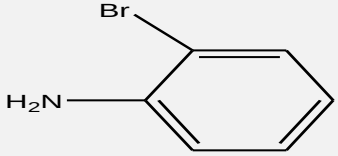
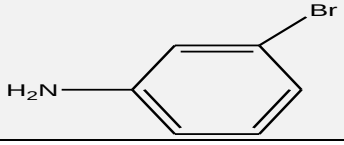
Materials and Methods: All the chemicals used in the synthesis of the intermediates and final derivatives were of A.R grade and procured from the Merck and LOBA chemicals. All the synthesized quinazolinone derivatives were characterized by melting point determination using Veergo digital melting point apparatus in open capillary tubes and were uncorrected.

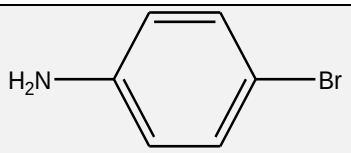
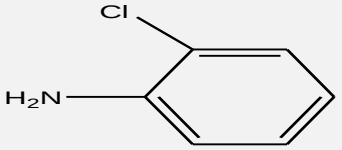
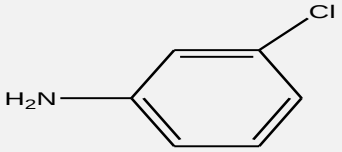
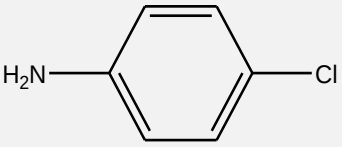
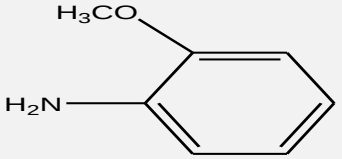
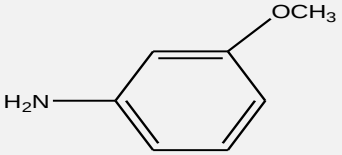
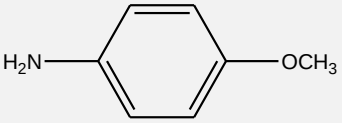
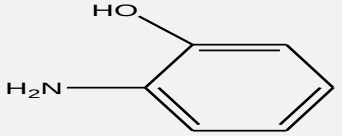
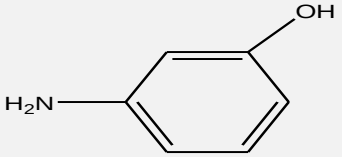
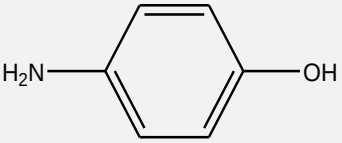
IR spectra were recorded using Perkin Elmer FTIR spectrophotometer using KBr pellets techniques, and ^{1}HNMR spectra of the synthesized compounds in deuteriated DMSO were recorded on BRUKER AVANCE II 400MHz NMR Spectrometer instrument using TMS as the internal standard. Mass spectra were recorded using LC-MSD-Tranp-SL2010A SHIMADZU using Dimethyl-sulphoxide (DMSO) as a solvent. TLC was performed using silica gel GF254 coated plates of 0.25 mm thickness. Ethyl acetate, petroleum ether, chloroform (0.6:0.8:8.6) were used as a solvent and iodine vapors as a visualizing agent.



SCHEME OF SYNTHESIS

TABLE 1: LIST OF VARIOUS AROMATIC AMINES

S. no.	Compounds Code	Substituted Aromatic Amine (Ar)	Structure of Aromatic Amine (Ar)
1	Q_1	Aniline	
2	Q_2	o-nitro aniline	
3	Q_3	m-nitro aniline	
4	Q_4	p-nitro aniline	
5	Q_5	o-bromo aniline	
6	Q_6	m-bromo aniline	

7	Q_7	p-bromo aniline	
8	Q_8	o-chloro aniline	
9	Q_9	m-chloro aniline	
10	Q_{10}	p-chloro aniline	
11	Q_{11}	o-methoxy aniline	
12	Q_{12}	m-methoxy aniline	
13	Q_{13}	p-methoxy aniline	
14	Q_{14}	o-hydroxy aniline	
15	Q_{15}	m-hydroxy aniline	
16	Q_{16}	p-hydroxy aniline	

The Experimental Work Comprises in Three Steps:

Step-I: Synthesis of 5-chloro-N-acetyl anthranilic acid from 5-chloro anthranilic acid.

Step-II: Synthesis of 4-[6-chloro-2-methyl-4-oxo quinazolin-3(4H)-yl] benzoic acid.

Step-III: Synthesis of various derivatives of quinazolinone by mannich reaction.

Step-I: General Procedure for the Synthesis of 5-Chloro-N-Acetyl Anthranilic Acid From 5-Chloro Anthranilic Acid (Intermediate-I): 5-Chloro anthranilic acid (0.02 moles) was mixed with equimolar quantities of anhydrous sodium acetate (0.03 moles) and acetic anhydride (0.04 moles in slight excess) and refluxed on sand bath under the anhydrous condition for 1 h. Then the reaction mixture was poured into ice-cold water,

and the crude product was filtered and dried. The dried crude product was recrystallized from ethanol. Yield: 81.34% M.P.: 188-190 ºC.

Step-II: General Procedure for the Synthesis of 4-[6-Chloro-2-Methyl-4-Oxo Quinazolin- 3(4H)-Yl] Benzoic Acid (Intermediate-II): 5-Chloro-N-acetyl anthranilic acid (0.01 moles) was added to a mixture of 4-Amino benzoic acid (0.02 moles), Phosphorus pentoxide (0.03 moles) and Glacial acetic acid (15 ml) and the mixture were refluxed under the anhydrous condition for 6 h. Then the reaction mixture was poured into 10% Sodium bicarbonate solution (50 ml), and the crude product was filtered and dried. The dried crude product was recrystallized from ethanol. Yield: 76.67% M.P.: 220-222 ºC.

Step-III: General Procedure for the Synthesis of Various Derivatives of Quinazolinone By Mannich Reaction (Q1-Q16) 4-[6-Chloro-2-Arylaminoethyl-4-Oxoquinazolin-3(4H)-Yl] Benzoic Acid: A mixture of 4-[6-Chloro-2-methyl-4-oxoquinazolin-3(4H)–yl] benzoic acid (0.01 mole), various aromatic amines (0.02 mole) and formaldehyde (0.02 mole) were taken in methanol (80 ml), and the reaction mixture was refluxed for 4 h. The completion of the reaction was monitored by TLC. The excess of the solvent was distilled off, and the residue was recrystallized from acetone to give the final product.

Q1: 4-(6-Chloro -4-Oxo- 2-(2-(Phenylamino) Ethyl) Quinazolin-3 (4H)-Yl) Benzoic Acid: Dark brown colored solid, Molecular formula: $C_{23}H_{18}ClN_3O_3$, Molecular weight: 419.86, Yield: 69.22%, M.P.: 176-178 °C, R_f value: 0.79, FT-IR (KBr, cm^{-1}): 3407.07 (N-H Str.), 2905.12 (C-H Str.), 1609.91 (C=C Str.), 1711.94 (C=O Str.), 1250.04 (C-N Str.), 734.56 (Ar C-H Bend.).

^{1}H-NMR (400 MHz, DMSO, δ ppm): 1.55 (t, 2H, CH_2), 3.22 (q, 2H, CH_2), 4.13 (t, ^{1}H, NH), 6.38-8.10 (m, 12H, Ar H), 11.10 (s, ^{1}H, COOH). Mass Spectra: m/z: 421.67 (M^{+2}). Elemental Analysis, % found (% required): C 65.64 (65.79); H 4.28 (4.32); N 9.93 (10.01); O 11.32 (11.43); Cl 8.38 (8.44).

Q2: 4-(6-Chloro -2-(2-(2- Nitrophenylamino) Ethyl)-4-Oxoquinazolin-3(4H)-Yl) Benzoic Acid: Yellowish brown colored solid, Molecular formula: $C_{23}H_{17}C_lN_4O_5$, Molecular weight: 464.86, Yield: 68.32%, M.P.: 152-154 °C, R_f value: 0.79, FT-IR (KBr, cm^{-1}): 3434.67 (N-H Str.), 2991.28 (C-H Str.), 1629.41 (C=C Str.), 1701.03 (C=O Str.), 1254.04 (C-N Str.), 743.35 (Ar C-H Bend.), 1497.09 (Ar N=O Str.). ^{1}H-NMR (400 MHz, DMSO, δ ppm): 1.57 (t, 2H, CH_2), 3.31 (q, 2H, CH_2), 4.20 (t, ^{1}H, NH), 6.70-8.14 (m, ^{11}H, Ar H), 11.12 (s, ^{1}H, COOH). Mass Spectra: m/z: 466.43 (M^{+2}). Elemental Analysis, % found (% required): C 59.34 (59.43); H 3.65 (3.69); N 11.96 (12.05); O 17.15 (17.21); Cl 7.55 (7.63).

Q3: 4-(6- Chloro-2-(2-(3- Nitrophenylamino) Ethyl)-4-Oxoquinazolin-3(4H)-Yl) Benzoic Acid: Creamish yellow colored solid, Molecular formula: $C_{23}H_{17}C_lN_4O_5$, Molecular weight: 464.86, Yield: 67.22%, M.P.: 168-170 °C, R_f value: 0.80, FT-IR (KBr, cm^{-1}): 3396.21 (N-H Str.), 2895.47 (C-H Str.), 1599.85 (C=C Str.), 1704.19 (C=O Str.), 1222.75 (C-N Str.), 742.59 (Ar C-H Bend.), 1452.39 (Ar N=O Str.). 1H-NMR (400 MHz, DMSO, δ ppm): 1.58 (t, 2H, CH_2), 3.29 (q, 2H, CH_2), 4.17 (t, 1H, NH), 6.75-8.12 (m, 11H, Ar H), 11.00 (s, ^{1}H, COOH). Mass Spectra: m/z: 466.57 (M^{+2}). Elemental Analysis, % found (% required): C 59.35 (59.43); H 3.51 (3.69); N 12.08 (12.05); O 17.12 (17.21); Cl 7.52 (7.63).

Q4: 4-(6-Chloro -2-(2-(4- Nitrophenylamino) Ethyl)-4-Oxoquinazolin-3(4H)-Yl) Benzoic Acid: Pale yellow colored solid, Molecular formula: $C_{23}H_{17}C_lN_4O_5$, Molecular weight: 464.86, Yield: 67.77%, M.P.: 178-180 ºC, R_f value: 0.76, FT-IR (KBr, cm^{-1}): 3434.65 (N-H Str.), 2917.59 (C-H Str.), 1657.18 (C=C Str.), 1754.78 (C=O Str.), 1259.94 (C-N Str.), 796.20 (Ar C-H Bend.), 1470.73 (Ar N=O Str.). 1H-NMR (400 MHz, DMSO, δ ppm): 1.61 (t, 2H, CH_2), 3.15 (q, 2H, CH_2), 4.11 (t, 1H, NH), 6.65-8.12 (m, 11H, Ar H), 11.15 (s, 1H, COOH). Elemental Analysis, % found (% required): C 59.37 (59.43); H 3.61 (3.69); N 11.95 (12.05); O 17.13 (17.21); Cl 7.60 (7.63).

Q5: 4-(2-(2-(2- Bromophenylamino) Ethyl)-6-Chloro-4- Oxoquinazolin-3(4h)-Yl) Benzoic Acid: Pale red colored solid, Molecular formula: $C_{23}H_{17}BrC_lN_3O_3$, Molecular weight: 498.76, Yield: 73.24%, M.P.: 155-157 ºC, R_f value: 0.76, FT-IR (KBr, cm^{-1}): 3363.76 (N-H Str.), 2898.37 (C-H Str.), 1599.58 (C=C Str.), 1679.43 (C=O Str.), 1258.02 (C-N Str.), 768.37 (Ar C-H Bend.), 678.46

(Ar C-Br Bend.). 1H-NMR (400 MHz, DMSO, δ ppm): 1.63 (t, 2H, CH2), 3.12 (q, 2H, CH2), 4.14 (t, 1H, NH), 6.34-8.11 (m, 11H, Ar H), 11.10 (s, 1H, COOH). Mass Spectra: m/z: 500.07 (M^{+2}). Elemental Analysis, % found (% required): C 55.32 (55.39); H 3.40 (3.44); N 8.35 (8.42); O 9.57 (9.62); Cl 7.07 (7.11); Br 15.99 (16.02).

Q6: 4-(2-(2-(3-Bromophenylamino) Ethyl) -6-Chloro-4- Oxoquinazolin-3 (4H)-Yl) Benzoic Acid: Light red colored solid, Molecular formula: $C_{23}H_{17}BrC_lN_3O_3$, Molecular weight: 498.76, Yield: 72.84%, M.P.: 158-160 ºC, R_f value: 0.74, FT-IR (KBr, cm^{-1}): 3320.88 (N-H Str.), 2809.58 (C-H Str.), 1589.43 (C=C Str.), 1666.88 (C=O Str.), 1258.46 (C-N Str.), 718.27 (Ar C-H Bend.), 650.43 (Ar C-Br Bend.). 1H-NMR (400 MHz, DMSO, δ ppm): 1.64 (t, 2H, CH_2), 3.10 (q, 2H, CH_2), 4.16 (t, 1H, NH), 6.37-8.13 (m, 11H, Ar H), 11.08 (s, 1H, COOH). Mass Spectra: m/z: 500.04 (M^{+2}). Elemental Analysis, % found (% required): C 55.32 (55.39); H 3.38 (3.44); N 8.36 (8.42); O 9.57 (9.62); Cl 7.10 (7.11); Br 15.98 (16.02).

Q7: 4-(2-(2-(4-Bromophenylamino) Ethyl) -6-Chloro-4- Oxoquinazolin-3 (4H)-Yl) Benzoic Acid: Greyish red colored solid, Molecular formula: $C_{23}H_17BrClN_3O_3$, Molecular weight: 498.76, Yield: 70.24%, M.P.: 160-162 ºC, R_f value: 0.70, FT-IR (KBr, cm^{-1}): 3334.67 (N-H Str.), 2849.31 (C-H Str.), 1597.36(C=C Str.), 1693.03 (C=O Str.), 1255.20 (C-N Str.), 717.26 (Ar C-H Bend.), 637.77 (Ar C-Br Bend.). 1H-NMR (400 MHz, DMSO, δ ppm): 1.57 (t, 2H, CH_2), 3.14 (q, 2H, CH_2), 3.94 (t, 1H, NH), 6.29-8.10 (m, 11H, Ar H), 11.05 (s, 1H, COOH). Elemental Analysis, % found (% required): C 55.36 (55.39); H 3.41 (3.44); N 8.34 (8.42); O 9.59 (9.62); Cl 7.02 (7.11); Br 15.94 (16.02).

Q8: 4-(6-Chloro-2-(2-(2- Chlorophenylamino) Ethyl)-4- Oxoquinazolin-3(4H)-Yl) Benzoic Acid: Dark Brown colored solid, Molecular formula: $C_{23}H_{17}C_{l2}N_3O_3$, Molecular weight: 454.31, Yield: 69.30%, M.P.: 204-206 ºC, R_f value: 0.71, FT-IR (KBr, cm^{-1}): 3371.64 (N-H Str.), 2863.85 (C-H Str.), 1572.74(C=C Str.), 1711.10 (C=O Str.), 1259.33 (C-N Str.), 713.32 (Ar C-H Bend.), 654.23 (Ar C-Cl Bend.). 1H-NMR (400 MHz, DMSO, δ ppm): 1.59 (t, 2H, CH2), 2.98 (q, 2H, CH2), 4.03 (t, 1H, NH), 6.37-8.12 (m, 11H, Ar H), 11.02 (s, 1H, COOH). Mass Spectra: m/z: 456.39 (M^{+2}). Elemental Analysis, % found (% required): C 60.77 (60.81); H 3.72 (3.77); N 9.20 (9.25); O 10.51 (10.57); Cl 15.58 (15.61).

Q9: 4-(6-Chloro-2-(2-(3- Chlorophenylamino) Ethyl)- 4-Oxoquinazolin-3 (4H)-Yl) Benzoic Acid: Pale Brown colored solid, Molecular formula: $C_{23}H_{17}C_{l2}N_3O_3$, Molecular weight: 454.31, Yield: 67.84%, M.P.: 210-212 ºC, R_f value: 0.78, FT-IR (KBr, cm^{-1}): 3394.98 (N-H Str.), 2858.37 (C-H Str.), 1504.84 (C=C Str.), 1724.98 (C=O Str.), 1209.33 (C-N Str.), 710.11 (Ar C-H Bend.), 673.29 (Ar C-Cl Bend.). 1H-NMR (400 MHz, DMSO, δ ppm): 1.62 (t, 2H, CH_2), 3.11 (q, 2H, CH_2), 4.08 (t, 1H, NH), 6.30-8.07 (m, 11H, Ar H), 11.01 (s, 1H, COOH). Elemental Analysis, % found (% required): C 60.77 (60.81); H 3.71 (3.77); N 9.22 (9.25); O 10.54 (10.57); Cl 15.58 (15.61).

Q10: 4-(6-Chloro-2-(2-(4-Chlorophenylamino) Ethyl)-4- Oxoquinazolin- 3(4H)-Yl) Benzoic Acid: Creamish Brown colored solid, Molecular formula: $C_{23}H_{17}C_{l2}N_3O_3$, Molecular weight: 454.31, Yield: 66.67%, M.P.: 209-211 ºC, R_f value: 0.69, FT-IR (KBr, cm^{-1}): 3375.68 (N-H Str.), 2719.68 (C-H Str.), 1531.28 (C=C Str.), 1717.59 (C=O Str.), 1207.04 (C-N Str.), 761.10 (Ar C-H Bend.), 640.39 (Ar C-Cl Bend.). 1H-NMR (400 MHz, DMSO, δ ppm): 1.60 (t, 2H, CH_2), 3.17 (q, 2H, CH_2), 4.10 (t, 1H, NH), 6.35-8.11 (m, 11H, Ar H), 10.89 (s, 1H, COOH). Mass Spectra: m/z: 456.69 (M^{+2}). Elemental Analysis, % found (% required): C 60.78 (60.81); H 3.74 (3.77); N 9.19 (9.25); O 10.50 (10.57); Cl 15.58 (15.61).

Q11: 4-(6-Chloro-2-(2-(2-Methoxyphenylamino) Ethyl)-4- Oxoquinazolin-3(4H)-Yl) Benzoic Acid: Yellowish White colored solid, Molecular formula: $C_{24}H_{20}C_lN_3O_4$, Molecular weight: 449.89, Yield: 64.54%, M.P.: 147-149 ºC, R_f value: 0.65, FT-IR (KBr, cm^{-1}): 3369.95 (N-H Str.), 2809.33 (C-H Str.), 1517.09(C=C Str.), 1694.15 (C=O Str.), 1217.93 (C-N Str.), 710.77 (Ar C-H Bend.). 1H-NMR (400 MHz, DMSO, δ ppm): 1.61 (t, 2H, CH_2), 3.22 (q, 2H, CH_2), 4.16 (t, 1H, NH), 3.71 (s, 3H, OCH3), 6.31-8.09 (m, 11H, Ar H), 11.04 (s, 1H, COOH). Mass Spectra: m/z: 451.27 (M^{+2}). Elemental Analysis, % found (% required): C 63.98 (64.07); H 4.45 (4.48); N 9.30 (9.34); O 14.19 (14.23); Cl 7.84 (7.88).

Q12: 4-(6-Chloro-2-(2-(3-Methoxyphenylamino) Ethyl)-4- Oxoquinazolin-3(4H)-Yl) Benzoic Acid: Creamish White colored solid, Molecular formula: $C^{24}H^{20}C^{l}N^{3}O^{4}$, Molecular weight: 449.89, Yield: 60.53%, M.P.: 152-154 ºC, R_f value: 0.68, FT-IR (KBr, cm^{-1}): 3396.75 (N-H Str.), 2898.47 (C-H Str.), 1531.07(C=C Str.), 1704.58 (C=O Str.), 1239.75 (C-N Str.), 719.43 (Ar C-H Bend.). 1H-NMR (400 MHz, DMSO, δ ppm): 1.58 (t, 2H, CH_2), 3.11 (q, 2H, CH2), 4.13 (t, 1H, NH), 3.75 (s, 3H, OCH_3), 5.91-8.0 (m, 11H, Ar H), 11.01 (s, 1H, COOH). Elemental Analysis, %found (% required): C 63.99 (64.07); H 4.45 (4.48); N 9.32(9.34); O 14.19 (14.23); Cl 7.81 (7.88).

Q13: 4-(6-Chloro-2-(2-(4-Methoxyphenylamino) Ethyl)-4 -Oxoquinazolin-3(4H)-Yl) Benzoic Acid: White Brown colored solid, Molecular formula: $C_{24}H_{20}C_lN_3O_4$, Molecular weight: 449.89, Yield: 70.79%, M.P.: 138-140 ºC, R_f value: 0.66, FT-IR (KBr, cm^{-1}): 3421.07 (N-H Str.), 2918.12 (C-H Str.), 1546.13(C=C Str.), 1719.41 (C=O Str.), 1208.08 (C-N Str.), 735.04 (Ar C-H Bend.). 1H-NMR (400 MHz, DMSO, δ ppm): 1.64 (t, 2H, CH_2), 3.14 (q, 2H, CH_2), 4.07 (t, 1H, NH), 3.72 (s, 3H, OCH_3), 6.30-8.03 (m, 11H, Ar H), 10.86 (s, 1H, COOH). Mass Spectra: m/z: 451.35 (M^{+2}). Elemental Analysis, % found (% required):C 64.01 (64.07); H 4.46 (4.48); N 9.30(9.34); O 14.18 (14.23); Cl 7.80 (7.88).

Q14: 4-(6-Chloro-2-(2-(2-Hydroxyphenylamino) Ethyl)-4 -Oxoquinazolin-3(4H)-Yl) Benzoic Acid: Greyish Black colored solid, Molecular formula: $C_{23}H_{18}C_lN_3O_4$, Molecular weight: 435.86, Yield: 72.11%, M.P.: 133-135 ºC, R_f value: 0.71, FT-IR (KBr, cm-1): 3478.22 (N-H Str.), 2934.89 (C-H Str.), 1530.86(C=C Str.), 1643.50 (C=O Str.), 1209.79 (C-N Str.), 737.35 (Ar C-H Bend.), 3446.18 (Ar C-OH Str.). 1H-NMR (400 MHz, DMSO, δ ppm): 1.69 (t, 2H, CH_2), 3.25 (q, 2H, CH_2), 4.01 (t, 1H, NH), 5.10 (s, 1H, OH), 6.24-8.12 (m, 11H, Ar H), 11.12 (s, 1H, COOH). Mass Spectra: m/z: 437.11 (M+2). Elemental Analysis, % found (% required): C 63.35 (63.38); H 4.10 (4.16); N 9.59(9.64); O 14.66 (14.68); Cl 8.09 (8.13).

Q15: 4-(6-Chloro-2-(2-(3-Hydroxyphenylamino) Ethyl)- 4-Oxoquinazolin-3(4H)-Yl) Benzoic Acid: Yellowish Black colored solid, Molecular formula: $C_{23}H_{18}C_lN_3O_4$, Molecular weight: 435.86, Yield: 74.38%, M.P.: 138-140 ºC, R_f value: 0.74, FT-IR (KBr, cm^{-1}): 3477.38 (N-H Str.), 2979.13 (C-H Str.), 1531.84(C=C Str.), 1622.98 (C=O Str.), 1207.57 (C-N Str.), 762.11 (Ar C-H Bend.), 3446.93 (Ar C-OH Str.). 1H-NMR (400 MHz, DMSO, δ ppm): 1.65 (t, 2H, CH_2), 3.19 (q, 2H, CH_2), 4.04 (t, 1H, NH), 5.07 (s, 1H, OH), 5.89-8.14 (m, 11H, Ar H), 11.03 (s, 1H, COOH). Elemental Analysis, % found (% required): C 63.33 (63.38); H 4.11 (4.16); N 9.60 (9.64); O 14.63 (14.68); Cl 8.08 (8.13).

Q16: 4-(6-Chloro-2-(2-(4-Hydroxyphenylamino) Ethyl)- 4-Oxoquinazolin-3(4H)-Yl) Benzoic Acid: Black Red colored solid, Molecular formula: $C_{23}H_{18}C_lN_3O_4$, Molecular weight: 435.86, Yield: 70.25%, M.P.: 144-146oC, R_f value: 0.69, FT-IR (KBr, cm^{-1}): 3377.38 (N-H Str.), 2979.49 (C-H Str.), 1572.38(C=C Str.), 1617.87 (C=O Str.), 1249.85 (C-N Str.), 733.59 (Ar C-H Bend.), 3315.87 (Ar C-OH Str.). 1H-NMR (400 MHz, DMSO, δ ppm): 1.63 (t, 2H, CH_2), 3.18 (q, 2H, CH_2), 4.11 (t, 1H, NH), 5.02 (s, 1H, OH), 6.25-8.11 (m, 11H, Ar H), 11.10 (s, 1H, COOH). Elemental Analysis, % found (% required): C 63.31 (63.38); H 4.11 (4.16); N 9.60 (9.64); O 14.66 (14.68); Cl 8.07 (8.13).

Biological Study:

Evaluation of Anti- Inflammatory Activity [15, 16]: Adult albino rats of both sexes weighing between 120 and 150 g were used. Rats were uniformly hydrated by giving 3 ml water/rat through gastric inoculation to reduce variability to oedema response. Animals were divided into 18 groups, each of five animals. The control group was given saline solution containing few drops of Tween 80. Diclofenac sodium (50 mg/kg) was taken as a standard drug for comparison, and compounds under examination (100 mg/kg) were suspended in distilled water with the aid of few drops of Tween 80 and were given orally 1 h before induction of inflammation. Induction of inflammation was performed by S.C. injection of 50 µl of 1% carrageenan-sodium gel into the sub-plantar region of the right hind paw. The dorsoventral diameter (thickness) of the right and left hind paw of each rat was measured using a pair of dial thickness gauge callipers accurate to 0.001 cm 0.5, 1, 2, and 3 hr after induction of inflammation. The left hind paw diameter served as a control for the degree of inflammation in the right hind paw. The percentage

of anti-inflammatory activity (% inhibition of inflammation) was calculated according to the following equation:

$$\% \text{ inhibition} = (Wc - Wt/Wc) \times 100$$

Wt: is the mean increase in paw thickness in rats treated with the tested compounds, Wc: is the mean increase in paw thickness in the control group.

Biochemical Estimation:

Estimation of Cytokines Tumor Necrosis Factor Alpha, Interleukin 6 and Interleukin 1 In Serum: After completion of the carrageenan-induced paw edema experiment, the rats were anesthetized, and blood samples were collected from the orbital sinus. The serum was separated by allowing blood to clot followed by centrifugation, and was stored at 20 ºC until use. TNF-α, IL-6, IL-1 from each sample were measured in duplicate with highly sensitive rat TNF-α Elisa Kit (Bio molecular Integrations), rat IL-6 Elisa Kit (Koma Biotech), rat IL-1 Elisa Kit (Boster Biological Technology Ltd) respectively, specifically designed for rats, according to manufacturer's instructions.

Determination of Tissue Lipid Peroxidation:

MDA Assay: The Malonylaldehyde from carrageenan-induced edema foot was evaluated by using the thiobarbituric acid reacting substance method. Briefly, the reaction mixture containing 1 ml 0.67% Thio Barbituric Acid (TBA), 1 ml 20% Tri Carboxylic Acid (TCA) and 100 ml serum were incubated at 100 ºC for 30 min, at this temperature, MDA reacted with thiobarbituric acid at acidic pH resulting in the formation of a red complex TBARS and centrifuged at 5000 rpm for 15 min. The absorbance of TBARS was determined at 532 nm.

RESULTS AND DISCUSSION:

Chemistry: All the novel quinazolinone derivatives were synthesized, purified, and separated by using column chromatography or recrystallization method. Synthesized compounds were characterized by using elemental analysis, FT-IR, ^{1}HNMR, and Mass Spectrometric studies. The integration curves fully support the orientation of protons in the analyzed compounds. Furthermore, all the compounds demonstrated the characteristic chemical shifts for the quinazolinone nucleus. Additionally, synthesized compounds were analyzed by mass spectra and indicated no difference in the fragmentation pattern among the set of synthesized series.

Anti- Inflammatory Activity: The anti-inflammatory activity was assessed by using Carrageenan-induced rat paw edema using Diclofenac sodium as the reference drug. The anti-inflammatory activity data were obtained as the thickness of edema at 0.5 h, 1 h, 2 h, and 3 h intervals and expressed in % inhibition as shown in **Tables 2** and **3**. Compounds Q3, Q8, and Q15 showed excellent anti-inflammatory activity at 74.46%, 72.34%, and 68.08% inhibition respectively at 3rd h, which were nearby 78.72% inhibition of the standard Diclofenac sodium used and also greater than the other quinazolinone derivatives.

TABLE 2: OEDEMA THICKNESS OF CONTROL, DICLOFENAC SODIUM AND TESTED COMPOUNDS.

Compounds	Oedema Thickness (mm) ± SEM			
	0.5 h	1 h	2 h	3 h
Control	0.380 ± 0.020	0.410 ± 0.022	0.440 ± 0.021	0.470 ± 0.021
Diclofenac Sodium	0.160 ± 0.008	0.150 ± 0.006	0.120 ± 0.004	0.100 ± 0.005***
Q_1	0.240 ± 0.013	0.220 ± 0.013	0.200 ± 0.011	0.170 ± 0.012**
Q_2	0.250 ± 0.014	0.210 ± 0.013	0.190 ± 0.015	0.160 ± 0.010***
Q_3	0.200 ± 0.007	0.180 ± 0.009	0.150 ± 0.008	0.120 ± 0.007***
Q_4	0.260 ± 0.016	0.250 ± 0.015	0.230 ± 0.011	0.200 ± 0.014**
Q_5	0.290 ± 0.007	0.280 ± 0.008	0.280 ± 0.008	0.270 ± 0.006**
Q_6	0.280 ± 0.011	0.260 ± 0.012	0.250 ± 0.011	0.230 ± 0.013**
Q_7	0.300 ± 0.004	0.290 ± 0.005	0.270 ± 0.003	0.260 ± 0.004**
Q_8	0.220 ± 0.009	0.190 ± 0.006	0.170 ± 0.008	0.130 ± 0.008***
Q_9	0.240 ± 0.010	0.220 ± 0.011	0.180 ± 0.012	0.160 ± 0.010***
Q_{10}	0.270 ± 0.008	0.240 ± 0.007	0.220 ± 0.009	0.190 ± 0.006**
Q_{11}	0.290 ± 0.014	0.270 ± 0.012	0.260 ± 0.013	0.240 ± 0.013**
Q_{12}	0.260 ± 0.015	0.260 ± 0.014	0.240 ± 0.015	0.220 ± 0.012**
Q_{13}	0.310 ± 0.005	0.300 ± 0.006	0.290 ± 0.006	0.250 ± 0.004**
Q_{14}	0.260 ± 0.009	0.240 ± 0.010	0.220 ± 0.011	0.210 ± 0.009**
Q_{15}	0.230 ± 0.011	0.210 ± 0.009	0.180 ± 0.008	0.150 ± 0.008***
Q_{16}	0.240 ± 0.012	0.230 ± 0.013	0.210 ± 0.012	0.180 ± 0.011**

Values are expressed as mean ± SEM of five animals in each group. **Statistically significant (P<0.05). ***Statistically significant (P<0.01)

TABLE 3: % INHIBITION OF COMPOUNDS ON CARRAGEENAN INDUCED PAW RAT PAW EDEMA

Compounds	Oedema inhibition (%)			
	0.5 h	1 h	2 h	3 h
Control	-	-	-	-
Diclofenac Sodium	57.89	63.41	72.72	78.72***
Q_1	36.84	46.34	54.54	63.82**
Q_2	34.21	48.78	56.81	65.95***
Q_3	47.36	56.09	65.90	74.46***
Q_4	31.57	39.02	47.72	57.44**
Q_5	23.68	31.70	36.36	42.55**
Q_6	26.31	36.58	43.18	51.06**
Q_7	21.05	29.26	38.63	44.68**
Q_8	42.10	53.65	61.36	72.34***
Q_9	36.84	46.34	59.09	65.95***
Q_{10}	28.94	41.46	50	59.57**
Q_{11}	23.68	34.14	40.90	48.93**
Q_{12}	31.57	36.58	45.45	53.19**
Q_{13}	18.42	26.82	34.09	46.80**
Q_{14}	31.57	41.46	50	55.31**
Q_{15}	39.47	48.78	59.09	68.08***
Q_{16}	36.84	43.90	42.27	61.70**

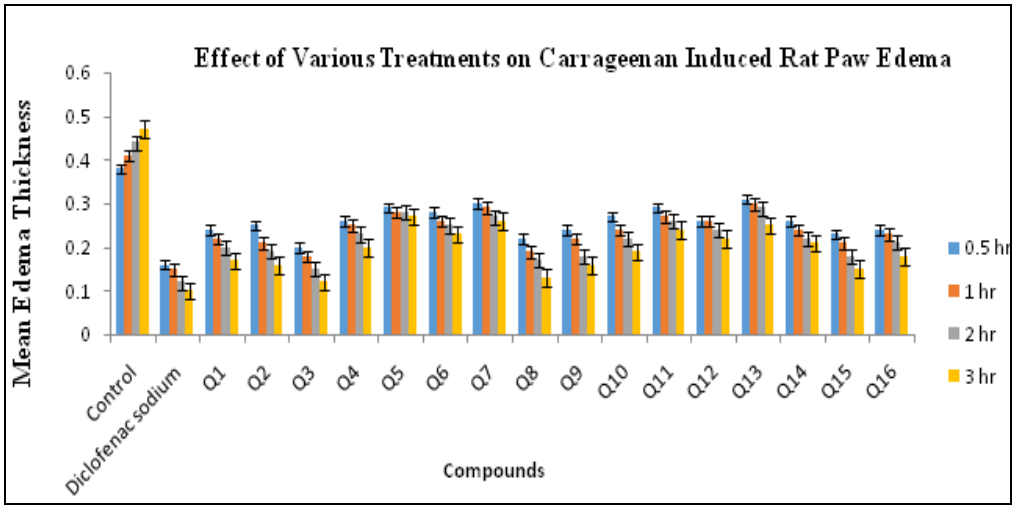


FIG. 1: EFFECT OF VARIOUS TREATMENTS ON MEAN EDEMA THICKNESS IN CARRAGEENAN INDUCED RAT PAW EDEMA

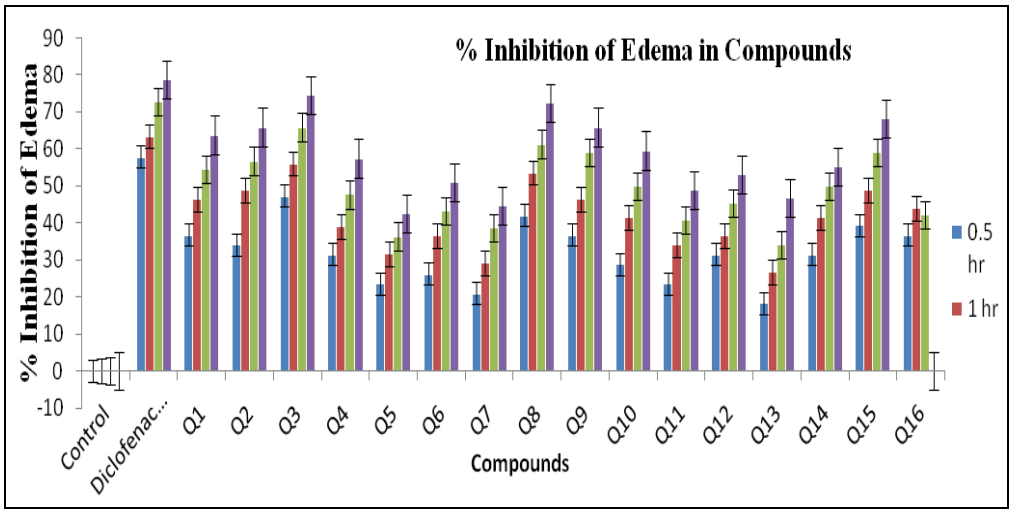


FIG. 2: EFFECT OF VARIOUS TREATMENTS ON MEAN EDEMA THICKNESS IN CARRAGEENAN INDUCED RAT PAW EDEMA

Biochemical Estimation: The potent quinazolinone derivatives Q3, Q8, and Q15 were chosen for further biochemical estimation of cytokines and tissue lipid peroxidation.

Estimation of Cytokines Tumor Necrosis Factor Alpha, Interleukin 6 and Interleukin 1 In Serum: Synthesized quinazolinone derivatives showed good significant ($p < 0.01$) and ($p < 0.05$) inhibition of cytokines likes Tumor Necrosis Factor Alpha (TNF-α), Interleukin-6 (IL-6) and Interleukin-1 (IL-1) compared to control group.

As shown in **Table 4**, there was a dose-dependent decrease in all three serum cytokines (TNF-α, IL-6, IL-1) in Q3, Q8 and Q15 groups at both 100 mg/kg.

TABLE 4: EFFECTS OF QUINAZOLINONE DERIVATIVES ON SERUM CYTOKINES IN CARRAGEENAN INDUCED PAW EDEMA

Groups	TNF-α (pg/ml)	IL-6 (pg/ml)	IL-1 (pg/ml)
Control	698.14 ± 18.67	589.12 ± 18.07	243.26 ± 19.21
Diclofenac Sodium	412.34 ± 17.26**	263.41 ± 14.64**	159.37 ± 16.10**
Q_3	521.14 ± 23.55**	386.37 ± 15.12**	157.14 ± 13.02**
Q_8	637.19 ± 23.63*	464.07 ± 22.84*	209.47 ± 17.34*
Q_{15}	588.23 ± 16.39**	451.09 ± 14.19**	181.78 ± 17.52**

Values are expressed as mean ± SEM. *P<0.05, **P<0.01. SEM: Standard error of the mean
TNF-α: Tumor necrosis factor-alpha, IL-Interleukin

Estimation of Tissue Lipid Peroxidation in Serum: As shown in **Table 5** there were a dose-dependent significant decrease in Malonylaldehyde (MDA) levels in all three groups. All three Compounds (Q3, Q8, and Q15) showed good significant ($p < 0.01$) and ($p < 0.05$) inhibition of lipid peroxidation (decrease in MDA) compared to the control group. The Diclofenac sodium showed 64.38 %, Q3 showed 45.17%, Q8 showed 19.86%, and Q15 showed a 31.27% decrease in MDA level in the brain. In this study, we found that titled compounds decreased the levels of MDA in serum after carrageenan injection.

TABLE 5: EFFECTS OF QUINAZOLINONE DERIVATIVES ON TISSUE LIPID PEROXIDATION (MDA) INCARRAGEENAN INDUCED PAW EDEMA

Groups	Dose (mg/kg)	MDA (nmol/mg protein)	Percentage Decreases in MDA
Control	2ml	0.81 ± 0.049	0
Diclofenac sodium	50	0.27 ± 0.061**	64.38
Q_3	100	0.48 ± 0.501**	45.17
Q_8	100	0.68 ± 0.698*	19.86
Q_{15}	100	0.51 ± 0.439**	31.27

Values are expressed as mean ± SEM. *P<0.05, **P<0.01. SEM: Standard error of mean MDA- Malonylaldehyde

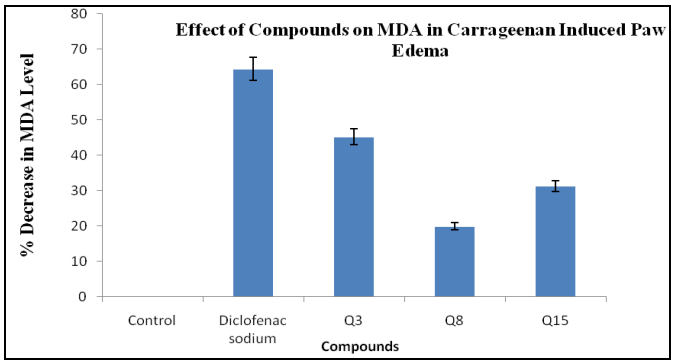


FIG. 3: EFFECT OF QUINAZOLINE DERIVATIVES ON MDA IN CARRAGEENAN INDUCED RAT PAW EDEMA

CONCLUSION: The main focus of this research work was to synthesize novel series of quinazolinone derivatives, purify, characterize and evaluate their anti-inflammatory activity. From the results, it can be concluded that the modified quinazolinone shows significant biological evaluation as anti-inflammatory agents. However, further evaluation of quinazolinone will be undertaken concerning the structural arrangements in ring for anti-inflammatory activity.

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