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Research Article

Evaluation of Analgesic Activity of Some Novel Quinazolinone Analogues

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Abstract

The synthesis, characterization and spectroscopic studies of new quinazolinone substituted analogues Q_1 - Q_{16} with analgesic activity were described. A series of novel quinazolinone derivatives were synthesized. In this view, 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 amino benzoic acid to yield 4-[6-chloro-2-methyl-4-oxoquinazolin-3(4H)-yl] benzoic acid as intermediate-II. This resulted intermediate-II undergo mannich base reaction to produce novel quinazolinone derivatives on reaction of formaldehyde with different aromatic amines. All the synthetic derivatives were fully characterized by spectral analytical data (elemental analysis, FTIR, 1H NMR and Mass) and the purity of the compounds was determined by TLC. Analgesic activities were tested via both hot plate and acetic acid induced writhing methods. The study concluded that the compound Q_5, Q_8 and Q_9 were found to exhibit significant analgesic activity when compared to Ibuprofen as standard drug while other derivatives exhibit moderate to good analgesic activity.

Keywords: 5-Chloro Anthranilic Acid; Quinazolinone; Analgesic Activity; Ibuprofen

Introduction

It is evident from literature that**,** Quinazolinone is a heterocyclic compound play vital role towards synthetic medicinal chemistry. The synthetic derivatives of quinazolinone are utilized as therapeutic agent for combating against different pathological conditions. 5-chloro anthranilic acid mainly employed for the synthesis of quinazolinone compounds as starting materials [1]. Quinazolinone and its derivatives possess a major class of biologically active compounds which exhibited large spectrum of therapeutic activities including; anti-malarial [2], analgesic [3], antioxidant [4], anticancer [5], antiviral [6], antifeedant [7], sedative-hypnotic [8], anticonvulsant [9], antimicrobial [10], antialgal [11], hypotensive [12] and anti-inflammatory [13]. Recently quinazolinone derivatives seek 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 analgesic activity [14].

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 deuterated 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 Dimethylsulphoxide (DMSO) as solvent. TLC was performed using silica gel GF_{254} coated platesof 0.25 mm thickness. Ethyl acetate, petroleum ether, chloroform (0.6:0.8:8.6) were used as solvent system and iodine vapors as visualizing agent.

Scheme of synthesis

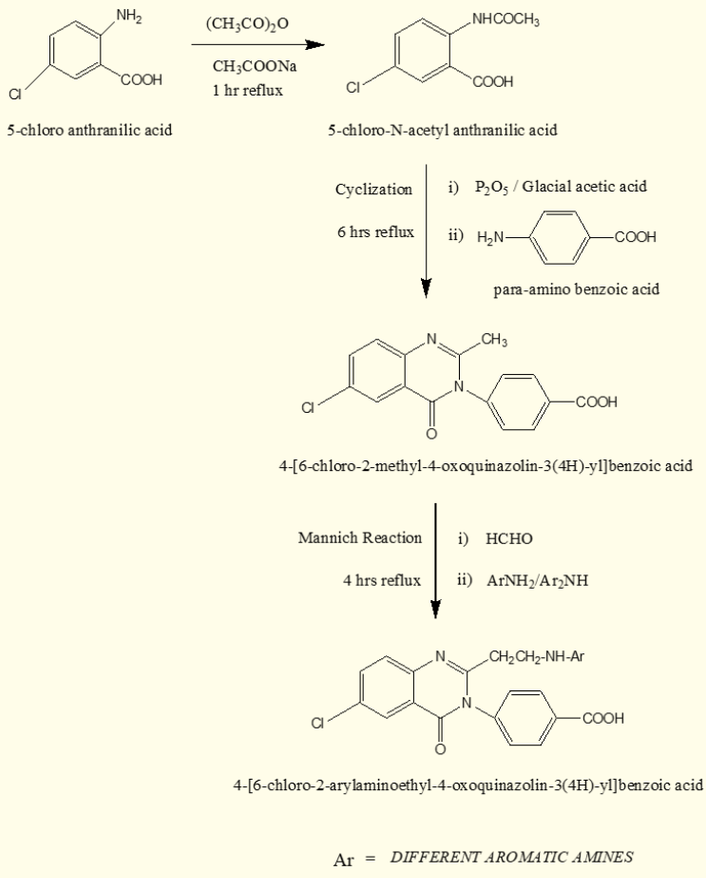
(Figure and Table 1)

The experimental work comprises in three steps:

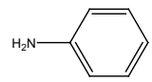
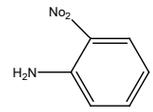
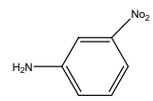
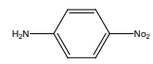
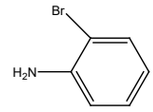
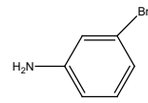
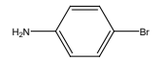
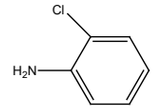
1. Step-I: Synthesis of 5-chloro-N-acetyl anthranilic acid from 5 -chloro anthranilic acid.
2. Step-II: Synthesis of 4-[6-chloro-2-methyl-4-oxo quinazolin-3(4H)-yl] benzoic acid.
3. Step-III: Synthesis of various derivatives of quinazolinone by mannich reaction.

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 an equimolar quantities of anhydrous sodium acetate (0.03 moles) and



Figure

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	

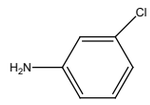
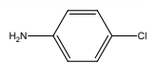
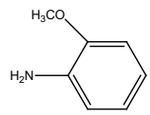
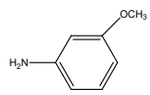
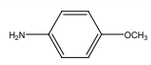
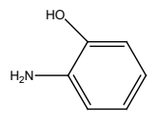
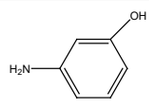
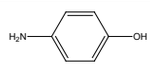
9	Q_9	m-chloro aniline	
10	Q_{10}	p-chloro aniline	
11	Q_{11}	o-methoxy aniline	
12	Q_{12}	m-methoxy aniline	
13	Q_{13}	p-methoxy aniline	
14	Q_{14}	o-hydroxy aniline	
15	Q_{15}	m-hydroxy aniline	
16	Q_{16}	p-hydroxy aniline	

Table 1: List of various aromatic amines.

acetic anhydride (0.04 moles in slight excess) and refluxed on sand bath under anhydrous condition for 1 hr. 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°C.

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 was refluxed under anhydrous condition for 6 hrs. Then the reaction mixture was poured into 10% Sodium bicarbonate solution (50 ml) and crude product was filtered and dried. The dried crude product was recrystallized from ethanol. Yield: 76.67% M.P.: 220 - 222°C.

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 hrs. The completion of reaction was monitored by TLC. The excess of the solvent was distilled off and the residue was recrystallized from acetone to give 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, 1H, NH), 6.38 - 8.10 (m, 12H, Ar H), 11.10 (s, 1H, 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: $C_{23}H_{17}ClN_4O_5$, 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, 1H, NH), 6.70 - 8.14 (m, 11H, Ar H), 11.12 (s, 1H, 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: $C_{23}H_{17}ClN_4O_5$, 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.). ^{1}H-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, 1H, 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: $C_{23}H_{17}ClN_4O_5$, Molecular weight: 464.86, Yield: 67.77%, M.P.: 178 - 180°C, 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.). ^{1}H-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: $C_{23}H_{17}BrClN_3O_3$, Molecular weight: 498.76, Yield: 73.24%, M.P.: 155 - 157°C, 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.). ^{1}H-NMR (400 MHz, DMSO, δ ppm): 1.63 (t, 2H, CH_2), 3.12 (q, 2H, CH_2), 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: $C_{23}H_{17}BrClN_3O_3$, Molecular weight: 498.76, Yield: 72.84%, M.P.: 158 - 160°C, 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.). ^{1}H-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: $C_{23}H_{17}BrClN_3O_3$, Molecular weight: 498.76, Yield: 70.24%, M.P.: 160 - 162°C, 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.). ^{1}H-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: $C_{23}H_{17}Cl_2N_3O_3$, Molecular weight: 454.31, Yield: 69.30%, M.P.: 204 - 206°C, 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.). ^{1}H-NMR (400 MHz, DMSO, δ ppm): 1.59 (t, 2H, CH_2), 2.98 (q, 2H, CH_2), 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: $C_{23}H_{17}Cl_2N_3O_3$, Molecular weight: 454.31, Yield: 67.84%, M.P.: 210 - 212°C, 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.). ^{1}H-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: $C_{23}H_{17}Cl_2N_3O_3$, Molecular weight: 454.31, Yield: 66.67%, M.P.: 209 - 211°C, 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.). ^{1}H-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: $C_{24}H_{20}ClN_3O_4$, Molecular weight: 449.89, Yield: 64.54%, M.P.: 147 - 149°C, 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.). ^{1}H-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, OCH_3), 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: $C_{24}H_{20}ClN_3O_4$, Molecular weight: 449.89, Yield: 60.53%, M.P.: 152 - 154°C, R_f val-

ue: 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.). ^{1}H-NMR (400 MHz, DMSO, δ ppm): 1.58 (t, 2H, CH_2), 3.11 (q, 2H, CH_2), 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: $C_{24}H_{20}ClN_3O_4$, Molecular weight: 449.89, Yield: 70.79%, M.P.: 138 - 140°C, 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.). ^{1}H-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: $C_{23}H_{18}ClN_3O_4$, Molecular weight: 435.86, Yield: 72.11%, M.P.: 133 - 135°C, 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.). ^{1}H-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: $C_{23}H_{18}ClN_3O_4$, Molecular weight: 435.86, Yield: 74.38%, M.P.: 138 - 140°C, 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.). ^{1}H-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: $C_{23}H_{18}ClN_3O_4$, Molecular weight: 435.86, Yield: 70.25%, M.P.: 144 - 146°C, 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.). ^{1}H-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 analgesic activity [15-17]

Animals

Adult male albino mice (20 - 25g) were used for studying the analgesic activity. The animals (five per cage) were maintained under standard laboratory conditions (light period of 12 hrs/day, temperature 27 ± 2°C with relative humidity of 45 - 55%). They were fed with standard animal feed and water *ad libitum.* The experimental procedures were carried out in strict compliance with the Institutional Animal Ethics Committee. All experiments were performed in the morning according to the guidelines for the care of laboratory animals.

The hot-plate method: Analgesic activity of the tested compounds was determined by the hot-plate method. A total number of 90 mice were divided into 18 groups of five animals each. The first group was administered DMSO orally (0.2 ml/mice) and kept as negative control. Ibuprofen was given as standard drug (50 mg/kg) to the second group and the tested compounds Q_1 to Q_{16} dissolved in DMSO were administered at a dose of 100 mg/kg body weight to the rest of the groups. Each animal was placed individually on a hot plate and maintained at 55°C. The time taken by the animals to lick the hind paw or jump out of the plate was taken as the reaction time, which was measured at 30 min., 1 hrs, 2 hrs and 3 hrs. A cut off period of 30s was considered as maximal latency to avoid paw injury. The pain inhibition percentage (PIP) was calculated according to the following formula:

$$\text{Pain inhibition percentage (PIP)} = (T_t - T_c/T_c) \times 100$$

Where T_c and T_t are the latency for the control and drug-treated animal groups.

The acetic acid-induced writhing test: This test was conducted using the method described by Collier., *et al* [18]. Muscle contractions were induced in 18 groups of mice (five animals per group) by intraperitoneal injection of 0.6% solution of acetic acid (10 ml/kg). Thirty minutes before this administration, the animals in the first group were treated orally with DMSO (0.2 ml/mice) and they served as negative controls. Ibuprofen as the reference standard (50 mg/kg) and the tested compounds Q_1 to Q_{16} dissolved in DMSO were administered orally (100 mg/kg) to the animals of the rest of the groups. Immediately after administration of acetic acid the animals were placed in glass cages and the number of 'stretching' per animal was recorded during the course of the next 15 minutes. Writhing movement was accepted as contraction of the abdominal muscles accompanied by stretching of hind limbs. There was significant reduction in the number of writhes in the drug-treated animals as compared with vehicle-treated animals. This was considered a positive analgesic response and the percentage inhibition of writhing was calculated according to the following formula: % Analgesic activity = [No. of writhings for control - No. of writhings for test compounds/No. of writhings for control] × 100

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.

Analgesic activity: The analgesic activity was assessed by using hot plate and acetic acid induced writhing methods using Ibuprofen as the standard drug. The analgesic activity data by hot plate method was obtained as mean latency time at 30 min., 1 hrs, 2 hrs and 3 hrs intervals and expressed in % inhibition as shown in table 2 and 3. Compounds Q_5, Q_8 and Q_9 showed excellent analgesic activity as 89.96%, 88.40% and 74.92% inhibition respectively at 3rd hrs, which were nearby 100% inhibition of the standard Ibuprofen drug used and also greater than the other quinazolinone derivatives. Compounds Q_5, Q_8 and Q_9 showed significant % of analgesic activity as 90.92%, 88.44% and 87.97% in acetic acid induced writhing method as shown in table 4.

Compounds	Mean Latency Time (s) ± SEM			
	0.5 hr	1 hr	2 hr	3 hr
Control	2.49 ± 0.010	2.57 ± 0.012	3.08 ± 0.008	3.19 ± 0.013
Ibuprofen	3.49 ± 0.015	4.02 ± 0.017	5.43 ± 0.017	6.38 ± 0.016***
Q_1	3.01 ± 0.011	3.42 ± 0.012	4.59 ± 0.009	5.40 ± 0.010**
Q_2	3.08 ± 0.015	3.39 ± 0.014	4.50 ± 0.013	5.22 ± 0.013**
Q_3	3.10 ± 0.018	3.47 ± 0.019	4.56 ± 0.019	5.29 ± 0.014**
Q_4	3.04 ± 0.013	3.36 ± 0.012	4.49 ± 0.011	5.16 ± 0.012**
Q_5	3.43 ± 0.005	4.05 ± 0.006	5.18 ± 0.004	6.06 ± 0.007***
Q_6	3.28 ± 0.018	4.00 ± 0.022	5.05 ± 0.017	5.45 ± 0.019**
Q_7	3.06 ± 0.014	3.44 ± 0.017	4.46 ± 0.011	5.35 ± 0.014**
Q_8	3.47 ± 0.007	4.01 ± 0.009	5.07 ± 0.010	6.01 ± 0.008***
Q_9	3.21 ± 0.011	3.59 ± 0.008	5.01 ± 0.010	5.58 ± 0.012***
Q_{10}	3.13 ± 0.008	3.58 ± 0.005	5.03 ± 0.006	5.50 ± 0.004**
Q_{11}	3.02 ± 0.011	3.40 ± 0.018	4.40 ± 0.009	5.01 ± 0.012**
Q_{12}	3.10 ± 0.017	3.33 ± 0.023	4.35 ± 0.019	5.05 ± 0.010**
Q_{13}	3.14 ± 0.012	3.38 ± 0.012	4.52 ± 0.017	5.25 ± 0.016**
Q_{14}	3.00 ± 0.008	3.25 ± 0.006	4.31 ± 0.012	4.58 ± 0.014**
Q_{15}	3.16 ± 0.024	3.56 ± 0.021	4.58 ± 0.020	5.19 ± 0.021**
Q_{16}	3.09 ± 0.009	3.30 ± 0.016	4.30 ± 0.013	4.55 ± 0.006**

Table 2: Analgesic activity of the tested compounds in mice using hot-plate method.

Values are expressed as mean ± SEM of five animals in each group.

**Statistically significant (P < 0.05).

***Statistically significant (P < 0.01).

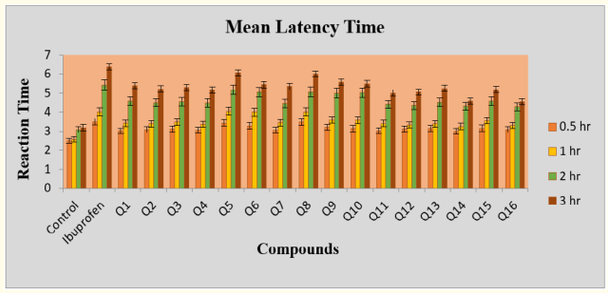


Figure 1: Effect of various treatments on mean latency time by using hot plate method.

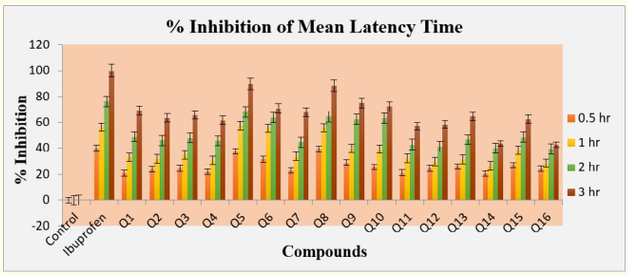


Figure 2: % inhibition of mean latency time by using hot plate method.

Compounds	Pain inhibition (%)			
	0.5 hr	1 hr	2 hr	3 hr
Control	-	-	-	-
Ibuprofen	40.16	56.42	76.29	100***
Q_1	20.88	33.07	49.02	69.27**
Q_2	23.69	31.90	46.10	63.63**
Q_3	24.49	35.01	48.05	65.83**
Q_4	22.08	30.73	45.77	61.75**
Q_5	37.75	57.58	68.18	89.96***
Q_6	31.72	55.64	63.96	70.84**
Q_7	22.89	33.85	44.80	67.71**
Q_8	39.35	56.03	64.61	88.40***
Q_9	28.91	39.68	62.66	74.92***
Q_{10}	25.70	39.29	63.31	72.41**
Q_{11}	21.28	32.29	42.85	57.05**
Q_{12}	24.49	29.57	41.23	58.30**
Q_{13}	26.10	31.51	46.75	64.57**
Q_{14}	20.48	26.45	39.93	43.57**
Q_{15}	26.90	38.52	48.70	62.69**
Q_{16}	24.09	28.40	39.61	42.63**

Table 3: % inhibition of analgesic activity of the tested compounds in mice using hot-plate method.

Compounds	No. of writhings in 15 minutes ± SEM	% Analgesic activity
Control	61.37 ± 3.41	00
Ibuprofen	4.31 ± 0.52	92.97***
Q_1	13.31 ± 1.18	78.31***
Q_2	14.07 ± 1.29	77.07***
Q_3	11.19 ± 1.05	81.76***
Q_4	16.57 ± 2.24	72.99**
Q_5	5.57 ± 0.59	90.92***
Q_6	12.46 ± 1.47	79.69***
Q_7	13.14 ± 1.28	78.58**
Q_8	7.09 ± 2.16	88.44***
Q_9	7.38 ± 2.44	87.97***
Q_{10}	11.41 ± 1.86	81.40***
Q_{11}	21.46 ± 2.11	65.09**
Q_{12}	18.33 ± 1.37	70.13**
Q_{13}	14.45 ± 1.26	76.45**
Q_{14}	20.03 ± 2.02	67.36**
Q_{15}	17.41 ± 2.47	71.63**
Q_{16}	23.20 ± 2.58	62.19**

Table 4: Acetic acid induced writhing response of the tested compounds and % analgesic activity.

Values are expressed as mean ± SEM of five animals in each group.

**Statistically significant (P < 0.05).

***Statistically significant (P < 0.01).

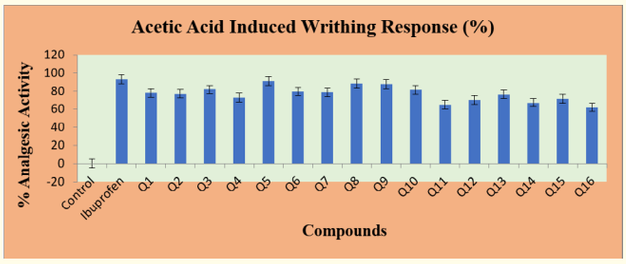


Figure 3: Acetic acid induced writhing test of compounds.

Conclusion

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

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Conflict of Interest

The authors declared no conflict of interest.

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