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Synthesis, Characterization and Biological Study of Novel Quinazolinone Derivatives.

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ABSTRACT

Condensation of 2-methyl-4*H*-1,3-benzoxazine-4-one (1) with *p* – amino acetophenone gave 3-(4-acetylphenyl)-2-methylquinazolin-4*(3H)*-one(2). Claisen-Schmidt condensation of compound **2** with different aromatic and heterocyclic aldehydes afforded different quinazolyl chalcones **3(a-h)** which on refluxing with hydroxylamine hydrochloride gave 3-(4-(5-(3-substitutedphenyl)-4,5-isoxazol-3-yl) phenyl)-2-methyl quinazolin -4*(3H)*-ones **4 (a-h)** in good yields. In anti-convulsant and anti-inflammatory screening, three compounds **4a, 4b & 4f** have shown excellent anti-convulsant activity and compounds **4c & 4f** showed significant anti-inflammatory activity.

Keywords: Quinazolines, Isoxazole, Claisen-Schmidt condensation, Anti-Convulsant, Anti-Inflammatory.

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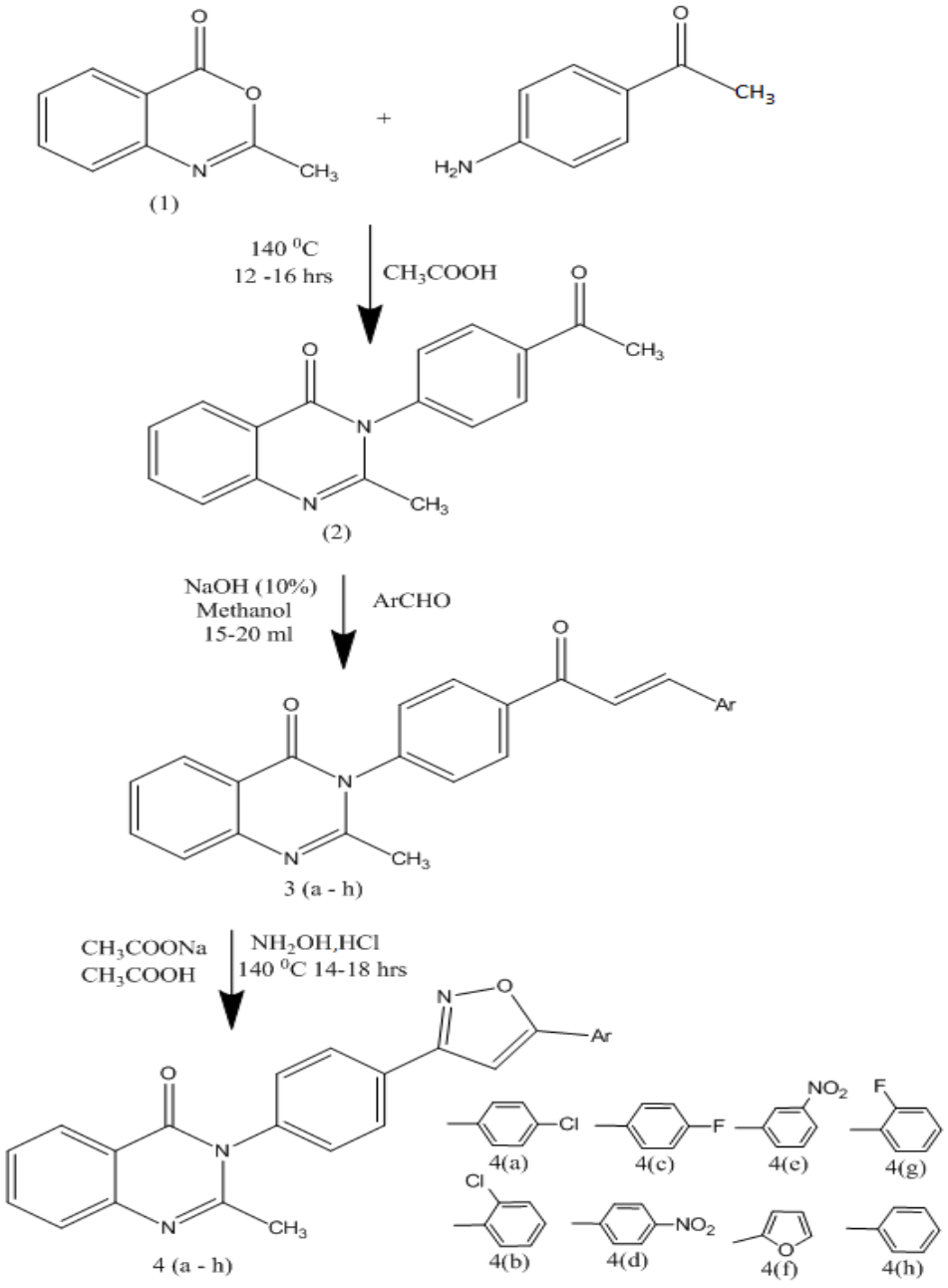
INTRODUCTION

Quinazolin-4-*(3H)*-one derivatives are of considerable interest due to their pharmacological properties like anti-convulsant[1], anti-depressant[2], anti-microbial[3], anti-fungal[4], anti tubercular[5], anti-inflammatory[6,], anti-allergic besides protein tyrosine kinase and cholecystokinin inhibitor activities. Derivatives of isoxazole have played a vital role in the history of heterocyclic chemistry. They are being used extensively in medicinal chemistry due to their versatile pharmacological properties such as insecticidal, anti-bacterial[7], antibiotic[8], anti-fungal, anti-tuberculosis[9], anti-cancer and anti-inflammatory activities[10]. In the present study, we tried to combine these two heterocycles into a single scaffold in order to obtain compounds with better and noteworthy pharmacological properties. The synthesized 3-(4-(5-(3-substitutedphenyl)-4,5-isoxazol-3-yl) phenyl)-2-methyl quinazolin -4*(3H)*-ones were characterized by physical and spectral data and screened for anti-convulsant and anti-inflammatory activities.

RESULTS AND DISCUSSION



Scheme 1

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Eight novel isoxazole incorporated quinazolinone derivatives were synthesized from 2-methyl-4*H*-1, 3 -benzoxazine-4-one (**1**) as per Scheme 1. Further Condensation of **1** with *p* – amino acetophenone in glacial acetic acid under reflux at 140^0 C for 12-16 h gave 3-(4-acetylphenyl)-2-methylquinazolin-4*(3H)*-one (**2**). The confirmation of compound **2** was done with the help of FTIR and mass spectral data. IR data of compound **2** showed two absorption peaks at 1689 cm^{-1} and 1666 cm^{-1} corresponding to C=O str. of quinazolinone and C=O str. of $COCH_3$ respectively. Molecular ion peak of 100% intensity was observed at m/z 279.1 in mass spectrum which clearly indicated the formation of compound **2**. Claisen-Schmidt condensation of compound **2** with various aromatic and heterocyclic aldehydes resulted into different quinazolyl chalcones **3(a-h)** and were confirmed by FTIR and mass spectral data. In IR spectra, the shifting of absorption peaks observed from 1666 cm^{-1} to around 1650 cm^{-1} indicated the formation of chalcones. Further confirmation was obtained in mass spectra which showed molecular ion peaks of 100% intensity corresponding to molecular weight of the compounds. Cyclization of quinazolyl chalcones into isoxazoles was carried out using hydroxylamine hydrochloride in acetic acid medium by refluxing at 120 ^{0}C for 16-18 h. In IR spectra, the disappearance of α, β unsaturated carbonyl absorption and appearance of new absorption peak around 1600 cm^{-1} due to C=N stretching and a molecular ion peaks corresponding to molecular weight indicated the formation of isoxazolyl quinazolinones. In ^{1}H NMR, the three protons of CH_3 group at position 2 on quinazolinone ring appeared as singlet at δ 2.1 -2.4 and aromatic protons appeared in the range of δ 7.0 – 8.4. All the synthesized compounds were screened for anti-convulsant and anti- inflammatory activities using standard procedures.

BIOLOGICAL ACTIVITY

ANTI-CONVULSANT ACTIVITY

In the present investigation, the anti-convulsant activitiy of eight derivatives was tested by maximal electroshock method[11] using phenytoin as standard drug. Results were expressed as mean ± standard error of measurement (SEM). Statistical analysis was performed using ANOVA, followed by Dunnett's test [12]; $P < 0.05$ was considered statistically significant. The results obtained in this investigation indicated that the compounds **4a, 4b** and **4f** have significant and protective effects on seizures when compared to vehicle treated rats. Among these three compounds, compound **4a** showed maximum protection against seizures and the effect was almost similar to standard drug, phenytoin and the results are shown in table 1.

Table 1 Anti-convulsant activity of synthesized compounds

Treatment	Flexion	Extension	E/F$^{\$}$	% Protection
control	5±0.013	17±0.020	3.4	-
Standard (phenytoin)	2±0.009	0	-	100
4(a)	7±0.008	2±0.011	0.4	91.7
4(b)	5±0.007	2±0.010	0.28	88.2
4(c)	4±0.005	6±0.007	1.5	55.8
4(d)	4±0.005	8±0.009	2.0	41.1
4(e)	5±0.004	12±0.008	2.4	29.4
4(f)	2±0.007	1±0.008	0.5	85.2
4(g)	4±0.006	7±0.007	1.75	47.2
4(h)	5±0.007	9±0.007	1.8	48.1

Values are expressed as mean±SE, n=6 animals (rats) in each group.
Standard and test compounds were administered orally at a dose of 25 mg/kg ; $^{\$}$ E/F = extension/flexion [decrease in ratio of extension phase (in seconds) / flexion phase (in seconds)]

ANTI-INFLAMMATORY ACTIVITY

The anti-inflammatory activity of eight derivatives was tested by carrageenan induced rat paw edema method as per the procedure described in our earlier work [6] using ibuprofen as standard drug. The mean edema volume and percentage inhibition were recorded and presented in Table 2. The results obtained in this investigation indicated that the percentage protection against edema formation with compounds **4c** and **4f** were significant, while the other compounds showed moderate protection. The test was done by using standard procedure against inflammation induced by carrageenan at 0.5, 1, 2, 3 and 4 h intervals.

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Table 2 Anti-inflammatory activity of synthesized compounds (4a-4h)

Compound[¥]	Paw edema(ml)(Mean±S.E)					% Inhibition After 3h
	30 min	1 h	2h	3h	4h	
Control	0.27±0.013	0.34±.020	0.61±.019	0.72±0.028	0.80±.0011	-
Standard	0.17±0.011	0.18±0.009	0.20±0.008	0.23±0.007	0.24±0.007	68
4a	0.18±.008	0.21±0.011	0.32±0.014	0.39±0.017	0.42±0.019	45
4b	0.19±0.007	0.22±0.010	0.35±0.012	0.38±0.010	0.41±0.009	47
4c	0.20±0.005	0.23±0.007	0.25±0.008	0.29±0.013	0.32±0.015	**59**
4d	0.21±0.005	0.24±0.009	0.35±0.009	0.40±0.011	0.55±0.015	44
4e	0.22±0.004	0.25±0.008	0.37±0.014	0.44±0.016	0.57±0.017	38
4f	0.21±0.007	0.23±0.008	0.26±0.007	0.30±0.009	0.36±0.010	**58**
4g	0.22±0.014	0.26±0.010	0.40±0.014	0.50±0.011	0.58±0.016	30
4h	0.23±0.004	0.27±0.018	0.39±0.014	0.48±0.016	0.53±0.014	33

[¥] Standard and test compounds were administered orally at a dose of 100 mg/kg Each value represents mean ± SE of six animals; *P < 0.05 was considered significant when compared to control

EXPERIMENTAL

All the solvents and chemicals used were of synthetic grade from SD fine chemicals Ltd., E.Merck, NR chemicals Ltd. and Aldrich chemicals. Completion of the reactions was monitored by analytical thin layer chromatography (TLC) using E- Merck 0.25 mm silica gel plates. Visualization was accomplished with UV light (256 nm) and iodine chamber. Purification of synthesized compounds was done by re-crystallization process. The purity of the compounds was checked by a single spot in TLC. Melting points were determined in open capillary tubes using ANALAB melting point apparatus and are uncorrected. All the ^{1}H NMR spectra were recorded on AVANCE 300 MHz spectrometer using DMSO-d_6 as solvent and tetra methyl silane (TMS) as an internal standard. Chemical shift values are listed in δ scale. The IR spectra were recorded on Schimadzu FTIR spectrophotometer by using 1% potassium bromide discs. Mass spectra of the compounds were recorded on Agilent 6430 triple quadruple LC-MS system and were given in mass units (m/z).

General procedure for synthesis of chalcones 3 (a-h)

Equimolar quantities of 3-(4-acetylphenyl)-2-methylquinazolin-4*(3H)*-one and substituted aromatic /heterocyclic aldehyde were dissolved in 15 - 20 ml of methanol and 3 to 5 ml of NaOH (10%) was added and stirred for 2-4 h in order to obtain the precipitate. The reaction was monitored by TLC and after the completion of the reaction; the precipitate was filtered, washed thoroughly with water and recrystallized from aq. methanol.

General procedure for synthesis of 3-(4-(5-(3-substitutedphenyl)-4,5-isoxazol-3-yl) phenyl)-2-methyl quinazolin -4*(3H)*-ones 4 (a-h)

To a mixture of 0.01 mol of chalcone and 0.03 mol of hydroxyl amine hyrdrochloride, was added 1 g of sodium acetate and 10 ml of acetic acid and refluxed for 14 – 16 hr at 120 ^{0}C. Completion of the reaction was monitored by TLC. Reaction mixture was poured into crushed ice to obtain the solid product which was filtered, washed thoroughly with water and recrystallised from aq. methanol. The structural confirmation of the compounds was done with the help of IR, mass and ^{1}H NMR spectral data which are given below.

(4a) : Cream coloured yellow solid; Yield:76%; M. p: 112-119; IR: (KBr)cm^{-1}: 3064.61 (aromatic C-H),1683 (C=O of quinazolinone) , 1593 (C=N) ,755 (C-Cl); ^{1}H NMR (300MHz, $CDCl_3$ +DMSO d_6) : 2.1 (s, 3H, CH_3), 7.3 – 8.1 (15H,Ar-H); Mass : m/z :415 (M^+1);[Found :C, 69.35; H, 3.78; N, 9.89 $C_{24}H_{17}N_3O_2Cl$ calculated C,69.65; H,3.90; N,10.15%].

(4b) : Cream coloured yellow solid; Yield:73%; m. p: 114-120; IR: (KBr)cm^{-1}: 3054.61 (aromatic C-H),1681 (C=O of quinazolinone),1588 (C=N), 753 (C-Cl); ^{1}H NMR(300MHz, $CDCl_3$ +DMSO d_6): 2.2 (s, 3H, CH_3), 7.4 – 8.1 (15H,Ar-H); Mass : m/z :415 (M^+1);[Found :C, 69.35; H, 3.78; N, 9.89 $C_{24}H_{17}N_3O_2Cl$ calculated C,69.65; H,3.90; N,10.15%].

(4c) : pale coloured yellow solid; Yield:82%; m. p: 120-123; IR: (KBr)cm^{-1}: 3066.6 (aromatic C-H),1683 (C=O of quinazolinone), 1593 (C=N); ^{1}H NMR(300MHz, $CDCl_3$ +DMSO d_6): 2.3 (s, 3H, CH_3) 6.8 – 7.9 (14H, Ar-H); Mass : m/z: 398 (M^+1);[Found :C,72.34; H, 3.96; N,10.37 $C_{24}H_{17}N_3O_2F$ calculated C,72.54; H, 4.06; N,10.57%].

(4d): Brownish coloured yellow solid; Yield:75%; m. p: 128-130; IR: (KBr)cm^{-1}: 3072.61 (aromatic C-H),1681 (C=O of quinazolinone), 1595 (C=N), 1535, 1346 (NO_2); ^{1}H NMR(300MHz, $CDCl_3$ +DMSO d_6): 2.1 (s, 3H, CH_3), 7.3 – 8.4 (14H,Ar-H); Mass : m/z :426 (M^+1);[Found :C,76.20; H, 4.76; N,10.37 $C_{24}H_{17}N_4O_4$ calculated C,76.32; H, 4.87; N,10.68%].

(4e) : Reddish coloured yellow solid; Yield:83%;m. p: 127-131; IR: (KBr)cm^{-1}: 3052.61(aromatic C-H), 1683 (C=O of quinazolinone), 1593 (C=N), 1540, 1346 (NO_2); ^{1}H NMR(300MHz, $CDCl_3$ +DMSO d_6): 2.3(s, 3H, CH_3) 7.2 – 8.0 (15H,Ar-H); Mass : m/z: 426(M^+1);[Found :C,76.20; H, 4.76; N,10.37 $C_{24}H_{17}N_4O_4$ calculated C,76.32; H, 4.87; N,10.68%].

(4f) : Black coloured solid; Yield:68%; m. p: 132-128; IR: (KBr)cm^{-1}: 2980.61(aromatic C-H), 1681 (C=O of quinazolinone), 1593 (C=N), 1230 (C-O-C) str; ^{1}H NMR(300MHz, $CDCl_3$ +DMSO d_6): 2.3 (s, 3H, CH_3)7.0 – 8.0 (14H, Ar-H); Mass : m/z :370 (M^+1);[Found :C,71.44; H, 3.96; N,11.17 $C_{22}H_{15}N_3O_3$ calculated C,71.54; H, 4.09; N,11.38%].

(4g) : pale coloured yellow solid; Yield:79%; m. p: 112-119; IR: (KBr)cm^{-1}: 30656.6 (aromatic C-H),1688 (C=O of quinazolinone), 1593 (C=N); ^{1}H NMR(300MHz, $CDCl_3$ +DMSO d_6): 2.1 (s, 3H, CH_3), 7.2– 8.2 (14H,Ar-H); Mass : m/z: 398 (M^+1);[Found :C,72.34; H, 3.96; N,10.37 $C_{24}H_{17}N_3O_2F$ calculated C,72.54; H, 4.06; N,10.57%].

(4h) : Cream coloured solid; Yield:60%; m. p: 121-124; IR: (KBr)cm^{-1}: 3046.6 (aromatic C-H),1681 (C=O of quinazolinone), 1593 (C=N); ^{1}H NMR: (300MHz, $CDCl_3$ +DMSO d_6) 2.0 (s, 3H, CH_3) 7.3 – 8.1 (15H,Ar-H); Mass : m/z: 379.5 (M^+); $C_{24}H_{17}N_3O_2$ [Found :C, 75.35; H, 4.38; N, 10.98 calculated C,75.98; H,4.52; N,11.08%].

ANTI-CONVULSANT ACTIVITY

Young adult male Wistar rats weighing 150-180 g were used, which were acclimatized to the laboratory conditions and maintained on standard laboratory rat feed and clean water. The rats were divided into groups of 6 animals each; the ear electrodes were placed on the ear by holding the animal properly and the current was applied at 150 mA for 0.02 Sec. The control group received only the vehicle, i.e. the CMC. The other groups were administered with standard and test drugs at a dose of 25 mg/kg body weight orally, 30 minutes after inducing electro convulsions. All the experimental groups were compared with the control.

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