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Research Article

SYNTHESIS OF SOME NOVEL 2, 3- DISUBSTITUTED QUINAZOLINONE DERIVATIVES AS ANALGESIC AND ANTI-INFLAMMATORY AGENTS

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ABSTRACT

A series of some novel 2, 3-disubstituted quinazolinone derivatives were synthesized by condensing 2-methyl/ 2-phenyl/6-bromo-2-methyl/6-bromo-2-phenyl/6, 8-dibromo-2-methyl/ 6, 8-dibromo-2-phenyl benzoxazines with compounds containing amino group. All the newly synthesized compounds chemical structures were confirmed by IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectral data. All the synthesized compounds have been tested for their analgesic and anti-inflammatory activities.

Keywords: Quinazolinone, Benzoxazine, Analgesic, Anti-inflammatory.

INTRODUCTION

Quinazolinone is a bicyclic compound consisting of a pyrimidine system fused at 5, 6 with benzene ring having broad spectrum of medicinal values such as anti-bacterial[1], anti-fungal[2], anti-cancer[3, 4], anti-inflammatory[5], analgesic[6], anti-diabetic[7], anti-oxidant[8], anti-HIV[9], anti-tubercular[10], CNS depressant[11], anti-convulsant[12], anti-parkinsonism[13]etc. Nonsteroidal anti-inflammatory drugs (NSAID) are commonly prescribed for the treatment of acute and chronic inflammation, pain and fever. However, long term clinical usage of NSAIDS is associated with significant side effects such as gastro intestinal lesions, bleeding and nephrotoxicity. Therefore discovery of new safer anti-inflammatory drugs represents a challenging goal in research area[14, 15]. Quinazolinone derivatives with 2, 3- substitution are reported to possess significant analgesic and anti-inflammatory activity[16, 17]. Looking at the biological significance of quinazolinone nucleus, it was thought to synthesize new quinazolinone derivatives and screen them for their analgesic and anti-inflammatory activity.

EXPERIMENTAL

Melting points were determined using an open ended capillary tube method and are uncorrected. The completion of the reaction was checked by TLC using a silica gel G as stationary phase and the spot is visualized by UV-chamber. FT-IR Spectra were recorded on a Perkin-Elmer 1800 FT-IR in KBr disc. ^{1}H-NMR spectra were recorded at 400 MHZ on a Brucker FT-NMR spectrophotometer using TMs as internal standard. Mass spectra were recorded using a Thermo Finnigan LCQ Advantage MAX 6000 ESI Mass spectrometer. The synthetic strategy to synthesize the target compounds is depicted in scheme 1 & 2 and the scheme details are given in Table 1.

Synthesis of 2-methyl-4*H*-Benzoxazine-4-one[18]

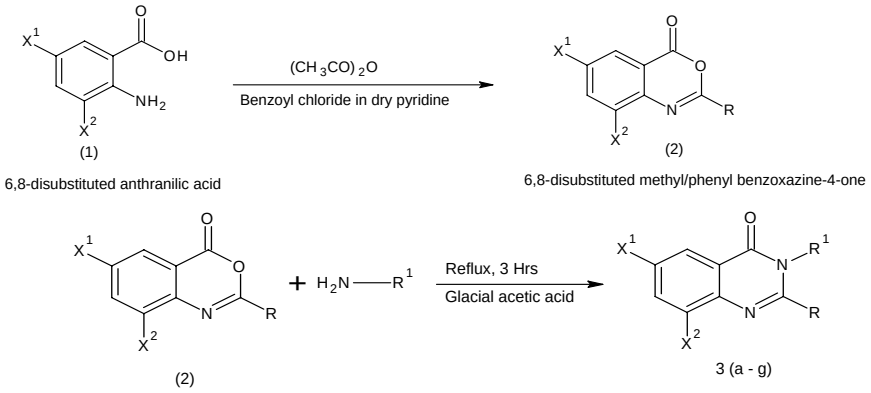
A mixture of disubstitued anthranilic acid, (1) (R^1, R^2= H, Br) (0.12 mol) in Acetic anhydride (0.2 mol) was refluxed for 4 Hrs. The excess solvents were then distilled off under reduced pressure. The reaction mixture was filtered, washed, dried and recrystallized with absolute ethanol.

Synthesis of 2-phenyl-4*H*-Benzoxazine-4-one[19]

A mixture of disubstitued anthranilic acid, (1) (X^1, X^2= H, Br) (0.1 mol) was dissolved in 50 ml of dry pyridine. To this solution, Benzoyl chloride (0.2mol) was added dropwise with constant stirring at low temperature. The reaction mixture was cooled. When the addition of Benzoyl chloride was completed, the resultant mixture was treated with 10% sodium bicarbonate. The reaction mixture was filtered and washed repeatedly with water to remove inorganic materials. The crude product obtained was recrystallized from ethanol.

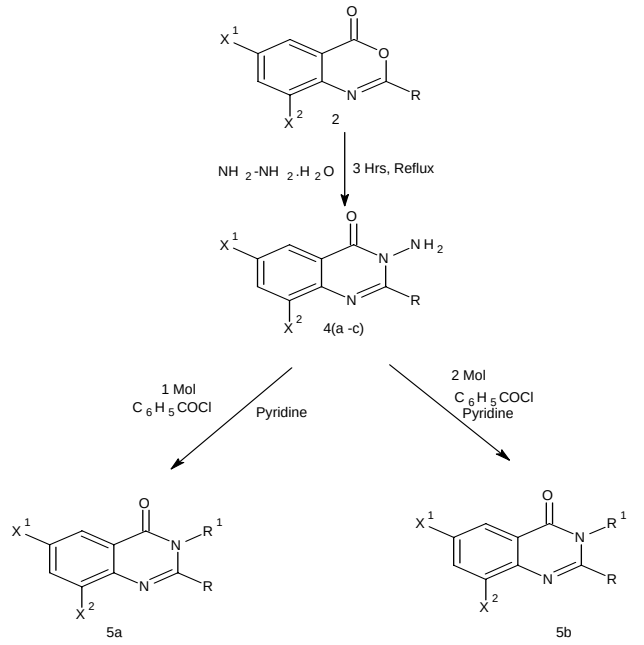
General procedure for the synthesis of title compounds

Various substituted benzoxazine-4-ones (2) (0.01 mol) and o-Toludine/ pyridine-4-carbohydrazide/ Hydrazine Hydrate (0.01 mol) were refluxed for 3 hours in presence of glacial acetic acid. The reaction mixture was allowed to cool at room temperature. The crude product was recrystallized using absolute alcohol. The pyhsico chemical parameters of the synthesized compounds were represented in table 2.



X^1, X^2 = H, Br; R= CH_3, C_6H_5; R^1 = -$C_6H_4CH_3$, -$NHCOC_6H_4N$

Scheme 1



X^1, X^2 = H, Br; R= CH_3, C_6H_5; R^1= -$NHCOC_6H_5$, -N $(COC_6H_5)_2$

Scheme 2

Table 1: Scheme details

Sl. No	Compound Code	X^1	X^2	R	R^1
1	3a	H	H	CH_3	-$C_6H_4CH_3$
2	3b	H	H	C_6H_5	-$C_6H_4CH_3$
3	3c	Br	H	C_6H_5	-$C_6H_4CH_3$
4	3d	Br	Br	CH_3	-$C_6H_4CH_3$
5	3e	Br	Br	C_6H_5	-$C_6H_4CH_3$
6	3f	H	H	CH_3	$NHCOC_6H_4N$
7	3g	H	H	C_6H_5	$NHCOC_6H_4N$
8	4a	H	H	CH_3	-NH_2
9	4b	H	H	C_6H_5	-NH_2
10	4c	Br	Br	C_6H_5	-NH_2
11	5a	H	H	C_6H_5	$NHCOC_6H_5$
12	5b	H	H	C_6H_5	-$N(COC_6H_5)_2$

Table 2: Physico chemical parameter of the synthesized compounds

Sl. No.	Compound code	Molecular formula	Molecular Weight (in gms)	Percentage yield	Rf value	Melting point (^{0}C)	Log p value
1	3a	$C_{16}H_{14}N_2O$	250.29	62	0.54	84	2.5
2	3b	$C_{21}H_{16}N_2O$	312.36	68	0.64	78	4.4
3	3c	$C_{21}H_{15}BrN_2O$	391.26	72	0.61	94	5.21
4	3d	$C_{16}H_{12}Br_2N_2O$	408.08	70	0.64	98	4
5	3e	$C_{21}H_{14}Br_2N_2O$	470.15	71	0.53	90	5.94
6	3f	$C_{15}H_{12}N_4O_2$	280.28	58	0.58	170	1.2
7	3g	$C_{20}H_{14}N_4O_2$	342.35	68	0.68	142	2.16
8	4a	$C_9H_9N_3O$	175.18	62	0.62	138	0.73
9	4b	$C_{14}H_{11}N_3O$	237.25	60	0.72	144	1.71
10	4c	$C_{14}H_9Br_2N_3O$	395.04	64	0.71	132	3.21
11	5a	$C_{21}H_{15}N_3O_2$	341.36	49	0.65	130	3.42
12	5b	$C_{28}H_{19}N_3O_3$	445.46	50	0.75	80	4.75

CHEMISTRY

IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectra were consistent with the assigned structure.

2-methyl-3-(2-methylphenyl) quinazolin-4 (3*H*)-one (3a). IR (KBr): 1672 (C=O str), 1152.7 (C=C), 1604.5(C=N), 1448.8 (CH_3), 3397.5,(C-H Ar); ^{1}H-NMR ($CDCl_3$): δ 6.64 (s, 1H, Ar-H), 6.6 (s, 1H, Ar-H), 7.4- 8(m,6H, Ar- H), 2.8 (s, 3H, CH_3), 2.23(s, 3H, CH_3); ^{13}C-NMR: δ 24.19, 25.52, 29.75, 44.63, 57.96, 120.3, 122.6, 123.9, 125.7, 126.8, 127, 128, 130.1, 131.66, 134.8, 139.9; EI-MS (M/Z): 250 (M+1).

3-(2-methylphenyl)-2-phenylquinazolin-4(3*H*)-one(3b). IR(KBr): 1666 (C=O str), 1517.3(C=C), 1595.5(C=N), 1026 (C-N),

1448.1 (C-H), 2923 (C-H, Ar); ^{1}H-NMR ($CDCl_3$): δ 7.0 (s, 1H, Ar-H), 7.1 (s, 1H, Ar-H), 7.2-7.8 (m, 11H, Ar-H), 2.3 (s, 3H, CH_3); ^{13}C-NMR: δ 87.5, 93.5, 98.2, 120.4, 122.7, 125.8, 126.5, 126.5, 126.8, 127.0, 127.2, 127.4, 128.8, 131.9; EI-MS (M/Z): 212 (M+1)

6 -bromo-3-(2-methylphenyl)-2-phenyl quinazolin-4(3*H*)-one (3c).IR (KBr): 1666.6 (C=O str), 1594, 1515 (C=C), 1447.3(C- H), 3047.1, 3568.4 (C-H, Ar), 531 (C-Br);

6, 8-dibromo-2-methyl-3-(2-methylphenyl) quinazolin-4(3*H*)-one (3d).

IR (KBr): 3373.4 (C-H, Ar), 1672(C=O str), 1493(C=C), 2535 (C- H), 1617(C=N), 530,656 (C-Br).

6, 8-dibromo-3-(2-methylphenyl)-2-phenylquinazolin-4(3*H*)-one (3e).IR (KBr): 3403.3 (C-H, Ar), 1671(C=O str), 1512(C=C), 1447(C-H), 1603(C=N), 548, 699(Br).

***N*-(2-methyl-4-oxoquinazolin-3(4*H*)-yl) pyridine-4-carboxamide (3f).**IR(KBr): 3428.8 (C-H, Ar), 1669(C=O str), 1543(C=C), 1605(C=N), 3428 (N-H str); ^{1}H-NMR ($CDCl_3$): δ 7.4-7.5 (m, 4H, Ar-H), 7.28-8.6 (4H, Ar-H in pyridine), 2.37 (s, 3H, CH_3), 2(s, 1H, NH).

3g: *N*-(4-oxo-2-phenylquinazolin-3(4*H*)-yl) pyridine-4-carboxamide:

IR(KBr): 3057.7 (C-H, Ar), 1685(C=O str), 1526(C=C), 1452(C-H), 1599(C=N), 1239(C-N), 3502(N-H str); ^{1}H-NMR ($CDCl_3$): δ 7.31-7.38 (m, 5H, Ar-H), 7.41 (s, 1H, Ar-H), 7.53-7.57 (m, 3H, Ar-H), 7.6-7.72 (m, 5H, Ar-H); EI-MS (M/Z): 342 (M+1)

3-amino-2-methylquinazolin-4(3*H*) - one (4a).IR (KBr): 3540.2(C-H, Ar), 1658.2 (C=O str), 1598.4 (C=C), 1427.8 (C-H), 3302 (N-H str), 1475.9 (C=N).

3-amino-2-phenylquinazolin-4(3*H*)-one (4b).IR(KBr): 3307.1 (C-H, Ar), 1661.2 (C=O str), 1566.9(C=C), 1472.1 (C- H), 3216.8 (N-H str); ^{1}H-NMR ($CDCl_3$): δ 5.02 (s, 2H, NH_2), 7.47-7.48 (d, 2H, Ar-H), 7.73-7.7 (m, 5H, Ar-H), 8.25-8.27 (d, 2H, Ar-H); ^{13}C-NMR: δ 120.19, 126.6, 127.06, 127.9, 128.2, 129.3, 130.2, 134.1, 134.49.

***N*-(4-oxo-2-phenylquinazolin-3(4*H*)-yl) benzamide (5a).**IR(KBr): 3307.7(C-H, Ar), 1660.7 (C=O str), 1566.4 (C=C), 3034.1 (C- H), 1252.1 (C-N), 3216.8 (N-H str); ^{1}H-NMR ($CDCl_3$): δ 7.47-7.51 (m, 5H, Ar-H), 7.73-7.78 (m, 6H, Ar-H), 5.01(s, 1H, Ar-H), 5.05 (s, 1H, Ar-H), 8.27 (s, 1H, N-H), 8.29 (s, 1H, Ar-H); ^{13}C-NMR: δ 120.2, 126.6, 127, 127.9, 128.2, 129.3, 130.2, 134.1, 134.5, 147, 154.6, 131.6.

***N*-(4-oxo-2-phenylquinazolin-3(4*H*)-yl) dibenzamide(5b).** IR (KBr): 3307.7 (C-H, Ar), 1660.7 (C=O str), 1566.4 (C=C), 3034.1 (C-H), 1252.1 (C-N), 3216.8 (N-H str).

Pharmacological activity

The animals were maintained under standardized environmental conditions (22-28^{o}C, 60-70% relative humidity, 12 hours dark/light cycle) and fed with standard mouse Chow and water *ad libitum*. All the animal experiments were conducted during the present study got permission from institutional animal Ethics Committee (IAEC approved) and followed the guidelines of IEAC having the Reference No. 149/99/CTCFEA.

Analgesic activity

The analgesic activity[20] was determined by tail-immersion method. Swiss mice (n=5) of either sex selected by random sampling technique. Diclofenac sodium at the dose of 20 mg/kg (i.p.) was used as a standard drug. The test compounds at a dose of 100 mg/kg in DMSO were administred i.p. The animals were held in position by a suitable restrainer with the tail extending out and the tail (upto 5 cm) was taken dipped in a beaker of water maintained at 55 ±0.5º C. The time in sec taken to withdraw the tail clearly out of water was taken as the reaction time. The first reading 0 min was taken immediately after the administration of the test compound and subsequent reaction time was recorded at 15, 30, 60 and 120 min after the administration of compounds. A cut off point of 15 sec was observed to prevent the tail damage. The percentage analgesic activity was calculated using the following formula and the results are presented in the table 3.

$$PAA = [(T_2-T_1)/T_2] \times 100$$

Where,

T_1 is the reaction time (in sec) before treatment

T_2 is the reaction time (in sec) after treatment

PAA is the percentage analgesic activity

Anti-inflammatory activity

Anti-inflammatory activity[21] was performed by carrageenan-induced paw oedema method in rats. Oedema was induced by sub-plantar injection of 0.1ml of freshly prepared 1% carrageenan into the right hind paw of the rats. The animals were divided into 13 groups of 5 each. Group-1 served as control and received carrageenan (1% W/V in saline). Group 2 served as standard and received Diclofenac sodium (20 mg/kg i.p) , group 3 to 13 received the test compound (100mg/kg). The volume of paw oedema was measured at 0, 15, 30, 60, 120 and 180 min after injection of carrageenan using plethysmograph. The % of oedema inhibition was calculated for each animal group using the formula and the results were represented in table 4.

$$\% \text{ Protection} = \frac{[\text{Control} - \text{Test}]}{\text{Control}} \times 100$$

T – Average increase in paw thickness in groups treated with test compounds; C – Average increase in paw thickness in control.

Statistical analysis

The results are expressed as Mean ± SEM, Students't' test was used to verify the statistical significance at p< 0.001 between the treated and control groups.

Table 3: Analgesic activity of the synthesized compounds

Sl. No	Compound code	Basal reaction time in sec								
		0 min	15 min		30 min		60 min		120 min	
		Mean± SEM	Mean±SEM	%	Mean±SEM	%	Mean±SEM	%	Mean±SEM	%
1	Control	1.24±0.01	1.12±0.01		1.14±0.01**	----	1.5±0.02	----	1.55±0.02	----
2	Standard	1.77±0.07	5.31±0.33	66.6	8.11±0.14**	78	8.62±0.12**	79.5	4.23±0.02**	58.15
3	3a	1.58±0.02	3.46±0.03**	54.3	4.07±0.009**	61.17	3.15±0.026**	49.84	2.28±0.008**	30.7
4	3b	1.09±0.02	2.4±0.023**	54.5	3.21±0.02**	66.04	2.42±0.03**	54.95	1.7±0.019**	35.8
5	3c	1.24±0.11	2.9±0.025**	57.4	4.41±0.02**	66.37	3.23±0.017**	61.6	2.28±0.008**	45.61
6	3d	1.17±0.02	2.27±0.018	48.5	3.1±0.012**	62.25	2.51±0.009**	53.5	1.92±0.012**	39.06
7	3e	1.42±0.02	3.15±0.026	54.9	4.14±0.03**	65.7	3.22±0.02**	55.7	2.27±0.018	37.44
8	3f	1.82±0.03	3.23±0.017	43.6	4.24±0.018**	57.07	3.15±0.026**	42.2	2.91±0.02**	37.45
9	3g	1.51±0.02	3.34±0.02**	54.7	4.23±0.018**	64.3	3.32±0.021**	54.5	2.93±0.01**	48.1
10	4a	1.31±0.02	2.51±0.013	47.8	3.22±0.01**	59.31	2.28±0.008**	42.5	1.91±0.01**	31.41
11	4b	1.71±0.01	3.39±0.018	49.5	4.36±0.018**	60.77	3.46±0.03**	50.57	2.82±0.01**	39.36
12	4c	1.55±0.02	3.75±0.023	58.6	4.23±0.013**	70.08	3.73±0.014**	58.44	2.62±0.01**	40.83
13	5a	1.14±0.01	2.6±0.008	56.5	3.9±0.02**	70.76	2.23±0.01**	48.87	2.19±0.04	47.94

All values are mean ± SD , n=5; ** P < 0.001 indicates the highly significant difference compared with control.

Table 4: Evaluation of anti-inflammatory activity

Sl. No	Compound	Time interval in minutes						% Inhibition
		0	15	30	60	120	180	
1	Control	0.16±0.004	0.18±0.0098	0.21±0.0054**	0.27±0.0059	0.28±0.007	0.3±0.007**	----
2	Standard (Diclofenac Sodium-20mg/kg)	0.14±0.004	0.17±0.006	0.22±0.0054**	0.2±0.007	0.2±0.007***	0.16±0.0039**	63
3	3a	0.14±0.0039	0.16±0.004	0.2±0.005**	0.22±0.0037	0.19±0.005***	0.18±0.006**	40
4	3b	0.16±0.006	0.19±0.006	0.22±0.009**	0.24±0.0067	0.2±0.0078***	0.19±0.016***	36
5	3c	0.12±0.009	0.17±0.002	0.19±0.0056**	0.23±0.006	0.2±0.0078***	0.18±0.008***	40
6	3d	0.11±0.01	0.18±0.004	0.18±0.004	0.21±0.0038	0.16±0.004***	0.14±0.004***	53
7	3e	0.15±0.005	0.17±0.0059	0.24±0.0067***	0.26±0.002	0.21±0.007***	0.17±0.004***	43
8	3f	0.15±0.0032	0.18±0.006	0.22±0.0037**	0.25±0.0039	0.21±0.007***	0.17±0.004***	43
9	3g	0.17±0.005	0.19±0.0051	0.21±0.0068**	0.23±0.0056	0.16±0.006	0.15±0.004***	50
10	4a	0.13±0.009	0.14±0.023	0.16±0.0045	0.2±0.007	0.19±0.005***	0.15±0.0037***	50
11	4b	0.16±0.0056	0.19±0.006	0.22±0.0085**	0.18±0.0045	0.15±0.006***	0.15±0.005***	50
12	4c	0.12±0.0066	0.16±0.0045	0.18±0.0075	0.22±0.006	0.17±0.006	0.13±0.009***	56
13	5a	0.13±0.009	0.15±0.005	0.19±0.0051**	0.2±0.006	0.14±0.004***	0.12±0.007***	60

All values are mean ± SD , n=5; *** - P<0.001, ** P<0.01.

RESULTS AND DISCUSSION

In the present study, we have evaluated the analgesic activity by tail immersion and anti-inflammatory activity by carageenan induced paw edema method. Among the synthesized compounds, compound 4c (70.08 & 56%) and 5a (70.76 & 60%) exhibited significant analgesic and anti-inflammatory activity compared to standard drug Diclofenac sodium (20mg/kg) respectively. The significant activity may be due to the presence of electron withdrawing group in 3 rd position of Quinazolinone nucleus. Compounds 3c, 3b, 3e and 3g exhibited moderate analgesic activity. Compounds 3d, 3g, 4a and 4b exhibited moderate anti-inflammatory activity.

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