Full Paper

Synthesis and Evaluation of Novel 2-Substituted-quinazolin-4(3*H*)-ones as Potent Analgesic and Anti-inflammatory Agents

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A novel series of 2-substituted-quinazolin-4(3*H*)-ones were synthesized by reacting 3,5-disubstituted-anthranilic acid with acetic anhydride/benzoyl chloride, which were further reacted with different primary amines to obtain 2,6,8-substituted-quinazolin-4(3*H*)-ones **6a–f**, **7**, **8**. All the synthesized compounds were characterized and screened for analgesic and anti-inflammatory activities. Compounds 6,8-dibromo-2-phenyl-3-(4′-carboxyl phenyl)quinazolin-4(3*H*)-one **7** and 6,8-dibromo-2-phenyl-3-(2′-phenylethanoic acid)quinazolin-4(3*H*)-one **8** displayed good analgesic and anti-inflammatory activity in comparison to the reference standards acetyl salicylic acid and indomethacin, respectively.

Keywords: Analgesic / Anti-inflammatory activity / Quinazolines / Quinazolinones

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Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly prescribed for the treatment of acute and chronic inflammation, pain, and fever. Most of the NSAIDs that are available in the market are known to inhibit isoforms of the enzyme cyclooxygenase (COX), a constitutive form, COX-1, and an inducible form, COX-2, to elicit therapeutic effect. However, long-term clinical use of NSAIDs is associated with significant side effects such as gastrointestinal lesions, bleeding, and nephrotoxicity. Development of analgesic and anti-inflammatory active drugs with less ulcerogenic side effects is still a distinct dream, therefore, discovery of new and safer anti-inflammatory drugs represents a challenging goal [1–2].

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Abbreviations: monoamine oxidase (MAO); percent analgesic activity (PAA)

Quinazolin-4(3*H*)-ones with 2,3-disubstitutions are reported to possess significant anticonvulsant [3, 4], antimicrobial [5–7], antihelminthic [8, 9], antiallergic [10], antitumor [11], anticancer [12], monoamine oxidase (MAO) inhibitor [13], and CNS activities [14], and, in particular, analgesic as well as anti-inflammatory activities [15–36]. Some important analgesic and anti-inflammatory active quinazolinones include 2-phenyl-3-[substituted-benzothiazol-2-yl]-4(3*H*)-quinazolinones **1a–c**, which are COX-1 and COX-2 enzyme inhibitors [22], 3-(4-methoxyphenyl)-2-substituted-amino-quinazolin-4(3*H*)-ones **2a–b** with lower gastrointestinal and ulcerogenic side effects in comparison to acetyl salicylic acid [23], and 4-(4-fluorophenyl)-1-isopropyl-7-methyl-1*H*-quinazolin-2-one **3** (Fig. 1). Substitution of the aromatic ring [24, 25] and substituted aryl moiety [26, 27] at the 3-position [28, 29] of the quinazolin-4(3*H*)-one nucleus are reported to generate potential analgesic and anti-inflammatory agents.

Recently, we had reported the synthesis and antimicrobial evaluation of 2-methyl-quinazolin-4(3*H*)-ones [26, 27]. Keeping in view the reported high analgesic and anti-inflammatory activity of the substituted quinazolin-4(3*H*)-ones, it was decided to synthesize a series of novel

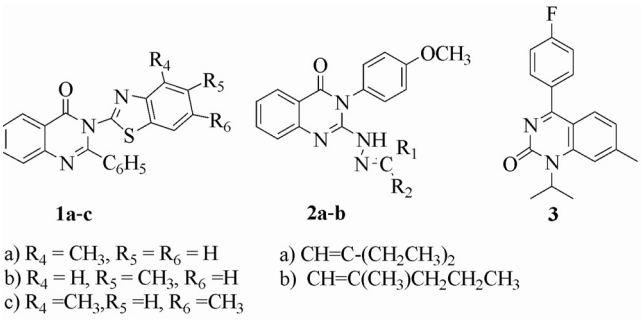


Figure 1. Some potential analgesic and anti-inflammatory agents.

2-substituted-quinazoline-4(3*H*)-ones and evaluate their analgesic and anti-inflammatory activities.

Results and discussion

Chemistry

The key intermediates, substituted-2-methyl-benzoxazin-4(3*H*)-ones **5a–c** were obtained in high yield when a mixture of 3,5-disubstituted-anthranilic acid **4** and acetic anhydride was refluxed under anhydrous conditions for 4 h. The excess of acetic anhydride was distilled off under reduced pressure and the reaction mixture was cooled to room temperature. Similarly, substituted-2-phenyl-benzoxazin-4(3*H*)-ones **5d–f** were obtained by reacting 3,5-disubstituted-anthranilic acid **4** dissolved in 120 mL of pyridine with benzoyl chloride, added dropwise with constant stirring at 0–5°C over a period of 1 h. After completion of the addition, the reaction mixture was stirred for half an hour at room temperature; at the end of the reaction, a solid mass of substituted-2-phenylbenzoxazin-4(3*H*)-ones **5d–f** was obtained. The solid mass was filtered, washed successively with sodium bicarbonate solution (10%) to remove the unreacted acid followed by water, dried, and recrystallized from alcohol. The intermediates **5a–f** obtained as solid masses were used immediately for the next step. Thus, 2,6,8-substituted-benzoxazin-4-ones **5a–f** were refluxed with different substituted aromatic amines *i.e.*, 4-(2-aminophenyl)morpholine, *p*-amino-benzoic acid, and α-amino-phenyl acetic acid in glacial acetic acid over a period of 6 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was allowed to cool and was then poured in ice cold water with constant stirring; it was kept overnight, in the refrigerator. The resulting solid compounds **6a–f**, **7**, and **8** were further washed with distilled water, dried, and recrystallized from ethanol. The purified compounds obtained after recrystallization (Table 1, Scheme 1) were characterized by detailed spectroscopic (^{1}H- and ^{13}C-NMR, IR, Mass) and elemental analysis.

Table 1. Reaction yields of products **6a–f**, **7**, **8** (in %).

Entry	Reactants		Products	Yield (%)
1	**5a**	**ii**	**6a**	80
2	**5b**	**ii**	**6b**	82
3	**5c**	**ii**	**6c**	82
4	**5d**	**ii**	**6d**	81
5	**5e**	**ii**	**6e**	80
6	**5f**	**ii**	**6f**	81
7	**5e**	**iii**	**7**	83
8	**5e**	**iv**	**8**	81

Table 2. Percent analgesic activity of the test compounds (acetic-acid-induced writhing technique).

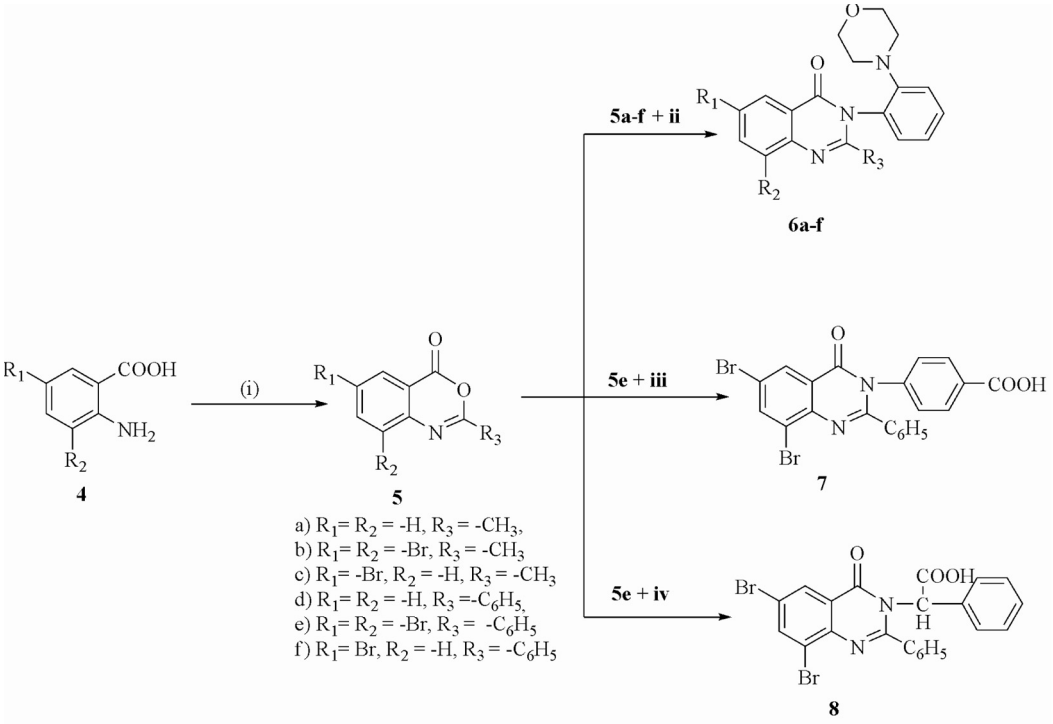
Compound	Dose (mg/kg)	Mean ± SD	Protection (%)
6a	200	28.83 ± 1.666§	18.38
	400	23.00 ± 0.894#	34.72
6b	200	28.50 ± 1.892§	19.95
	400	23.16 ± 0.909#	34.25
6c	200	30.66 ± 2.043§	19.48
	400	24.83 ± 0.666#	32.83
6d	200	29.66 ± 0.954§	16.02
	400	23.66 ± 1.201#	32.83
6e	200	30.66 ± 1.358	13.19
	400	24.83 ± 0.945#	29.61
6f	200	30.66 ± 1.763	13.82
	400	24.66 ± 0.802#	30.09
7	200	23.50 ± 0.885#	33.47
	400	22.00 ± 0.577#	37.55
8	200	24.66 ± 1.429#	30.17
	400	20.33 ± 0.557#	42.27
Control	–	35.33 ± 1.801	–
Acetyl salicylic acid	100	6.50 ± 0.662#	81.60

Each value is the mean ± SEM using six animals in each group. Significant differences with respect to the control group were evaluated by student's test.
$P < 0.05$; § $P < 0.01$; # $P < 0.001$

Pharmacology

All the synthesized products **6a–f**, **7**, **8** were evaluated for their analgesic [30] and anti-inflammatory activity [31].

Analgesic activity was evaluated using the acetic-acid-induced writhing reflex model in Wistar albino mice with acetyl salicylic acid as positive control. The evaluation of analgesic activity of all the test compounds was carried out at two dose levels of 200 and 400 mg/kg body weight (Table 2). After oral administration of the test compounds, control and positive control (acetyl salicylic acid), the writhings were counted for 20 min in an isolated individual cage. The %-protection was calculated



Reactions and reaction conditions: (i) Ac_2O / C_6H_5COCl; (ii) 2-(morpholino-4-yl)-aniline; (iii) *p*-aminobenzoic acid; (iv) a-amino-phenyl-acetic acid.

Scheme 1. Synthesis of some novel 2-substituted quinazolin-4(3*H*)-ones.

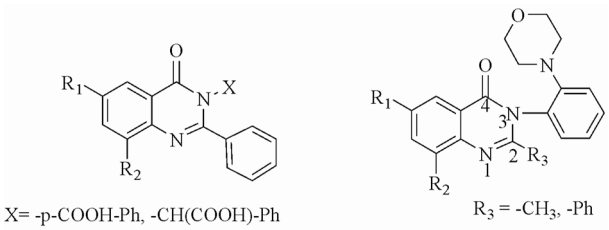


Figure 2. Structure–activity relationship.

using percent analgesic activity (PAA). Compounds **7** and **8** with 2-morpholino-phenyl and 2-phenylethanoic acid substitution showed maximum (PAA) protection of 33.47% and 30.17%, respectively. It is followed by 19.95% (**6b**), 19.48% (**6c**), 18.38% (**6a**), and 16.02% (**6d**) protection; these compounds were moderately analgesic at 200 mg/kg body weight, whereas, compound (**6e**) shows a very low analgesic activity of 13.19%.

Anti-inflammatory activity

The anti-inflammatory activity of the test compounds was evaluated by carrageenan-induced paw oedema test in rats using indomethacin as positive control. The anti-inflammatory inhibition (in %; Table 3) of all the test compounds and the positive control was observed at different time intervals of 1, 2, 4, and 5 h. The maximum inhibition was recorded at 5 h. Maximum inhibitory activity was observed for compound **7** (41.01%) and **8** (38.45%) at 2 h, which is followed by moderately active 35.03% (**6e**), 29.90% (**6c**), and 29.05% (**6a**, **d**). However, lowest %-inhibitory activity of 24.78% was observed for **6b**.

Structure-activity relationships

The preliminary structure-activity relationships for all the compounds was developed at the dose level of 100 mg/kg for both analgesic and anti-inflammatory activity (Fig. 2).

Analgesic activity

Maximum analgesic activity was observed for 6,8-dibromo-substituted compounds **7** and **8** bearing -*p*-Ph-COOH and -CH(COOH)-Ph moieties. Moderate activity was observed for compounds **6a**, **b**, **d** bearing methyl at C2. Very low activity was observed for 6, 8-dibromo-substituted compound **6e** bearing a $-C_6H_5$ moiety at C2.

Anti-inflammatory activity

Maximum anti-inflammatory activity was observed for 6,8-dibromo-substituted and C2 phenyl-substituted compounds **7**, **8**, and **6e**, bearing -*p*-Ph-COOH-, -CH(COOH)-Ph, and -*o*-morpholino-Ph moiety. Subsquently, replacement

Table 3. Percent anti-inflammatory activity of the test compounds (carrageenan-induced paw oedema method).

Compounds/ Standard	Dose (mg/kg)	30 min	% inhibition	1 h	% inhibition	2 h	% inhibition	3 h	% inhibition
6a	200	0.22 ± 0.009	7.77	0.29 ± 0.013	11.6	0.27 ± 0.013#	29.05	0.29 ± 0.019#	19.43
	400	0.22 ± 0.007	7.77	0.27 ± 0.009	10	0.25 ± 0.013#	35.03	0.26 ± 0.014#	25.92
6b	200	0.21 ± 0.20	8.88	0.30 ± 0.014	21.66	0.29 ± 0.015#	24.78	0.30 ± 0.017#	16.66
	400	0.20 ± 0.020	5.55	0.29 ± 0.001	16.66	0.26 ± 0.014#	32.47	0.28 ± 0.015#	21.29
6c	200	0.22 ± 0.016	14.44	0.20 ± 0.020	20	0.27 ± 0.016#	29.90	0.27 ± 0.014#	23.14
	400	0.19 ± 0.013	13.32	0.28 ± 0.019	11.66	0.25 ± 0.009#	35.03	0.26 ± 0.012#	27.77
6d	200	0.22 ± 0.013	4.44	0.29 ± 0.006	15	0.27 ± 0.009#	29.05	0.28 ± 0.009#	21.29
	400	0.20 ± 0.15	6.66	0.30 ± 0.008	15	0.24 ± 0.012#	36.74	0.26 ± 0.015#	26.84
6e	200	0.20 ± 0.011	8.88	0.27 ± 0.009	10	0.25 ± 0.009#	35.03	0.26 ± 0.008#	27.77
	400	0.19 ± 0.018	11.10	0.27 ± 0.013	15	0.22 ± 0.011#	41.87	0.24 ± 0.013#	33.32
6f	200	0.21 ± 0.013	7.77	0.27 ± 0.006	11.66	0.24 ± 0.010#	38.45	0.24 ± 0.011#	31.47
	400	0.20 ± 0.013	8.88	0.28 ± 0.012	11.66	0.23 ± 0.008#	41.02	0.24 ± 0.007#	33.32
7	200	0.22 ± 0.013	7.77	0.29 ± 0.001	11.66	0.23 ± 0.014#	41.01	0.24 ± 0.016#	32.40
	400	0.22 ± 0.012	12.21	0.27 ± 0.012	11.66	0.23 ± 0.014#	39.31	0.22 ± 0.011#	38.88
8	200	0.23 ± 0.016	9.99	0.30 ± 0.014	18.33	0.24 ± 0.012#	38.45	0.25 ± 0.015#	29.62
	400	0.22 ± 0.012	7.77	0.29 ± 0.015	16.66	0.22 ± 0.014#	43.58	0.22 ± 0.016#	37.03
Control		0.20 ± 0.013		0.30 ± 0.005		0.39 ± 0.012#		0.36 ± 0.014#	
Indomethacin	20	0.19 ± 0.008	8.33	0.28 ± 0.015	11.10	0.10 ± 0.008#	74.35	0.16 ± 0.005#	55.55

Each value is the mean ± SEM using six animals in each group. Significant differences with respect to the control group were evaluated by student's test.
$P < 0.05$; § $P < 0.01$; # $P < 0.001$

of the -C_6H_5 with CH_3 in case of **6a**, **d**, **c** resulted in a decrease in activity. Moderate activity was observed for compounds **6a**, **b**, **d** bearing methyl at C2. Very low activity was observed for 6, 8-dibromo-substituted compound **6e** bearing -C_6H_5 at C2.

Conclusion

A number of quinazolines were synthesized and evaluated for their analgesic and anti-inflammatory activities. 6,8-Dibromo-3-phenylquinazoline compounds **7**, **8**, bearing -*p*-COOH-Ph, -CH(COOH)-Ph at N3 displayed moderate analgesic and anti-inflammatory activity. Further structural modification *i. e.*, replacement of bromo at C6 and C8 position, C2 Phenyl/CH_3 and replacement of *o*-morpholino-Ph with -*p*-COOH-Ph, -CH(COOH)-Ph moieties at N3 position resulted in a decrease in both analgesic and anti-inflammatory activity. The high activity observed in case of compounds **7**, **8** may be due to the hydrogen bonding ability of -*p*-COOH-Ph, -CH(COOH)-Ph groups. These lead quinazolines may be subjected to further structural modifications.

Experimental

Chemistry

Melting points were taken in open capillaries on Thomas Hoover melting point apparatus (Thomas Hoover Capillary Apparatus, Philadelphia, PA, USA) and are uncorrected. The IR spectra were recorded in potassium bromide discs on a Perkin-Elmer 398 spectrometer (Perkin-Elmer, Norwalk, CT, USA). The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on 300 MHz-Bruker DPX 200 NMR spectrometer (Bruker Bioscience,USA). The chemical shifts were reported as parts per million (δ ppm) using tetramethyl silane (TMS) as internal standard. Mass spectra were obtained on Finnigan MAT 8230 MS instrument (Thermo Finnigan MAT GmbH, Bremen, Germany) using fast atom bombardment (FAB positive). Elemental analysis was performed on Perkin-Elmer 2400 C, H, N analyzer and values were within the acceptable limits (± 0.4%) of the calculated values. The purity of the synthesized compounds were checked by TLC using E-Merck TLC aluminum sheets silica gel 60 F_{254} (0.2 mm; Merck, Germany) using chloroform/methanol (9:1) as eluent and visualized in an iodine chamber. All the chemicals used were of analytical grade.

Synthesis of substituted benzoxazin-4(3H)-ones ***5a, b***

Unsubstituted/substituted anthranilic acid (0.05 mol) in 0.1 mol of acetic anhydride was refluxed under anhydrous conditions for 4–6 h. The excess of acetic anhydride was then distilled off under reduced pressure and cooled to room temperature. 2-Methyl-benzoxazin-4(3*H*)-one **5a** obtained was filtered and dried under vacuum. Likewise, 2-phenyl-benzoxazin-4(3*H*)-one **5b** was prepared by reacting unsubstituted/substituted anthranilic acid (1 M) dissolved in 120 mL of pyridine by adding benzoyl chloride (1 M) dropwise with constant stirring at 0–5°C over the period of 1 h. After completion of the addition, the reaction mixture was stirred for half an hour at room temperature. At the end of the reaction, a solid mass of 2-phenyl-benzoxazin-4(3*H*)-ones **5b** was obtained. It was filtered, washed successively with sodium bicarbonate solution (10%) to remove the unreacted acid and the water, then it was dried and recrystallized from spirit.

Synthesis of quinazolin-4(3H)-ones ***6a-f, 7, 8***

Equimolar quantities of unsubstituted/substituted 2-methyl-benzoxazin-4(3*H*)-one/2-phenyl-benzoxazin-4(3*H*)-one **5a**, **b** and

the corresponding amine in glacial acetic acid was refluxed for 6 h. After cooling, the contents were poured into crushed ice. The resulting solid was washed with distilled water, filtered, dried in vacuum, and recrystallized from warm ethanol.

2-Methyl-3-(2′-morpholinophenyl)quinazolin-4(3H)-one ***6a***

Yield: 64%; m.p.: 185–187°C; IR (KBr) (in cm^{-1}): 1652 (C=O), 1607 (C=C), 1694 (C=N), 1088 (C-O-C); ^{1}H-NMR ($CDCl_3$) δ: 6.51–7.95 (m, 8H, Ar-H), 3.63 (brs, 4H), 2.94 (brs, 4H), 0.91 (s, 3H, -CH_3); ^{13}C-NMR ($CDCl_3$) *d*: 160.2 (C_4), 151.2 (C_2), 147.2 (C_9'), 143.0 (C_2'), 133.5 (C_7), 128.6 (C_5), 127.6 (C_6), 125.0 (C_4'), 124.3 (C_1'), 122.6 (C_6'), 122.3 (C_8), 120.9 (C_{10}), 118.4 (C_5'), 114.7 (C_3'), 66.7 (C_3' & C_5'), 46.3 (C_2'' & C_6''), 22.4 (-CH_3); MS (*m/z*): 321 [M^+]. Anal. calcd. for $C_{19}H_{19}N_3O_2$: C, 71.01; H, 5.96; N, 13.08. Found: C, 70.99 ; H, 5.73; N, 12.92.

6,8-Dibromo-2-methyl-3-(2′-morpholinophenyl)quinazolin-4(3H)-one ***6b***

Yield: 68%; m.p.: 205–207°C; IR (KBr) (in cm^{-1}): 1772 (C=O), 1603 (C=C), 1670 (C=N), 1010 (C-O-C); ^{1}H-NMR ($CDCl_3$) δ: 8.17 (s, 1H, ArH), 7.63–6.25 (m, 5H, ArH), 3.67 (brs, 4H), 2.93 (brs, 4H), 0.93 (s, 3H, -CH_3); ^{13}C-NMR ($CDCl_3$) δ: 160.4 (C_4), 151.3 (C_2), 150.6 (C_9'), 142.2 (C_2'), 139.3 (C_7), 131.2 (C_5), 125.6 (C_{10}), 125.3 (C_4'), 124.4 (C_1'), 123.7 (C_6), 122.5 (C_6'), 118.6 (C_5'), 114.4 (C_3'), 113.4 (C_8), 66.3 (C_3'' & C_5''), 46.9 (C_2'' & C_6''), 22.3 (-CH_3); MS (*m/z*): 478 [M^+]. Anal. calcd. for $C_{19}H_{17}N_3Br_2O_2$: C, 47.63; H, 3.58; N, 8.77. Found: C, 47.56; H, 3.41; N, 8.61.

6-Bromo-2-methyl-3-(2′-morpholinophenyl)quinazolin-4(3H)-one ***6c***

Yield: 75%; m.p.: 198–200°C; IR(KBr) (in cm^{-1}): 1684 (C=O), 1601 (C=C), 1645 (C=N), 1010 (C-O-C); ^{1}H-NMR ($CDCl_3$) δ: 8.15 (s, 1H, ArH), 7.73–6.58 (m, 6H, ArH), 3.66 (brs, 4H), 2.90 (brs, 4H), 0.92 (s, 3H, -CH_3); ^{13}C-NMR ($CDCl_3$) δ: 160.8 (C_4), 152.2 (C_2), 146.1 (C_9'), 143.1 (C_2'), 136.5 (C_7), 132.4 (C_5), 125.7 (C_{10}), 125.3 (C_4'), 124.6 (C_1'), 123.2 (C_6), 122.6 (C_6'), 118.7 (C_5'), 114.9 (C_3'), 113.1 (C_8), 66.7 (C_3'' & C_5''), 46.4 (C2″ & C_6''), 22.1 (-CH_3); MS (*m/z*): 399 [M^+]. Anal. calcd. for $C_{19}H_{18}N_3BrO_2$: C, 57.01; H, 4.53; N, 10.50. Found: C, 56.88; H, 4.34; N, 10.30.

2-Phenyl-3-(2′-morpholinophenyl)quinazolin-4(3H)-one ***6d***

Yield: 62%; m.p.: 110–113°C; IR(KBr) (in cm^{-1}): 1685 (C=O), 1602 (C=C), 1673 (C=N), 1068 (C-O-C), 795(C-H); ^{1}H-NMR ($CDCl_3$) δ: 7.91–6.43 (m, 13H, ArH), 3.60 (brs, 4H), 2.98 (brs, 4H); ^{13}C-NMR ($CDCl_3$) δ: 164.2 (C_4), 160.3 (C_2), 151.4 (C_9), 142.3 (C_2'), 133.7 (C_6), 130.5 (C_7), 128.9 (C_1'''), 128.8 (C_4'''), 128.7 (C_5), 127.5 (C_3''' & C_5'''), 126.1 (C_{10}), 125.6 (C_2''' & C_6'''), 125.4 (C_4'), 122.6 (C_1'), 120.9 (C_5'),118.6 (C_4), 114.8 (C_3'), 113.1 (C_8), 66.7 (C_3'' & C_5''), 46.2 (C_2'' & C_6''); MS (*m/z*): 382 [M^+]. Anal. calcd. for $C_{24}H_{21}N_3O_2$: C, 75.18; H, 5.52; N, 10.96. Found: C, 75.08; H, 5.32; N, 10.01.

6,8-Dibromo-2-phenyl-3-(2′-morpholinophenyl)-quinazolin-4(3H)-one ***6e***

Yield: 78%; m.p.: 165–168°C; IR (KBr) (in cm^{-1}): 1653 (C=O), 1451 (C=C), 1653 (C=N), 1057 (C-O-C); ^{1}H-NMR ($CDCl_3$) δ: 8.12 (s, 1H, ArH), 7.86 (s, 1H, ArH), 7.67–6.53 (m, 9H, ArH), 3.64 (brs, 4H), 2.95 (brs, 4H); ^{13}C-NMR ($CDCl_3$) δ: 164.5 (C_4), 160.3 (C_2), 154.7 (C_9), 142.8 (C_2'), 139.7 (C_6), 130.3 (C_7), 131.3 (C_1'''), 128.8 (C_4'''), 128.7 (C_5), 126.4 (C_3''' & C_5'''), 126.2 (C_{10}), 125.6 (C_2''' & C_6'''), 125.4 (C_4'), 124.8 (C_1'), 124.2 (C_5'), 122.5 (C_4), 114.6 (C_3'), 113.6 (C_8), 67.1 (C_3'' & C_5''), 46.4 (C2″ & C_6''); MS (*m/z*): 540 [M^+]. Anal. calcd. for $C_{24}H_{19}N_3Br_2O_2$: C, 53.26; H, 5.54; N, 7.76. Found: C, 53.12; H, 3.34; N, 7.01.

6-Bromo-2-phenyl-3-(2′-morpholinophenyl)quinazolin-4(3H)-one ***6f***

Yield: 73%; m.p.: 132–135°C; IR (KBr) (in cm^{-1}): 1685 (C=O), 1602 (C=C), 1674 (C=N), 1065 (C-O-C), 795 (C-H), 554 (C-Br); ^{1}H-NMR ($CDCl_3$) δ: 8.14 (s, 1H, ArH), 6.52–8.01 (m, 11H, ArH), 3.61 (brs, 4H), 2.90 (brs, 4H); ^{13}C-NMR ($CDCl_3$) δ: 163.7 (C_4), 160.8 (C_2), 150.6 (C_9), 142.7 (C_2'), 136.6 (C_6), 133.1 (C_7), 130.2 (C_1'''), 128.7 (C_4'''), 126.5 (C_5), 126.3 (C_3''' & C_5'''), 125.4 (C_{10}), 124.9 (C_2''' & C_6'''), 123.6 (C_4'), 122.5 (C_1'), 121.7 (C_5'), 118.3 (C_4), 114.4 (C_3'), 113.6 (C_8), 66.7 (C_3'' & C_5'', 46.8 (C_2'' & C_6''); MS (*m/z*): 461 [M^+]. Anal. calcd. for $C_{24}H_{20}N_3BrO_2$: C, 62.35; H, 4.36; N, 9.09. Found: C, 62.22; H, 4.28; N, 8.93.

6,8-Dibromo-2-phenyl-3-(4′-carboxylphenyl)quinazolin-4(3H)-one ***7***

Yield: 71%; m.p.: 236–238°C; IR (KBr) (in cm^{-1}): 1681 (C=O), 1600 (C=C), 1660 (C=N), 1064 (C-O-C), 852 (C-H), 544 (C-Br); ^{1}H-NMR ($CDCl_3$) δ: 10.96 (s, 1H, -COOH), 7.23–7.81 (m, 9H, ArH), 7.87 (s, 1H, ArH), 8.11 (s, 1H, ArH); ^{13}C-NMR ($CDCl_3$) δ: 169.3 (-COOH), 164.3 (C_4), 161.2 (C_2), 153.9 (C_{10}), 139.6 (C_1'), 138.1 (C_7), 131.2 (C_1''), 130.6 (C_9), 130.2 (C_5), 129.5 (C_3' & C_5'), 129.1 (C_4''), 128.9 (C_3'' & C_5''), 128.2 (C_6), 126.1 (C_2'' & C_6''), 125.9 (C_8), 121.6 (C_6' & C_2'); MS (*m/z*): 496 [M^+]. Anal. calcd. for $C_{21}H_{12}N_2Br_2O_3$: C, 50.43; H, 2.42; N, 5.60. Found: C, 50.24; H, 2.18; N, 5.43.

6,8-Dibromo-2-phenyl-3-(2′-phenylethanoic acid) quinazolin-4(3H)-one ***8***

Yield: 86%; m.p.: 152–154°C; IR (KBr) (in cm^{-1}): 1681 (C=O), 1602 (C=C), 1651 (C=N), 876 (C-H), 562 (C-Br); ^{1}H-NMR ($CDCl_3$) δ: 10.92 (s, 1H, -COOH), 8.11 (s, 1H, ArH), 7.88 (s, 1H, Ar-H), 7.03–7.67 (m, 10H, ArH), 5.73 (s, 1H, -*CH*-COOH); ^{13}C-NMR ($CDCl_3$) δ: 176.2 (-COOH), 163.9 (C_4), 161.9 (C_2), 154.6 (C_{10}), 139.6 (C_1'), 136.2 (C_7), 131.5 (C_1''), 130.8 (C_9), 130.3 (C_5), 129.7 (C_3' & C_5'), 129.2 (C_4''), 128.8 (C_3'' & C_5''), 127.5 (C_6), 126.1 (C_2'' & C_6''), 128.7 (C_8), 125.3 (C_2'' & C_6''), 123.9 (C_8), 120.9 (C_6' & C_2'), 57.2 (-CH-COOH); MS (*m/z*): 513 [M^+]. Anal. calcd. for $C_{22}H_{14}N_2$ Br_2O_3: C, 51.39; H, 2.74; N, 5.45. Found: C, 51.23; H, 2.41; N, 5.31.

Pharmacology

The synthesized compounds were evaluated for analgesic and anti-inflammatory activities. The test compounds and the standard drugs were administered in form of a suspension (polyethylene glycol as vehicle) by *i. p.* route of administration for analgesic and anti-inflammatory activity. Each group consisted of six animals. The animals were maintained in colony cages at 25 ± 2°C, relative humidity of 45–55% under 12 h light and dark cycles. All the animals were acclimatized for a week before use. The animals were fed with standard animal feed and water *ad libitum*. The Institutional Animal Ethics Committee approved the protocol adopted for experimentation with animals.

Analgesic activity

Analgesic activity was performed by acetic-acid induced writhing reflux model in mice using Wistar albino mice (25–35 g; Tamil Nadu Veterinary College and Research Institute, Chennai)

of either sex selected by random sampling technique. At a dose level of 100 mg/kg, acetyl salicylic acid was administered *i. p.* as reference drug for comparison. Each group of animal received 0.1 mL/10 g body weight of 0.6% v/v of acetic acid after half an hour of administration of the title compounds in the two dose level of 200, 400 mg/kg body weight *p. o.* immediately after the administration of acetic acid, the animal was isolated in an individual cage and the writhing were counted for 20 min.

$$PAA = [T_2 - T_1/10 - T_1] \times 100 \quad (1)$$

in which T_1 is the reaction time (s) before treatment and T_2 is the reaction time (s) after treatment.

Anti-inflammatory activity

Anti-inflammatory activity was evaluated by carageenan-induced paw oedema test in rats [31]. Indomethacin 10–20 mg/kg was administered as a standard drug for comparison. The test compounds were administered at two dose levels: 200, 400 mg/kg body weight *p. o*. The paw volumes were measured using the mercury displacement technique with the help of a plethysmograph, immediately before and at 30 min, 1, 2, and 3 h after carageenan injection. The percent inhibition *I* of paw oedema was calculated.

$$I = 100\,[1 - (a - x)/(b - y)] \quad (2)$$

Where *x* is the mean paw volume of rats before the administration of carrageenan and test compounds or reference compound, *a* is the mean paw volume of rats before the administration of carrageenan in the test group (drug treated), *b* is the mean paw volume of rats before the administration of carrageenan in the control group, and *y* the mean paw volume of rats before the administration of carrageenan in the control group.

Statical analysis

Stastical analysis of the biological activity of the synthesized compounds on animals was evaluated using a one-way analysis of variance (ANOVA). In all cases, post hoc comparisons of the mean of the individual groups were performed using Tukey's test. A significance level of $p < 0.05$ denoted significance in all cases. All values are expressed as means ± SD (standard deviation).

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