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Synthesis, Characterization and Biological Activities of Novel 2-Methyl-quinazolin-4(3*H*)-ones

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In the present study, a novel series of 2-methyl-qninazolin-4(3*H*)-ones were synthesized and characterized by IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral analysis. The synthesized compounds were screened for analgesic (100, 200 and 400 mg/kg), antiinflammatory (200 and 400 mg/kg), antibacterial (*Bacillus subtilis, Bacillus cereus, Staphylococcus epidermidis, Micrococcus luteus* and *Escherichia coli*) and antifungal (*Candida albicans* and *Aspergillus niger*) activities. The minimum inhibitory concentrations of the compounds were also ascertained by agar dilution method. Graded dose response was observed with the compounds. 6,8-dibromo-2-methyl-3-(4'-morpholino-phenyl)-4(3*H*)-one (2) exhibited the highest analgesic and antiinflammatory activity. 6-Bromo-2-methyl-3-(4'-morpholino-phenyl)-quinazolin-4-(3*H*)-one (1) was found to exhibit the highest antimicrobial activity. All the compounds exhibited significant activity against the bacteria and fungi tested.

Quinazolin-4(3*H*)-one derivatives were reported to possess analgesic[1,2], antiinflammatory[3-5], antimicrobial[6-10], antihelminthic[11,12], antiallergic[13], antitumour[14], anticancer[15], MAO inhibitory[16] and central nervous system activities[17-19]. Quinazolin-4(3*H*)-ones with 3-substitution has been reported to be associated with antimicrobial properties[20-22]. The 3-substitution which were reported are various substituted phenyl ring moieties[23-25], bridged phenyl rings[26,27], heterocyclic rings[28-30] and aliphatic systems[31-33]. 2-substituted-3-(4'-morpholino-phenyl)quinazolin-4(3*H*)-ones[34] were reported to possess antibacterial properties.

These observation lead to the conception that a new series of 2-methyl-quinazolin-4(3*H*)-ones with bromo substitution in the benzene ring and a dihalogen/trihalogen substituted phenyl/morpholino-phenyl ring in 3rd position would possess potent biological activities. In continuation of our earlier work[22] on quinzolin-4(3*H*)-ones, the present study involves the synthesis of a new series of 2-methyl-quinzolin-4(3*H*)-ones and its characterization by IR, NMR (^{1}H and ^{13}C) and Mass spectral analysis. The synthesized compounds were screened for analgesic activity by tail immersion method, antiinflammatory activity by paw edema method, antibacterial (*Bacillus subtilis, Bacillus cereus, Staphylococcus epidermidis, Micrococcus luteus* and *Escherichia coli*) and antifungal (*Candida albicans* and *Aspergillus Niger*) activities. The minimum inhibitory concentrations of the compounds were also ascertained by agar dilution method.

MATERIALS AND METHODS

Melting points were determined in open capillary tubes and are uncorrected. IR spectra were recorded (in KBr) on an ABB Bomem MB-104. NMR spectra were recorded on 300 MHz -Bruker DPX200 using tetra methyl silane as internal standard. Mass spectra were recorded on a Shimadzu GC-MS QP5000. The purity of the synthesized compounds were checked by TLC using E-Merck TLC aluminum sheets - silica gel 60 F_{254} (0.2 mm) using ethyl acetate:hexane (1:4) as eluent and visualized in an iodine chamber. All the chemicals used were of analytical grade.

*For correspondence

General method of synthesis:

Unsubstituted/substituted anthranilic acid (0.05 mol) in 0.1 mol of acetic anhydride was refluxed under anhydrous condition for 4-6 h. The excess of acetic anhydride was then distilled off under reduced pressure and cooled to room temperature. The corresponding 2-methyl-benzoxazin-4-one so obtained was filtered and dried under vacuum. Equimolar quantities of unsubstituted/substituted-2-methyl-benzoxazin-4-one and the corresponding amine in glacial acetic acid was refluxed for 4-6 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 (Table 1).

6-Bromo-2-methyl-3-(4'-morpholino-phenyl)quinazolin-4(3H)-one (1):

Yield 62%, m.p. 254°. IR (KBr) cm^{-1}: 1652 (CO-N), 1624 (C=O), 1567 (C=N), 1150, 1127 (C-N), 1088 (C-O-C), 761, 694, 678 (Ar-H), 554 (Ar-Br). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.74 (d, *J*=8.2 Hz, 1H; 7-H), 7.25 (d, *J*=8.2 Hz, 1H; 8-H) 6.8-7.1 (m, 5H; 5,2',3',5',6'-H), 3.68 (t, *J*=7.8 Hz, 4H; 3",5"-H), 3.35 (t, *J*=7.8 Hz, 4H; 2",6"-H), 2.32 (s, 3H; 2-CH_3). ^{13}C-NMR ($CDCl_3$) δ ppm: 167.5, 141.8, 140.2, 139.6, 128.3, 127.2, 125.5, 123, 122.5, 121.6, 119.2, 52.5, 39.1, 24.3. MS (EI) m/z: 399 (M^+), 363, 337, 319, 262, 234, 205, 151, 131, 116, 102, 99, 69, 57, 54 (B).

6,8-Dibromo-2-methyl-3-(4'-morpholino-phenyl)quinazolin-4(3H)-one (2):

Yield 64%, m.p. 260°. IR (KBr) cm^{-1}: 1715 (C=O), 1670 (C=N), 1184 (C-N), 1114 (C-O-C), 735 (Ar-H), 594, 548 (Ar-Br). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.82 (s, 1H; 7-H), 7.76 (s, 1H; 5-H), 6.8-7.1 (m, 4H; 2',3',5',6'-H), 3.64 (t, *J*=7.8 Hz, 4H; 3",5"-H), 3.38 (t, *J*=7.8 Hz, 4H; 2",6"-H), 2.35 (s, 3H; 2-CH_3). ^{13}C-NMR ($CDCl_3$) δ ppm: 167.8, 140.1, 139.6, 136, 128.8, 127, 126.3, 125.6, 123.4, 122.8, 122.5, 119.1, 52.5, 39.1, 24.3. MS (EI) m/z: 479 (M^+), 429, 408, 386, 373, 352, 319, 296, 274, 250, 228, 207, 184, 171, 149, 125, 111, 97, 83, 69, 55, 43 (B).

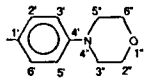
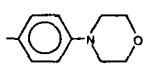
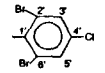
2-Methyl-3-(2',6'-dibromo-4'-chlorophenyl)quinazolin-4(3H)-one (3):

Yield 57%, m.p. 246°. IR (KBr) cm^{-1}: 1716 (C=O), 1683 (C=N), 1615 (CO-N), 1128, 1057 (C-N), 859, 731(Ar-H), 634 (Ar-Cl), 553 (Ar-Br). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.4.1 (s, 2H; 3',5'-H), 6.8-7.1 (m, 4H; 5,6,7,8-H), 2.18 (s, 3H; 2-CH_3). ^{13}C-NMR ($CDCl_3$) δ ppm: 168.2, 141.9, 140, 139.2, 136.2, 128.8, 127.1, 126.7, 123.6, 122.7, 122.3, 121.6, 120, 23.8. MS (EI) m/z: 428 (M^+), 397, 374, 352, 339, 317, 298, 285, 252, 230, 217, 206, 179, 163, 142, 125, 124, 90, 73, 62 (B), 44.

6,8-Dibromo-2-methyl-3-(2',6'-dibromo-4'-chlorophenyl)quinazolin-4(3H)-one (4):

Yield 61%, m.p. 263°. IR (KBr) cm^{-1}: 1689 (C=O), 1670 (CO-N), 1615 (C=N), 1209, 1056 (C-N), 859,731 (Ar-H), 611 (Ar-Cl), 552 Ar-Br). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.56 (s, 1H; 7-

TABLE 1: PHYSICAL CONSTANTS OF THE SYNTHESIZED COMPOUNDS.

Comp.	R_1	R_2	R_3	Molecular Formula	Molecular Weight	M.P. (°)	Yield (%)	R_f[a]
1	Br	H		$C_{19}H_{18}BrN_3O_2$	399.9	254	62	0.54
2	Br	Br		$C_{19}H_{17}Br_2N_3O_2$	478.8	260	64	0.74
3	H	H		$C_{15}H_9Br_2ClN_2O$	428.3	246	57	0.66
4	Br	Br		$C_{15}H_7Br_4ClN_2O$	568.1	263	61	0.84
5	Br	Br		$C_{15}H_8Br_2Cl_2N_2O$	462.7	270	59	0.77

[a] Eluent used in TLC was ethyl acetate:hexane (1:4).

H), 7.44(s, 1H; 5-H), 7.37 (s, 2H; 3',5'-H), 2.24 (s, 3H; 2-CH_3) ^{13}C-NMR ($CDCl_3$) δ ppm: 167.9, 151.2, 145.2, 144.1, 142, 135.9, 135.6, 135.1, 134.2, 129.8, 126.1, 120.4, 22.9. MS (EI) m/z: 568 (M^+), 544, 521, 498, 467, 444, 427, 400, 376, 354, 329, 308, 292, 275, 249, 232, 207, 189, 153, 129, 110, 88, 74, 61, 43 (B).

6,8-Dibromo-2-methyl-3-(3',4'-dichlorophenyl)quinazolin-4(3H)-one (5):

Yield 59%, m.p 270°. IR (KBr) cm^{-1}: 1671 (C=O), 1605 (CO-N), 1587 (C=N), 1182, 1115 (C-N), 820, 791 (Ar-H), 715, 735 (Ar-Cl), 593, 548 (Ar-Br). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.62(s, 1H; 7-H), 7.58 (s, 1H; 2'-H), 7.45 (s, 1H; 5-H), 7.25 (d, *J*=6.7 Hz, 2H; 5',6'-H), 2.31 (s, 3H; 2-CH_3). ^{13}C-NMR ($CDCl_3$) δ ppm: 168.4, 151.8, 145.4, 141.2, 140, 137.1, 136.8, 134.2, 129.8, 121.4, 120.6, 119.6, 24. MS (EI) m/z: 462 (M^+), 372, 334, 329, 313, 290, 275, 249, 213, 197, 183, 168, 153, 132, 116, 97, 88, 69, 61, 43 (B).

Pharmacology:

Swiss mice (15-25 g) and Wistar rats (150-200 g) were used for the study[35]. They were kept in colony cages at 25±2°, relative humidity of 45-55% under 12 h light and dark cycle. All the animals were acclimatized for a week before use. The animals were fed with standard animal feed and water *ad libitum*. The test compounds were administered orally using intragastric tube in the form of suspension using 1% carboxymethyl cellulose as suspending agent. The experimental dose was selected between the minimum effective dose and maximal non-lethal dose. All the animal experimentations were performed according to the protocols and recommendations of the Institutional Animal Ethics Committee. Unpaired student-t-test[36] was performed to ascertain the significance of the exhibited analgesic and antiinflammatory activities.

Analgesic Activity:

The analgesic activity[35] was determined by tail immersion method. Swiss mice (n=6) of either sex selected by random sampling technique was used for the study. Pentazocine at the dose of 10 mg/kg i.p was administered as standard drug for comparison. The test compounds at 3 dose levels (100, 200 and 400 mg/kg) were adminlstered orally. The animals were held in position by a suitable restrainer with the tail extending out and the tail (up to 5 cm) was then dipped in a beaker of water maintained at 55±0.5°. The time in seconds 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 30, 60, 120 and 180 min after the administration of compounds. A cut off point of 15 sec was observed to prevent the tail damage. The results are presented in Table 2.

Antiinflammatory Activity:

The antiinflammatory activity[37] was determined by egg albumin-induced paw edema method in Wistar rat (n=6) of either sex selected by random sampling technique. Indomethacin (20 mg/kg, i.p) was administered as standard drug. The test compounds were administered at 2 dose levels (200 and 400 mg/kg) orally 30 min prior to the administration of fresh egg white (0.1 ml) is the plantar region of the paw. The paw volumes were measured using vernier calliper at 15, 30, 60, 120 and 180 min after egg white administration. The results are presented in Table 3.

Antimicrobial Activity:

The antibacterial activity[38] of the test compounds was tested against *B. subtilis*, *B. cereus*, *S. epidermidis*, *M. luteus* and *E. coli* using tryptone soya agar medium. The antifungal activity of the compounds was tested against *C. albicans* and *A. niger* using Sabourand dextrose agar medium. The sterilized[38] (autoclaved at 120° for 30 min) medium (40-50°) was innoculated (1 ml/100 ml of medium) with the suspension of the microorganism (matched to McFarland barium sulphate standard) and poured into a petridish to give a depth of 3-4 mm. The paper impregnated with the test compounds (200 μg/ml in dimethylformamide) was placed on the solidified medium. The plates were preincubated for 1 h at room temperature and incubated at 37° for 24 h and 48 h for antibacterial and antifungal activity respectively. Ciprofloxacin (100 μg/disc) and fluconazole (100 μg/disc) was used as standard for antibacterial and antifungal activity respectively. The observed zone of inhibition is presented in Table 4.

Minimum Inhibitory concentration:

Minimum Inhibitory concentration (MIC)[39] of the test compounds were determined by agar dilution method. A stock solution of the test compounds (100 μg/ml) in dimethyl formamide was prepared and graded quantities of the test compounds were incorporated in specified quantity of molten sterile agar (nutrient agar for antibacterial activity and Sabourand dextrose agar medium for antifungal activity). A specified quantity of the medium (40-50°) containing the compound was poured into a petridish to give a depth of 3-4 mm and allowed to solidify. The microorganisms were then streaked on agar plate and the plates were incubated at 37° for 24 h and 48 h for bacteria and fungi respectively.

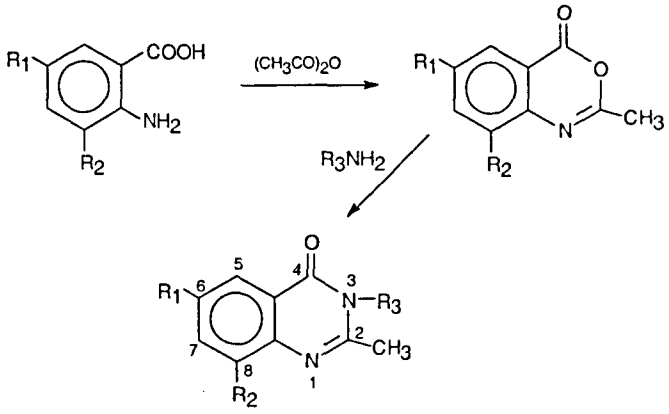
The MIC was considered to be the lowest concentration of the test substance exhibiting no growth of bacteria or fungi. The observed MIC values are presented in Table 4.

RESULTS AND DISCUSSION

All the spectral data was consistent with the assigned structure of the compounds. The ^{1}H-NMR spectral data of **1** consisted of one singlet (2-CH_3), two doublet (7-H and 8-H with J=7.8 Hz), two triplet (morpholino protons with J=7.8 Hz) and a multiplet (5-H and phenyl protons) peaks.

The spectral data of **2** consisted of three singlet (2-CH_3, 5-H and 7-H), two triplet (morpholino protons with J=7.8 Hz) and multiplet (phenyl protons) peaks. The spectral data of **3** consisted of two singlet (2-CH_3 and phenyl protons) and one multiplet (quinazolin-4(3H)-one protons) peaks. The spectral data of **4** consisted of four singlet (2-CH_3, 5-H, 7-H and phenyl protons), doublet, triplet and multiplet peaks. The spectral data of **5** consisted of four singlet (2-CH_3, 5-H, 7-H and 2'H) and one doublet (other phenyl protons with J=6.7 Hz) peaks.

The ^{13}C-NMR spectral data NMR spectral data of all the compounds consistently exhibited peaks for the com-



Scheme 1: Synthetic scheme of 2-methyl-quinazolin-4(3*H*)-ones from unsubstituted/substituted anthranilic acid.

TABLE 2: ANALGESIC ACTIVITY OF THE COMPOUNDS.

Compound	Dose (mg/kg)	Pain reaction time (min)				
		0	30	60	120	180
1	100	2.70±0.21	7.00±0.37*	8.30±0.33*	7.50±0.22*	4.50±0.43
	200	3.10±0.46	8.50±0.22*	8.50±0.61*	8.40±0.17*	6.00±0.61*
	400	3.00±0.24	9.00±0.14*	9.10±0.61*	10.0±0.52*	6.20±0.31*
2	100	3.30±0.21	8.30±0.74*	8.00±0.64*	7.70±0.96*	8.10±0.91*
	200	3.40±0.42	8.60±0.33*	9.00±0.42*	9.30±0.33*	10.7±0.33*
	400	3.30±0.24	9.30±0.90*	10.7±1.20*	12.0±1.12*	14.0±0.42*
3	100	2.90±0.70	7.70±0.33*	8.00±0.61*	8.30±0.33*	3.30±0.33
	200	2.70±0.33	7.90±0.45*	8.30±0.93*	9.30±0.74*	4.00±0.17
	400	2.40±0.51	8.30±0.33*	10.7±0.33*	12.7±0.33*	4.50±0.58
4	100	2.70±0.31	6.30±0.79*	6.30±0.33*	6.70±0.37*	3.30±0.16
	200	2.80±0.34	7.70±0.31*	7.80±0.92*	8.00±0.65*	3.30±0.11
	400	2.90±0.34	8.00±0.67*	11.7±0.92*	11.9±0.33*	3.70±0.68
5	100	3.00±0.61	6.30±0.33*	7.70±0.31*	7.90±0.18*	2.70±0.71
	200	3.10±0.22	7.30±0.71*	8.30±0.64*	9.30±0.71*	3.30±0.18
	400	2.90±0.34	8.70±0.33*	11.0±0.62*	11.7±0.31*	4.00±0.46
Pentazocine	10	3.10±0.26	9.40±0.90*	12.1±0.61*	14.9±0.73*	14.6±0.64*

Each value is mean±SEM (n=6), pain reaction time in seconds and $p<0.01$ compared to 0 min reaction time.

TABLE 3: ANTIINFLAMMATORY ACTIVITY OF THE COMPOUNDS.

Compound	Dose (mg/kg)	% Reduction of edema (min)				
		30	60	90	120	180
1	200	2.44±0.02	14.7±0.03	20.5±0.02	24.2±0.07	20.5±0.05
	400	8.53±0.03	24.6±0.02	34.2±0.01*	35.1±0.02*	32.4±0.10*
2	200	8.54±0.02	16.7±0.01	30.8±0.03*	34.8±0.05*	46.8±0.16*
	400	14.6±0.01	28.6±0.01	45.7±0.01*	57.5±0.04*	63.8±0.08*
3	200	2.44±0.05	14.8±0.01	34.8±0.02*	36.2±0.08*	41.5±0.01*
	400	14.6±0.04	25.6±0.03	35.5±0.03*	45.7±0.06*	46.8±0.11*
4	200	2.44±0.06	16.7±0.02	30.8±0.02*	36.1±0.01*	46.5±0.01*
	400	14.6±0.01	22.7±0.05	33.8±0.06*	39.9±0.01*	57.5±0.06*
5	200	14.6±0.02	22.7±0.01	30.8±0.08*	34.8±0.11*	41.5±0.04*
	400	16.7±0.03	26.7±0.05	33.8±0.12*	45.7±0.02*	46.8±0.05*
Indomethacin	20	16.7±0.02	63.1±0.04*	75.9±0.05*	79.4±0.13*	79.4±0.13*

Each value is mean(%)±SEM (n=6) and *p<0.01 compared to control.

mon functionalities present in the series of compounds such as 4-C, CH_3, quinazoline and phenyl side chain carbons. The peak corresponding to 4-C in **1**, **2**, **3**, **4** and **5** was 167.5, 167.8, 168.2, 167.9 and 168.4 respectively. The peak corresponding to CH_3 in **1**, **2**, **3**, **4** and **5** was 24.3, 24.3, 23.8, 22.9 and 24 respectively. The peak corresponding to quinazoline carbons (representative) in **1**, **2**, **3**, **4** and **5** was 140.2, 140.1, 140, 142 and 141.2 respectively. The peak corresponding to phenyl carbons in **1**, **2**, **3**, **4** and **5** was 128.3, 128.8, 128.8, 129.8 and 129.8 respectively. The peak corresponding to morpholino carbon in **1** and **2** was 52.5.

The mass spectral data revealed that all the compounds exhibited parent peak (M^+) consistent with the assigned molecular formula. The fragmentation peaks correspond to the hypothesis of the fragmentation pattern of the com-

TABLE 4: ANTIMICROBIAL ACTIVITY OF THE SYNTHESIZED COMPOUNDS.

Compound	Zone of inhibition (MIC)						
	B. subtilis	*B. cereus*	*S. epidermidis*	*M. luteus*	*E. coli*	*C. albicans*	*A. niger*
1	18 (24)	22 (24)	22 (25)	23 (27)	24 (24)	24 (66)	16 (72)
2	15 (36)	22 (35)	18 (45)	19 (45)	21(39)	22 (87)	14 (90)
3	13 (36)	22 (46)	21(33)	22 (31)	23 (36)	21(96)	13 (94)
4	17 (27)	21(36)	19 (41)	21(34)	23 (30)	22 (88)	15 (83)
5	17 (25)	23 (23)	18 (47)	20 (39)	23 (47)	23 (76)	12 (106)
Cipro (100 µg/disc)	24	24	23	25	25	-	-
Fluco (100 µg/disc)	-	-	-	-	-	24	18

Zone of inhibition in mm and MIC in µg/ml. Cipro-ciprofloxacin and fluco-fluconazole.

pounds. The base peak of **1**, **2**, **3**, **4** and **5** was 54, 43, 62, 43 and 43 respectively. The base peaks were found to be consistent with the proposed fragmentation hypothesis and according to the nature of the substituents.

All the compounds exhibited significant analgesic and anti inflammatory activity. 6,8-dibromo-2-methyl-3-(4'-morpholino-phenyl) quinazolin-4(3*H*)-one (**2**) possessed the highest analgesic and antiinflammatory activity of the series of compounds. Graded dose response (analgesic and antiinflammatory activity) was observed for all the compounds. The order of analgesic activity of the synthesized compounds was **2>1>3>5>4** and that of antiinflammatory activity was **2>4>3>5>1**.

All the synthesized compounds exhibited highly significant antibacterial and antifungal activity with an MIC range of 23-106 μg/ml. 6-bromo-2-methyl-3-(4'-morpholino-phenyl)-quinazolin-4-(3*H*)-one (**1**) was found to exhibit the highest antimicrobial activity against *B. subtilis* (MIC: 24 μg/ml), *S. epidermidis* (25 μg/ml), *M. luteus* (27 μg/ml), *E. coli* (24 μg/ml), *C. albicans* (66 μg/ml) and *A. niger* (72 μg/ml). 6,8-Dibromo-2-methyl-3-(3',4'-dichlorophenyl)quinazolin-4(3*H*)-one (**5**) exhibited highest activity against *B. cereus* (MIC: 23 μg/ml). The compounds were active against all the tested microorganism with a range of MIC values for *B. subtilis* (24-36 μg/ml), *B. cereus* (23-46 μg/ml), *S. epidermidis* (25-47 μg/ml), *M. luteus* (27-45 μg/ml), *E. coli* (24-47 μg/ml), *C. albicans* (66-96 μg/ml) and *A. niger* (72-106 μg/ml).

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