Synthesis, Analgesic, Anti-inflammatory and Antibacterial Activities of Some Novel 2-Methyl-3-substituted Quinazolin-4-(3*H*)-ones

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A series of novel 2-methyl-3-substituted quinazolin-4-(3*H*)-ones have been synthesized by treating (2-methyl-4-oxo-3*H*-quinazolin-3-yl)dithiocarbamic acid methyl ester with different amines, the starting material dithiocarbamate was synthesized from anthranilic acid. The compounds synthesized were investigated for analgesic, anti-inflammatory and antibacterial activities. All the test compounds exhibited significant activity, the compounds VA2, VA3 and VA4 shown more potent analgesic activity, and the compounds VA3 and VA4 shown more potent anti-inflammatory activity than the reference compound diclofinac sodium.

Key words quinazoline; thiourea; pyrimidine; analgesic; anti-inflammatory

Bacterial infections often produce pain and inflammation. In normal practice, two groups of agents (chemotherapeutic, analgesic and anti-inflammatory) are prescribed simultaneously. The compounds possessing all three activities are not common. Quinazolines and condensed quinazolines exhibit potent antimicrobial[1)] and CNS activities like analgesic,[2)] anti-inflammatory[3)] and anticonvulsant[4)] activities. In view of these facts and to develop our earlier reported 2-phenyl-3-substituted quinazolines series[5)] and 2,3-disubstituted quinazolines[6)] that shown good analgesic and anti-inflammatory activities, in the present study we aimed to synthesize some 2-methyl-3-substituted quinazolin-4(3*H*)-ones. The title compounds were synthesized by nucleophilic substitution of (2-methyl-4-oxo-3*H*-quinazolin-3-yl)dithiocarbamic acid methyl ester with variety of amines. The (2-methyl-4-oxo-3*H*-quinazolin-3-yl)dithiocarbamic acid methyl ester was synthesized by reacting the amino group of 3-amino-2-methyl quinazoline with carbondisulphide and dimethyl sulphate. The 3-amino-2-methyl quinazoline was synthesized from anthranilic acid (Chart 1). Spectral data (IR, NMR, and mass spectra) confirmed the structures of the synthesized compounds, the purity of these compounds was ascertained by microanalysis (Table 1). The synthesized compounds were tested for their analgesic, anti-inflammatory and antibacterial activities.

CHEMISTRY

Melting points were determined in open capillary tubes on a Thomas Hoover apparatus and are uncorrected. IR spectra were recorded in KBr on a Perkin Elmer-841 grating spectrometer (cm^{-1}), mass spectra on a varian Atlas CH-7 mass spectrometer at 70 eV and NMR spectra on a varian A-60 or EM-360 spectrometer, using tetramethylsilane as internal standard. Elemental analysis were performed on Carlo erba 1108.

Synthesis of 2-Methyl-benzo[*d*][1,3]oxazin-4-one A mixture of anthranilic acid 1.37 g (0.01 mol) and acetic anhydride 10.2 ml (0.1 mol) was refluxed on gentle flame for 1 h. The excess of acetic anhydride was distilled off under reduced pressure and the residue was dissolved in petroleum ether and kept aside for 1 h. The solid obtained was filtered and dried, yield=73%, mp 182 °C; IR (KBr) cm^{-1}: 3350 (NH), 1700 (C=O) and 1640 (C=N); NMR ($CDCl_3$) δ: 2.5 (s, 3H, CH_3) 6.9—7.4 (m, 4H, ArH), MS (*m/z*) 161 (M^+). *Anal.* Calcd for $C_9H_7NO_2$: C, 67.08; H, 4.34; N, 8.69. Found: C, 67.19; H, 4.39; N, 8.62.

Synthesis of 3-Amino-2-methyl-3*H*-quinazolin-4-one A mixture of 2-methyl-benzo[*d*][1,3]oxazin-4-one 1.61 g (0.01 mol) and hydrazine hydrate 1.5 g (0.03 mol) in ethanol was refluxed for 2 h and cooled. The separated solid was recrystallized from ethanol, yield=76%, mp 140—142 °C; IR (KBr) cm^{-1}; 3320, 3260 (NH_2), 1680 (cyclic C=O), 1640 (C=N) and 1600, (C=C); ^{1}H-NMR ($CDCl_3$) δ: 2.6 (s, 3H, CH_3) 4.6 (s, 2H, NH_2), 6.6—7.2 (m, 4H, ArH); MS (*m/z*) 175 (M^+). *Anal.* Calcd for $C_9H_9N_3O$: C, 61.71; H, 5.14; N, 24.00. Found: C, 61.65; H, 5.17; N, 24.16.

Synthesis of (2-Methyl-4-oxo-3*H*-quinazolin-3-yl)dithiocarbamic Acid Methyl Ester To a vigorously stirred solution of 3-amino-2-methyl-3*H*-quinazolin-4-one 3.51 g (0.02 mol) in dimethyl sulfoxide (10 ml) at room temperature carbondisulphide 1.6 ml (0.026 mol) and sodium hydroxide

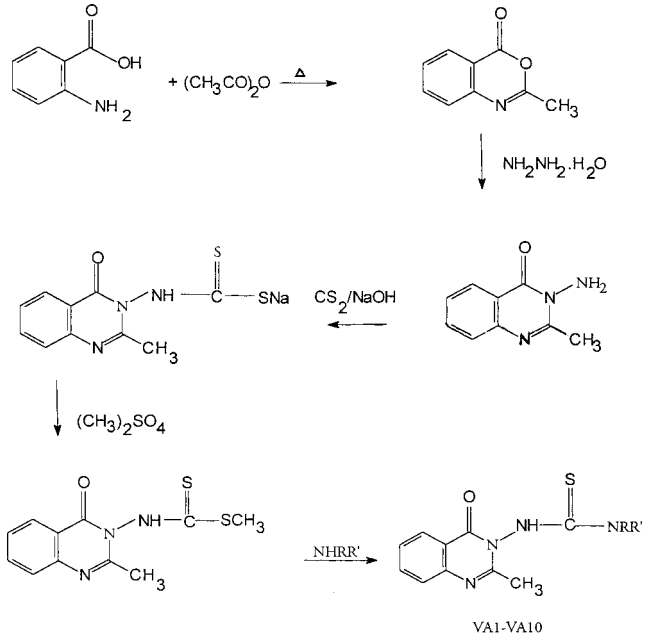

Chart 1. Synthesis of 1-Methyl-3-(2-methyl-4-oxo-3*H*-quinazolin-3-yl)-thiourea and Its Derivatives from Anthranilic Acid

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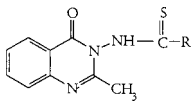
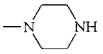
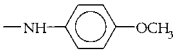
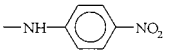
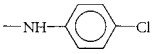
Table 1. Physical Data for 1-Substituted-3-(2-methyl-4-oxo-3*H*-quinazolin-3-yl)thioureas



Compound code	R	Molecular formula[a)]	Molecular weight[b)]	mp (°C)	Yield (%)
VA1	$-NHCH_3$	$C_{11}H_{12}N_4OS$	248	151—153	69
VA2	$-N(CH_3)_2$	$C_{12}H_{14}N_4OS$	262	189—192	77
VA3	$-N(C_2H_5)_2$	$C_{14}H_{18}N_4OS$	290	201—203	70
VA4		$C_{14}H_{16}N_4OS$	288	196—199	65
VA5		$C_{14}H_{16}N_4O_2S$	304	215—219	81
VA6		$C_{14}H_{17}N_5OS$	303	226—229	69
VA7		$C_{17}H_{16}N_4O_2S$	340	256—258	75
VA8		$C_{16}H_{13}N_5O_3S$	355	261—262	69
VA9		$C_{16}H_{13}N_4OSCl$	344	237—240	72
VA10	$-N(C_6H_5)_2$	$C_{22}H_{18}N_4OS$	386	246—247	76

a) All Compounds gave satisfactory elemental analysis (±0.4% of theoritical values). *b*) Molecular weight determination by mass spectra.

1.2 ml (20 mol solution) were added drop wise during 30 min, it was allowed to stirr for 30 min more. Dimethyl sulphate 2.5 g (0.02 mol) was added at 5—10 °C, stirring was continued for 2 h and the reaction mixture was poured into ice water, the solid, so obtained was filtered, washed with water, dried and recrystallized from ethanol, yield=71%, mp 103—106 °C, IR (KBr) cm^{-1}: 3260 (NH), 1690 (cyclic C=O), 1600 (C=N) and 1130 (C=S); ^{1}H-NMR ($CDCl_3$) δ: 2.9—3.0 (s, 3H, CH_3), 3.2—3.3 (s, 3H, CH_3), 6.9—7.3 (m, 4H, ArH), 8.6—8.7 (s, 1H, NH); MS (*m*/*z*) 265 (M^+). *Anal.* Calcd for $C_{11}H_{11}N_3OS_2$: C, 49.80; H, 4.15; N, 15.84. Found: C, 49.61; H, 4.21; N, 15.93.

Synthesis of 1-Methyl-3-(2-methlyl-4-oxo-3*H*-quinazolin-3-yl)thiourea (VA1) A mixture of (2-methyl-4-oxo-3*H*-quinazolin-3-yl)dithiocarbamic acid methyl ester 2.65 g (0.01 mol) and methylamine 0.62 g (0.02 mol) in *N*,*N*-dimethylformamide (20 ml) was refluxed for 19 h cooled and poured into ice water, the solid obtained was filtered, dried and recrystallized from ethanol, yield=69%, mp 151—153 °C, IR (KBr) cm^{-1}: 3260 (NH), 1680 (cyclic C=O), 1600 (C=N), 1310 (C–N), 1140 (C=S); ^{1}H-NMR ($CDCl_3$) δ: 2.2—2.4 (s, 3H, CH_3), 3.1—3.3 (s, 3H, –N–CH_3), 6.3—6.7 (m, 4H, ArH), 8.4—8.5 (s, 1H, NH); MS (*m*/*z*) 248 (M^+). *Anal.* Calcd for $C_{11}H_{12}N_4OS$, C, 53.22; H, 4.83; N, 22.58. Found: C, 53.26; H, 4.76; N, 22.35. Adopting the same procedure compounds VA2—VA10 were synthesized.

PHARMACOLOGY

The synthesized compounds were evaluated for analgesic, anti-inflammatory and antimicrobial activities. Student-*t*-test was performed for all the activities to ascertain the significance of the exhibited activities. The test compounds and the standard drugs were administered in the form of a suspension (1% carboxyl methyl cellulose as vehicle) in the same route of administration. Each group consisted of six animals.

Animals The animals were procured from "National Biological Center", Madurai, India, and were maintained in colony cages at 25±2 °C, relative humidity of 45—55%, maintained under 12 h light and dark cycle and were fed with standard animal feed. All the animals were acclimatized for a week before use.

Analgesic Activity[7,8] Test for analgesic activity was performed by tail-flick technique using Wistar albino mice (25—35 g) of either sex selected by random sampling technique Diclofenac sodium at a dose level of 10 mg/kg and 20 mg/kg was administered orally as reference drug for comparison. The test compounds at two dose levels (10, 20 mg/kg) were administered orally. The reaction time was recorded at 30 min, 1, 2 and 3 h after the treatment. The cut-off time was 10 s. The percent analgesic activity (PAA) was calculated by the following formula,

$$\mathrm{PAA}=\left[\frac{T_2-T_1}{10-T_1}\right]\times 100$$

Where T_1 is the reaction time(s) before treatment, T_2 is the reaction time(s) after treatment.

Anti-inflammatory Activity Anti-inflammatory activity was performed by carrageenan-induced paw oedema test in rats.[9] Diclofenac sodium 10, 20 mg/kg was administered as standard drug for comparison. The test compounds were administered at two dose levels (10, 20 mg/kg). The paw volumes were measured using the mercury displacement technique with the help of a plethysmograph immediately before and 30 min, 1, 2 and 3 h after carrageenan injection. The percent inhibition of paw oedema was calculated by using the following formula,

percent inhibition $I=100[1-(a-x)/(b-y)]$

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

Antibacterial Activity Evaluation of antibacterial activity by agar dilution method.[10] The standard strains were procured from the American Type Culture Collection (ATCC), Rockville, U.S.A., and the pathological strains were procured from the Department of Microbiology, Madurai Medical College and Research Institute, Madurai, India. The antibacterial activity of the synthesized compounds were screened against the following bacterial strains: *Proteus vulgaris* ATCC 9484, *Escherichia coli*, *Klebsiella pneumoniae* ATCC 13883, *Edwardsiella tarda*, *Staphylococcus aureus*, *Bacillus subtilis* ATCC 6051. All bacteria were grown on Muller–Hinton Agar (Hi-media) plates (37 °C, 24 h) then the minimum inhibitory concentration (MIC) was considered to be the lowest concentration that completely inhibited the growth on agar plates, disregarding a single colony or faint haze caused by the inoculums. The MIC of the test compounds were compared with the reference drug norfloxacin.

RESULTS AND DISCUSSION

The analgesic activity data reveals that all the test compounds exhibited significant activity (Table 2). The compound VA1 with methyl substitution showed good activity; with the increased lipophilicity (dimethyl group) compound VA2 shown increased activity. Further increase in liphopilicity (diethyl group) VA3 led to further increase in activity. Substitution with alicyclic amines VA4 retains the activity. Placement of alicyclic amines with additional heteroatoms VA5, VA6 led to decrease in activity. Aromatic substitution VA7 to VA10 shown still lower activity. The compounds with aliphatic substitution (VA1—VA4) shown the better activity. The compound VA4 was found to be the most active analgesic agent and it is more potent than diclofenac sodium and our earlier reported 2-phenyl-3-substituted quinazolines.

The anti-inflammatory activity data (Table 3) reveled that all the test compound protected rats from carrageenan-induced inflammation and are more potent than our earlier reported 2-phenyl-3-subtituted quinazolines. The compounds VA3 and VA4 showed more anti-inflammatory activity than that of diclofenac sodium, while the compound VA2 is equipotent with diclofenac sodium.

The result of antibacterial activity indicates that all the test compounds exhibited moderate activity against the tested bacteria. The compound VA3 showed good activity against *B. subtilis*, *E. tarda* and *S. aureus*; The compound VA8 exhibited good activity against *B. subtilis* and *S. aureus*; and the compound VA9 exhibited good activity against *P. vulgaris*, *K. pneumoniae* and *S. aureus*.

The results of analgesic and anti-inflammatory activities indicate that the replacement of C-2 phenyl group of 2-phenyl-3-substituted quinazolines by C-2 methyl group showed increase in activity. However the potency is lesser than our earlier reported 2, 3-disubstituted quinazolines. Hence further structural modification is planned to increase not only the analgesic and anti-inflammatory activities also the antibacterial activity.

Table 2. Analgesic Activity (Tail-Flick Technique)

Compound code	Dose (mg/kg)	Percentage analgesic activity			
		30 min	1 h	2 h	3 h
VA1	10	32±0.96**	35±0.63*	39±0.21*	30±0.19***
	20	45±0.16*	51±0.39**	57±0.41**	38±0.11***
VA2	10	37±0.91*	41±0.53*	47±0.43***	33±0.56**
	20	49±0.66**	58±0.45**	61±0.68**	45±0.46*
VA3	10	39±0.52**	43±0.51*	49±0.44**	35±0.42*
	20	52±0.46**	60±0.48**	64±0.83*	46±0.49**
VA4	10	41±0.49**	45±0.77**	50±0.57*	36±0.11**
	20	53±0.42**	62±0.63***	65±0.79*	48±0.26*
VA5	10	31±0.43**	32±0.73***	35±0.72**	27±0.49***
	20	43±0.65**	48±0.68**	51±0.98*	35±0.68***
VA6	10	30±0.18*	31±0.48**	35±0.19**	24±0.98**
	20	43±0.76**	48±0.78**	49±0.76*	37±0.43*
VA7	10	28±0.49**	30±0.79*	32±0.82**	20±0.19**
	20	41±0.83**	45±0.53*	46±0.68***	33±0.68***
VA8	10	29±0.28**	30±0.76*	35±0.47***	23±0.78**
	20	42±0.55**	47±0.49**	51±0.72**	36±0.94*
VA9	10	27±0.17**	29±0.48**	31±0.18***	17±0.12***
	20	37±0.18***	40±0.49*	41±0.58**	27±0.48**
VA10	10	22±0.11**	24±0.49*	26±0.44***	16±0.49***
	20	35±0.54*	36±0.28**	38±0.85**	24±0.65*
Control		2±0.35	6±0.49	4±0.59	4±0.91
Diclofenac	10	35±0.51*	40±0.91**	44±0.16*	32±0.45**
	20	47±0.46*	56±0.83***	60±0.54**	41±0.61*

Each value represents the mean±S.D. ($n=6$). Significance levels $*p<0.5$, $**p<0.01$ and $***p<0.001$ as compared with the respective control.

Table 3. Anti-inflammatory Activity (Carrageenan-Induced Rat Paw Oedema Method)

Compound code	Dose (mg/kg)	Percentage protection			
		30 min	1 h	2 h	3 h
VA1	10	24±0.25**	27±0.42**	33±0.41*	25±0.17**
	20	36±0.17**	41±0.86*	50±0.48*	37±0.26*
VA2	10	31±0.46**	38±0.28*	41±042**	36±0.37**
	20	42±0.55*	52±0.48**	56±0.36**	44±0.43**
VA3	10	32±0.17*	39±0.28**	46±0.29**	35±0.18**
	20	45±0.45**	54±0.36*	60±0.53**	45±0.38*
VA4	10	35±0.53**	38±0.67*	44±0.39**	35±0.56***
	20	44±0.49*	55±0.61**	60±0.48**	43±0.27*
VA5	10	23±0.36**	27±0.55***	29±0.84*	26±0.17**
	20	35±0.42*	39±0.39**	46±0.38**	33±0.25**
VA6	10	24±0.17**	26±0.35**	28±0.28*	21±0.17**
	20	34±0.32**	37±0.38**	46±0.37***	36±0.29**
VA7	10	21±0.33**	23±0.27**	30±0.57**	24±0.58***
	20	26±0.52*	34±0.55*	37±0.38**	29±0.26*
VA8	10	20±0.97**	24±0.74*	33±0.57*	23±0.54*
	20	24±0.52**	36±0.29*	37±0.62*	25±0.25*
VA9	10	23±0.42**	26±0.49*	34±0.57*	26±0.49**
	20	28±0.56**	35±0.48**	39±0.29*	31±0.72**
VA10	10	22±0.17**	25±0.46*	32±0.43*	22±0.26**
	20	25±0.39**	35±0.27***	35±0.36*	29±0.73**
Control		5.13±0.29	6.12±0.27	5.79±0.32	3.29±0.51
Diclofenac	10	29±0.19**	32±0.13*	36±0.56**	30±0.72***
	20	41±0.25***	51±0.56**	56±0.49*	39±0.45***

Each value represents the mean±S.D. ($n=6$). Significance levels $*p<0.5$, $**p<0.01$ and $***p<0.001$ as compared with the respective control.

Table 4. Antibacterial Activity (Agar Dilution Method) MIC Values (μg/ml)

Compound code	*E. coli*	*P. vulgaris*	*K. pneumoniae*	*B. subtilis*	*E. tarda*	*S. aureus*
VA1	19.53	156.25	78.12	39.06	78.12	78.12
VA2	156.25	39.06	78.12	19.53	156.25	39.06
VA3	19.53	78.12	19.53	9.76	9.76	9.76
VA4	78.12	156.25	78.12	156.25	39.06	19.53
VA5	39.06	156.25	19.53	78.12	156.25	78.12
VA6	156.25	19.06	78.12	19.06	78.12	19.06
VA7	19.06	78.12	78.12	19.06	156.25	39.06
VA8	156.25	78.12	19.53	9.76	19.53	9.76
VA9	39.06	9.76	9.76	19.53	78.12	9.76
VA10	156.25	78.12	156.25	19.53	78.12	39.06
Norfloxacin	0.60	2.44	0.60	4.88	9.76	1.22

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