Synthesis, Analgesic, Anti-inflammatory and Antibacterial Activities of some Novel 2-Methylthio-3-Substituted Quinazolin-4-(3*H*)-ones

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A variety of novel 2-methylthio-3-substituted quinazolin-4-(3*H*)-ones have been synthesized by reacting (2-methylthio-4-oxo-3*H*-quinazolin-3-yl)dithiocarbamic acid methyl ester with a variety of amines, the starting material dithiocarbamate was synthesized from methylanthranilate. The title compounds were investigated for analgesic, anti-inflammatory and antibacterial activities. While the test compounds exhibited significant activity, the compounds A1, A2, A3 and A4 shown more potent analgesic activity, and the compound A4 shown more potent anti-inflammatory activity than the reference diclofenac 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,[5)] 2,3-disubstituted quinazolines[6)] and 2-methyl-3-substituted quinazolin-4-(3*H*)-ones[7)] that exhibited good analgesic and anti-inflammatory activities, in the present study we aimed to synthesize some 2-methylthio-3-substituted quinazolin-4(3*H*)-ones. The title compounds were synthesized by nucleophilic substitution of (2-methylthio-4-oxo-3*H*-quinazolin-3-yl)dithiocarbamic acid methyl ester with different amines. The (2-methylthio-4-oxo-3*H*-quinazolin-3-yl)dithiocarbamic acid methyl ester was synthesized by reacting the amino group of 3-amino-2-methylthioquinazoline with carbondisulphide and dimethyl sulphate. The 3-amino-2-methylthio 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 3-Amino-2-mercaptoquinazolin-4(3*H*)-one To a vigorously stirred solution of methylanthranilate 3.02 g (0.02 mol) in dimethylsulfoxide (10 ml) at room temperature, carbondisulphide (1.6 ml, 0.026 mol) and aqueous sodium hydroxide 1.2 ml (20 mol solution) was added dropwise simultaneously during 30 min and it was stirred for 30 min more. Dimethyl sulphate 2.5 g (0.02 mol) was added dropwise under cooling with an ice bath. Stirring was continued for 3 h, the reaction mixture was poured into ice-water and then it was extracted with chloroform. The solvent was removed by distillation under reduced pressure. Thus the obtained crude methyl *N*-(2-methoxycarbonylphenyl)dithiocarbamate was used for further reaction without purification.

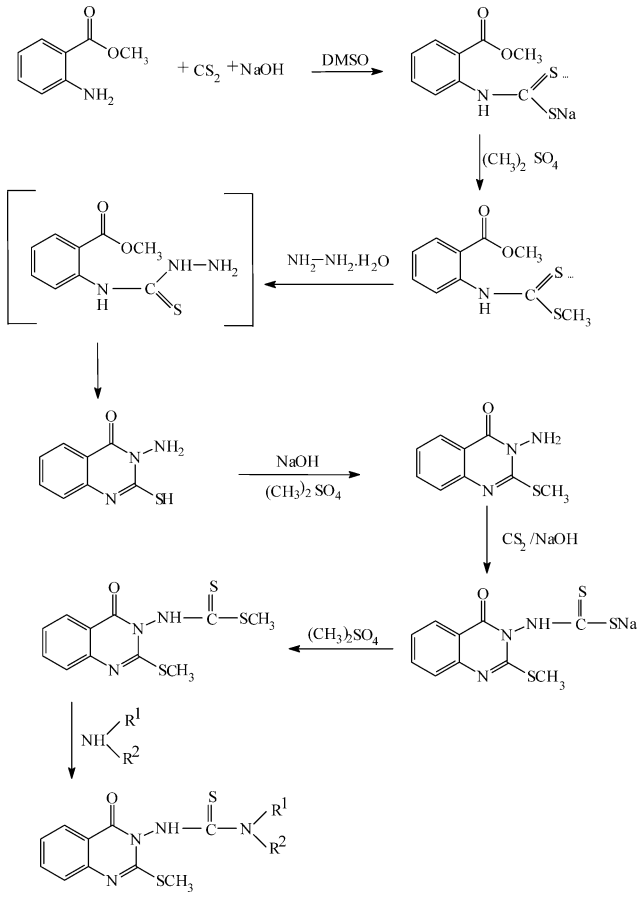

Chart 1. Synthesis of 1-Substituted-3-(2-methylthio-4-oxo-3*H*-quinazolin-3-yl)thiourea from Methylanthranilate

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Hydrazine hydrate 8.6 g (0.2 mol) was added dropwise to a stirred methyl *N*-(2-methoxycarbonylphenyl)dithiocarbamate 4.82 g (0.02 mol) in cold condition. After the completion of addition, stirring was continued for 1.5 h at 50 °C and the mixture was poured into ice-water. The solid obtained was filtered, washed with water, dried and recrystallized from dimethylformamide and ethanol, yield=90%, mp 236—237 °C; IR (KBr) cm^{-1}; 3300, 3220 (NH_2), 2560 (SH), 1680 (C=O); ^{1}H-NMR ($CDCl_3$) δ: 3.21 (s, 1H, SH), 5.12 (s, 2H, NH_2, D_2O exchangeable), 7.14 (m, 4H, ArH). *Anal.* Calcd for $C_8H_7N_3OS$: C, 49.74; H, 3.65; N, 21.77. Found: C, 49.26; H, 3.72; N, 21.94.

Synthesis of 3-Amino-2-methylthioquinazolin-4(3*H*)-one A solution of 3-amino-2-mercaptoquinazolin-4(3*H*)-one 1.93 g (0.01 mol) in sodium hydroxide 10 ml (20% w/v) was obtained by warming on a water bath. It was clarified by filteration while in warm condition, cooled and treated with dimethylsulphate 1.26 g (0.01 mol) under constant stirring. The solution was stirred at room temperature for 12 h. The solid obtained was filtered, washed with cold water, dried and recrystallized from chloroform–ethanol, yield=90%, mp 155—159 °C; IR (KBr) cm^{-1}: 3400, 3320 (NH_2), 1700 (C=O); ^{1}H-NMR (DMSO-d_6) δ: 2.51 (s, 3H, SCH_3), 6.6 (s, 2H, NH_2, D_2O exchangeable), 7.5—7.8 (m, 4H, ArH). *Anal.* Calcd for $C_9H_9N_3OS$: C, 52.22; H, 4.38; N, 20.3. Found: C, 52.46; H, 3.98; N, 20.52.

Synthesis of (2-Methylthio-4-oxo-3*H*-quinazolin-3-yl)-dithiocarbamic acid methyl ester To a vigorously stirred solution of 3-amino-2-methylthioquinazolin-4(3*H*)-one 3.86 g (0.02 mol) in dimethyl sulfoxide (10 ml) at room temperature carbondisulphide (1.6 ml, 0.026 mol) and sodium hydroxide (1.2 ml, 20 mol solution) were added dropwise 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 3 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=73%, mp 95—97 °C, IR (KBr) cm^{-1}: 3330 (NH), 1680 (cyclic C=O), 1610 (C=N), 1160 (C=S); ^{1}H-NMR ($CDCl_3$) δ: 2.6—2.7 (s, 3H, CH_3), 3.0—3.1 (s, 3H, CH_3), 6.6—7.1 (m, 4H, ArH), 8.5—8.7 (s, 1H, NH); MS (*m*/*z*) 297 (M^+). *Anal.* Calcd for $C_{11}H_{11}N_3OS_3$: C, 44.44; H, 3.70; N, 14.14. Found: C, 44.30; H, 3.66; N, 14.05.

Synthesis of 1-Methyl-3-(2-methlylthio-4-oxo-3*H*-quinazolin-3-yl)thiourea (A1) A mixture of (2-methylthio-4-oxo-3*H*-quinazolin-3-yl)dithiocarbamic acid methyl ester 3.27 g (0.01 mol) and methylamine 0.62 g (0.02 mol) in *N*,*N*-dimethyl formamide (20 ml) was refluxed for 20 h cooled and poured into ice water, the solid obtained was filtered, dried and recrystallized from ethanol, yield=71%, mp 166—169 °C, IR (KBr) cm^{-1}: 3350 (NH), 1670 (cyclic C=O), 1620 (C=N), 1310 (C–N), 1130 (C=S); ^{1}H-NMR ($CDCl_3$) δ: 2.3—2.5 (s, 3H, –NCH_3), 2.7—3.0 (s, 3H, –SCH_3), 6.5—7.0 (m, 4H, ArH), 8.2—8.3 (s, 1H, NH); MS (*m*/*z*) 280 (M^+). *Anal.* Calcd for $C_{11}H_{12}N_4OS_2$, C, 47.14; H, 4.28; N, 20.00. Found: C, 47.26; H, 4.21; N, 19.91. Adopting this procedure compounds **A2**—**A11** were prepared.

1,1-Dimethyl-3-(2-methlylthio-4-oxo-3*H*-quinazolin-3-yl)thiourea (A2) IR (KBr) cm^{-1}: 3310 (NH), 1680 (cyclic C=O), 1640 (C=N), 1330 (C–N), 1120 (C=S); ^{1}H-NMR ($CDCl_3$) δ: 2.2 (s, 6H, –$N(CH_3)_2$), 2.6—2.8 (s, 3H, –SCH_3), 6.9—7.5 (m, 4H, ArH), 8.4—8.5 (s, 1H, NH); MS (*m*/*z*) 294 (M^+). *Anal.* Calcd for $C_{12}H_{14}N_4OS_2$, C, 48.97; H, 4.76; N, 19.05. Found: C, 49.05; H, 4.69; N, 19.09.

1,1-Diethyl-3-(2-methlylthio-4-oxo-3*H*-quinazolin-3-yl)thiourea (A3) IR (KBr) cm^{-1}: 3330 (NH), 1670 (cyclic C=O), 1630 (C=N), 1310 (C–N), 1130 (C=S); ^{1}H-NMR ($CDCl_3$) δ: 1.1 (m, 4H, –$N(CH_2CH_3)_2$), 1.3 (m, 6H, –$N(CH_2CH_3)_2$, 2.5—2.7 (s, 3H, –SCH_3), 6.6—7.2 (m, 4H, ArH), 8.3—8.4 (s, 1H, NH); MS (*m*/*z*) 322 (M^+). *Anal.* Calcd for $C_{14}H_{18}N_4OS_2$, C, 52.17; H, 5.59; N, 17.39. Found: C, 52.23; H, 5.63; N, 17.31.

1-(Pyrrolidinyl)-3-(2-methlylthio-4-oxo-3*H*-quinazolin-3-yl)thiourea (A4) IR (KBr) cm^{-1}: 3320 (NH), 1680 (cyclic C=O), 1620 (C=N), 1330 (C–N), 1110 (C=S); ^{1}H-NMR ($CDCl_3$) δ: 1.6 (m, 4H, –$N(CH_2CH_2)_2$, 2.7 (m, 4H, –$N(CH_2CH_2)_2$, 3.0—3.2 (s, 3H, –SCH_3), 6.7—7.3 (m, 4H, ArH), 8.5—8.6 (s, 1H, NH); MS (*m*/*z*) 320 (M^+). *Anal.* Calcd for $C_{14}H_{16}N_4OS_2$, C, 52.50; H, 5.00; N, 17.50. Found: C, 52.59; H, 5.13; N, 17.42.

1-(Morpholinyl)-3-(2-methlylthio-4-oxo-3*H*-quinazolin-3-yl)thiourea (A5) IR (KBr) cm^{-1}: 3300 (NH), 1670 (cyclic C=O), 1630 (C=N), 1340 (C–N), 1120 (C=S); ^{1}H-NMR ($CDCl_3$) δ: 2.5 (m, 4H, –$(N(CH_2CH_2)_2$–O), 3.0 (m, 4H, –$(N(CH_2CH_2)_2$–O), 3.3—3.4 (s, 3H, –SCH_3), 6.9—7.5 (m, 4H, ArH), 8.5—8.6 (s, 1H, NH); MS (*m*/*z*) 336 (M^+). *Anal.* Calcd for $C_{14}H_{16}N_4O_2S_2$, C, 50.00; H, 4.76; N, 16.66. Found: C, 51.01; H, 4.47; N, 16.70.

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[8,9] 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 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.[10] Diclofenac sodium 10, 20 mg/kg was administered as standard drug for comparison. The test compounds were administered at two dose levels (10 mg, 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

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

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.

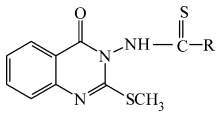
Antibacterial Activity Evaluation of antibacterial activity by agar dilution method.[11] The standard strains were procured from the American Type Culture Collection (ATCC), Rockville, U.S.A., and the pathological strains were procured from the Department of Microbiology, Madurai Medical College and Research Institute, Madurai, India. The antibacterial activity of the synthesized compounds were screened against the following bacterial strains: *Proteus vulgaris* ATCC 9484, *Salmonella typhimurium* ATCC 33068, *Klebsiella pneumoniae* ATCC 13883, *Edwardsiella tarda*, *Pseudomonas aeruginosa* ATCC 2853, *Bacillus subtilis* ATCC 6051, *Salmonella paratyphi*, 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 results of analgesic activity indicate that all the test compounds exhibited significant activity (Table 2). The compound **A1** with methyl substitution showed good activity, with the increased lipophilicity (dimethyl group) compound **A2** shown increase in activity. Further increase in lipophilicity (diethyl group) **A3** led to further increase in activity. Substitution with alicyclic amines **A4** retains the activity. Placement of alicyclic amines with additional heteroatoms **A5**, **A6** led to decrease in activity. Aromatic substitution **A7** to **A11** shown still lower activity. The compounds with aliphatic substitution (**A1**—**A4**) shown the better activity. The compound **A3** was found to be the most active analgesic agent and it is more potent than diclofenac sodium and our earlier reported 2,3-disubstituted quinazolin-4(3*H*)-ones.

The anti-inflammatory activity data (Table 3) indicated that all the test compound protected rats from carrageenan-induced inflammation and are more potent than our earlier reported 2,3-disubstituted quinazolin-4(3*H*)-ones. The compound **A4** showed more anti-inflammatory activity than that

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



Compound code	R	Molecular formula[a]	Molecular weight[b]	mp (°C)	Yield (%)
A1	—NHCH$_3$	$C_{11}H_{12}N_4OS_2$	280	166—169	71
A2	—N(CH$_3$)$_2$	$C_{12}H_{14}N_4OS_2$	294	191—192	73
A3	—N(Et)$_2$	$C_{14}H_{18}N_4OS_2$	322	199—202	71
A4	—N(pyrrolidine)	$C_{14}H_{16}N_4OS_2$	320	209—212	69
A5	—N(morpholine)	$C_{14}H_{16}N_4O_2S_2$	336	226—231	76
A6	—N(piperazine)	$C_{14}H_{17}N_5OS_2$	335	195—199	79
A7	—NH—C$_6$H$_4$—OCH$_3$	$C_{17}H_{16}N_4O_2S_2$	372	241—245	69
A8	—NH—C$_6$H$_4$—NO$_2$	$C_{16}H_{13}N_5O_3S_2$	387	216—219	75
A9	—NH—C$_6$H$_4$—Cl	$C_{16}H_{13}N_4OS_2Cl$	376	263—266	72
A10	—N(ph)$_2$	$C_{22}H_{18}N_4OS_2$	418	257—259	71
A11	—NH—C$_6$H$_5$	$C_{16}H_{14}N_4OS_2$	342	235—236	73

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

Table 2. Analgesic Activity (Tail-Flick Technique)

Compound code	Dose (mg/kg)	Percent analgesic activity			
		30 min	1 h	2 h	3 h
A1	10	37±0.15**	41±0.56*	47±0.51**	35±0.11**
	20	51±1.15**	56±2.12**	63±0.52**	46±0.72*
A2	10	42±1.16*	44±1.12**	49±1.13**	37±0.49*
	20	53±1.91**	60±0.46**	64±0.85**	49±0.96*
A3	10	44±0.89*	49±1.66**	56±1.12**	40±1.76*
	20	60±1.56**	66±2.13***	67±1.18***	51±0.38**
A4	10	43±0.59*	48±0.66**	52±0.56**	39±1.19*
	20	53±2.65**	62±2.54***	66±3.28***	48±1.39**
A5	10	31±2.38*	33±1.18**	36±1.26**	30±2.15*
	20	44±1.09**	51±1.56**	53±0.75***	35±0.19*
A6	10	35±0.45*	37±0.95**	40±0.95**	27±1.23*
	20	43±2.16**	51±1.95***	53±2.05**	35±0.16*
A7	10	30±1.38**	33±0.44*	34±1.26**	21±0.38**
	20	41±1.19**	44±0.48**	46±0.39**	33±1.11*
A8	10	36±2.24*	36±1.15**	40±2.23**	27±0.56*
	20	43±3.01**	47±1.18***	52±0.46**	36±0.39*
A9	10	29±1.07*	32±2.01**	33±1.34*	19±0.26*
	20	41±1.11**	45±0.93**	46±0.13*	30±1.16*
A10	10	23±0.85*	25±2.61*	29±0.03*	19±1.11*
	20	38±0.46**	39±0.33**	42±0.63***	26±1.16*
A11	10	23±2.11*	26±2.34*	31±0.43**	17±1.35*
	20	35±0.96**	38±0.49**	39±0.56*	24±1.10*
Control		2±0.35	6±0.49	4±0.59	4±0.91
Diclofenac	10	35±1.06**	40±0.92**	44±0.79***	32±1.01*
	20	47±0.95**	56±0.79***	60±1.01***	41±0.56*

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 Odema Method)

Compound code	Dose (mg/kg)	Percent protection			
		30 min	1 h	2 h	3 h
A1	10	26±0.51**	28±0.29*	31±1.15**	24±0.65*
	20	40±0.96**	49±0.39*	51±1.23**	39±1.19*
A2	10	27±0.19*	29±1.13**	33±0.45**	26±1.39*
	20	42±0.49*	50±0.56**	54±1.23**	40±0.55*
A3	10	30±1.59*	34±0.34*	36±1.13**	28±0.42*
	20	43±0.29**	55±1.35**	59±1.55**	42±0.46*
A4	10	33±0.81*	37±0.94**	40±1.43***	30±0.38**
	20	46±1.19**	57±0.36***	62±0.28**	39±0.18*
A5	10	26±1.23*	29±1.45**	30±0.76**	23±0.23*
	20	39±0.81**	46±1.18**	49±0.46**	36±1.17*
A6	10	25±0.66*	26±0.49*	29±0.63**	21±1.18*
	20	38±0.37**	45±0.59***	48±0.45**	37±1.25*
A7	10	24±0.67*	28±0.71*	28±0.63**	25±1.17*
	20	34±0.52**	40±0.79***	41±1.13***	33±1.29**
A8	10	26±1.16*	29±0.47*	30±0.83**	22±1.17*
	20	39±0.16**	49±0.94**	51±0.63***	35±0.21*
A9	10	23±0.93*	25±1.46*	26±0.73**	25±0.49*
	20	32±0.82*	39±0.63***	39±0.79**	30±1.43*
A10	10	21±1.19*	24±0.76**	22±0.81**	22±0.73**
	20	33±0.92*	39±0.63**	42±0.27**	29±1.89*
A11	10	24±1.06*	25±0.85***	26±1.06**	21±1.35*
	20	26±1.95**	36±0.22**	39±1.10***	25±0.06*
Control		5.13±0.29	6.12±0.27	5.79±0.32	3.29±0.51
Diclofenac	10	31±0.96**	35±1.13***	39±1.52***	31±1.69**
	20	42±1.19**	53±0.76***	56±0.79***	40±1.15*

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	*B. subtilis*	*S. paratyphi* B	*E. tarda*	*P. aeruginosa*	*S. typhimurium*	*P. vulgaris*	*K. pneumoniae*
A1	156.25	19.53	156.25	78.12	78.12	156.25	39.06
A2	78.12	156.25	78.12	39.06	19.53	19.53	78.12
A3	39.06	78.12	78.12	19.53	78.12	39.06	39.06
A4	156.25	39.06	156.25	39.06	78.12	78.12	39.06
A5	78.12	156.25	78.06	156.25	156.25	78.12	156.25
A6	78.12	78.12	312.50	156.25	156.25	39.06	39.06
A7	9.76	9.76	9.76	78.12	39.06	39.06	19.06
A8	39.06	9.76	9.76	39.06	9.76	39.06	78.12
A9	39.06	78.12	39.06	78.12	78.12	156.25	78.12
A10	312.50	39.06	156.25	78.12	78.12	156.25	312.50
A11	312.50	78.12	39.06	156.25	78.12	78.12	19.53
Norfloxacin	2.44	0.60	0.60	0.018	4.88	1.22	9.76

of diclofenac sodium.

The results of antibacterial activity indicate that all the test compounds exhibited weak activity against the tested bacteria. The compound **A7** showed good activity against *B. subtilis*, *S. paratyphi* B and *E. tarda*. The compound **A8** exhibited good activity against *S. paratyphi* B, *E. tarda* and *S. typhimurium*.

The results of analgesic and anti-inflammatory activities indicate that the replacement of C-2 phenyl group of 2-phenyl-3-substituted quinazolines and C-2 methyl group of 2-methyl-3-substituted quinazolines by C-2 methylthio group showed increase in activity. However there is no increase in antibacterial activity. Hence further structural modification is planned to increase not only the analgesic and anti-inflammatory activities also the antibacterial activity.

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