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

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A series of novel 2-phenyl-3-substituted quinazolin-4(3*H*)-ones have been synthesized by treating methyl-*N*-(2-phenyl quinazolin-3-yl-4(3*H*)-one) dithiocarbamate with different amines, the starting material dithiocarbamate was synthesized from anthranilic acid. The title compounds were investigated for analgesic, anti-inflammatory and antibacterial activities. All the test compounds exhibited significant activity, the compounds A1, A2 and A3 shown more potent analgesic activity, and the compound A3 shown more potent anti-inflammatory activity than the reference standard diclofenac sodium.

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

Bacterial infections often produce inflammation and pain. 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 central nervous system (CNS) activities like analgesic,[2] anti-inflammatory[3] and anticonvulsant[4] activities. In view of these facts and as a continuation of our previous efforts carried out in our laboratory,[5,6] 1-(2-phenyl quinazolin-3-yl-4(3*H*)-one)-3-substituted thioureas were synthesized. The title compounds were prepared by nucleophilic substitution of methyl-*N*-(2-phenyl quinazolin-3-yl-4(3*H*)-one dithiocarbamate with variety of amines. The starting material dithiocarbamate was synthesized by reacting the amino group of 3-amino-2-phenyl quinazolin-4(3*H*)-one with carbondisulphide, sodium hydroxide and dimethyl sulphate (Chart 1). The chemical structures of the synthesized compounds were confirmed by ^{1}H-NMR, IR and mass spectral data, the purity was ascertained by elemental analysis. The synthesized compounds were tested for their analgesic activity by tail-flick method, anti-inflammatory activity by carrageenan induced rat paw oedema method and antibacterial activity by agar dilution method.

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 (TMS) as internal standard. Elemental analysis were performed on Carlo erba 1108.

Synthesis of 2-Phenyl-3,l-benzoxazin-4-one To a solution of anthranilic acid (0.1 mol) dissolved in pyridine (60 ml), benzoyl chloride (0.2 mol) was added. The mixture was stirred for 30 min followed by treatment with 5% $NaHCO_3$ (15 ml). The solid obtained was crystallized from ethanol, yield=80%, mp 120 °C; IR (KBr) cm^{-1}: 3350 (NH), 1780 (C=O) 1680 (cyclic C=O) and 1620 (C=N); NMR ($CDCl_3$) δ: 6.8—7.5 (m, 9H, ArH); MS (*m*/*z*) 223 (M^+). *Anal.* Calcd for $C_{14}H_9NO_2$: C, 75.33; H, 4.03; N, 6.27. Found: C, 75.37; H, 3.99; N, 6.31.

Synthesis of 3-Amino-2-phenyl quinazolin-4(3*H*)-one A mixture of 2-phenyl-3-benzoxazin-4-one (0.05 mol) and hydrazine hydrate (0.05 mol) in ethanol was refluxed for 3 h and cooled. The separated solid was crystallized from ethanol, yield=85%, mp 196 °C; IR (KBr) cm^{-1}: 3300 (NH_2), 1680 (cyclic C=O), 1620 (C=N) and 1600 (C=C); ^{1}H-NMR ($CDCl_3$) δ: 4.5 (s, 2H, NH_2), 6.7—7.4 (m, 9H, ArH); MS (*m*/*z*) 237 (M^+). *Anal.* Calcd for $C_{14}H_{11}N_3O$: C, 70.88; H, 4.64; N, 17.72. Found: C, 70.92; H, 4.67; N, 17.68.

Synthesis of Methyl-*N*-(2-phenyl quinazolin-3-yl-4(3*H*)-one Dithiocarbamate To a vigorously stirred solution of 3-amino-2-phenyl quinazolin-4(3*H*)-one 4.74 g (0.02 mol) in dimethyl sulphoxide (10 ml) at room temperature carbondisulphide (1.6 ml, 0.026 mol) and sodium hydroxide (1.2 ml, 20 mol) were added dropwise during 30 min, it was allowed to stir 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

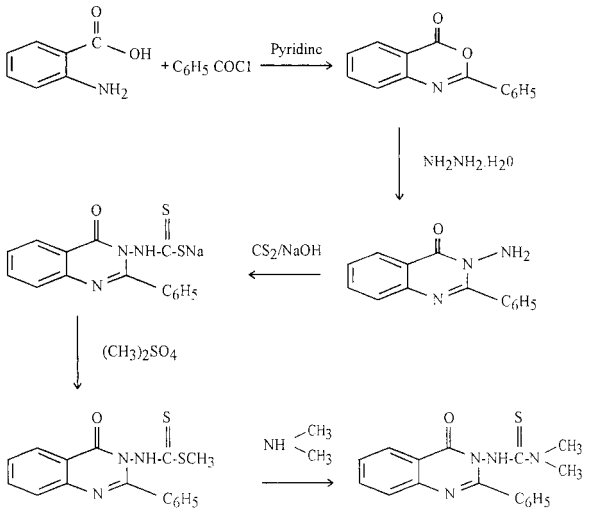

Chart 1

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solid, so obtained was filtered, washed with water, dried and recrystallized from ethanol–chloroform mixture, yield=78%, mp 125 °C; IR (KBr) cm^{-1}: 3310 (NH), 1680 (cyclic C=O), 1620 (C=N), 1600 (C=C), 1150 (C=S) and 650 cm^{-1} (C–S); ^{1}H-NMR ($CDCl_3$) δ: 3.5—3.6 (s, 3H, CH_3), 6.7—7.4 (m, 9H, ArH), 8.8—8.9 (s, 1H, NH); MS (*m*/*z*) 327 (M^+). *Anal.* Calcd for $C_{16}H_{13}N_3OS_2$: C, 58.71; H, 3.97; N, 12.84. Found: C, 58.68; H, 4.01; N, 12.87.

Synthesis of 1-(2-Phenyl quinazolin-3-yl-4(3*H*)-one)-3-dimethyl Thiourea A mixture of methyl-*N*-(2-phenyl-3-yl-4(3*H*)-one dithiocarbamate 3.27 g (0.01 mol) and dimethyl amine 0.9 g (0.02 mol) in dimethyl formamide (20 ml) was refluxed for 22 h cooled and poured into ice water, the solid obtained was filtered, dried and recrystallized from chloroform, yield=74%, mp 178 °C; IR (KBr) cm^{-1}: 3310 (NH), 2850 (CH), 1680 (cyclic C=O), 1620 (C=N), 1600 (C=C), 1300 (C–N), 1150 (C=S); ^{1}H-NMR ($CDCl_3$) δ: 3.2—3.5 (s, 6H, –N–$(CH_3)_2$), 6.7—7.4 (m, 9H, ArH), 8.6—8.7 (s, 1H, NH); MS (*m*/*z*) 324 (M^+). *Anal.* Calcd for $C_{17}H_{16}N_4OS$, C, 62.96; H, 4.93, N, 17.28. Found : 62.93, H, 4.89; N, 17.32.

PHARMACOLOGY

The synthesized compounds were evaluated for analgesic anti-inflammatory and antimicrobial acivities. 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 as standard 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,

$$\text{PAA}=(T_2/T_1)\times 100$$

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

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

Where x=the mean paw volume of rats before the administration of carrageenan and test compounds or standard compound, a stands for mean paw volume of rats after the administration of carrageenan in the control group, b is the mean paw volume of rats before the administration of carrageenan in the control group, y is mean paw volume of rats after 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: *Salmonella typhimurium* ATCC 33068, *Pseudomonas aeruginosa* ATCC 2853, *Salmonella paratyphi B*, *Proteus vulgaris* ATCC 9484, *Klebsiella pneumoniae* ATCC 13883, *Edwersiella tarda*, *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 inoculum. The MIC of the test compounds were compared with the reference drug norfloxacin.

RESULTS AND DISCUSSION

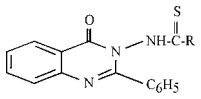
It has been observed that all the compounds tested showed significant analgesic activity. The compound **A1** with methyl substitution shown good activity, with the increased liphophilicity (dimethyl group) compound **A2** shown increased activity. Further increase in liphophilicity (diethyl group). **A3** led to further increase in activity. Substitution with alicyclic amines **A4** led to decrease in activity. Placement of alicyclic amines with additional hetero atoms **A5**, **A6** led to further decrease in activity. Aromatic substitution **A7**—**A11** shown still lower activity. In general the compounds with aliphatic open chain substitution shown the better activity. The compound **A3** was found to be the most active analgesic agent.

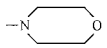
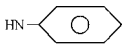
All the compounds showed significant anti-inflammatory activity, when compared with diclofenac sodium, the compounds **A1** and **A2** were equipotent and the compound **A3** was more potent.

The results of antibacterial activity reveals that all the test compounds showed moderate activity against the tested bacteria. The compound **A2** exhibited good activity against *Proteus vulgaris*, *K. pneumoniae* and *B. subtilis*; The compound **A3** exhibited good activity against *S. typhimurium*, *P. aeruginosa*, *S. paratyphi B*, *Proteus vulgaris*, *E. tarda* and *Bacillus subtilis*; and the compound **A9** exhibited good activity against *S. typhimurium*, *P. aeruginosa*, *S. paratyphi B* and *K. pneumoniae.*

Although the title compounds exhibited potent analgesic and anti-inflammatory activities, moderate antibacterial activity was found. Hence, necessary structural modifications are planned in the further study to increase the antibacterial activity. In general in the present study it is observed that

Table 1. Physical Data for 1-(2-Phenylquinazolin-3-yl-4-(3*H*)-one-3-substituted Thioureas



Code	R	Molecular formula[a)]	Molecular weight[b)]	mp (°C)	Yield (%)
A1	N–CH_3	$C_{16}H_{14}N_4OS$	310	140	72
A2	–N(Me)(Me)	$C_{17}H_{16}N_4OS$	324	178	74
A3	–N(Et)(Et)	$C_{19}H_{20}N_4OS$	352	189	69
A4		$C_{19}H_{15}N_4OS$	347	205	71
A5		$C_{19}H_{18}N_4O_2S$	366	200	72
A6		$C_{19}H_{19}N_5OS$	351	218	70
A7		$C_{22}H_{18}N_4O_2S$	402	223	68
A8		$C_{21}H_{15}N_5O_3S$	417	210	67
A9		$C_{21}H_{15}N_4O_3Cl$	406	207	69
A10	–N(Ph)(Ph)	$C_{27}H_{20}N_4OS$	448	231	71
A11		$C_{20}H_{15}N_5OS$	373	144	66

a) All compounds gave satisfactory elemental analysis (±0.04% of calculated values). *b*) Molecular weight determination by mass spectra.

Table 2. Analgesic Activity (Tail-flick Technique)

Compound	Dose (mg/kg)	Percent analgesic activity			
		30 min	1 h	2 h	3 h
A1	10	76±0.35*	89±0.30**	95±0.40***	84±0.38**
	20	146±0.38**	159±0.26***	165±0.37***	132±0.29**
A2	10	79±0.23*	91±0.29***	97±0.75***	85±0.25**
	20	159±0.73**	171±0.42***	183±0.38***	150±0.5***
A3	10	80±0.58**	95±0.62***	115±0.22***	87±0.31**
	20	165±0.82***	173±0.6***	189±0.42***	151±0.48***
A4	10	79±0.55*	80±0.42*	91±0.75***	72±0.65*
	20	128±0.25**	136±0.52**	144±0.37**	116±0.28**
A5	10	70±0.42*	80±0.29*	88±0.65**	69±0.34*
	20	115±0.82**	137±0.61**	142±0.41**	116±0.20**
A6	10	71±0.23*	79±0.76*	90±0.39***	68±0.16*
	20	113±0.47**	129±0.34**	131±0.21**	93±0.60*
A7	10	69±0.31*	73±0.10*	84±0.7**	74±0.41*
	20	102±0.12**	126±0.35**	132±0.24**	101±0.46**
A8	10	69±0.26*	74±0.42*	79±0.39*	62±0.63*
	20	105±0.52**	137±0.18**	135±0.80**	87±0.22*
A9	10	68±0.70*	72±0.26*	80±0.40**	65±0.52*
	20	97±0.66*	123±0.31**	128±0.23**	99±0.34*
A10	10	62±0.53*	75±0.62*	77±0.18*	69±0.24*
	20	104±0.42**	141±0.29**	143±0.82**	103±0.21**
A11	10	66±0.31*	73±0.26*	78±0.43*	62±0.52*
	20	99±0.63*	112±0.34**	136±0.49**	89±0.19*
Control		4.41±0.61	3.39±0.52	1.13±0.29	2.12±0.42
Diclofenac	10	72±0.19*	86±0.46*	95±0.31***	70±0.29*
	20	116.63±0.26**	139.41±0.32**	152.85±0.14***	90.56±0.6*

Significance levels: $*p<0.05$, $**p<0.01$ and $***p<0.001$.

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

Compound	Dose (mg/kg)	Percent protection			
		30 min	1 h	2 h	3 h
A1	10	22±0.42*	25±0.26**	31±0.19***	22.3±0.36*
	20	30±0.61**	50±0.38***	53±0.23***	35±0.41**
A2	10	23±0.16*	27±0.71**	32±0.21***	20±0.22*
	20	34±0.46**	55±0.39***	59±0.29***	37±0.36***
A3	10	26±0.22**	30±0.48***	37±0.34***	23±0.19*
	20	38±0.56***	56±0.24***	58±0.45***	35±0.29**
A4	10	21±0.42*	26±0.72**	30±0.28***	21±0.34*
	20	33±0.35**	42±0.46***	44±0.59***	30±0.13**
A5	10	22±0.29*	24±0.19*	33±0.36***	20±0.43*
	20	30±0.53**	43±0.28***	43±0.49***	29±0.36*
A6	10	21±0.36*	27±0.24**	29±0.6***	18±0.15*
	20	29±0.18*	36±0.65*	39±0.22**	24±0.41*
A7	10	20±0.24*	20±0.30*	27±0.49***	17±0.63*
	20	31±0.63**	39±0.32**	44±0.47***	31±0.24**
A8	10	23±0.19*	24±0.73**	28±0.26**	19±0.34*
	20	33±0.65*	45±0.16***	46±0.50***	32±0.43**
A9	10	19±0.39*	23±0.52**	23±0.36**	20±0.17*
	20	27±0.67*	29±0.23*	32±0.18**	25±0.34*
A10	10	20±0.05*	24±0.39**	26±0.92**	21±0.15*
	20	26±0.47*	32±0.36**	35±0.28**	27±0.59*
A11	10	21±0.63*	25±0.41**	27±0.23**	19±0.22*
	20	32±0.13**	37±0.43**	40±0.55***	29±0.18*
Control		4.15±0.60	4.19±0.48	3.31±0.27	2.47±0.40
Diclofenac	10	23±0.14*	26±0.52**	31±0.35***	21±0.26*
	20	37±0.29**	49±0.47***	53±0.35***	36±0.32**

Significance levels: $*p<0.05$, $**p<0.01$ and $***p<0.001$.

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

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

aliphatic open chain compounds (**A1**—**A3**) are more active than alicyclic compounds (**A4**—**A6**) which inturn is more potent than aryl amines. The compound **A3** was the most active agent and could therefore serve as a lead molecule for further modification to obtain clinically useful analgesic, anti-inflammatory and antibacterial agent.

REFERENCES

1) Alagarsamy V., Pathak U. S., Sriram D., Pandeya S. N., Clercq E. De., *Ind. J. Pharm. Sci.*, **62**, 433—437 (2000).
2) Nigam R., Swarup S., Saxena V. K., *Indian Drugs*, **27**, 238—243 (1990).
3) Koizomi M., Marakuni Y., *Japan Kokai*, **77**, 51 (1917).
4) Manabu H., Ryvichi I., Hideaki H., *Chem. Pharm. Bull.*, **38**, 618—622 (1990).
5) Alagarsamy V., Meena S., Vijaya Kumar S., Revathi R., *Farmaco*, **58**, (2003) in press.
6) Alagarsamy V., Pathak U. S., Goyal R. K., *Ind. J. Pharm. Sci.*, **62**, 63—66 (2000).
7) Kulkarni S. K., *Life Sci.*, **27**, 185—188 (1980).
8) Amour R. E., Smith D. L., *J. Pharm. Expt. Therap.*, **72**, 74—78 (1941).
9) Winter C. A., Risely E. A., Nu G. N., *Proc. Soc. Exp. Brol.*, **111**, 544—547 (1982).
10) Barry A., "Antibiotics in Laboratory Medicine," fifth ed., William and Wilkins, Baltimore, MD, 1991, p. 1.