Synthesis and Pharmacological Evaluation of Some 3-(4-Methoxyphenyl)-2-substitutedamino-quinazolin-4(3*H*)-ones as Analgesic and Anti-inflammatory Agents

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A variety of novel 3-(4-methoxyphenyl)-2-substitutedamino-quinazolin-4(3*H*)-ones were synthesized by reacting the amino group of 2-hydrazino-3-(4-methoxyphenyl)-quinazolin-4(3*H*)-one with a variety of alkyl and aryl ketones. The starting material 2-hydrazino-3-(4-methoxyphenyl)-quinazolin-4(3*H*)-one was synthesized from 4-methoxyaniline. The title compounds were investigated for analgesic, anti-inflammatory and ulcerogenic index activities. While the test compounds exhibited significant activity, compounds 2-(1-methylpropylidene)-hydrazino-3-(4-methoxyphenyl)-quinazolin-4(3*H*)-one (A1), 2-(1-ethylpropylidene)-hydrazino-3-(4-methoxyphenyl)-quinazolin-4(3*H*)-one (A2) and 2-(1-methylbutylidene)-hydrazino-3-(4-methoxyphenyl)-quinazolin-4(3*H*)-one (A3) showed moderately more potent analgesic activity and the compound 2-(1-methylbutylidene)-hydrazino-3-(4-methoxyphenyl)-quinazolin-4(3*H*)-one (A3) showed moderately more potent anti-inflammatory activity when compared to the reference standard diclofenac sodium. Interestingly the test compounds showed only mild ulcerogenic potential when compared to aspirin.

Key words quinazoline; analgesic; anti-inflammatory

Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly prescribed for the treatment of acute and chronic inflammation, pain, and fever. Most of NSAIDs that are available in market are known to inhibit isoforms, a constitutive form, COX-1 and an inducible form, COX-2 to offer therapeutic effect. However long-term clinical usage of NSAIDs are associated with significant side effects of gastrointestinal lesions, bleeding, and nephrotoxicity. Therefore the discovery of new safer anti-inflammatory drugs represents a challenging goal for such a research area.[1—4] On our going medicinal chemistry research program we found that quinazolines and condensed quinazolines exhibit potent central nervous system (CNS) activities like analgesic, anti-inflammatory[5] and anticonvulsant.[6] Quinazolin-4(3*H*)-ones with 2,3-disubstitution is reported to possess significant analgesic, anti-inflammatory[7,8] and anticonvulsant activities.[9] Earlier we have documented 2-phenyl-3-substituted quinazolines,[10] 2-methyl-3-substituted quinazolines,[11] 2-methylthio-3-substituted quinazolines,[12] 2,3-disubstituted quinazolines[13] they exhibited good analgesic and anti-inflammatory activities. The present work is an extension of our ongoing efforts towards the development and identification of new molecules for analgesic and anti-inflammatory activities with minimal gastrointestinal ulceration side effects. With this background in the present study we have synthesized a series of 3-(4-methoxyphenyl)-2-substitutedamino-quinazolin-4(3*H*)-one. The synthesized compounds were tested for their analgesic, anti-inflammatory and ulcerogenic index activities.

Chemistry

The key intermediate 3-(4-methoxyphenyl)-2-thioxo-2,3-dihydro-quinazolin-4(1*H*)-one **4** was obtained by reacting 4-methoxyaniline (**1**) with carbon disulphide and sodium hydroxide in dimethyl sulphoxide to give sodium dithiocarbamate, which was methylated with dimethyl sulphate to afford the dithiocarbamic acid methyl ester (**2**). Compound **2** on reflux with methyl anthranilate (**3**) in ethanol yielded the desired 3-(4-methoxyphenyl)-2-thioxo-2,3-dihydro-quinazolin-4(1*H*)-one (**4**) *via* the thiourea intermediate in good yield (80%). The product obtained was cyclic and not an open chain thiourea **3a**. It was confirmed by its low *Rf* value, high melting point and its solubility in sodium hydroxide solution. The IR spectrum of **4** show intense peaks at 3218 cm^{-1} for cyclic thio urea (NH), 1680 cm^{-1} for carbonyl (C=O) and 1200 cm^{-1} for thioxo (C=S) stretching. ^{1}H-NMR spectra of **4** showed singlet at δ 3.88 ppm due to OCH_3 group, a multiplet at δ 7—8.1 ppm for aromatic (8H) protons and a singlet at δ 10.36 ppm indicating the presence of NH. Data from the elemental analyses have been found to be in conformity with the assigned structure. Further the molecular ion recorded in the mass spectrum is also in agreement with the molecular weight of the compound.

The 2-methysulfanyl-3-(4-methoxyphenyl)-quinazolin-4(3*H*)-one **5** was obtained by dissolving **4** in 2% alcoholic sodium hydroxide solution and methylating with dimethyl sulphate with stirring at room temperature. The IR spectra of **5** showed disappearance of NH and C=S stretching signals of cyclic thiourea. It showed a peak for carbonyl (C=O) stretching at 1683 cm^{-1}. The ^{1}H-NMR spectra of compound **5** showed singlets at δ 2.5 ppm and 3.87 ppm due to SCH_3 and OCH_3 respectively, a multiplet at δ 7.0—8.26 ppm was observed for aromatic (8H) protons. Data from the elemental analyses and molecular ion recorded in the mass spectra further confirmed the assigned structure.

Nucleophilic displacement of methylthio group of **5** with hydrazine hydrate was carried out using ethanol as solvent to afford 2-hydrazino-3-(4-methoxyphenyl)-quinazolin-4(3*H*)-one **6**. The long duration of reaction (30 h) required might be due to the presence of bulky aromatic ring at the N-3 position, which might have reduced the reactivity of quinazoline ring system at C-2 position. The formation of **6** was confirmed by the presence of NH and NH_2 signals at 3350—3320 cm^{-1} in the IR spectrum. It also showed a peak for car-

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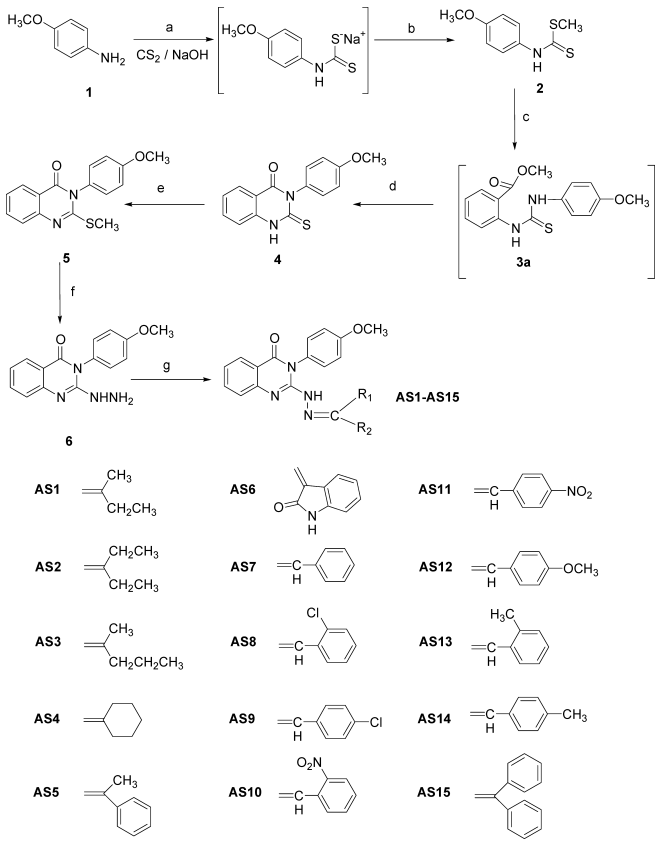

Reagents and conditions: (a) DMSO, rt, 30 min; (b) $(CH_3)_2SO_4$, 5—10 °C, 2 h; (c) methyl anthranilate (**3**), K_2CO_3, ethanol reflux for 21 h; (d) 10% alcoholic NaOH/dil.HCl, yield 80%; (e) 2% alcoholic NaOH, $(CH_3)_2SO_4$, rt, 1 h, yield 78%; (f) NH_2NH_2, K_2CO_3, ethanol reflux for 22 h, yield 74%; (g) $(R_2R_1)CO$; gla.CH_3COOH reflux, 33 h.

Chart 1. Synthesis of 2-(1-Methylpropylidene)-hydrazino-3-(4-methoxyphenyl)-quinazolin-4(3*H*)-one and Its Derivatives from 4-Methoxyaniline

bonyl (C=O) at 1674 cm^{-1}. The ^{1}H-NMR spectra of the compound **6** showed singlets at δ 3.79 ppm, 4.95 ppm and 8.56 ppm due to OCH_3, NH_2 and NH respectively, a multiplet at δ 6.82—8.06 ppm was observed for aromatic (8H) protons. Data from the elemental analyses have been found to be in conformity with the assigned structure. Further the molecular ion recorded in the mass spectra is also in agreement with the molecular weight of the compound.

The title compounds 3-(4-methoxyphenyl)-2-substituted-amino-quinazolin-4(3*H*)-ones **A1**—**A15** were obtained by the condensation of amino group of 2-hydrazino-3-(4-methoxy phenyl)-quinazolin-4(3*H*)-one (**6**) with a variety of alkyl and aryl ketones. The formation of title product is indicated by the disappearance of peak due to NH_2 of the starting material in IR and ^{1}H-NMR spectrum of all the compounds **A1**—**A15**. The IR and ^{1}H-NMR spectrum of these compounds showed the presence of peaks due to ($N=CR^1R^2$) carbonyl (C=O), NH and Aryl groups. The mass spectra of the title compounds are in confirmity with the assigned structure. The mass spectra of these compounds showed molecular ion peaks corresponding to their molecular formulae. A common peak at *m*/*z* 144 corresponding to quinazolin-4-one moiety appeared in all mass spectra of compounds **A1**—**A15**. Elemental (C, H, N) analysis satisfactorily confirmed elemental composition and purity of the synthesized compounds.

Table 1. Percent Analgesic Activity of Test Compounds (Tail-Flick Technique)

Compound code	Dose (mg/kg)	Percent analgesic activity			
		30 min	1 h	2 h	3 h
A1	10	45±1.76*	47±1.93*	52±1.27**	32±1.81*
	20	59±1.69***	63±1.83***	64±1.19***	44±1.36*
A2	10	52±1.68**	57±1.94**	59±1.37***	43±1.38*
	20	65±1.47***	70±1.81***	73±1.94***	48±1.04*
A3	10	46±1.92*	49±1.06*	53±1.94**	35±1.17*
	20	59±1.73***	66±1.82***	68±1.81***	46±1.72*
A4	10	35±1.05*	42±1.83*	47±160*	28±1.04*
	20	51±1.37**	54±1.20**	55±1.38**	36±1.29*
A5	10	39±1.61*	46±1.83*	48±1.47*	31±1.71*
	20	52±1.03**	55±1.82**	56±1.91**	38±1.73*
A6	10	37±1.98*	39±1.03*	41±1.44*	29±1.92*
	20	48±1.64*	54±1.93**	56±1.87**	34±1.05*
A7	10	36±1.01*	39±1.73*	45±1.62*	33±1.38*
	20	47±1.61*	54±1.24**	57±1.36**	38±1.03*
A8	10	27±1.41*	32±1.92*	36±1.71*	28±1.62*
	20	35±1.93*	40±1.38*	44±1.15*	38±1.81*
A9	10	34±1.15*	37±1.85*	41±1.22*	29±1.37*
	20	43±1.28*	47±1.94*	47±1.81*	33±1.72*
A10	10	33±1.08*	37±1.24*	42±1.74*	32±1.91*
	20	45±1.85*	49±1.74*	53±1.79*	36±1.95*
A11	10	32±1.42*	36±1.71*	40±195*	29±1.69*
	20	35±1.91*	46±1.85*	49±1.47*	35±1.54*
A12	10	35±1.63*	39±1.79*	44±1.54*	33±1.07*
	20	41±1.52*	44±1.96*	48±1.64**	38±1.24*
A13	10	37±1.42*	38±1.73*	43±1.95*	31±1.26*
	20	48±1.08*	51±1.76**	53±1.93*	38±1.84*
A14	10	38±1.08*	42±1.94*	43±1.54*	34±1.65*
	20	47±1.45*	49±1.16*	54±1.25**	41±1.76*
A15	10	36±1.42*	38±1.95*	43±1.83*	30±1.42*
	20	42±1.37*	45±1.18*	49±1.46*	38±1.60*
Control		2±0.35	6±0.49	4±0.59	4±0.91
Diclofenac	10	37±1.69*	43±1.42*	45±0.92*	33±0.96*
	20	46±0.95*	55±1.16**	62±1.49***	39±1.13*

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.

Results and Discussion

Test for analgesic activity was performed by tail-flick technique[14,15] using Wistar albino mice. The results of analgesic activity indicate that test compounds exhibited moderate analgesic activity at 30 min of reaction time; the activity increased at 1 h and it reached to peak level at 2 h. Declining in activity was observed at 3 h (Table 1). Compound **A1** with 1-methylpropylidene substituent showed good activity; with the increased lipophilicity (1-ethylpropylidene group, compound **A2**) showed increase in activity. Replacement of 1-ethylpropylidene group with its isomer 2-pentylidene group (compound **A3**) retains the activity. Replacement of an alkyl chain at the 2-position with a cycloalkyl group and an aralkyl group (compounds **A4** and **A5** respectively) leads to moderate decrease in activity. Placement of aryl group at the N-3 position (compounds **A6**, **A7** and **A13**—**A15**) also results in decreasing activity. Placement of electron withdrawing group at N-3 aryl ring (compounds **A8**—**A12**) leads to further decrease of activity. Compound 2-(1-ethylpropylidene)-hydrazino-3-(4-methoxyphenyl)-quinazolin-4(3*H*)-one (**A2**) emerged as the most active analgesic agent and it is more potent when compared to the reference standard diclofenac sodium.

Anti-inflammatory activity was evaluated by carrageenan-induced paw oedema test in rats.[16] The anti-inflammatory activity data (Table 2) indicated that all the test compounds protected rats from carrageenan-induced inflammation moderately at 30 min of reaction time; the activity increased at 1 h and it reached to peak level at 2 h. Declining in activity

Table 2. Percent Anti-inflammatory Activity of Test Compounds (Carrageenan-Induced Paw Oedema Test in Rats)

Compound code	Dose (mg/kg)	Percent protection			
		30 min	1 h	2 h	3 h
A1	10	35±1.15*	38±1.73*	40±1.81*	25±1.54*
	20	41±1.94*	46±1.26**	49±1.42**	38±1.31*
A2	10	37±1.93*	39±1.37*	43±1.05*	30±1.82*
	20	44±1.90**	57±1.47***	59±1.52***	38±1.26*
A3	10	37±1.17*	45±1.42*	49±1.83*	32±1.08*
	20	48±1.19**	60±1.63***	65±1.45***	40±1.38*
A4	10	30±1.94*	36±1.42*	39±1.63*	26±1.46*
	20	38±1.51*	46±1.94*	46±1.48*	31±1.63*
A5	10	34±1.18*	37±1.37*	39±1.19*	28±1.83*
	20	41±1.26*	43±1.37*	46±1.26**	33±1.45*
A6	10	29±1.15*	32±1.29*	35±1.16*	26±1.83*
	20	34±1.36*	37±1.15*	38±1.71*	33±1.15*
A7	10	27±1.84*	34±1.27*	38±1.06*	26±1.37*
	20	33±1.25*	42±1.47*	45±1.28*	31±1.93*
A8	10	24±1.41*	27±1.83*	29±1.52*	23±1.73*
	20	29±1.82*	36±1.63*	32±1.85*	28±1.14*
A9	10	28±1.23*	26±1.15*	32±1.26*	24±1.93*
	20	31±1.05*	34±1.24*	34±1.25*	30±1.93*
A10	10	29±1.73*	32±1.06*	36±1.93*	23±1.18*
	20	36±1.05*	37±1.73*	39±1.39*	34±1.53*
A11	10	24±1.74*	29±1.32*	33±1.85*	23±1.39*
	20	38±1.27*	38±1.93*	42±1.85*	30±1.27*
A12	10	26±1.18*	29±1.23*	34±1.93*	24±1.91*
	20	36±1.48*	38±1.92*	42±1.61*	31±1.83*
A13	10	32±1.72*	34±1.37*	37±1.93*	29±1.28*
	20	38±1.54*	41±1.51*	44±1.62*	33±1.93*
A14	10	29±1.71*	36±1.83	36±1.54*	27±1.42*
	20	41±1.71*	41±1.36**	42±1.81*	37±1.62*
A15	10	29±1.05*	30±1.41*	32±1.54*	28±1.39*
	20	38±1.54*	41±1.62*	45±1.61*	36±1.63*
Control		5.1±0.29	6.1±0.27	5.7±0.32	3.2±0.93
Diclofenac	10	32±0.63*	38±1.58*	39±1.97*	33±0.93*
	20	45±1.61**	52±0.92***	60±1.52***	42±1.36**

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.

was observed at 3 h. The compound 2-(1-methylbutylidene)-hydrazino-3-(4-methoxyphenyl)-quinazolin-4(3*H*)-one (**A3**) showed moderately more potent anti-inflammatory activity when compared to the reference standard diclofenac sodium. The compound 2-(1-ethylpropylidene)-hydrazino-3-(4-methoxyphenyl)-quinazolin-4(3*H*)-one (**A2**) showed equipotent anti-inflammatory activity when compared to the reference standard diclofenac sodium.

The ulcer index of the test compounds (Table 3) reveal that the compounds with open chain aliphatic substituents (compounds **A1**—**A3**) showed negligible ulcer index, whereas aryl substituents (compounds **A5**—**A7** and **A13**—**A15**) exhibited little increase in ulcer index and the aryl substituents containing electron withdrawing groups (compounds **A8**—**A12**) exhibited higher ulcer index over other test compounds. When compared to the reference standards aspirin (ulcer index 1.73±0.41) and diclofenac (ulcer index 1.65±0.59) the test compounds exhibited about 35 to 50% of the ulcer index of reference standards. Compounds 2-(1-ethylpropylidene)-hydrazino-3-(4-methoxyphenyl)-quinazolin-4(3*H*)-one (**A2**) and 2-(1-methylbutylidene)-hydrazino-3-(4-methoxyphenyl)-quinazolin-4(3*H*)-one (**A3**) exhibited least ulcer index (0.57±1.37 and 0.56±1.04 respectively) among the test compounds which is about one third of the ulcer index of reference standards aspirin and diclofenac. The compound 2-(*N'*-(4-chloro-benzylidene-hydrazino)-3-(4-methoxyphenyl)-quinazolin-4(3*H*)-one (**A9**) showed the highest ulcer index (0.96±1.64) among the test compounds which is about 50% of the ulcer index of aspirin and diclofenac.

Table 3. Evaluation of Ulcerogenicity Index

Compd. code	Ulcer index
A1	0.63±1.51*
A2	0.57±1.37*
A3	0.56±1.04*
A4	0.74±1.39*
A5	0.72±1.32*
A6	0.74±1.41*
A7	0.81±1.72*
A8	0.91±1.05*
A9	0.96±1.64
A10	0.94±1.65*
A11	0.92±1.47*
A12	0.95±1.59*
A13	0.66±1.07*
A14	0.88±1.27*
A15	0.69±1.36*
Control	1.05±0.32
Diclofenac	1.65±0.59**
Aspirin	1.73±0.41**

Dose 20 mg for test compounds, diclofenac and aspirin. Each value represents the mean±S.D. (n=6). Significance levels $*p<0.05$ and $**p<0.01$ as compared with the respective control.

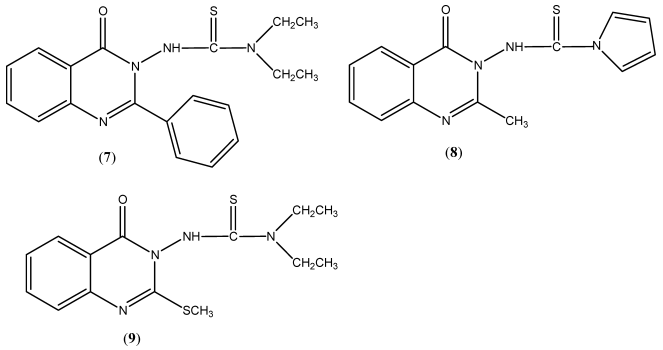

Fig. 1

Conclusions

In our earlier studies[10—13] we observed that the presence of alkyl groups exhibited more analgesic and anti-inflammatory activities over aryl groups at the N-3 position. Hence in the C-2 position also we made a substitution in such a way to increase lipophilicity of the molecule. The placement of such a group enhanced the analgesic and anti-inflammatory activities. The most active compound of the C-2 phenyl series 1-diethyl-3-(2-phenyl quinazolin-3-yl-4(3*H*)-one) thiourea (**7**) (Fig. 1) showed 44% and 58% analgesic and 38% and 53% anti-inflammatory activity at the dose of 10 and 20 mg/kg respectively, at the reaction time of 2 h.[10] Whereas the C-2 methyl series lead molecule 1-pyrrolidinyl-3-(2-methyl quinazolin-3-yl-4(3*H*)-one) thiourea (**8**) (Fig. 1) exhibited 50% and 65% analgesic and 44% and 60% anti-inflammatory activity at the dose of 10 and 20 mg/kg respectively at the reaction time of 2 h.[11] Introduction of sulphur atom at C-2 position in the above series *i.e.* by placing methylthio group at C-2 position[12] compound 1-diethyl-3-(2-methylthio quinazolin-3-yl-4(3*H*)-one) thiourea (**9**) (Fig. 1) exhibited 56%, 67% analgesic activity, 40% and 62% anti-inflammatory activity at 10 and 20 mg/kg respectively at the reaction time of 2 h. The results of the analgesic and anti-inflammatory activities of the present series showed that moderate enhancement of activity. The compound **A2** exhibited 59% and

73% analgesic activity at 10 and 20 mg/kg dose level respectively at the reaction time of 2 h. The compound **A3** showed 49% and 65% anti-inflammatory activity at the dose of 10 and 20 mg/kg respectively at the reaction of 2 h. Interestingly these compounds showed 35% of ulcer index of the reference NSAID's aspirin and diclofenac. Hence this series could be developed as a novel class of analgesic and anti-inflammatory agents. However further structural modification is planned to increase the analgesic and anti-inflammatory activities with the decreased ulcerogenic index.

Experimental

Melting points (mp) were taken in open capillaries on Thomas Hoover melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on an FT-IR Perkin-Elmer spectrometer. The ^{1}H-NMR spectra were recorded on a DPX-300 MHz Bruker FT-NMR spectrometer. The chemical shifts were reported as parts per million (δ ppm) tetramethylsilane (TMS) as an internal standard. Mass spectra were obtained on a JEOL-SX-102 instrument using fast atom bombardment (FAB positive). Elemental analysis was performed on a Perkin-Elmer 2400 C, H, N analyzer and values were within the acceptable limits of the calculated values. The progress of the reaction was monitored on readymade silica gel plates (Merck) using chloroform–methanol (9 : 1) as a solvent system. Iodine was used as a developing agent. Spectral data (IR, NMR and mass spectra) confirmed the structures of the synthesized compounds and the purity of these compounds was ascertained by microanalysis. Elemental (C, H, N) analysis indicated that the calculated and observed values were within the acceptable limits (±0.4%). All chemicals and reagents were obtained from Aldrich (U.S.A.), Lancaster (U.K.) or Spectrochem Pvt.Ltd (India) and were used without further purification.

3-(4-Methoxyphenyl)-2-thioxo-2,3-dihydro-quinazolin-4(1*H*)-one (4) A solution of 4-methoxyaniline **1** (2.46 g, 0.02 mol) in dimethyl sulfoxide (10 ml) was stirred vigorously. To this was added carbon disulphide (1.98 g, 0.026 mol) and 20 M aqueous sodium hydroxide solution (1.2 ml) drop wise during 30 min with stirring. Dimethyl sulphate (2.52 g, 0.02 mol) was added gradually keeping the reaction mixture stirring in freezing mixture for 2 h. The reaction mixture was then poured into ice water. The solid obtained was filtered, washed with water, dried and recrystallized from ethanol. Methyl anthranilate (1.51 g, 0.01mol) and the above prepared *N*-(4-methoxyphenyl)-methyl dithiocarbamic acid (2.13 g, 0.01 mol), were dissolved in ethanol (20 ml). To this anhydrous potassium carbonate (100 mg) was added and refluxed for 21 h. The reaction mixture was cooled in ice and the solid separated was filtered and purified by dissolving in 10% alcoholic sodium hydroxide solution and reprecipitated by treating with dilute hydrochloric acid. The solid obtained was filtered, washed with water, dried and recrystallized from ethanol. Yield=80%, mp 296—300 °C. IR (KBr) cm^{-1}: 3218 (NH), 1680 (C=O), 1593 (C=C), 1200 (C=S); ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.88 (s, 3H, OCH_3), 7.0—8.1 (m, 8H, ArH), 10.36 (s, 1H, NH); MS (*m*/*z*): 284 (M^+). *Anal.* Calcd for $C_{15}H_{12}N_2O_2S$: C, 63.36; H, 4.25; N, 9.85. Found: C, 63.29; H, 4.21; N, 9.91.

2-Methylsulfanyl-3-(4-methoxyphenyl)-quinazolin-4(3*H*)-one (5) The 3-(4-methoxyphenyl)-2-thioxo-2,3-dihydro-quinazolin-4(1*H*)-one **4** (2.84 g, 0.01 mol) was dissolved in 40 ml of 2% alcoholic sodium hydroxide solution. To this dimethyl sulphate (1.26 g, 0.01 mol) was added drop wise with stirring. The stirring was continued for 1 h, the reaction mixture was then poured into ice water. The solid obtained was filtered, washed with water, dried and recrystallized from ethanol–chloroform (75 : 25) mixture. Yield=78%, mp 142—145 °C; IR (KBr) cm^{-1}: 1683 (C=O), 1610 (C=C); ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.5 (s, 3H, SCH_3), 3.87 (s, 3H, OCH_3), 7.0—8.26 (m, 8H ArH); MS (*m*/*z*): 298 (M^+); *Anal.* Calcd for $C_{16}H_{14}N_2O_2S$: C, 64.41; H, 4.72; N, 9.38. Found: C, 64.53; H, 4.67; N, 9.45.

2-Hydrazino-3-(4-methoxyphenyl)-quinazolin-4(3*H*)-one (6) The 2-methylsulfanyl-3-(4-methoxyphenyl)-quinazolin-4(3*H*)-one **5** (2.98 g, 0.01 mol) was dissolved in ethanol (25 ml). To this hydrazine hydrate (99%) (5 g, 0.1 mol) and anhydrous potassium carbonate (100 mg) was added and refluxed for 30 h. The reaction mixture was cooled and poured into ice-water. The solid so obtained was filtered, washed with water, dried and recrystallized from chloroform–benzene (25 : 75) mixture. Yield=74%, mp 196—200 °C; IR (KBr) cm^{-1}: 3350, 3320 ($NHNH_2$), 1674 (C=O); ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.79 (s, 3H, OCH_3), 4.95 (s, 2H, NH_2), 6.82—8.06 (m, 8H, ArH), 8.56 (s, 1H, NH); MS (*m*/*z*): 282 (M^+); *Anal.* Calcd for $C_{15}H_{14}N_4O_2$: C, 63.82; H, 4.99; N, 19.84. Found: C, 63.71; H, 4.95; N, 19.93.

2-(1-Methylpropylidene)-hydrazino-3-(4-methoxyphenyl)-quinazolin-4(3*H*)-one (A1) A mixture of 2-hydrazino-3-(4-methoxyphenyl)-quinazolin-4(3*H*)-one (**6**) (1.128 g, 0.288 g, 0.004 mol) and ethylmethyl ketone (0.004 mol) in glacial acetic acid was refluxed for 36 h. The reaction mixture was poured into ice water. The solid obtained was recrystallized from ethanol. Yield=76%, mp 218—219 °C. IR (KBr) cm^{-1}: 3356 (NH), 1673 (C=O), 1610 (C=N). ^{1}H-NMR ($CDCl_3$) δ ppm: 1.2—1.3 (q, 2H, CH_2CH_3), 1.6—1.7 (t, 3H, CH_2CH_3), 2.0—2.1 (s, 3H, CH_3), 3.2—3.3 (s, 3H, OCH_3), 7.0—7.7 (m, 8H, ArH), 8.2 (br s, 1H, NH). MS (*m*/*z*): 336 (M^+). *Anal.* Calcd for $C_{19}H_{20}N_4O_2$: C, 67.83; H, 5.99; N, 16.65. Found: C, 67.86; H, 5.5.97; N, 16.61.

2-(1-Ethylpropylidene)-hydrazino-3-(4-methoxyphenyl)-quinazolin-4(3*H*)-one (A2) Compound **A2** was prepared by adopting the same procedure as for **A1** and obtained in 73% yield, mp 243—245 °C. IR (KBr) cm^{-1}: 3330 (NH), 1686 (C=O), 1616 (C=N). ^{1}H-NMR ($CDCl_3$) δ ppm: 1.0—1.2 (m, 4H, $(CH_2CH_3)_2$), 1.5—1.7 (m, 6H, $(CH_2CH_3)_2$), 3.0 (s, 3H, OCH_3), 7.5—8.2 (m, 8H, ArH), 8.4 (br s, 1H, NH). MS (*m*/*z*): 350 (M^+). *Anal.* Calcd for $C_{20}H_{22}N_4O_2$: C, 68.55; H, 6.32; N, 15.98. Found: C, 68.60; H, 6.36; N, 15.95.

2-(1-Methylbutylidene)-hydrazino-3-(4-methoxyphenyl)-quinazolin-4(3*H*)-one (A3) Compound **A3** was prepared by adopting the same procedure as for **A1** and obtained in 77% yield, mp 251—252 °C. IR (KBr) cm^{-1}: 3260 (NH), 1687 (C=O), 1618 (C=N). ^{1}H-NMR ($CDCl_3$) δ ppm: 1.3—1.4 (t, 2H, $CH_2CH_2CH_3$), 1.7—1.8 (sext, 2H, $CH_2CH_2CH_3$), 2.3—2.4 (t, 3H, $CH_2CH_2CH_3$), 2.8 (s, 3H, CH_3), 3.5 (s, 3H, OCH_3), 7.2—7.9 (m, 9H, ArH), 8.2 (br s, 1H, NH). MS (*m*/*z*): 350 (M^+). *Anal.* Calcd for $C_{20}H_{22}N_4O_2$: C, 68.55; H, 6.32; N, 15.98. Found: C, 68.52; H, 6.37; N, 17.96.

2-(*N*′-Cyclohexylidene-hydrazino)-3-(4-methoxyphenyl)-quinazolin-4(3*H*)-one (A4) Compound **A4** was prepared by adopting the same procedure as for **A1** and obtained in 77% yield, mp 247—248 °C. IR (KBr) cm^{-1}: 3230 (NH), 1690 (C=O), 1616 (C=N). ^{1}H-NMR ($CDCl_3$) δ ppm: 1.1—1.9 (m, 10H, cyclohexyl), 3.3 (s, 3H, OCH_3),7.1—7.8 (m, 8H, ArH), 8.5 (br s, 1H, NH). MS (*m*/*z*): 362 (M^+). *Anal.* Calcd for $C_{21}H_{22}N_4O_2$: C, 69.59; H, 6.11; N, 15.45. Found: C, 69.51; H, 6.08; N, 15.48.

2-(*N*′-1-Phenylethylidene-hydrazino)-3-(4-methoxyphenyl)-quinazolin-4(3*H*)-one (A5) Compound **A5** was prepared by adopting the same procedure as for **A1** and obtained in 77% yield, mp 273—274 °C. IR (KBr) cm^{-1}: 3290 (NH), 1686 (C=O), 1619 (C=N). ^{1}H-NMR ($CDCl_3$) δ ppm: 1.1 (s, 3H, CH_3), 3.4 (s, 3H, OCH_3), 7.3—8.2 (m, 13H, ArH), 8.6 (br s, 1H, NH). MS (*m*/*z*): 384 (M^+). *Anal.* Calcd for $C_{23}H_{20}N_4O_2$: C, 71.85; H, 5.24; N, 14.57. Found: C, 71.89; H, 5.26; N, 14.62.

2-(*N*′-2-Oxo-indolin-2-one-3-yl-idene-hydrazino)-3-(4-methoxyphenyl)-quinazolin-4(3*H*)-one (A6) Compound **A6** was prepared by adopting the same procedure as for **A1** and obtained in 79% yield, mp 224—225 °C. IR (KBr) cm^{-1}: 3280 (NH), 1680 (C=O), 1615 (C=N). ^{1}H-NMR ($CDCl_3$) δ ppm: 3.1 (s, 3H, OCH_3), 7.0—8.2 (m, 12H, ArH), 8.4 (br s, 1H, NH), 9.0 (br s, 1H, NH). MS (*m*/*z*): 411 (M^+). *Anal.* Calcd for $C_{23}H_{17}N_4O_3$: C, 67.14; H, 4.16; N, 13.61. Found: C, 67.17; H, 4.18; N, 13.64.

2-(*N*′-Benzylidene-hydrazino)-3-(4-methoxyphenyl)-quinazolin-4(3*H*)-one (A7) Compound **A7** was prepared by adopting the same procedure as for **A1** and obtained in 73% yield, mp 243—245 °C. IR (KBr) cm^{-1}: 3310 (NH), 1686 (C=O), 1610 (C=N). ^{1}H-NMR ($CDCl_3$) δ ppm: 3.3 (s, 3H, OCH_3), 6.5 (s, 1H, CH), 7.1—8.2 (m, 13H, ArH), 8.7 (br s, 1H, NH). MS (*m*/*z*): 370 (M^+). *Anal.* Calcd for $C_{22}H_{18}N_4O_2$: C, 71.33; H, 4.89; N, 15.12. Found: C, 71.36; H, 4.86; N, 15.14.

2-(*N*′-(2-Chloro-benzylidene-hydrazino))-3-(4-methoxyphenyl)-quinazolin-4(3*H*)-one (A8) Compound **A8** was prepared by adopting the same procedure as for **A1** and obtained in 79% yield, mp 263—264 °C. IR (KBr) cm^{-1}: 3320 (NH), 1683 (C=O), 1610 (C=N). ^{1}H-NMR ($CDCl_3$) δ ppm: 3.2 (s, 3H, OCH_3), 6.3 (s, 1H, CH), 7.1—8.3 (m, 12H, ArH), 8.6 (br s, 1H, NH). MS (*m*/*z*): 405 (M^+). *Anal.* Calcd for $C_{22}H_{17}N_4O_2Cl$: C, 65.27; H, 4.23; N, 13.84. Found: C, 65.21; H, 4.26; N, 13.88.

2-(*N*′-(4-Chloro-benzylidene-hydrazino)-3-(4-methoxyphenyl)-quinazolin-4(3*H*)-one (A9) Compound **A9** was prepared by adopting the same procedure as for **A1** and obtained in 78% yield, mp 260—261 °C. IR (KBr) cm^{-1}: 3270 (NH), 1687 (C=O), 1616 (C=N). ^{1}H-NMR ($CDCl_3$) δ ppm: 3.4 (s, 3H, OCH_3), 6.2 (s, 1H, CH), 7.0—8.1 (m, 12H, ArH), 8.4 (br s, 1H, NH). MS (*m*/*z*) : 405 (M^+). *Anal.* Calcd for $C_{22}H_{17}N_4O_2Cl$: C, 65.27; H, 4.23; N, 13.84. Found: C, 65.33; H, 4.21; N, 13.81.

2-(*N*′-(2-Nitro-benzylidene-hydrazino)-3-(4-methoxyphenyl)-quinazolin-4(3*H*)-one (A10) Compound **A10** was prepared by adopting the same procedure as for **A1** and obtained in 73% yield, mp 237—239 °C. IR (KBr) cm^{-1}: 3250 (NH), 1686 (C=O), 1620 (C=N). ^{1}H-NMR ($CDCl_3$) δ ppm: 3.2 (s, 3H, OCH_3), 6.1 (s, 1H, CH), 7.3—8.2 (m, 12H, ArH), 8.5 (br s,

1H, NH). MS (*m*/*z*) : 415 (M^+). *Anal.* Calcd for $C_{22}H_{17}N_5O_4$: C, 63.61; H, 4.12; N, 16.86. Found: C, 63.67; H, 4.15; N, 16.82.

2-(*N′*-(4-Nitro-benzylidene-hydrazino))-3-(4-methoxyphenyl)-quinazolin-4(3*H*)-one (A11) Compound **A11** was prepared by adopting the same procedure as for **A1** and obtained in 77% yield, mp 231—233 °C. IR (KBr) cm^{-1}: 3293 (NH), 1687 (C=O), 1616 (C=N). ^{1}H-NMR ($CDCl_3$) δ ppm: 3.4 (s, 3H, OCH_3), 6.3 (s, 1H, CH), 7.0—8.2 (m, 12H, ArH), 8.7 (br s, 1H, NH). MS (*m*/*z*): 415 (M^+). *Anal.* Calcd for $C_{22}H_{17}N_5O_4$: C, 63.61; H, 4.12; N, 16.86. Found: C, 63.65; H, 4.14; N, 16.83.

2-(*N′*-(4-Methoxy-benzylidene-hydrazino))-3-(4-methoxyphenyl)-quinazolin-4(3*H*)-one (A12) Compound **A12** was prepared by adopting the same procedure as for **A1** and obtained in 73% yield, mp 256—258 °C. IR (KBr) cm^{-1}: 3276 (NH), 1683 (C=O), 1611 (C=N). ^{1}H-NMR ($CDCl_3$) δ ppm: 2.7 (s, 3H, OCH_3), 3.3 (s, 3H, OCH_3), 6.5 (s, 1H, CH), 7.0—8.2 (m, 12H, ArH), 8.5 (br s, 1H, NH). MS (*m*/*z*): 400 (M^+). *Anal.* Calcd for $C_{23}H_{20}N_4O_3$: C, 68.99; H, 5.03; N, 13.99. Found: C, 68.94; H, 5.07; N, 13.96.

2-(*N′*-(2-Methyl-benzylidene-hydrazino))-3-(4-methoxyphenyl)-quinazolin-4(3*H*)-one (A13) Compound **A13** was prepared by adopting the same procedure as for **A1** and obtained in 76% yield, mp 230—232 °C. IR (KBr) cm^{-1}: 3318 (NH), 1684 (C=O), 1615 (C=N). ^{1}H-NMR ($CDCl_3$) δ ppm: 2.3 (s, 3H, CH_3), 3.1 (s, 3H, OCH_3), 6.2 (s, 1H, CH), 7.0—8.1 (m, 12H, ArH), 8.4 (s, 1H, NH). MS (*m*/*z*): 384 (M^+). *Anal.* Calcd for $C_{23}H_{20}N_4O_2$: C, 71.86; H, 5.24; N, 14.57. Found: C, 71.82; H, 5.25; N, 14.60.

2-(*N′*-(4-Methyl-benzylidene-hydrazino)-3-(4-methoxyphenyl)-quinazolin-4(3*H*)-one (A14) Compound **A14** was prepared by adopting the same procedure as for **A1** and obtained in 76% yield, mp 258—259 °C. IR (KBr) cm^{-1}: 3240 (NH), 1692 (C=O), 1616 (C=N). ^{1}H-NMR ($CDCl_3$) δ ppm: 2.1 (s, 3H, CH_3), 3.3 (s, 3H, OCH_3), 6.6 (s, 3H, CH_3), 7.3—8.4 (m, 12H, ArH), 8.6 (br s, 1H, NH). MS (*m*/*z*): 384 (M^+). *Anal.* Calcd for $C_{23}H_{20}N_4O_2$: C, 71.86; H, 5.24; N, 14.57. Found: C, 71.82; H, 5.27; N, 14.61.

2-(*N′*-Phenyl-benzylidene-hydrazino)-3-(4-methoxyphenyl)-quinazolin-4(3*H*)-one (A15) Compound **A15** was prepared by adopting the same procedure as for **A1** and obtained in 79% yield, mp 251—253 °C. IR (KBr) cm^{-1}: 3270 (NH), 1692 (C=O), 1613 (C=N). ^{1}H-NMR ($CDCl_3$) δ ppm: 3.5 (s, 3H, OCH_3), 7.1—8.4 (m, 18H, ArH), 8.7 (br s, 1H, NH). MS (*m*/*z*): 446 (M^+). *Anal.* Calcd for $C_{28}H_{22}N_4O_2$: C, 75.32; H, 4.97; N, 12.55. Found: C, 75.38; H, 4.92; N, 12.56.

Pharmacology The synthesized compounds were evaluated for analgesic, anti-inflammatory, ulcerogenicindex and antimicrobial activities. Student *t*-test was performed to ascertain the significance of all the exhibited activities. The test compounds and the standard drugs were administered in the form of a suspension (1% carboxy methyl cellulose as a vehicle) by oral route of administration for analgesic and anti-inflammatory but for ulcerogenicity studies by intra peritoneally as suspension in 10% v/v Tween. Each group consisted of six animals. The animals were procured from the Tetrex Biological Center, Madurai, India, and were maintained in colony cages at 25±2 °C, relative humidity of 45—55%, under a 12 h light and dark cycle; they were fed standard animal feed. All the animals were acclimatized for a week before use. The Institutional Animal Ethics committee approved the protocol adopted for the experimentation of animals.

Analgesic Activity Test for analgesic activity was performed by tail-flick technique[14,15] 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, and 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, and T_2 is the reaction time (s) after treatment.

Anti-inflammatory Activity Anti-inflammatory activity was evaluated by carrageenan-induced paw oedema test in rats.[16] Diclofenac sodium 10, 20 mg/kg was administered as a 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 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.

Evaluation of Ulcerogenicity Index Ulceration in rats was induced as described by Goyal *et al.*[17] Albino rats of wistar strain weighing 150—200 g of either sex were divided into various groups each of six animals. Control group of animals were administered only with 10% v/v Tween 80 suspension intraperitonially. One group was administered with Aspirin (German Remedies) intraperitoneally in a dose of 20 mg/kg once daily for 3 d. The remaining group of animals was administered with test compounds intraperitoneally in a dose of 20 mg/kg. On fourth day, pylorus was ligated as per the method of Shay *et al.*[18] Animals were fasted for 36 h before the pylorus ligation procedure. Four hours after the ligation, animals were sacrificed. The stomach was removed and opened along with the greater curvature. Ulcer index was determined by the method of Ganguly and Bhatnagar[19] and recorded in Table 3.

Statistical Analysis Statistical 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 means of 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 mean±S.D. (standard deviations). For statistical analysis we have used GraphPad Prism 3.0 version. (GraphPad Prism 3.0 version, GraphPad Software, Inc.11452 El Camino Real, #215, San Diego, CA 92130 U.S.A.).

References

1) Vane J. R., Botting R. M., *Inflamm. Res.*, **47**, 578—587 (1998).
2) Ryn J. V., Trummlitz G., Pairet M., *Curr. Med. Chem.*, **7**, 1145—1161 (2000).
3) Carter J. S., *Expert Opin. Ther. Pat.*, **10**, 1011—1020 (2000).
4) Beuck M., *Angew. Chem., Int. Ed.*, **38**, 631—633 (1999).
5) Alagarsamy V., Meena S., Revathi R., Vijayakumar S., Ramseshu K. V., *Pharmazie*, **58**, 4—8 (2003).
6) Alagarsamy V., Thanga Thirupathy A., Mandal S. C., Rajasekaran S., Vijayakumar S., Revathi R., Anburaj J., Arunkumar S., Rajesh S., *Indian J. Pharm. Sci.*, **68**, 108—111 (2006).
7) Srivastava B. M., Bhalla V. K., Shankar T. N., *Arzneim.-Forsch.*, **43**, 595—600 (1993).
8) Hitkari A., Saxena M., Verma A. K., Gupta M., Shankar M. P., *Bull. Chim. Farm.*, **134**, 609—615 (1995).
9) Ibrahim M. K., Al Azhar, *J. Pharm. Sci.*, **22**, 9—12 (1998).
10) Alagarsamy V., Raja Solomon V., Vanikavitha G., Paluchamy V., Ravichandran M., Arnald Sujin A., Thangathirupathy A., Amuthalakshmi S., Revathi R., *Biol. Pharm. Bull.*, **25**, 1432—1435 (2002).
11) Alagarsamy V., Murugananthan G., Venkateshperumal R., *Biol. Pharm. Bull.*, **26**, 1711—1714 (2003).
12) Alagarsamy V., Rajesh R., Meena R., Vijaykumar S., Ramseshu K. V., Durai Anandakumar T., *Biol. Pharm. Bull.*, **27**, 652—656 (2004).
13) Alagarsamy V., Muthukumar V., Pavalarani N., Vasanthanathan P., Revathi R., *Biol. Pharm. Bull.*, **26**, 557—559 (2003).
14) Kulkarni S. K., *Life Sci.*, **27**, 185—188 (1980).
15) Amour R. E., Smith D. L., *J. Pharmacol. Exp. Ther.*, **72**, 74—78 (1941).
16) Winter C. A., Risely E. A., Nu G. N., *Proc. Soc. Exp. Biol.*, **111**, 544—547 (1982).
17) Goyal R. K., Chakrabarthi A., Sanyal A. K., *Planta Medica*, **29**, 85—88 (1985).
18) Shay H., Komarov S. A., Fels S. E., Mmeraze D., Gruenstei M., Siplet H., *Gastroenterology*, **5**, 43—61 (1945).
19) Ganguly A. K., Bhatnagar O. P., *Canadian J. Physiol. Pharmacol.*, **51**, 748—750 (1973).