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Synthesis and pharmacological evaluation of some 3-phenyl-2-substituted-3*H*-quinazolin-4-one as analgesic, anti-inflammatory agents

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Abstract—A variety of novel 3-phenyl-2-substituted-3*H*-quinazolin-4-ones were synthesized by reacting the amino group of 2-hydrazino-3-phenyl-3*H*-quinazolin-4-one with different aldehydes and ketones. The starting material 2-hydrazino-3-phenyl-3*H*-quinazolin-4-one was synthesized from aniline. The title compounds were investigated for analgesic, anti-inflammatory and ulcerogenic index activities. While the test compounds exhibited significant activity, compounds, 2-(*N*′-2-butylidene-hydrazino)-3-phenyl-3*H*-quinazolin-4-one (**AS1**), 2-(*N*′-3-pentylidene-hydrazino)-3-phenyl-3*H*-quinazolin-4-one (**AS2**) and 2-(*N*′-2-pentylidene-hydrazino)-3-phenyl-3*H*-quinazolin-4-one (**AS3**), exhibited moderate analgesic activity. The compound 2-(*N*′-2-pentylidene-hydrazino)-3-phenyl-3*H*-quinazolin-4-one (**AS3**) showed more potent anti-inflammatory activity when compared to the reference standard diclofenac sodium. Interestingly, the test compounds showed only mild ulcerogenic side effect when compared to aspirin.
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1. Introduction

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 is 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 programme we have 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 are reported to possess significant analgesic and 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] and 2,3-disubstituted quinazolines[13] 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. On this basis, we have synthesized some 3-phenyl-2-substituted-3*H*-quinazolin-4-ones. The synthesized compounds were tested for their analgesic, anti-inflammatory and ulcerogenic index activities.

2. Chemistry

The key intermediate 3-phenyl-2-thioxo-2,3-dihydro-1*H*-quinazolin-4-one (**4**) was prepared by reacting aniline (**1**) with carbon disulfide and sodium hydroxide in dimethyl sulfoxide to give sodium dithiocarbamate, which was methylated with dimethyl sulfate to afford the dithiocarbamic acid methyl ester (**2**). Compound **2** on reflux with methyl anthranilate (**3**) in ethanol yielded the desired 3-phenyl-2-thioxo-2,3-dihydro-1*H*-quinazolin-4-one (**4**) via the thiourea intermediate in good yield (80%). The product obtained was cyclic and not an open chain thiourea **3a**. The IR spectra

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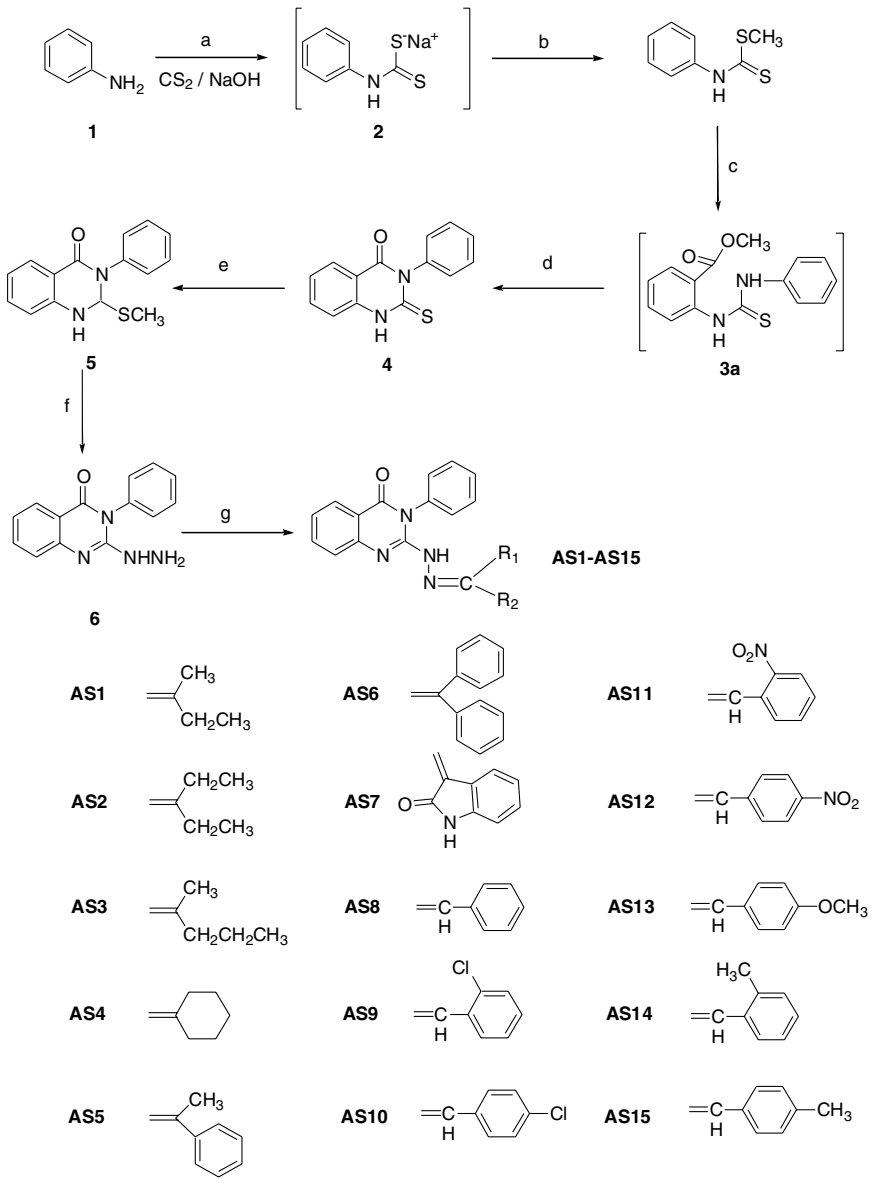

Figure 1. Reagents and conditions: (a) DMSO, rt, 30 min; (b) $(CH_3)_2SO_4$, 5–10 °C, 2 h; (c) methyl anthranilate (**3**), K_2CO_3, ethanol reflux for 18 h; (d) 10% alcoholic NaOH/dil. HCl (e) 2% alcoholic NaOH, $(CH_3)_2SO_4$, rt, 1 h ; (f) NH_2NH_2, K_2CO_3, ethanol reflux for 22 h; (g) $(R_2R_1)CO$; gla. CH_3COOH reflux, 33 h.

of **4** show intense peaks at 3220 cm^{-1} for cyclic thiourea (NH), 1660 cm^{-1} for carbonyl (C=O) and 1200 cm^{-1} for thioxo (C=S) stretching. ^1H NMR spectra of **4** showed multiplet at δ 7.0–9.0 for aromatic (9H) protons and a singlet at δ 10.5 indicating the presence of NH.

The 2-methylsulfanyl-3-phenyl-3*H*-quinazolin-4-one (**5**) was obtained by dissolving **4** in 2% alcoholic sodium hydroxide solution with methylating agent of dimethyl sulfate. 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 1680 cm^{-1}. The ^1H NMR spectra of compound **5** showed singlet at δ 2.5 due to SCH_3 and a multiplet at δ 7.0–8.6 was observed for aromatic (9H) protons.

Nucleophilic displacement of methylthio group of **5** with hydrazine hydrate was carried out using ethanol as solvent to afford 2-hydrazino-3-phenyl-3*H*-quinazolin-4-one **6**. The long duration of reaction (22 h) required might be due to the presence of bulky aromatic ring at position 3, 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 3334, 3280 cm^{-1} in the IR spectra. It also showed a peak for carbonyl (C=O) at 1680 cm^{-1}. The ^1H NMR spectra of the compound **6** showed singlet at δ 5.0 and 8.7 due to NH_2 and NH, respectively, a multiplet at δ 7.0–8.1 was observed for aromatic (9H) protons.

The title compounds 3-phenyl-2-substituted quinazolin-4(3*H*)-ones **AS1–AS15** were obtained by the condensation of amino group of 2-hydrazino-3-

phenyl-3*H*-quinazolin-4-one (**6**) with different aldehydes and ketones. The formation of title product is indicated by the disappearance of peak due to 3-NH_2 of the starting material in IR and ^{1}H NMR spectrum of all the compounds **AS1–AS15**. 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 compounds reported (Fig. 1) in this study have been thoroughly characterized by spectral data and elemental analysis.

3. Results and discussion

3.1. Analgesic activity

The analgesic activity was performed by tail-flick technique using Wistar albino mice.[14,15] The results of analgesic activity (Table 1) indicate that all the test compounds exhibited significant activity. Compound **AS1** with 2-butylidene substituent showed good activity; with the increased lipophilicity 3-pentylidene group (**AS2**) showed increase in activity. Replacement of 3-pentylidene group with isomer 2-pentylidene group (compound **AS3**) retains the activity. The presence of aliphatic group and cycloalkyl group (compounds **AS4** and **AS5**) leads to moderate decrease in activity. The presence of aryl group at N-3 position (compounds **AS6–AS8** and **A14**) also results in decreasing activity. The presence of electron-withdrawing group at N-3 aryl ring (compounds **AS9–AS13**) results in further decrease of activity. Compound 2-(*N*′-3-pentylidene-hydrazino)-3-phenyl-3*H*-quinazolin-4-one (**AS2**) emerged as the most active analgesic agent and it is more potent when compared to the reference standard diclofenac sodium (Table 1).

3.2. Anti-inflammatory activity

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. The compound 2-(*N*′-2-pentylidene-hydrazino)-3-phenyl-3*H*-quinazolin-4-one (**AS3**) showed more potent anti-inflammatory activity and the compound **AS2** showed equipotent anti-inflammatory activity when compared to the reference standard diclofenac sodium.

Table 1. Percent analgesic activity of test compounds (tail-flick technique)

Compound	Dose (mg/kg)	Percent analgesic activity[a]			
		30 min	1 h	2 h	3 h
AS1	10	41 ± 1.36*	43 ± 1.52*	50 ± 1.67**	31 ± 1.32*
	20	55 ± 1.71**	59 ± 1.31**	61 ± 1.92***	42 ± 1.62*
AS2	10	49 ± 1.23*	55 ± 1.25**	58 ± 1.39**	41 ± 1.06*
	20	63 ± 1.97***	69 ± 1.08***	72 ± 1.73***	49 ± 1.22*
AS3	10	43 ± 1.48*	48 ± 1.81*	50 ± 1.06**	33 ± 1.28*
	20	56 ± 1.72**	62 ± 1.02***	62 ± 1.24***	45 ± 1.26**
AS4	10	33 ± 1.41*	40 ± 1.17*	46 ± 1.81*	25 ± 1.63*
	20	50 ± 1.03**	51 ± 1.76**	53 ± 1.28**	37 ± 1.69*
AS5	10	38 ± 1.07*	43 ± 1.29*	43 ± 1.39*	29 ± 1.23*
	20	50 ± 1.26**	53 ± 1.34**	57 ± 1.65**	36 ± 1.26*
AS6	10	33 ± 1.51*	36 ± 1.46*	41 ± 1.26*	29 ± 1.30*
	20	39 ± 1.09*	41 ± 1.55*	46 ± 1.72*	36 ± 1.51*
AS7	10	36 ± 1.27*	37 ± 1.28*	40 ± 1.82*	30 ± 1.61*
	20	47 ± 1.63*	51 ± 1.72**	55 ± 1.42**	35 ± 1.83*
AS8	10	35 ± 1.23*	38 ± 1.39*	41 ± 1.93*	29 ± 1.97*
	20	46 ± 1.85*	50 ± 1.97**	53 ± 1.62**	31 ± 1.72*
AS9	10	29 ± 1.32*	30 ± 1.61*	37 ± 1.06*	25 ± 1.27*
	20	37 ± 1.43*	39 ± 1.90*	42 ± 1.83*	36 ± 1.26*
AS10	10	32 ± 1.08*	34 ± 1.28*	42 ± 1.73*	27 ± 1.71*
	20	40 ± 1.71*	45 ± 1.37*	48 ± 1.42*	35 ± 1.07*
AS11	10	35 ± 1.71*	36 ± 1.86*	39 ± 1.28*	30 ± 1.26*
	20	42 ± 1.22*	46 ± 1.92*	49 ± 1.31*	39 ± 1.06*
AS12	10	34 ± 1.62*	39 ± 1.35*	39 ± 1.22*	27 ± 1.27*
	20	38 ± 1.36*	45 ± 1.47*	47 ± 1.19*	33 ± 1.22*
AS13	10	38 ± 1.28*	42 ± 1.42*	43 ± 1.33*	30 ± 1.62*
	20	42 ± 1.47*	49 ± 1.71*	50 ± 1.06**	29 ± 1.77*
AS14	10	35 ± 1.92*	39 ± 1.27*	40 ± 1.26*	33 ± 1.91*
	20	45 ± 1.19*	46 ± 1.39**	48 ± 1.17*	39 ± 1.62*
AS15	10	37 ± 1.23*	40 ± 1.71*	40 ± 1.23*	33 ± 1.27*
	20	43 ± 1.36*	47 ± 1.82*	50 ± 1.71**	40 ± 1.26*
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*

Significance levels *$p < 0.5$, **$p < 0.01$ and ***$p < 0.001$ as compared with the respective control.

[a] Each value represents the means ± SD ($n = 6$).

Table 2. Percent anti-inflammatory activity of test compounds (Carrageenan-induced paw oedema test in rats)

Compound	Dose (mg/kg)	Percent protection			
		30 min	1 h	2 h	3 h
AS1	10	32 ± 1.33*	36 ± 1.29*	37 ± 1.12*	26 ± 1.23*
	20	38 ± 1.23*	45 ± 1.71**	47 ± 1.72**	36 ± 1.60*
AS2	10	34 ± 1.38*	35 ± 1.90*	39 ± 1.81*	32 ± 1.23*
	20	43 ± 1.27*	52 ± 1.56***	56 ± 1.36***	36 ± 1.82*
AS3	10	35 ± 1.96*	41 ± 1.06*	42 ± 1.23*	33 ± 1.22*
	20	46 ± 1.92**	58 ± 1.28***	60 ± 1.72***	41 ± 1.76*
AS4	10	29 ± 1.02*	33 ± 1.09*	36 ± 1.27*	27 ± 1.22*
	20	39 ± 1.19*	42 ± 1.42*	42 ± 1.51*	35 ± 1.70*
AS5	10	31 ± 1.52*	35 ± 1.47*	38 ± 1.72*	29 ± 1.41*
	20	38 ± 1.71*	42 ± 1.82*	46 ± 1.32**	33 ± 1.71*
AS6	10	27 ± 1.23*	33 ± 1.23*	35 ± 1.22*	26 ± 1.36*
	20	36 ± 1.72*	39 ± 1.26*	42 ± 1.30*	35 ± 1.55*
AS7	10	27 ± 1.72*	30 ± 1.53*	31 ± 1.90*	23 ± 1.27*
	20	32 ± 1.90*	36 ± 1.82*	39 ± 1.23*	30 ± 1.80*
AS8	10	28 ± 1.27*	32 ± 1.22*	35 ± 1.22*	29 ± 1.82*
	20	36 ± 1.91*	40 ± 1.97*	43 ± 1.81*	26 ± 1.06*
AS9	10	21 ± 1.08*	24 ± 1.19*	26 ± 1.08*	20 ± 1.22*
	20	27 ± 1.13*	32 ± 1.17*	35 ± 1.26*	25 ± 1.81*
AS10	10	26 ± 1.46*	28 ± 1.84*	30 ± 1.91*	23 ± 1.47*
	20	29 ± 1.84*	32 ± 1.92*	37 ± 1.87*	25 ± 1.26*
AS11	10	27 ± 1.29*	29 ± 1.93*	34 ± 1.27*	22 ± 1.32*
	20	32 ± 1.34*	33 ± 1.28*	36 ± 1.82*	30 ± 1.26*
AS12	10	25 ± 1.26*	28 ± 1.71*	30 ± 1.26*	20 ± 1.18*
	20	35 ± 1.17*	35 ± 1.26*	36 ± 1.23*	32 ± 1.71*
AS13	10	28 ± 1.27*	30 ± 1.80*	32 ± 1.22*	24 ± 1.06*
	20	33 ± 1.83*	37 ± 1.23*	37 ± 1.08*	30 ± 1.25*
AS14	10	30 ± 1.08*	32 ± 1.93*	33 ± 1.41*	28 ± 1.07*
	20	36 ± 1.42*	39 ± 1.26*	42 ± 1.17*	30 ± 1.25*
AS15	10	28 ± 1.29*	33 ± 1.73	34 ± 1.25*	25 ± 1.18*
	20	38 ± 1.35*	38 ± 1.08**	43 ± 1.92*	36 ± 1.15*
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**

[a]Each value represents the means ± SD ($n = 6$). Significance levels $^{*}p < 0.5$, $^{**}p < 0.01$ and $^{***}p < 0.001$ as compared with the respective control.

Table 3. Evaluation of ulcerogenicity index

Drug	Ulcer index
AS1	0.56 ± 1.32*
AS2	0.51 ± 1.26*
AS3	0.53 ± 1.53*
AS4	0.69 ± 1.18*
AS5	0.62 ± 1.25*
AS6	0.72 ± 1.15*
AS7	0.65 ± 1.28*
AS8	0.71 ± 1.32*
AS9	0.96 ± 1.19*
AS10	0.93 ± 1.22
AS11	0.85 ± 1.83*
AS12	0.81 ± 1.29*
AS13	0.79 ± 1.32*
AS14	0.76 ± 1.51*
AS15	0.79 ± 1.63*
Control	0.15 ± 0.32
Aspirin	1.73 ± 0.41**

[a]Each value represents the means ± SD ($n = 6$). Significance levels $^{*}p < 0.05$ and $^{**}p < 0.01$ as compared with the respective control.

3.3. Evaluation of ulcerogenicity index

The ulcer index of the test compounds (Table 3) reveals that the compounds **AS9–AS13** possessing electron-withdrawing groups exhibited higher ulcer index than the other test compounds. The high ulcer index score for these compounds may be due to the suppression of the prostaglandin synthesis.

4. Conclusion

In our earlier studies, we observed that the presence of alkyl groups exhibited more analgesic and anti-inflammatory activities over aryl groups at the N-3 position.[10–13] However, in the C-2 position also we made a substitution in such a way as to increase lipophilicity of the molecule. The presence of such a group enhanced the analgesic and anti-inflammatory activities. To compare the increase in activity we have taken the average of all the readings of reaction time noted for each compound for each pharmacological activity. The most active compound of the C-2 phenyl series showed 43% analgesic and 36% anti-inflammatory activity,[10] whereas the C-2 methyl series lead molecule showed 50% analgesic and 44% anti-inflammatory activity.[11] Introduction of sulfur atom at C-2 position in the above series, that is, by placing thiomethyl group at C-2 position showed 54% analgesic and 43% anti-inflammatory activity.[13] The result of the analgesic and anti-inflammatory

activities of the present series, one of the compound, **AS2**, showed that enhancement of activity (57% analgesic and 45% anti-inflammatory activity). Interestingly these compounds showed negligible ulcer index unlike other NSAIDs. 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.

5. Experimental

5.1. General

Melting points (mp) were taken in open capillaries on Thomas Hoover melting point apparatus and are uncorrected. The IR spectra were recorded in film or in potassium bromide disks on a Perkin-Elmer 398 spectrometer. The ^{1}H 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, and N) analysis indicated that the calculated and observed values were within the acceptable limits (±0.4%). All chemicals and reagents were obtained from Aldrich (USA), Lancaster (UK) or Spectrochem Pvt. Ltd (India) and were used without further purification.

5.1.1. 3-Phenyl-2-thioxo-2,3-dihydro-1*H*-quinazolin-4-one (4). A solution of aniline (**1**) (0.02 mol) in dimethylsulfoxide (10 ml) were stirred vigorously. To this was added carbon disulfide (1.6 ml) and aqueous sodium hydroxide 1.2 ml (20 M solution) dropwise during 30 min with stirring. Dimethyl sulfate (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 under high vacuum and recrystallized from ethanol. Methyl anthranilate (0.01 mol) and the above-prepared *N*-phenyl-methyl dithiocarbamic acid (0.01 mol) were dissolved in ethanol (20 ml). To this anhydrous potassium carbonate (100 mg) was added and refluxed for 18 h. The reaction mixture was cooled in ice and the solid separated was filtered and purified by dissolving in 10% alcoholic sodium hydroxide solution (95% alcohol was used for preparation) and re-precipitated by treating with dilute hydrochloric acid. The solid obtained was filtered, washed with water, dried and recrystallized from ethanol. Yield 86%, mp 305–306 °C; IR (KBr) cm^{-1}: 3220 (NH), 1660 (C=O), 1200 (C=S); ^{1}H NMR ($CDCl_3$): δ 7.0–9.0 (m, 9H, Ar-*H*), 10.5 (s, 1H, N*H*); MS (*m*/*z*): 254 [M$^+$]. Anal. Calcd for $C_{14}H_{10}N_2OS$: C, 66.12; H, 3.96; N, 11.02. Found: C, 66.15; H, 3.98; N, 11.07.

5.1.2. 2-Methylsulfanyl-3-phenyl-3*H*-quinazolin-4-one (5). The 3-phenyl-2-thioxo-2,3-dihydro-1*H*-quinazolin-4-one (**4**) (0.01 mol) was dissolved in 40 ml of 2% alcoholic sodium hydroxide solution (95% alcohol was used for preparation). To this dimethyl sulfate (0.01 mol) was added dropwise 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 under high vacuum and recrystallized from ethanol/chloroform (75:25) mixture. Yield 88%, mp 124–126 ° C; IR (KBr) cm^{-1}: 1680 (C=O), 1620 (C=C); ^{1}H NMR ($CDCl_3$): δ 2.5 (s, 3H, SCH_3), 7.0–8.6 (m, 9H Ar-*H*); MS (*m*/*z*): 268 [M$^+$]; Anal. Calcd for $C_{15}H_{12}N_2OS$: C, 67.14; H, 4.51; N, 10.44. Found: C, 67.19; H, 4.53; N, 10.41.

5.1.3. 2-Hydrazino-3-phenyl-3*H*-quinazolin-4-one (6). The 2-methylsulfanyl-3-phenyl-3*H*-quinazolin-4-one (**5**) (0.01 mol) was dissolved in ethanol (25 ml). To this hydrazine hydrate (0.1 mol) and anhydrous potassium carbonate (100 mg) were added and refluxed for 22 h. The reaction mixture was cooled and poured into ice-water. The solid obtained was filtered, washed with water, dried under high vacuum and recrystallized from chloroform/benzene (25:75) mixture. Yield 81%, mp 158–160 °C; IR (KBr) cm^{-1}: 3334, 3280 ($NHNH_2$), 1680 (C=O); ^{1}H NMR ($CDCl_3$): δ 5.1 (br s, 2H, NH_2), 7.0–8.1 (m, 9H, Ar-*H*), 8.7 (br s, 1H, N*H*); MS (*m*/*z*): 252 [M$^+$]; Anal. Calcd for $C_{14}H_{12}N_4O$: C, 66.65; H, 4.79; N, 22.21. Found: C, 66.62; H, 4.77; N, 22.26.

5.1.4. General synthetic procedure for compounds (AS1–AS15). A mixture of 2-hydrazino-3-phenyl-3*H*-quinazolin-4-one (**6**) (0.004 mol) and appropriate ketone/aldehyde (0.004 mol) in glacial acetic acid was refluxed for 33 h. The reaction mixture was poured into ice water. The solid obtained was filtered, washed with water, dried under high vacuum and recrystallized from ethanol.

5.1.5. 2-(*N*′-2-Butylidene-hydrazino)-3-phenyl-3*H*-quinazolin-4-one (AS1). Yield 73%; mp 239–241 °C; IR : 3310 (NH), 1679 (C=O), 1615 (C=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.0–1.1 (q, 2H, CH_2CH$_3$), 1.5–1.6 (t, 3H, CH$_2$CH_3), 1.9 (s, 3H, CH_3), 7.1–7.9 (m, 9H, Ar-*H*), 8.5 (br s, 1H, N*H*); MS (*m*/*z*): 306 (M$^+$); Anal. Calcd for $C_{18}H_{18}N_4O$: C, 70.56; H, 5.92; N, 18.28. Found: C, 70.59; H, 5.98; N, 18.26.

5.1.6. 2-(*N*′-3-Pentylidene-hydrazino)-3-phenyl-3*H*-quinazolin-4-one (AS2). Yield 79%; mp 261–263 °C; IR : 3360 (NH), 1682 (C=O), 1610 (C=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.3–1.5 (m, 4H, (CH_2CH$_3$)$_2$), 1.8–2.0 (m, 6H, (CH$_2$CH_3)$_2$), 7.3–8.1 (m, 9H, Ar-*H*), 8.7 (br s, 1H, N*H*); MS (*m*/*z*): 320 (M$^+$); Anal. Calcd for $C_{19}H_{20}N_4O$: C, 71,22; H, 6.29; N, 17.48. Found: C, 71.26; H, 6.25; N, 16.51.

5.1.7. 2-(*N*′-2-Pentylidene-hydrazino)-3-phenyl-3*H*-quinazolin-4-one (AS3). Yield 73%; mp 210–211 °C; IR: 3300 (NH), 1680 (C=O), 1613 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.1–1.2 (t, 2H, $CH_2CH_2CH_3$), 1.4–1.5 (sext, 2H, $CH_2CH_2CH_3$), 1.6–1.7 (t, 3H, $CH_2CH_2CH_3$), 2.1 (s, 3H, CH_3), 7.1–7.8 (m, 9H, Ar-*H*), 8.5 (br s, 1H, N*H*); MS (*m*/*z*): 320 (M^+); Anal. Calcd for $C_{19}H_{20}N_4O$: C, 71.22; H, 6.29; N, 17.48. Found: C, 71.19; H, 6.31; N, 17.54.

5.1.8. 2-(*N*′-Cyclohexylidene-hydrazino)-3-phenyl-3*H*-quinazolin-4-one (AS4). Yield 76%; mp 223–224 °C; IR: 3260 (NH), 1685 (C=O), 1610 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.9–1.7 (m, 10H, cyclohexanyl), 7.2–7.9 (m, 9H, Ar-*H*), 8.3 (br s, 1H, N*H*); MS (*m*/*z*): 332(M^+); Anal. Calcd for $C_{20}H_{20}N_4O$: C, 72.29; H, 6.02; N, 16.86. Found: C, 72.31; H, 6.07; N, 16.82.

5.1.9. 2-(*N*′-1-Phenylethylidene-hydrazino)-3-phenyl-3*H*-quinazolin-4-one (AS5). Yield 72%; mp 255–256 °C; IR: 3320 (NH), 1690 (C=O), 1616 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.3 (s, 3H, CH_3), 7.0–8.1 (m, 14H, Ar-*H*), 8.5 (br s, 1H, N*H*); MS (*m*/*z*): 354 (M^+); Anal. Calcd for $C_{22}H_{18}N_4O$: C, 74.55; H, 5.11; N, 15.80. Found: C, 74.58; H, 5.16; N, 15.77.

5.1.10. 2-(*N*′-Phenyl-benzylidene-hydrazino)-3-phenyl-3*H*-quinazolin-4-one (AS6). Yield 73%; mp 229–231 °C; IR: 3310 (NH), 1685 (C=O), 1616 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.2–8.5 (m, 19H, Ar-*H*), 8.5 (br s, 1H, NH); MS (*m*/*z*): 416 (M^+); Anal. Calcd for $C_{27}H_{20}N_4O$: C, 77.86; H, 4.84; N, 13.45. Found: C, 77.88; H, 4.89; N, 13.41.

5.1.11. 2-(*N*′-Indolin-2-one-3-yl-idene-hydrazino)-3-phenyl-3*H*-quinazolin-4-one (AS7). Yield 74%; mp 233–235 °C; IR: 3300 (NH), 1700 (C=O), 1612 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.2–8.3 (m, 13H, Ar-*H*), 8.5 (br s, 1H, N*H*), 9.3 (br s, 1H, N*H*); MS (*m*/*z*): 381 (M^+); Anal. Calcd for $C_{22}H_{15}N_5O_2$: C, 69.28; H, 3.96; N, 18.36. Found: C, 69.21; H, 3.3.94; N, 18.39.

5.1.12. 2-(*N*′-Benzylidene-hydrazino)-3-phenyl-3*H*-quinazolin-4-one (AS8). Yield 76%; mp 205–206 °C; IR: 3360 (NH), 1690 (C=O), 1615 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 6.3 (s, 1H, C*H*), 7.3–8.3 (m, 14H, Ar-*H*), 8.5 (br s, 1H, N*H*); MS (*m*/*z*): 340 (M^+); Anal. Calcd for $C_{21}H_{16}N_4O$: C, 74.10; H, 4.73; N, 16.46. Found: C, 74.17; H, 4.75; N, 16.49.

5.1.13. 2-(*N*′-(2-Chloro-benzylidene-hydrazino))-3-phenyl-3*H*-quinazolin-4-one (AS9). Yield 74%; mp 247–249 °C; IR: 3360 (NH), 1685 (C=O), 1616 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 6.5 (s, 1H, C*H*), 7.0–8.1 (m, 13H, Ar-*H*), 8.9 (br s, 1H, N*H*); MS (*m*/*z*): 375 (M^+); Anal. Calcd for $C_{21}H_{15}N_4OCl$: C, 67.29; H, 4.03; N, 14.94. Found: C, 67.26; H, 4.07; N, 14.99.

5.1.14. 2-(*N*′-(4-Chloro-benzylidene-hydrazino))-3-phenyl-3*H*-quinazolin-4-one (AS10). Yield 72%; mp 219–221 °C; IR: 3340 (NH), 1690 (C=O), 1612 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 6.5 (s, 1H, C*H*), 7.2–8.2 (m, 13H, Ar-*H*), 8.5 (br s, 1H, N*H*); MS (*m*/*z*): 375 (M^+); Anal. Calcd for $C_{21}H_{15}N_4OCl$: C, 67.29; H, 4.03; N, 14.94. Found: C, 67.22; H, 4.09; N, 14.91.

5.1.15. 2-(*N*′-(2-Nitro-benzylidene-hydrazino))-3-phenyl-3*H*-quinazolin-4-one (AS11). Yield 76%; mp 212–214 °C; IR: 3290 (NH), 1680 (C=O), 1616 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 6.4 (s, 1H, C*H*), 7.0–8.1 (m, 13H, Ar-*H*), 8.5–8.6 (br s, 1H, N*H*); MS (*m*/*z*): 385 (M^+); Anal. Calcd for $C_{21}H_{15}N_5O_3$: C, 65.44; H, 3.92; N, 18.17. Found: C, 65.49; H, 3.96; N, 18.21.

5.1.16. 2-(*N*′-(4-Nitro-benzylidene-hydrazino))-3-phenyl-3*H*-quinazolin-4-one (AS12). Yield 73%; mp 260–262 °C; IR: 3310(NH), 1690 (C=O), 1610 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 6.5 (s, 1H, C*H*), 7.3–8.4 (m, 13H, Ar-*H*), 8.4 (br s, 1H, N*H*). MS (*m*/*z*): 385 (M^+); Anal. Calcd for $C_{21}H_{15}N_5O_3$: C, 65.44; H, 3.92; N, 18.17. Found: C, 65.46; H, 3.96; N, 18.24.

5.1.17. 2-(*N*′-(4-Methoxy-benzylidene-hydrazino))-3-phenyl-3*H*-quinazolin-4-one (AS13). Yield 79%; mp 274–276 °C; IR: 3290 (NH), 1680 (C=O), 1616 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.5–2.6 (s, 3H, OCH_3), 6.3 (s, 1H, C*H*), 7.1–8.1 (m, 13H, Ar-*H*), 8.3 (br s, 1H, N*H*); 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, 4.95; N, 15.13.

5.1.18. 2-(*N*′-(2-Methyl-benzylidene-hydrazino))-3-phenyl-3*H*-quinazolin-4-one (AS14). Yield 73%; mp 263–265 °C; IR: 3260 (NH), 1680 (C=O), 1612 (C=N) $cm^{-}1$; ^1H NMR ($CDCl_3$): δ 2.1 (s, 3H, CH_3), 6.4 (s, 1H, C*H*), 7.3–8.4 (m, 13H, Ar-*H*), 8.6 (br s, 1H, N*H*). MS (*m*/*z*): 354 (M^+); Anal. Calcd for $C_{22}H_{18}N_4O$: C, 74.55; H, 5.11; N, 15.80. Found: C, 74.58; H, 5.16; N, 15.76.

5.1.19. 2-(*N*′-(4-Methyl-benzylidene-hydrazino))-3-phenyl-3*H*-quinazolin-4-one (AS15). Yield 70%; mp 272–273 °C; IR : 3290 (NH), 1690 (C=O), 1610 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.3 (s, 3H, CH_3), 6.3 (s, 3H, CH_3), 7.1–8.1 (m, 13H, Ar-*H*), 8.3 (br s, 1H, N*H*); MS (*m*/*z*): 354 (M^+); Anal. Calcd for $C_{22}H_{18}N_4O$: C, 74.55; H, 5.11; N, 15.80. Found: C, 74.62; H, 5.13; N, 15.86.

5.2. Pharmacology

The synthesized compounds were evaluated for analgesic, anti-inflammatory, ulcerogenic index and antimicrobial 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 intraperitoneally as suspension in 10% v/v Tween 20. 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.

5.2.1. Analgesic activity. The 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 and 20 mg/kg was administered orally as reference drug for comparison. The test compounds at two dose levels (10 and 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.

5.2.2. 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 (Model PLYAN; Buxco, USA). 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 and y is the mean paw volume of rats before the administration of carrageenan in the control group.

5.2.3. 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 three days. 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.

5.2.4. 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 means ± SD (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, USA).

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