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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and analgesic, anti-inflammatory activities of 3-(3-methoxyphenyl)-2-substituted amino-quinazolin-4 (3*H*)-ones

V. Alagarsamy · M. Gopinath · P. Parthiban · B. Subba Rao · K. Murali · V. Raja Solomon

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Abstract A variety of novel 3-(3-methoxyphenyl)-2-substituted amino-quinazolin-4(3*H*)-ones were synthesized by reacting the amino group of 2-hydrazino-3-(3-methoxyphenyl)-quinazolin-4(3*H*)-one with a variety of aldehydes and ketones. The starting material 2-hydrazino-3-(3-methoxyphenyl)-quinazolin-4(3*H*)-one was synthesized from 3-methoxy aniline. The title compounds were investigated for analgesic, anti-inflammatory, and ulcerogenic behavior. Among these the compound 2-(1-methyl butylidene-hydrazino)-3-(3-methoxyphenyl)-3*H*-quinazolin-4-one (**AS3**) emerged as the most active compound for the analgesic activity, while the compound 2-(1-ethyl propylidene-hydrazino)-3-(3-methyoxyphenyl)-3*H*-quinazolin-4-one (**AS2**) showed most potent anti-inflammatory activity of the series and these compounds are moderately more potent when compared to the reference standard diclofenac sodium. Interestingly the test compounds showed only mild ulcerogenic potential when compared to acetylsalicylic acid.

V. Alagarsamy (✉) · P. Parthiban · B. Subba Rao
Medicinal Chemistry Research Laboratory, MNR College of Pharmacy, MNR Nagar, Sangareddy, Gr. Hyderabad 502 294, India
e-mail: drvalagarsamy@gmail.com

M. Gopinath
Department of Pharmaceutical Chemistry, Ratnam Institute of Pharmacy, Pidathapolur, Nellore 524 346, India

K. Murali
Department of Pharmaceutical Chemistry, C.L. Baid Metha College of Pharmacy, Thorappakkam, Chennai 600 097, India

V. Raja Solomon
Medicinal and Process Chemistry Division, Central Drug Research Institute, Lucknow 226 001, India

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Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly prescribed for the treatment of acute and chronic inflammation, pain, and fever. 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 (Van Ryn, 1971; Van Ryn and Botting, 1995; Van Ryn *et al*., 2000; Beuck, 1999). As part of our ongoing medicinal chemistry research program we found that quinazolines and condensed quinazolines exhibit potent central nervous system (CNS) activities including analgesic, anti-inflammatory (Alagarsamy *et al*., 2003a), and anticonvulsant (Alagarsamy *et al*., 2006). Quinazolin-4(3*H*)-ones with 2,3-disubstitution are reported to possess significant analgesic, anti-inflammatory (Abdel-Rahman *et al*., 2003; Chambhare *et al*., 2003), and anticonvulsant effects (Santagati *et al*., 1995). Previously we have documented some lead compound 2-phenyl-3-substituted quinazolines (Alagarsamy *et al*., 2002), 2-methyl-3-substituted quinazolines (Alagarsamy *et al*., 2003b), 2-methylthio-3-substituted quinazolines (Alagarsamy *et al*., 2004), and 2, 3-disubstituted quinazolines (Alagarsamy *et al*., 2003c) that exhibited good analgesic and anti-inflammatory activities. The present work is an extension of our ongoing efforts toward the development and identification of new molecules for analgesic and anti-inflammatory activities with minimal gastrointestinal ulceration side effects. The present work is an extension of our ongoing efforts toward

the development and identification of new molecules for analgesic and anti-inflammatory activities with minimal gastrointestinal ulceration side effects. In the present study, we have synthesized a series of 3-(3-methoxyphenyl)-2-substituted amino-quinazolin-4(3*H*)-ones. The synthesized compounds were tested for their analgesic, anti-inflammatory, and ulcerogenic behavior.

Results and discussion

Synthetic route depicted in Scheme 1 outline the chemistry part of the present work. The key intermediate 3-(3-methoxyphenyl)-2-thioxo-2,3-dihydro-1*H*-quinazolin-4-one (**4**) was obtained by reacting 3-methoxy aniline (**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-(3-methoxyphenyl)-2-thioxo-2,3-dihydro-1*H*-quinazolin-4-one (**4**) via the thiourea intermediate in good yield (85%). It was confirmed by IR spectra of **4** show intense peaks at 3312 cm^{-1} for cyclic thio urea (NH), 1690 cm^{-1} for carbonyl (C=O), and 1210 cm^{-1} for thioxo (C=S) stretching. ^{1}H-NMR spectra of compound **4** showed singlet at δ 3.12 ppm due to OCH_3 group, a multiplet at δ 7.33–7.95 ppm for aromatic (8H) protons and a singlet at δ 10.52 ppm indicating the presence of NH. Data from the elemental analyses have been found to be in conformity with the assigned structure. Furthermore the molecular ion recorded in the mass spectra is also in agreement with the molecular weight of the compound.

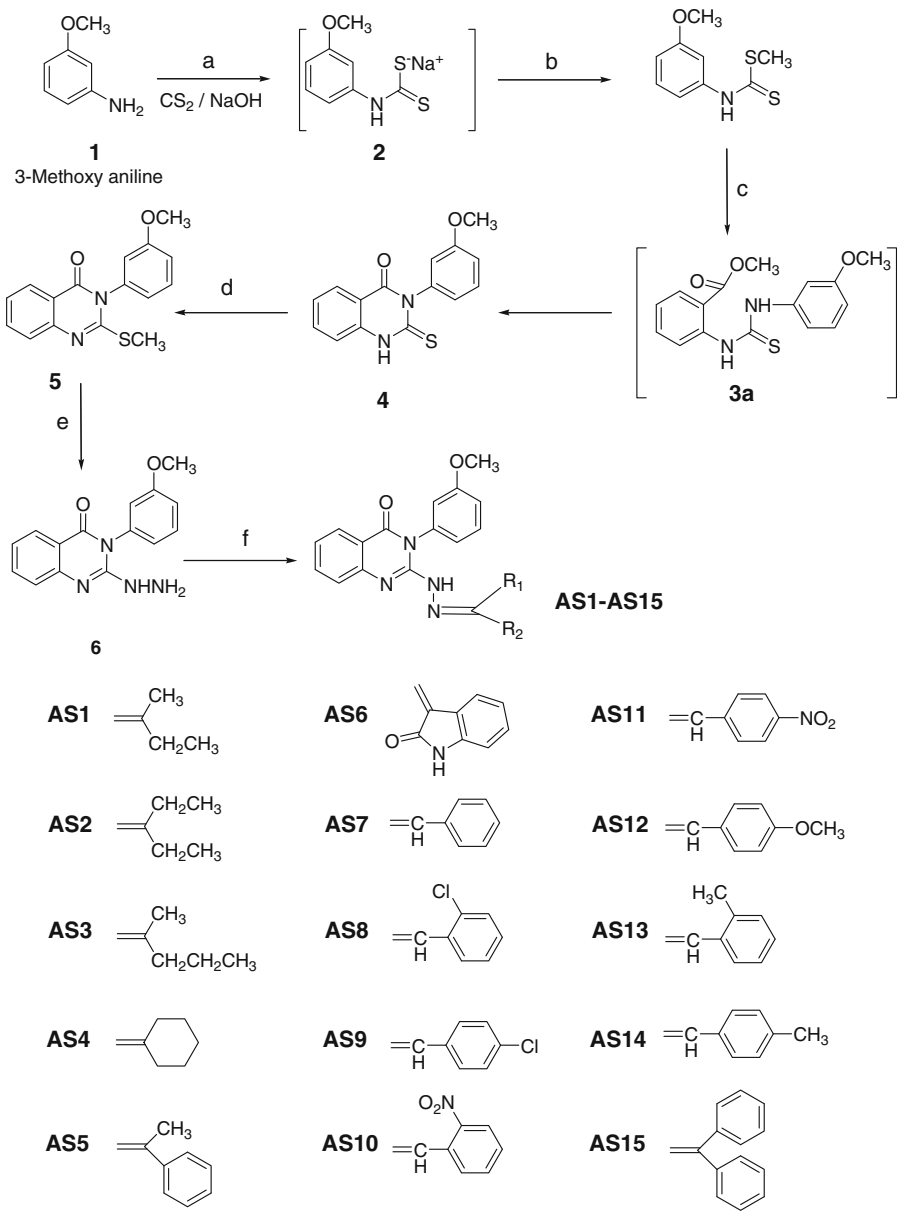
The 3-(3-methoxyphenyl)-2-methysulfanyl-3*H*-quinazolin-4-one **5** was obtained by dissolving **4** in 2% alcoholic sodium hydroxide solution and methylating with dimethyl sulphate with stirring at room temperature. The IR spectra of **5** showed disappearance of NH and C=S stretching signals of cyclic thiourea. It showed a peak for carbonyl (C=O) stretching at 1690 cm^{-1}. The ^{1}H-NMR spectra of compound **5** showed singlets at δ 2.84 and 3.35 ppm due to SCH_3 and OCH_3, respectively, a multiplet at δ 7.25–7.77 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 the methylthio group of **5** with hydrazine hydrate was carried out using ethanol as solvent to afford 2-hydrazino-3-(3-methoxyphenyl)-3*H*-quinazolin-4-one **6**. The long duration of reaction (35 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 compound **6** was confirmed by the presence of NH and NH_2 signals at 3368 and 3210 cm^{-1} in the IR spectra. It also showed a peak for carbonyl (C=O) at 1678 cm^{-1}. The ^{1}H-NMR spectra of the compound **6** showed singlets at δ 3.50, 5.31, and 9.35 ppm due to CH_3, NH_2, and NH, respectively, a multiplet at δ 7.33–7.84 ppm was observed for aromatic (8H) protons. Elemental analyses data and mass spectral data is also in agreement with the assigned structure of the compound.

The title compounds 3-(3-methoxyphenyl)-2-substituted amino-3*H*-quinazolin-4-ones **AS1–15** were obtained by the condensation of amino group of 2-hydrazino-3-(3-methoxyphenyl)-3*H*-quinazolin-4-one (**6**) with a variety of aldehydes and 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 **AS1–15**. 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 showed molecular ion peaks corresponding to their molecular formulae. In mass spectrum of compounds **AS1–15** a common peak at *m/z* 144 corresponding to quinazolin-4-one moiety appeared. The M^++2 peaks were observed in the spectra of compounds **AS8** and **AS9**, confirming the presence of a chlorine atom in the compounds. The relative intensities of these M^++2 peaks in comparison with M^+ peaks were in the ratio of 1:3. Elemental (C, H, and N) analyses satisfactorily confirmed elemental composition and purity of the synthesized compounds.

Evaluation of analgesic activity was performed by the tail-flick technique using Wistar albino mice (Kulkarni, 1980; Amour and Smith, 1941). The results of analgesic testing indicate that the test compounds exhibited moderate analgesic activity at 30 min of reaction time and an increase in activity at 1 h which reached a peak level at 2 h. Declining in activity was observed at 3 h (Table 1). Compound **AS1** with 1-methylpropylidene substituent showed good activity; with the increased lipophilicity (1-ethylpropylidene group, compound **AS2**) showed an increase in activity. Replacement of 1-ethylpropylidene group with its isomer 1-methylbutylidene group (compound **AS3**) retains the activity. Replacement of C-2 alkyl chain with a cycloalkyl group and an aralkyl group (compounds **AS4** and **AS5**, respectively) leads to a moderate decrease in activity. Placement of aryl group at the N-3 position (compounds **AS6, AS7**, and **AS13-15**) also results in decreasing the activity. Placement of electron withdrawing group at N-3 aryl ring (compounds **AS8–12**) leads to further decrease of activity. 2-(1-methyl butylidene-hydrazino)-3-(3-methoxyphenyl)-3*H*-quinazolin -4-one (**AS3**) emerged as the most active analgesic agent and it is moderately more potent when compared to the reference standard diclofenac.

Anti-inflammatory activity was evaluated by the carrageneenan-induced paw edema test in rats (Winter *et al*.,



Scheme 1 Synthesis of 3-(3-methoxyphenyl)-2-substituted amino-3*H*-quinazolin-4-ones. 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 23 h; (d) 2% alcoholic NaOH, $(CH_3)_2SO_4$, rt, 1 h; (e) NH_2NH_2, K_2CO_3, ethanol reflux for 35 h; and (f) $(R_2R_1)CO$; CH_3COOH reflux, 38 h

1962). 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 with increased activity at 1 h that reached a peak level at 2 h. Decline in activity was observed at 3 h. The compound 2-(1-ethyl propylidene-hydrazino)-3-(3-methyoxyphenyl)-3*H*-quinazolin-4-one (**AS2**) showed the most potent anti-inflammatory activity of the series and it is moderately more potent when compared to the reference standard diclofenac sodium.

The most potent compounds (**AS1**–**5**) of the series were evaluated for their ulcerogenic behavior. The ulcer index of the test compounds (Table 3) reveal that the compounds with aliphatic substituents (compounds **AS1**–**4**) showed negligible ulcer index, whereas aryl substituent (compound **AS5**) exhibited little increase in ulcer index. When compared to the reference standards ASA (ulcer index 1.73) and diclofenac (ulcer index 1.65) the test compounds exhibited about 35–50% of the ulcer index of the reference standards. Compounds 2-(1-methylpropylidene-hydrazino)-3-(3-methoxyphenyl)-3*H*-quinazolin-4-one (**AS1**) and 2-(1-ethylpropylidene-hydrazino)-3-(3-methyoxyphenyl)-3*H*-quinazolin-4-one (**AS2**) exhibited least ulcer index (0.53 and 0.51, respectively) among the test compounds which is about one-third of the ulcer index of reference standards aspirin and diclofenac.

Table 1 Analgesic activity of test compounds (tail-flick technique)

Compound code	Percent analgesic activity[a]			
	30 min	1 h	2 h	3 h
AS1	53 ± 1.43*	58 ± 1.15**	61 ± 1.24***	37 ± 1.46*
AS2	56 ± 1.45***	61 ± 1.73***	66 ± 1.13***	38 ± 1.57*
AS3	57 ± 1.52**	64 ± 1.23***	68 ± 1.87***	41 ± 1.78*
AS4	46 ± 1.53*	49 ± 1.14*	53 ± 1.77**	38 ± 1.68*
AS5	44 ± 1.94*	46 ± 1.26*	51 ± 1.76**	36 ± 1.32*
AS6	39 ± 1.42*	43 ± 1.78*	46 ± 1.54*	36 ± 1.28*
AS7	40 ± 1.16*	47 ± 1.29*	47 ± 1.83*	35 ± 1.35*
AS8	32 ± 1.89*	39 ± 1.55*	42 ± 1.75*	33 ± 1.83*
AS9	34 ± 1.29*	40 ± 1.45*	42 ± 1.87*	34 ± 1.97*
AS10	35 ± 1.70*	41 ± 1.50*	45 ± 1.28**	36 ± 1.13*
AS11	35 ± 1.84*	40 ± 1.36*	42 ± 1.63*	36 ± 1.78*
AS12	30 ± 1.77*	37 ± 1.49*	41 ± 1.25*	30 ± 1.19*
AS13	39 ± 1.59*	45 ± 1.83**	51 ± 1.77**	34 ± 1.15*
AS14	44 ± 1.13*	48 ± 1.08*	50 ± 1.23**	37 ± 1.35*
AS15	41 ± 1.99*	46 ± 1.63*	49 ± 1.39*	36 ± 1.53*
Control	2 ± 0.35	6 ± 0.49	4 ± 0.59	4 ± 0.91
Diclofenac	37 ± 1.69*	43 ± 1.42*	45 ± 0.92*	33 ± 0.96*

Significance levels * $p < 0.5$, ** $p < 0.01$, and *** $p < 0.001$ as compared with the respective control. Control refers to no treatment (vehicle only)

[a] Data expressed as mean ± SD from six different experiments done in duplicate

Table 2 Anti-inflammatory activity of test compounds (carrageenan-induced paw edema test in rats)

Compound code	Percent protection			
	30 min	1 h	2 h	3 h
AS1	41 ± 1.16**	45 ± 1.28**	46 ± 1.49**	32 ± 1.42*
AS2	45 ± 1.24**	48 ± 1.95**	53 ± 1.33***	34 ± 1.75*
AS3	42 ± 1.63**	47 ± 1.42***	49 ± 1.30***	34 ± 1.29*
AS4	37 ± 1.57*	40 ± 1.24**	43 ± 1.72**	31 ± 1.84*
AS5	39 ± 1.72*	42 ± 1.38**	45 ± 1.25**	32 ± 1.49*
AS6	35 ± 1.32*	37 ± 1.82*	40 ± 1.49**	31 ± 1.13*
AS7	35 ± 1.27*	38 ± 1.34*	47 ± 1.65**	31 ± 1.53*
AS8	29 ± 1.28*	33 ± 1.88*	37 ± 1.33*	27 ± 1.98*
AS9	33 ± 1.32*	36 ± 1.42*	37 ± 1.59*	27 ± 1.42*
AS10	35 ± 1.84*	35 ± 1.37*	38 ± 1.83*	27 ± 1.25*
AS11	33 ± 1.38*	35 ± 1.65*	37 ± 1.78*	29 ± 1.49*
AS12	29 ± 1.82*	35 ± 1.98*	37 ± 1.20*	27 ± 1.95*
AS13	36 ± 1.84*	41 ± 1.32*	42 ± 1.29*	30 ± 1.49*
AS14	32 ± 1.36*	35 ± 1.81**	40 ± 1.62**	28 ± 1.28*
AS15	33 ± 1.71*	38 ± 1.71*	41 ± 1.74**	30 ± 1.46*
Control	5.1 ± 0.29	6.1 ± 0.27	5.7 ± 0.32	3.2 ± 0.93
Diclofenac	32 ± 0.63*	38 ± 1.58*	39 ± 1.97*	33 ± 0.93*

Significance levels * $p < 0.5$, ** $p < 0.01$, and *** $p < 0.001$ as compared with the respective control. Control refers to no treatment (vehicle only)

[a] Data expressed as mean ± SD from six different experiments done in duplicate

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 (Alagarsamy *et al*., 2002, 2003a, 2004). 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 (Fig. 1, I) showed 44% analgesic and 38% anti-inflammatory activity at the dose of 10 mg/kg, at the reaction time of 2 h (Alagarsamy *et al*., 2002), whereas the C-2 methyl series lead molecule 1-pyrrolidinyl-3-(2-methyl quinazolin-3-yl-4(3*H*)-one) thiourea (Fig. 1, II) exhibited

Table 3 Evaluation of ulcerogenicity index

Compound code	Ulcer index[a]
AS1	0.53 ± 1.36*
AS2	0.51 ± 1.01*
AS3	0.58 ± 1.53*
AS4	0.61 ± 1.72*
AS5	0.62 ± 1.12*
Control	0.15 ± 0.32
Diclofenac	1.65 ± 0.59**
ASA	1.73 ± 0.41**

Significance levels * $p < 0.05$ and ** $p < 0.01$ as compared with the respective control

[a] Data expressed as mean ± SD from six different experiments done in duplicate

50% analgesic and 44% anti-inflammatory activity at the dose of 10 mg/kg, at the reaction time of 2 h (Alagarsamy *et al*., 2003a). Introduction of sulphur atom at C-2 position in the above series, i.e., by placing methylthio group at C-2 position compound 1-diethyl-3-(2-methylthio quinazolin-3-yl-4(3*H*)-one) thiourea (Fig. 1, III) exhibited 56% analgesic activity and 40% anti-inflammatory activity at 10 mg/kg, respectively, at the reaction time of 2 h. Results of analgesic and anti-inflammatory activities of the present series showed that moderate enhancement of activity, compound **AS3** exhibited 68% analgesic activity at 10 mg/kg dose level at the reaction time of 2 h. The compound **AS2** showed 53% anti-inflammatory activity at the dose of 10 mg/kg, at the reaction of 2 h. Interestingly these compounds showed one-third of ulcer index of the reference NSAIDs aspirin and diclofenac. Hence, this series could be developed as a novel class of analgesic and anti-inflammatory agents.

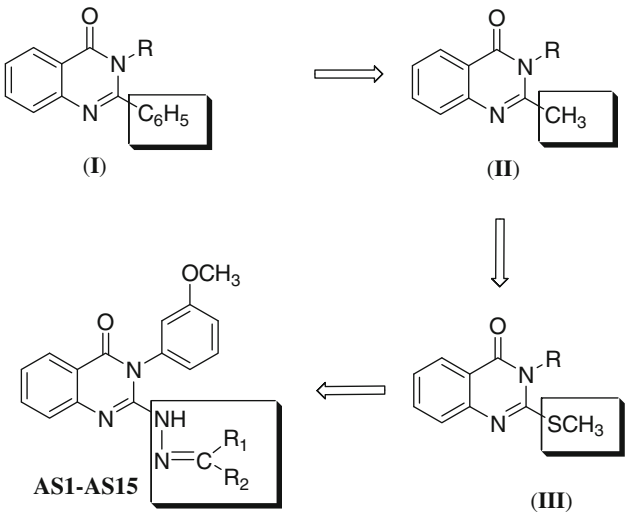


Fig. 1 Lead molecules of quinazolin-4-ones

Materials and methods

Chemistry

Melting points (mp) were taken in open capillaries on Thomas Hoover melting point apparatus (Thomas Hoover, USA) and are uncorrected. The IR spectra were recorded in film or in potassium bromide disks on a Perkin-Elmer 398 spectrometer (Perkin-Elmer, USA). The ^{1}H spectra were recorded on a DPX-300 MHz Bruker FT-NMR spectrometer (Bruker, USA). 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 (JEOL, Japan) using fast atom bombardment (FAB positive). Elemental analysis was performed on a Perkin-Elmer 2400 CHN analyzer (Perkin-Elmer, USA) and values were within the acceptable limits of the calculated values (±0.4%). The progress of the reaction was monitored on readymade silica gel plates (Merck, USA) using chloroform/methanol (9:1) as a solvent system. Iodine was used as a developing agent. All chemicals and reagents used in the synthesis were obtained from Aldrich (USA), Lancaster (USA), or Spectrochem (India) and were used without further purification.

3-(3-Methoxyphenyl)-2-thioxo-2,3-dihydro-1*H*-quinazolin-4-one (**4**)

A solution of 3-methoxy aniline **1** (0.02 mol) in dimethyl sulfoxide (10 ml) was stirred vigorously. To this reaction mixture carbon disulphide (1.6 ml) was added and aqueous sodium hydroxide 1.2 ml (2 mol) added drop wise during 30 min with stirring. Dimethyl sulphate (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 (0.01 mol) and the above prepared *N*-(3-methoxyphenyl)-methyl dithiocarbamic acid (0.01 mol), were dissolved in ethanol (20 ml). To this anhydrous potassium carbonate (100 mg) was added and refluxed for 23 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 re-precipitated by treating with dilute hydrochloric acid. The solid obtained was filtered, washed with water, dried, and recrystallized from ethanol. Yield = 85%, mp 255–256°C. IR: 3312 (NH), 1690 (C=O), 1210 (C=S) cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 3.12 (s, 3H, OCH_3), 7.33–7.95 (m, 8H, ArH), 10.52 (br 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, 63.38; H, 4.30; N, 9.88.

3-(3-Methoxyphenyl)-2-methylsulfanyl-3*H*-quinazolin-4-one (**5**)

The 3-(3-methoxyphenyl)-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. To this dimethyl sulphate (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 = 86%, mp 155–156°C. IR: 1690 (C=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 2.84 (s, 3H, SCH_3), 3.35 (s, 3H, OCH_3), 7.25–7.77 (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, 64.45; H, 4.74; N, 9.33.

2-Hydrazino-3-(3-methoxphenyl)-3*H*-quinazolin-4-one (**6**)

The 3-(3-methoxyphenyl)-2-methylsulfanyl-3*H*-quinazolin-4-one **5** (0.01 mol) was dissolved in ethanol (25 ml). To this hydrazine hydrate (99%) (0.1 mol) and anhydrous potassium carbonate (100 mg) was added and refluxed for 35 h. The reaction mixture was cooled and poured into ice water. The solid obtained was filtered, washed with water, dried, and recrystallized from chloroform–benzene (25:75) mixture. Yield = 79%, mp 211–213°C. IR: 3368, 3210 ($NHNH_2$), 1678 (C=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 3.50 (s, 3H, OCH_3), 5.31 (s, 2H, NH_2), 7.33–7.84 (m, 8H, ArH), 9.35 (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, 63.81; H, 4.96; N, 19.87.

2-(1-Methylpropylidene-hydrazino)-3-(3-methoxyphenyl)-3*H*-quinazolin-4-one (**AS1**)

A mixture of 2-hydrazino-3-(3-methoxyphenyl)-3*H*-quinazolin-4-one **(6)** (0.004 mol) and ethyl methyl ketone (0.004 mol) in glacial acetic acid was refluxed for 38 h. The reaction mixture was poured into ice water. The solid obtained was recrystallized from ethanol. Yield = 72%, mp 243–245°C; IR (KBr) cm^{-1}: 3210 (NH), 1685 (C=O), 1617 (C=N); ^{1}H-NMR ($CDCl_3$): δ 1.00–1.15 (q, 2H, CH_2CH_3), 1.32–1.45 (t, 3H, CH_2CH_3), 2.36 (s, 3H, CH_3), 3.01 (s, 3H, OCH_3), 7.44–8.06 (m, 8H, ArH), 8.55 (br s, 1H, NH D_2O exchangeable); 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, 67.88; H, 5.97; N, 16.68.

2-(1-Ethyl propylidene-hydrazino)-3-(3-methyoxyphenyl)-3*H*-quinazolin-4-one (**AS2**)

Compound **AS2** was prepared by adopting the same procedure as for **AS1** and obtained in 82% yield, mp 261–262°C; IR (KBr) cm^{-1}: 3265 (NH), 1700 (C=O), 1615 (C=N); ^{1}H-NMR ($CDCl_3$): δ 0.75–0.95 (m, 4H, $(CH_2CH_3)_2$), 1.56–1.78 (m, 6H, $(CH_2CH_3)_2$), 2.86 (s, 3H, OCH_3), 7.45–8.15 (m, 8H, ArH), 8.9 (br s, 1H, NH D_2O exchangeable); 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, 68.59; H, 6.30; N, 15.95.

2-(1-Methyl butylidene-hydrazino)-3-(3-methoxyphenyl)-3*H*-quinazolin-4-one (**AS3**)

Compound **AS3** was prepared by adopting the same procedure as for **AS1** and obtained in 78% yield, mp 253–254°C; IR (KBr) cm^{-1}: 3267 (NH), 1695 (C=O), 1612 (C=N); ^{1}H-NMR ($CDCl_3$): δ 1.2–1.3 (t, 2H, $CH_2CH_2CH_3$), 1.8–1.9 (sext, 2H, $CH_2CH_2CH_3$), 2.20–2.35 (t, 3H, $CH_2CH_2CH_3$), 2.67 (s, 3H, CH_3), 3.3 (s, 3H, OCH_3), 7.34–8.15 (m, 9H, ArH), 8.89 (br s, 1H, NH D_2O exchangeable); 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, 68.53; H, 6.28; N, 15.99.

2-(*N*′-Cyclohexylidene-hydrazino)-3-(3-methoxyphenyl)-3*H*-quinazolin-4-one (**AS4**)

Compound **AS4** was prepared by adopting the same procedure as for **AS1** and obtained in 73% yield, mp 237–239°C; IR (KBr) cm^{-1}: 3310 (NH), 1685 (C=O), 1610 (C=N); ^{1}H-NMR ($CDCl_3$): δ 0.90–1.81 (m, 10H, cyclohexyl), 2.92–3.02 (s, 3H, OCH_3), 7.24–7.85 (m, 8H, ArH), 8.33 (br s, 1H, NH D_2O exchangeable); 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, 69.63; H, 6.15; N, 15.44.

2-(*N*′-1-Phenylethylidene-hydrazino)-3-(3-methoxyphenyl)-3*H*-quinazolin-4-one (**AS5**)

Compound **AS5** was prepared by adopting the same procedure as for **AS1** and obtained in 73% yield, mp 263–264°C; IR (KBr) cm^{-1}: 3310 (NH), 1685 (C=O), 1610 (C=N); ^{1}H-NMR ($CDCl_3$): δ 1.63 (s, 3H, CH_3), 3.01 (s, 3H, OCH_3), 7.24–8.25 (m, 13H, ArH), 8.87 (br s, 1H, NH D_2O exchangeable); 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, 71.88; H, 5.22; N, 14.59.

2-(*N*′-2-Oxo-indolin-2-one-3-yl-idene-hydrazino)-3-(3-methoxyphenyl)-3*H*-quinazolin-4-one (**AS6**)

Compound **AS6** was prepared by adopting the same procedure as for **AS1** and obtained in 83% yield, mp

294–295°C; IR (KBr) cm^{-1}: 3285 (NH), 1670 (C=O), 1613 (C=N); ^{1}H-NMR ($CDCl_3$): δ 2.94 (s, 3H, OCH_3), 7.25–8.10 (m, 12H, ArH), 8.91 (br s, 1H, NH D_2O exchangeable), 9.34 (br s, 1H, NH D_2O exchangeable); 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, 67.15; H, 4.18; N, 13.60.

2-(*N*′-Benzylidene-hydrazino)-3-(3-methoxyphenyl)-3*H*-quinazolin-4-one (**AS7**)

Compound **AS7** was prepared by adopting the same procedure as for **AS1** and obtained in 77% yield, mp 267–268°C; IR (KBr) cm^{-1}: 3290 (NH), 1690 (C=O), 1614 (C=N); ^{1}H-NMR ($CDCl_3$): δ 3.32 (s, 3H, CH_3), 5.81 (s, 1H, CH), 7.22–8.25 (m, 13H, ArH), 8.86 (br s, 1H, NH D_2O exchangeable); 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, 71.37; H, 4.90; N, 15.10.

2-(*N*′-(2-Chloro-benzylidene-hydrazino))-3-(3-methoxyphenyl)-3*H*-quinazolin-4-one (**AS8**)

Compound **AS8** was prepared by adopting the same procedure as for **AS1** and obtained in 73% yield, mp 216–218°C; IR (KBr) cm^{-1}: 3360 (NH), 1700 (C=O), 1610 (C=N); ^{1}H-NMR ($CDCl_3$):δ 3.12 (s, 3H, OCH_3), 6.01 (s, 1H, CH), 7.21–8.13 (m, 12H, ArH), 8.65 (br s, 1H, NH D_2O exchangeable); MS (*m/z*): 405 [M^+], 407 [M^+ +2]; 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, 13.87.

2-(*N*′-(4-Chloro-benzylidene-hydrazino)-3-(3-methoxyphenyl)-3*H*-quinazolin-4-one (**AS9**)

Compound **AS9** was prepared by adopting the same procedure as for **AS1** and obtained in 74% yield, mp 273–274°C; IR (KBr) cm^{-1}: 3225 (NH), 1670 (C=O), 1615 (C=N); ^{1}H NMR ($CDCl_3$): δ 3.31 (s, 3H, OCH_3), 5.82 (s, 1H, CH), 7.34–8.26 (m, 12H, ArH), 8.97 (br s, 1H, NH D_2O exchangeable); MS (*m/z*): 405 [M^+], 407 [M^+ +2]. Anal. Calcd. for $C_{22}H_{17}N_4O_2Cl$: C, 65.27; H, 4.23; N, 13.84. Found: C, 65.24; H, 4.26; N, 13.85.

2-(*N*′-(2-Nitro-benzylidene-hydrazino)-3-(3-methoxyphenyl)-3*H*-quinazolin-4-one (**AS10**)

Compound **AS10** was prepared by adopting the same procedure as for **AS1** and obtained in 79% yield, mp 220–221°C; IR (KBr) cm^{-1}: 3310 (NH), 1687 (C=O), 1611 (C=N); ^{1}H-NMR ($CDCl_3$): δ 3.53 (s, 3H, CH_3), 6.04 (s, 1H, CH), 7.15–8.06 (m, 12H, ArH), 8.67 (br s, 1H, NH D_2O exchangeable); 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, 63.62; H, 4.11; N, 16.90.

2-(*N*′-(4-Nitro-benzylidene-hydrazino))-3-(3-methoxyphenyl)-3*H*-quinazolin-4-one (**AS11**)

Compound **AS11** was prepared by adopting the same procedure as for **AS1** and obtained in 70% yield, mp 199–201°C; IR (KBr) cm^{-1}: 3278 (NH), 1680 (C=O), 1610 (C=N); ^{1}H-NMR ($CDCl_3$): δ 2.93 (s, 3H, OCH_3), 6.35 (s, 1H, CH), 7.31–8.23 (m, 12H, ArH), 8.84 (br s, 1H, NH D_2O exchangeable); 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, 63.58; H, 4.14; N, 16.91.

2-(*N*′-(4-Methoxy-benzylidene-hydrazino))-3-(3-methoxyphenyl)-3*H*-quinazolin-4-one (**AS12**)

Compound **AS12** was prepared by adopting the same procedure as for **AS1** and obtained in 77% yield, mp 290–291°C; IR (KBr) cm^{-1}: 3260 (NH), 1687 (C=O), 1615 (C=N); ^{1}H-NMR ($CDCl_3$): δ 3.24 (s, 3H, CH_3), 3.43 (s, 3H, OCH_3), 6.32 (s, 1H, CH), 7.01–8.15 (m, 12H, ArH), 8.56 (br s, 1H, NH D_2O exchangeable); 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, 68.96; H, 5.04; N, 13.98.

2-(*N*′-(2-Methyl-benzylidene-hydrazino))-3-(3-methoxyphenyl)-3*H*-quinazolin-4-one (**AS13**)

Compound **AS13** was prepared by adopting the same procedure as for **AS1** and obtained in 80% yield, mp 248–249°C; IR (KBr) cm^{-1}: 3330 (NH), 1683 (C=O), 1612 (C=N); ^{1}H-NMR ($CDCl_3$): δ 1.32 (s, 3H, CH_3), 2.83 (s, 3H, OCH_3), 6.04 (s, 1H, CH), 7.02–8.03 (m, 12H, ArH), 8.75 (br s, 1H, NH D_2O exchangeable); 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, 71.85; H, 5.22; N, 15.59.

2-(*N*′-(4-Methyl-benzylidene-hydrazino)-3-(3-methoxyphenyl)-3*H*-quinazolin-4-one (**AS14**)

Compound **AS14** was prepared by adopting the same procedure as for **AS1** and obtained in 74% yield, mp 261–262°C; IR (KBr) cm^{-1}: 3310 (NH), 1670 (C=O), 1611 (C=N); ^{1}H-NMR ($CDCl_3$): δ 1.03 (s, 3H, CH_3), 2.8 (s, 3H, OCH_3), 6.04 (s, 1H, CH), 7.32–8.25 (m, 12H, ArH), 9.02 (br s, 1H, NH D_2O exchangeable); 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, 71.79; H, 5.25; N, 14.60.

2-(*N*′-Phenyl-benzylidene-hydrazino)-3-(3-methoxyphenyl)-3*H*-quinazolin-4-one (**AS15**)

Compound **AS15** was prepared by adopting the same procedure as for **AS1** and obtained in 71% yield, mp

230–231°C; IR (KBr) cm^{-1}: 3330 (NH), 1679 (C=O), 1610 (C=N); ^{1}H-NMR ($CDCl_3$): δ 3.32 (s, 3H, OCH_3), 7.11–8.44 (m, 18H, ArH), 8.75 (br s, 1H, NH D_2O exchangeable); 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, 75.36; H, 4.94; N, 12.56.

Pharmacology

The synthesized compounds were evaluated for analgesic, anti-inflammatory, and ulcerogenic index. The test compounds and the standard drugs were administered in the form of a suspension (using 1% carboxy methyl cellulose as a vehicle) by oral route of administration for analgesic and anti-inflammatory but for ulcerogenicity studies intraperitoneally as suspension in 10% v/v Tween 80. Each group consisted of six animals. The animals were maintained in colony cages at 25 ± 2°C, relative humidity 45–55%, under a 12 h light 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 (Kulkarni, 1980; Amour and Smith, 1941) 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 the 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 the cut-off time was 10 s. The percentage analgesic activity (PAA) was calculated by the following formula:

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

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 edema test in rats (Winter *et al.*, 1962). Diclofenac sodium 10 and 20 mg/kg was administered as a standard drug for comparison. The test compounds were administered at two dose levels (10 and 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 edema 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.

Evaluation of ulcerogenicity index

Ulceration in rats was induced as described by Goyal *et al.* (1985). 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 10% v/v Tween 80 suspension intraperitoneally. One group was administered with Aspirin (German Remedies) intraperitoneally at a dose of 20 mg/kg once daily for 3 days. The remaining group of animals was administered with test compounds intraperitoneally at a dose of 20 mg/kg. On the fourth day, pylorus was ligated as per the method (Shay *et al.*, 1945). Animals were fasted for 36 h before the pylorus ligation procedure. Four hours after the ligation, animals were killed. The stomach was removed and opened along with the greater curvature. Ulcer index was determined by the method of Ganguly and Bhatnagar (1973) and is 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 ± SD (standard deviations). For statistical analysis, we have used GraphPad Prism version 3.0. (GraphPad Prism version 3.0, GraphPad Software, Inc. San Diego, CA).

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