Full Paper

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

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A variety of 3-(4-methyl phenyl)-2-substituted amino-3*H*-quinazolin-4-ones were synthesized by reacting the amino group of 2-hydrazino-3-(4-methyl phenyl)-3*H*-quinazolin-4-one with a variety of aldehydes and ketones. The starting material 2-hydrazino-3-(4-methyl phenyl)-3*H*-quinazolin-4-one was synthesized from 4-methyl aniline. The title compounds were investigated for analgesic, anti-inflammatory, and ulcerogenic index activities. While the test compounds exhibited significant activity, compounds **A1**, **A2**, and **A3** showed more potent analgesic activity and the compound **A3** showed 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.

Keywords: Analgesic / Anti-inflammatory / Quinazoline

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Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly prescribed for the treatment of acute and chronic inflammation, pain, and fever. Most of the NSAIDs that are available on the market are known to inhibit isoforms, a constitutive form, COX-1 and an inducible form, COX-2 to offer a 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]. In our ongoing 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-di-substitution is reported to possess significant analgesic, anti-inflammatory [7, 8], and anticonvulsant activities [9]. Earlier, we have documented that 2-phenyl-3-substituted quinazolines [10], 2-methyl-3-substituted quinazolines [11], 2-methylthio-3-substituted quinazolines [12], 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. With this background in mind, in the present study, we have synthesized a series of 3-(4-methyl phenyl)-2-substituted amino-3*H*-quinazolin-4-ones. The synthesized compounds were tested for their analgesic, anti-inflammatory, and ulcerogenic index activities.

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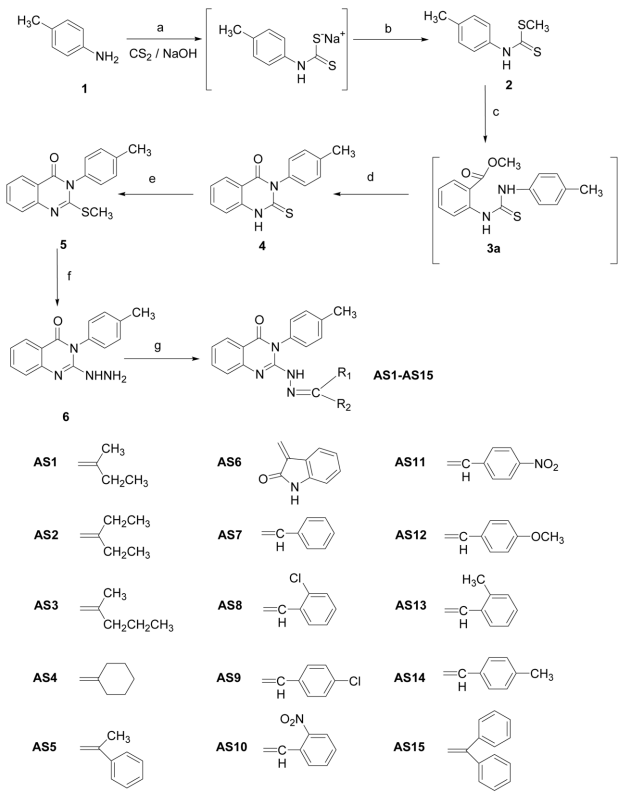
Results and discussion

The synthetic route depicted in Scheme 1 outlines the chemistry part of the present work. The key intermediate 3-(4-methylphenyl)-2-thioxo-2,3-dihydro-1*H*-quinazolin-4-one **4** was obtained by reacting 4-methyl 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-(4-methylphenyl)-2-thioxo-2,3-dihydro-1*H*-quinazolin-4-one **4** via a thiourea intermediate in good yield (75%). The product obtained was cyclic and not an open chain thiourea **3a**. It was confirmed by its low R_f value, high melting point, and its solubility in sodium hydroxide solution. The IR spectrum of **4** shows intense peaks at 3215 cm^{-1} for cyclic thio urea (NH), 1680 cm^{-1} for carbonyl (C=O) and 1210 cm^{-1} for thioxo (C=S) stretching. ^{1}H-NMR spectrum of **4** showed singlet at δ 1.2 – 1.3 ppm due to CH_3 group, a multiplet at δ 7.2 – 8.3 ppm for aromatic (8H) protons and a singlet at δ 10.23 ppm indicating the presence of NH. Elemental analyses confirm the elemental composition of the molecule. 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-methylphenyl)-3*H*-quinazolin-4-one **5** was obtained by dissolving **4** in 2% alcoholic sodium hydroxide solution and methylation with dimethyl sulphate under stirring at room temperature. The IR spectrum of **5** showed disappearance of NH and C=S stretching signals of cyclic thiourea. It showed a peak for carbonyl (C=O) stretching at 1679 cm^{-1}. The ^{1}H-NMR spectrum of compound **5** showed singlets at δ 2.4 ppm and 2.5 ppm due to CH_3 and SCH_3 respectively, a multiplet at δ 7.1 – 8.2 ppm was observed for the aromatic (8H) protons. Data from the elemental analyses and molecular ion recorded in the mass spectrum 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-methylphenyl)-3*H*-quinazolin-4-one **6**. The long duration (30 h) of the reaction required might be due to the presence of the bulky aromatic ring at position **3**, which might have reduced the reactivity of the quinazoline ring system at C-2 position. The formation of **6** was confirmed by the presence of NH and NH_2 signals at 3334 – 3314 cm^{-1} in the IR spectrum. It also showed a peak for carbonyl (C=O) at 1674 cm^{-1}. The ^{1}H-NMR spectrum of the compound **6** showed singlets at δ 2.01 ppm, 5.2 ppm, and 8.72 ppm due to CH_3, NH_2, and NH, respectively, a multiplet at δ 7.12 – 8.07 ppm was



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, 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) $(R_2R_1)CO$; glacial CH_3COOH, reflux, 33 h.

Scheme 1. Synthesis of 2-(1-methylpropylidene)-hydrazino-3-(4-methyl phenyl)-quinazoline 4(3*H*)-one and its derivatives from 4-methyl aniline.

observed for the 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 spectrum is also in agreement with the molecular weight of the compound.

The title compounds 3-(4-methylphenyl)-2-substituted amino-3*H*-quinazolin-4-ones **A1-A15** were obtained by the condensation of amino group of 2-hydrazino-3-(4-methyl phenyl)-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 spectra of all the compounds **A1 – A15**. The IR and ^{1}H-NMR spectra 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 conformity with the assigned structure. The mass spectra of these compounds showed molecular ion peaks corresponding to their molecular formula. In mass spectra of compounds **A1 – A15**, a common

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

Compound Code	Dose (mg/kg)	Percent Analgesic activity			
		30 min	1 h	2 h	3 h
A1	10	48 ± 1.53*	51 ± 1.82**	55 ± 1.14**	35 ± 1.53*
	20	61 ± 1.38***	64 ± 1.74***	67 ± 1.82***	46 ± 1.74*
A2	10	49 ± 1.52**	54 ± 1.64***	58 ± 1.94***	38 ± 1.35*
	20	63 ± 1.74***	69 ± 1.48***	72 ± 1.75***	49 ± 1.83*
A3	10	56 ± 1.36*	62 ± 1.72**	63 ± 1.53**	42 ± 1.73*
	20	67 ± 1.33***	73 ± 1.81***	75 ± 1.87***	49 ± 1.37*
A4	10	37 ± 1.62*	45 ± 1.85*	48 ± 1.37*	30 ± 1.93*
	20	55 ± 1.63**	57 ± 1.07**	56 ± 1.37**	38 ± 1.82*
A5	10	43 ± 1.63*	47 ± 1.17*	49 ± 1.53*	34 ± 1.71*
	20	56 ± 1.63**	58 ± 1.82**	59 ± 1.39**	39 ± 1.53*
A6	10	38 ± 1.07*	42 ± 1.53*	42 ± 1.47*	28 ± 1.82*
	20	50 ± 1.74*	56 ± 1.38**	59 ± 1.82**	37 ± 1.27*
A7	10	38 ± 1.51*	40 ± 1.59*	46 ± 1.52*	35 ± 1.83*
	20	48 ± 1.93*	56 ± 1.37**	59 ± 1.62**	43 ± 1.73*
A8	10	30 ± 1.62*	35 ± 1.73 *	39 ± 1.81*	29 ± 1.53*
	20	38 ± 1.53*	43 ± 1.27*	47 ± 1.47*	39 ± 1.62*
A9	10	36 ± 1.54*	38 ± 1.62*	46 ± 1.62*	30 ± 1.31*
	20	45 ± 1.83*	49 ± 1.61*	49 ± 1.49*	38 ± 1.27*
A10	10	36 ± 1.37*	40 ± 1.18*	46 ± 1.51*	31 ± 1.62*
	20	49 ± 1.48*	53 ± 1.41*	55 ± 1.52*	39 ± 1.61*
A11	10	34 ± 1.52*	39 ± 1.81 *	44 ± 1.57*	31 ± 1.34*
	20	37 ± 1.62*	48 ± 1.83*	48 ± 1.91*	39 ± 1.36*
A12	10	36 ± 1.07*	40 ± 1.38*	46 ± 1.72*	37 ± 1.61*
	20	44 ± 1.72*	45 ± 1.81*	49 ± 1.82**	39 ± 1.37*
A13	10	39 ± 1.52*	39 ± 1.61*	46 ± 1.47*	33 ± 1.57*
	20	49 ± 1.28*	54 ± 1.71**	55 ± 1.54*	39 ± 1.73*
A14	10	39 ± 1.71*	45 ± 1.05*	48 ± 1.48*	39 ± 1.72*
	20	49 ± 1.68*	53 ± 1.53*	58 ± 1.62**	46 ± 1.52*
A15	10	39 ± 1.61*	42 ± 1.63*	46 ± 1.72*	39 ± 1.06*
Control	20	47 ± 1.52*	50 ± 1.04*	53 ± 1.62*	46 ± 1.51*
		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*

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

peak at m/z 144 corresponding to quinazolin-4-one moiety appeared. Elemental (C, H, N) analyses satisfactorily confirmed elemental composition and purity of the synthesized compounds.

Test for analgesic activity was performed by the tail-flick technique [14, 15] using Wistar albino mice. The results of analgesic activity indicate that all the test compounds exhibited significant activity (Table 1). Compound **A1** with 2-butylidene substituent showed good activity; with the increased lipophilicity (3-pentylidene group), **A2** showed increase in activity. Replacement of the 3-pentylidene group with its isomer 2-pentylidene group (compound **A3**) retains the activity. Placement of alkyl group and cycloalkyl group (compounds **A4** and **A5**) leads to moderate decrease in activity. Placement of aryl group at N-3 position (compounds **A6, A7** and **A13 – A15**) also results in decreasing activity. Placement of an electron-withdrawing group at N-3 aryl ring (compounds **A8 – A12**) leads to a further decrease of activity. Compound **A3** 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. The compound **A3** showed more potent anti-inflammatory activity while the compounds **A1** and **A2** exhibited equipotent anti-inflammatory activity when compared to the reference standard diclofenac sodium.

The ulcer index of the test compounds (Table 3) reveals that the compounds **A8 – A12** possessing electron-withdrawing groups exhibited higher ulcer index than the

Table 2. Percent anti-inflammatory activity of test compounds (carrageenan-induced paw oedema test in rats)[a].

Compound Code	Dose (mg/kg)	Percent Protection			
		30 min	1 h	2 h	3 h
A1	10	39 ± 1.63*	41 ± 1.27*	45 ± 1.72*	27 ± 1.29*
	20	46 ± 1.31**	53 ± 1.51**	52 ± 1.78**	42 ± 1.62*
A2	10	39 ± 1.73*	43± 1.28*	48 ± 1.83**	34 ± 1.92*
	20	48 ± 1.62**	60± 1.51***	63 ± 1.73***	42 ± 1.83*
A3	10	39 ± 1.62*	48 ± 1.91**	53 ± 1.72**	36 ± 1.27*
	20	50 ± 1.69**	59 ± 1.62***	63 ± 1.73***	43 ± 1.62*
A4	10	32 ± 1.73*	37 ± 1.94*	41 ± 1.81*	28 ± 1.48*
	20	39 ± 1.73*	48 ± 1.16**	50 ± 1.38**	36 ± 1.80*
A5	10	37 ± 1.17*	39 ± 1.83*	42 ± 1.29*	29 ± 1.35*
	20	44 ± 1.34*	48 ± 1.18**	49 ± 1.46**	38 ± 1.53*
A6	10	31 ± 1.73*	35 ± 1.38*	38 ± 1.62*	29 ± 1.42*
	20	38 ± 1.37*	39 ± 1.63*	41 ± 1.84*	35 ± 1.67*
A7	10	29 ± 1.73*	35 ± 1.61*	39 ± 1.29*	28 ± 1.38*
	20	36 ± 1.64*	44 ± 1.37*	48 ± 1.91*	34 ± 1.83*
A8	10	27 ± 1.72*	29 ± 1.82*	33± 1.43*	26 ± 1.72*
	20	32 ± 1.16*	38 ± 1.633*	39 ± 1.28*	29 ± 1.184*
A9	10	29 ± 1.63*	30 ± 1.73*	33± 1.48*	36 ± 1.58*
	20	35 ± 1.73*	38 ± 1.90*	39 ± 1.72*	29 ± 1.23*
A10	10	31 ± 1.61*	34 ± 1.89*	38 ± 1.37*	25 ± 1.92*
	20	38 ± 1.18*	39 ± 1.54*	42 ± 1.74*	38 ± 1.38*
A11	10	27 ± 1.24*	28 ± 1.68*	32 ± 1.73*	26 ± 1.58*
	20	39 ± 1.51*	39 ± 1.84*	43 ± 1.93*	32 ± 1.27*
A12	10	28 ± 1.94*	30 ± 1.74*	37 ± 1.06*	26 ± 1.85*
	20	39 ± 1.62*	39 ± 1.71*	45 ± 1.95**	30 ± 1.94*
A13	10	35 ± 1.38	38 ± 1.84*	38 ± 1.03*	28 ± 1.27*
	20	40 ± 1.12*	45 ± 1.74**	49 ± 1.18**	34 ± 1.05*
A14	10	31 ± 1.38*	34 ± 1.04	39 ± 1.37*	29 ± 1.28*
	20	45 ± 1.47**	48 ± 1.17**	49 ± 1.63**	40 ± 1.63*
A15	10	31 ± 1.61*	35 ± 1.53*	36 ± 1.83*	29 ± 1.27*
	20	41 ± 1.84*	45 ± 1.56**	47 ± 1.73**	39 ± 1.56*
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 mean ± SD (n = 6).
Significance levels. * $p < 0.5$, ** $p < 0.01$ and *** $p < 0.001$ as compared with the respective control.

other test compounds. The high ulcer index score for these compounds may be due to the suppression of the prostaglandin synthesis.

Conclusions

In our earlier studies [10 – 15], 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 we also made a substitution in such a way as to increase lipophilicity of the molecule. The placement 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 a sulfur atom at C-2 position in the above series, *i.e.* by placing a methyl thio group at C-2 position, showed 54% analgesic and 43% anti-inflammatory activity [12]. The results of the analgesic and anti-inflammatory activities of the present series showed that enhancement of activity (61% analgesic and 49% anti-inflammatory activity). Interestingly, these compounds showed negligible ulcer index unlike other non-steroidal anti-inflammatory drugs (NSAIDs). Hence this series could be developed as a novel class of analgesic and anti-inflammatory agents. However, further structural modification is planned to

Table 3. Evaluation of ulcerogenicity index[a].

Compound Code	Ulcer index
A1	0.65 ± 1.33*
A2	0.57 ± 1.20*
A3	0.83 ± 1.32*
A4	0.69 ± 1.18*
A5	0.62 ± 1.06*
A6	0.59 ± 1.53*
A7	0.77 ± 1.28*
A8	0.95 ± 1.54*
A9	0.92 ± 1.59
A10	0.99 ± 1.21*
A11	0.97 ± 1.94*
A12	0.90 ± 1.57*
A13	0.72 ± 1.31*
A14	0.79 ± 1.73*
A15	0.59 ± 1.23*
Control	0.15 ± 0.32
Aspirin	1.73 ± 0.41**

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

increase the analgesic and anti-inflammatory activities with the decreased ulcerogenic index.

Experimental

Chemistry

Melting points (mp) were taken in open capillaries on Thomas Hoover melting point apparatus (Philadelphia, PA, USA) and are uncorrected. Infrared (IR) spectra were recorded in film or in potassium bromide disks on a Perkin-Elmer 398 spectrometer (Perkin Elmer). The ^{1}H-NMR spectra were recorded on a DPX-300 MHz Bruker FT-NMR spectrometer (Bruker Biosciences, 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, Tokyo, Japan) 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) analyses 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.

*2-Thioxo-3-(4-methylphenyl)quinazolin-4(3H)-one **4***

A solution of 4-methyl aniline (**1**) 2.14 g (0.02 mol) in dimethyl sulphoxide (DMSO) (10 mL) was stirred vigorously. To this was added carbon disulphide (1.6 mL, 0.026 mol) and aqueous sodium hydroxide (1.2 mL, 20 mol solution) drop-wise during 30 min with stirring. Dimethyl sulphate 2.5 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 (0.01 mol) and the above prepared *N*-(4-methylphenyl)-methyl dithiocarbamic acid 1.97 g (0.01 mol), were dissolved in ethanol (20 mL). To this anhydrous potassium carbonate (100 mg) was added and refluxed 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: 75%, mp 302 – 305°C; IR (KBr) cm^{-1}: 3215 (NH), 1680 (C=O), 1210 (C=S); ^{1}H-NMR ($CDCl_3$): δ 1.2 – 1.3 (s, 3H, CH_3), 7.2 – 8.3 (m, 8H, ArH) and 10.23 (s, 1H, NH); 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, 67.17; H, 4.53; N, 10.49.

*2-Methylthio-3-(4-methylphenyl)quinazolin-4(3H)-one **5***

The 2-thioxo-3-(4-methylphenyl) quinazolin-4(3*H*)-one (**4**) 2.68 g (0.01 mol) was dissolved in 40 mL of 2% alcoholic sodium hydroxide solution. To this, dimethyl sulphate 1.25 g (0.01 mol) was added drop-wise with stirring. The stirring was continued for 1 h, the reaction was then poured into ice water. The solid obtained was filtered, washed with water, dried, and recrystallized from ethanol-chloroform (75 : 25) mixture. Yield: 76%, mp 160 – 162°C; IR (KBr) cm^{-1}: 1679 (C=O); ^{1}H-NMR ($CDCl_3$): δ 2.4 (s, 3H, CH_3), 2.5 (s, 3H, SCH_3) and 7.1 – 8.2 (m, 8H ArH); MS (m/z) 282 [M$^+$]. Anal. Calcd. for $C_{16}H_{14}N_2OS$: C, 68.06; H, 5.00; N, 9.92. Found: C, 68.09; H, 5.06; N, 9.87.

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

The 2-methylthio-3-(4-methylphenyl) quinazolin-4(3*H*)-one (**5**) 2.82 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: 72%, mp 170°C; IR (KBr) cm^{-1}: 3334, 3314 ($NHNH_2$), 1674 (C=O); ^{1}H-NMR ($CDCl_3$): δ 2.01 (s, 3H, CH_3), 5.2 (s, 2H, NH_2), 7.12 – 8.07 (m, 8H, ArH), 8.72 (s, 1H, NH); MS (m/z) 266 [M$^+$]. Anal. Calcd. for $C_{15}H_{14}N_4O$: C, 67.65; H, 5.29; N, 21.04. Found: C, 67.69; H, 5.25; N, 21.07.

*2-(N′-2-Butylidene-hydrazino)-3-(4-methylphenyl)-3H-quinazolin-4-one **A1***

A mixture of 2-hydrazino-3-(4-methyl phenyl)-3*H*-quinazolin-4-one (**6**) (0.004 mol) and ethylmethyl ketone (0.004 mol) in glacial acetic acid was refluxed for 33 h. The reaction mixture was poured into ice water. The solid obtained was recrystallized from ethanol. Yield: 73%, mp 231 – 233°C; IR (KBr) cm^{-1}: 3345 (NH), 1670 (C=O), 1615 (C=N); ^{1}H-NMR ($CDCl_3$): δ 1.1 – 1.2 (q, 2H, CH_2CH_3), 1.5 – 1.6 (t, 3H, CH_2CH_3), 1.9 – 2.0 (s, 3H, CH_3), 2.5 – 2.6 (s, 3H, CH_3), 7.2 – 8.0 (m, 8H, ArH), 8.5 (brs, 1H, NH); 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, 6.30; N, 17.43. Compounds **A2 – A15** were prepared by adopting the same procedure.

Biological evaluation

The synthesized compounds were evaluated for analgesic, anti-inflammatory, and antimicrobial activities and the ulcerogenic index. 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 intraperitoneally 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 gm) 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 sec. 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:

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 intraperitoneally. 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 the 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 ± SD (standard deviations). For statistical analysis, we have used GraphPad Prism 3.0 version. (GraphPad Prism 3.0 version, GraphPad Software, Inc. San Diego, CA, USA).

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