JPP Journal of Pharmacy and Pharmacology

JPP 2007, 59: 669–677

Received October 9, 2006
Accepted January 29, 2007
DOI 10.1211/jpp.59.5.0007
ISSN 0022-3573

Synthesis and pharmacological investigation of novel 3-(3-methylphenyl)-2-substituted amino-3*H*-quinazolin-4-ones as analgesic and anti-inflammatory agents

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Abstract

A variety of novel 3-(3-methylphenyl)-2-substituted amino-3*H*-quinazolin-4-ones were synthesized by reacting the amino group of 2-hydrazino-3-(3-methylphenyl)-3*H*-quinazolin-4-one with a variety of aldehydes and ketones. The starting material 2-hydrazino-3-(3-methylphenyl)-3*H*-quinazolin-4-one was synthesized from 3-methyl aniline. The title compounds were investigated for analgesic, anti-inflammatory and ulcerogenic index activities. Compound 2-(1-ethylpropylidene-hydrazino)-3-(3-methylphenyl)-3*H*-quinazolin-4-one (**AS2**) was the most active analgesic agent. Compound 2-(1-methylbutylidene-hydrazino)-3-(3-methylphenyl)-3*H*-quinazolin-4-one (**AS3**) was the most active anti-inflammatory agent and was moderately more potent than the reference standard diclofenac sodium. The test compounds showed only mild ulcerogenic potential compared with aspirin.

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly prescribed for the treatment of acute and chronic inflammation, pain and fever. Most NSAIDs inhibit isoforms of cyclooxygenase (COX), a constitutive form, COX-1, and an inducible form, COX-2, to produce therapeutic effects. However long-term clinical use 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; Beuck 1999; Van Ryn et al 2000). On our ongoing medicinal chemistry research programme we have found that quinazolines and condensed quinazolines exhibit potent analgesic, anti-inflammatory (Alagarsamy et al 2003a) and anticonvulsant activities (Alagarsamy et al 2006). Quinazolin-4(3*H*)-ones with 2,3-disubstituents are reported to possess significant analgesic, anti-inflammatory (Bhalla et al 1993; Chao et al 1999; Alagarsamy et al 2003b) and anticonvulsant activities (Zappalà et al 2003). We have previously described 2-phenyl-3-substituted quinazolines (Alagarsamy et al 2002), 2-methyl-3-substituted quinazolines (Alagarsamy et al 2003c) and 2-methylthio-3-substituted quinazolines (Alagarsamy et al 2004), which exhibited good analgesic and anti-inflammatory activities. The current work is part of our ongoing efforts into the development and identification of new molecules that have analgesic and anti-inflammatory activities but with less propensity to cause gastrointestinal side-effects, particularly ulcers, than the currently used NSAIDs. We have synthesized some 3-(3-methylphenyl)-2-substituted-3*H*-quinazolin-4-ones and tested them for analgesic, anti-inflammatory and ulcerogenic activities.

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Materials and Methods

Chemistry

Melting point (mp) was measured in open capillaries using Thomas Hoover melting point apparatus (Philadelphia, PA, USA) and values are uncorrected. Infrared (IR) spectra were recorded on a Fourier transformation (FT)-IR spectrometer (Perkin Elmer, Roundtree Dairy Road, Woodbridge, Ontario, Canada). The ^{1}H-NMR spectra were recorded on a DPX-300 MHz Bruker FT-NMR spectrometer (Pacific Northwest, Richland, Washington, WA, USA). The chemical shifts were reported as parts per million (δ ppm), using tetramethylsilane as an internal standard. Mass spectra were obtained on a JEOL-SX-102 instrument (Maspec, Tokyo, Japan) using fast atom bombardment. Elemental analysis was performed on a Perkin-Elmer 2400 C, H, N analyser, and values were within the acceptable limits of the calculated values. The progress of the reaction was monitored on readymade silica gel plates (Merck & Co. Inc., Whitehouse Station, NJ, USA) using chloroform–methanol (9:1) as the solvent system. Iodine was used as a developing agent. Spectral data (IR, NMR and mass spectra (MS)) confirmed the structures of the synthesized compounds. The purity of the 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 Sigma-Aldrich (St Louis, MO, USA), Alfa Aesar (Johnson Matthey Company, Ward Hill, MA, USA) or Spectrochem Pvt. Ltd (Mumbai, India) and were used without further purification.

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

A solution of 3-methyl aniline (**1**) (0.02 mol) in DMSO (10 mL) was stirred vigorously. To this was added carbon disulfide (1.6 mL, 0.026 mol) and aqueous sodium hydroxide (1.2 mL, 20 M solution) drop wise over 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 and recrystallized from ethanol. Methyl anthranilate (0.01 mol) and the above prepared *N*-(3-methylphenyl)-methyl dithiocarbamic acid (0.01 mol) were dissolved in ethanol (20 mL). Anhydrous potassium carbonate (100 mg) was added to this, and the mixture refluxed for 20 h. The reaction mixture was cooled in ice and the separated solid was filtered and purified by dissolving in alcoholic sodium hydroxide (10% solution) and was reprecipitated by treating with dilute hydrochloric acid. The solid obtained was filtered, washed with water, dried and recrystallized from ethanol. Yield 75%; mp 286–289°C; IR (KBr) cm^{-1}: 3218 (NH), 1680 (C=O), 1200 (C=S); ^{1}H NMR ($CDCl_3$): δ (ppm) 1.3–1.36 (s, 3H, CH_3), 7.0–8.1 (m, 8H, ArH), 10.3 (s, 1H, NH); MS (m/z) 268 [M^+]. Analysis: calculated for $C_{15}H_{12}N_2OS$: C, 67.14; H, 4.51; N, 10.43; found: C, 67.36; H, 4.56; N, 10.52.

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

3-(3-Methylphenyl)-2-thioxo-2,3-dihydro-1*H*-quinazolin-4-one (**4**) (0.01 mol) was dissolved in alcoholic sodium hydroxide (2% 40 mL). Dimethyl sulfate (0.01 mol) was added drop wise to this reaction mixture with stirring, which 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 81%; mp 148–150°C; IR (KBr) cm^{-1}: 1678 (C=O), 1610 (C=C); ^{1}H NMR ($CDCl_3$): δ (ppm) 2.4 (s, 3H, CH_3), 2.5 (s, 3H, SCH_3) 7.1–8.2 (m, 8H, ArH); MS (m/z) 282 [M^+]. Analysis: calculated for $C_{16}H_{14}N_2OS$: C, 68.06; H, 4.99; N, 9.92; found: C, 68.31; H, 4.85; N, 9.86.

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

2-Methylsulfanyl-3-(3-methylphenyl)-3*H*-quinazolin-4-one (**5**) (0.01 mol) was dissolved in ethanol (25 mL). To this reaction mixture we added hydrazine hydrate (99%; 0.1 mol) and anhydrous potassium carbonate (100 mg) and refluxed the mixure for 32 h. The reaction mixture was then cooled and poured into ice water. The solid obtained was filtered, washed with water, dried and recrystallized from chloroform–benzene (25:75). Yield 76%; mp 195–197 °C; IR (KBr) cm^{-1}: 3320–3205 ($NHNH_2$), 1674 (C=O); ^{1}H NMR ($CDCl_3$): δ (ppm) 2.33 (s, 3H, CH_3), 4.93 (s, 2H, NH_2), 7.15–8.08 (m, 8H, ArH), 8.63 (s, 1H, NH); MS (m/z) 266 [M^+]. Analysis: calculated for $C_{15}H_{14}N_4O$: C, 67.65; H, 5.29; N, 21.04; found: C, 67.81 H, 5.36 N, 21.05.

General synthetic procedure for compounds (AS1–AS15)

A mixture of 2-hydrazino-3-(3-methylphenyl)-3*H*-quinazolin-4-one (**6**) (0.004 mol) and appropriate ketone/aldehyde (0.004 mol) in glacial acetic acid was refluxed for 41 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.

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

Yield 71%, mp 251–253°C; IR (KBr) cm^{-1}: 3290 (NH), 1679 (C=O), 1613 (C=N); ^{1}H NMR ($CDCl_3$): δ (ppm) 1.0–1.1 (q, 2H, CH_2CH_3), 1.3–1.4 (t, 3H, CH_2CH_3), 1.7–1.8 (s, 3H, CH_3), 2.1–2.2 (s, 3H, CH_3), 7.0–7.7 (m, 8H, ArH), 8.6 (br s, 1H, NH); MS (*m/z*): 320 [M^+]. Analysis: calculated composition for $C_{19}H_{20}N_4O$: C, 71.22; H, 6.29; N, 17.48; found: C, 71.20; H, 6.26; N, 17.50.

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

Yield 73%; mp 271–272 °C; IR (KBr) cm^{-1}: 3360 (NH), 1671 (C=O), 1616 (C=N); ^{1}H NMR ($CDCl_3$): δ (ppm) 1.1–1.3 (m, 4H, $(CH_2CH_3)_2$), 1.5–1.7 (m, 6H, $(CH_2CH_3)_2$), 1.9 (s, 3H, CH_3), 7.3–8.1 (m, 8H, ArH), 8.5 (br s, 1H, NH); MS (m/z):

334 [M^+]. Analysis: calculated composition for $C_{20}H_{22}N_4O$: C, 71.83; H, 6.63; N, 16.75; found: C, 71.85; H, 6.66; N, 16.71.

*2-(1-Methyl butylidene-hydrazino)-3-(3-methylphenyl)-3H-quinazolin-4-one (**AS3**)*
Yield 77%; mp 218–219 °C; IR (KBr) cm^{-1}: 3370 (NH), 1682 (C=O), 1610 (C=N); ^1H NMR ($CDCl_3$): δ (ppm) 1.3–1.4 (t, 2H, $CH_2CH_2CH_3$), 1.8–1.9 (sext, 2H, $CH_2CH_2CH_3$), 2.1–2.2 (t, 3H, $CH_2CH_2CH_3$), 2.4 (s, 3H, CH_3), 2.6 (s, 3H, CH_3), 7.2–8.0 (m, 9H, ArH), 8.7 (br s, 1H, NH); MS (m/z): 334 [M^+]. Analysis: calculated composition for $C_{20}H_{22}N_4O$: C, 71.83; H, 6.63; N, 16.75; found: C, 71.86; H, 6.68; N, 16.72.

*2-(N′-Cyclohexylidene-hydrazino)-3-(3-methylphenyl)-3H-quinazolin-4-one (**AS4**)*
Yield 77%; mp 258–259 °C; IR (KBr) cm^{-1}: 3320 (NH), 1680 (C=O), 1618 (C=N); ^1H NMR ($CDCl_3$): δ (ppm) 0.8–1.9 (m, 10H, cyclohexyl), 2.3–2.4 (s, 3H, CH_3), 7.0–7.8 (m, 8H, ArH), 8.5 (br s, 1H, NH); MS (m/z): 346 [M^+]. Analysis: calculated for $C_{21}H_{22}N_4O$: C, 72.80; H, 6.40; N, 16.17; found: C, 72.87; H, 6.38; N, 16.20.

*2-(N′-1-Phenylethylidene-hydrazino)-3-(3-methylphenyl)-3H-quinazolin-4-one (**AS5**)*
Yield 78%; mp 273–275 °C; IR (KBr) cm^{-1}: 3290 (NH), 1676 (C=O), 1610 (C=N); ^1H NMR ($CDCl_3$) δ (ppm): 1.1–1.2 (s, 3H, CH_3), 1.7–1.8 (s, 3H, CH_3), 7.0–8.1 (m, 13H, ArH), 8.4 (br s, 1H, NH); MS (m/z): 368 [M^+]. Analysis: calculated for $C_{23}H_{20}N_4O$: C, 74.97; H, 5.47; N, 15.20; found: C, 74.90; H, 5.48; N, 15.25.

*2-(N′-2-Oxo-indolin-2-one-3-yl-idene-hydrazino)-3-(3-methylphenyl)-3H-quinazolin-4-one (**AS6**)*
Yield 72%; mp 239–241 °C; IR (KBr) cm^{-1}: 3360 (NH), 1684 (C=O), 1617 (C=N); ^1H NMR ($CDCl_3$): δ (ppm) 1.7–1.8 (s, 3H, CH_3), 7.0–8.2 (m, 12H, ArH), 8.8 (br s, 1H, NH), 9.0 (br s, 1H, NH); MS (m/z): 395 [M^+]. Analysis: calculated for $C_{23}H_{17}N_4O_2$: C, 69.86; H, 4.33; N, 14.16; found: C, 69.88; H, 4.30; N, 14.19.

*2-(N′-Benzylidene-hydrazino)-3-(3-methylphenyl)-3H-quinazolin-4-one (**AS7**)*
Yield 78%; mp 268–269 °C; IR (KBr) cm^{-1}: 3277 (NH), 1686 (C=O), 1613 (C=N); ^1H NMR ($CDCl_3$): δ (ppm) 1.7–1.8 (s, 3H, CH_3), 6.1 (s, 1H, CH), 7.3–8.1 (m, 13H, ArH), 8.7 (br s, 1H, NH); MS (m/z): 354 [M^+]. Analysis: calculated for $C_{22}H_{18}N_4O$: C, 74.55; H, 5.11; N, 15.80; found: C, 74.50; H, 5.18; N, 15.85.

*2-(N′-(2-Chloro-benzylidene-hydrazino))-3-(3-methylphenyl)-3H-quinazolin-4-one (**AS8**)*
Yield 74%; mp 269–270 °C; IR (KBr) cm^{-1}: 3362 (NH), 1680 (C=O), 1614 (C=N); ^1H NMR ($CDCl_3$): δ (ppm) 2.0 (s, 3H, CH_3), 6.3 (s, 1H, CH), 7.1–8.2 (m, 12H, ArH), 8.7 (br s, 1H, NH); MS (m/z): 388 [M^+], 390 [M^+ +2]. Analysis: calculated for $C_{22}H_{17}N_4OCl$: C, 67.95; H, 4.40; N, 14.40; found: C, 67.99; H, 4.42; N, 14.36.

*2-(N′-(4-Chloro-benzylidene-hydrazino)-3-(3-methylphenyl)-3H-quinazolin-4-one (**AS9**)*
Yield 79%; mp 244–245 °C; IR (KBr) cm^{-1}: 3330 (NH), 1689 (C=O), 1610 (C=N); ^1H NMR ($CDCl_3$): δ (ppm) 1.6–1.7 (s, 3H, CH_3), 6.1 (s, 1H, CH), 7.2–8.3 (m, 12H, ArH), 8.7 (br s, 1H, NH); MS (m/z): 388 [M^+], 390 [M^+ +2]. Analysis: calculated for $C_{22}H_{17}N_4OCl$: C, 67.95; H, 4.40; N, 14.40; found: C, 67.90; H, 4.43; N, 14.36.

*2-(N′-(2-Nitro-benzylidene-hydrazino)-3-(3-methylphenyl)-3H-quinazolin-4-one (**AS10**)*
Yield 74%; mp 225–226 °C; IR (KBr) cm^{-1}: 3280 (NH), 1687 (C=O), 1617 (C=N); ^1H NMR ($CDCl_3$): δ (ppm) 1.3–1.4 (s, 3H, CH_3), 6.5 (s, 1H, CH), 7.2–8.3 (m, 12H, ArH), 8.6 (br s, 1H, NH); MS (m/z): 399 [M^+]. Analysis: calculated for $C_{22}H_{17}N_5O_3$: C, 66.15; H, 4.28; N, 17.53; found: C, 66.11; H, 4.34; N, 17.57.

*2-(N′-(4-Nitro-benzylidene-hydrazino))-3-(3-methylphenyl)-3H-quinazolin-4-one (**AS11**)*
Yield 78%; mp 233–235 °C; IR (KBr) cm^{-1}: 3294 (NH), 1679 (C=O), 1616 (C=N); ^1H NMR ($CDCl_3$): δ (ppm) 1.2–1.3 (s, 3H, CH_3), 6.5 (s, 1H, CH), 7.1–8.2 (m, 12H, ArH), 8.7 (br s, 1H, NH); MS (m/z): 399 [M^+]. Analysis: calculated for $C_{22}H_{17}N_5O_3$: C, 66.15; H, 4.28; N, 17.53; found: C, 66.11; H, 4.26; N, 17.54.

*2-(N′-(4-Methoxy-benzylidene-hydrazino))-3-(3-methylphenyl)-3H-quinazolin-4-one (**AS12**)*
Yield 81%; mp 262–263 °C; IR (KBr) cm^{-1}: 3310 (NH), 1687 (C=O), 1616 (C=N); ^1H NMR ($CDCl_3$): δ (ppm) 1.7–1.8 (s, 3H, CH_3), 3.1–3.2 (s, 3H, OCH_3), 6.6 (s, 1H, CH), 7.0–8.1 (m, 12H, ArH), 8.7 (br s, 1H, NH); MS (m/z): 384 [M^+)]. Analysis: calculated for $C_{23}H_{20}N_4O_2$: C, 71.85; H, 5.24; N, 14.57; found: C, 71.89; H, 5.28; N, 14.55.

*2-(N′-(2-Methyl-benzylidene-hydrazino))-3-(3-methylphenyl)-3H-quinazolin-4-one (**AS13**)*
Yield 76%; mp 213–215 °C; IR (KBr) cm^{-1}: 3314 (NH), 1685 (C=O), 1613 (C=N); ^1H NMR ($CDCl_3$): δ (ppm) 1.3–1.4 (s, 3H, CH_3), 1.6–1.7 (s, 3H, CH_3), 6.1 (s, 1H, CH), 7.0–8.1 (m, 12H, ArH), 8.4 (s, 1H, NH); MS (m/z): 368 [M^+]. Analysis: calculated for $C_{23}H_{20}N_4O$: C, 74.97; H, 5.47; N, 15.20; found: C, 74.98; H, 5.49; N, 15.25.

*2-(N′-(4-Methyl-benzylidene-hydrazino)-3-(3-methylphenyl)-3H-quinazolin-4-one (**AS14**)*
Yield 74%; mp 271–272 °C; IR (KBr) cm^{-1}: 3316 (NH), 1690 (C=O), 1617 (C=N); ^1H NMR ($CDCl_3$): δ (ppm) 1.1–1.2 (s, 3H, CH_3), 1.4–1.5 (s, 3H, CH_3), 6.3 (s, 1H, CH), 7.3–8.3 (m, 12H, ArH), 8.5 (br s, 1H, NH); MS (m/z): 368 [M^+]. Analysis: calculated for $C_{23}H_{20}N_4O$: C, 74.97; H, 5.47; N, 15.20; found: C, 74.90; H, 5.44; N, 15.23.

*2-(N′-Phenyl-benzylidene-hydrazino)-3-(3-methylphenyl)-3H-quinazolin-4-one (**AS15**)*
Yield 79%; mp 237–239 °C; IR (KBr) cm^{-1}: 3282 (NH), 1690 (C=O), 1613 (C=N); ^1H NMR ($CDCl_3$): δ (ppm) 3.1–3.2

(s, 3H, OCH_3), 7.3–8.7 (m, 18H, ArH), 9.0 (br s, 1H, NH); MS (*m/z*): 430 [M^+]. Analysis: calculated for $C_{28}H_{22}N_4O$: C, 78.11; H, 5.15; N, 13.01; found: C, 78.10; H, 5.19; N, 13.06.

Pharmacology

The synthesized compounds were evaluated for analgesic, anti-inflammatory and ulcerogenic activities. For measurement of analgesic and anti-inflammatory activity, test compounds and standard drugs were administered orally by gavage as suspensions in 1% carboxy methyl cellulose (vehicle). For studies of ulcerogenic activity, test compounds and standard drugs were administered i.p. in 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 45–55%, in a 12 h light–dark cycle; they were fed standard animal feed. All animals were acclimatized for a week before use. The institutional animal ethics committee approved the protocol for the experimentation of animals (Approval no. AKCP/45/19/2005–2006).

Analgesic activity

Analgesic activity was measured using the tail-flick method (Arulmozhi et al, 2004) in Wistar albino mice (25–35 g) of either sex, selected by a random sampling technique. Diclofenac sodium (10 and 20 mg kg^{-1}) was administered orally as a reference drug for comparison. The test compounds were administered orally at 10 and 20 mg kg^{-1}. The reaction time was recorded at 30 min and 1, 2 and 3 h after the treatment, and the cut-off time was 10 s. The percentage analgesic activity (PAA) was calculated using the following formula: $([T_2 - T_1] / [10 - T_1]) \times 100$, where T_1 and T_2 are the reaction time(s) before and after treatment, respectively.

Anti-inflammatory activity

Anti-inflammatory activity was evaluated using the carrageenan-induced paw oedema test in rats (Arulmozhi et al 2004). Diclofenac sodium and the test compounds were administered at 10 and 20 mg kg^{-1}. Paw volume was measured using the mercury displacement technique with the help of a plethysmograph, immediately before carrageenan injection and after 30 min and 1, 2 and 3 h. The percentage inhibition (I) of paw oedema was calculated using the formula $I = 100 \times [1 - (a - x) / (b - y)]$ where x is the mean paw volume before administration of carrageenan and test or reference compound (test group), a is the mean paw volume after administration of carrageenan in the test group (drug treated), b is the mean paw volume after administration of carrageenan in the control group, and y is the mean paw volume before administration of carrageenan in the control group.

Evaluation of ulcerogenic index

Ulcers were induced in rats using the method described by Goyal et al (1985). Albino Wistar rats weighing 150–200 g of either sex were divided into groups of six animals. Control animals were given 10% v/v Tween 80 suspension i.p. One group received aspirin (German Remedies, Mumbai, India), 20 mg kg^{-1} i.p. once daily for 3 days. The remaining groups received a test compound, 20 mg kg^{-1} i.p. On the fourth day, the pylorus was ligated as per the method of Shay et al (1945). Animals were fasted for 36 h before pylorus ligation. Animals were sacrificed 4 h after the ligation. The stomach was removed and opened along the greater curvature. The ulcerogenic index was determined by the method of Ganguly and Bhatnagar (1973).

Statistical analysis

The pharmacological activity of the synthesized compounds was evaluated using one-way analysis of variance. In all cases, post-hoc comparisons of the means of individual groups were performed using Tukey's test. A P value below 0.05 was considered to be significant. All values are expressed as mean ± s.d. Statistical analysis of the ulcerogenic index was determined using the Kruskal–Wallis nonparametric analysis of variance test. Significant results were analysed by two-group comparisons using Dunn's test. Ulcerogenic index values were expressed as mean ± s.d., and differences between the groups were determined using analysis of variance. A P value below 0.05 was considered significant. Statistical analysis was performed using GraphPad Prism version 3.0 (GraphPad Software, Inc. San Diego, CA, USA).

Results and Discussion

The synthetic route shown in Figure 1 outlines the chemistry of the present work. The key intermediate 3-(3-methylphenyl)-2-thioxo-2,3-dihydro-1*H*-quinazolin-4-one **(4)** was obtained by reacting 3-methyl aniline **(1)** with carbon disulfide and sodium hydroxide in DMSO 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-(3-methylphenyl)-2-thioxo-2,3-dihydro-1*H*-quinazolin-4-one **(4)** via the thiourea intermediate (75% yield). The product obtained was cyclic and was not an open-chain thiourea **(3a)**. The IR spectrum of **4** showed intense peaks at 3218 cm^{-1} for cyclic thiourea (NH), 1680 cm^{-1} for carbonyl (C = O) and 1200 cm^{-1} for thioxo (C = S) stretching. ^1H NMR spectrum of **4** showed a singlet at δ 1.3–1.36 ppm due to the CH_3 group, a multiplet at δ 7.0–8.1 ppm for aromatic (8H) protons and a singlet at δ 10.3 ppm, indicating the presence of NH. Data from the elemental analyses agreed with the assigned structure. Furthermore, the molecular ion peak recorded in the MS agreed with the molecular weight of the compound.

The 2-methysulfanyl-3-(3-methylphenyl)-3*H*-quinazolin-4-one **(5)** was obtained by dissolving **4** in 2% alcoholic sodium hydroxide solution and methylating with dimethyl sulfate with stirring at room temperature. The IR spectrum of compound **5** showed that the NH and C = S stretching signals of cyclic thiourea had disappeared and showed a peak for carbonyl (C = O) stretching at 1678 cm^{-1}. The ^1H

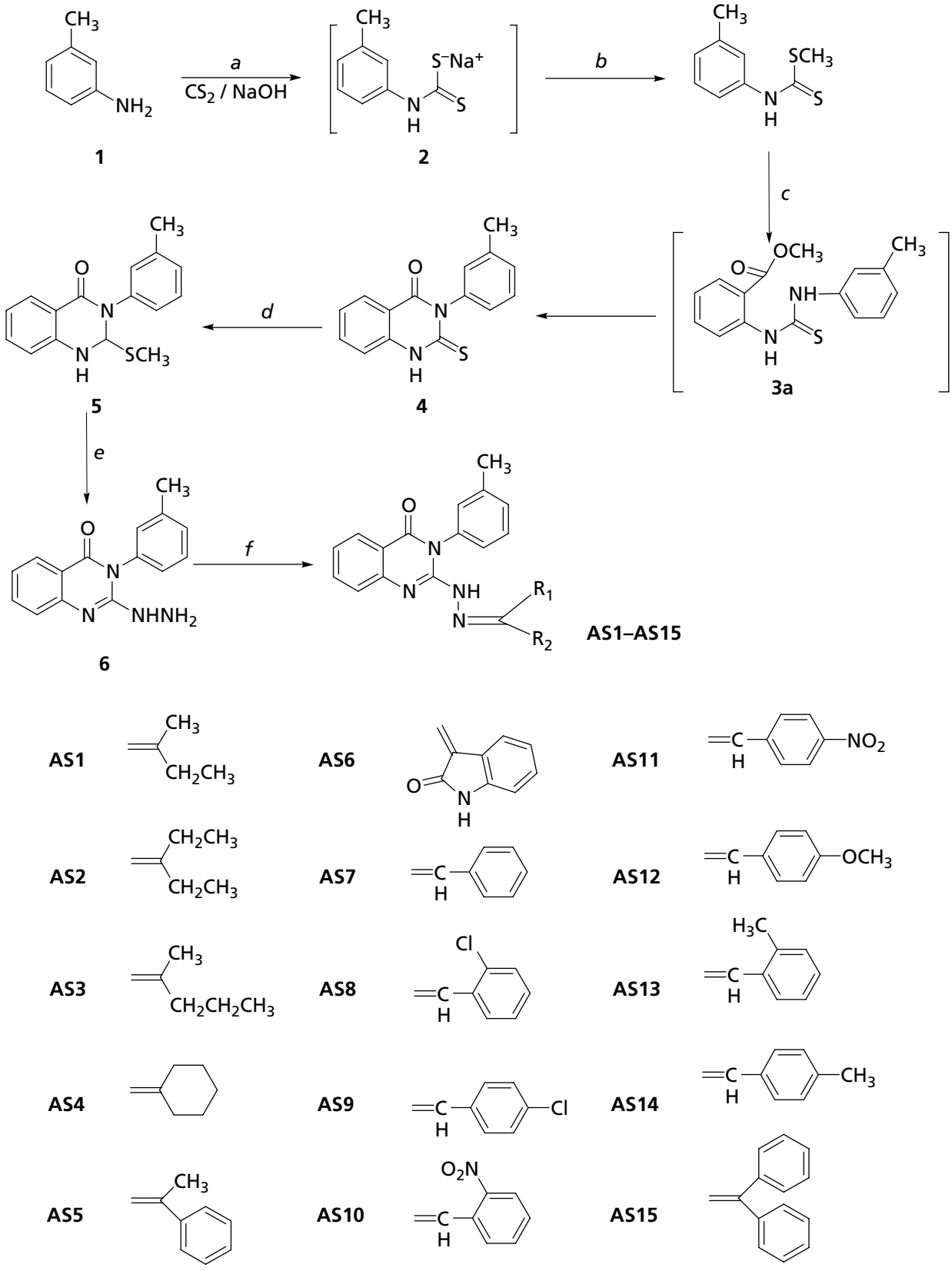

Figure 1 Synthesis of 3-(3-methylphenyl)-2-substituted amino-3*H*-quinazolin-4-ones. Reagents and conditions: ***a*** DMSO, room temperature, 30 min; ***b*** $(CH_3)_2SO_4$, 5–10 °C, 2 h; ***c*** methyl anthranilate ***3***, K_2CO_3, ethanol reflux for 32 h; ***d*** 2% alcoholic NaOH, $(CH_3)_2SO_4$, room temperature, 1 h; ***e*** NH_2NH_2, K_2CO_3, ethanol reflux for 22 h; ***f*** $(R_2R_1)CO$; CH_3COOH reflux, 41 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 aromatic (8H) protons. Data from the elemental analyses and molecular ion peak recorded in the MS further confirmed the assigned structure.

Nucleophilic displacement of the methylthio group of compound **5** with hydrazine hydrate was carried out using ethanol as the solvent to afford 2-hydrazino-3-(3-methylphenyl)-3*H*-quinazolin-4-one **6**. The long duration of reaction (32 h) required might be due to the presence of a bulky aromatic ring at position 3, which might have reduced the reactivity of the quinazoline ring system at the C-2 position. Formation of compound **6** was confirmed by the presence of NH and NH_2 signals at 3320–3205 cm^{-1} in the IR spectrum. It also showed a peak for carbonyl (C=O) at 1674 cm^{-1}. The ^1H NMR spectrum of compound **6** showed singlets at δ 2.33 ppm, 4.93 ppm and 8.63 ppm, due to CH_3, NH_2 and NH, respectively; a multiplet at δ 7.15–8.08 ppm was observed for aromatic (8H) protons. Data from the elemental analyses agreed with the assigned structure, and the molecular ion peak recorded in the MS is in agreement with the molecular weight of the compound.

The title compounds 3-(3-methylphenyl)-2-substituted amino-3*H*-quinazolin-4-ones **AS1–AS15** were obtained by condensation of the amino group of 2-hydrazino-3-(3-methylphenyl)-3*H*-quinazolin-4-one **(6)** with a variety of aldehydes and ketones. The formation of **AS1–AS15** was indicated by disappearance of the peak due to NH_2 of the starting material in the IR and ^1H NMR spectra for all compounds. The IR ^1H NMR spectra of these compounds showed the presence of peaks due to $N=CR^1R^2$, carbonyl (C=O), NH and aryl groups. The MS of the title compounds showed molecular ion peaks corresponding to their molecular formulae. The MS of compounds **AS1–A15** showed a common peak at m/z 144, corresponding to the quinazolin-4-one moiety. $M^+ + 2$ 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 compared with M^+ peaks were in the ratio of 1:3. Elemental (C, H, N) analysis satisfactorily confirmed the elemental composition and purity of the synthesized compounds.

Analgesic activity (Table 1) was measured using the tail-flick technique (Arulmozhi et al 2004) in mice. The

Table 1 Analgesic activity of the test compounds (measured using the tail-flick test in mice)

Compound	Dose (mg kg^{-1})	Percentage analgesic activity			
		30 min	1 h	2 h	3 h
AS1	10	56 ± 1.36**	59 ± 1.69**	63 ± 1.61**	39 ± 1.73*
	20	67 ± 1.79***	72 ± 1.52***	76 ± 1.29***	48 ± 1.90*
AS2	10	60 ± 1.74**	67 ± 1.05***	71 ± 1.36***	43 ± 1.94*
	20	72 ± 1.38***	77 ± 1.73***	79 ± 1.71***	52 ± 1.65*
AS3	10	59 ± 1.48**	61 ± 1.63***	65 ± 1.71***	41 ± 1.29*
	20	69 ± 1.83***	76 ± 1.65***	78 ± 1.61***	52 ± 1.28*
AS4	10	39 ± 1.84*	48 ± 1.53*	53 ± 1.84*	36 ± 1.51*
	20	50 ± 1.36*	56 ± 1.94**	59 ± 1.84**	42 ± 1.25*
AS5	10	47 ± 1.95*	49 ± 1.49*	54 ± 1.32*	38 ± 1.83*
	20	59 ± 1.73**	63 ± 1.28***	68 ± 1.34***	46 ± 1.42*
AS6	10	43 ± 1.74*	47 ± 1.92*	49 ± 1.48*	31 ± 1.81*
	20	52 ± 1.36*	55 ± 1.73**	60 ± 1.38*	38 ± 1.03*
AS7	10	42 ± 1.71*	46 ± 1.47*	49 ± 1.33*	37 ± 1.26*
	20	49 ± 1.46*	58 ± 1.94**	58 ± 1.53**	46 ± 1.05*
AS8	10	33 ± 1.62	39 ± 1.49*	42 ± 1.73	33 ± 1.27*
	20	42 ± 1.36*	47 ± 1.72*	50 ± 1.54*	38 ± 1.29*
AS9	10	39 ± 1.73	39 ± 1.20*	48 ± 1.48*	31 ± 1.42
	20	48 ± 1.63*	52 ± 1.69*	53 ± 1.52*	37 ± 1.73*
AS10	10	39 ± 1.53*	44 ± 1.72*	49 ± 1.16*	34 ± 1.72*
	20	51 ± 1.73*	55 ± 1.48**	58 ± 1.59*	36 ± 1.27*
AS11	10	36 ± 1.08*	41 ± 1.63*	46 ± 1.72*	35 ± 1.74*
	20	43 ± 1.74*	49 ± 1.36*	52 ± 1.74*	38 ± 1.95*
AS12	10	38 ± 1.83*	44 ± 1.57*	49 ± 1.85*	40 ± 1.84*
	20	47 ± 1.05*	49 ± 1.73*	53 ± 1.27*	46 ± 1.63*
AS13	10	42 ± 1.74*	46 ± 1.85	49 ± 1.72*	37 ± 1.27*
	20	51 ± 1.56*	56 ± 1.83**	58 ± 1.61**	40 ± 1.94*
AS14	10	45 ± 1.83*	49 ± 1.47*	52 ± 1.75*	44 ± 1.52*
	20	56 ± 1.84**	58 ± 1.67**	60 ± 1.43**	49 ± 1.72*
AS15	10	43 ± 1.51*	45 ± 1.79*	48 ± 1.52	41 ± 1.74*
	20	51 ± 1.45*	56 ± 1.85**	59 ± 1.53**	48 ± 1.36*
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*

Data are expressed as mean ± s.d. from six different experiments done in duplicate. *$P < 0.05$, **$P < 0.01$,***$P < 0.001$ compared with control.

compounds exhibited moderate analgesic activity at 30 min, which increased at 1 h and reached a peak at 2 h, after which it declined. Compound **AS1** with a 1-methylpropylidene substituent showed good activity; the increased lipophilicity associated with the 1-ethylpropylidene group in compound **AS2** increased the analgesic activity. Replacement of the 1-ethylpropylidene group with its isomer 1-methylbutylidene group (**AS3**) retained the activity. Replacement of the C-2 alkyl chain with a cycloalkyl group and an aralkyl group (compounds **AS4** and **AS5**, respectively) led to a moderate decrease in activity. Placement of an aryl group at the N-3 position (compounds **AS6, AS7** and **AS13–AS15**) also resulted in decreased activity. Placement of an electron-withdrawing group at the N-3 aryl ring (compounds **AS8–AS12**) leads to a further decrease in activity. The compound 2-(1-ethylpropylidene)-hydrazino-3-(3-methylphenyl)-quinazolin-4(3*H*)-one **(AS2)** was the most active analgesic agent, and was moderately more potent than the reference standard diclofenac.

Anti-inflammatory activity was evaluated using the carrageenan-induced paw oedema test in rats (Arulmozhi et al 2004) and is shown in Table 2. All the test compounds protected rats from carrageenan-induced inflammation moderately at 30 min, increasing at 1 h and reaching a peak at 2 h, declining thereafter. The compound 2-(1-methylbutylidene)-hydrazino-3-(3-methylphenyl)-quinazolin-4(3*H*)-one **(AS3)** was the most potent anti-inflammatory of the series and was moderately more potent than diclofenac sodium. Compound **AS2** 2-(1-ethylpropylidene)-hydrazino-3-(3-methylphenyl)-quinazolin-4(3*H*)-one was equipotent with diclofenac sodium in terms of anti-inflammatory activity.

The ulcerogenic index of the test compounds (Table 3) revealed that the compounds with aliphatic substituents (compounds **AS1–AS4**) showed negligible ulcerogenic index; those with aryl substituents (compounds **AS5–AS7** and **AS13–AS15**) exhibited little increase in ulcerogenic index; those with aryl substituents containing electron-withdrawing groups (compounds **AS8–AS12**) exhibited the highest ulcerogenic indices. The test compounds exhibited 35–50% of the ulcerogenic activity of the reference standards aspirin and diclofenac (ulcerogenic index 1.73 ± 0.41 and 1.65 ± 0.59, respectively). Among the test compounds,

Table 2 Anti-inflammatory activity of the test compounds (measured using the carrageenan-induced paw oedema test in rats)

Compound	Dose (mg kg^{-1})	Percentage protection			
		30 min	1 h	2 h	3 h
AS1	10	42 ± 1.51*	45 ± 1.36*	46 ± 1.52**	29 ± 1.63*
	20	49 ± 1.62**	58 ± 1.62**	59 ± 1.91**	43 ± 1.26*
AS2	10	45 ± 1.64**	49 ± 1.82*	55 ± 1.16**	37 ± 1.72*
	20	53 ± 1.48**	62 ± 1.18***	68 ± 1.39**	46 ± 1.02*
AS3	10	46 ± 1.54**	51 ± 1.72**	57 ± 1.61**	39 ± 1.93*
	20	57 ± 1.16**	65 ± 1.84***	69 ± 1.58***	47 ± 1.56*
AS4	10	35 ± 1.73*	38 ± 1.15	44 ± 1.84*	32 ± 1.63*
	20	44 ± 1.64*	50 ± 1.73**	56 ± 1.26**	38 ± 1.45*
AS5	10	39 ± 1.27*	43 ± 1.89*	45 ± 1.63**	30 ± 1.48*
	20	47 ± 1.25**	50 ± 1.68**	55 ± 1.56**	42 ± 1.83*
AS6	10	34 ± 1.79*	39 ± 1.33*	43 ± 1.62*	34 ± 1.31*
	20	39 ± 1.54*	43 ± 1.62*	45 ± 1.81**	38 ± 1.36*
AS7	10	33 ± 1.85*	38 ± 1.26*	43 ± 1.47*	29 ± 1.85*
	20	39 ± 1.45*	45 ± 1.26**	50 ± 1.64**	37 ± 1.84*
AS8	10	29 ± 1.25*	32 ± 1.12*	37 ± 1.37*	28 ± 1.64*
	20	35 ± 1.62*	39 ± 1.23*	43 ± 1.42*	32 ± 1.14*
AS9	10	36 ± 1.53*	37 ± 1.82*	39 ± 1.58*	37 ± 1.46*
	20	38 ± 1.47*	42 ± 1.82*	47 ± 1.54*	31 ± 1.89
AS10	10	35 ± 1.54*	37 ± 1.26*	41 ± 1.48*	28 ± 1.25*
	20	39 ± 1.24*	43 ± 1.74*	46 ± 1.52*	39 ± 1.57*
AS11	10	30 ± 1.45*	36 ± 1.57*	38 ± 1.24*	29 ± 1.84*
	20	42 ± 1.45*	46 ± 1.24*	48 ± 1.92*	35 ± 1.24*
AS12	10	29 ± 1.22*	36 ± 1.42*	36 ± 1.09*	28 ± 1.45*
	20	38 ± 1.24*	40 ± 1.56*	47 ± 1.42**	33 ± 1.84*
AS13	10	40 ± 1.74	43 ± 1.54*	47 ± 1.72*	31 ± 1.64*
	20	45 ± 1.24*	49 ± 1.45**	53 ± 1.27**	37 ± 1.84*
AS14	10	35 ± 1.45*	39 ± 1.28	43 ± 1.74*	31 ± 1.45*
	20	47 ± 1.42**	49 ± 1.64**	53 ± 1.56**	42 ± 1.56*
AS15	10	36 ± 1.54*	38 ± 1.42*	39 ± 1.58*	33 ± 1.74*
	20	46 ± 1.24**	48 ± 1.55**	49 ± 1.64**	41 ± 1.09*
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**

Data are expressed as mean ± s.d. from six experiments done in duplicate. *$P < 0.05$, **$P < 0.01$,***$P < 0.001$ compared with control.

Table 3 Ulcerogenicity index of the test compounds in rats

Compound	Ulcerogenicity index
AS1	0.53 ± 1.33*
AS2	0.59 ± 1.20*
AS3	0.56 ± 1.32*
AS4	0.60 ± 1.18*
AS5	0.63 ± 1.06*
AS6	0.65 ± 1.53*
AS7	0.69 ± 1.28*
AS8	0.89 ± 1.54*
AS9	0.83 ± 1.59*
AS10	0.85 ± 1.21*
AS11	0.82 ± 1.94*
AS12	0.80 ± 1.57*
AS13	0.69 ± 1.31*
AS14	0.71 ± 1.73*
AS15	0.68 ± 1.23*
Control	0.15 ± 0.32
Diclofenac	1.65 ± 0.59**
Aspirin	1.73 ± 0.41**

Data are expressed as mean ± s.d. from six experiments done in duplicate. *$P < 0.05$, **$P < 0.01$.

AS2 and **AS3** (2-(1-ethylpropylidene)-hydrazino-3-(3-methylphenyl)-quinazolin-4(3*H*)-one and 2-(1-methyl-butylidene)-hydrazino-3-(3-methylphenyl)-quinazolin-4(3*H*)-one) exhibited the lowest ulcerogenic indices (0.59 ± 1.20 and 0.56 ± 1.32, respectively), which is about one-third of the ulcerogenic index of aspirin and diclofenac. Compound **AS8** (2-(*N*′-(3-chloro-benzylidene-hydrazino)-3-(3-methyl-phenyl)-quinazolin-4(3*H*)-one) had the highest ulcerogenic index (0.89 ± 1.54), which is about 50% of that of aspirin and diclofenac.

Conclusion

In our earlier studies (Alagarsamy et al 2002, 2003b,c, 2004) we observed that the presence of alkyl groups on the C-2 position of the quinazoline ring resulted in higher analgesic and anti-inflammatory activities over aryl groups at the N-3 position of the quinazoline core. We have made a substitution in the C-2 position of the quinazoline ring in order to increase the lipophilicity of the molecule, which enhanced the analgesic and anti-inflammatory activities. The most active compound of the C-2 phenyl series was 1-diethyl-3-(2-phenylquinazolin-3-yl-4(3*H*)-one) thiourea (**7**) (Figure 2), which showed 44% and 58% PAA and 38% and 53% of maximum anti-inflammatory activity at doses of 10 and 20 mg kg^{-1} respectively, 2 h after administration (Alagarsamy et al 2002). By comparison, the lead molecule of the C-2 methyl series, 1-pyrrolidinyl-3-(2-methyl quinazolin-3-yl-4(3*H*)-one) thiourea (**8**) (Figure 2), exhibited 50% and 65% PAA and 44% and 60% anti-inflammatory activity at doses of 10 and 20 mg kg^{-1}, respectively, 2 h after administration (Alagarsamy et al 2003c). Introduction of a sulfur atom at the C-2 position in the above series (i.e. by placing a methylthio group at the C-2 position) (Alagarsamy et al 2004) gave 1-diethyl-3-(2-methylthio quinazolin-3-yl-4(3*H*)-one) thiourea (**9**) (Figure 2), which exhibited 56% and 67% PAA and 40% and 62% of maximum anti-inflammatory activity at 10 and 20 mg kg^{-1} respectively, 2 h after administration.

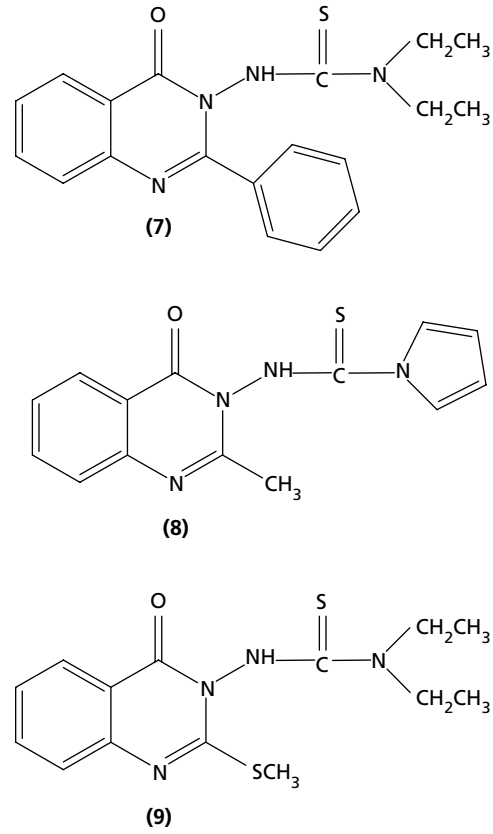

Figure 2 The most active compounds of the C-2 phenyl series (**7**) 1-diethyl-3-(2-phenyl quinazolin-3-yl-4(3*H*)-one) thiourea and the C-2 methyl series (**8**) 1-pyrrolidinyl-3-(2-methyl quinazolin-3-yl-4(3*H*) -one) thiourea. (**9**) 1-diethyl-3-(2-methylthio quinazolin-3-yl-4(3*H*)-one) thiourea.

The series reported here showed moderate enhancement of analgesic and anti-inflammatory activities: compound **AS2** exhibited 71% and 79% PAA at 10 and 20 mg kg^{-1}, respectively, 2 h after administration. At 10 and 20 mg kg^{-1}, compound **AS3** showed 57% and 69%, respectively, of maximum anti-inflammatory activity. Interestingly, these compounds showed one-third of the ulcerogenic activity of the reference NSAIDs aspirin and diclofenac. Hence this series could be developed as a novel class of analgesic and anti-inflammatory agents. Further structural modification of the C-2 position of the quinazoline ring is planned, to increase the analgesic and anti-inflammatory activities whilst decreasing the ulcerogenic index.

References

Alagarsamy, V., Raja Salomon, V., Vanikavitha, G., Paluchamy, V., Ravichandran, M., Arnald Sujin, A., Thangathirupathy, A., Amuthalakshmi, S., Revathi, R. (2002) Synthesis, analgesic, anti-inflammatory and antibacterial activities of some novel 2-phenyl-3-substituted quinazolin-4(3*H*)-ones. *Biol. Pharm. Bull*. **25**: 1432–1435

Alagarsamy, V., Revathi, R., Vijayakumar, S., Ramseshu, K. V. (2003a) Synthesis and pharmacological investigation of some novel 2,3-disubstituted quinazolin-4(3*H*)-ones as analgesic and anti-inflammatory agents. *Pharmazie* **58**: 4–8

Alagarsamy, V., Muthukumar, V., Pavalarani, N., Vasanthanathan, P., Revathi, R. (2003b) Synthesis, analgesic and anti-inflammatory activities of some novel 2,3-disubstituted quinazolin-4(3*H*)-ones. *Biol. Pharm. Bull*. **26**: 557–559

Alagarsamy, V., Murugananthan, G., Venkateshperumal, R. (2003c) Synthesis, analgesic, anti-inflammatory and antibacterial activities of some novel 2-methyl-3-substituted quinazolin-4(3*H*)-ones. *Biol. Pharm. Bull*. **26**: 1711–1714

Alagarsamy, V., Rajesh, R., Meena, R., Vijaykumar, S., Ramseshu, K. V., Durai Anandakumar, T. (2004) Synthesis, analgesic, anti-inflammatory and antibacterial activities of some novel 2-methylthio-3-substituted quinazolin-4(3*H*)-ones. *Biol. Pharm. Bull*. **27**: 652–656

Alagarsamy, V., Thangathiruppathy, A., Mandal, S. C., Rajasekaran, S., Vijayakumar, S., Revathi, R., Anburaj, J., Arun Kumar, S., Rajesh, S. (2006) Pharmacological evaluaion of 2-substituted (1,3,4) thiadiazolo quinazolines. *Indian J. Pharm. Sci*. **68**: 108–111

Arulmozhi, D. K., Veeranjaneyulu, A., Bodhankar, S. L., Arora, S. K. (2004) Investigations into the antinociceptive activity of Sapindus trifoliatus in various pain models. *J. Pharm. Pharmacol*. **56**: 655–661

Beuck, M. (1999) Nonsteroidal antiinflammatory drugs: a new generation of cyclooxygenase inhibitors. *Angew. Chem. Int. Ed*. **38**: 631–633

Bhalla, M., Srivatava, V. K., Bhalla, T. N., Shankar, K. (1993) Anti-inflammatory and analgesic activities of indolyl quinazolones and their congeners. *Arzneim. Forsch. Drug Res*. **43**: 595–600

Chao, Q., Deng, L., Shih, H., Leoni, L. M., Genini, D., Carson, D. A, Cottam, H. B. (1999) Substituted isoquinolines and quinazolines as potential anti-inflammatory agents. *J. Med. Chem*. **42**: 3860–3873

Ganguly, A. K., Bhatnagar, O. P. (1973) Effect of bilateral adrenalectomy on production of restraint ulcers in the stomach of albino rats. *Can. J. Physiol. Pharmacol*. **51**: 748–750

Goyal R. K., Chakrabarti, A., Sanyal, A. K. (1985) The effect of biological variables on the anti-ulcerogenic effect of vegetable plantain banana. *Planta Med.* **29**: 85–88

Shay, M., Komarov, S. A., Fels, D., Meranze, D., Grunstein, H., Siplet, H. (1945) A simple method for the uniform production of gastric ulceration in the rats. *Gastroenterology* **5**: 43–61

Van Ryn, J. (1971) Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature*: *New Biology* **231**: 232–235

Van Ryn, J., Botting, R. M. (1945) New insights into the mode of action of anti-inflammatory drugs. *Inflamm. Res*. **44**: 1–10

Van Ryn, J., Trummlitz. G., Pairet, M. (2000) COX-2 selectivity and inflammatory processes. *Curr. Med. Chem*. **7**: 1145–1161

Zappalà, M., Grasso, S., Micale, N., Zuccalà, G., Menniti, F. S., Ferreri, G., De Sarro, G., De Micheli, C. (2003) 1-Aryl-6,7-methylenedioxy-3H-quinazolin-4-ones as anticonvulsant agents. *Bioorg. Med. Chem. Lett*. **13**: 4427–4430