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Synthesis and pharmacological investigation of novel 1-substituted-4-(4-substituted phenyl)-4*H*-[1,2,4]triazolo[4,3-*a*]quinazolin-5-ones as a new class of H_1-antihistamine agents

V. Alagarsamy, Rajani Giridhar and M. R. Yadav

Abstract

A series of novel 1-substituted-4-(4-substituted phenyl)-4*H*-[1,2,4]triazolo[4,3-*a*]quinazolin-5-ones was synthesized by the cyclization of 2-hydrazino-3-(4-substituted phenyl)-3*H*-quinazolin-4-one with various one-carbon donors. The starting material, 2-hydrazino-3-(4-substituted phenyl)-3*H*-quinazolin-4-one, was synthesized from 4-substituted aniline by a novel innovative route. When tested for in-vivo H_1-antihistamine activity on conscious guinea-pigs, all the test compounds significantly protected the animals against histamine-induced bronchospasm. The compound 1-methyl-4-(4-chloro phenyl)-4*H*-[1,2,4]triazolo[4,3-*a*]quinazolin-5-one (**VII**) was more potent (72.71% protection), and 1-methyl-4-(4-methoxy phenyl)-4*H*-[1,2,4]triazolo[4,3-*a*]quinazolin-5-one (**II**) was equipotent (71% protection), when compared with the reference standard, chlorpheniramine maleate (71% protection). Compounds **II** and **VII** showed negligible sedation (5% and 8% respectively) when compared with chlorpheniramine maleate (25%). Compounds **II** and **VII** could serve as prototype molecules for further development as a new class of H_1-antihistamines.

Introduction

First generation antihistamines penetrate the blood–brain barrier and also possess anticholinergic properties; this has led to the development of a second generation of H_1-antagonists (Simons & Simons 1994) such as terfenadine, cetirizine and astemizole. A common feature of first generation compounds is two aryl or heteroaryl rings linked to an aliphatic tertiary amine via the side chain (Ellis et al 1985) (e.g. diphenhydramine and pheniramine). The second generation compounds (terfenadine and cetirizine) also contain many of the structural features of first generation compounds. The real breakthrough of non-sedative antihistamines came in the early 1980s with the discovery of modern antihistamines that exhibit potent antihistamine activity without sedative effects (Carr & Meyer 1982). Condensed heterocycles containing a new generation of H_1-antihistamines (e.g. loratadine, azelastine and flazelastine) that do not possess the above-mentioned pharmacophore for H_1-antihistamines gave way to the discovery of many novel antihistamines such as temelastine (Mc Call et al 1985) and mangostin (Chirungsrilerd et al 1987). Quinazolines and condensed quinazolines show excellent antihistamine activity (Wade 1984; West & July 1981). Continuing our search for quinazoline derivatives with potent antihistamine activity and reduced sedative effects (Alagarsamy et al 2002; Alagarsamy 2004), we aimed to prepare a series of 1,2,4-triazolo-4*H*-[4,3-*a*]quinazolin-5-ones containing 4-substituted phenyl substitution at position 4 and alkyl substitution at position 1. The title compounds were synthesized by the cyclization of 2-hydrazino-3-(4-substituted phenyl)-3*H*-quinazolin-4-one (**6**) with various one-carbon donors. The 2-hydrazino-3-(4-substituted phenyl)-3*H*-quinazolin-4-one (**6**) was synthesized from 4-substituted aniline (**1**) by a novel innovative route. Spectral data (IR, NMR and mass spectra) confirmed the structures of the synthesized compounds; the purity of the compounds was determined by microanalysis. The synthesized compounds were tested for their in-vivo H_1-antihistamine activity in conscious guinea-pigs. As sedation is one of the major side-effects associated with antihistamines, the test

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compounds were also evaluated for their sedative potential by measuring the reduction in locomotor activity using an actophotometer.

Materials and Methods

Chemistry

Melting points were determined in open capillary tubes on a Thomas Hoover apparatus and are uncorrected. IR spectra were recorded in KBr on a Shimadzu FT-IR, 8300 spectrometer (cm^{-1}), mass spectra were recorded on a MASPEC msw 9629 mass spectrometer at 70 eV, and ^{1}H NMR spectra were recorded on a Varian 300 MHz spectrometer, using tetramethylsilane as internal standard. Elemental analyses were performed on a Carlo erba 1108 instrument.

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

A solution of 4-methoxy aniline (**1**) (0.02 mol) in dimethylsulfoxide (DMSO) (10 mL) was stirred vigorously. To this was added carbon disulfide (1.6 mL) and aqueous sodium hydroxide (1.2 mL; 20 mol) dropwise during 30 min with stirring. Dimethylsulfate (0.02 mol) was added gradually, keeping the reaction mixture stirred 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*-(4-methoxyphenyl)-methyl dithiocarbamic acid (0.01 mol) were dissolved in ethanol (20 mL). Anhydrous potassium carbonate (100 mg) was then added and refluxed for 21 h. The reaction mixture was cooled in ice. The solid separated was filtered and purified by dissolving in 10% alcoholic sodium hydroxide solution and reprecipitated by treating with dilute hydrochloric acid. The solid obtained was filtered, washed with water, dried and recrystallized from ethanol. Yield 80%, mp 296–300°C; IR (KBr) cm^{-1}: 3218 (NH), 1680 (C=O), 1593 (C=C), 1200 (C=S); ^{1}H NMR ($CDCl_3$) δ (ppm): 3.88 (s, 3H, OCH_3), 7.0–8.1 (m, 8H, ArH), 10.36 (s, 1H, NH); MS (m/z): 284 [M^+]. Anal. calcd for $C_{15}H_{12}N_2O_2S$: C, 63.36; H, 4.25; N, 9.85. Found: C, 63.29; H, 4.21; N, 9.91.

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

This was prepared using the method described above. Yield 74%, mp 319–321°C. IR (KBr) cm^{-1}: 3210 (NH), 1690 (C=O), 1210 (C=S); ^{1}H NMR ($CDCl_3$) δ (ppm): 7.5–8.2 (m, 8H, ArH), 10.36 (s, 1H, NH); MS (m/z): 288 [M^+], 290 [M^++2]. Anal. calcd for $C_{14}H_9N_2OSCl$: C, 58.23; H, 03.14; N, 9.70. Found: C, 58.32; H, 03.11; N, 09.74.

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

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

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

This was prepared using the method described above. Yield 80%, mp 136–139°C; IR (KBr) cm^{-1}: 1680 (C=O); ^{1}H NMR ($CDCl_3$) δ (ppm): 2.7 (s, 3H, SCH_3), 7.3–8.4 (m, 8H ArH); MS (m/z): 302 [M^+], 304 [M^++2]; Anal. calcd for $C_{15}H_{11}N_2OSCl$: C, 59.50; H, 03.66; N, 9.25. Found: C, 59.53; H, 03.61; N, 9.31.

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

2-Methylsulfanyl-3-(4-methoxyphenyl)-3*H*-quinazolin-4-one (**5a**) (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 30 h. The reaction mixture was cooled and poured into ice water. The solid obtained was filtered, washed with water, dried and recrystallized from a chloroform/benzene (25:75) mixture. Yield 74%, mp 196–200°C; IR (KBr) cm^{-1}: 3350, 3320 ($NHNH_2$), 1674 (C=O); ^{1}H NMR ($CDCl_3$) δ (ppm): 3.79 (s, 3H, OCH_3), 4.95 (s, 2H, NH_2), 6.82–8.06 (m, 8H, ArH), 8.56 (s, 1H, NH); MS (m/z): 282 [M^+]; Anal. calcd for $C_{15}H_{14}N_4O_2$: C, 63.82; H, 4.99; N, 19.84. Found: C, 63.71; H, 4.95; N, 19.93.

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

This was prepared using the method described above. Yield 83%, mp 162–165°C; IR (KBr) cm^{-1}: 3360, 3300 ($NHNH_2$), 1680 (C=O); ^{1}H NMR ($CDCl_3$) δ (ppm): 4.98 (s, 2H, NH_2), 6.93–8.12 (m, 8H, ArH), 8.73 (s, 1H, NH); MS (m/z): 286 [M^+], 320 [M^++2]; Anal. calcd for $C_{14}H_{11}N_4OCl$: C, 58.64; H, 03.86; N, 19.54. Found: C, 58.60; H, 03.82; N, 19.59.

*4-(4-Methoxyphenyl)-4*H-*[1,2,4] triazolo [4,3-*a*] quinazolin-5-one (**I**)*

The 2-hydrazino-3-(4-methoxyphenyl)-3*H*-quinazolin-4-one (**6a**) (0.01 mol) and formic acid (25 mL) were mixed in a round-bottomed flask and refluxed for 34 h, cooled and poured into ice water. The solid obtained was filtered, washed with water, dried and recrystallized from ethanol. IR (KBr) cm^{-1}: 1684 (C=O), 1607 (C=N); ^{1}H NMR ($CDCl_3$) δ (ppm): 3.9 (s, 3H, OCH_3), 7.1–8.4 (m, 8H, ArH), 8.6–8.7 (s, 1H, ArH); MS (m/z): 292 [M^+]. Using this procedure, compounds **II**–**X** were prepared.

*1-Methyl-4-(4-methoxyphenyl)-4*H-*[1,2,4] triazolo [4,3-*a*] quinazolin-5-one (**II**)*

IR (KBr) cm^{-1}: 1707 (C=O), 1605 (C=N); ^{1}H NMR ($CDCl_3$) δ (ppm): 2.5–2.6 (s, 3H, CH_3), 3.6–3.7 (s, 3H, OCH_3), 7.0–8.1 (m, 8H, ArH); MS (m/z): 306 [M^+].

*1-Ethyl-4-(4-methoxyphenyl)-4*H*-[1,2,4] triazolo [4,3-*a*] quinazolin-5-one (**III**)*
IR (KBr) cm^{-1}: 1684 (C=O), 1601 (C=N); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.3–1.35 (t, 3H, CH_2CH_3), 2.69–2.76 (q, 2H, CH_2CH_3), 3.9 (s, 3H, OCH_3), 7.1–8.4 (m, 8H, ArH); MS (m/z): 320 [M^+].

*1-Propyl-4-(4-methoxyphenyl)-4*H*-[1,2,4] triazolo [4,3-*a*] quinazolin-5-one (**IV**)*
IR (KBr) cm^{-1}: 1683 (C=O), 1602 (C=N); ^{1}H NMR ($CDCl_3$) δ (ppm): 0.6–0.7 (t, 2H, $CH_2CH_2CH_3$), 1.2–1.3 (sext, 2H, $CH_2CH_2CH_3$), 2.0–2.1 (t, 3H, $CH_2CH_2CH_3$), 3.2–3.3 (s, 3H, OCH_3), 7.1–8.0 (m, 8H, ArH); MS (m/z): 334 [M^+].

*1-Chloromethyl-4-(4-methoxyphenyl)-4*H*-[1,2,4] triazolo [4,3-*a*] quinazolin-5-one (**V**)*
IR (KBr) cm^{-1}: 1709 (C=O), 1605 (C=N); ^{1}H NMR ($CDCl_3$) δ (ppm): 3.9 (s, 3H, OCH_3), 4.2–4.3 (s, 2H, CH_2), 7.3–8.2 (m, 8H, ArH); MS (m/z): 340 [M^+], 342 [M^++2].

*4-(4-Chlorophenyl)-4*H*-[1,2,4] triazolo [4,3-*a*] quinazolin-5-one (**VI**)*
IR (KBr) cm^{-1}: 1682 (C=O); ^{1}H NMR ($CDCl_3$) δ (ppm): 7.2–8.0 (m, 8H, ArH), 8.8–8.9 (s, 1H, ArH); MS (m/z): 296 [M^+], 298 [M^++2].

*1-Methyl-4-(4-chlorophenyl)-4*H*-[1,2,4] triazolo [4,3-*a*] quinazolin-5-one (**VII**)*
IR (KBr) cm^{-1}: 1688 (C=O), 1609 (C=N); ^{1}H NMR ($CDCl_3$) δ (ppm): 3.0–3.1 (s, 3H, CH_3), 7.3–7.4 (m, 4H, ArH), 7.5–7.8 (m, 4H, ArH); MS (m/z): 310 [M^+], 312 [M^++2].

*1-Ethyl-4-(4-chlorophenyl)-4*H*-[1,2,4] triazolo [4,3-*a*] quinazolin-5-one (**VIII**)*
IR (KBr) cm^{-1}: 1688 (C=O), 1631 (C=N); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.34–1.38 (t, 3H, CH_2CH_3), 2.72–2.79 (q, 2H, CH_2CH_3), 7.27–8.45 (m, 8H, ArH); MS (m/z): 324 [M^+], 326 [M^++2].

*1-Propyl-4-(4-chlorophenyl)-4*H*-[1,2,4] triazolo [4,3-*a*] quinazolin-5-one (**IX**)*
IR (KBr) cm^{-1}: 1689 (C=O), 1606 (C=N); ^{1}H NMR ($CDCl_3$) δ (ppm): 0.8–1.0 (t, 2H, $CH_2CH_2CH_3$), 1.6–1.8 (sext, 2H, $CH_2CH_2CH_3$) 2.6–2.8 (t, 3H, $CH_2CH_2CH_3$.), 7.2–8.5 (m, 8H, ArH); MS (m/z): 338 [M^+], 340 [M^++2].

*1-Chloromethyl-4-(4-chloro phenyl)-4*H*-[1,2,4] triazolo [4,3-*a*] quinazolin-5-one (**X**)*
IR (KBr) cm^{-1}: 1690 (C=O), 1609 (C=N); ^{1}H NMR ($CDCl_3$) δ (ppm): 4.6–4.7 (s, 2H, CH_2), 7.3–8.5 (m, 4H, ArH), 7.6–7.7 (m, 4H, ArH); MS (m/z): 345 [M^+], 347 [M^++2].

Pharmacology

The antihistamine and sedative/hypnotic activity protocols were approved by the institutional animal ethics committee (approval no. SBCP/56/15/2002-2003).

Antihistamine activity
A modification of the technique of Van Arman et al (1960) was adopted to determine the antihistamine potential of the synthesized compounds. Male Dunkin Hartley guinea-pigs (250–300 g) were fasted for 12 h. There were six animals in each group. The test compounds was administered orally at a dose of 10 mg kg^{-1} in 1% CMC and challenged with histamine aerosol (3 mL of a 0.2% aqueous solution of histamine acid chloride) in a vaponephrin pocket nebulizer sprayed into a closed transparent cage. The respiratory status, reflecting the increasing degree of bronchoconstriction, was recorded. The time of onset of convulsions (pre-convulsion) was recorded. Animals remaining stable for more than 6 min were considered protected against histamine-induced bronchospasm. The test animals were given an intraperitoneal injection of chlorpheniramine maleate (Avil; Hoechst, Mumbai, India) at a dose of 25 mg kg^{-1} for their recovery. The mean pre-convulsion time of animals treated with the test compounds was compared with the control and expressed in terms of percentage protection:

$$\% \text{ protection} = [1 - (T_1 / T_2)] \times 100$$

where T_2 is the pre-convulsive time of the test compound, and T_1 is the pre-convulsive time of the control. The activity of the test compounds was compared with the standard antihistamine chlorpheniramine maleate.

Sedative/hypnotic activity
Sedative/hypnotic activity was determined by measuring the reduction in locomotor activity using an actophotometer (Dews 1953; Khun & Van Mannen 1961). Swiss albino mice were used in groups of six animals. The basal activity score was taken and then compounds **I**–**X** and the standard chlorpheniramine maleate were administered orally at a dose of 5 mg kg^{-1} in 1% CMC. Scores were recorded at 0.5, 1, 2 and 3 h after the drug administration. The Student's *t*-test was performed to determine the significance of the exhibited activity. The percentage reduction in locomotor activity was calculated by the following formula:

$$\% \text{ reduction in motor activity} = [(A - B)/A] \times 100,$$

where A is the basal score, and B is the score after drug treatment.

Statistical analysis

Statistical analysis of the biological activity of the synthesized compounds was evaluated using a one-way analysis of variance. In all cases, post-hoc comparisons of the means of individual groups were performed using Tukey's test. A level of $P < 0.05$ denoted significance in all cases. All values are expressed as mean ± s.d. GraphPad Prism version 3.0 was used for statistical analysis (GraphPad Software, San Diego, CA, USA).

Results and Discussion

The key intermediate 3-(4-substituted phenyl)-2-thioxo-2,3-dihydro-1*H*-quinazolin-4-one (**4**) was prepared by refluxing methyl anthranilate with 4-substituted phenyl isothiocyanate in ethanol (Figure 1). However, the preparation of 4-substituted

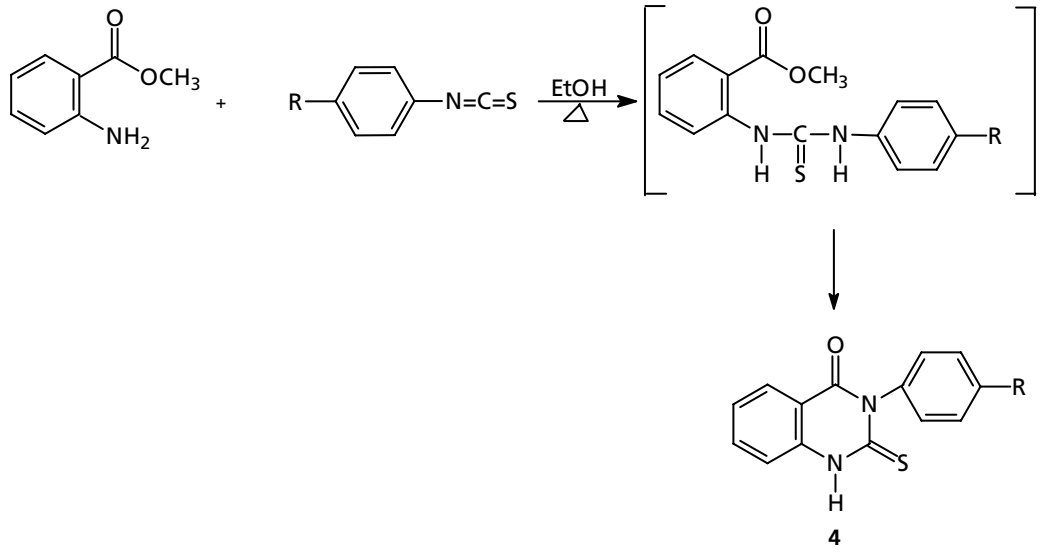


Figure 1 Synthesis of 3-(4-substituted phenyl)-2-thioxo-2,3-dihydro-1*H*-quinazolin-4-one from 4-(substituted phenyl) isothiocyanate.

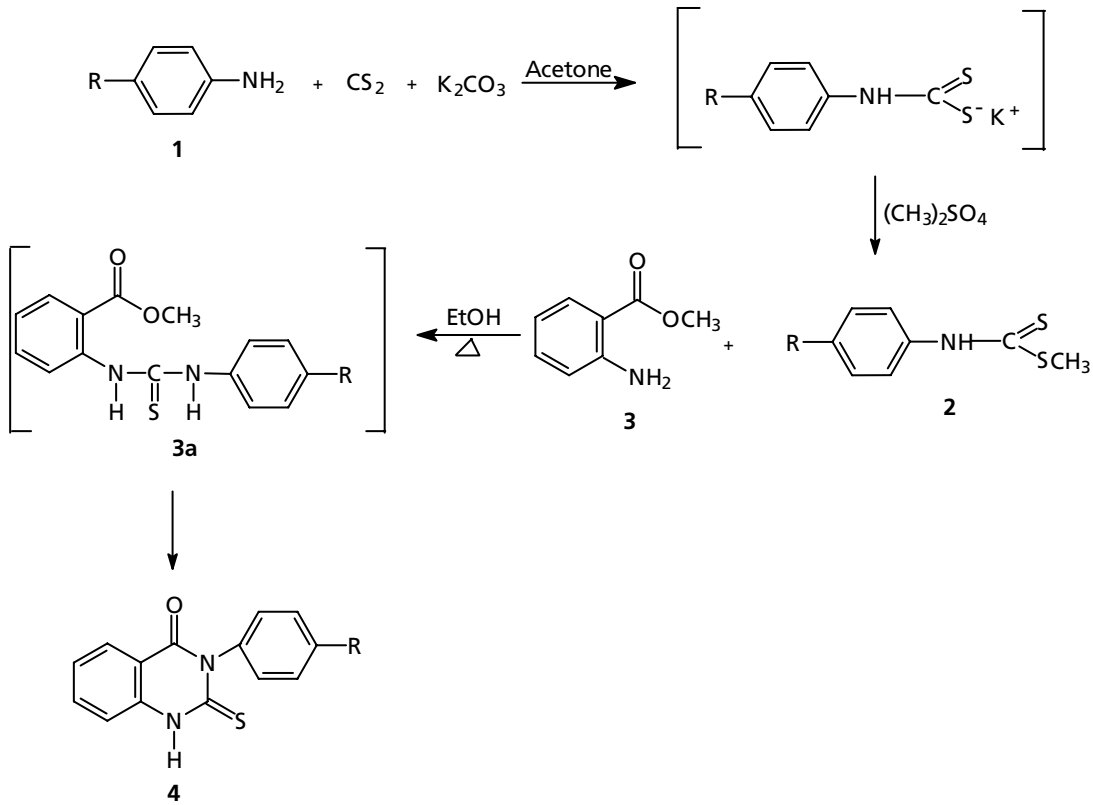


Figure 2 Synthesis of 3-(4-substituted phenyl)-2-thioxo-2,3-dihydro-1*H*-quinazolin-4-one from 4-substituted aniline.

phenyl isothiocyanate required for the reaction was a tedious and time-consuming process and the yield was also low (60%).

An alternate route to synthesize **4** was attempted (Figure 2). In this route, 4-substituted aniline (**1**) was reacted with carbon disulfide and anhydrous potassium carbonate in acetone to give potassium dithiocarbamate, which was methylated with dimethylsulfate to afford dithiocarbamic acid methyl ester (**2**). Compound **2** on reflux with methyl anthranilate (**3**) yielded **4**. This process of synthesizing **4** suffers from the following drawbacks: it is a multistep process, it requires prolonged reaction time (37 h) and the yield is also very low (30%). The method was therefore modified. Aqueous sodium hydroxide (20 mol solution) was used as a base instead of anhydrous K_2CO_3, and DMSO was substituted for acetone as

the reaction solvent (Figure 3). The use of DMSO as the reaction solvent enhanced the rate of reaction and the use of the alkali in a higher concentration helped to prevent hydrolysis of the intermediate, probably owing to less solvation. These modifications reduced the reaction time from 37 h to 23 h, and also increased the yield from 30% to 80%.

Thus, 4-substituted aniline (**1**) was treated 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-(4-substituted phenyl)-2-thioxo-2,3-dihydro-1*H*-quinazolin-4-one (**4**) via the thiourea intermediate in a good yield (80%). 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** showed intense peaks at 3218 cm^{-1} for cyclic thio urea (NH), 1680 cm^{-1} for carbonyl (C=O), and 1200 cm^{-1} for thioxo (C=S) stretching. The ^1H NMR spectrum of **4** showed a singlet at δ 3.88 ppm due to the OCH_3 group, a multiplet at δ 7–8.1 ppm for aromatic (8H) protons, and a singlet at δ 10.36 ppm, indicating the presence of NH. Data from the elemental analyses were found to be in accord with the assigned structure. Further, the molecular ion recorded in the mass spectrum was also in agreement with the molecular weight of the compound.

The 2-methylsulfanyl-3-(4-substituted phenyl)-3H-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 **5** showed disappearance of NH and C=S stretching signals of cyclic thiourea. It showed a peak for carbonyl (C=O) stretching at 1683 cm^{-1}. The ^1H NMR spectrum of compound **5** showed singlets at δ 2.5 ppm and 3.87 ppm due to SCH_3 and

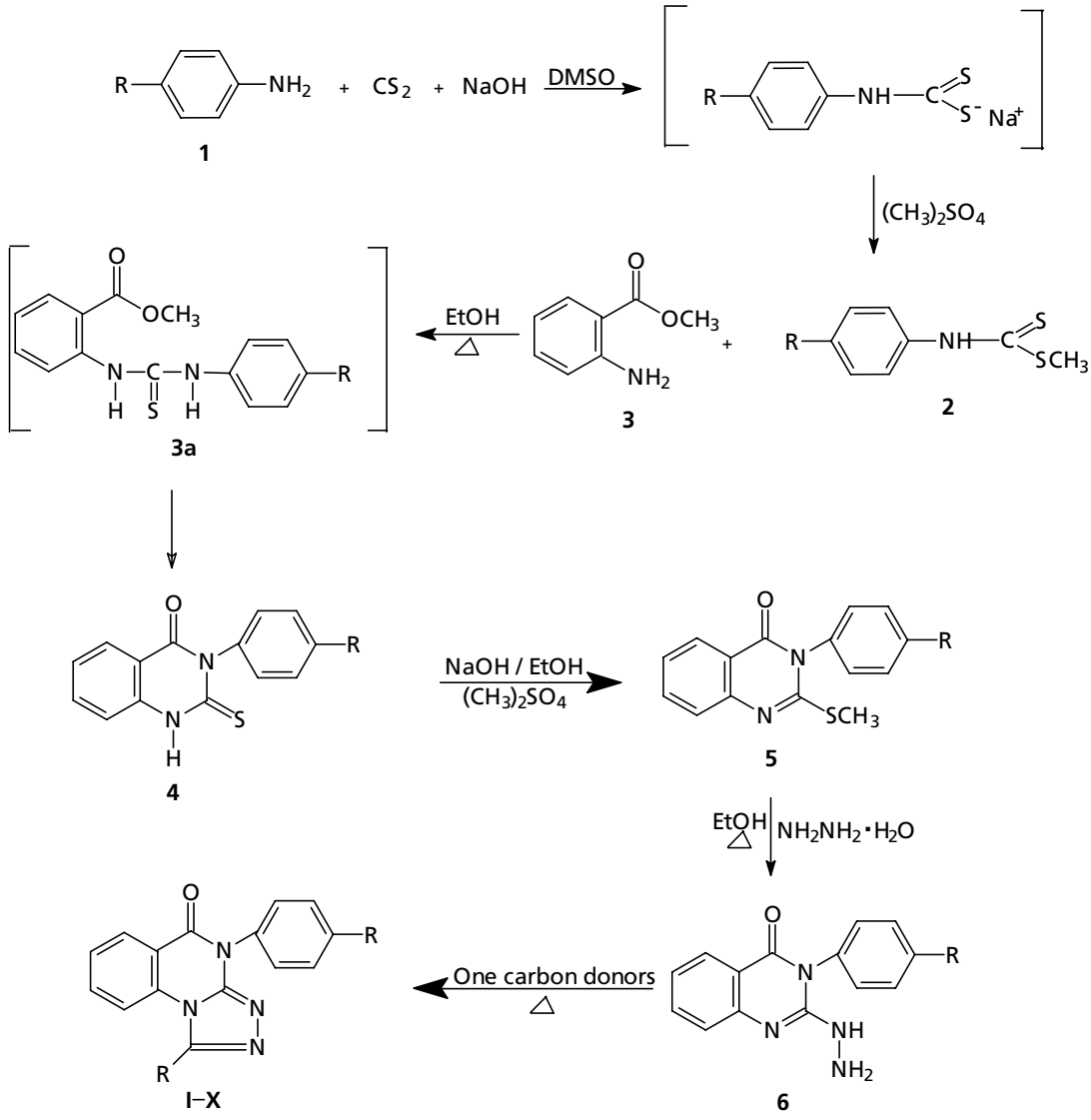


Figure 3 Synthesis of 1-substituted-4-(4-substituted phenyl)-4*H*-[1,2,4]triazolo[4,3-*a*] quinazolin-5-ones.

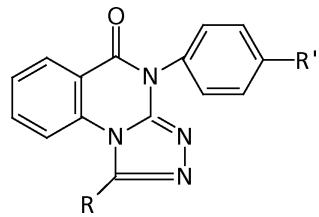
OCH_3, respectively, and a multiplet at δ 7.0–8.26 ppm was observed for aromatic (8H) protons. Data from the elemental analyses and molecular ion recorded in the mass spectrum further confirmed the assigned structure.

Nucleophilic displacement of the methylthio group of **5** with hydrazine hydrate was carried out using ethanol as solvent to afford 2-hydrazino-3-(4-substituted phenyl)-3*H*-quinazolin-4-one **6**. The long time required for the reaction (30 h) 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. The formation of **6** was confirmed by the presence of NH and NH_2 signals at 3350–3320 cm^{-1} in the IR spectrum. It also showed a peak for carbonyl (C=O) at 1674 cm^{-1}. The ^1H NMR spectrum of the compound **6** showed singlets at δ 3.79 ppm, 4.95 ppm and 8.56 ppm due to OCH_3, NH_2 and NH, respectively, and a multiplet at δ 6.82–8.06 ppm was observed for aromatic (8H) protons. Data from the elemental analyses were found to be in accord with the assigned structure. Further, the molecular ion recorded in the mass spectrum was also in agreement with the molecular weight of the compound.

The title compounds **I–X** were obtained in fair to good yields through the cyclization of **6** with a variety of one-carbon donors such as formic acid, acetic acid, propionic acid, butyric acid and chloroacetyl chloride at reflux. The formation of a cyclic product was indicated by the disappearance of peaks due to NH and NH_2 of the starting material at 3350–3320 cm^{-1} in IR spectra of all the compounds **I–X**. The ^1H NMR spectra of **I–X** showed the absence of NH and NH_2 signals. In the IR spectra, these compounds showed a peak for carbonyl (C=O) around 1680 cm^{-1}. The ^1H NMR spectra of compounds **I–X** showed a multiplet around δ 7.0–8.5 ppm integrating for aromatic protons. The mass spectra of the title compounds were in accord with the assigned structure. The mass spectra of these compounds showed molecular ion peaks corresponding to their molecular formulae. The M^++2 peaks were observed in the spectra of compounds **4b, 5b, 6b** and **V–X**, 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 1:3. In mass spectra of compounds **I–X**, the peak due to 1,2,4-triazolo [4,3-*a*] quinazoline cation appeared at m/z 168. In addition, a common peak at m/z 144, corresponding to the quinazolin-4-one moiety, appeared in all mass spectra. Elemental (C, H, N) analysis satisfactorily confirmed the elemental composition and purity of the synthesized compounds. The physical data of the title compounds is presented in Table 1.

Ten compounds containing the 1,4-disubstituted [1,2,4] triazolo quinazoline ring system were evaluated for their in-vivo antihistamine activity. Histamine causes bronchospasm and guinea-pigs are the most susceptible animals to histamine. The effect of the test compounds against histamine-induced bronchospasm in conscious guinea-pigs was therefore determined.

Table 1 Characterization data of 1-substituted-4-(4-substituted phenyl)-4*H*-[1,2,4]triazolo[4,3-*a*] quinazolin-5-ones



Compound	R	R′	% Yield	Mp (°C)	Elemental analysis calculated/found		
					%C	%H	%N
I	-H	$-OCH_3$	72	248–250	65.75	04.14	19.17
					65.31	04.42	19.46
II	$-CH_3$	$-OCH_3$	72	245–248	66.66	04.61	18.29
					66.23	04.62	18.10
III	$-CH_2CH_3$	$-OCH_3$	76	220–224	67.49	05.03	17.49
					67.41	05.06	17.51
IV	$-(CH_2)_2CH_3$	$-OCH_3$	72	210–214	68.25	05.43	16.76
					68.33	05.39	16.71
V	$-CH_2Cl$	$-OCH_3$	80	254–255	59.92	03.84	16.44
					60.66	04.61	16.84
VI	-H	-Cl	90	228–231	60.72	03.06	18.88
					60.97	03.09	18.19
VII	$-CH_3$	-Cl	89	316–319	61.84	03.57	18.03
					61.89	03.48	18.11
VIII	$-CH_2CH_3$	-Cl	77	267–270	62.87	04.03	17.25
					62.94	04.07	17.33
IX	$-(CH_2)_2CH_3$	-Cl	82	233–234	63.81	04.46	16.64
					63.86	04.51	16.57
X	$-CH_2Cl$	-Cl	80	244–247	55.67	02.92	16.23
					55.63	02.96	16.32

Table 2 Antihistamine and sedative/hypnotic activity of compounds **I–X**

Compound	Time of onset of convulsion (s)	Protection (%)	CNS depression (%)			
			30 min	1 h	2 h	3 h
I	382 ± 4.23^a	69.00 ± 1.48^a	2 ± 1.47^a	3 ± 1.37^a	6 ± 1.55^a	4 ± 1.41^b
II	388 ± 5.16^a	71.00 ± 1.25^a	3 ± 1.44^a	5 ± 1.39^b	7 ± 1.37^c	5 ± 1.61^b
III	387 ± 7.25^a	70.00 ± 1.69^a	3 ± 1.54^c	6 ± 1.70^c	7 ± 1.80^c	4 ± 1.74^c
IV	371 ± 5.08^a	68.00 ± 1.45^a	3 ± 1.90^a	6 ± 1.57^a	7 ± 1.39^b	6 ± 1.22^b
V	369 ± 4.24^a	68.00 ± 1.49^a	3 ± 1.90^a	4 ± 1.85^a	5 ± 1.58^a	4 ± 1.57^b
VI	394 ± 6.20^a	70.00 ± 1.19^a	3 ± 1.61^a	9 ± 1.71^a	10 ± 1.92^a	7 ± 1.61^b
VII	423 ± 12.25^a	72.00 ± 1.29^a	4 ± 1.83^a	9 ± 1.83^a	12 ± 2.06^a	8 ± 2.54^b
VIII	392 ± 6.79^a	70.00 ± 1.54^a	5 ± 1.90^a	11 ± 1.85^b	13 ± 1.48^c	10 ± 1.88^c
IX	387 ± 8.20^a	70.00 ± 1.38^a	6 ± 2.25^a	9 ± 1.87^b	10 ± 1.72^b	8 ± 1.89^b
X	380 ± 8.46^a	69.00 ± 1.60^a	4 ± 1.21^b	8 ± 1.19^c	13 ± 1.30^c	6 ± 1.26^c
Chlorpheniramine	398 ± 29.50^a	71.00 ± 1.36^a	11 ± 1.93^a	37 ± 1.80^d	32 ± 1.71^d	22 ± 1.97^d

Data are expressed as mean ± s.d. from six different experiments done in duplicate. $^aP < 0.5$, $^bP < 0.1$, $^cP < 0.05$ and $^dP < 0.001$. In all cases, a significance level of $P < 0.05$ was observed.

The advantage of this method is that it is non-invasive and the animals are recovered after the experiment.

All the test compounds were found to exhibit good antihistamine activity (Table 2). Percentage protection data showed that all compounds offered significant protection, over the range of 68–72%. Structure–activity relationship studies indicated that different substituents on the N-4 aromatic ring exerted varied biological activity. The electronic nature of the substituent group of the N-4 aromatic ring led to a significant variation in antihistamine activity. For example, 4-chloro substituents were more active than 4-methoxy substituents. Structure–activity relationship studies also indicated that different alkyl substituents over the first position of the triazoloquinazoline ring exerted varied biological activity. The presence of a methyl group (compound **II** $C_{log}P$ 2.13 and compound **VII** $C_{log}P$ 2.81) showed better activity over the unsubstituted compounds (compound **I** $C_{log}P$ 1.85 and compound **VI** $C_{log}P$ 2.54). With increased lipophilicity (i.e. ethyl compound **III** $C_{log}P$ 2.65 and compound **VIII** $C_{log}P$ 3.33), activity was retained; further increases in lipophilicity (i.e. propyl compound **IV** $C_{log}P$ 3.18 and compound **IX** $C_{log}P$ 3.86) lead to a decrease in activity. Replacement of a proton of the methyl group by chloro (compound **V** $C_{log}P$ 2.41 and compound **X** ClogP 3.10) showed a further decrease in activity. The order of activity of substituents at the first position was methyl > ethyl > unsubstituted > propyl > chloromethyl. As the test compounds could not be converted to a water-soluble form, in-vitro evaluation for antihistamine activity could not be performed.

Regarding sedative/hypnotic activity, all the test compounds were found to exhibit only negligible sedation (4–10%), whereas the reference standard chlorpheniramine maleate showed 25% sedation.

Among the series, 1-methyl-4-(4-chloro phenyl)-4*H*-[1,2,4]triazolo[4,3-*a*]quinazolin-5-one (**VII**) was found to be the most active compound (protection 72.71%) and was more potent than the reference standard chlorpheniramine maleate (protection 71%), while compound 1-methyl-4-(4-methoxy phenyl)-4*H*-[1,2,4]triazolo[4,3-*a*]quinazolin-5-one (**II**) was equipotent (protection 71%) to the standard chlorpheniramine maleate. Interestingly, compounds **II** and **VII** also showed negligible sedation (5% and 8%, respectively) compared with chlorpheniramine maleate (25%) and could therefore serve as lead molecules for further modification to obtain a clinically useful novel class of non-sedative antihistamines.

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