Synthesis and H_1-Antihistaminic Activity of Some Novel 1-Substituted-4-(3-methylphenyl)-1,2,4-triazolo[4,3-*a*]quinazolin-5(4*H*)-ones

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Received February 22, 2005; accepted May 16, 2005

A series of novel 1-substituted-4-(3-methylphenyl)-1,2,4-triazolo[4,3-*a*]quinazolin-5(4*H*)-ones were synthesized by the cyclization of 2-hydrazino-3-(3-methylphenyl) quinazolin-4(3*H*)-one with various one carbon donors. The starting material 2-hydrazino-3-(3-methylphenyl)quinazolin-4(3*H*)-one, was synthesized from 3-methylaniline by a novel innovative route. When tested for their *in vivo* H_1-antihistaminic activity on conscious guinea pigs, all the test compounds protected the animals from histamine induced bronchospasm significantly, whereas the compound 1-methyl-4-(3-methylphenyl)-1,2,4-triazolo[4,3-*a*]quinazolin-5(4*H*)-one II was found to be equipotent (percent protection 70.0%) with the reference standard chlorpheniramine maleate (percent protection 71%). Compound II show negligible sedation (7%) when compared to chlorpheniramine maleate (25%). Hence it could serve as prototype molecule for further development as a new class of H_1-antihistamines.

Key words quinazoline; triazole; triazoloquinazoline; pyrimidine; antihistamine

The prevalence of asthma and other allergic diseases is increasing[1—3] providing a rapidly expanding market for antiallergic drugs. The first generation anti-histamines penetrate the blood brain barrier and also possess anticholinergic properties and this has led to the development of a second generation[4] of H_1-antagonists such as terfenadine and astemizole. A common feature of first generation compounds includes two aryl or heteroaryl rings linked to an aliphatic tertiary amine *via* the side chain[5] (*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 eighties of twentieth century when the discovery of modern antihistamines, was found to exhibit potent antihistaminic activity without sleep-inducing effect.[6] Condensed heterocycles containing new generation of H_1-antihistamines (*e.g.* Loratadine, Azelastine and Flazelastine) that does not possessing the above mentioned pharmacophore for H_1-antihistamines gave way for the discovery of many novel antihistamines (temelastine[7] and mangostin[8]). A literature survey reveals excellent antihistaminic activity in quinazolines and condensed quinazolines.[9,10] In view of these facts and to continue our efforts[11,12] in the search of quinazolines derived potent antihistamines with least sedation, we aimed to synthesize a series of 1,2,4-triazolo[4,3-*a*]quinazolin-5(4*H*)-ones containing 3-methylphenyl substitution at position 4 and alkyl substitution at position 1. The title compounds were synthesized by the cyclization of 2-hydrazino-3-(3-methylphenyl)quinazolin-4(3*H*)-one (**6**) with various one carbon donors. The 2-hydrazino-3-(3-methylphenyl)quinazolin-4(3*H*)-one (**6**), was synthesized from 3-methylaniline **1**, by a novel route (Chart 1). Spectral data (IR, NMR and mass spectra) confirmed the structures of the synthesized compounds, the purity of these compounds was ascertained by microanalysis (Table 1). The synthesized compounds were tested for their *in vivo* H_1-antihistaminic activity on conscious guinea pigs. As sedation is one of the major side effects associated with antihistamines, the test compounds were also evaluated for their sedative potentials, by measuring the reduction in loco motor activity using actophotometer.

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 on a MASPEC msw 9629 mass spectrometer at 70 eV and NMR spectra on varian 300 MHz spectrometer, using tetramethylsilane as internal standard. Elemental analyses were performed on Carlo erba 1108.

Synthesis of 2-Thioxo-3-(3-methylphenyl)quinazolin-4(3*H*)-one (4) A solution of 3-methylaniline (**1**) 2.14 g (0.02 mol) in dimethyl sulphoxide (DMSO) (10 ml) was

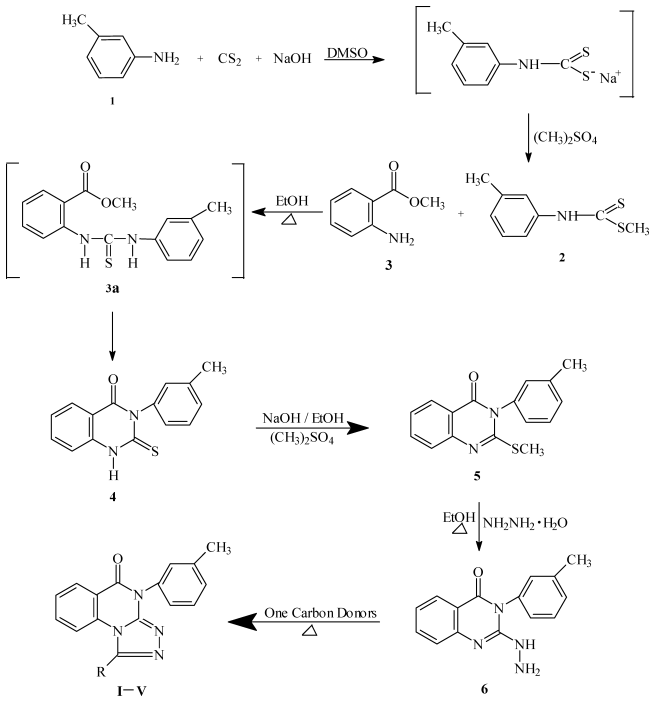

Chart 1. Synthesis of 1-Substituted-4-(3-methylphenyl)-1,2,4-triazolo-[4,3-*a*]quinazolin-5(4*H*)-ones from 3-Methylaniline

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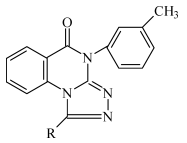
Table 1. Physical Data for 1-Substituted-4-(3-methylphenyl)-1,2,4-triazolo[4,3-*a*]quinazolin-5(4*H*)-ones



Compound code	R	Molecular formula[a)]	Molecular weight[b)]	mp (°C)	Yield (%)
I	–H	$C_{16}H_{12}N_4O$	276	256—258	78
II	$-CH_3$	$C_{17}H_{14}N_4O$	290	275—278	77
III	$-CH_2CH_3$	$C_{18}H_{16}N_4O$	304	258—262	77
IV	$-(CH_2)_2CH_3$	$C_{19}H_{18}N_4O$	318	260—265	70
V	$-CH_2Cl$	$C_{17}H_{13}N_4OCl$	324	266—268	76

a) All compounds gave satisfactory elemental analysis (±0.4% of theoretical values). *b*) Molecular weight determination by mass spectra.

stirred vigorously. To this was added carbon disulphide (1.6 ml, 0.026 mol) and aqueous sodium hydroxide (1.2 ml, 20 mol solution) dropwise 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*-(3-methylphenyl)-methyl dithiocarbamic acid 1.97 g (0.01 mol), were dissolved in ethanol (20 ml). To this anhydrous potassium carbonate (10 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 hydrocholoric 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: 1.3—1.36 (s, 3H, CH_3), 7.0—8.1 (m, 8H, ArH) and 10.3 (s, 1H, NH); MS (*m*/*z*) 268 (M^+). *Anal.* Calcd for $C_{15}H_{12}N_2OS$: C, 67.14; H, 4.51; N, 10.43. Found: C, 67.36; H, 4.56; N, 10.52.

Synthesis of 2-Methylthio-3-(3-methylphenyl)quinazolin-4(3*H*)-one (5) The 2-thioxo-3-(3-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 dropwise 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=81%, mp 148—150 °C; IR (KBr) cm^{-1}: 1678 (C=O), 1610 (C=C); ^{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, 4.99; N, 9.92. Found: C, 68.31; H, 4.85; N, 9.86.

Synthesis of 2-Hydrazino-3-(3-methylphenyl)quinazolin-4(3*H*)-one (6) The 2-methylthio-3-(3-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=76%, mp 195—197 °C; IR (KBr) cm^{-1}: 3320—3205 ($NHNH_2$), 1674 (C=O); ^{1}H-NMR ($CDCl_3$) δ: 2.33 (s, 3H, CH_3), 4.93 (s, 2H, NH_2), 7.15—8.08 (m, 8H, ArH) and 8.63 (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, 67.81; H, 5.36; N, 21.15.

Synthesis of 1-Ethyl-4-(3-methylphenyl)-1,2,4-triazolo-[4,3-*a*]quinazolin-5(4*H*)-one (III) The 2-hydrazino-3-(3-methylphenyl)quinazolin-4(3*H*)-one (**6**) 2.66 g (0.01 mol) and propionic acid (25 ml) was taken 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. Yield=77.4%, mp 258—262 °C; IR (KBr) cm^{-1}: 1680 (C=O), 1602 (C=N); ^{1}H-NMR ($CDCl_3$) δ: 1.3—1.5 (t, 3H, $CH_2C\underline{H}_3$), 2.7—2.8 (q, 2H, $C\underline{H}_2CH_3$), 2.5—2.6 (s, 3H, CH_3) and 7.2—8.5 (m, 8H, ArH); MS (*m*/*z*) 304 (M^+). *Anal.* Calcd for $C_{18}H_{16}N_4O$: C, 71.04; H, 05.30; N, 18.41. Found: C, 71.84; H, 5.36; N, 18.76.

PHARMACOLOGY

The synthesized compounds were evaluated for antihistaminic and sedative-hypnotic activities. Student-*t*-test was performed for all the activities to ascertain the significance of the exhibited activities. The test compounds and the standard drugs were administered in the form of a suspension (1% carboxyl methyl cellulose as vehicle) in the same route of administration. Each group consisted of six animals.

Animals The animals were procured from "National Biological Center", Madurai, India, and were maintained in colony cages at 25±2 °C, relative humidity of 45—55%, maintained under 12 h light and dark cycle and were fed with standard animal feed. All the animals were acclimatized for a week before use.

Antihistaminic Activity A modification of the technique of Van Arman[13] was adopted to determine the antihistaminic potential of the synthesized compounds. Male Dunkin Hartley Guinea pigs (250—300 g) were fasted for 12 h. The test compounds, was administered orally at a dose of 10 mg/kg in 1% CMC and challenged with histamine aerosol (0.2% aqueous solution of histamine acid chloride 3 ml) in a vaponephrin pocket nebulizer sprayed into a closed transparent cage. The respiratory status reflecting the increasing degree of bronchoconstriction was recorded. The time for onset of convulsions (preconvulsion) was recorded. Animals remaining stable for more than 6 min were considered protected against histamine-induced bronchospasm. An intraperitoneal injection of chlorpheniramine maleate (Avil; Hoechst, Mumbai, India) at a dose of 25 mg/kg was given for the recovery of the test animals. The mean preconvulsion time of test compounds treated animals was compared with control and is expressed in terms of percentage protection (Table 2).

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

T_2, preconvulsive time of test compound; T_1, preconvulsive time of control.

The activity of the test compounds was compared with the standard antihistamine chlorpheniramine maleate.

Sedative-Hypnotic Activity It was determined by mea-

Table 2. Antihistaminic and Sedative-Hypnotic Activity

Drug code	Time of onset of convulsion (in s)	% Protection	Percent CNS depression				
			30 min	1 h	2 h	3 h	Average
I	372±2.43*	68.82	5±1.02*	5±1.02*	8±2.01**	5±2.06**	6
II	383±8.99*	70.0	5±0.68****	9±1.07*****	9±0.77*****	5±1.26***	7
III	376±8.36*	69.15	5±1.50**	7±1.58***	10±1.50****	6±1.12*	7
IV	369±2.79*	68.56	5±1.22*****	9±1.51*****	9±1.39*****	7±1.85***	8
V	368±5.07*	68.48	3±1.49*	4±1.35*	6±1.25*	3±1.16*	4
Chlorpheniramine	400±29.50*	71.00	11±1.96*	37±1.82*****	32.0±1.73*****	22±1.98***	25

Each value represents the mean±S.E.M. ($n=6$). Significance levels $*p<0.5$, $**$ p<0.1, $***p<0.05$, $****p<0.01$ and $*****p<0.001$ as compared with the respective control.

suring the reduction in loco motor activity using actophotometer.[14,15] Mice were chosen as test animals. Basal activity score was taken and then compounds I—V and standard chlorpheniramine maleate were administered orally at the dose of 5 mg/kg. Scores were recorded at 0.5, 1, 2 and 3 h after the drug administration. The percent reduction in loco motor activity was calculated by the following formula and shown in Table 2.

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

Where A, basal score; B, score after drug treatment.

RESULTS AND DISCUSSION

The key intermediate 2-thioxo-3-(3-methylphenyl)quinazolin-4(3*H*)-one was prepared by treating carbon disulphide and sodium hydroxide solution with 3-methylaniline **1** in dimethyl sulphoxide to give dithiocarbamate salt, which was methylated with dimethyl sulphate to afford the dithiocarbomic acid methyl ester (**2**). Compound **2** on reflux with methyl anthranilate (**3**) in ethanol yielded the desired 2-thioxo-3-(3-methylphenyl)quinazoline (**4**) *via* the thiourea intermediate. The use of DMSO as the reaction solvent enhanced the rate of reaction and the use of alkali in higher concentration helped in preventing the hydrolysis of the intermediate probably, due to less salvation. This method of synthesis of 2-thioxo-3-(3-methylphenyl)quinazolin-4(3*H*)-one, not only curtails the reaction time also afforded good yield (75%). The product obtained was cyclic and not an open chain thiourea **3a**. The IR spectrum of **4** show intense peaks at 3218 cm^{-1} for amino (NH), 1680 cm^{-1} for carbonyl (C=O) and 1200 cm^{-1} for thioxo (C=S) stretching. NMR spectrum of 4 showed singlet at δ 1.3—1.36 due to methyl group, a multiplet at δ 7—8.1 for aromatic (8H) protons and a singlet at δ 10.3 indicating the presence of NH. 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 2-methylthio-3-(3-methylphenyl)quinazolin-4(3*H*)-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 spectrum of **5** showed disappearance of amino (NH) and thioxo (C=S) stretching signals of the starting material. It showed a peak for carbonyl (C=O) stretching at 1678 cm^{-1}. The NMR spectrum of compound **5** showed singlet at δ 2.4 and 2.5 due to CH_3 and SCH_3 respectively, a multiplet at δ 7.1—8.2 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 methylthio group of **5** with hydrazine hydrate was carried out using ethanol as solvent to afford 2-hydrazino-3-(3-methylphenyl)quinazolin-4(3*H*)-one **6**. The long duration of reaction (30 h) required might be due to the presence of bulky aromatic ring at position 3, which might have reduced the reactivity of quinazoline ring system at C-2 position. The formation of **6** was confirmed by the presence of NH and NH_2 signals around 3320—3205 cm^{-1} in the IR spectrum. It also showed a peak for carbonyl (C=O) at 1674 cm^{-1}. The NMR spectrum of the compound **6** showed singlet at δ 2.33, 4.96 and 8.63 due to CH_3, NH_2 and NH respectively, a multiplet at δ 7.15—8.08 was observed for 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 I—V 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 cyclic product is indicated by the disappearance of peaks due to NH and NH_2 of the starting material at 3400—3200 cm^{-1} in IR spectrum of all the compounds I—V. The NMR spectrum of I—V showed the absence of NH and NH_2 signals. In the IR spectrum, it showed a peak for carbonyl (C=O) at 1680 cm^{-1}. The NMR spectrum of the compounds I—V showed multiplet at δ 7.0—8.0 integrating for aromatic protons. The molecular ion recorded in the mass spectrum also in agreement with the molecular weight of the compounds. Elemental (C, H, N) analysis indicated that the calculated and observed values were within the acceptable limits (±0.4%). Physical data of the title compounds is represented in Table 1.

From the results of antihistaminic activity, it has been found that presence of methyl group (compound II) showed better activity over the unsubstituted compounds (compound I), with increased lipohilicity (*i.e.*, ethyl compound III) activity retained, further increase in lipophilicity (*i.e.*, propyl compound IV) leads to decrease in activity. Replacement of a hydrogen of the methyl group by chloro (compound V) showed further decrease in activity. While the order of activity of substituents at first position was methyl>ethyl>unsubsti-

tuted>propyl>chloromethyl. As the test compounds could not be converted to water soluble form, *in vitro* evaluation for antihistaminic activity could not be performed.

The results of sedative-hypnotic activity, indicate that all the test compounds were found to exhibit only negligible sedation (4—8%), whereas the reference standard chlorpheniramine maleate showed 25% sedation.

Among the series, 1-methyl-4-(3-methylphenyl)-1,2,4-triazolo[4,3-*a*]quinazolin-5(4*H*)-one II was the most potent with the percentage protection of 70.0%, which is equipotent with that of standard chlorpheniramine maleate (percentage protection 71). Compound II also showed mild sedation (7%) compared to chlorpheniramine maleate (25%) and could therefore serve as a lead molecule for further modification to obtain a clinically useful novel class of non-sedative antihistamines.

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