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Original article

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

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Abstract

A series of novel 1-substituted-4-(2-methylphenyl)-4*H*-[1,2,4]triazolo[4,3-*a*]quinazolin-5-ones were synthesized by the cyclization of 2-hydrazino-3-(2-methylphenyl)-3*H*-quinazolin-4-one with various one carbon donors. The starting material 2-hydrazino-3-(2-methylphenyl)-3*H*-quinazolin-4-one was synthesized from 2-methyl aniline by a novel innovative route. The title compounds were tested for their *in vivo* H_1-antihistaminic activity on guinea pigs; all the tested compounds protected the animals from histamine-induced bronchospasm significantly. Compound 1-methyl-4-(2-methylphenyl)-4*H*-[1,2,4]triazolo[4,3-*a*]quinazolin-5-one (**II**) emerged as the most active compound of the series and it is more potent (72.45%) when compared to the reference standard chlorpheniramine maleate (71%). Compound **II** showed negligible sedation (11%) when compared to chlorpheniramine maleate (30%). Hence it could serve as the prototype molecule for further development as a new class of H_1-antihistaminic agents.

Keywords: Quinazolin-5-ones; Triazoles; Triazoloquinazolines; Sedation; H_1-antihistaminic agents

1. Introduction

The first generation antihistamines penetrate the blood–brain barrier and also possess anticholinergic properties and this has led to the development of a second generation of H_1-antagonists such as terfenadine, cetirizine and astemizole [1]. A common feature of first generation compounds includes two aryl or heteroaryl rings linked to an aliphatic tertiary amine via the side chain (e.g. diphenhydramine and phenirамine) [2], 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 80s of the 20th century when the modern antihistamines discovered were found to exhibit potent antihistaminic activity without sleep-inducing effect [3]. Condensed heterocycles containing 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 for the discovery of many novel antihistamines such as temelastine and mangostin [4,5]. A literature survey reveals excellent antihistaminic activity in quinazolines and condensed quinazolines [6–8]. In view of these facts and to continue our efforts in the search of quinazoline derivatives as potent antihistamines with least sedation [9–11] we aimed to synthesize a series of 1,2,4-triazolo-4*H*-[4,3-*a*]quinazolin-5-one compounds containing 2-methylphenyl substitution at position 4 and alkyl/

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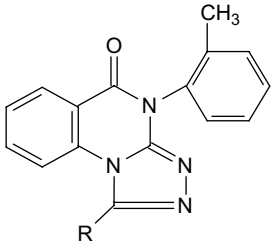
alicyclic amine substitution at position 1. The title compounds were synthesized by the cyclization of 2-hydrazino-3-(2-methylphenyl)-3*H*-quinazolin-4-one (**6**) with various one carbon donors. 2-Hydrazino-3-(2-methylphenyl)-3*H*-quinazolin-4-one (**6**) was synthesized from 2-methyl aniline (**1**) by a novel innovative route. 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 locomotor activity using actophotometer.

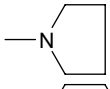
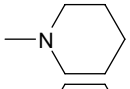
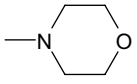
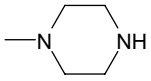
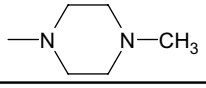
2. Chemistry

The key intermediate 3-(2-methylphenyl)-2-thioxo-2,3-dihydro-1*H*-quinazolin-4-one (**4**) was prepared by refluxing methyl anthranilate with 2-methylphenyl isothiocyanate in ethanol (Fig. 1). However, this route is not much attractive as the preparation of 2-methylphenyl isothiocyanate required for the reaction is a tedious and time consuming process, and the yield was also low (60%). An alternate route (Fig. 2) was attempted to synthesize compound **4**. In this route, 2-methyl aniline (**1**) was reacted with carbon disulphide and anhydrous potassium carbonate in acetone to give potassium dithiocarbamate, which was methylated with dimethyl sulphate to afford dithiocarbamic acid methyl ester (**2**). Compound **2** on reflux with methyl anthranilate (**3**) yielded compound **4**. The process of synthesizing compound **4** by this scheme suffers from the following drawbacks: it is a multi-step process, it requires prolonged reaction time (37 h) and the yield is also very low (30%). Hence, improvisation was carried out on this method, by using aqueous sodium hydroxide solution (20 mol) as a base instead of anhydrous K_2CO_3 and dimethyl sulphoxide (DMSO) was substituted for acetone as the reaction solvent (Fig. 3). The use of DMSO as the reaction solvent enhanced the rate of the reaction and the use of alkali in higher concentration helped in preventing the hydrolysis of the intermediate, probably due to less solvation. These modifications not only curtailed the reaction time from 37 h to 25 h, but also increased the yield from 30% to 89%.

Thus 2-methyl aniline (**1**) was treated 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**).

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



Compound code	R	Molecular formula	Mp, °C (% yield)	Elemental analysis, calculated/found		
				%C	%H	%N
I	−H	$C_{16}H_{12}N_4O$	265–266 (72)	69.55/69.51	4.38/4.43	20.08/20.11
II	$-CH_3$	$C_{17}H_{14}N_4O$	239–241 (77)	70.33/70.36	4.86/4.88	19.30/19.37
III	$-CH_2CH_3$	$C_{18}H_{16}N_4O$	273–274 (79)	71.04/71.08	5.30/5.35	18.41/18.36
IV	$-(CH_2)_2CH_3$	$C_{19}H_{18}N_4O$	231–232 (77)	71.68/71.61	5.70/5.74	17.60/17.58
V	$-CH_2Cl$	$C_{17}H_{13}ClN_4O$	289–291 (80)	62.87/62.91	4.03/4.07	17.25/17.20
VI		$C_{21}H_{21}N_5O$	229–230 (72)	70.17/70.10	5.89/5.94	19.48/19.42
VII		$C_{22}H_{23}N_5O$	241–242 (74)	70.76/70.74	6.21/6.26	18.75/18.68
VIII		$C_{21}H_{21}N_5O_2$	266–267 (71)	67.18/67.11	5.64/5.62	18.65/18.68
IX		$C_{21}H_{22}N_6O$	273–274 (70)	67.36/67.33	5.92/5.94	22.44/22.41
X		$C_{22}H_{24}N_6O$	266–267 (73)	68.02/68.11	6.23/6.32	21.63/21.58

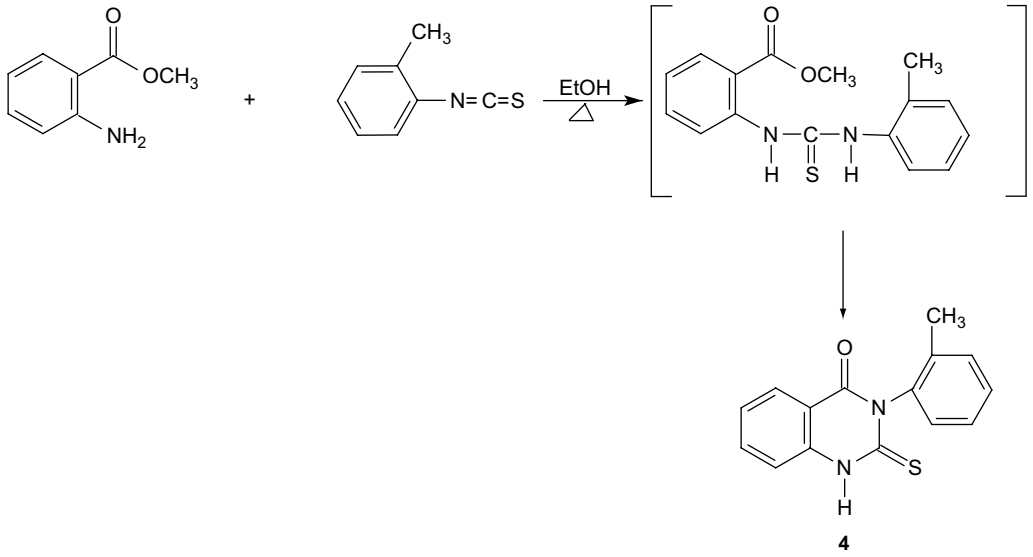


Fig. 1. Synthetic protocol of 3-(2-methylphenyl)-2-thioxo quinazolin-4(3*H*)-one from 2-methylphenyl isothiocyanate.

Compound **2** on reflux with methyl anthranilate (**3**) in ethanol yielded the desired 3-(2-methylphenyl)-2-thioxo-2,3-dihydro-1*H*-quinazolin-4-one (**4**) via the thiourea intermediate in good yield (80%). 3-(2-Methylphenyl)-2-methylsulfanyl-3*H*-quinazolin-4-one (**5**) was obtained by dissolving compound **4** in 2% alcoholic sodium hydroxide solution and methylating with dimethyl sulphate with stirring at room temperature. Nucleophilic displacement of the methylthio group of compound **5** with hydrazine hydrate was carried out using ethanol as solvent to afford 2-hydrazino-3-(2-methylphenyl)-3*H*-quinazolin-4-one (**6**). The long duration of reaction (33 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 title compounds **I**–**V** were obtained in fair to good yields through the cyclization of compound **6** with a variety of one carbon donors, such as formic

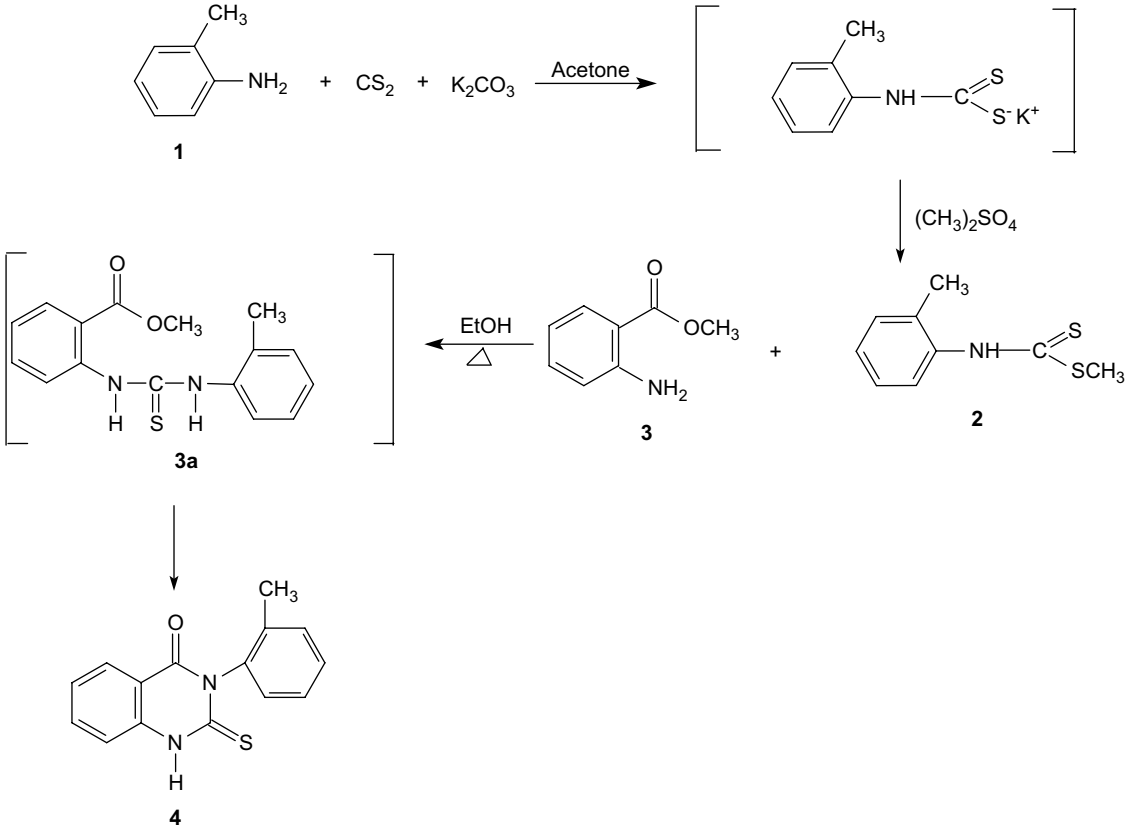


Fig. 2. Synthetic protocol of 3-(2-methylphenyl)-2-thioxo quinazolin-4(3*H*)-one from 2-methyl aniline.

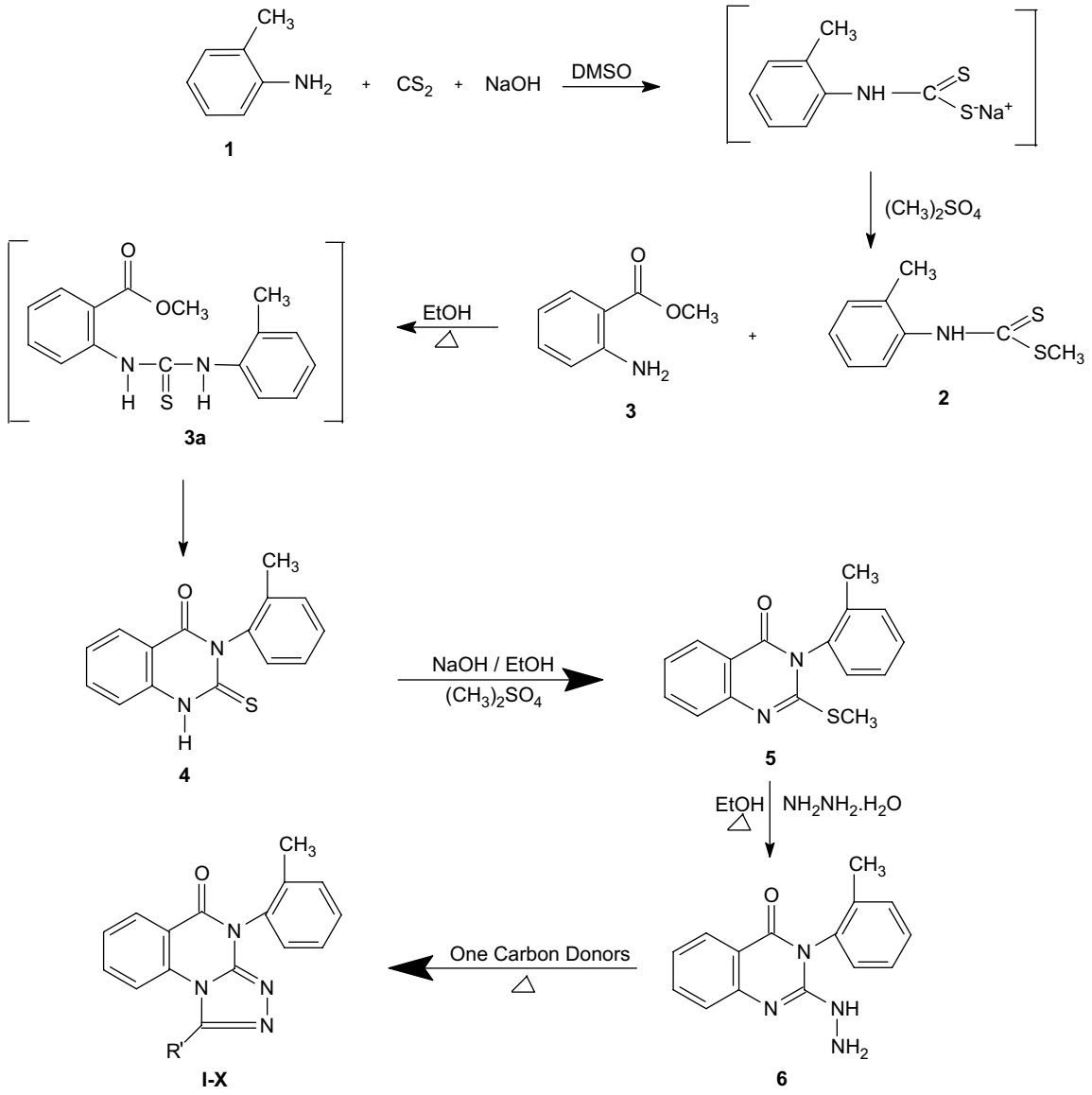


Fig. 3. Synthetic protocol of 1-substituted-4-(2-methylphenyl)-4*H*-[1,2,4]triazolo[4,3-*a*]quinazolin-5-ones.

acid, acetic acid, propionic acid, butyric acid and chloroacetyl chloride, at reflux. Compounds **VI**–**X** were obtained by the displacement of the chloro of compound **V** with various alicyclic amines like pyrrolidine, piperidine, morpholine, piperazine and 4-methylpiperazine. All the synthesized compounds were confirmed by spectral data (IR, NMR and mass spectra). Physical data of the title compounds are presented in Table 1.

3. Results and discussion

The compounds containing 1,4-disubstituted[1,2,4]triazoloquinazoline ring system (**I**–**X**) have been evaluated for their *in vivo* antihistaminic activity. Histamine causes bronchospasm and the guinea pigs are the most susceptible animals for histamine, hence the method of protection against histamine-induced bronchospasm on conscious guinea pigs was adopted to determine the antihistaminic potential of the test compounds [12]. The advantage of this method is that it is one of the non-invasive methods and the animals are recovered after the experiment.

All the test compounds were found to exhibit good antihistaminic activity (Table 2). Percentage protection data showed that all the compounds of the series show significant protection in the range of 69–72%. Pharmacological studies indicated that different substituents in the 1-position of triazoloquinazoline ring exerted varied biological activity. The presence of methyl group (compound **II**) showed better activity over the unsubstituted compound (compound **I**); with increased lipophilicity (i.e., ethyl compound **III**) the activity retained; further increase in lipophilicity (i.e., propyl compound **IV**) leads to decrease in activity. Replacement of a proton of the methyl group by chloro (compound **V**) showed further decrease in activity. Replacement of a proton of the methyl group by alicyclic amines (pyrrolidyl and piperidyl compounds **VI** and **VII**, respectively) showed increase in activity over the chloro substituent. Placement of an additional heteroatom in the alicyclic amines with (morpholinyl

Table 2
Antihistaminic and sedative–hypnotic activities of compounds **I–X**

Compound code	Time of onset of convulsion, s	% Protection	% CNS depression		
			1 h	2 h	3 h
I	385 ± 7.06*	69.87 ± 1.63*	9 ± 1.23*	11 ± 1.65*	5 ± 1.61^{NS}
II	421 ± 9.32*	72.45 ± 1.51*	11 ± 1.63*	14 ± 1.63**	8 ± 1.68*
III	397 ± 10.16*	70.78 ± 1.62*	13 ± 1.71*	14 ± 1.38**	9 ± 1.72*
IV	380 ± 9.13*	69.47 ± 1.43*	13 ± 1.45*	17 ± 1.27***	8 ± 1.25**
V	369 ± 6.41*	68.56 ± 1.72*	8 ± 1.32*	13 ± 1.73*	4 ± 1.62^{NS}
VI	372 ± 6.52*	68.81 ± 1.87*	10 ± 1.23*	14 ± 1.38**	7 ± 1.42*
VII	380 ± 8.29*	69.47 ± 1.61*	13 ± 1.27*	16 ± 1.72***	9 ± 1.51*
VIII	391 ± 5.72*	70.33 ± 1.37*	10 ± 1.47*	15 ± 1.35**	10 ± 1.52*
IX	396 ± 6.57*	70.70 ± 1.28*	12 ± 1.17*	13 ± 1.26**	9 ± 1.41*
X	415 ± 6.53*	72.05 ± 1.61*	12 ± 1.73*	18 ± 1.93***	10 ± 1.33*
Chlorpheniramine	400 ± 29.50*	71.00 ± 1.36*	37 ± 1.82***	32.0 ± 1.73***	22 ± 1.98***

Each value represents the mean ± SEM ($n = 6$). Significance levels $*p < 0.5$, $**p < 0.1$ and $***p < 0.05$ and NS indicates not significant, as compared with respective control.

compound **VIII**, piperazinyl compound **IX** and 4-methylpiperazinyl compound **X**) led to further increase in activity.

Sedative–hypnotic activity was determined by measuring the reduction in locomotor activity using actophotometer [13,14] on Albino Swiss mice. The results of sedative–hypnotic activity indicate that all the test compounds were found to exhibit only negligible sedation (9–13%), whereas the reference standard chlorpheniramine maleate showed 25% sedation.

4. Conclusion

In summary, the synthesis of a new series of 1-substituted-4-(2-methylphenyl)-4*H*-[1,2,4]triazolo[4,3-*a*]quinazolin-5-ones has been described. In this study, the intermediate compound 3-(2-methylphenyl)-2-thioxo-2,3-dihydro-1*H*-quinazolin-4-one has been synthesized by a novel innovative route with improved yield. These derivatives have exhibited promising antihistaminic activity against histamine-induced bronchospasm on conscious guinea pig model. In this series, 1-methyl-4-(2-methylphenyl)-4*H*-[1,2,4]triazolo[4,3-*a*]quinazolin-5-one (**II**) was found to be the most active antihistaminic agent, which is more potent than the reference standard chlorpheniramine maleate and could therefore serve as a lead molecule for further modification to obtain a clinically useful novel class of antihistaminic agents.

5. Experimental protocols

5.1. Chemistry

Melting points (mp) were recorded in open capillaries on Thomas Hoover melting point apparatus and are uncorrected. IR spectra were recorded in film or in potassium bromide disks on a Perkin–Elmer 398 spectrometer. ^{1}H NMR spectra were recorded on a DPX-300 MHz Bruker FT-NMR spectrometer. Chemical shifts were reported in parts per million (δ ppm) with tetramethylsilane (TMS) as an internal standard. Mass spectra were obtained on a JEOL-SX-102 instrument using fast atom bombardment (FAB+). Elemental analysis was performed on a Perkin–Elmer 2400 C,H,N analyzer and the values were within the acceptable limits of the calculated values. The progress of the reaction was monitored on ready-made 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) analysis 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.

5.1.1. Synthesis of 3-(2-methylphenyl)-2-thioxo-2,3-dihydro-1H-quinazolin-4-one (4)

A solution of 2-methyl aniline **1** (0.02 mol) in dimethyl sulphoxide (10 ml) was stirred vigorously. To this were added carbon disulphide (1.6 ml, 0.026 mol) and aqueous sodium hydroxide (1.2 ml, 20 M solution) dropwise for 30 min with stirring. Dimethyl sulphate (0.02 mol) was added gradually keeping the reaction mixture stirring in freezing mixture for 2 h. The reaction mixture was then poured into ice water. The solid obtained was filtered, washed with water, dried and recrystallized from ethanol. Methyl anthranilate (0.01 mol) and the above prepared *N*-(2-methylphenyl)-methyl dithiocarbamic acid (0.01 mol) were dissolved in ethanol (20 ml). To this anhydrous potassium carbonate (100 mg) was added and refluxed for 22 h. The reaction mixture was cooled in ice and the solid separated was filtered and purified by dissolving in 10% alcoholic sodium hydroxide solution and re-precipitated by treating with dilute hydrochloric acid. The solid obtained was filtered, washed with water, dried and recrystallized from ethanol. Yield = 86%, mp 240–242 °C. IR: 3211 (NH), 1686 (C=O), 1213 (C=S) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 2.4 (s, 3H, CH_3), 7.1–7.4 (m, 4H, ArH), 7.5–7.8 (m, 4H, ArH), 10.3 (br 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, 67.19; H, 4.57; N, 10.38.

*5.1.2. Synthesis of 3-(2-methylphenyl)-2-methylsulfanyl-3H-quinazolin-4-one (**5**)*

3-(2-Methylphenyl)-2-thioxo-2,3-dihydro-1*H*-quinazolin-4-one **4** (0.01 mol) was dissolved in 40 ml of 2% alcoholic sodium hydroxide solution. To this dimethyl sulphate (0.01 mol) was added dropwise with stirring. The stirring was continued for 1 h, the reaction mixture was then poured into ice water. The solid obtained was filtered, washed with water, dried and recrystallized from ethanol–chloroform (75:25) mixture. Yield = 88%, mp 130–132 °C. IR: 1685 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.1 (s, 3H, CH_3), 2.8 (s, 3H, SCH_3), 7.3–7.6 (m, 4H, ArH), 7.7–8.0 (m, 4H, 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, 68.10; H, 5.03; N, 9.95.

*5.1.3. Synthesis of 2-hydrazino-3-(2-methylphenyl)-3H-quinazolin-4-one (**6**)*

3-(2-Methylphenyl)-2-methylsulfanyl-3*H*-quinazolin-4-one **5** (0.01 mol) was dissolved in ethanol (25 ml). To this hydrazine hydrate (99%) (0.1 mol) and anhydrous potassium carbonate (100 mg) were added and refluxed for 33 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 = 81%, mp 200–202 °C. IR: 3356, 3293 ($NHNH_2$), 1672 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.6 (s, 3H, CH_3), 5.0 (s, 2H, NH_2), 7.1–7.4 (m, 4H, ArH), 7.5–7.8 (m, 4H, ArH), 9.6 (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, 67.58; H, 5.28; N, 21.02.

*5.1.4. Synthesis of 4-(2-methylphenyl)-4H-[1,2,4]triazolo-[4,3-a]quinazolin-5-one (**I**)*

2-Hydrazino-3-(2-methylphenyl)-3*H*-quinazolin-4-one (**6**) (0.01 mol) and formic acid (25 ml) were taken in a round bottomed flask and refluxed for 37 h and then cooled and poured into ice water. The solid obtained was filtered, washed with water, dried and recrystallized from ethanol. IR: 1680 (C=O), 1609 (C=N) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.0 (s, 3H, CH_3), 7.1–7.4 (m, 4H, ArH), 7.7 (s, 1H, ArH), 7.9–8.2 (m, 4H, ArH). ^{13}C NMR ($CDCl_3$) δ 13.8, 120.6, 121.0, 121.9 (2), 124.5, 127.8 (2), 129.3, 132.8 (2), 134.2, 137.8, 147.7, 148.9, 160.2. MS (*m/z*): 276 (M^+). Adopting this procedure compounds **II**–**V** were prepared.

*5.1.5. 4-(2-Methylphenyl)-1-methyl-4H-[1,2,4]triazolo-[4,3-a]quinazolin-5-one (**II**)*

IR: 1679 (C=O), 1605 (C=N) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.3 (s, 3H, CH_3), 2.5 (s, 3H, CH_3), 7.0–7.3 (m, 4H, ArH), 7.4–7.7 (m, 4H, ArH). ^{13}C NMR ($CDCl_3$) δ 10.2, 13.2, 120.8, 121.5, 121.8 (2), 124.5, 127.8 (2), 129.3, 132.8 (2), 134.2, 137.8, 148.7, 160.2, 162.8. MS (*m/z*): 290 (M^+).

*5.1.6. 1-Ethyl-4-(2-methylphenyl)-4H-[1,2,4]triazolo-[4,3-a]quinazolin-5-one (**III**)*

IR: 1685 (C=O), 1610 (C=N) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.1–1.2 (t, 3H, CH_2CH_3), 1.9 (s, 3H, CH_3), 2.5–2.6 (q, 2H, CH_2CH_3), 7.4–7.7 (m, 4H, ArH), 7.8–8.1 (m, 4H, ArH). MS (*m/z*): 304 (M^+).

*5.1.7. 4-(2-Methylphenyl)-1-propyl-4H-[1,2,4]triazolo-[4,3-a]quinazolin-5-one (**IV**)*

IR: 1680 (C=O), 1605 (C=N) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.6–0.7 (t, 2H, $CH_2CH_2CH_3$), 1.2–1.4 (sext, 2H, $CH_2CH_2CH_3$), 2.1 (s, 3H, CH_3), 2.4–2.6 (t, 3H, $CH_2CH_2CH_3$), 7.1–7.4 (m, 4H, ArH), 7.6–7.9 (m, 4H, ArH). MS (*m/z*): 318 (M^+).

*5.1.8. 1-Chloromethyl-4-(2-methylphenyl)-4H-[1,2,4]triazolo[4,3-a]quinazolin-5-one (**V**)*

IR: 1680 (C=O), 1607 (C=N) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.3 (s, 3H, CH_3), 3.6 (s, 2H, CH_2), 7.0–7.3 (m, 4H, ArH), 7.4–7.8 (m, 4H, ArH). MS (*m/z*): 324 (M^+), 326 ($M^+ + 2$).

*5.1.9. Synthesis of 4-(2-methylphenyl)-1-(pyrrolidyl)-4H-[1,2,4]triazolo[4,3-a]quinazolin-5-one (**VI**)*

A mixture of 1-chloromethyl-4-(2-methylphenyl)-4*H*-[1,2,4]triazolo[4,3-*a*]quinazolin-5-one (**V**) (0.01 mol) and pyrrolidine (0.05 mol) and anhydrous potassium carbonate (100 mg) in dioxan (25 ml) were taken in a round bottomed flask and refluxed for 31 h, cooled and poured into ice water. The solid obtained was filtered, washed with water, dried and recrystallized from ethanol–benzene (50:50). IR: 1685 (C=O), 1609 (C=N) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.0–1.2 (m, 4H, NCH_2CH_2), 1.5–1.7 (m, 4H, NCH_2CH_2), 2.5 (s, 3H, CH_3), 7.2–7.5 (m, 4H, ArH), 7.6–7.9 (m, 4H, ArH). MS (*m/z*): 359 (M^+). Adopting this procedure compounds **VII**–**X** were prepared.

*5.1.10. 4-(2-Methylphenyl)-1-(piperidyl)-4H-[1,2,4]triazolo[4,3-a]quinazolin-5-one (**VII**)*

IR: 1689 (C=O), 1604 (C=N) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.3–1.6 (m, 6H, CH_2–piperidyl), 1.6–1.8 (m, 4H, CH_2–piperidyl), 2.8 (s, 3H, CH_3), 7.1–7.4 (m, 4H, ArH), 7.5–7.8 (m, 4H, ArH). ^{13}C NMR ($CDCl_3$) δ 24.2, 25.7, 25.9, 54.7, 55.8, 120.1 (2), 121.8 (2), 124.5, 127.8 (2), 129.3 (2), 132.8 (2), 134.2, 137.8 (2), 148.7, 160.2. MS (*m/z*): 373 (M^+).

*5.1.11. 4-(2-Methylphenyl)-1-(morpholinyl)-4H-[1,2,4]triazolo[4,3-a]quinazolin-5-one (**VIII**)*

IR: 1680 (C=O), 1612 (C=N) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.7–1.9 (m, 4H, NCH_2CH_2O), 2.2–2.4 (m, 4H, NCH_2CH_2O), 2.7 (s, 3H, CH_3), 7.4–7.6 (m, 4H, ArH), 7.7–8.0 (m, 4H, ArH). MS (*m/z*): 375 (M^+).

*5.1.12. 4-(2-Methylphenyl)-1-(piperazinyl)-4H-[1,2,4]triazolo[4,3-a]quinazolin-5-one (**IX**)*

IR: 1693 (C=O), 1609 (C=N) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.5–1.7 (m, 4H, NCH_2CH_2NH), 2.0–2.2 (m, 4H,

NCH_2CH_2NH), 2.5 (s, 2H, CH_2), 7.3–7.6 (m, 4H, ArH), 7.6–8.0 (m, 4H, ArH), 9.6 (s, 1H, NH). MS (*m/z*): 374 (M^+).

*5.1.13. 4-(2-Methylphenyl)-1-(4-methylpiperazinyl)-4H-[1,2,4]triazolo[4,3-a]quinazolin-5-one (**X**)*

IR: 1681 (C=O), 1602 (C=N) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.7–1.9 (m, 4H, NCH_2CH_2N), 2.1–2.3 (m, 4H, NCH_2CH_2N), 2.5 (s, 3H, CH_3), 2.7 (s, 2H, CH_3), 7.4–7.8 (m, 4H, ArH), 7.9–8.2 (m, 4H, ArH). MS (*m/z*): 388 (M^+).

5.2. Pharmacological screening

The synthesized compounds were evaluated for antihistaminic and sedative–hypnotic activities. The animals 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 with 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.

5.2.1. Antihistaminic activity

A modification of the technique of Van Arman was adopted to determine the antihistaminic potential of the synthesized compounds [12]. Male Dunkin Hartley guinea pigs (250–300 g) were fasted for 12 h. Six animals were taken in each group. The test compounds were 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 animals treated with the test compounds was compared to control and is expressed in terms of percentage protection (Table 2).

$$\text{Percent protection} = [1 - (T1/T2)] \times 100$$

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.

5.2.2. Sedative–hypnotic activity

Sedative–hypnotic activity was determined by measuring the reduction in locomotor activity using actophotometer [12,13]. Albino Swiss mice were chosen as test animals in group of six. Basal activity score was recorded and then compounds **I–X** and standard chlorpheniramine maleate were administered orally at the dose of 5 mg/kg in 1% CMC. Scores were recorded at 1 h, 2 h and 3 h after the drug administration. Student-*t*-test was performed to ascertain the significance of the exhibited activity. The percent reduction in locomotor activity was calculated by the following formula and is shown in Table 2.

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

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

5.3. 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 Software, Inc., 11452 El Camino Real, #215, San Diego, CA 92130, USA).

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