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Design and Synthesis of 1-Substituted-4-(4-Nitrophenyl)-[1,2,4]triazolo[4,3-*a*]quinazolin-5(4*H*)-ones as a New Class of Antihistaminic Agents

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Abstract—Some new 1-substituted-4-(4-nitrophenyl)-[1,2,4]triazolo[4,3-*a*]quinazolin-5(4*H*)-ones were synthesized and screened for their H_1-antihistaminic activity. The structures of the newly synthesized compounds were confirmed by IR, ^{1}H NMR, and mass spectral data and purity of the compounds was determined by elemental analysis. Antihistaminic activity of synthesized compounds was determined by the protection against histamine-induced bronchospasm on conscious guinea pigs. Percentage protection data showed that all title compounds of the series show significant protection in the range of 68–71.56% when compared to reference drug chlorpheniramine maleate (70.17%). The sedative properties of the compounds were also evaluated and found to be negligible when compared to chlorpheniramine maleate.

Keywords: histamine, triazolo, quinazolin-5(4*H*)-ones, antihistaminic activity, sedative–hypnotic activity

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INTRODUCTION

Heterocyclic synthesis is a powerful technique for generating new chemical entities with clinically valuable pharmacophores. Quinazolines are classes of fused heterocycles and the various structural modifications on quinazoline nucleus showed wide range of biological activities like antihistaminic, antimicrobial, antitubercular, antiviral, antihypertensive, analgesic, anti-inflammatory, and anticancer activity. The histamine H_1-antagonists are used as the first-line treatment for patients with allergic rhinitis [1–3]. Currently used first generation antihistamines are effective and relatively inexpensive. But they cause sedation and dry mouth at therapeutic doses, due to their blood-brain barrier penetration and lack of receptor specificity. Significant percentage (10 to 40%) of patients using these drugs experienced somnolence and also impairment in driving and other types of performance [4–7]. Second-generation histamine H_1-receptor antagonists showed improved antihistaminic activity with less sedative potential. Unfortunately, some of the second-generation antihistamines produced fatal arrhythmogenic events through impairing cardiac muscle repolarization. As antihypertensive agents, like prazocin, terazocin, and doxazocin (specific α_1-blockers) derived from quinazoline have not shown any arrhythmogenic effect, compounds obtained with quinazoline are expected to have the advantage of lack of cardiotoxic potential unlike some of the second-generation antihistamines.

The present study was aimed to synthesize some novel 1,2,4-triazolo[4,3-*a*]quinazolin-5(4*H*)-ones containing aryl/hetero aryl substitution at position 4 and alkyl substitutions at position 1 [5, 7]. The synthetic scheme for the synthesis of title compounds is depicted in Scheme 1. The synthesized compounds were determined from spectral and physicochemical analysis. IR, ^{1}H NMR, and mass spectrometry analysis results confirmed the formation of desired products. The synthesized compounds were evaluated for their antihistaminic activity using protection against histamine-induced bronchospasm on conscious guinea pigs [8, 9]. Sedative potential was also evaluated by measuring the reduction in locomotor activity in actophotometer.

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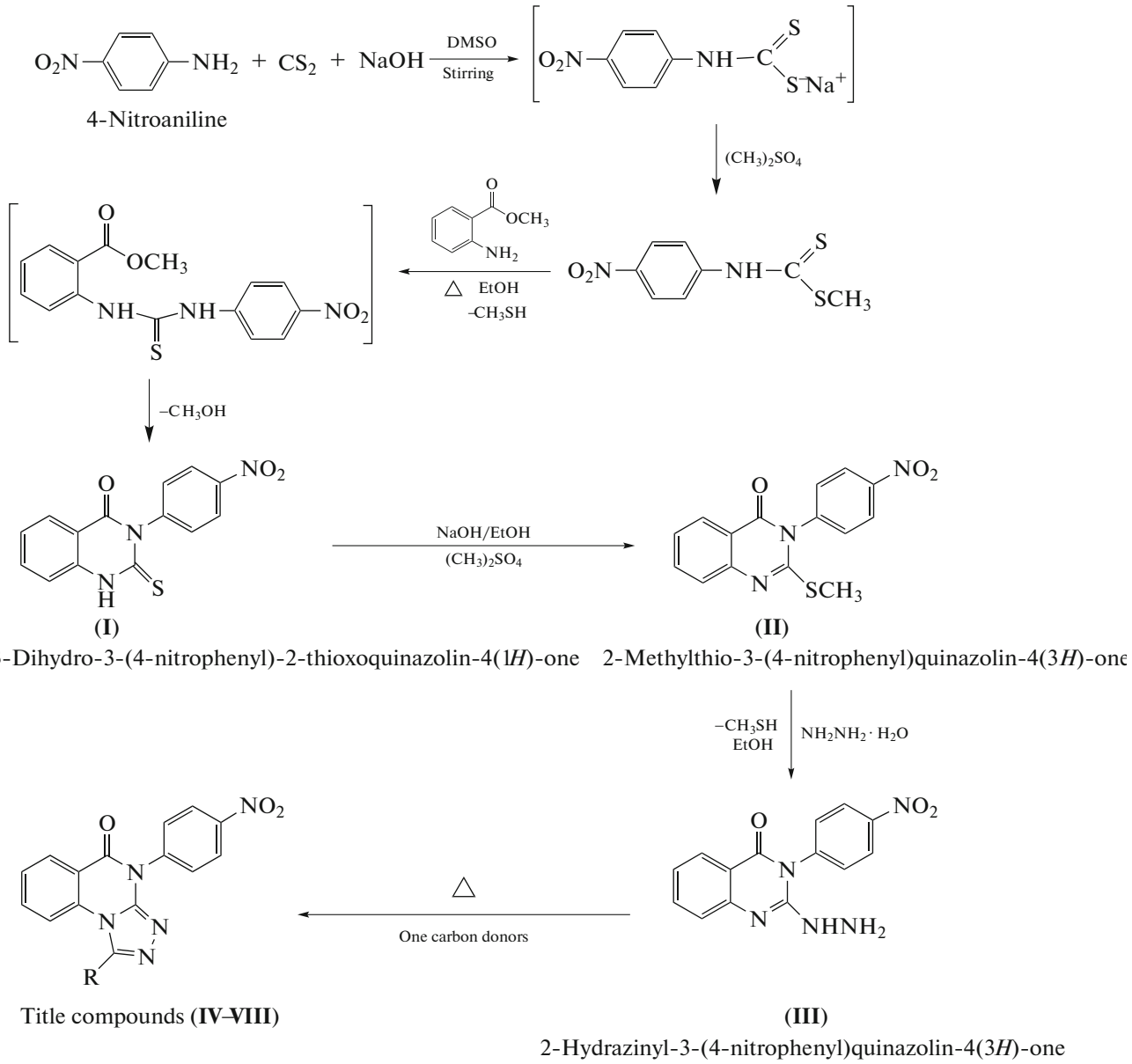

R = **(IV)** –H; **(V)** $-CH_3$; **(VI)** $-CH_2CH_3$; **(VII)** $-(CH_2)_2CH_3$; **(VIII)** $-CH_2Cl$

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

RESULTS AND DISCUSSION

Chemistry

The key intermediate 2,3-dihydro-3-(4-nitrophenyl)-2-thioxoquinazolin-4(1*H*)-one (**I**), was prepared by reacting 4-nitroaniline with carbon disulphide and sodium hydroxide in dimethyl sulfoxide to give sodium dithiocarbamate, which was methylated with dimethyl sulfate to afford the dithiocarbamic acid methyl ester, which upon reflux with methyl anthranilate in ethanol yielded the desired 2,3-dihydro-3-(4-nitrophenyl)-2-thioxoquinazolin-4(1*H*)-one (**I**) via the thiourea intermediate in good yield (85%). The product obtained was cyclic and not an open chain thiourea. The 2-methylthio-3-(4-nitrophenyl)-quinazolin-4(3*H*)-one (**II**) was obtained by dissolving compound (**I**) in 2% alcoholic sodium hydroxide solution and methylating with dimethyl sulfate with stirring at room temperature. Nucleophilic displacement of methylthio group of compound (**II**) with hydrazine hydrate was carried out using ethanol as solvent to afford 2-hydrazinyl-3-(4-nitrophenyl)-quinazolin-4(3*H*)-one (**III**). The long duration of reaction (24 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

Table 1. Antihistaminic and sedative–hypnotic activity of compounds **(IV)**–**(VIII)**

Compound code	Histamine-induced bronchospasm, s	% protection	Percent CNS depression		
			1 h	2 h	3 h
(IV)	396.00 ± 4.82**	70.20 ± 0.38**	8.94 ± 0.09**	8.12 ± 0.11**	7.23 ± 0.09**
(V)	415.00 ± 3.96**	71.56 ± 0.23**	10.80 ± 0.16**	09.62 ± 0.07**	8.35 ± 0.10**
(VI)	408.00 ± 4.13**	71.07 ± 0.38**	11.73 ± 0.24**	10.38 ± 0.11**	9.04 ± 0.09**
(VII)	374.33 ± 2.90**	68.47 ± 0.25**	11.90 ± 0.18**	10.10 ± 0.10**	9.40 ± 0.07**
(VIII)	369.50 ± 4.06**	68.06 ± 0.36**	07.66 ± 0.14**	08.71 ± 0.13**	6.76 ± 0.15**
Chlorpheniramine	394.00 ± 4.43*	70.09 ± 0.33*	38.80 ± 1.32**	34.80 ± 0.72**	29.58 ± 0.72**

Each value represents the mean ± SEM ($n = 6$). Statistical significance: **$p < 0.05$.

position. The title compounds **(IV)**–**(VIII)** were obtained in fair-to-good yields through the cyclization of compound **(III)** with a variety of one-carbon donors, such as formic acid, acetic acid, propionic acid, butyric acid, and chloroacetyl chloride, at reflux. The ^{1}H NMR spectra of compounds **(IV)**–**(VIII)** showed the absence of NH and NH_2 signals. All the synthesized compounds were confirmed by spectral data (IR, NMR, and mass spectra). Elemental (C, H, N) analysis satisfactorily confirmed elemental composition and purity of the synthesized compounds.

Antihistaminic Activity

The series of compounds containing triazolo[4,3-*a*]quinazolines ring system have been evaluated for their in vivo antihistaminic activity. The protection against histamine-induced bronchospasm on conscious guinea pigs method was adopted to determine the antihistaminic potential of the test compounds. All of them have been found to exhibit significant antihistaminic activity (Table 1). Percentage protection data showed that all test compounds of the series show significant protection in the range of 68–71.56%. Structure–activity relationship (SAR) studies indicated that different substituents at the C-1 atom exerted varied biological activity. It has been found that the presence of methyl group showed better activity over the unsubstituted compounds. When chain length increased from methyl to ethyl and propyl the activity decreased. Replacement of a proton of the methyl group by chloro group showed further decrease in activity. The order of activity was methyl > ethyl > unsubstituted > propyl > chloromethyl. Among the series, 1-methyl-4-(4-nitrophenyl)-[1, 2, 4]triazolo[4,3-*a*]quinazolin-5(4*H*)-one **(V)** was the most potent with the percentage protection of 71.56 and it is comparable to that of standard chlorpheniramine maleate (71.00%). As the test compounds could not be converted to water-soluble form, in vitro evaluation for antihistaminic activity could not be performed.

Sedative–Hypnotic Activity

As sedation is one of the major side effects associated with antihistamines, the test compounds were also evaluated for their sedative potentials. Sedative–hypnotic activity was determined by measuring the reduction in motor activity. The results of this study (Table 1) showed that almost all the test compounds were found to exhibit mild activity (less than 10%). Cetirizine and chlorpheniramine maleate were used as references, chlorpheniramine showed nearly 35% of sedation and cetirizine showed 10–13% of sedation approximately. Compared to the reference standard chlorpheniramine maleate (first-generation antihistamine), all the test compounds showed lower sedative potential; compared to cetirizine (non-sedative antihistamine) the test compounds showed equipotent sedative activity. Hence this series can be developed as clinically useful non-sedative antihistamines.

CONCLUSION

In summary, synthesis of a new series of 1-substituted-4-(4-nitrophenyl)-[1,2,4]triazolo[4,3-*a*]quinazolin-5(4*H*)-ones has been described. These derivatives exhibited promising antihistaminic activity against histamine-induced bronchospasm in the conscious guinea pigs model. Among the series, 1-methyl-4-(4-nitrophenyl)-[1,2,4]triazolo[4,3-*a*]quinazolin-5(4*H*)-one **(V)** was found to be the most active antihistaminic agent, which is more potent (71.56%) than the reference standard chlorpheniramine maleate (71.00%). Interestingly the sedative property of compound **(V)** was found to be negligible (9.59%) when compared to chlorpheniramine maleate (34.6%), therefore it could serve as a lead molecule for further modification to obtain a clinically useful novel class of antihistaminic agents.

EXPERIMENTAL

Chemistry

Starting material for the synthesis, reagents, and solvents were of analytical grade and were purchased

from Aldrich Chemical Co., Merck Chemical Co., and dried when necessary. Histamine (Sigma Chemicals, USA), aminophylline (Unichem, Mumbai), chlorpheniramine maleate (Hoechst, Mumbai), and cetirizine (Sun Pharma, Mumbai) were procured for the present biological study. All melting points reported were taken on a Veego make Silicone oil bath-type melting point apparatus and are uncorrected. NMR spectra (Bruker, USA, 500 MHz) were recorded in $CDCl_3$ (unless specified) with TMS as internal reference (chemical shift in δ, ppm). IR spectra (Shimadzu FT-IR, 8300) were registered in KBr (ν max in cm^{-1}). Mass spectra were recorded at 70 eV on a MASPEC msw 9629 instrument. Elemental analysis was performed in Carlo Erba apparatus. Silica gel-G (Merck) was used for TLC and iodine vapors used for exposure of TLC plates. Anhydrous sodium sulfate (S.D. fine chemicals) was used as drying agent.

2,3-Dihydro-3-(4-nitrophenyl)-2-thioxoquinazolin-4(1*H*)-one (I). A solution of the 4-nitroaniline (0.02 mol, 2.72 g) in dimethyl sulfoxide (10 mL) was stirred vigorously. To this carbon disulfide (1.6 mL) and aqueous sodium hydroxide (1.2 mL, 20 molar) was added dropwise during 30 min with stirring. Dimethyl sulfate (2.5 g, 0.02 mol) was added gradually with stirring by keeping the reaction mixture in freezing mixture and stirred 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 (1.5 g, 0.01 mol) and the above prepared *N*-(4-nitrophenyl)-methyl dithiocarbamic acid (0.01 mol) were dissolved in ethanol (20 mL). An anhydrous potassium carbonate (100 mg) was added to the above solution and refluxed for 18 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 in dilute hydrochloric acid. The solid obtained was filtered, washed with water, dried, and recrystallized from ethanol [4]. Yield 85%, mp 285–286°C, IR: 3268 (NH), 1670 (C=O), 1226 (C=S), 1520 (NO_2). ^{1}H NMR: 4.47 (br s, 1H, NH), 6.64 (d, J = 5.0 Hz, 1H, Ar-H), 6.76–6.78 (m, 1H, Ar-H), 7.25–7.27 (m, 1H, Ar-H), 7.70 (d, J = 5.0 Hz, 1H), 7.91 (d, J = 10.0 Hz, 2H, Ar-H), 8.15 (d, J = 2.0 Hz, 2H, Ar-H); ^{13}C NMR: 116.87 (2C), 118.75 (2C), 122.34, 124.23, 125.62, 126.75, 131.14, 137.56, 141.87, 141.32, 157.86, 177.91. MS (*m/z*): 299 [M^+]; Anal. calculated for $C_{14}H_9N_3O_3S$: C, 56.25; H, 3.05; N, 14.08. Found: C, 56.18; H, 3.03; N, 14.04.

2-Methylthio-3-(4-nitrophenyl)-quinazolin-4(3*H*)-one (II). The 2,3-dihydro-3-(4-nitrophenyl)-2-thioxoquinazolin-4(1*H*)-one (**I**) (0.01 mol, 2.98 g) was dissolved in 20 mL of 2% alcoholic sodium hydroxide solution. To this dimethyl sulfate (1.26 g, 0.01 mol) was added dropwise with stirring and stirring was continued for 1 h after completion of addition. 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 [10]. Yield 86%, mp 160–161°C, IR: 1680 (C=O), 1610 (C=N), 1510 (NO_2). ^{1}H NMR: 2.56 (s, 3H, SCH_3), 4.45 (br s, 1H, NH), 6.77–6.79 (m, 1H, Ar-H), 7.26–7.28 (m, 1H, Ar-H), 7.73 (d, J = 5.0 Hz, 1H, Ar-H), 7.95 (d, J = 10.0 Hz, 2H, Ar-H), 8.16 (d, J = 2.0 Hz, 2H, Ar-H); ^{13}C NMR: 14.75, 116.75 (2C), 118.65 (2C), 122.27, 124.21, 125.65, 126.77, 131.18, 137.66, 141.76, 141.56, 157.44, 161.65; MS (*m/z*): 313 [M^+]; Anal. calculated for $C_{15}H_{11}N_3O_3S$: C, 57.56; H, 3.55; N, 13.43. Found: C, 57.56; H, 3.54; N, 13.41.

2-Hydrazinyl-3-(4-nitrophenyl)-quinazolin-4(3*H*)-one (III). The 2-methylthio-3-(4-nitrophenyl)-quinazolin-4(3*H*)-one (**II**) (0.01 mol, 3.14 g) was dissolved in ethanol (25 mL). To this hydrazine hydrate (99%) (5.0 g, 0.1 mol) and anhydrous potassium carbonate (100 mg) were added and refluxed for 23 h. The reaction mixture was cooled and poured into ice-cold water. The solid obtained was filtered, washed with water, dried, and recrystallized from chloroform–benzene (50 : 50) mixture [11]. Yield 78%, mp 211–212°C, IR: 3332–3260 ($NHNH_2$), 1680 (C=O), 1516 (NO_2). ^{1}H NMR: 4.45 (br s, 2H, NH_2), 4.65 (br s, 1H, NH), 7.35 (d, J = 5.0 Hz, 1H, Ar-H), 7.37–7.39 (m, 1H, Ar-H), 7.46–7.48 (m, 1H, Ar-H), 7.85 (d, J = 5.0 Hz, 1H, Ar-H), 7.97 (d, J = 10.0 Hz, 2H, Ar-H), 8.26 (d, J = 2.0 Hz, 2H, Ar-H); ^{13}C NMR: 116.75 (2C), 118.65 (2C), 122.27, 124.21, 125.65, 126.77, 131.18, 137.66, 141.56, 157.44, 161.45, 162.71; MS (*m/z*): 297 [M^+]; Anal. calculated for $C_{14}H_{11}N_5O_3$: C, 56.51; H, 3.74; N, 23.53. Found: C, 56.56; H, 3.72; N, 23.55.

4-(4-Nitrophenyl)-[1,2,4]triazolo[4,3-*a*]quinazolin-5(4*H*)-one (IV). The 2-hydrazinyl-3-(4-nitrophenyl)-quinazolin-4(3*H*)-one (**III**) (0.01 mol, 2.92 g) and formic acid (15 mL) were taken in a round bottom flask and refluxed for 29 h, cooled, and poured into ice-cold water. The solid obtained was filtered, washed with water, dried, and recrystallized from chloroform–ethanol (75 : 25) mixture [12]. Yield 88%, mp 239–240°C; IR: 1682 (C=O), 1610 (C=N), 1518 (NO_2); ^{1}H NMR: 7.25 (d, J = 5.0 Hz, 1H, Ar-H), 7.35–7.37 (m, 1H, Ar-H), 7.44–7.46 (m, 1H, Ar-H), 7.86 (d, J = 5.0 Hz, 1H, Ar-H), 7.95 (d, J = 10.0 Hz, 2H, Ar-H), 8.35 (d, J = 2.0 Hz, 2H, Ar-H), 8.75 (s, 1H, Ar-H); ^{13}C NMR: 116.81 (2C), 118.77 (2C), 122.43, 124.56, 125.76, 126.58, 131.47, 137.85, 139.45, 141.89, 141.65, 148.76, 157.44; MS (*m/z*): 307 [M^+]; Anal. cald. for $C_{15}H_9N_5O_3$: C, 58.56; H, 2.93; N, 22.74. Found: C, 58.63; H, 2.95; N, 22.79.

1-Methyl-4-(4-nitrophenyl)-[1,2,4]triazolo[4,3-*a*]quinazolin-5(4*H*)-one (V). The 2-hydrazinyl-3-(4-nitrophenyl) quinazolin-4(3*H*)-one (**IV**) (0.01 mol, 2.92 g) and acetic acid (15 mL) were taken in a round-bottom flask and refluxed for 31 h, cooled, and poured into ice-cold water. The solid obtained was filtered, washed with water, dried, and recrystallized from ethanol [5]. Yield 86%, mp 255–256°C. IR: 1670 (C=O),

1602 (C=N), 1526 (NO_2). ^{1}H NMR: 2.15 (s, 3H, CH_3), 7.24 (d, J = 5.0 Hz, 1H), 7.36–7.38 (m, 1H, Ar-H), 7.45–7.46 (m, 1H, Ar-H), 7.85 (d, J = 5.0 Hz, 1H, Ar-H), 7.97 (d, J = 10.0 Hz, 2H, Ar-H), 8.36 (d, J = 2.0 Hz, 2H, Ar-H); ^{13}C NMR: 10.18, 116.92 (2C), 118.89 (2C), 122.43, 124.56, 125.76, 126.58, 131.47, 137.85, 139.45, 141.89, 148.76, 148.81, 158.65; MS (*m/z*): 321 [M^+]; Anal. calculated for $C_{16}H_{11}N_5O_3$: C, 59.74; H, 3.42; N, 21.80. Found: C, 59.81; H, 3.45; N, 21.79.

1-Ethyl-4-(4-nitrophenyl)-[1,2,4]triazolo[4,3-*a*]quinazolin-5(4*H*)-one (VI). The 2-hydrazinyl-3-(4-nitrophenyl) quinazolin-4(3*H*)-one (**IV**) (0.01 mol, 2.92 g) and propionic acid (15 mL) were taken in a round-bottom flask and refluxed for 25 h, cooled, and poured into ice-cold water. The solid obtained was filtered, washed with water, dried, and recrystallized from ethanol [13]. Yield 81%, mp 241–242°C, IR: 1690 (C=O), 1610 (C=N), 1522 (NO_2). ^{1}H NMR: 1.21 (t, J_1 = 10.0 Hz, J_1 = 5.0 Hz, CH_3), 2.84–2.88 (m, 2H, CH_2), 7.38 (d, J = 5.0 Hz, 1H, Ar-H), 7.40–7.41 (m, 1H, Ar-H) 7.45–7.46 (m, 1H, Ar-H), 7.87 (d, J = 5.0 Hz, 1H, Ar-H), 7.97 (d, J = 10.0 Hz, 2H, Ar-H), 8.17 (d, J = 2.0 Hz, 2H, Ar-H); ^{13}C NMR: 10.08, 18.56, 116.78 (2C), 118.85 (2C), 121.93, 124.56, 125.87, 126.77, 131.81, 137.91, 139.67, 141.89, 148.56, 148.96, 158.87; Mass: (*m/z*) 335 [M^+]; Anal. calculated for $C_{17}H_{13}N_5O_3$: C, 60.95; H, 3.91; N, 20.83. Found: C, 60.89; H, 3.90; N, 20.88.

4-(4-Nitrophenyl)-1-propyl-[1,2,4]triazolo[4,3-*a*]quinazolin-5(4*H*)-one (VII). The 2-hydrazinyl-3-(4-nitrophenyl) quinazolin-4(3*H*)-one (**IV**) (0.01 mol, 2.92 g) and butyric acid (15 mL) were taken in a round-bottom flask and refluxed for 23 h, cooled, and poured into ice-cold water. The solid obtained was filtered, washed with water, dried, and recrystallized from ethanol [14]. Yield 79%, mp 261–262°C, IR: 1710 (C=O), 1612 (C=N), 1518 (NO_2); ^{1}H NMR: 0.92 (t, J_1 = 10.0 Hz, J_1 = 5.0 Hz, CH_3), 1.51–1.59 (m, 2H, CH_2), 2.52–2.59 (m, 2H, CH_2), 7.36 (d, J = 5.0 Hz, 1H), 7.39–7.40 (m, 1H), 7.44–7.45 (m, 1H), 7.89 (d, J = 5.0 Hz, 1H), 7.97 (d, J = 10.0 Hz, 2H), 8.21 (d, J = 10.0 Hz, 2H); ^{13}C NMR: 10.89, 18.75, 22.56, 116.78 (2C), 118.85 (2C), 121.93, 124.56, 125.87, 126.77, 131.81, 137.89, 139.87, 141.77, 148.56, 148.96, 159.11; MS (*m/z*): 349 [M^+]; Anal. calculated for $C_{18}H_{15}N_5O_3$: C, 61.95; H, 4.30; N, 20.01. Found: C, 61.88; H, 4.32; N, 20.04.

1-(Chloromethyl)-4-(4-nitrophenyl)-[1,2,4]triazolo[4,3-*a*]quinazolin-5(4*H*)-one (VIII). The 2-hydrazinyl-3-(4-nitrophenyl) quinazolin-4(3*H*)-one (**IV**) (0.01 mol, 2.92 g) and chloroacetyl chloride (0.01 mol) in glacial acetic acid (15 mL) were taken in a round-bottom flask and refluxed for 13 h, cooled, and poured into ice water. The solid obtained was filtered, washed with water, dried, and recrystallized from chloroform–ethanol (75 : 25) mixture [15]. Yield 83%, mp 280–282°C, IR: 1680 (C=O), 1610 (C=N), 1516 (NO_2); ^{1}H NMR: 4.15 (s, 2H, CH_2Cl), 7.28 (d, J = 5.0 Hz, 1H, Ar-H), 7.41–7.48 (m, 1H, Ar-H), 7.51–7.53 (m, 1H, Ar-H), 7.95 (d, J = 5.0 Hz, 1H, Ar-H), 8.02 (d, J = 10.0 Hz, 2H, Ar-H), 8.27 (d, J = 2.0 Hz, 2H, Ar-H); ^{13}C NMR: 10.18, 116.92 (2C), 118.89 (2C), 122.43, 124.56, 125.76, 126.58, 131.47, 137.85, 139.45, 141.89, 148.76, 148.81, 158.65; MS (*m/z*): 355 [M^+] 357 [M + 2], Anal. calculated for $C_{16}H_{10}N_5O_3Cl$: C, 54.11; H, 2.85; N, 19.65. Found: C, 54.02; H, 2.83; N, 19.68.

Pharmacology

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 standard animal feed. All the animals were acclimatized for a week before use.

Antihistaminic activity. A modification of the technique of Van Arman was adopted to determine the antihistaminic potential of the synthesized compounds [9]. Male Dunkin Hartley Guinea pigs (250–300 g) were fasted for 12 h. Six animals were taken in each group. 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 [15, 16]. An intraperitoneal injection of chlorpheniramine maleate (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 1).

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

where T_1 is preconvulsive time of control; T_2, preconvulsive time of test compound. 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 actophotometer [17, 18]. Swiss albino mice were chosen as test animals in a group of six. Basal activity score was taken and then compounds (**IV**)–(**VIII**) and standard chlorpheniramine maleate were administered orally at the dose of 5 mg/kg in 1% CMC. Scores were recorded at 1, 2, and 3 h after the drug administration [19]. The percent reduction in locomotor activity was calculated by the following formula and shown in Table 1.

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

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

Statistical analysis. Statistical analysis of the biological activity of the test compounds on various animals was performed by one way ANOVA followed by Dunnett's test (manually). In all cases significance level of the means of individual groups were performed and compared with control. A significance level of $p < 0.5$ denoted significance in all cases.

COMPLIANCE WITH ETHICAL STANDARDS

The Institutional Animal Ethics committee approved the protocol adopted for the experimentation of animals. All applicable international, national, and institutional guidelines for the care and use of animals were followed.

Conflict of Interests

The authors report no conflicts of interest.

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