The first generation antihistamines penetrate the blood brain barrier and also possess anticholinergic properties; this has led to the development of a second generation of H1-antagonists such as terfenadine, cetirizine and astemizole (1). A common feature of the first generation compounds includes two aryl or heteroaryl rings linked to an ali-
phatic tertiary amine via the side chain (diphenhydramine and pheniramine) (2). The second generation compounds (terfenadine and cetirizine) contain many of the structural features of the first generation compounds. The real breakthrough of non-sedative antihistamines came in the early eighties of the twentieth century when modern antihistamines were found to exhibit potent antihistaminic activity without sedative effect (3). Condensed heterocycles containing the new generation of H₁-antihistamines (loratadine, azelastine and flazelastine) that do not possess the above mentioned pharmacophore for H₁-antihistamines paved the way for the discovery of many novel antihistamines, temelastine (4) and mangostin (5). Quinazolines and condensed quinazolines show excellent antihistaminic activity (6, 7). In continuation, we demonstrated (8, 9) the quinazoline derivatives as potent antihistamines with the least sedation. The present work is an extension of our ongoing efforts towards development and identification of new molecules. Therefore we undertook to synthesize a series of 1,2,4-triazolo-4H-[4,3-a]quinazolin-5-ones containing 3-ethylphenyl substituted at position 4 and alkyl/alicyclic amines substituted at position 1. The synthesized compounds were tested for their in vivo H₁-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 an actophotometer.

EXPERIMENTAL

Melting points were taken in open capillaries on a Thomas Hoover melting point apparatus (Thomas Hoover, USA) and are uncorrected. IR spectra were recorded in film or in potassium bromide disks on a Perkin-Elmer 398 spectrometer (Perkin-Elmer, USA). ¹H spectra were recorded on a DPX-300 MHz Bruker FT-NMR spectrometer (Bruker, USA). Chemical shifts were reported as parts per million (δ ppm) using tetramethylsilane (TMS) as an internal standard. Mass spectra were obtained on a Jeol-SX-102 instrument (Jeol, Japan) using fast atom bombardment (FAB positive). Elemental analysis was performed on a Perkin-Elmer 2400 CHN analyzer (Perkin-Elmer, USA); the values were found within the acceptable limits of the calculated values (± 0.4 %). Spectral data (IR, NMR and mass spectra) and elemental analysis data are presented in Tables I and II. The progress of the reaction was monitored on ready-made silica gel plates (Merck, Norway) using chloroform/methanol (9:1) as a solvent system. Iodine was used as a developing agent. All chemicals and reagents used in the syntheses were obtained from Aldrich (USA), Lancaster (USA) or Spectrochem (India). They were used without further purification.

Syntheses

3-(3-Ethylphenyl)-2-thioxo-2,3-dihydro-1H-quinazolin-4-one (I). – A solution of 3-ethyl aniline (0.02 mol) in dimethyl sulfoxide (10 mL) was stirred vigorously. To this, carbon-disulphide (1.6 mL) and aqueous sodium hydroxide 1.2 mL (2 mol L⁻¹) were added dropwise during 30 min under stirring. Dimethyl sulfate (0.02 mol) was added gradually keeping the reaction mixture 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 (0.01 mol) and the prepared N-(3-ethylphenyl)-methyl di-thiocarbamic acid (0.01 mol) were dissolved in ethanol (20 mL). To this, anhydrous potassium carbonate (100 mg) was added and refluxed for 23 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 hydrochloric
acid. The solid obtained was filtered, washed with water, dried and recrystallized from ethanol.

3-(3-Ethylphenyl)-2-methylsulfanyl-3H-quinazolin-4-one (2). – Compound 1 (0.01 mol) was dissolved in 40 mL of 2 % alcoholic sodium hydroxide solution. To this, dimethyl sulfate (0.01 mol) was added dropwise under 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 the ethanol/chloroform (75:25) mixture.

3-(3-Ethylphenyl)-2-hydrazino-3H-quinazolin-4-one (3). – Compound 2 (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 38 h. The reaction mixture was cooled and poured into ice water. The solid so obtained was filtered, washed with water, dried and recrystallized from the chloroform/benzene (25:75) mixture.

4-(3-Ethylphenyl)-1-substituted-4H-[1,2,4]triazolo[4,3-a]quinazolin-5-ones (4a-e). – 3-(4-Ethylphenyl)-2-hydrazino-3H-quinazolin-4-one (3) (0.01 mol) was poured into a round-bottomed flask and refluxed for 36 h, cooled and poured into ice water. The solid obtained 4a was filtered, washed with water, dried and recrystallized from ethanol. Adopting this procedure, compounds 4b-e were also prepared.

4-(3-Ethylphenyl)-1-substituted-4H-[1,2,4]triazolo[4,3-a]quinazolin-5-ones (4f-j). – A mixture of 1-chloromethyl-4-(3-ethylphenyl)-4H-[1,2,4]triazolo[4,3-a]quinazolin-5-one (4e) (0.01 mol) pyrrolidine (0.05 mol) and anhydrous potassium carbonate (100 mg) in dioxane (25 mL) was put in a round-bottom flaks and refluxed for 39 h, cooled and poured into ice water. The solid obtained 4f was filtered, washed with water, dried and recrystallized from ethanol/benzene (50:50). Implementing this protocol, compounds 4g-j were also prepared.

Pharmacology

Animals. – Antihistaminic activity was evaluated on male Dunkin Hartley guinea pigs (250–300 g), while sedative-hypnotic activity was tested on albino Swiss mice. 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 animals were acclimatized for a week before the experiment. The Institutional Animal Ethics Committee approved the protocol adopted for the experimentation on animals.

Antihistaminic activity. – A modification of the method of Van Arman (10) was adopted to determine the antihistaminic potential of the synthesized compounds. Six animals were allotted to each group and were fasted for 12 h. The test compounds and reference standard (chlorpheniramine maleate) were administered orally at a dose of 10 mg kg\(^{-1}\) in 1 % CMC (carboxymethylcellulose) and challenged with histamine aerosol (3 mL of 0.2 % aqueous solution of histamine hydrochloride) 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 the 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, India) at a dose of 25 mg kg\(^{-1}\) was given for the recovery of test animals.

Sedative-hypnotic activity. – Sedative-hypnotic activity was determined by measuring the reduction in locomotor activity using an actophotometer (11, 12). Six albino Swiss mice were allotted to each group. Basal activity score was taken and then compounds 4a-j and standard chlorpheniramine maleate were administered orally at a dose of 5 mg kg\(^{-1}\) in 1 % CMC. Scores were recorded 1, 2 and 3 h after the drug administration.
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, posthoc comparisons of the means of individual groups were performed using Tukey’s test. All values are expressed as mean ± SD (standard deviation). For statistical analysis, the GraphPad Prism 3.0 version was used.

RESULTS AND DISCUSSION

Chemistry

The key intermediate 3-(3-ethylphenyl)-2-thioxo-2,3-dihydro-1H-quinazolin-4-one (1) was prepared by refluxing methyl anthranilate with 3-ethylphenyl isothiocyanate in ethanol. However, the preparation of 3-ethylphenyl isothiocyanate required for the reaction was a tedious, time consuming process and the yield was also low (60 %). An alternate route was attempted to synthesize compound 1. In this route, 3-ethyl aniline was reacted with carbon disulphide and anhydrous potassium carbonate in acetone to give potassium dithiocarbamate, which was methylated with dimethyl sulfate to afford the dithiocarbamic acid methyl ester, which was refluxed with methyl anthranilate to yield 1. This way of synthesizing 1 suffers from the drawbacks such as the multi-step process, prolonged reaction time (37 h) and low yield (30 %). Hence, improvisation was carried out in this method by using aqueous sodium hydroxide (2 mol L⁻¹) instead of anhydrous K₂CO₃, and dimethyl sulphoxide (DMSO) (Scheme 1) as a solvent instead of acetone. The use of DMSO as the reaction solvent enhanced the rate of reaction and the use of alkali in higher concentration helped prevent hydrolysis of the intermediate, probably due to less solvation. These modifications not only curtailed the reaction time from 37 to 25 h, but also increased the yield from 30 to 80 %. The product obtained was cyclic and not open-chain thiourea. The structure was confirmed by the IR spectrum, which showed intense peaks at 3216 cm⁻¹ for cyclic thiourea (NH), 1692 cm⁻¹ for carbonyl (C=O) and 1210 cm⁻¹ for thioxo (C=S) stretching. ¹H NMR spectrum of 1 showed a triplet at δ 1.52–1.64 ppm due to the CH₃ group, a quartet at δ 2.43–2.56 ppm due to CH₂ and a multiplet at δ 7.15–7.79 ppm for aromatic (8H) protons and a singlet at δ 10.45 ppm indicating the presence of NH. Data from the elemental analyses were found to be in conformity with the assigned structure. Further, the molecular ions recorded in the mass spectrum are also in agreement with the molecular mass of compound 1.

3-(3-Ethylphenyl)-2-methylsulfanyl-3H-quinazolin-4-one (2) was obtained by dissolving product 1 in a 2 % alcoholic sodium hydroxide solution and methylating with dimethyl sulfate while stirring at room temperature. The IR spectrum of 2 showed disappearance of NH and C=S stretching signals of cyclic thiourea. It showed a peak for carbonyl (C=O) stretching at 1692 cm⁻¹. The ¹H NMR spectrum of compound 2 showed a triplet at δ 1.25–1.38 ppm due to CH₃, a quartet at δ 2.05–2.17 ppm due to CH₂, a singlet at δ 2.46 ppm due to SCH₃ and a multiplet at δ 7.33–8.05 ppm due to aromatic (8H) protons. Data from the elemental analyses and the molecular ion recorded in the mass spectrum further confirmed the assigned structure of 2.

Nucleophilic displacement of methylthio group of compound 2 with hydrazine hydrate was carried out using ethanol as solvent to afford 3-(3-ethylphenyl)-2-hydrazino-
The title compounds were synthesized by cyclization of 3-(3-ethylphenyl)-2-hydrazino-3H-quinazolin-4-one (3) with various one-carbon donors. The 3-(3-ethylphenyl)-2-hydrazino-3H-quinazolin-4-one was synthesized from 3-ethyl aniline by a new innovative route (Scheme 1). The title compounds 4a-j were obtained in fair to good yields through cyclization of 3 with a variety of one-carbon donors such as formic acid, acetic acid, propionic acid, butyric acid and chloroacetyl chloride at reflux. The cyclic product formation is indicated by the disappearance of peaks due to NH and NH$_2$ of the starting compound at 3390–3225 cm$^{-1}$ in IR spectra of all compounds 4a-e. The $^1$H NMR spectra of 4f-j showed the absence of NH and NH$_2$ signals. Compounds 4f-j were obtained by the displacement of chlorine of compound 4e with various alicyclic amines such as pyrro-
lidine, piperidine, morpholine, piperazine and 4-methylpiperazine. The IR spectra of compounds 4a-j showed a peak for carbonyl (C=O) around 1680 cm⁻¹. The ¹H NMR spectra of compounds 4a-j showed multiplets around δ 7.01–8.09 ppm integrating for aromatic protons. Mass spectra of the title compounds are in conformity with the assigned structure and showed molecular ion peaks corresponding to their molecular formula. The M⁺+2 peak was observed in the spectrum of compound 4e, confirming the presence of the chlorine atom in the compound. The relative intensity of this M⁺+2 peak compared to M⁺ peak is in a ratio of 1:3. The M⁺+2 peak observed in the spectrum of compound 4e disappeared in compounds 4f-j, confirming the displacement of chlorine. In mass spectra of compounds 4a-j, 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 quinazolin-4-one moiety appeared in all mass spectra. Elemental analyses confirmed the elemental composition and purity of the synthesized compounds.

Pharmacology

Compounds containing the 1,4-disubstituted [1,2,4]triazoloquinazoline ring system (4a-j) were evaluated for their in vivo antihistaminic activity. All the tested compounds were found to exhibit good antihistaminic activity (Table III). Protection data showed that all compounds of the series show significant protection in the range of 70–74 %. Biological studies indicated that different substituents over the first position of the triazoloquinazoline ring exerted varying biological activity. The presence of the methyl group (compound 4b, 74.6 % protection) showed better activity than the unsubstituted com-

<table>
<thead>
<tr>
<th>Compd.</th>
<th>Time of onset of convulsion (s)</th>
<th>Protection (%)a</th>
<th>CNS depression (%)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controlc</td>
<td>116 ± 2d</td>
<td>-</td>
<td>6 ± 1d</td>
</tr>
<tr>
<td>Chlorpheniramine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4a</td>
<td>400 ± 10</td>
<td>71 ± 1</td>
<td>37 ± 2</td>
</tr>
<tr>
<td>4b</td>
<td>415 ± 2f</td>
<td>72 ± 2</td>
<td>8 ± 1d</td>
</tr>
<tr>
<td>4c</td>
<td>456 ± 3d</td>
<td>75 ± 2e</td>
<td>9 ± 2d</td>
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<tr>
<td>4d</td>
<td>445 ± 9e</td>
<td>74 ± 1e</td>
<td>11 ± 2d</td>
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<td>398 ± 7f</td>
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<td>390 ± 7f</td>
<td>70 ± 1f</td>
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<tr>
<td>4g</td>
<td>395 ± 7f</td>
<td>71 ± 1f</td>
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<td>4h</td>
<td>403 ± 8</td>
<td>71 ± 2</td>
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<tr>
<td>4i</td>
<td>409 ± 7</td>
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<tr>
<td>4j</td>
<td>426 ± 6e</td>
<td>73 ± 2f</td>
<td>10 ± 1d</td>
</tr>
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</table>

a Control: oral dose of 10 mg kg⁻¹ in 1 % CMC.
b Control: oral dose of 5 mg kg⁻¹ in 1 % CMC.
c Control: animals were administered 1 % CMC orally.
Significant different relative to chlorpheniramine: d p < 0.0001, e p < 0.001, f p < 0.01.
pound (compound 4a, 72.0 % protection). With increased lipophilicity (i.e., ethyl compound 4c, 73.9 % protection) the activity remained but further increase in lipophilicity (i.e., propyl compound 4d, 70.8 % protection) led to a decrease in activity. Replacement of a proton of the methyl group by chlorine (compound 4e, 70.2 % protection) showed a further decrease in activity. Replacement of a proton of the methyl group by alicyclic amines (pyrrolidinyl and piperidinyl compound 4f 70.6 % protection and 4g 71.2 % protection, respectively) showed an increase in activity compared to the chloro substituent. Placement of alicyclic amines with additional hetero atom (morpholinyl compound 4h 71.6 % protection; piperazinyl compound 4i 72.8 % protection and 4-methyl piperazinyl compound 4j 73.5 % protection) led to further increase in activity. 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 exhibited negligible sedation (8–13 %) whereas the reference standard chlorpheniramine maleate showed 30 % sedation.

CONCLUSIONS

The present study describes the synthesis of a new series of 4-(3-ethylphenyl)-1-substituted-4H-[1,2,4]triazolo[4,3-a]quinazolin-5-ones (4a-j). The title compounds have exhibited promising antihistaminic activity against histamine-induced bronchospasm on conscious guinea pigs in the in vivo model. Among the series, 4-(3-ethylphenyl)-1-methyl-4H-[1,2,4]triazolo[4,3-a]quinazolin-5-one (4b) was found to be the most active compound. It was more potent than the reference standard chlorpheniramine maleate. Interestingly, compound 4b also showed negligible sedation and could therefore serve as a lead molecule for further modifications to obtain a clinically useful novel class of non-sedating antihistamines.

REFERENCES


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S A Ž E T A K

Sinteza i farmakološko ispitivanje novih 4-(3-etilfenil)-1-supstituiranih 4H-[1,2,4]triazolo[4,3-a]kinazolin-5-ona kao nove klase H₁-antihistaminika

VEERACHAMY ALAGARSAMY, KUNCHU KAVITHA, MANI RUPESHKUMAR, VISWAS RAJA SOLOMON, JAYA KUMAR, DINAKARAN SATHESH KUMAR i HEMANT KUMAR SHARMA

Ciklizacijom 3-(3-etilfenil)-2-hidrazino-3H-kinazolin-4-onu (3) s različitim donorima jednog C atoma sintetizirana je serija novih 4-(3-etilfenil)-1-supstituiranih 4H-[1,2,4]triazolo[4,3-a]kinazolin-5-ona (4a-j). Početni spoj 3 pripravljen je iz 3-etil anilina na novi, innovativni način, s poboljšanim iskorišćenjem. U testovima in vivo na zamorcima, s testirani spojevi pokazali su značajno zaštitno djelovanje protiv bronhospazma induciranog histaminom. Spoj 4-(3-etilfenil)-1-metil-4H-[1,2,4]triazolo[4,3-a]kinazolin-5-on (4b) najaktivniji je među testiranim spojevima (zaštita 74.6 %) i jači od referentnog standarda klorfeniramin maleata (zaštita 71 %). Spoj 4b pokazuje zanemarivu sedaciju (10 %) u usporedbi s klorfeniramin maleatom (30 %). Stoga spoj 4b može biti vodeći spoj za daljnji razvoj nove klase H₁-antihistaminika.

Ključne riječi: kinazolin-5-oni, sedacija, H₁-antihistaminici

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