Thiazolidinyl-triazinoquinazolines as potent anti-inflammatory agents

Ekta Bansal, Tilak Ram, Shalabh Sharma, Mirdula Tyagi, Kiran Bajaj, Ritu Tyagi, Bhawna Goel, V K Srivastava, J N Gurut & Ashok Kumar

Medicinal Chemistry Division, Department of Pharmacology, L.L.R.M. Medical College, Meerut 250 004, India

Received 1 April 1999; accepted (revised) 28 June 2000

Some new S-(S'-substituted-aryl-2'-oxo-4'-thiazolidin-1'-yl)amino-4-phenyl-2-methyl-10-bromo-[1,2,4]triazino[2,3-c]quinazolines have been synthesized by [1,5]cyclocondensation of thiolactic acid with S-arylidene hydrazino-4-phenyl-2-methyl-10-bromo-[1,2,4]triazino[2,3-c]quinazolines. All the compounds of the series have been screened for their anti-inflammatory activity. The most potent compound of the series S-(S'-p-dimethylaminophenyl-2'-oxo-4'-thiazolidin-1'-yl)amino-4-phenyl-2-methyl-10-bromo-[1,2,4]triazino[2,3-c]quinazolines has shown 48.93% activity at a dose of 50 mg/kg p.o. The structures of the products have been delineated by chemical reactions, elemental analysis and spectral studies.

Various quinazoline congeners possess diverse type of biological activities viz. anti-inflammatory, anti-parkinsonian, antihelmintic, anti-bacterial and hypotensive etc. Furthermore, a wide spectrum of biological activities including the anti-inflammatory activity has been reported in different heterocyclic derivatives of thiazolidinones and [1,2,4]triazino derivatives. No attempts have been made to incorporate [1,2,4]triazino and thiazolidinyl moieties in quinazoline nucleus in a single molecular framework.

In order to see the effect of incorporation of [1,2,4]triazino- and 4-thiazolidinyl moieties in quinazoline nucleus, on inflammation produced by carrageenan in albino rats, we have synthesized a new series 5-(5'-substituted-aryl-2'-oxo-4'-thiazolidin-1'-yl) amino-4-phenyl-2-methyl-10-bromo-[1,2,4]triazino[2,3-c]quinazolines. The starting compound 2-methyl-6-bromo-4H-3,1-benzoxazin-4-one has been prepared according to the reported method.

Compound 1 was treated with phenyl hydrazine in the presence of dry pyridine to give 3-anilino-2-methyl-6-bromoquinazolin-4-one. Its structure was derived from elemental analysis and spectral (IR,1H NMR) data. Among the significant features of its 1H NMR spectrum were the appearance of the proton of -NH of phenyl hydrazine moiety, downfield at δ 9.8 and a multiplet at δ 7.65-6.90 due to aromatic protons.

On the other hand, the reaction of 3-anilinoquinazolin-4-one with chloroacetyl chloride in the presence of DMF furnished a compound formulated as 3-(N-chloroacetyl)anilino-2-methyl-6-bromoquinazolin-4-one on the basis of its analytical and spectral (IR,1H NMR) data. Appearance of a singlet at δ 3.95 due to CH2 group of chloroacetyl moiety further confirms the structure of compound 3.

Compound 3 underwent cyclization in the presence of ammonium acetate to give 4-phenyl-2,3-dihydro-6-methyl-10-bromo-[1,2,4]triazino[2,3-c]quinazolin-5-one. Its structure was confirmed by IR and 1H NMR spectra.

The oxo-triazino compound 4 on treatment with POCI3 in ethanol gave 3-chloro-4-phenyl-6-methyl-10-bromo-[1,2,4]triazino[2,3-c]quinazolines. The appearance of C-Cl band (680 cm-1) with the disappearance of C=O (1720-1660 cm-1) clearly indicates that C=O group of oxo-triazino compound 4 has been changed into chloro group. The appearance of a multiplet for 6-aromatic protons in compound 5 instead of 5-aromatic protons in compound 4 in the 1H NMR spectrum of chlorotriazino compound 5 clearly shows the presence of one more aromatic proton of N-CH group and the change of C=O group into C-Cl.

Chlorotriazinoquinazolines on refluxing with hydrazine hydrate in DMF for 1 hr yielded 3-hydrazino-4-phenyl-6-methyl-10-bromo-[1,2,4]triazino[2,3-c]quinazolines. Its structure was confirmed by IR and 1H NMR spectra.

Compound 6 on reaction with various aldehydes in the presence of glacial acetic acid yielded various arylidene hydrazinoquinazolines 7a-e. The formation
of 7a-e was confirmed by the absence of a signal at δ 8.45 (bs, 2H, NH2) and appearance of a signal at δ 8.6 (s, 1H, =CH-Ar) in the 1H NMR spectrum. Also in the IR spectrum, disappearance of a band at 3340 cm⁻¹ (due to C=N of arylidene group) further supports the formation of 7a-e (Scheme I, Table I).

[1,2] Cyclocondensation of thiolactic acid with arylidene-hydrazino quinazolines 7a-e in the presence of 1,4 dioxane gave 3- (5'-substituted-aryl-2'-oxo-4'-thiazolidin-1'-yl) amino-4-phenyl-6-methyl-10-bromo-[1,2,4]triazino[2,3-c]quinazolines 8a-e. Its IR spectrum exhibited sharp absorption bands due to C=O (1640 cm⁻¹) and C=S (1140 cm⁻¹) which indicates the presence of β-thiolactum ring. Further, in 1H NMR spectrum a signal at δ 5.94 (s, 1H, =CH-Ar), 4.45 (q, 1H, =CH-CH₃) and δ 0.70 (d, 3H, -CH-CH₃) confirms the presence of thiazolidinone moiety.

Anti-inflammatory activity
This study was conducted on albino rats of either sex (120 to 180 g). The rats were divided into groups of six animals each. All the newly synthesized compounds were screened for their anti-inflammatory activity using rat paw oedema method of Winter et al.12 The percent anti-inflammatory activity was calculated according to the formula given below.

\[
\text{% anti-inflammatory effect} = 1 - \frac{D_D}{D_C} \times 100
\]
<table>
<thead>
<tr>
<th>Compd</th>
<th>R</th>
<th>R'</th>
<th>m.p. °C</th>
<th>Yield (%)</th>
<th>Recrystallisation solvent</th>
<th>Mol. Formula (Mol. wt.)</th>
<th>Found % (Calcd)</th>
<th>Anti-inflammatory activity (% inhibition)*</th>
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</thead>
<tbody>
<tr>
<td>2</td>
<td>-H</td>
<td>-</td>
<td>210</td>
<td>90</td>
<td>Methanol</td>
<td>C_{9}H_{11}N_{2}Br (330)</td>
<td>54.50 3.59 12.76</td>
<td>11.12</td>
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<tr>
<td>3</td>
<td>-H</td>
<td>-</td>
<td>120</td>
<td>80</td>
<td>Benzene/Pet.ether</td>
<td>C_{7}H_{12}N_{2}OBr (406.5)</td>
<td>50.22 3.23 10.29</td>
<td>15.34</td>
</tr>
<tr>
<td>4</td>
<td>-H</td>
<td>-</td>
<td>186</td>
<td>85</td>
<td>Ethanol/Water</td>
<td>C_{17}H_{14}N_{2}OCl (439)</td>
<td>55.32 3.52 15.17</td>
<td>17.67</td>
</tr>
<tr>
<td>5</td>
<td>-H</td>
<td>-</td>
<td>228</td>
<td>70</td>
<td>Benzene/Pet. ether</td>
<td>C_{17}H_{14}N_{2}BrCl (387.5)</td>
<td>52.60 3.13 14.49</td>
<td>24.23</td>
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<tr>
<td>6</td>
<td>-H</td>
<td>-</td>
<td>219</td>
<td>65</td>
<td>Ethanol</td>
<td>C_{21}H_{20}N_{2}Br (383)</td>
<td>53.30 3.87 21.89</td>
<td>10.12</td>
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<tr>
<td>7a</td>
<td>-H</td>
<td>-</td>
<td>232</td>
<td>55</td>
<td>Methanol/Water</td>
<td>C_{9}H_{11}N_{2}Br (471)</td>
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<tr>
<td>7b</td>
<td>-H</td>
<td>-</td>
<td>224</td>
<td>40</td>
<td>Benzene/Acetone</td>
<td>C_{9}H_{11}N_{2}OBr (461)</td>
<td>57.30 3.64 18.18</td>
<td>11.11</td>
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<td>7c</td>
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<td>-</td>
<td>248</td>
<td>50</td>
<td>THF</td>
<td>C_{21}H_{20}N_{2}Br (514)</td>
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<td>7d</td>
<td>-H</td>
<td>-</td>
<td>258</td>
<td>45</td>
<td>Acetic acid/water</td>
<td>C_{21}H_{20}N_{2}OBr (501)</td>
<td>59.82 4.23 16.80</td>
<td>30.55</td>
</tr>
<tr>
<td>7e</td>
<td>-H</td>
<td>-</td>
<td>252</td>
<td>60</td>
<td>Ethanol/Benzene</td>
<td>C_{21}H_{20}N_{2}OBr (501)</td>
<td>59.84 4.15 16.80</td>
<td>33.33</td>
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Table I — Characterisation data and anti-inflammatory activity of compounds 2-7
where $D_t$ and $D_c$ are the volume of oedema in drug treated and control group, respectively. Phenylbutazone was used as the reference drug for comparison.

**Experimental Section**

3-Anilino-2-methyl-6-bromoquinazolin-4-one 2. To a solution of compound 1 (0.01 mole) in dry pyridine (120 mL), phenyl hydrazine (0.01 mole) was added dropwise. The reaction mixture was refluxed for 6-8 hr, concentrated, cooled and poured onto iced HCl. The solid thus obtained, was recrystallised from methanol to give 2, mp 210°C, yield 90% (Found: C, 54.50; H, 3.59; N, 12.76. C$_{11}$H$_7$N$_5$OBr requires C, 54.54; H, 3.63; N, 12.72%); IR (KBr): 3340 (C-OH), 3290, 2960, 2880 (aliphatic CH), 1720-1660 (C=O), 1620 (C=C), 1610 (C=N), 1300 (NCN), 1230 (C=N), 850, 780, 690 (phenyl and aryl groups); $^1$H NMR (CDCl$_3$+DMSO-d$_6$): $\delta$ 1.30 (s, 3H, -CH$_3$), 3.65 (s, 2H, CH$_2$), 8.25 (dd, 1H, $J = 9.0$ Hz, H$_a$), 7.90 (dd, 1H, $J = 7.41$ Hz, H$_b$), 7.75 (dd, 1H, $J = 7.51$ Hz, H$_c$), 7.65-6.90 (m, 5H, Ar-H); MS: m/z 369 [M$^+$].

3-Chloro-4-phenyl-6-methyl-10-bromo[1,2,4]triazino[2,3-c]quinazolines 5. Compound 4 (0.01 mole) and POCl$_3$ (20 mL) in ethanol was heated on an oil-bath for 2 hr at 150-60°C and then cooled. The oily mixture obtained was poured onto ice with constant stirring. The solid thus obtained was washed with dil. NaOH followed by cold water and recrystallised from benzene/pet ether, mp 228°C, yield 70% (Found: C, 52.60; H, 3.13; N, 14.49. C$_{17}$H$_9$N$_3$OBr requires C, 52.64; H, 3.09; N, 14.45%); IR (KBr): 1640 (C=C), 1620 (C=N), 1320 (NCN), 680 (C-Cl); $^1$H NMR (CDCl$_3$+DMSO-d$_6$): $\delta$ 8.20 (dd, 1H, $J = 9.0$ Hz, H$_a$), 7.95 (dd, 1H, $J = 7.41$ Hz, H$_b$), 7.70 (dd, 1H, $J = 7.51$ Hz, H$_c$), 7.65-6.95 (m, 6H, Ar-H and -N=CH$_2$ of triazino ring), 1.35 (s, 3H, CH$_3$); MS: m/z 387.5 [M$^+$].

3-Hydrazino-4-phenyl-6-methyl-10-bromo[1,2,4]triazino[2,3-c]quinazolines 6. To a solution of compound 5 (0.01 mole) in DMF (25 mL) hydrazine hydrate (0.01 mole) was added slowly with stirring and the reaction mixture was refluxed for 2 hr, concentrated and cooled. The separated solid was filtered and recrystallised from ethanol, mp 219°C, yield 65% (Found: C, 53.30; H, 3.87; N, 21.89. C$_{17}$H$_9$N$_3$OBr requires C, 53.26; H, 3.91; N, 21.93%); IR (KBr): 3340 (NH$_2$), 3150 (NH$_2$), 1620 (C=C), 1340 (NCN), 1600 (C=N); $^1$H NMR (CDCl$_3$+DMSO-d$_6$): $\delta$ 8.20 (dd, 1H, $J = 9.0$ Hz, H$_a$), 7.95 (dd, 1H, $J = 7.41$ Hz, H$_b$), 7.70 (dd, 1H, $J = 7.51$ Hz, H$_c$), 7.65-6.90 (m, 6H, Ar-H and -N=CH$_2$ of triazino ring), 1.35 (s, 3H, CH$_3$); MS: m/z 383 [M$^+$].

4-Phenyl-2,3-dihydro-6-methyl-10-bromo[1,2,4]-triazino[2,3-c]quinazolin-5-one 4. A mixture of compound 3 (0.01 mole) in ethanol and ammonium acetate (5 g) in the presence of a few drops of gl. acetic acid was refluxed for 5 hr. The reaction mixture was concentrated, cooled and poured into cold water. The separated solid was filtered, washed with water and recrystallised from ethanol/water to yield compound 4, mp 186 °C, yield 85% (Found: C, 55.32; H, 3.48; N, 15.21. C$_{17}$H$_9$N$_3$OBr requires C, 55.28; H, 3.52; N, 15.17%); IR (KBr): 3070 (aromatic CH), 2960, 2880 (aliphatic CH), 1720-1660 (C=O), 1620 (C=C), 1610 (C=N), 1300 (NCN), 1230 (C=N), 850, 780, 690 (phenyl and aryl groups); $^1$H NMR (CDCl$_3$+DMSO-d$_6$): $\delta$ 1.30 (s, 3H, -CH$_3$), 3.65 (s, 2H, CH$_2$), 8.25 (dd, 1H, $J = 9.0$ Hz, H$_a$), 7.90 (dd, 1H, $J = 7.41$ Hz, H$_b$), 7.75 (dd, 1H, $J = 7.51$ Hz, H$_c$), 7.65-6.90 (m, 5H, Ar-H); MS: m/z 369 [M$^+$].

3-Arylidene hydrazino-4-phenyl-6-methyl-10-bromo[1,2,4]triazino[2,3-c]quinazolines 7a-e. A mixture of 6 (0.01 mole) in ethanol in the presence of a few drops of glacial acetic acid and some aldehydes namely, benzaldehyde, furfuraldehyde, p-(N, N-dimethylaminobenzaldehyde, p-anisaldehyde, o-anisaldehyde was refluxed for 6-8 hr. The reaction mixture was concentrated, cooled and poured onto ice. The separated solid was filtered and recrystallised from appropriate solvents. Physical and analytical data of compounds 7a-e are given in Table 1 Compound 7d: IR (KBr): 1650-1600 (C=C, C=N), 850 (phenyl group),
### Table II — Characterisation data and anti-inflammatory activity of compounds 8a-e

<table>
<thead>
<tr>
<th>Compd</th>
<th>R</th>
<th>R'</th>
<th>m.p.</th>
<th>Yield (%)</th>
<th>Recrystallisation solvent</th>
<th>Mol. formula</th>
<th>Found % (Calcld)</th>
<th>Dose mg/kg p.o</th>
<th>Anti-inflammatory activity (% inhibition)*</th>
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<tbody>
<tr>
<td>8a</td>
<td>-H</td>
<td></td>
<td>258</td>
<td>45</td>
<td>DMF</td>
<td>C₂₈H₂₈N₆O₂SBr (559)</td>
<td>57.92/4.07/15.06</td>
<td>50</td>
<td>25.53</td>
</tr>
<tr>
<td>8b</td>
<td>-H</td>
<td></td>
<td>244</td>
<td>35</td>
<td>Ethanol/benzene</td>
<td>C₂₈H₂₈N₆O₂SBr (549)</td>
<td>54.68/3.86/15.26</td>
<td>50</td>
<td>12.76</td>
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<tr>
<td>8c</td>
<td>-H</td>
<td>N(CH₃)₂</td>
<td>256</td>
<td>40</td>
<td>Benzene/hexane</td>
<td>C₂₈H₂₈N₆O₂SBr (602)</td>
<td>57.76/4.69/16.32</td>
<td>25</td>
<td>38.29</td>
</tr>
<tr>
<td>8d</td>
<td>-H</td>
<td>OCH₃</td>
<td>272</td>
<td>30</td>
<td>Methanol/toluene</td>
<td>C₂₈H₂₈N₆O₂SBr (589)</td>
<td>57.00/4.20/14.30</td>
<td>50</td>
<td>34.04</td>
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<tr>
<td>8e</td>
<td>-H</td>
<td>OCH₃</td>
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<td>Acetone/benzene</td>
<td>C₂₈H₂₈N₆O₂SBr (589)</td>
<td>57.00/4.28/14.22</td>
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<td>29.79</td>
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<td>Phenylbutazone</td>
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<td></td>
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<td>35</td>
<td></td>
<td></td>
<td></td>
<td>50</td>
<td>38.90</td>
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</table>

*All compounds were tested at a dose of 50 mg/Kg P.O
3110 (NH), 750 (aryl group), 3040 (e=C-H), 2870 (C-H), 1590 (C=N of N=CH-Ar group); \(^1\)H NMR (CDCl\(_3\)+DMSO-\(d_6\)): \(\delta\) 9.85 (s, 1H, NH attached to triazino ring, exchangeable with D\(_2\)O), 8.6 (s, 1H, =CH-Ar), 3.80 (s, 3H, Ar-OCH\(_3\)), 3.80 (s, 3H, Ar-OCH\(_3\)), 8.3 (dd, 1H, J = 9.0 Hz, H\(_6\)), 7.90 (dd, 1H, J = 7.41 Hz, H\(_5\)), 7.75 (dd, 1H, J = 7.51 Hz, H\(_7\)), 7.65-6.25 [m, 10H, (9H, Ar-H; 1H, N-CH=)](M)

3-(5'Substituted-aryl-2'oxo-4'thiazolidin-1'-yl)amino-4-phenyl-6methyl-10bromo[1,2,4]triazino[2,3-c]quinazolines 8a-c. To a cold solution of 7a-e (0.01 mole) in ethanol was added thiolactic acid (0.02 mole) dropwise with stirring at ambient temperature in the presence of 1,4-dioxane. The reaction mixture was refluxed for 18-20 hr, concentrated, cooled and poured into iced-water. The resulting solid was recrystallised from appropriate solvents. Physical and analytical data of compounds 8a-e are given in Table II. Compound 8e: \(^1\)H NMR (CDCl\(_3\)), 1.35 (s, 3H, -CH\(_3\)), 3.80 (s, 3H, Ar-OCH\(_3\)), 8.3 (dd, 1H, J = 9.0 Hz, H\(_6\)), 7.90 (dd, 1H, J = 7.41 Hz, H\(_5\)), 7.70 (dd, 1H, J = 5.41 Hz, H\(_7\)), 7.60-6.20 [m, 10H (9H, Ar-H; 1H, -N=CH-C- of triazino ring)]; MS: m/z 589 [M\(^+\)].

Anti-inflammatory activity against carrageenan induced oedema

Fifteen newly synthesized quinazolines were studied for their anti-inflammatory activity against carrageenan induced oedema. All the compounds were tested at the dose of 50 mg/kg oral unless otherwise stated (Tables I and II).

All the compounds of the series have shown varying degree (11.11% to 48.93%) of anti-inflammatory activity. However, five compounds (7c, 7d, 7e, 8c, 8d) exhibited statistically significant inhibition of the oedema. Compound 8e was found to possess most potent activity (48.93% inhibition), which was found to be more than that of the reference drug, phenylbutazone (38.9% inhibition).

Compound 8c and phenylbutazone were tested for the anti-inflammatory activity at three graded doses (25, 50 and 100 mg/kg oral). At all the three dose levels, 8c showed more inhibitory activity than that of phenylbutazone.

Structure activity relationship

SAR study of quinazoline has revealed that substitution at position 2 and 3 of quinazoline nucleus markedly enhanced the anti-inflammatory activity. Substitution at 3-position of quinazoline nucleus by phenylhydrazine group (compound 2) have shown noticeable anti-inflammatory activity. Furthermore, introduction of chloroethyl moiety to quinazoline nucleus (compound 3) showed a slight increase in the activity. Formation of triazino ring due to [3,4] cyclisation of quinazoline nucleus (compound 4) further revealed a remarkable increase in the activity. Compound 5 showed a greater increase in anti-inflammatory activity as it has got more electron negative atom i.e. Cl-atom.

Hence, from the present study, it can be concluded:
(a) that [3,4] cyclisation of quinazoline nucleus into triazino ring increases the anti-inflammatory activity;
(b) that the hydrazino-triazino quinazolines having methoxy substituent (at o- or p-position) in the phenyl group linked to hydrazino-triazino ring possess potent anti-inflammatory activity;
(c) that the substitution of p-dimethylamino group (7e and 8c) at p-position of phenyl group linked to azomethine group exhibits potent anti-inflammatory activity;
(d) furthermore, [1,5] cyclocondensation of thiolactic acid with hydrazinotriazino quinazolines resulting in the for-mation of thiazolidinyl-triazino-quinazolines showed a remarkable increase in anti-inflammatory activity.

References