SYNTHESIS OF NOVEL THIAZOLO-QUINAZOLINES AS ANTINOCICEPTIVE AND ANTI-INFLAMMATORY AGENTS

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ABSTRACT
A series of 6,7,8,9 tetrahydro-5H-5-(2'-hydroxy phenyl)-2-(4'-some substituted benzylidine) thiazolo (2,3-b) quinazolin-3(2H)-one (4a-4d) and 6,7,8,9 tetrahydro-5H-5-(2'-hydroxy phenyl)-2-(4'-some substituted benzylidine)-3-(4-nitrophenyl amino) thiazolo quinazoline (5a-5d) have been synthesized. All the newly synthesized compounds chemical structure were confirmed by IR, ¹H-NMR, mass spectroscopy and elemental analysis. The new compounds have been tested for their antinociceptive, anti-inflammatory activities. The results of studies indicate that the hydroxy substitution in the benzylidine ring increased the anti-inflammatory and antinociceptive activities.

Key words: Thiazolo quinazoline; Antinociceptive activity; Anti-inflammatory.

INTRODUCTION
Bacterial infections often produce pain and inflammation. In normal practice, two groups of agents (antinociceptive and anti-inflammatory) are prescribed simultaneously. Unfortunately, none of drug possesses these two activities in a single component. Therefore the discovery of new safer anti-inflammatory drugs represents a challenging goal for such a research area. On our ongoing medicinal chemistry research program we found that quinazolines and condensed quinazolines exhibit potent central nervous system (CNS) activities like antinociceptive, anti-inflammatory and anticonvulsant. Quinazolin-4(3H )ones with 2,3-disubstitution is reported to possess significant antinociceptive, anti-inflammatory and anticonvulsant activities. On the other hand, some thiazole derivatives also have various biological properties like anti-inflammatory, antimicrobial, anthelmintic and immunorestoration. These observation led to the conception that a novel series of 6,7,8,9 tetrahydro-5H-5-(2'-hydroxy phenyl)-2-(some substituted benzylidine) thiazolo(2,3-b) quinazolin-3-phenyl hydrazone derivatives were synthesized using different aromatic aldehydes by condensation with phenyl hydrazine in Schiff base mechanism and their chemical structure were confirmed by IR, ¹H-NMR, mass spectroscopy and elemental analysis. The main objective of the present study is to investigate the antinociceptive and antiinflammatory activity of different substitute of thiazoloquinazoline derivatives.

EXPERIMENTAL
General experimental procedures
In the present study the equimolar quantities of each (3.8g 0.039 mol) of cyclohexanone and salicylaldehyde (4.8g 0.039 mol) were taken in a beaker, to this sodium hydroxide solution was added to make the solution alkaline, shake and allow the mixture to stand tallises out or will so upon scratching the vessel with a glass rod. Filter off the solid, wash it with a little cold ethanol and recrystallise it from absoulte ethanol.
A mixture of -hydroxy benzylidine cyclohexanone ring 1 (7.9g 0.039 mol) thiourea (3.0g 0.03 mol) and potassium hydroxide (2.5g) in ethanol (100 ml) was heated under reflux for 3h. The reaction mixture was
concentrated to half of its volume, dilute with water, then acetified with dil acetic acid and kept over night. The solid thus obtained, was filtered, washed with water and crystallised from ethanol to give 4-hydroxy phenyl-3,4,5,6,7,8-hexahydro quinazolin-2-thione 2

The chloroacetic acid (9.0g 0.096mol) was melted on a water bath and thione (2.3g 0.009mol) added to it portionwise to maintain its homogeneity. The homogeneous melt was further heated on a water bath for 30 min and kept overnight. The solid thus obtained was washed with water until neutral and crystallised from ethanol to give 6,7,8,9 tetrahydro-5H-5-(2'-hydroxy phenyl) thiazolo (2,3-b)quinazolin-3(2H)-one 3.

**General method of synthesis 4a-4d**

A mixture of 3 (0.6g 0.002 mol), substituted benzaldehyde (0.002 mol) and anhydrous sodium acetate (0.2g 0.002 mol) in glacial acetic acid (10 ml) was heated under reflux for 4h. The reaction mixture was kept overnight and the solid, thus separated, was filtered, washed with water and recrystallized from ethanol to furnish 6,7,8,9 tetrahydro-5H-5-(2'-hydroxy phenyl)-2-(4'-some substituted benzylidine) thiazolo (2,3-b) quinazolin-3(2H)-one 4a-4d.

**General method of synthesis 5a-5d**

Equimolar quantities (0.004 mol) of compound 4a-4d treated with thionyl chloride and DMF to get chloro derivative and then coupled with substituted anilines in DMF at 80°C and quenched in ice-water to get the product were separated by filtration, vacuum dried and recrystallized from warm ethanol to yields 6,7,8,9 tetra hydro-5H-5-(2'-hydroxy phenyl)-2-(4'-some substituted benzylidine)-3-(4-nitrophenyl amino) thiazolo quinazoline (5a-5d).

**Pharmacological Procedures**

**Animals**

Male Swiss albino mice 25-35g were used. The animals were procured from C.L.Baid Metha College of Pharmacy, Chennai, 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. Animals were maintained under standard conditions in an animal house approved by committee for the purpose of control and supervision on experiments on animals (CPCSEA). Institutional Animal Ethics Committee approved the experimental protocol. The entire animals were acclimatized for a week before use.

**Antinociceptive Activity by Tail-flick method**

Test for antinociceptive activity was performed by tail-flick technique using Wistar albino mice (25-35g) of either sex selected by random sampling technique Diclofenac sodium at dose level of 10 mg/kg was administered orally as reference drug for comparison. The test compound at a dose level of (100 mg/kg, p.o). The reaction time was recorded at 30min, 1, 2 and 3 h after the treatment. The cut off time was 10s. The percent antinociceptive activity (PAA) was calculated by the following,

\[
PAA = \left( \frac{T_2-T_1}{10-T_1} \right) \times 100
\]

Where T₁ is the reaction time (s) before treatment, T₂ is the reaction time (s) after treatment.

**Carrageenan-induced hind paw edema model**

Carrageenan-induced hind paw edema model was used for determination of anti-inflammatory activity. Sixty minutes after the oral administration of synthesized compounds at one dose level (100 mg/kg), each mouse was injected with freshly prepared suspension of carrageenan (0.5mg/25µl) in physiological saline (154nM NaCl) into subplanter tissue of the right hind paw. As the control, 25µl saline solutions were injected into that of the left hind paw. Paw edema was the measured by a gauge calipers (C.L. Baid Metha, Chennai, India) Mean values of treated groups were compared with those of a control group and analyzed by using statistical methods. Indomethacin (10 mg/kg) was used as the reference drug.
Acute toxicity studies
Acute toxicity test was performed for the entire synthesized compound to ascertain the LD50 values as per OECD guidelines\(^1\). Mice are treated with doses of 10, 100, 1000, 1600, 2000 and 5000 mg/kg of the synthesized drugs. The animals were kept under close observation over a period of 14 days. Restlessness, respiratory distress, convulsion, diarrhoea, motor activity, posture and reflexes were qualitatively determined. In addition internal organ (stomach, heart, lung, liver, kidney, etc.) were removed and examined macroscopically to detect internal lesions finally the weight of animal was monitored throughout the experiment doses were selected between the minimum effective dose and minimum non lethal dose.

Statistical Analysis
Data obtained from experiments were expressed as the mean standard error (± S.E.M). Statistical difference between the treated and the control groups were evaluated by ANOVA and Student-Newman-Keuls post hoc tests. P,0.05 was considered to be significant (*p<0.05; **p<0.01; ***p<0.001)

RESULTS AND DISCUSSION
The melting points were taken in open capillary tube and are uncorrected. The IR spectra of the compounds were recorded on ABB Bomem FT-IR spectrometer MB 104 with KBr pellets. The \(^1\)H-NMR spectra were recorded on 300 MHz-Bruker DPX 200 NMR spectrometer (with TMS for \(^1\)H as internal references). Mass spectra were recorded on Shimadzu GC MS QP 5000. Microanalyses for C, H, N were performed in Heraeus CHN Rapid Analyzer. The purity of the compounds was checked by TLC on pre-coated SiO\(_2\) gel (HF\(_2\)54, 200 mesh) aluminum plates (E Merck) using benzene: petroleum ether (3:1) and visualized in UV chamber. IR, \(^1\)H-NMR, Mass spectra and Elemental analysis were consistent with the assigned structures.

The IR, \(^1\)H-NMR, Mass Spectroscopy and Elemental analysis Data were given below-

4a. 6,7,8,9 tetra hydro-5H-5-(2'-hydroxy phenyl)-2-(4'-hydroxy benzylidine) thiazolo (2, 3-b) quinazolin-3(2H)-one
IR : 3421 (O-H), 3098 (Ar-CH), 1722 (C=O), 1342 (N-H) cm\(^{-1}\); \(^1\)H-NMR (CDCl\(_3\)): 6 6.98-7.56 (m, 8H, Ar-H), 6.62 (s, 1H, =CH), 5.84 (s, 1H, H-5), 5.36 (s, 1H, H-2', Ar-OH), 5.02 (s, 1H, H-4", Ar-OH), 1.62-2.56 (m, 8H, 4×CH\(_2\)); EI-MS (m/z, %): 404(M+); (Calcd for C\(_{23}\)H\(_{20}\)N\(_2\)O\(_3\)S; 404.12); Anal. Calcd for C\(_{23}\)H\(_{20}\)N\(_2\)O\(_3\)S; C, 68.30; H, 4.98; N, 6.93; O, 11.86; S, 7.94; Found: C, 68.32; H, 4.96; N, 6.97; O, 11.84; S, 7.98.

4b. 6,7,8,9 tetra hydro-5H-5-(2'-hydroxy phenyl)-2-(4'-methoxy benzylidine) thiazolo (2, 3-b) quinazolin-3(2H)-one
IR : 3476 (O-H), 3096 (Ar-CH), 1728 (C=O), 1339 (N-H) cm\(^{-1}\); \(^1\)H-NMR (CDCl\(_3\)): 6 6.96-7.54 (m, 8H, Ar-H), 6.67 (s, 1H, =CH), 5.83 (s, 1H, H-5), 5.48 (s, 1H, Ar-OH), 3.75 (s, 3H –OCH\(_3\)), 1.58-2.62 (m, 8H, 4×CH\(_2\)); EI-MS (m/z, %): 418(M+); (Calcd for C\(_{24}\)H\(_{22}\)N\(_2\)O\(_3\)S; 418.51); Anal. Calcd for C\(_{24}\)H\(_{22}\)N\(_2\)O\(_3\)S; C, 68.88; H, 5.30; N, 6.69; O, 11.47; S, 7.66; Found: C, 68.90; H, 4.96; N, 6.72; O, 11.51; S, 7.69.

4c. 6,7,8,9 tetra hydro-5H-5-(2'-hydroxy phenyl)-2-(4'-methyl benzylidine) thiazolo (2, 3-b) quinazolin-3(2H)-one
IR : 3448 (O-H), 3049 (Ar-CH), 1694 (C=O), 1297 (N-H) cm\(^{-1}\); \(^1\)H-NMR (CDCl\(_3\)): 6 6.86-7.74 (m, 8H, Ar-H), 6.72 (s, 1H, =CH), 5.76 (s, 1H, H-5), 5.54 (s, 1H, Ar-OH), 2.20 (s, 3H –CH\(_3\)), 1.62-2.32 (m, 8H, 4×CH\(_2\)); EI-MS (m/z, %): 402(M+); (Calcd for C\(_{24}\)H\(_{22}\)N\(_2\)O\(_2\)S; 402.14); Anal. Calcd for C\(_{24}\)H\(_{22}\)N\(_2\)O\(_2\)S; C, 71.00; H, 5.51; N, 6.96; O, 7.95; S, 7.97; Found: C, 69.87; H, 5.32; N, 6.74; O, 7.58; S, 7.72.
4d. 6,7,8,9 tetra hydro-5H-5-(2'-hydroxy phenyl)-2-(4'-chloro benzylidine) thiazolo (2, 3-b) quinazolin-3(2H)-one
IR : 3415 (O-H), 3027 (Ar-CH), 1712 (C=O), 1503 (C=C), 1322 (N-H), 823 (C-Cl) cm⁻¹; ¹H-NMR (CDCl₃): δ 7.12-7.56 (m, 8H, Ar-H), 6.82 (s, 1H, =CH), 5.74 (s, 1H, H-5), 5.56 (s, 1H, Ar-OH), 1.26-2.65 (m, 8H, 4 × CH₂); EI-MS (m/z, %): 424 (M+2); (Calcd for C₂₃H₁₉N₂O₂SCl; 422.09). Anal. Calcd for C₂₃H₁₉N₂O₂SCl; C, 65.32; H, 4.53; N, 6.62; O, 7.57; Cl, 8.38; Found: C, 65.36; H, 4.55; N, 6.63; O, 7.57; Cl, 8.35.

5a. 6,7,8,9 tetra hydro-5H-5-(2'-hydroxy phenyl)-2-(4'-hydroxy benzylidine)-3-(4-nitrophenyl amino) thiazolo quinazoline
IR : 3415 (O-H), 3060 (Ar-CH), 1534 (C=C), 1312 (N-H), 3310 (N-NH) cm⁻¹; ¹H-NMR (CDCl₃): δ 6.98-7.36 (m, 12H, Ar-H), 6.53 (s, 1H, =CH), 5.82 (s, 1H, H-5), 5.40 (s, 1H, H-2', Ar-OH), 5.12 (s, 1H, H-4'', Ar-OH), 7.78 (s, 1H, N-H), 1.59-2.47 (m, 8H, 4 × CH₂); EI-MS (m/z, %): 526 (M+); (Calcd for C₂₉H₂₆N₄O₄S; 526.17). Anal. Calcd for C₂₉H₂₆N₄O₄S; C, 66.14; H, 4.98; N, 10.64; O, 12.15; S, 6.09; Found: C, 66.12; H, 4.96; N, 10.63; O, 12.15; S, 6.11.

5b. 6,7,8,9 tetra hydro-5H-5-(2'-hydroxy phenyl)-2-(4'-methoxy benzylidine)-3-(4-nitrophenyl amino) thiazolo quinazoline
IR : 3464 (O-H), 3027 (Ar-CH), 1494 (C=C), 1326 (N-H), 3284 (N-NH) cm⁻¹; ¹H-NMR (CDCl₃): δ 6.72-7.23 (m, 12H, Ar-H), 6.43 (s, 1H, =CH), 5.62 (s, 1H, H-5), 5.44 (s, 1H, Ar-OH), 3.78 (s, 3H, –OCH₃), 7.76 (s, 1H, N-H), 1.46-2.42 (m, 8H, 4 × CH₂); EI-MS (m/z, %): 540 (M+); (Calcd for C₃₀H₂₈N₄O₄S; 540.18). Anal. Calcd for C₃₀H₂₈N₄O₄S; C, 66.65; H, 5.22; N, 10.36; O, 11.84; S, 5.93; Found: C, 66.67; H, 5.25; N, 10.38; O, 11.85; S, 5.96.

5c. 6,7,8,9 tetra hydro-5H-5-(2'-hydroxy phenyl)-2-(4'-methyl benzylidine)-3-(4-nitrophenyl amino) thiazolo quinazoline
IR : 3438 (O-H), 3024 (Ar-CH), 1412 (C=C), 1332 (N-H), 3310 (N-NH) cm⁻¹; ¹H-NMR (CDCl₃): δ 6.69-7.24 (m, 12H, Ar-H), 6.36 (s, 1H, =CH), 5.72 (s, 1H, H-5), 5.39 (s, 1H, Ar-OH), 2.28 (s, 3H, –CH₃), 7.69 (s, 1H, N-H), 1.36-2.41 (m, 8H, 4 × CH₂); EI-MS (m/z, %): 524 (M+); (Calcd for C₃₀H₂₈N₄O₃S; 524.19). Anal. Calcd for C₃₀H₂₈N₄O₃S; C, 68.68; H, 5.22; N, 10.36; O, 11.84; S, 5.93; Found: C, 68.67; H, 5.25; N, 10.70; O, 9.18; S, 6.14.

5d. 6,7,8,9 tetra hydro-5H-5-(2'-hydroxy phenyl)-2-(4'-chloro benzylidine)-3-(4-nitrophenyl amino) thiazolo quinazoline
IR : 3446 (O-H), 3021 (Ar-CH), 1521 (C=C), 1324 (N-H), 3316 (N-NH), 820 (C-Cl) cm⁻¹; ¹H-NMR (CDCl₃): δ 6.79-7.34 (m, 12H, Ar-H), 6.46 (s, 1H, =CH), 5.82 (s, 1H, H-5), 5.49 (s, 1H, Ar-OH), 7.79 (s, 1H, N-H), 1.26-2.32 (m, 8H, 4 × CH₂); EI-MS (m/z, %): 544 (M+2); (Calcd for C₂₉H₂₅ClN₄O₃S; 544.13). Anal. Calcd for C₂₉H₂₅ClN₄O₃S; C, 63.90; H, 4.62; N, 10.28; O, 8.81; S, 5.88; Cl, 6.50; Found: C, 63.93; H, 4.65; N, 10.30; O, 8.83; S, 5.86; Cl, 6.53.

Anti-nociceptive and anti-inflammatory activity
In the present study, we have evaluated the antinociceptive activity by tail-flick and anti-inflammatory activity by carrageenan induced paw edema method. Among the synthesized compounds, compound 4d showed better antinociceptive and anti-inflammatory activity when compared to 6,7,8,9 tetra hydro-5H-5-(2'-hydroxy phenyl)-2-(4'-hydroxy benzylidine) thiazolo (2, 3-b) quinazolin-3(2H)-one 4a, 6,7,8,9 tetra hydro-5H-5-(2'-hydroxy phenyl)-2-(4'-methoxy benzylidine) thiazolo (2, 3-b) quinazolin-3(2H)-one 4b and 6,7,8,9 tetra hydro-5H-5-(2'-hydroxy phenyl)-2-(4'-methyl benzylidine) thiazolo (2, 3-b) quinazolin-3(2H)-one 4c because presence of electron with drawing chloro group in its structure. Among all the eight compound synthesized compound 6,7,8,9 tetra hydro-5H-5-(2'-hydroxy phenyl)-2-(4'-chloro benzylidine)-3-(4-nitrophenyl amino) thiazolo quinazoline 5d produced more potent antinociceptive, anti-inflammatory activity against standard diclofenac sodium (Table-1), and indomethacin respectively (Table-2). This is mainly because of the addition of electron with drawing nitro substituent in third position of thiazolo quinazoline.
SYNTHESIS OF NOVEL THIAZOLO-QUINAZOLINES

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Scheme-1
Table-1: Antinociceptive Activity (Tail-Flick Technique)

<table>
<thead>
<tr>
<th>Compound code</th>
<th>Dose (mg/kg)</th>
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<th>1h</th>
<th>2h</th>
<th>3h</th>
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<td>Control</td>
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<td>2±0.35</td>
<td>6±0.49</td>
<td>4±0.59</td>
<td>4±0.91</td>
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<tr>
<td>4a</td>
<td>100</td>
<td>43±1.83</td>
<td>49±0.29</td>
<td>54±2.54</td>
<td>35±0.47</td>
</tr>
<tr>
<td>4b</td>
<td>100</td>
<td>46±0.68</td>
<td>50±1.24</td>
<td>48±2.45</td>
<td>39±1.09</td>
</tr>
<tr>
<td>4c</td>
<td>100</td>
<td>43±0.34</td>
<td>46±1.15</td>
<td>49±0.96</td>
<td>39±0.67</td>
</tr>
<tr>
<td>4d</td>
<td>100</td>
<td>48±3.01</td>
<td>56±1.33</td>
<td>59±1.31</td>
<td>41±0.72</td>
</tr>
<tr>
<td>5a</td>
<td>100</td>
<td>58±1.38</td>
<td>59±1.26</td>
<td>61±1.45</td>
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</tr>
<tr>
<td>5b</td>
<td>100</td>
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<td>63±1.42</td>
<td>68±0.42</td>
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</tr>
<tr>
<td>5c</td>
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<td>59±1.68</td>
<td>40±0.83</td>
</tr>
<tr>
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<td>65±0.65</td>
<td>8±1.36</td>
<td>70±1.29</td>
<td>56±1.37</td>
</tr>
<tr>
<td>Diclofenac</td>
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<td>37±1.69</td>
<td>43±1.42</td>
<td>45±0.92</td>
<td>33±0.96</td>
</tr>
</tbody>
</table>

Each value represents the mean±S.D (n=6). Significance levels *P<0.5, **P<0.01 and ****P<0.001 as compared with the respective control.

Table-2: Effect of the synthesized compounds and active principles on carrageenan-induced hind paws edema in mice

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<th>Test samples</th>
<th>Dose (mg/kg)</th>
<th>90 min</th>
<th>180 min</th>
<th>270 min</th>
<th>360 min</th>
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<tr>
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<td>100</td>
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<td>35.0±2.5</td>
<td>37.5±3.3</td>
<td>41.5±2.8</td>
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<tr>
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<td>43.3±3.9</td>
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<tr>
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<tr>
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<td>32.7±2.7</td>
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<td>31.8±2.5</td>
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</tr>
</tbody>
</table>

Data represent Mean ± S.E.M (Standard Error Mean) (n = 6). *p < 0.05; **p < 0.01; ***p < 0.001

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