SYNTHESIS AND ANTI-INFLAMMATORY EVALUATION OF NEW 5-ARYLIDENE-3- METHYLSULPHONYL THIAZOLIDINE-2, 4-DIONES.

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ABSTRACT
A series of novel thiazolidine-2, 4-dione derivatives containing 3-methyl sulphonyl moiety were synthesized by following convenient synthetic protocols to investigate their potential anti-inflammatory activity. The chemical structures of the synthesized compounds were elucidated by spectral and elemental analyses. The evaluated compounds in the series exhibited significant anti-inflammatory activities when compared with reference drug indomethacin.

KEYWORDS
5-Arylidene 2, 4-thiazolidinone dione, anti-inflammatory, mice paw edema.

INTRODUCTION
Non-steroidal ant-inflammatory drugs (NSAID₃) are the most commonly used medications throughout the world. They are prescribed for the treatment of pain, fever and inflammation, particularly arthritis.¹² The most common prevalent side effects of the use of non-steroidal anti-inflammatory drugs are the occurrence of gastrointestinal damage³ with gastric upset⁴ and irritation. The search for newer and safer NSAID₃ is ever lasting as existing drugs do possess undesirable side effects. 5-Arylidene 2, 4-thiazolidinediones display significant pharmacological properties.⁵⁶ A library of 5-arylidene 2, 4-thiazolidinones are under clinical trials as potential phospholipase A₂ inhibitors, dual COX-2/5-LOX inhibitors⁷ and anti-inflammatory agents.

Thiazolidinedione binding with PPARγ⁸⁹ has been proved to play a down regulatory role in treatment of inflammatory disorders. Thiazolidine diones display potential anti-inflammatory activity by inhibiting monocyte/macrophage activation and expression of inflammatory...
molecules, i.e. interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF-α)\textsuperscript{[10]}, inducible nitric oxide synthase and gelatinase B.\textsuperscript{[11]} Thiazolidinediones also inhibit some other inflammatory molecules (IL-2, IL-8, and interferon-γ)\textsuperscript{[12]} and cell types (endothelial cell, colon cell) in vitro. In vitro and vivo anti-inflammatory effects of thiazolidinediones viz. troglitazone\textsuperscript{[14]} and rosiglitazone (Fig. 1)\textsuperscript{[15,16]} have been reported by Dandona \textit{et al.}\textsuperscript{[17]}

Recently Barros \textit{et al}.\textsuperscript{[18]} have synthesized eight new 5-arylidene-3-benzyl-thiazolidine-2, 4-diones with halide group on their benzyl rings and tested for their anti-inflammatory activity. Among synthesized compounds, 3-(2-bromo-benzyl)-5-(4-methanesulfonyl-benzylidene)-thiazolidine-2, 4-dione (Fig.1) showed higher activity than the rosiglitazone reference drug. The binding patterns observed for the docked compounds strongly support the idea that they are possible ligands of the PPARγ receptor. Competition binding assays using purified His-LBD- PPARγ confirmed that these arylidene-thiazolidinediones directly bind to LBD-hPPARγ in vitro and are low affinity ligands for the receptor. This suggests that the substituted 5-arylidene and 3-benzylidene groups play important roles in the anti-inflammatory activity of this class of compounds. 2, 4-Thiazolidinedione derivatives have been investigated as a template\textsuperscript{[19,20]} to design and synthesis novel and safe anti-inflammatory compounds. It has been reported that N-methyl 2, 4-thiazolidinediones are found to retain anti-inflammatory activity as observed in some Meclofenamic acid and Indomethacin\textsuperscript{[21]} derivatives. Sulfonamides are an important class of compounds in organic chemistry. Research is going on drug development, where the sulfonamides are proved to be a separate class of therapeutic agents, used in different areas as cholesterol modulator\textsuperscript{[22]}, anti-arrhythmic drugs and β-adrenergic blockers\textsuperscript{[23]}, anti-tumor agents against multi-drug resistant tumors\textsuperscript{[24]} and selective COX-2 inhibitors.\textsuperscript{[25,26]} Nimesulide, flosulide and NS-398 are the anti-inflammatory drugs having methane sulfonamido pharmacophore. Sondhi \textit{et al.}, have synthesized series of 4-aryl-3(2-or 4-picoryl)-2-imino-4-thiazoline (Fig.1) sulphonamide and amidine derivatives.\textsuperscript{[27]} These molecules have displayed anti-inflammatory activity. Promoted with the above mentioned studies and in continuation of our research interest in the synthesis and biological activities of novel 2, 4 thiazolidinone-dione derivatives\textsuperscript{[28,29,30,31,32]} the present study aimed together two bioactive entities, 5-benzylidene thiazolidinone 2, 4-diones and methyl sulphonamide in one molecular framework for the purpose of synergism, for obtaining enhanced anti-inflammatory activity.
RESULTS AND DISCUSSION

CHEMISTRY

5-Arylidene 2, 4-thiazolidinediones 3a-e were synthesized carrying safer Knoevenagel condensation of aromatic aldehydes 1a-e and 2, 4-thiazolidinedione 2 in PEG-400.\(^{33,34}\) Knoevenagel condensation generally gives mixture of Z and E isomers. It has been reported when the condensation of aromatic aldehydes and 2, 4-thiazolidinediones carried under Knoevenagel using bases yielded single Z-isomeric products.\(^{35}\) However, the products obtained by condensing 2, 4-thiazolidinedione and aromatic aldehydes in polyethylene glycol were single isomers confirmed by thin layer chromatography. It is well known that the E and Z isomers can be distinguished by the \(^1\)HNMR spectral characteristics. Benzylidine proton appears below 7.42 δ ppm in E isomer and above 7.90 δ ppm in Z isomer.\(^{36,37}\) The \(^1\)H NMR spectra of 3a displayed singlet at 8.04 δ ppm which confirmed that the product obtained is Z isomer. The spectral data of 5-Arylidene 2, 4-thiazolidinediones synthesized by this route are in good agreement with those reported in the literature.\(^{38,39}\) Mesylation of 5-arylidene-2, 4-thiazolidinediones (3a-e) with methyl sulphonyl chloride when carried in DCM at ambient temperature afforded target 5-arylidene-3-methylsulphonyl thiazolidine 2, 4-diones 4a-e with moderate yields (Scheme 1). In the \(^1\)H NMR spectra of 4a exchangable signal around 12 δ ppm is not observed which supported effective mesylation. Mass spectra of 4a also displays peak at 284 (M+H)\(^+\) and a peak at 205[(M+H)^+ -79] due to loss of –SO\(_2\)CH\(_3\) fragment. IR spectra of 4a also showed bands at 1342 (S=O asymm.) and 1169 (S=O symm.) cm\(^{-1}\) which also supported the proposed structure 4a.
Scheme 1. Synthesis of 5-arylidene-3-methylsulphonyl thiazolidine-2, 4-diones 4a-d, Ar:
(a) -C₆H₅; (b) 4-CH₃-C₆H₄; (c) 4-Cl-C₆H₄; (d) 4-(N,N-dimethyl)-C₆H₄; (e) 4-OCH₃-C₆H₄.

Table No. 1 Anti-inflammatory activity at different time intervals of compounds 4a, 4b, 4c, 4d and 4e using carrageenan-induced paw edema in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Paw Thickness (± SEM) (inhibition %) at different interval (Hrs)</th>
<th>P &lt; when control group compared with drug</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
<td>Control</td>
<td>3.690 (0.187)</td>
<td>4.305 (0.175)</td>
</tr>
<tr>
<td>STD</td>
<td>3.585 (0.273)</td>
<td>3.413 (0.180)</td>
</tr>
<tr>
<td>4a</td>
<td>4.128 (2.27)</td>
<td>3.763 (0.179)</td>
</tr>
<tr>
<td>4b</td>
<td>4.327 (0.100)</td>
<td>4.052 (0.120)</td>
</tr>
<tr>
<td>4c</td>
<td>3.943 (0.188)</td>
<td>3.428 (0.231)</td>
</tr>
<tr>
<td>4d</td>
<td>4.172 (0.226)</td>
<td>4.393 (0.175)</td>
</tr>
<tr>
<td>4e</td>
<td>4.410 (0.097)</td>
<td>4.190 (0.177)</td>
</tr>
</tbody>
</table>

Note: one way ANOVA test followed by student t test was applied to determine the significance of the difference between the control group and mice treated with the tested compounds. (n = 6), p <0.05significant difference from control group.

ANTHI-INFLAMMATORY ACTIVITY

All the synthesized 2, 4-thiazolidinone dione derivatives were evaluated for their in vivo anti-inflammatory activities. All the compounds have shown anti-inflammatory activity ranging from 2.67 to 32.54% at the dose of 100 mg/kg Table 1. Indomethacin was used as the
reference drug in a dose of 10 mg/kg. Compounds 4a-e, exhibit anti-inflammatory activities. The reference drug, indomethacin displayed maximum anti-inflammatory activity 29.35% after 4 h. The synthesized compounds exhibited considerable anti-inflammatory activity relative to indomethacin that increased significantly to a maximum after 2 h, and then the activity decreased gradually for the next 2 h for the majority of the synthesized compounds. Compound 4a, 4b, 4d and 4e showed maximum anti-inflammatory activity 21.43%, 12.12%, 19.76% and 32.90% respectively at 2 h. Compound 4e, in which phenyl ring is substituted with a methoxy group at position-4 displayed highest activity 32.90% which more than standard indomethacin (fig.2.1). The synthesized compound 4c, with a phenyl ring having 4-chloro substituent exhibited maximum highest activity 32.54% at 24 h. Overall compound 4c is equipotent to standard indomethacin and has significant activity lasting for 24 h (fig.2).

Fig. 2 Percent inhibition of drugs at various time intervals.

MATERIALS AND METHODS

CHEMISTRY

Reactions were monitored by TLC. Melting points were determined by open capillary method and are uncorrected. IR spectra were recorded on Bruker alpha ATR spectrophotometer. $^1$H NMR and $^{13}$C NMR spectra were carried out on Bruker apparatus at DRX-300 MHz, using TMS as internal reference and DMSO-$d_6$ as medium. Chemical shifts (δ values) are expressed in parts per million (ppm). Mass spectra have been scanned on DART-MS (ESI$^+$) and on JMS 100LC, AccuTOF spectrometers. Elemental analysis was performed on Perkin–Elmer 2400 CHNS Elemental analyzer at SAIF CDRI Lucknow, India.
General procedure for the synthesis of 5-arylidene 2, 4-thiazolidine-diones (3a-e)
A mixture of 2, 4-thiazolidinedione (50 mmol) and aromatic aldehydes (50 mmol) was dissolved in PEG-400 (20 mL) and the solution was heated on an oil bath at 120° C. The progress of the reaction was monitored by thin layer chromatography. After 3h the reaction mass was cooled and then poured on cold water. The obtained solid product was filtered and washed with water. The crude obtained was then crystallized from ethanol. The structure of thiazolidinone derivatives 3a-e was confirmed by mps and spectra and the data is in good agreement with those reported in the literature.[40]

5-Benzylidene thiazolidine 2, 4-dione (3a) as a representative.
Yellow crystals (ethanol) in (8.91 g, 87% yield); Mp 214-215° C; IR (cm⁻¹): 3281(-NH, stretch), 3114 (Ar-H stretch), 2926 (C-H, strech asmm.) 2857 (C-H, stretch symm.), 1743(C=O), 1695 (C=O), 1647 (C=C) and 1553 (N-H, bend). ¹H NMR (300MHz, DMSO-d₆, δ = ppm) δ = 12.60 (-NH,exchangable with D₂O), 7.77 (s, 1H, olefinic) 7.59–7.44 (m, 5H, Ar-H). ¹³C NMR (75 MHz, DMSO-d₆, δ = ppm) δ = 167.83, 167.27, 138.0, 131.7, 130.3, 129.9, 129.2, 123.5. MS (ESI⁺ mode): m/z (% intensity) 206 [(M+H)+, 100], 115.08 (34.37),117.09 (21.87), 99.08 (96.87). Anal. Calcd for C₁₀H₇NO₂S (205): C, 58.52; H, 3.44; N, 6.82; S, 15.62. Found: C, 58.50; H, 3.40; N, 6.81; S, 15.62.

5-[(4-Methyl) benzylidene] thiazolidine 2, 4-dione (3b). Mp 226-228° C [(29); 225-226° C].
5-[(4-Chloro) benzylidene] thiazolidine 2, 4-dione (3c). Mp 268-269° C [(40); 267-268° C].
5-[(4-N, N-dimethyl) benzylidene] thiazolidine 2, 4-dione (3d). Mp 280-282° C [(40); 281° C].
5-[(4-Methoxy) benzylidene] thiazolidine 2, 4-dione (3e). Mp 235-236° C [(40); 235° C].

General procedure for the synthesis 5-arylidene-3-methylsulphonyl thiazolidine 2, 4-diones(4a-h).
To the stirred solution of 5-arylidene 2, 4-thiazolidinedione (5 mmol) in DCM (20 ml), triethyl amine (7.5mmol) was added at r.t. and stirring was continued for 15 minutes. Then to this solution methyl sulphonyl chloride (7.5 mmol) was added dropwise with costant stirring. After complete addition the reaction mass was further stirred for 6 h.at r.t. Progress of the reaction was monitored by thin layer chromatography (n-hexane, ethyl acetate 4:6).On completion of the reaction DCM was removed by rota-evaporator and obtained crude solid product was poured in ice cold water. Then the solid thus obtained was filtered vacuum dried and crystallized from alcohol.
5- (Bezylidene)-3-methyl sulphonyl thiazolidine-2, 4-dione (4a)
Pale yellow crystals (ethanol) in (1.91 g, 90% yield); 184-186 °C; IR (cm⁻¹): 3025 (Ar-H stretch), 2789 (C-H strech aliphatic), 1744 (C=O), 1690 (C=O), 1601 (C=C), 1342 (S=O asymm.), 1169 (S=O symm.) ¹H NMR (300 MHz, DMSO-d₆, δ = ppm) δ = 7.79 (s,1H,olefinic), 7.45- 7.64 (m,5H, Ar-H), 4.07 (s, 3H,SO₂CH₃). ¹³C NMR (75 MHz, DMSO-d₆, δ = ppm) δ = 167.85, 167.28, 140.70, 131.78, 130.40, 129.99, 129.30, 123.53, 39.24. DART-MS (ESI⁺): m/z (% intensity) 284 [(M+H)+, 100], 205.01 (22.48), 102.13 (27.90). Anal. Calcd for C₁₁H₉NO₄S₂ (283): C, 46.60; H, 3.20; N, 4.94; S, 22.63; Found; C, 46.63; H, 3.14; N, 4.92; S, 22.61.

5-[(4-Methyl benzylidene)-3-methylsulphonyl thiazolidine 2, 4-dione (4b)
Pale yellow crystals (ethanol) in (1.75 g, 79% yield); 190-191°C; IR (cm⁻¹): 3125 (Ar-H stretch), 2927 (C-H strech aliphatic), 1744 (C=O), 1692 (C=O), 1603 (C=C), 1344 (S=O asymm.), 1164 (S=O symm.) ¹H NMR (300 MHz, DMSO-d₆, δ = ppm) δ = 7.94 (s, 1H, olefinic), 7.51 (d, J = 8.0 Hz 2H), 7.36 (d, J = 8.0 Hz, 2H), 3.65 (s, 3H,SO₂CH₃), 2.43 (s, 3H, Ar-CH₃). ¹³C NMR (75 MHz, DMSO-d₆, δ = ppm) δ = 167.86, 167.34, 140.70, 131.80, 130.27, 129.44, 122.29, 69.77, 21.07. DART-MS (ESI⁺ mode): m/z (% intensity) 298.02 [(M+H)+, 100], 297.01 (12), 219 (6.4),117.09 (8.8), 99.08 (32). Anal. Calcd for C₁₂H₁₁NO₄S₂ (297.01): C, 48.47; H, 3.73; N, 4.71; S, 21.57; Found; C, 48.03; H, 3.34; N, 4.82; S, 21.61.

5-[(4-Chloro benzylidene)-3-methylsulphonyl thiazolidine 2, 4-dione (4c)
Pale brown crystals (ethanol) in (2.06 g, 87% yield); 179-181°C; IR (cm⁻¹): 3115 (Ar-H stretch), 2925 (C-H strech aliphatic), 1743 (C=O), 1703 (C=O), 1648 (C=C), 1422 (S=O asymm.), 1172 (S=O symm.) ¹H NMR (300 MHz, DMSO-d₆, δ = ppm) δ = 7.77 (s,1H,olefinic), 7.66 (d, J = 8.4 Hz 2H), 7.54 (d, J = 8.6 Hz, 2H), 3.66 (s, 3H, -SO₂CH₃). ¹³C NMR (75 MHz, DMSO-d₆, δ = ppm) δ = 167.49, 166.89, 135.24, 133.94, 131.56, 130.16, 129.87, 127.91, 125.38, 123.14, 39.51. DART-MS (ESI⁺ mode): m/z (% intensity) 317.97 [(M+H)+ 100], 319.96 [(M+H)+2], 43.41, 115.08 (32.55), 117.09 (19.37), 99.08 (90). Anal. Calcd for C₁₁H₉ClNO₄S₂ (316.96): C, 41.58; H, 2.54; N, 4.41; S, 20.18; Found; C, 41.62; H, 2.50; N, 4.52; S, 20.31.

5-[(4-N, N-dimethyl benzylidene]-3-methylsulphonyl thiazolidine 2, 4-dione (4d)
Orange crystals (ethanol) in (2.02 g, 83% yield); 209-210°C; IR (cm⁻¹): 3023 (Ar-H stretch), 2926 (C-H strech aliphatic), 1742 (C=O), 1696 (C=O), 1648 (C=C), 1355 (S=O asymm.),
1167 (S=O symm.) $^1$H NMR (300 MHz, DMSO-d$_6$, $\delta$ = ppm) $\delta$ = 7.84 (s,1H,olefinic), 7.45 (d, $J$ = 8.7 Hz, 2H), 6.82 (d, $J$= 8.2 Hz, 2H), 3.64 (s, 3H,-SO$_2$ CH$_3$), 3.04 (s, 3H, N-CH$_3$). $^{13}$C NMR (75 MHz, DMSO-d$_6$, = ppm) $\delta$ = 168.10, 167.47, 151.88, 135.46, 132.89, 132.80, 132.10, 119.92, 119.52, 115.71, 110.45, 42.30. DART-MS (ESI$^+$ mode): m/z (% intensity) 327.04 [(M+H)$^+$ 100], 326.04 (19.10), 249.06 (60.67), 99 (38.20). Anal. Calcd for C$_{13}$H$_{14}$N$_2$O$_4$S$_2$ (326.04): C, 47.84; H, 4.32; N, 8.58; S, 19.65; Found; C, 47.71; H, 4.22; N, 8.63; S, 19.70.

5-[(4-Methoxy) benzylidene]-3-methylsulphonyl thiazolidine 2, 4-dione (4e)

Yellow crystals (ethanol) in (1.99 g, 85% yield); 172–174$^\circ$C; IR (cm$^{-1}$): 3005 (Ar-H stretch), 2921 (C-H strech aliphatic), 1703 (C=O), 1648 (C=O), 1615 (C=C), 1355 (S=O asymm.), 1164 (S=O symm.) $^1$H NMR (300 MHz, DMSO-d$_6$, $\delta$ = ppm) $\delta$ = 7.99 (s,1H,olefinic), 7.59 (d, $J$ = 8 Hz, 2H), 7.11 (d, $J$ = 8 Hz, 2H), 3.84 (s,3H,-OCH$_3$), 3.64 (s, 3H,-SO$_2$ CH$_3$). $^{13}$C NMR (75 MHz, DMSO-d$_6$, = ppm) $\delta$ = 168.39, 167.87, 161.46, 134.69, 133.07, 132.54, 132.31, 125.95, 120.73, 116.47, 55.95, 42.81. DART-MS (ESI$^+$ mode): m/z (% intensity) 314.01[(M+H)$^+$ 100], 236.03 (9.3), 235.02 (16.27), 117.09 (14.72) 115.08 (23.25), 99.08 (69.76). Anal. Calcd for C$_{12}$H$_{11}$N$_2$O$_5$S$_2$ (313.01): C, 46.00; H, 3.54; N, 4.47; S, 20.47; Found; C, 46.08; H, 3.43; N, 4.52; S, 20.39.

ANTI-INFLAMMATORY ACTIVITY

Healthy Swiss Albino mice (20 to 25 g) of either sex procured from the animal colony of the Institute (National Toxicological Centre, Pune (India) were used. The animals were housed in a group of six in a single polypropylene cage with paddy husk bedding. All animals were kept under uniform and controlled conditions of temperature and light/dark (12/12 h) cycles, fed with pellet diet and water ad libitum. All animals were fasted overnight before the experiments and during experimentation and free access to water ad libitum. The experimental tests on animals have been performed in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) formed by the Government of India in 1964. The institute has taken permission from the animal ethical committee for the work (Project. no. 122/11/ IAEC). The anti-inflammatory effect of newly synthesized compounds was evaluated by carrageenan-induced mice paw edema method$^{[41]}$ described earlier by Levy.$^{[42]}$ Seven groups of animals each consisting of six mice were selected. The first group was injected with 0.05 ml of 1% carrageenan in the sub planar tissue of the right hind paw and served as untreated control.
The positive control group was given 10 mg/kg i.p. indomethacin 0.5 h before carrageenan injection.

The test compounds were suspended in 0.5% carboxymethylcellulose (CMC) and given orally to the mice at a dose of 100 mg/kg i.p. 0.5 h before carrageenan injection. The inflammation was induced by injecting 1% solution of carrageenan lambda (sigma) dissolved in a 0.5% saline solution in the right hind paw mice. The paw thickness was measured by digital thickness gauge caliper (Mitutoyo Corp. Kawasaki-Japan) at a predetermined time interval i.e. 0.5, 1, 2, 4, 24 hrs. The mean values of drugs treated groups were compared with mean values of control group and analyzed using statistical methods. Data obtained from animal experiment were expressed as mean ± standard deviation (SD). Statistical differences between the treatment and the control were tested by one-way analysis of variance (ANOVA) and student’s t-test. A value of \( p < 0.05 \) was considered to be significant. The anti-inflammatory activity was expressed as percentage inhibition of edema thickness in treated animals in comparison with the control group (Table 1; Fig. 2): % Inhibition = \( \left(1 - \frac{V_t}{V_c}\right) \times 100 \) Where, \( V_t \) is edema thickness in the drug treated animal group. \( V_c \) is edema thickness in the control animal group.

**CONCLUSION**

A series of novel 5-arylidene 3-methylsulfonyl thiazolidine 2, 4-diones has been synthesized in good yield and screened for their anti-inflammatory activity. Most of the synthesized compounds showed significant anti-inflammatory activity. The result indicated that a compound 4c is equipotent with standard indomethacin. The newly synthesized hybrid molecules having safer and bio-available 5-arylidene thiazolidinone 2, 4-diones and COX-2 selective methyl sulphonamide pharmacophors in the molecular framework are found to be promising candidates to develop new leads.

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