Synthesis of novel 5-{[2-(4-fluorobenzyl)-6-arylimidazo[2,1-b] [1,3,4]thiadiazol-5-yl]methylene}thiazolidine-2,4-diones as potent Antidiabetic agents

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Abstract
A series of novel 5-{[2-(4-fluorobenzyl)-6-arylimidazo[2,1-b] [1,3,4]thiadiazol-5-yl]methylene}thiazolidine-2,4-dione derivatives (4a-d) were synthesized. These final compounds (4a-d) were synthesized by Knoevenagel condensation of 2-(4-fluorobenzyl)-6-arylimidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehydes (3a-d) with thiazolidine-2,4-dione. All the newly synthesized compounds were screened for their in vivo hypoglycemic and hypolipidemic activity in male Wister rats. The Structures of all the newly synthesized compounds were established by analytical and spectral data.

Keywords: Imidazothiadiazoles, Thiazolidinediones, Hypoglycemic activity, Hypolipidemic activity, Antidiabetic agents.

1. INTRODUCTION
Thiazolidinediones (TZDs) also known as glitazones, bind to PPARγ, a type of nuclear regulatory protein involved in transcription of genes regulating glucose and fat metabolism. These PPARs act on peroxysome proliferator responsive elements (PPRE). The PPREs influence insulin-sensitive genes, which enhance production of mRNAs of insulin-dependent enzymes. The final result is better use of glucose by the cells. Imidazo[2,1-b][1,3,4]thiadiazole derivatives have attracted the interest of medicinal chemists for many years because of their diverse pharmacological properties such as anticancer[1], antitubercular[2], antibacterial[3], antifungal [4], anticonvulsant, analgesic[5] and antisecretory[6] activities. They have been reported to selectively inhibit several therapeutic receptors and enzymes, extending their applications in modern drug design. Further, there are reports in the literature about the antidiabetic activity of the derivatives containing thiadiazole ring system[7].

Thiazolidinedione moiety is significant because of its pharmacophoric acidic group in a central flat ring. Structure activity relationship studies have revealed that better activity can be gained by linking a lipophilic fragment such as aromatic/heteroaromatic ring via one or two carbon atom spacer at C5-position of the thiazolidinedione moiety. Hence it is proposed to synthesize various thiazolidinedione derivatives by substituting pharmacophorically important group and as a lipophilic fragments like imidazothiadiazoles. Such molecules are expected to exhibit better antidiabetic properties for non-insulin dependent diabetes mellitus (NIDDM) as cited in the literature.
2. RESULTS AND DISCUSSION

During the present investigation required imidazo[2,1-b][1,3,4]thiadiazoles were prepared by the reaction of 2-amino-1,3,4-thiadiazole (I) with appropriately substituted α-halo ketones (phenacylbromides) in dry ethanol as hydrobromides, which on neutralization with aqueous sodium carbonate solution gave corresponding free bases (2a-d) in good yields. The absence of v\textsubscript{NH} band in IR spectra of the resulting compounds confirms the formation of product, which exhibits imidazole (C=H) proton in the region δ 7.96-8.31 in \textsuperscript{1}H NMR spectra.

Imidazo[2,1-b][1,3,4]thiadiazoles (2a-d) were further subjected to Vilsmeier Haack reaction, which resulted in the formation of expected imidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehydes (3a-d). The IR spectra of these compounds displayed the aldehydic carbonyl around 1674 cm\(^{-1}\) and v\textsubscript{C=H} around 2850 cm\(^{-1}\). The structures were further confirmed by the presence of a signal around δ 10.00 for aldehyde proton and absence of C=H of imidazole in the \textsuperscript{1}H NMR spectra apart from other aromatic protons.

The intermediate imidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehydes (3a-d) were exploited for the preparation of target molecules (structure analogues of englitazone) by Knoevenagel condensation with thiazolidine-2,4-dione. The formation of 5-[(2-alkyl/aryl-6-arylimidazo[2,1-b][1,3,4]thiadiazol-5-yl)methylene]-1,3-thiazolidine-2,4-dione (4a-d) was confirmed by their IR spectra, which displayed the v\textsubscript{C=O} bands around 1725 and 1690 cm\(^{-1}\). The v\textsubscript{NH} was observed in the region 3112-3320 cm\(^{-1}\). Further, they were confirmed by \textsuperscript{1}H NMR spectra, where aldehydic proton disappeared and the vinylic proton resonated in the region δ 7.60-7.92 as a singlet. This series of compounds is characterized by the presence of one carbon atom spacer between thiazolidine-2,4-dione moiety and fused heterocyclic ring.

Newly synthesized compounds were analyzed for their C, H and N compositions and the values are within the allowed limits. All the newly synthesized compounds were screened for their hypoglycemic and hypolipidemic activities.

**Reagents and Conditions:**
- I. Dry ethanol reflux, 18hr, Na\textsubscript{2}CO\textsubscript{3}.
- ii. DMF/POCl\textsubscript{3}, Na\textsubscript{2}CO\textsubscript{3}.
- iii. Piperidine acetate, toluene, reflux.

3. EXPERIMENTAL SCHEME

3.1. MATERIALS

All chemicals & reagents were purchased from Sigma-Aldrich Chemicals Pvt. Ltd India. Melting points were determined in open capillaries. The IR spectra were recorded on Nicolet Impact-FT-IR spectrophotometer,(Model-410,USA)using KBr pellet technique. \textsuperscript{1}HNMR experiments were performed on a 300 MHz Bruker AC-300F spectrometer (Model RX-300, Switzerland) using TMS as an internal standard in CDCl\textsubscript{3}.

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All chemical shifts were reported as δ (ppm) values. All the newly synthesized compounds were analyzed for C, H, N and results were found to be within the range of ±0.4% of the theoretical value.

3.2. METHODOLOGY

3.2.1. Synthesis of 2-Amino-5-(4-fluorobenzyl)-1,3,4-thiadiazole (1)

**General method:** A mixture of 4-fluorophenylacetic acid (0.1 mol) and thiourea (9.113 g, 0.1 mol) in phosphorous oxychloride (30 mL) was refluxed gently for 45 min. The reaction mixture was cooled and quenched (highly exothermic) with cold water (90 mL). The resulting solution was refluxed for additional 4 hrs and filtered hot. The filtrate was cooled and basified with aqueous potassium hydroxide solution to get free base. It was collected by filtration, suspended in water and neutralized and the solid hydrobromide salt that separated was filtered, washed with water, dried and recrystallized from ethanol.

3.2.2 Synthesis of 2-(4-alkyl/aryl)-6-arylimidazo[2,1-b][1,3,4]thiadiazoles : (2a-d)

**General method:** A mixture of equimolar quantities of 2-amino-5-(4-arylamino)-1,3,4-thiadiazole (0.01 mol) and bromoacetyl compound (0.01 mol) was refluxed in dry ethanol for 18 hrs. The excess of solvent was distilled off and the solid hydrobromide salt that separated was collected by filtration, suspended in water and neutralized by aqueous sodium carbonate solution to get free base. It was filtered, washed with water, dried and recrystallized from suitable solvent.

2-(4-Fluoro-benzyl)-6-p-tolyl-imidazo[2,1-b][1,3,4]thia diazole (2a)

Brown crystalline solid (ethanol), yield 75%, m.p. 168-170°C; IR (KBr) vcm⁻¹: 3124, 2923, 2853, 1602, 1507; ¹H NMR (300MHz, CDCl₃) δ: 2.38 (s, 3H, CH₃), 4.29 (s, 2H, CH₂), 7.06-7.44 (m, 6H, Ar-H), 7.83 (d, J=7.2Hz, 2H, Ar-H), 7.98 (s, 1H, C₃-H, imidazole). Anal. calcd. for C₁₃H₁₂FN₅S: C, 66.85; H, 4.36; N, 12.99. Found: C, 66.81; H, 4.32; N, 12.92%.

2-[(4-Fluoro-benzyl)-6-naphthalen-2-yl-imidazo[2,1-b][1,3,4]thiadiazole (2b)

White solid (ethanol), yield 80%, m.p. 223-225°C; IR (KBr) vcm⁻¹: 3015, 2856, 2816, 1506; ¹H NMR (300MHz, CDCl₃) δ: 4.29 (s, 2H, CH₂), 7.16-7.49 (m, 11H, Ar-H), 7.76 (d, J=7.5Hz, 2H, Ar-H), 7.98 (s, 1H, C₃-H, imidazole). Anal. calcd. for C₂₅H₁₈FN₅S: C, 71.67; H, 4.18; N, 10.90. Found: C, 71.65; H, 4.17; N, 10.83%.

2-(4-Fluoro-benzyl)-6-naphthalen-2-yl-imidazo[2,1-b][1,3,4]thiadiazole (2c)

Yellow crystalline solid (ethanol), yield 64%, m.p. 212-214°C; IR (KBr) vcm⁻¹: 3107, 2924, 2854, 1603, 1524, 1501; ¹H NMR (300MHz, CDCl₃) δ: 4.31 (s, 2H, CH₂), 7.09-7.66 (m, 10H, Ar-H), 8.1(s, 1H, C₃-H, imidazole), 8.31 (s, 1H, C₃-H, naphthalene). Anal. calcd. for C₂₁H₁₃FN₅S: C, 70.18; H, 3.93; N, 11.69. Found: C, 70.12; H, 3.88; N, 11.68%.

3-[2-(4-Fluoro-benzyl)-imidazo[2,1-b][1,3,4]thiadiazol-6-yl]-4a,8a-dihydro-chromen-2-one (2d)

White needles (ethanol), yield 75%, m.p. 151-153°C; IR (KBr) vcm⁻¹: 3010, 2834, 2812, 1718, 1610, 1509; ¹H NMR (300MHz, CDCl₃) δ: 4.26 (s, 2H, CH₂), 6.93-7.04 (m, 4H, Ar-H), 7.30 (d, J=7.6Hz, 2H, Ar-H), 7.74 (d, J=8.2Hz, 2H, Ar-H), 8.24 (s, 1H, C₃-H, coumarin), 8.66 (s, 1H, C₃-H, imidazole). Anal. calcd. for C₂₈H₂₁FN₅O₂S: C, 63.31; H, 3.72; N, 11.08. Found: C, 63.30; H, 3.66; N, 11.03%.
3.2.3. Synthesis of 2-(4-fluorobenzyl)-6-arylimidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehydes (3a-d):

General method: (Vilsmeier Haack reaction): Vilsmeier Haack reagent was prepared by adding phosphorous oxychloride (3mL) in dimethylformamide (20mL) at 0°C with stirring. At the same temperature 2-(4-fluorobenzyl)-6-arylimidazo[2,1-b][1,3,4]thiadiazole, (2a-d) (0.01mol) was added to the reagent and stirred at 0-5°C for 30 minutes. The mixture was further stirred for 2 hrs at room temperature and then at 60°C for additional 2 hrs. The reaction mixture was cooled in ice water bath and quenched with water(5mL). The reaction mixture was basified with aq. sodium carbonate (10%) solution with cooling and further stirred at 80-90°C for 2 hrs. After cooling, the mixture was diluted with water, extracted with chloroform (30mLx3). The combined extracts were washed with water (100mLx3), dried over anhydrous sodium sulphate. Solvent was removed by evaporation and solid obtained was further stirred at 80°C for 30 minutes. The mixture was collected by filtration, washed with hot benzene and methanol. The products were recrystallized from dimethylformamide in excellent yields.

2-(4-fluoro-benzyl)-6-p-tolyl-imidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde (3a).

Brown solid (Chloroform), Yield 90%, m.p.165°C; IR (KBr) ν cm⁻¹: 2923, 1612, 1683; ¹H NMR (300MHz, CDCl₃) δ: 2.23 (s, 3H, CH₃), 4.41 (s, 2H, CH₂), 6.9-7.84 (m, 8H, Ar-H), 10.02 (s, 1H, CHO); Anal. Calcd. for C₂₅H₁₄F₂N₇O₅S: C, 64.90; H, 4.01; N, 11.91%. Found: C, 64.90; H, 4.01; N, 11.91%.

2-(4-fluoro-benzyl)-6-(2-oxo-4a,8a-dihydro-2H-chromen-3-yl)-imidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde (3d).

White needles (ethanol), yield 86%, m.p. 175-176°C; IR (KBr) ν cm⁻¹: 2834, 2923, 1712, 1678, 1513; ¹H NMR (300MHz, CDCl₃) δ: 4.4 (s, 2H, CH₂), 6.93-7.04 (m, 4H, Ar-H), 7.30 (d, J=7.6Hz, 2H, Ar-H), 7.74 (d, J=8.2Hz, 2H, Ar-H), 8.34 (s, 1H, C=H, coumarin), 10.20 (s, 1H, CHO); Anal. Calcd. for C₂₇H₂₄F₂N₇O₅S: C, 61.91; H, 3.46; N, 10.31. Found: C, 61.90; H, 3.41; N, 10.30%.

3.2.4. Synthesis of 5-[(2-(4-alkyl/aryl)-6-arylimidazo[2,1-b][1,3,4][thiadiazol-5-yl]methylene]-1,3-thiazolidine-2,4-dione (4a-d)

General method: A mixture of 2-alkyl/aryl-6-arylimidazo[2,1-b][1,3,4] thiazidazole-5-carbaldehyde (0.001mol) and 1,3-thiazolidine-2,4-dione (0.001mol) was refluxed in toluene (25mL) with catalytic amount of piperidine-acetate for 2 hrs. The yellow solid separated was collected by filtration, washed with hot benzene and methanol. The products were recrystallized from dimethylformamide.

5-(2-(4-fluorobenzyl)-6-p-tolyl-imidazo[2,1-b][1,3,4]thiazidazole-5-ylmethylene] thiazolidine-2,4-dione (4a).

[Image]

Yellow granules (DMF), yield 95%, m.p. 295-296°C; IR (KBr) υ cm⁻¹: 3016, 2859, 2817, 1722, 1603, 1504; ¹H NMR (300MHz, DMSO, δ) δ: 2.34 (s, 3H, CH₃), 4.34 (s, 2H, CH₂), 7.31-7.76 (m, 6H, Ar-H), 7.88 (d, J=7.6Hz, 2H, Ar-H), 7.96 (s, 1H, vinylic proton), 12.20 (s, 1H, NH, D₂O exchangeable), Anal. Calcd. For C₂₇H₂₃F₃N₅O₅S: C, 58.65; H, 3.36; N, 12.44. Found: C, 58.63; H, 3.32; N, 12.41%.

5-(2-(4-fluorobenzyl)-6-(2-oxo-4a,8a-dihydro-2H-chromen-3-yl)-imidazo[2,1-b][1,3,4][thiadiazol-5-ylmethylene] thiazolidine-2,4-dione (4d).

[Image]

Yellow granules (DMF), yield 85%, m.p. >300°C; IR (KBr) υ cm⁻¹: 3016, 2859, 2817, 1722, 1603, 1504; ¹H NMR (300MHz, DMSO, δ) δ: 4.4 (s, 2H, CH₂), 6.93-7.04 (m, 4H, Ar-H), 7.30 (d, J=7.6Hz, 2H, Ar-H), 7.74 (d, J=8.2Hz, 2H, Ar-H), 8.34 (s, 1H, C=H, coumarin), 10.20 (s, 1H, CHO); Anal. Calcd. for C₂₇H₂₄F₂N₇O₅S: C, 61.91; H, 3.46; N, 10.31. Found: C, 61.90; H, 3.41; N, 10.30%.
7.30 (d, J=7.6Hz, 2H, Ar-H), 7.74 (d, J= 8.2Hz, 2H, Ar-H), 7.96 (s, 1H, vinylic proton), 8.34 (s, 1H, C=H, coumarin), 12.24 (s, 1H, NH, D$_2$O exchangeable). Anal. calcd. for C$_{24}$H$_{15}$FN$_2$O$_5$S$_2$: C, 56.91; H, 2.98; N, 11.06. Found: C, 56.90; H, 2.91; N, 11.01%.

4. PHARMACOLOGICAL EVALUATIONS.

4.1. Hypoglycemic & hypolipidemic activities.

Male Wister rats weighing 150–200 g were used for this study. All animals were maintained under 12 h light and 12 h dark cycle at 25±1°C. All animals were given standard chow (National Institute of Nutrition, India) and water ad libitum. The experiments were designed and conducted in accordance with the guidelines of institutional animal’s ethics committee. The acclimatized animals were kept fasting for 24 h with water ad libitum and alloxan monohydrate (120 mg/kg) in normal saline was then administered. Serum glucose level was checked after 72 h. Animals with serum glucose levels >250 mg/dL were considered diabetic and were used for the study. The animals were divided into two groups of six animals each. Group I animals were termed as control or untreated and group II animals were treated with 100 mg/kg drug. Group II animals were administered with compounds to be screened for euglycemic effect. The suspension of the compound was prepared in water with 1% carboxy methyl cellulose as suspending agent. All the test compounds were orally administered at different doses (10, 30, 100 mg/kg) for 15 days. Pioglitazone was used as standard drug.

On the final day, the blood samples were collected from the tail vein. Plasma was separated from whole blood of each group by centrifugation. Plasma glucose (PG) and triglyceride (TG) levels were estimated using commercial kit [8].

Table 1: Plasma glucose (PG) level of 4a–d at various drug doses

<table>
<thead>
<tr>
<th>Compound</th>
<th>% Decrease in plasma glucose level (PG) at various drug doses (mg/kg bodyweight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 mg</td>
</tr>
<tr>
<td>Pioglitazone</td>
<td>47.25±5.50</td>
</tr>
<tr>
<td>4a</td>
<td>20.15±1.14</td>
</tr>
<tr>
<td>4b</td>
<td>32.25±3.14</td>
</tr>
<tr>
<td>4c</td>
<td>42.48±3.25</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SEM (n = 6). Percentage reduction was calculated according to the formula: [(PG in control – PG in treated)/PG in control] *100; [(TG in control – TG in treated)/TG in control]*100.

Table 2: Triglyceride (TG) level of 4a–d at various drug doses

<table>
<thead>
<tr>
<th>Compound</th>
<th>% Decrease in triglyceride level (TG) at various drug doses (mg/kg body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 mg</td>
</tr>
<tr>
<td>Pioglitazone</td>
<td>35.25±20.42</td>
</tr>
<tr>
<td>4a</td>
<td>25.75±12.32</td>
</tr>
<tr>
<td>4b</td>
<td>27.79±18.32</td>
</tr>
<tr>
<td>4c</td>
<td>33.48±19.24</td>
</tr>
<tr>
<td>4d</td>
<td>32.68±12.36</td>
</tr>
</tbody>
</table>

Each value represents the mean ±SEM (n = 6). Percentage reduction was calculated according to the formula: [(TG in control – TG in treated)/TG in control]*100; [(TG in control – TG in treated)/TG in control]*100.

5. CONCLUSIONS

Thiazolidinediones (TZDs) with naphthyl & coumarinyl substitution ie 5-[2-(4-fluorobenzyl)-6-naphthlen-imidozo[2,1-b][1,3,4]thiadiazol-5-ylmethylene] thiazolidine-2,4-dione (4c), 5-[2-(4-fluoro-benzyl)-6-(2-oxo-4a,8a-dihydro-2H-chromen-3-yl)-imidazo[2,1-b][1,3,4]thiadiazol-5-ylmethylene]-thiazolidine-2,4-dione (4d) exhibited promising hypoglycemic & hypolipidemic activity.

6. REFERENCES


