CHAPTER-2

AN EFFICIENT AND NOVEL PROCESS FOR THE
PREPARATION OF PIOGLITAZONE HYDROCHLORIDE:
AN ANTI DIABETIC DRUG
2.1 INTRODUCTION

Diabetes is one of the oldest ailments and was first documented in Ebers Papyrus, which was written in 1552 BC. Physicians from India were discovered this disease at the same time and named as madhumeha or honey urine. Indian physicians were observed that ants and flies were attracted to urine of the people having diabetes and they had suggested that this is preliminary test for identification of diabetes. In 230 BC, the word “diabetes” was initially named by scientist from Greek, Aretaeus of Cappadocia and meaning of “diabetes” in Greek is “to pass through”.\(^1\) As per world health organization (WHO), about 285 million people are having diabetes worldwide and this number was expected to double by 2030. Recent estimates indicated that India having highest number of diabetic patients (50.8 million) when compared to other countries in the world\(^2\).

Metformin is widely prescribed oral anti diabetic drug in the world for the treatment of type-2 diabetes and in particular for the patients having normal kidney function.\(^3\)-\(^6\) Metformin was first reported in 1922\(^8\), approved by health Canada and United States food and drugs administration (US FDA) in 1972 and 1994 respectively. If metformin was not enough for treating type-2 diabetes, other class of medications available for treating type-2 diabetes is thiazolidinediones, DPP-IV inhibitors, sulfonylureas, alpha-glucosidase inhibitors, GLP-1 analogs and meglitinides alone or in combination with metformin.
Thiazolidinediones also known as glitazones are chemically 2, 4-thiazolidinediones derivatives that include pioglitazone, rosiglitazone, troglitazone, netoglitazone, rivoglitazone, ciglitazone and balaglitazone. These compounds were discovered in late 1990s for the management of type-2 diabetes. In 1997, first drug troglitazone was approved by US FDA but this was withdrawn from market in year 2000 due to potential liver toxicity. Among all the glitazones rosiglitazone and pioglitazone are the widely used drugs to manage type-2 diabetes. Rosiglitazone was approved by US FDA in May 25, 1999, later this was approved in Europe, Japan, New Zealand along with some other countries. Some reports indicate that rosiglitazone associated with high level of cardiovascular risks such as heart attacks. US FDA placed strict restrictions for use of the rosiglitazone in 2011 and this was withdrawn from Europe in 2010.

US FDA was approved pioglitazone in the form of its hydrochloride salt in July 15, 1999 for treatment of type-2 diabetes. Later, pioglitazone was approved in about 45 countries including Europe, Canada, Japan and India. This was marketed under the brand name of Actos® and it is available in 15 mg, 30 mg and 45 mg tablets. Pioglitazone is also available in combination with other anti diabetic agents such as metformin and glimepride. Worldwide sales of pioglitazone hydrochloride in year 2011 are about 5500 million USD (~INR 28,000 crores) with the consumption of 75,000 kgs. The major contribution is from USA (4000
million USD) and this was the one of the top 10 best selling drugs in USA.

Above mentioned particulars and importance of pioglitazone for treating diabetes motivated us to develop novel, efficient, cost effective and moderately greener process for the synthesis of pioglitazone hydrochloride.

2.1.1 PRODUCT PROFILE

1. Generic name : Pioglitazone hydrochloride
2. Chemical structure :

3. Chemical names : (±)-5-[[4-[2-(5-ethyl-2-pyridinyl) ethoxy phenyl]methyl]-2,4-thiazolidinedione hydrochloride.
4. Molecular formula : C_{19}H_{20}N_{2}O_{3}S.HCl
5. Molecular weight : 392.90
6. CAS No : 112529-15-4
7. Therapeutic category : Anti diabetic
8. Indication : Diabetes
9. Action : Insulin Sensitizer

2.1.2 PHYSICAL CHARACTERISTICS
1. Description of API : Oderless white crystalline powder
2. Melting point : 193-194°C
3. Solubility of API : N, N-Dimethylformamide (FDA Label)

2.1.3 MARKET INFORMATION
1. Applicant : Takeda pharms NA
2. Patentee : Takeda pharms NA
3. Brand name : ACTOS
4. USFDA Approval date : July 15, 1999

2.2 LITERATURE REVIEW

The literature survey revealed the following literature precedence for the synthesis of pioglitazone hydrochloride 1, HCl. Scientists from Takeda chemical industries Ltd have synthesized pioglitazone 1, where starting material 5-ethyl-2-pyridyl ethanol 6 was condensed with 4-fluoro nitro benzene 65 to obtain nitro compound 66. Hydrogenation of nitro compound 4 in methanol using 10% Pd/C afforded compound 67.

Scheme 2.1: Synthesis of Pioglitazone 1 (product patent route)
Amine compound 67 was reacted with sodium nitrate in presence of HBr to give diazo compound 68, which was reacted with methyl acrylate in presence of cuprous oxide by applying Meerwein arylation conditions afforded α-Bromo ester compound 69. Condensation of compound 69 with thio urea in presence of sodium acetate followed by acid catalysed hydrolysis resulted pioglitazone 1 (Scheme 2.1).

Kanji Meguro et al. reported alternative process for the synthesis of 1. This involves protection of 6 with p-toluene sulfonyl chloride in presence of phase transfer catalyst (benzyl tert-butyl ammonium chloride (BTBAC)) resulted intermediate 71. Subsequently, intermediate 71 was subjected to nucleophilic substitution. In particular, intermediate 71 was reacted with 4-hydroxy benzaldehyde 8 in the presence of NaOH to give aldehyde compound 9. The resulted 9 was reacted with 2, 4-thiazolidinedione 10 in presence of piperidine by employing knovengal reaction conditions to obtain benzylidene intermediate 11, which was hydrogenated using Pd/C in dioxane to obtain 1.

Scheme 2.2: Synthesis of Pioglitazone 1
Joel E. Huber\textsuperscript{9} reported synthesis of pioglitazone \textbf{1} involves the reduction of \textbf{11} using sodiumborohydride in presence of cobalt chloride/dimethylglyoxime catalyst system (scheme 2.3). An improved procedure by using similar reagents was described by Andrzej Les\textsuperscript{10} and co-workers for the preparation of \textbf{1}.

\begin{center}
\textbf{Scheme 2.3:} Synthesis of Pioglitazone \textbf{1}
\end{center}

Reduction of benzylidene intermediate \textbf{11} using microbial reductase, derived from suitable red yeast, was reported by scientists from Smithkline Beecham pharmaceuticals (scheme 2.4)\textsuperscript{11}.

\begin{center}
\textbf{Scheme 2.4:} Synthesis of Pioglitazone \textbf{1}
\end{center}

Bipin Pandey \textit{et al}\textsuperscript{12} described process for the synthesis of \textbf{1} involves the usage of halohydrin compounds as intermediates. Reaction of 5-ethyl-2-vinyl pyridine \textbf{14} with N-bromo succinamide provided bromohydrin compound \textbf{72}, which was reacted with \textbf{8} in presence of base (NaOH, K\textsubscript{2}CO\textsubscript{3} or NaH) to afford compound \textbf{73}. Condensation of 2-4 thiazolidinedione \textbf{10} with compound \textbf{73} by employing Knoevenagel
reaction conditions resulted in benzyldiene compound 74, which was hydrogenated with sodium borohydride in presence of cobalt chloride and dimethylglyoxime to furnish compound 75. Chlorination of 75 with PCl₅, POCl₃ or SOCl₂, followed by reaction with zinc and acetic acid resulted pioglitazone 1(scheme 2.5). Reactions were also performed by replacing OH group with other groups (Cl, Br, OMs, OTs and OSO₃H).

\[
\begin{align*}
\text{Et} & \quad \text{N} & \quad \text{Et} & \quad \text{OH} & \quad \text{Br} & \quad \text{NaOH, water} & \quad \text{Et} & \quad \text{N} & \quad \text{Et} & \quad \text{O} & \quad \text{CHO} \\
\text{14} & & & & & & \text{72} & & & & & \\
\text{NBS} & \quad \text{NaOH, water} & \quad \text{Et} & \quad \text{N} & \quad \text{Et} & \quad \text{O} & \quad \text{CHO} & \quad \text{8} & & & & & \\
\text{K₂CO₃ (or) NaH} & & & & & & \text{Et} & \quad \text{N} & \quad \text{Et} & \quad \text{O} & \quad \text{CHO} & & & & & \\
\text{10} & & & & & & \text{Et} & \quad \text{N} & \quad \text{Et} & \quad \text{O} & \quad \text{CHO} & & & & & \\
\text{Piperidine} & \quad \text{Acetic acid} & \quad \text{Etanol, Water} & & & & \text{Et} & \quad \text{N} & \quad \text{Et} & \quad \text{O} & \quad \text{CHO} & & & & & \\
\text{14} & & & & & & \text{Et} & \quad \text{N} & \quad \text{Et} & \quad \text{O} & \quad \text{CHO} & & & & & \\
\text{NaBH₄} & \quad \text{CoCl₂, DMG} & \quad \text{DMF} & & & & \text{Et} & \quad \text{N} & \quad \text{Et} & \quad \text{O} & \quad \text{CHO} & & & & & \\
\text{10} & & & & & & \text{Et} & \quad \text{N} & \quad \text{Et} & \quad \text{O} & \quad \text{CHO} & & & & & \\
\text{PCl₅} & \quad \text{CHCl₃} & & & & \text{Et} & \quad \text{N} & \quad \text{Et} & \quad \text{O} & \quad \text{CHO} & & & & & \\
\text{10} & & & & & & \text{Et} & \quad \text{N} & \quad \text{Et} & \quad \text{O} & \quad \text{CHO} & & & & & \\
\text{Zn, Acetic acid} & & & & & & \text{Et} & \quad \text{N} & \quad \text{Et} & \quad \text{O} & \quad \text{CHO} & & & & & \\
\end{align*}
\]

**Scheme 2.5: Synthesis of Pioglitazone 1**

Kanai tsutomu et al.⁰³ reported an alternative synthesis of pioglitazone 1, where 2, 4-thiazolidinedione 10 was condensed with 4-hydroxy benzaldehyde 8 in presence of sodium acetate, acetic anhydride and dimethylacetamide to obtain intermediate 77, which was hydrogenated with Pd/C and H₂ in acetic acid to furnish 78. The resultant compound 78 was subjected to N-alkylation with triphenyl methyl chloride in methylene chloride resulted 79. Hydrolysis of 79 using sodium methoxide in toluene afforded 80. Condensation of 80 and
tosylate intermediate 71 in basic medium (K₂CO₃) followed by deprotection of trityl group in presence of hydrochloric acid furnished compound 1 (scheme 2.6).

Scheme 2.6: Synthesis of Pioglitazone 1

Thijs Lambertus and co-workers¹⁴ have synthesized pioglitazone 1, where 4-benzyloxybenzaldehyde 82 was condensed with tert-butyl chloroacetate 83, employing Darzens condensation conditions, afforded α, β-epoxy ester 84, which was underwent for debenzylation using 10% Pd/C and hydrogen to obtain intermediate 85. Intermediate 85 was reacted with 5-ethylpyridine-2-ethyl mesylate 86 in presence of K₂CO₃ afforded α-hydroxy compound 87, which was mesylated using methanesulfonyl chloride in presence of TEA to provide mesylate compound 88. Requisite compound 1 was prepared by condensation of thio urea with mesylated intermediate 88 in presence of sodium acetate,
followed by hydrolysis of imine compound 70 using hydrochloric acid (scheme 2.7).

Scheme 2.7: Synthesis of Pioglitazone 1

The reported synthetic methods possess some disadvantages, involving cascade of reactions sequence using irritant and toxic methyl acrylate and α-bromo intermediates (scheme 2.1), enrichment of E2 elimination impurity during the condensation of tosylate intermediate 9 with para-hydroxy benzaldehyde 8 (scheme 2.2), involves the isolation of unstable non solid intermediates, incompletion of benzlidene compound 11 reduction using sodium borohydride (scheme 2.3) with the given conditions, multiple purifications for removal of unreacted compound 11 in 1, large number of steps (scheme 2.5, scheme 2.6), protections & deprotections (scheme 2.7) and longer reaction time. Apart from these disadvantages, the reported processes involves the use of (a) expensive palladium metal, (b) plant-unfriendly, pyrophoric non-nucleophilic base,
NaH and (d) partially recoverable and highly expensive solvents like dichloromethane, dioxane, tetrahydrofuran and ethyl acetate.

2.3 PRESENT WORK

2.3.1 OBJECTIVE

The process disadvantages of the reported synthetic approaches motivated us to design high-yielding, cost-effective, moderately greener and free from patent protection approaches for the synthesis of pioglitazone hydrochloride $\text{1.HCl}$. To study impurity profile of pioglitazone $\text{1}$, including identification, synthesis, characterization and root cause for formation of potential process related compounds in synthesis of $\text{1}$.

2.3.2 RESULTS AND DISCUSSION

2.3.2.1 FIRST SYNTHETIC APPROACH FOR SYNTHESIS OF $\text{1}$

Based on retro-synthesis (figure 2.2), the key building blocks for pioglitazone can be derived as (a) 5-ethyl-2-pyridine ethanol, (b) $p$-hydroxy benzaldehyde, (c) 2, 4-thiazolidinedione, (d) $p$-toluene sulfonyl chloride. Synthesis of pioglitazone involves majorly four reactions.

(a) Activation of alcohol with para-toluene sulfonyl chloride.

(b) Formation of aryl ether linkage.

(c) Knoevenagel condensation.

(d) Reduction of alkene group.
In this regard, new synthetic approach-1 (scheme 2.8) has been designed, which starts with the commercially available key starting material 5-ethyl-2-pyridine ethanol 6. The first step involves the activation of alcohol compound 6 with methanesulfonyl chloride in presence of triethyl amine in toluene to obtain mesylate compound 7. The major disadvantage of this step is formation of about 20% of E2 elimination impurity, 5-ethyl-2-vinyl pyridine 14 during the reaction. In addition to this, maximum conversion of mesylate compound 7 in to E2 elimination impurity 14 was observed upon storage of 7 at ambient temperature. These facts are clearly indicated that mesylate compound 7 is highly unstable intermediate. After screening the key process parameters, E2 elimination impurity 14 during the reaction was significantly reduced to below 5% and enrichment of 14 upon storage is controlled by avoided the isolation of 7 as intermediate.

**Figure 2.1:** Retro-synthetic analysis of pioglitazone
The resultant mesylate compound 7 was subjected to \textit{in situ} condensation with \textit{p}-hydroxy benzaldehyde 8 in presence of base to afford an aldehyde intermediate 9. This step suffers with the moderate yield (65.5\%) and purity (54\%). To improve the yield and purity, key reaction parameters such as base and mole ratio of 8 was studied. Base plays major role for the \textit{in situ} generation of phenoxide ion and its further reaction with mesylate compound 7. In view of this, various alkali and alkaline metal hydroxides and carbonates such as sodium hydroxide, potassium carbonates were explored to check the impact of the base during the synthesis of 9. Experimental results revealed that potassium carbonate delivered compound 9 with highest yield (83\%) and highest purity (86\%) when compared with the other bases. After chosen potassium carbonate as base, other key parameter quantity of 8 was examined. Optimal results were obtained when 1.1 mole ratios of 8 was used for the synthesis of 9 as depicted in entry 3 of Table 2.1.

\textbf{Table 2.1:} Synthesis of 9 using different mole ratios of 8

<table>
<thead>
<tr>
<th>S.No.</th>
<th>8 Mole ratio</th>
<th>9 Yield (%)</th>
<th>9 Purity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.9</td>
<td>79.3</td>
<td>76.14</td>
</tr>
<tr>
<td>2</td>
<td>1.0</td>
<td>78.4</td>
<td>80.9</td>
</tr>
<tr>
<td>3</td>
<td>1.1</td>
<td>83.1</td>
<td>86.1</td>
</tr>
<tr>
<td>4</td>
<td>1.2</td>
<td>77.2</td>
<td>84.2</td>
</tr>
</tbody>
</table>
In the reported processes, the resulted crude product 9 was purified by (i) using column chromatography and (ii) by isolate as an acid addition salt using acids such as hydrochloric acid, trifluoro acetic acid, oxalic acid and maleic acid, the obtained salt was recrystallized followed by treated with base to obtain 9\textsuperscript{15}. Multiple purifications and manifold isolations of 9 make process expensive and less viable in commercial scale. At our end, we have avoided the multiple isolations, purification of 9 and preceded to next stage since the resulted compound 9 is an unstable and non-solid intermediate.

![Chemical structure](image)

**Scheme 2.8:** Efficient and novel synthesis of pioglitazone hydrochloride

Subsequent reaction involves the condensation of compound 9 with 2, 4-thiazolidinedione 10 under knoevenagel reaction condensations using piperidine as base. An advantageous process was developed in high purity (~99%) for the synthesis of 11 using piperidine
as base in methanol solvent system. Lengthy work up procedures in this reaction was simplified by adopted simple crystallization at neutral pH and usage of multiple solvent systems for the reaction and purification of crude product was replaced with the single solvent methanol.

Having developed an efficient and scalable process for synthesis of 11, our attention turned towards the reduction of benzylidene compound 11. Reduction of 11 was reported using Pd/C, NaBH₄, CoCl₂.6H₂O/dimethylglyoxime catalyst system and microbial reductase, derived from suitable red yeast. Maximum conversion was reported for the synthesis of 1 using yeast cells is 87%. In view of cost, safety, commercial availability and industrial applicability, NaBH₄ is chosen as preferable reducing agent for reduction of 11. Reduction of benzylidene compounds in particular compound 11 using NaBH₄ as reducing agent in presence of catalyst system comprising cobalt ion and a ligand was generically and specifically covered in United States patent US5585495 and its family equivalent patents, filed by The Upjohn Company. The said patent was further defined the cobalt ions that are used for the reduction is in the form of cobaltous or cobaltic chloride or cobaltous diacetate. To develop a novel process, free from patent protection, cobalt ions which were not covered by said patent such as cobalt nitrate hexahydrate, cobalt ammonium sulfate, cobalt oxalate, cobalt hydroxide and cobalt sulfate were identified and reactivity of these reagents during the synthesis of 1 was studied. Experimental results revealed that cobalt
nitrate hexahydrate and cobalt chloride hexahydrate offered highest yield and purity when compared with other reagents.

**Table 2.2:** Synthesis of 1 using different catalytic system

<table>
<thead>
<tr>
<th>S.No.</th>
<th>NaBH₄ Mole ratio</th>
<th>1 Yield (%)</th>
<th>1 Purity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Co(Cl)₂.₆H₂O</td>
<td>94.9</td>
<td>98.14</td>
</tr>
<tr>
<td>2</td>
<td>Co(NO₃)₂.₆H₂O</td>
<td>93.5</td>
<td>98.52</td>
</tr>
<tr>
<td>3</td>
<td>(NH₄)₂Co(SO₄)₂.₆H₂O</td>
<td>70.6</td>
<td>90.96</td>
</tr>
<tr>
<td>4</td>
<td>Co(OH)₂</td>
<td>73.6</td>
<td>95.82</td>
</tr>
<tr>
<td>5</td>
<td>CoCO₃</td>
<td>72.6</td>
<td>92.08</td>
</tr>
<tr>
<td>6</td>
<td>CoC₂O₄</td>
<td>68.6</td>
<td>95.10</td>
</tr>
</tbody>
</table>

Yield and quality of the end product in reduction reactions always depend upon the reducing agent and amount of the reducing agent used for the particular transformation. To check the impact of the NaBH₄ quantity on the yield and purity of the 1 during the transformation of 11 to 1, various mole ratios (1.0, 1.2, 1.4, 1.5, 1.7, 1.9 and 2.3) of NaBH₄ were studied. Removal of unreacted starting material in end product 1 is found to be difficult even at 0.2-0.5% level since the solubility of both starting material and end product was almost similar. To ensure the complete conversion of starting material into end product excess mole ratio of NaBH₄ was studied rather than the lesser mole ratio. Optimal results were obtained when 1.7 Mole ratio of NaBH₄ used as reducing agent (entry 5, Table 2.3).
Table 2.3: Synthesis of 1 using different mole ratios of NaBH₄

<table>
<thead>
<tr>
<th>S.No.</th>
<th>NaBH₄ Mole ratio</th>
<th>1 Yield (%)</th>
<th>1 Purity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0</td>
<td>90.4</td>
<td>88.77</td>
</tr>
<tr>
<td>2</td>
<td>1.2</td>
<td>91.9</td>
<td>90.73</td>
</tr>
<tr>
<td>3</td>
<td>1.4</td>
<td>91.9</td>
<td>94.16</td>
</tr>
<tr>
<td>4</td>
<td>1.5</td>
<td>94.4</td>
<td>97.21</td>
</tr>
<tr>
<td>5</td>
<td><strong>1.7</strong></td>
<td><strong>94.9</strong></td>
<td><strong>98.14</strong></td>
</tr>
<tr>
<td>6</td>
<td>1.9</td>
<td>90.4</td>
<td>98.69</td>
</tr>
<tr>
<td>7</td>
<td>2.3</td>
<td>89.4</td>
<td>98.08</td>
</tr>
</tbody>
</table>

As per ICH guidelines, any known related compound in the finished active pharmaceutical ingredient (API) should be less than or equal to 0.15%. In similar way any un-known related compound in API should be less than or equal to 0.10%. In view of this, the obtained compound 1 after reduction of benzylidene compound 11 was subjected to purification. To identify the suitable solvent for the purification of crude compound 1, various solvents such as 1, 4-dioxane, N, N-Dimethyl formamide (DMF), acetone, isopropyl alcohol (IPA), methanol, chloroform and ethyl acetate (EA) were screened using input crude compound 1 having purity about 92%. Experimental results indicated that N, N-Dimethyl formamide as solvent system furnished compound 1 with highest yield and purity when compared with the other solvents (entry 2, Table 2.4).
Table 2.4: Purification of 1 using different solvents

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name of the solvent</th>
<th>1 Yield (%)</th>
<th>Purity of 1 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1,4-dioxane</td>
<td>84.4</td>
<td>98.9</td>
</tr>
<tr>
<td>2</td>
<td>DMF</td>
<td>90.0</td>
<td>99.3</td>
</tr>
<tr>
<td>3</td>
<td>Acetone</td>
<td>90.0</td>
<td>94.4</td>
</tr>
<tr>
<td>4</td>
<td>IPA</td>
<td>90.0</td>
<td>95.7</td>
</tr>
<tr>
<td>5</td>
<td>Methanol</td>
<td>75.0</td>
<td>94.4</td>
</tr>
<tr>
<td>6</td>
<td>Chloroform</td>
<td>85.0</td>
<td>90.5</td>
</tr>
<tr>
<td>7</td>
<td>Ethyl acetate</td>
<td>85.0</td>
<td>92.9</td>
</tr>
</tbody>
</table>

The obtained pioglitazone 1 was converted into its hydrochloride salt since the approved product containing active pharmaceutical ingredient in the form of hydrochloride salt of 1. The requisite compound hydrochloride salt of 1 was prepared by treating compound 1 with aqueous hydrochloric acid in water medium. To meet the stringent regulatory requirements the obtained salt was subjected to purification in mixture of methanol and acetone to afforded hydrochloride salt of 1 with about 99.9% purity.

2.3.2.2 Second synthetic approach for synthesis of 1

Though there were good number of synthetic approaches reported in literature for synthesis of 1, there is always need for an alternative synthetic route for preparation of 1. In this context, new synthetic approach has been designed, which is commenced with activation of
alcohol group of compound 6 with methanesulfonyl chloride to provide mesylate compound 7, which upon in-situ reaction with 8 in presence of potassium carbonate afforded aldehyde compound 9. Reduction of aldehyde compound 9 using NaBH₄ in methanol afforded benzyl alcohol intermediate 12. The obtained alcohol intermediate 12 was chlorinated by using hydrochloric acid in dichloromethane to obtain benzyl chloride compound 13, followed by reacted with 2, 4-thiazolidinedione 10 in LiAlH₄ to afford pioglitazone 1. The resultant compound 1 was converted into its hydrochloride salt by using hydrochloric acid in water.

**Scheme 2.9:** Alternative synthesis of pioglitazone hydrochloride

Compound 12 was alternatively synthesized by reacting mesylate intermediate 7 with 4-hydroxy benzyl alcohol 89 in presence of potassium carbonate.
Scheme 2.10: Alternative synthesis of 12

2.4 Related substances of pioglitazone

In our previous chapter importance of related substances in pharmaceutical compounds was described. Related substances also known as impurities that are usually intermediates, starting materials, by-products, metals, residual solvents and degradation products can be formed during the synthesis or in storage of pharmaceutical compounds. Comprehensive study on impurity profile of active pharmaceutical ingredients, its key intermediates and key starting materials, including identification, characterization and root cause for formation is required for many of the regulatory authorities. Different pharmacopoeias, such as the British Pharmacopoeia (BP) and the United States Pharmacopoeia (USP), are slowly incorporating limits to allowable levels of impurities in the APIs or formulations.

In this context, we have taken up an extensive study on impurity profile of pioglitazone 1. To find out the possible impurities, crude compound 1 and key intermediate 11 was analyzed by Liquid Chromatographic-Mass Spectrometry (LC-MS). Based on molecular weight and reaction conditions probable impurities were anticipated and their structures were described in figure 2.2. These impurities were
synthesized, characterized by spectral data such as Mass, IR and NMR. Root cause for formation of these impurities were identified and controlled to below 0.15% in final active pharmaceutical ingredient and as well as in finished pharmaceuticals.

![Structures of pioglitazone related substances](image)

**Figure 2.2:** Structures of pioglitazone related substances

### 2.4.1 Synthesis and root cause for formation of related substance 14.

Related compound 14 is process related impurity formed during the activation of alcohol group of compound 6 and it was increased in subsequent reaction of 7 with 8. This impurity was controlled by avoiding isolation of highly unstable intermediate 7 and restricted to below 0.10% in 11. Related compound 14 was synthesized by stirring mesylated compound 7 in presence of triethyl amine and toluene at 80-
90 °C for 10 hours. It was also observed that compound 7 was converted to compound 14 upon storage of compound 7 at ambient temperature.

Scheme 2.11: synthesis of 14

The ESI-MS spectrum of 14 (Fig.2.3) displayed a protonated molecular ion at 134 m/z with positive segment polarity. The IR spectrum (Fig.2.4), absorption at 2966 & 2928 cm\(^{-1}\) for aliphatic alkyl group and 1599 & 1577 cm\(^{-1}\) for alkene group were observed. The \(^1\)H NMR spectrum (figure 2.5) shows the methyl protons at δ 1.2 (t, 3H, CH\(_3\)), methylene protons at δ 2.6 (q, 2H, CH\(_2\)), ethylene protons at δ 5.3-5.5 (dd, 1H, =CH\(_2\)), δ 6.1-6.2 (dd, 1H, =CH\(_2\)), vinyl proton attached to pyridine at δ 7.1 (t, 1H, =C-H) and aromatic protons at δ 6.8-7.4 (m, 3H, Ar-H).

Figure 2.3: ESI mass spectrum of compound 14
Figure 2.4: IR spectrum of compound 14

Figure 2.5: $^1$H NMR spectrum of compound 14
2.4.2 Synthesis and root cause for formation of related substance 15.

Compound 15 is process related impurity observed during synthesis of key starting material 6 and it was controlled to below 0.10% in benzyldiene compound 11 by adopted purification to the crude compound 11 in methanol. This impurity was synthesized by treating readily available 5-ethyl-2-methyl pyridine (EMP) with formalin solution at elevated temperature (Scheme 2.12) and it was confirmed by $^1$H NMR, mass and IR spectral data.

![Scheme 2.12: synthesis of 15](image)

The ESI-MS spectrum of 15 (Fig.2.6) displayed a protonated molecular ion at 182.1 $m/z$ and sodium adduct as a base peak at 204.1 $m/z$ with positive segment polarity. In the FT-IR spectrum (Fig.2.7), one broad peak at 3349 for alcoholic group, absorption at 2965 & 2931 cm$^{-1}$ for aliphatic alkyl group and 1603 & 1569 cm$^{-1}$ for pyridine C=C group were observed. The $^1$H NMR spectrum (figure 2.8) shows the methyl protons at $\delta$ 1.2 (t, 3H, CH$_3$), methylene protons at $\delta$ 2.6 (q, 2H, CH$_2$), tertiary proton at $\delta$ 3.0 (m, 1H, -C-H), methylene protons attached to alcohol at $\delta$ 4.0 (m, 4H, -CH$_2$), alcoholic protons at $\delta$ 4.7 (s, 2H, OH) and aromatic protons at $\delta$ 7.0-8.3 (m, 3H, Ar-H).
Figure 2.6: ESI mass spectrum of compound 15

Figure 2.7: IR spectrum of compound 15
2.4.3 Synthesis and root cause for formation of related substance 16.

Compound 16 was likely to be derived from small amount of 4-hydroxybenzaldehyde 8 present in the aldehyde compound 9. Related compound 16 was controlled by adopted basic water washings to the organic layer (obtained after completion of reaction between 7 and 8) for removal of unreacted 8 present in 9. Related compound 16 was synthesized by treating compound 8 with 10 in presence of piperidine and methanol (Scheme 2.13) and it was confirmed by $^1$H NMR, mass and IR spectral data.
Scheme 2.13: synthesis of 16

The ESI-MS spectrum of 16 (Fig. 2.9) displayed molecular ion at 220.0 m/z a with negative segment polarity. In the FT-IR spectrum (Fig. 2.10), one sharp NH signal appeared at 3405 cm\(^{-1}\) along with two carbonyl signals at 1719 cm\(^{-1}\) and 1679 cm\(^{-1}\). The \(^1\)H NMR spectrum (figure 2.11) shows the alkene protons at \(\delta\) 7.7 (s, 1H, =CH), aromatic protons at \(\delta\) 6.8-7.5 (m, 4H, Ar-H), alcoholic proton at \(\delta\) 10.3 (s, 1H, OH) and secondary amine proton at \(\delta\) 12.5 (s, 1H, -NH).

Figure 2.9: ESI mass spectrum of compound 16
Figure 2.10: IR spectrum of compound 16

Figure 2.11: $^1$H NMR spectrum of compound 16
2.4.4 Synthesis and root cause for formation of related substance 18.

Compound 18 is process related impurity resulted from the reaction of penultimate intermediate 11 with the mesylated compound 7. This can be controlled by managing the content of mesylated compound 7 in aldehyde compound 9 rather than the purification of 11. Compound 18 was prepared by treating benzylidene intermediate 11 with mesylated compound 7 in presence of K$_2$CO$_3$ and toluene (Scheme 2.14), which was confirmed by $^1$H NMR, mass and IR spectral data.

Scheme 2.14: synthesis of 18

The ESI-MS spectrum of 15 (Fig.2.12) displayed a protonated molecular ion at 488.3 $m/z$ with positive segment polarity. In the FT-IR spectrum (Fig.2.13) shows bands at 1743 & 1682 cm$^{-1}$ for carbonyl group and 1347 cm$^{-1}$ for C-N group. The $^1$H NMR spectrum (figure 2.14) shows the two methyl groups protons at $\delta$ 1.2 (t, 6H, CH$_3$), methylene protons at $\delta$ 2.6 (q, 4H, CH$_2$), methylene protons attached to pyridine and thiazolidinedione linkage moiety at $\delta$ 3.1 (t, 1H, CH$_2$), $\delta$ 4.1 (t, 1H, CH$_2$) and aromatic protons at $\delta$ 7.0-8.4 (m, 10H, Ar-H). The $^{13}$C NMR spectrum (figure 2.15) showed peaks at $\delta$ 35.3 & 41.6 corresponding to methylene group carbons attached to pyridine and thiazolidinedione.
linkage and other carbons appeared in their respective regions further confirmed the assigned structure $18$.

Figure 2.12: ESI mass spectrum of compound $18$

Figure 2.13: IR spectrum of compound $18$
Figure 2.14: $^1$H NMR spectrum of compound 18

Figure 2.15: $^{13}$CNMR spectrum of compound 18
2.4.5 *Synthesis and root cause for formation of related substance 17.*

Synthesis of pioglitazone 1 involves the reduction of benzylidene compound 11 using NaBH₄. In similar conditions compound 16 (process related impurity formed in synthesis of 11) undergoes reduction to form benzyl compound 17. Formation of this impurity was controlled by managing the corresponding benzylidene compound 16 at below 0.15% in penultimate stage. Related compound 17 was prepared from 16 by employing conditions used for reduction of 11 (Scheme 2.15) and it was confirmed by ¹H NMR and mass spectral data.

\[ \text{HO} \quad \text{S} \quad \text{NH} \quad \text{O} \]
\[ \text{HO} \quad \text{S} \quad \text{NH} \quad \text{O} \]

**Scheme 2.15: synthesis of 17**

The ESI-MS spectrum of 19 (Fig.2.16), protonated ammonium adduct appeared at 240.9 m/z with positive segment polarity.

**Figure 2.16: ESI mass spectrum of compound 17**
The $^1$H NMR spectrum (figure 2.17) shows the methylene group protons at $\delta$ 3.0-3.3 (dd, 2H, CH$_2$), aromatic protons at $\delta$ 6.7-7.0 (m, 4H, Ar-H), alcoholic proton at $\delta$ 9.3 (s, 1H, OH) and amide proton at $\delta$ 12.0 (s, 1H, -NH).

![Figure 2.17: $^1$H NMR spectrum of compound 17](image)

2.4.6 Synthesis and root cause for formation of related substance 19.

The origin of impurity 19 was contamination of impurity 18 in penultimate benzylidene compound 11. Compound 18 is process related impurity formed during synthesis of 11. Formation of this impurity was controlled by manage the corresponding benzylidene compound 18 at below 0.15% in penultimate stage. Related compound 19 was prepared from 18 by employing conditions used for reduction of 11 (Scheme 2.16) and it was confirmed by $^1$H NMR, mass and IR spectral data.
Scheme 2.16: synthesis of 19

The ESI-MS spectrum of 19 (Fig.2.18), protonated molecular ion appeared at 490.3 \( m/z \) with positive segment polarity. The FT-IR spectrum (Fig.2.19) showed bands at 1743 & 1686 cm\(^{-1}\) for carbonyl group and 1379 cm\(^{-1}\) for C-N group. The \(^1\)H NMR spectrum (figure 2.20) shows the two methyl groups protons at \( \delta \) 1.2 (t, 6H, CH\(_3\)), methylene protons in ethyl group at \( \delta \) 2.6 (q, 4H, CH\(_2\)), methylene protons attached to pyridine and thiazolidinedione linkage moiety at \( \delta \) 2.9 (t, 1H, CH2), \( \delta \) 4.3 (t, 1H, CH2) and aromatic protons at \( \delta \) 6.8-8.4 (m, 10H, Ar-H).

Figure 2.18: ESI mass spectrum of compound 19
**Figure 2.19:** IR spectrum of compound 19

**Figure 2.20:** $^1$H NMR spectrum of compound 19
2.5 CONCLUSION

In conclusion, an efficient, novel and robust synthetic routes for the preparation of pioglitazone 1 is developed and these are more compatible on commercial scale. The possible potential impurities in pioglitazone 1 and its key intermediate 11 are identified and root cause for their formation also discussed. All impurities were synthesized and characterized by using spectroscopic techniques (Mass, IR and NMR).

2.6 EXPERIMENTAL SECTION

2.6.1 High Performance Liquid Chromatography HPLC)

A Waters Model Alliance 2690-separation module equipped with a waters 996-photo diode array detector was used. The analysis was carried out on intertsil ODS3V columns, 250 mm × 4.6 mm, 5 µ.m particle size with a mobile phase consisting of (degassed buffer) 0.68 g of KH$_2$PO$_4$ 1.0 g of sodium salt of 1-hexanesulphonicacid in 1000 ml of milli-Q water and pH adjusted to 2.6 with diluted phosphoric acid (1.0 g in 10.0 ml water) and acetonitrile in the ratio of 70:30 was used with UV detection at 225 nm at a flow rate of 1.0 ml/min. The column temperature was maintained at 27 °C. The data was recorded using Waters Millennium software. This LC method was able to detect all the isomers and impurities ranged from 0.05 % to 0.10 % in the presence of parent compound.

2.6.2 Process description

2.6.2.1 2-(5-ethylpyridin-2-yl)ethanol (6)
5-Ethyl -2-methyl pyridine (200 g, 1.652 mol) and formalin (134 g, 1.652 mol) are charged into an autoclave vessel and then stirred at 150-155 °C for 2-3 hours. The obtained reaction mixture was distilled at 80-90 °C under reduced pressure to afford 58 g of title compound with 97.5% purity.

**IR (KBr, cm⁻¹):** 3272 (OH), 2965 (Ali, CH), 1604 & 1568 (C═C, aromatic), 1053 (C-O alcohol).

**¹H NMR (400 MHz, DMSO-d₆):** δ_H 7.0-8.3 (m, 3H, Ar-H), 4.5 (s, 1H, OH), 4.0 (t, 2H, CH₂), 3.0 (t, 2H, CH₂), 2.6 (q, 2H, CH₂), 1.2 (t, 3H, CH₃).

**M/S (m/z):** 152.1 (M⁺ + H).

**2.6.2.2 5-((4-(5-ethyl-2-pyridyl)ethoxy)benzylidene)-2,4-thiazolidinedione (11)**

Methanesulfonyl chloride (42.5 g, 0.371 mol) was added slowly to a stirring solution of 2-(5-ethyl-2-pyridyl)ethanol 6 (50 g, 0.331 mol), toluene (200 mL) and triethylamine (57.6 mL, 0.413 mol) at about 25-35 °C. After stirring for 3 h at 25-35 °C, the separated unwanted solid was filtered and washed with toluene (60 mL). The resultant filtrate was
washed with 4% NaHCO₃ solution (60 mL). The aqueous layer was extracted with toluene (50 mL), and the total organic layers were washed with water (2 × 60 mL). 4-hydroxybenzaldehyde 8 (43.2 g, 0.354 mol) and potassium carbonate (80 g, 0.579 mol) were added to the organic layer containing intermediate 7 at 25-35 °C. After stirring for 13 h at 90 °C, the reaction mass was allowed to cool down to 50 °C and water (250 mL) was added. The layers were separated, aqueous layer was extracted with toluene (2 × 150 mL), and the total organic layers were washed with 5% NaOH solution (250 mL). The total organic layer was distilled completely under vacuum at below 65 °C to afford the crude compound 9 (68.6 g). Methanol (450 mL), piperidine (23.3 g, 0.274 mol) and 2,4-thiozolidinedione 10 (29.1 g, 0.248 mol) were added to the crude compound 9 at 25 °C. The resultant reaction mixture was heated to 65 °C and stirred at 65 °C for 14 hours. The reaction mass was allowed to cool to 25-35 °C and diluted with methanol (195 mL). Reaction mass pH was adjusted to about 6-6.5 by using acetic acid (35 mL) and methanol (130 mL) was added. After stirring for 1.5 h at 65 °C, the reaction mass was allowed to cool to 25-35 °C. The separated solid was collected by filtration, washed with methanol (65 mL) and dried under vacuum at 65 °C for 4 h to afford title compound (58.6 g) in 50% yield and >98.9% purity.

**IR (KBr, cm⁻¹):** 2965 (Ali, CH), 1725 & 1697 (C=O, amide), 1599 & 1569 (C=C, aromatic), 1258 (C-O-C ether).
**1H NMR (400 MHz, DMSO-\textit{d}_6):** $\delta$=12.5 (s, 1H, NH), 7.0-8.4 (m, 7H, Ar-H), 7.7 (s, 1H, CH), 4.4(t, 2H, CH$_2$), 3.3 (t, 2H, CH$_2$), 3.2 (t, 2H, CH$_2$), 2.6 (q, 2H, CH$_2$), 1.2 (t, 3H, CH$_3$).

**M/S (m/z):** 355.1 (M$^+$ + H).

### 2.6.2.3 5-((4-(5-ethyl-2-pyridyl)ethoxy)benzyl)-2,4-thiazolidine dione (1)

![Chemical Structure](image)

Cobalt nitrate hexahydrate (0.75 g, 0.025 mol) and dimethyl glyoxime (7.8 g, 0.067 mol) in dimethyl formamide (90 mL) solution was added to a stirring solution of compound **11** (60 g, 0.169 mol) in water (42 mL), 4% NaOH solution (78 mL) and methanol (120 mL) at 25-35 °C. Mixture of sodium borohydride solution (11.1 g dissolved in water 120 mL) and 4% NaOH solution (27 mL) was added slowly at 20-25 °C. The resultant reaction mixture was stirred for 3 h at 20-25 °C, charcoal (3 g) was added to the reaction mass and stirred for 30 min at 20 °C. The reaction mixture was passed through the Celite bed, and the pH of the filtrate was neutralized with acetic acid (24 mL). After stirring for 45 min at 25 °C, the separated solid was collected by filtration, washed with water (60 mL) followed by methanol (2 × 75 mL). The obtained wet compound was dried under vacuum at 70 °C for 8 h to afford compound **1** (59.2 kg) in 98% yield and >98% purity.
IR (KBr, cm⁻¹): 2964 (Ali, CH), 1735 & 1705 (C=O, amide), 1610 & 1515 (C=C, aromatic), 1254 (C-O-C).

¹H NMR (400 MHz, DMSO-d₆): δH 12.0 (s, 1H), 6.8-8.2 (m, 7H, Ar-H), 4.8 (t, 1H, CH), 4.3 (t, 2H, CH₂), 3.1 (t, 2H, CH₂), 2.6 (q, 2H, CH₂), 1.2 (t, 3H, CH₃)

M/S (m/z): 357.1 [M⁺ + H].

2.6.2.4 5-((4-(5-ethyl-2-pyridyl)ethoxy)benzyl)-2,4-thiazolidinedione hydrochloride salt (1.HCl)

Hydrochloric acid (75 mL) was slowly added to a stirring solution of pioglitazone 1 (50 g, 0.141 mol) in water (250 mL) at 25 °C and heated to 80 °C to get clear solution. The resultant clear solution was allowed to cool down to about 25 °C and stirred at 25 °C for 45 minutes. The separated solid was filtered, washed with water (50 mL). Methanol (100 mL) and wet compound was heated to 60 °C to get the clear solution. Acetone (250 mL) was added to the clear solution at about 35 °C and then allowed to cool down to 25 °C. The separated solid was collected by filtration, washed with acetone (50 mL) and dried under vacuum at 70 °C for 4 h to afford 1·HCl (49.6 g) in 90% yield and >99.7% purity.
**IR (KBr, cm⁻¹):** 2928 (Ali, CH), 2742 (NH, amide), 1743 & 1694 (C=O, amide), 1615 & 1510 (C=C, aromatic), 1461 & 1313 (C-H, Ali), 1243 & 1038 (C-O-C), 850 & 712 (C-H, aromatic).

**¹H NMR (400 MHz, DMSO-d₆):** δH 12.08 (s, 1H), 8.73 (d, 1H, J = 1.6 Hz), 8.43 (dd, 1H, J = 2.0, 8.0 Hz), 8.01 (d, 1H, J = 8.0 Hz), 7.16 (d, 2H, J = 8.8 Hz), 6.89 (d, 2H, J = 8.8 Hz), 4.88 (dd, 1H, J = 4.4, 8.8 Hz), 4.34 (t, 2H, J = 6.2 Hz), 3.55 (t, 2H, J = 6.2 Hz), 3.30 (dd, 1H, J = 4.4, 14.0 Hz), 3.06 (dd, 1H, J = 8.8, 14.0 Hz), 2.80 (q, 2H, J = 7.6 Hz), 1.24 (t, 3H, J = 7.6 Hz);

**¹³C NMR (400 MHz, DMSO-d₆):** δC 175.6, 171.6, 157.0, 151.0, 145.2, 141.3, 139.9, 130.1, 129.0, 127.2, 114.4, 65.4, 52.9, 40.1, 39.9, 39.7, 39.5, 39.3, 39.1, 38.9, 36.2, 32.1, 24.6, 14.5.

**M/S (m/z):** 357.5 [M⁺ + H].

**2.6.2.5 (4-(2-(5-ethylpyridin-2-yl)ethoxy)phenyl)methanol (30)**

![Chemical Structure]

To a stirring solution of 9 (50 g, 0.196 mol) and methanol (250 mL) was added slowly sodium borohydride (14.9 g, 0.392 mol) at 25-30 °C. After stirring for 2 hours at 25-30 °C, the reaction mass solvent was distilled at below 60 °C under reduced pressure. To the resultant crude water (200 mL) and dichloromethane (200 mL) was added and stirred for 15 minutes. The layers were separated, aqueous layer was extracted
with dichloromethane (2x50 mL), combined organic layer washed with water (2x50 mL) and dried using sodium sulfate. The organic layer was distilled completely at below 45 °C under reduced pressure to afford title compound in 90.7% yield.

\[ ^1H \text{ NMR (400 MHz, DMSO-}d_6)\]: 7.0-8.4 (m, 7H, Ar-H), 4.9 (s, 1H, OH), 4.9 (s, 2H, CH\(_2\)), 4.3 (t, 2H, CH\(_2\)), 3.3 (t, 2H, CH\(_2\)), 2.6 (q, 2H, CH\(_2\)), 1.2 (t, 3H, CH\(_3\)).

\[ M/S (m/z): 258.1 \text{ (M}^+ + \text{ H).} \]

2.6.2.6 2-(2-(4-(chloromethyl)phenoxy)ethyl)-5-ethylpyridine (31)

To a stirring solution of 30 (50 g, 0.194 mol) in dichloromethane (250 mL), dry hydrochloric acid gas was passed for 4 hours at 25-35 °C. The resultant reaction mass solvent was evaporated at below 45 °C under reduced pressure to afforded title compound in 95% yield.

\[ ^1H \text{ NMR (400 MHz, DMSO-}d_6)\]: 6.8-8.4 (m, 7H, Ar-H), 4.9 (s, 2H, CH\(_2\)), 4.3 (t, 2H, CH\(_2\)), 3.3 (t, 2H, CH\(_2\)), 2.6 (q, 2H, CH\(_2\)), 1.2 (t, 3H, CH\(_3\)).

\[ M/S (m/z): 276.5 \text{ (M}^+ + \text{ H).} \]

2.6.2.7 5-((4-(5-ethyl-2-pyridyl)ethoxy)benzyl)-2,4-thiazolidinedione (1)
To a stirring solution of 2, 4-thiazolidinedione 10 (4.2 g, 0.036 mol) in tetrahydrofuran (50 mL) at about -40 °C was added slowly lithium aluminium hydride (5.4 g, 0.143 mol) and stirred at -40 °C for 30-45 minutes. To the reaction mixture, solution of compound 31 (10 g, 0.036 mol) in tetrahydrofuran (30 mL) was added slowly at -40 °C over 30-45 minutes and stirred at -40 °C for 12-14 hours. Water (50 mL) was added slowly to the reaction mass and pH was adjusted to 6.5-7.0 with hydrochloric acid. The separated solid was collected by filtration, washed with methanol (20 mL) and dried at 60 °C to afford title compound.

**IR (KBr, cm⁻¹):** 2964 (Ali, CH), 1735 & 1705 (C=O, amide), 1610 & 1515 (C=C, aromatic), 1254 (C-O-C).

**¹H NMR (400 MHz, DMSO-ｄ₆):** δH 12.0 (s, 1H), 6.8-8.2 (m, 7H, Ar-H), 4.8 (t, 1H, CH), 4.3 (t, 2H, CH₂), 3.1 (t, 2H, CH₂), 2.6 (q, 2H, CH₂), 1.2 (t, 3H, CH₃)

**M/S (m/z):** 357.1 [M⁺ + H].

2.6.2.8 2-(5-ethylpyridin-2-yl)propane-1,3-diol (15)
5-Ethyl-2-methyl pyridine (200 g 1.652 mol) and formalin (134 g, 1.652 mol) are charged into autoclave vessel and then stirred at 150-155 °C for 2-3 hours. The obtained reaction mixture was distilled at 80-90 °C under reduced pressure. The resultant crude compound was purified by using column chromatography to afford title compound.

**IR (KBr, cm⁻¹):** 3349 (OH, alcohol), 2965 & 2931 (Ali, CH), 1603 & 1569 (C=C, aromatic), 1037 (C-O-C).

**¹H NMR (400 MHz, DMSO-d₆):** δH 7.0-8.3 (m, 3H, Ar-H), 4.7 (s, 2H, OH), 4.0 (m, 4H, -CH₂), 3.0 (m, 1H, -C-H), 2.6 (q, 2H, CH₂), 1.2 (t, 3H, CH₃). **M/S (m/z):** 182.1 [M+ + H].

### 2.6.2.9 5-(4-hydroxybenzylidene)thiazolidine-2,4-dione (16)

4-hydroxy benzaldehyde 8 (10 g, 0.081 mol), 2,4-thiazolidinedione 10 (9.6 g, 0.081 mol), piperidine (7.7 g, 0.09 mol) and methanol (70 mL) was heated to 65 °C. After stirring at 65 °C for 12 hours, reaction mass pH was adjusted to 6.0-6.5 using acetic acid at 25-35 °C and stirred for 45 minutes. The separated solid was filtered, washed with methanol (10 mL) and dried at 50 °C under vacuum to afford 13 g of title compound.

**IR (KBr, cm⁻¹):** 3405 (NF, amine), 1719 & 1679 (C=O, amide), 1592 & 1573 (C=C, aromatic), 1155 (C-O, alcohol).
$^1$H NMR (400 MHz, DMSO–d$_6$): $\delta$H 12.5 (s, 1H, -NH), 10.3 (s, 1H, OH), 7.7 (s, 1H, =CH), 6.8-7.5 (m, 4H, Ar-H).

**M/S (m/z):** 220.0 [M$^+$ - H].

### 2.6.2.10 5-ethyl-2-vinylpyridine (14)

Mesylated compound 7 ((37 g, 0.161 mol), 4-hydroxy benzaldehyde 8 (21.6 g, 0.177 mol), triethyl amine (29.3 g, 0.289 mol) and toluene (285 mL) was heated to 80-90 °C. After stirring at 80-90 °C for 15 hours, reaction mass was cooled to 30 °C and washed with 5% NaOH solution (50 mL). The resultant reaction mass solvent was evaporated at below 70 °C under vacuum to afford 23.4 g of title compound.

**IR (KBr, cm$^{-1}$):** 2966 & 2928 (Ali, CH), 1599 & 1577 (C=C, aromatic), 846 (=C-H, aromatic).

$^1$H NMR (400 MHz, DMSO–d$_6$): $\delta$H 6.8-7.4 (m, 3H, Ar-H), 7.1 (t, 1H, =C-H), 6.1-6.2 (dd, 1H, =CH$_2$), 5.3-5.5 (dd, 1H, =CH$_2$), 2.6 (q, 2H, CH$_2$).

**M/S (m/z):** 134 [M$^+$ + H].

### 2.6.2.11 5-(4-(2-(5-ethylpyridin-2-yl)ethoxy)benzylidene)-3-(2-(5-ethylpyridin-2-yl)ethyl)thiazolidine-2,4-dione (18)
To a stirred solution of benzylidene compound 11 (5 g, 0.014 mol) and methanol (50 mL), piperidine (2.4 g, 0.028 mol) was added at 30 °C and stirred for 15 minutes. Compound 7 (8.1 g, 0.035 mol) was added at 30 °C and then heated to 65 °C. After stirring at 65 °C for 30 hours, reaction mixture was cooled to 30 °C and stirred for 15-30 minutes. The separated solid was filtered, washed with methanol (5 mL) and dried at 50 °C under vacuum to afford title compound.

**IR (KBr, cm⁻¹):** 2959 & 2931 (Ali, CH), 1743 & 1682 (C=O, amide), 1597 & 1567 (C=C, aromatic), 1347 (C-N group).

**¹H NMR (400 MHz, DMSO-d₆):** δH 7.0-8.4 (m, 10H, Ar-H), 4.1 (t, 1H, CH₂), 3.1 (t, 1H, CH₂), 2.6 (q, 4H, CH₂), 1.2 (t, 6H, CH₃).

**M/S (m/z):** 488.3 [M⁺ + H].

**2.6.2.12 5-(4-hydroxybenzyl)thiazolidine-2,4-dione (17)**

Cobalt nitrate hexahydrate (0.18 g, 0.0006 mol) and dimethyl glyoxime (2.1 g, 0.018 mol) in dimethyl formamide (30 mL) solution was added to a stirring solution of compound 16 (10 g, 0.045 mol) in water (7 mL), 4% NaOH solution (13 mL) and methanol (20 mL) at 25-35 °C.
Mixture of sodium borohydride solution (4 g dissolved in water 20 mL) and 4% NaOH solution (5 mL) was added slowly at 20-25 °C. The resultant reaction mixture was stirred for 3 h at 20-25 °C, and the pH was neutralized with acetic acid (24 mL). After stirring for 45 min at 25 °C, the separated solid was collected by filtration, washed with water (10 mL) followed by methanol (2 × 10 mL). The obtained wet compound was dried under vacuum at 70 °C for 8 h to afford title compound.

**IR (KBr, cm\(^{-1}\))**: 2963 & 2928 (Ali, CH), 1742 & 1682 (C=O, amide), 1588 & 1553 (C=C, aromatic).

**\(^1\)H NMR (400 MHz, DMSO–d\(_6\))**: \(\delta_{H} 12.0 \text{ (s, 1H, -NH)}, 9.3 \text{ (s, 1H, OH)}, 6.7-7.0 \text{ (m, 4H, Ar-H)}, 3.0-3.3 \text{ (dd, 2H, CH\(_2\))}.

**M/S (m/z)**: 240.9 [M\(^+\) + H].

2.6.2.13 5-(4-(2-(5-ethylpyridin-2-yl)ethoxy)benzyl)-3-(2-(5-ethylpyridin-2-yl)ethyl)thiazolidine-2,4-dione (19)

Cobalt nitrate hexahydrate (0.08 g, 0.0003 mol) and dimethyl glyoxime (0.9 g, 0.0078 mol) in dimethyl formamide (30 mL) solution was added to a stirring solution of compound 16 (10 g, 0.020 mol) in water (7 mL), 4% NaOH solution (15 mL) and methanol (20 mL) at 25-35 °C. Mixture of sodium borohydride solution (4 g dissolved in water 20 mL) and 4% NaOH solution (10 mL) was added slowly at 20-25 °C. The
resultant reaction mixture was stirred for 3 h at 25 °C, pH was adjusted to 6.5-7.0 with acetic acid (24 mL). After stirring for 30 minutes at 25 °C, the separated solid was filtered, washed with water (10 mL) followed by methanol (2 × 10 mL). The obtained wet compound was dried under vacuum at 70 °C for 8 h to afford title compound.

**IR (KBr, cm⁻¹):** 2962 & 2929 (Ali, CH), 1743 & 1686 (C=O, amide), 1610 & 1568 (C=C, aromatic), 1379 (C-N group), 1252 (C-O, ether).

**¹H NMR (400 MHz, DMSO–d₆):** δH 6.8-8.4 (m, 10H, Ar-H), 4.3 (t, 1H, CH₂), 2.9 (t, 1H, CH₂), 2.6 (q, 4H, CH₂), 1.2 (t, 6H, CH₃).

**M/S (m/z):** 490.3 [M⁺ + H].

### 2.7 REFERENCES


