4.1. Designing of molecules

Molecular docking studies have been carried out for a group of designed molecules using VLlife MDS 4.2 software. The molecules with good dock score have been selected for synthesis.

4.1.1. Molecular Docking studies

Molecular docking studies were carried out by using VLlife MDS 4.2 software. The PLP function was incorporated by the MDS VLlife Science software in the GRIP docking method, which calculates the ligand-receptor binding affinity in terms of the PLP score. The PLP score was designed to enable flexible docking of ligands to perform a full conformational and positional search within a rigid binding site. All the optimized conformers were docked into active binding site of EACP reductase target protein (protein data bank, PDB entry 1ZID) and Integrase target protein (protein data bank, PDB entry 1BI4) which were considered as the reference to define the active binding site in the present investigation. Water molecules and HET ATOM-like bound ligand data were removed from the PDB file of EACP Reductase and Integrase protein during docking study. The crystal structure was refined using VLlife Science’s MDS 4.2 software. The refinement of the crude PDB structure of receptor was done by completing the incomplete residues. The co-crystallized ligand lying within the receptor was modified by assigning missing bond order and hybridization states. The side chain hydrogens were then added to the crystal structure and their positions were optimized up to the rms gradient 1
by aggregating the other part of the receptor. The optimized receptor was then saved as mol file and used for docking simulation. The 2D structure of the compounds were built and then converted into the 3D with the help of VLife MDS4.2 software. The 3D structures were then energetically minimized up to the rms gradient of 0.01 using Merck Molecular Force Field (MMFF). Conformers of compounds were then generated by Monte Carlo method. In doing so, all rotatable bonds of the ligand were selected and number of seeds used for searching the conformational space was set 5. All the conformers were then energetically minimized up to the rms gradient of 0.01 and then saved in separate folder. The active site selection was done by choosing the cavity having maximum hydrophobic surface area. The docking simulation was done using GRIP batch docking. In this, all generated conformers of one ligand were put as one batch in GRIP docking wizard. Likewise, the batches for all other ligands were put. All the conformers were virtually docked at the defined cavity of the receptor. The parameters fixed for docking simulation was like this-number of placement: 50, rotation angle of: 10°, exhaustive method, scoring function: dock score. By rotation angle, ligand would be rotated inside the receptor cavity to generate different ligand poses inside the receptor cavity. By placements, the method will check all the 50 possible placements into the active site pocket and will result out few best placements out of 50. For each ligand, all the conformers with their best placements and their dock score will be saved in output folder. The method also highlights the best placements of best conformer of one particular ligand which is having best (minimum) dock score. The ligand forming most stable drug-receptor complex is the one which is having minimum dock score. After docking simulation, the best docked conformer of each ligand and receptor were merged and their
complex was then energetically optimized by defining radius of 10 Å measured from the docked ligand. Stepwise energy optimization was done by first hydrogens; second side chains and finally the backbone of receptor. The optimized complexes were then checked for various interaction of ligand with receptor like hydrogen bonding, hydrophobic bonding and van der Waals' interaction. The binding affinity was evaluated by the binding free energy ($\Delta G_b$, kcal/mol), hydrogen bonding interaction, hydrophobic interaction and RMSD values (Noolvi 2013). Docking interaction of synthesized compounds is discussed in chapter V.

4.1.2. Calculation of pKa

The ADME of ionizable drugs is greatly influenced by their pKa values, which influence their transport properties (absorption and distribution), and the excretion of metabolites. The pKa values are often measured during a process of physicochemical profiling that occurs during the selection of newly discovered molecules for further development (Clark, 2003; Avdeef, 2001). They are also measured to a high standard of accuracy and GLP during later-stage development and for regulatory purposes. pKa of all the test compounds were calculated using ChemAxon Marvin sketch software.

4.1.3. Evaluation of the test compounds using Lipinski’s rule

Lipinski’s rule of five is a rule of thumb to evaluate the drug-likeness (Lipinski, 2001). This rule describe the important molecular properties required for drug’s pharmacokinetics, viz., absorption, distribution, metabolism and excretion (ADME).
Lipinski’s rule states that the drug to have good pharmacokinetic profile, should not be more than one violation of the following criteria:

- Not more than 5 hydrogen bond donors (like OH & NH)
- Not more than 10 hydrogen bond acceptors (electronegative atoms like O & N)
- The molecular weight should be less than 500 daltons
- The partition coefficient, log $P$, (octanol-water) not greater than 5.

All the test compounds were evaluated for their pharmacokinetic profile based on Lipinski’s rule using chemspider software.

4.1.4. Evaluation of the test compounds using Veber’s rule

Veber’s rule is the extension of the Lipinski’s rule to predict the oral bioavailability of molecules (Veber, 2002). Veber’s rule for good oral bioavailability in rats are as follows:

- Number of rotatable bonds = 10
- Polar surface area (PSA) = 140 Å²

All the test compounds were evaluated based on Veber’s rule using chemspider software.
4.2. Materials and Methods:

Melting points were determined in open capillaries using Toshniwal or Shital scientific industries apparatus without correction. The purity of the compounds was checked by TLC using silica gel coated Aluminium plates ((Merck 60 F254, 0.25mm)) and the blots were envisaged under ultra violet light at 254 and 366 nm. Each compound was dried under high vacuum and the yield was determined. IR spectra were recorded in KBr pellets on an IR Schimadzu FT-IR spectrophotometer (cm⁻¹), ¹H-NMR spectra on a Bruker Ascend 400MHz spectrometer using tetramethylsilane as internal standard (chemical shifts in d, ppm), mass spectra on a shimadzuQp 2010 ultra-mass spectrometer and elemental analysis on a Perkin Elmer 2400 CHN elemental analyzer. Unless otherwise indicated, all reagents were purchased from commercial suppliers and were used without further purification.

4.3. Synthesis

Synthesis of the present work is discussed under the following heads.

**Series I** : 1-(1H-benzimidazol-2-ylmethyl)piperidin-4-substituted imines (PB1 – PB25)

**Series II** : 1-(1H-benzimidazol-2-ylmethyl)-3,5-bis(furan-2-ylmethylidene)piperidin-4-substituted imines (B1 – B25)

**Series III** : 3,5-bis(furan-2-ylmethylidene)piperidin-4-substituted imines (R1-R25)
4.3.1. Series I: 1-(1H-benzimidazol-2-ylmethyl)piperidin-4-substituted imines (PB1–PB25)

Fig. 4.1. Synthetic scheme of 1-(1H-benzimidazol-2-ylmethyl)piperidin-4-substituted imines (PB1 – PB25)

4.3.1.1. Reaction Mechanisms

The compound PB1, 1-(1H-benzimidazol-2-ylmethyl)piperidin-4-one, was synthesized by the reaction of piperidin-4-one (1) with 2-chloromethylbenzimidazole (2) in the presence of an acid scavenger.
Reaction:

\[
\text{1} \quad \overset{\text{O}}{\text{N}} \quad \overset{\text{H}}{\text{H}} \quad + \quad \overset{\text{Cl}}{\text{N}} \quad \overset{\text{H}}{\text{H}} \quad \overset{\text{N}}{\text{Cl}} \quad \overset{\text{TEA}}{\text{PB1}}
\]

Mechanism involved:

The chloro group present in the 2-chloromethylbenzimidazole is a good leaving group, makes the methylene carbon for nucleophilic attack by the 2° amine of 4-piperdione. The HCl formed during this reaction is neutralized with triethylamine.
PB1 was condensed with appropriate substituted amines in the presence of sodium acetate to obtain PB2 - PB23 by Schiff’s reaction.

Reaction:

Mechanism involved:

Step I:

Nucleophilic attack of carbonyl carbon by 1° amine forms zwitterion.
Step II:

The zwitterion rearranges to form unstable carbinolamine.

\[
\text{N} \quad \text{H} \\
\text{O} \\
\text{N} \quad \text{H} \\
\text{R} \\
\text{\textcolor{red}{\textbf{\textit{zwitterion}}}} \\
\text{N} \quad \text{H} \\
\text{O} \\
\text{N} \quad \text{H} \\
\text{R} \\
\text{\textcolor{blue}{\textbf{\textit{carbinolamine}}}}
\]

Step III:

The acetate ion deprotonate the amino group in carbinolamine and protonate the \( \text{OH}^- \) ion leads to the formation of imine (Schiff's base).

\[
\text{N} \quad \text{H} \\
\text{O} \\
\text{N} \quad \text{H} \\
\text{R} \\
\text{\textcolor{red}{\textbf{\textit{carbinolamine}}}} \\
\text{N} \quad \text{H} \\
\text{O} \\
\text{N} \quad \text{H} \\
\text{R} \\
\text{\textcolor{blue}{\textbf{\textit{PB2 - PB23}}}}
\]
The compound, \( \text{PB1} \), undergoes reduction in the presence of sodium tetra borate to yield the alcohol, \( \text{PB24} \).

**Reaction**

\[
\text{NaBH}_4 + \text{PB1} \rightarrow \text{PB24} + \text{BH}_3 + \text{Na}^+
\]

**Mechanism involved:**

**Step I:**

Nucleophilic attack of carbonyl carbon of \( \text{PB1} \) with hydride ion forms alkoxide ion.

\[
\text{PB1} + \text{Na}^+ \rightarrow \text{alkoxide ion}
\]
Step II:

The alkoxide ion on protonation yields the alcohol (PB24).

\[
\begin{array}{c}
\text{alkoxide ion} \quad \xrightarrow{\text{H}^+} \quad \text{alcohol (PB24)}
\end{array}
\]

The compound PB24, on treatment with benzene sulphonyl chloride yields PB25.

Reaction:

\[
\begin{array}{c}
\text{PB24} \quad \xrightarrow{\text{C}_6\text{H}_5\text{SO}_2\text{Cl}} \quad \text{PB25}
\end{array}
\]

Mechanism involved:

The chloro group present in the benzene sulphonyl chloride is a good leaving group, which makes the sulphonyl group for nucleophilic attack by the oxygen of PB24, followed by elimination of HCl forms the compound PB25.
4.3.1.2. Synthesis of 1-(1H-benzimidazol-2-ylmethyl)piperidin-4-one (PB1)

To a reaction mixture of 4-piperidone hydrochloride (0.76 gm, 0.005 mol) and 2-chloromethylbenzimidazole (0.8 gm, 0.005 mol) in dimethyl formamide (10 ml), triethylamine (0.7 ml, 0.005 mol) was added and heated under reflux for 7 hr. The completion of the reaction was confirmed by TLC, then the contents were cooled and poured into crushed ice. This mixture was refrigerated for 12 hr and the precipitate was filtered, vacuum dried after washing with water. Finally, recrystallization was carried out using methanol to yield the pure product.

4.3.1.3. 1-(1H-benzimidazol-2-ylmethyl)-N-hydroxypiperidin-4-imine (PB2)

The reaction was carried out based on the reported method (Banerjee 2011). To an ethanolic mixture of 1-(1H-benzimidazol-2-ylmethyl)piperidin-4-one (2.29 gm, 0.01 mol) with hydroxylamine hydrochloride (0.69 gm, 0.01 mol), sodium acetate (0.82 gm, 0.01mol) was added with stirring and it was heated under reflux for 3.5 hr with constant stirring. After completion of the reaction, which was monitored using TLC, the contents were cooled to room temperature and poured into ice cold water. The resulting product was filtered under suction and vacuum dried.
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after washing thoroughly with water. It was then recrystallized from the mixture of methanol and dimethyl formamide (2:8) to yield the pure compound.

4.3.1.4. Synthesis of hydroxy thiosemicarbazide

Hydroxy thiosemicarbazide was prepared based on the reported method (Banerjee 2011). The mixture of hydroxylamine hydrochloride (0.69 gm, 0.01 mol) in ethanol (20 ml) and KOH (0.56 gm, 0.01 mol) and carbon disulphide (0.75 ml) was stirred at 0-5 °C for 1 hr to form corresponding dithiocarbamate. To the stirred mixture of dithiocarbamate, hydrazine hydrate (0.5 ml, 0.01 mol) was added and stirring was continued at 80 °C for 1 hr. Then the reaction mixture was poured into crushed ice. The product formed was converted to its hydrochloride salt. Yield, 68.5%; melting point, 259-260 °C.

4.3.1.5. Synthesis of thiocarbohydrazide

This compound was prepared as per the literature method (Malone 1979). Hydrazine hydrate (2.9 ml, 0.05 mol) was placed in three necked flask equipped with thermometer, dropping funnel and reflux condenser which was connected to the caustic trap. The temperature was lowered to 10 °C and carbon disulphide (0.6 ml, 0.01 mol) was added, while maintaining the temperature below 15 °C. Water (7.5 ml) was added and the temperature was raised to 85 °C and held there for 1.5 hr. The temperature was then lowered to 10 °C. The precipitated product was filtered, washed with water and recrystallised using methanol.

4.3.1.6. General method of procedure for the synthesis of PB2 - PB23

The reaction was performed based on the literature method (Banerjee 2011). To an ethanolic mixture of 1-(1H-benzimidazol-2-ylmethyl)piperidin-4-one (2.29 gm,
0.01 mol) with the appropriate amine (0.01 mol), sodium acetate (0.82 gm, 0.01 mol) was added with stirring and it was heated under reflux for 3.5 hr with constant stirring. After completion of the reaction, which was confirmed using TLC, the contents were cooled to room temperature and poured into ice cold water. The resulting product was filtered under suction and vacuum dried after washing thoroughly with water. It was then recrystallized from the mixture of methanol and dimethyl formamide (2:8) to yield the pure compound. The other compounds, PB3-PB23 were prepared by the similar method using appropriate amine.

4.3.1.7. Synthesis of 1-(1H-benzimidazol-2-ylmethyl)piperidin-4-ol (PB24)

The reduction reaction was carried out based on the literature method (Aridoss 2008). To the compound PB1 (0.458 gm, 0.002 mol) in methanol (5 ml), sodium borohydride (0.76 gm, 0.02 mol) was added at 0 °C with stirring. Methanol was removed and aqueous solution of ammonium chloride (2ml) was added to the residue and the product was extracted with ethyl acetate. Then ethyl acetate was evaporated and the compound was recrystallised using methanol and DMF.

4.3.1.8. Synthesis of 1-(1H-benzimidazol-2-ylmethyl)piperidin-4-yl benzenesulfonylate (PB25)

This reaction was carried out as per the reported method (Chernyshov 2011). A solution of benzenesulphonyl chloride (0.1 ml, 0.001 mol) in pyridine (5 ml) was heated to 110-115 °C with vigorous agitation and thoroughly ground compound PB24 (0.23 gm, 0.001 mol) was added. The mixture was boiled with reflux under agitation for 30 min. and then 2M NaOH solution (150 ml) was poured in and the mixture was boiled for additional 5-8 min. The resulting solution was neutralized
with glacial acetic acid and cooled to 20 °C. The product formed was filtered and recrystallised using methanol and DMSO.

4.3.1.9. Characterization of 1-(1H-benzimidazol-2-ylmethyl)piperidin-4-substituted imines (PB1–PB25)

1-(1H-benzimidazol-2-ylmethyl)piperidin-4-one (PB1)

Yield : 79 %

Melting Point : 300 - 303 °C

Rf : 0.62 (benzene : ethanol, 8:2)

IR (KBr) cm\(^{-1}\) : 3250 (NH); 3041, 3007, 2985 (C-H); 1704 (C=O).

\(^1\)H-NMR (DMSO-d\(_6\)) d : 2.12 (t, 4H, J=6.8 Hz, H\(_3\)&H\(_5\)piperidone), 2.50 (t, 4H, J=6.8 Hz, H\(_2\)&H\(_6\) piperidone), 3.42 (s, 2H, -N-CH\(_2\)), 7.12 (d, 2H, J=8.4Hz, H\(_5\)&H\(_6\) benzimidazole), 7.81 (d, 2H, J=8.4 Hz, H\(_4\)&H\(_7\) benzimidazole), 12.21 (s,1H, NH)

\(^{13}\)C-NMR (DMSO-d\(_6\)) d : 41.61, 52.94 (4C, piperidone), 55.76 (CH\(_2\)), 115.24, 123.07, 139.11 (6C, Ar), 141.55 (imidazole), 210.30 (C=O).

MS m/z : 229 (M\(^+\)), 213, 173, 153, 131

Elemental Analysis (%) : Calculated: C, 68.10; H, 6.59; N, 18.33.

Found: C, 67.85; H, 6.20; N, 18.66.

1-(1H-benzimidazol-2-ylmethyl)-N-hydroxypiperidin-4-imine (PB2)

Yield : 82%

Melting Point : 319 - 320 °C

Rf : 0.60 (chloroform : methanol, 7:3)

IR (KBr) cm\(^{-1}\) : 3384 (OH); 3295 (NH); 3080, 2845 (CH), 1580 (C=N).
\( ^1H\)-NMR (DMSO-\textit{d}_6) d: 1.40 (t, 4H, \( J=6.8\) Hz, \( H_3\&H_5\) piperidine), 2.42 (t, 4H, \( J=6.8\) Hz, \( H_2\&H_6\) piperidine), 3.42 (s, 2H, -\textit{N-CH}_2), 7.19 (d, 2H, \( J=8.4\) Hz, \( H_6\&H_5\) benzimidazole), 7.70 (d, 2H, \( J=8.4\) Hz, \( H_6\&H_7\) benzimidazole), 11.07 (s, 1H, OH), 12.30 (s, 1H, NH benzimidazole).

\( ^{13}C\)-NMR (DMSO-\textit{d}_6) d: 25.63, 53.32 (4C, piperidine), 54.93 (CH_2), 115.56, 122.76, 139.45 (6C, Ar), 142.04 (imidazole), 160.68 (C=N).

\textit{MS m/z}: 244(M)^+ , 227, 213, 173, 131

Elemental Analysis (%): Calculated: C, 63.91; H, 6.60; N, 22.93

Found: C, 63.61; H, 6.32; N, 23.17.

\textit{2-[(4-hy}drazinylidenepiperidin-1-yl)methyl]-1H-benzimidazole (PB3)}

Yield: 75%

Melting Point: 315 - 316 °C

\( R_f \): 0.65 (benzene : ethanol, 8:2)

\textit{IR (KBr) cm}^{-1}: 3314, 3247 (NH$_2$, NH), 3057, 2975, 2884 (CH), 1585 (C=N).

\( ^1H\)-NMR (DMSO-\textit{d}_6) d: 1.43 (t, 4H, \( J=6.8Hz\), \( H_3\&H_5\) piperidine), 2.45 (t, 4H, \( J=6.8Hz\), \( H_2\&H_6\) piperidine), 3.57 (s, 2H, -\textit{N-CH}_2), 5.95 (s, 2H, =N-NH$_2$); 7.23 (d, 2H, \( J=8.4\) Hz, \( H_6\&H_5\) benzimidazole), 7.59 (d, 2H, \( J=8.4\) Hz, \( H_6\&H_7\) benzimidazole), 12.19 (s, 1H, NH benzimidazole).

\( ^{13}C\)-NMR (DMSO-\textit{d}_6) d: 23.51, 53.71 (4C, piperidine), 55.54 (CH_2), 114.96, 122.93, 138.75 (6C, Ar), 142.07 (imidazole), 161.58 (C=N).

\textit{MS m/z}: 243 (M)^+ 

Elemental Analysis (%): Calculated: C, 64.17; H, 7.04; N, 28.78.

Found: C, 63.87, H, 6.65; N, 29.11.
2-[1-(1H-benzimidazol-2-ylmethyl)piperidin-4-ylidene]hydrazinecarbothioamide (PB4)

Yield : 89%

Melting Point : 292 - 294°C

Rf : 0.54 (chloroform : methanol, 7:3)

IR (KBr) cm\(^{-1}\) : 3347, 3277 (NH\(_2\), NH); 3074, 2956, 2874 (CH), 1633 (C=N); 1205 (C=S).

\(^1\)H-NMR (DMSO-d\(_6\)) d : 1.44 (t, 4H, J=6.8 Hz, H\(_3\)&H\(_5\) piperidine), 2.62 (t, 4H, J=6.8 Hz, H\(_2\)&H\(_6\) piperidine), 3.53 (s, 2H, -N-CH\(_2\)), 6.38 (s, 2H, NH\(_2\)), 7.25 (d, 2H, J=8.4 Hz, H\(_5\)&H\(_6\) benzimidazole), 7.55 (d, 2H, J=8.4 Hz, H\(_4\)&H\(_7\) benzimidazole), 9.11 (s, 1H, =N-NH), 12.21 (s, 1H, NH benzimidazole)

\(^{13}\)C-NMR (DMSO-d\(_6\)) d : 25.07, 53.14 (4C, piperidine), 55.47 (CH\(_2\)), 115.56, 122.84, 139.36 (6C, Ar), 142.04 (imidazole), 162.04 (C=N), 187.54 (C=S).

MS m/z : 302 (M)\(^+\)

Elemental Analysis (%) : Calculated: C, 55.61; H, 6.00; N, 27.79

Analysis (%) : Found: C, 55.29, H, 5.66; N, 28.18.

2-[1-(1H-benzimidazol-2-ylmethyl)piperidin-4-ylidene]-N-hydroxyhydrazinecarbothioamide (PB5)

Yield : 83 %

Melting Point : 279 - 281°C

Rf : 0.58 (chloroform : methanol, 7:3)

IR (KBr) cm\(^{-1}\) : 3357 (OH); 3284 (NH), 3057, 2965 (CH), 1597 (C=N), 1186 (C=S).
$^1$H-NMR (DMSO-d$_6$) d: 1.30 (t, 4H, J=6.8 Hz, H$_3$&H$_5$ piperidine), 2.24 (t, 4H, J=6.8 Hz, H$_2$&H$_6$ piperidine), 3.50 (s, 2H, -N-CH$_2$), 7.39 (d, 2H, J=8.4 Hz, H$_5$&H$_6$ benzimidazole), 7.79 (d, 2H, J=8.4 Hz, H$_4$&H$_7$ benzimidazole), 8.41 (s, 1H, -N-NH), 9.20 (s, 1H, -C-NH-OH), 10.26 (s, 1H, -C-NH-OH), 12.13 (s, 1H, NH benzimidazole).

$^{13}$C-NMR (DMSO-d$_6$) d: 23.13, 53.36 (4C, piperidine), 55.03 (CH$_2$), 115.76, 124.37, 139.69 (6C, Ar), 141.86 (imidazole), 161.45 (C=N), 185.48 (C=S).

MS m/z: 318 (M$^+$), 242, 213, 187, 173, 131

Elemental Analysis (%): Calculated: C, 52.81; H, 5.70; N, 26.39. Found: C, 52.62; H, 5.44; N, 26.21

$N$-[1-(1H-benzimidazol-2-ylmethyl)piperdin-4-ylidene]-4-fluoroaniline (PB6)

Yield: 87%

Melting Point: 287 – 290 °C

$R_f$: 0.50 (petether : ethyl acetate, 7:3)

IR (KBr) cm$^{-1}$: 3253 (NH), 3059, 2974, 2884 (CH), 1639 (C=N).

$^1$H-NMR (DMSO-d$_6$) d: 1.45 (t, 4H, J=6.8 Hz, H$_3$&H$_5$ piperidine), 2.40 (t, 4H, J=6.8 Hz H$_2$&H$_6$ piperidine), 3.60 (s, 2H, -N-CH$_2$), 7.01-7.60 (m, 8H, Ar-H), 12.15 (s, 1H, NH benzimidazole).

$^{13}$C-NMR (DMSO-d$_6$) d: 25.25, 53.07 (4C, piperidine), 55.37 (CH$_2$), 115.55, 123.07, 138.33, (6C, Ar), 116.90 (2C, C3&C5 fluorophenyl), 130.01 (2C, C2&C6 fluorophenyl), 144.57 (1C, C1 fluorophenyl), 161.45 (1C, C4 fluorophenyl), 141.56 (imidazole), 187.79 (C=N).

MS m/z: 322 (M$^+$).
Elemental Analysis (%):  
Calculated: C, 70.79; H, 5.94; N, 17.38

Experimentally:

**N**-[1-(1H-benzimidazol-2-ylmethyl)piperidin-4-ylidene]-2,4-dimethoxyaniline (PB7)

Yield: 75%

Melting Point: 275 - 276 °C

Rf: 0.48 (benzene : ethyl acetate, 8:2)

IR (KBr) cm⁻¹: 3234 (NH), 3089, 2986, 2904 (CH), 1622 (C=N).

¹H-NMR (DMSO-d₆) d: 1.43 (t, 4H, J=6.8 Hz, H₃&H₅ piperidine), 2.29 (t, 4H, J=6.8 Hz, H₂&H₆ piperidine), 3.67 (s, 2H, -N-CH₂), 7.01-7.55 (m, 9H, Ar-H), 11.19 (s, 1H, -N-NH), 12.21 (s, 1H, NH benzimidazole).

¹³C-NMR (DMSO-d₆) d: 21.16, 53.46 (4C, piperidine), 55.04 (CH₂), 115.24, 116.67, 118.53, 123.07, 129.57, 139.11, 144.03 (12C, Ar), 142.47 (imidazole), 160.92 (C=N).

MS m/z: 319 (M)⁺

Elemental Analysis (%):  
Calculated: C, 71.45; H; 6.63; N, 21.93

Experimentally:

**N**-[1-(1H-benzimidazol-2-ylmethyl)piperidin-4-ylidene]-2,4-dimethoxyaniline (PB7)

Yield: 84%

Melting Point: 312 – 315 °C

Rf: 0.45 (benzene : ethyl acetate, 8:2)

IR (KBr) cm⁻¹: 3281 (NH), 3067, 2913 (CH); 1597 (C=N).
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$^1$H-NMR (DMSO-$d_6$) d: 1.46 (t, 4H, $J$=6.8 Hz, H$_3$&H$_5$ piperidine), 2.44 (t, 4H, $J$=6.8 Hz, H$_2$&H$_6$ piperidine), 3.46 (s, 2H, -N-CH$_2$), 3.75 (s, 6H, -O-CH$_3$), 6.92-7.44 (m, 7H, Ar-H), 12.19 (s, 1H, NH benzimidazole).

$^{13}$C-NMR (DMSO-$d_6$) d: 24.57, 53.47 (4C, piperidine), 54.94 (CH$_2$), 55.93 (2C, 2OCH$_3$), 102.02, 107.34, 114.74, 123.47, 124.45, 127.38, 139.11, 153.67, 159.94 (12C, Ar), 142.13 (imidazole), 187.13 (C=N).

$N$-[1-(1H-benzimidazol-2-ylmethyl)piperidin-4-ylidene]-2,3-dimethylaniline (PB9)

Yield: 87 %

Melting Point: 305 – 307 °C

R$_f$: 0.49 (petether : ethyl acetate, 7:3)

IR (KBr) cm$^{-1}$: 3276 (NH), 3058, 2943, 2892 (CH), 1602 (C=N).

$^1$H-NMR (DMSO-$d_6$) d: 1.43 (t, 4H, $J$=6.8 Hz, H$_3$&H$_5$ piperidine), 2.31 (s, 6H, 2CH$_3$), 2.56 (t, 4H, $J$=6.8 Hz, H$_2$&H$_6$ piperidine), 3.67 (s, 2H, -N-CH$_2$), 6.96-7.53 (m,7H, Ar-H), 12.22 (s, 1H, NH benzimidazole).

$^{13}$C-NMR (DMSO-$d_6$) d: 17.24, 21.87 (2C, 2CH$_3$), 24.74, 53.18 (4C, piperidine), 55.34 (CH$_2$), 115.47, 119.75, 123.16, 127.04, 127.55128.94, 138.12, 139.11, 146.83(12C, Ar), 141.55 (imidazole), 210.30 (C=O).

MS $m/z$: 332 (M)$^+$

$N$-[1-(1H-benzimidazol-2-ylmethyl)piperidin-4-ylidene]-3-chloro-2-methylaniline (PB10)

Yield: 79 %

Melting Point: 319 – 320 °C

R$_f$: 0.43 (petether : ethyl acetate, 7:3)

IR (KBr) cm$^{-1}$: 3233 (NH), 3036, 2914, (CH), 1617 (C=N)
**N-[1-(1H-benzimidazol-2-ylmethyl)piperidin-4-ylidene]-2,4-dichloroaniline (PB11)**

Yield : 75 %

Melting Point : > 360 °C

R<sub>f</sub> : 0.41 (petether : ethyl acetate, 7:3)

IR (KBr) cm<sup>-1</sup> : 3227 (NH), 3073, 2954, 2893 (CH), 1604 (C=N).

**N-[1-(1H-benzimidazol-2-ylmethyl)piperidin-4-ylidene]-2,4-difluoroaniline (PB12)**

Yield : 80 %

Melting Point : 316 – 317 °C

R<sub>f</sub> : 0.47 (petether : ethyl acetate, 7:3)

IR (KBr) cm<sup>-1</sup> : 3238 (NH), 3027, 2966, 2894 (CH), 1628 (C=N).

<sup>1</sup>H-NMR (DMSO-d<sub>6</sub>) d : 1.40 (t, 4H, J=6.8 Hz, H<sub>3</sub>&H<sub>5</sub> piperidine), 2.39 (t, 4H, J=6.8 Hz, H<sub>2</sub>&H<sub>6</sub> piperidine), 3.66 (s, 2H, -N-CH<sub>2</sub>), 6.72-7.52 (m, 7H, Ar-H), 12.17 (s, 1H, NH benzimidazole).

<sup>13</sup>C-NMR (DMSO-d<sub>6</sub>) d : 25.64, 53.18 (4C, piperidine), 55.47 (CH<sub>2</sub>), 115.39, 123.15, 139.02, (6C, Ar), 106.06 (1C, C3 difluorophenyl), 112.54 (1C, C5 difluorophenyl), 125.77 (1C, C6 difluorophenyl), 131.51 (1C, C1 difluorophenyl), 155.24 (1C, C2 difluorophenyl), 163.06 (1C, C4 difluorophenyl), 141.43 (imidazole), 187.96 (C=N).

MS m/z : 340 (M)<sup>+</sup>

Elemental Analysis (%) : Calculated: C, 67.05; H, 5.33; N, 16.46

Analysis (%) : Found: C, 66.77, H, 5.54; N, 16.11
1-[1-(1H-benzimidazol-2-ylmethyl)piperidin-4-ylidene]guanidine (PB13)

Yield : 90 %

Melting Point : 279 – 281 °C

Rf : 0.73 (benzene : ethanol, 8:2)

IR (KBr) cm⁻¹ : 3294, 3216 (NH₂, NH), 3078, 2975 (CH), 1559 (C=N).

¹H-NMR (DMSO-d₆) d : 1.51 (t, 4H, J=6.8 Hz, H₃&H₅ piperidine), 2.53 (t, 4H, J=6.8 Hz, H₂&H₆ piperidine), 3.67 (s, 2H, -N-CH₂), 5.43 (s, 2H, NH₂), 6.05 (s,1H, C=NH), 7.35 (d, 2H, J=8.4 Hz, H₅&H₆ benzimidazole), 7.74 (d, 2H, J=8.4 Hz, H₄&H₇ benzimidazole), 12.29 (s, 1H, NH benzimidazole).

¹³C-NMR (DMSO-d₆) d : 24.56, 53.06 (4C, piperidine), 55.39 (CH₂), 115.91, 123.48, 139.17 (6C, Ar), 141.89 (imidazole), 161.93 (C=NH),164.34 (C=N)

MS m/z : 270 (M)⁺

Elemental Analysis (%): Calculated: C, 62.20; H, 6.71; N, 31.09
Found: C, 61.89; H, 6.34; N, 31.46

N-[1-(1H-benzimidazol-2-ylmethyl)piperidin-4-ylidene]aniline (PB14)

Yield : 86 %

Melting Point : 295 - 297 °C

Rf : 0.57 (petether : ethyl acetate, 7:3)

IR (KBr) cm⁻¹ : 3219 (NH), 3044, 2980, 2881 (CH), 1587 (C=N).

N-[1-(1H-benzimidazol-2-ylmethyl)piperidin-4-ylidene]-4-chloroaniline (PB15)

Yield : 74 %
Melting Point : 307 – 310 °C

R<sub>f</sub> : 0.53 (petether : ethyl acetate, 7:3)

IR (KBr) cm<sup>-1</sup> : 3243 (NH), 3064, 2914, 2884 (CH), 1621 (C=N).

**1-[1-(1H-benzimidazol-2-ylmethyl)piperidin-4-ylidene]urea (PB16)**

Yield : 82 %

Melting Point : 298 – 299 °C

R<sub>f</sub> : 0.75 (chloroform : methanol, 7:3)

IR (KBr) cm<sup>-1</sup> : 3307, 3241 (NH<sub>2</sub>, NH), 3074, 2968 (CH), 1684 (C=O), 1613 (C=N).

<sup>1</sup>H-NMR (DMSO-d<sub>6</sub>) δ : 1.62 (t, 4H, J=6.8 Hz, H<sub>3</sub>&H<sub>5</sub> piperidine), 2.61 (t, 4H, J=6.8 Hz, H<sub>2</sub>&H<sub>6</sub> piperidine), 3.62 (s, 2H, -N-CHH<sub>2</sub>), 6.62 (s, 2H, NH<sub>2</sub>), 7.23 (d, 2H, J=8.4 Hz, H<sub>5</sub>&H<sub>6</sub> benzimidazole), 7.57 (d, 2H, J=8.4 Hz, H<sub>4</sub>&H<sub>7</sub> benzimidazole), 12.18 (s, 1H, NH benzimidazole).

<sup>13</sup>C-NMR (DMSO-d<sub>6</sub>) δ : 25.07, 53.47 (4C, piperidine), 55.25 (CH<sub>2</sub>), 116.94, 123.35, 138.05 (6C, Ar), 141.33 (imidazole), 150.02 (C=O), 164.34 (C=N).

MS m/z : 271 (M)<sup>+</sup>

Elemental Analysis (%) : Calculated: C, 61.98; H, 6.32; N, 25.81

Analysis (%) : Found: C, 62.37; H, 5.94; N, 26.21

**N-[1-(1H-benzimidazol-2-ylmethyl)piperidin-4-ylidene]-2,4-dinitroaniline (PB17)**

Yield : 85 %

Melting Point : > 360 °C
**Experimental**

Rf: 0.40 (petether : ethyl acetate, 7:3)

**IR (KBr) cm⁻¹**
- 3249 (NH), 3029, 2964, 2875 (CH), 1590 (C=N).

**¹H-NMR**
- 1.47 (t, 4H, J=6.8 Hz, H₃&H₅ piperidine), 2.45 (t, 4H, J=6.8 Hz, H₂&H₆ piperidine), 3.63 (s, 2H, -N-CH₂), 7.26-8.67 (m, 7H, Ar-H), 12.14 (s, 1H, NH benzimidazole).

**¹³C-NMR**
- 25.07, 53.60 (4C, piperidine), 55.44 (CH₂), 115.35, 121.47, 123.17, 124.19, 131.66, 139.04, 144.18, 147.43, 148.40 (12C, Ar), 141.64 (imidazole), 187.96 (C=N).

**MS m/z**
- 394 [M]+

**Elemental Analysis (%)**
- Calculated: C, 57.86; H, 4.60; N, 21.31
- Found: C, 58.17, H, 4.84; N, 20.97

*1-[1-(1H-benzimidazol-2-ylmethyl)piperidin-4-ylidene]thiourea (PB18)*

**Yield**
- 86 %

**Melting Point**
- 290 – 292 °C

Rf: 0.69 (chloroform : methanol, 7:3)

**IR (KBr) cm⁻¹**
- 3284, 3247 (NH₂, NH), 3076, 3004, 2968 (CH), 1238 (C=S).

**¹H-NMR**
- 1.57 (t, 4H, J=6.8 Hz, H₃&H₅ piperidine), 2.74 (t, 4H, J=6.8 Hz, H₂&H₆ piperidine), 3.59 (s, 2H, -N-CH₂), 5.17(s, 2H, NH₂), 7.15 (d, 2H, J=8.4 Hz, H₅&H₆ benzimidazole), 7.48 (d, 2H, J=8.4 Hz, H₄&H₇ benzimidazole), 12.18 (s, 1H, NH benzimidazole).

**¹³C-NMR**
- 25.05, 52.89 (4C, piperidine), 55.67 (CH₂), 116.06, 123.17, 139.74 (6C, Ar), 142.07 (imidazole), 164.62 (C=N), 186.57 (C=S).

**MS m/z**
- 287 (M)+
Elemental Analysis (%): Calculated: C, 58.51; H, 5.96; N, 24.37
Found: C, 58.28; H, 5.59; N, 24.04

1-(1H-benzimidazol-2-ylmethyl)-N-methoxypiperidin-4-imine (PB19)

Yield: 80%
Melting Point: 258 – 261 °C
Rf: 0.66 (benzene : ethylacetate, 8:2)
IR (KBr) cm⁻¹: 3254 (NH), 3036, 2987, 2890 (CH), 1620 (C=N).

2-[1-(1H-benzimidazol-2-ylmethyl)piperidin-4-ylidene]hydrazinecarboxamide (PB20)

Yield: 76%
Melting Point: 267 – 270 °C
Rf: 0.65 (chloroform : methanol, 7:3)
IR (KBr) cm⁻¹: 3294, 3257 (NH₂, NH), 3053, 3011, 2965 (CH), 1681 (C=O).

¹H-NMR (DMSO-d₆) d: 1.49 (t, 4H, J=6.8 Hz, H₃&H₅ piperidine), 2.51 (t, 4H, J=6.8 Hz, H₂&H₆ piperidine), 3.74 (s, 2H, -N-CH₂), 6.54 (s, 2H, NH₂), 7.23 (d, 2H, J=8.4 Hz, H₃&H₅ benzimidazole), 7.57 (d, 2H, J=8.4 Hz, H₄&H₇ benzimidazole), 9.39 (s, 1H, =N-NH), 12.18 (s, 1H, NH benzimidazole).

¹³C-NMR (DMSO-d₆) d: 24.74, 53.27 (4C, piperidine), 55.11 (CH₂), 116.46, 122.84, 139.04 (6C, Ar), 143.48 (imidazole), 162.11 (C=O), 164.74 (C=N).

N-[1-(1H-benzimidazol-2-ylmethyl)piperidin-4-ylidene]thiocarbohydrazide (PB21)

Yield: 79%
Melting Point : 263 – 264 °C

Rf : 0.70 (chloroform : methanol, 7:3)

IR (KBr) cm⁻¹ : 3290, 3244 (NH₂, NH), 3042, 2965, 2897 (CH), 1631 (C=N), 1213 (C=S).

¹H-NMR (DMSO-d₆) d : 1.45 (t, 4H, J=6.8 Hz, H₃&H₅ piperidine), 2.53 (t, 4H, J=6.8 Hz, H₂&H₆ piperidine), 3.72 (s, 2H, -N-CH₂), 7.22 (d, 2H, J=8.4 Hz, H₅&H₆ benzimidazole), 7.63 (d, 2H, J=8.4 Hz, H₄&H₇ benzimidazole), 8.37 (1H, s, -N-NH), 8.91 (1H, s, -C-NH-NH₂), 9.45 (2H, s, -C-NH-NH₂), 12.21 (1H, s, NH benzimidazole).

¹³C-NMR (DMSO-d₆) d : 24.44, 53.35 (4C, piperidine), 55.41 (CH₂), 115.62, 123.18, 138.62 (6C, Ar), 142.56 (imidazole), 161.87 (C=O), 183.06 (C=S).

MS m/z : 317 (M)⁺

Elemental Analysis (%) : Calculated: C, 52.98; H, 6.03; N, 30.89
Found: C, 53.24, H, 5.86; N, 30.55

N-[1-(1H-benzimidazol-2-ylmethyl)piperidin-4-ylidene]-2-fluoroaniline (PB22)

Yield : 73 %

Melting Point : 331 – 332 °C

Rf : 0.44 (petether : ethyl acetate, 7:3)

IR (KBr) cm⁻¹ : 3242 (NH), 3043, 2982, 2906 (CH), 1635 (C=N).

N-[1-(1H-benzimidazol-2-ylmethyl)piperidin-4-ylidene]-3,4-difluoroaniline (PB23)

Yield : 75 %

Melting Point : > 360 °C
RF: 0.46 (petether : ethyl acetate, 7:3)

IR (KBr) cm\(^{-1}\): 3234 (NH), 3081, 2980, 2912 (CH), 1630 (C=N).

\(^1\)H-NMR (DMSO-\(d_6\)) d: 1.40 (t, 4H, J=6.8 Hz, H3&H5 piperidine), 2.41 (t, 4H, J=6.8 Hz, H2&H6 piperidine), 3.63 (s, 2H, -N-CH\(_2\)), 6.70-7.59 (m, 7H, Ar-H), 12.20 (s, 1H, NH benzimidazole).

\(^{13}\)C-NMR (DMSO-\(d_6\)) d: 25.33, 53.56 (4C, piperidine), 55.02 (CH\(_2\)), 115.61, 122.75, 118.77 (1C, C2 difluorophenyl), 119.82 (1C, C6 difluorophenyl), 141.62 (imidazole), 187.68 (C=N).

**1-(1H-benzimidazol-2-ylmethyl)piperidine-4-ol (PB24)**

Yield: 83 %

Melting Point: 323 – 326 °C

RF: 0.64 (chloroform : methanol, 7:3)

IR (KBr) cm\(^{-1}\): 3342 (OH), 3237 (NH), 3053, 3046, 2984 (CH).

\(^1\)H-NMR (DMSO-\(d_6\)) d: 1.61 (q, 4H, J=6.8 Hz, H3&H5 piperidine), 2.55 (t, 4H, J=6.8 Hz, H2&H6 piperidine), 2.81 (t, 1H, J=6.8 Hz, H4 Piperidine), 3.51 (s, 2H, -N-CH\(_2\)), 4.75 (s, 1H, OH), 7.02 (d, 2H, J=8.4 Hz, H5&H6 benzimidazole), 7.75 (d, 2H, J=8.4 Hz, H4&H7 benzimidazole), 12.30 (s, 1H, -NH).

\(^{13}\)C-NMR (DMSO-\(d_6\)) d: 34.02, 47.86 (4C, piperidone), 55.52 (CH\(_2\)), 67.62 (1C, CH-OH), 114.96, 123.16, 138.77 (6C, Ar), 141.46 (imidazole).

MS m/z: 231 (M)\(^+\), 214, 201, 173, 131

Elemental Analysis (%): Calculated: C, 67.51; H, 7.41; N, 18.17

Analysis (%): Found: C, 67.19, H, 7.72; N, 17.82
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1-(1H-benzimidazol-2-ylmethyl)piperidin-4-yl benzenesulfonate (PB25)

Yield : 76 %

Melting Point : 343 – 346 °C

Rf : 0.52 (petether : ethyl acetate, 7:3)

IR (KBr) cm$^{-1}$ : 3261 (NH), 3072, 3015, 2942 (CH).

$^{1}$H-NMR (DMSO-d$_6$) d : 1.45 (q, $J$=6.8 Hz, 4H, H$_3$&H$_5$ piperidine), 2.18 (t, 4H, $J$=6.8 Hz, H$_2$&H$_6$ piperidine), 3.55 (s, 2H, -N-CH$_2$), 4.46 (t, $J$=6.8 Hz, 1H, H$_4$ Piperidine), 7.09-8.20 (m, 10H, ArH), 12.18 (s, 1H, -NH).

$^{13}$C-NMR (DMSO-d$_6$) d : 29.85, 47.96 (4C, piperidone), 55.42 (CH$_2$), 62.33 (1C, C-O), 115.16, 123.34, 129.56, 130.63, 134.70, 138.61, 140.94 (12C, Ar), 141.32 (imidazole).

MS m/z : 371 (M$^+$), 294, 230, 214, 173, 131, 77
4.3.2. Series II. 1-(1H-benzimidazol-2-ylmethyl)-3,5-bis(furan-2-ylmethylidene)piperidin-4-substituted imines (B1 – B25)

Fig. 4.2. Synthetic scheme of 1-(1H-benzimidazol-2-ylmethyl)-3,5-bis(furan-2-ylmethylidene)piperidin-4-substituted imines (B1 – B25)
4.3.2.1. Reaction Mechanisms

**PB1** undergoes aldol condensation on reaction with 2-furfural in the presence of NaOH yield aldol product, which on removal of water yield the compound, **B1**.

**Reaction:**

\[
\text{PB1 + OHC-} + \text{NaOH} \rightarrow \text{B1}
\]

**Mechanism involved:**

**Step I**

A base removes an α-proton to form an enolate ion to serve as a nucleophile.

**Step II**

The enolate ion, which is a nucleophile, attacks the carbonyl carbon of 2-furfural to form an alkoxide.
**Step III**

Protonation of alkoxide gives the β-hydroxyketone (aldol product).

**Step IV**

Removal of water from the aldol product yield the bis furylidene compound, $B_1$. 
**B1** was condensed with appropriate substituted amines in the presence of sodium acetate to obtain **B2 - B23** by Schiff's reaction.

**Reaction:**

![Reaction](image)

**Mechanism involved:**

**Step I:**

Nucleophilic attack of carbonyl carbon by 1° amine forms zwitterion.
Step II:

The zwitterion rearranges to form unstable carbinolamine.

\[ \text{zwitterion} \rightarrow \text{carbinolamine} \]

Step III:

The acetate ion deprotonate the amino group in carbinolamine and protonate the OH\(^-\) ion leads to the formation of imine (Schiff’s base).

\[ \text{carbinolamine} \rightarrow \text{B2 - B23} \]
The compound, B1, undergoes reduction in the presence of sodium tetra borate to yield the alcohol, B24.

**Reaction**

![Chemical structure of B1 and B24 with NaBH4 and [H] reagents](image)

**Mechanism involved:**

**Step I:**

Nucleophilic attack of carbonyl carbon of B1 with hydride ion forms alkoxide ion.
**Chapter IV**

**Step II:**

The alkoxide ion on protonation yields the alcohol (B24).

![Reaction diagram](attachment:image1.png)

The compound B24, on treatment with benzene sulphonyl chloride yields B25.

**Reaction:**

![Reaction diagram](attachment:image2.png)

**Mechanism involved:**

The chloro group present in the benzene sulphonyl chloride is a good leaving group, which makes the sulphonyl group for nucleophilic attack by the oxygen of B24, followed by elimination of HCl forms the compound B25.
4.3.2.2. Synthesis of $(3E,5E)$-1-$(1H$-benzimidazol-2-ylmethyl)$)$-3,5-bis(furan-2-ylmethylidene)piperidin-4-one (B1)

Synthesis of B1 was carried out as per the reported method (Katsori 2011; Kalai 2011). A mixture of PB1 (2.29 gm, 0.01 mol) and furfural (1.8 ml, 0.022 mol) was taken in a Erlenmeyer flask. A solution of ethanol (12 ml) and 10% NaOH (20 ml) was prepared, cooled to 20 °C in an ice-cold water, and this solution was added to the above mixture with stirring. Stirring was continued at room temperature for 2 hr. When the reaction was completed, which was confirmed by TLC, the reaction
mixture was cooled in an ice bath. The precipitated product was filtered under vacuum, washed with water, dried and recrystallised using a mixture of acetonitrile and methanol (1:1).

4.3.2.3. General method of procedure for the synthesis of B2-B23

The reaction was carried out based on the reported method (Banerjee 2011). To an ethanolic mixture of \((3E,5E)-1-(1H\text{-}\text{benzimidazol-2-ylmethyl})-3,5\text{-}\text{bis(furan-2-ylmethylidene)piperidin-4-one B1 (3.85 gm, 0.01 mol)}\) with the appropriate amine (0.01 mol), sodium acetate (0.82 gm, 0.01 mol) was added with stirring and it was heated under reflux for 3.5 hr with constant stirring. After completion of the reaction, which was confirmed using TLC, the contents were cooled to room temperature and poured into ice cold water. The resulting product was filtered under suction and vacuum dried after washing thoroughly with water. It was then recrystallized from the mixture of methanol and dimethyl formamide (2:8) to yield the pure compound.

4.3.2.4. Synthesis of 1-(1H-benzimidazol-2-ylmethyl)piperidin-4-ol (B24)

The reduction reaction was carried out based on the literature method (Aridoss 2008). To the compound B1 (0.77 gm, 0.002 mol) in methanol (5 ml), sodium borohydride (0.76 gm, 0.02 mol) was added at 0 °C with stirring. Methanol was removed and aqueous solution of ammonium chloride (2 ml) was added to the residue and the product was extracted with ethyl acetate. Then ethyl acetate was evaporated and the compound was recrystallised using methanol and DMF.
4.3.2.5. **Synthesis of 1-(1H-benzimidazol-2-ylmethyl)piperidin-4-ylbenzenesulfonate (B25)**

This reaction was carried out as per the reported method (Chernyshov 2011). A solution of benzenesulphonyl chloride (0.1 ml, 0.001 mol) in pyridine (5 ml) was heated to 110-115 °C with vigorous agitation and thoroughly ground compound B24 (0.38 gm, 0.001 mol) was added. The mixture was boiled with reflux under agitation for 30 min. and then 2M NaOH solution (150 ml) was poured in and the mixture was boiled for additional 5-8 min. The resulting solution was neutralized with glacial acetic acid and cooled to 20 °C. The product formed was filtered and recrystallised using methanol and DMSO.

4.3.2.6. **Characterization of 1-(1H-benzimidazol-2-ylmethyl)-3,5-bis(furan-2-ylmethylidene)piperidin-4-substituted imines (B1 – B25)**

*(3E,5E)-1-(1H-benzimidazol-2-ylmethyl)-3,5-bis(furan-2-ylmethylidene)piperidin-4-one (B1)*

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield</td>
<td>82 %</td>
</tr>
<tr>
<td>Melting Point</td>
<td>254 - 256 °C</td>
</tr>
<tr>
<td>R&lt;sub&gt;f&lt;/sub&gt;</td>
<td>0.75 (benzene : ethylacetate, 8:2)</td>
</tr>
<tr>
<td>IR (KBr) cm&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>3267 (NH); 3101, 3056, 2973 (C-H); 1710 (C=O).</td>
</tr>
<tr>
<td>&lt;sup&gt;1&lt;/sup&gt;H-NMR (DMSO-&lt;em&gt;d&lt;/em&gt;&lt;sub&gt;6&lt;/sub&gt;) d</td>
<td>3.05 (4H, s, H&lt;sub&gt;2&lt;/sub&gt; &amp; H&lt;sub&gt;piperidone&lt;/sub&gt;), 3.59 (s, 2H, N-CH&lt;sub&gt;2&lt;/sub&gt;), 6.94 – 8.09 (m, 12H, ArH &amp; =CH), 12.20 (1H, s, NH).</td>
</tr>
<tr>
<td>&lt;sup&gt;13&lt;/sup&gt;C-NMR (DMSO-&lt;em&gt;d&lt;/em&gt;&lt;sub&gt;6&lt;/sub&gt;) d</td>
<td>48.14 (2C, 2,6-piperidone), 55.92 (CH&lt;sub&gt;2&lt;/sub&gt;), 111.21, 113.05, 146.</td>
</tr>
</tbody>
</table>

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Experimental

**MS m/z** : 385 (M)*, 369, 343, 301, 258, 212, 173, 131

**Elemental Analysis (%)**

Calculated: C, 71.67; H, 4.97; N, 10.90

Found: C, 71.93; H, 5.25; N, 10.77

(3E,5E)-1-(1H-benzimidazol-2-ylmethyl)-3,5-bis(furan-2-ylmethylidene)-N-hydroxypiperidin-4-imine (B2)

**Yield** : 76 %

**Melting Point** : 275 - 278 °C

**Rf** : 0.63 (chloroform : methanol, 7:3)

**IR (KBr) cm**⁻¹ : 3364 (OH); 3287 (NH); 3047, 2942 (CH), 1565 (C=N).

**¹H-NMR** (DMSO-d₆) d : 3.13 (4H, s, H₂ & H₅ piperidone), 3.51 (s, 2H, N-CH₂), 6.93 – 7.96 (m, 12H, ArH & =CH), 11.10 (1H, s, OH), 12.24 (1H, s, NH).

**¹³C-NMR** (DMSO-d₆) d : 49.02 (2C, 2,6-piperidone), 56.15 (CH₂), 111.33, 112.76, 115.06, 122.54, 138.90 (6C, Ar), 118.91 (2C, =CH), 141. 93 (imidazole), 137.20 (2C, 3,5-piperidone), 165.27 (1C, C=N).

**MS m/z** : 400(M)*

**Elemental Analysis (%)**

Calculated: C, 68.99; H, 5.03; N, 13.99

Found: C, 69.20; H, 4.73; N, 14.30

(3E,5E)-1-(1H-benzimidazol-2-ylmethyl)-3,5-bis(furan-2-ylmethylidene)-4-hydrazinylidene piperidine (B3)

**Yield** : 73 %

**Melting Point** : 264 – 265 °C

**Rf** : 0.7 (benzene : ethanol, 8:2)

**IR (KBr) cm**⁻¹ : 3359, 3264 (NH₂, NH); 3114, 3024, 2948, (CH), 1657 (C=N).
\textbf{N-[(3E,5E)-1-(1H-benzimidazol-2-ylmethyl)-3,5-bis(furan-2-ylmethylidene)piperidin-4-ylidene]hydrazinecarbothioamide (B4)}

Yield : 77 %  
Melting Point : 302 – 305 °C  
\( R_f \) : 0.54 (chloroform : methanol, 7:3)  
IR (KBr) cm\(^{-1}\) : 3314, 3284 (NH\(_2\), NH), 3086, 2957 (CH), 1565 (C=N) 1208 (C=S).  
\(^1\)H-NMR (DMSO-\(d_6\)) d : 3.17 (4H, s, H\(_2\) & H\(_5\) piperidone), 3.50 (s, 2H, N-CH\(_2\)), 6.31 (2H, s, NH\(_2\)), 6.85 – 7.91 (m, 12H, ArH & =CH), 9.15 (1H, s, =N-NH), 12.18 (1H, s, NH benzimidazole).  
\(^{13}\)C-NMR (DMSO-\(d_6\)) d : 48.81 (2C, 2,6-piperidone), 55.77 (CH\(_2\)), 111.35, 112.91, 146.06, 151.43 (8C, furan) 115.26, 122.83, 139.14 (6C, Ar), 115.79 (2C, =CH), 141.42 (imidazole), 136.04 (2C, 3,5-piperidone), 155.64 (1C, C=N), 180.53 (1C, C=S).

\textbf{N-[(3E,5E)-1-(1H-benzimidazol-2-ylmethyl)-3,5-bis(furan-2-ylmethylidene)piperidin-4-ylidene]-N-hydroxyhydrazinecarbothioamide (B5)}

Yield : 79 %  
Melting Point : 315 - 318 °C  
\( R_f \) : 0.51 (chloroform : methanol, 7:3)  
IR (KBr) cm\(^{-1}\) : 3367 (OH); 3251 (NH), 3108, 3049, 2907 (CH), 1636 (C=N).  
\(^1\)H-NMR (DMSO-\(d_6\)) d : 2.85 (4H, s, H\(_2\) & H\(_5\) piperidone), 3.72 (s, 2H, N-CH\(_2\)), 6.85 – 8.19 (m, 12H, ArH & =CH), 8.52 (1H, s, -N-NH), 9.29 (1H, s, -C-NH-OH), 10.40 (1H, s, -C-NH-OH), 12.29 (1H, s, NH benzimidazole).  
\(^{13}\)C-NMR (DMSO-\(d_6\)) d : 49.06 (2C, 2,6-piperidone), 56.22 (CH\(_2\)), 111.07, 113.20, 146.21, 151.32 (8C, furan) 115.54, 123.34, 138.65 (6C, Ar), 116.67 (2C, =CH), 141.87 (imidazole), 135.68 (2C, 3,5-
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\[ \text{N-[(3E,5E)-1-(1H-benzimidazol-2-ylmethyl)-3,5-bis(furan-2-ylmethylidene)piperidin-4-ylidene]-4-fluoroaniline (B6)} \]

**Yield**: 80%
**Melting Point**: 319 – 320 °C
**Rf**: 0.52 (petether : ethyl acetate, 7:3)
**IR (KBr) cm\(^{-1}\)**: 3244 (NH), 3074, 2937 (CH), 1651 (C=N).

\[ \text{(3E,5E)-1-(1H-benzimidazol-2-ylmethyl)-3,5-bis(furan-2-ylmethylidene)-4-(2-phenylhydrazinylidene)piperidine (B7)} \]

**Yield**: 71%
**Melting Point**: 325 – 326 °C
**Rf**: 0.57 (benzene : ethanol, 8:2)
**IR (KBr) cm\(^{-1}\)**: 3276 (NH), 3102, 2986, 2885 (CH), 1571 (C=N).

\[ \text{N-[(3E,5E)-1-(1H-benzimidazol-2-ylmethyl)-3,5-bis(furan-2-ylmethylidene)piperidin-4-ylidene]-2,4-dimethoxyaniline (B8)} \]

**Yield**: 74%
**Melting Point**: > 360 °C
**Rf**: 0.45 (benzene : ethylacetate, 8:2)
**IR (KBr) cm\(^{-1}\)**: 3224 (NH), 3053, 2947, 2883 (CH), 1626 (C=N).

**\(^{1}\text{H-NMR}\)**: 3.13 (4H, s, H\(_2\&H_5\) piperidone), 3.57 (s, 2H, N-CH\(_2\)), 3.70 (6H, s, -O-CH\(_3\)), 6.90-7.95 (15H, m, Ar-H & =CH), 12.18 (1H, s, NH)
(DMSO-d$_6$) d benzimidazole).

$^{13}$C-NMR : 49.30 (2C, 2,6-piperidone), 55.63 (2C, 2OCH$_3$), 56.35 (CH$_2$),
(DMSO-d$_6$) d 111.07, 112.86, 146.07, 151.24 (8C, furan) 101.90, 108.27, 115.40, 123.06, 124.47, 128.03, 138.67, 153.26, 159.70, (12C, Ar), 114.81 (2C, =CH), 141.27 (imidazole), 134.64 (2C, 3,5-piperidone), 164.42 (1C, C=N).

MS m/z : 520 (M)$^+$

$N$-[(3E,5E)-1-(1H-benzimidazol-2-ylmethyl)-3,5-bis(furan-2-ylmethylidene)piperidin-4-ylidene]-2,3-dimethylaniline (B9)

Yield : 70 %
Melting Point : 290 – 291 °C
$R_l$ : 0.49 (petether : ethyl acetate, 7:3)
IR (KBr) cm$^{-1}$ : 3273 (NH), 3112, 3038, 2922 (CH), 1637 (C=N).

$^1$H-NMR : 2.35 (6H, s, 2CH$_3$), 3.09 (4H, s, H$_2$&H$_5$ piperidone), 3.52 (s, (DMSO-d$_6$) d 2H, N-CH$_2$), 6.93-7.91 (15H, m, Ar-H & =CH), 12.20 (1H, s, NH benzimidazole).

$^{13}$C-NMR : 17.16, 22.04 (2C, 2CH$_3$), 49.17 (2C, 2,6-piperidone), 55.90 (DMSO-d$_6$) d (CH$_2$), 111.53, 112.49, 145.65, 151.30 (8C, furan) 114.86, 118.87, 122.91, 127.03, 127.70, 128.52, 137.73, 139.04, 146.50 (12C, Ar), 115.27 (2C, =CH), 141.61 (imidazole), 135.40 (2C, 3,5-piperidone), 164.33 (1C, C=N).

$N$-[(3E,5E)-1-(1H-benzimidazol-2-ylmethyl)-3,5-bis(furan-2-ylmethylidene)piperidin-4-ylidene]-2-fluoroaniline (B10)

Yield : 74 %
Melting Point : 279 – 280 °C
$R_l$ : 0.38 (petether : ethyl acetate, 7:3)
IR (KBr) cm$^{-1}$ : 3250 (NH), 3085, 3014, 2857 (CH), 1638 (C=N).

$^1$H-NMR (DMSO-d$_6$) d $^1$H (4H, s, H$_2$&H$_5$ piperidone), 3.62 (s, 2H, N-CH$_2$), 6.85-7.80 (16H, m, Ar-H & =CH), 12.20 (1H, s, NH benzimidazole).

$^{13}$C-NMR (DMSO-d$_6$) d 49.34 (2C, 2,6-piperidone), 55.68 (CH$_2$), 111.52, 113.07, 115.04, 122.37, 138.11, 145.53, 151.06 (8C, furan), 125.91 (1C, C5 fluorophenyl), 129.12 (1C, C4 fluorophenyl), 135.76 (1C, C1 fluorophenyl), 154.25 (1C, C2 fluorophenyl), 115.21 (2C, =CH), 142.17 (imidazole), 135.48 (2C, 3,5-piperidone), 164.83 (1C, C=N).

MS m/z : 478 (M$^+$)

$N$-[(3E,5E)-1-(1H-benzimidazol-2-ylmethyl)-3,5-bis(furan-2-ylmethylidene)piperidin-4-ylidene]-2,4-dichloroaniline (B11)

Yield : 78 %
Melting Point : 352 – 353 °C
R$_f$ : 0.36 (petether : ethyl acetate, 7:3)
IR (KBr) cm$^{-1}$ : 3240 (NH), 3077, 2958 (CH), 1604 (C=N).

$N$-[(3E,5E)-1-(1H-benzimidazol-2-ylmethyl)-3,5-bis(furan-2-ylmethylidene)piperidin-4-ylidene]-2,4-difluoroaniline (B12)

Yield : 81 %
Melting Point : 333 – 335 °C
R$_f$ : 0.42 (petether : ethyl acetate, 7:3)
IR (KBr) cm$^{-1}$ : 3267 (NH), 3072, 3005, 2944 (CH), 1623 (C=N).
Experimental

(DMSO-d$_6$) d 145.73, 151.29 (8C, furan), 114.80, 122.74, 138.67, (6C, Ar), 105.83 (1C, C3 difluorophenyl), 112.08 (1C, C5 difluorophenyl), 125.15 (1C, C6 difluorophenyl), 131.91 (1C, C1 difluorophenyl), 155.80 (1C, C2 difluorophenyl), 162.77 (1C, C4 difluorophenyl), 114.83 (2C, =CH), 141.36 (imidazole), 136.24 (2C, 3,5-piperidone), 164.37 (1C, C=N).

MS m/z : 496 (M$^+$)

1-[(3E,5E)-1-(1H-benzimidazol-2-ylmethyl)-3,5-bis(furan-2-ylmethylidene)piperidin-4-ylidene]guanidine (B13)

Yield : 79 %
Melting Point : 298 – 299 °C
R$_f$ : 0.62 (chloroform : methanol, 7:3)
IR (KBr) cm$^{-1}$ : 3287, 3244 (NH$_2$, NH), 3064, 3007, 2987 (CH), 1588 (C=N).

N-[(3E,5E)-1-(1H-benzimidazol-2-ylmethyl)-3,5-bis(furan-2-ylmethylidene)piperidin-4-ylidene]aniline (B14)

Yield : 72 %
Melting Point : 293 – 294 °C
R$_f$ : 0.59 (petether : ethyl acetate, 7:3)
IR (KBr) cm$^{-1}$ : 3249 (NH), 3072, 2981, 2874 (CH), 1644 (C=N).

N-[(3E,5E)-1-(1H-benzimidazol-2-ylmethyl)-3,5-bis(furan-2-ylmethylidene)piperidin-4-ylidene]-4-chloroaniline (B15)

Yield : 78 %
Melting Point : 321 – 323 °C
R$_f$ : 0.44 (petether : ethyl acetate, 7:3)
IR (KBr) cm\(^{-1}\) : 3260 (NH), 3071, 3022, 2961 (CH), 1633 (C=N).

MS m/z : 494 (M)+

1-[(3E,5E)-1-(1H-benzimidazol-2-ylmethyl)-3,5-bis(furan-2-ylmethylidene)piperidin-4-ylidene]urea (B16)

Yield : 76 %

Melting Point : 310 – 311 °C

R\(_f\) : 0.77 (chloroform : methanol, 7:3)

IR (KBr) cm\(^{-1}\) : 3286, 3247 (NH\(_2\), NH), 3063, 2964, 2880 (CH), 1686 (C=O), 1621 (C=N).

\(^1\)H-NMR (DMSO-d\(_6\)) d : 3.19 (4H, s, H\(_2\) \& H\(_5\) piperidone), 3.53 (s, 2H, N-CH\(_2\)), 6.60 (2H, s, NH\(_2\)), 6.89 – 7.79 (m, 12H, ArH \& =CH), 12.24 (1H, s, NH benzimidazole).

\(^{13}\)C-NMR (DMSO-d\(_6\)) d : 48.34 (2C, 2,6-piperidone), 56.20 (CH\(_2\)), 110.06, 112.60, 145.77, 151.55 (8C, furan), 115.43, 122.86, 139.33 (6C, Ar), 115.57 (2C, =CH), 141.82(imidazole), 134.56(2C, 3,5-piperidone), 148.77 (1C, C=O), 164.27 (1C, C=N).

N-[(3E,5E)-1-(1H-benzimidazol-2-ylmethyl)-3,5-bis(furan-2-ylmethylidene)piperidin-4-ylidene]-3,4-difluoroaniline (B17)

Yield : 75 %

Melting Point : 328 – 329 °C

R\(_f\) : 0.31 (petether : ethyl acetate, 7:3)

IR (KBr) cm\(^{-1}\) : 3250 (NH), 3071, 2953, 2879 (CH), 1633 (C=N).

\(^1\)H-NMR (DMSO-d\(_6\)) d : 3.04 (4H, s, H\(_2\) \& H\(_5\) piperidone), 3.57 (s, 2H, N-CH\(_2\)), 6.89-8.10 (15H, m, ArH \& =CH), 12.11 (1H, s, NH benzimidazole).

\(^{13}\)C-NMR (DMSO-d\(_6\)) d : 50.45 (2C, 2,6-piperidone), 55.61 (CH\(_2\)), 111.72, 113.04, 145.
122.64, 138.26, 146.47, 148.51, 150.52 (12C, Ar) 115.13 (2C, =CH), 142.41 (imidazole), 134.48 (2C, 3,5-piperidone), 164.44 (1C, C=N).

MS m/z : 496 (M)+, 412, 383, 365, 323, 173, 145, 131

Elemental Analysis (%) : Calculated: C, 70.15; H, 4.47; N, 11.28
Found: C, 69.84, H, 4.20; N, 11.51

\[ N-[(3E,5E)-1-(1H-benzimidazol-2-ylmethyl)-3,5-bis(furan-2-ylmethylidene)piperidin-4-ylidene]-4-chloro-2-fluoroaniline (B18) \]

Yield : 72 %
Melting Point : 339 – 340 °C
Rf : 0.34 (petether : ethyl acetate, 7:3)
IR (KBr) cm\(^{-1}\) : 3234 (NH), 3081, 2974, 2893 (CH), 1627 (C=N).

\[ 1-[(3E,5E)-1-(1H-benzimidazol-2-ylmethyl)-3,5-bis(furan-2-ylmethylidene)piperidin-4-ylidene] thiourea (B19) \]

Yield : 79 %
Melting Point : 288 – 289 °C
Rf : 0.6 (chloroform : methanol, 7:3)

\[ (3E,5E)-1-(1H-benzimidazol-2-ylmethyl)-4-[2-(2,4-dinitrophenyl)hydrazinylidene]-3,5-bis(furan-2-ylmethylidene)piperidine (B20) \]

Yield : 80 %
Melting Point : >360 °C
Rf : 0.39 (benzene : ethylacetate, 8:2)
IR (KBr) cm\(^{-1}\) : 3250 (NH), 3092, 3014, 2947 (CH), 1624 (C=N).
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$^1$H-NMR (DMSO-$d_6$) \textit{d}: 3.14 (4H, s, H$_2$&H$_5$ piperidone), 3.58 (s, 2H, N-CH$_2$), 6.78-7.81 (13H, m, Ar-H & =CH), 8.30 (1H, d, H5 dinitrophenyl), 8.81 (1H, s, H3 dinitrophenyl), 10.84 (1H, s, =N-NH), 12.25 (1H, s, NH benzimidazole).

$^{13}$C-NMR (DMSO-$d_6$) \textit{d}: 49.94 (2C, 2,6-piperidone), 56.17 (CH$_2$), 111.54, 112.57, 146.02, 150.71 (8C, furan), 114.92, 117.53, 123.07, 126.54, 128.61, 137.41, 138.61, 139.65, 144.53, 146.47, 148.51, 150.52 (12C, Ar), 115.72 (2C, =CH), 141.92 (imidazole), 136.24 (2C, 3,5-piperidone), 155.84 (1C, C=N).

MS m/z : 565 (M$^+$)

Elemental Analysis (%): Calculated: C, 61.59; H, 4.10; N, 17.34

Found: C, 61.33, H, 4.25; N, 17.57

\textbf{N-[(3E,5E)-1-(1H-benzimidazol-2-ylmethyl)-3,5-bis(furan-2-ylmethylidene)piperidin-4-ylidene]thiocarbohydrazide (B21)}

\textbf{Yield} : 75%

\textbf{Melting Point} : 344 – 346 $^\circ$C

\textbf{Rf} : 0.55 (benzene : ethanol, 8:2)

\textbf{IR (KBr) cm$^{-1}$} : 3306, 3249 (NH$_2$, NH), 3063, 2965, 2883 (CH), 1623 (C=N), 1217 (C=S).

$^1$H-NMR (DMSO-$d_6$) \textit{d}: 3.03 (4H, s, H$_2$&H$_6$piperidone), 3.63 (s, 2H, N-CH$_2$), 6.88 – 7.90 (m, 12H, ArH & =CH), 8.40 (1H, s, -N-NH), 8.94 (1H, s, -C-NH-NH$_2$), 9.50 (2H, s, -C-NH-NH$_2$), 12.22 (1H, s, NH benzimidazole).

$^{13}$C-NMR (DMSO-$d_6$) \textit{d}: 48.66 (2C, 2,6-piperidone), 55.75 (CH$_2$), 110.89, 112.45, 145.57, 151.60 (8C, furan) 114.83, 122.69, 139.11 (6C, Ar), 115.64 (2C, =CH), 141.27 (imidazole), 136.02 (2C, 3,5-piperidone), 157.03 (1C, C=N), 182.75 (1C, C=S).

MS m/z : 473 (M$^+$), 389, 342, 369, 300, 173, 131,
(3E,5E)-1-(1H-benzimidazol-2-ylmethyl)-3,5-bis(furan-2-ylmethylidene)-N-methoxypiperidin-4-imine (B22)

Yield : 84 %
Melting Point : 281 – 283 °C
Rf : 0.40 (benzene : ethylacetate, 8:2)
IR (KBr) cm\(^{-1}\) : 3245 (NH), 3090, 3024, 2947 (CH), 1630 (C=N).

N-[(3E,5E)-1-(1H-benzimidazol-2-ylmethyl)-3,5-bis(furan-2-ylmethylidene)piperidin-4-ylidene]hydrazinecarboxamide (B23)

Yield : 80 %
Melting Point : 319 – 320 °C
Rf : 0.7 (benzene : ethylacetate, 8:2)
IR (KBr) cm\(^{-1}\) : 3307, 3261 (NH\(_2\), NH), 3069, 2987, 2886 (CH), 1683 (C=O), 1615 (C=N).

(3E,5E)-1-(1H-benzimidazol-2-ylmethyl)-3,5-bis(furan-2-ylmethylidene)piperidin-4-ol (B24)

Yield : 85 %
Melting Point : 267 – 269 °C
Rf : 0.58 (chloroform : methanol, 7:3)
IR (KBr) cm\(^{-1}\) : 3341 (OH), 3244 (NH), 3083, 2978, 2906 (CH).

\(^1\)H-NMR (DMSO-d\(_6\)) d : 3.01 (s, 4H, H\(_2\)&H\(_6\)piperidine), 3.38 (s, 2H, N-CH\(_2\)), 4.48 (s, 1H, H\(_4\)piperidine), 5.12 (s, 1H, OH), 6.89 –7.98 (m, 12H, ArH & =CH), 12.30 (s, 1H, NH, benzimidazole).

\(^{13}\)C-NMR (DMSO-d\(_6\)) d : 48.06 (2C, 2,6-piperidone), 56.18 (CH\(_2\)), 77.05 (1C, C-OH), 110.73, 112.38, 146.06, 150.86 (8C, furan), 114.07 (2C, =CH), 115.84, 123.65, 138.60, (6C, Ar), 141.40 (imidazole), 102
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143.95 (2C, 3,5-piperidone).

MS m/z : 387 (M)⁺

Elemental Analysis (%) : Calculated: C, 71.30; H, 5.46; N, 10.85

Found: C, 71.43, H, 5.80; N, 11.09

\((3E,5E)-1-(1H-benzimidazol-2-ylmethyl)-3,5\text{-bis(furan-2-ylmethylidene)piperidin-4-yl benzenesulfonate (B25)}\)

Yield : 79 %

Melting Point : 354 – 355 °C

Rf : 0.47 (petether : ethyl acetate, 7:3)

IR (KBr) cm⁻¹ : 3265 (NH), 3086, 3017, 2948 (CH).

\(^1\text{H-NMR}\) (DMSO-d₆) d : 2.89 (s, 4H, H₂&H₆ piperidine), 3.65 (s, 2H, -N-CH₂), 5.80 (s, 1H, H₄ piperidine), 6.81 – 8.10 (m, 17H, ArH&=CH), 12.10 (s, 1H, NH benzimidazole).

\(^1\text{C-NMR}\) (DMSO-d₆) d : 48.78 (2C, 2,6-piperidone), 56.80 (CH₂), 70.75 (1C, C-O), 111.76, 113. 31, 145.56, 151.64 (8C, furan), 113.63 (2C, =CH), 115.67, 122.78, 129.40, 130.71, 135.04, 138.38, 140.70 (12C, Ar), 141. 83 (imidazole), 143.60 (2C, 3,5-piperidone).

MS m/z : 527 (M)⁺, 443, 396, 354, 173, 131, 77

Elemental Analysis (%) : Calculated: C, 66.02; H, 4.78; N, 7.96

Found: C, 65.84, H, 5.01; N, 8.28
4.3.3. Series III. 3,5-Bis(furan-2-ylmethylidene)piperidin-4-substituted imines (R1 - R25)

![Synthetic scheme of 3,5-bis(furan-2-ylmethylidene)piperidin-4-substituted imines](image)

Fig. 4.3. Synthetic scheme of 3,5-bis(furan-2-ylmethylidene)piperidin-4-substituted imines

4.3.3.1. Reaction Mechanisms

Piperidin-4-one (1) undergoes aldol condensation on reaction with 2-furfural in the presence of NaOH yield aldol product, which on removal of water yield the compound, R1.

Reaction:

![Reaction scheme](image)
Mechanism involved:

Step I

A base removes an α-proton to from an enolate ion to serve as a nucleophile.

![Diagram showing the mechanism of step I](image)

Step II

The enolate ion, which is a nucleophile, attacks the carbonyl carbon of 2-furfural to form an alkoxide.

![Diagram showing the mechanism of step II](image)
Step III

Protonation of alkoxide gives the β-hydroxyketone (aldol product).

\[
\text{alkoxide} \xrightarrow{H^+} \text{aldol product}
\]

Step IV

Removal of water from the aldol product yield the bis methylidene compound, R1.

\[
\text{aldol product} \xrightarrow{-\text{H}_2\text{O}} \text{R}_1
\]

R1 was condensed with appropriate substituted amines in the presence of sodium acetate to obtain R2 - R23 by Schiff’s reaction.

Reaction:

\[
\text{R1} + \text{R} \cdot \text{NH}_2 \xrightarrow{\text{CH}_3\text{COONa}} \text{R2 - R23}
\]
Mechanism involved:

Step I:

Nucleophilic attack of carbonyl carbon by $1^o$ amine forms zwitterion.

Step II:

The zwitterion rearranges to form unstable carbinolamine.

Step III:

The acetate ion deprotonate the amino group in carbinolamine and protonate the $\text{OH}^-$ ion leads to the formation of imine (Schiff's base).
The compound, \( R_1 \), undergoes reduction in the presence of sodium tetra borate to yield the alcohol, \( R_{24} \).

**Reaction**

\[
\begin{align*}
\text{carbinolamine} & \rightarrow R_1 \\
R_1 & \xrightarrow{\text{NaBH}_4, [H]} \text{alkoxide ion} \\
\text{alkoxide ion} & + \text{BH}_3 + \text{Na}^+ \\
\end{align*}
\]

**Mechanism involved:**

**Step I:**

Nucleophilic attack of carbonyl carbon of \( R_1 \) with hydride ion forms alkoxide ion.
Step II:

The alkoxide ion on protonation yields the alcohol (R24).

\[
\text{alkoxide ion} \xrightarrow{\text{protonation}} \text{alcohol (R24)}
\]

The compound R24, on treatment with benzene sulphonylchloride yields R25.

Reaction:

\[
\text{R24} \xrightarrow{\text{C}_6\text{H}_5\text{SO}_2\text{Cl}} \text{R25}
\]

Mechanism involved:

The chloro group present in the benzene sulphonyl chloride is a good leaving group, which makes the sulphonyl group for nucleophilic attack by the oxygen of R24, followed by elimination of HCl forms the compound R25.
4.3.3.2. Synthesis of (3E,5E)-3,5-bis(furan-2-ylmethylidene)piperidin-4-one (R1)

Synthesis of R1 was carried out as per the reported method (Katsori 2011; Kalai 2011). A mixture of 4-piperidone hydrochloride (1.35 gm, 0.01 mol) and furfural (1.8 ml, 0.022 mol) was taken in a Erlenmeyer flask. A solution of ethanol (12 ml) and 10% NaOH (20 ml) was prepared, cooled to 20 °C in ice-cold water, and this solution was added to the above mixture with stirring. Stirring was continued at room temperature. for 2 hrs. When the reaction was completed, which was monitored using TLC, the reaction mixture was cooled in an ice bath. The precipitated product was filtered under vacuum, washed with water, dried and recrystallised using a mixture of acetonitrile and methanol (1:1)
4.3.3.3. General Procedure for the Synthesis of R2 - R23

The reaction was carried out based on the reported method (Banerjee 2011). To an ethanolic mixture of \((3E,5E)-3,5\)-bis(furan-2-ylmethylidene)piperidin-4-one \(\text{R1}\) (2.55 gm, 0.01 mol) with the appropriate amine (0.01 mol), sodium acetate (0.82 gm, 0.01 mol) was added with stirring and it was heated under reflux for 3.5 hr with constant stirring. After completion of the reaction, which was monitored using TLC, the contents were cooled to room temperature and poured into ice cold water. The resulting product was filtered under suction and vacuum dried after washing thoroughly with water. It was then recrystallized from the mixture of methanol and dimethyl formamide (2:8) to yield the pure compound.

4.3.3.4. Synthesis of 1-(1H-benzimidazol-2-ylmethyl)piperidin-4-ol (R24)

The reduction reaction was carried out based on the literature method (Aridoss 2008). To the compound \(\text{R1}\) (0.51 gm, 0.002 mol) in methanol (5 ml), sodium borohydride (0.76 gm, 0.02 mol) was added at 0 °C with stirring. Methanol was removed and aqueous solution of ammonium chloride (2ml) was added to the residue and the product was extracted with ethyl acetate. Then ethyl acetate was evaporated and the compound was recrystallised using methanol and DMF.

4.3.3.5. Synthesis of 1-(1H-benzimidazol-2-ylmethyl)piperidin-4-yl benzenesulfonate (R25)

This reaction was carried out as per the reported method (Chernyshov 2011). A solution of benzenesulphonyl chloride (0.1 ml, 0.001 mol) in pyridine (5 ml) was heated to 110-115 °C with vigorous agitation and thoroughly ground compound \(\text{R24}\) (0.257 gm, 0.01 mol) was added. The mixture was boiled with reflux under
agitation for 30 min. and then 2M NaOH solution (150 ml) was poured in and the mixture was boiled for additional 5-8 min. The resulting solution was neutralized with glacial acetic acid and cooled to 20 °C. The product formed was filtered and recrystallised using methanol and DMSO.

4.3.3.6. Characterization of 3,5-Bis(furan-2-ylmethylidene)piperidin-4-substituted imines (R1 - R25)

(3E,5E)-3,5-bis(furan-2-ylmethylidene)piperidin-4-one (R1)

Yield : 86 %
Melting Point : 220 - 222 °C
Rf : 0.77 (chloroform : methanol, 7:3)
IR (KBr) cm⁻¹ : 3238 (NH); 3071, 2968 (C-H); 1707 (C=O).
¹H-NMR (DMSO-d₆) d : 2.52 (1H, s, NH), 3.39 (4H, s, H₂&H₅ piperidone), 6.93 – 8.17 (m, 8H, ArH & =CH).
¹³C-NMR (DMSO-d₆) d : 43.84 (2C, 2,6-piperidone), 110.73, 112.35, 146.27, 151.86 (8C, furan), 130.05 (2C, =CH), 146.98 (2C, 3,5-piperidone), 185.81 (1C, C=O).
MS m/z : 255 (M)^+, 239, 212, 171
Elemental Analysis (%) : Calculated: C, 70.50; H, 5.13; N, 5.49
Found: C, 71.77; H, 5.40; N, 5.17

(3E,5E)-3,5-bis(furan-2-ylmethylidene)-N-hydroxypiperidin-4-imine (R2)

Yield : 81 %
Melting Point : 297 – 299 °C
Rf : 0.67 (chloroform : methanol, 7:3)
IR (KBr) cm⁻¹ : 3364 (OH); 3253 (NH); 3072, 3014, 2948 (CH), 1572 (C=N).
Chapter IV

Experimental

$^1$H-NMR (DMSO-$d_6$) d: 2.48 (1H, s, NH), 3.25 (4H, s, H$_2$&H$_5$ piperidine), 6.75 – 8.04 (m, 8H, ArH & =CH) 11.04 (1H, s, OH).

$^{13}$C-NMR (DMSO-$d_6$) d: 43.62 (2C, 2,6-piperidine), 111.54, 112.51, 146.04, 151.73 (8C, furan), 119.71 (2C, =CH), 137.53 (2C, 3,5-piperidine), 164.46 (1C, C=N).

MS m/z : 270 (M)$^+$

Elemental Analysis (%): Calculated: C, 66.66; H, 5.22; N, 10.36
Found: C, 66.95; H, 4.92; N, 10.61

$(3E,5E)$-3,5-bis(furan-2-ylmethylidene)-4-hydrazinylidene piperidine (R3)

Yield : 77 %

Melting Point : 305 – 308 ºC

R$_f$ : 0.64 (benzene : ethanol, 8:2)

IR (KBr) cm$^{-1}$ : 3359, 3264 (NH$_2$, NH); 3114, 3024, 2948, (CH), 1657 (C=N).

$^1$H-NMR (DMSO-$d_6$) d: 2.50 (1H, s, NH), 3.17 (4H, s, H$_2$ & H$_5$ piperidine), 6.03 (2H, s, NH$_2$), 6.77 – 8.01 (m, 8H, ArH & =CH)

$^{13}$C-NMR (DMSO-$d_6$) d: 44.34 (2C, 2,6-piperidine), 111.60, 113.04, 145.71, 151.24 (8C, furan), 116.25 (2C, =CH), 135.38 (2C, 3,5-piperidine), 155.20 (1C, C=N).

MS m/z : 269 (M)$^+$, 239, 226, 185

Elemental Analysis (%): Calculated: C, 66.90; H, 5.61; N, 15.60
Found: C, 66.71; H, 5.92; N, 15.35

2-[(3E,5E)-3,5-bis(furan-2-ylmethylidene)piperidin-4-ylidene]hydrazinecarbothioamide (R4)

Yield : 79 %

Melting Point : 287 – 290 ºC
Rf  :  0.6 (chloroform : methanol, 7:3)

IR (KBr) cm⁻¹  :  3347, 3256 (NH₂, NH), 3075, 2976, 2907 (CH), 1563 (C=N) 1210 (C=S).

2-[(3E,5E)-3,5-bis(furan-2-ylmethylidene)piperidin-4-ylidene]-N-
hydroxyhydrazinecarbothioamide (R5)

Yield  :  80 %
Melting Point  :  144 – 147 °C
Rf  :  0.62 (chloroform : methanol, 7:3)
IR (KBr) cm⁻¹  :  3357 (OH); 3237 (NH), 3085, 3011, 2949 (CH), 1642 (C=N).

¹H-NMR (DMSO-d₆) d  :  2.50 (1H, s, NH), 3.19 (4H, s, H₂ & H₅ piperidine), 6.81 – 7.91 (m, 8H, ArH & =CH), 8.54 (1H, s, -N-NH), 9.31 (1H, s, -C-NH-OH), 10.44 (1H, s, -C-NH-OH).

¹³C-NMR (DMSO-d₆) d  :  44.92 (2C, 2,6-piperidine), 111.54, 112.86, 145.65, 151.82 (8C, furan) 116.23 (2C, =CH), 135.38 (2C, 3,5-piperidine), 155.75 (1C, C=N), 182.74 (1C, C=S).

MS m/z  :  344 (M⁺)

N-[(3E,5E)-3,5-bis(furan-2-ylmethylidene)piperidin-4-ylidene]-4-fluoroaniline
(R6)

Yield  :  75 %
Melting Point  :  285 – 288 °C
Rf  :  0.55 (petether : ethyl acetate, 7:3)
IR (KBr) cm⁻¹  :  3255 (NH), 3081, 2985, 2917 (CH), 1644 (C=N).

¹H-NMR (DMSO-d₆) d  :  2.48 (1H, s, NH), 3.23 (4H, s, H₂ & H₅ piperidine), 6.84-7.86 (12H, m, Ar-H & =CH).

¹³C-NMR (DMSO-d₆) d  :  45.26 (2C, 2,6-piperidine), 111.04, 112.42, 146.32, 151.61 (8C, furan), 116.84 (2C, C3&C5 fluorophenyl), 129.46 (2C, C2&C6 fluorophenyl), 144.83, (1C, C1 fluorophenyl), 160.92
(1C, C4 fluorophenyl), 115.54 (2C, =CH), 136.43 (2C, 3,5-piperidone), 164.44 (1C, C=N).

\((3E,5E)-3,5\text{-bis(furan-2-ylmethylidene)}-4-(2\text{-phenylhydrazinylidene})\text{piperidine}\) (R7)

Yield : 74 %
Melting Point : 229 – 232 °C
\(R_f\) : 0.57 (benzene : ethylacetate, 8:2)
IR (KBr) cm\(^{-1}\) : 3264 (NH), 3082, 2975, 2904 (CH), 1562 (C=N).
\(^1\)H-NMR (DMSO-\(d_6\)) d : 2.51 (1H, s, NH), 3.25 (4H, s, \(H_2\&H_5\) piperidine), 6.79-7.80 (13H, m, Ar-H & =CH), 11.18 (1H, s, -N-NH).
\(^{13}\)C-NMR (DMSO-\(d_6\)) d : 44.47 (2C, 2,6-piperidine), 111.62, 112.65, 146.26, 151.34 (8C, furan), 116.40, 119.04, 129.45, 143.26 (6C, Ar), 115.82 (2C, =CH), 135.96 (2C, 3,5-piperidine), 155.91 (1C, C=N).
MS m/z : 345 (M\(^+\)), 302, 268, 261, 239, 77
Elemental Analysis (%) : Calculated: C, 73.03; H, 5.54; N, 12.17
Analysis (%) : Found: C, 72.75; H, 5.67; N, 12.40

\(N-[(3E,5E)-3,5\text{-bis(furan-2-ylmethylidene})\text{piperidin-4-ylidene}-2,4\text{-methoxyaniline}\) (R8)

Yield : 77 %
Melting Point : 261 – 264 °C
\(R_f\) : 0.45 (benzene : ethylacetate, 8:2)
IR (KBr) cm\(^{-1}\) : 3245 (NH), 3071, 2981, 2908 (CH), 1642 (C=N).


N-[(3E,5E)-3,5-bis(furan-2-ylmethylidene)piperidin-4-ylidene] 2,3-dimethylaniline (R9)

Yield : 79 %
Melting Point : 249 – 252 °C
Rf : 0.42 (petether : ethyl acetate, 7:3)
IR (KBr) cm⁻¹ : 3246 (NH), 3072, 3016, 2945 (CH), 1642 (C=N).

N-[(3E,5E)-3,5-bis(furan-2-ylmethylidene)piperidin-4-ylidene] 2-fluoroaniline (R10)

Yield : 83 %
Melting Point : 270 – 272 °C
Rf : 0.4 (petether : ethyl acetate, 7:3)
IR (KBr) cm⁻¹ : 3243 (NH), 3091, 3005, 2914 (CH), 1656 (C=N).

¹H-NMR (DMSO-d₆) d : 2.49 (1H, s, NH), 3.25 (4H, s, H₂⁻H₅piperidone), 6.79 – 8.12 (m, 12H, ArH & =CH).
¹³C-NMR (DMSO-d₆) d : 45.30 (2C, 2,6-piperidone), 111.61, 112.45, 146.36, 151.82 (8C, furan), 115.67 (2C, =CH), 117. 04, 124. 34, 126.12, 129. 37, 135.89, 154.46 (6C, Ar), 131.05 (2C, 3,5-piperidone), 164. 17 (1C, C=N).

N-[(3E,5E)-3,5-bis(furan-2-ylmethylidene)piperidin-4-ylidene] 2,4-dichloroaniline (R11)

Yield : 78 %
Melting Point : 241 – 244 °C
Rf : 0.43 (petether : ethyl acetate, 7:3)
IR (KBr) cm⁻¹ : 3258 (NH), 3066, 3002, 2962 (CH), 1586 (C=N).
**Chapter IV**

**Experimental**

**N-[(3E,5E)-3,5-bis(furan-2-ylmethylidene)piperidin-4-ylidene]-2,4-difluoroaniline (R12)**

Yield : 82 %  
Melting Point : 283 – 286 °C  
Rf : 0.36 (petether : ethyl acetate, 7:3)  
IR (KBr) cm⁻¹ : 3251 (NH), 3069, 3023, 2972 (CH), 1614 (C=N).  
¹H-NMR (DMSO-d₆) d : 2.27 (1H, s, NH), 3.38 (4H, s, H₂piperidone), 6.78 – 7.80 (m, 11H, ArH & =CH).  
¹³C-NMR (DMSO-d₆) d : 46.21 (2C, 2,6-piperidine), 110.44, 113.87, 146.54, 150.63 (8C, furan), 116.75 (2C, =CH), 135.49 (2C, 3,5-piperidone), 105.48, 114.07, 124.42, 131.76, 157.06, 162.24 (6C, Ar), 165.16 (1C, C=N).  
MS m/z : 366 (M)⁺

**1-[(3E,5E)-3,5-bis(furan-2-ylmethylidene)piperidin-4-ylidene]guanidine (R13)**

Yield : 82 %  
Melting Point : 310 – 312 °C  
Rf : 0.59 (benzene : ethanol, 8:2)

**N-[(3E,5E)-3,5-bis(furan-2-ylmethylidene)piperidin-4-ylidene]aniline (R14)**

Yield : 78 %  
Melting Point : 223 – 224 °C  
Rf : 0.68 (benzene : ethylacetate, 8:2)  
IR (KBr) cm⁻¹ : 3257 (NH), 3086, 2986, 2907 (CH), 1657 (C=N).
**N-[(3E,5E)-3,5-bis(furan-2-ylmethylidene)piperidin-4-ylidene]-4-chloroaniline (R15)**

Yield : 75 %
Melting Point : 255 – 258 °C
R<sub>f</sub> : 0.52 (petether : ethyl acetate, 7:3)
IR (KBr) cm<sup>-1</sup> : 3248 (NH), 3065, 2976, 2912 (CH), 1648 (C=N).

**1-[(3E,5E)-3,5-bis(furan-2-ylmethylidene)piperidin-4-ylidene]urea (R16)**

Yield : 73 %
Melting Point : 268 – 269 °C
R<sub>f</sub> : 0.63 (chloroform : methanol, 7:3)
IR (KBr) cm<sup>-1</sup> : 3290, 3261 (NH<sub>2</sub>, NH), 3082, 3016, 2935 (CH), 1675 (C=O), 1611 (C=N).

**N-[(3E,5E)-3,5-bis(furan-2-ylmethylidene)piperidin-4-ylidene]-3,4-difluoroaniline (R17)**

Yield : 74 %
Melting Point : 295 – 298 °C
R<sub>f</sub> : 0.31 (petether : ethyl acetate, 7:3)
IR (KBr) cm<sup>-1</sup> : 3268 (NH), 3055, 3013, 2958 (CH), 1645 (C=N).

<sup>1</sup>H-NMR (DMSO-d<sub>6</sub>) d : 2.21 (1H, s, NH), 3.24 (4H, s,H<sub>2</sub>&H<sub>5</sub>piperidine), 6.79 – 7.72 (m, 11H, ArH & =CH).
<sup>13</sup>C-NMR (DMSO-d<sub>6</sub>) d : 45.70 (2C, 2,6-piperidine), 111.36, 113.17, 146.42, 151.41 (8C, furan), 110.73, 118.17, 119.30, 147.27, 148.21, 150.15 (6C, Ar), 115.54 (2C, =CH), 134.85 (2C, 3,5-piperidone), 164.11 (1C, C=O).
MS m/z : 366 (M)<sup>+</sup>
Chapter IV

Experimental

Elemental Analysis (%): Calculated: C, 68.85; H, 4.40; N, 7.65

Found: C, 69.11; H, 4.11; N, 7.30

**N-[(3E,5E)-3,5-bis(furan-2-ylmethylidene)piperidin-4-ylidene]-4-chloro-2-fluoroaniline (R18)**

Yield : 71 %

Melting Point : 278 – 280 °C

R<sub>f</sub> : 0.34 (petether : ethyl acetate, 7:3)

IR (KBr) cm<sup>-1</sup> : 3260 (NH), 3076, 3019, 2980 (CH), 1657 (C=N). MS m/z: 382 (M)<sup>+</sup>

**1-[(3E,5E)-3,5-bis(furan-2-ylmethylidene)piperidin-4-ylidene]thiourea (R19)**

Yield : 75 %

Melting Point : 301 – 304 °C

R<sub>f</sub> : 0.58 (chloroform : methanol, 7:3)

IR (KBr) cm<sup>-1</sup> : 3325, 3264 (NH<sub>2</sub>, NH), 3045, 2986, 2914 (CH), 1655 (C=N). 1231 (C=S).

<sup>1</sup>H-NMR (DMSO-d<sub>6</sub>) d : 2.05 (1H, s, NH), 3.25 (4H, s,H<sub>2</sub>&H<sub>5</sub>piperidine), 5.02 (2H, s, NH<sub>2</sub>), 6.84 – 7.68 (m, 8H, ArH & =CH).

<sup>13</sup>C-NMR (DMSO-d<sub>6</sub>) d : 46.08 (2C, 2,6-piperidine), 111.40, 112.26, 145.82, 151.66 (8C, furan), 115.45 (2C, =CH), 135.26 (2C, 3,5-piperidine), 164.71 (1C, C=N), 187.34 (1C, C=S).

MS m/z : 313 (M)<sup>+</sup>, 270, 239, 229,

Elemental Analysis (%): Calculated: C, 61.32; H, 4.82; N, 13.41

Found: C, 65.97; H, 4.50; N, 13.14
*(3E,5E)-4-[2-(2,4-dinitrophenyl)hydrazinylidene]-3,5-bis(furan-2-ylmethylidene)piperidine (R20)*

Yield : 73 %
Melting Point : 274 – 276 °C
R<sub>f</sub> : 0.3 (benzene : ethylacetate, 8:2)
IR (KBr) cm<sup>-1</sup> : 3241 (NH), 3064, 2974, 2923 (CH), 1637 (C=N).
<sup>1</sup>H-NMR (DMSO-d<sub>6</sub>) d : 2.46 (1H, s, NH), 3.27 (4H, s, H<sub>2</sub>&H<sub>5</sub> piperidine), 6.78-7.81 (11H, m, Ar-H & =CH), 8.28 (1H, d, H5 dinitrophenyl), 8.78 (1H, s, H3 dinitrophenyl), 10.85 (1H, s, =N-NH).
<sup>13</sup>C-NMR (DMSO-d<sub>6</sub>) d : 45.65 (2C, 2,6-piperidine), 111.46, 112.38, 145.75, 150.42 (8C, furan), 117.84, 121.17, 131.24, 137.65, 138.94, 145.27, (6C, Ar), 115.47 (2C, =CH), 136.50 (2C, 3,5-piperidone), 156.17 (1C, C=N).
MS m/z : 435 (M)<sup>+</sup>
Elemental Analysis (%): Calculated: C, 57.93; H, 3.94; N, 16.09
Found: C, 58.31; H, 4.22; N, 16.42

*N''-[(3E,5E)-3,5-bis(furan-2-ylmethylidene)piperidin-4-ylidene]thiocarbonohydrazide (R21)*

Yield : 73 %
Melting Point : 246 – 248 °C
R<sub>f</sub> : 0.47 (benzene : ethanol, 8:2)
IR (KBr) cm<sup>-1</sup> : 3234 (NH), 3083, 3016, 2928 (CH), 1647 (C=N).

*(3E,5E)-3,5-bis(furan-2-ylmethylidene)-N-methoxypiperidin-4-imine (R22)*

Yield : 78 %
Melting Point : 306 – 308 °C
R_f : 0.61 (benzene : ethylacetate, 8:2)
IR (KBr) cm\(^{-1}\) : 3234 (NH), 3083, 3016, 2928 (CH), 1647 (C=N).

\(N-[\{3E,5E\}-3,5\text{-bis(furan-2-ylmethylidene})\text{piperidin-4-ylidene}]\text{hydrazinecarboxamide} \) (R23)

Yield : 82 %
Melting Point : 291 – 293 °C
R_f : 0.54 (chloroform : methanol, 7:3)
IR (KBr) cm\(^{-1}\) : 3337, 3258 (NH\(_2\), NH), 3076, 3017, 2920 (CH), 1672 (C=O), 1625 (C=N).

\((3E,5E)-3,5\text{-bis(furan-2-ylmethylidene})\text{piperidin-4-ol} \) (R24)

Yield : 84 %
Melting Point : 237 – 238 °C
R_f : 0.5 (chloroform : methanol, 7:3)
IR (KBr) cm\(^{-1}\) : 3357 (OH), 3256 (NH), 3078, 3015, 2946 (CH).

\(^1\text{H-NMR} \) (DMSO-\(d_6\)) d : 2.19 (1H, s, NH), 3.49 (4H, s, \(H_2\&H_5\) piperidine), 4.40 (1H, s, \(CH-\text{OH}\)), 5.02 (1H, s, OH), 6.20 (2H, s, = CH), 6.68 – 7.91 (m, 6H, ArH).
\(^{13}\text{C-NMR} \) (DMSO-\(d_6\)) d : 40.16 (2C, 2,6-piperidine), 75.90 (1C, C=OH), 110.90, 112.43, 146.39, 151.71 (8C, furan), 113.62 (2C, =CH), 143.83 (2C, 3,5-piperidine).

MS m/z : 257 (M\(^+\)), 240, 214, 173

\((3E,5E)-3,5\text{-bis(furan-2-ylmethylidene})\text{piperidin-4-yl benzenesulfonate} \) (R25)

Yield : 76 %
Melting Point : 191 – 194 °C
\[ R_f : 0.46 \text{ (benzene : ethylacetate, 8:2)} \]

\[
\text{IR (KBr) cm}^{-1} : 3250 \text{ (NH)}, 3081, 2983, 2920 \text{ (CH)}. 
\]

\[
^1\text{H-NMR (DMSO-d}_6\text{)} d : 2.40 \text{ (1H, s, NH)}, 3.26 \text{ (4H, s, H}_2\text{&H}_5\text{piperidine)}, 6.11 \text{ (1H, s, H}_4\text{piperidine)}, 6.46 \text{ (2H, s, = CH)}, 6.73 - 8.30 \text{ (m, 6H, ArH)}. 
\]

\[
^{13}\text{C-NMR (DMSO-d}_6\text{)} d : 39.44 \text{ (2C, 2,6-piperidine)}, 70.76 \text{ (1C, C-OH)}, 110.90, 113.04, 145.61, 151.20 \text{ (8C, furan)}, 114.43 \text{ (2C, =CH)}, 129.45, 130.70, 135.14, 140.79 \text{ (6C, Ar)}, 143.19 \text{ (2C, 3,5-piperidine)}. 
\]

**4.4. Antitubercular Evaluation**

**4.4.1. Agar Dilution Method**

Two-fold serial dilutions (50.0, 25.0, 12.5, 6.25, 3.13, 1.56 and 0.78 μg/ml) of each test compounds, and standard drugs were made and included into Middlebrook 7H11 agar medium with OADC (oleic acid, albumin, dextrose and catalase; Difco) growth addendum. Inoculant of *M. tuberculosis* H37Rv ATCC 27294 was made from fresh Middlebrook 7H11 agar slants with OADC growth addendum oriented to 1 mg/ml (wet weight) in Tween 80 (0.05%) saline diluted to $10^{-2}$ in order to get ~$10^7$ cfu/ml concentration. 5 μl of this mycobacterial suspension was blotted into 7H11 agar tubes containing varying concentrations of the test and standard drugs as mentioned above. The tubes were incubated at 37 °C, and the readings were recorded after 28 days (Hall, 2012; Addla, 2014). This method is similar to that recommended by the National Committee for Clinical Laboratory Standards for the determination of MIC in triplicate. The MIC values of the test and standard drugs (pyrazinamide, ethambutol and INH) are furnished in Table 5.3, 5.7 and 5.11 (chapter V).
4.5. Cytotoxicity assay

4.5.1. MTT Method

Vero cell lines (African green monkey kidney cells) cultures were procured from National Centre for Cell Sciences (NCCS), Pune, India. The cells were cultured in Dulbecco’s modified essential medium (DMEM) which is supplemented with penicillin (100 IU/ml), amphotericin-B (5 µg/ml), streptomycin (100 µg/ml) and 10% heat inactivated Fetal bovine serum (FBS) in a 5% CO₂ atmosphere at 37°C until confluent. TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS) was used to trypsinize the cells. The cultures were grown in 25 cm² flat bottles and the studies were performed in 96 well plates. Viability of the cells were assessed on the basis of the conversion of MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] into a formazan product, which was measured at 540 nm (Mosmann, 1983, Denizot, 1986).

The percentage growth was calculated using the formula below:

\[
\text{% growth inhibition} = \left( \frac{\text{control absorbance} - \text{test absorbance}}{\text{control absorbance}} \right) \times 100
\]

IC₅₀ (the compound concentration that reduces the number of cells by 50%) values were calculated (Cheng, 1973) and selectivity index (SI) was obtained from the formula below:

\[
\text{SI} = \frac{\text{IC}₅₀}{\text{MIC}}
\]

SI value of more than 10 is considered to be nontoxic for the in-vitro antitubercular evaluation (Luo, 2013; Protopopova, 2005).
4.6. Anti-HIV Evaluation Assay

4.6.1. Single-cycle infection assay

The inhibitory activity of small molecules was tested on HIV-1 pseudovirus expressing Env of the HIV-1HXB2 (X4) as described in the literature (Curreli 2012). NBD-556 was used as control. Briefly, 100 µl of TZM-bl cells at 1 \times 10^5 cells/ml was added to the wells of a 96-well tissue culture plate and cultured at 37°C overnight. 50 µl of a test compound at graded concentrations was mixed with 50 µl of the HXB2 pseudovirus at about 100 TCID_{50}. After incubation at 37°C for 30 min, the mixtures were added to the cells and incubated at 37°C for 3 days. The cells were washed 2 times with PBS and lysed with 50 µl of cell culture lysis reagent. 20 µl of lysates were transferred to a white 96-well plate and mixed with 100 µl of luciferase assay reagent. The luciferase activity was immediately measured with a Tecan Infinite M1000 reader and the percent inhibition and IC_{50} values were calculated using the GraphPad Prism software (Kong, 2012).

4.6.2. Multi-cycle infection assay

The inhibitory activity of test compounds on infection by laboratory-adapted HIV-1 IIIB was determined as described in the literature (Zhang 2008). In brief, 1 x 10^4 MT-2 cells were infected with HIV-1 at 100 TCID_{50} (0.01 mol) in the presence or absence of test compounds at graded concentrations overnight. As control, MT-2 cells were infected in the presence of zidovudine, a nucleoside reverse-transcriptase inhibitor (NRTI). The culture supernatants were then removed and fresh media was added. On the fourth day of post-infection, 100 µl of culture supernatants were collected from each well, mixed with equal volume of 5% Triton X-100 and tested for p24 antigen by "sandwich" ELISA (Kamada, 2006).
percent inhibition of p24 production and IC\textsubscript{50} values were calculated by the GraphPad Prism software.

4.7. Cytotoxicity Assay

4.7.1. MT-2 cells.

Cytotoxicity of test compounds in MT-2 cells was measured by colorimetric method using XTT [(sodium 3'-(1-(phenylamino)-carbonyl)-3,4-tetrazolium-bis(4-methoxy-6-nitro) bezenesulfonic acid hydrate)] (PolySciences) as described in the literature (Zhang 2008). Briefly, 100 µl of a small molecule at graded concentrations was added to an equal volume of MT-2 cells (1x10\textsuperscript{5} cells/ml) in 96-well plates followed by incubation at 37°C for 4 days, which ran parallel to the neutralization assay in MT-2. After addition of XTT the soluble intracellular formazan was quantitated colorimetrically at 450 nm 4 hr later (Koh, 2009). The percent of cytotoxicity and the CC\textsubscript{50} (the concentration for 50% cytotoxicity) values were calculated with the GraphPad Prism software.

4.7.2. TZM-bl cells.

The cytotoxicity of test compounds in TZM-bl cells was also measured by the XTT method as described in the literature (Curreli 2012). Briefly, 100 µl of test compounds at graded concentrations were added to an equal volume of cells (10\textsuperscript{5}/ml) in wells of 96-well plates followed by incubation at 37 °C for 3 days and addition of XTT. The soluble intracellular formazan was quantitated colorimetrically at 450 nm 4 hr later (Matoba, 2010). The percent of cytotoxicity and the CC\textsubscript{50} values were calculated as mentioned above.