4. EXPERIMENTAL PROTOCOLS

4.1. Chemistry

The chemicals used were obtained from various chemical units E. Merck India Ltd., CDH, and s.d. Fine Chem. and Qualigens. These solvents and reagents were of LR grade and purified before their use. The silica gel G (160-120 mesh) used for analytical chromatography (TLC) was obtained from E. Merck India Ltd. Two solvent systems were used Benzene: Acetone (8:2) and (6:4), Toluene: Ethyl acetate: Formic acid (5:4:1). All the melting points were determined in open glass capillary using Kjeldahl flask containing paraffin and are uncorrected. $^1$H NMR and $^{13}$C NMR spectra were recorded on Bruker model DRX 400 NMR spectrometer in DMSO-$d_6$ using tetramethylsilane (TMS) as an internal standard with $^1$H resonant frequency of 400 MHz and $^{13}$C resonant frequency of 100 MHz. All the Fourier transform infra red (FT-IR) spectra were recorded in KBr pellets on a Jasco FT/IR 410 spectrometer. Elemental analyses were realized on a Perkin-Elmer model 240c analyzer and were within ±0.4 % of the theoretical values. The mass spectra were recorded using Qq TOF mass spectrometer. The ultraviolet absorbance was taken on Schimadzu (UV-1601). For the molecular mechanics calculations, the ACD/Chemskech/3-D viewer Freeware version program was used for employing the Chemistry at Harvard Macromolecular Mechanics (CHARMM) force field.
**Reagent and conditions.** (a) Heat on waterbath, 1.5h; (b) anhyd. K$_2$CO$_3$, substituted C$_6$H$_5$CH$_2$.Cl, CH$_3$COCH$_3$, stirring, 8h; (c) Arylthio ureas, CH$_3$C O CH$_3$, reflux, 3h.

**Fig. 5: Synthetic route to the compounds 3a-p – Scheme 1**
Synthetic Methods- Scheme 1

*General procedure for the synthesis of titled compounds 3a-p*

**2-(Chloromethyl)-1H-benzo[d]imidazole (1)**

A mixture of *o-*phenylene diamine (0.01 mol) and 2-chloroacetic acid (0.01mol) were taken in a dry conical flask and mouth of the flask was plugged with cotton. The mixture was heated on a boiling water bath for 1.5 h. Flask was cooled down under tap water and the product was basified with 10% NaOH solution, filtered, washed with cold water and compound 1 obtained was recrystallized with hot water.

**2-(Chloromethyl)-1-(2-substituted benzyl)-1H-benzo[d]imidazole (2a-b)**

To the solution of compound 1 (0.01 mol) and anhydrous potassium carbonate (0.01 mol) in acetone (30 ml), substituted benzyl chloride (0.01 mol) was added dropwise. The mixture was stirred at room temp for about 8 h. The mixture was then poured into water and extracted with ethyl acetate, dried over anhydrous sodium sulfate and concentrated under vacuum to give the pure compound. Desired compound 2a-b was finally recrystallized with ethanol.

**2-[(1-Substituted benzyl-1H-benzo[d]imidazol-2-yl) methyl]-1-arylisothiourea (3a-p)**

A mixture of compounds 2a-b (0.01mol) and arylthioureas (0.01mol) in acetone were refluxed for 3 h. Reaction mixture was then poured onto crushed ice and the resulting solid compounds 3a-p were dried and recrystallized with ethanol. The physicochemical parameters of all the final compounds are presented in Table 3.

**2-[(1-Benzyl-1H-benzo[d]imidazol-2-yl)methyl]-1-phenylisothiourea (3a)**. Yield 58 %, mp 180 °C. IR (KBr) \( \nu_{\text{max}} \) cm\(^{-1}\): 3365 (NH str), 2900 (CH str), 1594 (C=N str), 617 (C-S-C str); \(^1\)H-NMR (DMSO-\(d_6\)) \( \delta \) ppm: 4.71(s,2H,CH\(_2\)-Ar), 5.27(s, 2H, CH\(_2\)), 6.96 (s,1H, NH=C), 7.02(s,1H, NH-Ar), 7.04-7.50(m,10H,Ar-H), 7.13-7.70(m,2H,Bz-H), 7.18-7.40(d,1H,Bz-H), 7.30-7.50(d,1H, Bz-H). ; MS: \( m/z \) 371(M-1)

**2-[(1-Benzyl-1H-benzo[d]imidazol-2-yl)methyl]-1-o-tolylisothiourea (3b)**. Yield 57 %, mp 210 °C. IR (KBr) \( \nu_{\text{max}} \) cm\(^{-1}\): 3397 (NH str), 2850 (CH str), 1835 (C=N str), 640 (C-S-C str); \(^1\)H-NMR (CDCl\(_3\)) \( \delta \) ppm: 2.40(s,3H,CH\(_3\)), 3.78(s,2H,CH\(_2\)-Ar), 5.56(s,2H,CH\(_2\)), 7.21 (s,1H, NH=C), 7.24-7.74 (m,9H,Ar-H), 7.23-7.74(m,2H,Bz-H), 7.29( s, 1H, NH-Ar), 7.32-7.73(d,1H,Bz-H), 7.73-7.74(d,1H, Bz-H).

**2-[(1-Benzyl-1H-benzo[d]imidazol-2-yl)methyl]-1-m-tolylisothiourea (3c)**. Yield 49 %, mp 240 °C. IR (KBr) \( \nu_{\text{max}} \) cm\(^{-1}\): 3397 (NH str), 2850 (CH str), 1835 (C=N str), 7.45 (s, 1H, NH-Ar), 7.32-7.74(d,1H,Bz-H), 7.73-7.74(d,1H, Bz-H).
738 (C-S-C str); \(^1\)H-NMR (DMSO-\(d_6\)) δ ppm: 2.43(s,3H,CH\(_3\)), 3.56(s,2H,CH\(_2\)-Ar), 5.26(s,2H,CH\(_2\)), 7.13 (s,1H, NH=C), 7.21-7.73 (m,9H,Ar-H), 7.25-7.73(m,2H,Bz-H), 7.26 (s, 1H, NH-Ar), 7.34-7.71(d,1H,Bz-H), 7.75-7.78(d,1H, Bz-H).

2-[(1-Benzyl-1H-benzo[d]imidazol-2-yl)methyl]-1-p-tolylisothiourea (3d). Yield 44 %, mp 215 °C. IR (KBr) \(V_{\text{max}}\) cm\(^{-1}\): 3185 (NH str), 3105 (CH str), 1685 (C=N str), 760 (C-S-C str); \(^1\)H-NMR (DMSO-\(d_6\)) δ ppm: 3.70(s,3H,CH\(_3\)), 5.13(s,2H,CH\(_2\)-Ar), 5.36(s,2H,CH\(_2\)), 7.21 (s,1H, NH=C), 7.25-7.78 (m,9H,Ar-H), 7.24-7.78(m,2H,Bz-H), 7.31 (s, 1H, NH-Ar), 7.32-7.76(d,1H,Bz-H), 7.77-7.82(d,1H, Bz-H).

2-[(1-Benzyl-1H-benzo[d]imidazol-2-yl)methyl]-1-(2-methoxyphenyl)isothiourea (3e). Yield 51 %, mp 268 °C. IR (KBr) \(V_{\text{max}}\) cm\(^{-1}\): 3564 (NH str), 3260 (CH str), 1825 (C=N str), 1100 (OCH\(_3\)), 690(C-S-C str); \(^1\)H-NMR (DMSO-\(d_6\)) δ ppm: 3.70(s,3H,CH\(_3\)), 5.13(s,2H,CH\(_2\)-Ar), 5.75(s,2H,CH\(_2\)), 6.70 (s,1H, NH=C), 6.73-7.40 (m,9H,Ar-H), 6.76-7.42(m,2H,Bz-H), 6.79-7.38(d,1H,Bz-H), 7.19(s,1H,NH-Ar), 7.25-7.42(d,1H, Bz-H).

2-[(1-Benzyl-1H-benzo[d]imidazol-2-yl)methyl]-1-(3-methoxyphenyl)isothiourea (3f). Yield 59 %, mp 185 °C. IR (KBr) \(V_{\text{max}}\) cm\(^{-1}\): 3510 (NH str), 2710 (CH str), 1710 (C=N str), 1090(OCH\(_3\)), 710(C-S-C str); \(^1\)H-NMR (DMSO-\(d_6\)) δ ppm: 3.56(s,3H,CH\(_3\)), 5.23(s,2H,CH\(_2\)-Ar), 5.77(s,2H,CH\(_2\)), 6.72 (s,1H, NH=C), 6.74-7.47 (m,9H,Ar-H), 6.75-7.47(m,2H,Bz-H), 6.76-7.37(d,1H,Bz-H), 7.23(s,1H,NH-Ar), 7.34-7.56(d,1H, Bz-H).

2-[(1-Benzyl-1H-benzo[d]imidazol-2-yl)methyl]-1-(4-methoxyphenyl)isothiourea (3g). Yield 66 %, mp 216 °C. IR (KBr) \(V_{\text{max}}\) cm\(^{-1}\): 3450 (NH str), 2825 (CH str), 1685 (C=N str), 1105(OCH\(_3\)), 723(C-S-C str); \(^1\)H-NMR (DMSO-\(d_6\)) δ ppm: 3.25(s,3H,CH\(_3\)), 5.67(s,2H,CH\(_2\)-Ar), 5.37(s,2H,CH\(_2\)), 6.84 (s,1H, NH=C), 6.63-7.72 (m,9H,Ar-H), 6.71-7.48(m,2H,Bz-H), 6.73-7.56(d,1H,Bz-H), 7.25(s,1H,NH-Ar), 7.21-7.72(d,1H, Bz-H).

2-[(1-Benzyl-1H-benzo[d]imidazol-2-yl)methyl]-1-(naphthalen-3-yl)isothiourea (3h). Yield 58 %, mp 174 °C. IR (KBr) \(V_{\text{max}}\) cm\(^{-1}\): 3137 (NH str), 2805 (CH str), 1672 (C=N str), 724 (C-S-C str); \(^1\)H-NMR (DMSO-\(d_6\)) δ ppm: 4.39(s,2H,CH\(_2\)-Ar), 6.58(s,
2H, CH₂), 6.59 (s, 1H, NH=C), 7.24 (s, 1H, NH-Ar), 7.61-7.94 (m, 12H, Ar-H), 7.62-7.96 (m, 2H, Bz-H), 7.84-7.94 (d, 1H, Bz-H), 7.94-7.97 (d, 1H, Bz-H).

2-[(1-(2-Chlorobenzyl)-1H-benzo[d]imidazol-2-yl)methyl]-1-phenylisothiourea (3l). Yield 49%, mp 212 °C. IR (KBr) \( V_{\text{max}} \) cm\(^{-1} \): 3255 (NH str), 2899 (CH str), 1688 (C=N str), 832 (C-Cl), 778 (C-S-C str); \(^1\text{H-NMR (DMSO-}d_6\) \( \delta \) ppm: 5.24 (s, 2H, CH₂-Ar), 6.21 (s, 2H, CH₂), 6.45-7.91 (m, 9H, Ar-H), 6.75 (s, 1H, NH=C), 6.74-6.76 (m, 2H, Bz-H), 7.43 (s, 1H, NH-Ar), 7.51-7.54 (d, 1H, Bz-H), 7.93-7.96 (d, 1H, Bz-H).

2-[(1-(2-Chlorobenzyl)-1H-benzo[d]imidazol-2-yl)methyl]-1-o-tolylisothiourea (3f). Yield 53%, mp 170 °C. IR (KBr) \( V_{\text{max}} \) cm\(^{-1} \): 3488 (NH str), 2900 (CH str), 1674 (C=N str), 816 (C-Cl), 689 (C-S-C str); \(^1\text{H-NMR (DMSO-}d_6\) \( \delta \) ppm: 1.25 (s, 3H, CH₃), 4.65 (s, 2H, CH₂-Ar), 5.41 (s, 2H, CH₂), 6.56 (s, 1H, NH=C), 7.26-8.51 (m, 8H, Ar-H), 7.43 (s, 1H, NH-Ar), 7.44-8.51 (d, 1H, Bz-H), 7.45-8.52 (d, 1H, Bz-H), 7.82-8.51 (m, 2H, Bz-H).

2-[(1-(2-Chlorobenzyl)-1H-benzo[d]imidazol-2-yl)methyl]-1-m-tolylisothiourea (3k). Yield 69%, mp 185 °C. IR (KBr) \( V_{\text{max}} \) cm\(^{-1} \): 3390 (NH str), 2950 (CH str), 1650 (C=N str), 810 (C-Cl), 725 (C-S-C str); \(^1\text{H-NMR (DMSO-}d_6\) \( \delta \) ppm: 1.34 (s, 3H, CH₃), 4.45 (s, 2H, CH₂-Ar), 5.65 (s, 2H, CH₂), 6.67 (s, 1H, NH=C), 7.25-8.53 (m, 8H, Ar-H), 7.32 (s, 1H, NH-Ar), 7.42-8.53 (d, 1H, Bz-H), 7.47-8.56 (d, 1H, Bz-H), 7.81-8.58 (m, 2H, Bz-H).

2-[(1-(2-Chlorobenzyl)-1H-benzo[d]imidazol-2-yl)methyl]-1-p-tolylisothiourea (3l). Yield 65%, mp 216 °C. IR (KBr) \( V_{\text{max}} \) cm\(^{-1} \): 3370 (NH str), 2890 (CH str), 1610 (C=N str), 834 (C-Cl), 763 (C-S-C str); \(^1\text{H-NMR (DMSO-}d_6\) \( \delta \) ppm: 1.65 (s, 3H, CH₃), 4.84 (s, 2H, CH₂-Ar), 5.85 (s, 2H, CH₂), 6.38 (s, 1H, NH=C), 7.45-8.82 (m, 8H, Ar-H), 7.73 (s, 1H, NH-Ar), 7.45-8.87 (d, 1H, Bz-H), 7.44-8.95 (d, 1H, Bz-H), 7.84-8.90 (m, 2H, Bz-H).

2-[(1-(2-Chlorobenzyl)-1H-benzo[d]imidazol-2-yl)methyl]-1-(2-methoxyphenyl)isothiourea (3m). Yield 53%, mp 180 °C. IR (KBr) \( V_{\text{max}} \) cm\(^{-1} \): 3361 (NH str), 2858 (CH str), 1670 (C=N str), 1128 (OCH₃), 822 (C-Cl), 690 (C-S-C str); \(^1\text{H-NMR (DMSO-}d_6\) \( \delta \) ppm: 3.51 (s, 3H, OCH₃), 5.41 (s, 2H, CH₂-Ar), 6.15-7.96 (m, 8H, Ar-H), 6.31 (s, 2H, CH₂), 6.78 (s, 1H, NH=C), 7.21-7.25 (m, 2H, Bz-H), 7.85 (s, 1H, NH-Ar), 7.87-7.89 (d, 1H, Bz-H), 7.92-7.94 (d, 1H, Bz-H).
Chapter 4

Experimental Protocols

2-[(1-(2-Chlorobenzyl)-1H-benzo[d]imidazol-2-yl)methyl]-1-(3-methoxyphenyl) isothiourea (3n). Yield 58\%, mp 218 °C. IR (KBr) $V_{\text{max}}$ cm$^{-1}$: 3250 (NH str), 2670 (CH str), 1610 (C=N str), 1100 (OCH$_3$), 814 (C-Cl), 738 (C-S-C str); $^1$H-NMR (DMSO-$d_6$) $\delta$ ppm: 3.55(s,3H,OCH$_3$), 5.46(s,2H,CH$_2$-Ar), 6.12-7.97 (m,8H,Ar-H), 6.35(s, 2H, CH$_2$), 6.81 (s,1H, NH=C), 7.24-7.75(m,2H,Bz-H), 7.88( s,1H,NH-Ar), 7.89-8.12(d,1H,Bz-H), 7.92-7.98(d,1H, Bz-H).

2-[(1-(2-Chlorobenzyl)-1H-benzo[d]imidazol-2-yl)methyl]-1-(4-methoxyphenyl) isothiourea (3o). Yield 57\%, mp 162 °C. IR (KBr) $V_{\text{max}}$ cm$^{-1}$: 3070 (NH str), 2795 (CH str), 1680 (C=N str), 1090 (OCH$_3$), 823 (C-Cl), 725 (C-S-C str); $^1$H-NMR (DMSO-$d_6$) $\delta$ ppm: 3.56(s,3H,OCH$_3$), 5.48(s,2H,CH$_2$-Ar), 6.15-7.94 (m,8H,Ar-H), 6.39(s, 2H, CH$_2$), 6.86 (s,1H, NH=C), 7.22-7.77(m,2H,Bz-H), 7.86( s,1H,NH-Ar), 7.83-8.11(d,1H,Bz-H), 7.90-7.94(d,1H, Bz-H).

2-[(1-(2-Chlorobenzyl)-1H-benzo[d]imidazol-2-yl)methyl]-1-(naphthalen-3-yl) isothiourea (3p). Yield 53\%, mp 167 °C. IR (KBr) $V_{\text{max}}$ cm$^{-1}$: 3464 (NH str), 2900 (CH str), 1666 (C=N str), 822 (C-Cl), 814 (C-S-C str); $^1$H-NMR (DMSO-$d_6$) $\delta$ ppm: 5.14(s,2H,CH$_2$-Ar), 6.74(s, 2H, CH$_2$), 6.31-7.95 (m,8H,Ar-H), 6.47-6.49(m,2H, Bz-H), 6.98 (s,1H, NH=C), 7.20( s,1H,NH-Ar), 7.21-7.25(d,1H,Bz-H), 8.52-8.54(d,1H, Bz-H).
### Table 3- Physicochemical parameters of compounds 3a-p - Scheme 1

<table>
<thead>
<tr>
<th>Compd</th>
<th>R</th>
<th>R’</th>
<th>Mol. Formula</th>
<th>Log P</th>
<th>R_f (R_m)</th>
<th>Elemental analysis %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Calculated</td>
</tr>
<tr>
<td>3a</td>
<td>H</td>
<td>C6H5</td>
<td>C_{22}H_{20}N_{4}S</td>
<td>5.75(4.54)</td>
<td>0.77(-0.52)</td>
<td>70.97</td>
</tr>
<tr>
<td>3b</td>
<td>H</td>
<td>2-CH₃C₆H₅</td>
<td>C_{23}H_{22}N_{4}S</td>
<td>6.21(5.04)</td>
<td>0.84(-0.72)</td>
<td>71.25</td>
</tr>
<tr>
<td>3c</td>
<td>H</td>
<td>3-CH₃C₆H₅</td>
<td>C_{23}H_{22}N_{4}S</td>
<td>6.20(5.04)</td>
<td>0.86(-0.78)</td>
<td>71.67</td>
</tr>
<tr>
<td>3d</td>
<td>H</td>
<td>4-CH₃C₆H₅</td>
<td>C_{23}H_{22}N_{4}S</td>
<td>6.22(5.04)</td>
<td>0.90(-0.95)</td>
<td>71.03</td>
</tr>
<tr>
<td>3e</td>
<td>H</td>
<td>2-OCH₃C₆H₅</td>
<td>C_{23}H_{22}N_{4}OS</td>
<td>5.63(4.46)</td>
<td>0.91(-1.00)</td>
<td>68.27</td>
</tr>
<tr>
<td>3f</td>
<td>H</td>
<td>3-OCH₃C₆H₅</td>
<td>C_{23}H_{22}N_{4}OS</td>
<td>5.61(4.46)</td>
<td>0.79(-0.57)</td>
<td>68.97</td>
</tr>
<tr>
<td>3g</td>
<td>H</td>
<td>4-OCH₃C₆H₅</td>
<td>C_{23}H_{22}N_{4}OS</td>
<td>5.60(4.46)</td>
<td>0.77(-0.52)</td>
<td>68.78</td>
</tr>
<tr>
<td>3h</td>
<td>H</td>
<td>C_{10}H_{7}</td>
<td>C_{26}H_{22}N_{4}S</td>
<td>6.70(5.71)</td>
<td>0.82(-0.65)</td>
<td>73.25</td>
</tr>
<tr>
<td>3i</td>
<td>2-Cl</td>
<td>C₆H₅</td>
<td>C_{22}H_{19}ClN_{4}S</td>
<td>6.28(5.25)</td>
<td>0.80(-0.60)</td>
<td>64.23</td>
</tr>
<tr>
<td>3j</td>
<td>2-Cl</td>
<td>2-CH₃C₆H₅</td>
<td>C_{23}H_{21}ClN_{4}S</td>
<td>6.73(5.75)</td>
<td>0.94(-1.19)</td>
<td>65.34</td>
</tr>
<tr>
<td>3k</td>
<td>2-Cl</td>
<td>3-CH₃C₆H₅</td>
<td>C_{23}H_{21}ClN_{4}S</td>
<td>6.70(5.75)</td>
<td>0.78(-0.54)</td>
<td>65.24</td>
</tr>
<tr>
<td>3l</td>
<td>2-Cl</td>
<td>4-CH₃C₆H₅</td>
<td>C_{23}H_{21}ClN_{4}S</td>
<td>6.68(5.75)</td>
<td>0.91(-1.00)</td>
<td>65.39</td>
</tr>
<tr>
<td>3m</td>
<td>2-Cl</td>
<td>2-OCH₃C₆H₅</td>
<td>C_{23}H_{21}ClN_{4}OS</td>
<td>6.00(5.17)</td>
<td>0.83(-0.68)</td>
<td>62.95</td>
</tr>
<tr>
<td>3n</td>
<td>2-Cl</td>
<td>3-OCH₃C₆H₅</td>
<td>C_{23}H_{21}ClN_{4}OS</td>
<td>6.01(5.17)</td>
<td>0.94(-1.19)</td>
<td>63.47</td>
</tr>
</tbody>
</table>
### Experimental Protocols

| 3o  | 2-Cl | 4-OCH$_3$C$_6$H$_5$ | C$_{23}$H$_{21}$ClN$_4$OS | 5.57(5.17) | 0.77(-0.52) | 62.98 | 4.79 | 12.57 |
| 3p  | 2-Cl | C$_{10}$H$_7$     | C$_{26}$H$_{21}$ClN$_4$S | 7.12(6.42) | 0.76(-0.50) | 67.92 | 4.32 | 12.74 |

*Solvent of crystallization—Ethanol.*

*Log P was determined by octanol:phosphate buffer method; Log P was calculated using software ChemDraw Ultra 8.0*

*Solvent system- Toluene: Ethyl acetate: Formic acid (5:4:1).*

*A logarithmic function of $R_f$ value was also calculated; $R_m = \log (1/R_f).$*

*Elemental analysis for C, H, N were within ± 0.4 % of the theoretical value.*
Reagents and conditions: (i) Heated for 1.5h, cooled and basified with 10% NaOH sol® (ii) Anhy. K₂CO₃, acetone, substituted benzyl chloride, stirred for 8h (iii) NH₂NH₂·H₂O, C₂H₅OH, refluxed for 20-22 h (iv) ArNCS, absolute ethanol, refluxed for 5-6h

Fig. 6: Synthetic route to the compounds 4a-p – Scheme 2
Synthetic Methods- Scheme 2

**General procedure for the synthesis of titled compounds 4a-p**

2-(Chloromethyl)-1H-benzo[d]imidazole (1). A mixture of o-phenylene diamine (0.01 mol) and 2-chloroacetic acid (0.01 mol) were taken in a dry conical flask and mouth of the flask was plugged with cotton. The mixture was heated on a boiling water bath for 1.5 h. Flask was cooled down under tap water and the product was basified with 10% NaOH solution, filtered, washed with cold water and compound 1 obtained was recrystallized with hot water.

2-(Chloromethyl)-1-(2-substitutedbenzyl)-1H-benzo[d]imidazoles (2a,b). To a solution of compound 1 (0.01 mol) and anhydrous potassium carbonate (0.01 mol) in acetone (30 ml), substituted benzyl chloride (0.01 mol) was added dropwise. The mixture was stirred at room temp for about 8 h. The mixture was then poured into water and extracted with ethyl acetate, dried over sodium sulfate anhydrous and concentrated under vacuum to give the pure compound. Desired compound 2a, b was finally recrystallized with ethanol.

1-[(1-Substituted benzyl-1H-benzo[d]imidazol-2-yl)methyl]hydrazines (3a,b). Hydrazine hydrate (10 mL) was placed in a round bottom flask, and compound 2a, b (0.01 mol) was added. Contents were diluted with a sufficient quantity of dry ethanol till clear solution was obtained and the reaction mixture was refluxed for 20–22 h. After completion of the reaction, ethanol was distilled off till a small volume was left. On cooling, crystals of compounds (3a, b) were formed and were filtered and recrystallized with ethanol.

2-[(1-(2-Substitutedbenzyl)-1H-benzo[d]imidazol-2-yl)methyl]-N-substituted phenyl hydrazine carbothioamides (4a-p). A mixture of compound 3a, b (0.01 mol) and substituted phenylisothiocyanates (0.01 mol) in 20 mL of absolute ethanol was refluxed for 5–6 h. After completion of the reaction the reaction, mixture was concentrated and kept overnight at room temperature. The needle shaped crystals thus obtained were purified by repeated washing with petroleum ether. The physicochemical parameters of all the final compounds are presented in Table 4.
2-[(1-Benzyl-1H-benzo[d]imidazol-2-yl) methyl]-N-phenylhydrazinecarbothioamide (4a). Yield 64%, mp 180 °C. IR (KBr) cm\(^{-1}\): 3576 (NH\(_{str}\)), 3373 (NH\(_{str}\)), 3200 (NH\(_{str}\)-thioamide), 3089 (Ar-CH\(_{str}\)), 2957 (CH\(_{str}\)), 1602 (C=N, cyclic), 1528 (C=S\(_{str}\)); \(^1\)H NMR (CDCl\(_3\)): δ 3.66 (s, 2H, CH\(_2\)), 4.05 (s, 2H, CH\(_2\)-Ar), 6.59-7.37 (m, 10H, Ar-H), 7.01-7.67 (m, 4H, Bz-H), 8.46 (s, 1H, NH), 8.67 (s, 1H, NH), 10.50 (s, 1H, NH-Ar, D\(_2\)O exchangeable); \(^{13}\)C NMR (DMSO-d\(_6\)) δ (ppm): 42.24, 45.63, 109.41, 117.32, 120.24, 122.62, 123.53, 124.64, 126.42, 127.12, 127.35, 127.63, 128.23, 129.52, 130.54, 130.87, 134.23, 135.12, 138.54, 148.46, 153.26, 161.24; MS: m/z 386 (M-1).

2-[(1-Benzyl-1H-benzo[d]imidazol-2-yl) methyl]-N-(2-chlorophenyl) hydrazine carbothioamide (4b). Yield 52%, mp 190 °C. IR (KBr) cm\(^{-1}\): 3525 (NH\(_{str}\)), 3345 (NH\(_{str}\)), 3265 (NH\(_{str}\)-thioamide), 3008 (Ar-CH\(_{str}\)), 2985 (CH\(_{str}\)), 1608 (C=N, cyclic), 1537 (C=S\(_{str}\)), 805 (C-Cl); \(^1\)H NMR (CDCl\(_3\)): δ 3.58 (s, 2H, CH\(_2\)), 4.25 (s, 2H, CH\(_2\)-Ar), 6.64-7.86 (m, 9H, Ar-H), 7.04-7.36 (m, 4H, Bz-H), 8.24 (s, 1H, NH, D\(_2\)O exchangeable), 8.95 (s, 1H, NH, D\(_2\)O exchangeable), 10.24 (s, 1H, NH-Ar, D\(_2\)O exchangeable); \(^{13}\)C NMR (DMSO-d\(_6\)) δ (ppm): 43.44, 45.53, 110.28, 118.22, 119.82, 122.82, 123.63, 125.25, 126.19, 127.28, 127.39, 127.84, 128.81, 129.28, 130.10, 130.92, 133.43, 135.27, 137.34, 148.28, 152.16, 163.82; MS: m/z 403 (M+2).

2-[(1-Benzyl-1H-benzo[d]imidazol-2-yl) methyl]-N-o-tolylhydrazinecarbothioamide (4c). Yield 51%, mp 175 °C. IR (KBr) cm\(^{-1}\): 3371 (NH\(_{str}\)), 3194 (NH\(_{str}\)), 3065 (NH\(_{str}\)-thioamide), 2990 (Ar-CH\(_{str}\)), 2943 (CH\(_{str}\)), 1685 (C=N, cyclic), 1540 (C=S\(_{str}\)); \(^1\)H NMR (CDCl\(_3\)): δ 2.48 (s, 3H, CH\(_3\)) 3.89 (s, 2H, CH\(_2\)), 4.09 (s, 2H, CH\(_2\)-Ar), 7.04-7.97 (m, 9H, Ar-H), 7.22-7.91 (m, 4H, Bz-H), 8.00 (s, 1H, NH, D\(_2\)O exchangeable), 9.25 (s, 1H, NH, D\(_2\)O exchangeable), 10.01 (s, 1H, NH-Ar, D\(_2\)O exchangeable); \(^{13}\)C NMR (DMSO-d\(_6\)) δ (ppm): 18.24, 41.27, 43.48, 110.17, 118.29, 119.83, 121.29, 124.10, 125.53, 126.82, 127.16, 127.23, 127.72, 128.73, 129.18, 130.28, 130.96, 133.14, 134.47, 136.17, 149.27, 151.92, 164.92; MS: m/z 403 (M+2).

2-[(1-Benzyl-1H-benzo[d]imidazol-2-yl)methyl]-N-m-tolylhydrazinecarbothioamide (4d). Yield 44%, mp 210 °C. IR (KBr) cm\(^{-1}\): 3345 (NH\(_{str}\)), 3173 (NH\(_{str}\)), 3065 (NH\(_{str}\)-thioamide), 2985 (Ar-CH\(_{str}\)), 2932 (CH\(_{str}\)), 1647 (C=N, cyclic), 1564 (C=S\(_{str}\)); \(^1\)H NMR (CDCl\(_3\)): δ 2.45 (s, 3H, CH\(_3\)) 3.58 (s, 2H, CH\(_2\)), 4.05 (s, 2H, CH\(_2\)-Ar), 7.04-7.85 (m, 9H, Ar-H), 7.12-7.99 (m, 4H, Bz-H), 8.05 (s, 1H, NH, D\(_2\)O exchangeable),
9.29 (s, 1H, NH, D$_2$O exchangeable), 10.17 (s, 1H, NH-Ar, D$_2$O exchangeable); $^{13}$C NMR (DMSO-$d_6$) δ (ppm): 17.26, 42.47, 44.29, 112.21, 117.13, 120.15, 121.72, 124.27, 125.17, 126.73, 127.33, 127.74, 127.85, 128.83, 129.92, 130.12, 130.83, 133.26, 134.18, 136.92, 147.22, 153.12, 162.61; MS: $m/z$ 401 (M+1).

2-[(1-Benzyl-1H-benzo[d]imidazol-2-yl) methyl]-N-p-tolylhydrazinocarbothioamide (4e). Yield 65%, mp 215 °C. IR (KBr) cm$^{-1}$: 3321 (NH str.), 3173 (NH str.), 3054 (NH$_{str}$-thioamide), 2974 (Ar-CH$_{str}$), 2965 (CH$_{str}$), 1627 (C=N,cyclic), 1538 (C=S str.); $^1$H NMR (CDCl$_3$): δ 2.44 (s, 3H, CH$_3$), 3.48 (s, 2H, CH$_2$), 4.04 (s, 2H, CH$_2$-Ar), 7.06-7.84 (m, 9H, Ar-H), 7.14-7.47 (m, 4H, Bz-H), 8.45 (s, 1H, NH, D$_2$O exchangeable), 9.04 (s, 1H, NH, D$_2$O exchangeable), 10.14 (s, 1H, NH-Ar, D$_2$O exchangeable); $^{13}$C NMR (DMSO-$d_6$) δ (ppm): 18.25, 40.23, 43.18, 113.41, 118.92, 120.62, 121.16, 124.68, 125.15, 126.92, 127.17, 127.82, 127.44, 128.21, 129.34, 130.28, 131.83, 132.23, 134.35, 138.32, 147.45, 152.39, 160.91; MS: $m/z$ 402 (M+1).

2-[(1-Benzyl-1H-benzo[d]imidazol-2-yl) methyl]-N-(2-methoxyphenyl) hydrazinecarbothioamide (4f). Yield 63%, mp 170 °C. IR (KBr) cm$^{-1}$: 3424 (NH str.), 3299 (NH$_{str}$), 3117 (NH$_{str}$-thioamide), 2961 (Ar-CH$_{str}$), 2901 (CH$_{str}$), 1601 (C=N,cyclic), 1536 (C=S str.), 1169 (OCH$_3$); $^1$H NMR (CDCl$_3$): δ 3.17 (s, 2H, CH$_2$), 3.40 (s, 3H, OCH$_3$), 4.15 (s, 2H, CH$_2$-Ar), 6.58-8.15 (m, 9H, Ar-H), 7.22-8.76 (m, 4H, Bz-H), 8.82 (s, 1H, NH, D$_2$O exchangeable), 9.19 (s, 1H, NH, D$_2$O exchangeable), 11.11 (s, 1H, NH-Ar, D$_2$O exchangeable); $^{13}$C NMR (DMSO-$d_6$) δ (ppm): 39.24, 42.84, 55.25, 114.26, 119.28, 120.27, 121.38, 124.84, 125.28, 126.94, 127.21, 127.23, 127.49, 128.48, 129.28, 130.47, 131.23, 133.40, 134.35, 138.24, 147.36, 150.58, 163.41; MS: $m/z$ 419 (M+2).

2-[(1-Benzyl-1H-benzo[d]imidazol-2-yl)methyl]-N-(3-methoxyphenyl) hydrazinocarbothioamide (4g). Yield 61%, mp 151 °C. IR (KBr) cm$^{-1}$: 3458 (NH$_{str}$), 3273 (NH$_{str}$), 3123 (NH$_{str}$-thioamide), 2984 (Ar-CH$_{str}$), 2928 (CH$_{str}$), 1648 (C=N,cyclic), 1521 (C=S$_{str}$), 1114 (OCH$_3$); $^1$H NMR (CDCl$_3$): δ 3.25 (s, 2H, CH$_2$), 3.63 (s, 3H, OCH$_3$), 4.27 (s, 2H, CH$_2$-Ar), 6.24-8.56 (m, 9H, Ar-H), 7.02-8.21 (m, 4H, Bz-H), 8.15 (s, 1H, NH, D$_2$O exchangeable), 9.01 (s, 1H, NH, D$_2$O exchangeable), 11.23 (s, 1H, NH-Ar, D$_2$O exchangeable); $^{13}$C NMR (DMSO-$d_6$) δ (ppm): 41.25, 43.95, 57.28, 111.37,
Chapter 4

Experimental Protocols

119.83, 120.73, 121.49, 122.39, 125.25, 126.29, 127.35, 127.57, 127.21, 128.24, 129.35, 130.64, 131.24, 133.87, 137.25, 141.23, 148.26, 150.35, 165.45.

2-[(1-Benzyl-1H-benzo[d]imidazol-2-yl)methyl]-N-(4-methoxyphenyl) hydrazine carbothioamide (4h). Yield 59 %, mp 200 °C. IR (KBr) cm⁻¹: 3445 (NH str.), 3273 (NH str.-thioamide), 1147 (OCH₃), 148.26, 150.35, 165.45.

Yield 59 %, mp 200 °C. IR (KBr) cm⁻¹: 3445 (NH str.), 3273 (NH str.-thioamide), 1147 (OCH₃), 148.26, 150.35, 165.45.

1H NMR (CDCl₃): δ 3.26 (s, 2H, CH₂), 3.58 (s, 3H, OCH₃), 4.15 (s, 2H, CH₂-Ar), 6.14-8.56 (m, 9H, Ar-H), 7.05-8.12 (m, 4H, Bz-H), 8.17 (s, 1H, NH, D₂O exchangeable), 9.06 (s, 1H, NH, D₂O exchangeable), 11.31 (s, 1H, NH-Ar, D₂O exchangeable); ¹³C NMR (DMSO-d₆) δ (ppm): 40.34, 45.24, 53.26, 114.26, 118.26, 120.23, 121.24, 123.34, 125.36, 127.56, 127.68, 127.48, 128.23, 129.42, 129.62, 132.54, 133.35, 139.24, 140.24, 146.36, 149.85, 164.43.

1H NMR (CDCl₃): δ 3.26 (s, 2H, CH₂), 3.58 (s, 3H, OCH₃), 4.15 (s, 2H, CH₂-Ar), 6.14-8.56 (m, 9H, Ar-H), 7.05-8.12 (m, 4H, Bz-H), 8.17 (s, 1H, NH, D₂O exchangeable), 9.06 (s, 1H, NH, D₂O exchangeable), 11.31 (s, 1H, NH-Ar, D₂O exchangeable); ¹³C NMR (DMSO-d₆) δ (ppm): 40.34, 45.24, 53.26, 114.26, 118.26, 120.23, 121.24, 123.34, 125.36, 127.56, 127.68, 127.48, 128.23, 129.42, 129.62, 132.54, 133.35, 139.24, 140.24, 146.36, 149.85, 164.43.

2-[(1-Benzyl-1H-benzo[d]imidazol-2-yl)methyl]-N-(4-methoxyphenyl) hydrazine carbothioamide (4h). Yield 59 %, mp 200 °C. IR (KBr) cm⁻¹: 3445 (NH str.), 3273 (NH str.-thioamide), 1147 (OCH₃), 148.26, 150.35, 165.45.

Yield 59 %, mp 200 °C. IR (KBr) cm⁻¹: 3445 (NH str.), 3273 (NH str.-thioamide), 1147 (OCH₃), 148.26, 150.35, 165.45.

1H NMR (CDCl₃): δ 3.26 (s, 2H, CH₂), 3.58 (s, 3H, OCH₃), 4.15 (s, 2H, CH₂-Ar), 6.14-8.56 (m, 9H, Ar-H), 7.05-8.12 (m, 4H, Bz-H), 8.17 (s, 1H, NH, D₂O exchangeable), 9.06 (s, 1H, NH, D₂O exchangeable), 11.31 (s, 1H, NH-Ar, D₂O exchangeable); ¹³C NMR (DMSO-d₆) δ (ppm): 40.34, 45.24, 53.26, 114.26, 118.26, 120.23, 121.24, 123.34, 125.36, 127.56, 127.68, 127.48, 128.23, 129.42, 129.62, 132.54, 133.35, 139.24, 140.24, 146.36, 149.85, 164.43.

1H NMR (CDCl₃): δ 3.26 (s, 2H, CH₂), 3.58 (s, 3H, OCH₃), 4.15 (s, 2H, CH₂-Ar), 6.14-8.56 (m, 9H, Ar-H), 7.05-8.12 (m, 4H, Bz-H), 8.17 (s, 1H, NH, D₂O exchangeable), 9.06 (s, 1H, NH, D₂O exchangeable), 11.31 (s, 1H, NH-Ar, D₂O exchangeable); ¹³C NMR (DMSO-d₆) δ (ppm): 40.34, 45.24, 53.26, 114.26, 118.26, 120.23, 121.24, 123.34, 125.36, 127.56, 127.68, 127.48, 128.23, 129.42, 129.62, 132.54, 133.35, 139.24, 140.24, 146.36, 149.85, 164.43.
Chapter 4

Experimental Protocols

2-[(1-(2-Chlorobenzyl)-1H-benzo[d]imidazol-2-yl)methyl]-N-o-tolylhydrazinecarbothioamide (4k). Yield 64 %, mp 300 °C. IR (KBr) cm⁻¹: 3506 (NH, NH), 3484 (NH, NH), 3463 (NH, NH), 3063 (Ar-CH), 2950 (CH), 1610 (C=N, cyclic), 1409 (C=S, 801 (C-Cl); ¹H NMR (CDCl₃): δ 2.50 (s, 3H, CH₃), 3.37 (s, 2H, CH₂), 5.24 (s, 2H, CH₂-Ar), 6.47–7.96 (m, 8H, Ar-H), 7.02–7.97 (m, 4H, Bz-H), 9.85 (s, 1H, NH, D₂O exchangeable), 10.39 (s, 1H, NH, D₂O exchangeable), 10.67 (s, 1H, NH-Ar, D₂O exchangeable); ¹³C NMR (DMSO-d₆) δ (ppm): 22.36, 39.02, 47.74, 118.87, 119.37, 120.34, 121.72, 124.87, 125.53, 126.57, 127.45, 127.93, 127.95, 128.49, 130.86, 131.09, 132.46, 133.67, 136.42, 144.67, 153.37, 158.94.

2-[(1-(2-Chlorobenzyl)-1H-benzo[d]imidazol-2-yl)methyl]-N-m-tolylhydrazinecarbothioamide (4l). Yield 56 %, mp 300 °C. IR (KBr) cm⁻¹: 3545 (NH, NH), 3444 (NH, NH), 3412 (NH, NH), 3085 (Ar-CH), 2953 (CH), 1641 (C=N, cyclic), 1485 (C=S, 805 (C-Cl); ¹H NMR (CDCl₃): δ 2.55 (s, 3H, CH₃), 3.42 (s, 2H, CH₂), 5.66 (s, 2H, CH₂-Ar), 6.63–7.95 (m, 8H, Ar-H), 7.69–7.14 (m, 4H, Bz-H), 9.12 (s, 1H, NH, D₂O exchangeable), 10.74 (s, 1H, NH, D₂O exchangeable), 10.34 (s, 1H, NH-Ar, D₂O exchangeable); ¹³C NMR (DMSO-d₆) δ (ppm): 24.35, 40.95, 44.57, 117.96, 119.85, 120.74, 121.73, 124.42, 125.58, 126.37, 127.15, 127.27, 127.34, 128.43, 129.79, 130.23, 131.42, 132.47, 135.86, 136.98, 143.35, 155.27, 158.23.

2-[(1-(2-Chlorobenzyl)-1H-benzo[d]imidazol-2-yl)methyl]-N-p-tolylhydrazinecarbothioamide (4m). Yield 53 %, mp 180 °C. IR (KBr) cm⁻¹: 3565 (NH, NH), 3484 (NH, NH), 3412 (NH, NH), 3085 (Ar-CH), 2953 (CH), 1641 (C=N, cyclic), 1442 (C=S, 805 (C-Cl); ¹H NMR (CDCl₃): δ 2.54 (s, 3H, CH₃), 3.45 (s, 2H, CH₂), 5.32 (s, 2H, CH₂-Ar), 6.52–7.85 (m, 8H, Ar-H), 7.56–7.46 (m, 4H, Bz-H), 9.85 (s, 1H, NH, D₂O exchangeable), 10.32 (s, 1H, NH, D₂O exchangeable), 10.47 (s, 1H, NH-Ar, D₂O exchangeable); ¹³C NMR (DMSO-d₆) δ (ppm): 26.67, 41.24, 46.43, 118.57, 119.34, 120.34, 121.48, 124.97, 125.97, 126.45, 127.24, 127.74, 127.64, 128.23, 129.96, 130.35, 131.75, 132.95, 135.86, 136.98, 144.78, 154.26, 157.22.

2-[(1-(2-Chlorobenzyl)-1H-benzo[d]imidazol-2-yl)methyl]-N-(2-methoxyphenyl) hydrazinecarbothioamide (4n). Yield 57 %, mp 185 °C. IR (KBr) cm⁻¹: 3497 (NH, NH), 3484 (NH, NH), 3474 (NH, NH), 3074 (Ar-CH), 2826 (CH), 1670 (C=N, cyclic), 1488 (C=S, 1105 (OCH₃), 733 (C-Cl); ¹H NMR (CDCl₃): δ 3.31 (s,
2-[1-(2-Chlorobenzyl)-1H-benzo[d]imidazol-2-yl)methyl]-N-(3-methoxyphenyl) hydrazinecarbothioamide (4o). Yield 54 %, mp 300 °C. IR (KBr) cm⁻¹: 3466 (NH_str.), 3454 (NH_str.), 3412 (NH_str.-thioamide), 3021 (Ar-CH_str.), 1654 (C=N,cyclic), 1484 (C=S_str.), 1131 (OCH₃), 745 (C-Cl); ¹H NMR (CDCl₃): δ 3.32 (s, 2H, CH₂), 3.75 (s, 3H, OCH₃), 3.56 (s, 2H, CH₂-Ar), 6.54-7.86 (m,8H, Ar-H), 7.05-7.32 (m, 4H, Bz-H), 7.85 (s, 1H, NH, D₂O exchangeable), 9.12 (s, 1H, NH, D₂O exchangeable), 10.41 (s, 1H, NH-Ar, D₂O exchangeable). ¹³C NMR (DMSO-d₆) δ(ppm): 37.24, 44.45, 59.37, 109.43, 118.32, 118.35, 120.42, 123.54, 125.53, 126.56, 127.17, 127.32, 127.35, 128.47, 129.09, 130.85, 132.56, 133.75, 137.64, 140.43, 147.32, 149.32, 165.35.

2-[1-(2-Chlorobenzyl)-1H-benzo[d]imidazol-2-yl)methyl]-N-(4-methoxyphenyl) hydrazinecarbothioamide (4p). Yield 57 %, mp 190 °C. IR (KBr) cm⁻¹: 3423 (NH_str.), 3412 (NH_str.), 3358 (NH_str.-thioamide), 3054 (Ar-CH_str.), 2896 (CH_str.), 1612 (C=N,cyclic), 1431 (C=S_str.), 1154 (OCH₃), 736 (C-Cl); ¹H NMR (CDCl₃): δ 3.12 (s, 2H, CH₂), 3.14 (s, 2H, CH₂-Ar), δ 3.50 (s, 3H, OCH₃), 6.65-7.84 (m,8H, Ar-H), 7.01-7.32 (m, 4H, Bz-H), 7.45 (s, 1H, NH, D₂O exchangeable), 9.22 (s, 1H, NH, D₂O exchangeable), 10.51 (s, 1H, NH-Ar, D₂O exchangeable). ¹³C NMR (DMSO-d₆) δ(ppm): 37.36, 44.56, 56.47, 103.38, 117.56, 118.43, 120.37, 121.23, 124.45, 125.46, 127.23, 127.37, 127.87, 128.16, 129.48, 131.48, 132.47, 133.65, 136.67, 138.36, 144.26, 146.32, 167.84.
Table 4- Physicochemical parameters of compounds 4a-p - Scheme 2

<table>
<thead>
<tr>
<th>Compd</th>
<th>R</th>
<th>R'</th>
<th>Mol. Formula</th>
<th>Log P&lt;sup&gt;b&lt;/sup&gt;</th>
<th>R&lt;sub&gt;f&lt;/sub&gt; (R&lt;sub&gt;m&lt;/sub&gt;)&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Elemental analysis %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Found</td>
<td></td>
<td>C</td>
</tr>
<tr>
<td>4a</td>
<td>H</td>
<td>H</td>
<td>C&lt;sub&gt;22&lt;/sub&gt;H&lt;sub&gt;21&lt;/sub&gt;N&lt;sub&gt;5&lt;/sub&gt;S</td>
<td>4.21(3.99)</td>
<td>0.79(-0.57)</td>
<td>68.19</td>
</tr>
<tr>
<td>4b</td>
<td>H</td>
<td>2-Cl</td>
<td>C&lt;sub&gt;22&lt;/sub&gt;H&lt;sub&gt;20&lt;/sub&gt;ClN&lt;sub&gt;5&lt;/sub&gt;S</td>
<td>4.57(4.70)</td>
<td>0.82(-0.65)</td>
<td>62.62</td>
</tr>
<tr>
<td>4c</td>
<td>H</td>
<td>2-CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>C&lt;sub&gt;23&lt;/sub&gt;H&lt;sub&gt;23&lt;/sub&gt;N&lt;sub&gt;5&lt;/sub&gt;S</td>
<td>4.20(4.48)</td>
<td>0.80(-0.60)</td>
<td>68.80</td>
</tr>
<tr>
<td>4d</td>
<td>H</td>
<td>3-CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>C&lt;sub&gt;23&lt;/sub&gt;H&lt;sub&gt;23&lt;/sub&gt;N&lt;sub&gt;5&lt;/sub&gt;S</td>
<td>4.38(4.48)</td>
<td>0.90(-0.95)</td>
<td>68.62</td>
</tr>
<tr>
<td>4e</td>
<td>H</td>
<td>4-CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>C&lt;sub&gt;23&lt;/sub&gt;H&lt;sub&gt;23&lt;/sub&gt;N&lt;sub&gt;5&lt;/sub&gt;S</td>
<td>4.21(4.48)</td>
<td>0.91(-1.00)</td>
<td>68.95</td>
</tr>
<tr>
<td>4f</td>
<td>H</td>
<td>2-OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>C&lt;sub&gt;23&lt;/sub&gt;H&lt;sub&gt;23&lt;/sub&gt;N&lt;sub&gt;5&lt;/sub&gt;OS</td>
<td>4.19(3.90)</td>
<td>0.94(-1.19)</td>
<td>66.16</td>
</tr>
<tr>
<td>4g</td>
<td>H</td>
<td>3-OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>C&lt;sub&gt;23&lt;/sub&gt;H&lt;sub&gt;23&lt;/sub&gt;N&lt;sub&gt;5&lt;/sub&gt;OS</td>
<td>4.14(3.90)</td>
<td>0.83(-0.68)</td>
<td>66.56</td>
</tr>
<tr>
<td>4h</td>
<td>H</td>
<td>4-OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>C&lt;sub&gt;23&lt;/sub&gt;H&lt;sub&gt;23&lt;/sub&gt;N&lt;sub&gt;5&lt;/sub&gt;OS</td>
<td>4.10(3.90)</td>
<td>0.94(-1.19)</td>
<td>66.34</td>
</tr>
<tr>
<td>4i</td>
<td>2-Cl</td>
<td>H</td>
<td>C&lt;sub&gt;22&lt;/sub&gt;H&lt;sub&gt;20&lt;/sub&gt;ClN&lt;sub&gt;5&lt;/sub&gt;S</td>
<td>4.67(4.70)</td>
<td>0.83(-0.68)</td>
<td>62.60</td>
</tr>
<tr>
<td>4j</td>
<td>2-Cl</td>
<td>2-Cl</td>
<td>C&lt;sub&gt;22&lt;/sub&gt;H&lt;sub&gt;19&lt;/sub&gt;Cl&lt;sub&gt;2&lt;/sub&gt;N&lt;sub&gt;5&lt;/sub&gt;S</td>
<td>4.33(4.41)</td>
<td>0.94(-1.19)</td>
<td>57.90</td>
</tr>
<tr>
<td>4k</td>
<td>2-Cl</td>
<td>2-CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>C&lt;sub&gt;23&lt;/sub&gt;H&lt;sub&gt;22&lt;/sub&gt;ClN&lt;sub&gt;5&lt;/sub&gt;S</td>
<td>5.16(5.20)</td>
<td>0.77(-0.52)</td>
<td>63.36</td>
</tr>
<tr>
<td>4l</td>
<td>2-Cl</td>
<td>3-CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>C&lt;sub&gt;23&lt;/sub&gt;H&lt;sub&gt;22&lt;/sub&gt;ClN&lt;sub&gt;5&lt;/sub&gt;S</td>
<td>5.41(5.20)</td>
<td>0.76(-0.50)</td>
<td>63.72</td>
</tr>
<tr>
<td>4m</td>
<td>2-Cl</td>
<td>4-CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>C&lt;sub&gt;23&lt;/sub&gt;H&lt;sub&gt;22&lt;/sub&gt;ClN&lt;sub&gt;5&lt;/sub&gt;S</td>
<td>5.52(5.20)</td>
<td>0.94(-1.19)</td>
<td>63.52</td>
</tr>
<tr>
<td>4n</td>
<td>2-Cl</td>
<td>2-OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>C&lt;sub&gt;23&lt;/sub&gt;H&lt;sub&gt;22&lt;/sub&gt;ClN&lt;sub&gt;5&lt;/sub&gt;OS</td>
<td>4.25(4.62)</td>
<td>0.77(-0.52)</td>
<td>61.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>4o</td>
<td>2-Cl</td>
<td>3-OCH₃</td>
<td>C₂₃H₂₂ClN₅OS</td>
<td>4.44(4.62)</td>
<td>0.94(-1.19)</td>
<td>61.36</td>
</tr>
<tr>
<td>4p</td>
<td>2-Cl</td>
<td>4-OCH₃</td>
<td>C₂₃H₂₂ClN₅OS</td>
<td>4.51(4.62)</td>
<td>0.78(-0.54)</td>
<td>61.02</td>
</tr>
</tbody>
</table>

**a** Solvent of crystallization—Ethanol.

**b** Log P was determined by octanol:phosphate buffer method; CLog P was calculated using software ChemDraw Ultra 8.0

**c** Solvent system—Toluene: Ethyl acetate: Formic acid (5:4:1).

**d** A logarithmic function of Rᵢ value was also calculated; \( Rₘ = \log (1/Rᵢ) \).

**e** Elemental analysis for C, H, N were within ± 0.4% of the theoretical value.
Reagents and conditions: (i) HNO₃, H₂SO₄, heat at 60 °C, 1h (ii) Anhy. K₂CO₃, acetone, sub. benzyl chloride, stirring, 8h (iii) Iron powder, HCl, Heat at 80 °C, stirring 4h (iv) GAA, NaOCN, cool in ice (v) Aryl isothiocyanates, Anhy.K₂CO₃, abs. ethanol, reflux, 5-6h

Fig. 7: Synthetic route to the compounds 5a-p – Scheme 3
Synthetic Methods- Scheme 3

General procedure for the synthesis of titled compounds 5a-p

5-Nitroindoline-2, 3-dione (1)

Compound indoline-2,3-dione (48.5 g, 0.33 mol) was added into a mixture of 50g (35 mL, 0.5 mol) of conc. nitric acid and 74g (40 mL, 0.75 mol) conc. sulfuric acid with frequent shaking in 500 mL round bottomed flask, keeping the mixture cool by immersing the flask in ice cold water. After adding all indoline-2,3-dione, flask was fitted with reflux condenser and the mixture was heated on water bath maintaining the temperature at 60°C for 1h to obtain the desired compound 5-nitroindoline-2, 3-dione. Content was then poured into a beaker containing 500 mL cold water, stirred in order to wash out as much as acid from the desired compound. When compound (1) completely settled to the bottom, the upper acid liquor was completely poured off from the mixture. Then the residual liquid was transferred to the separating funnel and shaked vigorously with about 50 mL of water. Bottom layer was collected, dried with anhydrous calcium chloride and finally filtered to obtain the pure compound (1). Yield 62%, mp 230 °C. IR (KBr) cm⁻¹: 3306 (NH str.), 3015 (Ar-CH str.), 1725 (C=O, Isatin), 1563 (NO str.-sym.), 1325 (NO str.-asym.), ¹H NMR (CDCl₃) δ(ppm): 7.12-7.89 (m, 3H, Ar-H), 9.53 (s, 1H, NH-Isatin); ¹³C NMR (DMSO-d₆) δ(ppm): 107.34, 114.46, 127.24, 129.83, 144.24, 152.73, 160.26, 178.45.

5-Nitro-1-substituted benzylindoline-2, 3-dione (2a,b)

To a solution of compound 1 (0.01 mol) and anhydrous potassium carbonate (0.01mol) in acetone (30 ml), substituted benzyl chloride (0.01 mol) was added drop wise. The mixture was stirred at room temp for about 8 h. The mixture was then poured into water and extracted with ethyl acetate, dried over sodium sulfate anhydrous and concentrated under vacuum to give the pure compound. Desired compound 2a,b was finally recrystallized with ethanol.

5-Nitro-1- benzylindoline-2, 3-dione (2a) Yield 60%, mp 260 °C. IR (KBr) cm⁻¹: 3063 (Ar-CH str.), 2825 (CH str.), 1723 (C=O, Isatin), 1548 (NO str.-sym.), 1352 (NO str.-asym.), ¹H NMR (CDCl₃) δ(ppm): 4.23 (s, 2H, CH₂-Ar), 7.01-7.78 (m, 8H, Ar-H), ¹³C NMR (DMSO-d₆) δ(ppm): 48.32, 120.43, 122.78, 124.26, 124.28, 125.10, 127.25, 129.23, 129.54, 130.13, 138.52, 143.27, 156.38, 158.34, 183.35.
1-(2-Chlorobenzyl)-5-nitroindoline-2,3-dione (2b) Yield 62%, mp 275 °C. IR (KBr) cm\(^{-1}\): 3037 (Ar-\text{CH} \text{str.}), 2818 (CH\text{str.}), 1690 (C=O, Isatin), 1540 (NO\text{str.-sym.}), 1348 (NO\text{str.-asym.}), 725 (C-Cl); \(^1\)H NMR (CDCl\(_3\)) δ(ppm): 4.15 (s, 2H, CH\(_2\)-Ar), 7.23-7.85 (m, 7H, Ar-H); \(^13\)C NMR (DMSO-d\(_6\)) δ(ppm): 47.45, 122.48, 123.36, 124.24, 126.48, 127.12, 129.37, 129.01, 131.27, 132.37, 137.72, 142.67, 157.38, 158.92, 183.35.

5-Amino-1-substituted benzylindoline-2, 3-dione (3a,b)
Compound 2a,b (0.01 mol) and iron powder (0.01 mol) was placed in round bottom flask containing 200 mL ethanol. The mixture was heated on oil bath till temperature attained to 80-85 °C. Then 4 mL of HCl (1.2 M) was added and content was further stirred for 4h while maintaining the temperature. Content was then filtered at reduced pressure and the filtrate was adjusted to pH 7-8 using NaHCO\(_3\) to get the precipitate of compound 3a,b. It was then recrystallized with ethanol.

5-Amino-1-benzylindoline-2,3-dione (3a) Yield 71%, mp 265 °C. IR (KBr) cm\(^{-1}\): 3265 (NH\text{str.}), 3125 (Ar-\text{CH} \text{str.}), 2848 (CH\text{str.}), 1764 (C=O, Isatin); \(^1\)H NMR (CDCl\(_3\)) δ(ppm): 3.76 (s, 1H, NH), 4.15 (s, 2H, CH\(_2\)-Ar), 7.21-7.89 (m, 8H, Ar-H); \(^13\)C NMR (DMSO-d\(_6\)) δ(ppm): 46.27, 121.46, 123.59, 124.38, 124.75, 125.79, 126.28, 129.38, 129.41, 130.27, 138.56, 144.28, 157.48, 159.14, 182.65.

1-(2-Chlorobenzyl)-5-aminoindoline-2,3-dione (3b) Yield 68%, mp 272 °C. IR (KBr) cm\(^{-1}\): 3356 (NH\text{str.}), 3234 (Ar-\text{CH} \text{str.}), 2858 (CH\text{str.}), 1726 (C=O, Isatin), 738 (C-Cl); \(^1\)H NMR (CDCl\(_3\)) δ(ppm): 3.65 (s, 1H, NH), 4.02 (s, 2H, CH\(_2\)-Ar), 6.84-7.85 (m, 7H, Ar-H); \(^13\)C NMR (DMSO-d\(_6\)) δ(ppm): 45.48, 121.26, 123.59, 124.38, 124.75, 125.79, 126.28, 129.38, 129.47, 129.46, 130.28, 138.18, 144.29, 157.27, 159.17, 184.15.

1-(1-Substituted benzyl-2, 3-dioxoindolin-5-yl) urea (4a,b)
Compound 3a,b (0.01 mol) was dissolved in 10 mL glacial acetic acid and was diluted to 100 mL in a conical flask. To it a solution of NaOCN (0.01 mol) and 50 mL warm water was added with continuous shaking. Content was allowed to stand for 30 min, then cooled in ice, allowed to stand for further 30 min, filtered at the pump, washed with water and dried at 100°C. The desired compound 4a,b were formed and recrystallized with ethanol.
Chapter 4

Experimental Protocols

1-(1-Benzyl-2,3-dioxoindolin-5-yl)urea (4a) Yield 74%, mp 282 °C. IR (KBr) cm⁻¹: 3325 (NHstr.), 3137 (Ar-CH,str.), 2839 (CHstr.), 1785 (C=O, Isatin), 1625 (C=O, Urea); ¹H NMR (CDCl₃) δ(ppm): 4.26 (s, 1H, NH-Urea, D₂O exchangeable), 4.85 (s, 2H, CH₂-Ar), 6.57-7.83 (m, 8H, Ar-H), 8.42 (s, 1H, NH-Isatin, D₂O exchangeable); ¹³C NMR (DMSO-d₆) δ(ppm): 49.25, 121.28, 122.38, 124.27, 124.36, 125.48, 127.38, 129.25, 130.42, 132.21, 137.52, 144.47, 156.43, 157.44, 162.18, 184.15.

1-[1-(2-Chlorobenzyl)-2,3-dioxoindolin-5-yl]urea (4b) Yield 78%, mp 295 °C. IR (KBr) cm⁻¹: 3364 (NHstr.), 3128 (Ar-CH,str.), 2848 (CHstr.), 1728 (C=O, Isatin), 1612 (C=O, Urea), 710 (C-Cl); ¹H NMR (CDCl₃) δ(ppm): 4.12 (s, 1H, NH-Urea, D₂O exchangeable), 4.63 (s, 2H, CH₂-Ar), 6.78-7.84 (m, 7H, Ar-H), 8.37 (s, 1H, NH-Isatin, D₂O exchangeable); ¹³C NMR (DMSO-d₆) δ(ppm): 52.67, 120.46, 123.78, 124.25, 124.94, 125.28, 127.17, 129.37, 130.51, 134.61, 138.92, 146.42, 156.74, 159.47, 168.37, 186.35.

1-(Amino-N-arylmethanethio)-3-(1-substituted benzyl-2, 3-dioxoindolin-5-yl) urea (5a-p)

A mixture of compound 4a,b (0.01 mol), anhydrous potassium carbonate and substituted phenylisothiocyanates (0.01 mol) in 20 mL of absolute ethanol was refluxed for 5–6 h. After completion of the reaction the reaction, mixture was concentrated and kept overnight at room temperature. The needle shaped crystals thus obtained were purified by repeated washing with petroleum ether. Desired titled compounds 5a-p was recrystallized with ethanol. The physicochemical parameters of all the final compounds are presented in Table 5.

1-(Amino-N-phenylmethanethio)-3-(1-benzyl-2, 3-dioxoindolin-5-yl) urea (5a) Yield 67%, mp 300 °C. IR (KBr) cm⁻¹: 3505 (NHstr.), 2999 (NHstr.), 2955 (NHstr.), 2918 (Ar-CHstr.), 2850 (CHstr.), 1758 (C=O, Isatin), 1458 (C=O, amide), 1427 (C=Sstr.); ¹H NMR (CDCl₃) δ(ppm): 3.85 (s, 2H, CH₂-Ar), 7.00-7.89 (m,10H, Ar-H), 6.97-7.96 (m, 3H, ArH-Isatin), 7.98 (s, 1H, NH, D₂O exchangeable), 9.08 (s, 1H, NH-Ar, D₂O exchangeable), 10.72 (s, 1H, NH-Isatin, D₂O exchangeable); ¹³C NMR (DMSO-d₆) δ(ppm): 46.21, 109.51, 115.82, 119.54, 121.56, 123.62, 124.21, 124.28, 126.24, 126.47, 128.02, 128.21, 128.34, 129.71, 129.73, 134.72, 137.21, 141.32, 149.51, 152.32, 158.84, 182.36, 183.67; MS: m/z 429(M-1)
Experimental Protocols

1-[(Amino-N-(2-chlorophenyl) methanethio)-3-(1-benzyl-2, 3-dioxoindolin-5-yl) urea (5b) Yield 59%, mp 230 °C. IR (KBr) cm⁻¹: 3525 (NH_str.), 3095 (NH_str.), 3055 (NH_str.), 2958 (Ar-CH_str.), 2950 (CH_str.), 1752 (C=O, Isatin), 1658 (C=O, amide), 1627 (C=S_str.), 665 (C-Cl); ¹H NMR (CDCl₃) δ(ppm): 3.82 (s, 2H, CH₂-Ar), 7.25-7.88 (m, 9H, Ar-H), 6.93-7.68 (m, 3H, ArH-Isatin), 8.96 (s, 1H, NH, D₂O exchangeable), 9.78 (s, 1H, NH-Ar, D₂O exchangeable), 10.89 (s, 1H, NH-Isatin, D₂O exchangeable); ¹³C NMR (DMSO-d₆) δ(ppm): 45.25, 108.32, 114.72, 118.57, 120.45, 122.67, 123.27, 124.51, 126.32, 126.48, 127.14, 128.16, 128.34, 129.32, 129.58, 133.74, 136.46, 140.27, 148.12, 151.37, 157.59, 181.46, 182.64.

1-(Amino-N-o-tolylmethanethio)-3-(1-benzyl-2,3-dioxoindolin-5-yl)urea (5c) Yield 77%, mp 120 °C. IR (KBr) cm⁻¹: 3386 (NH_str.), 3268 (NH_str.), 3071 (NH_str.), 2924 (Ar-CH_str.), 2854 (CH_str.), 1707 (C=O, Isatin), 1640 (C=O, amide), 1606 (C=S_str.); ¹H NMR (CDCl₃) δ(ppm): 2.27 (s, 3H, CH₃), 3.80 (s, 2H, CH₂-Ar), 7.01-7.80 (m, 9H, Ar-H), 6.98-7.93 (m, 3H, ArH-Isatin), 8.83 (s, 1H, NH, D₂O exchangeable), 9.09 (s, 1H, NH-Ar, D₂O exchangeable), 9.88 (s, 1H, NH-Isatin, D₂O exchangeable); ¹³C NMR (DMSO-d₆) δ(ppm): 17.68, 46.54, 107.24, 115.47, 118.32, 121.52, 123.52, 123.87, 124.42, 125.24, 126.84, 127.01, 127.54, 128.34, 129.28, 132.34, 135.67, 141.74, 148.12, 150.23, 157.59, 181.14, 181.54.

1-(Amino-N-m-tolylmethanethio)-3-(1-benzyl-2,3-dioxoindolin-5-yl)urea (5d) Yield 47%, mp 110 °C. IR (KBr) cm⁻¹: 3356 (NH_str.), 3224 (NH_str.), 3053 (NH_str.), 2901 (Ar-CH_str.), 2824 (CH_str.), 1704 (C=O, Isatin), 1612 (C=O, amide), 1604 (C=S_str.); ¹H NMR (CDCl₃) δ(ppm): 2.22 (s, 3H, CH₃), 3.76 (s, 2H, CH₂-Ar), 7.23-7.89 (m, 9H, Ar-H), 6.35-7.98 (m, 3H, ArH-Isatin), 8.87 (s, 1H, NH, D₂O exchangeable), 9.64 (s, 1H, NH-Ar, D₂O exchangeable), 9.69 (s, 1H, NH-Isatin, D₂O exchangeable); ¹³C NMR (DMSO-d₆) δ(ppm): 16.54, 45.64, 106.24, 114.26, 117.56, 122.24, 122.42, 123.48, 123.84, 124.34, 125.12, 125.23, 128.45, 128.63, 128.85, 129.02, 131.34, 134.37, 142.14, 147.15, 151.22, 156.57, 182.17, 183.54.

1-(Amino-N-p-tolylmethanethio)-3-(1-benzyl-2,3-dioxoindolin-5-yl)urea (5e) Yield 63%, mp 57 °C. IR (KBr) cm⁻¹: 3352 (NH_str.), 3202 (NH_str.), 3053 (NH_str.), 2942 (Ar-CH_str.), 2814 (CH_str.), 1715 (C=O, Isatin), 1614 (C=O, amide), 1602 (C=S_str.); ¹H
NMR (CDCl₃) δ(ppm): 2.12 (s, 3H, CH₃), 3.89 (s, 2H, CH₂-Ar), 7.23-7.78 (m, 9H, Ar-H), 6.89-7.69 (m, 3H, ArH-Isatin), 8.89 (s, 1H, NH, D₂O exchangeable), 9.35 (s, 1H, NH-Ar, D₂O exchangeable), 9.97 (s, 1H, NH-Isatin, D₂O exchangeable); ¹³C NMR (DMSO-d₆) δ(ppm): 16.24, 44.14, 105.27, 113.54, 118.03, 121.84, 122.64, 123.21, 123.87, 125.24, 125.42, 126.24, 127.26, 128.74, 128.87, 129.46, 130.41, 133.37, 143.14, 146.54, 151.84, 156.14, 183.74, 184.34.

1-[Amino-N-(2-methoxyphenyl) methanethio]-3-(1-benzyl-2,3-dioxoindolin-5-yl)urea (5f) Yield 87%, mp 132 °C. IR (KBr) cm⁻¹: 3390 (NH str.), 2924 (NH str.), 2853 (NH str.), 2828 (Ar-CH str.), 2815 (CH str.), 1710 (C=O, Isatin), 1692 (C=O, amide), 1639 (C=S str.), 1115 (OCH₃); ¹H NMR (CDCl₃) δ(ppm): 3.32 (s, 3H, OCH₃), 3.36 (s, 2H, CH₂-Ar), 6.73-7.82 (m, 9H, Ar-H), 6.70-7.97 (m, 3H, ArH-Isatin), 7.98 (s, 1H, NH, D₂O exchangeable), 9.01 (s, 1H, NH-Ar, D₂O exchangeable), 10.59 (s, 1H, NH-Isatin, D₂O exchangeable); ¹³C NMR (DMSO-d₆) δ(ppm): 42.56, 56.42, 108.24, 114.34, 120.05, 122.74, 122.81, 123.01, 123.86, 125.27, 125.84, 126.22, 127.24, 128.07, 128.14, 129.01, 130.37, 135.31, 145.04, 148.53, 150.54, 159.17, 182.72, 186.37.

1-[Amino-N-(3-methoxyphenyl)methanethio]-3-(1-benzyl-2,3-dioxoindolin-5-yl)urea (5g) Yield 68 %, mp 205 °C. IR (KBr) cm⁻¹: 3385 (NH str.), 2945 (NH str.), 2885 (NH str.), 2824 (Ar-CH str.), 2815 (CH str.), 1710 (C=O, Isatin), 1692 (C=O, amide), 1621 (C=S str.), 1118 (OCH₃); ¹H NMR (CDCl₃) δ(ppm): 3.52 (s, 3H, OCH₃), 3.57 (s, 2H, CH₂-Ar), 6.58-7.87 (m, 9H, Ar-H), 6.86-7.69 (m, 3H, ArH-Isatin), 7.69 (s, 1H, NH, D₂O exchangeable), 9.38 (s, 1H, NH-Ar, D₂O exchangeable), 10.48 (s, 1H, NH-Isatin, D₂O exchangeable); ¹³C NMR (DMSO-d₆) δ(ppm): 40.25, 54.34, 106.45, 116.37, 121.95, 122.34, 122.94, 123.45, 123.83, 125.28, 125.35, 126.25, 127.54, 128.64, 128.54, 129.21, 130.34, 134.62, 144.03, 146.54, 148.24, 158.13, 181.77, 185.34.

1-[Amino-N-(4-methoxyphenyl) methanethio]-3-(1-benzyl-2,3-dioxoindolin-5-yl)urea (5h) Yield 55 %, mp 206 °C. IR (KBr) cm⁻¹: 3384 (NH str.), 2936 (NH str.), 2895 (NH str.), 2858 (Ar-CH str.), 2814 (CH str.), 1712 (C=O, Isatin), 1643 (C=O, amide), 1612 (C=S str.), 1165 (OCH₃); ¹H NMR (CDCl₃) δ(ppm): 3.56 (s, 3H, OCH₃), 3.89 (s, 2H, CH₂-Ar), 6.45-7.89 (m, 9H, Ar-H), 6.58-7.76 (m, 3H, ArH-Isatin), 7.57 (s, 1H, NH, D₂O exchangeable), 9.54 (s, 1H, NH-Ar, D₂O exchangeable), 10.37 (s, 1H, NH-Isatin, D₂O exchangeable); ¹³C NMR (DMSO-d₆) δ(ppm): 38.24, 56.38, 104.46, 117.32,
121.04, 122.32, 122.74, 123.36, 123.82, 125.11, 125.34, 126.26, 127.01, 127.65,
128.24, 129.45, 130.34, 134.14, 142.01, 145.53, 146.24, 156.37, 182.74, 183.35.

1-[1-(2-Chlorobenzyl)-2,3-dioxoindolin-5-yl]-3-(amino-N-phenylmethanethio)urea (5i)
Yield 68%, mp 185 °C. IR (KBr) cm⁻¹: 3451 (NH str.), 3259 (NH str.), 3086 (NH str.),
2924 (Ar-CH str.), 2950 (CH str.), 1753 (C≡O, Isatin), 1631 (C≡O, amide), 1527
(C≡S str.), 646 (C-Cl); ¹H NMR (CDCl₃) δ (ppm): 3.37 (s, 2H, CH₂–Ar), 6.50–7.97
(m, 9H, Ar–H), 6.63–7.96 (m, 3H, ArH–Isatin), 9.85 (s, 1H, NH, D₂O exchangeable),
10.39 (s, 1H, NH–Ar, D₂O exchangeable), 10.67 (s, 1H, NH–Isatin, D₂O exchangeable);
¹³C NMR (DMSO-d₆) δ (ppm): 51.37, 102.64, 115.56, 120.57, 121.24, 122.54, 123.76,
123.87, 124.31, 125.45, 126.37, 127.41, 127.85, 128.31, 129.01, 130.24, 133.42,
141.24, 144.57, 145.38, 150.08, 180.77, 186.65; MS: m/z 463(M-1).

1-[1-(2-Chlorobenzyl)-2,3-dioxoindolin-5-yl]-3-(amino-N-(2-chlorophenyl)
methanethio) urea (5j) Yield 68%, mp 185 °C. IR (KBr) cm⁻¹: 3456 (NH str.), 3245
(NH str.), 3062 (NH str.), 2936 (Ar-CH str.), 2912 (CH str.), 1723 (C≡O, Isatin), 1648
(C≡O, amide), 1623 (C≡S str.), 684 (C-Cl); ¹H NMR (CDCl₃) δ (ppm): 3.59 (s, 2H,
CH₂–Ar), 6.54–7.59 (m, 8H, Ar–H), 6.59–7.48 (m, 3H, ArH–Isatin), 9.39 (s, 1H, NH,
D₂O exchangeable), 10.48 (s, 1H, NH–Ar, D₂O exchangeable), 11.67 (s, 1H, NH–Isatin,
D₂O exchangeable); ¹³C NMR (DMSO-d₆) δ (ppm): 52.24, 98.54, 110.25, 118.53, 120.53,
121.14, 123.06, 123.32, 124.14, 125.27, 126.19, 127.38, 127.48, 128.51, 129.34,
130.25, 133.40, 142.27, 143.25, 145.37, 151.24, 182.37, 188.39; MS: m/z 463(M-1).

1-[1-(2-Chlorobenzyl)-2,3-dioxoindolin-5-yl]-3-(amino-N-o-tolylmethanethio) urea (5k)
Yield 82%, mp 215 °C. IR (KBr) cm⁻¹: 3456 (NH str.), 3245 (NH str.), 3090
(NH str.), 2925 (Ar-CH str.), 2728 (CH str.), 1707 (C≡O, Isatin), 1633 (C≡O, amide), 1534
(C≡S str.), 644 (C-Cl); ¹H NMR (CDCl₃) δ (ppm): 2.50 (s, 3H, CH₃), 3.31 (s, 2H, CH₂–
Ar), 7.16-8.20 (m, 8H, Ar–H), 7.16-8.23 (m, 3H, ArH–Isatin), 8.76 (s, 1H, NH, D₂O
exchangeable), 10.42 (s, 1H, NH–Ar, D₂O exchangeable), 11.52 (s, 1H, NH–Isatin,
D₂O exchangeable); ¹³C NMR (DMSO-d₆) δ (ppm): 18.54, 55.32, 96.46, 108.12,
117.34, 121.34, 122.39, 123.01, 123.34, 124.44, 125.02, 126.67, 127.23, 127.65,
128.24, 129.03, 130.54, 132.41, 143.24, 143.85, 145.24, 152.65, 183.87, 189.34.
Chapter 4

Experimental Protocols

1-[(2-Chlorobenzyl)-2,3-dioxoindolin-5-yl]-3-(amino-N-m-tolylmethanethio)urea (5l) Yield 58%, mp 300 °C. IR (KBr) cm⁻¹: 3452 (NH_str.), 3212 (NH_str.), 3035 (NH_str.), 2985 (Ar-CH_str.), 2941 (CH_str.), 1732 (C=O, Isatin), 1645 (C=O, amide), 1627 (C=S_str.), 656 (C-Cl); ¹H NMR (CDCl₃) δ(ppm): 2.69 (s, 3H, CH₃), 3.78 (s, 2H, CH₂-Ar), 7.34-8.53 (m, 8H, Ar-H), 7.25-8.57 (m, 3H, ArH-Isatin), 8.69 (s, 1H, NH, D₂O exchangeable), 10.38 (s, 1H, NH-Ar, D₂O exchangeable), 11.12 (s, 1H, NH-Isatin, D₂O exchangeable); ¹³C NMR (DMSO-d₆) δ(ppm): 17.32, 54.75, 92.32, 105.14, 116.94, 120.54, 121.32, 123.23, 123.54, 124.14, 125.24, 126.64, 127.24, 127.65, 128.24, 129.34, 130.57, 133.71, 143.64, 144.25, 145.74, 150.64, 185.37, 188.32.

1-[(2-Chlorobenzyl)-2,3-dioxoindolin-5-yl]-3-(amino-N-p-tolylmethanethio)urea (5m) Yield 57%, mp 260 °C. IR (KBr) cm⁻¹: 3453 (NH_str.), 3254 (NH_str.), 3068 (NH_str.), 2941 (Ar-CH_str.), 2935 (CH_str.), 1782 (C=O, Isatin), 1635 (C=O, amide), 1595 (C=S_str.), 638 (C-Cl); ¹H NMR (CDCl₃) δ(ppm): 2.35 (s, 3H, CH₃), 3.58 (s, 2H, CH₂-Ar), 7.34-8.68 (m, 8H, Ar-H), 7.24-8.58 (m, 3H, ArH-Isatin), 8.79 (s, 1H, NH, D₂O exchangeable), 10.48 (s, 1H, NH-Ar, D₂O exchangeable), 11.27 (s, 1H, NH-Isatin, D₂O exchangeable); ¹³C NMR (DMSO-d₆) δ(ppm): 16.22, 55.73, 96.56, 111.15, 117.34, 121.24, 122.33, 123.53, 123.64, 124.04, 125.45, 126.85, 127.32, 127.58, 128.21, 129.02, 130.37, 132.51, 144.14, 144.27, 145.64, 151.65, 186.17, 189.31.

1-[(2-Chlorobenzyl)-2,3-dioxoindolin-5-yl]-3-(amino-N-(2-methoxyphenyl)methanethio)urea (5n) Yield 75%, mp 123 °C. IR (KBr) cm⁻¹: 3454 (NH_str.), 3394 (NH_str.), 3080 (NH_str.), 2924 (Ar-CH_str.), 2912 (CH_str.), 1712 (C=O, Isatin), 1692 (C=O, amide), 1633 (C=S_str.), 1175 (OCH₃), 709 (C-Cl); ¹H NMR (CDCl₃) δ(ppm): 3.40 (s, 2H, CH₂-Ar), 3.73 (s, 3H, OCH₃), 7.22-8.36 (m, 8H, Ar-H), 6.58-8.76 (m, 3H, ArH-Isatin), 8.82 (s, 1H, NH, D₂O exchangeable), 9.19 (s, 1H, NH-Ar, D₂O exchangeable), 11.11 (s, 1H, NH-Isatin, D₂O exchangeable); ¹³C NMR (DMSO-d₆) δ(ppm): 41.85, 54.73, 94.31, 110.14, 116.42, 120.27, 122.34, 123.25, 123.84, 124.21, 125.34, 126.51, 127.33, 127.57, 128.64, 129.31, 130.35, 133.54, 144.24, 144.28, 145.24, 153.65, 184.13, 188.41.

1-[(2-Chlorobenzyl)-2,3-dioxoindolin-5-yl]-3-(amino-N-(3-methoxyphenyl)methanethio)urea (5o) Yield 52%, mp 240 °C. IR (KBr) cm⁻¹: 3456 (NH_str.), 3342 (NH_str.), 3036 (NH_str.), 2985 (Ar-CH_str.), 2957 (CH_str.), 1732 (C=O, Isatin), 1684
(C=O, amide), 1612 (C=S str.), 1123 (OCH₃), 704 (C-Cl); ¹H NMR (CDCl₃): δ(ppm): 3.57 (s, 2H, CH₂-Ar), 3.70 (s, 3H, OCH₃), 7.56-8.79 (m, 8H, Ar-H), 6.23-8.69 (m, 3H, ArH-Isatin), 8.58 (s, 1H, NH, D₂O exchangeable), 9.48 (s, 1H, NH-Ar, D₂O exchangeable), 11.35 (s, 1H, NH-Isatin, D₂O exchangeable); ¹³C NMR (DMSO-d₆): δ(ppm): 43.32, 56.53, 96.41, 112.16, 115.52, 121.67, 122.44, 123.45, 123.49, 124.20, 125.34, 126.21, 127.51, 127.58, 128.21, 129.34, 130.75, 132.64, 143.44, 144.35, 145.28, 154.67, 189.33, 189.47.

1-[1-(2-Chlorobenzyl)-2,3-dioxoindolin-5-yl]-3-[amino-N-(4-methoxyphenyl) methanethio] urea (5p) Yield 58%, mp 197 °C. IR (KBr) cm⁻¹: 3484 (NH str.), 3336 (NH str.), 3085 (NH str.), 2942 (Ar-CH str.), 2910 (CH str.), 1714 (C=O, Isatin), 1658 (C=O, amide), 1623 (C=S str.), 1141 (OCH₃), 712 (C-Cl); ¹H NMR (CDCl₃): δ(ppm): 3.52 (s, 3H, OCH₃), 3.58 (s, 2H, CH₂-Ar), 7.45-8.69 (m, 8H, Ar-H), 6.12-8.49 (m, 3H, ArH-Isatin), 8.37 (s, 1H, NH, D₂O exchangeable), 9.57 (s, 1H, NH-Ar, D₂O exchangeable), 11.58 (s, 1H, NH-Isatin, D₂O exchangeable); ¹³C NMR (DMSO-d₆): δ(ppm): 41.72, 55.43, 99.31, 113.56, 114.58, 122.68, 123.47, 123.65, 123.75, 124.32, 125.44, 126.31, 127.57, 127.59, 128.01, 129.44, 131.74, 132.74, 143.74, 144.39, 148.18, 154.68, 188.53, 189.45.
<table>
<thead>
<tr>
<th>Compd</th>
<th>R</th>
<th>R'</th>
<th>$^a$Mol. Formula</th>
<th>Log P$^b$</th>
<th>$^c$R$_f$ (R$_m$)$^d$</th>
<th>$^e$Elemental analysis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5a</td>
<td>H</td>
<td>H</td>
<td>C$<em>{23}$H$</em>{18}$N$_4$O$_3$S</td>
<td>2.48 (2.57)</td>
<td>0.94(-1.19)</td>
<td>64.17  4.21  13.01</td>
</tr>
<tr>
<td>5b</td>
<td>H</td>
<td>2-Cl</td>
<td>C$<em>{23}$H$</em>{17}$ClN$_4$O$_3$S</td>
<td>3.01 (3.29)</td>
<td>0.83(-0.68)</td>
<td>59.42  3.69  12.05</td>
</tr>
<tr>
<td>5c</td>
<td>H</td>
<td>2-CH$_3$</td>
<td>C$<em>{24}$H$</em>{20}$N$_4$O$_3$S</td>
<td>2.98 (3.07)</td>
<td>0.77(-0.52)</td>
<td>64.85  4.54  12.60</td>
</tr>
<tr>
<td>5d</td>
<td>H</td>
<td>3-CH$_3$</td>
<td>C$<em>{24}$H$</em>{20}$N$_4$O$_3$S</td>
<td>2.92 (3.07)</td>
<td>0.94(-1.19)</td>
<td>64.55  4.72  12.89</td>
</tr>
<tr>
<td>5e</td>
<td>H</td>
<td>4-CH$_3$</td>
<td>C$<em>{24}$H$</em>{20}$N$_4$O$_3$S</td>
<td>2.96 (3.07)</td>
<td>0.77(-0.52)</td>
<td>64.68  4.38  12.50</td>
</tr>
<tr>
<td>5f</td>
<td>H</td>
<td>2-OCH$_3$</td>
<td>C$<em>{24}$H$</em>{20}$N$_4$O$_4$S</td>
<td>2.38 (2.49)</td>
<td>0.83(-0.68)</td>
<td>62.60  4.38  12.17</td>
</tr>
<tr>
<td>5g</td>
<td>H</td>
<td>3-OCH$_3$</td>
<td>C$<em>{24}$H$</em>{20}$N$_4$O$_4$S</td>
<td>2.40 (2.49)</td>
<td>0.94(-1.19)</td>
<td>62.95  4.62  12.39</td>
</tr>
<tr>
<td>5h</td>
<td>H</td>
<td>4-OCH$_3$</td>
<td>C$<em>{24}$H$</em>{20}$N$_4$O$_4$S</td>
<td>2.39 (2.49)</td>
<td>0.94(-1.19)</td>
<td>62.84  4.32  11.97</td>
</tr>
<tr>
<td>5i</td>
<td>2-Cl</td>
<td>H</td>
<td>C$<em>{23}$H$</em>{17}$ClN$_4$O$_3$S</td>
<td>3.18 (3.29)</td>
<td>0.78(-0.54)</td>
<td>59.40  3.67  12.01</td>
</tr>
<tr>
<td>5j</td>
<td>2-Cl</td>
<td>2-Cl</td>
<td>C$<em>{23}$H$</em>{16}$Cl$_2$N$_4$O$_3$S</td>
<td>3.98 (4.00)</td>
<td>0.76(-0.50)</td>
<td>55.32  3.23  11.22</td>
</tr>
<tr>
<td>5k</td>
<td>2-Cl</td>
<td>2-CH$_3$</td>
<td>C$<em>{24}$H$</em>{19}$ClN$_4$O$_3$S</td>
<td>3.70 (3.79)</td>
<td>0.90(-0.95)</td>
<td>60.18  4.00  11.70</td>
</tr>
<tr>
<td>5l</td>
<td>2-Cl</td>
<td>3-CH$_3$</td>
<td>C$<em>{24}$H$</em>{19}$ClN$_4$O$_3$S</td>
<td>3.72 (3.79)</td>
<td>0.91(-1.00)</td>
<td>60.46  4.34  11.97</td>
</tr>
<tr>
<td>5m</td>
<td>2-Cl</td>
<td>4-CH$_3$</td>
<td>C$<em>{24}$H$</em>{19}$ClN$_4$O$_3$S</td>
<td>3.73 (3.79)</td>
<td>0.94(-1.19)</td>
<td>59.95  3.98  11.58</td>
</tr>
<tr>
<td>5n</td>
<td>2-Cl</td>
<td>2-OCH$_3$</td>
<td>C$<em>{24}$H$</em>{19}$ClN$_4$O$_4$S</td>
<td>3.18 (3.21)</td>
<td>0.79(-0.57)</td>
<td>58.24  3.87  11.32</td>
</tr>
<tr>
<td></td>
<td>2-Cl</td>
<td>3-OCH₃</td>
<td>C₂₄H₁₉ClN₄O₄S</td>
<td>3.19 (3.21)</td>
<td>0.82(-0.65)</td>
<td>58.45</td>
</tr>
<tr>
<td>---</td>
<td>------</td>
<td>-------</td>
<td>----------------</td>
<td>------------</td>
<td>-------------</td>
<td>-------</td>
</tr>
<tr>
<td>5p</td>
<td>2-Cl</td>
<td>4-OCH₃</td>
<td>C₂₄H₁₉ClN₄O₄S</td>
<td>3.24 (3.21)</td>
<td>0.80(-0.60)</td>
<td>58.35</td>
</tr>
</tbody>
</table>

*a* Solvent of crystallization—Ethanol.

*b* Log P was determined by octanol: phosphate buffer method; CLog P was calculated using software ChemDraw Ultra 8.0

*c* Solvent system- Toluene: Ethyl acetate: Formic acid (5:4:1).

*d* A logarithmic function of R₁ value was also calculated ; Rᵢ = log (1-1/R₁).

*e* Elemental analysis for C, H, N were within ± 0.4 % of the theoretical value.
Reagents and conditions: (i) GAA, bromine, cool at 10 °C (ii) Benzene, triethylamine, chloroacetyl chloride, stirring in cool (iii) Hydrazine hydrate, dry ethanol, stirring 20-22h (iv) GAA, ethanol, aromatic aldehyde, reflux, 5h

Fig. 8: Synthetic route to the compounds 4a-y – Scheme 4
Synthetic Methods- Scheme 4

General procedure for the synthesis of titled compounds 4a-y

6-Substituted benzo[d]thiazol-2-amine (1a-e)

A mixture of substituted aniline (0.01 mol) and potassium thiocyanate (0.01 mol) in glacial acetic acid was cooled and stirred. To this solution, bromine (0.01 mol) was added dropwise at such a rate to keep the reaction temperature below 10 °C throughout the addition. Stirring was continued to additional 3 h and the separated hydrochloride salt was filtered, washed with acetic acid and dried. It was dissolved in hot water and neutralized with aqueous ammonia solution (25%). The precipitate obtained was filtered, washed with water, dried and recrystallized to afford the 6-substituted benzo[d]thiazol-2-amine (1a-e).

2-Chloro -N-(6-substituted benzo[d]thiazol-2-yl)acetamide (2a-e)

Compounds (1a-e) (0.01 mol) were dissolved in 100 mL of benzene and 0.01 mol of triethylamine was added. This mixture was allowed to stir on ice bath. 0.01 mol of chloroacetyl chloride and 10 mL of benzene mixture were added drop by drop. After completion of dropping, reaction mixture was stirred for 1 h at room temperature. Benzene was vaporized and brown residue was recrystallized from methanol to give 2-chloro -N-(6-substituted benzo[d]thiazol-2-yl) acetamide (2a-e).

N-(6-substituted benzo[d]thiazol-2-yl)-2-hydrazinylacetamide (3a-e)

Hydrazine hydrate (10 mL) was placed in a round bottom flask, and compound 2a-e (0.01 mol) was added. Contents were diluted with a sufficient quantity of dry ethanol till clear solution was obtained and the reaction mixture was refluxed for 20–22 h. After completion of the reaction, ethanol was distilled off till a small volume was left. On cooling, crystals of compounds (3a-e) were formed and were filtered and recrystallized with ethanol.

2-[2-(4-Substituted benzylidene) hydrazinyl]-N-(6-substituted benzo[d]thiazol-2-yl) acetamide (4a-y)

The solution of compounds (3a-e) (0.01 mol) in glacial acetic acid (5 mL) and ethanol (10 mL) were heated to boiling and refluxed with aromatic aldehydes (0.01 mol) for 5 h. Refluxed solution was cooled to room temperature and kept overnight. The solid was collected out, washed with methanol, dried and recrystallized from ethanol to get
the pure compounds (4a-y). The physicochemical parameters of all the final compounds are presented in Table 6.

2-(2-Benzylidenehydrazinyl)-N-(benzo[d]thiazol-2-yl) acetamide (4a). Yield 72%, mp 240°C. IR (KBr) cm\(^{-1}\): 3456, 3370 (NH\(_{str.}\)), 3089 (Ar-CH\(_{str.}\)), 1684 (C=O\(_{str.}\)), 1598 (C=N\(_{str.\ cyclic}\)); \(^1\)H NMR (CDCl\(_3\)) \(\delta\) (ppm): 3.83 (s, 2H, CH\(_2\)), 6.20 (s, 1H, CH), 6.72-7.44 (m, 9H, Ar-H), 8.51 (s, 1H, NH, D\(_2\)O exchangeable), 8.54 (s, 1H, NH-Amide, D\(_2\)O exchangeable).

2-(2-(4-Methylbenzylidene) hydrazinyl)-N-(benzo[d]thiazol-2-yl) acetamide (4b). Yield 75%, mp 256°C. IR (KBr) cm\(^{-1}\): 3424, 3299 (NH\(_{str.}\)), 3117 (Ar-CH\(_{str.}\)), 2961 (CH\(_{str.}\)), 1667 (C=O\(_{str.}\)), 1601 (C=N\(_{str.\ cyclic}\)); \(^1\)H NMR (CDCl\(_3\)) \(\delta\) (ppm): 2.33 (s, 3H, CH\(_3\)), 4.63 (s, 2H, CH\(_2\)), 5.16 (s, 1H, CH), 6.55-7.84 (m, 8H, Ar-H), 7.96 (s, 1H, NH, D\(_2\)O exchangeable), 8.41 (s, 1H, NH-Amide, D\(_2\)O exchangeable).

2-(2-(4-Methoxybenzylidene)hydrazinyl)-N-(benzo[d]thiazol-2-yl)acetamide (4c). Yield 68%, mp 278°C. IR (KBr) cm\(^{-1}\): 3576, 3471 (NH\(_{str.}\)), 3323 (OH\(_{str.}\)), 3320 (Ar-CH\(_{str.}\)), 2924 (CH\(_{str.}\)), 1625 (C=O\(_{str.}\)), 1595 (C=N\(_{str.\ cyclic}\), 1147 (OCH\(_3\)); \(^1\)H NMR (DMSO-\(d_6\)) \(\delta\) (ppm): 3.87 (s, 3H, OCH\(_3\)), 4.16 (s, 2H, CH\(_2\)), 5.34 (s, 1H, CH), 6.61-7.64 (m, 8H, Ar-H), 7.97 (s, 1H, NH, D\(_2\)O exchangeable), 10.64 (s, 1H, NH-Amide, D\(_2\)O exchangeable).

2-(2-(4-Hydroxybenzylidene)hydrazinyl)-N-(benzo[d]thiazol-2-yl)acetamide (4d). Yield 60%, mp 272°C. IR (KBr) cm\(^{-1}\): 3570, 3456 (NH\(_{str.}\)), 3332 (OH\(_{str.}\)), 3320 (Ar-CH\(_{str.}\)), 2924 (CH\(_{str.}\)), 1612 (C=O\(_{str.}\)), 1605 (C=N\(_{str.\ cyclic}\)); \(^1\)H NMR (CDCl\(_3\)) \(\delta\) (ppm): 4.23 (s, 2H, CH\(_2\)), 5.58 (s, 1H, CH), 6.17-7.79 (m, 8H, Ar-H), 8.21 (s, 1H, NH, D\(_2\)O exchangeable), 10.13 (s, 1H, NH-Amide, D\(_2\)O exchangeable); MS: m/z 325 (M-1)

2-(2-(4-Nitrobenzylidene)hydrazinyl)-N-(benzo[d]thiazol-2-yl)acetamide (4e). Yield 68%, mp 280°C. IR (KBr) cm\(^{-1}\): 3527, 3453 (NH\(_{str.}\)), 3362 (Ar-CH\(_{str.}\)), 2982 (CH\(_{str.}\)), 1674 (C=O\(_{str.}\)), 1625 (C=N\(_{str.\ cyclic}\), 1325 (NO\(_2\)); \(^1\)H NMR (CDCl\(_3\)) \(\delta\) (ppm): 4.35 (s, 2H, CH\(_2\)), 5.37 (s, 1H, CH), 6.17-7.79 (m, 8H, Ar-H), 8.58 (s, 1H, NH, D\(_2\)O exchangeable), 9.48 (s, 1H, NH-Amide, D\(_2\)O exchangeable).
2-(2-Benzylidenehydrazinyl)-N-(6-methylbenzo[d]thiazol-2-yl)acetamide (4f). Yield 75%, mp 282°C. IR (KBr) cm\(^{-1}\): 3563, 3476 (NH\(_\text{str.}\)), 3323 (Ar-CH\(_\text{str.}\)), 2937 (CH\(_\text{str.}\)), 1638 (C=O\(_\text{str.}\)), 1621 (C=N\(_\text{str.}\) cyclic); \(^1\)H NMR (CDCl\(_3\)) \(\delta\) (ppm): 2.18 (s, 3H, CH\(_3\)), 3.30 (s, 2H, CH\(_2\)), 5.76 (s, 1H, CH), 6.88-7.31 (m, 8H, Ar-H), 8.44 (s, 1H, NH, D\(_2\)O exchangeable), 8.48 (s, 1H, NH-Amide, D\(_2\)O exchangeable).

2-(2-(4-Methylbenzylidene)hydrazinyl)-N-(6-methylbenzo[d]thiazol-2-yl)acetamide (4g). Yield 85%, mp 288°C. IR (KBr) cm\(^{-1}\): 3586, 3418 (NH\(_\text{str.}\)), 3327 (Ar-CH\(_\text{str.}\)), 2972 (CH\(_\text{str.}\)), 1637 (C=O\(_\text{str.}\)), 1595 (C=N\(_\text{str.}\) cyclic); \(^1\)H NMR (CDCl\(_3\)) \(\delta\) (ppm): 2.12 (s, 3H, CH\(_3\)), 4.10 (s, 2H, CH\(_2\)), 5.43 (s, 1H, CH), 6.32-7.76 (m, 7H, Ar-H), 8.98 (s, 1H, NH, D\(_2\)O exchangeable), 9.45 (s, 1H, NH-Amide, D\(_2\)O exchangeable).

2-(2-(4-Methoxybenzylidene)hydrazinyl)-N-(6-methylbenzo[d]thiazol-2-yl)acetamide (4h). Yield 66%, mp 279°C. IR (KBr) cm\(^{-1}\): 3556, 3485 (NH\(_\text{str.}\)), 3332 (Ar-CH\(_\text{str.}\)), 2943 (CH\(_\text{str.}\)), 1614 (C=O\(_\text{str.}\)), 1134 (OCH\(_3\)\(_\text{str.}\)), 1134 (OCH\(_3\)), 4.42 (s, 2H, CH\(_2\)), 5.76 (s, 1H, CH), 6.28-7.89 (m, 7H, Ar-H), 8.76 (s, 1H, NH, D\(_2\)O exchangeable), 9.73 (s, 1H, NH-Amide, D\(_2\)O exchangeable).

2-(2-(4-Hydroxybenzylidene)hydrazinyl)-N-(6-methylbenzo[d]thiazol-2-yl)acetamide (4i). Yield 64%, mp 276°C. IR (KBr) cm\(^{-1}\): 3536, 3418 (NH\(_\text{str.}\)), 3327 (OH\(_\text{str.}\)), 3318 (Ar-CH\(_\text{str.}\)), 2916 (CH\(_\text{str.}\)), 1614 (C=O\(_\text{str.}\)), 1134 (OCH\(_3\)), 4.42 (s, 2H, CH\(_2\)), 5.18 (s, 1H, Ar-OH), 6.15-7.72 (m, 7H, Ar-H), 8.84 (s, 1H, NH, D\(_2\)O exchangeable), 9.85 (s, 1H, NH-Amide, D\(_2\)O exchangeable).

2-(2-(4-Nitrobenzylidene)hydrazinyl)-N-(6-methylbenzo[d]thiazol-2-yl)acetamide (4j). Yield 64%, mp 282°C. IR (KBr) cm\(^{-1}\): 3518, 3426 (NH\(_\text{str.}\)), 3316 (Ar-CH\(_\text{str.}\)), 2983 (CH\(_\text{str.}\)), 1677 (C=O\(_\text{str.}\)), 1622 (C=N\(_\text{str.}\) cyclic), 1316 (NO\(_2\))\(_\text{str.}\)); \(^1\)H NMR (CDCl\(_3\)) \(\delta\) (ppm): 2.43 (s, 3H, CH\(_3\)), 4.84 (s, 2H, CH\(_2\)), 5.27 (s, 1H, CH), 6.43-7.86 (m, 7H, Ar-H), 8.64 (s, 1H, NH, D\(_2\)O exchangeable), 9.82 (s, 1H, NH-Amide, D\(_2\)O exchangeable).

2-(2-Benzylidenehydrazinyl)-N-(6-methoxybenzo[d]thiazol-2-yl)acetamide (4k). Yield 66%, mp 276°C. IR (KBr) cm\(^{-1}\): 3607, 3593 (NH\(_\text{str.}\)), 3370 (Ar-CH\(_\text{str.}\)), 3010 (CH\(_\text{str.}\)), 1632 (C=O\(_\text{str.}\)), 1585 (C=N\(_\text{str.}\) cyclic), 1120 (OCH\(_3\)), \(^1\)H NMR (CDCl\(_3\)) \(\delta\) (ppm): 3.30...
(s, 3H, OCH₃), 3.34 (s, 2H, CH₂), 6.21 (s, 1H, CH), 6.86-7.28 (m, 8H, Ar-H), 7.86 (s, 1H, NH, D₂O exchangeable), 8.13 (s, 1H, NH-Amide, D₂O exchangeable).

2-(2-(4-Methylbenzylidene)hydrazinyl)-N-(6-methoxybenzo[d]thiazol-2-yl) acetamide (4d). Yield 61%, mp 272°C. IR (KBr) cm⁻¹: 3517, 3426 (NH str.), 3362 (Ar-CH str.), 2964 (CH₃ str.), 1605 (C=O str.), 1572 (C=N str. cyclic), 1113 (OCH₃); ¹H NMR (CDCl₃) δ (ppm): 2.20 (s, 3H, CH₃), 3.35 (s, 3H, OCH₃), 4.04 (s, 2H, CH₂), 6.23 (s, 1H, CH), 6.36-7.76 (m, 7H, Ar-H), 8.37 (s, 1H, NH, D₂O exchangeable), 9.65 (s, 1H, NH-Amide, D₂O exchangeable).

2-(2-(4-Methoxybenzylidene)hydrazinyl)-N-(6-methoxybenzo[d]thiazol-2-yl) acetamide (4m). Yield 58%, mp 274°C. IR (KBr) cm⁻¹: 3538, 3472 (NH str.), 3316 (Ar-CH str.), 2938 (CH₂ str.), 1614 (C=O str.), 1548 (C=N str. cyclic), 1126 (OCH₃); ¹H NMR (CDCl₃) δ (ppm): 2.78, 3.35 (s, 3H, OCH₃), 4.53 (s, 2H, CH₂), 6.46 (s, 1H, CH), 6.37-7.89 (m, 7H, Ar-H), 8.54 (s, 1H, NH, D₂O exchangeable), 9.87 (s, 1H, NH-Amide, D₂O exchangeable).

2-(2-(4-Hydroxybenzylidene)hydrazinyl)-N-(6-methoxybenzo[d]thiazol-2-yl)acetamide (4n). Yield 58%, mp 274°C. IR (KBr) cm⁻¹: 3517, 3435 (OH str.), 3326 (Ar-CH str.), 3326 (OH str.), 2935 (CH₂ str.), 1639 (C=O str.), 1600 (C=N str. cyclic), 1116 (OCH₃); ¹H NMR (CDCl₃) δ (ppm): 3.37 (s, 3H, OCH₃), 4.56 (s, 2H, CH₂), 5.76 (s, 1H, CH), 6.42 (s, 1H, Ar-OH), 6.24-7.86 (m, 7H, Ar-H), 8.96 (s, 1H, NH, D₂O exchangeable), 9.98 (s, 1H, NH-Amide, D₂O exchangeable).

2-(2-(4-Nitrobenzylidene)hydrazinyl)-N-(6-methoxybenzo[d]thiazol-2-yl)acetamide (4o). Yield 63%, mp 288°C. IR (KBr) cm⁻¹: 3527, 3412 (NH str.), 3382 (Ar-CH str.), 2951 (CH₂ str.), 1625 (C=O str.), 1612 (C=N str. cyclic), 1353 (NO₂), 1164 (OCH₃); ¹H NMR (CDCl₃) δ (ppm): 3.40 (s, 3H, OCH₃), 4.37 (s, 2H, CH₂), 5.85 (s, 1H, CH), 6.24-7.35 (m, 7H, Ar-H), 8.13 (s, 1H, NH, D₂O exchangeable), 9.74 (s, 1H, NH-Amide, D₂O exchangeable).

2-(2-Benzylidenehydrazinyl)-N-(6-chlorobenzo[d]thiazol-2-yl)acetamide (4p). Yield 74%, mp 243°C. IR (KBr) cm⁻¹: 3445, 3384 (NH str.), 3024 (Ar-CH str.), 2916 (CH₂ str.), 1632 (C=O str.), 1563 (C=N str. cyclic), 818 (C-Cl); ¹H NMR (CDCl₃) δ (ppm): 4.65 (s,
Chapter 4

Experimental Protocols

2H, CH₂), 5.92 (s, 1H, CH), 6.26-7.87 (m, 8H, Ar-H), 8.83 (s, 1H, NH, D₂O exchangeable), 9.83 (s, 1H, NH-Amide, D₂O exchangeable).

2-(2-(4-Methylbenzylidene)hydrazinyl)-N-(6-chloro benzo[d]thiazol-2-yl) acetamide (4q). Yield 71%, mp 248°C. IR (KBr) cm⁻¹: 3464, 3374 (NH str.), 3054 (Ar-CH str.), 2939 (CH₃), 1618 (C=O str.), 1583 (C=N str. cyclic), 753 (C-Cl) ; ¹H NMR (CDCl₃) δ(ppm): 2.32 (s, 3H, CH₃), 4.45 (s, 2H, CH₂), 5.63 (s, 1H, CH), 6.92-8.73 (m, 7H, Ar-H), 9.23 (s, 1H, NH, D₂O exchangeable), 9.97 (s, 1H, NH-Amide, D₂O exchangeable).

2-(2-(4-Methoxybenzylidene)hydrazinyl)-N-(6-chloro benzo[d]thiazol-2-yl) acetamide (4r). Yield 65%, mp 244°C. IR (KBr) cm⁻¹: 3436, 3385 (NH str.), 3038 (Ar-CH str.), 2903 (CH str.), 1698 (C=O str.), 1612 (C=N str. cyclic), 1123 (OCH₃), 785 (C-Cl) ; ¹H NMR (CDCl₃) δ(ppm): 3.45 (s, 3H, OCH₃), 4.87 (s, 2H, CH₂), 5.83 (s, 1H, CH), 6.26-8.93 (m, 7H, Ar-H), 9.83 (s, 1H, NH, D₂O exchangeable), 10.17 (s, 1H, NH-Amide, D₂O exchangeable).

2-(2-(4-Hydroxybenzylidene)hydrazinyl)-N-(6-chloro benzo[d]thiazol-2-yl) acetamide (4s). Yield 65%, mp 244°C. IR (KBr) cm⁻¹: 3387, 3334 (NH str.), 3241 (OH str.), 3046 (Ar-CH str.), 2964 (CH₃), 1606 (C=O str.), 1534 (C=N str. cyclic), 765 (C-Cl) ; ¹H NMR (CDCl₃) δ(ppm): 4.26 (s, 2H, CH₂), 5.73 (s, 1H, CH), 6.14-8.28 (m, 7H, Ar-H), 9.53 (s, 1H, NH, D₂O exchangeable), 10.47 (s, 1H, NH-Amide, D₂O exchangeable).

2-(2-(4-Nitrobenzylidene)hydrazinyl)-N-(6-chloro benzo[d]thiazol-2-yl) acetamide (4t). Yield 74%, mp 274°C. IR (KBr) cm⁻¹: 3375, 3312 (NH str.), 3084 (Ar-CH str.), 2934 (CH₃), 1612 (C=O str.), 1584 (C=N str. cyclic), 1320 (NO₂), 715 (C-Cl) ; ¹H NMR (CDCl₃) δ(ppm): 4.76 (s, 2H, CH₂), 5.80 (s, 1H, CH), 6.25 (s, 1H, Ar-OH), 6.14-8.28 (m, 7H, Ar-H), 9.43 (s, 1H, NH, D₂O exchangeable), 10.68 (s, 1H, NH-Amide, D₂O exchangeable).

2-(2-Benzylidenehydrazinyl)-N-(6-bromobenzo[d]thiazol-2-yl) acetamide (4u). Yield 76%, mp 248°C. IR (KBr) cm⁻¹: 3438, 3385 (NH str.), 3073 (Ar-CH str.), 2986 (CH₃), 1612 (C=O str.), 1507 (C=N str. cyclic), 563 (C-Br) ; ¹H NMR (CDCl₃) δ(ppm): 4.47 (s, 2H, CH₂), 5.32 (s, 1H, CH), 6.17-8.92 (m, 8H, Ar-H), 9.82 (s, 1H, NH, D₂O exchangeable), 10.21 (s, 1H, NH-Amide, D₂O exchangeable).
2-(2-(4-Methylbenzylidene)hydrazinyl)-N-(6-bromobenzo[d]thiazol-2-yl)acetamide (4v). Yield 82%, mp 237°C. IR (KBr) cm⁻¹: 3415, 3316 (NH str.), 3153 (Ar-CH str.), 2913 (CH₃ str.), 1627 (C=O str.), 1572 (C=N str. cyclic), 572 (C-Br); \(^1\)H NMR (CDCl₃) δ(ppm): 2.53 (s, 3H, CH₃), 4.82 (s, 2H, CH₂), 5.62 (s, 1H, CH), 6.67-8.82 (m, 7H, Ar-H), 9.83 (s, 1H, NH, D₂O exchangeable), 10.72 (s, 1H, NH-Amide, D₂O exchangeable).

2-(2-(4-Methoxybenzylidene)hydrazinyl)-N-(6-bromobenzo[d]thiazol-2-yl)acetamide (4w). Yield 84%, mp 226°C. IR (KBr) cm⁻¹: 3427, 3352 (NH str.), 3136 (Ar-CH str.), 2921 (CH₃ str.), 1624 (C=O str.), 1583 (C=N str. cyclic), 1126 (OCH₃), 603 (C-Br); \(^1\)H NMR (CDCl₃) δ(ppm): 3.33 (s, 3H, OCH₃), 4.73 (s, 2H, CH₂), 5.74 (s, 1H, CH), 6.37-8.72 (m, 7H, Ar-H), 9.43 (s, 1H, NH, D₂O exchangeable), 10.83 (s, 1H, NH-Amide, D₂O exchangeable).

2-(2-(4-Hydroxybenzylidene)hydrazinyl)-N-(6-bromobenzo[d]thiazol-2-yl)acetamide (4x). Yield 87%, mp 260°C. IR (KBr) cm⁻¹: 3387, 3275 (NH str.), 3144 (Ar-CH str.), 2982 (CH₃ str.), 1614 (C=O str.), 1527 (C=N str. cyclic), 623 (C-Br); \(^1\)H NMR (CDCl₃) δ(ppm): 4.54 (s, 2H, CH₂), 5.83 (s, 1H, CH), 6.32-8.93 (m, 7H, Ar-H), 9.73 (s, 1H, NH, D₂O exchangeable), 10.72 (s, 1H, NH-Amide, D₂O exchangeable).

2-(2-(4-Nitrobenzylidene)hydrazinyl)-N-(6-bromobenzo[d]thiazol-2-yl)acetamide (4y). Yield 70%, mp 283°C. IR (KBr) cm⁻¹: 3382, 3253 (NH str.), 3144 (Ar-CH str.), 2982 (CH₃ str.), 1662 (C=O str.), 1528 (C=N str. cyclic), 1286 (NO₂), 676 (C-Br); \(^1\)H NMR (CDCl₃) δ(ppm): 4.65 (s, 2H, CH₂), 5.74 (s, 1H, CH), 6.33-8.93 (m, 7H, Ar-H), 9.84 (s, 1H, NH, D₂O exchangeable), 10.74 (s, 1H, NH-Amide, D₂O exchangeable).
Table 6- Physicochemical parameters of compounds 4a-y - Scheme 4

<table>
<thead>
<tr>
<th>Compd</th>
<th>R</th>
<th>R’</th>
<th>^aMol. Formula</th>
<th>Log Pb</th>
<th>^cRf (Rm) d</th>
<th>^eElemental analysis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Calculated)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4a</td>
<td>H</td>
<td>H</td>
<td>C_{16}H_{14}N_{4}O S</td>
<td>4.10(3.79)</td>
<td>0.99(-1.99)</td>
<td>61.92 4.55 18.05</td>
</tr>
<tr>
<td>4b</td>
<td>H</td>
<td>4-CH3</td>
<td>C_{17}H_{16}N_{4}O S</td>
<td>4.46(4.60)</td>
<td>0.84(-0.72)</td>
<td>62.94 4.97 17.27</td>
</tr>
<tr>
<td>4c</td>
<td>H</td>
<td>4-OCH3</td>
<td>C_{17}H_{16}N_{4}O_{2} S</td>
<td>4.10(4.39)</td>
<td>0.90(-0.95)</td>
<td>59.98 4.74 16.46</td>
</tr>
<tr>
<td>4d</td>
<td>H</td>
<td>4-OH</td>
<td>C_{16}H_{14}N_{4}O_{2} S</td>
<td>4.38(4.48)</td>
<td>0.92(-1.06)</td>
<td>58.88 4.32 17.17</td>
</tr>
<tr>
<td>4e</td>
<td>H</td>
<td>4-NO2</td>
<td>C_{16}H_{13}N_{5}O_{2} S</td>
<td>4.22(4.44)</td>
<td>0.96(-1.38)</td>
<td>54.08 3.69 19.71</td>
</tr>
<tr>
<td>4f</td>
<td>4-CH3</td>
<td>H</td>
<td>C_{17}H_{16}N_{4}O S</td>
<td>4.18(3.80)</td>
<td>0.98(-1.69)</td>
<td>62.90 4.94 17.25</td>
</tr>
<tr>
<td>4g</td>
<td>4-CH3</td>
<td>4-CH3</td>
<td>C_{18}H_{18}N_{4}O S</td>
<td>4.14(3.90)</td>
<td>0.83(-0.68)</td>
<td>63.88 5.36 16.56</td>
</tr>
<tr>
<td>4h</td>
<td>4-CH3</td>
<td>4-OCH3</td>
<td>C_{18}H_{18}N_{4}O_{2} S</td>
<td>4.08(3.80)</td>
<td>0.91(-1.00)</td>
<td>61.00 5.12 15.81</td>
</tr>
<tr>
<td>4i</td>
<td>4-CH3</td>
<td>4-OH</td>
<td>C_{17}H_{16}N_{4}O_{2} S</td>
<td>4.67(4.70)</td>
<td>0.89(-0.90)</td>
<td>59.98 4.74 16.46</td>
</tr>
<tr>
<td>4j</td>
<td>4-CH3</td>
<td>4-NO2</td>
<td>C_{17}H_{15}N_{5}O_{2} S</td>
<td>4.24(4.47)</td>
<td>0.82(-0.65)</td>
<td>55.27 4.09 18.96</td>
</tr>
<tr>
<td>4k</td>
<td>4-OCH3</td>
<td>H</td>
<td>C_{17}H_{16}N_{4}O_{2} S</td>
<td>5.15(5.21)</td>
<td>0.97(-1.50)</td>
<td>59.98 4.74 16.46</td>
</tr>
<tr>
<td>4l</td>
<td>4-OCH3</td>
<td>4-CH3</td>
<td>C_{18}H_{18}N_{4}O_{2} S</td>
<td>5.42(5.22)</td>
<td>0.95(-1.27)</td>
<td>61.00 5.12 15.81</td>
</tr>
<tr>
<td>4m</td>
<td>4-OCH3</td>
<td>4-OCH3</td>
<td>C_{18}H_{18}N_{4}O_{3} S</td>
<td>5.51(5.21)</td>
<td>0.81(-0.62)</td>
<td>58.36 4.90 15.12</td>
</tr>
<tr>
<td>4n</td>
<td>4-OCH3</td>
<td>4-OH</td>
<td>C_{17}H_{16}N_{4}O_{3} S</td>
<td>4.24(4.63)</td>
<td>0.93(-1.12)</td>
<td>57.29 4.52 15.72</td>
</tr>
<tr>
<td>4o</td>
<td>4-OCH3</td>
<td>4-NO2</td>
<td>C_{17}H_{15}N_{5}O_{3} S</td>
<td>4.45(4.63)</td>
<td>0.88(-1.18)</td>
<td>52.98 3.92 18.17</td>
</tr>
</tbody>
</table>
### Experimental Protocols

<table>
<thead>
<tr>
<th>Compound</th>
<th>Substitution</th>
<th>Structure</th>
<th>Molecular Formula</th>
<th>Log P (Octanol:Phosphate Buffer)</th>
<th>Rf Value</th>
<th>Log (1 - 1/Rf)</th>
<th>Percentage Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>4p</td>
<td>4-Cl</td>
<td>H</td>
<td>C₁₆H₁₃ClN₂O₂S</td>
<td>4.52 (4.61)</td>
<td>0.94 (-1.19)</td>
<td>55.73</td>
<td>3.80</td>
</tr>
<tr>
<td>4q</td>
<td>4-Cl</td>
<td>4-CH₃</td>
<td>C₁₇H₁₅ClN₂O₂S</td>
<td>4.22 (3.98)</td>
<td>0.86 (-1.18)</td>
<td>56.90</td>
<td>4.21</td>
</tr>
<tr>
<td>4r</td>
<td>4-Cl</td>
<td>4-OCH₃</td>
<td>C₁₇H₁₅ClN₂O₂S</td>
<td>4.54 (4.70)</td>
<td>0.89 (-0.90)</td>
<td>54.47</td>
<td>4.03</td>
</tr>
<tr>
<td>4s</td>
<td>4-Cl</td>
<td>4-OH</td>
<td>C₁₆H₁₃ClN₂O₂S</td>
<td>4.23 (4.44)</td>
<td>0.82 (-0.65)</td>
<td>53.26</td>
<td>3.63</td>
</tr>
<tr>
<td>4t</td>
<td>4-Cl</td>
<td>4-NO₂</td>
<td>C₁₆H₁₂ClN₃O₃S</td>
<td>4.34 (4.45)</td>
<td>0.93 (-1.12)</td>
<td>49.30</td>
<td>3.10</td>
</tr>
<tr>
<td>4u</td>
<td>4-Br</td>
<td>H</td>
<td>C₁₆H₁₃BrN₄O₂S</td>
<td>4.24 (4.47)</td>
<td>0.88 (-1.18)</td>
<td>49.37</td>
<td>3.37</td>
</tr>
<tr>
<td>4v</td>
<td>4-Br</td>
<td>4-CH₃</td>
<td>C₁₇H₁₅BrN₄O₂S</td>
<td>4.34 (4.46)</td>
<td>0.94 (-1.19)</td>
<td>50.63</td>
<td>3.75</td>
</tr>
<tr>
<td>4w</td>
<td>4-Br</td>
<td>4-OCH₃</td>
<td>C₁₇H₁₅BrN₄O₂S</td>
<td>5.16 (5.20)</td>
<td>0.86 (-1.18)</td>
<td>48.70</td>
<td>3.61</td>
</tr>
<tr>
<td>4x</td>
<td>4-Br</td>
<td>4-OH</td>
<td>C₁₆H₁₃BrN₄O₂S</td>
<td>5.42 (5.21)</td>
<td>0.94 (-1.19)</td>
<td>47.42</td>
<td>3.23</td>
</tr>
<tr>
<td>4y</td>
<td>4-Br</td>
<td>4-NO₂</td>
<td>C₁₆H₁₂BrN₃O₃S</td>
<td>5.51 (5.24)</td>
<td>0.86 (-1.18)</td>
<td>44.25</td>
<td>2.79</td>
</tr>
</tbody>
</table>

**Notes:**

- Solvent of crystallization – Ethanol.
- Log P was determined by octanol:phosphate buffer method; CLog P was calculated using software ChemDraw Ultra 8.0.
- A logarithmic function of Rf value was also calculated; Rm = log (1 - 1/Rf).
- Elemental analysis for C, H, N were within ± 0.4 % of the theoretical value.
Reagents and conditions: (i) GAA, bromine, stirring, 10 °C (ii) Benzene, triethylamine, chloroacetyl chloride, stirring, 1h (iii) HCl, heat (iv) Acetone, reflux, 3h

Fig. 9: Synthetic route to the compounds 4a-y – Scheme 5
Synthetic Methods- Scheme 5

General procedure for the synthesis of titled compounds 4a-y

6-Substituted benzo[d]thiazol-2-amine (1a-e)

A mixture of substituted aniline (0.01 mol) and potassium thiocyanate (0.01 mol) in glacial acetic acid was cooled and stirred. To this solution, bromine (0.01 mol) was added dropwise at such a rate to keep the reaction temperature below 10 °C throughout the addition. Stirring was continued to additional 3 h and the separated hydrochloride salt was filtered, washed with acetic acid and dried. It was dissolved in hot water and neutralized with aqueous ammonia solution (25%). The precipitate obtained was filtered, washed with water, dried and recrystallized to afford the 6-substituted benzo[d]thiazol-2-amine (1a-e).

2- Chloro -N-(6-substituted benzo[d]thiazol-2-yl)acetamide (2a-e)

Compounds 1a-e (0.01 mol) were dissolved in 100 mL of benzene and 0.01 mol of triethylamine was added. This mixture was allowed to stir on ice bath. 0.01 mol of chloroacetyl chloride and 10 mL of benzene mixture were added drop by drop. After completionof dropping, reaction mixture was stirred for 1h at room temperature. Benzene was vaporized and brown residue was recrystallized from methanol to give 2- chloro -N-(6-substituted benzo[d]thiazol-2-yl) acetamide (2a-e)

1-(4-Substituted phenyl) thiourea (3a-e)

Aniline (0.10 mol) was mixed with concentrated hydrochloric acid (2.4 mL, 0.10 mol) and the resulting mixture was heated on a water bath till the content just became half of the original volume. Ammonium thiocyanate (7.6 g, 0.10 mol) was added with constant stirring and the reaction mixture was heated on a water bath till a semisolid mass was formed, which on pouring in to ice cold water gave phenylthiourea (3a). In a similar way other arylthioureas (3b-e) were also prepared using appropriate aromatic amines, ammonium thiocyanate and recrystallized from ethanol.

2-[(6-Substituted benzo[d]thiazol-2-ylcarbamoyl) methyl]-1-(4-substituted phenyl) isothiourea (4a-y)

A mixture of compound 2a-e (0.01mol) and arylthiourea 3a-e (0.01mol) in acetone was refluxed for 3 h. It was then poured onto crushed ice and the resulting solid
compound 4a-y was dried and recrystallized with ethanol. The physicochemical parameters of all the final compounds are presented in Table 7.

2-[(Benzo[d]thiazol-2-ylcarbamoyl) methyl]-1-phenylisothiourea (4a). Yield 76%, mp 240 °C. IR (KBr) cm⁻¹: 3576, 3373, 3200 (NH str.), 3089 (Ar-CH str.), 2957 (CH str.), 1760 (C=O str.), 1643 (C=N str. cyclic), 682 (C-S-C str.); ¹H NMR (CDCl₃) δ: 4.10 (s, 2H, CH₂), 7.05-7.48 (m, 9H, Ar-H), 7.46 (s, 1H, NH= C, D₂O exchangeable), 7.48 (s, 1H, NH-Ar, D₂O exchangeable), 9.21 (s, 1H, NH-Amide, D₂O exchangeable); MS: m/z 344(M+2).

2-[(Benzo[d]thiazol-2-ylcarbamoyl) methyl]-1-p-tolylisothiourea (4b). Yield 68%, mp 253 °C. IR (KBr) cm⁻¹: 3445, 3374, 3273 (NH str.), 3053 (Ar-CH str.), 2974 (CH str.), 1743 (C=O str.), 1621 (C=N str. cyclic), 690 (C-S-C str.); ¹H NMR (CDCl₃) δ: 2.92 (s, 3H, CH₃), 4.52 (s, 2H, CH₂), 6.71-7.64 (m, 8H, Ar-H), 7.65 (s, 1H, NH= C, D₂O exchangeable), 7.66 (s, 1H, NH-Ar, D₂O exchangeable), 9.80 (s, 1H, NH-Amide, D₂O exchangeable).

2-[(Benzo[d]thiazol-2-ylcarbamoyl) methyl]-1-(4-methoxyphenyl) isothiourea (4c). Yield 78%, mp 274 °C. IR (KBr) cm⁻¹: 3427, 3374, 3273 (NH str.), 3053 (Ar-CH str.), 2855 (CH str.), 1783 (C=O str.), 1684 (C=N str. cyclic), 1193 (OCH₃), 684 (C-S-C str.); ¹H NMR (CDCl₃) δ: 3.33 (s, 3H, OCH₃), 4.45 (s, 2H, CH₂), 6.34-7.45 (m, 8H, Ar-H), 7.34 (s, 1H, NH= C, D₂O exchangeable), 7.58 (s, 1H, NH-Ar, D₂O exchangeable), 9.80 (s, 1H, NH-Amide, D₂O exchangeable).

2-[(Benzo[d]thiazol-2-ylcarbamoyl) methyl]-1-(4-chlorophenyl) isothiourea (4d). Yield 64%, mp 265 °C. IR (KBr) cm⁻¹: 3462, 3247, 3173 (NH str.), 2924 (Ar-CH str.), 2835 (CH str.), 1783 (C=O str.), 1620 (C=N str. cyclic), 817 (C-Cl), 676 (C-S-C str.); ¹H NMR (CDCl₃) δ: 4.32 (s, 2H, CH₂), 6.36-7.89 (m, 8H, Ar-H), 7.21 (s, 1H, NH= C, D₂O exchangeable), 7.34 (s, 1H, NH-Ar, D₂O exchangeable), 9.34 (s, 1H, NH-Amide, D₂O exchangeable).

2-[(Benzo[d]thiazol-2-ylcarbamoyl) methyl]-1-(4-bromophenyl) isothiourea (4e). Yield 62%, mp 294 °C. IR (KBr) cm⁻¹: 3435, 3228, 3193 (NH str.), 2934 (Ar-CH str.), 2886 (CH str.), 1713 (C=O str.), 1652 (C=N str. cyclic), 656 (C-S-C str.), 606 (C-Br); ¹H NMR (CDCl₃) δ: 4.36 (s, 2H, CH₂), 6.12-7.97 (m, 8H, Ar-H), 7.23 (s, 1H, NH= C,
D$_2$O exchangeable), 7.46 (s, 1H, NH-Ar, D$_2$O exchangeable), 9.67 (s, 1H, NH-Amide, D$_2$O exchangeable).

2-[(6-Methylbenzo[d]thiazol-2-ylcarbamoyl) methyl]-1-phenylisothiourea (4f). Yield 74%, mp 284 °C. IR (KBr) cm$^{-1}$: 3371, 3194, 3091 (NH, str.), 2990 (Ar-CH$_2$-str.), 2943 (CH$_3$-str.), 1685 (C=O, str.), 1602 (C=N, cyclic), 702 (C-S-C, str.); $^1$H NMR (CDCl$_3$) δ: 2.46 (s, 3H, CH$_3$), 4.31 (s, 2H, CH$_2$), 7.14-7.60 (m, 8H, Ar-H), 7.66 (s, 1H, NH=Ar, D$_2$O exchangeable), 9.67 (s, 1H, NH-Amide, D$_2$O exchangeable).

2-[(6-Methylbenzo[d]thiazol-2-ylcarbamoyl) methyl]-1-p-tolylisothiourea (4g). Yield 72%, mp 273 °C. IR (KBr) cm$^{-1}$: 3363, 3162, 3074 (NH, str.), 2972 (Ar-CH$_2$-str.), 2981 (CH$_3$-str.), 1692 (C=O, str.), 1643 (C=N, cyclic), 725 (C-S-C, str.); $^1$H NMR (CDCl$_3$) δ: 2.46, 2.76 (s, 3H, CH$_3$), 4.45 (s, 2H, CH$_2$), 7.21-7.34 (m, 7H, Ar-H), 7.45 (s, 1H, NH=C, D$_2$O exchangeable), 7.67 (s, 1H, NH-Ar, D$_2$O exchangeable), 9.78 (s, 1H, NH-Amide, D$_2$O exchangeable).

2-[(6-Methylbenzo[d]thiazol-2-ylcarbamoyl)methyl]-1-(4-methoxyphenyl)isothiourea (4h). Yield 67%, mp 282 °C. IR (KBr) cm$^{-1}$: 3265, 3173, 3074 (NH, str.), 2936 (Ar-CH$_2$-str.), 2981 (CH$_3$-str.), 1722 (C=O, str.), 1662 (C=N, cyclic), 690 (C-S-C, str.); 1184 (OCH$_3$); $^1$H NMR (CDCl$_3$) δ: 2.23 (s, 3H, CH$_3$), 3.43 (s, 3H, OCH$_3$), 4.32 (s, 2H, CH$_2$), 7.23-7.67 (m, 7H, Ar-H), 7.69 (s, 1H, NH=C, D$_2$O exchangeable), 7.73 (s, 1H, NH-Ar, D$_2$O exchangeable), 9.88 (s, 1H, NH-Amide, D$_2$O exchangeable).

2-[(6-Methylbenzo[d]thiazol-2-ylcarbamoyl)methyl]-1-(4-chlorophenyl)isothiourea (4i). Yield 74%, mp 275 °C. IR (KBr) cm$^{-1}$: 3287, 3174, 3084 (NH, str.), 2983 (Ar-CH$_2$-str.), 2935 (CH$_3$-str.), 1737 (C=O, str.), 1647 (C=N, cyclic), 845 (C-Cl), 685 (C-S-C, str.); $^1$H NMR (CDCl$_3$) δ: 2.45 (s, 3H, CH$_3$), 4.57 (s, 2H, CH$_2$), 7.21-7.84 (m, 7H, Ar-H), 7.62 (s, 1H, NH=C, D$_2$O exchangeable), 7.84 (s, 1H, NH-Ar, D$_2$O exchangeable), 10.13 (s, 1H, NH-Amide, D$_2$O exchangeable).

2-[(6-Methylbenzo[d]thiazol-2-ylcarbamoyl)methyl]-1-(4-bromophenyl)isothiourea (4j). Yield 60%, mp 234 °C. IR (KBr) cm$^{-1}$: 3234, 3156, 3068 (NH, str.), 2983 (Ar-CH$_2$-str.), 2974 (CH$_3$-str.), 1783 (C=O, str.), 1684 (C=N, cyclic), 680 (C-S-C, str.), 612
(C-Br); \(^1\)H NMR (CDCl\(_3\)) \(\delta\): 2.67 (s, 3H, CH\(_3\)), 4.24 (s, 2H, CH\(_2\)), 7.31-7.78 (m, 7H, Ar-H), 7.84 (s, 1H, NH=C, D\(_2\)O exchangeable), 7.89 (s, 1H, NH-Ar, D\(_2\)O exchangeable), 10.32 (s, 1H, NH-Amide, D\(_2\)O exchangeable).

2-[(6-Methoxybenzo[d]thiazol-2-ylcarbamoyl) methyl]-1-phenylisothiourea (4k). Yield 53\%, mp 273 °C. IR (KBr) cm\(^{-1}\): 3283, 3182, 3096 (NH\(_{str}\)), 2968 (Ar-CH\(_{str}\)), 2934 (CH\(_{str}\)), 1785 (C=O\(_{str}\)), 1632 (C=N\(_{str}\) cyclic), 1123 (OCH\(_3\)), 705 (C-S-C\(_{str}\)); \(^1\)H NMR (CDCl\(_3\)) \(\delta\): 3.96 (s, 3H, OCH\(_3\)), 4.37 (s, 2H, CH\(_2\)), 7.24-8.03 (m, 8H, Ar-H), 8.21 (s, 1H, NH=C, D\(_2\)O exchangeable), 8.24 (s, 1H, NH-Ar, D\(_2\)O exchangeable), 8.27 (s, 1H, NH-Amide, D\(_2\)O exchangeable).

2-[(6-Methoxybenzo[d]thiazol-2-ylcarbamoyl) methyl]-1-p-tolylisothiourea (4l). Yield 62\%, mp 286 °C. IR (KBr) cm\(^{-1}\): 3295, 3190, 3003 (NH\(_{str}\)), 2994 (Ar-CH\(_{str}\)), 2903 (CH\(_{str}\)), 1793 (C=O\(_{str}\)), 1605 (C=N\(_{str}\) cyclic), 1195 (OCH\(_3\)), 728 (C-S-C\(_{str}\)); \(^1\)H NMR (CDCl\(_3\)) \(\delta\): 2.34 (s, 3H, CH\(_3\)), 3.86 (s, 3H, OCH\(_3\)), 4.23 (s, 2H, CH\(_2\)), 7.24-8.46 (m, 7H, Ar-H), 8.48 (s, 1H, NH=C, D\(_2\)O exchangeable), 8.52 (s, 1H, NH-Ar, D\(_2\)O exchangeable), 9.69 (s, 1H, NH-Amide, D\(_2\)O exchangeable).

2-[(6-Methoxybenzo[d]thiazol-2-ylcarbamoyl) methyl]-1-(4-methoxyphenyl) isothiourea (4m). Yield 73\%, mp 294 °C. IR (KBr) cm\(^{-1}\): 3225, 3156, 3084 (NH\(_{str}\)), 2943 (Ar-CH\(_{str}\)), 2923 (CH\(_{str}\)), 1728 (C=O\(_{str}\)), 1649 (C=N\(_{str}\) cyclic), 1138, 1076 (OCH\(_3\)), 678 (C-S-C\(_{str}\)); \(^1\)H NMR (CDCl\(_3\)) \(\delta\): 3.35, 3.76 (s, 3H, OCH\(_3\)), 4.34 (s, 2H, CH\(_2\)), 7.26-8.74 (m, 7H, Ar-H), 8.35 (s, 1H, NH=C, D\(_2\)O exchangeable), 8.98 (s, 1H, NH-Ar, D\(_2\)O exchangeable), 9.93 (s, 1H, NH-Amide, D\(_2\)O exchangeable).

2-[(6-Methoxybenzo[d]thiazol-2-ylcarbamoyl)methyl]-1-(4-chlorophenyl)isothiourea (4n). Yield 64\%, mp 248 °C. IR (KBr) cm\(^{-1}\): 3238, 3103, 3035 (NH\(_{str}\)), 2938 (Ar-CH\(_{str}\)), 2921 (CH\(_{str}\)), 1783 (C=O\(_{str}\)), 1694 (C=N\(_{str}\) cyclic), 1023 (OCH\(_3\)), 832 (C-Cl), 656 (C-S-C\(_{str}\)); \(^1\)H NMR (CDCl\(_3\)) \(\delta\): 3.34 (s, 3H, OCH\(_3\)), 4.21 (s, 2H, CH\(_2\)), 7.10-8.27 (m, 7H, Ar-H), 8.67 (s, 1H, NH=C, D\(_2\)O exchangeable), 9.18 (s, 1H, NH-Ar, D\(_2\)O exchangeable), 10.95 (s, 1H, NH-Amide, D\(_2\)O exchangeable).

2-[(6-Methoxybenzo[d]thiazol-2-ylcarbamoyl)methyl]-1-(4-bromophenyl)isothiourea (4o). Yield 67\%, mp 243 °C. IR (KBr) cm\(^{-1}\): 3239, 3133, 3039 (NH\(_{str}\)), 2929 (Ar-CH\(_{str}\)), 2917 (CH\(_{str}\)), 1783 (C=O\(_{str}\)), 1694 (C=N\(_{str}\) cyclic), 1023 (OCH\(_3\)), 832 (C-Br), 656 (C-S-C\(_{str}\)); \(^1\)H NMR (CDCl\(_3\)) \(\delta\): 3.34, 3.76 (s, 3H, OCH\(_3\)), 4.21 (s, 2H, CH\(_2\)), 7.10-8.27 (m, 7H, Ar-H), 8.67 (s, 1H, NH=C, D\(_2\)O exchangeable), 9.18 (s, 1H, NH-Ar, D\(_2\)O exchangeable), 10.95 (s, 1H, NH-Amide, D\(_2\)O exchangeable).
2993 (CH\textsubscript{str.}), 1783 (C=O\textsubscript{str.}), 1657 (C=N\textsubscript{str. cyclic}), 1048 (OCH\textsubscript{3}), 756 (C-S-C\textsubscript{str.}), 613 (C-Br); \textsuperscript{1}H NMR (CDCl\textsubscript{3}) \( \delta \): 3.54 (s, 3H, OCH\textsubscript{3}), 4.35 (s, 2H, CH\textsubscript{2}), 7.13-8.46 (m, 7H, Ar-H), 8.64 (s, 1H, NH=C, D\textsubscript{2}O exchangeable), 9.16 (s, 1H, NH-Ar, D\textsubscript{2}O exchangeable), 10.35 (s, 1H, NH-Amide, D\textsubscript{2}O exchangeable).

2-[(6-Chlorobenzo[d]thiazol-2-ylcarbamoyl)methyl]-1-phenylisothiourea (4p). Yield 86%, mp 245 °C. IR (KBr) cm\(^{-1}\): 3247, 3167, 3068 (NH\textsubscript{str.}), 2959 (Ar-CH\textsubscript{str.}), 2957 (CH\textsubscript{str.}), 1796 (C=O\textsubscript{str.}), 1647 (C=N\textsubscript{str. cyclic}), 839 (C-Cl), 697 (C-S-C\textsubscript{str.}); \textsuperscript{1}H NMR (CDCl\textsubscript{3}) \( \delta \): 3.38 (s, 2H, CH\textsubscript{2}), 6.75-7.46 (m, 8H, Ar-H), 7.57 (s, 1H, NH=C, D\textsubscript{2}O exchangeable), 7.59 (s, 1H, NH-Ar, D\textsubscript{2}O exchangeable), 9.91 (s, 1H, NH-Amide, D\textsubscript{2}O exchangeable).

2-[(6-Chlorobenzo[d]thiazol-2-ylcarbamoyl)methyl]-1-p-tolylisothiourea (4q). Yield 83%, mp 238 °C. IR (KBr) cm\(^{-1}\): 3238, 3149, 3056 (NH\textsubscript{str.}), 2945 (Ar-CH\textsubscript{str.}), 2947 (CH\textsubscript{str.}), 1754 (C=O\textsubscript{str.}), 1665 (C=N\textsubscript{str. cyclic}), 833 (C-Cl), 687 (C-S-C\textsubscript{str.}); \textsuperscript{1}H NMR (CDCl\textsubscript{3}) \( \delta \): 2.24 (s, 3H, CH\textsubscript{3}), 3.34 (s, 2H, CH\textsubscript{2}), 6.15-7.24 (m, 7H, Ar-H), 7.57 (s, 1H, NH=C, D\textsubscript{2}O exchangeable), 8.83 (s, 1H, NH-Ar, D\textsubscript{2}O exchangeable), 9.95 (s, 1H, NH-Amide, D\textsubscript{2}O exchangeable).

2-[(6-Chlorobenzo[d]thiazol-2-ylcarbamoyl)methyl]-1-(4-methoxyphenyl)isothiourea (4r). Yield 75%, mp 257 °C. IR (KBr) cm\(^{-1}\): 3256, 3184, 3038 (NH\textsubscript{str.}), 2948 (Ar-CH\textsubscript{str.}), 2994 (CH\textsubscript{str.}), 1725 (C=O\textsubscript{str.}), 1647 (C=N\textsubscript{str. cyclic}), 1028 (OCH\textsubscript{3}), 842 (C-Cl), 695 (C-S-C\textsubscript{str.}); \textsuperscript{1}H NMR (CDCl\textsubscript{3}) \( \delta \): 3.34 (s, 3H, OCH\textsubscript{3}), 3.44 (s, 2H, CH\textsubscript{2}), 6.25-7.54 (m, 7H, Ar-H), 7.89 (s, 1H, NH=C, D\textsubscript{2}O exchangeable), 8.85 (s, 1H, NH-Ar, D\textsubscript{2}O exchangeable), 9.76 (s, 1H, NH-Amide, D\textsubscript{2}O exchangeable).

2-[(6-Chlorobenzo[d]thiazol-2-ylcarbamoyl) methyl]-1-(4-chlorophenyl)isothiourea (4s). Yield 65%, mp 254 °C. IR (KBr) cm\(^{-1}\): 3273, 3145, 3056 (NH\textsubscript{str.}), 2956 (Ar-CH\textsubscript{str.}), 2956 (CH\textsubscript{str.}), 1765 (C=O\textsubscript{str.}), 1677 (C=N\textsubscript{str. cyclic}), 856, 844 (C-Cl), 724 (C-S-C\textsubscript{str.}); \textsuperscript{1}H NMR (CDCl\textsubscript{3}) \( \delta \): 4.15 (s, 2H, CH\textsubscript{2}), 6.12-7.79 (m, 7H, Ar-H), 7.99 (s, 1H, NH=C, D\textsubscript{2}O exchangeable), 8.98 (s, 1H, NH-Ar, D\textsubscript{2}O exchangeable), 9.96 (s, 1H, NH-Amide, D\textsubscript{2}O exchangeable).
2-[(6-Chlorobenzo[d]thiazol-2-ylcarbamoyl) methyl]-l-(4-bromophenyl)isothiourea (4t). Yield 68%, mp 287 °C. IR (KBr) cm⁻¹: 3298, 3167, 3056 (NH₃), 2989 (Ar-CH₃), 2954 (CH₃), 1754 (C=O), 1623 (C=N cyclic), 878 (C-Cl), 728 (C-S-C), 624 (C-Br); ¹H NMR (CDCl₃) δ: 4.23 (s, 2H, CH₂), 6.32-7.88 (m, 7H, Ar-H), 7.98 (s, 1H, NH=C, D₂O exchangeable), 8.95 (s, 1H, NH-Ar, D₂O exchangeable), 10.26 (s, 1H, NH-Amide, D₂O exchangeable).

2-[(6-Bromobenzo[d]thiazol-2-ylcarbamoyl) methyl]-l-phenylisothiourea (4u). Yield 59%, mp 234 °C. IR (KBr) cm⁻¹: 3616, 3595, 3532 (NH₃), 2992 (Ar-CH₃), 2805 (CH₃), 1878 (C=O), 1681 (C=N cyclic), 697 (C-S-C), 654 (C-Br); ¹H NMR (CDCl₃) δ: 4.36 (s, 2H, CH₂), 6.73-7.75 (m, 8H, Ar-H), 7.87 (s, 1H, NH=C, D₂O exchangeable), 7.91 (s, 1H, NH-Ar, D₂O exchangeable), 8.00 (s, 1H, NH-Amide, D₂O exchangeable).

2-[(6-Bromobenzo[d]thiazol-2-ylcarbamoyl) methyl]-l-p-tolylisothiourea (4v). Yield 83%, mp 258 °C. IR (KBr) cm⁻¹: 3244, 3132, 3056 (NH₃), 2944 (Ar-CH₃), 2954 (CH₃), 1732 (C=O), 1666 (C=N cyclic), 686 (C-S-C), 664 (C-Br); ¹H NMR (CDCl₃) δ: 2.43 (s, 3H, CH₃), 4.73 (s, 2H, CH₂), 6.84-7.79 (m, 7H, Ar-H), 7.88 (s, 1H, NH=C, D₂O exchangeable), 8.95 (s, 1H, NH-Ar, D₂O exchangeable), 9.20 (s, 1H, NH-Amide, D₂O exchangeable).

2-[(6-Bromobenzo[d]thiazol-2-ylcarbamoyl) methyl]-l-(4-methoxyphenyl)isothiourea (4w). Yield 78%, mp 245 °C. IR (KBr) cm⁻¹: 3256, 3167, 3078 (NH₃), 2965 (Ar-CH₃), 2967 (CH₃), 1754 (C=O), 1645 (C=N cyclic), 1023 (OCH₃), 698 (C-S-C), 634 (C-Br); ¹H NMR (CDCl₃) δ: 3.53 (s, 3H, OCH₃), 4.37 (s, 2H, CH₂), 6.28-7.83 (m, 7H, Ar-H), 7.85 (s, 1H, NH=C, D₂O exchangeable), 8.96 (s, 1H, NH-Ar, D₂O exchangeable), 9.47 (s, 1H, NH-Amide, D₂O exchangeable).

2-[(6-Bromobenzo[d]thiazol-2-ylcarbamoyl) methyl]-l-(4-chlorophenyl) isothiourea (4x). Yield 56%, mp 267 °C. IR (KBr) cm⁻¹: 3245, 3176, 3045 (NH₃), 2978 (Ar-CH₃), 2956 (CH₃), 1755 (C=O), 1677 (C=N cyclic), 879 (C-Cl), 756 (C-S-C), 644 (C-Br); ¹H NMR (CDCl₃) δ: 4.26 (s, 2H, CH₂), 6.43-7.46 (m, 7H, Ar-H), 7.96 (s, 1H, NH=C, D₂O exchangeable), 8.94 (s, 1H, NH-Ar, D₂O exchangeable), 9.68 (s, 1H, NH-Amide, D₂O exchangeable).
2-[(6-Bromobenzothiazol-2-ylcarbamoyl)methyl]-1-(4-bromophenyl)isothiourea (4y). Yield 75%, mp 254 °C. IR (KBr) cm⁻¹: 3278, 3123, 3054 (NH str.), 2936 (Ar-CH str.), 2921 (CH str.), 1734 (C=O str.), 1678 (C=N str. cyclic), 696 (C-S-C str.), 684, 637 (C-Br); ¹H NMR (CDCl₃) δ: 4.46 (s, 2H, CH₂), 6.27-7.18 (m, 7H, Ar-H), 7.92 (s, 1H, NH=C, D₂O exchangeable), 8.85 (s, 1H, NH-Ar, D₂O exchangeable), 9.86 (s, 1H, NH-Amide, D₂O exchangeable).
Table 7- Physicochemical parameters of compounds 4a-y - Scheme 5

<table>
<thead>
<tr>
<th>Compd</th>
<th>R</th>
<th>R'</th>
<th>*Mol. Formula</th>
<th>Log P&lt;sup&gt;b&lt;/sup&gt; (Calculated)</th>
<th>*R&lt;sub&gt;t&lt;/sub&gt;(R&lt;sub&gt;m&lt;/sub&gt;)&lt;sup&gt;d&lt;/sup&gt;</th>
<th>*Elemental analysis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>H</td>
<td>H</td>
<td>C&lt;sub&gt;16&lt;/sub&gt;H&lt;sub&gt;16&lt;/sub&gt;N&lt;sub&gt;4&lt;/sub&gt;OS&lt;sub&gt;2&lt;/sub&gt;</td>
<td>4.32 (3.17)</td>
<td>0.95(-1.27)</td>
<td>56.12 4.12 16.36</td>
</tr>
<tr>
<td>4b</td>
<td>H</td>
<td>4-CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>C&lt;sub&gt;17&lt;/sub&gt;H&lt;sub&gt;16&lt;/sub&gt;N&lt;sub&gt;4&lt;/sub&gt;OS&lt;sub&gt;2&lt;/sub&gt;</td>
<td>4.79 (3.67)</td>
<td>0.81(-0.62)</td>
<td>57.28 4.52 15.72</td>
</tr>
<tr>
<td>4c</td>
<td>H</td>
<td>4-OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>C&lt;sub&gt;17&lt;/sub&gt;H&lt;sub&gt;16&lt;/sub&gt;N&lt;sub&gt;4&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;S&lt;sub&gt;2&lt;/sub&gt;</td>
<td>4.10 (3.09)</td>
<td>0.93(-1.12)</td>
<td>54.82 4.33 15.04</td>
</tr>
<tr>
<td>4d</td>
<td>H</td>
<td>4-Cl</td>
<td>C&lt;sub&gt;16&lt;/sub&gt;H&lt;sub&gt;13&lt;/sub&gt;ClN&lt;sub&gt;4&lt;/sub&gt;OS&lt;sub&gt;2&lt;/sub&gt;</td>
<td>4.81 (3.88)</td>
<td>0.91(-1.00)</td>
<td>50.99 3.48 14.87</td>
</tr>
<tr>
<td>4e</td>
<td>H</td>
<td>4-Br</td>
<td>C&lt;sub&gt;16&lt;/sub&gt;H&lt;sub&gt;13&lt;/sub&gt;BrN&lt;sub&gt;4&lt;/sub&gt;OS&lt;sub&gt;2&lt;/sub&gt;</td>
<td>5.01 (4.03)</td>
<td>0.89(-0.90)</td>
<td>45.61 3.11 13.30</td>
</tr>
<tr>
<td>4f</td>
<td>4-CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>H</td>
<td>C&lt;sub&gt;17&lt;/sub&gt;H&lt;sub&gt;16&lt;/sub&gt;N&lt;sub&gt;4&lt;/sub&gt;OS&lt;sub&gt;2&lt;/sub&gt;</td>
<td>4.62 (3.67)</td>
<td>0.82(-0.65)</td>
<td>57.26 4.50 15.70</td>
</tr>
<tr>
<td>4g</td>
<td>4-CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>4-CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>C&lt;sub&gt;18&lt;/sub&gt;H&lt;sub&gt;18&lt;/sub&gt;N&lt;sub&gt;4&lt;/sub&gt;OS&lt;sub&gt;2&lt;/sub&gt;</td>
<td>5.30 (4.16)</td>
<td>0.83(-0.68)</td>
<td>58.35 4.90 15.12</td>
</tr>
<tr>
<td>4h</td>
<td>4-CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>4-OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>C&lt;sub&gt;18&lt;/sub&gt;H&lt;sub&gt;18&lt;/sub&gt;N&lt;sub&gt;4&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;S&lt;sub&gt;2&lt;/sub&gt;</td>
<td>4.58 (3.58)</td>
<td>0.99(-1.99)</td>
<td>55.94 4.69 14.50</td>
</tr>
<tr>
<td>4i</td>
<td>4-CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>4-Cl</td>
<td>C&lt;sub&gt;17&lt;/sub&gt;H&lt;sub&gt;13&lt;/sub&gt;ClN&lt;sub&gt;4&lt;/sub&gt;OS&lt;sub&gt;2&lt;/sub&gt;</td>
<td>5.21 (4.38)</td>
<td>0.84(-0.72)</td>
<td>52.23 3.87 14.33</td>
</tr>
<tr>
<td>4j</td>
<td>4-CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>4-Br</td>
<td>C&lt;sub&gt;17&lt;/sub&gt;H&lt;sub&gt;13&lt;/sub&gt;BrN&lt;sub&gt;4&lt;/sub&gt;OS&lt;sub&gt;2&lt;/sub&gt;</td>
<td>5.14 (4.13)</td>
<td>0.90(-0.95)</td>
<td>46.90 3.47 12.87</td>
</tr>
<tr>
<td>4k</td>
<td>4-OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>H</td>
<td>C&lt;sub&gt;17&lt;/sub&gt;H&lt;sub&gt;16&lt;/sub&gt;N&lt;sub&gt;4&lt;/sub&gt;OS&lt;sub&gt;2&lt;/sub&gt;</td>
<td>3.89 (3.47)</td>
<td>0.99(-1.99)</td>
<td>54.80 4.31 15.01</td>
</tr>
<tr>
<td>4l</td>
<td>4-OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>4-CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>C&lt;sub&gt;18&lt;/sub&gt;H&lt;sub&gt;18&lt;/sub&gt;N&lt;sub&gt;4&lt;/sub&gt;OS&lt;sub&gt;2&lt;/sub&gt;</td>
<td>4.42 (3.97)</td>
<td>0.86(-1.18)</td>
<td>55.92 4.67 14.51</td>
</tr>
<tr>
<td>4m</td>
<td>4-OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>4-OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>C&lt;sub&gt;18&lt;/sub&gt;H&lt;sub&gt;18&lt;/sub&gt;N&lt;sub&gt;4&lt;/sub&gt;O&lt;sub&gt;3&lt;/sub&gt;S&lt;sub&gt;2&lt;/sub&gt;</td>
<td>3.80 (3.39)</td>
<td>0.94(-1.19)</td>
<td>53.71 4.51 13.92</td>
</tr>
<tr>
<td>4n</td>
<td>4-OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>4-Cl</td>
<td>C&lt;sub&gt;17&lt;/sub&gt;H&lt;sub&gt;13&lt;/sub&gt;ClN&lt;sub&gt;4&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;S&lt;sub&gt;2&lt;/sub&gt;</td>
<td>3.87 (4.18)</td>
<td>0.86(-1.18)</td>
<td>50.18 3.72 13.77</td>
</tr>
<tr>
<td>4o</td>
<td>4-OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>4-Br</td>
<td>C&lt;sub&gt;17&lt;/sub&gt;H&lt;sub&gt;13&lt;/sub&gt;BrN&lt;sub&gt;4&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;S&lt;sub&gt;2&lt;/sub&gt;</td>
<td>4.92 (4.33)</td>
<td>0.88(-1.18)</td>
<td>45.24 3.35 12.41</td>
</tr>
</tbody>
</table>
### Experimental Protocols

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>4p</strong></td>
<td>4-Cl</td>
<td>H</td>
<td>C\textsubscript{16}H\textsubscript{13}Cl\textsubscript{4}N\textsubscript{4}OS\textsubscript{2}</td>
<td>4.68 (3.89)</td>
</tr>
<tr>
<td><strong>4q</strong></td>
<td>4-Cl</td>
<td>4-CH\textsubscript{3}</td>
<td>C\textsubscript{17}H\textsubscript{15}Cl\textsubscript{4}N\textsubscript{4}OS\textsubscript{2}</td>
<td>5.12 (4.39)</td>
</tr>
<tr>
<td><strong>4r</strong></td>
<td>4-Cl</td>
<td>4-OCH\textsubscript{3}</td>
<td>C\textsubscript{17}H\textsubscript{15}Cl\textsubscript{4}N\textsubscript{4}OS\textsubscript{2}</td>
<td>4.62 (3.81)</td>
</tr>
<tr>
<td><strong>4s</strong></td>
<td>4-Cl</td>
<td>4-Cl</td>
<td>C\textsubscript{16}H\textsubscript{12}Cl\textsubscript{2}N\textsubscript{4}OS\textsubscript{2}</td>
<td>5.41 (4.60)</td>
</tr>
<tr>
<td><strong>4t</strong></td>
<td>4-Cl</td>
<td>4-Br</td>
<td>C\textsubscript{16}H\textsubscript{12}BrCl\textsubscript{2}N\textsubscript{4}OS\textsubscript{2}</td>
<td>5.62 (4.75)</td>
</tr>
<tr>
<td><strong>4u</strong></td>
<td>4-Br</td>
<td>H</td>
<td>C\textsubscript{16}H\textsubscript{12}BrN\textsubscript{4}OS\textsubscript{2}</td>
<td>4.93 (4.04)</td>
</tr>
<tr>
<td><strong>4v</strong></td>
<td>4-Br</td>
<td>4-CH\textsubscript{3}</td>
<td>C\textsubscript{17}H\textsubscript{15}BrN\textsubscript{4}OS\textsubscript{2}</td>
<td>5.54 (4.54)</td>
</tr>
<tr>
<td><strong>4w</strong></td>
<td>4-Br</td>
<td>4-OCH\textsubscript{3}</td>
<td>C\textsubscript{17}H\textsubscript{15}BrN\textsubscript{4}OS\textsubscript{2}</td>
<td>4.97 (3.96)</td>
</tr>
<tr>
<td><strong>4x</strong></td>
<td>4-Br</td>
<td>4-Cl</td>
<td>C\textsubscript{16}H\textsubscript{12}BrCl\textsubscript{2}N\textsubscript{4}OS\textsubscript{2}</td>
<td>5.52 (4.75)</td>
</tr>
<tr>
<td><strong>4y</strong></td>
<td>4-Br</td>
<td>4-Br</td>
<td>C\textsubscript{16}H\textsubscript{12}Br\textsubscript{2}N\textsubscript{4}OS\textsubscript{2}</td>
<td>5.91 (4.90)</td>
</tr>
</tbody>
</table>

* Solvent of crystallization—Ethanol.
* Log P was determined by octanol:phosphate buffer method; Log P was calculated using software ChemDraw Ultra 8.0.
* Solvent system- Toluene: Ethyl acetate: Formic acid (5:4:1).
* A logarithmic function of R\textsubscript{f} value was also calculated; R\textsubscript{m} = log (1 \textdiv R\textsubscript{f}).
* Elemental analysis for C, H, N were within ± 0.4 % of the theoretical value.
4.2. Pharmacology

The pharmacological testing of the final compounds of series 2 and 3 have been performed by National Institute of Neurological Disorders and Stroke (NINDS), USA under Anticonvulsant screening program (ASP), following the protocol adopted by the Antiepileptic Drug Development (ADD) program. The pharmacological screenings of rest of the compounds of the series 1, 4 and 5 were carried out on Swiss albino mice (20-25 g) or Adult Wistar rats (130-160 g) of either sex. Animals were obtained from Central Animal House Facility, Jamia Hamdard, and were housed under standard normal laboratory conditions (12 h light-dark cycles) in groups of six in Perspex cages in the laboratory three days prior to the experiments. They were kept at an ambient temperature of 25 ± 2°C and allowed free access to food and water except at the time they were brought out of the cage. The experimental sessions were during the light phase of the cycle between 8 am to 4 pm. Test drugs were dissolved in polyethylene glycol (PEG). All the procedures described in the study were reviewed and approved by the Institutional Animal Ethics Committee Form no. 502 (173/CPCSEA, 28th Jan-2000).

The Pharmacological evaluation includes:

4.2.1. Anticonvulsant Screening
   4.2.1.1. Maximal Electroshock Induced Seizure (MES) test
   4.2.1.2. Subcutaneous pentylenetetrazole induced seizure (scPTZ) test
   4.2.1.3. Minimal clonic seizure (6 Hz) test

4.2.2. Minimal motor impairment test

4.2.3. Acute toxicity studies (LD\textsubscript{50})

4.2.4. Antidepressant Screening

4.2.5. Antinociceptive Screening

4.2.6. Biochemical estimation

4.2.7. Neurochemical study
   4.2.7.1. Estimation of GABA level in rat brain
4.2.1. Anticonvulsant Screening
Initially all compounds were administered ip at doses of 30, 100 and 300 mg/kg to mice. Activity was established using the MES and scPTZ tests according to the protocol by Antiepileptic Drug Development Program (ADD), Epilepsy Branch, National Institute of Health, Bethesda, MD, USA

4.2.1.1. Maximal Electroshock Induced Seizure (MES) test
Maximal electroshock seizure test was carried out according to the standard protocol. Mice were prescreened 24 h before by delivering maximal electroshock (50 mA; 60 Hz and 0.2 s duration) by means of corneal electrodes. A drop of 0.9% sodium chloride was instilled in each eye prior to the application of electrodes in order to prevent death of the animal. Test solution of all compounds was prepared in 30 % v/v polyethylene glycol 400 and the animals were dosed intraperitoneally 30 min prior to testing. In the preliminary screening, each compound was administered as an i.p. injection at three dose levels (30, 100 and 300 mg/kg body mass) and the anticonvulsant activity was assessed after 0.5 h and 4.0 h intervals of administration. Abolition of hind limb tonic extensor component of the seizure in half or more of the animals is defined as protection.

4.2.1.2. Subcutaneous pentylenetetrazole induced seizure (scPTZ) test
The subcutaneous pentylenetetrazole test was performed according to the known protocol. This method utilizes pentylenetetrazole (75 mg/kg) that produces seizures in >95% of animals as a 0.5% solution subcutaneously in the posterior midline. The animal was observed for 30 min., failure to observe even a threshold seizure (a single episode of clonic spasms of at least 5s duration) was defined as protection.

4.2.1.3. Minimal clonic seizure (6 Hz) test
Some clinically useful AEDs are ineffective in the standard MES and scPTZ tests but still have anticonvulsant activities in vivo. In order to identify potential AEDs with this profile, some compounds were tested in the minimal clonic seizure (6 Hz or psychomotor) test. Like the maximal electroshock (MES) test, the minimal clonic seizure (6 Hz) test was used to assess compound’s efficacy against electrically induced seizures but used a lower frequency (6 Hz) and longer duration of stimulation (3 s). Test compounds were pre-administered to mice via i.p. injection. At varying
times, individual mice (four mice per time point) were challenged with sufficient current delivered through corneal electrodes to elicit a psychomotor seizure in 97% of animals (32 mA for 3 s). The untreated mice would display seizures characterized by a minimal clonic phase followed by stereotyped, automatistic behaviors, described originally as being similar to the aura of human patients with partial seizures. Animals not displaying this behavior were considered to be protected.

4.2.2. Minimal motor impairment test
The minimal motor impairment was measured in mice by the rotorod test. The mice were trained to stay on an accelerating rotorod of diameter 3.2 cm that rotates at 10 rpm. Trained animals were given i.p. injection of the test compounds 30, 100 and 300 mg/kg. Neurotoxicity was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min. in each of the trials.

4.2.3. Acute toxicity studies (LD<sub>50</sub>)
The most active compounds were tested for their acute toxic effects on mice. In this test, the animals were administered a range of intraperitoneal doses of compounds and the toxic effects, if any, were studied. The median lethal dose, LD<sub>50</sub> (abbreviation for “Lethal Dose, 50 %”) of a toxic substance or radiation is the dose required to kill half the members of a tested population after a specified test duration. The most active compounds were dissolved in PEG-400 and different doses were administered intraperitoneally to mice and the behavioral changes are seen and compared with phenytoin, carbamazepine and phenobarbital.

4.2.4. Antidepressant Screening
The most active compounds were tested for their antidepressant effects on rats. The forced swim pool method described previously by Porsolt et al. was followed. Wistar rats were placed in chamber (diameter 45 cm, height 20 cm) containing water up to a height of 15 cm at 25 ± 2 °C. Two swim sessions were conducted an initial 15 minutes pretest, followed by a 5-minute test session 24 hours later. The animals were drug administrated (100 mg/kg) the test compound i.p. 30 minutes before the test session. The period of immobility (passive floating without struggling, making only those movements that are necessary to keep its head above the surface of water) during the 5-minute test period were measured.
4.2.5. Antinociceptive Screening

The most active compounds were tested for their antinociceptive effects on mice. The antinociceptive activity was assessed by using Thermal stimulus technique. Investigations were performed on albino mice in groups of 6 each which were kept under standard laboratory conditions. Test compounds were suspended in methyl cellulose-water (0.5 %) mixture. Each compound was administered orally at a dose of 20 mg/kg. The antinociceptive activity was assessed after 4 h interval of the administration. Tail of each mice was gently immersed into thermostatically controlled water at 55 °C. The parameter measured was the time that elapsed between immersion and the attempt to withdraw the tail from hot water for control as well as treated group of animals.

4.2.6. Biochemical estimation

The most active compounds were tested for their biochemical estimation on rats. The biochemical parameters have been measured to assess the magnitude of hepatotoxicity that may be caused by the synthesized compounds. There are mainly three tests by which the liver function tests are assessed. These include the estimation of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and alkaline phosphatase.

**Animals:** Healthy albino rats of either sex weighing 200-250 g were used as experimental animals. The animals were divided into groups of 6 each. They were kept on the normal diet and were housed under normal laboratory conditions (23 ± 2 °C, 60-70% RH, 12 h light cycle).

4.2.6.1. Estimation of Serum glutamate oxaloacetate transaminase (SGOT) or Aspartate transaminase (AST)

It is estimated using Reitman and Frankel method. It is a mitochondrial enzyme present in large quantities in liver, heart, skeletal muscles and kidney that gets released whenever these tissues are destroyed

**Principle**

SGOT (AST) is the enzyme that is responsible for the catalysis of the reaction:

\[ \alpha \text{-Ketoglutarate} + L\text{-Aspartate} = L\text{-Glutamate} + Oxaloacetate \]
The oxaloacetate thus released gets coupled with 2, 4-dinitrophenyl hydrazine (2, 4-DNPH) to form the corresponding hydrazone derivative that gives color in alkaline medium and can be estimated colorimetrically.

**Reagents:**

1) Reagent 1: Buffered aspartate α-keto glutarate substrate, pH 7.4
2) Reagent 2: DNPH color reagent
3) Reagent 3: 4N NaOH
4) Reagent 4: 2mM working pyruvate standard

**Solutions Preparation**

**Solution 1:** 1 mL of the Reagent 3 was diluted to 10 mL with distilled water. Other reagents were used as such.

**Procedure**

**Preparation of Standard Plot**

Serial dilutions for standard plot were prepared as shown in the Table 8.

**Table 8- Serial dilution for SGOT standard curve**

<table>
<thead>
<tr>
<th>Tube no.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assigned Enzymatic activity (IU/mL)</td>
<td>0</td>
<td>24</td>
<td>61</td>
<td>114</td>
<td>190</td>
</tr>
<tr>
<td>Reagents to pipette</td>
<td>Volume in mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reagent 1</td>
<td>0.5</td>
<td>0.45</td>
<td>0.40</td>
<td>0.35</td>
<td>0.30</td>
</tr>
<tr>
<td>Reagent 4</td>
<td>-</td>
<td>0.05</td>
<td>0.1</td>
<td>0.15</td>
<td>0.2</td>
</tr>
<tr>
<td>Purified water</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Reagent 2</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Mixed well and allowed to stand at room temperature (15-30 °C) for 20 min

| Solution 1 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |

Mixed well by inversion and allowed to stand at room temperature for 10 min and the optical density (O.D.) of all the five tubes were measured against distilled water. The optical densities that were obtained for various dilutions are summarized in Table 9. A standard curve was plotted using enzyme activity (units/mL) on X-axis and O.D. on Y-axis. It was observed that the O.D. increases with the increase in the enzymatic
activity but with a decreasing rate. This is because the products formed as the reaction proceeds, inhibits the enzyme and thus its activity by feedback mechanism.

**Table 9** - Optical density for the enzyme activity

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>Enzyme activity (units/mL)</th>
<th>Optical density</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0.328</td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td>0.448</td>
</tr>
<tr>
<td>3</td>
<td>61</td>
<td>0.591</td>
</tr>
<tr>
<td>4</td>
<td>114</td>
<td>0.621</td>
</tr>
<tr>
<td>5</td>
<td>190</td>
<td>0.653</td>
</tr>
</tbody>
</table>

**Test samples**

The animals were divided into groups of six, and the control group received a basal diet and vehicle. Other groups were administered the test drug in a dose of 30 mg/kg/day p.o. (in methylcellulose) for two weeks. After the stipulated period, each animal was anesthetized by anesthetic ether, blood was collected and centrifuged at 6000 rpm for 10 min to get the serum which was then processed further immediately after collection. After making the standard plot the test samples were prepared by using the reagent 1, 2 and solution 3 to the serum obtained from the experimental animal. The sequence of addition of the reagents is shown in Table 10.

**Table 10** - Serial dilution for measurement of the optical density

<table>
<thead>
<tr>
<th>Pipette into tube marked</th>
<th>Test (T) Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent 1</td>
<td>0.25 mL</td>
</tr>
<tr>
<td>Incubated at 37 °C for 5 min</td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>0.05 mL</td>
</tr>
<tr>
<td>Mixed well and incubated at 37 °C for 60 min</td>
<td></td>
</tr>
<tr>
<td>Reagent 2</td>
<td>0.25 mL</td>
</tr>
<tr>
<td>Mixed well and allowed to stand at room temperature for 20 min</td>
<td></td>
</tr>
<tr>
<td>Solution 3</td>
<td>2.5 mL</td>
</tr>
</tbody>
</table>

The final mixture was mixed well and allowed to stand at room temperature for 10 min and the optical density was measured against distilled water at 505 nm.
Calculations
O.D. of test (T) was marked on the Y-axis of the standard curve and extrapolated to get the corresponding enzyme activity.

4.2.6.2. Estimation of Serum glutamate pyruvate transaminase (SGPT) or Alanine transaminase (AST)
It is estimated using Reitman and Frankel method. It is a cytosolic enzyme present abundantly in liver cells, the level of which is elevated in liver diseases.

Principle
SGPT (ALT) is the enzyme that is responsible for the catalysis of the reaction:
$$\alpha-Ketoglutarate + L-Alanine = L-Glutamate + Pyruvate$$
The pyruvate thus released gets coupled with 2,4-dinitrophenyl hydrazine (2,4-DNPH) to form the corresponding hydrazone derivative that gives color in alkaline medium and can be estimated colorimetrically.

Reagents:
1) Reagent 1: Buffered alanine $\alpha$-keto glutarate substrate, pH 7.4
2) Reagent 2: DNPH color reagent
3) Reagent 3: 4N NaOH
4) Reagent 4: 2mM working pyruvate standard

Preparation of Test solutions
Solution 1: 1 mL of the Reagent 3 was diluted to 10 mL with distilled water. Other reagents were used as such.

Procedure
Preparation of Standard Plot
Serial dilutions were prepared as shown in the Table 11.
Mixed well by inversion and allowed to stand at room temperature for 10 min and the optical density (O.D.) of all the five tubes were measured against distilled water.
The optical densities that were obtained for various dilutions are summarized in Table 12. A standard curve was plotted using enzyme activity (units/mL) on X-axis and O.D. on Y-axis. It was observed that the O.D. increases with the increase in the enzymatic activity but with a decreasing rate. This is because the products formed as
the reaction proceeds, inhibits the enzyme and thus its activity by feedback mechanism.

**Table 11: Serial dilution for SGPT standard curve**

<table>
<thead>
<tr>
<th>Tube no.</th>
<th>Assigned Enzymatic activity (IU/mL)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>28</td>
<td>57</td>
<td>97</td>
<td>150</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reagents to pipette</th>
<th>Volume in mL</th>
<th>Reagent 1</th>
<th>Reagent 4</th>
<th>Purified water</th>
<th>Reagent 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent 1</td>
<td>0.5</td>
<td>0.45</td>
<td>0.40</td>
<td>0.15</td>
<td>0.30</td>
</tr>
<tr>
<td>Reagent 4</td>
<td>-</td>
<td>0.05</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Purified water</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Reagent 2</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Mixed well and allowed to stand at room temperature (15-30 °C) for 20 min

| Solution 1 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |

**Table 12: Optical density obtained for enzyme activity**

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>Enzyme activity (units/mL)</th>
<th>Optical density</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0.359</td>
</tr>
<tr>
<td>2</td>
<td>28</td>
<td>0.463</td>
</tr>
<tr>
<td>3</td>
<td>57</td>
<td>0.582</td>
</tr>
<tr>
<td>4</td>
<td>97</td>
<td>0.619</td>
</tr>
<tr>
<td>5</td>
<td>150</td>
<td>0.686</td>
</tr>
</tbody>
</table>

**Test samples**

The animals were divided into groups of six, and the control group received a basal diet and vehicle. Other groups were administered the test drug in a dose of 30 mg/kg/day po (in methylcellulose) for two weeks. After the stipulated period, each animal was anesthetized by anesthetic ether, blood was collected and centrifuged at 6000 rpm for 10 min to get the serum which was then processed further immediately after collection.
After making the standard plot the test samples were prepared by using the reagent 1, 2 and solution 3 to the serum obtained from the experimental animal. The sequence of addition of the reagents is shown in Table 13.

**Table 13- Serial dilution for measurement of the optical density**

<table>
<thead>
<tr>
<th>Pipette into tube marked</th>
<th>Test (T) Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent 1</td>
<td>0.25 mL</td>
</tr>
<tr>
<td>Incubated at 37 °C for 5 min</td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>0.05 mL</td>
</tr>
<tr>
<td>Mixed well and incubated at 37 °C for 60 min</td>
<td></td>
</tr>
<tr>
<td>Reagent 2</td>
<td>0.25 mL</td>
</tr>
<tr>
<td>Mixed well and allowed to stand at room temperature for 20 min</td>
<td></td>
</tr>
<tr>
<td>Solution 3</td>
<td>2.5 mL</td>
</tr>
</tbody>
</table>

The final mixture was mixed well and allowed to stand at room temperature for 10 min and the optical density was measured against distilled water at 505 nm.

**Calculations**

O.D. of test (T) was marked on the Y-axis of the standard curve and extrapolated to get the corresponding enzyme activity.

**4.2.6.3. Estimation of alkaline phosphatase**

Phosphatases are the category of enzymes that remove phosphoric acid from certain monophosphoric esters. There are several body processes that utilize this enzyme and therefore its change of concentration acts as a marker for various diseases. It was estimated using King and Armstrong’s method.\(^ \text{10} \)

**Principle**

Alkaline phosphatase from serum converts phenyl phosphates to inorganic phosphate and phenol at pH 10. Phenol thus formed reacts with 4-amino antipyrine in presence of the oxidizing agent potassium ferric cyanide in alkaline medium and forms an orange red colored complex that can be measured colorimetrically. The intensity of color is directly proportional to the enzyme activity.
Reagents:
1) Reagent 1: Buffered substrate, pH 10.0
2) Reagent 2: Chromogen reagent
3) Reagent 3: Phenol standard, 10 mg, 10%

Preparation of test solutions
Solution 1: The contents of the vial of reagent 1 (buffered substrate) was reconstituted with 4-5 mL of distilled water. Other reagents were used as such.

Procedure
Experimental procedure and sequence of addition of reagents for the estimation of alkaline phosphatase is summarized in Table 14.

Table 14: Estimation of alkaline phosphatase in serum

<table>
<thead>
<tr>
<th></th>
<th>Blank (B)</th>
<th>Standard (S)</th>
<th>Control (C)</th>
<th>Test (T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working buffered substrate</td>
<td>1.0 mL</td>
<td>1.0 mL</td>
<td>1.0 mL</td>
<td>1.0 mL</td>
</tr>
<tr>
<td>Distilled water</td>
<td>3.0 mL</td>
<td>3.0 mL</td>
<td>3.0 mL</td>
<td>3.0 mL</td>
</tr>
<tr>
<td>Serum</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.0 mL</td>
</tr>
<tr>
<td>Phenol Standard</td>
<td>-</td>
<td>1.0 mL</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chromogen Reagent</td>
<td>2.0 mL</td>
<td>2.0 mL</td>
<td>2.0 mL</td>
<td>2.0 mL</td>
</tr>
<tr>
<td>Serum</td>
<td>-</td>
<td>-</td>
<td>1.0 mL</td>
<td>-</td>
</tr>
</tbody>
</table>

All the solutions were mixed well by inversion and the optical density (O.D.) was measured for blank (B), standard (S), control (C) and test (T) against distilled water at 510 nm.

Calculations
The enzyme activity was calculated by using following formula,

\[
Serum\text{ alkaline phosphatase} = \frac{O.D_{\text{test}} - O.D_{\text{control}}}{O.D_{\text{std}} - O.D_{\text{blank}}} \times 10 \text{ KA Units}
\]
4.2.7. Neurochemical study

4.2.7.1. Estimation of GABA level

The most active compounds of Scheme 3 and 4 were tested for estimation of GABA level in rat brain. The neurochemical investigation was carried out to investigate the effect of the synthesized compounds to the GABA levels in various regions of rat brain. Various methods for the estimation of GABA in tissue extracts have been reported. Some of the methods are:

i. Enzymatic UV method
ii. Enzymatic fluorimetric method
iii. Paper chromatographic method
iv. Receptor inhibition method
v. Column chromatographic method

Among these methods, enzymatic UV method is very selective, specific and can be used successfully to determine the concentration of GABA in tissue extracts. In the present study, the enzymatic ultraviolet (UV) method has been employed.

1. Glass apparatus: Borosil
2. Micropipettes: Accupippette
3. Refrigerated centrifuge: Remi cooling compufuge
4. Refrigerator/deep freezer: Vest frost
5. Vacuum centrifuge: Maxi dry Iyo
6. Water bath: Remi
7. UV Spectrophotometer: Shimadzu
8. Sonicator: Biosonic 1

Reagents—All the reagents used were of AR grade

1. Dipotassium hydrogen orthophosphate; K$_2$HPO$_4$
2. Potassium dihydrogen phosphate; KH$_2$PO$_4$
3. Sodium pyrophosphate; Na$_4$P$_2$O$_7$. 10H$_2$O
4. 2-Mercaptoethanol
5. Nicotinamide adenine dinucleotide (β-form) (NAD)
6. Nicotinamide adenine dinucleotide reduced (β-form) (NADH)
7. α-Ketoglutaric acid
8. γ-Aminobutyric acid
9. Sodium hydroxide
10. Hydrochloric acid

Collection of samples:
Animals: Wistar rats (130-160 g) in group of six
Route of administration: Intraperitoneal (ip)

Schematic depiction of the UV enzymatic method for the determination of GABA in tissue samples are given in Fig. 10.
After 2 hrs of drug administration, animal sacrificed

Brain dissected off (whole brain & different regions)

Brain tissue homogenized in 80% v/v ice-cold ethanol.

Centrifuged (7000 rpm, 10 min, 0°C)

Residue

Resuspended in 75% v/v ice-cold ethanol

Centrifuged (twice, 7000 rpm 20 min, 0°C)

Residue (discarded)

Supernatant (collected)

Dried in vacuum centrifuge

Centrifuged (10,000 rpm, 20 min, 0°C)

1 mL of distilled water for every 100 mg of fresh weight of tissue

Supernatant

Assay

Residue (discarded)

Fig. 10: Flow chart for the collection of samples from the rat brain tissue
UV method
The most rapid, specific and scientific determination of GABA in biological extracts is based on the use of the UV enzymatic method. The procedure described below was employed for the determination of GABA in extracts from brain, but obviously can be modified for extracts from other types of tissues.

Composition of reagents
1. Sodium pyrophosphate buffer (0.1 M, pH 8.1);
   Weighed 4.46 g of Na$_4$P$_2$O$_7$.10H$_2$O in 70 mL of distilled water, adjusted to pH 8.1 and diluted to 100 mL with distilled water.
2. Mercaptoethanol (20 mg/mL)
   70 μL of mercaptoethanol (20 mg) was diluted to 10 mL in sodium pyrophosphate buffer.
3. NAD;
   13.2 mg of NAD dissolved in 2 mL of distilled water
4. α-Ketoglutarate;
   0.146 g of α-ketoglutaric acid was dissolved in 5 mL of distilled water. It was neutralized with 1N sodium hydroxide and diluted to 10 mL with distilled water.
5. Reagent mixture: 1mL of solution 2, 3 and 4 were mixed.

Stability of solution
Solution 5 was freshly prepared and stored in ice cold condition. Also the enzyme solution was stored in ice cold condition.

Procedure
For the study, Wistar rats weighing 130-160 g were used. The control group was treated only with the vehicle (30% v/v polyethylene glycol 400). The compounds were dosed at their minimal anticonvulsant activity doses. After 2 h of drug administration the animal was sacrificed by cervical dislocation and the brains were separated immediately and weighed. The whole brain or different brain regions like mid brain, cerebellum, medulla oblongata and olfactory lobe were dropped into separate vials containing 4-6 mL of 80% ice cold ethanol and processed further under frozen condition as presented in Fig.10.
Standard GABA solution and calibration curve

Standard GABA solutions were prepared to give a concentration range of 1-12 μg/mL in distilled water and processed as follows. The reaction was started by the addition of 0.3 mL of pyrophosphate buffer (pH = 8.1), 0.3 mL of the reagent mixture and 1.53 mL of the enzyme solution and the sample was transferred to an optical tube. Optical density at 340 nm was read immediately after transferring the reaction mixture.

All spectrophotometric readings were made with the Shimadzu UV spectrophotometer. A standard curve was obtained by plotting different concentration of standard GABA with the corresponding O.D. observed.

Observation and calculations

All the spectrophotometric readings were recorded at 340 nm. The optical density was converted to concentration of GABA for the test samples from the standard calibration curve.

<table>
<thead>
<tr>
<th>Concentration (μg/mL)</th>
<th>Optical Density (O.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.021</td>
</tr>
<tr>
<td>2</td>
<td>0.039</td>
</tr>
<tr>
<td>4</td>
<td>0.077</td>
</tr>
<tr>
<td>6</td>
<td>0.112</td>
</tr>
<tr>
<td>8</td>
<td>0.146</td>
</tr>
<tr>
<td>10</td>
<td>0.190</td>
</tr>
</tbody>
</table>

Optical density values at different concentration of GABA
4.3. References