5. EXPERIMENTAL

5.1 Chemical Work

All the reagents and solvents required for synthesis of the compounds were purified by general laboratory techniques before use. Melting points were determined using silicon oil bath type (Veego) and heating block type (Lab India) melting point apparatus and are uncorrected. Completion of the reactions were monitored by thin layer chromatography (TLC) using silica gel pre-coated plates (60F254, Merck, 0.25 mm thickness) visualizing in ultraviolet light (254 nm) or iodine vapours. Yields reported here are un-optimized. The IR spectra (wave numbers in cm\(^{-1}\)) were recorded on a BRUKER ALPHA-T (Germany) FT-IR spectrophotometer using potassium bromide discs. \(^1\)H-NMR and \(^{13}\)C-NMR spectra were recorded using Bruker Advance-II 400 MHz spectrometer in CDCl\(_3\) or DMSO-d\(_6\) solvents; chemical shift has been expressed as δ ppm and coupling constant (\(J\)) in Hz. Mass spectra were recorded using Thermo Fisher mass spectrometer with EI as ion source or Advion mass spectrometer with ESI as ion source and also using AB Sciex 3200 Q Trap mass analyzer for the compounds. Elemental analyses were performed on a ThermoFisher FLASH 2000 organic elemental analyzer.

HPLC analysis was performed with an Ekspert ultraLC -100XL(As a part of Sciex) quaternary pump which delivered the gradient mobile phase at a flow rate of 0.8 ml/min, the mobile phase composition was phase A - water with 0.1 % formic acid and B – Methanol : Acetonitrile (50:50) in different ratios . The gradient program followed was, 0 min to 3min (B 10%), 12 min (B 80%), 16 min (B 10%), 20 min (B 10%) with an Ekspert auto-sampler, degasser and column compartment. The auto-sampler was equipped with a 108 well plate and was used to inject 20 μl samples into the HPLC column. The Ekspert auto-sampler cooling device was set at 15 °C. Chromatography was performed on a Phenomenex® Luna C18 (100 x 2.0 mm id, 5 μm) analytical column fitted with a Phenomenex® Security Guard™ System containing a C18 (4 x 3 mm) pre-column. The column was kept at 40°C with an Ekspert 100 column oven compartment.

Analysis was performed on an AB SCIEX API 3200 triple quadrupole mass spectrometer (AB SCIEX, ) equipped with an electrospray ionization (ESI\(^+\)) source operated at 550 °C and set in the positive ion mode for ion production. MRM method is a world widely used a gold standard method for quantitation and gives better selectivity and
sensitivity. Moreover it increases the confidence level of analysis. Transition of the protonated precursor ions (Q1MS) and their fragment ions (Q2MS) were monitored at unit resolution in the multiple reaction monitoring (MRM) mode with a dwell time in ms as per number of transition. The curtain, nebulizer, turbo, and collision gases were set at 25, 50, 50 and 5 psi, respectively, while the ion spray voltage and the source temperature were set at 5500 V and 550°C, respectively. The declustering potential, collision energy, entrance potential, and collision cell exit potential were optimized. The instrument was interfaced to a workstation running Analyst™ version 1.6.2 software and all the generated data were captured and stored on the work station’s hard disc drive.

5.1.1 1,2-Di(p-tolyl)ethanone\textsuperscript{127} (4)

2-p-Tolylacetic acid (1) (2 g, 13.33 mM) was converted to acid chloride using thionyl chloride (3 ml) under refluxing for 3 hrs under anhydrous conditions. Excess of thionyl chloride was removed by vacuum. In another RBF, dry DCM (10 ml) and anhydrous AlCl\textsubscript{3} (1.74 g) were taken. The contents were stirred under anhydrous conditions. To this solution (1.26 ml) of toluene was added dropwise. The acid chloride was added to the contents of the RBF and stirring was continued for 2 hrs maintaining the temperature between -5 to -10 °C. The contents were finally poured over crushed ice containing conc. HCl and then extracted with successive quantities of chloroform. The organic extract was washed with sodium bicarbonate solution (5%) and water. It was then dried over anhydrous sodium sulfate, filtered and subjected to solvent recovery. The crude product so obtained was recrystallised from methanol to afford 1,2-di(p-tolyl)ethanone (4) as white crystals, (1.7 gm, 85%); m.p. 98-102 °C (Lit.\textsuperscript{128} 102-103 °C).

Anal:

\begin{align*}
\text{TLC} & : R_f 0.54 (20 \% \text{ EtOAc in } n\text{-hexane}) . \\
\text{IR (KBr, cm}^{-1}) & : 3028, 2898, 1688, 1600, 813 . \\
\text{MS (m/z)} & : 224.61 (M)^+ .
\end{align*}

5.1.2 1,2-Bis(4-chlorophenyl)ethanone\textsuperscript{127} (5)

The title compound was prepared from 2-(4-chlorophenyl)acetic acid (2) (2 g, 11.722 mM) and chlorobenzene (1.20 ml) following the method described for the synthesis of compound (4). The title compound (5) was obtained as white crystals, (1.9 gm, 96 %); m.p. 111-113 °C (Lit.\textsuperscript{129} 113-114 °C).
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Anal:

TLC : R_f 0.35 (20 % EtOAc in n-hexane).

IR (KBr, cm^{-1}) : 3093, 2896, 1689, 1584, 820.

MS (m/z) : 265.14 (M)^+.

5.1.3 1,2-Bis(4-methoxyphenyl)ethanone\textsuperscript{130} (6)

The title compound was prepared from 2-(4-methoxyphenyl)acetic acid (3) (2 g, 12.33 mM) and anisole (1.26 ml) following the method described for the synthesis of compound (4). The title compound (6) was obtained as white crystals, (1.7 gm, 85%); m.p. 115-117 \degree C (Lit\textsuperscript{131} 110-112 \degree C).

Anal:

TLC : R_f 0.54 (20 % EtOAc in n-hexane).

IR (KBr, cm^{-1}) : 3035, 2961, 1679, 1512, 1172, 828.

MS (m/z) : 256.38 (M)^+.

5.1.4 2-Bromo-1,2-di(p-tolyl)ethanone\textsuperscript{127} (7)

1,2-Di(p-tolyl)ethanone (4) (2.0 g, 8.92 mM) was taken in a 100 ml RBF and dissolved in sufficient quantity of glacial acetic acid (10 ml) by warming. Bromine (0.5 ml) was added drop-wise into the stirred solution and the reaction was monitored by TLC until completion. The reaction mixture was poured into the ice cold water (200 ml) containing sodium metabisulphite to neutralize the excess bromine. The white precipitate so obtained was extracted with chloroform (3 x 20 ml) and the separated chloroform layer was dried, the solvent distilled off and the resulting residue was crystallised in methanol to yield pure white crystals of compound (7), (1.5 gm, 75%); m.p. 98-100 \degree C (Lit.\textsuperscript{132} 96 \degree C).

Anal:

TLC : R_f 0.41 (20 % EtOAc in n-hexane).

IR (KBr, cm^{-1}) : 3013, 2858, 1676, 1603, 841.

MS (m/z) : 303.29 (M)^+.
5.1.5 2-Bromo-1,2-bis(4-chlorophenyl)ethanone\textsuperscript{127} (8)

The title compound was prepared from 1,2-bis(4-chlorophenyl)ethanone (2.0 g, 3.77 mM) (5) and bromine (0.8 ml) following the method described for the synthesis of compound (7). The title compound (8) was obtained as white crystals, (1.4 gm, 86 %); m.p. 86-88 °C (Lit.\textsuperscript{133} 84 °C).

Anal:
TLC : R\textsubscript{f} 0.50 (20 % EtOAc in n-hexane).
IR (KBr, cm\textsuperscript{-1}) : 3093, 2982, 1680, 1586, 839.
MS (m/z) : 344.77 (M)+.

5.1.6 2-Bromo-1,2-bis(4-methoxyphenyl)ethanone\textsuperscript{134} (9)

The title compound was prepared from 1,2-bis(4-methoxyphenyl)ethanone (2.0 g, 7.80 mM) (6) and bromine (0.5 ml) following the method described for the synthesis of compound (7). The title compound was obtained as white crystals (9), (1.5 gm, 76 %); m.p. 104-106 °C (Lit.\textsuperscript{135} 104-105 °C).

Anal:
TLC : R\textsubscript{f} 0.48 (20 % EtOAc in n-hexane).
IR (KBr, cm\textsuperscript{-1}) : 3067, 2936, 1658, 1598, 1161, 836.
MS (m/z) : 335.31 (M)+.

5.1.7 4,5-Di(p-tolyl)thiazol-2-ylamine\textsuperscript{136, 137} (10)

2-Bromo-1,2-di(p-tolyl)ethanone (7) (1.0 g, 3.29 mM) was dissolved in sufficient quantity of methanol in a 100 ml round bottom flask. Thiourea (0.35 g, 4.59 mM) and 3-4 drops of water were added into the reaction mixture and refluxed for 4-6 hrs. The reaction was monitored by TLC. After completion of the reaction, it was poured onto ice-cold water and the resulting solution was basified with ammonia. The product so precipitated was filtered, dried and crystallized in methanol to obtain the desired compound (10) as a white solid, (0.85 gm, 85 %); m.p. 165-167 °C (Lit.\textsuperscript{138} 167 °C).

Anal:
TLC : R\textsubscript{f} 0.40 (20 % EtOAc in n-hexane).
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IR (KBr, cm$^{-1}$) : 3436, 3250, 2920, 2859, 1615, 1081, 849.

$^1$H-NMR (CDCl$_3$) : 7.34-7.32 (d, 2H, J = 8.0 Hz), 7.16-7.14 (d, 2H, J = 8.0 Hz), 7.05-7.03 (d, 4H, J = 8.0 Hz), 5.49 (s, 1H), 2.31 (s, 6H).

MS (m/z) : 280.19 (M$^+$).

5.1.8 4,5-Bis(4-chlorophenyl)thiazol-2-ylamine $^{136,137}$ (11)

The title compound was prepared from 2-bromo-1,2-bis(4-chlorophenyl)ethanone (1.0 g, 1.75 mM) (8) and thiourea (2.5 g, 2.08 mM) following the method described for the synthesis of compound (10). The title compound (11) was obtained as white crystals, (0.85 gm, 94%), m.p. 162-164 °C, (Lit.$^{137}$ 160 °C).

Anal:

TLC : $R_f$ 0.54 (20% EtOAc in n-hexane).

IR (KBr, cm$^{-1}$) : 3444, 3274, 2939, 1630, 1528, 1086, 827.

MS (m/z) : 321.32 (M$^+$).

5.1.9 4,5-Bis(4-methoxyphenyl)thiazol-2-ylamine $^{136,137}$ (12)

The title compound was prepared from 2-bromo-1,2-bis(4-methoxyphenyl)ethanone (1.0 g, 2.98 mM) (9) and thiourea (0.35 g, 4.59 mM) following the method described for the synthesis of compound (10). The title compound (12) was obtained as white crystals, (0.85 gm, 87%), m.p. 210-212 °C (Lit.$^{139}$ 210-212 °C).

Anal:

TLC : $R_f$ 0.58 (20% EtOAc in n-hexane).

IR (KBr, cm$^{-1}$) : 3390, 3292, 1638, 1531, 1176, 833.

MS (m/z) : 312.24 (M$^+$).

5.1.10 1-(t-Butoxycarbonyl)piperidine-4-carboxylic acid $^{140}$ (13)

To a solution of piperidine-4-carboxylic acid (200 mg, 1.66 mM) in THF (5 ml), 1N/NaOH (2 ml) was added till a clear solution was obtained. To this clear solution, BOC anhydride (372 mg, 170 mM) in THF was added. The reaction mixture was allowed to stir for overnight. THF was distilled out and the resulting compound was acidified with dilute HCl to
get white solid (13) which was filtered and dried, (0.08 gm, 94 %), m.p. 152-154 °C (Lit\textsuperscript{140}. 150-152 °C).

Anal:

\textbf{TLC} : R\textsubscript{f} 0.44 (20% EtOAc in n-hexane).

\textbf{IR (KBr, cm}^{-1} ) : 3217, 2974, 2864, 1736, 1660, 1285, 820.

\textbf{\textsuperscript{1}H-NMR (CDCl\textsubscript{3})} : 10.37 (bs, 1H), 4.01 (bs 2H), 2.89-2.83 (t, 2H), 2.51-2.45 (m, 1H), 1.92-1.89 (m, 2H), 1.69-1.59 (m, 2H), 1.46 (d, 9H).

\textbf{MS (m/z)} : 229.05 (M)\textsuperscript{+}.

5.1.11 \textit{t}.Butyl 4-[4,5-bis(p-tolyl)thiazol-2-ylcarbamoyl]piperidine-1-carboxylate (14)

1-\textit{t}-(Butyloxy carbonyl)piperidine-4-carboxylic acid (13) (0.85 gm, 3.73 mmol) and BOP reagent (2.4 gm, 3.73 mmol) were dissolved in dry acetonitrile (20 ml) maintaining a reaction temperature of 0 °C. To this reaction mixture, DIPEA (1.5 ml, 5.59 mmol) was added slowly followed by addition of 4,5-di(p-tolyl)thiazol-2-ylamine (10) (0.5 gm) and stirring was carried out for overnight. TLC analysis (EtOAc : n-hexane 2:8) was used to confirm completion of the reaction. Acetonitrile was removed, cold water added and the reaction mixture was basified with sodium hydroxide followed by extraction with DCM to get the pure compound (14), as white solid (0.38 gm, 79 %); m.p. 184-186 °C.

Anal:

\textbf{TLC} : R\textsubscript{f} 0.35 (20% EtOAc in n-hexane).

\textbf{IR (KBr, cm}^{-1} ) : 3435, 3134, 1688, 1536, 1161, 825.

\textbf{MS (m/z)} : 491.07 (M)\textsuperscript{+}.

5.1.12 \textit{t}.Butyl 4-[4,5-bis(4-chlorophenyl)thiazol-2-ylcarbamoyl]piperidine-1-carboxylate (15)

The title compound was prepared from 4,5-bis(4-chlorophenyl)thiazol-2-ylamine (11) (0.5 gm) following the method described for the synthesis of compound (14). The title compound (15) was obtained as white crystals, (0.43 gm, 85 %); m.p. 188-190 °C.

Anal:

\textbf{TLC} : R\textsubscript{f} 0.47 (20% EtOAc in n-hexane).

\textbf{IR (KBr, cm}^{-1} ) : 3438, 3141, 1687, 1565, 980.
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MS (m/z) : 532.00 (M)^+.

5.1.13 t-Butyl 4-[4,5-bis(4-methoxyphenyl)thiazol-2-ylcarbamoyl]piperidine-1-carboxylate (16)

The title compound was prepared from 4,5-bis(4-methoxyphenyl)thiazol-2-ylamine (12) (0.5 gm) following the method described for the synthesis of compound (14). The title compound (16) was obtained as white crystals, (0.35 gm, 83 %); m.p. 138-140 °C.

Anal:
TLC : R_f 0.44 (20% EtOAc in n-hexane).
IR (KBr, cm^{-1}) : 3435, 2933, 2856, 1691, 1544, 1248.
MS (m/z) : 523.14 (M)^+.

5.1.14 N-[4,5-Bis(p-tolyl)thiazol-2-yl]piperidine-4-carboxamide (17)

To an RBF containing t-butyl 4-[4,5-bis(p-tolyl)thiazol-2-ylcarbamoyl]piperidine-1-carboxylate (14) (0.5 gm) in DCM (2 ml), a mixture of trifluoroacetic acid and DCM (70:30) (3 ml) was added and stirred for 2 hrs. DCM was distilled off and diethyl ether was added to the reaction mixture in cold conditions slowly with continuous stirring to get a white solid compound (17), (0.35 gm, 88 %); m.p. 186-188 °C.

Anal:
TLC : R_f 0.35 (20% EtOAc in n-hexane).
IR (KBr, cm^{-1}) : 3193, 3027, 2817, 1674, 1548, 1132.
MS (m/z) : 391.33 (M)^+.

5.1.15 N-[4,5-Bis(4-chlorophenyl)thiazol-2-yl]piperidine-4-carboxamide (18)

The title compound was prepared from t-butyl 4-[4,5-bis(4-chlorophenyl)thiazol-2-ylcarbamoyl]piperidine-1-carboxylate (15) (0.5 gm) following the method described for the synthesis of compound (17). The title compound (18) was obtained as white solid, (0.38 gm, 90 %); m.p. 240-242 °C.

Anal:
TLC : R_f 0.44 (20% EtOAc in n-hexane).
IR (KBr, cm\(^{-1}\)) : 3163, 2856, 1677, 1552, 833.

MS (m/z) : 431.70 (M)^+

5.1.16 \(N-\text{[4,5-Bis(4-methoxyphenyl)thiazol-2-yl]piperidine-4-carboxamide (19)}\)

The title compound was prepared from \(t\)-butyl 4-[4,5-bis(4-methoxyphenyl)thiazol-2-ylcarbamoyl]piperidine-1-carboxylate (16) (0.5 gm) following the method described for the synthesis of compound (17). The title compound (19) was obtained as white solid, (0.40 gm, 95 %); m.p. 168-170 °C.

Anal:

TLC : \(R_f\) 0.48 (20% EtOAc in n-hexane).

IR (KBr, cm\(^{-1}\)) : 3425, 3176, 2839, 1675, 1549, 1201.

MS (m/z) : 423.17 (M)^+

5.1.17 \(1\)-(2-Methylbenzyl)-\(N\)-[4,5-bis(p-tolyl)thiazol-2-yl]piperidine-4-carboxamide (20)

In a round-bottomed flask, \(N\)-[4,5-bis(p-tolyl)thiazol-2-yl]piperidine-4-carboxamide (17) (0.5 gm) and \(K_2\)CO\(_3\) (0.36 gm, 3.15 mmol) were dissolved in Dry DMF. Suitable quantity of 2-methylbenzyl bromide (0.15 ml) was added to the contents of the flask. The mixture was stirred at 60 °C for 1 hr. After completion of the reaction, the mixture was poured into water. The precipitated off-white coloured solid was filtered, washed with cold water and dried. Further purification was carried by column chromatography with the help of petroleum ether and ethyl acetate as eluent to get compound (20) as a white solid (0.42 gm, 80 %); m.p. 156-158 °C.

Anal:

TLC : \(R_f\) 0.16 (20% EtOAc in n-hexane).

IR (KBr, cm\(^{-1}\)) : 3152, 3024, 2858, 1683, 1541, 1445, 1266, 817.

\(^1\)H-NMR (CDCl\(_3\)) : 11.05 (s, 1H), 7.40-7.38 (d, 2H, \(J = 8.1\) Hz), 7.26-7.24 (d, 2H, \(J = 8.1\) Hz), 7.18-7.07 (m, 8H), 3.33 (s, 2H), 2.72-2.70 (d, 2H, \(J = 9.3\) Hz), 2.36 (s, 3H), 2.30 (s, 6H), 1.68-1.65 (m, 5H), 1.49-1.46 (d, 2H, \(J = 9.3\) Hz).
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$^{13}$C-NMR (CDCl$_3$) : 173.37, 156.54, 143.60, 137.69, 137.66, 137.54, 131.95, 130.26, 129.72, 129.49, 129.35, 129.20, 129.13, 128.80, 127.01, 126.60, 125.45, 60.94, 52.62, 29.72, 28.51, 21.26, 19.23.

MS (m/z) : 495.85 (M$^+$).

LC-MS/MS : $t_R$ 5.95 min, 496.29 (M+H).

Calcd for C$_{31}$H$_{33}$N$_3$OS: C, 75.12; H, 6.71; N, 8.48. Found: C, 74.95; H, 6.87; N, 8.29 %.

5.1.18 1-(2-Trifluoromethylbenzyl)-N-[4,5-bis(p-tolyl)thiazol-2-yl]piperidine-4-carboxamide (21)

The title compound was prepared from $N$-[4,5-bis(p-tolyl)thiazol-2-yl]piperidine-4-carboxamide (17) (0.5 gm) and 2-trifluoromethylbenzyl bromide (0.35 gm) following the method described for the synthesis of compound (20). The title compound (21) was obtained as white solid, (0.34 gm, 79 %); m.p. 194-196 °C.

Anal:

TLC : $R_f$ 0.21 (20% EtOAc in n-hexane).

IR (KBr, cm$^{-1}$) : 3159, 3029, 2858, 1685, 1542, 1312, 1267, 1119, 769.

$^1$H-NMR (CDCl$_3$) : 11.74 (s, 1H), 7.74-7.72 (d, 1H, $J$ = 8.0 Hz), 7.59-7.57 (d, 1H, $J$ = 8.0 Hz), 7.49-7.42 (m, 3H), 7.30-7.25 (m, 3H), 7.15-7.13 (d, 2H, $J$ = 8.0 Hz), 7.11-7.09 (d, 2H, $J$ = 8.0 Hz), 3.49 (s, 2H), 2.64-2.62 (d, 2H, $J$ = 8.6 Hz), 2.37 (s, 3H), 2.28 (s, 3H), 1.67-1.58 (m, 4H), 1.54-1.51 (m, 1H), 1.38-1.36 (d, 2H, $J$ = 8.6 Hz).

$^{13}$C-NMR (CDCl$_3$) : 173.84, 157.52, 143.44, 138.09, 137.85, 137.77, 131.86, 131.77, 130.13, 129.54, 129.35, 129.04, 128.93, 128.22, 126.61, 126.54, 125.55, 125.50, 58.13, 52.55, 41.99, 28.45, 21.22.

MS (m/z) : 549.80 (M$^+$).

LC-MS/MS : $t_R$ 7.01 min, 550.23 (M+H).

Calcd for C$_{31}$H$_{30}$F$_3$N$_3$OS: C, 67.74; H, 5.50; N, 7.64. Found: C, 67.46; H, 5.67; N, 7.83 %.
5.1.19 1-(3-Fluorobenzyl)-N-[4,5-bis(p-tolyl)thiazol-2-yl]piperidine-4-carboxamide (22)

The title compound was prepared from N-[4,5-bis(p-tolyl)thiazol-2-yl]piperidine-4-carboxamide (17) (0.5 gm) and 3-fluorobenzyl bromide (0.14 ml) following the method described for the synthesis of compound (20). The title compound (22) was obtained as white solid, (0.37 gm, 75 %); m.p. 165-167 °C.

Anal:

TLC : Rf 0.23 (20% EtOAc in n-hexane).

IR (KBr, cm⁻¹) : 3148, 2926, 2816, 1681, 1543, 1445, 1263, 815.

¹H-NMR (CDCl₃) : 11.68 (s, 1H), 7.39-7.37 (d, 2H, J = 8.0 Hz), 7.26-7.24 (d, 2H, J = 8.1 Hz), 7.21-7.19 (m, 1H), 7.15-7.13 (d, 2H, J = 8.0 Hz), 7.07-7.05 (d, 2H, J = 8.1 Hz), 6.98-6.95 (m, 2H), 6.93-6.88 (m, 1H), 3.35 (s, 2H), 2.66-2.64 (d, 2H, J = 11.5 Hz), 2.37 (s, 3H), 2.29 (s, 3H), 1.65-1.49 (m, 5H), 1.39-1.36 (d, 2H, J = 11.5 Hz).

¹³C-NMR (CDCl₃) : 173.72, 164.12, 161.67, 157.50, 143.37, 141.02, 140.95, 137.86, 131.86, 129.60, 129.33, 129.28, 129.05, 128.90, 126.50, 124.43, 115.76, 113.74, 62.43, 52.22, 41.90, 28.27, 21.25.

MS (m/z) : 499.47 (M)⁺.

LC-MS/MS : tR 5.93 min, 500.25 (M+H).

Calcd for C₃₀H₃₀FN₃OS: C, 72.12; H, 6.05; N, 8.41. Found: C, 72.35; H, 6.18; N, 8.27%.

5.1.20 1-(3,5-Difluorobenzyl)-N-[4,5-bis(p-tolyl)thiazol-2-yl]piperidine-4-carboxamide (23)

The title compound was prepared from N-[4,5-bis(p-tolyl)thiazol-2-yl]piperidine-4-carboxamide (17) (0.5 gm) and 3,5-difluorobenzyl bromide (0.16 ml) following the method described for the synthesis of compound (20). The title compound (23) was obtained as white solid, (0.29 gm, 78 %); m.p. 153-155 °C.

Anal:

TLC : Rf 0.22 (20% EtOAc in n-hexane).
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IR (KBr, cm⁻¹) : 3155, 2924, 2807, 1686, 1547, 1453, 1268, 1117, 810.

¹H-NMR (CDCl₃) : 11.48 (s, 1H), 7.40-7.38 (d, 2H, J = 8.1 Hz), 7.26-7.24 (d, 2H, J = 8.0 Hz), 7.17-7.13 (d, 2H, J = 8.1 Hz), 7.09-7.07 (d, 2H, J = 8.0 Hz), 6.81-6.77 (m, 2H), 6.67-6.62 (m, 1H), 3.33 (s, 2H), 2.66-2.63 (d, 2H, J = 9.8 Hz), 2.39 (s, 3H), 2.30 (s, 3H), 1.70-1.54 (m, 5H), 1.43-1.37 (d, 2H, J = 9.8 Hz).

¹³C-NMR (CDCl₃) : 173.69, 164.20, 161.74, 157.59, 143.35, 142.92, 142.78, 137.80, 131.87, 130.80, 129.32, 129.02, 128.54, 126.54, 111.16, 102.30, 62.13, 52.26, 41.79, 29.37, 21.28.

MS (m/z) : 517.10 (M⁺).

LC-MS/MS : tₗ 6.21 min, 518.21 (M+H).

Calcd for C₃₀H₂₉F₂N₃O:S: C, 69.61; H, 5.65; N, 8.12; Found: C, 69.74; H, 5.78; N, 8.25%.

5.1.21 1-(2-Chloro-4-fluorobenzyl)-N-[4,5-bis(p-tolyl)thiazol-2-yl]piperidine-4-carboxamide (24)

The title compound was prepared from N-[4,5-bis(p-tolyl)thiazol-2-yl]piperidine-4-carboxamide (17) (0.5 gm) and 2-chloro-4-fluorobenzyl bromide (0.27 gm) following the method described for the synthesis of compound (20). The title compound (24) was obtained as white solid, (0.45 gm, 89 %); m.p. 178-180 °C.

Anal:

TLC : Rₜ 0.14 (20% EtOAc in n-hexane).

IR (KBr, cm⁻¹) : 3145, 2926, 2857, 1679, 1543, 1444, 1265, 815.

¹H-NMR (CDCl₃) : 11.16 (s, 1H), 7.40-7.38 (d, 3H, J = 8.0 Hz), 7.26-7.24 (m, 2H) 7.15-7.13 (d, 2H, J = 8.0 Hz), 7.09-7.07 (m, 3H), 6.94-6.90 (m, 1H), 3.45 (s, 2H), 2.72-2.70 (d, 2H), 2.37 (s, 3H), 2.30 (s, 3H), 1.75-1.67 (m, 7H).

¹³C-NMR (CDCl₃) : 173.52, 157.15, 143.47, 137.77, 134.59, 134.48, 131.89, 131.59, 131.50, 129.52, 129.33, 129.05, 128.87, 126.58, 116.66, 116.41,
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113.94, 113.73, 58.48, 52.34, 42.07, 28.37, 21.25.

MS (m/z) : 533.04 (M)+.

LC-MS/MS : \( t_R \ 2.68 \text{ min}, 534.27 (\text{M+H}). \)

Calcd for \( \text{C}_{30}\text{H}_{29}\text{ClFN}_3\text{OS} \): C, 67.46; H, 5.47; N, 7.87; Found: C, 67.62; H, 5.57; N, 7.81%.

5.1.22 1-(4-Fluorobenzyl)-N-[4,5-bis(\(p\)-toly)thiazol-2-yl]piperidine-4-carboxamide (25)

The title compound was prepared from \( \text{N-[4,5-bis(\(p\)-toly)thiazol-2-yl]piperidine-4-carboxamide (17)} \) (0.5 gm) and 4-fluorobenzylbromide (0.20 ml) following the method described for the synthesis of compound (20). The title compound (25) was obtained as white solid, (0.23 gm, 85 %); m.p. 167-169 °C.

Anal:

TLC : \( R_f \ 0.17 \) (20% EtOAc in \( n\)-hexane).

IR (KBr, cm\(^{-1}\)) : 3201, 2852, 1675, 1539, 1401, 1190, 809, 655.

5.1.23 1-(2-Chloro-6-fluorobenzyl)-N-[4,5-bis(\(p\)-toly)thiazol-2-yl]piperidine-4-carboxamide (26)

The title compound was prepared from \( \text{N-[4,5-bis(\(p\)-toly)thiazol-2-yl]piperidine-4-carboxamide (17)} \) (0.5 gm) and 2-chloro-6-fluorobenzyl bromide (0.36 gm) following the method described for the synthesis of compound (20). The title compound (26) was obtained as white solid, (0.35 gm, 85 %); m.p. 194-196 °C.

Anal:

TLC : \( R_f \ 0.21 \) (20% EtOAc in \( n\)-hexane).

IR (KBr, cm\(^{-1}\)) : 3144, 2930, 2854, 1680, 1543, 1450, 1267, 819.

\(^1\)H-NMR (CDCl\(_3\)) : 11.35 (s, 1H), 7.35-7.33 (d, 2H, \( J = 8.0 \) Hz), 7.26-7.24 (d, 2H, \( J = 8.0 \) Hz), 7.15-7.13 (d, 4H, \( J = 8.0 \) Hz), 7.04-7.02 (d, 2H, \( J = 8.0 \) Hz), 6.95-6.91 (m, 1H), 3.60 (s, 2H), 2.77-2.74 (d, 2H, \( J =11.1 \) Hz), 2.37 (s, 3H), 2.29 (s, 3H), 1.77-1.52 (m, 3H), 1.65- 1.62 (m, 2H), 1.42-1.39 (d, 2H, \( J = 11.1 \) Hz).
5.1.24 1-(4-Cyanobenzyl)-N-[4,5-bis(p-tolyl)thiazol-2-yl]piperidine-4-carboxamide (27)

The title compound was prepared from N-[4,5-bis(p-tolyl)thiazol-2-yl]piperidine-4-carboxamide (17) (0.5 gm) and 4-cyanobenzyl bromide (0.32 gm) following the method described for the synthesis of compound (20). The title compound (27) was obtained as white solid, (0.41 gm, 85 %); m.p. 92-94 °C.

Anal:

TLC : R_f 0.19 (20% EtOAc in n-hexane).

IR (KBr, cm^{-1}) : 3149, 3029, 2924, 2227, 1659, 1559, 1445, 1266, 822.

^1H-NMR (CDCl_3) : 11.46 (bs, 1H), 7.58-7.56 (d, 2H, J = 8.0 Hz), 7.39-7.37 (d, 2H, J = 8.0 Hz), 7.38-7.36 (d, 2H, J = 8.0 Hz), 7.26-7.24 (d, 2H, J = 8.0 Hz), 7.15-7.13 (d, 2H, J = 8.0 Hz), 7.08-7.06 (d, 2H, J = 8.0 Hz), 3.52 (s, 2H), 2.66-2.64 (d, 2H, J = 8.6 Hz), 2.38 (s, 3H), 2.29 (s, 3H), 1.66-1.65 (m, 5H), 1.50-1.42 (bs, 2H).

^13C-NMR (CDCl_3) : 173.24, 156.83, 143.48, 137.82, 137.77, 132.11, 131.86, 129.54, 129.35, 129.30, 129.23, 129.00, 128.82, 118.90, 116.25, 111.01, 62.44, 52.46, 42.03, 29.72, 28.30, 21.29.

MS (m/z) : 506.92 M^+.

LC-MS/MS : t_R 5.97 min, 507.24 (M+H).

Calcd for C_{31}H_{30}N_{4}OS: C, 73.49; H, 5.97; N, 11.06; Found: C, 73.24; H, 5.76; N, 10.83 %.
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5.1.25 1-(4-Nitrobenzyl)-N-[4,5-bis(p-tolyl)thiazol-2-yl]piperidine-4-carboxamide (28)

The title compound was prepared from N-[4,5-bis(p-tolyl)thiazol-2-yl]piperidine-4-carboxamide (17) (0.5 gm) and 4-nitrobenzyl bromide (0.26 gm) following the method described for the synthesis of compound (20). The title compound (28) was obtained as white solid, (0.36 gm, 81 %); m.p. 203-205 °C.

Anal:

TLC : Rf 0.19 (20% EtOAc in n-hexane).

IR (KBr, cm⁻¹) : 3267, 3023, 2858, 1680, 1552, 1449, 1270, 816.

¹H-NMR (CDCl₃) : 11.75 (bs, 1H), 8.14-8.12 (d, 2H, J = 8.6 Hz), 7.43-7.38 (d, 4H, J = 8.0 Hz), 7.26-7.24 (d, 2H, J = 8.6 Hz), 7.16-7.14 (d, 2H, J = 8.0 Hz), 7.08-7.06 (d, 2H, J = 8.0 Hz), 3.47 (s, 2H), 2.64-2.62 (d, 2H, J = 6.4 Hz), 2.38 (s, 3H), 2.29 (s, 3H), 1.66-1.59 (m, 4H), 1.54-1.52 (d, 1H), 1.27-1.24 (d, 2H).

¹³C-NMR (CDCl₃) : 173.44, 157.22, 147.13, 146.39, 143.39, 137.86, 137.82, 131.84, 129.56, 129.32, 129.29, 128.97, 128.87, 126.61, 125.25, 123.51, 62.14, 52.42, 41.80, 28.28, 21.29.

MS (m/z) : 527.2 (M+1)⁺.

LC-MS/MS : tR 6.06 min.

Calcd for C₃₀H₂₈N₄O₃S: C, 68.42; H, 5.74; N, 10.64; Found: C, 68.73; H, 5.63; N, 10.76%.

5.1.26 1-(4-(Trifluoromethyl)benzyl)-N-[4,5-bis(p-tolyl)thiazol-2-yl]piperidine-4-carboxamide (29)

The title compound was prepared from N-[4,5-bis(p-tolyl)thiazol-2-yl]piperidine-4-carboxamide (17) (0.5 gm) and 4-trifluoromethylbenzyl bromide (0.36gm) following the method described for the synthesis of compound (20). The title compound (29) was obtained as white solid, (0.27 gm, 71 %); m.p. 130-132 °C.

Anal:

TLC : Rf 0.21 (20% EtOAc in n-hexane).
**Experimental**

**IR (KBr, cm\(^{-1}\))**: 3160, 2926, 2823, 1687, 1545, 1446, 1263, 852.

\( ^1\)H-NMR (CDCl\(_3\)) : 11.48 (s, 1H), 7.38-7.36 (d, 2H, J = 8.0 Hz), 7.26-7.24 (d, 4H, J = 8.0 Hz), 7.15-7.10 (d, 4H, J = 8.0 Hz), 7.05-7.03 (d, 2H, J = 9.7 Hz), 3.37 (s, 2H), 2.66-2.64 (d, 2H, J = 9.7 Hz), 2.37 (s, 3H), 2.28 (s, 3H), 1.66-1.55 (m, 5H), 1.41-1.39 (m, 2H, J = 9.7 Hz).

\( ^{13}\)C-NMR (CDCl\(_3\)) : 173.68, 157.47, 148.23, 143.36, 137.79, 136.92, 131.85, 130.18, 129.53, 129.31, 129.26, 129.04, 128.90, 126.50, 120.68, 119.21, 62.10, 52.13, 41.89, 28.27, 21.23.

**MS (m/z)** : 549.52 (M\(^+\)).

**LC-MS/MS** : t\(_R\) 7.01 min, 500.23 (M+H).

Calcd for C\(_{31}\)H\(_{30}\)F\(_3\)N\(_3\)O\(_2\): C, 67.74; H, 5.50; N, 7.64; Found: C, 67.87; H, 5.62; N, 7.56%.

5.1.27 1-(2-Methylbenzyl)-N-[4,5-bis(4-chlorophenylthiazol-2-yl)piperidine-4-carboxamide (30)

The title compound was prepared from N-[4,5-bis(4-chlorophenyl)thiazol-2-yl] piperidine-4-carboxamide (18) (0.5 gm) and 2-methylbenzyl bromide (0.39 ml) following the method described for the synthesis of compound (20). The title compound (30) was obtained as white solid, (0.42 gm, 84%); m.p. 179-181 °C.

Anal:

TLC : R\(_f\) 0.22 (20% EtOAc in \(n\)-hexane).

**IR (KBr, cm\(^{-1}\))** : 3158, 3047, 2927, 1688, 1545, 1297, 825.

\( ^1\)H-NMR (CDCl\(_3\)) : 10.13 (s, 1H), 7.41-7.38 (d, 2H, J = 8.5 Hz), 7.32-7.30 (d, 2H, J = 8.5 Hz), 7.28-7.26 (d, 4H, J = 8.5 Hz), 7.21-7.19 (d, 1H), 7.16-7.12 (m, 3H), 3.39 (s, 2H), 2.84-2.81 (d, 2H, J = 11.2 Hz), 2.33 (s, 3H), 1.99-1.93 (m, 1H), 1.85-1.77 (m, 2H), 1.75-1.65 (m, 4H).

\( ^{13}\)C-NMR (CDCl\(_3\)) : 173.08, 156.48, 143.29, 137.57, 136.36, 134.15, 132.92, 130.72, 130.32, 130.24, 130.12, 129.75, 129.22, 128.81, 127.09, 126.23, 125.48, 60.97, 52.71, 42.95, 28.54, 19.23.
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MS (m/z) : 535.93 (M)^+.  
LC-MS/MS : t_R 6.04 min, 538.43 (M+H).

Calcd for C_{29}H_{27}Cl_{2}N_{3}OS: C, 64.92; H, 5.07; N, 7.83; Found: C, 65.04; H, 5.22; N, 7.75%.

5.1.28 1-(2-Trifluoromethyl)benzyl)-N-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidine-4-carboxamide (31)

The title compound was prepared from N-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidine-4-carboxamide (18) (0.5 gm) and 2-trifluoromethylbenzyl bromide (0.44 ml) following the method described for the synthesis of compound (20). The title compound (31) was obtained as white solid, (0.27 gm, 83%); m.p. 162-164 °C.

Anal:
TLC : R_f 0.24 (20% EtOAc in n-hexane).

IR (KBr, cm^{-1}) : 3155, 2927, 2856, 1690, 1540, 1313, 1113, 825.

^1H-NMR (CDCl_3) : 10.62 (s, 1H), 7.75-7.73 (d, 1H, J = 7.8 Hz), 7.61-7.59 (d, 1H, J = 7.8 Hz), 7.51-7.43 (m, 1H), 7.41-7.40 (m, 2H), 7.34-7.24 (m, 7H), 3.56 (s, 2H), 2.79-2.76 (d, 2H, J = 10.6 Hz), 1.89-1.71 (m, 5H), 1.62-1.58 (d, 2H).

MS (m/z) : 589.33 (M)^+.
LC-MS/MS : t_R 5.30 min.

Calcd for C_{28}H_{24}ClFN_{3}OS: C, 58.99; H, 4.10; N, 7.12; Found: C, 59.13; H, 4.32; N, 7.28%.

5.1.29 1-(3-Fluorobenzyl)-N-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidine-4-carboxamide (32)

The title compound was prepared from N-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidine-4-carboxamide (18) (0.5 gm) and 3-fluorobenzyl bromide (0.52 gm) following the method described for the synthesis of compound (20). The title compound (32) was obtained as white solid, (0.30 gm, 90%); m.p. 166-168 °C.
Anal:

TLC : $R_f$ 0.22 (20% EtOAc in n-hexane).

IR (KBr, cm$^{-1}$) : 3144, 2925, 2763, 1681, 1547, 1297, 1092, 826.

$^1$H-NMR (CDCl$_3$) : 12.25 (s, 1H), 7.42-7.39 (d, 2H, $J = 8.5$ Hz), 7.38 (s, 1H), 7.35-7.33 (d, 2H, $J = 8.5$ Hz), 7.29-7.28 (d, 2H, $J = 6.5$ Hz), 7.27-7.26 (d, 2H, $J = 6.5$ Hz), 7.18-7.13 (m, 2H), 7.03-6.99 (m, 1H), 3.78 (s, 2H), 3.02-3.00 (d, 2H, $J = 10.8$ Hz), 2.64 (s, 1H), 2.00 (bs, 2H), 1.92-1.80 (m, 4H).

$^{13}$C-NMR (CDCl$_3$) : 173.34, 163.40, 160.97, 156.21, 142.97, 133.22, 132.84, 132.55, 130.69, 129.97, 129.84, 128.88, 128.22, 124.98, 124.48, 115.77, 114.20, 114.00, 60.93, 51.87, 29.01, 27.40.

MS (m/z) : 539.50 (M$^+$).

5.1.30 1-(3,5-Difluorobenzyl)-N-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidine-4-carboxamide (33)

The title compound was prepared from N-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidine-4-carboxamide (18) (0.5 gm) and 3,5-difluorobenzyl bromide (0.35 ml) following the method described for the synthesis of compound (20). The title compound (33) was obtained as white solid, (0.42 gm, 88 %); m.p. 196-198 °C.

Anal:

TLC : $R_f$ 0.23 (20% EtOAc in n-hexane).

IR (KBr, cm$^{-1}$) : 3138, 3023, 2852, 1688, 1536, 1264, 1093, 826.

$^1$H-NMR (CDCl$_3$) : 10.35 (s, 1H), 7.37-7.35 (d, 2H, $J = 8.5$ Hz), 7.33-7.31 (d, 2H, $J = 8.5$ Hz), 7.26-7.24 (d, 4H, $J = 8.5$ Hz), 7.19-7.17 (m, 2H), 6.99-6.94 (m, 1H), 3.66 (s, 2H), 2.91-2.88 (d, 2H, $J = 11.4$ Hz), 2.04-1.94 (m, 2H), 1.76-1.62 (m, 5H).

$^{13}$C-NMR (CDCl$_3$) : 173.41, 163.31, 160.84, 157.61, 142.97, 136.75, 134.21, 132.76, 130.71, 130.17, 129.25, 129.11, 128.92, 126.13, 125.46, 114.05, 113.82, 52.36, 42.38, 29.71, 28.31.
Calcd for C\textsubscript{28}H\textsubscript{23}Cl\textsubscript{2}F\textsubscript{2}N\textsubscript{3}OS: C, 60.22; H, 4.15; N, 7.52 10.23; Found: C, 60.05; H, 4.28; N, 7.69 %.

5.1.31 1-(2-Chloro-4-fluorobenzyl)-N-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidine-4-carboxamide (34)

The title compound was prepared from N-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidine-4-carboxamide (18) (0.5 gm) and 2-chloro-4-fluorobenzyl bromide (0.61 gm) following the method described for the synthesis of compound (20). The title compound (34) was obtained as white solid, (0.52 gm, 78 %); m.p. 179-181°C.

Anal:

TLC : R\textsubscript{f} 0.17 (20% EtOAc in n-hexane).

IR (KBr, cm\textsuperscript{-1}) : 3155, 2948, 2763, 1686, 1537, 1488, 1262, 1093, 828.

\textsuperscript{1}H-NMR (CDCl\textsubscript{3}) : 10.94 (s, 1H), 7.42-7.40 (d, 2H, J = 8.5 Hz), 7.38 (s, 1H), 7.34-7.31 (d, 2H, J = 8.5 Hz ), 7.29-7.25 (d, 4H, J = 8.5 Hz), 7.09-7.06 (d, 1H, J = 8.5 Hz), 6.96-6.91 (d, 1H, J = 8.5 Hz), 3.47 (s, 2H), 2.79-2.77 (d, 2H), 1.89-1.70 (m, 4H), 1.55-1.53 (m, 2H), 1.30-1.25 (m, 1H).

\textsuperscript{13}C-NMR (CDCl\textsubscript{3}) : 173.26, 164.32, 160.84, 157.28, 143.10, 134.26, 134.20, 132.83, 130.69, 130.18, 130.11, 129.27, 128.92, 126.25, 116.74, 116.50, 114.50, 113.79, 58.52, 52.46, 42.44, 28.40.

MS (m/z) : 573.54 (M\textsuperscript{+}).

LC-MS/MS : t\textsubscript{R} 6.38 min, 574.10 (M+H).

Calcd for C\textsubscript{28}H\textsubscript{23}Cl\textsubscript{2}F\textsubscript{2}N\textsubscript{3}OS: C, 58.49; H, 4.03; N, 7.31; Found: C, 58.78; H, 3.89; N, 7.23 %.

5.1.32 1-(2-Cyanobenzyl)-N-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidine-4-carboxamide (35)

The title compound was prepared from N-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidine-4-carboxamide (18) (0.5 gm) and 2-cyanobenzyl bromide (0.60 gm) following the method described for the synthesis of compound (20). The title compound (35) was obtained as white solid, (0.31 gm, 85 %); m.p. 196-198°C.
Section I

Experimental

Anal:

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<th>Method</th>
<th>Value</th>
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<td>TLC</td>
<td>R&lt;sub&gt;f&lt;/sub&gt; 0.25 (20% EtOAc in n-hexane).</td>
</tr>
<tr>
<td>IR (KBr, cm&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>3249, 2953, 2923, 2231, 1685, 1544, 1291, 824.</td>
</tr>
<tr>
<td>&lt;sup&gt;1&lt;/sup&gt;H-NMR (CDCl&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>10.89 (s, 1H), 7.63-7.61 (d, 1H, J = 7.6 Hz), 7.54-7.52 (d, 2H, J = 8.0 Hz), 7.42-7.40 (d, 2H, J = 8.0 Hz), 7.34-7.32 (m, 3H), 7.29-7.27 (d, 4H, J = 8.0 Hz), 3.61 (s, 2H), 2.79-2.76 (d, 2H, J = 10.6 Hz), 1.85-1.68 (m, 5H), 1.57-1.55 (d, 2H).</td>
</tr>
<tr>
<td>&lt;sup&gt;13&lt;/sup&gt;C-NMR (CDCl&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>172.99, 156.79, 143.21, 142.43, 134.21, 134.09, 132.85, 132.67, 130.72, 130.15, 129.87, 129.25, 128.86, 127.60, 126.25, 117.80, 112.84, 60.46, 52.54, 42.51, 29.71, 28.41.</td>
</tr>
<tr>
<td>MS (m/z)</td>
<td>546.82 (M)&lt;sup&gt;+&lt;/sup&gt;.</td>
</tr>
<tr>
<td>LC-MS/MS</td>
<td>t&lt;sub&gt;R&lt;/sub&gt; 5.72 min.</td>
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Calcd for C<sub>29</sub>H<sub>24</sub>Cl<sub>2</sub>N<sub>4</sub>O<sub>5</sub>: C, 63.62; H, 4.42; N, 10.23; Found: C, 63.74; H, 4.31; N, 10.18 %.

5.1.33 1-(4-Fluoro-2-trifluoromethylbenzyl)-N-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidine-4-carboxamide (36)

The title compound was prepared from N-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidine-4-carboxamide (18) (0.5 gm) and 4-fluoro-2-trifluoromethylbenzyl bromide (0.35 ml) following the method described for the synthesis of compound (20). The title compound (36) was obtained as white solid, (0.87 gm, 73 %); m.p. 154-156 °C.

Anal:

<table>
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<th>Method</th>
<th>Value</th>
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<tr>
<td>TLC</td>
<td>R&lt;sub&gt;f&lt;/sub&gt; 0.28 (20% EtOAc in n-hexane).</td>
</tr>
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<td>IR (KBr, cm&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>3213, 2917, 2754, 2764, 1665, 1578, 1232, 1121, 863.</td>
</tr>
</tbody>
</table>

5.1.34 1-(4-Trifluoromethoxybenzyl)-N-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidine-4-carboxamide (37)

The title compound was prepared from N-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidine-4-carboxamide (18) (0.5 gm) and 4-trifluoromethoxybenzyl bromide (0. 47 ml) following the method described for the synthesis of compound (20). The title compound (37) was obtained as white solid, (0.89 gm, 63 %); m.p. 134-136 °C.
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**Experimental**

Anal:

TLC : R_f 0.17 (20% EtOAc in n-hexane).

IR (KBr, cm^{-1}) : 3154, 2924, 2853, 2764, 1688, 1540, 1259, 1092, 827.

^1^H-NMR (CDCl\textsubscript{3}) : 9.92 (bs, 1H), 7.31-7.29 (d, 2H, J = 8.5 Hz), 7.25-7.23 (d, 4H), 7.20-7.16 (m, 4H), 7.09-7.07 (d, 2H, J = 8.5 Hz), 3.39 (s, 2H), 2.79-2.76 (d, 2H, J = 11.2 Hz), 2.02-1.97 (m, 1H), 1.85-1.78 (m, 6H).

^1^C-NMR (CDCl\textsubscript{3}) : 173.14, 157.02, 148.28, 143.13, 136.87, 134.23, 132.81, 130.68, 130.19, 130.16, 130.13, 129.25, 128.87, 126.21, 121.76, 120.78, 119.21, 62.15, 52.43, 42.52, 31.94.

MS (m/z) : 607.1(M)^+.

LC-MS/MS : t\textsubscript{R} 2.62 min, 606.13 (M+H).

Calcd for C\textsubscript{29}H\textsubscript{24}Cl\textsubscript{2}F\textsubscript{3}N\textsubscript{3}O\textsubscript{2}S: C, 57.43; H, 3.99; N, 6.93; Found: C, 57.61; H, 3.65; N, 6.82 %.

5.1.35 1-(2-Methylbenzyl)-N-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]piperidine-4-carboxamide (38)

The title compound was prepared from N-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]piperidine-4-carboxamide (19) (0.5 gm) and 2-methylbenzyl bromide (0.40 ml) following the method described for the synthesis of compound (20). The title compound (38) was obtained as white solid, (0.65 gm, 54 %); m.p. 158-160 °C.

Anal:

TLC : R_f 0.18 (20% EtOAc in n-hexane).

IR (KBr, cm^{-1}) : 3162, 3010, 2940, 2836, 1665, 1537, 1250, 1176, 837.

^1^H-NMR (CDCl\textsubscript{3}) : 11.11 (bs, 1H), 7.58 (s, 1H), 7.44-7.42 (d, 2H), 7.19-7.13 (m, 6H), 6.87-6.82 (m, 3H), 3.92 (s, 3H), 3.78 (s, 3H), 3.34 (s, 2H), 2.73-2.71 (d, 2H), 2.31 (s, 3H), 1.68-1.63 (m, 5H), 1.49 (bs, 2H).

^1^C-NMR (CDCl\textsubscript{3}) : 173.69, 159.29, 159.25, 156.90, 142.92, 137.53, 136.48, 130.74, 130.25, 130.16, 129.72, 127.28, 126.99, 125.55, 125.42, 124.34,
114.29, 113.95, 60.93, 55.32, 52.58, 42.44, 28.50, 19.23.

**MS (m/z)** : 527.82 (M)^+. 

**LC-MS/MS** : t<sub>R</sub> 5.59 min, 528.23 (M+H).

Calcd for C<sub>31</sub>H<sub>33</sub>N<sub>3</sub>O<sub>3</sub>S: C, 70.56; H, 6.30; N, 7.96; Found: C, 70.78; H, 6.61; N, 7.87%.

5.1.36 1-(2-Trifluoromethyl)benzyl)-N-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]piperidine-4-carboxamide (39)

The title compound was prepared from N-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]piperidine-4-carboxamide (19) (0.5 gm) and 2-trifluoromethylbenzyl bromide (0.45 ml) following the method described for the synthesis of compound (20). The title compound (39) was obtained as white solid, (0.91 gm, 70%); m.p. 194-196°C.

Anal:

**TLC** : R<sub>f</sub> 0.21 (20% EtOAc in n-hexane).

**IR (KBr, cm<sup>-1</sup>)** : 3150, 2949, 2843, 1685, 1540, 1311, 1255, 837.

**<sup>1</sup>H-NMR (CDCl<sub>3</sub>)** : 11.32 (bs, 1H), 7.76-7.75 (m, 1H), 7.60-7.56 (m, 2H), 7.51-7.43 (m, 3H), 7.32-7.24 (m, 3H), 6.90-6.81 (m, 3H), 3.92 (s, 3H), 3.78 (s, 3H), 3.59 (s, 2H) 2.70 (bs, 2H), 1.87-1.84 (m, 4H), 1.49-1.42 (m, 2H), 1.25-0.97 (m, 1H).

**<sup>13</sup>C-NMR (CDCl<sub>3</sub>)** : 173.59, 159.50, 159.30, 157.18, 155.59, 143.66, 134.12, 131.83, 130.74, 130.15, 129.73, 128.24, 126.83, 125.80, 123.89, 114.32, 113.99, 112.03, 111.92, 56.29, 55.29, 52.65, 42.23, 28.44.

**MS (m/z)** : 581.46 (M)^+.

**LC-MS/MS** : t<sub>R</sub> 2.39 min, 582.18 (M+H).

Calcd for C<sub>31</sub>H<sub>35</sub>F<sub>3</sub>N<sub>3</sub>O<sub>3</sub>S: C, 64.01; H, 5.20; N, 7.22; Found: C, 64.38; H, 5.29; N, 7.13%.

5.1.37 1-(3-Fluorobenzyl)-N-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]piperidine-4-carboxamide (40)

The title compound was prepared from N-(4,5-bis[4-methoxyphenyl]thiazol-2-yl]piperidine-4-carboxamide (19) (0.5 gm) and 3-fluorobenzyl bromide (0.35 ml) following the

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method described for the synthesis of compound (20). The title compound (40) was obtained as white solid, (0.89 gm, 74 %); m.p. 158-160 °C.

Anal:

TLC : Rf 0.21 (20% EtOAc in n-hexane).

IR (KBr, cm\(^{-1}\)) : 3141, 3034, 2929, 1677, 1609, 1539, 1298, 1250, 1032, 832.

\(^1\)H-NMR (CDCl\(_3\)) : 11.46 (bs, 1H), 7.42-7.40 (d, 2H, \(J = 8.7\) Hz), 7.29-7.26 (d, 2H, \(J = 8.7\) Hz), 7.23-7.20 (m, 1H) 7.02-6.99 (m, 2H), 6.97-6.91 (m, 1H), 6.89-6.87 (d, 2H, \(J = 8.7\) Hz), 6.82-6.80 (d, 2H, \(J = 8.7\) Hz), 3.84 (s, 3H), 3.77 (s, 3H), 3.39 (s, 2H), 2.71 (bs, 2H), 1.67 (bs, 5H), 1.47 (bs, 2H).

\(^{13}\)C-NMR (CDCl\(_3\)) : 173.50, 164.11, 161.67, 159.33, 157.01, 142.84, 130.72, 130.14, 129.64, 129.56, 127.21, 125.58, 124.50, 124.26, 115.83, 115.62, 114.31, 113.95, 62.37, 55.31, 52.26, 42.02, 28.25.

MS (m/z) : 532.3 (M+1).

LC-MS/MS : \(t_R\) 5.59 min, 532.22 (M+H).

Calcd for C\(_{30}\)H\(_{30}\)FN\(_3\)O\(_3\)S: C, 67.78; H, 5.69; N, 7.90; Found: C, 67.64; H, 5.95; N, 7.98 %.

5.1.38 1-(3,5-Difluorobenzyl)-N-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]piperidine-4-carboxamide (41)

The title compound was prepared from N-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]piperidine-4-carboxamide (19) (0.5 gm) and 3,5-difluorobenzyl bromide (0.36 ml) following the method described for the synthesis of compound (20). The title compound (41) was obtained as white solid, (0.97 gm, 80 %); m.p. 175-177 °C.

Anal:

TLC : Rf 0.18 (20% EtOAc in n-hexane).

IR (KBr, cm\(^{-1}\)) : 3141, 3032, 2929, 1680, 1542, 1251, 1177, 1032, 833.

\(^1\)H-NMR (CDCl\(_3\)) : 11.45 (bs, 1H), 7.45-7.43, (d, 2H, \(J = 8.7\) Hz), 7.29-7.27 (d, 2H, \(J = 8.7\) Hz), 6.89-6.87 (d, 2H, \(J = 8.7\) Hz), 6.82-6.80 (d, 2H, \(J =
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8.7 Hz), 6.79-6.77 (m, 2H), 6.68-6.63 (m, 1H), 3.84 (s, 3H), 3.78 (s, 3H), 3.34 (s, 2H), 2.68-2.65 (d, 2H), 1.65-1.60 (bs, 5H), 1.45-1.43 (d, 2H).

$^{13}$C-NMR (CDCl$_3$): 173.55, 164.27, 161.80, 161.08, 159.36, 157.16, 142.81, 130.72, 130.15, 127.23, 125.61, 124.23, 114.33, 113.98, 111.38, 111.20, 102.58, 102.07, 62.13, 55.31, 52.33, 41.95, 28.29.

MS (m/z) : 549.73(M$^+$).

LC-MS/MS : t$_R$ 2.57 min, 549.19 (M+H).

Calcd for C$_{30}$H$_{29}$F$_2$N$_3$O$_3$S: C, 65.56; H, 5.32; N, 7.65; Found: C, 65.73; H, 5.39; N, 7.49 %.

5.1.39 1-(4-Fluorobenzyl)-N-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]piperidine-4-carboxamide (42)

The title compound was prepared from N-[4,5-bis(4-methoxyphenyl)thiazol-2-yl] piperidine-4-carboxamide (19) (0.5 gm) and 4-fluorobenzyl bromide (0.35 ml) following the method described for the synthesis of compound (20). The title compound (42) was obtained as white solid, (0.95 gm, 79%); m.p. 124-126 °C.

Anal:

TLC : R$_f$ 0.18 (20% EtOAc in n-hexane).

IR (KBr, cm$^{-1}$) : 3154, 2940, 2762, 1688, 1541, 1177, 1033, 832.

$^1$H-NMR (CDCl$_3$) : 10.95 (bs, 1H), 7.34-7.32 (d, 2H, $J = 8.5$ Hz), 7.21-7.19 (d, 2H, $J = 8.5$ Hz), 7.16-7.12 (m, 2H), 6.91-6.87 (m, 2H), 6.81-6.79 (d, 2H, $J = 8.5$ Hz), 6.73-6.71 (d, 2H, $J = 8.5$ Hz), 3.71 (s, 6H), 3.32 (s, 2H), 2.66 (bs, 2H), 1.69-1.61 (m, 4H), 1.48 (bs, 3H).

$^{13}$C-NMR (CDCl$_3$) : 173.59, 163.28, 160.85, 159.32, 157.04, 142.92, 133.76, 130.78, 130.56, 127.29, 125.65, 124.34, 115.14, 114.93, 114.13, 112.12, 62.22, 55.38, 52.26, 42.22, 29.78.

MS (m/z) : 531.23(M$^+$).

LC-MS/MS : t$_R$ 5.56 min, 532.22 (M+H).
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Calcd for C_{30}H_{30}FN_{3}O_{3}S: C, 67.78; H, 5.69; N, 7.90; Found: C, 67.43; H, 5.87; N, 7.75%.

5.1.40 1-(2-Chloro-6-fluorobenzyl)-N-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]piperidine-4-carboxamide (43)

The title compound was prepared from N-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]piperidine-4-carboxamide (19) (0.5 gm) and 2-chloro-6-fluorobenzyl bromide (0.40 ml) following the method described for the synthesis of compound (20). The title compound (43) was obtained as white solid, (0.8 gm, 62%); m.p. 187-189 °C.

Anal:

TLC : R_f 0.18 (20% EtOAc in n-hexane).

IR (KBr, cm^{-1}) : 3157, 2931, 2841, 1683, 1539, 1255, 1113, 805.

^1H-NMR (CDCl_3) : 11.08 (bs, 1H), 7.35-7.33 (d, 2H), 7.29-7.16 (m, 2H), 6.84-6.74 (m, 6H), 6.64-6.57(m, 1H), 3.85 (s, 2H), 3.71 (s, 6H), 3.31 (bs, 2H), 2.65 (bs, 3H), 1.18 (bs, 4H).

MS (m/z) : 564.75 (M)^+.

Calcd for C_{30}H_{29}ClFN_{3}O_{3}S: C, 63.65; H, 5.16; N, 7.42; Found: C, 63.58; H, 5.29; N, 7.27%.

5.1.41 1-(2-Cyanobenzyl)-N-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]piperidine-4-carboxamide (44)

The title compound was prepared from N-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]piperidine-4-carboxamide (19) (0.5 gm) and 2-cyanobenzyl bromide (0.55 gm) following the method described for the synthesis of compound (20). The title compound (44) was obtained as white solid, (0.75 gm, 62%); m.p. 187-189 °C.

Anal:

TLC : R_f 0.20 (20% EtOAc in n-hexane).

IR (KBr, cm^{-1}) : 3229, 3176, 3006, 2953, 2219, 1684, 1531, 1290, 1029, 830.

^1H-NMR (CDCl_3) : 11.34 (bs, 1H), 7.61-7.59 (d, 1H, J = 7.6 Hz), 7.51-7.49 (d, 2H, J = 8.6 Hz), 7.45-7.42(d, 2H, J = 8.6 Hz), 7.34-7.26 (m, 3H), 6.89-6.87 (d, 2H, J = 8.6 Hz), 6.83-6.81 (d, 2H, J = 8.6 Hz), 3.84 (s,
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3H), 3.78 (s, 3H), 3.59 (s, 2H), 2.73-2.71 (d, 2H), 1.78-1.70 (m, 5H), 1.50 (bs, 2H).

$^{13}$C-NMR (CDCl$_3$) : 173.31, 159.31, 159.26, 156.72, 142.92, 142.52, 132.73, 132.65, 130.73, 130.13, 129.82, 127.48, 127.20, 125.64, 124.21, 117.78, 114.31, 113.96, 112.70, 60.39, 55.29, 52.44, 42.13, 29.70, 28.38.

MS (m/z) : 538.44 (M$^+$).

LC-MS/MS : t$_R$ 6.13 min, 540.15 (M+H).

Calcd for C$_{31}$H$_{30}$N$_4$O$_3$: C, 69.12; H, 5.61; N, 10.40; Found: C, 69.35; H, 5.45; N, 10.63 %.

5.1.42 1-(4-Fluoro-2-trifluoromethylbenzyl)-N-[4,5-bis(4-methoxyphenyl)thiazol-2-yl] piperidine-4-carboxamide (45)

The title compound was prepared from N-[4,5-bis(4-methoxyphenyl)thiazol-2-yl] piperidine-4-carboxamide (19) (0.5 gm) and 4-fluoro-2-trifluorobenzylbromide (0.40 ml) following the method described for the synthesis of compound (20). The title compound (45) was obtained as white solid, (0.75 gm, 55 %); m.p. 166-168 °C.

Anal:

TLC : R$_f$ 0.18 (20% EtOAc in n-hexane).

IR (KBr, cm$^{-1}$) : 3146, 3004, 2948, 1682, 1543, 1251, 1038, 834.

$^1$H-NMR (CDCl$_3$) : 11.60 (s, 1H), 7.74-7.71 (m, 1H), 7.47-7.45 (d, 2H, $J$ = 8.7 Hz), 7.30-7.24 (m, 3H), 7.20-7.18 (d, 1H, $J$ = 8.3 Hz), 6.89-6.87 (d, 2H, $J$ = 8.7 Hz), 6.84-6.82 (d, 2H, $J$ = 8.3 Hz), 3.84 (s, 3H), 3.77 (s, 3H), 3.46 (s, 2H), 2.65 (bs, 2H), 1.67-1.57 (m, 5H), 1.49 (bs, 2H).

$^{13}$C-NMR (CDCl$_3$) : 173.56, 162.15, 159.49, 157.25, 156.99, 155.60, 142.91, 134.12, 133.70, 132.33, 130.73, 129.72, 127.24, 125.66, 124.23, 118.81, 114.22, 113.99, 112.04, 57.53, 55.29, 52.56, 42.13, 28.47.

MS (m/z) : 599.29 (M$^+$).

LC-MS/MS : t$_R$ 6.83 min, 600.29 (M+H).
Calcd for C$_{31}$H$_{35}$N$_{3}$S: C, 77.30; H, 7.32; N, 8.72; Found: C, 77.58; H, 7.13; N, 8.85 %.

5.1.43 *N*-[(1-(2-Methylbenzyl)piperidin-4-yl)methyl]-4,5-bis(p-tolyl)thiazol-2-ylamine (46)

1-(2-methylbenzyl)-*N*-[4,5-bis(p-tolyl)thiazol-2-yl]piperidine-4-carboxamide (1 gm) (20) was dissolved in dry THF under nitrogen atmosphere. Borane-dimethyl sulfide solution (BH$_3$-DMS) was added to the reaction mixture at ice-cold conditions and allowed to stir for overnight. After completion of the reaction, THF was removed and the reaction mixture was acidified with conc. HCl and again refluxed for 2-3 hrs. The reaction mixture was basified using NaHCO$_3$. The slurry was extracted using DCM and further purification was carried out by column chromatography using chloroform and methanol as eluents and silica gel (100-200) as the adsorbent to get a white solid compound (46), (0.87 gm, 89 %); m.p. 142-144 °C.

Anal:

TLC : $R_f$ 0.26 (10% Methanol in chloroform).

IR (KBr, cm$^{-1}$) : 3186, 3023, 2942, 1557, 1271, 819.

$^1$H-NMR (CDCl$_3$) : 7.36-7.34 (d, 2H, $J = 8.0$ Hz), 7.27-7.25 (d, 2H, $J = 8.0$ Hz), 7.16-7.11 (m, 4H), 7.06-7.04 (d, 4H, $J = 8.0$ Hz), 5.70 (bs, 1H), 3.44 (s, 2H), 3.09 (s, 2H), 2.90-2.87 (d, 2H, $J = 11.8$ Hz), 2.34-2.31 (s, 9H), 2.00-1.94 (m, 3H), 1.71-1.62 (d, 2H, $J = 11.8$ Hz), 1.32-1.25 (t, 2H).

$^{13}$C-NMR (CDCl$_3$) : 167.81, 145.73, 137.51, 137.14, 136.71, 132.85, 130.29, 130.17, 129.84, 129.27, 129.16, 128.91, 128.78, 127.12, 125.55, 119.69, 60.99, 53.49, 36.15, 30.16, 21.34, 19.34.

MS (m/z) : 481.61 (M$^+$).

LC-MS/MS : $t_R$ 6.83 min, 600.29 (M+H).
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5.1.44  N-[(1-(2-(Trifluoromethylbenzyl)piperidin-4-yl)methyl)-4,5-bis(p-tolyl)thiazol-2-yl]amine (47)

The title compound was prepared from 1-(2-(trifluoromethyl)benzyl-N-[4,5-bis(p-tolyl)thiazol-2-yl]piperidine-4-carboxamide (21) (1.00 gm) following the method described for the synthesis of compound (46). The title compound (47) was obtained as white solid, (0.65 gm, 67 %); m.p. 142-144 °C.

Anal:

TLC : Rf 0.17 (10% Methanol in chloroform).

IR (KBr, cm\(^{-1}\)) : 3200, 3023, 2957, 1584, 1313, 1161, 1109, 818.

\(^{1}\)H-NMR (CDCl\(_3\)) : 7.81-7.80 (d, 1H, J =7.7 Hz), 7.62-7.60 (d, 2H, J =7.7 Hz), 7.53-7.49 (m, 1H), 7.36-7.29 (m, 3H), 7.17-7.15 (d, 2H, J = 8.1 Hz), 7.06-7.04 (d, 4H, J = 8.1 Hz), 3.64 (s, 2H), 3.17-3.15 (d, 2H, J = 6.28 Hz), 2.89-2.86 (d, 2H), 2.32 (s, 6H), 2.09-2.03 (m, 2H), 1.74-1.67 (m, 5H).

MS (m/z) : 536.10 (M\(^{+}\)).

Calcd for C\(_{31}\)H\(_{32}\)F\(_3\)N\(_3\)S: C, 69.51; H, 6.02; N, 7.84; Found: C, 69.67; H, 6.11; N, 7.74 %.

5.1.45  N-[(1-(3,5-Difluorobenzyl)piperidin-4-yl)methyl]-4,5-bis(p-tolyl)thiazol-2-yl]amine (48)

The title compound was prepared from 1-(3,5-difluorobenzyl)-N-[4,5-bis(p-tolyl)thiazol-2-yl]piperidine-4-carboxamide (23) (1.00 gm) following the method described for the synthesis of compound (46). The title compound (48) was obtained as white solid, (0.85 gm, 88 %); m.p. 152-154 °C.

Anal:

TLC : Rf 0.23 (10% Methanol in chloroform).

IR (KBr, cm\(^{-1}\)) : 3198, 2925, 2856, 1583, 1459, 1332, 1117, 851.

\(^{1}\)H-NMR (CDCl\(_3\)) : 7.29-7.27 (d, 2H, J = 7.6 Hz), 7.08-7.06 (d, 2H, J = 7.6 Hz), 6.99-6.95 (d, 4H, J = 7.6 Hz), 6.77-6.76 (d, 2H), 6.60-6.56 (m, 1H), 6.20 (bs, 1H), 3.33 (s, 2H), 2.95 (s, 2H), 2.73-2.70 (d,
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2H, J = 12.0 Hz), 2.23 (s, 6H), 1.85-1.79 (m, 2H), 1.59-1.56 (d, 2H, J = 12.0 Hz), 1.18-1.10 (m, 3H).

\(^{13}\)C-NMR (CDCl\textsubscript{3}): 167.97, 164.29, 161.83, 145.69, 143.35, 137.11, 136.63, 132.86, 130.10, 129.21, 128.89, 119.40, 111.39, 111.21, 102.49, 101.98, 62.36, 53.32, 35.85, 30.01, 21.29.

MS (m/z): 503.84 (M\(^{+}\)).

LC-MS/MS: t\textsubscript{R} 2.61 min, 504.20 (M+H).

Calcd for C\textsubscript{30}H\textsubscript{31}F\textsubscript{3}N\textsubscript{3}S: C, 71.54; H, 6.20; N, 8.34; Found: C, 71.48; H, 6.35; N, 8.45%.

5.1.46 \(N\)-[(1-(4-Fluorobenzyl)piperidin-4-yl)methyl]-4,5-bis(p-tolyl)thiazol-2-ylamine (49)

The title compound was prepared from 1-(4-fluorobenzyl)-\(N\)-[4,5-bis(p-tolyl)thiazol-2-yl]piperidine-4-carboxamide (25) (1.00 gm) following the method described for the synthesis of compound (46). The title compound (49) was obtained as white solid (0.76 gm, 78 %); m.p. 178-180 °C.

Anal:

TLC : R\textsubscript{f} 0.23 (10% Methanol in chloroform).

IR (KBr, cm\textsuperscript{-1}): 3196, 3091, 2818, 2791, 1582, 1425, 1331, 818.

\(^{1}\)H-NMR (CDCl\textsubscript{3}): 7.36-7.34 (d, 2H, J = 8.1 Hz), 7.26-7.24 (m, 2H), 7.15-7.14 (d, 2H, J = 8.1 Hz), 7.06-7.04 (d, 4H, J = 8.1 Hz), 7.01-6.96 (m, 2H), 5.58 (s, 1H), 3.45 (s, 2H), 3.13-3.10 (m, 2H), 2.88-2.86 (d, 2H, J = 12.0 Hz), 2.35 (s, 6H), 1.91-1.90 (m, 3H), 1.64-1.62 (d, 2H, J = 12.0 Hz), 1.36-1.25 (m, 2H).

\(^{13}\)C-NMR (CDCl\textsubscript{3}): 166.02, 144.65, 136.19, 135.97, 132.76, 130.35, 129.96, 129.01, 128.67, 128.36, 128.31, 117.73, 114.73, 114.52, 61.45, 52.76, 40.20, 39.37, 38.95, 29.61, 20.78.

MS (m/z): 486.01 (M\(^{+}\)).

Calcd for C\textsubscript{30}H\textsubscript{32}FN\textsubscript{3}S: C, 74.19; H, 6.64; N, 8.65; Found: C, 74.05; H, 6.56; N, 8.57%.
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5.1.47 \(N-[(1-(2-Methylbenzyl)piperidin-4-yl)methyl]-4,5-bis(4-chlorophenyl)thiazol-2-ylamine (50)\)

The title compound was prepared from \(1-(2-methylbenzyl)-N-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidine-4-carboxamide (30)\) (1.00 gm) following the method described for the synthesis of compound (46). The title compound (50) was obtained as white solid (0.75 gm, 77%); m.p. 155-157°C.

Anal:

TLC : \(R_f\) 0.20 (10% Methanol in chloroform).

IR (KBr, cm\(^{-1}\)) : 3200, 2920, 2799, 2756, 1580, 1330, 1093, 824.

\(^1\)H-NMR (CDCl\(_3\)): 7.39-7.36 (m, 2H), 7.26-7.21 (m, 5H), 7.18-7.13 (m, 5H), 5.53 (bs, 1H), 3.44 (s, 2H), 3.15- 3.12 (t, 2H), 2.91-2.89 (d, 2H), 2.35 (s, 3H), 2.01- 1.96 (t, 2H), 1.70-1.64 (m, 2H), 1.35-1.25 (m, 3H).

\(^{13}\)C-NMR (CDCl\(_3\)): 167.90, 145.26, 137.44, 133.57, 133.50, 133.05, 131.09, 130.44, 130.24, 130.21, 129.79, 128.87, 128.48, 127.02, 125.50, 119.16, 60.82, 53.35, 51.97, 36.08, 30.02, 19.27.

MS (m/z) : 521.86 (M\(^+\)).

LC-MS/MS : \(t_R\) 6.04 min, 522.15 (M+H).

Calcd for C\(_{29}\)H\(_{29}\)Cl\(_2\)N\(_3\)S: C, 66.66; H, 5.59; N, 8.04; Found: C, 66.42; H, 5.66; N, 8.22 %.

5.1.48 \(N-[(1-(3,5-Difluorobenzyl)piperidin-4-yl)methyl]-4,5-bis(4-chlorophenyl)thiazol-2-yl amine (51)\)

The title compound was prepared from \(1-(3,5-difluorobenzyl)-N-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidine-4-carboxamide (33)\) (1.00 gm) following the method described for the synthesis of compound (46). The title compound (51) was obtained as white solid (0.79 gm, 79%); m.p. 134-136 °C.

Anal:

TLC : \(R_f\) 0.18 (10% Methanol in chloroform).

IR (KBr, cm\(^{-1}\)) : 3195, 3087, 2924, 2846, 1579, 1331, 1092, 827.
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**1H-NMR (CDCl<sub>3</sub>):** 7.38-7.35 (m, 2H), 7.26-7.14 (m, 8H), 7.00-6.95 (m, 1H), 5.53 (s, 1H), 3.70 (s, 2H), 3.14-3.11 (t, 2H), 2.99-2.96 (d, 2H), 2.17-2.13 (m, 2H), 1.74-1.71 (d, 2H), 1.37-1.27 (m, 3H).

**MS (m/z):** 544.32 (M)<sup>+</sup>.

**LC-MS/MS:** t<sub>R</sub> 2.47 min, 545.36 (M+H).

Calcd for C<sub>28</sub>H<sub>25</sub>Cl<sub>3</sub>N<sub>3</sub>S: C, 61.76; H, 4.63; N, 7.72; Found: C, 66.42; H, 5.66; N, 8.22 %.

### 5.1.49 N-[(1-(2-Chloro-4-fluorobenzyl)piperidin-4-ylmethyl-4,5-bis(4-chlorophenyl)thiazol-2-yl]amine (52)

The title compound was prepared from 1-(2-chloro-4-fluorobenzyl)-N-[(4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidine-4-carboxamide (34) (1.00 gm) following the method described for the synthesis of compound (46). The title compound (52) was obtained as white solid (0.76 gm, 78 %); m.p. 134-136<sup>o</sup>C.

**Anal:**

**TLC** : R<sub>f</sub> 0.21 (10% Methanol in chloroform).

**IR (KBr, cm<sup>-1</sup>):** 3199, 3091, 2940, 1579, 1490, 1328, 1046, 819.

**1H-NMR (CDCl<sub>3</sub>):** 7.93-7.90 (m, 1H), 7.43-7.39 (m, 2H), 7.28-7.23 (m, 4H), 7.22-7.19 (m, 2H), 7.16-7.15 (m, 1H), 6.96-6.93 (m, 1H), 5.62 (bs, 1H), 3.57 (s, 2H), 3.16-3.14 (m, 2H), 2.93-2.90 (d, 2H), 2.17-2.08 (m, 2H), 1.76-1.69 (d, 2H), 1.66-1.64 (m, 1H), 1.37-1.36 (m, 2H).

**MS (m/z):** 561.03 (M+1)<sup>+</sup>.

**LC-MS/MS:** t<sub>R</sub> 2.66 min

Calcd for C<sub>28</sub>H<sub>25</sub>Cl<sub>3</sub>N<sub>3</sub>S: C, 59.95; H, 4.49; N, 7.49; Found: C, 59.78; H, 4.57; N, 7.63 %. 
5.1.50  \(N\)-(1-(4-Fluoro-2-(trifluoromethylbenzyl)piperidin-4-yl)methyl-4,5-bis(4-chlorophenyl)thiazol-2-yl)amine (53)

The title compound was prepared from \(N\)-(4,5-bis(4-chlorophenyl)thiazol-2-yl)-1-[4-fluoro-2-(trifluoromethyl)benzyl]piperidine-4-carboxamide (36) (1.00 gm) following the method described for the synthesis of compound (46). The title compound obtained as white solid (53) (0.94 gm, 91 %); m.p. 172-174 °C.

Anal:

TLC : Rf 0.20 (10% Methanol in chloroform).

IR (KBr, cm\(^{-1}\)) : 3195, 2924, 2803, 1579, 1366, 1092, 865.

\(^1\)H-NMR (CDCl\(_3\)) : 7.79-7.76 (m, 1H), 7.39-7.36 (m, 2H), 7.33-7.30 (d, 1H), 7.26-7.20 (m, 5H), 7.18-7.15 (m, 2H), 5.61 (bs, 1H), 3.58 (s, 2H), 3.17-3.14 (t, 2H), 2.85-2.82 (d, 2H, \(J = 11.5\) Hz), 2.17-2.08 (t, 2H), 1.75-1.72 (d, 2H), 1.43-1.27 (m, 3H).

\(^13\)C-NMR (CDCl\(_3\)) : 168.14, 162.13, 145.30, 134.03, 133.66, 133.56, 133.06, 131.09, 130.59, 130.26, 129.82, 128.96, 128.53, 124.42, 122.21, 119.07, 118.71, 113.22, 57.75, 53.38, 52.09, 35.95, 29.71.

MS (m/z) : 594.12 (M+1).

LC-MS/MS : \(t_R\) 2.93 min, 594.12 (M+H).

Calcd for C\(_{29}\)H\(_{25}\)Cl\(_2\)F\(_4\)N\(_3\)S: C, 58.59; H, 4.24; N, 7.07; Found: C, 58.47; H, 4.17; N, 7.23%.

5.1.51  \(N\)-(1-(2-Methylbenzyl)piperidin-4-yl)methyl]-4,5-bis(4-methoxyphenyl)thiazol-2-yl)amine (54)

The title compound was prepared from 1-(2-methylbenzyl)-\(N\)-(4,5-bis(4-methoxyphenyl)thiazol-2-yl)piperidine-4-carboxamide (1.00 gm) (38) (1.00 gm) following the method described for the synthesis of compound (46). The title compound (54) was obtained as white solid (0.86 gm, 88 %); m.p. 138-140 °C.

Anal:

TLC : Rf 0.22 (10% Methanol in chloroform).
IR (KBr, cm$^{-1}$) : 3200, 2957, 2900, 1579, 1456, 1295, 1243, 1035, 829.

$^1$H-NMR (CDCl$_3$) : 7.40-7.38 (m, 2H), 7.20-7.19 (m, 2H), 7.18-7.17 (m, 4H), 6.81-6.75 (m, 4H), 5.49 (s, 1H), 3.79 (s, 3H), 3.78 (s, 3H), 3.44 (s, 2H), 3.12 (bs, 2H), 2.77-2.75 (d, 2H), 2.35 (s, 3H), 2.04-1.98 (m, 2H), 1.71-1.67 (m, 2H), 1.36-1.22 (m, 3H).

$^{13}$C-NMR (CDCl$_3$) : 167.49, 158.82, 144.93, 137.40, 136.67, 133.82, 130.49, 129.71, 129.48, 128.65, 128.18, 126.89, 125.44, 118.67, 113.95, 111.74, 60.92, 55.22, 53.43, 52.09, 36.09, 30.13, 19.25.

MS (m/z) : 513.62 (M$^+$).

LC-MS/MS : $t_R$ 2.51 min, 514.21(M+H).

Calcd for C$_{31}$H$_{35}$N$_3$O$_2$: C, 72.48; H, 6.87; N, 8.18; Found: C, 72.73; H, 6.39; N, 8.32%.

5.1.52 N-[(1-(2-Trifluoromethyl)benzyl)piperidin-4-yl)methyl]-4,5-bis(4-methoxyphenyl)thiazol-2-ylamine (55)

The title compound was prepared from 1-(2-(trifluoromethyl)benzyl)-N-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]piperidine-4-carboxamide (1.00 gm) (39) (1.00 gm) following the method described for the synthesis of compound (46). The title compound (55) was obtained as white solid (0.89 gm, 91%); m.p. 179-181 °C.

Anal:

TLC : $R_f$ 0.21 (10% Methanol in chloroform).

IR (KBr, cm$^{-1}$) : 3208, 2998, 2842, 1584, 1313, 1221, 836.

$^1$H-NMR (CDCl$_3$) : 7.81-7.79 (d, 1H, $J = 7.8$ Hz), 7.62-7.60 (d, 1H, $J = 7.8$ Hz), 7.53-7.49 (m, 2H), 7.41-7.37 (m, 2H), 7.33-7.26 (m, 1H), 7.15-7.13 (m, 1H), 6.82-6.76 (m, 4H), 5.70 (s, 1H), 3.89 (s, 3H), 3.79 (s, 3H), 3.64 (s, 2H), 3.15-3.13 (m, 2H), 2.88-2.85 (d, 2H), 2.07-2.02 (m, 2H), 1.75-1.67 (m, 3H), 1.42-1.25 (m, 2H).
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$^{13}$C-NMR (CDCl$_3$) : 167.64, 159.09, 154.85, 145.82, 133.90, 131.74, 130.20, 129.61, 128.61, 128.31, 127.75, 126.96, 125.66, 116.95, 114.01, 113.66, 111.83, 67.92, 58.30, 56.25, 55.25, 35.97, 30.14.

MS (m/z) : 568.4(M+1).

LC-MS/MS : t$_R$ 2.58 min, 568.22 (M+H).

Calcd for C$_{31}$H$_{32}$F$_3$N$_3$: C, 65.59; H, 5.68; N, 7.40; Found: C, 58.47; H, 4.17; N, 7.23 %.

5.1.53 N-[(1-(2-Chloro-6-fluorobenzyl)piperidin-4-yl)methyl]-4,5-bis(4-methoxyphenyl)thiazol-2-ylamine (56)

The title compound was prepared from 1-(2-chloro-6-fluorobenzyl)-N-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]piperidine-4-carboxamide (1.00 gm) (43) (1.00 gm) following the method described for the synthesis of compound (46). The title compound (56) was obtained as white solid (0.85 gm, 84 %); m.p. 145-147 °C.

Anal:

TLC : R$_f$ 0.25 (10% Methanol in chloroform).

IR (KBr, cm$^{-1}$) : 3206, 3100, 2929, 2799, 1595, 1506, 1250, 1113, 836.

$^1$H-NMR (CDCl$_3$) : 7.49-7.48 (d, 1H), 7.39-7.37 (m, 2H), 7.13-7.12 (m, 1H), 6.88-6.87 (d, 2H), 6.81-6.76 (m, 4H), 6.70-6.65 (m, 1H), 5.68 (s, 1H), 3.88 (s, 3H), 3.79 (s, 3H), 3.47 (s, 2H), 3.16-3.14 (d, 2H, J = 5.9 Hz), 2.90-2.84 (d, 2H, J = 12.2 Hz), 2.03-1.98 (m, 2H), 1.77-1.74 (d, 2H, J = 12.2 Hz), 1.64-1.61 (m, 3H).

MS (m/z) : 551.29 (M)$^+$.  

Calcd for C$_{30}$H$_{31}$ClF$_3$N$_3$O$_2$: C, 65.26; H, 5.66; N, 7.61; Found: C, 65.08; H, 5.73; N, 7.93 %.

5.1.54 1-Benzylpiperidine-4-carboxamide (57)

To a solution of 4-piperidinecarboxamide (2 gm, 1.28 mM) in methanol, potassium carbonate (4.3 gm, 2.56 mM) and benzyl bromide (0.89 ml) were added and the reaction mixture was refluxed for 5-6 hrs. Solvent was distilled off and the residue so obtained on
addition of crushed ice led to formation of white colored precipitate of 1-benzylpiperidine-4-carboxamide (57), (0.89 gm, 85%); m.p. 161-163°C (Lit m.p. 162°C).

Anal:
- TLC : Rf 0.28 (30% EtOAc in n-hexane).
- IR (KBr, cm⁻¹) : 3348, 2946, 2756, 1635, 1463, 1043, 810.

5.1.55 1-(4-Methylbenzyl)piperidine-4-carboxamide (58)

The title compound was prepared from 4-methylbenzyl bromide (0.64 ml) following the method described for the synthesis of compound (57). The title compound (58) was obtained as white solid (3 gm, 83%); m.p. 169-170°C.

Anal:
- TLC : Rf 0.18 (30% EtOAc in n-hexane).
- IR (KBr, cm⁻¹) : 3342, 3170, 2921, 1630, 1430, 1145.
- MS (m/z) : 232.01 (M)^+.

5.1.56 1-(4-Methoxybenzyl)piperidine-4-carboxamide (59)

The title compound was prepared from 4-methoxybenzyl bromide (1.05 ml) following the method described for the synthesis of compound (57). The title compound (59) was obtained as white solid (2.9 gm, 75%); Mp: 144-146°C.

Anal:
- TLC : Rf 0.26 (30% EtOAc in n-hexane).
- IR (KBr, cm⁻¹) : 3343, 3160, 3013, 2790, 1636, 1511, 1245, 814.
- MS (m/z) : 248.92 (M)^+.

5.1.57 1-(2-Chloro-4-fluorobenzyl)piperidine-4-carboxamide (60)

The title compound was prepared from 2-chloro-4-fluorobenzyl bromide (1.6 gm) following the method described for the synthesis of compound (57). The title compound (60) was obtained as white solid (3.2 gm, 76%); m.p. 156-157°C.

Anal:
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TLC : R_f 0.21 (30% EtOAc in n-hexane).

IR (KBr, cm⁻¹) : 3381, 3188, 2938, 1654, 1491, 1042, 726.

MS (m/z) : 270.19 (M)⁺.

5.1.58 1-(4-Cyanobenzyl)piperidine-4-carboxamide (61)

The title compound was prepared from 4-cyanobenzyl bromide (1.53 ml) following the method described for the synthesis of compound (57). The title compound (61) was obtained as white solid (3.21 gm, 85 %); m.p. 162-164 °C.

Anal:

TLC : R_f 0.25 (30% EtOAc in n-hexane).

IR (KBr, cm⁻¹) : 3445, 3197, 2943, 2224, 1673, 1503, 1045, 788.

MS (m/z) : 243.94 (M)⁺.

5.1.59 1-(4-Trifluoromethylbenzyl)piperidine-4-carboxamide (62)

The title compound was prepared from 4-trifluoromethylbenzyl bromide (1.20 ml) following the method described for the synthesis of compound (57). The title compound (62) was obtained as white solid (4.2 gm, 93 %); m.p. 132-134 °C.

Anal:

TLC : R_f 0.26 (30% EtOAc in n-hexane).

IR (KBr, cm⁻¹) : 3334, 3163, 2949, 2793, 1636, 1439, 1247, 932, 830.

MS (m/z) : 286.46 (M)⁺.

5.1.60 1-Benzylpiperidin-4-ylthiourea (63)

A solution of bis(trifluoroacetoxy)iodobenzene (2.07 gm, 4.82 mM ) in acetonitrile (8 ml) and water (4 ml) was added to 1-benzylpiperidine-4-carboxamide (57). This mixture was heated at 65 °C for overnight. The reaction mixture was cooled in an ice bath and water (10 ml) added to it. Conc HCl (2 ml) was added to the mixture and washed twice with diethyl ether, rejecting the ether layer. The aqueous layer was concentrated in vacuo, and the residue was dissolved in water (40 ml). The resulting solution was saturated with solid potassium carbonate and the resulting slurry was extracted with dichloromethane. The combined organic
extract was dried over anhydrous sodium sulfate, filtered and concentrated in vacuo to get a yellow coloured oil of the amine, which was dissolved in dry dichloromethane and benzoyl isothiocyanate (0.72 ml, 4.11 mM) was added to it. The reaction mixture was stirred at room temp for overnight. The solvent was removed and the residue so obtained was dissolved in THF/1N sodium hydroxide (1:1) and refluxed for 5 hr. The organic solvent was removed under reduced pressure to get the desired thiourea (63) as brown solid. (0.64 gm, 58 %); m.p. 124-126°C.

Anal:
   TLC : Rf 0.29 (20% Methanol in chloroform).
   IR (KBr, cm\(^{-1}\)) : 3406, 3195, 1620, 1441, 795.

5.1.61 1-(4-Methylbenzyl)piperidin-4-ylthiourea (64)

The title compound was prepared from 1-(4-methylbenzyl)piperidine-4-carboxamide (58) (1 gm) following the method described for the synthesis of compound (63). The title compound (64) was obtained as white solid (0.78 gm, 71 %); m.p. 130-132 °C.

Anal:
   TLC : Rf 0.20 (20% Methanol in chloroform).
   IR (KBr, cm\(^{-1}\)) : 3298, 3089, 2769, 1630, 1437, 813.
   MS (m/z) : 249.48 (M\(^+\)).

5.1.62 1-(4-Methoxybenzyl)piperidin-4-ylthiourea (65)

The title compound was prepared from 1-(4-methoxybenzyl)piperidine-4-carboxamide (59) (1 gm) following the method described for the synthesis of compound (63). The title compound (65) was obtained as white solid (0.89 gm, 79 %); m.p. 151-154 °C.

Anal:
   TLC : Rf 0.25 (20% Methanol in chloroform).
   IR (KBr, cm\(^{-1}\)) : 3394, 3296, 2939, 1630, 1566, 1437, 1102, 812.
5.1.63 1-(2-Chloro-4-fluorobenzyl)piperidin-4-ylthiourea (66)

The title compound was prepared from 1-(2-chloro-4-fluorobenzyl)piperidine-4-carboxamide (60) (1 gm) following the method described for the synthesis of compound (63). The title compound (66) was obtained as white solid (0.77 gm, 71%); m.p. 114-116 °C.

Anal:

TLC : Rf 0.21 (20% Methanol in chloroform).

IR (KBr, cm⁻¹) : 3405, 3293, 2825, 1627, 1491, 831.

MS (m/z) : 301.17 (M)⁺.

5.1.64 1-(4-Cyanobenzyl)piperidin-4-ylthiourea (67)

The title compound was prepared from 1-(4-cyanobenzyl)piperidine-4-carboxamide (61) (1 gm) following the method described for the synthesis of compound (63). The title compound (67) was obtained as a white solid (0.86 gm, 76%); m.p. 151-153 °C.

Anal:

TLC : Rf 0.25 (20% Methanol in chloroform).

IR (KBr, cm⁻¹) : 3405, 3163, 2230, 1686, 1565, 977.

MS (m/z) : 274.90 (M)⁺.

5.1.65 1-(4-Trifluoromethylbenzyl)piperidin-4-ylthiourea (68)

The title compound was prepared from 1-(4-trifluoromethylbenzyl)piperidine-4-carboxamide (62) (1 gm) following the method described for the synthesis of compound (63). The title compound (68) was obtained as a semisolid (0.76 gm, 69%).

Anal:

TLC : Rf 0.23 (20% Methanol in chloroform).

IR (KBr, cm⁻¹) : 3404, 3296, 2939, 1630, 1576, 1439, 1104, 751.

5.1.66 1-Benzyl-N-[4,5-bis(p-toly)thiazol-2-yl]piperidin-4-ylamine (70)

Substituted 2-bromo-1,2-di(p-toly)ethanonone (7) (1 gm 3.29 mM) was dissolved in sufficient quantity of methanol in a 25 ml round bottom flask. 1-Benzylpiperidin-4-ylthiourea
(63) (0.98 gm) and 3-4 drops of water were added into the reaction mixture and refluxed for 4-6 hrs. The reaction was monitored by TLC. After completion of the reaction, the reaction mixture was poured onto ice-cold water and the resulting solution was basified with ammonia. The product so precipitated was filtered, dried and purified using column chromatograph using n-hexane: ethyl acetate (30%) as the eluent to get a white solid (70), (1.2 gm, 85 %); m.p. 158-160 °C.

Anal:

TLC : Rₐ 0.22 (20% EtOAc in petroleum ether).

IR (KBr, cm⁻¹) : 3396, 3197, 2944, 1534, 1208, 818.

¹H-NMR (CDCl₃) : 7.29-7.27 (d, 2H), 7.23-7.21 (d, 2H), 7.19-7.15 (m, 2H), 7.09-7.07 (m, 3H), 6.97-6.95 (m, 4H), 5.21-5.19 (d, 1H, J = 6.76 Hz), 3.42 (s, 2H), 3.30 (bs, 1H), 2.74-2.71 (d, 2H), 2.22 (s, 3H), 2.22 (s, 3H), 2.11-2.06 (t, 2H), 2.02-1.99 (d, 2H), 1.54-1.49 (m, 2H).

¹³C-NMR (CDCl₃) : 165.91, 145.61, 138.19, 137.08, 136.76, 132.71, 130.10, 129.25, 129.18, 128.84, 128.80, 128.28, 127.13, 119.93, 63.68, 52.96, 51.97, 32.20, 21.30, 21.23.

MS (m/z) : 453.05 (M)⁺.

LC-MS/MS : tᵣ 2.57 min, 454.21 (M+H).

Calcd for C₂₉H₃₁N₃S: C, 76.78; H, 6.89; N, 9.26; Found: C, 76.64; H, 6.76; N, 9.35 %.

5.1.67 4-Methylbenzyl-N-[4,5-bis(p-tolyl)thiazol-2yl]piperidin-4-ylamine (71)

The title compound was prepared from 1-(4-methylbenzyl)piperidin-4ylthiourea (64) (0.86 gm) and 2-bromo-1,2-bis(p-tolyl)ethanone (7) (0.5 gm) following the method described for the synthesis of compound (70). The title compound (71) was obtained as white solid (0.98 gm, 63 %); m.p. 179-181 °C.

Anal:

TLC : Rₐ 0.17 (20% EtOAc in petroleum ether).

IR (KBr, cm⁻¹) : 3201, 2918, 2849, 1553, 1209, 818.
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$^1$H-NMR (CDCl$_3$) : 7.37-7.35 (d, 2H, $J = 8.0$ Hz), 7.19-7.17 (d, 2H, $J = 8.0$ Hz), 7.17-7.15 (d, 2H, $J = 8.0$ Hz), 7.13-7.11 (d, 2H, $J = 8.0$ Hz), 7.05-7.03 (d, 4H, $J = 8.0$ Hz), 5.17-5.15 (d, 1H, $J = 7.5$ Hz), 3.47 (s, 2H), 3.38 (bs, 1H), 2.81-2.78 (d, 2H), 2.33 (s, 3H), 2.31 (s, 6H), 2.18-2.13 (m, 2H), 2.10-2.07 (d, 2H), 1.62-1.54 (m, 2H).

$^{13}$C-NMR (CDCl$_3$) : 165.84, 145.63, 137.06, 136.75, 135.01, 132.71, 130.10, 129.19, 128.95, 128.82, 119.96, 62.79, 52.96, 51.88, 32.23, 29.73, 14.15.

MS (m/z) : 467.17 (M$^+$).

LC-MS/MS : $t_R$ 5.97 min, 467.24 (M+H).

Calcd for C$_{30}$H$_{33}$N$_3$S: C, 77.05; H, 7.11; N, 8.99; Found: C, 77.14; H, 7.26; N, 8.86%.

5.1.68 1-Benzyl-N-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidin-4-ylamine (72)

The title compound was prepared from 1-benzylpiperidin-4-ylthiourea (63) (0.87gm) and 2-bromo-1,2-bis(4-chlorophenyl)ethanone (8) (0.5 gm) following the method described for the synthesis of compound (70). The title compound (72) was obtained as white solid (1.22 gm, 85 %); m.p. 169-171 °C.

Anal:

TLC : $R_f$ 0.25 (20% EtOAc in petroleum ether).

IR (KBr, cm$^{-1}$) : 3394, 3192, 2934, 1561, 1330, 1089.

$^1$H NMR (CDCl$_3$) : 7.32-7.31 (m, 3H), 7.25-7.23 (m, 2H), 7.17-7.14 (m, 6H), 7.09-7.07 (m, 2H) 5.18-5.16 (d, 1H, $J = 7.2$ Hz), 3.46 (s, 2H), 3.32 (bs, 1H), 2.78-2.75 (d, 2H), 2.05-2.01 (d, 2H), 1.58-1.49 (m, 2H).

$^{13}$C-NMR (CDCl$_3$) : 166.30, 145.22, 138.00, 133.57, 133.51, 133.12, 131.11, 130.51, 130.23, 129.19, 128.92, 128.49, 128.30, 127.19, 119.30, 63.04, 51.90, 32.12.

MS (m/z) : 494.62 (M$^+$).

LC-MS/MS : $t_R$ 2.53 min, 494.12 (M+H).
Calcd for C_{27}H_{25}Cl_{2}N_{3}S: C, 65.58; H, 5.10; N, 8.50; Found: C, 65.41; H, 5.34; N, 8.57 %.

5.1.69 4-Methylbenzyl-N-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidin-4-ylamine (73)

The title compound was prepared using 1-(4-methylbenzyl)piperidin-4-ylthiourea (64) (0.91gm) and 2-bromo-1,2-bis(4-chlorophenyl)ethanone (8) (0.5 gm) following the method described for the synthesis of compound (70). The title compound (73) was obtained as white solid (1.15 gm, 78 %); m.p. 182-184 °C.

Anal:

TLC : R_f 0.28 (20% EtOAc in petroleum ether).

IR (KBr, cm\(^{-1}\)) : 3182, 2919, 2827, 1551, 1090, 826.

\(^1\)H-NMR (CDCl\(_3\)) : 7.38-7.36 (d, 2H, \(J = 8.5\) Hz), 7.25-7.23 (m, 5H), 7.22-7.20 (d, 2H, \(J = 8.5\) Hz), 7.14-7.12 (m, 3H), 5.22-5.20 (d, 1H, \(J = 7.9\) Hz), 3.49 (s, 2H), 3.39-3.37 (bs, 1H), 2.83-2.80 (d, \(2\)H), 2.34 (s, 3H), 2.19-2.14 (m, 2H), 2.11-2.07 (d, 2H), 1.63-1.55 (m, 2H).

\(^13\)C-NMR (CDCl\(_3\)) : 166.29, 145.25, 136.79, 134.88, 133.60, 133.50, 133.12, 131.14, 130.51, 130.22, 129.17, 128.96, 128.91, 128.47, 119.30, 62.75, 53.05, 32.14, 29.72, 21.13.

MS (m/z) : 507.33 (M\(^+\)).

LC-MS/MS : \(t_R\) 2.56 min, 508.12 (M+H).

Calcd for C\(_{28}\)H\(_{27}\)Cl\(_2\)N\(_3\)S: C, 66.13; H, 5.35; N, 8.26; Found: C, 66.02; H, 5.71; N, 8.42 %.

5.1.70 4-Methoxybenzyl-N-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidin-4-ylamine (74)

The title compound was prepared from 1-(4-methoxybenzyl)piperidin-4-ylthiourea (65) (0.97gm) and 2-bromo-1,2-bis(4-chlorophenyl)ethanone (8) (0.5 gm) following the method described for the synthesis of compound (70). The title compound (74) was obtained as white solid (1.20 gm, 79 %); m.p. 138-140 °C.

Anal:
**Experimental**

TLC : Rₜ 0.29 (20% EtOAc in petroleum ether).

IR (KBr, cm⁻¹) : 3399, 3207, 2929, 1565, 1243, 1088.

¹H NMR (CDCl₃) : 7.49-7.47 (d, 2H), 7.25-7.21 (m, 6H), 7.15-7.14 (d, 2H), 6.87-6.85 (d, 2H), 5.27 (bs, 1H), 3.80 (s, 3H), 3.49-3.47 (s, 2H), 3.40 (bs, 1H), 2.85-2.83 (d, 2H), 2.12-2.09 (d, 2H), 1.66-1.58 (m, 2H).

MS (m/z) : 524.54 (M)⁺.

Calcd for C₂₈H₂₇Cl₂N₃OS: C, 64.12; H, 5.19; N, 8.01; Found: C, 64.38; H, 5.10; N, 7.94 %.

**5.1.71 4-Cyanobenzyl-N-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidin-4-ylamine (75)**

The title compound was prepared from 1-(4-cyanobenzyl)piperidin-4-ylthiourea (66) (0.97 gm) and 2-bromo-1,2-bis(4-chlorophenyl)ethanone (8) (0.5 gm) following the method described for the synthesis of compound (70). The title compound (75) was obtained as white solid (1.13 gm, 75 %); m.p. 166-168 °C.

Anal:

TLC : Rₜ 0.22 (20% EtOAc in petroleum ether).

IR (KBr, cm⁻¹) : 3394, 3352, 2921, 2223, 1598, 1541, 1090, 818.

¹H-NMR (CDCl₃) : 7.62-7.60 (d, 2H, J = 8.3 Hz), 7.46-7.44 (d, 2H, J = 8.1Hz), 7.38-7.36 (d, 2H, J = 8.3 Hz), 7.26-7.24 (d, 2H, J = 8.3 Hz), 7.23-7.21 (d, 2H, J = 8.1 Hz), 7.17-7.15 (d, 2H, J = 8.3Hz), 5.36 (bs, 1H), 3.57 (s, 2H), 3.43 (bs, 1H), 2.82-2.79 (d, 2H), 2.26-2.24 (m, 2H), 2.11-2.08 (m, 2H), 1.67-1.59 (m, 2H).

MS (m/z) : 519.84 (M)⁺.

LC-MS/MS : tᵣ 2.68 min.

Calcd for C₂₈H₂₄Cl₂N₄S: C, 64.74; H, 4.66; N, 10.79; Found: C, 64.63; H, 4.38; N, 10.92 %.
5.1.72 1-(2-Chloro-4-fluorobenzyl)-N-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidin-4-ylamine (76)

The title compound was prepared from 1-(2-chloro-4-fluorobenzyl)piperidin-4-ylthiourea (67) (1.05 gm) and 2-bromo-1,2-bis(4-chlorophenyl)ethanone (8) (0.5 gm) following the method described for the synthesis of compound (70). The title compound (76) was obtained as white solid (1.25 gm, 79 %); m.p. 151-153 °C.

Anal:

TLC : Rf 0.22 (20% EtOAc in petroleum ether).

IR (KBr, cm\(^{-1}\)) : 3210, 2925, 2818, 1545, 1090, 828.

\(^1\)H-NMR (CDCl\(_3\)) : 7.50-7.44 (m, 1H), 7.37-7.35 (m, 2H), 7.25-7.21 (m, 4H), 7.18-7.16 (m, 2H), 7.12-7.09 (d, 1H), 6.99-6.94 (m, 1H), 5.31-5.30 (d, 1H, J = 6.7 Hz), 3.59 (s, 2H), 3.44 (bs, 1H), 2.86-2.83 (d, 2H), 2.31-2.26 (m, 2H), 2.12-2.10 (d, 2H), 1.65-1.56 (m, 2H).

\(^13\)C-NMR (CDCl\(_3\)) : 166.23, 162.68, 160.21, 145.16, 134.78, 134.68, 133.50, 131.11, 130.46, 129.49, 128.89, 128.45, 119.30, 116.80, 113.98, 58.48, 51.88, 31.56, 29.68.

Calcd for C\(_{27}\)H\(_{23}\)Cl\(_3\)FN\(_3\)OS: C, 59.29; H, 4.24; N, 7.68; Found: C, 59.46; H, 4.69; N, 7.78%.

5.1.73 4-Methylbenzyl-N-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]piperidin-4-ylamine (77)

The title compound was prepared from 1-(4-methylbenzyl)piperidin-4-ylthiourea (65) (0.94 gm) and 2-bromo-1,2-bis(4-methoxyphenyl)ethanone (9) (0.5 gm) following the method described for the synthesis of compound (70). The title compound (77) was obtained as white solid (1.02 gm, 68 %); m.p. 110-112 °C.

Anal:

TLC : Rf 0.24 (20% EtOAc in petroleum ether).

IR (KBr, cm\(^{-1}\)) : 3391, 2924, 2850, 1550, 1249, 1023, 808.
Section I

Experimental

\(^1\)H-NMR (CDCl\(_3\)) : 7.54-7.52 (d, 1H), 7.48-7.47 (d, 2H), 7.21-7.19 (d, 2H), 7.14-7.12 (d, 4H), 6.79-6.75 (m, 3H), 5.24 (bs, 1H), 3.85 (s, 3H), 3.73 (s, 3H), 3.52 (s, 2H), 3.39 (bs , 1H), 2.87-2.84 (d, 2H), 2.30 (s, 3H), 2.28-2.12 (m, 2H), 2.10-2.072 (d, 2H), 1.67-1.60 (m, 2H).

MS (m/z) : 499.58 (M\(^+\)).

LC-MS/MS : tr 5.92 min, 500.25 (M+H).

Calcd for C\(_{30}\)H\(_{33}\)N\(_3\)O\(_2\)S: C, 72.11; H, 6.66; N, 8.41; Found: C, 72.32; H, 6.43; N, 8.52 %.

5.1.74 4-Methoxybenzyl-N-[4,5-bis(4-methoxyphenylthiazol-2-yl)piperidin-4-ylamino]methane (78)

The title compound was prepared from 1-(4-methoxybenzyl)piperidin-4-ylthiourea (66) (1 gm) and 2-bromo1,2-bis(4-methoxyphenyl)ethanone (9) (0.5 gm) following the method described for the synthesis of compound (70). The title compound (78) was obtained as white solid (1.02 gm, 73 %); m.p. 135-137 °C.

Anal:

TLC : R\(_f\) 0.25 (20% EtOAc in petroleum ether).

IR (KBr, cm\(^{-1}\)) : 3153, 2925, 2854, 1570, 1462, 1247, 1055.

\(^1\)H-NMR (CDCl\(_3\)) : 7.49-7.48 (d, 1H), 7.40-7.36 (m, 2H), 7.26-7.22 (m, 3H), 5.27 (bs, 1H), 3.88 (s, 3H), 3.80 (s, 6H), 3.49 (s, 2H), 3.39 (bs ,1H), 2.85-2.83 (d, 2H), 2.28-2.16 (m, 2H), 2.13-2.10 (d, 2H), 1.66-1.61 (m, 2H).

\(^{13}\)C-NMR (CDCl\(_3\)) : 165.81, 159.15, 154.89, 145.64, 133.92, 132.49, 130.82, 130.60, 129.61, 128.99, 127.60, 126.83, 117.19, 114.40, 113.80, 111.82, 62.02, 56.25, 55.29, 52.28, 51.49, 31.94, 29.71.

MS (m/z) : 515.01 (M\(^+\)).

LC-MS/MS : tr 2.59 min, 516.21 (M+H).

Calcd for C\(_{30}\)H\(_{33}\)F\(_3\)N\(_3\)O\(_2\)S: C, 69.87; H, 6.45; N, 8.15; Found: C, 69.71; H, 6.57; N, 8.07%.
5.1.75 4-Trifluoromethylbenzyl-N-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]piperidin-4-yl amine (79)

The title compound was prepared from 1-(4-trifluoromethylbenzyl)piperidin-4-ylthiourea (68) (1.12 gm) and 2-bromo-1,2-bis(4-methoxyphenyl)ethanone (9) (0.5 gm) following the method described for the synthesis of compound (70). The title compound was obtained as white solid (79) (1.25 gm, 76 %); m.p. 102-103 oC.

Anal:
TLC : Rf 0.24 (20% EtOAc in petroleum ether).
IR (KBr, cm\(^{-1}\)) : 3384, 3210, 2926, 2799, 1550, 1250, 833.
\(^1\)H-NMR (CDCl\(_3\)) : 7.49-7.48 (d, 1H), 7.40-7.36 (m, 2H), 7.29-7.27 (m, 2H), 7.15-7.12 (m, 1H), 7.03-6.97 (m, 2H), 5.27 (bs, 1H), 3.86 (s, 3H), 3.79 (s, 3H), 3.48 (s, 2H), 3.39 (bs ,1H), 2.82-2.79 (d, 2H), 2.20-2.17 (d, 2H), 2.14-2.10 (t, 2H), 1.64-1.55 (m, 2H).
\(^1^3\)C-NMR (CDCl\(_3\)) : 165.94, 160.85, 159.06, 154.88, 145.69, 133.92, 132.49, 130.69, 129.20, 113.63, 111.83, 62.51, 56.25, 52.91, 51.83, 32.65, 29.72.
MS (m/z) : 553.40 (M)+.
Calcd for C\(_{30}\)H\(_{30}\)F\(_3\)N\(_3\)O\(_2\)S: C, 65.08; H, 5.46; N, 7.59; Found: C, C, 65.22; H, 5.57; N, 7.41%.

5.2 Biological Work

(All biological work has been carried out by the researchers of pharmacology section and not by the candidate himself)

5.2.1 In vitro AChE and BuChE inhibition assays: The assays were performed according to the method described by Ellman et al.\(^\text{94}\) as reported earlier.\(^\text{142}\) AChE from human erythrocyte and BuChE from equine serum, 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB-Ellman’s reagent), acetylthiocholine iodide (ATCI) and butyrylthiocholine iodide (BTCI) were purchased from Sigma. Tacrine and donepezil were used as reference drugs (sigma). All the experiments were conducted in 50 mM Tris-HCl buffer at pH 8. Five different concentrations (0.001–100 µM) of each compound were used to determine enzyme inhibition
activity. Briefly, 50 µL of AChE (0.22 U mL\(^{-1}\)) or 50 µL of BuChE (0.06 U mL\(^{-1}\)) and 10 µL of the test or standard compound were incubated in 96-well plates at room temperature for 30 min. Further, 30 µL of the substrate viz. ATCI (15 mM) or BTCI (15 mM) was added and incubated for additional 30 min. Finally, 160 µL of DTNB (1.5 mM) was added and the absorbance was measured at 415 nm wavelength using microplate reader 680 XR (BIO-RAD, India). The IC\(_{50}\) value was calculated from the absorbance recorded for individual compounds. IC\(_{50}\) value depicts the concentration of the drug resulting in 50 % inhibition of the enzyme activity. All the determinations were performed in triplicate and at least in three independent runs.

### 5.2.2 Thioflavin T (ThT) assay:

Inhibition of AChE induced A\(_{\beta1-42}\) aggregation was evaluated using the thioflavin T (ThT) fluorescence assay as described earlier.\(^{98,99}\) A\(_{\beta1-42}\) (Sigma) was dissolved in phosphate buffer saline (PBS) and was further diluted with 0.215 M sodium phosphate buffer (\(pH\) 8). Test compounds were dissolved in DMSO and were further diluted with 0.215 M sodium phosphate buffer (\(pH\) 8). Briefly, 2 µl of the A\(_{\beta1-42}\) was incubated with 16 µl AChE in the presence of 2 µl of the test compound to obtain final concentration of 50 µM A\(_{\beta1-42}\), 230 µM AChE and 10 µM test compound. The mixture was co-incubated at room temperature for 24 hr. After incubation, 180 µl of 20 µM ThT (prepared in 50 mM glycine-NaOH buffer; \(pH\) 8.5) was added. The fluorescence intensity was read at 442 nm excitation and 490 nm emission wavelengths using Synergy HTX fluorescence microplate reader. The percentage inhibition of the AChE induced A\(_{\beta1-42}\) aggregation was calculated using the formula: 100-(IF\(_{i}\)/IF\(_{o}\)×100), where IF\(_{i}\) and IF\(_{o}\) are fluorescence intensities in the presence and absence of the test compound respectively. Each assay was conducted in triplicate and each experiment was repeated at least three times independently.

### 5.2.3 In vitro Blood-Brain Barrier permeation assay:

To predict the possible in vivo blood-brain barrier (BBB) permeation of selected hybrid diarylthiazole-benzylpiperidine derivatives, a parallel artificial membrane permeation assay of the blood-brain barrier (PAMPA-BBB) was performed as described by Di et al.\(^{100-102}\) Commercial drugs and dodecane were obtained from Sigma. Porcine brain lipid (PBL) was purchased from Avanti Polar Lipids. The donor microplates (PVDF membrane, pore size 0.45 mm) and the acceptor microplates were obtained from Millipore. The acceptor microplate was filled with 200 µL phosphate buffer saline (PBS):ethanol (70:30) and the filter surface of donor microplate was impregnated with 4 µL of porcine brain lipid in dodecane (20 mg mL\(^{-1}\)). The test compounds were dissolved in DMSO at 5 mg mL\(^{-1}\) and diluted in PBS/ethanol (70:30) to get the final
concentration of 100 µg mL\(^{-1}\). 200 µL of the solution was filled in the donor well. The donor plate was carefully placed on the acceptor plate to form a sandwich, keeping it undisturbed for 120 min at 25°C. After incubation period, the donor plate was removed and the concentration of test compounds in the acceptor wells was determined using UV spectroscopy. Each sample was analysed at five different wavelengths, in four wells, and at least in three independent runs. The results are expressed as mean ± SEM. In the experiment, nine quality standard commercial drugs of known BBB permeability were selected to validate the analysis results.

5.2.4 Cell culture: The human neuroblastoma SH-SY5Y cell line was obtained from National Centre for Cell Science (NCCS) (Pune, India). Cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1 mM glutamine, 50 U mL\(^{-1}\) penicillin and 50 µg mL\(^{-1}\) streptomycin (reagents from Gibco) at 37°C in a humidified incubator at 5% CO\(_2\). All the cells used in the study were of low passage number (<15).

5.2.5 Determination of cell viability and neuroprotection: To determine the cytotoxicity of the selected test compounds, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed. SH-SY5Y cells were seeded in 96-well plate at a density of 5×10\(^4\) cells per well. After 24 hr, the medium was replaced with relatively higher concentrations of test compounds (40 µM and 80 µM) for another 24 hr at 37°C. After incubation period, the cell viability was determined using MTT assay. In another set of experiment, test compounds were assessed for their ability to protect SH-SY5Y cells against oxidative damage induced by H\(_2\)O\(_2\). The cells were exposed to the test compounds at relatively lower concentrations (5 µM, 10 µM and 20 µM) and incubated for 2 hr. After the incubation period, the test compounds were replaced with media containing cytotoxic insult, i.e. H\(_2\)O\(_2\) (100 µM) which was left for an additional 24 hr period. Thereafter, cell viability was assessed using MTT assay. Briefly, the medium was replaced with 80 µL of fresh medium and 20 µL of MTT (0.5 mg mL\(^{-1}\), final concentration; Sigma) in PBS. After 4 hr, MTT was removed and crystals of the formazan were dissolved in DMSO. Formazan concentrations were quantified at 570 nm with 630 nm reference wavelengths using a microplate reader 680 XR (BIO-RAD, India). Percentage protection against H\(_2\)O\(_2\) insult was calculated by considering absorbance of control cells as 100% of the cell viability.
5.2.6 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH) assay: The 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) assay is based on the reduction of DPPH, a purple colored stable free radical. DPPH gets paired off and reduced to a yellow colored diphenylpicrylhydrazine by the antioxidant. Thus the assay measures an electron (or hydrogen atom) donating activity and hence provides assessment of antioxidant activity of a compound which may be attributed to its free radical scavenging ability.\textsuperscript{84, 104} The spectrophotometric DPPH assay was carried out as described earlier.\textsuperscript{143} Concentrations at which the selected test derivatives showed promising neuroprotective effects against H\textsubscript{2}O\textsubscript{2} insult were selected for the DPPH assay. In brief, 10 µL of a test compound (10 and 20 µM, in Tris-HCl buffer–pH 7.4) was mixed with 20 µL of DPPH (from 10 mM stock, in methanol) (Hi-Media) in the 96 well plate. Finally, the volume was adjusted to 200 µL using methanol. After 30 sec incubation at room temperature and protection from light, the absorbance was read at 520 nm wavelength using a microplate reader 680 XR (BIO-RAD, India). The free radical scavenging activity was determined as the reduction percentage (RP) of DPPH using the equation: \(\text{RP} = 100\left[\frac{A_0 - A_C}{A_0}\right]\), where \(A_0\) is the untreated DPPH absorbance and \(A_C\) is the absorbance value for added sample concentration \(C\). Ascorbic acid was used as the standard antioxidant.

5.2.7 ROS estimation using primary rat hippocampal culture: The intracellular ROS level was estimated in primary rat hippocampal neuronal culture using 2′,7′-dichlorofluorescin diacetate (DCFH-DA) assay.\textsuperscript{67, 109} Amongst all the tested hybrid diarylthiazole-benzylpiperidine derivatives, the most potent compound (48) was assessed for ROS scavenging activity. Aβ\textsubscript{1-42} was used to induce ROS generation in the primary rat hippocampal neuronal culture which was prepared as described earlier.\textsuperscript{144} Briefly, hippocampal tissues were dissected out from 18 days old rat foetuses, washed with cold HBSS, minced and incubated in 0.1% trypsin for 30 min at 37°C. Trituration was carried out to form a single cell suspension. Cells were plated in 96 well plates at a density of 5×10\textsuperscript{4} viable cells mL\textsuperscript{-1}. Cells were grown as neurons in serum free neurobasal medium (Invitrogen) containing N-2 supplement (1%) (Invitrogen), B-27 supplement (2%) (Invitrogen) and antibiotic-antimycotic solution (1%) (Sigma). Cultures of neurons were placed in a humidified incubator at 37°C and 5% CO\textsubscript{2}. Medium was changed every 3 days. Formation of small proliferating neurons started after 1 week, and mature neurons were observed in twenty days \textit{in vitro}. On the twenty first day, cells were exposed to the compound (48) (10 µM and 20 µM) for 2 hr, followed by Aβ\textsubscript{1-42} (10 µM; Sigma)\textsuperscript{145} treatment for 24 hr. Later on, the cells

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were incubated with 10 µM DCFH-DA in PBS (Sigma) at 37ºC for 30 min. After rinsing with PBS, the plate was read at excitation (485 nm) and emission (530 nm) wavelengths using the Synergy HTX multi-mode microplate reader. The fluorescence intensities in the presence and absence of inhibitors were compared using appropriate controls. Percentage inhibition of ROS generation was determined.

5.2.8 Assessment of apoptosis by flow cytometry: Flow cytometric assessment of apoptosis was performed using Annexin V-FITC and propidium iodide (PI) staining. Briefly, the rat hippocampal neuronal cells were seeded in six well plate which were exposed to Aβ1-42 (10 µM) for 24 hr. To determine anti-apoptotic potential of the test compound, cells were pre-treated with compound (48) (20 µM) for 2 hr followed by Aβ1-42 treatment. After incubation period, cells were harvested and suspended in 500 µl Annexin V binding buffer. Later, 5 µl Annexin V-FITC (BD Biosciences) and 10 µl PI (Sigma) were added and incubated with cells for 5 min in the dark. Untreated cells were used as the control for double staining. The stained cells were directly analysed using a FAC Scan flow cytometer.

5.2.9 Behavioural study: The experiments were performed in adult male Swiss Albino mice weighing 20-25 gm. The study protocol was approved by IAEC (Institutional Animal Ethics Committee) and experiments were performed as per CPCSEA (Committee for the purpose of Supervision of Experiments on Animals) guidelines (Approval No. MSU/IAEC/2014-15/1401). Scopolamine hydrochloride and donepezil hydrochloride were purchased from Sigma.

Scopolamine rodent model was adopted to induce AD like phenotype, especially amnesia. Mice were divided into four experimental groups of six animals each: (i) vehicle, (ii) scopolamine, (iii) scopolamine plus donepezil and (iv) scopolamine plus compound (48). Scopolamine (1.4 mg kg⁻¹) was dissolved in saline and administered intraperitoneally (i.p.) to all the groups except vehicle-treated control group that received equal volume of saline. Donepezil was suspended in 0.5% sodium carboxymethyl cellulose (CMC-Na) and was given at a dose of 5 mg kg⁻¹ orally 30 min before administration of scopolamine. Equivalent dose of test compound (48) corresponding to donepezil suspended in CMC-Na was also administered orally 30 min prior to the scopolamine treatment. All the treatments were continued for nine consecutive days. During the last five days of treatment period, spatial learning and memory was assessed using the Morris Water Maze (MWM) test.
The maze consists of a circular pool (65 cm diameter; 30 cm height) filled with water (26±1°C) up to 20 cm depth. The inside walls of the pool were painted black. The pool was divided into four quadrants and the escape platform was placed 1 cm below the water surface in the middle of any one quadrant. Individual experiments were carried out to determine the time required by the animal reaching the hidden platform (i.e. escape latency time-ELT) and the number of platform area crossings during 2 min of the training session to assess spatial learning and memory. All the experiments were carried out in a sound proof room and supervised by a blind observer.

5.2.10 Neurochemical analysis: At the end of MWM test, the mice were sacrificed. Whole brains were isolated from the skull and homogenized in glass teflon homogenizer in 12.5 mM sodium phosphate buffer (pH 7). The homogenates were centrifuged at 15,000 rpm for 15 min at 4°C. The supernatants were utilized for estimation of different biochemical parameters.

The cholinergic biomarkers AChE and BuChE, were estimated in the mice brain using Ellman’s method. 94, 115 100 µL of the supernatant was incubated with 2.7 mL of phosphate buffer and 100 µL of freshly prepared ATCI or BTCI (15 mM) for 5 min. Finally, 100 µL of DTNB (1.5 mM) was added and the absorbance was read at 415 nm wavelength spectrophotometrically.

MDA, an indicator of lipid peroxidation, was evaluated using thiobarbituric acid reacting substance (TBARS) method reported as earlier 116, 147 MDA reacted with thiobarbituric acid in acidic medium at high temperature and formed a red complex TBARS which was analysed spectrophotometrically. Briefly, 200 µL of supernatant was mixed with 1 mL of 50 % trichloroacetic acid in 0.1 M HCl and 1 mL of 26 mM thiobarbituric acid. After vortex mixing, samples were heated at 95°C for 20 min. Later on the samples were centrifuged at 15,000 rpm for 10 min and the supernatants were read at 532 nm wavelength.

Catalase (CAT) is an enzyme mediating breakdown of toxic form of oxygen metabolite, H₂O₂ into oxygen and water. CAT activity was determined following the method described by Sinha. 148 Briefly, 100 µL of the supernatant was mixed with 150 µL of 0.01 M phosphate buffer (pH 7). Reaction was started by addition of 250 µL of H₂O₂ (0.16 M), and the contents incubated at 37°C for one min and the reaction was stopped by addition of 1 mL of dichromate:acetic acid reagent (5% K₂Cr₂O₇:glacial acetic acid; 1:3; V/V). The reaction mixture was immediately kept on a boiling water bath for 15 min that resulted in
development of green color. Finally, the mixture was analysed at 570 nm wavelength spectrophotometrically.

5.2.11 ICV rat model of AD: Adult male Wister rats (200-250 gm) were divided into four experimental groups of six animals each: (a) vehicle, (b) Aβ1-42, (c) Aβ1-42 plus donepezil and (d) Aβ1-42 plus (48). Animals were anaesthetised with ketamine (100 mg/kg, i.p.) and xylazine (30 mg/kg, i.p.) and mounted on a stereotaxic apparatus (Stoelting, USA). All the groups (except the vehicle-treated control group which received equal volume of normal saline) were injected with 4 µl of Aβ1-42 (2 µM/µl in normal saline) unilaterally at the following co-ordinates: -4.0 mm anteroposterior, -2.5 mm mediolateral and -3.5 mm dorsoventral from Bregma. Compound (48) was administered at an equivalent dose of donepezil (5 mg/kg, p.o.) in 0.1% CMC to the respective experimental group animals for 15 consecutive days after five days of surgical recovery. 149, 150

5.2.12 Y maze test: The Y-maze test was adopted for assessment of immediate working memory. 151 The test was carried out during last five days of the treatment period in the animals which underwent ICV injection of Aβ1-42. Each animal from the treated groups was kept at the end of any one arm of the maze and allowed to explore all the three arms. The sequence and the number of arm entries were recorded visually for each rat over a period of 5 min. An actual “alteration” was defined as entries in all three arms in consecutive choices (i.e. ABC, BCA or CAB but not BAB). Repeat arm entry was considered as a sign of memory impairment. The number of arm entries indicated locomotor activity. The “alteration score” for each rat was calculated using the equation:

\[
\% \text{ Alternation} = \left( \frac{\text{(Number of alternations)}}{\text{(Total arm entries}-2)} \right) \times 100
\]

5.2.13 Western blot analysis: Hippocampal regions from different experimental rat brains were homogenized in tissue lysis buffer supplemented with protease and phosphatase inhibitors (Sigma). Homogenized samples were sonicated for 5 s, and centrifuged at 4°C at 15,000 rpm for 30 min. Equal amounts of proteins (100 µg) were loaded on 10% Tris-glycine gel. Membranes were blocked for 1 hr at room temperature using Tris-buffered saline/Tween-20 (TBST) (50 mM Tris-HCl, 150 mM NaCl, pH 7.4, 1% Tween-20) containing 5% non-fat-dried milk. Membranes were incubated overnight at 4°C with rabbit anti-Aβ1-42 (1:500, Santa Cruz), goat anti-p-Tau (1:500, Santa Cruz), rabbit anti-cleaved caspase-3 (active) (1:500, Sigma) and rabbit anti-cleaved poly (ADP-ribose) polymerase-1 (PARP) (1:1000, Cell Signalling) primary antibodies. After incubation, membranes were washed thrice with TBST
and incubated for 1 hr with HRP–conjugated secondary antibody (Sigma). Immunoreactive proteins were detected using the ECL Plus chemiluminescent kit (Invitrogen) according to the manufacturer’s instructions. Protein bands were quantified using Scion Image for Windows.

5.2.14 **Acute toxicity study:** Total twenty male Swiss Albino mice (20-25 gm) were used to determine acute toxicity of the test compound (48). During the experiment, animals were maintained with free access of food and water *ad libitum*. Compound (48) was suspended in 0.5% CMC-Na and given orally to the divided experimental groups (at 0, 677, 1333 and 2000 mg kg⁻¹, n = 5 per group). After the test compound administration, the animals were observed continuously for the first 4 hr for any abnormal behaviour and mortality. Later on the animals were observed intermittently for the next 24 hr and occasionally for 14 consecutive days after administration of compound (48). After 14 days mice were sacrificed and macroscopically examined for possible damage to the heart, liver and kidneys.⁶⁷ ⁶⁸