## 5. EXPERIMENTAL

The experimental work carried out to achieve the proposed aims and objectives has been detailed under three main headings:

**5.1.** Chemical studies,

**5.2.** Biological studies and

**5.3.** Computational studies.

### 5.1. Chemical studies

All of the commercial reagents and solvents required for synthesis of the compounds were purchased from Spectrochem, Sigma-Aldrich, S. d. fine chemicals, and Avra chemicals and were purified by general laboratory techniques whenever needed. Reaction monitoring was performed by thin layer chromatography (TLC), using silica gel precoated plates (60F254, Merck, 0.25 mm thickness) and visualizing in ultraviolet (UV) light ($\lambda$ = 254 nm) or in an iodine chamber. Chromatographic purification was performed by flash column chromatography with a Teledyne ISCO CombiFlash Rf system using RediSep Rf columns. Yields reported here are unoptimized. Melting points were determined in glass capillary tubes using a silicon oil-bath type melting point apparatus (Veego) or by differential scanning calorimetry (DSC) using a Shimadzu DSC-60 instrument, and are uncorrected. The purity of the compounds was assessed by HPLC, with all the compounds exhibiting a $\geq$95% purity level. The IR spectra were recorded on a Bruker ALPHA-T (Germany) FT-IR spectrophotometer for all the reported compounds and are consistent with the assigned structures. $^{1}$H NMR and $^{13}$C NMR spectra were recorded on a Bruker Advance-II 400 MHz spectrometer in $CDCl_3$ or DMSO-$d_6$ solvents. Chemical shifts ($\delta$) are expressed in parts per million (ppm) relative to the standard TMS, and the peak patterns are indicated as s (singlet), d (doublet), t (triplet), m (multiplet), and br (broad signal). Mass spectra were recorded using a Thermo Fisher mass spectrometer with an EI ion source. Elemental analyses were performed on a Thermo Fisher FLASH 2000 organic elemental analyzer. The elemental compositions of the compounds were within ±0.4% range of the calculated values. All the procedures performed on the animals during this work were in accordance with CPCSEA established guidelines and regulations and were reviewed and approved by IAEC (Institutional Animal Ethics Committee) (Approval No. MSU/IAEC/2016-17/1636).

The experimental work carried out has been discussed under the following two main heads:

**5.1.1.** Triazinoindole-based multifunctional anti-AD agents and,

**5.1.2.** Carbazole-based multifunctional anti-AD agents

## 5.1.1. Triazinoindole-based multifunctional anti-AD agents

### 5.1.1.1. 5*H*-[1,2,4]Triazino[5,6-*b*]indole-3-thiol (68)

To a stirred suspension of isatin (1.0 g, 6.80 mmol) in aqueous potassium carbonate (2.82 g in 50 mL water) was added thiosemicarbazide (0.62 g, 6.80 mmol). The mixture was refluxed for 5 hrs. After cooling down to room temperature, the solution was acidified with glacial acetic acid and left overnight. The obtained precipitate was filtered and washed with a mixture of water/acetic acid (24:1 v/v). The resulting solid was triturated with hot DMF, filtered, and dried to yield pale yellow colored titled compound (**68**) (1.26 g, 92 %), m.p. >350 °C (lit[112] m.p. >300 °C).

**Anal.:**

**TLC** : $R_f$ 0.33 (Chloroform-Methanol 19:1);

**IR (KBr, cm$^{-1}$)** : 3410, 3038, 1609, 1427, 1345 and1160;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 14.58 (br, 1H, -S*H*), 12.35 (br, 1H, -N*H*), 7.98 (d, 1H, Ar*H*), 7.58-7.62 (m, 1H, Ar*H*), 7.43 (d, 1H, Ar*H*) and 7.31-7.34 (m, 1H, Ar*H*);

**MS (*m/z*)** : 203 $[M+H]^+$.

### 5.1.1.2. 3-(Methylthio)-5*H*-[1,2,4]triazino[5,6-*b*]indole (69)

To a suspension of 5*H*-[1,2,4]triazino[5,6-*b*]indole-3-thiol (**68**) (1.0 g, 4.34 mmol) in DMF (10 mL), potassium carbonate (0.683 g, 4.94 mmol) and methyl iodide (0.31 mL, 4.94 mmol) were added. The reaction mixture was allowed to stir at 60 °C for 6–8 hrs. After completion of the reaction, the reaction mixture was poured into crushed ice. The precipitated product so formed was filtered, washed with water and recrystallized to yield the titled compound (**69**) (0.74 g, 69 %), m.p. 306.73 °C (DSC) (lit[112] m.p. >300 °C).

**Anal.:**

**TLC** : $R_f$ 0.58 (Chloroform-Methanol 19:1);

**IR (KBr, cm$^{-1}$)** : 3204, 3056, 2801, 1604, 1342 and 1184;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 12.62 (br,1H, -N*H*), 8.28 (d, 1H, Ar*H*), 7.64-7.67 (m, 1H, Ar*H*), 7.55 (d, 1H,Ar*H*), 7.39-7.42 (m, 1H, Ar*H*) and 2.66 (s, 3H, -C$H_3$);

**$^{13}$C NMR (DMSO-$d_6$)** : $\delta$ 168.1, 147.2, 141.3, 140.8, 131.3, 122.9, 121.9, 118.2, 113.2 and 13.9;

**MS (*m/z*)** : 216 $[M]^+$.

### 5.1.1.3. 3-(Methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (70)

To a stirred solution of 3-(methylthio)-5*H*-[1,2,4]triazino[5,6-*b*]indole (1.0 g, 4.62 mmol) in anhydrous methylene chloride (40 mL) at 0 °C, *m*CPBA (80-85 % tech solid, 2.42 g, 4.62 mmol) was added as a solid in small portions over a period of a few minutes. The resulting reaction mixture was stirred at room temperature with the exclusion of moisture for 24 hrs. The progress of the reaction was monitored by TLC. After completion of the reaction, the organic phase was washed several times with saturated sodium bicarbonate solution and then with the brine solution. The collected organic phase was dried over anhydrous magnesium sulfate, filtered, and evaporated to give light yellowish solid which was used in next step without further purification.[113]

**Anal.:**

**TLC** : $R_f$ 0.4 (*n*-Hexane-Ethyl acetate 10:10);

**IR (KBr, cm$^{-1}$)** : 3447, 3307, 1621, 1591, 1138, 1090 and 758;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 13.25 (bs, 1H, -N*H*), 8.45 (d, 1H, Ar*H*), 7.77-7.81 (m, 1H, Ar*H*), 7.68 (d, 1H, Ar*H*), 7.49-7.53 (m, 1H, Ar*H*) and 3.50 (s, 3H, -$SO_2CH_3$);

**MS (*m/z*)** : 249 $[M+H]^+$.

### 5.1.1.4. Synthesis of *N*-Substituted 5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine derivatives (71-80)

**Method A.** To 3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**70**) (0.5 g, 2.014 mmol) in THF (20 mL), an appropriate amine (10 mmol) was added and refluxed on a water-bath for approximately 4 hrs. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was poured into ice cold water (20 mL). The aqueous phase was extracted with ethyl acetate (3 × 30 mL), the organic layer was combined and washed with sodium bicarbonate solution (5%) and brine, and the combined organic layer was dried over anhydrous sodium sulfate and evaporated to give a crude product which was further purified by flash chromatography.[113]

### 5.1.1.4a. *N*-Propyl-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (71)

The title compound (**71**) was synthesized from 3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**70**) (0.5 g, 2.01 mmol), and *n*-propylamine (0.83 mL, 10.05 mmol) as per **Method A**. The title compound (**71**) was obtained as pale yellow solid (0.32 g, 70 %), m.p. 252-253 °C.

**Anal.:**

**TLC** : $R_f$ 0.66 (*n*-Hexane-Ethyl acetate 10:10);

**IR (KBr, cm$^{-1}$)** : 3447, 3060, 2963, 1614, 1533, 1459, 1393 and 749;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 11.73 (bs, 1H, -N*H*), 8.07 (d, 1H, Ar*H*), 7.22-7.44 (m, 3H, Ar*H*, 1H, -N*H*), 3.37-3.40 (m, 2H, -NHC$H_2$), 1.63-1.69 (m, 2H, -C$H_2$) and 0.97 (t, 3H, -$CH_2CH_3$);

**$^{13}$C NMR (DMSO-$d_6$)** : $\delta$ 161.7, 149.0, 139.5, 128.5, 121.9, 119.9, 119.7, 116.2, 112.3, 43.0, 22.6 and 11.9;

**MS (*m/z*)** : 228.1 $[M+H]^+$;

**RP-HPLC** : Purity: 96.1 %, $t_R$ = 4.65 min (Mobile phase: acetonitrile-water 50:50 (0.01 % TFA)).

### 5.1.1.4b. *N*-*Iso*butyl-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (72)

The title compound (**72**) was synthesized from 3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**69**) (0.5 g, 2.01 mmol), and isobutylamine (1.0 mL, 10.05 mmol) as per **Method A**. The title compound (**72**) was obtained as pale yellow solid (0.40 g, 82 %), m.p. 243-245 °C.

**Anal.:**

**TLC** : $R_f$ 0.61 (*n*-Hexane-Ethyl acetate 10:10);

**IR (KBr, cm$^{-1}$)** : 3429, 3069, 2960, 1609, 1524, 1467 and 750;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 11.81 (bs, 1H, -N*H*), 8.08 (d, 1H, Ar*H*), 7.25-7.45 (m, 3H, Ar*H*), 3.24-3.28 (m, 2H, -NHC$H_2$), 1.95-2.02 (m, 1H, -C*H*) and 0.94 (d, 6H, -C$H_3$);

**$^{13}$C NMR (DMSO-$d_6$)** : δ 161.7, 149.0, 139.5, 128.5, 121.9, 119.9, 119.7, 116.2, 112.3, 49.4, 28.1 and 20.8;

**MS (*m/z*)** : 242.1 $[M+H]^+$;

**RP-HPLC** : Purity: 96.7 %, $t_R$ = 3.39 min (Mobile phase: acetonitrile-water 50:50 (0.01 % TFA)).

### 5.1.1.4c. *N*-Benzyl-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (73)

The title compound (**73**) was synthesized from 3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**69**) (0.5 g, 2.01 mmol), and benzylamine (1.1 mL, 10.05 mmol) as per **Method A**. The title compound (**73**) was obtained as light yellow solid (0.5 g, 81 %), m.p. 294 °C (DSC).

**Anal.:**

**TLC** : $R_f$ 0.52 (*n*-Hexane-Ethyl acetate 10:10);

**IR (KBr, cm$^{-1}$)** : 3440, 3076, 2966, 1606, 1528 and 694;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 11.79 (bs, 1H, -N*H*), 8.07 (d, 1H, Ar*H*), 7.18-7.45 (m, 8H, Ar*H*) and 4.67 (d, 2H, -NHC$H_2$);

**$^{13}$C NMR (DMSO-$d_6$)** : $\delta$ 161.7, 148.9, 140.5, 139.6, 128.9, 128.7, 127.5, 127.2, 122.0, 120.0, 119.6, 116.2, 112.3 and 44.4;

**MS (*m/z*)** : 276.1 $[M+H]^+$;

**RP-HPLC** : Purity: 96.9 %, $t_R$ = 7.32 min (Mobile phase: acetonitrile-water 50:50 (0.01 % TFA)).

### 5.1.1.4d. *N*-(4-Chlorobenzyl)-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (74)

The title compound (**74**) was synthesized from 3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**69**) (0.5 g, 2.01 mmol), and 4-chlorobenzylamine (1.22 mL, 10.05 mmol) as per **Method A**. The title compound (**74**) was obtained as pale yellow solid (0.42 g, 68 %), m.p. 218-220 °C.

**Anal.:**

**TLC** : $R_f$ 0.45 (*n*-Hexane-Ethyl acetate 10:10);

**IR (KBr, cm$^{-1}$)** : 3414, 3062, 2921, 1620, 1592, 1092 and 758;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 11.82 (bs, 1H, -N*H*), 8.07 (d, 1H, Ar*H*), 7.24-7.46 (m, 7H, Ar*H*, 1H, -N*H*) and 4.62 (d, 2H, -NHC$H_2$);

**$^{13}$C NMR (DMSO-$d_6$)** : $\delta$ 161.5, 149.0, 139.6, 139.0, 131.6, 129.3, 128.8, 128.7, 122.4, 120.1, 119.5, 116.0, 112.4 and 43.9;

**MS (*m/z*)** : 310.1 [M+H]$^+$;

**RP-HPLC** : Purity: 96.4 %, $t_R$ = 5.94 min (Mobile phase: acetonitrile-water 50:50 (0.01 % TFA)).

### 5.1.1.4e. *N*-(4-Methoxybenzyl)-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (75)

The title compound (**75**) was synthesized from 3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**69**) (0.5 g, 2.01 mmol), and 4-methoxybenzyl amine (1.32 mL, 10.05 mmol) as per **Method A**. The title compound (**75**) was obtained as pale yellow solid (0.46 g, 75 %), m.p. 241-243 °C.

**Anal.:**

**TLC** : $R_f$ 0.52 (*n*-Hexane-Ethyl acetate 10:10);

**IR (KBr, cm$^{-1}$)** : 3371, 3058, 2961, 1610 and 756;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 11.83 (bs, 1H, -N*H*), 8.07 (d, 1H, Ar*H*), 7.96 (bs, 1H, -N*H*), 7.24-7.46 (m, 5H, Ar*H*), 6.82-6.86 (m, 2H, Ar*H*), 4.57 (d, 2H, -NHC$H_2$) and 3.73 (s, 3H, -OC$H_3$);

**MS (*m/z*)** : 306.1 [M+H]$^+$;

**RP-HPLC** : Purity: 97.3 %, $t_R$ = 4.11 min (Mobile phase: acetonitrile-water 50:50 (0.01 % TFA)).

### 5.1.1.4f. *N*-(3,4-Dimethoxybenzyl)-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (76)

The title compound (**76**) was synthesized from 3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**69**) (0.5 g, 2.01 mmol), and 3,4-dimethoxybenzyl amine (1.51 mL, 10.05 mmol) as per **Method A**. The title compound (**76**) was obtained as pale yellow solid (0.47 g, 70 %), m.p. 201-203 °C.

**Anal.:**

**TLC** : $R_f$ 0.6 (*n*-Hexane-Ethyl acetate 10:10);

**IR (KBr, cm$^{-1}$)** : 3374, 1615, 1518 and 806;

**$^{1}$H NMR (DMSO-$d_6$)** : *δ* 8.07 (d, 1H, Ar*H*), 6.82-7.46 (m, 6H, Ar*H*, 1H, -N*H*), 4.57 (d, 2H, -NHC$H_2$), 3.76 (s, 3H, -OC$H_3$) and 3.74 (s, 3H, -OC$H_3$);
**$^{13}$C NMR (DMSO-$d_6$)** : *δ* 161.2, 149.1, 148.9, 148.2, 139.7, 132.9, 128.7, 122.0, 120.1, 119.7, 116.0, 112.4, 112.5, 111.9, 56.0, 55.9 and 44.3;
**MS (*m/z*)** : 336.1 [M+H]$^+$;
**RP-HPLC** : Purity: 97.7 %, $t_R$ = 3.63 min (Mobile phase: acetonitrile-water 50:50 (0.01 % TFA)).

### 5.1.1.4g. *N*-(2-Picolyl)-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (77)

The title compound (**77**) was synthesized from 3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**69**) (0.5 g, 2.01 mmol), and 2-picolylamine (1.04 mL, 10.05 mmol) as per **Method A**. The title compound (**77**) was obtained as light yellow solid (0.46 g, 82 %), m.p. 242-244 °C.

**Anal.:**

**TLC** : $R_f$ 0.3 (Chloroform-Methanol 18:2);
**IR (KBr, cm$^{-1}$)** : 3396, 3069, 2972, 1615, 1562 and 750;
**$^{1}$H NMR (DMSO-$d_6$)** : *δ* 11.79 (bs, 1H,-N*H*), 8.52 (d, 1H, Ar*H*), 8.13-7.18 (m, 7H, Ar*H*) and 4.77 (d, 2H, -NHC$H_2$);
**$^{13}$C NMR (DMSO-$d_6$)** : *δ* 159.8, 149.3, 148.9, 139.6, 137.2, 137.1, 128.8, 122.5, 121.1, 120.9, 120.2, 119.5, 116.0, 112.4 and 46.5;
**MS (*m/z*)** : 277.1 [M+H]$^+$;
**RP-HPLC** : Purity: 98.3 %, $t_R$ = 3.36 min (Mobile phase: acetonitrile-water 50:50 (0.01 % TFA)).

### 5.1.1.4h. *N*-((Furan-2-yl)methyl)-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (78)

The title compound (**78**) was synthesized from 3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**69**) (0.5 g, 2.01 mmol), and furfurylamine (0.88 mL, 10.05 mmol) as per **Method A**. The title compound (**78**) was obtained as pale yellow solid (0.34 g, 63 %), m.p. 234-236 °C.

**Anal.:**

**TLC** : $R_f$ 0.5 (*n*-Hexane-Ethyl acetate 8:12);

**IR (KBr, cm$^{-1}$)** : 3406, 3118, 2967, 1615, 1559 and 751;

**$^{1}$H NMR (DMSO-$d_6$)** : $\delta$ 11.83 (bs, 1H, -N*H*), 8.08-8.12 (m, 1H, Ar*H*), 6.26-7.92 (m, 6H, Ar*H*, 1H, -N*H*) and 4.64 (d, 2H, -NHC$H_2$);

**$^{13}$C NMR (DMSO-$d_6$)** : $\delta$ 155.8, 153.5, 148.9, 142.5, 139.7, 128.8, 122.1, 120.9, 120.2, 119.5, 116.0, 110.9, 107.3 and 38.3;

**MS (*m/z*)** : 266.1 $[M+H]^+$;

**RP-HPLC** : Purity: 98.0 %, $t_R$ = 3.55 min (Mobile phase: acetonitrile-water 50:50 (0.01 % TFA)).

### 5.1.1.4i. *N*-(2-(Piperidin-1-yl)ethyl)-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (79)

The title compound (**79**) was synthesized from 3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**69**) (0.5 g, 2.01 mmol), and 1-(2-aminoethyl) piperidine (1.43 mL, 10.05 mmol) as per **Method A**. The title compound (**79**) was obtained as pale yellow solid (0.41 g, 69 %), m.p. 217-219 °C.

**Anal.:**

**TLC** : R$_f$ 0.35 (Chloroform-Methanol 19:1);

**IR (KBr, cm$^{-1}$)** : 3399, 3231, 3113, 1618, 1524, 1126, 1092 and 754;

**$^{1}$H NMR (DMSO-$d_6$)** : $\delta$ 11.84 (bs, 1H, -N*H*), 8.07 (d, 1H, Ar*H*), 7.24-7.46 (m, 3H, Ar*H*, 1H, -N*H*), 3.50-3.52 (m, 2H, -NHC$H_2$CH$_3$), 2.52-2.55 (m, 2H, -C$H_2$), 2.40-2.43 (m, 4H, -NC$H_2$), 1.51-1.55 (m, 4H, -C$H_2$) and 1.41-1.42 (m, 2H, -C$H_2$);

**$^{13}$C NMR (DMSO-$d_6$)** : $\delta$ 155.8, 149.0, 139.5, 128.4, 122.0, 120.0, 119.7, 116.0, 112.3, 57.9, 54.6, 41.4, 26.0 and 24.5;

**MS (*m/z*)** : 297.1 $[M+H]^+$;

**RP-HPLC** : Purity: 98.5 %, $t_R$ = 5.33 min (Mobile phase: acetonitrile-water 30:70 (0.01 % TFA)).

### 5.1.1.4j. *N*-(2-Morpholinoethyl)-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (80)

The title compound (**80**) was synthesized from 3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**69**) (0.5 g, 2.01 mmol), and 4-(2-aminoethyl) morpholine (1.32 mL, 10.05 mmol) as per **Method A**. The title compound (**80**) was obtained as light yellow solid (0.41 g, 67 %), m.p. >270 °C.

**Anal.:**

**TLC** : $R_f$ 0.3 (Chloroform-Methanol 19:1);

**IR (KBr, cm$^{-1}$)** : 3337, 3070, 2967, 1607, 1526, 1132 and 757;

**$^1$H NMR (DMSO-*d*$_6$)** : $\delta$ 11.74 (s, 1H, -N*H*), 8.08 (d, 1H, Ar*H*), 7.03-7.45 (m, 3H, Ar*H*, 1H, -N*H*), 3.62-3.64 (m, 4H, -OC$H_2$), 3.55-3.57 (m, 2H, -NHC$H_2$), 2.59-2.62 (m, 2H, -NC$H_2$) and 2.47- 2.49 (m, 4H, -NC$H_2$);

**$^{13}$C NMR (DMSO-*d*$_6$)** : $\delta$ 161.7, 149.0, 139.5, 128.6, 122.0, 120.0, 119.6, 116.2, 112.4, 66.7, 57.7, 53.8 and 38.3;

**MS (*m/z*)** : 299.1 $[M+H]^+$;

**RP-HPLC** : Purity: 99.1 %, $t_R$ = 4.55 min (Mobile phase: acetonitrile-water 30:70 (0.01 % TFA)).

### 5.1.1.5. Synthesis of *N*-substituted 5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine derivatives (117-126)

#### 5.1.1.5.1. Synthesis of *N*-substituted isatins (81-89)

*N*-substituted isatins (**81-89**) were synthesized from isatin by procedure reported by Kanhed A. *et. al*. The analytical data i.e. melting points, $R_f$ values and IR spectra of the *N*-substituted isatins are in accordance with the reported values.[118]

#### 5.1.1.5.2. Synthesis of 5-substituted 5*H*-[1,2,4]triazino[5,6-*b*]indol-3-thiol derivatives (90-98)

5-substituted 5*H*-[1,2,4]triazino[5,6-*b*]indol-3-thiol derivatives (**90-98**) were synthesized from *N*-substituted isatins (**81-89**) and thiosemicarbazide by procedure reported by Kanhed A. *et. al*. The analytical data i.e. melting points, $R_f$ values and IR spectra of the compound (**90-98**) are in accordance with the reported values.[118]

### 5.1.1.5.3. Synthesis of 5-substituted 3-(methylthio)-5*H*-[1,2,4] triazino[5,6-*b*]indole derivatives (99-107)

5-Substituted 3-(methylthio)-5*H*-[1,2,4]triazino[5,6-*b*]indole derivatives (**99-107**) were synthesized by methylation of the respective thiol derivatives (**90-98**) with methyl iodide as per the method described for compound (**69**).

### 5.1.1.5.3a. 5-Methyl-3-(methylthio)-5*H*-[1,2,4]triazino[5,6-*b*]indole (99)

The title compound (**99**) was synthesized from 5-methyl-5*H*-[1,2,4] triazino[5,6-*b*]indole-3-thiol (**90**) (1.0 g, 4.63 mmol) and methyl iodide (0.33 mL, 5.28 mmol) as per the method described for compound (**69**). The title compound (**99**) was obtained as a greenish yellow solid (0.9 g, 85 %), m.p. 160-162 °C.

**Anal.:**

**TLC** : $R_f$ 0.63 (Chloroform-Methanol 19:1);

**IR (KBr, cm$^{-1}$)** : 3052, 3022, 2968, 2925, 1578, 1179, 1072 and 762;

**MS (*m/z*)** : 231 $[M+H]^+$.

### 5.1.1.5.3b. 5-Ethyl-3-(methylthio)-5*H*-[1,2,4]triazino[5,6-*b*]indole (100)

The title compound (**100**) was synthesized from 5-ethyl-5*H*-[1,2,4] triazino[5,6-*b*]indole-3-thiol (**91**) (1.0 g, 4.34 mmol) and methyl iodide (0.31 mL, 4.95 mmol) as per the method described for compound (**69**). The title compound (**100**) was obtained as a greenish yellow solid (0.87 g, 82 %), m.p. 148-150 °C.

**Anal.:**

**TLC** : $R_f$ 0.63 (Chloroform-Methanol 19:1);

**IR (KBr, cm$^{-1}$)** : 3054, 2970, 2928, 2872, 1580, 1188, 1078 and 749;

**MS (*m/z*)** : 245 $[M+H]^+$.

### 5.1.1.5.3c. 5-Propyl-3-(methylthio)-5*H*-[1,2,4]triazino[5,6-*b*]indole (101)

The title compound (**101**) was synthesized from 5-propyl-5*H*-[1,2,4] triazino[5,6-*b*]indole-3-thiol (**92**) (1.0 g, 4.09 mmol) and methyl iodide (0.29 mL, 4.66 mmol) as per the method described for compound (**69**). The title compound (**101**) was obtained as yellow solid (0.85 g, 80 %), m.p. 128-130 °C.

**Anal.:**

**TLC** : R$_f$ 0.64 (Chloroform-Methanol 19:1);
**IR (KBr, cm$^{-1}$)** : 3053, 3026, 2966, 2924, 1575, 1190, 1073 and 746;
**MS (*m/z*)** : 259 [M+H]$^+$.

### 5.1.1.5.3d. 5-Butyl-3-(methylthio)-5*H*-[1,2,4]triazino[5,6-*b*]indole (102)

The title compound (**102**) was synthesized from 5-butyl-5*H*-[1,2,4] triazino[5,6-*b*]indole-3-thiol (**93**) (1.0 g, 3.87 mmol) and methyl iodide (0.27 mL, 4.41 mmol) as per the method described for compound (**69**). The title compound (**102**) was obtained as a pale yellow solid (0.89 g, 84 %), m.p. 140-142 °C.

**Anal.:**

**TLC** : R$_f$ 0.64 (Chloroform-Methanol 19:1);
**IR (KBr, cm$^{-1}$)** : 3054, 2953, 2925, 1578, 1187, 1075 and 742;
**MS (*m/z*)** : 273 [M+H]$^+$.

### 5.1.1.5.3e. 5-Benzyl-3-(methylthio)-5*H*-[1,2,4]triazino[5,6-*b*]indole (103)

The title compound (**103**) was synthesized from 5-benzyl-5*H*-[1,2,4] triazino[5,6-*b*]indole-3-thiol (**94**) (1.0 g, 3.42 mmol) and methyl iodide (0.24 mL, 3.90 mmol) as per the method described for compound (**69**). The title compound (**103**) was obtained as yellow solid (0.91 g, 87 %), m.p. 168-170 °C.

**Anal.:**

**TLC** : R$_f$ 0.66 (Chloroform-Methanol 19:1);
**IR (KBr, cm$^{-1}$)** : 3058, 3028, 2955, 2923, 1578, 1186, 1074, 745 and 694;
**MS (*m/z*)** : 307 [M+H]$^+$.

### 5.1.1.5.3f. 5-(2-Methylbenzyl)-3-(methylthio)-5*H*-[1,2,4]triazino[5,6-*b*] indole (104)

The title compound (**104**) was synthesized from 5-(2-methylbenzyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole-3-thiol (**95**) (1.0 g, 3.26 mmol) and methyl iodide (0.23 mL, 3.71 mmol) as per the method described for compound (**69**). The title compound (**104**) was obtained as pale yellow solid (0.86 g, 82 %), m.p. 161-163 °C.

**Anal.:**

**TLC** : $R_f$ 0.68 (Chloroform-Methanol 19:1);
**IR (KBr, cm$^{-1}$)** : 3059, 3029, 2974, 2924, 1574, 1181, 1073 and 748;
**MS (*m/z*)** : 321 $[M+H]^+$.

### 5.1.1.5.3g. 5-(4-Methylbenzyl)-3-(methylthio)-5*H*-[1,2,4]triazino[5,6-*b*] indole (105)

The title compound (**105**) was synthesized from 5-(4-methylbenzyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole-3-thiol (**96**) (1.0 g, 3.26 mmol) and methyl iodide (0.23 mL, 3.71 mmol) as per the method described for compound (**69**). The title compound (**105**) was obtained as yellow solid (0.85 g, 81 %), m.p. 195-197 °C.

**Anal.:**

**TLC** : $R_f$ 0.68 (Chloroform-Methanol 19:1);
**IR (KBr, cm$^{-1}$)** : 3056, 2922, 1581, 1182, 1083 and 749;
**MS (*m/z*)** : 321 $[M+H]^+$.

### 5.1.1.5.3h. 5-(4-Chlorobenzyl)-3-(methylthio)-5*H*-[1,2,4]triazino[5,6-*b*] indole (106)

The title compound (**106**) was synthesized from 5-(4-chlorobenzyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole-3-thiol (**97**) (1.0 g, 3.06 mmol) and methyl iodide (0.22 mL, 3.48 mmol) as per the method described for compound (**69**). The title compound (**106**) was obtained as yellow solid (0.82 g, 79 %), m.p. 181-183 °C.

**Anal.:**

**TLC** : $R_f$ 0.67 (Chloroform-Methanol 19:1);
**IR (KBr, cm$^{-1}$)** : 3059, 3026, 2927, 1583, 1184, 1088 and 748;
**MS (*m/z*)** : 341 $[M+H]^+$.

### 5.1.1.5.3i. 5-(4-Fluorobenzyl)-3-(methylthio)-5*H*-[1,2,4]triazino[5,6-*b*] indole (107)

The title compound (**107**) was synthesized from 5-(4-fluorobenzyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole-3-thiol (**98**) (1.0 g, 3.22 mmol) and methyl iodide (0.23 mL, 3.67 mmol) as per the method described for compound (**69**). The title compound (**107**) was obtained as a yellow solid (0.89 g, 85 %), m.p. 176-178 °C.

**Anal.:**

| | |
|---|---|
| **TLC** | : $R_f$ 0.65 (Chloroform-Methanol 19:1); |
| **IR (KBr, cm$^{-1}$)** | : 3055, 2924, 1581, 1185, 1078 and 751; |
| **MS (*m/z*)** | : 325 $[M+H]^+$. |

### 5.1.1.5.4. Synthesis of 5-substituted 3-(methylsulfonyl)-5*H*-[1,2,4]triazino [5,6-*b*]indole derivatives (108-116)

The sulfone derivatives (**108-116**) were synthesized by the oxidation of respective thiomethyl derivatives (**99-107**) with *m*CPBA as per the method described for the compound (**70**).[113] The obtained products were used in the next step without further purification.

### 5.1.1.5.5. Synthesis of 5-substituted 5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine derivatives (117-126)

Following the general method B, 5-substituted 5*H*-[1,2,4]triazino[5,6-*b*] indol-3-amine derivatives (**117-126**) were synthesized by the reaction of the respective sulfone derivatives (**70**, **108-116**) with aqueous ammonia solution.

**Method B:** To a solution of sulfone derivative (0.5 g, 1 equiv.) in THF (20 mL) in a sealed tube, aqueous ammonia solution (10 equiv.) was added and then the tube was tightly closed. The reaction mixture was heated to 80 ºC for approximately 7-8 hrs. Progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was poured into ice cold water (20 mL). The aqueous phase was extracted with ethyl acetate (30 mL × 3), the organic layer was combined, washed with sodium bicarbonate solution (5 %) and brine, and the collected organic layer was dried over anhydrous sodium sulfate and evaporated to give a crude product which was further purified by flash chromatography.

### 5.1.1.5.5a. 5-Methyl-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (117)

The title compound (**117**) was synthesized from 5-methyl-3-(methyl sulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**108**) (0.5 g, 1.91 mmol) and aqueous ammonia solution (25 %, 2.94 mL, 19.1 mmol) as per **Method B**. The title compound (**117**) was obtained as brown solid (0.25 g, 64 %), m.p. >270 °C.

**Anal.:**

| | |
|---|---|
| **TLC** | : $R_f$ 0.48 (Chloroform-Methanol 23:2); |

**IR (KBr, cm$^{-1}$)** : 3383, 3307, 3212, 1549, 1106 and 770;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 8.09 (d, 1H, Ar*H*), 7.60 (d, 1H, Ar*H*), 7.50-7.54 (m, 1H, Ar*H*), 7.31-7.35 (m, 1H, Ar*H*), 7.26 (bs, 2H, -N$H_2$) and 3.72 (s, 3H, -NC$H_3$);

**MS (*m/z*)** : 200 [M+H]$^+$;

**RP-HPLC** : Purity: 95.2 %, $t_R$ = 4.32 min (Mobile phase: acetonitrile: water 50:50 (0.01 % TFA)).

### 5.1.1.5.5b. 5-Ethyl-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (118)

The title compound (**118**) was synthesized from 5-ethyl-3-(methyl sulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**109**) (0.5 g, 1.81 mmol) and aqueous ammonia solution (25 %, 2.78 mL, 18.1 mmol) as per **Method B**. The title compound (**118**) was obtained as brown solid (0.26 g, 67 %), m.p. >270 °C.

**Anal.:**

**TLC** : $R_f$ 0.51 (Chloroform-Methanol 23:2);

**IR (KBr, cm$^{-1}$)** : 3384, 3308, 3211, 2937, 1549, 1019 and 755;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 8.02 (d, 1H, Ar*H*), 7.62-7.71 (m, 2H, Ar*H*), 7.37-7.41 (m, 1H, Ar*H*), 6.84 (bs, 2H, -N$H_2$), 4.24-4.30 (m, 2H, -NC$H_2$C$H_3$) and 1.76-1.82 (m, 3H, -NCH$_2$C$H_3$);

**MS (*m/z*)** : 214.2 [M+H]$^+$;

**RP-HPLC** : Purity: 99 %, $t_R$ = 4.68 min (Mobile phase: acetonitrile: water 50:50 (0.01 % TFA)).

### 5.1.1.5.5c. 5-Propyl-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (119)

The title compound (**119**) was synthesized from 5-propyl-3-(methyl sulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**110**) (0.5 g, 1.72 mmol) and aqueous ammonia solution (25 %, 2.65 mL, 17.2 mmol) as per **Method B**. The title compound (**119**) was obtained as greenish yellow solid (0.26 g, 67 %), m.p. 265-267 °C.

**Anal.:**

**TLC** : $R_f$ 0.52 (Chloroform-Methanol 23:2);

**IR (KBr, cm$^{-1}$)** : 3384, 3308, 3212, 2938, 1588, 1105 and 755;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 8.11 (d, 1H, Ar*H*), 7.65 (d, 1H, Ar*H*), 7.48-7.52 (m, 1H, Ar*H*), 7.30-7.34 (m, 1H, Ar*H*), 7.27 (bs, 2H, -N$H_2$), 4.21 (t, 2H, -NC$H_2$), 1.78-1.85 (m, 2H, -C$H_2\mathrm{CH_3}$) and 0.90 (t, 3H, -$\mathrm{CH_2C}H_3$);

**MS (*m/z*)** : 227 $[\mathrm{M}]^+$;

**RP-HPLC** : Purity: 99.1 %, $t_R$ = 4.88 min (Mobile phase: acetonitrile: water 50:50 (0.01 % TFA)).

### 5.1.1.5.5d. 5-Butyl-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (120)

The title compound (**120**) was synthesized from 5-butyl-3-(methyl sulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**111**) (0.5 g, 1.64 mmol) and aqueous ammonia solution (25 %, 2.52 mL, 16.4 mmol) as per **Method B**. The title compound (**120**) was obtained as brown solid (0.28 g, 70 %), m.p. 208-210 °C.

**Anal.:**

**TLC** : $R_f$ 0.52 (Chloroform-Methanol 23:2);

**IR (KBr, cm$^{-1}$)** : 3377, 3298, 3210, 2961, 1525, 1035 and 741;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 8.10 (d, 1H, Ar$H$), 7.51-7.52 (m, 2H, Ar$H$), 7.27-7.31 (m, 1H, Ar$H$), 6.90 (bs, 2H, -N$H_2$), 4.19-4.22 (m, 2H, -NC$H_2$), 1.74-1.81 (m, 2H, -$\mathrm{NCH_2C}H_2$), 1.30-1.36 (m, 2H, -C$H_2\mathrm{CH_3}$) and 0.92 (t, 3H, -$\mathrm{CH_2C}H_3$);

**MS (*m/z*)** : 242.3 $[\mathrm{M+H}]^+$;

**RP-HPLC** : Purity: 98.0 %, $t_R$ = 5.65 min (Mobile phase: acetonitrile: water 50:50 (0.01 % TFA)).

### 5.1.1.5.5e. 5-Benzyl-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (121)

The title compound (**121**) was synthesized from 5-benzyl-3-(methyl sulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**112**) (0.5 g, 1.48 mmol) and aqueous ammonia solution (25 %, 2.28 mL, 14.8 mmol) as per **Method B**. The title compound (**121**) was obtained as reddish brown solid (0.28 g, 68 %), m.p. 265-267 °C.

**Anal.:**

**TLC** : $R_f$ 0.51 (Chloroform-Methanol 23:2);

**IR (KBr, cm$^{-1}$)** : 3396, 3329, 3029, 2929, 1542, 1030 and 746;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 7.93-8.14 (m, 1H, Ar$H$), 7.22-7.49 (m, 8H, Ar$H$) and 5.45 (s, 2H, -NC$H_2$);

**MS ($m/z$)** : 276.2 $[M+H]^+$;

**RP-HPLC** : Purity: 99.1 %, $t_R$ = 8.92 min (Mobile phase: acetonitrile: water 50:50 (0.01 % TFA)).

### 5.1.1.5.5f. 5-(2-Methylbenzyl)-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (122)

The title compound (**122**) was synthesized from 5-(2-methylbenzyl)-3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**113**) (0.5 g, 1.42 mmol) and aqueous ammonia solution (25 %, 2.18 mL, 14.2 mmol) as per **Method B**. The title compound (**122**) was obtained as light brown solid (0.29 g, 71 %), m.p. 253-254 °C.

**Anal.:**

**TLC** : $R_f$ 0.54 (Chloroform-Methanol 23:2);

**IR (KBr, cm$^{-1}$)** : 3462, 3334, 3009, 2922, 1553, 1107 and 744;

**$^1$H NMR (CDCl$_3$)** : $\delta$ 8.40 (d, 1H, Ar*H*), 7.35-7.44 (m, 2H, Ar*H*), 7.20-7.28 (m, 2H, Ar*H*), 7.13 (d, 1H, Ar*H*), 7.06-7.09 (m, 1H, Ar*H*), 6.72 (d, 1H, Ar*H*), 5.44 (bs, 2H, -N$H_2$), 5.21 (s, 2H, -NC$H_2$) and 2.44 (s, 3H, -C$H_3$);

**MS ($m/z$)** : 290 $[M+H]^+$;

**RP-HPLC** : Purity: 95.9 %, $t_R$ = 5.54 min (Mobile phase: acetonitrile: water 50:50 (0.01 % TFA)).

### 5.1.1.5.5g. 5-(4-Methylbenzyl)-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (123)

The title compound (**123**) was synthesized from 5-(4-methylbenzyl)-3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**114**) (0.5 g, 1.42 mmol) and aqueous ammonia solution (25 %, 2.18 mL, 14.2 mmol) as per **Method B**. The title compound (**123**) was obtained as yellow solid (0.26 g, 64 %), m.p. 245-247 °C.

**Anal.:**

**TLC** : $R_f$ 0.53 (Chloroform-Methanol 23:2);

**IR (KBr, cm$^{-1}$)** : 3471, 3271, 3057, 2963, 1536, 1098 and 743;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 7.93-7.95 (m, 1H, Ar*H*), 7.14-7.55 (m, 5H, Ar*H*), 6.88-6.90 (m, 2H, Ar*H*), 5.27 (s, 2H, -NC$H_2$) and 3.72 (s, 3H, -C$H_3$);

**MS ($m/z$)** : 290.2 $[M+H]^+$;

| | | |
|---|---|---|
| **RP-HPLC** | : | Purity: 96.6 %, $t_R$ = 5.73 min (Mobile phase: acetonitrile: water 50:50 (0.01 % TFA)). |

### 5.1.1.5.5h. 5-(4-Chlorobenzyl)-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (124)

The title compound (**124**) was synthesized from 5-(4-chlorobenzyl)-3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**115**) (0.5 g, 1.34 mmol) and aqueous ammonia solution (25 %, 2.06 mL, 13.4 mmol) as per **Method B**. The title compound (**124**) was obtained as light brown solid (0.28 g, 67 %), m.p. 215-217 °C.

**Anal.:**

| | | |
|---|---|---|
| **TLC** | : | $R_f$ 0.57 (Chloroform-Methanol 23:2); |
| **IR (KBr, cm$^{-1}$)** | : | 3417, 3298, 2925, 1540, 1039 and 746; |
| **$^1$H NMR (CDCl$_3$)** | : | *δ* 8.39 (d, 1H, Ar*H*), 7.43-7.47 (m, 1H, Ar*H*), 7.26-7.39 (m, 6H, Ar*H*), 5.47 (s, 2H, -NC$H_2$) and 5.16 (bs, 2H, -N$H_2$); |
| **MS (*m/z*)** | : | 310 [M+H]$^+$; |
| **RP-HPLC** | : | Purity: 97.4%, $t_R$ = 5.99 min (Mobile phase: acetonitrile: water 50:50 (0.01 % TFA)). |

### 5.1.1.5.5i. 5-(3-Fluorobenzyl)-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (125)

The title compound (**125**) was synthesized from 5-(3-fluorobenzyl)-3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**116**) (0.5 g, 1.40 mmol) and aqueous ammonia solution (25 %, 2.15 mL, 14.0 mmol) as per **Method B**. The title compound (**125**) was obtained as brown solid (0.24 g, 59 %), m.p. 230-232 °C.

**Anal.:**

| | | |
|---|---|---|
| **TLC** | : | $R_f$ 0.53 (Chloroform-Methanol 23:2); |
| **IR (KBr, cm$^{-1}$)** | : | 3418, 3299, 2924, 1550, 1039 and 748; |
| **$^1$H NMR (DMSO-$d_6$)** | : | *δ* 7.94-7.96 (m, 1H, Ar*H*), 7.46-7.55 (m, 4H, Ar*H*), 7.30-7.32 (m, 1H, Ar*H*), 7.16-7.18 (m, 2H, Ar*H*) and 5.33 (s, 2H, -NC$H_2$); |
| **MS (*m/z*)** | : | 294 [M+H]$^+$; |
| **RP-HPLC** | : | Purity: 97.4%, $t_R$ = 6.93 min (Mobile phase: acetonitrile: water 50:50 (0.01 % TFA)). |

### 5.1.1.5.5j. 5*H*-[1,2,4]Triazino[5,6-*b*]indol-3-amine (126)

The title compound (**126**) was synthesized from 3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**70**) (0.5 g, 2.01 mmol) and aqueous ammonia solution (25 %, 3.09 mL, 20.01 mmol) as per **Method B**. The title compound (**126**) was obtained as light brown solid (0.19 g, 52 %), m.p. >270 °C.

**Anal.:**

| | | |
|---|---|---|
| **TLC** | : | $R_f$ 0.31 (Chloroform-Methanol 23:2); |
| **IR (KBr, $cm^{-1}$)** | : | 3383, 3307, 3212, 1558, 1021 and 755; |
| **$^1$H NMR (DMSO-$d_6$)** | : | $\delta$ 8.30 (d, 1H, Ar*H*), 7.63-7.67 (m, 1H, Ar*H*), 7.56 (d, 1H, Ar*H*), 7.39-7.43 (m, 1H, Ar*H*) and 6.90 (bs, 2H, -N$H_2$); |
| **MS (*m/z*)** | : | 186.09 $[M+H]^+$; |
| **RP-HPLC** | : | Purity: 98.7%, $t_R$ = 3.45 min (Mobile phase: acetonitrile: water 50:50 (0.01 % TFA)). |

### 5.1.1.6. Synthesis of *N*-(aminoalkyl)-5*H*-[1,2,4]triazino[5,6-b]indol-3-amine derivatives (179-202)

### 5.1.1.6.1. Synthesis of *N*-(bromoalkyl)phthalimides (128-130)

**Method C.** Phthalimide (1.0 g, 6.80 mmol), potassium carbonate (3.76 g, 27.2 mmol) and benzyltriethylammonium chloride (154 mg, 0.68 mmol) were suspended in acetone (50 mL). Dibromoalkane (27.2 mmol) was added to the suspension and the reaction mixture was stirred at room temperature for 24 hrs. The solvent was evaporated under vacuum and the residue was dissolved in water (50 mL) and dichloromethane (50 mL). The organic layer was separated, and the aqueous solution was further extracted with dichloromethane (50 mL × 2). The combined organic solution was dried over sodium sulfate, filtered, and concentrated. The crude product was purified by column chromatography to provide *N*-(bromoalkyl)phthalimides as colorless solids.[114]

### 5.1.1.6.1a. *N*-(Bromobutyl)phthalimide (128)

The title compound (**128**) was synthesized from phthalimide (**127**) (1.0 g, 6.80 mmol) and 1,4-dibromobutane (3.25 mL, 27.2 mmol) as per **Method C**. The title compound (**128**) was obtained as white colored solid (1.82 g, 95 %), m.p. 75-78 °C. (lit[114] m.p. 72-74 °C)

**Anal.:**

**TLC** : $R_f$ 0.44 (*n*-Hexane-Ethyl acetate 3:2);

**IR (KBr, cm$^{-1}$)** : 2988, 2862, 1768, 1712, 1610 and 717.

### 5.1.1.6.1b. *N*-(Bromopentyl)phthalimide (129)

The title compound (**129**) was synthesized from phthalimide (**127**) (1.0 g, 6.80 mmol) and 1,5-dibromopentane (3.71 mL, 27.2 mmol) as per **Method C**. The title compound (**129**) was obtained as white colored solid (1.93 g, 96 %), m.p. 61-63 °C (lit$^{171}$ m.p. 59-61 °C).

**Anal.:**

**TLC** : $R_f$ 0.45 (*n*-Hexane-Ethyl acetate 3:2);

**IR (KBr, cm$^{-1}$)** : 2932, 2862, 1769, 1710, 1613 and 717.

### 5.1.1.6.1c. *N*-(Bromohexyl)phthalimide (130)

The title compound (**130**) was synthesized from phthalimide (**127**) (1.0 g, 6.80 mmol) and 1,6-dibromohexane (4.18 mL, 27.2 mmol) as per **Method C**. The title compound (**130**) was obtained as white colored solid (2.0 g, 94 %), m.p. 57-59 °C (lit$^{172}$ m.p. 59-60 °C).

**Anal.:**

**TLC** : $R_f$ 0.44 (*n*-Hexane-Ethyl acetate 3:2);

**IR (KBr, cm$^{-1}$)** : 2983, 2927, 2860, 1764, 1704, 1610 and 720.

### 5.1.1.6.2. Synthesis of *N, N*-(disubstituted-amino)alkylamines (155-178)

To a solution of *N*-(bromoalkyl)phthalimide (4 mmol) in methanol (50 mL), secondary amines (4.8 mmol) and triethylamine (4.8 mmol) were added. The reaction mixture was refluxed overnight. After completion of the reaction, the solvent was removed under reduced pressure. The residue was dissolved in chloroform and washed with water and brine. The collected organic layer was dried over magnesium sulfate, filtered, and evaporated under reduced pressure to give yellowish brown oils which were used in next step without further purification.

The phthalimides thus obtained were dissolved in methanol, and hydrazine monohydrate (16 mmol) was added dropwise. The mixture was refluxed for about 4 hrs. The reaction mixture was cooled to room temperature, the insoluble phthalhydrazide was filtered off, and the filtrate was evaporated under reduced pressure. The obtained oil was dissolved in chloroform and filtered to remove some more phthalhydrazide (this procedure was repeated until complete disappearance of phthalhydrazide was observed). The filtrate was concentrated to give pure product as viscous yellowish oil which was used as such without further purification.[114]

### 5.1.1.6.3. Synthesis of desired *N*-(aminoalkyl)-5*H*-[1,2,4]triazino[5,6-*b*] indol-3-amine derivatives (179-202)

#### 5.1.1.6.3a. *N*-(4-(Dimethylamino)butyl)-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (179)

The title compound (**179**) was synthesized from 3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**70**) (0.5 g, 2.01 mmol) and *N,N*-dimethylbutane-1,4-diamine (**155**) (0.9 g, 8.06 mmol) as per **Method A**. The title compound (**179**) was obtained as light yellow solid (0.38 g, 67 %), m.p. 161-163 °C.

**Anal.:**

| | | |
|---|---|---|
| **TLC** | : | $R_f$ 0.23 (Chloroform-Methanol 19:1); |
| **IR (KBr, cm$^{-1}$)** | : | 3443, 3327, 3048, 2944, 1615, 1556, 1463, 1393 and 742; |
| **$^1$H NMR (DMSO-$d_6$)** | : | $\delta$ 11.75 (bs, 1H, N*H*), 8.07-8.09 (m, 1H, Ar*H*), 7.39-7.46 (m, 2H, Ar*H*), 7.23-7.27 (m, 1H, Ar*H*), 3.49-3.50 (m, 2H, -NHC$H_2$), 2.78-2.80 (m, 2H, -NC$H_2$), 2.56 (s, 6H, -NC$H_3$), 1.80-1.82 (m, 2H, -NHCH$_2$C$H_2$) and 1.68-1.75 (m, 2H, -C$H_2$); |
| **$^{13}$C NMR (DMSO-$d_6$)** | : | $\delta$ 155.9, 149.0, 139.6, 128.6, 122.1, 120.03, 119.6, 116.1, 112.4, 56.9, 46.1, 42.6, 26.3 and 26.2; |
| **MS (*m/z*)** | : | 285.1 $[M+H]^+$; |
| **RP-HPLC** | : | Purity: 98.1 %, $t_R$ = 3.45 min (Mobile phase: acetonitrile-water 30:70 (0.01 % TFA)). |

#### 5.1.1.6.3b. *N*-(4-(Diethylamino)butyl)-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (180)

The title compound (**180**) was synthesized from 3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**70**) (0.5 g, 2.01 mmol), and *N,N*-diethylbutane-

1,4-diamine (**156**) (1.16 g, 8.06 mmol) as per **Method A**. The title compound (**180**) was obtained as light yellow solid (0.43 g, 69 %), m.p. 190-192 °C.

**Anal.:**

**TLC** : $R_f$ 0.26 (Chloroform-Methanol 19:1);

**IR (KBr, cm$^{-1}$)** : 3442, 3336, 2966, 1616, 1580, 1391 and 748;

**$^1$H NMR (CDCl$_3$)** : $\delta$ 8.24 (d, 1H, Ar*H*), 7.44-7.48 (m, 1H, Ar*H*), 7.36 (d, 1H, Ar*H*), 7.29-7.33 (m, 1H, Ar*H*), 6.04 (bs, 1H, -N*H*), 3.50-3.55 (m, 2H, -NHC$H_2$), 2.42-2.51 (m, 6H, -NC$H_2$), 1.54-1.74 (m, 4H, -$CH_2CH_2$) and 1.04 (t, 6H, C$H_3$);

**MS (*m/z*)** : 313.3 [M+H]$^+$;

**RP-HPLC** : Purity: 97.4 %, $t_R$ = 3.43 min (Mobile phase: acetonitrile-water 30:70 (0.01 % TFA)).

### 5.1.1.6.3c. *N*-(4-(Dipropylamino)butyl)-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (181)

The title compound (**181**) was synthesized from 3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**70**) (0.5 g, 2.01 mmol), and *N,N*-dipropylbutane-1,4-diamine (**157**) (1.39 g, 8.06 mmol) as per **Method A**. The title compound (**181**) was obtained as light yellow solid (0.47 g, 69 %), m.p. 198-200 °C.

**Anal.:**

**TLC** : $R_f$ 0.13 (Chloroform-Methanol 19:1);

**IR (KBr, cm$^{-1}$)** : 3444, 3333, 3003, 2956, 1615, 1531, 1461, 1390 and 746;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 11.72 (bs, 1H, N*H*), 8.06 (d, 1H, Ar*H*), 7.36-7.44 (m, 2H, Ar*H*), 7.22-7.26 (m, 1H, Ar*H*), 3.36-3.38 (m, 2H, -NHC$H_2$), 2.38-2.41 (m, 2H, -NC$H_2$), 2.30-2.33 (m, 4H, -NC$H_2$), 1.62-1.66 (m, 2H, -NHCH$_2$C$H_2$), 1.48-1.51 (m, 2H, -C$H_2$), 1.35-1.44 (m, 4H, -C$H_2$CH$_3$) and 0.84 (t, 6H, -C$H_3$);

**$^{13}$C NMR (DMSO-$d_6$)** : $\delta$ 155.8, 149.1, 139.5, 128.5, 121.9, 119.9, 119.7, 116.2, 112.3, 56.0, 53.8, 41.1, 27.3, 24.8, 20.0 and 12.3;

**MS (*m/z*)** : 341.1 [M+H]$^+$;

**RP-HPLC** : Purity: 97.6 %, $t_R$ = 3.72 min (Mobile phase: acetonitrile-water 30:70 (0.01 % TFA)).

### 5.1.1.6.3d. *N*-(4-(Dibutylamino)butyl)-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (182)

The title compound (**182**) was synthesized from 3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**70**) (0.5 g, 2.01 mmol), and *N,N*-dibutylbutane-1,4-diamine (**158**) (1.61 g, 8.06 mmol) as per **Method A**. The title compound (**182**) was obtained as light yellow solid (0.48 g, 64 %), m.p. 180-182 °C.

**Anal.:**

**TLC** : $R_f$ 0.2 (Chloroform-Methanol 19:1);

**IR (KBr, cm$^{-1}$)** : 3442, 3222, 3003, 2955, 1614, 1523, 1460, 1388 and 743;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 8.25 (d, 1H, Ar*H*), 7.44-7.48 (m, 1H, Ar*H*), 7.38 (d, 1H, Ar*H*), 7.29-7.33 (m, 1H, Ar*H*), 5.89 (bs, 1H, N*H*), 3.50-3.53 (m, 2H, -NHC$H_2$), 2.50-2.54 (m, 2H, -NC$H_2$), 2.40-2.48 (m, 4H, -NC$H_2$), 1.66-1.73 (m, 2H, -NHCH$_2$C$H_2$), 1.56-1.63 (m, 2H, -NCH$_2$C$H_2$), 1.39-1.47 (m, 4H, -NCH$_2$C$H_2$), 1.23-1.32 (m, 4H, -C$H_2$CH$_3$) and 0.89 (t, 6H, -C$H_3$);

**$^{13}$C NMR (DMSO-$d_6$)** : $\delta$ 155.9, 149.1, 139.5, 128.4, 121.9, 119.9, 119.7, 116.2, 112.3, 53.8, 53.6, 41.1, 29.4, 27.3, 24.8, 20.5 and 14.3;

**MS (*m/z*)** : 369.2 $[M+H]^+$;

**RP-HPLC** : Purity: 96.2 %, $t_R$ = 3.51 min (Mobile phase: acetonitrile-water 30:70 (0.01 % TFA)).

### 5.1.1.6.3e. *N*-(4-(Pyrrolidin-1-yl)butyl)-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (183)

The title compound (**183**) was synthesized from 3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**70**) (0.5 g, 2.01 mmol), and 4-(pyrrolidin-1-yl) butan-1-amine (**159**) (1.15 g, 8.06 mmol) as per **Method A**. The title compound (**183**) was obtained as light yellow solid (0.44 g, 71 %), m.p. 220-222 °C.

**Anal.:**

**TLC** : $R_f$ 0.35 (Chloroform-Methanol-Triethylamine 19:1:1);

**IR (KBr, cm$^{-1}$)** : 3434, 3219, 3000, 2934, 1617, 1522, 1459, 1382 and 745;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 8.06 (d, 1H, Ar*H*), 7.36-7.44 (m, 2H, Ar*H*), 7.22-7.26 (m, 1H, Ar*H*), 3.34-3.41 (m, 2H, -NHC$H_2$), 2.43-2.47 (m, 6H, -NC$H_2$), 1.64-1.71 (m, 6H, -NCH$_2$C$H_2$) and 1.54-1.59 (m, 2H,C$H_2$);

**MS (*m/z*)** : 311.1 $[M+H]^+$;

**RP-HPLC** : Purity: 97.6 %, $t_R$ = 3.42 min (Mobile phase: acetonitrile-water 30:70 (0.01 % TFA)).

### 5.1.1.6.3f. *N*-(4-(Piperidin-1-yl)butyl)-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (184)

The title compound (**184**) was synthesized from 3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**70**) (0.5 g, 2.01 mmol), and 4-(piperidin-1-yl) butan-1-amine (**160**) (1.26 g, 8.06 mmol) as per **Method A**. The title compound (**184**) was obtained as light yellow solid (0.43 g, 66 %), m.p. 199-201 °C.

**Anal.:**

**TLC** : $R_f$0.13 (Chloroform-Methanol 19:1);

**IR (KBr, cm$^{-1}$)** : 3445, 3326, 3061, 2933, 1613, 1558, 1460, 1393 and 748;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 11.74 (bs, 1H, -N*H*), 8.06 (d, 1H, Ar*H*), 7.22-7.44 (m, 3H, Ar*H*), 3.40-3.41 (m, 2H, NHC$H_2$), 2.25-2.32 (m, 6H, -NC$H_2$), 1.59-1.66 (m, 2H, -NHCH$_2$C$H_2$), 1.49-1.58 (m, 6H, -NCH$_2$C$H_2$) and 1.39-1.40 (m, 2H, -C$H_2$);

**$^{13}$C NMR (DMSO-$d_6$)** : $\delta$ 155.78, 149.05, 139.54, 128.44, 121.9, 119.9, 119.7, 116.1, 112.3, 58.8, 54.5, 41.2, 27.4, 26.0, 24.7 and 24.4;

**MS (*m/z*)** : 325.1 $[M+H]^+$;

**RP-HPLC** : Purity: 98.2 %, $t_R$ = 3.43 min (Mobile phase: acetonitrile-water 30:70 (0.01 % TFA)).

### 5.1.1.6.3g. *N*-(4-Morpholinobutyl)-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (185)

The title compound (**185**) was synthesized from 3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**70**) (0.5 g, 2.01 mmol), and 4-morpholinobutan-1-amine (**161**) (1.27 g, 8.06 mmol) as per **Method A**. The title compound (**185**) was obtained as light yellow solid (0.42 g, 63 %), m.p. 191-193 °C.

**Anal.:**

**TLC** : $R_f$ 0.32 (Chloroform-Methanol-Triethylamine 19:1:1);

**IR (KBr, cm$^{-1}$)** : 3430, 3222, 3007, 2944, 1612, 1526, 1457, 1387 and 747;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 11.63 (bs, 1H, N*H*), 8.07 (d, 1H, Ar*H*), 7.36-7.44 (m, 2H, Ar*H*), 7.22-7.26 (m, 1H, Ar*H*), 3.62-3.64 (m, 4H, -OC$H_2$), 3.44-3.46 (m, 2H, -NHC$H_2$), 2.32-2.39 (m, 6H, -NC$H_2$), 1.62-1.71 (m, 2H, -C$H_2$) and 1.54-1.71 (m, 4H, -C$H_2$);

**$^{13}$C NMR (DMSO-$d_6$)** : $\delta$ 155.8, 149.1, 139.5, 128.5, 121.9, 119.9, 119.7, 116.2, 112.3, 66.7, 58.5, 53.8, 41.1, 27.2 and 24.0;

**MS (*m/z*)** : 327.3 $[M+H]^+$;

**RP-HPLC** : Purity: 97.2 %, $t_R$ = 3.49 min (Mobile phase: acetonitrile-water 30:70 (0.01 % TFA)).

### 5.1.1.6.3h. *N*-(4-(4-Methylpiperazin-1-yl)butyl)-5*H*-[1,2,4]triazino[5,6-*b*] indol-3-amine (186)

The title compound (**186**) was synthesized from 3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**70**) (0.5 g, 2.01 mmol), and 4-(4-methyl piperazin-1-yl)butan-1-amine (**162**) (1.38 g, 8.06 mmol) as per **Method A**. The title compound (**186**) was obtained as light yellow solid (0.42 g, 65 %), m.p. 175-177 °C.

**Anal.:**

| | | |
|---|---|---|
| **TLC** | : | $R_f$ 0.4 (Chloroform-Methanol-Triethylamine 19:1:1); |
| **IR (KBr, $cm^{-1}$)** | : | 3425, 3222, 3052, 2935, 1615, 1581, 1457, 1389 and 741; |
| **$^1$H NMR (DMSO-$d_6$)** | : | $\delta$ 11.68 (bs, 1H, -N*H*), 8.03 (d, 1H, Ar*H*), 7.32-7.41 (m, 2H, Ar*H*), 7.19-7.23 (m, 1H, Ar*H*), 3.38-3.39 (m, 2H, -NHC$H_2$), 2.27-2.31 (m, 10H, -NC$H_2$), 2.15 (s, 3H, -NC$H_3$), 1.59-1.64 (m, 2H, -NHCH$_2$C$H_2$) and 1.48-1.55 (m, 2H, -C$H_2$); |
| **$^{13}$C NMR (DMSO-$d_6$)** | : | $\delta$ 155.9, 149.1, 139.6, 128.5, 121.9, 119.9, 119.7, 116.2, 112.3, 58.0, 55.2, 53.2, 46.2, 41.2, 27.2 and 24.4; |
| **MS (*m/z*)** | : | 340.3 $[M+H]^+$; |
| **RP-HPLC** | : | Purity: 99.5 %, $t_R$ = 3.45 min (Mobile phase: acetonitrile-water 30:70 (0.01 % TFA)). |

### 5.1.1.6.3i. *N*-(5-(Dimethylamino)pentyl)-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (187)

The title compound (**187**) was synthesized from 3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**70**) (0.5 g, 2.01 mmol), and *N,N*-dimethylpentane-1,5-diamine (**163**) (1.05 g, 8.06 mmol) as per **Method A**. The title compound (**187**) was obtained as light yellow solid (0.37 g, 62 %), m.p. 198-200 °C.

**Anal.:**

| | | |
|---|---|---|
| **TLC** | : | $R_f$ 0.32 (Chloroform-Methanol-Triethylamine 19:1:1); |
| **IR (KBr, $cm^{-1}$)** | : | 3222, 3111, 3007, 2945, 1612, 1527, 1457, 1388 and 748; |
| **$^1$H NMR (DMSO-$d_6$)** | : | $\delta$ 8.10-8.05 (m, 1H, Ar*H*), 7.35-7.44 (m, 2H, Ar*H*), 7.21-7.26 (m, 1H, Ar*H*), 3.30-3.35 (m, 2H, -NHC$H_2$), 2.20 (t, 2H, -NC$H_2$), 2.14 (s, 6H, -NC$H_3$), 1.60-1.68 (m, 2H, -NHCH$_2$C$H_2$) and 1.34-1.46 (m, 4H, -C$H_2$); |

**$^{13}$C NMR (DMSO-$d_6$)** : *δ* 155.9, 149.0, 139.5, 128.4, 121.9, 119.9, 119.7, 116.2, 112.3, 59.6, 45.7, 41.2, 29.2, 27.4 and 24.9;

**MS (*m/z*)** : 299.3 [M+H]$^+$;

**RP-HPLC** : Purity: 97.8 %, t$_R$ = 3.25 min (Mobile phase: acetonitrile-water 30:70 (0.01 % TFA)).

### 5.1.1.6.3j. *N*-(5-(Diethylamino)pentyl)-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (188)

The title compound (**188**) was synthesized from 3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**70**) (0.5 g, 2.01 mmol), and *N,N*-diethylpentane-1,5-diamine (**164**) (1.28 g, 8.06 mmol) as per **Method A**. The title compound (**188**) was obtained as light yellow solid (0.46 g, 70 %), m.p. 191-193 °C.

**Anal.:**

**TLC** : R$_f$ 0.55 (Chloroform-Methanol-Triethylamine 19:1:1);

**IR (KBr, cm$^{-1}$)** : 3442, 3333, 3059, 2967, 1615, 1554, 1460, 1370 and 745;

**$^1$H NMR (DMSO-$d_6$)** : *δ* 11.62 (bs, 1H, N*H*), 8.03-8.06 (m, 1H, Ar*H*), 7.32-7.37 (m, 2H, Ar*H*), 7.19-7.23 (m, 1H, Ar*H*), 3.29-3.36 (m, 2H, -NHC$H_2$), 2.39-2.44 (m, 4H, -NC$H_2$), 2.34 (t, 2H, -NC$H_2$), 1.57-1.63 (m, 2H, -NHCH$_2$C$H_2$), 1.30-1.41 (m, 4H, -C$H_2$) and 0.92 (t, 6H, -C$H_3$);

**$^{13}$C NMR (DMSO-$d_6$)** : *δ* 155.9, 149.0, 139.5, 128.4, 121.9, 119.9, 119.7, 116.2, 112.3, 52.7, 46.7, 41.2, 29.3, 26.9, 24.9 and 12.2;

**MS (*m/z*)** : 327.1 [M+H]$^+$;

**RP-HPLC** : Purity: 98.4 %, t$_R$ = 4.18 min (Mobile phase: acetonitrile-water 30:70 (0.01 % TFA)).

### 5.1.1.6.3k. *N*-(5-(Dipropylamino)pentyl)-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (189)

The title compound (**189**) was synthesized from 3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**70**) (0.5 g, 2.01 mmol), and *N,N*-dipropylpentane-

1,5-diamine (**165**) (1.50 g, 8.06 mmol) as per **Method A**. The title compound (**189**) was obtained as light yellow solid (0.47 g, 66 %), m.p. 200-202 °C.

**Anal.:**

**TLC** : $R_f$ 0.29 (Chloroform-Methanol 19:1);

**IR (KBr, cm$^{-1}$)** : 3443, 3329, 3057, 2956, 1614, 1532, 1461, 1390 and 747;

**$^1$H NMR (DMSO-*d*$_6$)** : $\delta$ 8.25 (d, 1H, Ar*H*), 7.45-7.49 (m, 1H, Ar*H*), 7.37 (d, 1H, Ar*H*), 7.30-7.33 (m, 1H, Ar*H*), 5.54 (bs, 1H, -N*H*), 3.49-3.54 (m, 2H, -NHC*H*$_2$), 2.38-2.47 (m, 6H, -NC*H*$_2$), 1.66-1.71 (m, 2H,-NHCH$_2$C*H*$_2$), 1.39-1.55 (m, 8H, -C*H*$_2$) and 0.86 (t, 6H, -C*H*$_3$);

**$^{13}$C NMR (DMSO-*d*$_6$)** : $\delta$ 155.9, 149.1, 139.6, 128.4, 121.9, 119.9, 119.7, 116.2, 112.3, 56.1, 53.9, 41.1, 29.2, 27.1, 24.9, 20.4 and 12.2;

**MS (*m/z*)** : 355.1 $[M+H]^+$;

**RP-HPLC** : Purity: 99.2 %, $t_R$ = 5.0 min (Mobile phase: acetonitrile-water 30:70 (0.01 % TFA)).

### 5.1.1.6.3l. *N*-(5-(Dibutylamino)pentyl)-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (190)

The title compound (**190**) was synthesized from 3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**70**) (0.5 g, 2.01 mmol), and *N,N*-dibutylpentane-1,5-diamine (**166**) (1.73 g, 8.06 mmol) as per **Method A**. The title compound (**190**) was obtained as light yellow solid (0.56 g, 72 %), m.p. 184-186 °C.

**Anal.:**

**TLC** : $R_f$0.36 (Chloroform-Methanol 19:1);

**IR (KBr, cm$^{-1}$)** : 3444, 3332, 3059, 2931, 1614, 1535, 1460, 1390 and 746;

**$^1$H NMR (CDCl$_3$)** : $\delta$ 8.18 (d, 1H, Ar*H*), 7.37-7.41 (m, 1H, Ar*H*), 7.29-7.31 (m, 1H, Ar*H*), 7.22-7.25 (m, 1H, Ar*H*), 5.54 (bs, 1H, -N*H*), 3.36-3.40 (m, 2H,-NHC*H*$_2$), 2.36-2.42 (m, 6H, -NC*H*$_2$), 1.54-1.65 (m, 2H, -NHCH$_2$C*H*$_2$), 1.42-1.50 (m, 2H,

$-\mathrm{C}H_2$), 1.31-1.39 (m, 6H, $-\mathrm{NCH_2C}H_2$), 1.16-1.25 (m, 4H, $-\mathrm{C}H_2$) and 0.82 (t, 7.3 Hz, 6H, $-\mathrm{C}H_3$);

**$^{13}$C NMR (DMSO-*$d_6$*)** : *δ* 155.8, 149.0, 139.5, 128.4, 121.9, 119.9, 119.7, 116.1, 112.3, 53.9, 53.6, 41.2, 29.4, 29.2, 27.0, 24.9, 20.5 and 14.4;

**MS (*m/z*)** : 383.2 $[\mathrm{M + H}]^+$;

**RP-HPLC** : Purity: 98.8%, $t_R$ = 3.07 min (Mobile phase: acetonitrile-water 30:70 (0.01 % TFA)).

### 5.1.1.6.3m. *N*-(5-(Pyrrolidin-1-yl)pentyl)-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (191)

The title compound (**191**) was synthesized from 3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**70**) (0.5 g, 2.01 mmol), and 5-(pyrrolidin-1-yl) pentan-1-amine (**167**) (1.26 g, 8.06 mmol) as per **Method A**. The title compound (**191**) was obtained as a light yellow solid (0.48 g, 73 %), m.p. 191-193 °C.

**Anal.:**

**TLC** : R*f* 0.4 (Chloroform-Methanol-Triethylamine 19:1:1);

**IR (KBr, cm$^{-1}$)** : 3459, 3306, 3059, 2937, 1615, 1526, 1456, 1383 and 743;

**$^1$H NMR (DMSO-*$d_6$*)** : *δ* 11.66 (bs, 1H, -N*H*), 8.02-8.06 (m, 1H, Ar*H*), 7.32-7.40 (m, 2H, Ar*H*), 7.18-7.22 (m, 1H, Ar*H*), 3.37-3.38 (m, 2H, $-\mathrm{NHC}H_2$), 2.34-2.40 (m, 6H, $-\mathrm{NC}H_2$), 1.57-1.68 (m, 6H, $-\mathrm{NCH_2C}H_2$), 1.45-1.52 (m, 2H, $-\mathrm{C}H_2$) and 1.33-1.41 (m, 2H, $-\mathrm{C}H_2$);

**$^{13}$C NMR (DMSO-*$d_6$*)** : 155.8, 149.1, 139.5, 128.5, 121.9, 119.7, 119.6, 116.2, 112.2, 54.1, 53.2, 41.1, 29.3, 28.7, 25.0 and 23.5;

**MS (*m/z*)** : 325.4 $[\mathrm{M+H}]^+$;

**RP-HPLC** : Purity: 98.3 %, $t_R$ = 2.84 min (Mobile phase: acetonitrile-water 30:70 (0.01 % TFA)).

### 5.1.1.6.3n. *N*-(5-(Piperidin-1-yl)pentyl)-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (192)

The title compound (**192**) was synthesized from 3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**70**) (0.5 g, 2.01 mmol), and 5-(piperidin-1-yl) pentan-1-amine (**168**) (1.37 g, 8.06 mmol) as per **Method A**. The title compound (**192**) was obtained as yellow solid (0.43 g, 63 %), m.p. 196-198 °C.

**Anal.:**

**TLC** : $R_f$ 0.65 (Chloroform-Methanol-Triethylamine 19:1:1);

**IR (KBr, cm$^{-1}$)** : 3423, 3223, 3007, 2935, 1614, 1526, 1455, 1383 and 741;

**$^{1}$H NMR (DMSO-$d_6$)** : *δ* 11.58 (bs, 1H, -N*H*), 8.04 (d, 1H, Ar*H*), 7.39-7.43 (m, 1H, Ar*H*), 7.35 (d, 1H, Ar*H*), 7.21-7.25 (m, 1H, Ar*H*), 3.40-3.41 (m, 2H, -NHC$H_2$), 2.18-2.27 (m, 6H, -NC$H_2$), 1.59-1.64 (m, 2H, -NHCH$_2$C$H_2$), 1.41-1.49 (m, 6H, -NCH$_2$C$H_2$) and 1.34-1.37 (m, 4H, -C$H_2$);

**$^{13}$C NMR (DMSO-$d_6$)** : *δ* 155.9, 149.1, 139.6, 128.5, 121.9, 119.9, 119.7, 116.2, 112.3, 59.2, 54.6, 41.2, 29.3, 26.7, 26.1, 25.0 and 24.7;

**MS (*m/z*)** : 339.3 [M+H]$^{+}$;

**RP-HPLC** : Purity: 96.8 %, $t_R$ = 5.43 min (Mobile phase: acetonitrile-water 30:70 (0.01 % TFA)).

### 5.1.1.6.3o. *N*-(5-Morpholinopentyl)-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (193)

The title compound (**193**) was synthesized from 3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**70**) (0.5 g, 2.01 mmol), and 5-morpholinopentan-1-amine (**169**) (1.39 g, 8.06 mmol) as per **Method A**. The title compound (**193**) was obtained as yellow solid (0.43 g, 62 %), m.p. 199-201 °C.

**Anal.:**

**TLC** : $R_f$0.29 (Chloroform-Methanol 19:1);

**IR (KBr, cm$^{-1}$)** : 3426, 3222, 3050, 2925, 1613, 1524, 1455, 1384 and 749;

**$^{1}$H NMR (DMSO-$d_6$)** : $\delta$ 11.41 (bs, 1H, -N*H*), 8.03 (d, 1H, Ar*H*), 7.32-7.40 (m, 2H, Ar*H*), 7.18-7.22 (m, 1H, Ar*H*), 3.54-3.56 (m, 4H, -OC$H_2$), 3.37-3.39 (m, 2H, NHC$H_2$), 2.29-2.33 (m, 4H, -NC$H_2$), 2.23-2.27 (m, 2H, -NC$H_2$), 1.58-1.65 (m, 2H, -NHCH$_2$C$H_2$), 1.43-1.50 (m, 2H, -C$H_2$) and 1.32-1.40 (m, 2H, -C$H_2$);

**$^{13}$C NMR (DMSO-$d_6$)** : $\delta$ 155.8, 149.1, 139.5, 128.4, 122.0, 119.9, 119.7, 116.1, 112.3, 66.7, 58.8, 53.9, 41.1, 29.2, 26.2 and 24.9;

**MS (*m/z*)** : 341.1 [M+H]$^+$;

**RP-HPLC** : Purity: 98.5 %, $t_R$ = 3.20 min (Mobile phase: acetonitrile-water 30:70 (0.01 % TFA)).

### 5.1.1.6.3p. *N*-(5-(4-Methylpiperazin-1-yl)pentyl)-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (194)

The title compound (**194**) was synthesized from 3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**70**) (0.5 g, 2.01 mmol), and 5-(4-methylpiperazin-1-yl)pentan-1-amine (**170**) (1.49 g, 8.06 mmol) as per **Method A**. The title compound (**194**) was obtained as yellow solid (0.48 g, 68 %), m.p. 162-164 °C.

**Anal.:**

**TLC** : R$_f$ 0.5 (Chloroform-Methanol-Triethylamine 19:1:1);

**IR (KBr, cm$^{-1}$)** : 3439, 3234, 3061, 2931, 1614, 1530, 1460, 1389 and 745;

**$^{1}$H NMR (CDCl$_3$)** : $\delta$ 9.76 (bs, 1H, -N*H*), 8.25 (d, 1H, Ar*H*), 7.44-7.49 (m, 2H, Ar*H*), 7.31-7.37 (m, 1H, Ar*H*), 5.57 (bs, 1H, -N*H*), 3.47-3.53 (m, 2H, -NHC$H_2$), 2.42-2.60 (m, 6H, -NC$H_2$), 2.35-2.39 (m, 4H, -NC$H_2$), 2.29 (s, 3H, -NC$H_3$), 1.65-1.72 (m, 2H, -NHCH$_2$C$H_2$), 1.53-1.62 (m, 2H, -NCH$_2$C$H_2$) and 1.37-1.46 (m, 2H, -C$H_2$);

**$^{13}$C NMR (DMSO-$d_6$)** : $\delta$ 155.6, 149.1, 139.7, 128.5, 121.9, 119.9, 119.3, 116.2, 112.3, 58.4, 55.2, 53.2, 46.2, 41.1, 29.2, 26.6 and 24.9;

**MS (*m/z*)** : 354.3 $[M+H]^+$;

**RP-HPLC** : Purity: 98.4 %, $t_R$ = 3.13 min (Mobile phase: acetonitrile-water 30:70 (0.01 % TFA)).

### 5.1.1.6.3q. *N*-(6-(Dimethylamino)hexyl)-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (195)

The title compound (**195**) was synthesized from 3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**70**) (0.5 g, 2.01 mmol), and *N,N*-dimethylhexane-1,6-diamine (**171**) (1.16 g, 8.06 mmol) as per **Method A**. The title compound (**195**) was obtained as yellow solid (0.43 g, 69 %), m.p. 180-182 °C.

**Anal.:**

**TLC** : $R_f$ 0.6 (Chloroform-Methanol-Triethylamine 19:1:1);

**IR (KBr, cm$^{-1}$)** : 3321, 3223, 3058, 2937, 1615, 1523, 1462, 1386 and 744;

**$^{1}$H NMR (DMSO-$d_6$)** : $\delta$ 11.76 (bs, 1H, -N*H*), 8.06 (d, 1H, Ar*H*), 7.36-7.43 (m, 2H, Ar*H*), 7.25-7.27 (m, 1H, Ar*H*), 3.32-3.39 (m, 2H, -NHC$H_2$), 2.19 (t, 2H, -NC$H_2$), 2.12 (s, 6H, -NC$H_3$), 1.61-1.63 (m, 2H, -NHCH$_2$C$H_2$) and 1.32-1.44 (m, 6H, -C$H_2$);

**$^{13}$C NMR (DMSO-$d_6$)** : $\delta$ 155.7, 149.1, 139.5, 128.4, 121.9, 119.9, 119.7, 116.1, 112.3, 59.6, 45.6, 41.1, 29.3, 27.5, 27.1 and 26.9;

**MS (*m/z*)** : 313.1 $[M+H]^+$;

**RP-HPLC** : Purity: 99.8 %, $t_R$ = 3.52 min (Mobile phase: acetonitrile-water 30:70 (0.01 % TFA)).

### 5.1.1.6.3r. *N*-(6-(Diethylamino)hexyl)-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (196)

The title compound (**196**) was synthesized from 3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**70**) (0.5 g, 2.01 mmol), and *N,N*-diethylhexane-

1,6-diamine (**172**) (1.39 g, 8.06 mmol) as per **Method A**. The title compound (**196**) was obtained as yellow solid (0.49 g, 71 %), m.p. 170-172 °C.

**Anal.:**

**TLC** : R$_f$ 0.77 (Chloroform-Methanol-Triethylamine 19:1:1);

**IR (KBr, cm$^{-1}$)** : 3443, 3333, 3060, 2929, 1614, 1557, 1461, 1374 and 747;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 8.25 (d, 1H, Ar*H*), 7.35-7.47 (m, 3H, Ar*H*), 5.75 (bs, 1H, -N*H*), 3.43-3.50 (m, 2H, -NHC$H_2$), 2.59-2.65 (m, 4H, -NC$H_2$), 2.48-2.52 (m, 2H, NC$H_2$), 1.60-1.71 (m, 4H, -C$H_2$), 1.37-1.46 (m, 4H, -C$H_2$) and 1.06 (t, 6H, -C$H_3$);

**$^{13}$C NMR (DMSO-$d_6$)** : $\delta$ 155.9, 149.1, 139.6, 128.4, 121.9, 119.9, 119.7, 116.2, 112.3, 52.6, 46.7, 41.2, 29.3, 27.3, 27.2, 26.9 and 12.04;

**MS (*m/z*)** : 341.1 [M+H]$^+$;

**RP-HPLC** : Purity: 99.5 %, t$_R$ = 4.70 min (Mobile phase: acetonitrile-water 30:70 (0.01 % TFA)).

### 5.1.1.6.3s. *N*-(6-(Dipropylamino)hexyl)-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (197)

The title compound (**197**) was synthesized from 3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**70**) (0.5 g, 2.01 mmol) and *N,N*-dipropylhexane-1,6-diamine (**173**) (1.61 g, 8.06 mmol) as per **Method A**. The title compound (**197**) was obtained as yellow solid (0.48 g, 65 %), m.p. 195-197 °C.

**Anal.:**

**TLC** : R$_f$0.2 (Chloroform-Methanol 19:1);

**IR (KBr, cm$^{-1}$)** : 3445, 3332, 3060, 2929, 1614, 1533, 1461, 1374 and 747;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 11.67 (bs, 1H, -N*H*), 8.03 (d, 1H, Ar*H*), 7.38-7.40 (m, 1H, Ar*H*), 7.33-7.35 (m, 1H, Ar*H*), 7.20-7.24 (m, 1H, Ar*H*), 3.32 (m, 2H, -NHC$H_2$), 2.24-2.32 (m, 6H, -NC$H_2$), 1.58-1.62 (m, 2H, $-NHCH_2CH_2$), 1.29-1.39 (m, 10H, $-CH_2$) and 0.80 (t, 6H, $-CH_3$);

**$^{13}$C NMR (DMSO-$d_6$)** : $\delta$ 155.8, 149.1, 139.6, 128.5, 121.9, 119.9, 119.7, 116.2, 112.3, 56.1, 53.9, 41.1, 29.3, 27.2, 27.1, 26.9, 20.4 and 12.3;

**MS (*m/z*)** : 369.1 [M+H]$^+$;

**RP-HPLC** : Purity: 99.1 %, $t_R$ = 9.72 min (Mobile phase: acetonitrile-water 30:70 (0.01 % TFA)).

### 5.1.1.6.3t. *N*-(6-(Dibutylamino)hexyl)-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (198)

The title compound (**198**) was synthesized from 3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**70**) (0.5 g, 2.01 mmol) and *N,N*-dibutylhexane-1,6-diamine (**174**) (1.84 g, 8.06 mmol) as per **Method A**. The title compound (**198**) was obtained as yellow solid (0.54 g, 68 %), m.p. 192-194 °C.

**Anal.:**

**TLC** : $R_f$ 0.29 (Chloroform-Methanol 19:1);

**IR (KBr, cm$^{-1}$)** : 3445, 3322, 3058, 2955, 1615, 1533, 1461, 1391 and 747;

**$^{1}$H NMR (DMSO-$d_6$)** : $\delta$ 11.74 (bs, 1H, $-NH$), 8.07-8.09 (m, 1H, Ar$H$), 7.24-7.40 (m, 3H, Ar$H$), 3.40-3.42 (m, 2H, $-NHCH_2$), 2.33-2.54 (m, 6H, $-NCH_2$), 1.58-1.68 (m, 2H, $-NHCH_2CH_2$), 1.24-1.44 (m, 14H, $-CH_2$) and 0.87 (t, 6H, $-CH_3$);

**$^{13}$C NMR (DMSO-$d_6$)** : $\delta$ 155.8, 149.1, 139.6, 128.4, 121.9, 119.9, 119.7, 116.1, 112.3, 53.9, 53.6, 41.2, 29.4, 29.3, 27.2, 26.9, 26.0, 20.5 and 14.4;

**MS (*m/z*)** : 397.2 [M+H]$^+$;

**RP-HPLC** : Purity: 98.6 %, $t_R$ = 8.67 min (Mobile phase: acetonitrile-water 30:70 (0.01 % TFA)).

### 5.1.1.6.3u. *N*-(6-(Pyrrolidin-1-yl)hexyl)-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (199)

The title compound (**199**) was synthesized from 3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**70**) (0.5 g, 2.01 mmol) and 6-(pyrrolidin-1-yl) hexan-1-amine (**175**) (1.37 g, 8.06 mmol) as per **Method A**. The title compound (**199**) was obtained as yellow solid (0.51 g, 73 %), m.p. 177-179 °C.

**Anal.:**

**TLC** : $R_f$ 0.58 (Chloroform-Methanol-Triethylamine 19:1:1);

**IR (KBr, cm$^{-1}$)** : 3442, 3330, 3058, 2930, 1614, 1527, 1458, 1390 and 743;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 11.66 (bs, 1H, -N$H$), 8.01-8.05 (m, 1H, Ar$H$), 7.31-7.39 (m, 2H, Ar$H$), 7.17-7.21 (m, 1H, Ar$H$), 3.35-3.37 (m, 2H, -NHC$H_2$), 2.32-2.38 (m, 6H, -NC$H_2$), 1.57-1.66 (m, 6H, -NCH$_2$C$H_2$), 1.40-1.45 (m, 2H, -NHCH$_2$C$H_2$) and 1.33-1.41 (m, 4H, -C$H_2$);

**$^{13}$C NMR (DMSO-$d_6$)** : $\delta$ 155.8, 149.0, 139.6, 128.5, 121.9, 119.9, 119.7, 116.1, 112.3, 56.2, 54.1, 41.2, 29.3, 28.9, 27.4, 26.9 and 23.5;

**MS (*m/z*)** : 339.1 $[M+H]^+$;

**RP-HPLC** : Purity: 97.5 %, $t_R$ = 4.35 min (Mobile phase: acetonitrile-water 30:70 (0.01 % TFA)).

### 5.1.1.6.3v. *N*-(6-(Piperidin-1-yl)hexyl)-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (200)

The title compound (**200**) was synthesized from 3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**70**) (0.5 g, 2.01 mmol) and 6-(piperidin-1-yl) hexan-1-amine (**176**) (1.48 g, 8.06 mmol) as per **Method A**. The title compound (**200**) was obtained as yellow solid (0.49 g, 70 %), m.p. 178-180 °C.

**Anal.:**

**TLC** : $R_f$0.23 (Chloroform-Methanol 19:1);

**IR (KBr, cm$^{-1}$)** : 3445, 3322, 3058, 2955, 1615, 1534, 1461, 1391 and 747;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 11.68 (bs, 1H, -N$H$), 8.07 (d, 1H, Ar$H$), 7.35-7.44 (m, 2H, Ar$H$), 7.22-7.26 (m, 1H, Ar$H$), 3.32-3.42 (m, 2H, -NHC$H_2$), 2.21-2.31 (m, 6H, -NC$H_2$), 1.61-1.68 (m, 2H, -NHCH$_2$C$H_2$), 1.48-1.52 (m, 6H, -C$H_2$) and 1.34-1.37 (m, 6H, -C$H_2$);

**$^{13}$C NMR (DMSO-$d_6$)** : $\delta$ 155.8, 149.0, 139.5, 128.4, 121.9, 119.9, 119.7, 116.1, 112.3, 59.1, 54.6, 41.1, 29.3, 27.3, 26.9, 26.8, 26.1 and 24.7;

**MS (*m/z*)** : 353.1 [M+H]$^+$;

**RP-HPLC** : Purity: 97.1 %, $t_R$ = 4.66 min (Mobile phase: acetonitrile-water 30:70 (0.01 % TFA)).

### 5.1.1.6.3w. *N*-(6-Morpholinohexyl)-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (201)

The title compound (**201**) was synthesized from 3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**70**) (0.5 g, 2.01 mmol) and 6-morpholinohexan-1-amine (**177**) (1.50 g, 8.06 mmol) as per **Method A**. The title compound (**201**) was obtained as yellow solid (0.46 g, 65 %), m.p. 163-165 °C.

**Anal.:**

**TLC** : $R_f$ 0.323 (Chloroform-Methanol 19:1);

**IR (KBr, cm$^{-1}$)** : 3460, 3334, 3057, 2931, 1614, 1535, 1459, 1392 and 748;

**$^1$H NMR (CDCl$_3$)** : $\delta$ 9.20 (bs, 1H, -N$H$), 8.26 (d, 1H, Ar$H$), 7.46-7.50 (m, 1H, Ar$H$), 7.38-7.41 (m, 1H, Ar$H$), 7.30-7.34 (m, 1H, Ar$H$), 5.70 (bs, 1H, -N$H$), 3.72-3.74 (m, 4H, -OC$H_2$), 3.48-3.53 (m, 2H, -NHC$H_2$), 2.44-2.48 (m, 4H, -NC$H_2$), 2.33-2.36 (m, 2H, -NC$H_2$), 1.64-1.70 (m, 2H, -NHCH$_2$C$H_2$), 1.49-1.54 (m, 2H, -C$H_2$) and 1.36-1.45 (m, 4H, -C$H_2$);

**$^{13}$C NMR (DMSO-$d_6$)** : $\delta$ 155.9, 149.1, 139.6, 128.5, 121.9, 119.9, 119.7, 116.2, 112.3, 66.6, 58.7, 53.8, 41.1, 29.3, 27.1, 26.9 and 26.8;

**MS (*m/z*)** : 355.1 [M + H]$^+$;

**RP-HPLC** : Purity: 98.9 %, $t_R$ = 3.70 min (Mobile phase: acetonitrile-water 30:70 (0.01 % TFA)).

### 5.1.1.6.3x. *N*-(6-(4-Methylpiperazin-1-yl)hexyl)-5*H*-[1,2,4]triazino[5,6-*b*] indol-3-amine (202)

The title compound (**203**) was synthesized from 3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**70**) (0.5 g, 2.01 mmol) and 6-(4-methylpiperazin-1-yl)hexan-1-amine (**178**) (1.50 g, 8.06 mmol) as per **Method A**. The title compound (**202**) was obtained as yellow solid (0.50 g, 67 %), m.p. 160-162 °C.

**Anal.:**

| | | |
|---|---|---|
| **TLC** | **:** | $R_f$ 0.645 (Chloroform-Methanol-Triethylamine 19:1:1); |
| **IR (KBr, cm$^{-1}$)** | **:** | 3460, 3336, 3056, 2928, 1614, 1459, 1391 and 747; |
| **$^1$H NMR (DMSO-*d*$_6$)** | **:** | *δ* 11.71 (bs, 1H, -N*H*), 8.03 (d, 1H, Ar*H*), 7.33-7.41 (m, 2H, Ar*H*), 7.19-7.23 (m, 1H, Ar*H*), 3.34-3.36 (m, 2H, -NHC$H_2$), 2.28-2.32 (m, 6H, -NC$H_2$), 2.20-2.24 (m, 2H, -NC$H_2$), 2.13 (s, 3H, -NC$H_3$), 1.56-1.62 (m, 2H, -NHC$\mathrm{H}_2$C$H_2$) and 1.29-1.42 (m, 6H, -C$H_2$); |
| **$^{13}$C NMR (DMSO-*d*$_6$)** | **:** | *δ* 155.1, 149.1, 139.6, 128.5, 121.9, 119.9, 119.7, 116.4, 112.3, 58.3, 55.2, 53.2, 46.2, 41.1, 29.3, 27.2, 26.9 and 26.8; |
| **MS (*m/z*)** | **:** | 368.1 [M+H]$^+$; |
| **RP-HPLC** | **:** | Purity: 99.1 %, $t_R$ = 3.26 min (Mobile phase: acetonitrile-water 30:70 (0.01 % TFA)). |

### 5.1.1.7. 3- And 6-/7-/8-/9-disubstituted [1,2,4]triazino[5,6-*b*]indole derivatives (249-257, 261, 262)

### 5.1.1.7.1. Synthesis of substituted isatins (212-221)

Substituted isatins (**212-220**) were synthesized by two steps Sandmeyer isatin synthesis process (**Method D**).[148] In the first step, condensation of substituted anilines with chloral hydrate and hydroxylamine gives α-isonitroso-acetanilides. These α-isonitrosoacetanilides, in the second step, cyclized into substituted isatins by concentrated sulfuric acid.

**Method D:**

**Step 1:** In a round-bottomed flask, sodium sulfate (6 equiv.), and chloral hydrate (1 equiv.) were dissolved in water (100 mL). To it, substituted aniline (5.0 g, 1 equiv.) dissolved in concentrated HCl (40 mL) was added and stirred vigorously for 30 mins at room temperature. Hydroxylamine HCl (3 equiv.) solution in water (10 mL) was added to the reaction mixture. The reaction mixture was heated to 70 °C for 6-8 hrs. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was poured into the ice-cold water. The precipitates of $\alpha$-isonitrosoacetanilide so obtained was filtered, washed with water, dried and used in next step without further purification.

**Step 2:** To a round-bottomed flask charged with concentrated sulfuric acid (10 mL) and water (1 mL), $\alpha$-isonitrosoacetanilide (1.0 g) was added over a period of few minutes. The resulting deep red solution was heated to 80 °C for 4 hrs and then cooled to room temperature. The reaction mixture was added to a vigorously stirred mixture of ice water (100 mL) and ethyl acetate (50 mL). The organic phase was separated and the aqueous phase was extracted with ethyl acetate (50 mL × 2). The combined red organic phase was dried over anhydrous magnesium sulfate, filtered and evaporated under reduced pressure to obtain the titled compound.

### 5.1.1.7.1a. 5-Chloroisatin (212)

The title compound (**212**) was synthesized from 4-chloroaniline (5.0 g, 39.19 mmol) following **Method D**. Compound (**212**) was obtained as dark orange colored solid (4.41 g, 62 %), m.p. 255-257 °C (lit[173] m.p. 244-245 °C).

**Anal.:**

**TLC** : $R_f$ 0.45 (*n*-Hexane-Ethyl acetate 12:8);

**IR (KBr, cm$^{-1}$)** : 3094, 1751, 1704, 1616, 1310 and 846;

**MS (*m/z*)** : 182.02 $[M+H]^+$.

### 5.1.1.7.1b. 5,7-Dichloroisatin (213)

The title compound (**213**) was synthesized from 2,4-dichloroaniline (5.0 g, 30.86 mmol) following **Method D**. Compound (**213**) was obtained as reddish yellow colored solid (3.45 g, 52 %), m.p. 221-223 °C (lit[174] m.p. 228-230 °C).

**Anal.:**

**TLC** : $R_f$ 0.42 (*n*-Hexane-Ethyl acetate 12:8);

**IR (KBr, $cm^{-1}$)** : 3068, 1751, 1704, 1615, 1309, 846 and 747;

**MS (*m/z*)** : 217 $[M+H]^+$.

### 5.1.1.7.1c. 5-Bromoisatin (214)

The title compound (**214**) was synthesized from 4-bromoaniline (5.0 g, 29.07 mmol) following **Method D**. Compound (**214**) was obtained as orange colored solid (4.1 g, 62 %), m.p. 247-249 °C (lit[175] m.p. 248-250 °C).

**Anal.:**

**TLC** : $R_f$ 0.47 (*n*-Hexane-Ethyl acetate 12:8);

**IR (KBr, $cm^{-1}$)** : 3454, 3180, 3104, 1743, 1613, 1318 and 688;

**MS (*m/z*)** : 224 $[M-2]^+$.

### 5.1.1.7.1d. 7-Fluoroisatin (215)

The title compound (**215**) was synthesized from 2-fluoroaniline (5.0 g, 44.97 mmol) following **Method D**. Compound (**215**) was obtained as yellowish orange colored solid (3.86 g, 52 %), m.p. 192-194 °C (lit[176] m.p. 186-190 °C).

**Anal.:**

**TLC** : $R_f$ 0.39 (*n*-Hexane-Ethyl acetate 12:8);

**IR (KBr, $cm^{-1}$)** : 3169, 3031, 1736, 1638, 1260 and 1037;

**MS (*m/z*)** : 166 $[M+H]^+$.

### 5.1.1.7.1e. 5-Fluoroisatin (216)

The title compound (**216**) was synthesized from 4-fluoroaniline (5.0 g, 44.97 mmol) following **Method D**. Compound (**216**) was obtained as dark red colored solid (4.30 g, 58 %), m.p. 226-228 °C (lit[177] m.p. 223-225 °C).

**Anal.:**

**TLC** : $R_f$ 0.43 (*n*-Hexane-Ethyl acetate 12:8);

**IR (KBr, $cm^{-1}$)** : 3095, 1753, 1705, 1616 and 1309;

**MS (*m/z*)** : 166.04 $[M+H]^+$.

### 5.1.1.7.1f. 5-Methylisatin (217)

The title compound (**217**) was synthesized from 4-methylaniline (5.0 g, 46.66 mmol) following **Method D**. Compound (**217**) was obtained as reddish brown colored solid (4.89 g, 65 %), m.p. 184-187 °C (lit[175] m.p. 183-184 °C).

**Anal.:**

**TLC** : $R_f$ 0.47 (*n*-Hexane-Ethyl acetate 12:8);

**IR (KBr, $cm^{-1}$)** : 3288, 1749, 1704, 1625, 1301, 828 and 737;

**MS (*m/z*)** : 162.16 $[M+H]^+$.

### 5.1.1.7.1g. 4,6-Dimethylisatin (218)

The title compound (**218**) was synthesized from 3,5-dimethylaniline (5.0 g, 41.26 mmol) following **Method D**. Compound (**218**) was obtained as red colored solid (3.25 g, 45 %), m.p. 228-231 °C (lit[178] m.p. 239-241 °C).

**Anal.:**

**TLC** : $R_f$ 0.51 (*n*-Hexane-Ethyl acetate 12:8);

**IR (KBr, $cm^{-1}$)** : 3201, 1756, 1722, 1626, 1269 and 747;

**MS (*m/z*)** : 176.06 $[M+H]^+$.

### 5.1.1.7.1h. 4,7-Dimethylisatin (219)

The title compound (**219**)[179] was synthesized from 2,5-dimethylaniline (5.0 g, 41.26 mmol) following **Method D**. Compound (**219**) was obtained as red colored solid (3.04 g, 42 %), m.p. 190-192 °C.

**Anal.:**

**TLC** : $R_f$ 0.62 (*n*-Hexane-Ethyl acetate 12:8);

**IR (KBr, $cm^{-1}$)** : 3207, 3107, 1730, 1594, 1320, 957, 809 and 712;

**MS (*m/z*)** : 176.09 $[M+H]^+$.

### 5.1.1.7.1i. 5-Ethylisatin (220)

The title compound (**220**)[180] was synthesized from 4-ethylaniline (5.0 g, 41.26 mmol) following **Method D**. Compound (**220**) was obtained as red colored solid (3.90 g, 53 %), m.p. 131-133 °C.

**Anal.:**

**TLC** : $R_f$ 0.54 (*n*-Hexane-Ethyl acetate 12:8);

**IR (KBr, $cm^{-1}$)** : 3284, 1746, 1709, 1619, 1488, 1317 and 694;

**MS (*m/z*)** : 176.05 $[M+H]^+$.

### 5.1.1.7.1j. 5-(Piperidin-1-yl)isatin (221)

To a solution of piperidine (0.44 mL, 4.24 mmol) in DMF (10 mL), potassium carbonate (0.74 g, 5.31 mmol) and 5-bromoisatin (**214**) (1.0 g, 4.24 mmol) were added. The reaction mixture was allowed to stir at 60 °C for 6–8 hrs. After completion of the reaction, the reaction mixture was poured into crushed ice. The precipitated product so formed was filtered, washed with water and recrystallized to obtain red colored the title compound (**221**) (0.57 g, 56 %), m.p. 150-152 °C (lit[149] m.p. 154-156 °C).

**Anal.:**

**TLC** : $R_f$ 0.43 (*n*-Hexane-Ethyl acetate 12:8);

**IR (KBr, cm$^{-1}$)** : 3419, 2938, 1623, 1541, 1240, 825 and 748;

**MS (*m/z*)** : 231 $[M+H]^+$.

### 5.1.1.7.2. Synthesis of substituted 5*H*-[1,2,4]triazino[5,6-*b*]indol-3-thiol derivatives (222-230, 258)

### 5.1.1.7.2a. 8-Chloro-5*H*-[1,2,4]triazino[5,6-*b*]indole-3-thiol (222)

The title compound (**222**) was synthesized from 5-chloroisatin (**212**) (1.0 g, 5.51 mmol) and thiosemicarbazide (0.50 g, 5.51 mmol) as per the method described for compound (**68**). The crude product was triturated with hot DMF, filtered, and dried to yield yellow colored titled compound (**222**) (0.99 g, 76 %), m.p. >250 °C.

**Anal.:**

**TLC** : $R_f$ 0.513 (Chloroform-Methanol 19:1);

**IR (KBr, cm$^{-1}$)** : 3095, 2992, 1617, 1458, 1310, 1166 and 847;

**MS (*m/z*)** : 237 $[M]^+$.

### 5.1.1.7.2b. 6,8-Dichloro-5*H*-[1,2,4]triazino[5,6-*b*]indole-3-thiol (223)

The title compound (**223**) was synthesized from 5,7-dichloroisatin (**213**) (1.0 g, 4.63 mmol) and thiosemicarbazide (0.42 g, 4.63 mmol) as per the method described for compound (**68**). The crude product was triturated with hot DMF, filtered, and dried to yield light yellow colored titled compound (**223**) (0.85 g, 68 %), m.p. >250 °C.

**Anal.:**

**TLC** : $R_f$ 0.594 (Chloroform-Methanol 19:1);

**IR (KBr, cm$^{-1}$)** : 3289, 3061, 1605, 1433, 1318, 1146 and 841;
**MS (*m/z*)** : 271 $[M]^+$, 273 $[M+2]^+$.

### 5.1.1.7.2c. 8-Bromo-5*H*-[1,2,4]triazino[5,6-*b*]indole-3-thiol (224)

The title compound (**224**) was synthesized from 5-bromoisatin (**214**) (1.0 g, 4.42 mmol) and thiosemicarbazide (0.40 g, 4.42 mmol) as per the method described for compound (**68**). The crude product was triturated with hot DMF, filtered, and dried to yield yellow colored titled compound (**224**) (0.95 g, 76 %), m.p. >250 °C (lit[112] m.p. >250 °C).

**Anal.:**

**TLC** : $R_f$0.43 (Chloroform-Methanol 19:1);
**IR (KBr, cm$^{-1}$)** : 3359, 3091, 1600, 1449, 1313, 1167 and 813;
**MS (*m/z*)** : 281 $[M]^+$.

### 5.1.1.7.2d. 6-Fluoro-5*H*-[1,2,4]triazino[5,6-*b*]indole-3-thiol (225)

The title compound (**225**) was synthesized from 7-fluoroisatin (**215**) (1.0 g, 6.06 mmol) and thiosemicarbazide (0.55 g, 6.06 mmol) as per the method described for compound (**68**). The crude product was triturated with hot DMF, filtered, and dried to yield yellow colored titled compound (**225**) (0.92 g, 69 %), m.p. >250 °C.

**Anal.:**

**TLC** : $R_f$0.5 (Chloroform-Methanol 19:1);
**IR (KBr, cm$^{-1}$)** : 3424, 3063, 1621, 1480, 1320, 1141 and 1015;
**MS (*m/z*)** : 221 $[M+H]^+$.

### 5.1.1.7.2e. 8-Fluoro-5*H*-[1,2,4]triazino[5,6-*b*]indole-3-thiol (226)

The title compound (**226**) was synthesized from 5-fluoroisatin (**215**) (1.0 g, 6.06 mmol) and thiosemicarbazide (0.55 g, 6.06 mmol) as per the method described for compound (**68**). The crude product was triturated with hot DMF, filtered, and dried to yield yellow colored titled compound (**226**) (0.96 g, 72 %), m.p. >250 °C.

**Anal.:**

**TLC** : $R_f$0.54 (Chloroform-Methanol 19:1);
**IR (KBr, cm$^{-1}$)** : 3425, 3014, 1620, 1477, 1320, 1140 and 1015;
**MS (*m/z*)** : 221 $[M+H]^+$.

### 5.1.1.7.2f. 8-Methyl-5*H*-[1,2,4]triazino[5,6-*b*]indole-3-thiol (227)

The title compound (**227**) was synthesized from 5-methylisatin (**217**) (1.0 g, 6.2 mmol) and thiosemicarbazide (0.56 g, 6.2 mmol) as per the method described for compound (**68**). The crude product was triturated with hot DMF, filtered, and dried to yield yellow colored titled compound (**227**) (1.1 g, 82 %), m.p. >250 °C.

**Anal.:**

**TLC** : $R_f$ 0.53 (Chloroform-Methanol 19:1);

**IR (KBr, cm$^{-1}$)** : 3438, 3030, 2884, 1609, 1477, 1323, 1190 and 1142;

**MS (*m/z*)** : 217 $[M+H]^+$.

### 5.1.1.7.2g. 7,9-Dimethyl-5*H*-[1,2,4]triazino[5,6-*b*]indole-3-thiol (228)

The title compound (**228**) was synthesized from 4,6-dimethylisatin (**218**) (1.0 g, 6.2 mmol) and thiosemicarbazide (0.52 g, 5.71 mmol) as per the method described for compound (**68**). The crude product was triturated with hot DMF, filtered, and dried to yield yellow colored titled compound (**228**) (0.82 g, 63 %), m.p. >250 °C.

**Anal.:**

**TLC** : $R_f$ 0.48 (Chloroform-Methanol 19:1);

**IR (KBr, cm$^{-1}$)** : 3021, 2871, 1611, 1432, 1303 and 1163;

**MS (*m/z*)** : 231 $[M+H]^+$.

### 5.1.1.7.2h. 6,9-Dimethyl-5*H*-[1,2,4]triazino[5,6-*b*]indole-3-thiol (229)

The title compound (**229**) was synthesized from 4,7-dimethylisatin (**219**) (1.0 g, 6.2 mmol) and thiosemicarbazide (0.52 g, 5.71 mmol) as per the method described for compound (**68**). The crude product was triturated with hot DMF, filtered, and dried to yield yellow colored titled compound (**229**) (0.89 g, 68 %), m.p. >250 °C.

**Anal.:**

**TLC** : $R_f$ 0.525 (Chloroform-Methanol 19:1);

**IR (KBr, cm$^{-1}$)** : 3391, 3034, 2935, 1586, 1439, 1325 and 1149;

**MS (*m/z*)** : 231 $[M+H]^+$.

### 5.1.1.7.2i. 8-Ethyl-5*H*-[1,2,4]triazino[5,6-*b*]indole-3-thiol (230)

The title compound (**229**) was synthesized from 5-ethylisatin (**220**) (1.0 g, 5.71 mmol) and thiosemicarbazide (0.52 g, 5.71 mmol) as per the method described for compound (**68**). The crude product was triturated with hot DMF, filtered, and dried to yield yellow colored titled compound (**230**) (0.84 g, 68 %), m.p. >250 °C.

**Anal.:**

**TLC** : $R_f$ 0.4 (Chloroform-Methanol 19:1);

**IR (KBr, cm$^{-1}$)** : 3433, 3029, 2877, 1604, 1478, 1320, 1186, and 1141;

**MS (*m/z*)** : 231 $[M+H]^+$.

### 5.1.1.7.2j. 8-(Piperidin-1-yl)-5*H*-[1,2,4]triazino[5,6-*b*]indole-3-thiol (258)

The title compound (**258**) was synthesized from 5-(piperidin-1-yl)isatin (**221**) (1.0 g, 4.34 mmol) and thiosemicarbazide (0.40 g, 4.34 mmol) as per the method described for compound (**68**). The crude product was triturated with hot DMF, filtered, and dried to yield yellow colored titled compound (**258**) (0.83 g, 67 %), m.p. >250 °C.

**Anal.:**

**TLC** : $R_f$ 0.45 (Chloroform-Methanol 19:1);

**IR (KBr, cm$^{-1}$)** : 3361, 3089, 1599, 1449, 1312, 1239 and 1167;

**MS (*m/z*)** : 286 $[M+H]^+$.

### 5.1.1.7.3. Synthesis of substituted 3-(methylthio)-5*H*-[1,2,4]triazino [5,6-*b*] indole derivatives (231-239, 259)

### 5.1.1.7.3a. 8-Chloro-3-(methylthio)-5*H*-[1,2,4]triazino[5,6-*b*]indole (231)

The title compound (**231**) was synthesized from 8-chloro-5*H*-[1,2,4] triazino[5,6-*b*]indole-3-thiol (**222**) (1.0 g, 4.22 mmol) and methyl iodide (0.26 mL, 4.22 mmol) as per the method described for compound (**69**). Compound (**231**) was obtained as greenish yellow colored solid (0.82 g, 77 %), m.p. >250 °C.

**Anal.:**

**TLC** : $R_f$ 0.59 (Chloroform-Methanol 19:1);

**IR (KBr, cm$^{-1}$)** : 3446, 3048, 2933, 1606, 1454, 1186, 825 and 780;

**MS (*m/z*)** : 251 [M]$^+$, 253 [M+2]$^+$.

### 5.1.1.7.3b. 6,8-Dichloro-3-(methylthio)-5*H*-[1,2,4]triazino[5,6-*b*]indole (232)

The title compound (**232**) was synthesized from 6,8-dichloro-5*H*-[1,2,4] triazino[5,6-*b*]indole-3-thiol (**223**) (1.0 g, 4.22 mmol) and methyl iodide (0.23 mL, 3.69 mmol) as per the method described for compound (**69**). Compound (**232**) was obtained as greenish yellow colored solid (0.75 g, 71 %), m.p. >250 °C.

**Anal.:**

**TLC** : $R_f$ 0.62 (Chloroform-Methanol 19:1);

**IR (KBr, cm$^{-1}$)** : 3047, 2962, 2860, 1606, 1452, 1183, 820 and 775;

**MS (*m/z*)** : 285 [M]$^+$, 287 [M+2]$^+$.

### 5.1.1.7.3c. 8-Bromo-3-(methylthio)-5*H*-[1,2,4]triazino[5,6-*b*]indole (233)

The title compound (**233**) was synthesized from 8-bromo-5*H*-[1,2,4] triazino[5,6-*b*]indole-3-thiol (**224**) (1.0 g, 3.56 mmol) and methyl iodide (0.23 mL, 3.56 mmol) as per the method described for compound (**69**). Compound (**233**) was obtained as light yellow colored solid (0.77 g, 73 %), m.p. >250 °C.

**Anal.:**

**TLC** : $R_f$ 0.64 (Chloroform-Methanol 19:1);

**IR (KBr, cm$^{-1}$)** : 3047, 2961, 1607, 1316, 1183, 1091, 820 and 776;

**MS (*m/z*)** : 295 [M]$^+$, 297 [M+2]$^+$.

### 5.1.1.7.3d. 6-Fluoro-3-(methylthio)-5*H*-[1,2,4]triazino[5,6-*b*]indole (234)

The title compound (**234**) was synthesized from 6-fluoro-5*H*-[1,2,4] triazino[5,6-*b*]indole-3-thiol (**225**) (1.0 g, 3.56 mmol) and methyl iodide (0.28 mL, 4.54 mmol) as per the method described for compound (**69**). Compound (**234**) was obtained as light yellow colored solid (0.73 g, 68 %), m.p. >250 °C.

**Anal.:**

**TLC** : $R_f$ 0.513 (Chloroform-Methanol 19:1);
**IR (KBr, cm$^{-1}$)** : 3106, 3054, 2955, 1611, 1495, 1321 and 1155;
**MS (*m/z*)** : 235 $[M+H]^+$.

### 5.1.1.7.3e. 8-Fluoro-3-(methylthio)-5*H*-[1,2,4]triazino[5,6-*b*]indole (235)

The title compound (**235**) was synthesized from 8-fluoro-5*H*-[1,2,4] triazino[5,6-*b*]indole-3-thiol (**226**) (1.0 g, 3.56 mmol) and methyl iodide (0.28 mL, 4.54 mmol) as per the method described for compound (**69**). Compound (**235**) was obtained as light yellow colored solid (0.76 g, 72 %), m.p. >250 °C.

**Anal.:**

**TLC** : $R_f$ 0.54 (Chloroform-Methanol 19:1);
**IR (KBr, cm$^{-1}$)** : 3054, 2954, 1609, 1323, 1156 and 820;
**MS (*m/z*)** : 235 $[M+H]^+$.

### 5.1.1.7.3f. 8-Methyl-3-(methylthio)-5*H*-[1,2,4]triazino[5,6-*b*]indole (236)

The title compound (**236**) was synthesized from 8-methyl-5*H*-[1,2,4] triazino[5,6-*b*]indole-3-thiol (**227**) (1.0 g, 4.62 mmol) and methyl iodide (0.29 mL, 4.62 mmol) as per the method described for compound (**69**). Compound (**236**) was obtained as light yellow colored solid (0.70 g, 66 %), m.p. >250 °C.

**Anal.:**

**TLC** : $R_f$ 0.59 (Chloroform-Methanol 19:1);
**IR (KBr, cm$^{-1}$)** : 3061, 2976, 1603, 1471, 1319, 1208, 977 and 814;
**MS (*m/z*)** : 231 $[M+H]^+$.

### 5.1.1.7.3g. 7,9-Dimethyl-3-(methylthio)-5*H*-[1,2,4]triazino[5,6-*b*]indole (237)

The title compound (**237**) was synthesized from 7,9-dimethyl-5*H*-[1,2,4] triazino[5,6-*b*]indole-3-thiol (**227**) (1.0 g, 4.34 mmol) and methyl iodide (0.27 mL, 4.34 mmol) as per the method described for compound (**69**). Compound (**237**) was obtained as light yellow colored solid (0.77 g, 73 %), m.p. >250 °C.

**Anal.:**

**TLC** : $R_f$ 0.62 (Chloroform-Methanol 19:1);

**IR (KBr, $cm^{-1}$)** : 3049, 2966, 2919, 1589, 1426, 1313, 1179, 844 and 752;

**MS (*m/z*)** : 245 $[M+H]^+$.

### 5.1.1.7.3h. 6,9-Dimethyl-3-(methylthio)-5*H*-[1,2,4]triazino[5,6-*b*]indole (238)

The title compound (**238**) was synthesized from 6,9-dimethyl-5*H*-[1,2,4]triazino[5,6-*b*]indole-3-thiol (**229**) (1.0 g, 4.34 mmol) and methyl iodide (0.27 mL, 4.34 mmol) as per the method described for compound (**69**). Compound (**238**) was obtained as light yellow colored solid (0.79 g, 75 %), m.p. 212-214 °C.

**Anal.:**

**TLC** : $R_f$ 0.64 (Chloroform-Methanol 19:1);

**IR (KBr, $cm^{-1}$)** : 3093, 2965, 2920, 1588, 1334, 1180, 809 and 752;

**MS (*m/z*)** : 245 $[M+H]^+$.

### 5.1.1.7.3i. 8-Ethyl-3-(methylthio)-5*H*-[1,2,4]triazino[5,6-*b*]indole (239)

The title compound (**239**) was synthesized from 8-ethyl-5*H*-[1,2,4] triazino[5,6-*b*]indole-3-thiol (**230**) (1.0 g, 4.34 mmol) and methyl iodide (0.27 mL, 4.34 mmol) as per the method described for compound (**69**). Compound (**239**) was obtained as light yellow colored solid (0.73 g, 69 %), m.p. >250 °C.

**Anal.:**

**TLC** : $R_f$ 0.59 (Chloroform-Methanol 19:1);

**IR (KBr, $cm^{-1}$)** : 3062, 2961, 1599, 1480, 1318, 1204, 972, 806 and 739;

**MS (*m/z*)** : 245 $[M+H]^+$.

### 5.1.1.7.3j. 3-(Methylthio)-8-(piperidin-1-yl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (259)

The title compound (**259**) was synthesized from 8-(piperidin-1-yl)-5*H*-[1,2,4]triazino[5,6-*b*]indole-3-thiol (**258**) (1.0 g, 3.5 mmol) and methyl iodide

(0.22 mL, 3.5 mmol) as per the method described for compound (**69**). Compound (**259**) was obtained as light yellow colored solid (0.71 g, 67 %), m.p. >250 °C.

**Anal.:**

**TLC** : $R_f$ 0.58 (Chloroform-Methanol 19:1);

**IR (KBr, cm$^{-1}$)** : 3087, 2966, 1607, 1453, 1317, 1183, 1092, 976, 819 and 778;

**MS (*m/z*)** : 300 $[M+H]^+$.

### 5.1.1.7.4. Synthesis of substituted-3-(methylsulfonyl)-5*H*-[1,2,4]triazino [5,6-*b*]indole derivatives (240-248, 260)

The sulfone derivatives (**240-248, 260**) were synthesized by the oxidation of respective thiomethyl derivatives (**231-239, 259**) with *m*CPBA as per the method described for the compound (**70**). The obtained products were used in the next step without further purification.

### 5.1.1.7.5. Synthesis of $C_6$-$C_9$-substituted *N*-(aminoalkyl)-5*H*-[1,2,4]triazino [5,6-*b*]indol-3-amine derivatives (249-257, 261, 262)

### 5.1.1.7.5a. 8-Chloro-*N*-(6-(pyrrolidin-1-yl)hexyl)-5*H*-[1,2,4]triazino[5,6-*b*] indol-3-amine (249)

The title compound (**249**) was synthesized from 8-chloro-3-(methyl sulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**240**) (0.5 g, 1.77 mmol) and 6-(pyrrolidin-1-yl)hexan-1-amine (3.01 g, 17.7 mmol) as per **Method A**. The crude product was purified by flash chromatography to yield the titled compound (**249**) as yellow solid (0.46 g, 70 %), m.p. >250 °C.

**Anal.:**

**TLC** : $R_f$ 0.35 (Chloroform-Methanol-Triethylamine 14:0.5:0.5);

**IR (KBr, cm$^{-1}$)** : 3421, 3075, 2930, 1618, 1547, 1365, 1142, 1107 and 816;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 11.84 (bs, 1H, -N*H*), 8.02 (d, $J$ = 2.1 Hz, 1H, Ar*H*), 7.43 (dd, $J$ = 8.5, 2.2 Hz, 1H, Ar*H*), 7.36 (d, $J$ = 8.5 Hz, 1H, Ar*H*), 3.34-3.42 (m, 2H,

-NHC$H_2$), 2.42-2.54 (m, 6H, -NC$H_2$), 1.59-1.71 (m, 4H, -NCH$_2$C$H_2$), 1.52-1.57 (m, 2H, -NCH$_2$C$H_2$), 1.39-1.45 (m, 2H, -NHCH$_2$C$H_2$) and 1.25-1.34 (m, 4H, -C$H_2$);

**MS (*m/z*)** : 373 $[M]^+$, 375 $[M+2]^+$;

**RP-HPLC** : Purity: 96.7 %, $t_R$ = 4.08 min (Mobile phase: acetonitrile-water 30:70 (0.01 % TFA)).

### 5.1.1.7.5b. 6,8-Dichloro-*N*-(6-(pyrrolidin-1-yl)hexyl)-5*H*-[1,2,4]triazino [5,6-*b*]indol-3-amine (250)

The title compound (**250**) was synthesized from 6,8-dichloro-3-(methyl sulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**241**) (0.5 g, 1.58 mmol) and 6-(pyrrolidin-1-yl)hexan-1-amine (2.69 g, 15.8 mmol) as per **Method A**. The crude product was purified by flash chromatography to yield the titled compound (**250**) as yellow solid (0.44 g, 68 %), m.p. >250 °C.

**Anal.:**

**TLC** : R$_f$ 0.47 (Chloroform-Methanol-Triethylamine 14:0.5:0.5);

**IR (KBr, cm$^{-1}$)** : 3405, 3062, 2931, 1608, 1528, 1376, 1312, 1076, 834 and 724;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 7.43 (d, $J$ = 2.1 Hz, 1H, Ar$H$), 7.39 (d, $J$ = 2.1 Hz, 1H, Ar$H$), 3.35-3.45 (m, 2H, -NHC$H_2$), 2.36-2.51 (m, 6H, -NC$H_2$), 1.56-1.71 (m, 6H, -NCH$_2$C$H_2$), 1.42-1.49 (m, 2H, -NHCH$_2$C$H_2$) and 1.31-1.40 (m, 4H, -C$H_2$);

**MS (*m/z*)** : 407.31 $[M]^+$, 409.25 $[M+2]^+$;

**RP-HPLC** : Purity: 97.6 %, $t_R$ = 6.883 min (Mobile phase: acetonitrile-water 30:70 (0.01 % TFA)).

### 5.1.1.7.5c. 8-Bromo-*N*-(6-(pyrrolidin-1-yl)hexyl)-5*H*-[1,2,4]triazino[5,6-*b*] indol-3-amine (251)

The title compound (**251**) was synthesized from 8-bromo-3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**242**) (0.5 g, 1.53 mmol) and 6-(pyrrolidin-1-yl)hexan-1-amine (2.61 g, 15.3 mmol) as per **Method A**. The crude product was purified by flash chromatography to yield the titled compound (**251**) as yellow solid (0.41 g, 64 %), m.p. >250 °C.

**Anal.:**

**TLC** : R$_f$ 0.38 (Chloroform-Methanol-Triethylamine 14:0.5:0.5);

**IR (KBr, cm$^{-1}$)** : 3415, 3073, 2929, 1615, 1524, 1453, 1142, 1106 and 738;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 8.18 (d, $J$ = 2.0 Hz, 1H, Ar$H$), 7.58 (dd, $J$ = 8.5, 2.0 Hz, 1H, Ar$H$), 7.34 (d, $J$ = 8.5 Hz, 1H, Ar$H$), 3.35-3.40 (m, 2H, -NHC$H_2$), 2.30-2.38 (m, 6H, -NC$H_2$), 1.55-1.66 (m, 6H, -NCH$_2$C$H_2$) and 1.28-1.46 (m, 2H, -NHCH$_2$C$H_2$, 4H, -C$H_2$);

**MS (*m/z*)** : 417.31 [M]$^+$, 419.09 [M+2]$^+$;

**RP-HPLC** : Purity: 99.1 %, t$_R$ = 4.787 min (Mobile phase: acetonitrile-water 30:70 (0.01 % TFA)).

### 5.1.1.7.5d. 6-Fluoro-*N*-(6-(pyrrolidin-1-yl)hexyl)-5*H*-[1,2,4]triazino[5,6-*b*] indol-3-amine (252)

The title compound (**252**) was synthesized from 6-fluoro-3-(methyl sulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**243**) (0.5 g, 1.88 mmol) and 6-(pyrrolidin-1-yl)hexan-1-amine (3.20 g, 18.8 mmol) as per **Method A**. The crude product was purified by flash chromatography to yield the titled compound (**252**) as yellow solid (0.42 g, 61 %), m.p. 221-220 °C.

**Anal.:**

**TLC** : R$_f$ 0.35 (Chloroform-Methanol-Triethylamine 14:0.5:0.5);

**IR (KBr, cm$^{-1}$)** : 3235, 3108, 2935, 1584, 1526, 1361, 1128 and 1026;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 11.82 (bs, 1H, -N$H$), 7.82-7.87 (m, 1H, Ar$H$), 7.34-7.41 (m, 1H, Ar$H$), 7.25-7.33 (m, 1H, Ar$H$), 3.32-3.37 (m, 2H, -NHC$H_2$), 2.30-2.38 (m, 6H, -NC$H_2$), 1.55-1.67 (m, 6H,

-NCH$_2$C*H*$_2$) and 1.26-1.46 (m, 2H, -NHCH$_2$C*H*$_2$, 4H, -C*H*$_2$);

**MS (*m/z*)** : 356 [M]$^+$, 358 [M+2]$^+$;

**RP-HPLC** : Purity: 98.5 %, t$_R$ = 3.617 min (Mobile phase: acetonitrile-water 30:70 (0.01 % TFA)).

### 5.1.1.7.5e. 8-Fluoro-*N*-(6-(pyrrolidin-1-yl)hexyl)-5*H*-[1,2,4]triazino[5,6-*b*] indol-3-amine (253)

The title compound (**253**) was synthesized from 8-fluoro-3-(methyl sulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**244**) (0.5 g, 1.88 mmol) and 6-(pyrrolidin-1-yl)hexan-1-amine (3.20 g, 18.8 mmol) as per **Method A**. The crude product was purified by flash chromatography to yield the titled compound (**253**) as yellow solid (0.42 g, 63 %), m.p. 242-245 °C.

**Anal.:**

**TLC** : R$_f$ 0.45 (Chloroform-Methanol-Triethylamine 14:0.5:0.5);

**IR (KBr, cm$^{-1}$)** : 3233, 3106, 2935, 1528, 1360, 1293, 1128, 1025, 798 and 735;

**$^1$H NMR (DMSO-*d*$_6$)** : $\delta$ 11.87 (bs, 1H, -N*H*), 7.83-7.86 (m, 1H, Ar*H*), 7.36-7.39 (m, 1H, Ar*H*), 7.26-7.31 (m, 1H, Ar*H*), 3.34-3.36 (m, 2H, -NHC*H*$_2$), 2.28-2.41 (m, 6H, -NC*H*$_2$), 1.53-1.67 (m, 6H, -NCH$_2$C*H*$_2$) and 1.25-1.46 (m, 2H, -NHCH$_2$C*H*$_2$, 4H, -C*H*$_2$);

**MS (*m/z*)** : 356 [M]$^+$, 358 [M+2]$^+$;

**RP-HPLC** : Purity: 98.4 %, t$_R$ = 3.653 min (Mobile phase: acetonitrile-water 30:70 (0.01 % TFA)).

### 5.1.1.7.5f. 8-Methyl-*N*-(6-(pyrrolidin-1-yl)hexyl)-5*H*-[1,2,4]triazino[5,6-*b*] indol-3-amine (254)

The title compound (**254**) was synthesized from 8-methyl-3-(methyl sulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**245**) (0.5 g, 1.91 mmol) and 6-(pyrrolidin-1-yl)hexan-1-amine (3.25 g, 19.1 mmol) as per **Method A**. The crude product was purified by flash chromatography to yield the titled compound (**254**) as yellow solid (0.49 g, 73 %), m.p. 178-181 °C.

**Anal.:**

| | | |
|---|---|---|
| **TLC** | : | $R_f$ 0.5 (Chloroform-Methanol-Triethylamine 14:0.5:0.5); |
| **IR (KBr, cm$^{-1}$)** | : | 3225, 3104, 2931, 1613, 1523, 1282, 1209, 1100, 801 and 737; |
| **$^1$H NMR (DMSO-$d_6$)** | : | $\delta$ 11.61 (bs, 1H, -N*H*), 7.80-7.91 (m, 1H, Ar*H*), 7.15-7.36 (m, 2H, Ar*H*), 3.29-3.40 (m, 2H, -NHC$H_2$), 2.43 (s, 3H, ArC$H_3$), 2.30-2.41 (m, 6H, -NC$H_2$), 1.54-1.74 (m, 6H, -NCH$_2$C$H_2$) and 1.10-1.49 (m, 2H, -NHCH$_2$C$H_2$, 4H, -C$H_2$); |
| **MS (*m/z*)** | : | 352.93 [M]$^+$; |
| **RP-HPLC** | : | Purity: 98.7 %, $t_R$ = 3.813 min (Mobile phase: acetonitrile-water 30:70 (0.01 % TFA)). |

### 5.1.1.7.5g. 7,9-Dimethyl-*N*-(6-(pyrrolidin-1-yl)hexyl)-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (255)

The title compound (**255**) was synthesized from 7,9-dimethyl-3-(methyl sulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**246**) (0.5 g, 1.81 mmol) and 6-(pyrrolidin-1-yl)hexan-1-amine (3.08 g, 18.1 mmol) as per **Method A**. The crude product was purified by flash chromatography to yield the titled compound (**255**) as yellow solid (0.41 g, 62 %), m.p. 168-170 °C.

**Anal.:**

| | | |
|---|---|---|
| **TLC** | : | $R_f$ 0.53 (Chloroform-Methanol-Triethylamine 14:0.5:0.5); |
| **IR (KBr, cm$^{-1}$)** | : | 3372, 2931, 1619, 1312, 1136, 841 and 769; |
| **$^1$H NMR (DMSO-$d_6$)** | : | $\delta$ 11.72 (bs, 1H, -N*H*), 7.00 (s, 1H, Ar*H*), 6.90 (s, 1H, Ar*H*), 3.36-3.40 (m, 2H, -NHC$H_2$), 2.78 (s, 3H, ArC$H_3$), 2.37-2.49 (m, 6H, -NC$H_2$, 3H, ArC$H_3$), 1.55-1.71 (m, 6H, -NC$H_2$), 1.42-1.49 (m, 2H, -NHCH$_2$C$H_2$) and 1.30-1.40 (m, 4H, -C$H_2$); |
| **MS (*m/z*)** | : | 367 [M+H]$^+$; |

**RP-HPLC** : Purity: 98.9 %, $t_R$ = 4.060 min (Mobile phase: acetonitrile-water 30:70 (0.01 % TFA)).

### 5.1.1.7.5h. 6,9-Dimethyl-*N*-(6-(pyrrolidin-1-yl)hexyl)-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (256)

The title compound (**256**) was synthesized from 6,9-dimethyl-3-(methyl sulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**247**) (0.5 g, 1.81 mmol) and 6-(pyrrolidin-1-yl)hexan-1-amine (3.08 g, 18.1 mmol) as per **Method A**. The crude product was purified by flash chromatography to yield the titled compound (**256**) as yellow solid (0.46 g, 69 %), m.p. 154-156 °C.

**Anal.:**

**TLC** : $R_f$ 0.55 (Chloroform-Methanol-Triethylamine 14:0.5:0.5);

**IR (KBr, cm$^{-1}$)** : 3228, 3010, 2930, 1599, 1518, 1264, 1121, 1032, 799 and 751;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 11.44 (bs, 1H, -N*H*), 7.14 (d, *J* = 7.5 Hz, 1H, Ar*H*), 6.95 (d, *J* = 7.5 Hz, 1H, Ar*H*), 3.32-3.34 (m, 2H, -NHC$H_2$), 2.77 (s, 3H, ArC$H_3$), 2.41 (s, 3H, ArC$H_3$), 2.29-2.38 (m, 6H, -NC$H_2$), 1.56-1.66 (m, 6H, -NCH$_2$C$H_2$) and 1.26-1.46 (m, 2H, -NHCH$_2$C$H_2$, 4H, -C$H_2$);

**MS (*m/z*)** : 366.94 $[M]^+$;

**RP-HPLC** : Purity: 97.5 %, $t_R$ = 4.050 min (Mobile phase: acetonitrile-water 30:70 (0.01 % TFA)).

### 5.1.1.7.5i. 8-Ethyl-*N*-(6-(pyrrolidin-1-yl)hexyl)-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (257)

The title compound (**257**) was synthesized from 8-ethyl-3-(methyl sulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**248**) (0.5 g, 1.81 mmol) and 6-(pyrrolidin-1-yl)hexan-1-amine (3.08 g, 18.1 mmol) as per **Method A**. The crude product was purified by flash chromatography to yield the titled compound (**257**) as yellow solid (0.49 g, 74 %), m.p. 167-169 °C.

**Anal.:**

**TLC** : $R_f$ 0.5 (Chloroform-Methanol-Triethylamine 14:0.5:0.5);

**IR (KBr, cm$^{-1}$)** : 3226, 3012, 2933, 1613, 1525, 1379, 1100, 877 and 742;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 13.21 (bs, 1H, -N*H*), 8.31 (d, *J* = 2.1 Hz, 1H, Ar*H*), 7.71 (dd, *J* = 2.1 Hz, 8.4 Hz, 1H, Ar*H*), 7.64 (d, *J* = 8.4 Hz, 1H, Ar*H*), 3.36-3.37 (m, 2H, -NHC$H_2$), 2.82-2.87 (m, 2H, ArC$H_2$CH$_3$), 2.31–2.38 (m, 6H, -NC$H_2$), 1.54-1.66 (m, 6H, -NCH$_2$C$H_2$) and 1.28-1.44 (m, 2H, -NHCH$_2$C$H_2$, 4H, -C$H_2$, 3H, Ar-CH$_2$C$H_3$);

**MS (*m/z*)** : 367 [M+H]$^+$;

**RP-HPLC** : Purity: 97.0 %, $t_R$ = 4.383 min (Mobile phase: acetonitrile-water 30:70 (0.01 % TFA)).

### 5.1.1.7.5j. 8-(Piperidin-1-yl)-*N*-(6-(pyrrolidin-1-yl)hexyl)-5*H*-[1,2,4] triazino[5,6-*b*]indol-3-amine (261)

The title compound (**261**) was synthesized from (8-(piperidin-1-yl)-3-(methylsulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**260**) (0.5 g, 1.51 mmol) and 6-(pyrrolidin-1-yl)hexan-1-amine (2.57 g, 15.1 mmol) as per **Method A**. The crude product was purified by flash chromatography to yield the titled compound (**261**) as yellow solid (0.85 g, 67 %), m.p. 237-241 °C.

**Anal.:**

**TLC** : R$_f$ 0.66 (Chloroform-Methanol-Triethylamine 19:1:1);

**IR (KBr, cm$^{-1}$)** : 3416, 2930, 1617, 1538, 1363, 1143, 1107, 813 and 737;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 11.98 (bs, 1H, -N*H*), 8.18 (d, *J* = 2.1 Hz, 1H, Ar*H*), 7.58 (dd, *J* = 8.5, 2.1 Hz, 1H, Ar*H*), 7.34 (d, *J* = 2.1 Hz, 1H, Ar*H*), 3.10-3.53 (m, 2H, -NHC$H_2$, 4H, -NC$H_2$), 2.27-2.40 (m, 6H, -NC$H_2$), 1.50-1.66 (m, 6H, -NCH$_2$C$H_2$) and 1.25-1.48 (m, 2H, -NHCH$_2$C$H_2$, 4H, -C$H_2$, 6H, -NCH$_2$C$H_3$, 6H, -NCH$_2$C$H_2$C$H_2$);

**MS (*m/z*)** : 422 [M+H]$^+$;

**RP-HPLC** : Purity: 98.9 %, $t_R$ = 4.827 min (Mobile phase: acetonitrile-water 30:70 (0.01 % TFA)).

### 5.1.1.7.5k. 8-Bromo-*N*-methyl-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine (262)

The title compound (**262**) was synthesized from 8-bromo-3-(methyl sulfonyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (**242**) (0.5 g, 1.53 mmol) and methyl amine (2.0 M in THF, 7.72 mL, 15.3 mmol) as per **Method A**. The crude product was purified by flash chromatography to yield the titled compound (**262**) as yellow solid (0.26 g, 61 %), m.p. >250 °C.

**Anal.:**

**TLC** : $R_f$ 0.51 (Chloroform-Methanol 18:2);

**IR (KBr, cm$^{-1}$)** : 3426, 3076, 2972, 1632, 1552, 1193, 1048, 866 and 786;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 8.16 (d, $J$ = 2.0 Hz, 1H, Ar*H*), 7.56 (dd, $J$ = 8.5, 2.0 Hz, 1H, Ar*H*), 7.33 (d, $J$ = 8.5 Hz, 1H, Ar*H*) and 2.87 (s, 3H, -NHC$H_3$);

**MS (*m/z*)** : 278 $[M]^+$, 280 $[M+2]^+$.

### 5.1.2. Carbazole-based multifunctional anti-AD agent

### 5.1.2.1. Carbazole-based stilbene derivatives

Carbazole-based stilbene derivatives have been divided into two series i.e. Series-1 where the attachment was at carbazole A ring and Series-2 where the attachment was at phenyl B ring.

### 5.1.2.1.1. Synthesis of carbazole-based stilbene derivative (Series-1)

### 5.1.2.1.1.1. 9-Ethyl-9*H*-carbazole (267)

To a rapidly stirring solution of carbazole (**266**) (0.5 g, 2.99 mmol) in DMSO (10 mL), a few drops of aqueous sodium hydroxide (0.25 g) were added and stirred for 5 mins. To it ethyl iodide (0.3 mL, 3.58 mmol) was added slowly. After complete of addition the reaction mixture was stirred for further 4-5 hrs to complete the reaction. After completion of the reaction, the reaction mixture was poured in crushed ice, and solid precipitates so obtained was collected and washed with water to remove residual solvent and dried to obtain titled compound (**267**) (0.54 g, 94 %), m.p. 64-66 °C (lit[181] m.p. 67-69 °C).

**Anal.:**

| | | |
|---|---|---|
| **TLC** | : | $R_f$ 0.58 (*n*-Hexane-Ethyl acetate 19:1); |
| **IR (KBr, cm$^{-1}$)** | : | 3049, 2978, 2869, 1596, 1018, 753 and 700. |

### 5.1.2.1.1.2. 9-Ethyl-9*H*-carbazole-3-carbaldehyde (268)

Phosphorus oxychloride (0.47 mL, 5.12 mmol) was added, over a period of 10 mins to an ice cooled stirred solution of 9-ethyl-9*H*-carbazole (**267**) (1.0 g, 5.13 mmol) and dimethylformamide (0.38 mL, 5.12 mmol) in 10 mL of chloroform. The resulting reaction mixture was refluxed for overnight. The reaction mixture was then poured into crushed ice. After warming to RT, the resultant product was extracted into chloroform and organic phase was washed with water and brine, dried over magnesium sulphate and evaporated at reduced pressure. The obtained residue was purified by column chromatography on silica gel using petroleum ether-ethyl acetate (15%) to obtain the titled compound (**268**) (0.96 g, 88 %), m.p. 84-86 °C (lit[182] m.p. 84-86 °C).

**Anal.:**

| | | |
|---|---|---|
| **TLC** | : | $R_f$0.37 (*n*-Hexane-Ethyl acetate 4:1); |
| **IR (KBr, cm$^{-1}$)** | : | 2971, 2929, 2822, 2743, 1679, 1588 and 620; |
| **$^1$H NMR (DMSO-$d_6$)** | : | $\delta$ 10.12 (s, 1H, C*H*O), 8.64 (s, 1H, Ar*H*), 8.18 (d, $J$ = 8 Hz, 1H, Ar*H*), 8.04 (d, $J$ = 8 Hz, 1H, Ar*H*), 7.37-7.58 (m, 4H, Ar*H*), 4.43 (q, $J$ = 7.2 Hz, 2H, -$NCH_2CH_3$) and 1.49 (t, $J$ = 7.2 Hz, 3H, -$NCH_2CH_3$); |
| **MS (*m/z*)** | : | 224 $[M+H]^+$. |

### 5.1.2.1.1.3. 9-Ethyl-6-nitro-9*H*-carbazole-3-carbaldehyde (269)

Nitric acid (0.44 mL) was added drop-wise to a stirring solution of 9-ethyl-9*H*-carbazole-3-carbaldehyde (**268**) (1.0 g, 4.47 mmol) in acetic acid (10 mL) under cold conditions. After completion of addition, the reaction mixture was further stirred for additional 1 hr. The solid so precipitated was collected by filtration and washed with acetic acid (10 mL). Excess acetic acid was removed by washing with water. The solid so obtained was dried to obtain greenish colored compound (**269**) (1.1 g, 92 %), m.p. 241-243 °C (lit[183] m.p. 247-248 °C).

**Anal.:**

**TLC** : $R_f$ 0.53 (*n*-Hexane-Ethyl acetate 3:1);

**IR (KBr, cm$^{-1}$)** : 3081, 2967, 2870, 2822, 2728, 1685, 1591, 1021 and 752;

**$^1$H NMR (CDCl$_3$)** : $\delta$ 10.16 (s, 1H, -C*H*O), 9.09 (d, *J* = 2.4 Hz, 1H, Ar*H*), 8.69 (d, *J* = 1.2 Hz, 1H, Ar*H*), 8.47 (dd, *J* = 2.4 Hz, 9.2 Hz, 1H, Ar*H*), 8.15 (dd, *J* =1.6, 8.4 Hz, 1H, Ar*H*), 7.61 (d, *J* = 8.4 Hz, 1H, Ar*H*), 7.53 (d, *J* = 9.2 Hz, 1H, Ar*H*), 4.68 (q, *J* = 7.2 Hz, 2H, -NC$H_2$CH$_3$) and 1.54 (t, *J* = 7.2 Hz, 3H, -NCH$_2$C$H_3$);

**MS (*m/z*)** : 269 [M+H]$^+$.

### 5.1.2.1.1.4. (*E*)-9-Ethyl-3-nitro-6-styryl-9*H*-carbazole (270)

To a stirred solution of benzyltriphenylphosphonium bromide (2.4 g, 5.58 mmol) in isopropyl alcohol (25 mL), lithium hydroxide (0.18 g, 7.44 mmol) was added. The reaction mixture was stirred further for 30 mins at room temperature. To it 9-ethyl-6-nitro-9*H*-carbazole-3-carbaldehyde (**269**) (1.0 g, 3.72 mmol) was added in small portions over a period of a few minutes. The resulting reaction mixture was heated to 80 °C for 5-6 hrs. The progress of the reaction was monitored by TLC. After completion of the reaction, the solid so precipitated was collected by filtration and washed with isopropyl alcohol (15 mL). The obtained solid contained mixture of *cis* and *trans* isomers. This isomeric mixture was dissolved in benzene (50 mL) and catalytic amount of iodine was added to it. The reaction mixture was heated to 70 °C for 4-5 hrs. Conversion of all isomeric mixture to single *trans* isomer product was confirmed by TLC. After completion of the reaction, the organic phase was washed with aqueous sodium thiosulfate solution (10 %) to quench the remaining quantity of iodine. The organic layer was collected, washed with water and brine, dried over sodium sulfate, filtered and evaporated to give the titled compound as yellow solid (**270**) (0.99 g, 78.5%), m.p. 151-154 °C.

**Anal.:**

**TLC** : $R_f$ 0.4 (*n*-Hexane-Ethyl acetate 15:5);

**IR (KBr, cm$^{-1}$)** : 2986, 2939, 1592, 1482, 1315, 1088, 804 and 748;

**$^{1}$H NMR (CDCl$_3$)** : $\delta$ 9.00 (d, $J$ = 2 Hz, 1H, Ar*H*), 8.36-8.39 (dd, $J$ = 7.2 Hz, 2 Hz, 1H, Ar*H*), 8.23 (s, 1H, Ar*H*), 7.74-7.76 (m,1H, Ar*H*), 7.57-7.59 (m, 2H, Ar*H*), 7.16-7.45 (m, 5H, Ar*H,* 2H*,* vinylic-*H*), 4.40 (q, $J$ = 7.2 Hz, 2H, -NC$H_2$CH$_3$) and 1.48 (t, $J$ = 7.2 Hz, 3H, -NCH$_2$C$H_3$);

**MS (*m/z*)** : 343 [M+H]$^+$.

### 5.1.2.1.1.5. (*E*)-9-Ethyl-6-styryl-9*H*-carbazol-3-amine (271)

To a solution of (*E*)-9-ethyl-3-nitro-6-styryl-9*H*-carbazole (**270**) (1.0 g, 2.92 mmol) in 1:1 THF/MeOH mixture (50 mL), stannous chloride (1.10 g, 5.84 mmol) was added in small portions over a period of a few minutes. After completion of addition, the reaction mixture was refluxed for 6-7 hrs. The progress of reaction was monitored by TLC. After completion of reaction, *p*H of the mixture was adjusted to eight with NaOH solution (10 %), and then extracted with ethyl acetate (20 mL × 3). The collected organic layer was washed with water and brine, dried over magnesium sulfate, filtered and evaporated to give crude product. It was further purified by recrystallization with methanol to give yellowish green colored crystals of the titled compound (**271**) (0.7 g, 77 %), m.p. 132-134 °C.

**Anal.:**

**TLC** : R$_f$ 0.3 (*n*-Hexane-Ethyl acetate 13:7);

**IR (KBr, cm$^{-1}$)** : 3401, 3304, 3053, 3023, 2971, 2933, 1592, 1491, 797, 688;

**$^{1}$H NMR (CDCl$_3$)** : $\delta$ 8.15 (s, 1H, Ar*H*), 7.65-7.67 (m, 1H, Ar*H*), 7.57-7.59 (m, 2H, Ar*H*), 7.23-7.48 (m, 5H, Ar*H*, 2H, vinylic-*H*), 7.12-7.16 (m, 1H, Ar*H*), 6.94-6.96 (m, 1H, Ar*H*), 4.31-4.33 (m, 2H, -NC$H_2$CH$_3$), 1.42 (t, 3H, -NCH$_2$C$H_3$);

**MS (*m/z*)** : 313.5 [M+H]$^+$.

### 5.1.2.1.1.6. General method for the synthesis of (*E*)-*N*-(9-ethyl-6-styryl-9*H*-carbazol-3-yl)alkylamides (272-274)

**Method E:** To a stirring solution of 9-ethyl-6-styryl-9*H*-carbazole-3-amine (**271**) (1.0 g, 3.20 mmol) in dry acetone (25 mL), potassium carbonate (1.12 g, 7.98 mmol) was added. To it, respective acid chloride (4.8 mmol) was added dropwise under cold condition. After completion of addition, the reaction mixture was kept on stirring for additional 1 hr. The progress of the reaction was monitored by TLC. After completion of the starting material, the reaction mixture was poured in ice-cold water and extracted with ethyl acetate (20 mL × 3). The organic layer was washed with saturated sodium bicarbonate solution followed by water and brine, dried with sodium sulfate, and evaporated to give the title compound.

### 5.1.2.1.1.6a. (*E*)-3-Chloro-*N*-(9-ethyl-6-styryl-9*H*-carbazol-3-yl)propanamide (272)

The title compound (**272**) was synthesized from 9-ethyl-6-styryl-9*H*-carbazole-3-amine (**271**) (1.0 g, 3.20 mmol) and 3-chloropropionyl chloride (0.465 mL, 4.8 mmol) as per **Method E**. Compound (**272**) was obtained as brown colored solid (0.96 g, 74 %), m.p. 154-157 °C.

**Anal.:**

**TLC** : $R_f$ 0.36 (*n*-Hexane-Ethyl acetate 15:5);

**IR (KBr, cm$^{-1}$)** : 3297, 3024, 2972, 1652, 1595, 1554, 1231, 799 and 699;

**MS (*m/z*)** : 403.6 $[M]^+$, 405.6 $[M+2]^+$.

### 5.1.2.1.1.6b. (*E*)-4-Chloro-*N*-(9-ethyl-6-styryl-9*H*-carbazol-3-yl)butanamide (273)

The title compound (**273**) was synthesized from 9-ethyl-6-styryl-9*H*-carbazole-3-amine (**271**) (1 g, 3.20 mmol) and 4-chlorobutyryl chloride (0.53 mL, 4.8 mmol) as per **Method E**. Compound (**273**) was obtained as brown colored solid (0.99 g, 74%), m.p. 161-164 °C.

**Anal.:**

**TLC** : $R_f$ 0.38 (*n*-Hexane-Ethyl acetate 15:5);

**`IR (KBr, cm$^{-1}$)** : 3291, 3118, 2969, 1648, 1542, 1486, 1229, 956, 799 and 692;

**MS (*m/z*)** : 417.3 $[M]^+$, 419.3 $[M+2]^+$.

### 5.1.2.1.1.6c. (*E*)-5-Bromo-*N*-(9-ethyl-6-styryl-9*H*-carbazol-3-yl)pentanamide (274)

The title compound (**274**) was synthesized from 9-ethyl-6-styryl-9*H*-carbazole-3-amine (**271**) (1.0 g, 3.20 mmol) and 5-bromovaleryl chloride (0.96 g, 4.8 mmol) as per **Method E**. Compound (**274**) was obtained as brown colored solid (0.90 g, 72 %), m.p. 147-150 °C.

**Anal.:**

**TLC** : $R_f$ 0.45 (*n*-Hexane-Ethyl acetate 10:10);

**IR (KBr, cm$^{-1}$)** : 3288, 3025, 2967, 1647, 1593, 1540, 1486 and 793;

**MS (*m/z*)** : 476.4 $[M+H]^+$, 477.4 $[M+2]^+$.

### 5.1.2.1.1.7. General procedure for the synthesis of (*E*)-*N*-(9-ethyl-6-styryl-9*H*-carbazol-3-yl)aminoalkylamide (275-280)

**Method F:** Pyrrolidine/piperidine (5 equiv.) was added to a solution of (*E*)-*N*-(9-ethyl-6-styryl-9*H*-carbazol-3-yl)alkylamides (**272-274**) (0.5 g) in THF (10 mL). The reaction mixture was refluxed under nitrogen atmosphere till completion of the reaction as judged by TLC. The reaction mixture was then evaporated under reduced pressure and the residue was dissolved in 20 mL of water and extracted with ethyl acetate (20 mL × 3). The combined organic phase was then dried and evaporated to obtain the crude product, which was further purified by column chromatography using a mixture of chloroform and methanol as eluent to obtain off-white colored compound.

### 5.1.2.1.1.7a. (*E*)-*N*-(9-Ethyl-6-styryl-9*H*-carbazol-3-yl)-3-(pyrrolidin-1-yl) propanamide (275)

The title compound (**275**) was synthesized from 3-chloro-*N*-(9-ethyl-3-styryl-9*H*-carbazol-6-yl)propanamide (**272**) (0.5 g, 1.24 mmol) and pyrrolidine (0.5 mL, 6.20 mmol) as per **Method F**. Compound (**275**) was obtained as off-white colored solid (0.36 g, 67 %), m.p. 129-131 °C.

**Anal.:**

**TLC** : $R_f$ 0.33 (Chloroform-Methanol 19:1);

**IR (KBr, $cm^{-1}$)** : 3366, 3056, 3018, 2966, 1596, 1488, 1229, 963, 806 and 791;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 10.11 (s, 1H, -N*H*CO), 8.53 (d, 1H, Ar*H*), 8.31 (d, 1H, Ar*H*), 7.72-7.74 (dd, 1H, Ar*H*), 7.22-7.63 (m, 8H, Ar*H* and 2H, vinylic-*H*), 4.41 (q, $J$ = 7.2 Hz, 2H, -N$CH_2CH_3$), 2.76 (t, 2H, -NHCO$CH_2$), 2.45-2.54 (m, 6H, -N$CH_2CH_2$), 1.68-1.71 (m, 4H, -N$CH_2CH_2$) and 1.30 (t, $J$ = 7.2 Hz, 3H, -N$CH_2CH_3$);

**LCMS (*m/z*)** : 438.26 $[M+H]^+$; Purity: nearly 100 %.

### 5.1.2.1.1.7b. (*E*)-*N*-(9-Ethyl-6-styryl-9*H*-carbazol-3-yl)-3-(piperidin-1-yl) propanamide (276)

The title compound (**276**) was synthesized from 3-chloro-*N*-(9-ethyl-3-styryl-9*H*-carbazol-6-yl)propanamide (**272**) (0.5 g, 1.24 mmol) and piperidine (0.61 mL, 6.20 mmol) as per **Method F**. Compound (**276**) was obtained as off-white colored solid (0.38 g, 70 %), m.p. 148-151 °C.

**Anal.:**

**TLC** : $R_f$ 0.35 (Chloroform-Methanol 19:1);

**IR (KBr, $cm^{-1}$)** : 3309, 3023, 2967, 1690, 1587, 1520, 1478, 1233 and 749;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 10.20 (s, 1H, -N*H*CO), 8.53 (d, 1H, Ar*H*), 8.30 (d, 1H, Ar*H*), 7.73 (dd, 1H, Ar*H*), 7.45-7.63 (m, 3H, Ar*H*, 2H, vinylic-*H*), 7.36-7.41 (m, 3H, Ar*H*), 7.24-7.30 (m, 2H, Ar*H*), 4.40 (q, $J$ = 7.2 Hz, 2H, -N$CH_2CH_3$), 2.64 (t, 2H, -NHCO$CH_2$), 2.48-2.52 (m, 4H, -N$CH_2CH_2$), 2.35-2.42 (m, 2H, -N$CH_2CH_2$), 1.49-1.55 (m, 4H, -N$CH_2CH_2$), 1.39-1.40 (m, 2H, -$CH_2$) and 1.30 (t, $J$ = 7.2 Hz, 3H, -N$CH_2CH_3$);

**LCMS (*m/z*)** : 452.16 $[M+H]^+$; Purity: 98.58%.

### 5.1.2.1.1.7c. (*E*)-*N*-(9-Ethyl-6-styryl-9*H*-carbazol-3-yl)-4-(pyrrolidin-1-yl) butanamide (277)

The title compound (**277**) was synthesized from 4-chloro-*N*-(9-ethyl-3-styryl-9*H*-carbazol-6-yl)butanamide (**273**) (0.5 g, 1.20 mmol) and pyrrolidine (0.5 mL, 6.20 mmol) as per **Method F**. Compound (**277**) was obtained as off-white colored solid (0.34 g, 63 %), m.p. 127-130 °C.

**Anal.:**

**TLC** : R$_f$ 0.32 (Chloroform-Methanol 19:1);

**IR (KBr, cm$^{-1}$)** : 3292, 3118, 3027, 2961, 2876, 2795, 1651, 1596, 1532, 1486, 1305 and 797;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 9.94 (s, 1H, -N*H*CO), 8.53 (d, 1H, Ar*H*), 8.30 (s, 1H, Ar*H*), 7.72-7.74 (dd, 1H, Ar*H*), 7.24-7.63 (m, 8H, Ar*H,* 2H, vinylic-*H*), 4.41 (q, *J* = 7.2 Hz, 2H, -NC*H*$_2$CH$_3$), 2.37-2.46 (m, 6H, -NC*H*$_2$CH$_2$, 2H, -NHCOC*H*$_2$), 1.78-1.82 (m, 2H, -NCH$_2$C*H*$_2$), 1.66-1.69 (m, 4H, -NCH$_2$C*H*$_2$) and 1.30 (t, *J* = 7.2 Hz, 3H, -NCH$_2$C*H*$_3$);

**LCMS (*m/z*)** : 452.26 [M+H]$^+$; Purity: nearly 100 %.

### 5.1.2.1.1.7d. (*E*)-*N*-(9-Ethyl-3-styryl-9*H*-carbazol-6-yl)-4-(piperidin-1-yl) butanamide (278)

The title compound (**278**) was synthesized from 4-chloro-*N*-(9-ethyl-3-styryl-9*H*-carbazol-6-yl)butanamide (**273**) (0.5 g, 1.20 mmol) and piperidine (0.59 mL, 6.00 mmol) as per **Method F**. Compound (**278**) was obtained as off-white colored solid (0.38 g, 70 %), m.p. 114-118 °C.

**Anal.:**

**TLC** : R$_f$ 0.37 (Chloroform-Methanol 19:1);

**IR (KBr, cm$^{-1}$)** : 3286, 3117, 3025, 2928, 2770, 1644, 1594, 1536, 1486, 1152, 791 and 691;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 9.89 (s, 1H, -N*H*CO), 8.35 (d, 1H, Ar*H*) 8.15-8.18 (m, 1H, Ar*H*), 7.70 (dd, 1H, Ar*H*), 7.17-7.62 (m, 8H, Ar*H,* 2H, vinylic-*H*), 4.44 (q, *J* = 7.2 Hz, 2H, -NC*H*$_2$CH$_3$), 2.24-2.34 (m, 6H,

$\text{-NC}H_2\text{CH}_2$, 2H, $\text{-NHCOC}H_2$), 1.69-1.77 (m, 2H, $\text{-NCH}_2\text{C}H_2$), 1.44-1.50 (m, 4H, $\text{-NCH}_2\text{C}H_2$), 1.35-1.38 (m, 3H, $\text{-NCH}_2\text{CH}_2$ $\text{C}H_2$) and 1.32 (t, $J$ = 7.2 Hz, 3H, $\text{-NCH}_2\text{C}H_3$);

**LCMS (*m/z*)** : 466.4 $[M+H]^+$; Purity: nearly 100%.

### 5.1.2.1.1.7e. (*E*)-*N*-(9-Ethyl-6-styryl-9*H*-carbazol-3-yl)-5-(pyrrolidin-1-yl) pentanamide (279)

The title compound (**279**) was synthesized from 5-bromo-*N*-(9-ethyl-3-styryl-9*H*-carbazol-6-yl)pentanamide (**274**) (0.5 g, 1.05 mmol) and pyrrolidine (0.43 mL, 5.25 mmol) as per **Method F**. Compound (**279**) was obtained as off-white colored solid (0.34 g, 63 %), m.p. 161-164 °C.

**Anal.:**

**TLC** : $R_f$ 0.34 (Chloroform-Methanol 19:1);

**IR (KBr, cm$^{-1}$)** : 3289, 3026, 2962, 2933, 1648, 1596, 1484, 1082, 796 and 687;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 9.91 (s, 1H, -N*H*CO), 8.52 (d, 1H, Ar*H*), 8.30 (s, 1H, Ar*H*), 7.73 (dd, 1H, Ar*H*), 7.24-7.63 (m, 8H, Ar*H,* 2H, vinylic-*H*), 4.40 (q, $J$ = 7.2 Hz, 2H, $\text{-NC}H_2\text{CH}_3$), 2.34-2.42 (m, 6H, $\text{-NC}H_2$, 2H, $\text{-NHCOC}H_2$), 1.65-1.69 (m, 6H, $\text{-NCH}_2$ $\text{C}H_2$), 1.49-1.52 (m, 2H, $\text{-C}H_2$) and 1.30 (t, $J$ = 7.2 Hz, 3H, $\text{-NCH}_2\text{C}H_3$);

**LCMS (*m/z*)** : 466.26 $[M+H]^+$; Purity: nearly 100%.

### 5.1.2.1.1.7f. (*E*)-*N*-(9-Ethyl-6-styryl-9*H*-carbazol-3-yl)-5-(piperidin-1-yl) pentanamide (280)

The title compound (**280**) was synthesized from 5-bromo-*N*-(9-ethyl-3-styryl-9*H*-carbazol-6-yl)pentanamide (**274**) (0.5 g, 1.05 mmol) and piperidine (0.45 mL, 5.25 mmol) as per **Method F**. Compound (**280**) was obtained as off-white colored solid (0.36 g, 66 %), m.p. 159-161 °C.

**Anal.:**

**TLC** : $R_f$ 0.35 (Chloroform-Methanol 19:1);

**IR (KBr, cm$^{-1}$)** : 3290, 3025, 2929, 2856, 1647, 1540, 1487, 1228, 799 and 689;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 9.91 (s, 1H, -N*H*CO), 8.52 (d, 1H, Ar*H*), 8.30 (d, 1H, Ar*H*), 7.73 (dd, 1H, Ar*H*), 7.22-7.63 (m, 8H, Ar*H*, 2H, vinylic-*H*), 4.41 (q, *J* = 7.2 Hz, 2H, -NC$H_2$CH$_3$), 2.23-2.37 (m, 6H, -NC$H_2$CH$_2$, 2H, -NHCOC$H_2$), 1.61-1.65 (m, 2H, -NHCOCH$_2$C$H_2$), 1.45-1.49 (m, 6H, -NCH$_2$C$H_2$), 1.35-1.37 (m, 2H, -NCH$_2$CH$_2$ C$H_2$) and 1.30 (t, *J* = 7.2 Hz, 3H, -NCH$_2$C$H_3$);

**LCMS (*m/z*)** : 480.17 $[M+H]^+$; Purity: 98.79%.

### 5.1.2.1.1.8. General method E for the synthesis of (*E*)-1-(9-ethyl-6-styryl-9*H*-carbazol-3-yl)aminoalkylurea (281-286)

**Method G:** To a stirring solution of (*E*)-9-ethyl-6-styryl-9*H*-carbazol-3-amine (**271**) (1 g, 3.20 mmol) and triethylamine (0.5 mL, 3.52 mmol) in dry THF (20 mL), 4-nitrophenyl chloroformate (710 mg, 3.52 mmol) in dry THF (10 mL) was added dropwise at 0-5 °C over a period of 15 min. The resulting reaction mixture was allowed to stir at room temperature for 2 hrs. The progress of the reaction was monitored by TLC. After complete consumption of the starting material, respective amine (4.0 mmol) was added to the reaction mixture and the reaction was further stirred for 30 min. After the completion of the reaction, solvent was recovered at reduced pressure. The residues so obtained was triturated with chilled methanol (20 mL), filtered and dried to obtain the title compound.

### 5.1.2.1.1.8a. (*E*)-*N*-(9-Ethyl-6-styryl-9*H*-carbazol-3-yl)pyrrolidine-1-carboxamide (281)

The title compound (**281**) was synthesized from (*E*)-9-ethyl-6-styryl-9*H*-carbazol-3-amine (**271**) (1.0 g, 3.20 mmol) and pyrrolidine (0.33 mL, 4.00 mmol) as per **Method G**. Compound (**281**) was obtained as off-white colored solid (1.02 g, 78 %), m.p. 193-195 °C.

**Anal.:**

**TLC** : R$_f$ 0.48 (Chloroform-Methanol 15:0.5);

**IR (KBr, cm$^{-1}$)** : 3313, 3058, 3023, 2960, 2868, 1635, 1551, 1481, 1151, 956, 801 and 691;

**$^1$H NMR (DMSO-$d_6$)** : δ 8.29-8.27 (m, 2H, Ar*H*), 8.14 (s, 1H, -N*H*CO), 7.70-7.72 (m, 1H, Ar*H*), 7.61-7.63 (m, 2H, Ar*H*), 7.55-7.57 (m, 1H, Ar*H*), 7.21-7.52 (m, 5H, Ar*H*, 2H, vinylic-*H*), 4.40 (q, *J* = 7.2 Hz, 2H, $-NCH_2CH_3$), 3.39-3.43 (m, 4H, $-NCH_2CH_2$), 1.86-1.90 (m, 4H, $-NCH_2CH_2$) and 1.31 (t, *J* = 7.2 Hz, 3H, $-NCH_2CH_3$);

**LCMS (*m/z*)** : 410.30 $[M+H]^+$; Purity: nearly 100%.

### 5.1.2.1.1.8b. (*E*)-*N*-(9-Ethyl-6-styryl-9*H*-carbazol-3-yl)piperidine-1-carboxamide (282)

The title compound (**282**) was synthesized from (*E*)-9-ethyl-6-styryl-9*H*-carbazol-3-amine (**271**) (1.0 g, 3.20 mmol) and piperidine (0.40 mL, 4.00 mmol) as per **Method G**. Compound (**282**) was obtained as off-white colored solid (1.08 g, 82%), m.p. 217-219°C.

**Anal.:**

**TLC** : $R_f$ 0.6 (Chloroform-Methanol 15:0.5);

**IR (KBr, cm$^{-1}$)** : 3346, 3026, 2929, 2847, 1627, 1532, 1486, 1232, 1140, 860 and 750;

**$^1$H NMR (DMSO-$d_6$)** : δ 8.47 (s, 1H, -N*H*CO), 8.26-8.29 (m, 2H, Ar*H*), 7.69-7.72 (m, 1H, Ar*H*), 7.61-7.63 (m, 2H, Ar*H*), 7.55-7.57 (m, 1H, Ar*H*), 7.22-7.49 (m, 5H, Ar*H*, 2H, vinylic-*H*), 4.40 (q, *J* = 7.2 Hz, 2H, $-NCH_2CH_3$), 3.45-3.48 (m, 4H, $-NCH_2CH_2$), 1.52-1.60 (m, 6H, $-NCH_2 CH_2CH_2$) and 1.30 (t, *J* = 7.2 Hz, 3H, $-NCH_2CH_3$);

**LCMS (*m/z*)** : 424.25 $[M+H]^+$; Purity: nearly 100%.

### 5.1.2.1.1.8c. (*E*)-1-(9-Ethyl-3-styryl-9*H*-carbazol-6-yl)-3-(2-(pyrrolidin-1-yl)ethyl)urea (283)

The title compound (**283**) was synthesized from (*E*)-9-ethyl-6-styryl-9*H*-carbazol-3-amine (**271**) (1.0 g, 3.20 mmol) and 2-(aminoethyl)pyrrolidine (0.40 mL, 4.00 mmol) as per **Method G**. Compound (**283**) was obtained as off-white colored solid (1.03 g, 75 %), m.p. 176-178 °C.

**Anal.:**

**TLC** : $R_f$ 0.35 (Chloroform-Methanol-Triethylamine 19:1:1);

**IR (KBr, cm$^{-1}$)** : 3322, 3022, 2961, 2872, 1630, 1562, 1486, 1132, 952, 799 and 691;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 10.23 (s, 1H, -N*H*CO), 8.52 (s, 1H, -N*H*CO) 8.31 (s, 1H, Ar*H*), 7.24-7.57 (m, 10H, Ar*H*, 2H, vinylic-*H*), 4.41 (q, *J* = 7.2 Hz, 2H, -NC$H_2$CH$_3$), 2.62-2.67 (m, 2H, -NHC$H_2$), 2.35-2.45 (m, 6H, -NC$H_2$) and 1.31 (t, *J* = 7.2 Hz, 3H, -NCH$_2$C$H_3$);

**LCMS (*m/z*)** : 453.3 [M+H]$^+$; Purity: nearly 100 %.

### 5.1.2.1.1.8d. (*E*)-1-(9-Ethyl-6-styryl-9*H*-carbazol-3-yl)-3-(2-(piperidin-1-yl)ethyl)urea (284)

The title compound (**284**) was synthesized from (*E*)-9-ethyl-6-styryl-9*H*-carbazol-3-amine (**271**) (1.0 g, 3.20 mmol) and 2-(aminoethyl)piperidine (0.57 mL, 4.00 mmol) as per **Method G**. Compound (**284**) was obtained as off-white colored solid (1.06 g, 71 %), m.p. 187-189 °C.

**Anal.:**

**TLC** : $R_f$ 0.5 (Chloroform-Methanol-Triethylamine 19:1:0.5);

**IR (KBr, cm$^{-1}$)** : 3325, 3024, 2932, 2777, 1635, 1561, 1484, 1137, 952, 796 and 748;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 8.61 (s, 1H, -N*H*CO), 8.29 (s, 1H, Ar*H*), 7.21-7.71 (m, 9H, Ar*H*, 2H, vinylic-*H*), 6.01 (bs, 1H, -N*H*CO), 4.39 (q, *J* = 7.2 Hz, 2H, -NC$H_2$), 3.22-3.24 (m, 2H, -NHC$H_2$), 2.35-2.39 (m, 6H, -NC$H_2$), 1.50-1.52 (m, 4H, -NCH$_2$C$H_2$), 1.40-1.39 (m, 2H, -NCH$_2$CH$_2$C$H_2$) and 1.29 (t, *J* = 7.2 Hz, 3H, -NCH$_2$C$H_3$);

**LCMS (*m/z*)** : 467.26 [M+H]$^+$; Purity: 99.59 %.

### 5.1.2.1.1.8e. (*E*)-1-(9-Ethyl-3-styryl-9*H*-carbazol-6-yl)-3-(3-(pyrrolidin-1-yl)propyl) urea (285)

The title compound (**285**) was synthesized from (*E*)-9-ethyl-6-styryl-9*H*-carbazol-3-amine (**271**) (1.0 g, 3.20 mmol) and 3-(aminopropyl)pyrrolidine (0.51 g, 4.00 mmol) as per **Method G**. Compound (**285**) was obtained as off-white colored solid (1.1 g, 75 %), m.p. 164-166 °C.

**Anal.:**

**TLC** : $R_f$ 0.55 (Chloroform-Methanol-Triethylamine 19:1:1);

**IR (KBr, cm$^{-1}$)** : 3304, 3024, 2962, 2873, 1628, 1590, 1486, 1150, 957, 800 and 692;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 8.43 (s, 1H, -N*H*CO), 8.26-8.28 (m, 2H, Ar*H*), 7.21-7.71 (m, 9H, Ar*H*, 2H, vinylic-*H*), 6.18 (t, 1H, -N*H*CO), 4.39 (q, *J* = 7.2 Hz, 2H, -NC$H_2$), 3.14-3.19 (m, 2H, -NHC$H_2$), 2.52-2.55 (m, 6H, -NC$H_2$), 1.62-1.74 (m, 6H, -NCH$_2$C$H_2$) and 1.29 (t, *J* = 7.2 Hz, 3H, -NCH$_2$C$H_3$);

**LCMS (*m/z*)** : 467.4 [M+H]$^+$; Purity: nearly 100 %.

### 5.1.2.1.1.8f. (*E*)-1-(9-Ethyl-3-styryl-9*H*-carbazol-6-yl)-3-(3-(piperidin-1-yl)propyl) urea (286)

The title compound (**286**) was synthesized from (*E*)-9-ethyl-6-styryl-9*H*-carbazol-3-amine (**271**) (1.0 g, 3.20 mmol) and 3-(aminopropyl)piperidine (0.57 g, 4.00 mmol) as per **Method G**. Compound (**286**) was obtained as off-white colored solid (1.2 g, 79 %), m.p. 168-170 °C.

**Anal.:**

**TLC** : $R_f$ 0.6 (Chloroform-Methanol-Triethylamine 18:1:1);

**IR (KBr, cm$^{-1}$)** : 3289, 3024, 2927, 1645, 1595, 1485, 1285, 1083, 951, 798 and 688;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 8.36 (s, 1H, -N*H*CO), 8.26-8.29 (m, 2H, Ar*H*), 7.21-7.71 (m, 9H, Ar*H*, 2H, vinylic-*H*), 6.01 (t, 1H, -N*H*CO), 4.39 (q, *J* = 7.2 Hz, 2H,

-NC$H_2$CH$_3$), 3.11-3.16 (m, 2H, -NHC$H_2$), 2.27-2.33 (m, 6H, -NC$H_2$CH$_2$), 1.57-1.64 (m, 4H, -NCH$_2$C$H_2$), 1.46-1.51 (m, 4H, -NCH$_2$C$H_2$), 1.34-1.39 (m, 2H, -C$H_2$) and 1.30 (t, *J* = 7.2 Hz, 3H, -NCH$_2$C$H_3$);

**LCMS (*m/z*)** : 481.4 $[M+H]^+$; Purity: nearly 100%.

### 5.1.2.1.2. Synthesis of carbazole-based stilbene derivative (Series-2)

### 5.1.2.1.2.1. 2-(4-Nitrophenyl)acetic acid (287)

A mixture of concentrated nitric acid (1.47 mL) and an equal volume of concentrated sulphuric acid (1.47 mL) was placed in a two-neck flask fitted with a thermometer and a dropping funnel. The mixture was cooled to 10 ºC in ice bath and benzyl cyanide (1.0 g, 8.53 mmol) was run at such a rate that the temperature was maintained around 10 ºC and did not rise above 20 ºC. The solution was further stirred for 1 hr at room temperature and then poured into crushed ice. The mass was filtered under vacuum and pressed well to remove as much oil as possible. The crude product was recrystallized from methanol to obtain 2-(4-nitrophenyl)acetonitrile (1.21 g, 88 %), m.p. 110-112 °C.

A solution of sulphuric acid (50 %) was prepared by adding concentrated sulphuric acid cautiously to water. Two third of the sulphuric acid was added into a round bottom flask containing 2-(4-nitrophenyl)acetonitrile (1.0 g) and the nitrile adhering to the walls of the flask was washed down with the remaining acid. The content was boiled under reflux for 15 min and diluted with 25 mL of ice-cold water. The resulting pale-yellow solid mass was filtered, washed, decolorized and recrystallized from hot water to yield titled compound (**287**) (0.87 g, 78 %), m.p. 148-150 °C (lit[162] m.p. 151-152 °C).

**Anal.:**

**TLC** : $R_f$ 0.4 (Chloroform-Methanol 19:1);

**IR (KBr, $cm^{-1}$)** : 3450, 3084, 2931, 1708, 1515, 1346 and 709.

### 5.1.2.1.2.2. (*E*)-9-Ethyl-3-(4-nitrostyryl)-9*H*-carbazole (288)

A mixture of 2-(4-nitrophenyl)acetic acid (**287**) (1.63 g, 8.96 mmol) and 9-ethyl-9*H*-carbazole-3-carbaldehyde (**268**) (1.0 g, 4.47 mmol) in the presence of piperidine (1.33 mL, 13.44 mmol) was irradiated under microwave at 800 W in a microwave reactor. Reaction progress was monitored by TLC. After completion of reaction, the reaction mixture was cooled and methanol (15 mL) was added to it. The solid so obtained was filtered and dried to obtain the orange colored crystals of the titled compound (**288**) (1.25 g, 82 %), m.p. 215-217 °C.

**Anal.:**

**TLC** : $R_f$ 0.51 (*n*-Hexane-Ethyl acetate 16:4);

**IR (KBr, cm$^{-1}$)** : 3048, 2976, 1621, 1593, 1505, 1333 and 749;

**$^1$H NMR (DMSO-*d*$_6$)** : *δ* 8.49 (s, 1H, Ar*H*), 8.24 (d, 2H, Ar*H*), 8.18 (d, 1H, Ar*H*), 7.86 (d, 2H, Ar*H*), 7.81-7.83 (m, 1H, Ar*H*), 7.62-7.74 (m, 3H, Ar*H*), 7.42-7.50 (m, 1H, Ar*H,* 1H, Vinylic-*H*), 7.22-7.26 (m, 1H, Vinylic-*H*), 4.46 (q, *J* = 7.2 Hz, 2H, -NC$H_2$) and 1.32 (t, *J* = 7.2 Hz, 3H, -NCH$_2$C$H_3$);

**MS (*m/z*)** : 343 [M+H]$^+$.

### 5.1.2.1.2.3. (*E*)-4-(2-(9-Ethyl-9*H*-carbazol-3-yl)vinyl)aniline (289)

To a solution of (*E*)-9-ethyl-3-(4-nitrostyryl)-9*H*-carbazole (**288**) (1.0 g, 2.92 mmol) in tetrahydrofuran: methanol (1:1) mixture (50 mL), stannous chloride (1.11 g, 5.84 mmol) was added in portion. After completion of addition, the reaction mixture was refluxed for 6-7 hrs. The progress of reaction was monitored by TLC. After completion of reaction, *p*H of the mixture was adjusted to basic with aqueous NaOH solution (10 %), and then extracted with ethyl acetate (20 mL × 3). The collected organic layer was washed with water and brine, dried and evaporated to give crude product, which was further purified by recrystallization with methanol to give white colored crystals of the titled compound (**289**) (0.88 g, 92 %), m.p. 162-164 °C.

**Anal.:**

**TLC** : $R_f$ 0.67 (*n*-Hexane-Ethyl acetate 14:6);

**IR (KBr, cm$^{-1}$)** : 3417, 3368, 3029, 2966, 2926, 1615, 1513, 958, 819 and 748;

**$^1$H NMR (DMSO-*d*$_6$)** : *δ* 8.26 (s, 1H, Ar*H*), 8.14-8.16 (m, 1H, Ar*H*), 7.62-7.65 (m, 1H, Ar*H*), 7.54-7.59 (m, 2H, Ar*H*), 7.41-7.48 (m, 1H, Ar*H*), 7.27-7.30 (m, 2H, Ar*H*), 7.17-7.21 (m, 1H, Ar*H*), 7.05-7.07

(m, 2H, vinylic-*H*), 6.56-6.59 (m, 2H, Ar*H*), 5.20 (bs, 2H, -N*H*$_2$), 4.42 (q, *J* = 7.2 Hz, 2H, -NC*H*$_2$CH$_3$) and 1.31 (t, *J* = 7.2 Hz, 3H, -NCH$_2$C*H*$_3$);

**MS (*m/z*)** : 313 [M+H]$^+$.

### 5.1.2.1.2.4. General method H for the synthesis of (*E*)-chloro-*N*-(4-(2-(9-ethyl-9*H*-carbazol-3-yl)vinyl)phenyl)alkylamide (290-292)

**Method H.** To a stirring solution of (*E*)-4-(2-(9-ethyl-9*H*-carbazol-3-yl)vinyl)aniline (**289**) (1.0 g, 3.20 mmol) in dry acetone (25 mL), anhydrous potassium carbonate (1.12 g, 7.98 mmol) was added. To it an appropriate acid chloride (4.8 mmol) was added at 0-5 °C over a period of 15 min. The resulting reaction mixture was allowed to stir at room temperature for 2 hrs. The progress of the reaction was monitored by TLC. After completion of reaction, the reaction mixture was poured in ice cold water and extracted with ethyl acetate (3 × 20 mL). The organic layer was washed with saturated sodium bicarbonate solution followed by water and brine, dried over sodium sulphate, filtered and evaporated to give titled compound.

### 5.1.2.1.2.4a. (*E*)-2-Chloro-*N*-(4-(2-(9-ethyl-9*H*-carbazol-3-yl)vinyl)phenyl) acetamide (290)

The title compound (**290**) was synthesized from (*E*)-4-(2-(9-ethyl-9*H*-carbazol-3-yl)vinyl)aniline (**289**) (1.0 g, 3.20 mmol) and 2-chloroacetyl chloride (0.38 mL, 4.8 mmol) as per **Method H**. Compound (**290**) was obtained as off-white colored solid (0.91 g, 71 %), m.p. 207-209 °C.

**Anal.:**

**TLC** : R$_f$ 0.5 (*n*-Hexane-Ethyl acetate 12:8);

**IR (KBr, cm$^{-1}$)** : 3249, 3038, 2974, 1664, 1593 and 738;

**MS (*m/z*)** : 388.2 [M]$^+$, 390.2 [M+2]$^+$.

### 5.1.2.1.2.4b. (*E*)-3-Chloro-*N*-(4-(2-(9-ethyl-9*H*-carbazol-3-yl)vinyl)phenyl) propanamide (291)

The title compound (**291**) was synthesized from (*E*)-4-(2-(9-ethyl-9*H*-carbazol-3-yl)vinyl)aniline (**289**) (1.0 g, 3.20 mmol) and 3-chloropropionyl chloride (0.46 mL, 4.8 mmol) as per **Method H**. Compound (**290**) was obtained as off-white colored solid (0.87 g, 65 %), m.p. 219-222 °C.

**Anal.:**

| | | |
|---|---|---|
| **TLC** | **:** | $R_f$ 0.35 (*n*-Hexane-Ethyl acetate 12:8); |
| **IR (KBr, cm$^{-1}$)** | **:** | 3273, 3036, 2970, 1644, 1594 and 744; |
| **MS (*m/z*)** | **:** | 403.3 $[M]^+$, 405.3 $[M+H]^+$. |

### 5.1.2.1.2.4c. (*E*)-4-Chloro-*N*-(4-(2-(9-ethyl-9*H*-carbazol-3-yl)vinyl)phenyl) butanamide (292)

The title compound (**292**) was synthesized from (*E*)-4-(2-(9-ethyl-9*H*-carbazol-3-yl)vinyl)aniline (**289**) (1.0 g, 3.20 mmol) and 4-chlorobutyryl chloride (0.54 mL, 4.8 mmol) as per **Method H**. Compound (**292**) was obtained as off-white colored solid (0.88 g, 66 %), m.p. 193-195 °C.

**Anal.:**

| | | |
|---|---|---|
| **TLC** | **:** | $R_f$ 0.45 (*n*-Hexane-Ethyl acetate 12:8); |
| **IR (KBr, cm$^{-1}$)** | **:** | 3295, 3028, 2968, 1653, 1589 and 746; |
| **MS (*m/z*)** | **:** | 417.3 $[M]^+$, 419.3 $[M+H]^+$. |

### 5.1.2.1.2.5. General method for the synthesis of (*E*)-*N*-(4-(2-(9-ethyl-9*H*-carbazol-3-yl)vinyl)phenyl)aminoalkylamides (293-298)

**Method I.** Pyrrolidine/piperidine (5 equiv.) was added to a solution of (*E*)-chloro-*N*-(4-(2-(9-ethyl-9*H*-carbazol-3-yl)vinyl)phenyl)alkylamide (**290-292**) (0.5 g) in THF (10 mL). The reaction mixture was refluxed under nitrogen atmosphere till completion of reaction as judged by TLC. The reaction mixture was then evaporated under reduced pressure and the residue was poured in 20 mL of water and extracted with ethyl acetate (20 mL × 3). The organic layer was dried and evaporated to obtain the crude product, which was further purified by column chromatography using a mixture of chloroform and methanol as eluent to obtain off-white colored compounds.

### 5.1.2.1.2.5a. (*E*)-*N*-(4-(2-(9-Ethyl-9*H*-carbazol-3-yl)vinyl)phenyl)-2-(pyrrolidin-1-yl)acetamide (293)

The title compound (**293**) was synthesized from (*E*)-2-chloro-*N*-(4-(2-(9-ethyl-9*H*-carbazol-3-yl)vinyl)phenyl)acetamide (**290**) (0.5 g, 1.29 mmol) and pyrrolidine (0.54 mL, 6.45 mmol) as per **Method I**. Compound (**293**) was obtained as off-white colored solid (0.35 g, 65 %), m.p. 120-122 °C.

**Anal.:**

**TLC** : $R_f$ 0.63 (Chloroform-Methanol 19:1);

**IR (KBr, cm$^{-1}$)** : 3306, 3022, 2867, 1691, 1584, 1521, 1233 and 749;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 9.76 ( s, 1H, -N*H*CO), 8.37 (s, 1H, Ar*H*), 8.17 (d, 1H, Ar*H*), 7.67-7.72 (m, 3H, Ar*H*), 7.54-7.60 (m, 4H, Ar*H*), 7.43-7.47 (m, 1H, Ar*H*), 7.31-7.35 (m, 1H, Ar*H*), 7.19-7.23 (m, 2H, Vinylic-*H*), 4.43 (q, J = 7.2 Hz, 2H, -NC$H_2$), 3.25 (s, 2H, -NHCOC$H_2$), 2.56-2.61 (m, 4H, -NC$H_2$), 1.69-1.81 (m, 4H, -NCH$_2$C$H_2$) and 1.31 (t, *J* = 7.2 Hz, 3H, -NCH$_2$C$H_3$);

**$^{13}$C NMR (DMSO-$d_6$)** : $\delta$ 169.00, 140.33, 139.66, 138.15, 133.32, 128.85, 128.61, 126.85, 126.34, 125.69, 125.01, 123.03, 122.71, 120.87, 120.10, 119.38, 118.78, 109.78, 109.73, 59.97, 54.20, 37.53, 23.95 and 14.21;

**LCMS (*m/z*)** : 424.25 [M+H]$^+$; Purity: 99.71%.

### 5.1.2.1.2.5b. (*E*)-*N*-(4-(2-(9-Ethyl-9*H*-carbazol-3-yl)vinyl)phenyl)-2-(piperidin-1-yl)acetamide (294)

The title compound (**294**) was synthesized from (*E*)-2-chloro-*N*-(4-(2-(9-ethyl-9*H*-carbazol-3-yl)vinyl)phenyl)acetamide (**290**) (0.5 g, 1.29 mmol) and piperidine (0.64 mL, 6.45 mmol) as per **Method I**. Compound (**294**) was obtained as off-white colored solid (0.33 g, 60 %), m.p. 131-134 °C.

**Anal.:**

**TLC** : $R_f$ 0.47 (*n*-Hexane-Ethyl acetate 10:10);

**IR (KBr, cm$^{-1}$)** : 3326, 3016, 2938, 2795, 1693, 1582, 1516, 1231, 815 and 749;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 9.72 (s, 1H, -N*H*CO), 8.37 (d, 1H, Ar*H*), 8.17 (d, 1H, Ar*H*), 7.55-7.70 (m, 7H, Ar*H*), 7.43-7.47 (m, 1H, Ar*H*), 7.31-7.35 (m, 1H, Ar*H*), 7.19-7.23 (m, 2H, Vinylic-*H*), 4.43 (q, *J* = 7.2

Hz, 2H, -NC*H*$_2$CH$_3$), 3.07 (s, 2H, -NHCOC*H*$_2$), 2.46-2.49 (m, 4H, -NC*H*$_2$CH$_2$), 1.54-1.60 (m, 4H, -NCH$_2$C*H*$_2$), 1.37-1.42 (m, 2H, -NCH$_2$CH$_2$C*H*$_2$) and 1.32 (t, *J* = 7.2 Hz, 3H, -NCH$_2$C*H*$_3$);

**$^{13}$C NMR (DMSO-*d*$_6$)** : *δ* 168.96, 140.44, 139.67, 138.00, 133.39, 128.84, 128.64, 126.89, 126.34, 125.67, 125.01, 123.04, 122.72, 120.87, 120.04, 119.39, 118.80, 109.79, 109.73, 63.16, 54.57, 37.52, 25.95, 24.03 and 14.21;

**LCMS (*m/z*)** : 438.26 [M+H]$^+$; Purity: 99.81%.

### 5.1.2.1.2.5c. (*E*)-*N*-(4-(2-(9-Ethyl-9*H*-carbazol-3-yl)vinyl)phenyl)-3-(pyrrolidin-1-yl)propanamide (295)

The title compound (**295**) was synthesized from (*E*)-3-chloro-*N*-(4-(2-(9-ethyl-9*H*-carbazol-3-yl)vinyl)phenyl)propanamide (**291**) (0.5 g, 1.24 mmol) and pyrrolidine (0.52 mL, 6.20 mmol) as per **Method I**. Compound (**295**) was obtained as off-white colored solid (0.32 g, 60 %), m.p. 158-160 °C.

**Anal.:**

**TLC** : R$_f$ 0.63 (Chloroform-Methanol-Triethylamine 18:1:1);

**IR (KBr, cm$^{-1}$)** : 3281, 3175, 3102, 3025, 2963, 2930, 1653, 1595 and 745;

**$^1$H NMR (DMSO-*d*$_6$)** : *δ* 10.14 ( s, 1H, -N*H*CO), 8.36 (s, 1H, Ar*H*), 8.17 (d, 1H, Ar*H*), 7.69-7.72 (m, 1H, Ar*H*), 7.53-7.63 (m, 6H, Ar*H*), 7.43-7.47 (m, 1H, Ar*H*), 7.29-7.33 (m, 1H, Ar*H*), 7.18-7.23 (m, 2H, Vinylic-*H*) 4.43 (q, *J* = 7.2 Hz, 2H, -NC*H*$_2$CH$_3$), 2.71 (t, 2H, -NHCOC*H*$_2$), 2.46-2.50 (m, 6H, -C*H*$_2$ , -NC*H*$_2$CH$_2$), 1.66-1.69 (m, 4H, -NCH$_2$C*H*$_2$) and 1.31 (t, *J* = 7.2 Hz, 3H, -NCH$_2$C*H*$_3$);

**$^{13}$C NMR (DMSO-*d*$_6$)** : *δ* 170.57, 140.43, 139.65, 138.77, 133.01, 128.88, 128.46, 126.94, 126.33, 125.74, 124.99, 123.04, 122.71, 120.88, 119.66, 119.38, 118.77, 109.77, 109.71, 53.88, 52.04, 37.52, 36.64, 23.65 and 14.20;

**LCMS (*m/z*)** : 438.21 [M+H]$^+$, Purity: nearly 100 %.

### 5.1.2.1.2.5d. (*E*)-*N*-(4-(2-(9-Ethyl-9*H*-carbazol-3-yl)vinyl)phenyl)-3-(piperidin-1-yl)propanamide (296)

The title compound (**296**) was synthesized from (*E*)-3-chloro-*N*-(4-(2-(9-ethyl-9*H*-carbazol-3-yl)vinyl)phenyl)propanamide (**291**) (0.5 g, 1.24 mmol) and piperidine (0.61 mL, 6.20 mmol) as per **Method I**. Compound (**296**) was obtained as off-white colored solid (0.32 g, 58 %), m.p. 173-176 °C.

**Anal.:**

**TLC** : $R_f$ 0.56 (Chloroform-Methanol 19:1);

**IR (KBr, cm$^{-1}$)** : 3018, 2974, 2931, 2839, 2799, 1683, 1595, 1537, 817 and 747;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 10.24 (s, 1H, -N*H*CO), 8.36 (s, 1H, Ar*H*), 8.17 (d, 1H, Ar*H*), 7.70-7.72 (m, 1H, Ar*H*), 7.53-7.61 (m, 6H, Ar*H*), 7.44-7.48 (m, 1H, Ar*H*), 7.30-7.34 (m, 1H, Ar*H*), 7.18-7.22 (m, 2H, Vinylic-*H*), 4.44 (q, *J* = 7.2 Hz, 2H, -$NCH_2CH_3$), 2.58-2.61 (m, 2H, -$NHCOCH_2$), 2.45-2.49 (m, 2H, -$NCH_2$), 2.37-2.40 (m, 4H, -$NCH_2$), 1.49-1.52 (m, 4H, -$NCH_2CH_2$), 1.36-1.41 (m, 2H, -$NCH_2CH_2CH_2$) and 1.32 (t, *J* = 7.2 Hz, 3H, -$NCH_2CH_3$);

**$^{13}$C NMR (DMSO-$d_6$)** : $\delta$ 170.70, 140.43, 196.65, 138.73, 133.0, 128.8, 128.47, 126.96, 126.34, 125.73, 125.01, 123.03, 122.71, 120.88, 119.63, 119.37, 118.75, 109.78, 109.72, 54.93, 54.13, 37.52, 34.57, 26.10, 24.48 and 14.21;

**LCMS (*m/z*)** : 452.21 [M+H]$^+$, Purity: nearly 100%.

### 5.1.2.1.2.5e. (*E*)-*N*-(4-(2-(9-Ethyl-9*H*-carbazol-3-yl)vinyl)phenyl)-4-(pyrrolidin-1-yl)butanamide (297)

The title compound (**297**) was synthesized from ((*E*)-4-chloro-*N*-(4-(2-(9-ethyl-9*H*-carbazol-3-yl)vinyl)phenyl)butanamide (**292**) (0.5 g, 1.20 mmol) and pyrrolidine (0.50 mL, 6.0 mmol) as per **Method I**. Compound (**297**) was obtained as off-white colored solid (0.32 g, 60 %), m.p. 179-181 °C.

**Anal.:**

**TLC** : R$_f$ 0.43 (Chloroform-Methanol-Triethylamine 18:1:1);

**IR (KBr, cm$^{-1}$)** : 3294, 3023, 2960, 2874, 2791, 1657, 1523, 818 and 745;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 9.96 (s, 1H, -N*H*CO), 8.36 (d, 1H, Ar*H*), 8.17 (d, 1H, Ar*H*), 7.71 (dd, 1H, Ar*H*), 7.59-7.63 (m, 4H, Ar*H*), 7.53-7.55 (m, 2H, Ar*H*), 7.46-7.48 (m, 1H, Ar*H*), 7.29-7.33 (m, 1H, Ar*H*), 7.18-7.23 (m, 2H, Vinylic-*H*), 4.44 (q, *J* = 7.2 Hz, 2H, $-\mathrm{NC}H_2\mathrm{CH}_3$), 2.34-2.42 (m, 2H, $-\mathrm{NHCOC}H_2$, 6H, $-\mathrm{NC}H_2\mathrm{CH}_2$), 1.73-1.77 (m, 2H, $-\mathrm{NCH}_2\mathrm{C}H_2$), 1.65-1.68 (m, 4H, $-\mathrm{NCH}_2\mathrm{C}H_2$) and 1.32 (t, *J* = 7.2 Hz, 3H, $-\mathrm{NCH}_2\mathrm{C}H_3$);

**$^{13}$C NMR (DMSO-$d_6$)** : $\delta$ 171.59, 140.43, 139.64, 138.87, 132.90, 128.89, 128.40, 126.90, 126.33, 125.76, 124.99, 123.03, 122.71, 120.88, 119.65, 119.37, 118.75, 109.78, 109.72, 55.63, 54.00, 37.52, 35.00, 24.85, 23.60 and 14.20;

**LCMS (*m/z*)** : 452.21 [M+H]$^+$, Purity: 99.05%.

### 5.1.2.1.2.5f. (*E*)-*N*-(4-(2-(9-Ethyl-9*H*-carbazol-3-yl)vinyl)phenyl)-4-(piperidin-1-yl)butanamide (298)

The title compound (**298**) was synthesized from ((*E*)-4-chloro-*N*-(4-(2-(9-ethyl-9*H*-carbazol-3-yl)vinyl)phenyl)butanamide (**292**) (0.5 g, 1.20 mmol) and piperidine (0.59 mL, 6.0 mmol) as per **Method I**. Compound (**298**) was obtained as off-white colored solid (0.35 g, 63 %), m.p. 173-176 °C.

**Anal.:**

**TLC** : R$_f$ 0.36 (Chloroform-Methanol-Triethylamine 19:1:1)

**IR (KBr, cm$^{-1}$)** : 3296, 3023, 2928, 2850, 1657, 1594, 1523, 1409, 961, 858 and 745;

**$^1$H NMR (DMSO-*d*$_6$)** : $\delta$ 9.93 (s, 1H, -N*H*CO), 8.36 (d, 1H, Ar*H*), 8.17 (d, 1H, Ar*H*), 7.71 (dd, 1H, Ar*H*), 7.58-7.63 (m, 4H, Ar*H*), 7.44-7.54 (m, 3H, Ar*H*), 7.29-7.33 (m, 1H, Ar*H*), 7.18-7.21 (m, 2H, Vinylic-*H*), 4.44 (q, $J$ = 7.2 Hz, 2H,-NC$H_2$CH$_3$), 2.24-2.44 (m, 2H, -NHCOC$H_2$, 6H, -NC$H_2$CH$_2$), 1.72-1.75 (m, 2H, -NCH$_2$C$H_2$), 1.44-1.50 (m, 4H, -NCH$_2$C$H_2$), 1.33-1.36 (m, 2H, -C$H_2$) and 1.31 (t, $J$ = 7.2 Hz, 3H, -NCH$_2$C$H_3$);

**$^{13}$C NMR (DMSO-*d*$_6$)** : $\delta$ 171.61, 140.43, 139.64, 138.88, 132.87, 128.90, 128.39, 126.89, 126.34, 125.77, 124.99, 123.03, 122.71, 120.88, 119.65, 119.37, 118.74, 109.78, 109.72, 58.54, 54.52, 37.52, 34.99, 26.08, 24.66, 22.85 and 14.21;

**LCMS (*m/z*)** : 466.21 [M+H]$^+$, Purity: 99.88%.

### 5.1.2.1.2.6. General method for synthesis of (*E*)-*N*-(4-(2-(9-ethyl-9*H*-carbazol-3-yl)vinyl)phenyl)aminoalkylureas (299-304)

**Method J.** To a stirring solution of (*E*)-4-(2-(9-ethyl-9*H*-carbazol-3-yl) vinyl)aniline (**271**) (1 g, 3.20 mmol) and triethylamine (0.5 mL, 3.52 mmol) in dry THF (20 mL), 4-nitrophenyl chloroformate (710 mg, 3.52 mmol) in dry THF (10 mL) was added dropwise at 0-5 °C over a period of 15 min. The resulting reaction mixture was allowed to stir at room temperature for 2 hrs. The progress of the reaction was monitored by TLC. After complete consumption of the starting material, the respective amine (4.0 mmol) was added to the reaction mixture and the reaction was further stirred for 30 min. After the completion of the reaction, the solvent was removed at reduced pressure. The residue so obtained was triturated with chilled methanol (20 mL), filtered and dried to obtain the title compound.

### 5.1.2.1.2.6a. (*E*)-*N*-(4-(2-(9-Ethyl-9*H*-carbazol-3-yl)vinyl)phenyl)pyrrolidine-1-carboxamide (299)

The title compound (**299**) was synthesized from (*E*)-4-(2-(9-ethyl-9*H*-carbazol-3-yl)vinyl)aniline (**289**) (1.0 g, 3.20 mmol) and pyrrolidine (0.33 mL, 4.00 mmol) as per **Method J**. Compound (**299**) was obtained as off-white colored solid (0.76 g, 58 %), m.p. 144-146 °C.

**Anal.:**

**TLC** : $R_f$ 0.59 (Chloroform-Methanol 19:1);

**IR (KBr, cm$^{-1}$)** : 3334, 3018, 2974, 2873, 1630, 1522, 1234, 965 and 753;

**$^{1}$H NMR (DMSO-$d_6$)** : $\delta$ 8.23 (d, 1H, Ar*H*), 8.15 (d, 1H, Ar*H*), 7.67-7.69 (dd, 1H, Ar*H*), 7.38-7.52 (m, 7H, Ar*H*), 7.10-7.47 (m, 1H, Ar*H,* 2H, Vinylic-*H*), 6.28 (bs, 1H, -N*H*CO), 4.39 (q, *J* = 7.2 Hz, 2H, -NC$H_2$), 3.48-3.52 (m, 4H, -NC$H_2$), 1.98-2.02 (m, 4H, -NCH$_2$C$H_2$) and 1.46 (t, *J* = 7.2 Hz, 3H, -NCH$_2$C$H_3$);

**$^{13}$C NMR (DMSO-$d_6$)** : $\delta$ 140.39, 131.58, 129.01, 127.68, 126.64, 126.37, 125.97, 124.92, 123.00, 122.66, 120.89, 120.11, 120.01, 119.38, 118.61, 109.76, 109.68, 46.17, 37.51, 25.46 and 14.16;

**MS (*m/z*)** : 410.3 $[M+H]^+$.

### 5.1.2.1.2.6b. (*E*)-*N*-(4-(2-(9-Ethyl-9*H*-carbazol-3-yl)vinyl)phenyl)piperidine-1-carboxamide (300)

The title compound (**300**) was synthesized from (*E*)-4-(2-(9-ethyl-9*H*-carbazol-3-yl)vinyl)aniline (**289**) (1.0 g, 3.20 mmol) and piperidine (0.40 mL, 4.00 mmol) as per **Method J**. Compound (**300**) was obtained as off-white colored solid (0.58 g, 55 %), m.p. 214-216 °C.

**Anal.:**

**TLC** : $R_f$ 0.4 (*n*-Hexane-Ethyl acetate 10:10);

**IR (KBr, cm$^{-1}$)** : 3305, 3026, 2972, 2931, 2854, 1633, 1589, 1515, 1234, 951 and 746;

**$^1$H NMR (DMSO-*d*$_6$)** : *δ* 8.24 (s, 1H, Ar*H*), 8.15 (d, 1H, Ar*H*), 7.67-7.69 (m, 1H, Ar*H*), 7.39-7.52 (m, 8H, Ar*H*), 7.24-7.27 (m, 1H, Ar*H,* 2H, Vinylic-*H*), 7.10-7.14 (m, 1H, Ar*H*), 6.47 (bs, 1H, -N*H*CO), 4.39 (q, *J* = 7.2 Hz, 2H,-NC$H_2$CH$_3$), 3.49-3.51 (m, 4H, -NC$H_2$), 1.64-1.71 (m, 6H, -NCH$_2$C$H_2$) and 1.46 (t, *J* = 7.2 Hz, 3H, -NCH$_2$C$H_3$);

**$^{13}$C NMR (DMSO-*d*$_6$)** : *δ* 155.26, 140.41, 140.38, 139.55, 131.51, 129.05, 127.65, 126.61, 126.32, 126.00, 124.92, 123.02, 122.70, 120.87, 120.09, 119.35, 118.59, 109.76, 109.70, 45.17, 37.51, 26.00, 24.58 and 14.20;

**MS (*m/z*)** : 424.3 $[M+H]^+$.

### 5.1.2.1.2.6c. (*E*)-1-(4-(2-(9-Ethyl-9*H*-carbazol-3-yl)vinyl)phenyl)-3-(2-(pyrrolidin-1-yl)ethyl)urea (301)

The title compound (**301**) was synthesized from (*E*)-4-(2-(9-ethyl-9*H*-carbazol-3-yl)vinyl)aniline (**289**) (1.0 g, 3.20 mmol) and 2-(aminoethyl) pyrrolidine (0.40 mL, 4.00 mmol) as per **Method J**. Compound (**301**) was obtained as off-white colored solid (0.88 g, 60 %), m.p. >250 °C.

**Anal.:**

**TLC** : R$_f$ 0.48 (Chloroform-Methanol-Triethylamine 18:1:1);

**IR (KBr, cm$^{-1}$)** : 3299, 3025, 2969, 2931, 1645, 1593, 1234, 960 and 743;

**$^1$H NMR (DMSO-*d*$_6$)** : *δ* 8.73 (s, 1H, -N*H*CONH), 8.34 (s, 1H, Ar*H*), 8.17 (d, 1H, Ar*H*), 7.69 (d, 1H, Ar*H*), 7.56-7.60 (m, 2H, Ar*H*), 7.41-7.50 (m, 5H, Ar*H*), 7.15-7.27 (m, 1H, Ar*H,* 2H, Vinylic-*H*), 6.15 (t, 1H, -NHCON*H*), 4.42 (q, *J* = 7.2 Hz, 2H, -NC$H_2$), 3.20-3.24 (m, 2H, -NHCONHC$H_2$), 2.43-2.49 (m, 6H, -NC$H_2$), 1.66-1.72 (m, 4H, -NCH$_2$C$H_2$) and 1.31 (t, *J* = 7.2 Hz, 3H, -NCH$_2$C$H_3$);

**$^{13}$C NMR (DMSO-$d_6$)** : $\delta$ 155.64, 140.43, 139.60, 139.03, 132.00, 129.10, 127.94, 127.40, 127.03, 125.86, 124.98, 123.03, 122.71, 120.89, 119.37, 118.87, 118.68, 118.14, 109.78, 55.83, 53.97, 38.06, 37.52, 23.61 and 14.02;

**LCMS (*m/z*)** : 453.16 [M+H]$^+$, Purity: nearly 100%.

### 5.1.2.1.2.6d. (*E*)-1-(4-(2-(9-Ethyl-9*H*-carbazol-3-yl)vinyl)phenyl)-3-(2-(piperidin-1-yl)ethyl)urea (302)

The title compound (**302**) was synthesized from (*E*)-4-(2-(9-ethyl-9*H*-carbazol-3-yl)vinyl)aniline (**289**) (1.0 g, 3.20 mmol) and 2-(aminoethyl) piperidine (0.57 mL, 4.00 mmol) as per **Method J**. Compound (**302**) was obtained as off-white colored solid (0.89 g, 60 %), m.p. 213-216 °C.

**Anal.:**

**TLC** : R$_f$ 0.6 (Chloroform-Methanol-Triethylamine 18:1:1);

**IR (KBr, cm$^{-1}$)** : 3307, 3023, 2962, 2853, 1641, 1581,1237, 962 and 743;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 8.22 (d, 1H, Ar*H*), 8.13 (d, 1H, Ar*H*), 7.67 (dd, 1H, Ar*H*), 7.10-7.54 (m, 10H, Ar*H*, 2H, Vinylic-*H*), 6.0 (bs, 1H, -NHCON*H*), 4.37 (q, *J* = 7.2 Hz, 2H, -NC$H_2$CH$_3$), 3.43-3.47 (m, 2H, -NHCONHC$H_2$); 2.50-2.69 (m, 6H, -NC$H_2$), 1.70-1.76 (m, 4H, -NCH$_2$C$H_2$), 1.51-1.59 (m, 2H, -NCH$_2$CH$_2$C$H_2$) and 1.45 (t, *J* = 7.2 Hz, 3H, -NCH$_2$C$H_3$);

**$^{13}$C NMR (DMSO-$d_6$)** : $\delta$ 155.54, 140.41, 140.19, 139.53, 131.02, 129.07, 127.41, 127.41, 126.31, 126.00, 124.90, 123.02, 122.71, 120.88, 119.34, 118.56, 118.18, 109.76, 109.70, 58.60, 54.50, 37.51, 36.92, 26.01, 24.57 and 14.02;

**LCMS (*m/z*)** : 467.21 [M+H]$^+$, Purity: 99.88%.

### 5.1.2.1.2.6e. (*E*)-1-(4-(2-(9-Ethyl-9*H*-carbazol-3-yl)vinyl)phenyl)-3-(3-(pyrrolidin-1yl)propyl)urea (303)

The title compound (**303**) was synthesized from (*E*)-4-(2-(9-ethyl-9*H*-carbazol-3-yl)vinyl)aniline (**289**) (1.0 g, 3.20 mmol) and 3-(aminopropyl) pyrrolidine (0.51 g, 4.00 mmol) as per **Method J**. Compound (**303**) was obtained as off-white colored solid (0.97 g, 65 %), m.p. 215-217 °C.

**Anal.:**

| | | |
|---|---|---|
| **TLC** | : | R$_f$ 0.58 (Chloroform-Methanol-Triethylamine 18:1:1); |
| **IR (KBr, cm$^{-1}$)** | : | 3322, 3022, 2982, 2803, 1634, 1588, 1235, 959 and 745; |
| **$^1$H NMR (DMSO-*d*$_6$)** | : | *δ* 8.74 (s, 1H, -N*H*CONH), 8.34 (d, 1H, Ar*H*), 8.17 (d, 1H, Ar*H*),7.69 (dd, 1H, Ar*H*), 7.57-7.60 (m, 2H, Ar*H*), 7.40-7.49 (m, 5H, Ar*H*), 7.15-7.27 (m, 1H, Ar*H,* 2H, Vinylic-*H*), 6.07 (t, 1H, -NHCON*H*), 4.44 (q, *J* = 7.2 Hz, 2H, -NC$H_2$CH$_3$), 3.17-3.22 (m, 2H, -NHC$H_2$), 2.33-2.36 (m, 6H, -NC$H_2$), 1.49-1.54 (m, 4H, -NCH$_2$C$H_2$), 1.36-1.42 (m, 2H, -C$H_2$) and 1.31 (t, *J* = 7.2 Hz, 3H, -NCH$_2$C$H_3$); |
| **$^{13}$C NMR (DMSO-*d*$_6$)** | : | *δ* 155.64, 140.41, 140.18, 139.58, 131.03, 129.07, 127.42, 127.01, 126.31, 126.00, 124.89, 123.02, 122.71, 120.87, 119.34, 118.57, 118.26, 109.75, 109.69, 54.09, 53.75, 38.06, 37.51, 29.51, 23.56 and 14.20; |
| **LCMS (*m/z*)** | : | 467.21 [M+H]$^+$, Purity: 97.38%. |

### 5.1.2.1.2.6f. (*E*)-1-(4-(2-(9-Ethyl-9*H*-carbazol-3-yl)vinyl)phenyl)-3-(3-(piperidin-1-yl)propyl)urea (304)

The title compound (**304**) was synthesized from (*E*)-4-(2-(9-ethyl-9*H*-carbazol-3-yl)vinyl)aniline (**289**) (1.0 g, 3.20 mmol) and 3-(aminopropyl) piperidine (0.57 g, 4.00 mmol) as per **Method G**. Compound (**304**) was obtained as off-white colored solid (1.0 g, 67 %), m.p. 206-207 °C.

**Anal.:**

**TLC** : R*f* 0.61 (Chloroform-Methanol-Triethylamine 18:1:1);

**IR (KBr, cm$^{-1}$)** : 3308, 3027, 2961, 2870, 1641, 1591, 1233, 959 and 745;

**$^1$H NMR (DMSO-*d*$_6$)** : *δ* 8.53 (s, 1H, -N*H*CONH), 8.34 (d, 1H, Ar*H*), 8.17 (d, 1H, Ar*H*), 7.69 (dd, 1H, Ar*H*), 7.58-7.60 (m, 2H, Ar*H*), 7.41-7.49 (m, 5H, Ar*H*), 7.15-7.27 (m, 1H, Ar*H,* 2H, Vinylic-*H*), 6.17 (t, 1H, -NHCON*H*), 4.43 (q, *J* = 7.2 Hz, 2H, -NC*H*$_2$CH$_3$), 3.08-3.13 (m, 2H, -NHC*H*$_2$), 2.23-2.29 (m, 6H, -NC*H*$_2$), 1.55-1.59 (m, 2H, -NHCH$_2$C*H*$_2$), 1.46-1.51 (m, 4H, -NCH$_2$C*H*$_2$), 1.35-1.40 (m, 2H, -NCH$_2$CH$_2$C*H*$_2$) and 1.31 (t, *J* = 7.2 Hz, 3H, -NCH$_2$C*H*$_3$);

**$^{13}$C NMR (DMSO-*d*$_6$)** : *δ* 155.64, 140.41, 140.19, 139.52, 131.03, 129.07, 127.41, 127.01, 126.30, 126.00, 124.88, 123.03, 122.71, 120.87, 119.34, 118.58, 118.28, 109.74, 109.63, 56.64, 54.57, 38.09, 37.50, 27.52, 26.01, 24.62 and 14.19;

**LCMS (*m/z*)** : 481.17 [M+H]$^+$, Purity: nearly 100%.

### 5.1.2.1.2.7. General method for the synthesis of (*E*)-1-(9-ethyl-6-styryl-9*H*-carbazol-3-yl)-3-(aminoalkyl)thioureas (305-307)

**Method K.** To a stirring solution of (*E*)-4-(2-(9-ethyl-9*H*-carbazol-3-yl) vinyl)aniline (**289**) (1.0 g, 3.20 mmol) and triethylamine (0.5 mL, 3.52 mmol) in dry 1:1 THF/DCM mixture (20 mL), thiocarbonyldiimidazole (627 mg, 3.52 mmol) in 1:1 THF/DCM mixture (10 mL) was added drop wise at 0-5 °C over a period of 15 min. The resulting reaction mixture was allowed to stir at room temperature for 2 h. The progress of the reaction was monitored by TLC. After complete consumption of the starting material, the appropriate amine (4 mmol) was added to the reaction mixture and the reaction was further stirred for 30 min. After the completion of the reaction, the solvent was removed at reduced pressure. The residue so obtained was triturated with chilled methanol (20 mL).

The solid was collected by filtration, washed again with chilled methanol (10 mL) and dried to the give titled compound.

### 5.1.2.1.2.7a. (*E*)-1-(4-(2-(9-Ethyl-9*H*-carbazol-3-yl)vinyl)phenyl)-3-(2-(pyrrolidin-1-yl)ethyl)thiourea (305)

The title compound (**305**) was synthesized from (*E*)-4-(2-(9-ethyl-9*H*-carbazol-3-yl)vinyl)aniline (**289**) (1.0 g, 3.20 mmol) and 2-(aminoethyl) pyrrolidine (0.40 mL, 4.00 mmol) as per **Method K**. Compound (**305**) was obtained as off-white colored solid (1.13 g, 74 %), m.p. 114-116 °C.

**Anal.:**

**TLC** : R$_f$ 0.55 (Chloroform-Methanol-Triethylamine 19:1:1);

**IR (KBr, cm$^{-1}$)** : 3248, 3026, 2966, 2804, 1597, 1512, 1480, 1339, 1057, 961 and 746;

**$^1$H NMR (DMSO-*d*$_6$)** : $\delta$ 9.71 (s, 1H, -N*H*CSNH), 8.36 (d, 1H, Ar*H*), 8.17 (d, 1H, Ar*H*), 7.69-7.73 (m, 1H, Ar*H*), 7.54-7.61 (m, 4H, Ar*H*), 7.41-7.48 (m, 3H, Ar*H*), 7.19-7.32 (m, 1H, Ar*H*, 2H, Vinylic-*H*), 4.44 (q, *J* = 7.2 Hz, 2H, -NC*H*$_2$CH$_3$), 3.54-3.61 (m, 2H, -NHC*H*$_2$), 2.61 (t, 2H, -NC*H*$_2$CH$_2$), 2.47-2.51 (m, 4H, -NC*H*$_2$CH$_2$), 1.74-1.67 (m, 4H, -NCH$_2$C*H*$_2$) and 1.30 (t, *J* = 7.2 Hz, 3H, -NCH$_2$C*H*$_3$);

**$^{13}$C NMR (DMSO-*d*$_6$)** : $\delta$ 139.96, 139.22, 133.43, 128.48, 128.32, 126.27, 125.90, 125.10, 124.62, 122.55, 122.23, 120.44, 118.94, 118.39, 109.35, 109.30, 53.43, 42.90, 37.06, 23.20 and 13.77;

**LCMS (*m/z*)** : 469.3 [M+H]$^+$, Purity: nearly 100 %.

### 5.1.2.1.2.7b. (*E*)-1-(4-(2-(9-Ethyl-9*H*-carbazol-3-yl)vinyl)phenyl)-3-(2-(piperidin-1-yl)ethyl) thiourea (306)

The title compound (**306**) was synthesized from(*E*)-4-(2-(9-ethyl-9*H*-carbazol-3-yl)vinyl)aniline (**289**) (1.0 g, 3.20 mmol) and 2-(aminoethyl) piperidine (0.57 mL, 4.00 mmol) as per **Method K**. Compound (**306**) was obtained as off-white colored solid (1.08 g, 70 %), m.p. 183-185 °C.

**Anal.:**

**TLC** : R$_f$ 0.52 (Chloroform-Methanol-Triethylamine 25:1:1);

**IR (KBr, cm$^{-1}$)** : 3290, 3029, 2934, 2846, 2801, 1599, 1513, 1478, 1236, 961, 821 and 750;

**$^1$H NMR (DMSO-*d*$_6$)** : $\delta$ 9.72 (s, 1H, -N*H*CSNH), 8.37 (d, 1H,Ar*H*), 8.17 (d, 1H, Ar*H*), 7.71-7.73 (m, 1H, Ar*H*), 7.55-7.61 (m, 5H, Ar*H*), 7.43-7.48 (m, 3H, Ar*H*), 7.19-7.37 (m, 1H, Ar*H,* 2H, Vinylic-*H*), 4.44 (q, *J* = 7.2 Hz, 2H, -NC*H*$_2$CH$_3$), 3.52-3.58 (m, 2H, -NHC*H*$_2$), 2.47 (t, 2H, -NC*H*$_2$CH$_2$), 2.33-2.38 (m, 4H, -NC*H*$_2$CH$_2$), 1.45-1.52 (m, 2H, -NCH$_2$C*H*$_2$), 1.34-1.41 (m, 2H, -NCH$_2$CH$_2$C*H*$_2$) and 1.30 (t, *J* = 7.2 Hz, 3H, -NCH$_2$C*H*$_3$);

**$^{13}$C NMR (DMSO-*d*$_6$)** : $\delta$ 139.96, 139.24, 133.61, 128.59, 128.30, 126.41, 125.91, 125.06, 124.64, 122.85, 122.55, 122.23, 120.44, 118.95, 118.42, 109.35, 109.30, 53.87, 41.20, 37.06, 25.63, 24.08 and 13.76;

**LCMS (*m/z*)** : 483.4 [M+H]$^+$, Purity: nearly 100%.

### 5.1.2.1.2.7c. (*E*)-1-(4-(2-(9-Ethyl-9*H*-carbazol-3-yl)vinyl)phenyl)-3-(3-(pyrrolidin-1yl)propyl) thiourea (307)

The title compound (**307**) was synthesized from (*E*)-4-(2-(9-ethyl-9*H*-carbazol-3-yl)vinyl)aniline (**289**) (1.0 g, 3.20 mmol) and 3-(aminopropyl) pyrrolidine (0.51 g, 4.00 mmol) as per **Method K**. Compound (**307**) was obtained as off-white colored solid (1.11 g, 72 %), m.p. 188-190 °C.

**Anal.:**

**TLC** : R$_f$ 0.57 (Chloroform-Methanol-Triethylamine 19:1:1);

**IR (KBr, cm$^{-1}$)** : 3186, 2967, 2871, 2816, 1594, 1524, 1307, 1236, 958 and 747;

**$^1$H NMR (DMSO-*d*$_6$)** : $\delta$ 9.55 (s, 1H, -N*H*CSNH), 8.37 (d, 1H, Ar*H*), 8.17 (d, 1H, Ar*H*), 7.96-8.00 (m, 1H, Ar*H*), 7.71-7.73 (m, 1H, Ar*H*), 7.55-7.60 (m, 4H, Ar*H*), 7.19-7.47 (m, 3H, Ar*H,* 2H, Vinylic-*H*), 4.44 (q, $J$ = 7.2 Hz, 2H, -NC*H*$_2$CH$_3$), 3.51-3.55 (m, 2H, -NHC*H*$_2$), 2.37-2.45 (m, 6H, -NC*H*$_2$), 1.66-1.75 (m, 2H, -NCH$_2$C*H*$_2$), 1.55-1.59 (m, 4H, -NCH$_2$C*H*$_2$) and 1.32 (t, $J$ = 7.2 Hz, 3H, -NCH$_2$C*H*$_3$);

**$^{13}$C NMR (DMSO-*d*$_6$)** : $\delta$ 179.9, 139.96, 139.24, 133.69, 128.60, 128.29, 126.40, 125.04, 124.64, 123.09, 122.55, 122.23, 120.44, 118.95, 118.42, 109.35, 109.30, 53.59, 37.06, 27.34, 23.03 and 13.77;

**LCMS (*m/z*)** : 483.4 [M+H]$^+$, Purity: nearly 100%.

### 5.1.2.2. Carbazole-based azahelicene derivatives (316-329)

#### 5.1.2.2.1. (4-Bromobenzyl)triphenylphosphonium bromide (309)

To a solution of 4-bromobenzyl bromide (**308**) (1.0 g, 4.0 mmol) in toluene (20 mL), triphenylphosphine (1.15 g, 4.4 mmol) was added. The resulting reaction mixture was stirred at 100 ºC for 6-7 hrs. The progress of the reaction was monitored by TLC. After complete consumption of the starting material, white precipitates so obtained were filtered, washed with *n*-hexane and dried to obtain the white colored title compound (**309**) (1.9 g, 92 %), m.p. >250 °C (lit[184] m.p. >250 °C).

#### 5.1.2.2.2. 3-(4-Bromostyryl)-9-ethyl-9*H*-carbazole (310)

To a stirring solution of 9-ethyl-9*H*-carbazole-3-carbaldehyde (**268**) (1.0 g, 4.46 mmol) and 4-bromobenzyltriphenylphosphonium bromide (**309**) (2.30 g, 4.46 mmol) in chloroform (20 mL), a few drops of 50 % aqueous sodium hydroxide solution was added. The reaction mixture was stirred for 5-6 hrs at room temperature. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was diluted with water and organic phase was separated. The aqueous phase was again washed with chloroform (20 mL × 2). The combined organic phase was dried over anhydrous sodium sulphate, filtered and evaporated to give crude product. The crude residue was purified by silica-gel column-chromatography with *n*-hexane-ethyl acetate as eluent to obtain white colored title compound (**310**) (1.40 g, 83 %) which contained both *cis* and *trans* isomers.

**Anal.:**

**TLC** : $R_f$ 0.62, 0.63 (*n*-Hexane-Ethyl acetate 16:4);

**IR (KBr, $cm^{-1}$)** : 3047, 3008, 2976, 2929, 2867, 1596, 1481, 1073, 841 and 749;

### 5.1.2.2.3. 2-Bromo-9-ethyl-9*H*-naphtho[2,1-*c*]carbazole (311)

A solution of 3-(4-bromostyryl)-9-ethyl-9*H*-carbazole (**310**) (200 mg, 0.53 mmol), iodine (148 mg, 0.58 mmol), THF (0.76 mL, 10.6 mmol) in toluene (1.2 lit) was irradiated using HPMV lamp (125 Watt) for 24 hrs. The progress of reaction was monitored by TLC. After completion of the reaction, the reaction mixture was washed with aqueous sodium thiosulfate solution (10 %) to remove the unreacted iodine. The organic phase was collected and washed with water and brine solution, dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude residue was purified by silica-gel column-chromatography with *n*-hexane-ethyl acetate as eluent to obtain light yellow colored titled compound (**311**) (0.11 g, 55 %), m.p. 126-128 °C.

**Anal.:**

**TLC** : $R_f$ 0.42 (*n*-Hexane-Ethyl acetate 16:4);

**IR (KBr, $cm^{-1}$)** : 3042, 2968, 2928, 1582, 1511, 1024, 835 and 745;

**$^1H$ NMR ($CDCl_3$)** : $\delta$ 9.60 (d, 1H, Ar*H*), 8.82 (d, 1H, Ar*H*), 7.28-7.95 (m, 9H, Ar*H*), 4.58 (q, $J$ = 7.2 Hz, 2H, $-NCH_2$) and 1.55 (t, $J$ = 7.2 Hz, 3H, $-NCH_2CH_3$);

**MS (*m/z*)** : 374.17 $[M]^+$, 376.15 $[M+2]^+$

### 5.1.2.2.4. 9-Ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-amine (312)

To a solution of 2-bromo-9-ethyl-9*H*-naphtho[2,1-*c*]carbazole (**311**) (1.0 g, 2.67 mmol) in dry dioxane (20 mL), ethanolamine (0.41 mL, 6.67 mmol), trimethylsilylazide (0.62 g, 5.34 mmol) and copper powder (0.34 g, 5.34 mmol) were added. The reaction mixture was stirred at 95 ºC for 24 hrs. The progress of reaction was monitored by TLC. After completion of the reaction, the reaction mixture was diluted with ethyl acetate (10 mL) and then filtered through a celite pad. The pad was successively washed with ethyl acetate (20 mL), water (25 mL) and aqueous ammonia solution (10 mL). After the two layers were separated, the aqueous layer was extracted with ethyl acetate (2 × 10 mL). The combined organic layers were washed with brine (20 mL), dried over dry $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue was purified by silica-gel column-chromatography with *n*-hexane-ethyl acetate as eluent to obtain light brown colored titled compound (**312**) (0.61 g, 74 %), m.p. 145-147 °C.

**Anal.:**

**TLC** : $R_f$ 0.45 (*n*-Hexane-Ethyl acetate 15:5);

**IR (KBr, cm$^{-1}$)** : 3417, 3307, 3042, 2973, 2894, 1600, 1331, 1148, 822 and 739;

**$^1$H NMR (CDCl$_3$)** : $\delta$ 8.84 (dd, 1H, Ar*H*), 8.65 (d, 1H, Ar*H*), 7.87 (d, 1H, Ar*H*), 7.79 (d, 1H, Ar*H*), 7.55-7.69 (m, 3H, Ar*H*), 7.52-7.53 (m, 1H, Ar*H*), 7.48-7.51 (m, 1H, Ar*H*), 7.24-7.28 (m, 1H, Ar*H*), 7.08 (dd, 1H, Ar*H*), 4.55 (q, $J$ = 7.2 Hz, 2H, -$NCH_2$), 3.95 (bs, 2H, -$NH_2$) and 1.52 (t, $J$ = 7.2 Hz, 3H, -$NCH_2CH_3$);

**MS (*m/z*)** : 311.33 $[M+H]^+$.

### 5.1.2.2.5. General method for the synthesis of *N*-(9-ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-yl)alkylamides (313-315)

**Method L:** To stirring solution of 9-ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-amine (**312**) (1.0 g, 3.22 mmol) in dry acetone (25 mL), potassium carbonate (1.12 g, 8.05 mmol) was added. To it, respective acid chloride (4.83 mmol) was added dropwise under cold conditions. After completion of addition, the reaction mixture was stirred for additional 1 hr. The progress of the reaction was monitored by TLC. After completion of the starting material, the reaction mixture was poured in ice-cold water and extracted with ethyl acetate (20 mL × 3). The organic layer was washed with saturated sodium bicarbonate solution followed by water and brine, dried with sodium sulfate, and evaporated to give the title compound.

### 5.1.2.2.5a. 4-Chloro-*N*-(9-ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-yl)butanamide (313)

The title compound (**272**) was synthesized from 9-ethyl-9*H*-naphtho [2,1-*c*]carbazol-2-amine (**312**) (1.0 g, 3.22 mmol) and 4-chlorobutyryl chloride (0.54 mL, 4.83 mmol) as per **Method L**. Compound (**272**) was obtained as brown colored solid (1.09 g, 82 %), m.p. 105-107 °C.

**Anal.:**

**TLC** : $R_f$0.45 (*n*-Hexane-Ethyl acetate 8:12);

**IR (KBr, $cm^{-1}$)** : 3281, 3043, 2970, 2930, 2861, 1660, 1641, 1328, 1153, 835 and 746;

**MS (*m/z*)** : 415 $[M]^+$, 417 $[M+2]^+$.

### 5.1.2.2.5b. 5-Bromo-*N*-(9-ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-yl)pentanamide (314)

The title compound (**314**) was synthesized from 9-ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-amine (**312**) (1.0 g, 3.22 mmol) and 5-bromovaleryl chloride (0.96 g, 4.83 mmol) as per **Method L**. Compound (**314**) was obtained as brown colored solid (1.21 g, 79 %), m.p. 112-114 °C.

**Anal.:**

**TLC** : $R_f$0.51 (*n*-Hexane-Ethyl acetate 8:12);

**IR (KBr, $cm^{-1}$)** : 3294, 3042, 2965, 1658, 1326, 1155, 835 and 744;

**MS (*m/z*)** : 473 $[M]^+$, 475 $[M+2]^+$.

### 5.1.2.2.5c. 6-Bromo-*N*-(9-ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-yl)hexanamide (315)

The title compound (**315**) was synthesized from 9-ethyl-9*H*-naphtho [2,1-*c*]carbazol-2-amine (**312**) (1.0 g, 3.22 mmol) and 6-bromohexanoyl chloride (1.03 g, 4.83 mmol) as per **Method L**. Compound (**314**) was obtained as brown colored solid (1.18 g, 75 %), m.p. 125-127 °C.

**Anal.:**

**TLC** : $R_f$0.59 (*n*-Hexane-Ethyl acetate 8:12);

**IR (KBr, cm$^{-1}$)** : 3299, 3043, 2970, 1661, 1329, 1154, 836 and 746;

**MS (*m/z*)** : 487 [M]$^+$, 489 [M+2]$^+$.

### 5.1.2.2.6. General procedure for the synthesis of 1-(9-ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-yl)aminoalkylamides (316-321)

**Method M:** Pyrrolidine/piperidine (5 equiv.) was added to a solution of *N*-(9-ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-yl)alkylamides (**313-315**) (0.5 g, 1 equiv.) in THF (10 mL). The reaction mixture was refluxed under nitrogen atmosphere until completion of the reaction as judged by TLC. The reaction mixture was then evaporated under reduced pressure and the residue was dissolved in 20 mL of water and extracted with ethyl acetate (20 mL × 3). The organic layer was then dried and evaporated to obtain the crude product, which was further purified by column chromatography using a mixture of chloroform and methanol as eluent to obtain off-white colored compound.

### 5.1.2.2.6a. *N*-(9-Ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-yl)-4-(pyrrolidin-1-yl)butanamide (316)

The title compound (**316**) was synthesized from 4-chloro-*N*-(9-ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-yl)butanamide (**313**) (0.5 g, 1.20 mmol), and pyrrolidine (0.51 mL, 6.0 mmol) as per **Method M**. Compound (**316**) was obtained as off-white colored solid (0.42 g, 76 %), m.p. 102-104 °C.

**Anal.:**

**TLC** : R$_f$ 0.45 (Chloroform-Methanol 15:1);

**IR (KBr, cm$^{-1}$)** : 3272, 3171, 3043, 2930, 2862, 1649, 1587, 1549, 1303, 821and 752;

**$^1$H NMR (CDCl$_3$)** : $\delta$ 9.65 (s, 1H, -N*H*CO), 9.36 (s, 1H, Ar*H*), 8.90 (d, 1H, Ar*H*), 8.07-8.09 (m, 1H, Ar*H*), 7.92-7.98 (m, 2H, Ar*H*), 7.70-7.82 (m, 3H, Ar*H*), 7.52-7.60 (m, 2H, Ar*H*), 7.29-7.33 (m, 1H, Ar*H*), 4.59 (q, $J$ = 7.2 Hz, 2H, -NC$H_2$CH$_3$), 2.80-2.83 (m, 6H, -NC$H_2$), 2.68-2.72 (m, 2H, -NHCOC$H_2$), 1.82-1.84 (m, 6H, -NCH$_2$C$H_2$) and 1.55 (t, $J$ = 7.2 Hz, 3H, -NCH$_2$C$H_3$);

**MS (*m/z*)** : 450 [M+H]$^+$.

**RP-HPLC** : Purity: 98.8 %, $t_R$ = 4.11 min (Mobile phase: acetonitrile-water 80:20 (0.01 % TFA)).

### 5.1.2.2.6b. *N*-(9-Ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-yl)-4-(piperidin-1-yl) butanamide (317)

The title compound (**317**) was synthesized from 4-chloro-*N*-(9-ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-yl)butanamide (**313**) (0.5 g, 1.20 mmol), and piperidine (0.60 mL, 6.0 mmol) as per **Method M**. Compound (**317**) was obtained as off-white colored solid (0.4 g, 72 %), m.p. 163-165 °C.

**Anal.:**

**TLC** : $R_f$ 0.5 (Chloroform-Methanol 19:1);

**IR (KBr, cm$^{-1}$)** : 3293, 3240, 3042, 2931, 2851, 1665, 1612, 1586, 1326, 838 and 742;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 9.21 (s, 1H, Ar*H*), 8.90 (d, 1H, Ar*H*), 8.13 (d, 1H, Ar*H*), 7.93-7.99 (m, 2H, Ar*H*), 7.72-7.83 (m, 3H, Ar*H*), 7.53-7.61 (m, 2H, Ar*H*), 7.29-7.33 (m, 1H, Ar*H*), 4.60 (q, 2H, -NC$H_2$CH$_3$), 2.55-2.58 (m, 2H, -NHCOC$H_2$), 2.41-2.49 (m, 6H, -NC$H_2$), 1.95-2.00 (m, 2H, -COCH$_2$C$H_2$), 1.36-1.58 (m, 4H, -NCH$_2$C$H_2$, 3H, -NCH$_2$C$H_3$) and 1.31-1.41 (m, 2H, -CH$_2$C$H_2$);

**MS (*m/z*)** : 464 [M+H]$^+$.

**RP-HPLC** : Purity: 98.9 %, $t_R$ = 4.02 min (Mobile phase: acetonitrile-water 80:20 (0.01 % TFA)).

### 5.1.2.2.6c. *N*-(9-Ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-yl)-5-(pyrrolidin-1-yl) pentanamide (318)

The title compound (**318**) was synthesized from 5-bromo-*N*-(9-ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-yl)pentanamide (**314**) (0.5 g, 1.20 mmol), and pyrrolidine (0.45 mL, 5.25 mmol) as per **Method M**. Compound (**318**) was obtained as off-white colored solid (0.34 g, 69 %), m.p. 161-163 °C.

**Anal.:**

**TLC** : $R_f$ 0.43 (Chloroform-Methanol 15:1);

**IR (KBr, cm$^{-1}$)** : 3288, 3234, 3041, 2932, 1661, 1610, 1579, 1507, 1323, 837 and 742;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 10.22 (s, 1H, -N*H*CO), 9.55 (s, 1H, Ar*H*), 8.72 (d, 1H, Ar*H*), 8.00-8.02 (m, 3H, Ar*H*), 7.74-7.88 (m, 4H, Ar*H*), 7.51-7.55 (m, 1H, Ar*H*), 7.21-7.24 (m, 1H, Ar*H*), 4.68 (q, *J* = 7.2 Hz, 2H, -NC$H_2$CH$_3$), 2.61-2.90 (m, 6H, -NC$H_2$), 2.42-2.45 (m, 2H, -NHCOC$H_2$), 1.77-1.82 (m, 4H, -NCH$_2$C$H_2$), 1.60-1.71 (m, 4H, -COCH$_2$C$H_2$C$H_2$) and 1.41 (t, *J* = 7.2 Hz, 3H, -NCH$_2$C$H_3$);

**MS (*m/z*)** : 464.01 $[M+H]^+$.

**RP-HPLC** : Purity: 99.5 %, $t_R$ = 4.14 min (Mobile phase: acetonitrile-water 80:20 (0.01 % TFA)).

### 5.1.2.2.6d. *N*-(9-Ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-yl)-5-(piperidin-1-yl) pentanamide (319)

The title compound (**319**) was synthesized from 5-bromo-*N*-(9-ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-yl)pentanamide (**314**) (0.5 g, 1.20 mmol), and piperidine (0.53 mL, 5.25 mmol) as per **Method M**. Compound (**318**) was obtained as off-white colored solid (0.36 g, 71 %), m.p. 141-143 °C.

**Anal.:**

**TLC** : $R_f$ 0.67 (Chloroform-Methanol 18:2);

**IR (KBr, cm$^{-1}$)** : 3295, 3235, 3041, 2931, 1662, 1584, 1508, 1322, 836 and 742;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 9.27 (s, 1H, Ar*H*), 8.87 (d, 1H, Ar*H*), 8.11-8.13 (m, 1H, Ar*H*), 7.54-7.98 (m, 7H, Ar*H*), 7.30-7.33 (m, 1H, Ar*H*), 4.59 (q, *J* = 7.2 Hz, 2H, -NC$H_2$CH$_3$), 2.45-2.54 (m, 6H, -NC$H_2$), 2.13-2.24 (m, 2H, -NHCOC$H_2$), 1.78-1.90 (m, 2H, -NHCOCH$_2$C$H_2$), 1.62-1.77 (m, 6H, -NCH$_2$C$H_2$), 1.56 (t, *J* = 7.2 Hz, 3H, -NCH$_2$C$H_3$) and 1.41-1.51 (m, 2H, CH$_2$C$H_2$);

**MS (*m/z*)** : 478.03 $[M+H]^+$.

**RP-HPLC** : Purity: 98.1 %, $t_R$ = 4.05 min (Mobile phase: acetonitrile-water 80:20 (0.01 % TFA)).

### 5.1.2.2.6e. *N*-(9-Ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-yl)-6-(pyrrolidin-1-yl) hexanamide (320)

The title compound (**320**) was synthesized from 6-bromo-*N*-(9-ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-yl)hexanamide (**315**) (0.5 g, 1.02 mmol) and pyrrolidine (0.42 mL, 5.25 mmol) as per **Method M**. Compound (**320**) was obtained as off-white colored solid (0.37 g, 75.51%), m.p. 96-98 °C.

**Anal.:**

**TLC** : $R_f$ 0.43 (Chloroform-Methanol 16:1);

**IR (KBr, cm$^{-1}$)** : 3225, 3043, 2930, 2863, 1664, 1611, 1573, 1327, 839 and 745;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 10.19 (s, 1H, -N*H*CO), 9.55 (s, 1H,Ar*H*), 8.72 (d, 1H, Ar*H*), 7.22-8.24 (m, 9H, Ar*H*), 4.68-4.71 (m, 2H, $-NCH_2CH_3$), 2.41-2.79 (m, 6H, $-NCH_2$, 2H, $-NHCOCH_2$) and 1.25-2.07 (m, 6H, $-NCH_2CH_2$, 4H, $-COCH_2CH_2CH_2$, 3H, $-NCH_2CH_3$);

**MS (*m/z*)** : 478.03 $[M+H]^+$.

**RP-HPLC** : Purity: 98.9 %, $t_R$ = 4.20 min (Mobile phase: acetonitrile-water 80:20 (0.01 % TFA)).

### 5.1.2.2.6f. *N*-(9-Ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-yl)-6-(piperidin-1-yl) hexanamide (321)

The title compound (**321**) was synthesized from 6-bromo-*N*-(9-ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-yl)hexanamide (**315**) (0.5 g, 1.02 mmol) and piperidine (0.51 mL, 5.25 mmol) as per **Method M**. Compound (**321**) was obtained as off-white colored solid (0.37 g, 76 %), m.p. 98-100 °C.

**Anal.:**

**TLC** : $R_f$ 0.7 (Chloroform-Methanol 16:1);

**IR (KBr, cm$^{-1}$)** : 3222, 3044, 2930, 1663, 1611, 1572, 1328, 838 and 744;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 10.17 (s, 1H, -NHCO), 9.54 (s, 1H, Ar*H*), 8.71 (d, 1H, Ar*H*), 7.96-8.06 (m, 3H, Ar*H*), 7.74-7.88 (m, 4H, Ar*H*), 7.51-7.55 (m, 1H, Ar*H*), 7.19-7.23 (m, 1H, Ar*H*), 4.68 (q, 2H,

-$NCH_2CH_3$), 2.49-2.54 (m, 2H, -$NCH_2$), 2.32-2.47 (m, 4H, -$NCH_2$), 1.61-1.71 (m, 2H, -$COCH_2CH_2$), 1.45-1.59 (m, 4H, -$NCH_2CH_2$, 2H, -$NCH_2CH_2CH_2$) and 1.32-1.44 (m, 3H, -$NCH_2CH_3$, 4H, -$NCH_2CH_2CH_2$);

**MS (*m/z*)** : 492 $[M+H]^+$.

**RP-HPLC** : Purity: 97.6 %, $t_R$ = 4.52 min (Mobile phase: acetonitrile-water 80:20 (0.01 % TFA)).

### 5.1.2.2.7. General method E for the synthesis of 1-(9-ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-yl)aminoalkylurea (322-327)

**Method N:** To a stirring solution of 9-ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-amine (**312**) (0.5 g, 1.61 mmol) and triethylamine (0.25 mL, 1.77 mmol) in dry THF (20 mL), 4-nitrophenyl chloroformate (0.36 g, 1.77 mmol) in dry THF (10 mL) was added dropwise at 0-5 °C over a period of 15 min. The resulting reaction mixture was allowed to stir at room temperature for 2 hrs. The progress of the reaction was monitored by TLC. After complete consumption of the starting material, the respective amine (2.0 mmol) was added to the reaction mixture and the reaction was further stirred for 30 min. After the completion of the reaction, the solvent was removed at reduced pressure. The residue so obtained was triturated with chilled methanol (20 mL), filtered and dried to obtain the title compound.

### 5.1.2.2.7a. 1-(9-Ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-yl)-3-(2-(pyrrolidin-1-yl)ethyl)urea (322)

The title compound (**322**) was synthesized from 9-ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-amine (**312**) (0.5 g, 1.61 mmol) and 2-(aminoethyl) pyrrolidine (0.23 g, 2.01 mmol) as per **Method N**. Compound (**322**) was obtained as off-white colored solid (0.51 g, 70 %), m.p. 173-175 °C.

**Anal.:**

**TLC** : $R_f$ 0.51 (Chloroform-Methanol-Triethylamine 19:1:1);

**IR (KBr, cm$^{-1}$)** : 3305, 3046, 2930, 1694, 1652, 1592, 1323, 837 and 743;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 9.06 (s, 1H, Ar*H*), 8.83 (d, 1H, Ar*H*), 7.89-8.00 (m, 3H. Ar*H*), 7.87-7.98 (m, 3H, Ar*H*), 7.70-7.80 (m, 3H, Ar*H*), 7.49-7.57 (m, 2H, Ar*H*), 7.27-7.30 (m, 1H, Ar*H*), 5.52 (bs, 1H, -NHCON*H*), 4.56 (q, *J* = 7.2 Hz, 2H, -NC$H_2$), 3.38-3.42 (m, 2H, -NHCONHC$H_2$), 2.68 (t, 2H, -NC$H_2$), 2.51-2.58 (m, 4H, -NC$H_2$), 1.61-1.70 (m, 4H, -NCH$_2$C$H_2$) and 1.54 (t, *J* = 7.2 Hz, 3H, -NCH$_2$C$H_3$);

**MS (*m/z*)** : 451 $[M+H]^+$.

**RP-HPLC** : Purity: 98.6 %, $t_R$ = 3.82 min (Mobile phase: acetonitrile-water 80:20 (0.01 % TFA)).

### 5.1.2.2.7b. 1-(9-Ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-yl)-3-(2-(piperidin-1-yl)ethyl)urea (323)

The title compound (**323**) was synthesized from 9-ethyl-9*H*-naphtho [2,1-*c*]carbazol-2-amine (**312**) (0.5 g, 1.61 mmol) and 2-(aminoethyl)piperidine (0.26 g, 2.01 mmol) as per **Method N**. Compound (**323**) was obtained as off-white colored solid (0.58 g, 77.54%), m.p. 191-193 °C.

**Anal.:**

**TLC** : $R_f$ 0.62 (Chloroform-Methanol-Triethylamine 19:1:1);

**IR (KBr, cm$^{-1}$)** : 3317, 3044, 2932, 1691, 1649, 1592, 1323, 837 and 744;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 9.11 (s, 1H, Ar*H*), 8.83 (d, 1H, Ar*H*), 7.89-8.00 (m, 3H. Ar*H*), 7.81 (d, 1H, Ar*H*), 7.71-7.74 (m, 2H, Ar*H*), 7.50-7.58 (m, 2H, Ar*H*), 7.27-7.29 (m, 1H, Ar*H*), 5.60 (bs, 1H, -NHCON*H*), 4.56 (q, *J* = 7.2 Hz, 2H, -NC$H_2$), 3.35-3.39 (m, 2H, -NHCONHC$H_2$), 2.46 (t, 2H, -NC$H_2$), 2.34-2.38 (m, 4H, -NC$H_2$), 1.87-1.91 (m, 4H, -NCH$_2$C$H_2$), 1.54 (t, *J* = 7.2 Hz, 3H, -NCH$_2$C$H_3$) and 1.32-1.36 (m, 2H, -NCH$_2$CH$_2$C$H_2$);

**MS (*m/z*)** : 465.03 $[M+H]^+$.

**RP-HPLC** : Purity: 99.1 %, $t_R$ = 4.31 min (Mobile phase: acetonitrile-water 80:20 (0.01 % TFA)).

### 5.1.2.2.7c. 1-(9-Ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-yl)-3-(3-(pyrrolidin-1-yl)propyl)urea (324)

The title compound (**324**) was synthesized from 9-ethyl-9*H*-naphtho [2,1-*c*]carbazol-2-amine (**312**) (0.5 g, 1.61 mmol) and 3-(aminopropyl) pyrrolidine (0.26 g, 2.01 mmol) as per **Method N**. Compound (**324**) was obtained as off-white colored solid (0.51 g, 70 %), m.p. 105-107 °C.

**Anal.:**

**TLC** : R*f* 0.43 (Chloroform-Methanol-Triethylamine 19:1:1);

**IR (KBr, cm$^{-1}$)** : 3302, 3044, 2963, 1658, 1327, 1237, 837 and 745;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 9.12 (d, 1H, Ar*H*), 8.84 (d, 1H, Ar*H*), 7.88-7.97 (m, 3H. Ar*H*), 7.81 (d, 1H, Ar*H*), 7.71-7.76 (m, 2H, Ar*H*), 7.51-7.59 (m, 2H, Ar*H*), 7.27-7.30 (m, 1H, Ar*H*), 5.95 (bs, 1H, -NHCON*H*), 4.58 (q, *J* = 7.2 Hz, 2H, $-NCH_2$), 3.42-3.45 (m, 2H, $-NHCONHCH_2$), 2.55-2.60 (m, 2H, $-NCH_2$), 2.39-2.42 (m, 4H, $-NCH_2$), 1.70-1.74 (m, 6H, $-NCH_2CH_2$) and 1.55 (t, *J* = 7.2 Hz, 3H, $-NCH_2CH_3$);

**MS (*m/z*)** : 465.03 $[M+H]^+$.

**RP-HPLC** : Purity: 98.7 %, $t_R$ = 3.91 min (Mobile phase: acetonitrile-water 80:20 (0.01 % TFA)).

### 5.1.2.2.7d. 1-(9-Ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-yl)-3-(3-(piperidin-1-yl)propyl)urea (325)

The title compound (**325**) was synthesized from 9-ethyl-9*H*-naphtho [2,1-*c*]carbazol-2-amine (**312**) (0.5 g, 1.61 mmol) and 3-(aminopropyl) piperidine (0.29 g, 2.01 mmol) as per **Method N**. Compound (**325**) was obtained as off-white colored solid (0.5 g, 65 %), m.p. 99-101 °C.

**Anal.:**

**TLC** : $R_f$ 0.51 (Chloroform-Methanol-Triethylamine 19:1:1);

**IR (KBr, cm$^{-1}$)** : 3297, 3045, 2930, 1658, 1572, 1325, 1236, 836 and 745;

**$^1$H NMR (DMSO-*d*$_6$)** : $\delta$ 9.13 (s, 1H, Ar*H*), 8.83 (d, 1H, Ar*H*), 7.88-7.96 (m, 3H. Ar*H*), 7.80 (d, 1H, Ar*H*), 7.69-7.74 (m, 2H, Ar*H*), 7.45-7.57 (m, 2H, Ar*H*), 7.26-7.29 (m, 1H, Ar*H*), 6.24 (bs, 1H, -NHCON*H*), 4.55 (q, *J* = 7.2 Hz, 2H, $-\mathrm{NC}H_2$), 3.36-3.39 (m, 2H, $-\mathrm{NHC}H_2$), 2.40-2.43 (m, 2H, $-\mathrm{NC}H_2$), 2.22-2.32 (m, 4H, $-\mathrm{NC}H_2$), 1.90-2.00 (m, 6H, $-\mathrm{NCH_2C}H_2$) and 1.24-1.38 (m, 3H, $-\mathrm{NCH_2C}H_3$, 2H, $-\mathrm{NCH_2CH_2C}H_2$);

**MS (*m/z*)** : 479.02 [M+H]$^+$.

**RP-HPLC** : Purity: 98.1 %, $t_R$ = 4.42 min (Mobile phase: acetonitrile-water 80:20 (0.01 % TFA)).

### 5.1.2.2.7e. 1-(9-Ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-yl)-3-(4-(pyrrolidin-1-yl)butyl)urea (326)

The title compound (**326**) was synthesized from 9-ethyl-9*H*-naphtho [2,1-*c*]carbazol-2-amine (**312**) (0.5 g, 1.61 mmol) and 4-(aminobutyl) pyrrolidine (0.29 g, 2.01 mmol) as per **Method N**. Compound (**326**) was obtained as off-white colored solid (0.51 g, 70 %), m.p. 117-119 °C.

**Anal.:**

**TLC** : $R_f$ 0.49 (Chloroform-Methanol-Triethylamine 19:1:1);

**IR (KBr, cm$^{-1}$)** : 3305, 3046, 2961, 1647, 1554, 1307, 1236, 827 and 751;

**$^1$H NMR (DMSO-*d*$_6$)** : $\delta$ 9.13 (d, 1H, Ar*H*), 8.70-8.73 (m, 1H, Ar*H*), 7.87-8.00 (m, 4H. Ar*H*), 7.78-7.81 (m, 1H, Ar*H*), 7.70 (d, 1H, Ar*H*), 7.50-7.55 (m, 1H, Ar*H*), 7.22-7.26 (m, 1H, Ar*H*), 6.25 (t, 1H, -NHCON*H*), 4.67 (q, *J* = 7.2 Hz, 2H, $-\mathrm{NC}H_2$), 3.10-3.15 (m, 2H, -NHCONHC$H_2$), 2.53-2.62 (m, 2H, -NC$H_2$), 2.30-2.40 (m, 4H, -NC$H_2$), 1.46-1.50 (m, 6H, -NCH$_2$C$H_2$) and 1.36-1.42 (m, 3H, -NCH$_2$C$H_3$, 2H, -NCH$_2$CH$_2$C$H_2$);

**MS (*m/z*)** : 479.03 $[M+H]^+$.

**RP-HPLC** : Purity: 99.2 %, $t_R$ = 3.72 min (Mobile phase: acetonitrile-water 80:20 (0.01 % TFA)).

### 5.1.2.2.7f. 1-(9-Ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-yl)-3-(4-(piperidin-1-yl)butyl)urea (327)

The title compound (**327**) was synthesized from 9-ethyl-9*H*-naphtho [2,1-*c*]carbazol-2-amine (**312**) (0.5 g, 1.61 mmol) and 4-(aminobutyl) piperidine (0.32 g, 2.01 mmol) as per **Method N**. Compound (**327**) was obtained as off-white colored solid (0.56 g, 70 %), m.p. 127-129 °C.

**Anal.:**

**TLC** : $R_f$ 0.57 (Chloroform-Methanol-Triethylamine 19:1:1);

**IR (KBr, cm$^{-1}$)** : 3289, 3045, 2931, 1658, 1556, 1327, 1236, 836 and 744;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 9.06 (s, 1H, Ar*H*), 8.80 (d, 1H, Ar*H*), 7.04-7.94 (m, 9H, Ar*H*), 4.55 (q, $J$ = 7.2 Hz, 2H, -NC$H_2$CH$_3$), 3.25-3.28 (m, 2H, -NHC$H_2$), 2.29-2.43 (m, 6H, -NC$H_2$), 1.90-1.95 (m, 6H, -NCH$_2$C$H_2$), 1.53-1.59 (m, 3H, -NCH$_2$C$H_3$, 2H, -NCH$_2$CH$_2$C$H_2$) and 1.39-1.44 (m, 2H, -C$H_2$);

**MS (*m/z*)** : 493.10 $[M + H]^+$.

**RP-HPLC** : Purity: 98.3 %, $t_R$ = 4.02 min (Mobile phase: acetonitrile-water 80:20 (0.01 % TFA)).

### 5.1.2.2.8. General method for the synthesis of 1-(9-ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-yl)aminopropylthioureas (328, 329)

**Method O.** To a stirring solution of 9-ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-amine (**312**) (0.5 g, 1.61 mmol) and triethylamine (0.25 mL, 1.77 mmol) in dry 1:1 THF/DCM mixture (20 mL), thiocarbonyldiimidazole (0.29 g, 1.77 mmol) in 1:1 THF/DCM mixture (10 mL) was added drop wise at 0-5 °C over a period of 15 min. The resulting reaction mixture was allowed to stir at room temperature for 2 hrs. The progress of the reaction was monitored by TLC. After complete consumption of the starting material, appropriate amine (2 mmol) was added to the reaction mixture and the reaction was further stirred for 30 min. After the completion of the reaction, the solvent was recovered at reduced pressure. The residue so obtained was triturated with chilled methanol (20 mL). The solid was collected by filtration, washed again with chilled methanol (10 mL) and dried to give the titled compound.

### 5.1.2.2.8a. 1-(9-Ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-yl)-3-(3-(pyrrolidin-1-yl)propyl)thiourea (328)

The title compound (**328**) was synthesized from 9-ethyl-9*H*-naphtho [2,1-*c*]carbazol-2-amine (**312**) (0.5 g, 1.61 mmol) and 3-(aminopropyl) pyrrolidine (0.26 g, 2.01 mmol) as per **Method O**. Compound (**328**) was obtained as off-white colored solid (0.62 g, 79 %), m.p. 199-201 °C.

**Anal.:**

**TLC** : R$_f$ 0.46 (Chloroform-Methanol 19:1);

**IR (KBr, cm$^{-1}$)** : 3171, 3042, 2692, 2868, 1586, 1526, 1328, 1239, 837 and 750;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 9.73 (s, 1H, -N$H$CSNH), 9.06 (d, 1H, Ar$H$), 8.67 (s, 1H, -N$H$CSNH), 7.96-8.05 (m, 4H, Ar$H$), 7.89 (d, 1H, Ar$H$), 7.74-7.77 (m, 2H, Ar$H$), 7.66 (dd, 1H, Ar$H$), 7.47-7.50 (m, 1H, Ar$H$), 7.17-7.20 (m, 1H, Ar$H$), 4.63 (q, 2H, -NC$H_2$CH$_3$), 3.48-3.55 (m, 2H, -NHC$H_2$), 2.27-2.37 (m, 2H, -NC$H_2$), 2.12-2.26 (m, 4H, -NC$H_2$), 1.60-1.62 (m, 2H, -NCH$_2$C$H_2$) and 1.34-1.38 (m, 3H, -NCH$_2$C$H_3$, 4H, -NCH$_2$C$H_2$);

**MS (*m/z*)** : 481 [M+H]$^+$.

**RP-HPLC** : Purity: 99.0 %, t$_R$ = 3.78 min (Mobile phase: acetonitrile-water 80:20 (0.01 % TFA)).

### 5.1.2.2.8b. 1-(9-Ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-yl)-3-(3-(piperidin-1-yl)propyl)thiourea (329)

The title compound (**329**) was synthesized from 9-ethyl-9*H*-naphtho [2,1-*c*]carbazol-2-amine (**312**) (0.5 g, 1.61 mmol) and 3-(aminopropyl) piperidine (0.29 g, 2.01 mmol) as per **Method O**. Compound (**329**) was obtained as off-white colored solid (0.59 g, 74 %), m.p. 148-151 °C.

**Anal.:**

**TLC** : $R_f$ 0.65 (Chloroform-Methanol-Triethylamine 19:1:1);

**IR (KBr, cm$^{-1}$)** : 3136, 3043, 2930, 1583, 1328, 1245, 838 and 750;

**$^1$H NMR (DMSO-$d_6$)** : $\delta$ 9.74 (s, 1H, -N*H*CSNH), 9.07 (s, 1H, Ar*H*), 8.67 (d, 1H, Ar*H*), 7.95-8.02 (m, 2H. Ar*H*), 7.66-7.89 (m, 4H, Ar*H*), 7.66 (d, 1H, Ar*H*), 7.47-7.50 (m, 1H, Ar*H*), 7.17-7.20 (m, 1H, Ar*H*), 4.63 (q, *J* = 7.2 Hz, 2H, $-NCH_2CH_3$), 3.42-3.48 (m, 2H, $-NHCSNHCH_2$), 2.15-2.19 (m, 6H, $-NCH_2$), 1.59-1.62 (m, 2H, $-NHCH_2CH_2$), 1.35 (t, *J* = 7.2 Hz, 3H, $-NCH_2CH_3$) and 1.20-1.33 (m, 6H, $-CH_2$);

**MS (*m/z*)** : 495 $[M+H]^+$.

**RP-HPLC** : Purity: 98.3 %, $t_R$ = 4.11 min (Mobile phase: acetonitrile-water 80:20 (0.01 % TFA)).

## 5.2. Biological studies

### 5.2.1. Inhibition studies on AChE and BuChE

The potential of the test compounds to inhibit ChEs was assessed using the Ellman's method as detailed in our earlier report.[115-117] Human AChE (product number C1682), equine serum BuChE (product number C1057), 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB, product number D8130), acetylthiocholine iodide (ATCI, product number A5751), and butyrylthiocholine iodide (BTCI, product number B3253) were purchased from Sigma-Aldrich. Donepezil hydrochloride and tacrine hydrochloride hydrate were used as standard drugs. All the experiments were carried out in 50 mM

Tris-HCl buffer at *p*H 8. Five different concentrations (0.001−100 μM) of each test compound were used to determine the enzyme inhibition activity. Briefly, 50 μL of AChE (0.22 U/mL) or 50 μL of BuChE (0.06 U/mL) and 10 μL of the test or standard compounds were incubated in 96-well plates at room temperature for 30 min. Further, 30 μL of the substrate, namely, ATCI (15 mM) or BTCI (15 mM), was added, and the solution was incubated for additional 30 min. Finally, 160 μL of DTNB (1.5 mM) was added to it, and the absorbance was measured at 415 nm wavelength using the microplate reader 680 XR (BIO-RAD, India). The $IC_{50}$ values were determined from the absorbance obtained for various concentrations of the test and the standard compounds. The $IC_{50}$ value recounts the concentration of the drug required to inhibit the enzyme activity by 50 %. All determinations were performed in triplicate and at least in three independent runs.

### 5.2.2. Antioxidant activity [1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity]

The spectrophotometric DPPH assay was carried out as described earlier.[120] Concentrations of the selected test compounds that exhibited promising neuroprotective effects against $H_2O_2$ 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 (10 mM in methanol) in a 96-well plate. Finally, the volume was adjusted to 200 μL using methanol. After a 30 s incubation at room temperature protected from light, the absorbance was noted at 520 nm wavelength using a microplate reader 680 XR (BIORAD, India). The free radical scavenging activity was determined as the reduction percentage (RP) of DPPH using the equation $RP = 100[(A_0 - A_C)/A_0]$, where $A_0$ is the untreated DPPH absorbance and $A_C$ is the absorbance value for the added sample concentration. Ascorbic acid was used as the standard antioxidant.

### 5.2.3. Self-induced $A\beta_{1\text{-}42}$ aggregation inhibition study

The potential of compounds to inhibit self-mediated $A\beta_{1\text{-}42}$ aggregation was assessed by a thioflavin T (ThT)-based fluorescence assay.[165] 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP)-pretreated $A\beta_{1-42}$ peptide (Sigma Aldrich) was resolubilized with a 50 mM phosphate buffer (*p*H = 7.4) to obtain a stable stock solution (100 μM).The assay was conducted in Costar, black-surround, clearbottom 96-well plates. For the experiment, the A$\beta_{1-42}$ stock solution was further diluted to 50 μM with a 50 mM phosphate buffer (*p*H = 7.4) before use. A mixture of the peptide (10 μL) with or without the test compounds (10 μL) at 50 μM and 100 μM concentrations were incubated at 37 °C temperature for 48 hrs with frequent shaking. Blanks using 50 mM phosphate buffer (pH = 7.4) instead of a peptide with or without test compounds were also carried out. After incubation, the samples were diluted with 180 μL of thioflavin T (5 μM, in 50 mM glycine-NaOH buffer, *p*H = 8). The fluorescence intensities were measured on a SpectraMax iD3 multi-mode microplate reader with 446 nm excitation wavelength and 490 nm emission wavelengths. Each test compound was examined in duplicate. The fluorescence intensities were compared and the percent inhibition due to the presence of the test compound was calculated by the equation: % inhibition = $(1-IF_i/IF_0) \times 100$, where $IF_i$ and $IF_0$ are the fluorescence intensities obtained for A$\beta_{1-42}$ in the presence and the absence of test compound, respectively.

### 5.2.4. Metal-chelating study

The metal chelating ability of selected compounds was assessed using UV spectrophotometry.[166] The absorption spectra of test compounds and 8-hydroxyquinoline (25 μM, final concentration) alone or in the presence of $CuSO_4$, $ZnCl_2$, $FeSO_4$, $FeCl_3$, $AlCl_3$, $MgSO_4$ or $CaCl_2$ (25 μM, final concentration) in methanol for 30 min were recorded at room temperature at wavelengths ranging from 200 to 600 nm.

### 5.2.5. Cell Culture Studies[115]

The human neuroblastoma SH-SY5Y cell line was procured from the National Centre for Cell Science (NCCS) (Pune, India). Cells were routinely cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1 mM glutamine, 50 U/mL penicillin, and 50 μg/mL streptomycin (reagents from Gibco) at 37 °C in a humidified incubator at 5% $CO_2$. All cells used in the study were of the low passage number (<15).

### 5.2.6. 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.[115] SH-SY5Y cells were seeded in 96-well plate at a density of 5 × $10^4$ cells per well. After 24 hrs, the medium was replaced with relatively higher concentrations of test compound (40 μM and 80 μM) for another 24 hrs at 37 °C. After the incubation period, the cell viability was determined using MTT assay. In another set of experiments, the test compounds were assessed for their ability to protect SH-SY5Y cells against oxidative damage induced by $H_2O_2$ and $A\beta_{1-42}$. Cells were exposed to the test compounds at relatively low concentrations (5, 10 and 20 μM) and incubated for 2 hrs. After the incubation period, the test compounds were replaced with a medium containing cytotoxic insult, i.e., $H_2O_2$ (100 μM) or $A\beta_{1-42}$ (25 μM) and left for an additional 24 hrs period. Thereafter, the 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, final concentration; Sigma) in PBS. After 4 hrs, MTT was removed and the crystals of formazan were dissolved by adding DMSO in the medium. Formazan concentrations were quantified at 570 nm with 630 nm reference wavelengths using a microplate reader 680 XR (BIO-RAD, India). Percentage protection against $H_2O_2$ and $A\beta_{1-42}$ insults was calculated by considering absorbance of the control cells as 100% of the cell viability.

### 5.2.7. *In vitro* blood-brain barrier permeation assay

The PAMPA assay was performed to predict the BBB permeation of the most potent compounds. The donor microplates (PVDF membrane, pore size 0.45 μm) and the acceptor microplates were obtained from the Millipore. Porcine brain lipid (PBL) was purchased from Avanti Polar Lipids. The filter surface of the donor microplate was first impregnated with 4 μL of porcine brain lipid (20 mg in 1 mL dodecane). The acceptor microplate was filled with 200 μL of phosphate buffer saline (PBS)/ethanol (70:30). The test compounds (5 mg/mL) were dissolved in DMSO and diluted with PBS/ethanol (70:30) to get a final concentration of 100 μg/mL. 200 μL of the solution was filled in the donor well and the donor plate was carefully placed on the acceptor plate to form a sandwich and incubated for 120 min at 25 °C. After the incubation period, the donor plate was removed and the concentration of the test compounds in the acceptor wells was determined using UV spectrophotometry.[115] Each sample was analyzed at five different wavelengths, in four wells, and at least in three independent runs. The results are expressed as mean ± SEM. Pe was calculated using the following equation:

$$Pe = -\left(\frac{Vd \times Va}{(Vd + Va)A \times t}\right) \times \ln\left(1 - \frac{(\text{drug})acceptor}{(\text{drug})equilibrium}\right)$$

where $V_d$ and $V_a$ are the volumes in the donor well and acceptor well, respectively, A is the filter area, t is the permeation time, $(\text{drug})_{\text{acceptor}}$ is the concentration of the test compound in the acceptor well, and $(\text{drug})_{\text{equilibrium}}$ is the theoretical equilibrium concentration. In the experiment, seven commercial drugs of known BBB permeability were included to validate the analysis set. A plot of the $P_e$(exp) versus $P_e$(ref) values gave a strong linear correlation, $P_e$(exp) = 1.16$P_e$(ref) + 0.1668 ($R^2$ = 0.9781).

### 5.2.8. Assessment of Cognitive Improvement in an Animal Model of AD

#### 5.2.8.1. Morris Water Maze Test[116]

Adult male Swiss Albino mice (20-25 g) were divided into five groups of seven animals each as per the given treatment (normal control, vehicle-treated control, donepezil at 5 mg/kg, compound at 5 and 10 mg/kg). Scopolamine hydrochloride (1.4 mg/kg) was dissolved in saline and administered intraperitoneally (ip) to the animals of all groups except the vehicle-treated control group, which received an equal volume of saline. Donepezil at 5 mg/kg and compound at 5 and 10 mg/kg in 0.5 % CMC were administered orally 30 min prior to administration of scopolamine to the respective experimental group animals. All these treatments were continued for 14 consecutive days. During the last 5 days of the treatment period, spatial learning and memory were assessed using the Morris water maze (MWM) test. A maze consisting of a circular pool (65 cm diameter; 30 cm height) was filled with turbid 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. Experiments on the individual animals were carried out to determine the time required by the animal to reach the hidden platform (i.e., escape latency time, ELT) which assesses the spatial learning and memory. All of the experiments were carried out in a soundproof room and supervised by a blind observer.

### 5.2.8.2. Neurochemical analysis[116]

After completing the MWM test, the animals were sacrificed and the whole brain was isolated from the skull and homogenized in a glass Teflon homogenizer in 12.5 mM sodium phosphate buffer (*p*H 7). The homogenates were centrifuged at 15,000 rpm for 15 min at 4 °C. The supernatants were utilized for estimations of different biochemical parameters. The cholinergic biomarkers AChE and BuChE were estimated in the mice brain using Ellman's method. A volume of 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 noted at 415 nm wavelength spectrophotometrically. MDA, an indicator of lipid peroxidation, was estimated using the thiobarbituric acid reacting substance (TBARS) method as described earlier.

MDA reacted with thiobarbituric acid in acidic medium at high temperature and formed a red complex TBARS which was analyzed spectrophotometrically. Briefly, 200 µL of the 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 15000 rpm for 10 min and the supernatants were read at 532 nm wavelength. Catalase (CAT) is an enzyme mediating breakdown of $H_2O_2$, a toxic form of oxygen metabolite into oxygen and water.

CAT activity was determined following the method described by Sinha et al. Briefly, 100 µL of the supernatant was mixed with 150 µL of 0.01 M phosphate buffer (*p*H 7). Reaction was started by the addition of 250 µL of $H_2O_2$ (0.16 M), the medium was incubated at 37 °C for 1 min, and the reaction was stopped by the addition of 1 mL of dichromate/acetic acid reagent (5% $K_2Cr_2O_7$/glacial acetic acid; 1:3 v/v). The reaction mixture was immediately kept in a boiling water bath for 15 min that resulted in the development of a green color. Finally, the mixture was analyzed at 570 nm wavelength spectrophotometrically.

In order to extract glycine and glutamate, 10 µL aliquots of hypothalamus homogenate were thawed and then mixed with 0.17 M perchloric acid (10 % w/v). The mixture was left for 20 min at 4 °C. The resulting supernatant was decanted in a separate Eppendorf tube and centrifuged at 4 °C for 20 min at

15000 rpm. After centrifugation, the supernatant fraction was separated and maintained at -70 °C until determination of free amino acids by HPLC coupled with an electrochemical detector (model no. Waters 2465, Waters Corporation, Milford, MA). Mobile phase was prepared by dissolving monosodium phosphate (0.1 M), EDTA (0.5 mM) and potassium chloride (2 mM) in HPLC grade methanol (25% v/v), diluted with distillated water. The solution *p*H was adjusted to 4.5. The mobile phase was filtered twice and degassed in an ultrasonic bath for 20 min, before circulation in the HPLC system. Analysis was performed on a Welchrom C18 column with 1.2 mL/min flow rate, 3000 psi pressure, and 850 mV working potential of the detector. The internal standards were prepared by mixing 200 μL of 1 mM stock of glycine/glutamate in 200 μL of pooled supernatant and diluted with 600 μL of mobile phase. It was used with an equimolar concentration of derivatizing agent [22 mg of *o*-phthalaldehyde dissolved in 500 μL of absolute ethanol, 500 μL of 1 M sodium sulfite, and 900 μL of 0.1 M tetraborate buffer, adjusted to *p*H 10.4 with 5 M sodium hydroxide] to make final concentrations of 2, 1, 0.5, 0.4, 0.2, and 0.1 μmol/L, and 200 μL of which was injected into the HPLC column. The sample solutions were prepared by diluting 200 μL of homogenate in 800 μL of mobile phase; 10 μL of it was mixed with 10 μL of derivatizing agent and diluted in 880 μL of mobile phase. The concentration of neurotransmitter in the brain was estimated by comparing the area with the calibration curve.

#### 5.2.8.3. Y-Maze test[116]

Adult male Wistar rats (150−200 g) were divided into five experimental groups of seven animals each as per the given treatment: normal control, vehicle-treated control, donepezil at 5 mg/kg, and compound at 5 and 10 mg/kg. The animals were anesthetized with ketamine (100 mg/kg, ip) and xylazine (30 mg/kg, ip) and mounted in a stereotaxic apparatus (Stoelting). All of the groups (except the vehicle-treated control group which received an equal volume of normal saline) were injected with 2 μL of A$\beta_{1-42}$ (4 μM/μL in normal saline) unilaterally at the following coordinates: -4.0 mm anteroposterior, -2.5 mm mediolateral, and -3.5 mm dorsoventral from bregma. Donepezil at 5 mg/kg and the test compound at 5 and 10 mg/kg in 0.1% CMC were administered perorally to the respective experimental group animals for 15 consecutive days after 5 days of surgical recovery. The Y-maze test was adopted for the assessment of immediate working memory. The test was performed during the last 5 days of the treatment period in the animals which were subjected to icv injection of $A\beta_{1-42}$. 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:

% spontaneous alteration performance = [No of altered entries/ No. of repeated entries-2] × 100

### 5.2.9. Acute toxicity study[131]

Wistar female rats (200-250 g) were used to determine the acute toxicity of the test compound. During the experiment, animals were maintained with free access of food and water ad libitum. Test compound was suspended in 0.5 % CMC-Na and given orally to the experimental groups. After administration of the test compound, the animals were observed continuously for the first 4 hrs for any abnormal behavior and mortality. Afterward, the animals were observed intermittently for the next 24 hrs and occasionally for 14 consecutive days after administration of test compound. After 14 days, the animals were sacrificed and examined macroscopically for possible damage to the heart, liver, and kidneys.

## 5.3. Computational Studies

### 5.3.1. Docking Studies

#### 5.3.1.1. Docking studies of the compounds with ChEs

Docking studies were performed with Glide module of Schrodinger Suite. Glide is projected for screening of probable ligands based on binding mode and affinity for a given receptor molecule. It performs grid-based ligand docking and searches for promising interactions between ligand molecules and a macromolecule, typically a protein. The 3D structures of ligand molecules were built within Maestro93 using the Build module, and a single low energy conformation search was carried out for all molecules under study using the OPLS-2005 force field at physiological *p*H conditions using the LigPrep module of Schrödinger, keeping all parameters at standard values. The 3D crystallographic structures of AChE (PDB code: 2CKM, 1B41) and of BuChE (PDB code: 4BDS) were obtained from the RCSB Protein Data Bank[132] and prepared for docking with the protein preparation wizard of Schrödinger. The grid was generated on the active site of the respective receptor structure and was validated by redocking the preexisting cocrystallized ligand structures. Docking calculations for the minimized 3D ligand structures were performed in extra precision (XP) mode within the active sites of the receptor structures. This docking protocol was validated by comparing the interactions of the docked conformer of donepezil in the active site of AChE with the reported literature.

### 5.3.1.2. Docking studies of compound with $A\beta_{1-42}$

The docking studies were carried out by using AutoDock4.2. $A\beta_{1-42}$ peptide structure was obtained from RCSB (PDB Code: 1IYT)[132] and was cleaned and prepared for docking in AutoDock. The docking study of the compound was carried out by the blind docking method. Grid was generated over the entire protein structure and ligand under study was allowed to interact with the entire sequence to know the most stable/possible interactions between the ligand and the target protein. For the ligand-receptor complex, 10 docking experiments were carried out using the Lamarckian genetic algorithm. The maximum number of energy evaluations of 25 million was applied for each docking experiment.

### 5.3.2. Molecular dynamics simulations

Maestro-Desmond (based on the OPLS-2005 force field) was used to perform the molecular dynamics simulation study of the compound (**199**). To begin, the system was solvated with the TIP3P water model in an orthorhombic box of 10 $Å^3$ and neutralized by $Na^+$ ions for AChE and by $Cl^-$ ions for BuChE. The long-range electrostatic interactions were studied by smooth particle-mesh Ewald (PME) approximation, and the nonbonded interactions were analyzed by using a MSHAKE algorithm with a cutoff of 9 Å. The default “six step relaxation protocol” was followed to relax the system, which was involved with both restrained as well as unrestrained minimizations (2 steps) chased by the equilibration processes (4 steps). The productive MD was carried out for 20 ns with the NPT ensemble at 300 K and 1 atm pressure using a Nose-Hoover thermostat and Martyna-Tuckerman-Klein barostat. The coordinates and energies were noted at every 10 and 1.2 ps, respectively. The ligand−protein complex stability was analyzed by determining the root-mean-square deviation of the protein (RMSDP), root-mean-square fluctuation of the protein (RMSF-P), root mean-square deviation of the ligand (RMSD-L), molecular surface area (MolSA), solvent-accessible surface area (SASA), radius-of gyration (rGyr), and polar surface area (PSA).

### 5.3.3. *In silico* prediction of physicochemical and pharmacokinetics parameters of the compounds

*In silico* prediction of physicochemical and pharmacokinetic properties was carried out with QikProp program v 3.5 (Schrodinger LLC, New York).[136] The structures of the ligand molecules were built within Maestro using the Build module, and a single low energy conformation search was carried out for all molecules under study using OPLS_2005 force field at physiological pH condition using LigPrep module of Schrödinger. These structures were used for Qik-Prop to determine various physicochemical and pharmacokinetic descriptors. There were 51 properties predicted by the software. The major descriptors that were considered in this study were Lipinski’s rule of five (Rule of 5); NRB, number of rotatable bonds; PSA, polar surface area; SASA, total solvent accessible surface area; CNS, predicted central nervous system activity; QPPMDCK, predicted apparent MDCK cell permeability; QPPCaco, Caco-2 cell permeability in nm/s; QPlogBB, brain/blood partition coefficient; QPlogKhsa, binding to human serum albumin; QPlogS, predicted aqueous solubility; and percent human-oral Absorption, human oral absorption.