5. EXPERIMENTAL

All the required chemicals were procured from Sigma, Spectrochem or Avra synthesis Pvt. Ltd. and used as per requirement. Completion of the reactions was monitored by thin layer chromatography on Merck pre-coated silica gel F_{254} plates. Compounds were purified by column chromatography using appropriate eluent. The melting points of the synthesized compounds were checked on Veego VMP – D digital melting point apparatus by open capillary method and are uncorrected. Representative compounds from the synthesized series with melting point above 250 °C were checked by DSC method. The IR spectra of were recorded on Bruker FT-IR (IR affinity-1 ATR MIRACLE-10) in potassium bromide. The ^{1}H-NMR spectra were recorded in DMSO-d_6 using Bruker AVANCE II 400 NMR spectrometer with tetramethylsilane (TMS) as internal standard and the values are reported in δ ppm. ^{13}C-NMR spectra were recorded using a JEOL-ECX 500 FT (125 MHz) instrument in DMSO-d_6. The mass spectra were recorded on 410 Prostar Binary LC with 500 MS IT PDA detector. Elemental analysis of first series was performed on FLASH EA 1112, Thermo-Finnigan and indicated with the element symbol. The elemental analyses were within ± 0.4% of the theoretical values.

The experimental work has been divided into three parts:

5.1: Chemical work
5.2: Pharmacological work
5.3: Docking studies

5.1: Chemical work

5.1.1. Synthesis of isoalloxazine derivatives (10-substituted benzo[*g*]pteridine-2,4(3*H*,10*H*)-dione) (Series I) (38 – 55)

Synthesis of alloxan monohydrate (2)

Barbituric acid (0.01 M) was added portion-wise to a solution of chromium trioxide (0.015 M) in glacial acetic acid (9 ml) and water (10 ml) at room temperature over a period of half an hour with stirring. Temperature was not allowed to rise 50 °C during addition. Crystals of alloxan monohydrate started appearing in the reaction mixture during the addition of barbituric acid. The

reaction mixture was raised to 50 °C for next 30 minutes. After it was cooled to 10 °C, the precipitated product was filtered, washed with 50% acetic acid in water till the filtrate became colorless, followed by washing with solvent ether. The product so obtained was dried to afford the desired compound (**2**), yield 70%, m.p. 252-54 °C (Lit[132a] 254 °C).

Synthesis of *N*-alkyl/arylalkyl 2-nitroanilines (4 – 20)

Suitable amine (0.01 M) was added to a mixture of K_2CO_3 (0.015 M) in DMF (10 ml). To this mixture on stirring, 1-fluoro-2-nitrobenzene (0.01 M) was added with stirring and the reaction was allowed to complete at 60 °C. With different amines, the reactions were completed in 3-5 hours. The reaction mixture was allowed to cool to room temperature and poured into ice cold water; the precipitated solid product was filtered and washed with water. For *n*-propyl and *n*-butyl amines the reaction temperature was kept at 40 °C and workup of the reaction mixtures were carried out by extraction with ethyl acetate (3x20ml). The combined organic portion was washed 4-5 times with 50 ml of water to completely remove any traces of DMF and the organic layer after drying and evaporation afforded the desired products.

***N*-Propyl-2-nitroaniline (4)**

Anal.:

Yield : 73%

TLC : R_f 0.47 (5:1, *n*-Hexane: Ethyl acetate)

IR : 3392, 3052, 1583, 1502, 1354, 1268 and 776 cm^{-1}

***N*-Butyl-2-nitroaniline (5)**

Anal.:

Yield : 69%

TLC : R_f 0.53 (5:1, *n*-Hexane: Ethyl acetate)

IR : 3342, 2932, 1604, 1570, 1516, 1347, 1272 and 744 cm^{-1}

***N*-Allyl-2-nitroaniline (6)**

Anal.:

Yield : 76%

M.P. : 53-54 °C (Lit.[132b]: 53 °C)

TLC : R_f 0.55 (5:1, *n*-Hexane: Ethyl acetate)

IR : 3389, 2925, 1608, 1533, 1355, 1142 and 747 cm^{-1}

_N_-Benzyl-2-nitroaniline (7)

Anal.:

Yield : 83%

M.P. : 68-70 °C (Lit.[132c]: 69-72 °C)

TLC : R_f 0.48 (5:1, *n*-Hexane: Ethyl acetate)

IR : 3389, 2980, 1616, 1571, 1510, 1328, 1151 and 740 cm^{-1}

_N_-(3-Methylbenzyl)-2-nitroaniline (8)

Anal.:

Yield : 86%

M.P. : 70-72 °C

TLC : R_f 0.68 (5:1, *n*-Hexane: Ethyl acetate)

IR : 3390, 2980, 2889, 1618, 1570, 1510, 1327, 1236 and 742 cm^{-1}

_N_-(4-Methylbenzyl)-2-nitroaniline (9)

Anal.:

Yield : 81%

M.P. : 69-71 °C (Lit.[132d]: 65 °C)

TLC : R_f 0.71 (5:1, *n*-Hexane: Ethyl acetate)

IR : 3387, 2980, 1614, 1570, 1502, 1328, 1149 and 746 cm^{-1}

_N_-(2-Fluorobenzyl)-2-nitroaniline (10)

Anal.:

Yield : 79%

M.P. : 83-85 °C

TLC : R_f 0.7 (3:1, *n*-Hexane: Ethyl acetate)

IR : 3385, 2980, 1612, 1571, 1483, 1327, 1153 and 742 cm^{-1}

_N_-(3-Fluorobenzyl)-2-nitroaniline (11)

Anal.:

Yield : 84%

M.P. : 68-70 °C

TLC : R_f 0.56 (5:1, *n*-Hexane: Ethyl acetate)

IR : 3364, 2980, 1614, 1571, 1510, 1338, 1153 and 740 cm^{-1}

_N_-(3-Chlorobenzyl)-2-nitroaniline (12)

Anal.:

Yield : 82%

M.P. : 70-71 °C

TLC : R_f 0.53 (5:1, *n*-Hexane: Ethyl acetate)

IR : 3376, 2981, 1614, 1571, 1507, 1342, 1153 and 740 cm^{-1}

_N_-(4-Chlorobenzyl)-2-nitroaniline (13)

Anal.:

Yield : 87%

M.P. : 70-72 °C

TLC : R_f 0.57 (5:1, *n*-Hexane: Ethyl acetate)

IR : 3379, 2980, 2889, 1614, 1571, 1508, 1363, 1153 and 742 cm^{-1}

_N_-(2-Methoxybenzyl)-2-nitroaniline (14)

Anal.:

Yield : 78%

M.P. : 101-102 °C

TLC : R_f 0.57 (5:1, *n*-Hexane: Ethyl acetate)

IR : 3380, 2980, 1612, 1566, 1500, 1367, 1147 and 748 cm^{-1}

_N_-(4-Methoxybenzyl)-2-nitroaniline (15)

Anal.:

Yield : 76%

M.P. : 96-98 °C (Lit.[132e]: 94-95 °C)

TLC : R_f 0.46 (3:1, *n*-Hexane: Ethyl acetate)

IR : 3381, 2980, 1618, 1566, 1508, 1363, 1151 and 740 cm^{-1}

_N_-(2,3-Dimethoxybenzyl)-2-nitroaniline (16)

Anal.:

Yield : 80%

M.P. : 102-104 °C

TLC : R_f 0.47 (3:1, *n*-Hexane: Ethyl acetate)

IR : 3361, 2951, 1612, 1570, 1508, 1350, 1149 and 742 cm^{-1}

_N_-(3,4-Dimethoxybenzyl)-2-nitroaniline (17)

Anal.:

Yield : 79%

M.P. : 102-104 °C

TLC : R_f 0.51 (3:1, *n*-Hexane: Ethyl acetate)

IR : 3383, 2980, 1614, 1564, 1504, 1364, 1155 and 748 cm^{-1}

_N_-Phenethyl-2-nitroaniline (18)

Anal.:

Yield : 87%

M.P. : 68-70 °C

TLC : R_f 0.58 (5:1, *n*-Hexane: Ethyl acetate)

IR : 3385, 2980, 2970, 1612, 1566, 1500, 1327, 1141 and 744 cm^{-1}

_N_-(4-Chlorophenethyl)-2-nitroaniline (19)

Anal.:

Yield : 90%

M.P. : 80-82 °C.

TLC : R_f 0.5 (5:1, *n*-Hexane: Ethyl acetate)

IR : 3382, 2983, 1620, 1577, 1508, 1364, 1149 and 742 cm^{-1}

_N_-(3,4-Dimethoxyphenethyl)-2-nitroaniline (20)

Anal.:

Yield : 79%

M.P. : 134-136 °C (Lit.[132f]: 134 °C)

TLC : R_f 0.6 (5:1, *n*-Hexane: Ethyl acetate)

IR : 3380, 2980, 1614, 1570, 1498, 1354, 1153 and 742 cm^{-1}

Synthesis of *N*1-substituted 1,2-diamine intermediates (21 – 37)

N-Alkyl/arylalkyl 2-nitroaniline (2 mM) was added to a mixture of zinc (8 mM) and acetic acid (0.01 M) in methanol (15 ml). The reaction was allowed to stir for next 3-4 hours. The reaction mixture was filtered on hyflosupercel to remove inorganic residues. The filtrate so obtained was evaporated under rotary vacuum at temperature no more than 50 °C. The obtained viscous mass was neutralized using $NaHCO_3$ solution (10%) and the product was recovered by extraction with DCM (3x20 ml). The combined organic layer after drying and evaporation gave the desired products which were used as such immediately for the next step.

Synthesis of isoalloxazine and its derivatives (38 – 55)

Alloxan monohydrate (**2**) (3 mM) was reacted with *o*-phenylenediamine (OPD) (3 mM) in a mixture of boric acid (4 mM) and acetic acid (15 ml) at room temperature to obtain compound (**38**). To synthesize compounds (**39-55**), the respective *N*1-substituted 1,2-diamine intermediates obtained by reduction of the nitro group were reacted with alloxan monohydrate (**2**). As it was difficult to calculate the exact yield of the reduced intermediates, the obtained yields were considered 100% and to this equivalent amount of alloxan monohydrate, 1.2 eq. of boric acid in acetic acid (15 ml) as solvent were added. The reaction mixture was allowed to stir overnight and excess of alloxan monohydrate and boric acid were removed as water soluble components by pouring the reaction mixture into water. The highly hydrophobic solid got separated out as product. It was filtered, dried and purified by column chromatography with neutral alumina as stationary phase and *n*-hexane–ethyl acetate as mobile phase. (Melting points for all compounds were found to be above 250 °C).

Benzo[*g*]pteridine-2,4(3*H*,10*H*)-dione (38)

Anal.:

Yield	: 90%
TLC	: R_f 0.39 (*n*-Hexane : Ethyl acetate 50%)
IR	: 3410, 3177, 3085, 1736, 1692 and 1582 cm^{-1}
^{1}H-NMR	: 11.89 (br, 2H, -N*H*), 8.18-8.16 (d, 1H, Ar*H*), 7.94-7.92 (m, 2H, Ar*H*), 7.80-7.76 (m, 1H, Ar*H*)
^{13}C-NMR	: 160.69, 150.34, 147.00, 142.80, 139.34, 133.58, 131.73, 130.27,

128.65, 127.12

MS (ESI) : m/z 215 $(M+H)^+$

$C_{10}H_6N_4O_2$: Calcd. C, 56.08; H, 2.82; N, 26.16; found: C, 56.12; H, 2.81; N, 26.11.

10-Propylbenzo[*g*]pteridine-2,4(3*H*,10*H*)-dione (39)

Anal.:

Yield : 83%

TLC : R_f 0.43 (*n*-Hexane : Ethyl acetate 50%)

IR : 3432, 3195, 3071, 1722, 1670 and 1549 cm^{-1}

^{1}H-NMR : 11.35 (s, 1H, -N*H*), 8.10-8.08 (d, 1H, Ar*H*), 7.96-7.88 (m, 2H, Ar*H*), 7.63-7.59 (m, 1H, Ar*H*), 4.52-4.48 (t, 2H, N-CH_2-), 1.80-1.65 (m, 2H, -CH_2-), 1.02-0.98 (t, 3H, -CH_3)

MS (ESI) : m/z 257 $(M+H)^+$

$C_{13}H_{12}N_4O_2$: Calcd. C, 60.93; H, 4.72; N, 21.86; found: C, 60.87; H, 4.76; N, 21.79.

10-Butylbenzo[*g*]pteridine-2,4(3*H*,10*H*)-dione (40)

Anal.:

Yield : 83%

TLC : R_f 0.42 (*n*-Hexane : Ethyl acetate 50%)

IR : 3431, 3199, 3070, 2870, 1721, 1668 and 1548 cm^{-1}

^{1}H-NMR : 11.35 (s, 1H, -N*H*); 8.10-8.08 (d, 1H, Ar*H*); 7.92-7.90 (m, 2H, Ar*H*); 7.61-7.59 (m, 1H, Ar*H*), 4.56-4.52 (t, 2H, -N-CH_2-), 1.70-1.63 (m, 2H, -CH_2-), 1.46-1.39 (m, 2H, -CH_2-), 0.94-0.90 (t, 3H, -CH_3)

^{13}C-NMR : 159.82, 155.80, 150.41, 138.70, 135.03, 134.92, 132.41, 131.87, 126.01, 116.40, 44.08, 28.60, 19.57, 13.80

MS (ESI) : m/z 271 $(M+H)^+$

$C_{14}H_{14}N_4O_2$: Calcd. C, 62.21; H, 5.22; N, 20.73; found: C, 62.17; H, 5.23; N, 20.80.

10-Allylbenzo[*g*]pteridine-2,4(3*H*,10*H*)-dione (41)

Anal.:

Yield : 84%

TLC : R_f 0.43 (*n*-Hexane : Ethyl acetate 50%)

IR : 3434, 3198, 3075, 1721, 1673 and 1548 cm^{-1}

^{1}H-NMR : 11.38 (s, 1H, -N*H*), 8.11-8.09 (d, 1H, Ar*H*), 7.89-7.85 (m, 2H, Ar*H*), 7.62-7.60 (m, 1H, Ar*H*), 6.00-5.90 (m, 1H, =C*H*-), 5.23-5.09 (m, 4H, -CH_2- and=CH_2)

^{13}C-NMR : 159.75, 155.70, 150.52, 138.97, 134.80, 132.32, 131.70, 130.39, 126.01, 117.85, 116.74, 46.23

MS (ESI) : m/z 255 $(M+H)^+$

$C_{13}H_{10}N_4O_2$: Calcd. C, 61.41; H, 3.96; N, 22.04; found: C, 61.35; H, 3.95; N, 21.96.

10-Benzylbenzo[*g*]pteridine-2,4(3*H*,10*H*)-dione (42)

Anal.:

Yield : 83%

TLC : R_f 0.46 (*n*-Hexane : Ethyl acetate 50%)

IR : 3433, 3197, 3077, 1717, 1686 and 1551 cm^{-1}

^{1}H-NMR : 11.42 (s, 1H, -N*H*), 8.12-8.09 (m, 1H, Ar*H*), 7.78-7.56 (m, 3H, Ar*H*), 7.30-7.25 (m, 5H, Ar*H*), 5.86 (s, 2H, -CH_2-)

^{13}C-NMR : 159.81, 155.81, 151.18, 139.39, 134.75, 132.32, 131.77, 128.66, 127.50, 126.85, 126.06, 116.66, 47.13

MS (ESI) : m/z 305 $(M+H)^+$

$C_{17}H_{12}N_4O_2$: Calcd. C, 67.10; H, 3.97; N, 18.41; found: C, 67.22; H, 3.98; N, 18.47.

10-(3-Methylbenzyl)benzo[*g*]pteridine-2,4(3*H*,10*H*)-dione (43)

Anal.:

Yield : 87%

TLC : R_f 0.45 (*n*-Hexane : Ethyl acetate 50%)

IR : 3433, 3189, 3071, 1722, 1675 and 1546 cm^{-1}

^{1}H-NMR : 11.40 (s, 1H, -N*H*), 8.15-8.13 (d, 1H, Ar*H*), 7.77-7.75 (m, 1H, Ar*H*), 7.60-7.56 (m, 1H, Ar*H*), 7.44-7.42 (m, 1H, Ar*H*), 7.27-7.25 (m, 1H, Ar*H*), 7.15-7.14 (m, 1H, Ar*H*), 6.95-6.95 (m, 1H, Ar*H*), 6.58-6.56 (m, 1H, Ar*H*), 5.72 (s,2H, -CH_2-), 2.1 (s, 3H, -CH_3)

^{13}C-NMR : 159.87, 155.80, 151.12, 139.31, 135.22, 134.91, 132.50, 132.09, 131.78, 130.30, 127.21, 126.16, 126.06, 124.57, 116.79, 45.96, 18.88

MS (ESI) : m/z 319 $(M+H)^+$

$C_{18}H_{14}N_4O_2$: Calcd. C, 67.91; H, 4.43; N, 17.60; found: C, 68.09; H, 4.44; N, 17.58.

10-(4-Methylbenzyl)benzo[*g*]pteridine-2,4(3*H*,10*H*)-dione (44)

Anal.:

Yield : 82%

TLC : R_f 0.44 (*n*-Hexane : Ethyl acetate 50%)

IR : 3433, 3201, 3079, 1717, 1680 and 1552 cm^{-1}

^{1}H-NMR : 11.48 (s, 1H, -N*H*), 8.15-8.12 (m, 1H, Ar*H*), 7.82-7.78 (m, 1H, Ar*H*), 7.69-7.67 (m, 1H, Ar*H*), 7.60-7.56 (m, 1H, Ar*H*), 7.24-7.22 (d, 2H, Ar*H*), 7.11-7.09 (d, 2H, Ar*H*), 5.88 (s, 2H, -CH_2-), 2.26 (s, 3H, -CH_3)

MS (ESI) : m/z 319 $(M+H)^+$

$C_{18}H_{14}N_4O_2$: Calcd. C, 67.91; H, 4.43; N, 17.60; found: C, 68.06; H, 4.44; N, 17.63.

10-(2-Fluorobenzyl)benzo[*g*]pteridine-2,4(3*H*,10*H*)-dione (45)

Anal.:

Yield : 89%

TLC : R_f 0.42 (*n*-Hexane : Ethyl acetate 50%)

IR : 3415, 3187, 3069, 1724, 1674 and 1547 cm^{-1}

^{1}H-NMR : 11.43 (s, 1H, -N*H*), 8.14-8.12 (d, 1H, Ar*H*), 7.83-7.79 (m, 1H, Ar*H*), 7.61-7.57 (m, 2H, Ar*H*), 7.31-7.26 (m, 2H, Ar*H*), 7.00-6.95 (m, 2H, Ar*H*), 5.84 (s, 2H, -CH_2-)

MS (ESI) : m/z 323 $(M+H)^+$

$C_{17}H_{11}FN_4O_2$: Calcd. C, 63.35; H, 3.44; N, 17.38; found: C, 63.43; H, 3.43; N, 17.41.

10-(3-Fluorobenzyl)benzo[*g*]pteridine-2,4(3*H*,10*H*)-dione (46)

Anal.:

Yield : 81%

TLC : R_f 0.44 (*n*-Hexane : Ethyl acetate 50%)

IR : 3429, 3189, 3072, 1723, 1674 and 1548 cm^{-1}

^{1}H-NMR : 11.48 (s, 1H, -N*H*), 8.16-8.14 (d, 1H, Ar*H*), 7.83-7.79 (m, 1H, Ar*H*), 7.66-7.58 (m, 2H, Ar*H*), 7.37-7.35 (m, 1H, Ar*H*), 7.24-7.20 (m, 2H, Ar*H*), 7.08-7.06 (m, 1H, Ar*H*), 5.92 (s, 2H, -CH_2-)

MS (ESI) : m/z 323 $(M+H)^+$

$C_{17}H_{11}FN_4O_2$: Calcd. C, 63.35; H, 3.44; N, 17.38; found: C, 63.40; H, 3.43; N, 17.34.

10-(3-Chlorobenzyl)benzo[*g*]pteridine-2,4(3*H*,10*H*)-dione (47)

Anal.:

Yield : 82%

TLC : R_f 0.43 (*n*-Hexane : Ethyl acetate 50%)

IR : 3431, 3189, 3075, 1723, 1672 and 1548 cm^{-1}

^{1}H-NMR : 11.43 (s, 1H, -N*H*), 8.18-8.16 (d, 1H, Ar*H*), 7.80-7.76 (m, 1H, Ar*H*), 7.61-7.57 (m, 2H, Ar*H*), 7.55 (s, 1H, Ar*H*), 7.43-7.31 (m, 3H, Ar*H*), 5.85 (s, 2H, -CH_2-)

MS (ESI) : m/z 339 $(M+H)^+$; 341 $(M+2+H)^+$

$C_{17}H_{11}ClN_4O_2$: Calcd. C, 60.28; H, 3.27; N, 16.54; found: C, 60.33; H, 3.26; N, 16.56.

10-(4-Chlorobenzyl)benzo[*g*]pteridine-2,4(3*H*,10*H*)-dione (48)

Anal.:

Yield : 83%

TLC : R_f 0.44 (*n*-Hexane : Ethyl acetate 50%)

IR : 3433, 3186, 3074, 1723, 1674 and 1546 cm^{-1}

^{1}H-NMR : 11.47 (s, 1H, -N*H*), 8.15-8.13 (d, 1H, Ar*H*), 7.82-7.80 (m, 1H, Ar*H*), 7.69-7.67 (m, 1H, Ar*H*), 7.60-7.55 (m, 1H, Ar*H*), 7.44-7.41 (m, 2H, Ar*H*), 7.17- 7.13 (m, 2H, Ar*H*), 5.87 (s, 2H, -CH_2-)

^{13}C-NMR : 159.81, 155.81, 151.18, 139.45, 134.78, 132.22, 131.81, 130.92, 129.20, 129.12, 126.09, 116.57, 115.55, 115.34, 46.47

MS (ESI) : m/z 339 $(M+H)^+$; 341 $(M+2+H)^+$

$C_{17}H_{11}ClN_4O_2$: Calcd. C, 60.28; H, 3.27; N, 16.54; found: C, 60.38; H, 3.25; N, 16.58.

10-(2-Methoxybenzyl)benzo[*g*]pteridine-2,4(3*H*,10*H*)-dione (49)

Anal.:

Yield : 85%

TLC : R_f 0.40 (*n*-Hexane : Ethyl acetate 50%)

IR : 3434, 3272, 3085, 1710, 1690 and 1558 cm^{-1}

^{1}H-NMR : 11.43 (s, 1H, -N*H*), 8.18-8.16 (d, 1H, Ar*H*), 7.84-7.80 (m, 1H, Ar*H*), 7.63-7.59 (m, 1H, Ar*H*), 7.48-7.46 (d, 1H, Ar*H*), 7.30-7.26

(m, 1H, Ar*H*), 7.14-7.12 (d, 1H, Ar*H*), 6.77-6.75 (m, 2H, Ar*H*), 5.74 (s, 2H, -CH_2-), 3.97 (s, 3H, -OCH_3)

MS (ESI) : m/z 335 $(M+H)^+$

$C_{18}H_{14}N_4O_3$: Calcd. C, 64.66; H, 4.22; N, 16.76; found: C, 64.49; H, 4.21; N, 16.81.

10-(4-Methoxybenzyl)benzo[*g*]pteridine-2,4(3*H*,10*H*)-dione (50)

Anal.:

TLC : R_f 0.41 (*n*-Hexane : Ethyl acetate 50%)

IR : 3433, 3191, 3078, 1718, 1675 and 1552 cm^{-1}

^{1}H-NMR : 11.23 (s, 1H, -N*H*), 7.73-7.38 (m, 4H, Ar*H*), 7.13-7.11 (d, 2H, Ar*H*), 6.68-6.66 (d, 2H, Ar*H*), 5.63 (s, 2H, -CH_2-), 3.51 (s, 3H, -OCH_3)

MS (ESI) : m/z 335 $(M+H)^+$

$C_{18}H_{14}N_4O_3$: Calcd. C, 64.66; H, 4.22; N, 16.76; found: C, 64.56; H, 4.22; N, 16.79.

10-(2,3-Dimethoxybenzyl)benzo[*g*]pteridine-2,4(3*H*,10*H*)-dione (51)

Anal.:

Yield : 84%

M.P. : 266 °C (DSC)

TLC : R_f 0.44 (*n*-Hexane : Ethyl acetate 50%)

IR : 3434, 3171, 3051, 1712, 1688 and 1553 cm^{-1}

^{1}H-NMR : 11.38 (s, 1H, -N*H*), 8.09-8.07 (d, 1H, Ar*H*), 7.85-7.83 (m, 2H, Ar*H*), 7.60-7.56 (m, 1H, Ar*H*), 6.93-6.91 (m, 1H, Ar*H*), 6.83-6.77 (m, 2H, Ar*H*), 4.76 (s, 2H, -CH_2-), 3.67 (s, 6H, -OCH_3)

MS (ESI) : m/z 365 $(M+H)^+$

$C_{19}H_{16}N_4O_4$: Calcd. C, 62.63; H, 4.43; N, 15.38; found: C, 62.68; H, 4.44; N, 15.41.

10-(3,4-Dimethoxybenzyl)benzo[*g*]pteridine-2,4(3*H*,10*H*)-dione (52)

Anal.:

Yield : 84%

TLC : R_f 0.44 (*n*-Hexane : Ethyl acetate 50%)

IR : 3432, 3182, 3068, 1722, 1680 and 1546 cm^{-1}

^{1}H-NMR : 11.48 (s, 1H, -N*H*), 8.20-8.18 (m, 1H, Ar*H*), 7.90-7.86 (m, 2H, Ar*H*), 7.67-7.63 (m, 1H, Ar*H*), 7.16 (s, 1H, Ar*H*), 6.89-6.83 (m, 2H, Ar*H*), 5.86

(s, 2H, -CH_2-), 3.77 (s, 3H, -OCH_3), 3.75 (s, 3H, -OCH_3)

MS (ESI) : m/z 365 $(M+H)^+$

$C_{19}H_{16}N_4O_4$: Calcd. C, 62.63; H, 4.43; N, 15.38; found: C, 62.65; H, 4.43; N, 15.33.

10-Phenethylbenzo[*g*]pteridine-2,4(3*H*,10*H*)-dione (53)

Anal.:

Yield : 85%

TLC : R_f 0.37 (*n*-Hexane : Ethyl acetate 50%)

IR : 3433, 3198, 3075, 1719, 1678 and 1551 cm^{-1}

^{1}H-NMR : 8.55 (S, 1H, -N*H*), 8.34-8.32 (d, 1H, Ar*H*), 7.87-7.83 (m, 1H, Ar*H*), 7.65-7.61 (m, 1H, Ar*H*), 7.57-7.55 (d, 1H, Ar*H*), 7.33-7.23 (m, 5H, Ar*H*), 4.96- 4.92 (t, 2H, -CH_2-), 3.21-3.17 (t, 2H, -CH_2-)

^{13}C-NMR : 159.79, 155.74, 150.32, 137.75, 134.90, 134.77, 131.75, 128.99, 128.50, 126.73, 125.97, 116.37, 45.31, 32.01

MS (ESI) : m/z 319 $(M+H)^+$

$C_{18}H_{14}N_4O_2$: Calcd. C, 67.91; H, 4.43; N, 17.60; found: C, 68.01; H, 4.42; N, 17.58.

10-(4-Chlorophenethyl)benzo[*g*]pteridine-2,4(3*H*,10*H*)-dione (54)

Anal.:

Yield : 88%

TLC : R_f 0.46 (*n*-Hexane : Ethyl acetate 50%)

IR : 3401, 3198, 3078, 1721, 1677 and 1551 cm^{-1}

^{1}H-NMR : 11.40 (s, 1H, -N*H*), 8.09-8.07 (d, 1H, Ar*H*), 7.84-7.82 (m, 1H, Ar*H*), 7.60-7.58 (m, 1H, Ar*H*), 7.38-7.31 (m, 5H, Ar*H*), 4.77-4.73 (t, 2H, -CH_2-), 3.01-2.97 (t, 2H, -CH_2-)

MS (ESI) : m/z 353 $(M+H)^+$; 355 $(M+2+H)^+$

$C_{18}H_{13}ClN_4O_2$: Calcd. C, 61.28; H, 3.71; N, 15.88; found: C, 61.19; H, 3.72; N, 15.92.

10-(3,4-Dimethoxyphenethyl)benzo[*g*]pteridine-2,4(3*H*,10*H*)-dione (55)

Anal.:

Yield : 84%

TLC : R_f 0.46 (*n*-Hexane : Ethyl acetate 50%)

IR : 3434, 3170, 3050, 1713, 1647 and 1553 cm^{-1}

^{1}H-NMR : 11.51 (s, 1H, -N*H*), 8.15-8.13 (m, 1H, Ar*H*), 7.86-7.84 (m, 2H, Ar*H*), 7.62-7.58 (m, 1H, Ar*H*), 6.94 (s, 1H, Ar*H*), 6.82-6.79 (m, 2H, Ar*H*), 4.87-4.83 (t, 2H, -C*H*$_2$-), 3.78-3.75 (s, 6H, -OCH$_3$), 3.05-3.01 (t, 2H, -C*H*$_2$-)

^{13}C-NMR : 159.87, 155.79, 150.33, 148.75, 147.71, 138.74, 134.81, 132.48, 131.71, 130.21, 125.97, 120.95, 116.48, 113.02, 111.96, 55.61, 45.56, 31.77

MS (ESI) : m/z 379 $(M+H)^+$

$C_{20}H_{18}N_4O_4$: Calcd. C, 63.48; H, 4.79; N, 14.81; found: C, 63.61; H, 4.78; N, 14.86.

5.1.2. Synthesis of 1,2,4-triazino[5,6-*b*]indole-3-thiol (3-(Substituted thio)-5*H*-[1,2,4]triazino [5,6 -*b*]indole) derivatives (58 – 70) (series II)

Synthesis of 5*H*-[1,2,4]triazino[5,6-*b*]indole-3-thiol (58)

Isatin (**56**) (5 mM) was added to a solution of potassium carbonate (0.02 M) in water (300 ml). Thiosemicarbazide (**57**) (5 mM) was added to this mixture. The reaction mixture was refluxed for 4 hours during which the initial intense red colour turned to orange solution. Upon cooling to room temperature, the solution was filtered and the filtrate was acidified with glacial acetic acid to precipitate out the yellow colored product (**58**).[132g] The precipitated product was filtered washed with water, dried and subsequently recrystallized by DMF-water to get the final product.

Anal.:

Yield : 64%

M.P. : >350 °C (DSC)

TLC : R_f 0.25 (*n*-Hexane: Ethyl acetate 30%)

IR : 3410, 3038, 1609, 1427, 1345 and 1160 cm^{-1}

^{1}H-NMR : 14.58 (br, 1H, -S*H*), 12.35 (br, 1H, -N*H*), 7.99-7.97 (d, 1H, Ar*H*), 7.62-7.58 (m, 1H, Ar*H*), 7.44-7.42 (d, 1H, Ar*H*), 7.35-7.31 (m, 1H, Ar*H*)

MS (ESI) : m/z 203 $(M+H)^+$

Synthesis of 3-(Substituted thio)-5*H*-[1,2,4]triazino[5,6-*b*]indole (59 – 70)

To a solution of 5*H*-[1,2,4]triazino[5,6-*b*]indole-3-thiol (**58**) (5 mM) in DMF (20 ml) K_2CO_3 (0.01 M) and required alkyl bromide (5 mM) were added. The reaction mixture was

allowed to stir at 60 °C for 6-8 hours, was allowed to cool to room temperature and then poured into water with stirring. The precipitated product was filtered, washed with water and recrystallized from DMF-water to yield the entitled compounds (**59** – **70**).

3-Methylthio-5*H*-[1,2,4]triazino[5,6-*b*]indole (59)

Anal.:

Yield : 69%

M.P. : 306.73 °C (DSC)

TLC : R_f 0.39 (*n*-Hexane: Ethyl acetate 30%)

IR : 3204, 3056, 2801, 1604, 1342 and 1184 cm^{-1}

^{1}H-NMR : 12.62 (br, 1H, -N*H*), 8.29-8.27 (d, 1H, Ar*H*), 7.67-7.64 (m, 1H, Ar*H*), 7.56-7.54 (d, 1H, Ar*H*), 7.42-7.39 (m, 1H, Ar*H*), 2.66 (s, 3H, -CH_3)

^{13}C-NMR : 168.12, 147.21, 141.32, 140.75, 131.28, 122.95, 121.95, 118.18, 113.18, 13.90

MS (ESI) : m/z 216 $(M)^+$

3-Ethylthio-5*H*-[1,2,4]triazino[5,6-*b*]indole (60)

Anal.:

Yield : 71%

M.P. : 287.45 °C (DSC)

TLC : R_f 0.41 (*n*-Hexane: Ethyl acetate 30%)

IR : 3210, 3059, 2802, 1606, 1336 and 1187 cm^{-1}

^{1}H-NMR : 12.57 (br, 1H, -N*H*), 8.29-8.27 (d, 1H, -Ar*H*), 7.68-7.64 (m, 1H, Ar*H*), 7.56-7.54 (d, 1H, Ar*H*), 7.42-7.39 (m, 1H, Ar*H*), 3.31-3.24 (m, 2H, S-CH_2-), 1.45-1.41 (t, 3H, -CH_3)

^{13}C-NMR : 167.69, 147.24, 141.39, 140.76, 131.29, 122.94, 121.93, 118.19, 113.16, 24.28, 15.08

MS (ESI) : m/z 230 $(M)^+$

3-Isopropylthio-5*H*-[1,2,4]triazino[5,6-*b*]indole (61)

Anal.:

Yield : 67%

M.P. : 282.63 °C (DSC)

TLC : R_f 0.39 (*n*-Hexane: Ethyl acetate 30%)

IR : 3200, 3058, 2865, 2800, 1603, 1339 and 1182 cm^{-1}

^{1}H-NMR : 12.53 (br, 1H, -N*H*), 8.29-8.27 (d, 1H, -Ar*H*), 7.66-7.62 (m, 1H, Ar*H*), 7.55-7.53 (d, 1H, Ar*H*), 7.42-7.38 (m, 1H, Ar*H*), 4.13-4.06 (m, 1H, S-C*H*-), 1.48-1.46 (d, 6H, -CH_3)

^{13}C-NMR : 167.76, 147.22, 141.34, 140.78, 131.32, 122.95, 121.95, 118.19, 113.16, 35.72, 23.29

MS (ESI) : m/z 244 $(M)^+$

3-Butylthio-5*H*-[1,2,4]triazino[5,6-*b*]indole (62)

Anal.:

Yield : 68%

M.P. : 262.83 °C (DSC)

TLC : R_f 0.35 (*n*-Hexane: Ethyl acetate 30%)

IR : 3209, 3057, 2867, 2800, 1606, 1336 and 1187 cm^{-1}

^{1}H-NMR : 12.57 (br, 1H, -N*H*), 8.28-8.26 (d, 1H, -Ar*H*), 7.66-7.62 (m, 1H, Ar*H*), 7.55-7.53 (d, 1H, Ar*H*), 7.41-7.37 (m, 1H, Ar*H*), 3.28-3.25 (t, 2H, -S-CH_2-), 1.80-1.72 (m, 2H, -CH_2-), 1.55-1.45 (m, 2H, - CH_2-), 0.98-0.94 (t, 3H, -CH_3)

^{13}C-NMR : 167.81, 147.19, 141.38, 140.75, 131.28, 122.94, 121.92, 118.19, 113.15, 31.44, 30.13, 22.03, 14.09

MS (ESI) : m/z 258 $(M)^+$

3-Benzylthio-5*H*-[1,2,4]triazino[5,6-*b*]indole (63)

Anal.:

Yield : 70%

M.P. : 275.15 °C (DSC)

TLC : R_f 0.37 (*n*-Hexane: Ethyl acetate 30%)

IR : 3203, 3055, 2930, 2797, 1600, 1338 and 1180 cm^{-1}

^{1}H-NMR : 12.54 (br, 1H, -N*H*), 8.30-8.28 (d, 1H, -Ar*H*), 7.67-7.63 (m, 1H, Ar*H*), 7.57-7.55 (d, 1H, Ar*H*), 7.51-7.49 (d, 2H, Ar*H*), 7.43-7.39 (m, 1H, Ar*H*),

7.33-7.29 (m, 2H, Ar*H*), 7.26-7.22 (m, 1H, Ar*H*), 4.56 (s, 2H, -CH_2-)

^{13}C-NMR : 167.25, 147.12, 141.63, 140.83, 138.09, 131.43, 129.61, 128.99, 127.75, 123.03, 122.02, 118.16, 113.24, 34.55

MS (ESI) : m/z 292 $(M)^+$

3-Phenethylthio-5*H*-[1,2,4]triazino[5,6-*b*]indole (64)

Anal.:

Yield : 74%

M.P. : 246.05 °C (DSC)

TLC : R_f 0.33 (*n*-Hexane: Ethyl acetate 30%)

IR : 3204, 3058, 2965, 2801, 1606, 1334 and 1186 cm^{-1}

^{1}H-NMR : 12.55 (br, 1H, -N*H*), 8.31-8.29 (d, 1H, -Ar*H*), 7.69-7.64 (m, 1H, Ar*H*), 7.58-7.56 (d, 1H, Ar*H*), 7.44-7.40 (m, 1H, Ar*H*), 7.36-7.30 (m, 4H, Ar*H*), 7.26-7.20 (m, 1H, Ar*H*), 3.53-3.49 (m, 2H, S-CH_2-), 3.10-3.06 (m, 2H, -CH_2-Ar)

^{13}C-NMR : 167.54, 147.23, 141.47, 140.74, 131.34, 129.18, 128.94, 126.86, 122.99, 121.99, 118.21, 113.20, 35.46, 31.87

MS (ESI) : m/z 306 $(M)^+$

3-(2-Methylbenzylthio)-5*H*-[1,2,4]triazino[5,6-*b*]indole (65)

Anal.:

Yield : 66%

M.P. : 254.96 °C (DSC)

TLC : R_f 0.39 (*n*-Hexane: Ethyl acetate 30%)

IR : 3201, 3059, 2969, 2801, 1600, 1340 and 1175 cm^{-1}

^{1}H-NMR : 12.53 (br, 1H, -N*H*), 8.28-8.26 (m, 1H, Ar*H*), 7.66-7.61 (m, 1H, Ar*H*), 7.53-7.51 (m, 1H, Ar*H*), 7.47-7.45 (m, 1H, Ar*H*), 7.41-7.37 (m, 1H, Ar*H*), 7.19-7.10 (m, 3H, Ar*H*), 4.54 (s, 2H, S-CH_2-), 2.42 (s, 3H, -CH_3)

MS (ESI) : m/z 306.09 $(M)^+$

3-(3-Fluorobenzylthio)-5*H*-[1,2,4]triazino[5,6-*b*]indole (66)

Anal.:

Yield : 71%

M.P. : 262.80 °C (DSC)

TLC : R_f 0.41 (*n*-Hexane: Ethyl acetate 30%)

IR : 3210, 3059, 2944, 2802, 1606, 1335 and 1182 cm^{-1}

^{1}H-NMR : 12.55 (br, 1H, -N*H*), 8.30-8.28 (d, 1H, Ar*H*), 7.67-7.63 (m, 1H, Ar*H*), 7.57-7.55 (d, 1H, Ar*H*), 7.43-7.39 (m, 1H, Ar*H*), 7.35-7.29 (m, 3H, Ar*H*), 7.03-6.98 (m, 1H, Ar*H*), 4.57 (s, 2H, S-CH_2-)

^{13}C-NMR : 166.92, 163.51, 161.58, 147.12, 141.73, 140.89, 131.46, 130.91, 125.70, 123.03, 122.04, 118.13, 116.39, 114.62, 113.25, 33.90

MS (ESI) : m/z 309.99 $(M)^+$

3-(4-Methylbenzylthio)-5*H*-[1,2,4]triazino[5,6-*b*]indole (67)

Anal.:

Yield : 72%

M.P. : 290.38 °C (DSC)

TLC : R_f 0.43 (*n*-Hexane: Ethyl acetate 30%)

IR : 3202, 3052, 2964, 2798, 1604, 1340 and 1181 cm^{-1}

^{1}H-NMR : 12.62 (br, 1H, -N*H*), 8.31-8.29 (d, 1H, Ar*H*), 7.71-7.67 (m, 1H, Ar*H*), 7.59-7.57 (d, 1H, Ar*H*), 7.45-7.38 (m, 3H, Ar*H*), 7.14-7.12 (m, 2H, Ar*H*), 4.51 (s, 2H, S-CH_2-), 2.26 (s, 3H, -CH_3)

^{13}C-NMR : 166.78, 146.56, 141.02, 140.29, 136.37, 134.31, 130.83, 128.97, 122.44, 121.44, 117.61, 112.67, 33.80, 20.66

MS (ESI) : m/z 306.19 $(M)^+$

3-(4-Bromobenzylthio)-5*H*-[1,2,4]triazino[5,6-*b*]indole (68)

Anal.:

Yield : 74%

M.P. : 294.73 °C (DSC)

TLC : R_f 0.40 (*n*-Hexane: Ethyl acetate 30%)

IR : 3203, 3054, 2965, 2798, 1603, 1340 and 1180 cm^{-1}

^{1}H-NMR : 12.66 (br, 1H, -N*H*), 8.31-8.29 (d, 1H, Ar*H*), 7.71-7.67 (m, 1H, Ar*H*), 7.59-7.57 (d, 1H, Ar*H*), 7.53-7.47 (m, 4H, Ar*H*), 7.45-7.41

(m, 1H, Ar*H*), 4.53 (s, 2H, S-CH_2-)

^{13}C-NMR : 166.35, 146.56, 141.15, 140.34, 137.39, 131.24, 130.89, 122.46, 121.48, 120.23, 117.58, 112.70, 33.21

MS (ESI) : m/z 371 $(M+H)^+$, 373 $(M+2+H)^+$

3-(4-Cyanobenzylthio)-5*H*-[1,2,4]triazino[5,6-*b*]indole (69)

Anal.:

Yield : 76%

M.P. : 288.48 °C (DSC)

TLC : R_f 0.44 (*n*-Hexane: Ethyl acetate 30%)

IR : 3200, 3151, 2978, 2929, 2860, 2226, 1599, 1340 and 1179 cm^{-1}

^{1}H-NMR : 12.62 (br, 1H, -N*H*), 8.30-8.28 (d, 1H, Ar*H*), 7.78-7.66 (m, 5H, Ar*H*), 7.58-7.56 (d, 1H, Ar*H*), 7.44-7.40 (m, 1H, Ar*H*), 4.63 (s, 2H, S-CH_2-)

^{13}C-NMR : 166.60, 147.11, 144.68, 141.81, 140.91, 132.83, 131.54, 130.57, 123.07, 122.07, 119.32, 118.09, 113.27, 110.36, 34.03

MS (ESI) : m/z 317.09 $(M)^+$

3-(4-Methoxybenzylthio)-5*H*-[1,2,4]triazino[5,6-*b*]indole (70)

Anal.:

Yield : 64%

M.P. : 254.93 °C (DSC)

TLC : R_f 0.36 (*n*-Hexane: Ethyl acetate 30%)

IR : 3054, 2961, 2931, 2834, 1609, 1250 and 1178 cm^{-1}

^{1}H-NMR : 12.57 (br, 1H, -N*H*), 8.30-8.28 (m, 1H, Ar*H*), 7.68-7.64 (m, 1H, Ar*H*), 7.57-7.55 (d, 1H, Ar*H*), 7.46-7.38 (m, 3H, Ar*H*), 6.87-6.82 (m, 2H, Ar*H*), 4.50 (s, 2H, S-CH_2-), 3.73 (s, 3H, - OCH_3)

MS (ESI) : m/z 322.09 $(M)^+$

5.1.3. Synthesis of 5-Substituted 5*H*-[1,2,4]triazino[5,6-*b*]indole-3-thiol derivatives (82 – 92) (series III)

Synthesis of *N*-substituted isatins (71 – 81)

Isatin (3 mM) was dissolved in a mixture of K_2CO_3 (6 mM) and DMF (10 ml). To this stirring mixture required alkyl bromide (3 mM) was added. The mixture was stirred at 45-50 °C for 2-4 hours. After completion of the reaction, the mixture was poured into water with continuous stirring. The precipitated solid was filtered, dried and purified using column chromatography to obtain the desired compounds.

1-Methyl-1*H*-indole-2,3-dione (71)

Anal.:

Yield : 82%

M.P. : 130-131 °C (Lit.[132h]: 130 °C)

TLC : R_f 0.49 (*n*-Hexane: Ethyl acetate 50%)

IR : 1724, 1607, 1467, 1366, 1325, 1116, 1089, 759 and 699 cm^{-1}

1-Ethyl-1*H*-indole-2,3-dione (72)

Anal.:

Yield : 94%

M.P. : 132-134 °C

TLC : R_f 0.52 (*n*-Hexane: Ethyl acetate 50%)

IR : 1731, 1609, 1465, 1352, 1289, 1126, 1092, 758 and 699 cm^{-1}

1-Isopropyl-1*H*-indole-2,3-dione (73)

Anal.:

Yield : 92%

M.P. : 80-82 °C

TLC : R_f 0.56 (*n*-Hexane: Ethyl acetate 50%)

IR : 1718, 1601, 1465, 1386, 1348, 1180, 1116, 1088 and 754 cm^{-1}

1-Butyl-1*H*-indole-2,3-dione (74)

Anal.:

Yield : 88%

M.P. : 40-42 °C (Lit.[132i]: 36 °C)

TLC : R_f 0.60 (*n*-Hexane: Ethyl acetate 50%)

IR : 1727, 1611, 1469, 1354, 1294, 1131, 1094, 750 and 706 cm^{-1}

1-Benzyl-1*H*-indole-2,3-dione (75)

Anal.:

Yield : 88%

M.P. : 132-134 °C (Lit.[132j]: 133-135 °C)

TLC : R_f 0.67 (*n*-Hexane: Ethyl acetate 50%)

IR : 1733, 1611, 1469, 1348, 1177, 756 and 697 cm^{-1}

1-(4-Methylbenzyl)-1*H*-indole-2,3-dione (76)

Anal.:

Yield : 83%

M.P. : 136-138 °C

TLC : R_f 0.65 (*n*-Hexane: Ethyl acetate 50%)

IR : 1733, 1611, 1468, 1347, 1177, 1093 and 757 cm^{-1}

1-(3-Fluorobenzyl)-1*H*-indole-2,3-dione (77)

Anal.:

Yield : 92%

M.P. : 168-169 °C

TLC : R_f 0.69 (*n*-Hexane: Ethyl acetate 50%)

IR : 1727, 1614, 1471, 1356, 1249, 1142, 1096, 755 and 688 cm^{-1}

1-(4-*tert*-Butylbenzyl)-1*H*-indole-2,3-dione (78)

Anal.:

Yield : 84%

M.P. : 148-150 °C

TLC : R_f 0.70 (*n*-Hexane: Ethyl acetate 50%)

IR : 1730, 1612, 1467, 1373, 1178 and 763 cm^{-1}

1-(2-Bromobenzyl)-1*H*-indole-2,3-dione (79)

Anal.:

Yield : 82%

M.P. : 179-181 °C

TLC : R_f 0.64 (*n*-Hexane: Ethyl acetate 50%)

IR : 1753, 1608, 1465, 1369, 1176 and 754 cm^{-1}

1-(4-Chlorobenzyl)-1*H*-indole-2,3-dione (80)

Anal.:

Yield : 86%

M.P. : 136-139 °C

TLC : R_f 0.48 (*n*-Hexane: Ethyl acetate 50%)

IR : 1732, 1610, 1467, 1340, 1168 and 756 cm^{-1}

1-(4-Methoxybenzyl)-1*H*-indole-2,3-dione (81)

Anal.:

Yield : 78%

M.P. : 135-137 °C

TLC : R_f 0.56 (*n*-Hexane: Ethyl acetate 50%)

IR : 1732, 1608, 1512, 1465, 1244 and 759 cm^{-1}

Synthesis of 5-substituted 5*H*-[1,2,4]triazino[5,6-*b*]indole-3-thiol derivatives

N_1-substituted isatin (3 mM), thiosemicarbazide (3 mM) and K_2CO_3 (0.012 M) were taken in water (250 ml). The reaction mixture was refluxed for 4 hours. Upon cooling to room temperature, the solution was filtered and the filtrate was acidified with glacial acetic acid to precipitate out the yellow coloured product. The precipitate was filtered, washed with water, dried and recrystallized by DMF-water to get the final product.

5-Methyl[1,2,4]triazino[5,6-*b*]indole-3-thiol (82)

Anal.:

Yield : 68%

M.P. : 290.34 °C (DSC)

TLC : R_f 0.51 (*n*-Hexane: Ethyl acetate 40%)

IR : 2975, 1601, 1566, 1362, 1139 and 750 cm^{-1}

^{1}H-NMR : 14.61 (bs, 1H, -S*H*), 8.02-8.00 (d, 1H, Ar*H*), 7.71-7.67 (m, 1H, Ar*H*), 7.60-7.58 (d, 1H, Ar*H*), 7.41-7.37 (m, 1H, Ar*H*), 3.70 (s, 3H, -CH_3)

MS (ESI) : m/z 217.20 $(M+H)^+$

5-Ethyl[1,2,4]triazino[5,6-*b*]indole-3-thiol (83)

Anal.:

Yield : 73%

M.P. : 304.17 °C (DSC)

TLC : R_f 0.56 (*n*-Hexane: Ethyl acetate 40%)

IR : 2855, 1574, 1347, 1143 and 743 cm^{-1}

^{1}H-NMR : 14.61 (bs, 1H, -S*H*), 8.03-8.01 (d, 1H, Ar*H*), 7.71-7.63 (m, 2H, Ar*H*), 7.41-7.37 (m, 1H, Ar*H*), 4.30-4.25 (m, 2H, -CH_2), 1.39-1.35 (t, 3H, -CH_3)

^{13}C-NMR : 179.03, 147.61, 143.19, 135.34, 131.83, 123.45, 121.87, 117.51, 111.63, 35.92, 12.98

MS (ESI) : m/z 231.10 $(M+H)^+$

5-Isopropyl[1,2,4]triazino[5,6-*b*]indole-3-thiol (84)

Anal.:

Yield : 66%

M.P. : 307.01 °C (DSC)

TLC : R_f 0.53 (*n*-Hexane: Ethyl acetate 40%)

IR : 2941, 1602, 1559, 1349, 1146 and 742 cm^{-1}

^{1}H-NMR : 14.73(bs, 1H, -S*H*), 8.06-8.04 (d, 1H, Ar*H*), 7.81-7.79 (d, 1H, Ar*H*), 7.70-7.65 (m, 1H, Ar*H*), 7.41-7.37 (m, 1H, Ar*H*), 5.08-5.05 (m, 1H, -C*H*-), 1.61-1.59 (d, 6H, -CH_3)

^{13}C-NMR : 178.85, 147.70, 142.61, 135.33, 131.70, 123.76, 121.91, 117.93, 112.93, 45.80, 19.52

MS (ESI) : m/z 245.20 $(M+H)^+$

5-Butyl[1,2,4]triazino[5,6-*b*]indole-3-thiol (85)

Anal.:

Yield : 71%

M.P. : 274.32 °C (DSC)

TLC : R_f 0.59 (*n*-Hexane: Ethyl acetate 40%)

IR : 2925, 1603, 1561, 1330, 1137 and 757 cm^{-1}

^{1}H-NMR : 14.42(bs, 1H, -S*H*), 7.87-7.85 (d, 1H, Ar*H*), 7.53-7.52 (m, 2H, Ar*H*), 7.24-7.20 (m, 1H, Ar*H*), 4.03-4.00 (m, 2H, CH_2), 1.59-1.53 (m, 2H, CH_2), 1.20-1.14 (m, 2H, -CH_2), 0.76-0.72 (t, 3H, - CH_3)

^{13}C-NMR : 179.03, 147.95, 143.56, 135.22, 131.82, 123.44, 121.81, 117.42, 111.75, 42.78, 29.58, 19.60, 13.65

MS (ESI) : m/z 259.20 $(M+H)^+$

5-Benzyl[1,2,4]triazino[5,6-*b*]indole-3-thiol (86)

Anal.:

Yield : 69%

M.P. : 293.24 °C (DSC)

TLC : R_f 0.50 (*n*-Hexane: Ethyl acetate 40%)

IR : 2841, 1599, 1569, 1163 and 744 cm^{-1}

^{1}H-NMR : 14.73(bs, 1H,-S*H*), 8.03-8.01 (d, 1H, Ar*H*), 7.60-7.56 (m, 1H, Ar*H*), 7.45-7.43 (d, 1H, Ar*H*), 7.38-7.25 (m, 6H, Ar*H*), 5.47 (s, 2H, -CH_2-)

^{13}C-NMR : 179.33, 148.36, 143.31, 135.39, 131.80, 128.80, 127.84, 127.32, 123.70, 121.92, 117.65, 111.99, 44.08

MS (ESI) : m/z 291.68 $(M+H)^+$

5-(4-Methylbenzyl)[1,2,4]triazino[5,6-*b*]indole-3-thiol (87)

Anal.:

Yield : 72%

M.P. : 292.88 °C (DSC)

TLC : R_f 0.61 (*n*-Hexane: Ethyl acetate 40%)

IR : 2924, 1599, 1572, 1347, 1145 and 752 cm^{-1}

^{1}H-NMR : 14.69 (bs, 1H, -S*H*), 8.04-8.02 (d, 1H, Ar*H*), 7.61-7.57 (m, 1H, Ar*H*),

7.46-7.44 (d, 1H, Ar*H*), 7.38-7.34 (m, 1H, Ar*H*), 7.26-7.24 (d, 2H, Ar*H*), 7.13-7.11 (d, 2H, Ar*H*), 5.41 (s, 2H, CH_2), 2.27 (s, 3H,-CH_3)

^{13}C-NMR : 179.33, 148.30, 143.30, 137.13, 135.38, 132.38, 132.78, 129.34, 127.39, 123.68, 121.90, 117.66, 112.07, 43.87, 20.71

MS (ESI) : m/z 307.20 $(M+H)^+$

5-(3-Fluorobenzyl)[1,2,4]triazino[5,6-*b*]indole-3-thiol (88)

Anal.:

Yield : 64%

M.P. : 282.15 °C (DSC)

TLC : R_f 0.44 (*n*-Hexane: Ethyl acetate 40%)

IR : 2925, 1597, 1571, 1349, 1146 and 745 cm^{-1}

^{1}H-NMR : 14.71 (bs, 1H, -S*H*), 8.19 (s, 1H, Ar*H*), 8.05-8.03 (d, 1H, Ar*H*), 7.62-7.58 (m, 1H, Ar*H*), 7.47-7.45 (d, 1H, Ar*H*), 7.40-7.33 (m, 1H, Ar*H*), 7.20-7.18 (d, 2H, Ar*H*), 7.08-7.03 (m, 2H, Ar*H*), 5.48 (s, 2H, CH_2)

MS (ESI) : m/z 311.20 $(M+H)^+$

5-(4-*tert*-Butylbenzyl)[1,2,4]triazino[5,6-*b*]indole-3-thiol (89)

Anal.:

Yield : 81%

M.P. : 249.58 °C (DSC)

TLC : R_f 0.67 (*n*-Hexane: Ethyl acetate 40%)

IR : 3236, 3145, 2970, 2900, 1606, 1465, 1334 and 1140 cm^{-1}

^{1}H-NMR : 12.46 (bs, 1H, -S*H*), 7.73-7.72 (m, 1H, Ar*H*), 7.34-7.27 (m, 5H, Ar*H*), 7.12-7.08 (m, 1H, Ar*H*), 7.02-7.00 (m, 1H, Ar*H*), 4.91 (s, 2H, CH_2), 1.26 (s, 9H, *tert*-Butyl –CH_3)

MS (ESI) : m/z 350.09 $(M)^+$

5-(2-Bromobenzyl)[1,2,4]triazino[5,6-*b*]indole-3-thiol (90)

Anal.:

Yield : 84%

M.P. : 298.92 °C (DSC)

TLC : R_f 0.46 (*n*-Hexane: Ethyl acetate 40%)

IR : 2980, 2889, 1575, 1180 and 748 cm^{-1}

^{1}H-NMR : 14.72 (bs, 1H, -S*H*), 8.10-8.08 (m, 1H, Ar*H*), 7.72-7.70 (m, 1H, Ar*H*), 7.63-7.59 (m, 1H, Ar*H*), 7.43-7.39 (m, 1H, Ar*H*), 7.36-7.34 (d, 1H, Ar*H*), 7.27-7.22 (m, 2H, Ar*H*), 6.93-6.91 (m, 1H, Ar *H*), 5.45 (s, 2H, CH_2)

MS (ESI) : m/z 370.09 $(M)^+$, 372 $(M+2)^+$

5-(4-Chlorobenzyl)[1,2,4]triazino[5,6-*b*]indole-3-thiol (91)

Anal.:

Yield : 76%

M.P. : 300.43 °C (DSC)

TLC : R_f 0.42 (*n*-Hexane: Ethyl acetate 40%)

IR : 2980, 2885, 1571, 1377, 1141 and 754 cm^{-1}

^{1}H-NMR : 14.69 (bs, 1H, -S*H*), 8.05-8.03 (d, 1H, Ar-*H*), 7.63-7.59 (m, 1H, Ar-*H*), 7.49-7.47 (d, 1H, Ar-*H*), 7.41-7.35 (m, 5H, Ar-*H*), 5.45 (s, 2H, CH_2)

MS (ESI) : m/z 325.99 $(M)^+$, 328 $(M+2)^+$

5-(4-Methoxybenzyl)[1,2,4]triazino[5,6-*b*]indole-3-thiol (92)

Anal.:

Yield : 78%

M.P. : Degrades above 250 °C (DSC)

TLC : R_f 0.57 (*n*-Hexane: Ethyl acetate 40%)

IR : 2980, 1608, 1346, 1246 and 759 cm^{-1}

^{1}H-NMR : 14.63 (bs, 1H, -S*H*), 7.57-7.53 (m, 2H, Ar*H*), 7.34-7.32 (m, 2H, Ar*H*), 7.12-7.08 (m, 1H, Ar*H*), 6.98-6.96 (m, 1H, Ar*H*), 6.88-6.86 (m, 2H, Ar*H*), 4.84 (s, 2H, -CH_2), 3.73 (s, 3H, -OCH_3)

MS (ESI) : m/z 321.09 $(M)^+$

5.1.4. Synthesis of 6-Substituted 6*H*-indolo[2,3-*b*]quinoxaline derivatives (93, 96 – 106) (series IV)

1-(3-Trifluoromethylbenzyl)-1*H*-indole-2,3-dione (94)

Prepared by the procedure as described for *N*-substituted isatins (**71** – **79**).

Anal.:

Yield : 64%

M.P. : 95-97 °C

TLC : R_f 0.54 (*n*-Hexane: Ethyl acetate 50%)

IR : 1730, 1692, 1606, 1467, 1326, 1169 and 753 cm^{-1}

1-(4-Cyanobenzyl)-1*H*-indole-2,3-dione (95)

Prepared by the procedure as described for *N*-substituted isatins (**71** – **79**).

Anal.:

Yield : 78%

M.P. : 174-176 °C

TLC : R_f 0.61 (*n*-Hexane: Ethyl acetate 50%)

IR : 2227, 1735, 1611, 1467, 1350, 1175 and 757 cm^{-1}

Synthesis of 6-substituted 6*H*-indolo[2,3-*b*]quinoxaline derivatives (93, 96 – 106)

Isatin (1mM) and OPD (1mM) in acetic acid under microwave condition, 450 W for 8 minutes, were irradiated to obtain compound (**93**), whereas *N*-substituted isatins (1mM) and OPD (1mM) in acetic acid were irradiated under microwave condition at 450 W for 8-10 minutes to obtain the desired products (**96** – **106**). After completion, the reaction mixture was poured in ice cold water; the precipitated product was filtered, dried and purified using column chromatography with silica as stationary phase.

Indolo[2,3-*b*]quinoxaline (93)

Anal.:

Yield : 79%

M.P. : 289.93 °C (DSC)

TLC : R_f 0.42 (*n*-Hexane: Ethyl acetate 10%)

IR : 3138, 2960, 2926, 1597, 1404 and 744 cm^{-1}

^{1}H-NMR : 11.94 (bs, 1H, -N*H*), 8.35-8.33 (d, 1H, Ar*H*), 8.25-8.21 (m, 1H, Ar*H*), 8.07-8.05 (m, 1H, Ar*H*), 7.79-7.75 (m, 1H, Ar*H*), 7.71-7.65 (m, 2H, Ar*H*), 7.58-7.56 (d, 1H, Ar*H*), 7.37-7.33 (m, 1H, Ar-*H*)

MS (ESI) : m/z 220.10 $(M+H)^+$

6-Methyl-6*H*-indolo[2,3-*b*]quinoxaline (96)

Anal.:

Yield : 76%

M.P. : 150-152 °C

TLC : R_f 0.31 (*n*-Hexane: Ethyl acetate 10%)

IR : 3055, 2966, 1583, 1388, 1112 and 748 cm^{-1}

^{1}H-NMR : 8.39-8.37 (d, 1H, Ar*H*), 8.26-8.23 (m, 1H, Ar*H*), 8.12-8.10 (m, 1H, Ar*H*), 7.82-7.75 (m, 2H, Ar*H*), 7.73-7.69 (m, 2H, Ar*H*), 7.43- 7.39 (m, 1H, Ar*H*), 3.96 (s, 3H, -CH_3)

MS (ESI) : m/z 234.20 $(M+H)^+$

6-Ethyl-6*H*-indolo[2,3-*b*]quinoxaline (97)

Anal.:

Yield : 75%

M.P. : 136-138 °C

TLC : R_f 0.43 (*n*-Hexane: Ethyl acetate 10%)

IR : 3055, 2970, 1608, 1585, 1409, 1116 and 742 cm^{-1}

^{1}H-NMR : 8.40-8.38 (d, 1H, Ar*H*), 8.27-8.24 (m, 1H, Ar*H*), 8.13-8.11 (m, 1H, Ar*H*), 7.83-7.69 (m, 4H, Ar*H*), 7.43-7.39 (m, 1H, Ar*H*), 4.57-4.56 (m, 2H, -CH_2-), 1.47-1.43 (t, 3H, -CH_3)

^{13}C-NMR : 145.08, 144.30, 140.41, 140.07, 139.05, 131.94, 129.53, 127.97, 126.54, 122.80, 121.45, 119.06, 110.78, 36.31, 13.88

MS (ESI) : m/z 248.20 $(M+H)^+$

6-Isopropyl-6*H*-indolo[2,3-*b*]quinoxaline (98)

Anal.:

Yield : 70%

M.P. : 144-146 °C

TLC : R_f 0.65 (*n*-Hexane: Ethyl acetate 10%)

IR : 2985, 2968, 1608, 1579, 1382, 1234 and 748 cm^{-1}

^{1}H-NMR : 8.41-8.39 (d, 1H, Ar*H*), 8.24-8.22 (m, 1H, Ar*H*), 8.10-8.08 (m, 1H, Ar*H*), 7.80-7.67 (m, 4H, Ar*H*), 7.40-7.36 (m, 1H, Ar*H*), 5.42-5.39 (m, 1H, -C*H*$(CH_3)_2$), 1.78-1.77 (d, 6H, -CH(C*H*$_3)_2$)

MS (ESI) : m/z 262.20 $(M+H)^+$

6-Butyl-6*H*-indolo[2,3-*b*]quinoxaline (99)

Anal.:

Yield : 68%

M.P. : 115-117 °C

TLC : R_f 0.64 (*n*-Hexane: Ethyl acetate 10%)

IR : 3055, 2960, 2887, 1606, 1581, 1371, 1112 and 746 cm^{-1}

^{1}H-NMR : 8.39-8.37 (d, 1H, Ar*H*), 8.26-8.24 (m, 1H, Ar*H*), 8.11-8.09 (m, 1H, Ar*H*), 7.80-7.67 (m, 4H, Ar*H*), 7.41-7.37 (m, 1H, Ar*H*), 4.53-4.49 (t, 2H, -C*H*$_2$-), 1.94-1.86 (m, 2H, -C*H*$_2$-), 1.42-1.37 (m, 2H, -C*H*$_2$-), 0.97-0.94 (t, 3H, -C*H*$_3$)

^{13}C-NMR : 145.51, 144.73, 140.44, 139.93, 139.05, 131.95, 129.54, 128.03, 126.57, 122.75, 121.45, 118.98, 110.96, 41.20, 30.54, 20.19, 14.15

MS (ESI) : m/z 276.20 $(M+H)^+$

6-Benzyl-6*H*-indolo[2,3-*b*]quinoxaline (100)

Anal.:

Yield : 74%

M.P. : 172-174 °C

TLC : R_f 0.44 (*n*-Hexane: Ethyl acetate 10%)

IR : 3084, 2980, 2960, 1581, 1408, 1197 and 742 cm^{-1}

^{1}H-NMR : 8.41-8.39 (d, 1H, Ar*H*), 8.28-8.26 (m, 1H, Ar*H*), 8.13-8.11 (m, 1H, Ar*H*), 7.81-7.77 (m, 1H, Ar*H*), 7.73-7.65 (m, 2H, Ar*H*), 7.58-7.56 (d, 1H, Ar*H*), 7.41-7.35 (m, 3H, Ar*H*), 7.30-7.21 (m, 3H, Ar*H*), 5.75 (s, 2H, -C*H*$_2$-)

^{13}C-NMR : 145.66, 144.53, 140.49, 140.02, 139.35, 137.36, 132.00, 129.76, 129.62,

129.24, 128.03, 127.64, 126.85, 122.85, 121.86, 119.27, 111.12, 44.72

MS (ESI) : m/z 310.14 $(M+H)^+$

6-(4-Methylbenzyl)-6*H*-indolo[2,3-*b*]quinoxaline (101)

Anal.:

Yield : 72%

M.P. : 206-210 °C

TLC : R_f 0.70 (*n*-Hexane: Ethyl acetate 10%)

IR : 3053, 2990, 1581, 1469, 1197 and 742 cm^{-1}

^{1}H-NMR : 8.40-8.38 (d, 1H, Ar*H*), 8.28-8.26 (m, 1H, Ar*H*), 8.13-8.11 (m, 1H, Ar*H*), 7.81-7.79 (m, 1H, Ar*H*), 7.74-7.66 (m, 2H, Ar*H*), 7.61-7.59 (d, 1H, Ar*H*), 7.41-7.37 (m, 1H, Ar*H*), 7.26-7.24 (d, 2H, Ar*H*), 7.09-7.07 (d, 2H, Ar*H*), 5.70 (s, 2H, -CH_2-), 2.24 (s, 3H, -CH_3)

MS (ESI) : m/z 324.15 $(M+H)^+$

6-(3-Fluorobenzyl)-6*H*-indolo[2,3-*b*]quinoxaline (102)

Anal.:

Yield : 76%

M.P. : 137-140 °C

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

IR : 3062, 2980, 1587, 1408, 1253 and 748 cm^{-1}

^{1}H-NMR : 8.42-8.40 (d, 1H, Ar*H*), 8.30-8.28 (m, 1H, Ar*H*), 8.14-8.12 (m, 1H, Ar*H*), 7.84-7.59 (m, 4H, Ar*H*), 7.43-7.39 (m, 1H, Ar*H*), 7.32-7.28 (m, 1H, Ar*H*), 7.21-7.16 (m, 2H, Ar*H*), 7.05-7.00 (m, 1H, Ar*H*), 5.59 (s, 2H, -CH_2-)

MS (ESI) : m/z 328.20 $(M+H)^+$

6-(4-*tert*-Butylbenzyl)-6*H*-indolo[2,3-*b*]quinoxaline (103)

Anal.:

Yield : 77%

M.P. : 164-166 °C

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

IR : 3057, 2964, 1585, 1406, 1114 and 746 cm^{-1}

^{1}H-NMR : 8.40-8.38 (d, 1H, Ar*H*), 8.27-8.25 (d, 1H, Ar*H*), 8.12-8.10 (d, 1H, Ar*H*), 7.81-7.77 (m, 1H, Ar*H*), 7.73-7.61 (m, 3H, Ar*H*), 7.40-7.28 (m, 5H, Ar*H*), 5.69 (s, 2H, -CH_2-), 1.21 (s, 9H, -C(CH_3)$_3$)

MS (ESI) : m/z 366.20 $(M+H)^+$

6-(2-Bromobenzyl)-6*H*-indolo[2,3-*b*]quinoxaline (104)

Anal.:

Yield : 71%

M.P. : 194-196 °C

TLC : R_f 0.62 (*n*-Hexane: Ethyl acetate 10%)

IR : 3057, 2980, 1587, 1404, 1154 and 756 cm^{-1}

^{1}H-NMR : 8.45-8.43 (d, 1H, Ar*H*), 8.29-8.27 (m, 1H, Ar*H*), 8.08-8.06 (m, 1H, Ar*H*), 7.80-7.66 (m, 4H, Ar*H*), 7.46-7.41 (m, 2H, Ar*H*), 7.22-7.18 (m, 1H, Ar*H*), 7.15-7.11 (m, 1H, Ar*H*), 6.73-6.71 (m, 1H, Ar*H*), 5.76 (s, 2H, -CH_2-)

MS (ESI) : m/z 388.20 $(M+H)^+$, 390.20 $(M+ 2+H)^+$

6-(3-Trifluoromethylbenzyl)-6*H*-indolo[2,3-*b*]quinoxaline (105)

Anal.:

Yield : 68%

M.P. : 183-185 °C

TLC : R_f 0.55 (*n*-Hexane: Ethyl acetate 10%)

IR : 3034, 2980, 1585, 1327, 1166 and 750 cm^{-1}

^{1}H-NMR : 8.42-8.40 (d, 1H, Ar*H*), 8.29-8.27 (m, 1H, Ar*H*), 8.12-8.09 (m, 1H, Ar*H*), 7.82-7.78 (m, 1H, Ar*H*), 7.75-7.67 (m, 2H, Ar*H*), 7.62-7.54 (m, 5H, Ar*H*), 7.43-7.39 (m, 1H, Ar*H*), 5.85 (s, 2H, -CH_2-)

MS (ESI) : m/z 378.20 $(M+H)^+$

6-(4-Cynobenzyl)-6*H*-indolo[2,3-*b*]quinoxaline (106)

Anal.:

Yield : 74%

M.P. : 148-150 °C

TLC : R_f 0.55 (*n*-Hexane: Ethyl acetate 10%)

IR : 2972, 2306, 1587, 1409, 1253 and 750 cm^{-1}

^{1}H-NMR : 8.40-8.38 (d, 1H, Ar*H*), 8.27-8.25 (m, 1H, Ar*H*), 8.12-8.10 (m, 1H, Ar*H*), 7.80-7.76 (m, 1H, Ar*H*), 7.73-7.66 (m, 2H, Ar*H*), 7.59-7.57 (d, 1H, Ar*H*), 7.41-7.37 (m, 1H, Ar*H*) 7.32-7.27 (m, 1H, Ar*H*), 7.17-7.14 (m, 2H, Ar*H*), 7.01-6.97 (m, 1H, Ar*H*), 5.74 (s, 2H, -CH_2-)

^{13}C-NMR : 145.62, 144.40, 140.44, 140.07, 139.42, 132.01, 131.33, 131.24, 129.70, 129.62, 128.06, 126.85, 123.62, 122.86, 121.93, 119.33, 114.99, 114.78, 114.69, 114.47, 111.17, 44.22

5.1.5. Synthesis of 5-substituted 5*H*-indolo[2,3-*b*]quinoxaline derivatives (107 – 117) (Series V)

To synthesize the titled compounds N_1-substituted 1,2-diamines (**24**, **26**-**29**, **31**-**32**, **34**-**37**) were prepared from *N*-arylalkyl 2-nitroaniline intermediates (**7**, **9**-**12**, **14**-**15**, **17**-**20**) as mentioned in series I. These *N*1-substituted 1,2-diamines were treated with isatin (1 eq.) in presence of H_3BO_3 (1 eq.) and acetic acid (10 ml) to obtain the final compounds (**107** – **117**). The procedure followed here is same as mentioned in series I except in that in the place of alloxan monohydrate, isatin in equivalent quantities were used. The reaction mixture was allowed to stir overnight and the product was collected by pouring the reaction mixture into water. The precipitated product was filtered, dried and purified by using column chromatography with silica gel (100-200) as the stationary phase to get the final red coloured products.

5-Benzyl-5*H*-indolo[2,3-*b*]quinoxaline (107)

Anal.:

Yield : 67%

M.P. : 215-218 °C

TLC : R_f 0.57 (*n*-Hexane: Ethyl acetate 30%)

IR : 3064, 2980, 2962, 1579, 1438, 1288 and 746 cm^{-1}

^{1}H-NMR : 8.29-8.27 (m, 1H, Ar*H*), 8.24-8.22 (m, 1H, Ar*H*), 7.90-7.88 (m, 1H, Ar*H*) 7.76-7.72 (m, 1H, Ar*H*), 7.68-7.64 (m, 1H, Ar*H*), 7.61-7.56 (m, 2H, Ar*H*), 7.35-7.25 (m, 6H, Ar*H*), 6.14 (s, 2H, -CH_2-)

MS (ESI) : m/z 310.10 $(M+H)^+$

5-(4-Methylbenzyl)-5*H*-indolo[2,3-*b*]quinoxaline (108)

Anal.:

Yield : 68%

M.P. : 209-211 °C

TLC : R_f 0.63 (*n*-Hexane: Ethyl acetate 30%)

IR : 2980, 2887, 1579, 1566, 1438, 1290 and 754 cm^{-1}

^{1}H-NMR : 8.28-8.26 (m, 1H, Ar*H*), 8.24-8.22 (m, 1H, Ar*H*), 7.90-7.88 (d, 1H, Ar*H*), 7.75-7.71 (m, 1H, Ar*H*), 7.68-7.64 (m, 1H, Ar*H*), 7.61-7.55 (m, 2H, Ar*H*), 7.32-7.28 (m, 1H, Ar*H*), 7.25-7.23 (d, 2H, Ar*H*), 7.11-7.09 (d, 2H, Ar*H*), 6.08 (s, 2H, -CH_2-), 2.24 (s, 3H, -CH_3)

MS (ESI) : m/z 324.20 $(M+H)^+$

5-(2-Fluorobenzyl)-5*H*-indolo[2,3-*b*]quinoxaline (109)

Anal.:

Yield : 63%

M.P. : 196-198 °C.

TLC : R_f 0.63 (*n*-Hexane: Ethyl acetate 30%)

IR : 3032, 2960, 1579, 1438, 1138 and 748 cm^{-1}

^{1}H-NMR : 8.28-8.21 (m, 2H, Ar*H*), 7.92-7.90 (m, 1H, Ar*H*), 7.76-7.72 (m, 1H, Ar *H*), 7.68-7.64 (m, 1H, Ar*H*), 7.61-7.56 (m, 2H, Ar*H*), 7.45-7.42 (m, 2H, Ar*H*), 7.32-7.28 (m, 1H, Ar*H*), 7.10-7.06 (m, 2H, Ar*H*), 6.11 (s, 2H, -CH_2-)

MS (ESI) : m/z 328.10 $(M+H)^+$

5-(3-Fluorobenzyl)-5*H*-indolo[2,3-*b*]quinoxaline (110)

Anal.:

Yield : 59%

M.P. : 202-204 °C.

TLC : R_f 0.51 (*n*-Hexane: Ethyl acetate 30%)

IR : 2980, 2889, 1579, 1438, 1138 and 748 cm^{-1}

^{1}H-NMR : 8.29-8.27 (m, 1H, Ar*H*), 8.24-8.22 (m, 1H, Ar*H*), 7.89-7.87 (m, 1H, Ar

H), 7.77-7.73 (m, 1H, Ar*H*), 7.68-7.64 (m, 1H, Ar*H*), 7.61-7.57 (m, 2H, Ar*H*), 7.36-7.28 (m, 2H, Ar*H*), 7.24-7.22 (d, 1H, Ar*H*), 7.16-7.14 (d, 1H, Ar*H*), 7.08-7.04 (m, 1H, Ar*H*), 6.13 (s, 2H, -CH_2-)

MS (ESI) : m/z 328.10 $(M+H)^+$

5-(3-Chlorobenzyl)-5*H*-indolo[2,3-*b*]quinoxaline (111)

Anal.:

Yield : 66%

M.P. : 198-200 °C

TLC : R_f 0.66 (*n*-Hexane: Ethyl acetate 30%)

IR : 3057, 2980, 1579, 1436, 1288 and 746 cm^{-1}

^{1}H-NMR : 8.30-8.28 (m, 1H, Ar*H*), 8.24-8.22 (m, 1H, Ar*H*), 7.89-7.87 (m, 1H, Ar *H*), 7.77-7.75 (m, 1H, Ar*H*), 7.66-7.59 (m, 1H, Ar*H*), 7.61-7.59 (m, 2H, Ar*H*), 7.49 (s, 1H, Ar*H*), 7.32-7.25 (m, 4H, Ar*H*), 6.13 (s, 2H, -CH_2-)

MS (ESI) : m/z 344.10 $(M+H)^+$, 346.10 $(M+2+H)^+$

5-(2-Methoxybenzyl)-5*H*-indolo[2,3-*b*]quinoxaline (112)

Anal.:

Yield : 53%

M.P. : 218-220 °C

TLC : R_f 0.50 (*n*-Hexane: Ethyl acetate 30%)

IR : 2980, 2972, 1577, 1429, 1246 and 752 cm^{-1}

^{1}H-NMR : 8.31-8.29 (m, 1H, Ar*H*), 8.24-8.22 (m, 1H, Ar*H*), 7.73-7.56 (m, 5H, Ar *H*), 7.31-7.23 (m, 2H, Ar*H*), 7.13-7.11 (m, 1H, Ar*H*), 6.72-6.68 (m, 1H, Ar*H*), 6.59-6.57 (m, 1H, Ar*H*), 6.02 (s, 2H, -CH_2-), 4.10 (s, 3H, -OCH_3)

MS (ESI) : m/z 340.10 $(M+H)^+$

5-(4-Methoxybenzyl)-5*H*-indolo[2,3-*b*]quinoxaline (113)

Anal.:

Yield : 56%

M.P. : 216-218 °C

TLC : R_f 0.37 (*n*-Hexane: Ethyl acetate 30%)

IR : 3051, 2968, 1579, 1438, 1247 and 742 cm^{-1}

^{1}H-NMR : 8.29-8.22 (m, 2H, Ar*H*), 7.98-7.96 (m, 1H, Ar*H*), 7.78-7.74 (m, 1H, Ar *H*), 7.67-7.57 (m, 2H, Ar*H*), 7.35-7.28 (m, 3H, Ar*H*), 6.86-6.84 (m, 3H, Ar*H*), 6.06 (s, 2H, -CH_2-), 3.69 (s, 3H, -OCH_3)

MS (ESI) : m/z 340.20 $(M+H)^+$

5-(3,4-Dimethoxybenzyl)-5*H*-indolo[2,3-*b*]quinoxaline (114)

Anal.:

Yield : 59%

M.P. : 224-226 °C

TLC : R_f 0.36 (*n*-Hexane: Ethyl acetate 30%)

IR : 2970, 2902, 1579, 1438, 1259 and 752 cm^{-1}

^{1}H-NMR : 8.28-8.22 (m, 1H, Ar*H*), 7.98-7.96 (m, 1H, Ar*H*), 7.77-7.73 (m, 1H, Ar *H*), 7.68-7.56 (m, 3H, Ar*H*), 7.31-7.28 (m, 1H, Ar*H*), 7.21-7.07 (m, 1H, Ar*H*), 6.80-6.74 (m, 3H, Ar*H*), 6.05 (s, 2H, -CH_2-), 3.78(s, 3H, -OCH_3), 3.76(s, 3H, -OCH_3)

^{13}C-NMR : 159.29, 153.45, 149.36, 147.04, 133.36, 130.95, 130.80, 128.30, 124.30, 123.08, 121.55, 119.60, 118.90, 116.13, 112.44, 112.07, 55.98, 48.56, 40.56

MS (ESI) : m/z 370.20 $(M+H)^+$

5-Phenylethyl-5*H*-indolo[2,3-*b*]quinoxaline (115)

Anal.:

Yield : 68%

M.P. : 184-186 °C

TLC : R_f 0.50 (*n*-Hexane: Ethyl acetate 30%)

IR : 2980, 2885, 1564, 1454, 1244 and 746 cm^{-1}

^{1}H-NMR : 8.28-8.26 (m, 1H, Ar*H*), 8.21-8.19 (m, 1H, Ar*H*), 8.07-8.05 (m, 1H, Ar *H*), 7.83-7.79 (m, 1H, Ar*H*), 7.66-7.58 (m, 3H, Ar*H*), 7.41-7.39 (m, 2H, Ar*H*), 7.33-7.22 (m, 4H, Ar*H*), 5.09-5.05 (m, 2H, -CH_2-), 3.27-3.23 (m, 2H, -CH_2-)

^{13}C-NMR : 159.19, 153.08, 146.14, 136.30, 134.77, 133.25, 130.93, 130.89, 129.45, 129.00, 127.20, 124.17, 123.15, 122.93, 121.41, 118.91, 115.64, 46.67, 33.32

MS (ESI) : m/z 324.20 $(M+H)^+$

5-(4-Chlorophenylethyl)-5*H*-indolo[2,3-*b*]quinoxaline (116)

Anal.:

Yield : 63%

M.P. : 185-187 °C

TLC : R_f 0.32 (*n*-Hexane: Ethyl acetate 30%)

IR : 2980, 2889, 1566, 1444, 1290 and 752 cm^{-1}

^{1}H-NMR : 8.26-8.24 (m, 1H, Ar*H*), 8.20-8.18 (m, 1H, Ar*H*), 8.01-7.99 (m, 1H, Ar *H*), 7.81-7.77 (m, 1H, Ar*H*), 7.64-7.56 (m, 3H, Ar-*H*), 7.41-7.39 (m, 2H, Ar-*H*), 7.32-7.25 (m, 3H, Ar-*H*), 5.07-5.03 (m, 2H, -CH_2-), 3.28-3.24 (m, 2H, -CH_2-)

MS (ESI) : m/z 358.20 $(M+H)^+$, 360 $(M+2+H)^+$

5-(3,4-Dimethoxyphenylethyl)-5*H*-indolo[2,3-*b*]quinoxaline (117)

Anal.:

Yield : 57%

M.P. : 159-160 °C

TLC : R_f 0.28 (*n*-Hexane: Ethyl acetate 30%)

IR : 2980, 2972, 1564, 1460, 1136 and 746 cm^{-1}

^{1}H-NMR : 8.25-8.23 (m, 1H, Ar*H*), 8.19-8.17 (m, 1H, Ar*H*), 7.98-7.96 (m, 1H, Ar *H*), 7.79-7.75 (m, 1H, Ar*H*), 7.65-7.55 (m, 3H, Ar*H*), 7.27-7.24 (m, 1H, Ar*H*), 6.94 (s, 1H, Ar*H*), 6.85-6.79 (m, 2H, Ar*H*), 5.07-5.03 (m, 2H, -CH_2-), 3.76 (s, 3H, -OCH_3), 3.74 (s, 3H, -OCH_3), 3.21-3.17 (m, 2H, -CH_2-)

MS (ESI) : m/z 384.20 $(M+H)^+$

5.2: Pharmacological Work

5.2.1. Cholinesterase inhibition assay (Ellman's method)

Ellman's method[133] was used to find out the human AChE (product number C1682, Sigma-Aldrich) and equine serum BuChE (product number C1057, Sigma-Aldrich) inhibition profile of the compounds synthesized in **series I** to **series V** (IC_{50} values, µM). Suitable drugs like donepezil hydrochloride and tacrine hydrochloride hydrate (item number A79922 Sigma-Aldrich)

were used as standards for this study. Buffer solution (50 mM Tris–HCl, *p*H 8.0, 0.1 M NaCl, 0.02 M $MgCl_2.6H_2O$) was used to dilute the stock solutions of the test compounds dissolved in minimum volume of DMSO (1%). First of all 50 µL of AChE (0.22 U/mL prepared in 50 mM Tris–HCl, *p*H 8.0, 0.1% w/v bovine serum albumin, BSA) or 50 µL of BuChE (0.06 U/mL prepared in 50 mM Tris–HCl, *p*H 8.0, 0.1% w/v BSA) and 10 µL of different concentrations of the test and standard compounds (0.001–100 µM) were incubated in 96-well plates at room temperature for 30 min. Further, 30 µL of the substrate viz. acetylthiocholine iodide [ATCI (15 mM)] or butyrylthiocholine iodide [BTCI (15 mM)] was added and incubated for 30 min. Finally 160 µL DTNB (1.5 mM) was added and absorbance was measured at 415 nm wavelength using Biorad microplate reader 680XR. Percentage inhibition was calculated by comparing the test compounds treated to various control incubations that included 1% DMSO. The 50% inhibition (IC_{50}, µM) of the test compound was calculated from the concentration–inhibition response curve on logarithmic scale. All determinations were performed in triplicate.

5.2.2. *h*AChE induced $A\beta_{1\text{-}42}$ aggregation inhibition assay (Thioflavin-T assay)

The stock solution of $A\beta_{1\text{-}42}$ peptide (Sigma-Aldrich) (1 mM) was prepared in DMSO and further diluted to 50 µM solution with 0.215 M sodium phosphate buffer (*p*H 8.0). hAChE (Sigma-Aldrich) was prepared in sodium phosphate buffer at a final concentration of 230 µM. Aliquots of 2 µL of $A\beta_{1–42}$ (50 µM) and 16 µL of hAChE (230 µM) were incubated together. Co-incubation experiments were performed by incubating 2 µL of the test or the reference compounds (10 µM; prepared in 0.215 M sodium phosphate buffer, *p*H 8.0) with 2µL of $A\beta_{1–42}$ (50 µM) and 16 µL of hAChE (230 µM). This reaction mixture was incubated for 24 h at room temperature. After incubation, 100 µL of thioflavin-T (ThT) (Sigma-Aldrich) (20 µM; prepared in 50 mM glycine-NaOH buffer, *p*H 8.5) was added. Finally the volume was adjusted to 2 ml using 50 mM glycine-NaOH buffer, *p*H 8.5. Aggregation inhibition was quantified by monitoring the fluorescence using excitation at 442 nm and emission at 490 nm using spectroflourometer (RF-5301 PC, Shimadzu, Japan). Each assay was run in triplicate and each reaction was repeated at three independent time periods. Tacrine and donepezil were used as reference compounds. Percentage of aggregation inhibition was calculated using the equation: 100 - (IFi/IFo × 100) where IFi and IFo are the fluorescence intensities obtained for $A\beta_{1–42}$ + hAChE in the presence and absence of inhibitor, respectively.[134,135]

5.2.3. Congo red assay

The $A\beta_{1-42}$ aggregation was measured using Congo red (CR) binding assay. Stock solution of CR (Hi-Media) (5 mM) was prepared in PBS (*p*H 7.4) which was further diluted with PBS to get the final concentration of 5 µM. Stock solution of $A\beta_{1-42}$ (1 mM) was prepared in DMSO, which was further diluted with PBS to get the final concentration of 20 µM. 100µl of the test or reference compound (10 µM; prepared in PBS, *p*H 7.4) was incubated with 100µl of 20 µM $A\beta_{1-42}$ for 6 hr at 37 ºC. Later the mixture was further incubated with 100µl of 5 µM CR for 30 min at room temperature. Final volume was adjusted to 500µl using PBS. After the incubation, CR spectra were measured using a UV-spectrophotometer (UV-1700, Shimadzu, Japan) at 480 nm and 540nm. Tacrine and donepezil were used as reference compounds. CR binding was calculated according to the following formula: CB (M) = (OD at 540nm/25,295) - (OD at 480nm/46,306) where CB (M) is amount of CR bound with β sheet of $A\beta_{1-42}$ and OD is optical density.[136,137]

5.2.4. Cytotoxicity studies

The cytotoxicity of the test and reference compounds was assessed using SH-SY5Y human neuroblastoma cell line obtained from National Centre for Cell Science, Pune. The SH-SY5Y cells were routinely cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with (10% v/v) fetal bovine serum (FBS), 100 U/ml penicillin and 100 U/ml streptomycin at 37ºC with 5% CO_2. The cells, cultured in 75 cm^2 flasks, were plated in 96 well plates at a density of about 50,000 cells per well in 100 µl of fresh medium and incubated for 24 h. Subsequently, the growth medium was replaced with fresh normal media (control cultures) or with media supplemented with test or reference compounds (40µM in 0.1% DMSO) and again incubated for 24 h. Tacrine and donepezil was used as the reference compounds. After the incubation period, cell viability was assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. In brief, 20 µL of MTT (Sigma-Aldrich) solution (5 mg/ml stock solution) was added into each well and incubated for 4 h at 37 ºC. Later, the culture medium was replaced by 200 µL DMSO to dissolve the formazan. The optical density was measured at 570 nm with 630 nm reference wavelengths using a micro plate reader 680 XR (BIO-RAD, India). The absorbance of the control was considered as 100% of the cell viability.[138]

5.3. Docking Studies

Molecular docking studies were performed using Glide (Schrodinger 2009)[139]. The ligand structures were built within Maestro using the Build module and a single low energy conformation search was performed for molecules under study using OPLS_2005 force field at physiological *p*H condition using LigPrep[140] module of Schrödinger. Docking calculations were performed in extra precision (XP) mode with the active sites of receptor (enzyme) structures.