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

General methods

Solvents and reagents were purchased from commercial sources (Spectrochem, Sigma-Aldrich, S. D. Fine Chemicals and Avra) and purified and dried as per standard laboratory methods. Thin layer chromatography (TLC) was performed for monitoring the reaction progress using precoated silica gel plates (60F254, Merck, 0.25 mm thickness) and visualizing in ultraviolet (UV) light (λ = 254 nm) or in an iodine chamber. Melting points were determined in glass capillary tube using Veego programmable melting point apparatus. IR peak positions (in cm^{-1}) spectra in KBr pellets were recorded using Bruker ALPHA-T instrument. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker Advance II spectrometer (400 and 100 MHz) in $CDCl_3$ and DMSO-d_6. The chemical shifts (δ) are represented in parts per million (ppm) relative to internal standard TMS where signal multiplicities are designated as s, d, dd, dt, t, bs and m for singlet, doublet, doublet of doublet, doublet of triplet, triplet, broad singlet and multiplet, respectively. Mass spectra were recorded on Thermoscientific DSQ-II mass spectrometer. The synthesized compounds were purified by recrystallization and Flash column chromatography. Flash column chromatography was performed on Combiflash RF 200 (Teledyne Esco) using flash grade silica gel (230-400 mesh). Yields reported here are unoptimized. The purity of the compounds was assessed by HPLC, with all the compounds exhibiting a ≥95% purity level. 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. Human FXa and FIIa were purchased from

Haematologic Technologies (Essex Junction, VT), Enzyme Research Laboratories (South Bend, IN) and Molecular Innovations. Chromogenic substrate Spectrozyme TH was purchased from American Diagnostica (Greenwich, CT), while S-2222 and S-2765 were purchased from DiaPharma Group, Inc (West Chester, OH). 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/2016-17/1641 and MSU/IAEC/2019-20/1905).

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

5.1.1. 2-Aminobenzamide-based FXa Inhibitors,

5.1.2. 1,3,4-Thiadiazole-based FXa inhibitors and

5.1.3. Carbazole derivatives as FXa inhibitors.

5.1.1. 2-Aminobenzamide-based FXa Inhibitors

5.1.1.1. General method for the synthesis of *N*-substituted isatoic anhydride (52-63)

Method A: In a 50 mL round-bottom flask, isatoic anhydride (**51**) (1.0 g, 6.13 mmol) was dissolved in DMA (10 mL), and DIPEA (15.32 mmol, 2.6 mL) was added to it. Alkyl/arylalkyl halide (6.128 mmol) was added and the mixture was stirred overnight at RT. Completion of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was poured into ice-cold water with continuous stirring. The precipitated off-white to brownish colored solid was filtered, dried and used for the next step.

5.1.1.1a. *N*-Methylisatoic anhydride (52)

The title compound (**52**) was synthesized from isatoic anhydride (**51**) (1.0 g, 6.13 mmol) and methyl iodide (0.38 mL, 6.13 mmol) as per **Method A**. The title compound (**52**) was obtained as brownish solid (0.98 g, 90 %), m.p. 160-162 °C (lit.[90] m.p. 164-166 °C).

Analytical data

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

IR (KBr, cm^{-1}) : 1771, 1719, 1324, 1068, 1024, 762 and 742.

5.1.1.1b. *N*-Ethylisatoic anhydride (53)

The title compound (**53**) was synthesized from isatoic anhydride (**51**) (1.0 g, 6.13 mmol) and ethyl iodide (0.49 mL, 6.13 mmol) as per **Method A**. The title compound (**53**) was obtained as brownish solid (1.08 g, 92 %), m.p. 111-113 °C (lit.[91] m.p. 115-117 °C).

Analytical data

TLC : R_f 0.44 (*n*-Hexane-Ethyl acetate 12:8);

IR (KBr, cm^{-1}) : 1773, 1726, 1323, 1050 and 745.

5.1.1.1c. *N*-Allylisatoic anhydride (54)

The title compound (**54**) was synthesized from isatoic anhydride (**51**) (1.0 g, 6.13 mmol) and allyl bromide (0.53 mL, 6.13 mmol) as per **Method A**. The title compound (**54**) was obtained as yellowish solid (1.18 g, 95 %), m.p. 97-99 °C (lit.[92] m.p. 102-104 °C).

Analytical data

TLC : R_f 0.57 (*n*-Hexane-Ethyl acetate 12:8);

IR (KBr, cm^{-1}) : 1770, 1722, 1602, 1474, 1379, 1319, 1027 and 759.

5.1.1.1d. *N*-Benzylisatoic anhydride (55)

The title compound (**55**) was synthesized from isatoic anhydride (**51**) (1.0 g, 6.13 mmol) and benzyl bromide (0.73 mL, 6.13 mmol) as per **Method A**. The title compound (**55**) was obtained as off white solid (1.41 g, 91 %), m.p. 140-142 °C (lit.[90] m.p. 139-141 °C).

Analytical data

TLC : R_f 0.46 (*n*-Hexane-Ethyl acetate 12:8);

IR (KBr, cm^{-1}) : 1779, 1719, 1603, 1473, 1379, 1317, 1025 and 758

5.1.1.1e. *N*-(4-Bromobenzyl)isatoic anhydride (56)

The title compound (**56**) was synthesized from isatoic anhydride (**51**) (1.0 g, 6.13 mmol) and 4-bromobenzyl bromide (1.4 g, 6.13 mmol) as per **Method A**. The title compound (**56**) was obtained as white solid (1.88 g, 92 %), m.p. 172-174 °C (lit.[92] m.p. 175-177 °C).

Analytical data

TLC : R_f 0.60 (*n*-Hexane-Ethyl acetate 12:8);

IR (KBr, cm^{-1}) : 1777, 1719, 1606, 1476, 1379, 1324, 1027 and 754.

5.1.1.1f. *N*-(4-Methylbenzyl)isatoic anhydride (57)

The title compound (**57**) was synthesized from isatoic anhydride (**51**) (1.0 g, 6.13 mmol) and 4-methylbenzyl bromide (1.13 g, 3.06 mmol) as per **Method A**. The title compound (**57**) was obtained as white solid (1.48 g, 90 %), m.p. 131-133 °C (lit.[92] m.p. 133-135 °C).

Analytical data

TLC : R_f 0.50 (*n*-Hexane-Ethyl acetate 12:8);

IR (KBr, cm^{-1}) : 1786, 1716, 1474, 1376, 1325, 1025, 825 and 761.

5.1.1.1g. *N*-(4-Methoxybenzyl)isatoic anhydride (58)

The title compound (**58**) was synthesized from isatoic anhydride (**51**) (1.0 g, 6.13 mmol) and 4-methoxybenzyl bromide (0.88 mL, 6.13 mmol) as per **Method A**. The title compound (**58**) was obtained as white solid (1.58 g, 91 %), m.p. 137-139 °C (lit.[92] m.p. 138-140 °C).

Analytical data

TLC : R_f 0.55 (*n*-Hexane-Ethyl acetate 12:8);

IR (KBr, cm^{-1}) : 1777, 1717, 1477, 1380, 1310, 1250, 1028 and 759.

5.1.1.1h. *N*-(4-Cyanobenzyl)isatoic anhydride (59)

The title compound (**59**) was synthesized from isatoic anhydride (**51**) (1.0 g, 6.13 mmol) and 4-cyanobenzyl bromide (1.20 g, 6.13 mmol) as per

Method A. The title compound (**59**) was obtained as white solid (1.44 g, 85 %), m.p. 117–119 °C.

Analytical data

TLC : R_f 0.40 (*n*-Hexane-Ethyl acetate 12:8);

IR (KBr, cm^{-1}) : 2226, 1792, 1717, 1606, 1473, 1376, 1032 and 759.

5.1.1.1i. *N*-(4-*tert*-Butylbenzyl)isatoic anhydride (60)

The title compound (**60**) was synthesized from isatoic anhydride (**51**) (1.0 g, 6.13 mmol) and 4-*tert*-butylbenzyl bromide (1.13 mL, 6.13 mmol) as per **Method A**. The title compound (**60**) was obtained as white solid (1.8 g, 95 %), m.p. 164-166 °C.

Analytical data

TLC : R_f 0.70 (*n*-Hexane-Ethyl acetate 12:8);

IR (KBr, cm^{-1}) : 1777, 1722, 1472, 1376, 1323, 1025, 805 and 762.

5.1.1.1j. *N*-(3-Fluorobenzyl)isatoic anhydride (61)

The title compound (**61**) was synthesized from isatoic anhydride (**51**) (1.0 g, 6.13 mmol) and 3-fluorobenzyl bromide (0.76 mL, 3.06 mmol) as per **Method A**. The title compound (**61**) was obtained as white solid (1.5 g, 89 %), m.p. 130-132 °C (lit.[92] m.p. 133-135 °C).

Analytical data

TLC : R_f 0.63 (*n*-Hexane-Ethyl acetate 12:8);

IR (KBr, cm^{-1}) : 1785, 1713, 1604, 1475, 1377, 1321, 1029 and 759.

5.1.1.1k. *N*-(2-Bromobenzyl)isatoic anhydride (62)

The title compound (**62**) was synthesized from isatoic anhydride (**51**) (1.0 g, 6.13 mmol) and 2-bromobenzyl bromide (0.82 mL, 3.06 mmol) as per **Method A**. The title compound (**62**) was obtained as white solid (1.88 g, 92 %), m.p. 136-138 °C.

Analytical data

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

IR (KBr, cm^{-1}) : 1785, 1720, 1606, 1474, 1360, 1328, 1033 and 761.

5.1.1.1l. *N*-(2-Methylbenzyl)isatoic anhydride (63)

The title compound (**63**) was synthesized from isatoic anhydride (**51**) (1.0 g, 6.13 mmol) and 2-methylbenzyl bromide (0.82 mL, 6.13 mmol) as per **Method A**. The title compound (**63**) was obtained as white solid (1.48 g, 90 %), m.p. 130-132 °C.

Analytical data

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

IR (KBr, cm^{-1}) : 1774, 1722, 1603, 1474, 1377, 1315, 1028 and 768.

5.1.1.2. General method for the synthesis of substituted 2-amino-*N*-(5-chloropyridin-2-yl)benzamides (64-76)

Method B: To a rapidly stirred solution of potassium *tert*-butoxide (2 equiv.) in dry THF (10 mL), 2-amino-5-chloropyridine (1 equiv.) was added. After 10 min of stirring, isatoic anhydride (**51**)/*N*-substituted isatoic anhydrides (**52-63**) (0.50 g, 1 equiv.) were added in portions. Stirring was allowed to continue for additional 10 min. When the mixture became gelatinous, the reaction was quenched with water (10 mL). The aqueous part was extracted with ethyl acetate (15 mL × 3), dried over anhydrous sodium sulphate, filtered and evaporated to give a crude solid product which was purified by column chromatography over silica gel using petroleum ether-ethyl acetate as eluent.

5.1.1.2a. 2-Amino-*N*-(5-chloropyridin-2-yl)benzamide (64)

The title compound (**64**) was synthesized from isatoic anhydride (**51**) (0.50 g, 4.31 mmol) and 2-amino-5-chloropyridine (0.55 g, 4.31 mmol), as per **Method B**. The title compound (**64**) was obtained as yellowish solid (0.48 g, 78 %), m.p. 188-190 °C.

Analytical data

TLC : R_f 0.60 (*n*-Hexane-Ethyl acetate 16:4);

IR (KBr, cm^{-1}) : 3486, 3376, 1657, 1552, 1515, 851 and 747;

^{1}H-NMR ($CDCl_3$) : δ 8.61 (s, 1H, CON*H*), 8.29 (d, *J* = 8.8 Hz, 1H, Ar*H*), 8.18 (d, *J* = 2.5 Hz, 1H, Ar*H*), 7.68 (dd, *J* = 8.8, 2.5 Hz, 1H, Ar*H*), 7.49 (dd, 1H, Ar*H*), 7.24-7.28 (m, 1H, Ar*H*), 6.68-6.73 (m, 2H, Ar*H*) and 5.62 (bs, 2H, -N*H*);

MS (m/z) : 247.9 $(M)^+$, 119.9 $(M-127)^+$.

5.1.1.2b. *N*-(5-Chloropyridin-2-yl)-2-methylaminobenzamide (65)

The title compound (**65**) was synthesized from *N*-methylisatoic anhydride (**52**) (0.50 g, 2.82 mmol) and 2-amino-5-chloropyridine (0.36 g, 2.82 mmol), as per **Method B**. The title compound (**65**) was obtained as yellowish white solid (0.42 g, 71 %), m.p. 143-145 °C.

Analytical data

TLC : R_f 0.40 (*n*-Hexane-Ethyl acetate 14:6);

IR (KBr, cm^{-1}) : 3387, 3219, 1654, 1572, 1514, 832 and 743;

^{1}H-NMR ($CDCl_3$) : δ 8.58 (bs, 1H, -CON*H*), 8.26 (d, *J* = 9.2 Hz, 1H, Ar*H*), 8.19 (d, *J* = 2.4 Hz, 1H, Ar*H*), 7.68 (dd, *J* = 9.2, 2.4 Hz, 1H, Ar*H*), 7.51 (bs, 2H, Ar*H*, -N*H*), 7.36-7.40 (m, 1H, Ar*H*), 6.72 (dd, 1H, Ar*H*), 6.61-6.65 (m 1H, Ar*H*) and 2.9 (s, 3H, -CH_3);

^{13}C-NMR ($CDCl_3$) : δ 168.1, 151.2, 150, 146.1, 138.1, 134, 127.8, 126.4, 114.8, 113.6, 111.6 and 29.73;

MS (m/z) : 261.9 $(M)^+$, 134.9 $(M-127)^+$.

5.1.1.2c. *N*-(5-Chloropyridin-2-yl)-2-ethylaminobenzamide (66)

The title compound (**66**) was synthesized from *N*-ethylisatoic anhydride (**53**) (0.50 g, 2.62 mmol) and 2-amino-5-chloropyridine (0.34 g, 2.62 mmol), as per **Method B**. The title compound (**66**) was obtained as yellowish solid (0.54 g, 75 %), m.p. 126-128 °C.

Analytical data

TLC : R_f 0.50 (*n*-Hexane-Ethyl acetate 14:6);

IR (KBr, cm^{-1}) : 3387, 3219, 1654, 1572, 1514, 832 and 743;

^{1}H-NMR ($CDCl_3$) : δ 8.65 (bs, 1H, -CON*H*), 8.26 (d, *J* = 8.8 Hz, 1H, Ar*H*), 8.16 (d, *J* = 2.4 Hz, 1H, Ar*H*), 7.67 (dd, *J* = 8.8, 2.4 Hz, 1H, Ar*H*), 7.51 (dd, 1H, Ar*H*), 7.45 (bs, 1H, -N*H*), 7.33-7.37 (m, 1H, Ar*H*), 6.72 (d, 1H, Ar*H*), 6.59-6.63 (m, 1H, Ar*H*), 3.18-3.25 (m, 2H, -CH_2) and 1.31 (t, *J* = 7.2 Hz, 3H, -CH_3);

^{13}C-NMR ($CDCl_3$) : δ 168.1, 150.2, 150, 146, 138.2, 134, 127.9, 126.4, 114.9, 113.5, 112.2, 37.78 and 14.50;

MS (m/z) : 148.3 $(M-127)^+$.

5.1.1.2d. 2-Allylamino-*N*-(5-chloropyridin-2-yl)benzamide (67)

The title compound (**67**) was synthesized from *N*-allylisatoic anhydride (**54**) (0.50 g, 2.46 mmol) and 2-amino-5-chloropyridine (0.32 g, 2.46 mmol), as per **Method B**. The title compound (**67**) was obtained as yellowish solid (0.54 g, 76 %), m.p. 102-104 °C.

Analytical data

TLC : R_f 0.40 (*n*-Hexane-Ethyl acetate 14:6);

IR (KBr, cm^{-1}) : 3356, 3216, 1657, 1571, 1512, 829 and 745;

^{1}H-NMR ($CDCl_3$) : δ 8.66 (bs, 1H, -CON*H*), 8.27 (d, *J* = 8.8 Hz, 1H, Ar*H*), 8.16 (d, *J* = 2.8 Hz, 1H, Ar*H*), 7.74 (bs, 1H, -N*H*) 7.67 (dd, *J* = 8.8, 2.8 Hz, 1H, Ar*H*), 7.52 (dd, 1H, Ar*H*), 7.32-7.36 (m, 1H, Ar*H*), 6.71 (d, 1H, Ar*H*), 6.61-6.65 (m, 1H, Ar*H*), 5.9-6.0 (m, 1H, -C*H*=CH_2), 5.28-5.33 (m, 1H, -CH=CH_2), 5.17-5.20 (m, 1H, CH=CH_2) and 3.18-3.25 m(, 2H, -NHCH_2);

MS (m/z) : 287.9 $(M)^+$, 160.9 $(M-127)^+$, 132 $(M-155)^+$.

5.1.1.2e. 2-Benzylamino-*N*-(5-chloropyridin-2-yl)benzamide (68)

The title compound (**68**) was synthesized from *N*-benzylisatoic anhydride (**55**) (0.50 g, 1.97 mmol) and 2-amino-5-chloropyridine (0.25 g,

1.97 mmol), as per **Method B**. The title compound (**68**) was obtained as yellowish solid (0.47 g, 71 %), m.p. 152-154 °C.

Analytical data

TLC : R_f 0.43 (*n*-Hexane-Ethyl acetate 14:6);

IR (KBr, cm^{-1}) : 3356, 3257, 1653, 1571, 1509, 830 and 748;

^{1}H-NMR ($CDCl_3$) : δ 9.06 (bs, 1H, -CON*H*), 8.38 (d, *J* = 9.2 Hz, 1H, Ar*H*), 8.23 (d, *J* = 2.4 Hz, 1H, Ar*H*), 7.76 (dd, *J* = 9.2, 2.4 Hz, 1H, Ar*H*), 7.67 (dd, 1H, Ar*H*), 7.27-7.41 (m, 7H, Ar*H* and -N*H*), 6.67-6.73 (m, 2H, Ar*H*) and 4.48 (s, 2H, -CH_2);

MS (m/z) : 337.9 $(M)^+$, 210.9 $(M-127)^+$.

5.1.1.2f. 2-(4-Bromobenzyl)amino-*N*-(5-chloropyridin-2-yl)benzamide (**69**)

The title compound (**69**) was synthesized from *N*-(4-bromobenzyl) isatoic anhydride (**56**) (0.50 g, 1.51 mmol) and 2-amino-5-chloropyridine (0.20 g, 1.51 mmol), as per **Method B**. The title compound (**69**) was obtained as yellowish solid (0.46 g, 74 %), m.p. 130-132 °C.

Analytical data

TLC : R_f 0.48 (*n*-Hexane-Ethyl acetate 14:6);

IR (KBr, cm^{-1}) : 3396, 3208, 1649, 1574, 1511, 834 and 748;

^{1}H-NMR ($CDCl_3$) : δ 8.59 (bs, 1H, -CON*H*), 8.28 (d, *J* = 8.8 Hz, 1H, Ar*H*), 8.26 (d, *J* = 2.4 Hz, 1H, Ar*H*), 8.12 (t, 1H, -N*H*), 7.70 (dd, *J* = 8.8, 2.4 Hz, 1H, Ar*H*), 7.57 (dd, 1H, Ar*H*), 7.48 (d, 2H, Ar*H*), 7.25-7.34 (m, 3H, Ar*H*), 6.63-6.71 (m, 2H, Ar*H*) and 4.42 (d, 2H, -CH_2);

MS (m/z) : 416.6 $(M)^+$, 418.5 $(M+2)^+$, 290.4 $(M-127)^+$.

5.1.1.2g. *N*-(5-Chloropyridin-2-yl)-2-(4-methylbenzyl)aminobenzamide (**70**)

The title compound (**70**) was synthesized from *N*-(4-methylbenzyl) isatoic anhydride (**57**) (0.50 g, 1.87 mmol) and 2-amino-5-chloropyridine

(0.24 g, 1.87 mmol), as per **Method B**. The title compound (**70**) was obtained as yellowish solid (0.48 g, 73 %), m.p. 142-144 °C.

Analytical data

TLC : R_f 0.46 (*n*-Hexane-Ethyl acetate 14:6);

IR (KBr, cm^{-1}) : 3350, 3260, 1655, 1570, 1509, 825 and 746;

^{1}H-NMR ($CDCl_3$) : δ 8.52 (bs, 1H, CON*H*), 8.26 (d, *J* = 8.8 Hz, 1H, Ar*H*), 8.23 (d, *J* = 2.8 Hz, 1H, Ar*H*), 8.03 (t, 1H, -N*H*), 7.67 (dd, 1H, Ar*H*), 7.54 (dd, 1H, Ar*H*), 7.24-7.32 (m, 4H, Ar*H*), 7.13-7.15 (d, 1H, Ar*H*), 6.69 (d, 1H, Ar*H*), 6.62-6.66 (m, 1H, Ar*H*), 4.40 (d, *J* = 5.6 Hz, 2H, -CH_2) and 2.33 (s, 3H, -CH_3);

MS (m/z) : 351.6 $(M)^+$, 224.2 $(M-127)^+$.

5.1.1.2h. *N*-(5-Chloropyridin-2-yl)-2-(4-methoxybenzyl)aminobenzamide (71)

The title compound (**71**) was synthesized from *N*-(4-methoxybenzyl) isatoic anhydride (**58**) (0.50 g, 1.77 mmol) and 2-amino-5-chloropyridine (0.23 g, 1.77 mmol), as per **Method B**. The title compound (**71**) was obtained as yellowish solid (0.47 g, 72 %), m.p. 144-146 °C.

Analytical data

TLC : R_f 0.50 (*n*-Hexane-Ethyl acetate 14:6);

IR (KBr, cm^{-1}) : 3359, 3225, 1655, 1568, 1510, 835 and 746;

^{1}H-NMR ($CDCl_3$) : δ 8.54 (bs, 1H, CON*H*), 8.25 (d, *J* = 8.8 Hz, 1H, Ar*H*), 8.22 (d, *J* = 2.4 Hz, 1H, Ar*H*), 7.98 (t, 1H, -N*H*), 7.67 (dd, *J* = 8.8, 2.4 Hz 1H, Ar*H*), 7.54 (dd, 1H, Ar*H*), 7.25-7.33 (m, 4H, Ar*H*), 6.87-6.89 (m, 1H, Ar*H*), 6.70 (d, 1H, Ar*H*), 6.62-6.66 (m, 1H, Ar*H*), 4.38 (d, 2H, -CH_2) and 3.79 (s, 3H, -OCH_3);

MS (m/z) : 389.9 $(M+Na)^+$, 241 $(M-127)^+$, 212 $(M-156)^+$.

5.1.1.2i. *N*-(5-Chloropyridin-2-yl)-2-(4-cyanobenzyl)aminobenzamide (72)

The title compound (**72**) was synthesized from *N*-(4-cyanobenzyl) isatoic anhydride (**59**) (0.50 g, 1.80 mmol) and 2-amino-5-chloropyridine (0.23 g, 1.80 mmol), as per **Method B**. The title compound (**72**) was obtained as yellowish solid (0.46 g, 70 %), m.p. 140-142 °C.

Analytical data

TLC : R_f 0.50 (*n*-Hexane-Ethyl acetate 14:6);

IR (KBr, cm^{-1}) : 3391, 3310, 2232, 1654, 1571, 1521, 836 and 743;

^{1}H-NMR ($CDCl_3$) : δ 11.58 (bs, 1H, -CON*H*), 8.84 (d, *J* = 9.6 Hz, 1H, Ar*H*), 8.22 (d, *J* = 2.4 Hz, 1H, Ar*H*), 8.11 (dd, *J* = 9.6, 2.4 Hz, 1H, Ar*H*), 7.32-7.69 (m, 6H, Ar*H* and -N*H*), 6.81-6.85 (m, 1H, Ar*H*), 6.59 (d, 1H, Ar*H*) and 4.55-4.57 (d, 2H, -CH_2);

MS (m/z) : 362.5 $(M)^+$, 235.2 $(M-127)^+$.

5.1.1.2j. 2-(4-*tert*-Butylbenzyl)amino-*N*-(5-chloropyridin-2-yl)benzamides (73)

The title compound (**73**) was synthesized from *N*-(4-cyanobenzyl) isatoic anhydride (**60**) (0.50 g, 1.62 mmol) and 2-amino-5-chloropyridine (0.21 g, 1.62 mmol), as per **Method B**. The title compound (**73**) was obtained as yellowish solid (0.48 g, 76 %), m.p. 136-138 °C.

Analytical data

TLC : R_f 0.43 (*n*-Hexane-Ethyl acetate 14:6);

IR (KBr, cm^{-1}) : 3359, 3225, 1655, 1568, 1510, 835 and 746;

^{1}H-NMR ($CDCl_3$) : δ 8.59 (bs, 1H, -CON*H*), 8.25 (d, *J* = 8.8 Hz, 1H, Ar*H*), 8.20 (d, *J* = 2.4 Hz 1H, Ar*H*), 8.01 (t, 1H, -N*H*), 7.67 (dd, *J* = 8.8, 2.4 Hz, 1H, Ar*H*), 7.54 (d, 1H, Ar*H*), 7.29-7.37 (m, 5H, Ar*H*), 6.72 (d, 1H, Ar*H*), 6.62-6.66 (m, 1H, Ar*H*), 4.39 (d, 2H, -CH_2) and 1.31 (s, 9H, -CH_3);

MS (m/z) : 394.6 $(M)^+$, 267.6 $(M-127)^+$.

5.1.1.2k. *N*-(5-Chloropyridin-2-yl)-2-(3-fluorobenzyl)aminobenzamide (74)

The title compound (**74**) was synthesized from *N*-(3-fluorobenzyl) isatoic anhydride (**61**) (0.50 g, 1.85 mmol) and 2-amino-5-chloropyridine (0.24 g, 1.85 mmol), as per **Method B**. The title compound (**74**) was obtained as yellowish solid (0.47 g, 71 %), m.p. 118-120 °C.

Analytical data

TLC : R_f 0.65 (*n*-Hexane-Ethyl acetate 14:6);

IR (KBr, cm^{-1}) : 3406, 3212, 1652, 1574, 1512 and 750;

^{1}H-NMR ($CDCl_3$) : δ 8.56 (bs, 1H, -CON*H*), 8.27 (d, *J* = 8.8 Hz, 1H, Ar*H*), 8.23 (d, *J* = 2.4 Hz, 1H, Ar*H*), 8.14 (t, 1H, -N*H*), 7.69 (dd, *J* = 8.8, 2.4 Hz, 1H, Ar*H*), 7.55 (dd, *J* = 8.0 Hz, 1H, Ar*H*), 7.25-7.32 (m, 2H, Ar*H*), 7.14 (d, 1H, Ar*H*), 7.07 (d, 1H, Ar*H*), 6.92-6.96 (m, 1H, Ar*H*), 6.61-6.69 (m, 2H, Ar*H*), 4.45 (d, *J* = 6.0 Hz, 2H, -CH_2);

^{13}C-NMR ($CDCl_3$) δ 168.1, 161.9, 150.4, 149.5, 142.4, 141.5, 141.4, 140.8, 134.7, 130.2, 128.4, 126.4, 122.5, 116, 114.2, 113.8, 112.5, 46.66 and 14.50;

MS (m/z) : 377.9 $(M+Na)^+$, 355.9 $(M)^+$ and 227 $(M-127)^+$.

5.1.1.2l. 2-(2-Bromobenzyl)amino-*N*-(5-chloropyridin-2-yl)benzamide (75)

The title compound (**75**) was synthesized from *N*-(2-bromobenzyl) isatoic anhydride (**62**) (0.50 g, 1.5 mmol) and 2-amino-5-chloropyridine (0.24 g, 1.5 mmol), as per **Method B**. The title compound (**75**) was obtained as yellowish solid (0.43 g, 68 %), m.p. 119-121 °C.

Analytical data

TLC : R_f 0.70 (*n*-Hexane-Ethyl acetate 12:8);

IR (KBr, cm^{-1}) : 3416, 3319, 1659, 1572, 1513 and 739;

^{1}H-NMR ($CDCl_3$) : δ 9.86 (bs, 1H, CON*H*), 8.58 (t, 1H, -N*H*), 8.47 (d, *J* = 9.2 Hz, 1H, Ar*H*), 8.15 (d, *J* = 2.4 Hz, 1H, Ar*H*), 7.82 (dd, *J* = 9.2, 2.4 Hz, 1H, Ar*H*), 7.77 (dd, 1H, Ar*H*), 7.50 (d, 1H, Ar*H*), 7.25-7.34 (m, 3H, Ar*H*), 7.04-7.08 (m, 1H, Ar*H*), 6.64-6.68 (m, 1H, Ar*H*), 6.53 (d, 1H, Ar*H*) and 4.44 (d, 2H, -CH_2);

MS (m/z) : 416.5 $(M)^+$, 418.6 $(M+2)^+$.

5.1.1.2m. *N*-(5-Chloropyridin-2-yl)-2-(2-methylbenzyl)aminobenzamide (76)

The title compound (**76**) was synthesized from *N*-(2-methylbenzyl) isatoic anhydride (**63**) (0.50 g, 1.85 mmol) and 2-amino-5-chloropyridine (0.24 g, 1.85 mmol), as per **Method B**. The title compound (**76**) was obtained as yellowish solid (0.49 g, 71 %), m.p. 128-130 °C.

Analytical data

TLC : R_f 0.40 (*n*-Hexane-Ethyl acetate 14:6);

IR (KBr, cm^{-1}) : 3437, 3336, 1656, 1571, 1513 and 740;

^{1}H-NMR ($CDCl_3$) : δ 8.49 (bs, 1H, -CON*H*), 8.25 (d, *J* = 8.4 Hz, 1H, Ar*H*), 8.23 (d, *J* = 3.2 Hz, 1H, Ar*H*), 7.92 (t, 1H, -N*H*), 7.67 (dd, *J* = 8.8, 2.4 Hz, 1H, Ar*H*), 7.55 (d, 1H, Ar*H*), 7.14-7.35 (m, 5H, Ar*H*), 6.64- 6.70 (m, 2H, Ar*H*), 4.37 (d, 2H, -CH_2) and 2.38 (s, 3H, -CH_3);

MS (m/z) : 351.9 $(M)^+$, 353.9 $(M+2)^+$, 373.9 $(M+Na)^+$, 223.9 $(M-127)^+$, 104.9 $(M-247)^+$.

5.1.1.3. 2-(2-Chloroacetamido)-*N*-(5-chloropyridin-2-yl) benzamide (77)

To a stirring solution of 2-amino-*N*-(5-chloropyridin-2-yl)benzamide (**64**) (1.0 g, 4 mmol) and potassium carbonate (0.82 g, 6 mmol) in dry DCM was added 2-chloroacetyl chloride (0.4 mL, 5 mmol) drop-wise by maintaining the temperature at 0-10 °C. After stirring at 25-30 °C for 1.5 h, the reaction mixture was finally poured over crushed ice and extracted with

successive quantities of chloroform. The organic extract was washed with sodium bicarbonate solution (5 %) and water. It was then dried over anhydrous sodium sulphate, filtered and subjected to solvent recovery to get compound (**77**) as white solid (0.98 g, 75 %), m.p. 163-165 °C.

Analytical data

TLC : R_f 0.62 (*n*-Hexane-Ethyl acetate 16:4);

IR (KBr, cm^{-1}) : 3401, 3285, 1663, 1580, 1511, 839 and 750;

^{1}H-NMR ($CDCl_3$) : δ 11.57 (s, 1H, -CON*H*), 8.73 (bs, 1H, -CON*H*), 8.61 (d, *J* = 9.2 Hz, 1H, Ar*H*), 8.32 (dd, *J* = 9.2, 2.4 Hz, 1H, Ar*H*), 8.25 (d, *J* = 2.4, 1H, Ar*H*), 7.69-7.75 (m, 2H, Ar*H*), 7.54-7.59 (m, 1H, Ar*H*), 7.19-7.26 (m, 1H, Ar*H*) and 4.20 (s, 2H, -CH_2);

MS (m/z) : 324 $(M)^+$, 326 $(M+2)^+$.

5.1.1.4. General method for the synthesis of 2-(2-(*N*-substituted piperazin-1-yl)acetamido)-*N*-(5-chloropyridin-2-yl)benzamides (78-83)

Method C: 2-(2-Chloroacetamido)-*N*-(5-chloropyridin-2-yl)benzamide (**77**) (1.0 g, 3.0 mmol) and substituted piperazines (6 mmol) were dissolved in DMF (15 mL). The reaction mixture was stirred at 120 °C for 4 h. After completion of the reaction, the contents were finally poured over crushed ice. The solid so obtained was filtered and the desired compound was purified by column chromatography to remove unreacted piperazines.

5.1.1.4a. *N*-(5-Chloropyridin-2-yl)-2-(2-(4-phenylpiperazin-1-yl)acetamido)benzamide (78)

The title compound (**78**) was synthesized from 2-(2-chloroacetamido)-*N*-(5-chloropyridin-2-yl)benzamide (**77**) (0.50 g, 1.5 mmol) and 1-phenyl piperazine (0.49 g, 3 mmol), as per **Method C**. The title compound (**78**) was obtained as yellowish solid (0.35 g, 62 %), m.p. 148-150 °C.

Analytical data

TLC : R_f 0.49 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3431, 3200, 1693, 1666, 1573, 842 and 754;

^{1}H-NMR ($CDCl_3$) : δ 11.70 (s, 1H, -N*H*), 8.83 (s, 1H, -N*H*), 8.70 (d, 1H, Ar*H*), 8.24 (d, *J* = 8.8 Hz, 1H, Ar*H*), 8.14 (dd, *J* = 8.8, 2.4 Hz, 1H, Ar*H*), 7.61 (d, *J* = 2.4 Hz, 1H, Ar*H*), 7.50-7.54 (m, 1H, Ar*H*), 7.28-7.37 (m, 3H, Ar*H*), 7.10-7.14 (m, 1H, Ar*H*), 6.90-6.94 (m, 3H, Ar*H*), 3.23 (s, 2H, -CH_2), 3.33 (t, 4H, -CH_2) and 2.78 (t, 4H, -CH_2);

MS (m/z) : 450.1 $(M)^+$, 452.1 $(M+2)^+$.

5.1.1.4b. *N*-(5-Chloropyridin-2-yl)-2-(2-(4-(2-methoxyphenyl)piperazin-1-yl)acetamido)benzamide (**79**)

The title compound (**79**) was synthesized from 2-(2-Chloroacetamido)-*N*-(5-chloropyridin-2-yl)benzamide (**77**) (0.50 g, 1.5 mmol) and 1-(2-methoxyphenyl)piperazine (577 mg, 3 mmol), as per **Method C**. The title compound (**79**) was obtained as yellowish solid (0.36 g, 50 %), m.p. 140-142 °C.

Analytical data

TLC : R_f 0.46 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3430, 3223, 1666, 1578, 1503, 834 and 751;

^{1}H-NMR ($CDCl_3$) : δ 11.76 (s, 1H, -N*H*), 8.70-8.74 (m, 2H, Ar*H*, N*H*), 8.36 (d, *J* = 8.8 Hz, 1H, Ar*H*), 8.21 (d, *J* = 2.8 Hz, 1H, Ar*H*), 7.64 (dd, *J* = 8.0, 1.2 Hz, 1H, Ar*H*), 7.52-7.56 (m, 2H, Ar*H*), 7.13-7.17 (m, 1H, Ar*H*), 7.04-7.08 (m, 1H, Ar*H*), 6.95-6.99 (m, 1H, Ar*H*), 6.90-6.92 (m, 2H, Ar*H*), 3.87 (s, 3H, -OCH_3), 3.24 (s, 2H, -CH_2), 3.22-3.24 (m, 4H, -CH_2) and 2.83-2.85 (m, 4H, -CH_2);

MS (m/z) : 480.7 $(M)^+$, 482.7 $(M+2)^+$.

5.1.1.4c. *N*-(5-Chloropyridin-2-yl)-2-(2-(4-(4-methoxyphenyl)piperazin-1-yl)acetamido)benzamide (80)

The title compound (**80**) was synthesized from 2-(2-chloroacetamido)-*N*-(5-chloropyridin-2-yl)benzamide (**77**) (0.50 g, 1.5 mmol) and 1-(4-methoxyphenyl)piperazine (0.58 g, 3 mmol), as per **Method C**. The title compound (**80**) was obtained as yellowish solid (0.35 g, 49 %), m.p. 100-102 °C.

Analytical data

TLC : R_f 0.46 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3443, 3200, 1683, 1604, 1572, 1510, 827 and 773;

^{1}H-NMR ($CDCl_3$) : δ 8.55 (d, 1H, Ar*H*), 8.30 (dd, 1H, Ar*H*), 7.74-7.84 (m, 3H, Ar*H*), 7.50-7.54 (m, 1H, Ar*H*), 7.42 (dd, 1H, Ar*H*), 6.80-6.82 (m, 4H, Ar*H*), 3.75 (s, 3H, -OCH_3), 3.63 (s, 2H, -CH_2), 2.78-2.80 (m, 4H, -CH_2) and 2.39-2.41 (m, 4H, -CH_2);

^{13}C-NMR ($CDCl_3$) : δ 162.2, 152.3, 149, 147.6, 146.8, 137.2, 134.9, 132.2, 127.6, 127.5, 127, 126.25, 121.2, 118.2, 114.4, 61.19, 55.5, 52.61 and 50.46;

MS (m/z) : 480.7 $(M)^+$, 482.7 $(M+2)^+$.

5.1.1.4d. *N*-(5-Chloropyridin-2-yl)-2-(2-(4-*p*-tolylpiperazin-1-yl)acetamido)benzamide (81)

The title compound (**81**) was synthesized from 2-(2-Chloroacetamido)-*N*-(5-chloropyridin-2-yl)benzamide (**77**) (0.50 g, 1.5 mmol) and 1-(*p*-tolyl) piperazine (0.53 g, 3 mmol), as per **Method C**. The title compound (**81**) was obtained as yellowish solid (0.29 g, 42 %), m.p. 183-185 °C.

Analytical data

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

IR (KBr, cm^{-1}) : 3274, 1687, 1580, 1509, 843 and 765;

^{1}H-NMR ($CDCl_3$) : δ 11.74 (s, 1H, -N*H*), 8.73 (dd, 1H, Ar*H*), 8.55

(s, 1H, -N*H*), 8.24 (d, *J* = 8.8 Hz, 1H, Ar*H*), 8.22 (d, *J* = 2.4 Hz, 1H, Ar*H*), 7.63 (dd, *J* = 8.8, 2.4 Hz, 1H, Ar*H*), 7.52-7.56 (m, 1H, Ar*H*), 7.32-7.35 (m, 1H, Ar*H*), 7.14-7.18 (m, 1H, Ar*H*), 7.11 (d, 2H, Ar*H*), 6.83 (d, 2H, Ar*H*), 3.28 (t, 4H, -CH_2), 3.24 (s, 2H, -CH_2), 2.77-2.80 (m, 4H, -CH_2) and 2.32 (s, 3H, -CH_3);

MS (m/z) : 464.8 $(M)^+$, 466.8 $(M+2)^+$.

5.1.1.4e. 2-(2-(4-Benzylpiperazin-1-yl)acetamido)-*N*-(5-chloropyridin-2-yl) benzamide (82)

The title compound (**82**) was synthesized from 2-(2-chloroacetamido)-*N*-(5-chloropyridin-2-yl)benzamide (**77**) (0.50 g, 1.5 mmol) and 1-benzyl piperazine (0.53 g, 3 mmol), as per **Method C**. The title compound (**82**) was obtained as yellowish solid (0.45 g, 65 %), m.p. 150-152 °C.

Analytical data

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

IR (KBr, cm^{-1}) : 3435, 3233, 1667, 1576, 1511, 830 and 751;

^{1}H-NMR ($CDCl_3$) : δ 11.51 (s, 1H, -N*H*), 8.74 (s, 1H, -N*H*), 8.64 (dd, 1H, Ar*H*), 8.44 (d, *J* = 8.8 Hz, 1H, Ar*H*), 8.24 (d, *J* = 2.4 Hz, 1H, Ar*H*), 7.63 (dd, *J* = 8.8, 2.4 Hz, 1H, Ar*H*), 7.78 (dd, 1H, Ar*H*), 7.48-7.53 (m, 1H, Ar*H*), 7.24-7.26 (m, 5H, Ar*H*), 7.11-7.15 (m, 1H, Ar*H*), 3.52 (s, 2H, -CH_2), 3.16 (s, 2H, -CH_2) and 2.59-2.63 (m, 8H, -CH_2);

MS (m/z) : 463.8 $(M)^+$, 465 $(M+2)^+$.

5.1.1.4f. *N*-(5-Chloropyridin-2-yl)-2-(2-(4-(4-methylbenzyl)piperazin-1-yl) acetamido)benzamide (83)

The title compound (**83**) was synthesized from 2-(2-Chloroacetamido)-*N*-(5-chloropyridin-2-yl)benzamide (**77**) (0.50 g, 1.5 mmol) and 1-(4-methyl

benzyl)piperazine (0.57 g, 3 mmol), as per **Method C**. The title compound (**83**) was obtained as yellowish solid (0.45 g, 48 %), m.p. 154-156 °C.

Analytical data

TLC	:	R_f 0.47 (Chloroform-Methanol 19:1);
IR (KBr, cm^{-1})	:	3434, 3190, 1693, 1665, 1579, 1515, 840 and 762;
^{1}H-NMR ($CDCl_3$)	:	δ 11.50 (s, 1H, -N*H*), 8.73 (s, 1H, -N*H*), 8.64 (dd, 1H, Ar*H*), 8.44 (d, *J* = 8.8 Hz, 1H, Ar*H*), 8.23 (d, *J* = 2.4 Hz, 1H, Ar*H*), 7.62 (dd, *J* = 8.8, 2.4 Hz 1H, Ar*H*), 7.76 (dd, 1H, Ar*H*), 7.48-7.53 (m, 1H, Ar*H*), 7.11-7.20 (m, 5H, Ar*H*), 3.48 (s, 2H, -CH_2), 3.16 (s, 2H, -CH_2), 2.58-2.62 (m, 8H, -CH_2) and 2.34 (s, 3H, -CH_3);
MS (m/z)	:	478 $(M)^+$.

5.1.1.5. Synthesis of substituted 4-bromomethyl-1,1'-biphenyls (93-101)

Step-I: To a stirring mixture of sodium carbonate (0.21 g, 2 mmol), PEG 4000 (3.5 g) and water (3.0 mL) was added palladium acetate (2 mg, 1 mol %). The mixture was heated at 50 °C. Afterward, X-substituted (X = H, F) 4-bromotoluene (1 mmol) and Y-substituted phenylboronic acid (1.5 mmol) were added to the above solution, and the reaction was carried out at 50 °C for 30 to 60 min. After the reaction solution was cooled to room temperature, the resulting mixture was extracted with diethyl ether (15 mL × 4). The combined organic phase was dried over anhydrous magnesium sulfate, filtered, and concentrated to provide biphenyls as colorless solids. Characterization data for all the biphenyl compounds (**84-92**) were in accordance with the reported compounds.[93-97]

Step-II: To a solution of *N*-bromosuccinimide (1 equiv.) in carbon tetrachloride (20 mL), the biphenyls (**84-92**) (1.0 g, 1 equiv.) were added. To it catalytical amount of azobisisobutyronitrile (AIBN) was added and refluxed for 10 hrs. The progress of the reaction was monitored by TLC. After

completion of the reaction, solvent was evaporated and the residue was diluted with water and extracted with ethyl acetate (20 mL × 3). The combined organic phase was dried over anhydrous magnesium sulfate, filtered and evaporated to give a product (**93-101**) that was directly used for the next step.

5.1.1.6. General method for the synthesis of 2-(4-(2- or 4-substituted phenyl)benzylamino)-*N*-(5-chloropyridin-2-yl)benzamides (102-110)

Method D: To a stirred solution of 2-amino-*N*-(5-chloropyridin-2-yl) benzamide (**64**) (0.62 g, 2.5 mmol) and potassium carbonate (0.45 g, 3.75 mmol) in DMF (10 mL), substituted 4-bromomethyl-1,1'-biphenyl (3 mmol) in DMF (5 mL) was added drop-wise and the reaction mixture was stirred for 4-6 h at 120 °C. After completion of the reaction, the reaction mixture was diluted with water and extracted with DCM (15 mL × 3). The organic layer was dried over anhydrous sodium sulphate, filtered and evaporated to afford the desired compounds (**102-110**). All the synthesized compounds were purified by column chromatography on silica gel using petroleum ether-ethyl acetate as eluent.

5.1.1.6a. *N*-(5-Chloropyridin-2-yl)-2-(4-phenylbenzylamino)benzamide (102)

The title compound (**102**) was synthesized from 2-amino-*N*-(5-chloropyridin-2-yl)benzamide (**64**) (0.62 g, 2.5 mmol) and 4-(bromomethyl)-1,1'-biphenyl (0.74 g, 3 mmol), as per **Method D**. The title compound (**102**) was obtained as white solid (0.60 g, 58 %), m.p. 190-192 °C.

Analytical data

TLC : R_f 0.45 (*n*-Hexane-Ethyl acetate 18:2);

IR (KBr, cm^{-1}) : 3435, 3350, 1649, 1572, 1509 and 742;

^{1}H-NMR ($CDCl_3$) : δ 8.47 (s, 1H, -CON*H*), 8.20 (d, *J* = 8.8 Hz, 1H, Ar*H*), 8.17 (d, 2.4 Hz, 1H, Ar*H*), 8.06 (t, 1H, -N*H*), 7.61 (dd, *J* = 8.8, 2.4 Hz, 1H, Ar*H*), 7.48-7.52 (m, 5H, Ar*H*), 7.33-7.39 (m, 4H, Ar*H*), 7.23-7.28 (m, 2H, Ar*H*), 6.58-6.66 (m, 2H, Ar*H*) and 4.42 (d, *J* = 5.6 Hz, 2H, -CH_2);

MS (m/z) : 414.7 $(M)^+$, 416.5 $(M+2)^+$.

5.1.1.6b. 2-(4-(4-*tert.*Butylphenyl)benzylamino)-*N*-(5-chloropyridin-2-yl)benzamide (103)

The title compound (**103**) was synthesized from 2-amino-*N*-(5-chloro pyridin-2-yl)benzamide (**64**) (0.62 g, 2.5 mmol) and 4-(bromomethyl)-4'-(*tert*-butyl)-1,1'-biphenyl (0.91 g, 3 mmol), as per **Method D**. The title compound (**103**) was obtained as white solid (0.65 g, 55 %), m.p. 167-169 °C.

Analytical data

TLC : R_f 0.49 (*n*-Hexane-Ethyl acetate 18:2);

IR (KBr, cm^{-1}) : 3372, 3226, 2962, 1653, 1573, 1510, 814 and 749;

^{1}H-NMR ($CDCl_3$) : δ 8.53 (s, 1H, -CONH), 8.27 (d, *J* = 8.8 Hz, 1H, Ar*H*), 8.24 (d, *J* = 2.4 Hz, 1H, Ar*H*), 8.11 (t, 1H, -NH), 7.68 (dd, *J* = 8.8, 2.4 Hz, 1H, Ar*H*), 7.51-7.57 (m, 5H, Ar*H*), 7.41-7.46 (m, 4H, Ar*H*), 7.30-7.32 (m, 1H, Ar*H*), 6.73 (d, 1H, Ar*H*), 6.64-6.68 (m, 1H, Ar*H*),), 4.48 (d, 2H, -CH_2) and 1.35 (s, 9H, -CH_3);

MS (m/z) : 470.7 $(M)^+$, 472.7 $(M+2)^+$.

5.1.1.6c. *N*-(5-Chloropyridin-2-yl)-2-(4-(3,5-difluorophenyl)benzylamino) benzamide (**104**)

The title compound (**104**) was synthesized from 2-amino-*N*-(5-chloro pyridin-2-yl)benzamide (**64**) (0.62 g, 2.5 mmol) and 4'-(bromomethyl)-3,5-difluoro-1,1'-biphenyl (0.85 g, 3 mmol), as per **Method D**. The title compound (**104**) was obtained as white solid (0.67 g, 60%), m.p. 162-164 °C.

Analytical data

TLC : R_f 0.47 (*n*-Hexane-Ethyl acetate 18:2);

IR (KBr, cm^{-1}) : 3396, 3212, 3082, 1651, 1573, 1513, 837 and 750;

^{1}H-NMR ($CDCl_3$) : δ 8.54 (s, 1H, -CON*H*), 8.27 (d, *J* = 8.8 Hz, 1H, Ar*H*), 8.25 (d, *J* = 2.4 Hz, 1H, Ar*H*), 8.15

(t, 1H, -N*H*), 7,69 (dd, *J* = 8.8, 2.4 Hz, 1H, Ar*H*), 7.57 (dd, 1H, Ar*H*), 7.51 (d, 2H, Ar*H*) 7.45 (d, 2H, Ar*H*), 7.29-7.33 (m, 1H, Ar*H*), 7.06-7.11 (m, 2H, Ar*H*), 6.74-6.79 (m, 1H, Ar*H*), 6.62-6.69 (m, 2H, Ar*H*) and 4.49-4.50 (d, 2H, -CH_2);

MS (m/z) : 450.8 $(M)^+$

5.1.1.6d. 2-(4-(2-Chlorophenyl)benzylamino)-*N*-(5-chloropyridin-2-yl) benzamide (105)

The title compound (**105**) was synthesized from 2-amino-*N*-(5-chloro pyridin-2-yl)benzamide (**64**) (0.62 g, 2.5 mmol) and 4'-(bromomethyl)-2-chloro-1,1'-biphenyl (0.88 g, 3 mmol), as per **Method D**. The title compound (**105**) was obtained as white solid (0.63 g, 62 %), m.p. 110-112 °C.

Analytical data

TLC : R_f 0.43 (*n*-Hexane-Ethyl acetate 18:2);

IR (KBr, cm^{-1}) : 3368, 3229, 2227, 1650, 1572, 1511, 828 and 760;

^{1}H-NMR ($CDCl_3$) : δ 8.53 (s, 1H, -CON*H*), 8.28 (d, *J* = 9.2 Hz, 1H, Ar*H*), 8.24 (d, *J* = 2.4 Hz, 1H, Ar*H*), 8.14 (t, 1H, -N*H*), 7.68 (dd, *J* = 8.8, 2.4 Hz, 1H, Ar*H*), 7.57 (dd, 1H, Ar*H*), 7.40-7.47 (m, 5H, Ar*H*), 7.41-7.46 (m, 4H, Ar*H*), 6.75 (d, 1H, Ar*H*), 6.66-6.70 (m, 1H, Ar*H*) and 4.50 (d, 2H, -CH_2);

MS (m/z) : 448.8 $(M)^+$, 450.8 $(M+2)^+$

5.1.1.6e. *N*-(5-Chloropyridin-2-yl)-2-(4-(2-methoxyphenyl)benzylamino) benzamide (106)

The title compound (**106**) was synthesized from 2-amino-*N*-(5-chloro pyridin-2-yl)benzamide (**64**) (0.62 g, 2.5 mmol) and 4'-(bromomethyl)-2-methoxy-1,1'-biphenyl (0.83 g, 3 mmol), as per **Method D**. The title compound (**106**) was obtained as white solid (0.69 g, 62 %), m.p. 174-176 °C.

Analytical data

TLC : R_f 0.48 (*n*-Hexane-Ethyl acetate 18:2);

IR (KBr, cm^{-1}) : 3368, 3218, 1650, 1578, 1516, 836 and 749;

^{1}H-NMR ($CDCl_3$) : δ 10.46 (s, 1H, -CON*H*), 8.08 (t, *J* = 5.6 Hz, 1H, -N*H*), 8.17 (d, 1H, Ar*H*), 8.31 (d, 1H, Ar*H*), 7.82 (dd, 1H, Ar*H*), 7.76 (dd, 1H, Ar*H*), 7.46 (d, 2H, Ar*H*), 7.39 (d, 2H, Ar*H*), 7.25-7.32 (m, 3H, Ar*H*), 6.97-7.03 (m, 2H, Ar*H*), 6.72 (d, 1H, Ar*H*), 6.61-6.65 (m, 1H, Ar*H*), 4.45 (d, 2H, -CH_2) and 3.78 (s, 3H, -OCH_3);

MS (m/z) : 444.1 $(M)^+$, 317.2 $(M-127)^+$.

5.1.1.6f. *N*-(5-Chloropyridin-2-yl)-2-(4-(2-cyanophenyl)benzylamino) benzamide (107)

The title compound (**107**) was synthesized from 2-amino-*N*-(5-chloro pyridin-2-yl)benzamide (**64**) (0.62 g, 2.5 mmol) and 4'-(bromomethyl)-[1,1'-biphenyl]-2-carbonitrile (0.82 g, 3 mmol), as per **Method D**. The title compound (**107**) was obtained as white solid (0.82 g, 73 %), m.p. 185-187 °C.

Analytical data

TLC : R_f 0.44 (*n*-Hexane-Ethyl acetate 18:2);

IR (KBr, cm^{-1}) : 3368, 3229, 2227, 1650, 1572, 1511, 828 and 760;

^{1}H-NMR ($CDCl_3$) : δ 8.56 (s, 1H, -CON*H*), 8.28 (d, *J* = 8.8 Hz, 1H, Ar*H*), 8.23 (d, *J* = 2.4 Hz, 1H, Ar*H*), 8.18 (t, 1H, -N*H*), 7.75 (dd, 1H, Ar*H*), 7.69 (dd, *J* = 8.8, 2.4 Hz, 1H, Ar*H*), 7.62-7.65 (m, 1H, Ar*H*), 7.48-7.58 (m, 6H, Ar*H*), 7.41-7.45 (m, 1H, Ar*H*), 7.31-7.35 (m, 1H, Ar*H*), 6.66-6.72 (m, 2H, Ar*H*) and 4.52 (d, 2H, -CH_2);

^{13}C-NMR ($CDCl_3$) δ 168, 150.4, 149.6, 145.1, 143.1, 140.3, 139.3, 137.1, 134.6, 133.8, 132.8, 130, 129.4,

129.1, 128.3, 127.5, 127.4, 126.4, 118.8, 115.9, 113.1, 112.7, 111.1 and 46.90;

MS (m/z) : 438 $(M)^+$, 440.9 $(M+2)^+$, 460.9 $(M+Na)^+$.

5.1.1.6g. *N*-(5-Chloropyridin-2-yl)-2-(4-(4-methoxyphenyl)benzylamino) benzamide (108)

The title compound (**108**) was synthesized from 2-amino-*N*-(5-chloro pyridin-2-yl)benzamide (**64**) (0.62 g, 2.5 mmol) and 4'-(bromomethyl)-4-methoxy-1,1'-biphenyl (0.83 g, 3 mmol), as per **Method D**. The title compound (**108**) was obtained as white solid (0.80 g, 60 %), m.p. 187-189 °C.

Analytical data

TLC : R_f 0.47 (*n*-Hexane-Ethyl acetate 18:2);

IR (KBr, cm^{-1}) : 3368, 3218, 1650, 1578, 1516, 836 and 749;

^{1}H-NMR ($CDCl_3$) : δ 10.45 (s, 1H, -CON*H*), 8.32 (d, *J* = 2.4 Hz, 1H, Ar*H*), 8.17 (d, *J* = 8.4 Hz, 1H, Ar*H*), 8.08 (t, 1H, -N*H*), 7.82 (dd, 1H, Ar*H*), 7.76 (dd, *J* = 8.4, 2.4 Hz, 1H, Ar*H*), 7.52-7.54 (m, 4H, Ar*H*), 7.41 (d, *J* = 8.0, 2H, Ar*H*), 7.26-7.29 (m, 1H, -Ar*H*), 6.96 (d, 2H, Ar*H*), 6.69 (d, 1H, Ar*H*), 6.60-6.64 (m, 1H, Ar*H*), 4.45 (d, 2H, -CH_2) and 3.81 (s, 3H, -OCH_3);

MS (m/z) : 444 $(M)^+$, 317 $(M-127)^+$.

5.1.1.6h. *N*-(5-Chloropyridin-2-yl)-2-(2-fluoro-4-phenylbenzylamino) benzamide (109)

The title compound (**109**) was synthesized from 2-amino-*N*-(5-chloro pyridin-2-yl)benzamide (**64**) (0.62 g, 2.5 mmol) and 4'-(bromomethyl)-2-fluoro-1,1'-biphenyl (0.79 g, 3 mmol), as per **Method D**. The title compound (**109**) was obtained as white solid (0.72 g, 51 %), m.p. 162-164 °C.

Analytical data

TLC : R_f 0.47 (*n*-Hexane-Ethyl acetate 18:2);

IR (KBr, cm^{-1}) : 3400, 3210, 1651, 1572, 1514, 829 and 742;

^{1}H-NMR ($CDCl_3$) : δ 8.53 (s, 1H, -CON*H*), 8.28 (d, *J* = 8.8 Hz,

1H, Ar*H*), 8.25 (d, *J* = 2.4 Hz, 1H, Ar*H*), 8.12 (t, 1H, -N*H*), 7.69 (dd, *J* = 8.8, 2.4 Hz, 1H, Ar*H*), 7.54-7.58 (m, 3H, Ar*H*), 7.40-7.45 (m, 3H, Ar*H*), 7.29-7.37 (m, 4H, Ar*H*), 6.67-6.75 (m, 2H, Ar*H*) and 4.54 (d, *J* = 6.0 Hz, 2H, -CH_2);

MS (m/z) : 431.78 $(M)^+$.

5.1.1.6i. 2-(4-(4-*tert.*Butylphenyl)-2-fluorobenzylamino)-*N*-(5-chloro pyridin-2-yl)benzamide (110)

The title compound (**110**) was synthesized from 2-amino-*N*-(5-chloro pyridin-2-yl)benzamide (**64**) (0.62 g, 2.5 mmol) and 4-(bromomethyl)-4'-(*tert*-butyl)-3-fluoro-1,1'-biphenyl (0.96 g, 3 mmol), as per **Method D**. The title compound (**110**) was obtained as white solid (0.63 g, 52 %), m.p. 135-137 °C.

Analytical data

TLC : R_f 0.47 (*n*-Hexane-Ethyl acetate 18:2);

IR (KBr, cm^{-1}) : 3398, 3226, 2960, 1657, 1576, 1511, 824 and 746;

^{1}H-NMR ($CDCl_3$) : δ 8.52 (s, 1H, -CON*H*), 8.28 (d, *J* = 8.8 Hz, 1H, Ar*H*), 8.25 (d, *J* = 2.4 Hz, 1H, Ar*H*), 8.10 (t, 1H, -N*H*), 7.69 (dd, *J* = 8.8, 2.4 Hz, 1H, Ar*H*), 7.57 (dd, 1H, Ar*H*), 7.44-7.50 (m, 4H, Ar*H*), 7.38-7.42 (m, 1H, Ar*H*), 7.27-7.36 (m, 3H, Ar*H*), 6.74 (dd, 1H, Ar*H*), 6.66-6.70 (m, 1H, Ar*H*), 4.54 (d, 2H, -CH_2) and 1.33 (s, 9H, -CH_3);

MS (m/z) : 488.9 $(M)^+$, 490.9 $(M+2)^+$.

5.1.2. 1,3,4-Thiadiazole-based FXa inhibitors

5.1.2.1. Designing of 1,3,4-thiadiazole derivatives as FXa inhibitors

The protein data bank was searched for 3D structures of FXa from Homosapiens. Out of 224 entries, 47 PDB structures (**Table 5.1**) were selected based on their resolution, protein and co-crystallized ligand consistency and co-crystallized ligand reported biological activity. The co-crystallized ligands of all these selected PDB structures were reported to have activities of 10 nM or better. The selected PDB structures were prepared to ensure structural correctness for hydrogen consistency, bond orders, steric clashes, and charges using Protein Preparation Wizard in Schrodinger Suite supported by the OPLS2005 force field. After preparation, all the PDB structures were aligned over each other considering the backbone of protein structures to obtain all the co-crystallized ligand aligned based on structural features of structurally diverse scaffolds. Then all the receptor structures were selected and deleted to get active site based three-dimensionally aligned co-crystallized structures. These pre-aligned conformations were used for pharmacophore development.

Table 5.1. PDB structures selected for the alignment

Sr No.	PDB-ID	Resolution	PDB-ligand	IC_{50} or K_i
1	1FJS	1.92	Z34	0.11 nM (K_i)
2	1KSN	2.1	FXV	0.4 nM (K_i)
3	1MQ5	2.1	XLC	0.76 nM (K_i)
4	1MQ6	2.1	XLD	0.007-0.28 nM (K_i)
5	1NFW	2.1	RRR	1.1 nM (K_i)
6	1NFX	2.15	RDR	3 nM (K_i)
7	1NFY	2.1	RTR	1-1.13 nM (K_i)
8	1Z6E	1.8	IK8	0.19 nM (K_i)
9	2BOH	2.2	IIA	3 nM (K_i)
10	2BQ6	3.0	IIB	0.07 nM (K_i)

Sr No.	PDB-ID	Resolution	PDB-ligand	IC_{50} or K_i
11	2FZZ	2.2	5QC	0.03 nM (K_i)
12	2G00	2.1	4QC	0.18 nM (K_i)
13	2P3T	1.92	993	0.005-0.06 nM (K_i)
14	2P16	2.3	GG2	0.075-0.72 nM (K_i)
15	2P93	1.9	ME1	0.15 nM (K_i)
16	2P94	1.8	ME4	0.67 nM (K_i)
17	2P95	2.2	ME5	0.43 nM (K_i)
18	2PHB	2.3	230	0.32-0.57 nM (IC_{50})
19	2PR3	1.5	237	0.16-0.17 nM (IC_{50})
20	2Q1J	1.9	FXI	0.8 nM (IC_{50})
21	2UWO	1.75	701	2 nM (K_i)
22	2VH0	1.7	GSI	1 nM (K_i)
23	2VH6	1.95	GSV	0.2 nM (K_i)
24	2VVC	1.95	LZF	3 nM (K_i)
25	2W3I	1.9	L1C	0.1 nM (IC_{50})
26	2W3K	2.05	L1D	0.08 nM (K_i)
27	2W26	2.08	RIV	0.7- 700 nM (IC_{50}) 0.4-0.66 nM (K_i)
28	2WYG	1.88	461	2 nM (K_i)
29	2WYJ	2.38	898	0.8 – 1 nM (K_i)
30	2Y5F	1.29	XWG	2 nM (K_i)
31	2Y7X	1.9	MZA	1.3 nM (K_i)
32	2Y7Z	1.84	C0Z	2 nM (K_i)
33	2Y80	1.9	439	3-9 nM (K_i)
34	2Y81	1.7	931	2 nM (K_i)

Sr No.	PDB-ID	Resolution	PDB-ligand	IC_{50} or K_i
35	3ENS	2.3	ENS	8.9 nM (IC_{50})
36	3FFG	1.54	FFG	0.84 nM (K_i)
37	3KL6	1.45	443	2.2 -3.5 nM (IC_{50})
38	3KQB	2.25	LGJ	0.5 nM (K_i)
39	3KQC	2.2	LGK	2.2 nM (K_i)
40	3KQD	2.75	LGL	0.4 nM (K_i)
41	3M36	2.15	M35	2.4 nM (IC_{50})
42	3M37	1.9	M37	0.91-0.29 nM (K_i)
43	3TK5	2.2	D1Q	1.3 nM (IC_{50})
44	3TK6	1.8	D46	1.7 nM (IC_{50})
45	4A7I	2.4	A7I	2 nM (K_i)
46	4BTI	2.3	7R9	0.2 nM (IC_{50})
47	4BTT	2.3	6XS	0.5 nM (IC_{50})

The “Clean Drug-Like” molecules data base (13,195,609 molecules) was retrieved from the Zinc-12 library[125], structures were prepared using LigPrep module of Schrodinger and primary screening was carried out considering Lipinski rule of five. The obtained molecules were directed to next pharmacophore based filters.

PHASE module of Schrodinger[126] was used to generate pharmacophore models on database under study. PHASE presents a standard set of six pharmacophore features, namely hydrogen bond acceptor (A), hydrogen bond donor (D), hydrophobic (H) group, negatively ionizable (N) group, positively ionizable (P) group and aromatic ring (R). Using tree based partitioning algorithm, PHASE systematically identifies the arrangements of functional groups/features in space that are common and essential for the biological activity for a set of high-affinity ligands. All the aligned 3D structures were obtained by knocking-out receptor from finally selected pre-

aligned 47 PDB structures. Considering these pre-aligned ligand structures as bioactive conformations, Pharmacophore models were generated. Number of features in hypothesis generation was selected from 3 to 6 and criteria of at-least 60% molecules should match the hypothesis was considered. The pharmacophore models were generated using standard scoring function in PHASE module.

After the pharmacophore based filter, identified top 10,000 molecules were carried forward to a considerably fast but raw screening technique of Glide i.e. HTVS and the top 10% molecules were singled out for standard precision (SP) docking in Glide, and from that top 10% were carried forward for extra-precision (XP) analysis to identify the promising hit(s).

5.1.2.2. Synthesis of designed 1,3,4-thiadiazole derivatives

5.1.2.2.1. Synthetic methods for the synthesis of thiosemicarbazide derivatives (126-140)

Method-E: For the synthesis of compounds (**126-134**)

To a solution of aniline (1.0 g, 1 equiv.) in DMF (15 mL), sodium hydroxide (1.2 equiv.) and carbon disulphide (1 equiv.) were added. The reaction mixture was stirred at 20-35 °C for 1 h. To, the stirred mixture, hydrazine hydrate (3 equiv.) was added and stirring continued at 60-70 °C for another 1 h. Progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was poured into water, the solid so precipitated out was filtered, dried and used for the next step.[101]

Method-F: For the synthesis of compounds (**135-140**)

To a stirred solution of benzylamine (1.0 mL, 1 equiv.) and triethylamine (1.5 equiv.) in methanol (10 mL), a solution of carbon disulphide (1.2 equiv.) in methanol (5 mL) was added gradually. The reaction mixture was stirred at 15-30 °C for 75 min. After an additional 1 h at room temperature, the reaction mixture was cooled at -10 °C, iodomethane (1 equiv.) in methanol (5 mL) was added drop-wise over about a period of 20 min. After removal of the cooling bath, stirring was continued for additional 2 hrs at room temperature. It was then concentrated in vacuum to a volume of approximately 10 mL and partitioned between 30 mL of ether and 30 mL of

0.2 N HCl. The ethereal phase was washed with saturated aqueous sodium chloride solution, dried over anhydrous magnesium sulfate, filtered, and concentrated to give a solid. The solid so obtained was dissolved in methanol and hydrazine hydrate (3 equiv.) was added. The reaction mixture was refluxed for 2-4 h. After completion of the reaction, the mixture was cooled and allowed to stand at room temperature, a solid precipitated out which was filtered, dried and washed with methanol to get the desired product.[127]

5.1.2.2.1a. *N*-Phenylhydrazinecarbothioamide (126)

The title compound (**126**) was prepared from aniline (1.0 g, 10.73 mmol) following **Method-E**. It was obtained as a white solid (1.13 g, 63 %), m.p. 136-138 °C (lit.[100] m.p. 138-140 °C).

Analytical data

TLC : R_f 0.40 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3304, 3166, 1638, 1528, 1287, 1070 and 898.

5.1.2.2.1b. *N*-(4-Methylphenyl)hydrazinecarbothioamide (127)

The title compound (**127**) was prepared from *p*-toluidine (1.0 g, 9.33 mmol) following **Method-E**. It was obtained as white solid (1.16 g, 69 %), m.p. 137-139 °C (lit.[100] m.p. 140-142 °C).

Analytical data

TLC : R_f 0.42 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3252, 3193, 3032, 1619, 1544, 1276, 1063 and 806.

5.1.2.2.1c. *N*-(4-Methoxyphenyl)hydrazinecarbothioamide (128)

The title compound (**128**) was prepared from *p*-anisidine (1.0 g, 8.12 mmol) following **Method-E**. It was obtained as white solid (1.12 g, 70 %), m.p. 157-159 °C (lit.[100] m.p. 154-156 °C).

Analytical data

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

IR (KBr, cm^{-1}) : 3317, 3167, 1636, 1528, 1244, 1033 and 833.

5.1.2.2.1d. *N*-(4-Fluorophenyl)hydrazinecarbothioamide (129)

The title compound (**129**) was prepared from 4-fluoroaniline (1.0 g, 9.0 mmol) following **Method-E**. It was obtained as white solid (1.11 g, 67 %), m.p. 173-175 °C (lit.[100] m.p. 176-178 °C).

Analytical data

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

IR (KBr, cm^{-1}) : 3313, 3163, 2971, 1638, 1529, 1289, 1071 and 840.

5.1.2.2.1e. *N*-(4-Chlorophenyl)hydrazinecarbothioamide (130)

The title compound (**130**) was prepared from 4-chloroaniline (1.0 g, 7.84 mmol) following **Method-E**. It was obtained as white solid (1.0 g, 66 %), m.p. 166-168 °C (lit.[101] m.p. 162-164 °C).

Analytical data

TLC : R_f 0.38 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3360, 3249, 3083, 1550, 1263 and 831.

5.1.2.2.1f. *N*-(2,4-Dichlorophenyl)hydrazinecarbothioamide (131)

The title compound (**131**) was prepared from 2,4-dichloroaniline (1.0 g, 6.17 mmol) following **Method-E**. It was obtained as white solid (1.0 g, 72 %), m.p. 167-169 °C (lit.[100] m.p. 169-171 °C).

Analytical data

TLC : R_f 0.43 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3244, 3129, 1608, 1538, 1472, 1204, 1015 and 820.

5.1.2.2.1g. *N*-(Pyridin-2-yl)hydrazinecarbothioamide (132)

The title compound (**132**) was prepared from pyridin-2-amine (1.0 g, 10.6 mmol) following **Method-E**. It was obtained as white solid (1.16 g, 65 %), m.p. 188-190 °C.

Analytical data

TLC : R_f 0.51 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3241, 3158, 1607, 1510, 1207, 1008 and 761;

MS (m/z) : 169.0 $(M)^+$.

5.1.2.2.1h. *N*-(5-Methylpyridin-2-yl)hydrazinecarbothioamide (133)

The title compound (**133**) was prepared from of 5-methylpyridin-2-amine (1.0 g, 9.24 mmol) following **Method-E**. It was obtained as white solid (1.09 g, 65 %); m.p. 153-155 °C.

Analytical data

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

IR (KBr, cm^{-1}) : 3252, 3006, 1617, 1525, 1237, 1017 and 819;

MS (m/z) : 183.0 $(M)^+$.

5.1.2.2.1i. *N*-(5-Chloropyridin-2-yl)hydrazinecarbothioamide (134)

The title compound (**134**) was prepared from 5-chloropyridin-2-amine (1.0 g, 7.77 mmol) following **Method-E**. It was obtained as white solid (1.0 g, 68 %), m.p. 212-214 °C.

Analytical data

TLC : R_f 0.50 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3243, 3128, 1609, 1538, 1203, 1014 and 820;

MS (m/z) : 203.0 $(M)^+$, 205.0 $(M+2)^+$.

5.1.2.2.1j. *N*-Benzylhydrazinecarbothioamide (135)

The title compound (**135**) was prepared from benzylamine (1.0 mL, 9.33 mmol) following **Method-F**. It was obtained as white solid (1.0 g, 62 %), m.p. 126-128 °C.

Analytical data

TLC : R_f 0.40 (Chloroform-Methanol 18:2);

IR (KBr, cm^{-1}) : 3328, 3292, 3026, 1617, 1537, 1225, 1084 and 791;

MS (m/z) : 182.0 $(M)^+$.

5.1.2.2.1k. *N*-(4-Methylbenzyl)hydrazinecarbothioamide (136)

The title compound (**136**) was prepared from 4-methylbenzylamine (1.0 mL, 8.25 mmol) following **Method-F**. It was obtained as white solid (1.0 g, 66 %), m.p. 154-156 °C.

Analytical data

TLC	: R_f 0.48 (Chloroform-Methanol 18:2);
IR (KBr, cm^{-1})	: 3294, 2918, 1615, 1541, 1270, 1075 and 823;
MS (m/z)	: 195.75 $(M)^+$.

5.1.2.2.1l. *N*-(4-Methoxybenzyl)hydrazinecarbothioamide (137)

The title compound (**137**) was prepared from 4-methoxybenzylamine (1.0 mL, 7.65 mmol) following **Method-F**. It was obtained as white solid (1.0 g, 65 %), m.p. 112-114 °C.

Analytical data

TLC	: R_f 0.50 (Chloroform-Methanol 18:2);
IR (KBr, cm^{-1})	: 3332, 3230, 2914, 1615, 1538, 1248, 1029 and 843;
MS (m/z)	: 212.0 $(M)^+$.

5.1.2.2.1m. *N*-(4-Fluorobenzyl)hydrazinecarbothioamide (138)

The title compound (**138**) was prepared from 4-fluorobenzylamine (1.0 mL, 8.75 mmol) following **Method-F**. It was obtained as white solid (1.0 g, 65 %), m.p. 138-140 °C.

Analytical data

TLC	: R_f 0.50 (Chloroform-Methanol 18:2);
IR (KBr, cm^{-1})	: 3328, 3292, 2920, 1617, 1541, 1223, 1074 and 847;
MS (m/z)	: 200.0 $(M)^+$.

5.1.2.2.1n. *N*-(4-Chlorobenzyl)hydrazinecarbothioamide (139)

The title compound (**139**) was prepared from 4-chlorobenzylamine (1.0 mL, 8.19 mmol) following **Method-F**. It was obtained as white solid (0.94 g, 62 %), m.p. 178-180 °C.

Analytical data

TLC	: R_f 0.42 (Chloroform-Methanol 18:2);
IR (KBr, cm^{-1})	: 3317, 3200, 2925, 1615, 1549, 1238, 1083 and 808;

MS (m/z) : 216.0 $(M)^+$, 218.0 $(M+2)^+$.

5.1.2.2.1o. *N*-(4-Chlorobenzyl)hydrazinecarbothioamide (140)

The title compound (**140**) was prepared from 2,4-dichlorobenzylamine (1.0 mL, 7.38 mmol) following **Method-F**. It was obtained as white solid (0.84 g, 69 %), m.p. 140-142 °C.

Analytical data

TLC : R_f 0.37 (Chloroform-Methanol 18:2);

IR (KBr, cm^{-1}) : 3311, 3194, 2987, 1621, 1551, 1236, 1083 and 869;

MS (m/z) : 292.0 $(M)^+$, 294.0 $(M+2)^+$.

5.1.2.2.2. General synthetic method for the synthesis of 5-(substituted amino)-1,3,4-thiadiazole-2-thiols (141-155)

Method-G: To a solution of thiosemicarbazide derivative (1.0 g, 1 equiv.) in methanol (30 mL), potassium hydroxide (1.5 equiv.) and carbon disulphide (2 equiv.) were added. The reaction mixture was refluxed for 6-8 h and progress of the reaction was monitored by TLC. After completion of the reaction, the solvent was evaporated under reduced pressure and the residue was dissolved in water. The *p*H of mixture was made up to 3-4 by adding 2M HCl solution. The precipitated off-white colored solid was filtered, dried and was used as such for the next step.

5.1.2.2.2a. 5-(Phenylamino)-1,3,4-thiadiazole-2-thiol (141)

The title compound (**141**) was prepared from *N*-phenylhydrazine carbothioamide (1.0 g, 5.97 mmol) following **Method-G**. It was obtained as white solid (0.85 g, 68 %), m.p. 209-211 °C (lit.[102] m.p. 214-216 °C).

Analytical data

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

IR (KBr, cm^{-1}) : 3215, 3128, 3027, 1600, 1569, 1056 and 871;

MS (m/z) : 210.0 $(M)^+$.

5.1.2.2.2b. 5-(*p*-Tolylamino)-1,3,4-thiadiazole-2-thiol (142)

The title compound (**142**) was prepared from *N*-(*p*-tolyl)hydrazine carbothioamide (1.0 g, 5.50 mmol) following **Method-G**. It was obtained as white solid (0.87 g, 71 %), m.p. 218-220 °C (lit.[102] m.p. 220-222 °C).

Analytical data

TLC : R_f 0.50 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3230, 3114, 2910, 1606, 1564, 1326, 1056 and 812;

MS (m/z) : 224.0 $(M)^+$.

5.1.2.2.2c. 5-((4-Methoxyphenyl)amino)-1,3,4-thiadiazole-2-thiol (143)

The title compound (**143**) was prepared from *N*-(4-methoxyphenyl) hydrazinecarbothioamide (1.0 g, 5.0 mmol) following **Method-G**. It was obtained as white solid (0.81 g, 67 %), m.p. 160-162 °C.

Analytical data

TLC : R_f 0.43 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3201, 3077, 2948, 1605, 1567, 1496, 1056 and 852;

MS (m/z) : 240.0 $(M)^+$.

5.1.2.2.2d. 5-((4-Fluorophenyl)amino)-1,3,4-thiadiazole-2-thiol (144)

The title compound (**144**) was prepared from *N*-(4-fluorophenyl) hydrazinecarbothioamide (1.0 g, 5.39 mmol) following **Method-G**. It was obtained as white solid (0.84 g, 69 %), m.p. 204-206 °C.

Analytical data

TLC : R_f 0.40 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3239, 3110, 2928, 1615, 1570, 1057 and 824;

MS (m/z) : 228.0 $(M)^+$.

5.1.2.2.2e. 5-((4-Chlorophenyl)amino)-1,3,4-thiadiazole-2-thiol (145)

The title compound (**145**) was prepared from *N*-(4-chlorophenyl) hydrazinecarbothioamide (1.0 g, 4.95 mmol) following **Method-G**. It was obtained as white solid (0.80 g, 66 %), m.p. 201-203 °C (Lit.[102] m.p. 200-202).

Analytical data

TLC : R_f 0.40 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3230, 3115, 2926, 1602, 1565, 1057 and 820;

MS (m/z) : 243.41 $(M)^+$, 245.43 $(M+2)^+$.

5.1.2.2.2e. 5-((2,4-Dichlorophenyl)amino)-1,3,4-thiadiazole-2-thiol (146)

The title compound (**146**) was prepared from *N*-(2,4-dichlorophenyl) hydrazinecarbothioamide (1.0 g, 4.23 mmol) following **Method-G**. It was obtained as white solid (0.82 g, 70 %), m.p. 209-211 °C.

Analytical data

TLC : R_f 0.50 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3230, 3115, 2927, 1603, 1564, 1326, 1057 and 843;

MS (m/z) : 278.0 $(M)^+$, 280.0 $(M+2)^+$.

5.1.2.2.2f. 5-(Pyridin-2-ylamino)-1,3,4-thiadiazole-2-thiol (147)

The title compound (**147**) was prepared from *N*-(pyridin-2-yl)hydrazine carbothioamide (1.0 g, 5.94 mmol) following **Method-G**. It was obtained as white solid (0.82 g, 66 %), m.p. 237-239 °C.

Analytical data

TLC : R_f 0.40 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3251, 3114, 2929, 1619, 1558, 1479 and 865;

MS (m/z) : 211.0 $(M)^+$.

5.1.2.2.2g. 5-((5-Methylpyridin-2-yl)amino)-1,3,4-thiadiazole-2-thiol (148)

The title compound (**148**) was prepared from *N*-(5-methylpyridin-2-yl) hydrazinecarbothioamide (1.0 g, 5.48 mmol) following **Method-G**. It was obtained as white solid (0.76 g, 62 %), m.p. 243-245 °C.

Analytical data

TLC : R_f 0.43 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3228, 3134, 2973, 1617, 1492 and 818;

MS (m/z) : 225.0 $(M)^+$.

5.1.2.2.2h. 5-((5-Chloropyridin-2-yl)amino)-1,3,4-thiadiazole-2-thiol (149)

The title compound (**149**) was prepared from *N*-(5-chloropyridin-2-yl) hydrazinecarbothioamide (1.0 g, 4.93 mmol) following **Method-G**. It was obtained as white solid (0.78 g, 65 %), m.p. >250 °C.

Analytical data

TLC : R_f 0.38 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3223, 3115, 2964, 1611, 1555, 1475 and 819;

MS (m/z) : 245.0 $(M)^+$, 247.0 $(M+2)^+$.

5.1.2.2.2i. 5-(Benzylamino)-1,3,4-thiadiazole-2-thiol (150)

The title compound (**150**) was prepared from *N*-benzylhydrazine carbothioamide (1.0 g, 5.50 mmol) following **Method-G**. It was obtained as white solid (0.85 g, 69 %), m.p. 132-134 °C (lit.[103] m.p. 136-138 °C).

Analytical data

TLC : R_f 0.40 (Chloroform-Methanol 18:2);

IR (KBr, cm^{-1}) : 3255, 3033, 2871, 1552, 1458, 1299, 1045 and 746;

MS (m/z) : 224.0 $(M)^+$.

5.1.2.2.2j. 5-((4-Methylbenzyl)amino)-1,3,4-thiadiazole-2-thiol (151)

The title compound (**151**) was prepared from *N*-(4-methylbenzyl) hydrazinecarbothioamide (1.0 g, 5.12 mmol) following **Method-G**. It was obtained as white solid (0.86 g, 72 %), m.p. 155-157 °C.

Analytical data

TLC : R_f 0.44 (Chloroform-Methanol 18:2);

IR (KBr, cm^{-1}) : 3255, 3033, 2871, 1552, 1458, 1299, 1045 and 746;

MS (m/z) : 238.0 $(M)^+$.

5.1.2.2.2k. 5-((4-Methoxybenzyl)amino)-1,3,4-thiadiazole-2-thiol (152)

The title compound (**152**) was prepared from *N*-(4-methoxybenzyl) hydrazinecarbothioamide (1.0 g, 4.73 mmol) following **Method-G**. It was obtained as white solid (0.78 g, 65 %); m.p. 119-121 °C.

Analytical data

TLC : R_f 0.36 (Chloroform-Methanol 18:2);

IR (KBr, cm^{-1}) : 3209, 3077, 2886, 1566, 1467, 1311, 1251 and 824;

MS (m/z) : 253.0 $(M)^+$.

5.1.2.2.2l. 5-((4-Fluorobenzyl)amino)-1,3,4-thiadiazole-2-thiol (153)

The title compound (**153**) was prepared from *N*-(4-fluorobenzyl) hydrazinecarbothioamide (1.0 g, 5.0 mmol) following **Method-G**. It was obtained as white solid (0.8 g, 66 %), m.p. 165-167 °C.

Analytical data

TLC : R_f 0.30 (Chloroform-Methanol 18:2);

IR (KBr, cm^{-1}) : 3296, 3048, 2875, 1557, 1463, 1302, 1228 and 827;

MS (m/z) : 242.0 $(M)^+$.

5.1.2.2.2m. 5-((4-Chlorobenzyl)amino)-1,3,4-thiadiazole-2-thiol (154)

The title compound (**154**) was prepared from *N*-(4-chlorobenzyl) hydrazinecarbothioamide (1.0 g, 4.63 mmol) following **Method-G**. It was obtained as white solid (0.8 g, 67 %), m.p. 152-154 °C.

Analytical data

TLC : R_f 0.35 (Chloroform-Methanol 18:2);

IR (KBr, cm^{-1}) : 3276, 3050, 2874, 1562, 1484, 1095 and 810;

MS (m/z) : 258.0 $(M)^+$, 260.0 $(M+2)^+$.

5.1.2.2.2n. 5-((2,4-Dichlorobenzyl)amino)-1,3,4-thiadiazole-2-thiol (155)

The title compound (**155**) was prepared from *N*-(2,4-dichlorobenzyl) hydrazinecarbothioamide (1.0 g, 4.0 mmol) following **Method-G**. It was obtained as white solid (0.82 g, 70 %), m.p. 175-177 °C.

Analytical data

TLC : R_f 0.32 (Chloroform-Methanol 18:2);

IR (KBr, cm^{-1}) : 3276, 3050, 2874, 1562, 1484, 1095 and 810;

MS (m/z) : 292.0 $(M)^+$, 294.0 $(M+2)^+$.

5.1.2.2.3. Synthesis of lead molecule (158)

Synthesis of compound (**158**) was carried out by a three step method as described below.

5.1.2.2.3a. 2-Amino-3-carbethoxy-4,5,6,7-tetrahydrobenzo[*b*]thiophene (156)

Cyclohexanone (1.0 mL, 10 mmol), powdered sulfur (0.32 g, 10 mmol), ethyl cyanoacetate (1.0 mL, 10 mmol) and methanol (20 mL) were mixed and stirred together at room temperature. To this well stirred mixture, diethylamine (1.0 mL, 12.5 mmol) was added dropwise in 30 min and stirring continued for another 3 h at ambient temperature. The reaction mixture was allowed to attain room temperature and thereafter kept in refrigerator overnight. The solid so separated out was filtered at suction and washed with 20 mL chilled 50 % aq. methanol to get the titled compound (**156**) as pale yellow solid (1.6 g, 74 %), m.p. 113-115 ˚C (lit.[104] 112-115 °C).

Analytical data

TLC : R_f 0.55 (*n*-Hexane-Ethyl acetate 15:5);

IR (KBr, cm^{-1}) : 3405, 3298, 3168, 2937, 1647, 1491, 1026 and 783.

5.1.2.2.3b. 2-(((5-((4-Chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)thio) methyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (157)

A stream of dry HCl gas was bubbled through an ice-cold mixture of compound (**156**) (1.0 g, 4.44 mmol) and chloroacetonitrile (0.42 mL, 6.66 mmol) in dry dioxane (60 mL) for 1-2 h. The reaction mixture was allowed to stand at room temperature for 12 h. The reaction mixture was thereafter heated on a water bath for 2 to 3 h and progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was cooled to room temperature and poured onto ice-water mixture (15-20 mL) and neutralized with strong aq. NH_4OH solution (30 %). The solid so separated was filtered, washed with water and dried to afford the titled compound (**157**) (0.9 g, 80 %), m.p. 271-273 ˚C (lit.[105] 274-276 °C).

Analytical data

TLC	: R_f 0.35 (Chloroform-Methanol 19:1);
IR (KBr, cm^{-1})	: 3182, 3074, 1681, 1467 and 836;
^{1}H-NMR (DMSO-d_6)	: δ 12.15 (s, 1H, -N*H*), 4.17 (s, 2H, -CH_2), 2.90-2.85 (m, 2H, -CH_2), 2.74-2.70 (m, 2H, -CH_2), 1.84-1.78 (m, 4H, -CH_2);
MS (m/z)	: 254.79 $(M)^+$.

5.1.2.2.3c. 2-(((5-((4-Chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)thio) methyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (158)

To a solution of 5-(4-chlorophenylamino)-1,3,4-thiadiazole-2-thiol (**145**) (0.5 g, 2.05 mmol) in DMF (6 mL), potassium carbonate (0.85 g, 6.15 mmol) and compound (**157**) (0.52 g, 2.051 mmol) were added. The reaction mixture was stirred at room temperature for 3 to 4 h and progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was poured into ice-cold water with continuous stirring. The precipitate thus obtained was filtered under vacuum and dried. The crude product was purified by column chromatography to afford beige solid (0.24 g, 48 %), m.p. >250 °C.

Analytical data

TLC	: R_f 0.41 (Chloroform-Methanol 19:1);
IR (KBr, cm^{-1})	: 3185, 1664, 1562, 1495 and 1562;
^{1}H-NMR (DMSO-d_6)	: δ 12.42 (s, 1H, -N*H*), 10.52 (s, 1H, -N*H*), 7.59 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.33 (d, *J* = 8.8 Hz, 2H, Ar*H*), 4.27 (s, 2H, -SCH_2), 2.82 (t, 1H, -CH_2), 2.72 (s, 1H, -CH_2), 1.76-1.81 (m, 4H, -CH_2);
MS (m/z)	: 462.60 $(M)^+$, 464.60 $(M+2)^+$.

5.1.2.2.4. Synthesis of 5-(substituted-benzylthio)-*N*-(4-chlorophenyl)-1,3,4-thiadiazol-2-amines (159-165)

5.1.2.2.4a. 5-(Benzylthio)-*N*-(4-chlorophenyl)-1,3,4-thiodiazole-2-amine (159)

To a solution of 5-(4-chlorophenylamino)-1,3,4-thiadiazole-2-thiol (**145**) (0.5 g, 2.05 mmol) in DMF (6 mL), potassium carbonate (0.85 g, 6.15 mmol) and benzyl bromide (0.35 mL, 2.05 mmol) were added. The reaction mixture was stirred at room temperature for 3 to 4 h and progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was poured into ice-cold water with continuous stirring. The precipitate thus obtained was filtered under vacuum and dried. The crude product was purified by column chromatography using silica gel as stationary phase and *n*-hexane-ethyl acetate as an eluent to afford the titled compound (**159**) as off-white solid (0.46 g, 67 %), m.p. 185-187 °C.

Analytical data

TLC	: R_f 0.46 (*n*-Hexane-Ethyl acetate 15:5);
IR (KBr, cm^{-1})	: 3258, 3057, 2837, 1658, 1599, 1491, 1401 and 806;
^{1}H-NMR (DMSO-d_6)	: δ 10.34 (s, 1H, -N*H*), 7.60 (d, 2H, Ar*H*), 7.39 (d, 2H, Ar*H*), 7.25-7.34 (m, 5H, Ar*H*) and 4.39 (s, 2H, -SCH_2);
MS (m/z)	: 334.37 $(M)^+$, 336.36 $(M+2)^+$;
RP-HPLC	: Purity: 97.1 %, t_R = 6.03 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60).

5.1.2.2.4b. 5-((4-Chlorobenzyl)thio)-*N*-(4-chlorophenyl)-1,3,4-thiadiazol-2-amine (160)

The title compound (**160**) was prepared as per the method described for compound (**159**) using 5-(4-chlorophenylamino)-1,3,4-thiadiazole-2-thiol (**145**) (0.5 g, 2.05 mmol) and 4-chlorobenzylchloride (0.28 mL, 2.05 mmol).

The compound (**160**) was obtained as off-white solid (0.46 g, 64 %), m.p. 183-185 °C.

Analytical data

TLC : R_f 0.50 (*n*-Hexane-Ethyl acetate 15:5);

IR (KBr, cm^{-1}) : 3252, 3025, 2819, 1609, 1565, 1489, 1413 and 819;

^{1}H-NMR (DMSO-d_6) : δ 10.29 (bs, 1H, -N*H*), 7.59 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.38 (d, *J* = 8.4 Hz, 2H, Ar*H*), 7.30 (d, *J* = 8.4 Hz, 2H, Ar*H*), 7.26 (d, *J* = 8.8 Hz, 2H, Ar*H*), 4.36 (s, 2H, -SCH_2);

MS (m/z) : 368.38 $(M)^+$, 370.39 $(M+2)^+$;

RP-HPLC : Purity: 97.9 %, t_R = 5.37 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60)

5.1.2.2.4c. 4-(((5-((4-Chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)thio) methyl)benzonitrile (161)

The title compound (**161**) was prepared as per the method described for compound (**159**) using 5-(4-chlorophenylamino)-1,3,4-thiadiazole-2-thiol (**145**) (0.5 g, 2.05 mmol) and 4-cyanobenzyl bromide (0.40 g, 2.05 mmol). The compound (**161**) was obtained as off-white solid (0.45 g, 62 %), m.p. 201-203 °C.

Analytical data

TLC : R_f 0.43 (*n*-Hexane-Ethyl acetate 15:5);

IR (KBr, cm^{-1}) : 3250, 3039, 2917, 2227, 1619, 1494, 1411 and 820;

^{1}H-NMR (DMSO-d_6) : δ 10.35 (s, 1H, -NH), 7.70 (d, *J* = 8.0 Hz, 2H, Ar*H*), 7.58-7.60 (m, 4H, Ar*H*), 7.26 (d, *J* = 8.0 Hz, 2H, Ar*H*) and 4.36 (s, 2H, -SCH_2);

MS (m/z) : 359.43 $(M)^+$, 361.44 $(M+2)^+$;

RP-HPLC : Purity: 98.2 %, t_R = 4.50 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60).

5.1.2.2.4d. *N*-(4-Chlorophenyl)-5-((4-methoxybenzyl)thio)-1,3,4-thia diazol-2-amine (162)

The title compound (**162**) was prepared as per the method described for compound (**159**) using 5-(4-chlorophenylamino)-1,3,4-thiadiazole-2-thiol (**145**) (0.5 g, 2.05 mmol) and 4-methoxybenzyl bromide (0.29 mL, 2.05 mmol). The compound (**162**) was obtained as off-white solid (0.48 g, 64 %), m.p. 190-192 °C.

Analytical data

TLC : R_f 0.40 (*n*-Hexane-Ethyl acetate 15:5);

IR (KBr, cm^{-1}) : 3262, 3024, 2836, 1607, 1548, 1494, 1404, 1175, 1030 and 814;

^{1}H-NMR (DMSO-d_6) : δ 10.52 (bs, 1H, -N*H*), 7.62 (d, *J* = 8.4 Hz, 2H, Ar*H*), 7.40 (d, *J* = 8.4 Hz, 2H, Ar*H*), 7.34 (d, *J* = 8.4 Hz, 2H, Ar*H*), 6.91 (d, *J* = 8.4 Hz, 2H, Ar*H*), 4.38 (s, 2H, -SCH_2), 3.74 (s, 3H, -OCH_3);

MS (m/z) : 364.38 $(M)^+$, 366.39 $(M+2)^+$;

RP-HPLC : Purity: 97.4 %, t_R = 4.21 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60)

5.1.2.2.4e. 5-((4-Bromobenzyl)thio)-*N*-(4-chlorophenyl)-1,3,4-thiadiazol-2-amine (163)

The title compound (**163**) was prepared as per the method described for compound (**159**) using 5-(4-chlorophenylamino)-1,3,4-thiadiazole-2-thiol (**145**) (0.5 g, 2.05 mmol) and 4-bromobenzyl bromide (0.51 g, 2.05 mmol). The compound (**163**) was obtained as off-white solid (0.54 g, 64 %), m.p. 179-181 °C.

Analytical data

TLC : R_f 0.53 (*n*-Hexane-Ethyl acetate 15:5);

IR (KBr, cm^{-1}) : 3249, 3036, 2822, 1618, 1494, 1412 and 827;

^{1}H-NMR (DMSO-d_6) : δ 10.52 (s, 1H, -N*H*), 7.60 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.52 (d, *J* = 8.4 Hz, 2H, Ar*H*), 7.39 (d, *J*

= 8.8 Hz, 2H, Ar*H*), 7.37 (d, *J* = 8.4 Hz, 2H, Ar*H*) and 4.40 (s, 2H, -SCH_2);

MS (m/z) : 412.43 $(M)^+$, 414.48 $(M+2)^+$;

RP-HPLC : Purity: 96.1 %, t_R = 5.16 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60)

5.1.2.2.4f. *N*-(4-Chlorophenyl)-5-((4-fluorobenzyl)thio)-1,3,4-thiadiazol-2-amine (164)

The title compound (**164**) was prepared as per the method described for compound (**159**) using 5-(4-chlorophenylamino)-1,3,4-thiadiazole-2-thiol (**145**) (0.5 g, 2.05 mmol) and 4-fluorobenzyl bromide (0.39 g, 2.05 mmol). The compound (**164**) was obtained as off-white solid (0.55 g, 62 %), m.p. 178-180 °C.

Analytical data

TLC : R_f 0.45 (*n*-Hexane-Ethyl acetate 15:5);

IR (KBr, cm^{-1}) : 3246, 3189, 2824, 1891, 1618, 1455, 1224 and 823;

^{1}H-NMR (DMSO-d_6) : δ 10.52 (bs, 1H, -N*H*), 7.61 (d, 2H, Ar*H*), 7.39-7.48 (m, 4H, Ar*H*), 7.16-7.21 (m, 2H, Ar*H*), 4.40 (s, 2H, -SCH_2);

MS (m/z) : 352.36 $(M)^+$, 354.35 $(M+2)^+$;

RP-HPLC : Purity: 99.5 %, t_R = 4.82 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60)

5.1.2.2.4g. 4'-(((5-((4-Chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)thio) methyl)-[1,1'-biphenyl]-2-carbonitrile (165)

The title compound (**165**) was prepared as per the method described for compound (**159**) using 5-(4-chlorophenylamino)-1,3,4-thiadiazole-2-thiol (**145**) (0.5 g, 2.05 mmol) and 4'-(bromomethyl)-[1,1'-biphenyl]-2-carbonitrile (0.56 g, 2.05 mmol). The compound (**165**) was obtained as off-white solid (0.52 g, 58 %), m.p. 171-173 °C.

Analytical data

TLC	: R_f 0.43 (Chloroform-Methanol 19:1);
IR (KBr, cm^{-1})	: 3303, 3084, 2849, 2233, 1605, 1486, 1400 and 810;
^{1}H-NMR (DMSO-d_6)	: δ 10.43 (s, 1H, -NH), 7.87 (d, *J* = 8.8 and 1.2 Hz, 1H, Ar*H*), 7.74 (dt, *J* = 8.8 and 1.2 Hz, 1H, Ar*H*), 7.52-7.62 (m, 8H, Ar*H*), 7.29-7.32 (m, 2H, Ar*H*), 4.50 (s, 2H, -SCH_2);
^{13}C-NMR (DMSO-d_6)	: δ 164.45, 152.63, 143.93, 139.10, 137.62, 136.86, 133.76, 133.41, 129.97, 129.27, 128.80, 128.77, 128.13, 125.30, 118.82, 118.41, 109.99 and 37.26;
MS (m/z)	: 435.52 $(M+1)^+$, 437.52 $(M+2)^+$;
RP-HPLC	: Purity: 98.5 %, t_R = 6.56 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60)

5.1.2.2.5. Synthesis of amidoalkyls (169-177)

To a stirring solution of secondary amine (1.0 g, 1 equiv.) and potassium carbonate (3 equiv.) in dry DCM was added chloroacetyl chloride (1.2 equiv.) drop-wise maintaining the temperature at 0-10 ^oC. After stirring at 25-30 ^oC for 4 h, the reaction mixture was finally poured over crushed ice and extracted with successive quantities of chloroform. The organic extract was washed with sodium bicarbonate solution and water. It was then dried over anhydrous sodium sulphate and subjected to solvent recovery to get the products (**169-177**) that were directly used for the next step without further purification.

5.1.2.2.6. Synthesis of 5-(thiosubstituted)-*N*-(4-chlorophenyl)-1,3,4-thiadiazol-2-amines (178-186)

5.1.2.2.6a. 2-((5-((4-Chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)thio)-1-(pyrrolidin-1-yl)ethanone (178)

The title compound (**178**) was prepared as per the method described for compound (**159**) using 5-(4-chlorophenylamino)-1,3,4-thiadiazole-2-thiol (**145**) (0.5 g, 2.05 mmol) and 2-chloro-1-(pyrrolidin-1-yl)ethanone (**169**) (0.30 g, 2.05 mmol). The compound (**178**) was obtained as off-white solid (0.42 g, 58 %), m.p. 228-230 °C.

Analytical data

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

IR (KBr, cm^{-1}) : 3246, 3048, 2881, 1642, 1516, 1406, 1244 and 815;

^{1}H-NMR (DMSO-d_6) : δ 10.53 (bs, 1H, -N*H*), 7.62 (d, *J* = 8.0 Hz, 2H, Ar*H*), 7.40 (d, *J* = 8.0 Hz, 2H, Ar*H*), 4.22 (s, 2H, -SCH_2), 3.30-3.50 (m, 4H, -NCH_2), 1.87-1.94 (m, 2H, -CH_2) and 1.76-1.94 (m, 2H, -CH_2);

MS (m/z) : 355.4 $(M)^+$, 357.4 $(M+2)^+$;

RP-HPLC : Purity: 97.0 %, t_R = 3.36 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60).

5.1.2.2.6b. 2-((5-((4-Chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)thio)-1-(piperidin-1-yl)ethanone (179)

The title compound (**179**) was prepared as per the method described for compound (**159**) using 5-(4-chlorophenylamino)-1,3,4-thiadiazole-2-thiol (**145**) (0.5 g, 2.05 mmol) and 2-chloro-1-(piperidin-1-yl)ethanone (**170**) (0.33 g, 2.05 mmol). The compound (**179**) was obtained as off-white solid (0.44 g, 58 %), m.p. 201-203 °C.

Analytical data

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

IR (KBr, cm^{-1}) : 3259, 3038, 2856, 1632, 1495, 1215 and 823;

^{1}H-NMR (DMSO-d_6) : δ 10.53 (s, 1H, -N*H*), 7.60 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.39 (d, *J* = 8.8 Hz, 2H, Ar*H*), 4.30 (s, 2H, -SCH_2), 3.35-3.43 (m, 4H, -NCH_2) and 1.44-1.56 (m, 6H, -CH_2);

MS (m/z) : 369.5 $(M)^+$, 371.5 $(M+2)^+$;

RP-HPLC : Purity: 96.8 %, t_R = 3.65 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60)

5.1.2.2.6c. 2-((5-((4-Chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)thio)-1-morpholino ethanone (180)

The title compound (**180**) was prepared as per the method described for compound (**159**) using 5-(4-chlorophenylamino)-1,3,4-thiadiazole-2-thiol (**145**) (0.5 g, 2.05 mmol) and 2-chloro-1-morpholinoethanone (**171**) (0.34 g, 2.05 mmol). The compound (**180**) was obtained as off-white solid (0.36 g, 71 %), m.p. 200-202 °C.

Analytical data

TLC : R_f 0.40 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3250, 3050, 2865, 1641, 1494, 1407, 1216 and 818;

^{1}H-NMR (DMSO-d_6) : δ 10.54 (bs, 1H, -N*H*), 7.62 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.40 (d, *J* = 8.8 Hz, 2H, Ar*H*), 4.32 (s, 2H, -SCH_2), 3.52-3.62 (m, 4H, -OCH_2) and 3.36-3.51 (m, 4H, -NCH_2);

MS (m/z) : 371.49 $(M)^+$, 373.49 $(M+2)^+$;

RP-HPLC : Purity: 97.9 %, t_R = 3.07 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60)

5.1.2.2.6d. 3-((5-((4-Chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)thio)-1-(pyrrolidin-1-yl)propan-1-one (181)

The title compound (**181**) was prepared as per the method described for compound (**159**) using 5-(4-chlorophenylamino)-1,3,4-thiadiazole-2-thiol

(**145**) (0.5 g, 2.05 mmol) and 3-chloro-1-(pyrrolidin-1-yl)propan-1-one (**172**) (0.33 g, 2.05 mmol). The compound (**181**) was obtained as off-white solid (0.43 g, 57 %), m.p. 194-196 °C.

Analytical data

TLC	: R_f 0.48 (Chloroform-Methanol 19:1);
IR (KBr, cm^{-1})	: 3254, 3055, 2875, 1638, 1517, 1410 and 846;
^{1}H-NMR (DMSO-d_6)	: δ 10.54 (s, 1H, -N*H*), 7.62 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.40 (d, *J* = 8.8 Hz, 2H, Ar*H*), 3.34-3.39 (m, 4H, -NCH_2), 3.29 (t, *J* = 6.8 Hz, 2H, -SCH_2), 2.74 (t, *J* = 6.8 Hz, 2H, -COCH_2), 1.83-1.88 (m, 2H, -CH_2) and 1.75-1.80 (m, 2H, -CH_2);
MS (m/z)	: 369.0 $(M)^+$, 371.0 $(M+2)^+$;
RP-HPLC	: Purity: 95.9 %, t_R = 4.04 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60)

5.1.2.2.6e. 3-((5-((4-Chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)thio)-1-(piperidin-1-yl)propan-1-one (182)

The title compound (**182**) was prepared as per the method described for compound (**159**) using 5-(4-chlorophenylamino)-1,3,4-thiadiazole-2-thiol (**145**) (0.5 g, 2.05 mmol) and 3-chloro-1-(piperidin-1-yl)propan-1-one (**173**) (0.36 g, 2.05 mmol). The compound (**182**) was obtained as off-white solid (0.46 g, 58 %), m.p. 178-180 °C.

Analytical data

TLC	: R_f 0.58 (Chloroform-Methanol 19:1);
IR (KBr, cm^{-1})	: 3242, 3035, 2857, 1646, 1608, 1412 and 825;
^{1}H-NMR (DMSO-d_6)	: δ 10.48 (bs, 1H, -N*H*), 7.60 (d, *J* = 9.2 Hz, 2H, Ar*H*), 7.37 (d, *J* = 9.2 Hz, 2H, Ar*H*), 3.42 (t, 2H, -SCH_2), 3.29-3.35 (m, 4H, -NCH_2), 2.78 (t, 2H, -COCH_2), 1.54-1.58 (m, 2H, -CH_2) and 1.46-1.37 (m, 2H, -CH_2);
MS (m/z)	: 383.5 $(M)^+$, 385.4 $(M+2)^+$;

RP-HPLC : Purity: 97.2 %, t_R = 4.77 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60)

5.1.2.2.6f. 3-((5-((4-Chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)thio)-1-morpholino propan-1-one (183)

The title compound (**183**) was prepared as per the method described for compound (**159**) using 5-(4-chlorophenylamino)-1,3,4-thiadiazole-2-thiol (**145**) (0.5 g, 2.05 mmol) and 3-chloro-1-morpholinopropan-1-one (**174**) (0.36 g, 2.05 mmol). The compound (**183**) was obtained as off-white solid (0.49 g, 62 %), m.p. 214-216 °C.

Analytical data

TLC : R_f 0.51 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3236, 3035, 2865, 1647, 1523, 1412 and 847;

^{1}H-NMR (DMSO-d_6) : δ 10.53 (bs, 1H, -N*H*), 7.62 (d, *J* = 8.4 Hz, 2H, Ar*H*), 7.39 (d, *J* = 8.4 Hz, 2H, Ar*H*), 3.55 (t, 4H, -OCH_2), 3.40 (t, 4H, -NCH_2), 3.37 (t, *J* = 6.4 Hz, 2H, -SCH_2) and 2.83 (t, *J* = 6.4 Hz, 2H, -COCH_2);

MS (m/z) : 385.51 $(M)^+$, 387.54 $(M+2)^+$;

RP-HPLC : Purity: 98.1 %, t_R = 3.24 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60).

5.1.2.2.6g. 4-((5-((4-Chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)thio)-1-(pyrrolidin-1-yl)butan-1-one (184)

The title compound (**184**) was prepared as per the method described for compound (**159**) using 5-(4-chlorophenylamino)-1,3,4-thiadiazole-2-thiol (**145**) (0.5 g, 2.05 mmol) and 4-chloro-1-(pyrrolidin-1-yl)butan-1-one (**175**) (0.36 g, 2.05 mmol). The compound (**184**) was obtained as off-white solid (0.55 g, 63 %), m.p. 156-158 °C.

Analytical data

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

IR (KBr, cm^{-1}) : 3245, 3048, 1634, 1517 and 829;

^{1}H-NMR (DMSO-d_6) : δ 10.54 (bs, 1H, -N*H*), 7.62 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.37 (d, *J* = 8.8 Hz, 2H, Ar*H*), 3.24-3.34 (m, 4H, -NCH_2), 3.19 (t, 2H, -SCH_2), 2.32 (t, 2H, -COCH_2), 2.02-2.09 (m, 4H, -CH_2) and 1.91-1.94 (m, 2H, -CH_2);

^{13}C NMR (DMSO-d_6) : 169.23, 164.20, 153.36, 139.18, 128.81, 125.29, 118.81, 45.68, 45.12, 33.74, 32.0, 23.47, 24.42 and 23.80;

MS (m/z) : 383.50 $(M)^+$, 385.54 $(M+2)^+$;

RP-HPLC : Purity: 99.7 %, t_R = 3.56 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60)

5.1.2.2.6h. 4-((5-((4-Chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)thio)-1-(piperidin-1-yl)butan-1-one (185)

The title compound (**185**) was prepared as per the method described for compound (**159**) using 5-(4-chlorophenylamino)-1,3,4-thiadiazole-2-thiol (**145**) (0.5 g, 2.05 mmol) and 4-chloro-1-(piperidin-1-yl)butan-1-one (**176**) (0.39 g, 2.05 mmol). The compound (**185**) was obtained as off-white solid (0.51 g, 63 %), m.p. 145-147 °C.

Analytical data

TLC : R_f 0.37 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3245, 3048, 1634, 1517 and 829;

^{1}H-NMR (DMSO-d_6) : δ 10.51 (bs, 1H, -N*H*), 7.60 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.40 (d, *J* = 8 Hz, 2H, Ar*H*), 3.33-3.40 (m, 4H, -NCH_2), 3.17 (t, 2H, -SCH_2), 2.42 (t, 2H, -COCH_2), 1.84-1.92 (m, 2H, -CH_2), 1.51-1.56 (m, 2H, -CH_2) and 1.38-1.45 (m, 4H, -CH_2);

MS (m/z) : 397.50 $(M)^+$, 399.51 $(M+2)^+$;

RP-HPLC : Purity: 96.3 %, t_R = 3.93 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol

12:28:60)

5.1.2.2.6i. 4-((5-((4-Chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)thio)-1-morpholino butan-1-one (186)

The title compound (**186**) was prepared as per the method described for compound (**159**) using 5-(4-chlorophenylamino)-1,3,4-thiadiazole-2-thiol (**145**) (0.5 g, 2.05 mmol) and 4-chloro-1-morpholinobutan-1-one (**177**) (0.39 g, 2.05 mmol). The compound (**186**) was obtained as off-white solid (0.46 g, 56 %), m.p. 178-180 °C.

Analytical data

TLC : R_f 0.31 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3237, 3038, 1641, 1521, 1441 and 819;

^{1}H-NMR (DMSO-d_6) : δ 10.55 (bs, 1H, N*H*), 7.61 (d, *J* = 8.4 Hz, 2H, Ar*H*), 7.38 (d, *J* = 8.4 Hz, 2H, Ar*H*), , 3.48-3.53 (m, 4H, OCH_2), 3.35-3.41 (m, 4H, NCH_2), 3.18 (t, 2H, SCH_2), 2.45 (t, 2H, COCH_2), 1.88-1.92 (m, 2H, CH_2);

MS (m/z) : 399.56 $(M)^+$, 401.55 $(M+2)^+$;

RP-HPLC : Purity: 96.8 %, t_R = 3.25 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60)

5.1.2.2.7. Synthesis of substituted anilines (197-204, 208, 210)

5.1.2.2.7.1. General methods for the synthesis of substituted nitro compounds (189-196)

Method H: Synthesis of compounds (**189-192**)

In stirring solution of secondary amine (42 mmol) in DMF (15 mL), K_2CO_3 (84 mmol) was added. The reaction mixture was stirred at 120 °C for 30 min. To the reaction mixture, 1-fluoro-4-nitrobenzene (**187**) (29 mmol, 3 mL) was added with continuous stirring. Progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was poured into crushed ice (100 mL). Precipitated yellow solid so obtained was

filtered and dried to obtain a crude product which was further purified by recrystallization.

Method I: Synthesis of compounds (**193-196**)

1-Bromo-4-nitrobenzene (**188**) (2.0 g, 10 mmol), secondary amine (12 mmol) and CuI (0.38 g, 2 mmol) were taken in 1,4-dioxane, transferred to pressure tube and stirred for 30 min in an inert environment. DMEDA (0.43 mL, 4 mmol) and K_2CO_3 (3.45 g, 25 mmol) were added in the reaction mixture and the mixture was heated at 140-150 °C for 4-5 h. After completion of the reaction, the reaction mixture was filtered through a pad of Celite and washed with ethyl acetate (15 mL). The filtrate thus obtained was concentrated under reduced pressure and residues were dissolved in chloroform (50 mL). The organic phase was washed with water and brine and the collected organic layer was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to obtain a product which was further purified by recrystallization.

5.1.2.2.7.1a. 1-(4-Nitrophenyl)pyrrolidine (189)

The title compound (**189**) was prepared from 1-fluoro-4-nitrobenzene (**187**) (3.0 mL, 29 mmol) and pyrrolidine (3.51 mL, 42 mmol) following **Method-H**. Compound (**189**) was obtained as a light yellow solid (3.37 g, 62 %), m.p. 163-165 °C (lit.[106] 167-169 °C).

Analytical data:

TLC : R_f 0.6 (*n*-Hexane-Ethyl acetate 15:5);

IR (KBr, cm^{-1}) : 2972, 2859, 1520, 1297 and 821.

5.1.2.2.7.1b. 1-(4-Nitrophenyl)piperidine (190)

The title compound (**190**) was prepared from 1-fluoro-4-nitrobenzene (**187**) (3.0 mL, 29 mmol) and piperidine (4.20 mL, 42 mmol) following **Method-H**. Compound (**190**) was obtained as a light yellow solid (3.98 g, 68 %), m.p. 100-102 °C (lit.[107] 104-106 °C).

Analytical data

TLC : R_f 0.67 (*n*-Hexane-Ethyl acetate 15:5);

IR (KBr, cm^{-1}) : 2940, 2846, 1598, 1321 and 818.

5.1.2.2.6.1c. 4-(4-Nitrophenyl)morpholine (191)

The title compound (**191**) was prepared from 1-fluoro-4-nitrobenzene (**187**) (3.0 mL, 29 mmol) and morpholine (3.67 mL, 42 mmol) following **Method-H**. Compound (**191**) was obtained as a light yellow solid (3.29 g, 55 %), m.p. 154-156 (lit.[108] 152-154 °C).

Analytical data

TLC : R_f 0.57 (*n*-Hexane-Ethyl acetate 15:5);

IR (KBr, cm^{-1}) : 3111, 1583, 1057 and 848.

5.1.2.2.7.1d. 1-Methyl-4-(4-nitrophenyl)piperazine (192)

The title compound (**192**) was prepared from 1-fluoro-4-nitrobenzene (**187**) (3.0 mL, 29 mmol) and *N*-methylpiperazine (4.7 mL, 42 mmol) following **Method-H**. Compound (**192**) was obtained as a light yellow solid (3.69 g, 59 %), m.p. 101-103 (lit.[109] 102-104 °C).

Analytical data

TLC : R_f 0.44 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 2937, 2888, 1594, 1328 and 851.

5.1.2.2.7.1e. 1-(4-Nitrophenyl)pyrrolidin-2-one (193)

The title compound (**193**) was prepared from 1-bromo-4-nitrobenzene (**188**) (2.0 g, 10 mmol) and 2-pyrrolidinone (0.90 mL, 12 mmol) following **Method-I**. Compound (**193**) was obtained as a yellow solid (1.22 g, 60 %), m.p. 128-130 °C (lit.[110] 129-131 °C).

Analytical data

TLC : R_f 0.40 (*n*-Hexane-Ethyl acetate 10:10);

IR (KBr, cm^{-1}) : 3119, 2893, 1703, 1596, 1388 and 849.

5.1.2.2.7.1f. 1-(4-Nitrophenyl)piperidin-2-one (194)

The title compound (**194**) was prepared from 1-bromo-4-nitrobenzene (**188**) (2.0 g, 10 mmol) and δ-valerolactam (1.11 mL, 12 mmol) following **Method-I**. Compound (**194**) was obtained as a yellow solid (1.35 g, 62 %), m.p. 97-99 °C (lit.[80] 96-98 °C).

Analytical data

TLC : R_f 0.53 (*n*-Hexane-Ethyl acetate 10:10);

IR (KBr, cm^{-1}) : 3099, 2954, 1657, 1591 1344 and 858.

5.1.2.2.7.1g. 1-(4-Nitrophenyl)azepan-2-one (195)

The title compound (**196**) was prepared from 1-bromo-4-nitrobenzene (**188**) (2.0 g, 10 mmol) and caprolactam (1.36 g, 12 mmol) following **Method-I**. Compound (**196**) was obtained as a yellow solid (1.44 g, 62 %), m.p. 157-159 °C.

Analytical data

TLC : R_f 0.76 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 2932, 1659, 1522, 1346 and 862.

5.1.2.2.7.1h. 1-(4-Nitrophenyl)pyridin-2(1*H*)-one (196)

The title compound (**195**) was prepared from 1-bromo-4-nitrobenzene (**188**) (2.0 g, 10 mmol) and 2-hydroxypyridine (1.14 g, 12 mmol) following **Method-I**. Compound (**195**) was obtained as a yellow solid (1.58 g, 61 %), m.p. 187-189 °C (lit.[111] 188-190 °C).

Analytical data

TLC : R_f 0.57 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3099, 2954, 1657, 1591 1344 and 858.

5.1.2.2.7.2. General procedure for the synthesis of compounds (197-204, 208)

Method J: In a solution of previously synthesized nitro compound (**189-196**, **207**) (1.0 g, 1 equiv.) in ethanol (20 mL), catalytic amount of Pd/C was added. The reaction mixture was refluxed for 10 mins. Hydrazine hydrate (8 equiv.) was added dropwise to it and the reaction mixture refluxed for next 3-4 h. Progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was filtered through a pad of Celite, washed with chloroform and the filtrate was concentrated under reduced pressure. The residue so obtained was dissolved in chloroform (50 mL) and the organic phase was washed with water and brine. The collected organic phase was dried

over anhydrous magnesium sulphate, filtered and concentrated under reduced pressure to obtain a crude product which was purified by recrystallization.

5.1.2.2.7.2a. 4-(Pyrrolidin-1-yl)benzenamine (197)

The title compound (**197**) was synthesized from 1-(4-nitrophenyl)pyrrolidine(**189**) (1.0 g, 5.20 mmol) as described in **Method J**. Compound (**197**) was obtained as brown semisolid (0.51 g, 61 %).

Analytical data

TLC : R_f 0.44 (*n*-Hexane-Ethyl acetate 12:8);

IR (KBr, cm^{-1}) : 3423, 3320, 2926,1517 and 1266.

5.1.2.2.7.2b. 4-(Piperidin-1-yl)benzenamine (198)

The title compound (**198**) was synthesized from 1-(4-nitrophenyl) piperidine(**190**) (1.0 g, 5.32 mmol) as described in **Method J**. Compound (**198**) was obtained as a brown semisolid (0.59 g, 63 %).

Analytical data

TLC : R_f 0.40 (*n*-Hexane-Ethyl acetate 12:8);

IR (KBr, cm^{-1}) : 3430, 3350, 2931,1512 and 823.

5.1.2.2.7.2c. 4-Morpholinobenzenamine (199)

The title compound (**199**) was synthesized from 4-(4-nitrophenyl) morpholine(**191**) (1.0 g, 4.08 mmol) as described in **Method J**. Compound (**199**) was obtained as white solid (0.58 g, 68 %), m.p. 124-126 °C (lit.[113] 124-126).

Analytical data

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

IR (KBr, cm^{-1}) : 3384, 3326, 3012, 2893, 1517, 1264, 1110 and 832.

5.1.2.2.7.2d. 4-(4-Methylpiperazin-1-yl)benzenamine (200)

The title compound (**200**) was synthesized from 1-methyl-4-(4-nitrophenyl)piperazine(**192**) (1.0 g, 4.52 mmol) as described in **Method J**. Compound (**200**) was obtained as white solid (0.48 g, 55 %), m.p. 85-87 °C (lit.[114] 88-90 °C).

Analytical data

TLC : R_f 0.28 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3358, 3030, 2929, 1512, 1278 and 821.

5.1.2.2.7.2e. 1-(4-Aminophenyl)pyrrolidin-2-one (201)

The title compound (**201**) was synthesized from 1-(4-nitrophenyl) pyrrolidin-2-one (**193**) (1.0 g, 4.54 mmol) as described in **Method J**. Compound (**201**) was obtained as white solid (0.56 g, 66 %), m.p. 122-124 °C.

Analytical data

TLC : R_f 0.43 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3428, 3334, 3214, 2982, 1665, 1511, 1291 and 840.

5.1.2.2.7.2f. 1-(4-Aminophenyl)piperidin-2-one (202)

The title compound (**202**) was synthesized from 1-(4-nitrophenyl) piperidin-2-one (**194**) (1.0 g, 4.54 mmol) as described in **Method J**. Compound (**202**) was obtained as pale yellow solid (0.55 g, 65 %), m.p. 115-117 °C (lit.[115] 118-120 °C).

Analytical data

TLC : R_f 0.47 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3440, 3325, 3212, 2953, 1608, 1516,1298 and 823.

5.1.2.2.7.2g. 1-(4-Aminophenyl)azepan-2-one (203)

The title compound (**204**) was synthesized from 1-(4-nitrophenyl) azepan-2-one (**195**) (1.0 g, 5.32 mmol) as described in **Method J**. Compound (**203**) was obtained as off-white solid (0.56 g, 64 %), m.p. 157-159 °C.

Analytical data

TLC : R_f 0.37 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3408,3329, 3230, 2931, 1626, 1513, 1282 and 826.

5.1.2.2.7.2h. 1-(4-Aminophenyl)pyridin-2(1*H*)-one (204)

The title compound (**203**) was synthesized from 1-(4-nitrophenyl) pyridin-2(1*H*)-one (**196**) (1.0 g, 4.62 mmol) as described in **Method J**. Compound (**204**) was obtained as off-white solid (0.54 g, 63 %), m.p. 125-127 °C (lit.[115] 128-130 °C).

Analytical data

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

IR (KBr, cm^{-1}) : 3462, 3307, 3201, 1654, 1573 and 825.

5.1.2.2.7.2i. 4-Phenylmorpholin-3-one (206)

To a rapidly stirring solution of *2*-anilinoethanol (**205**) (6 mL, 48 mmol) in IPA (20 mL) in 3 neck RBF, 2-chloroacetyl chloride (11.4 mL, 16.27 mmol) and aqueous NaOH solution (10 N, 30 mL, 0.72 mol) were added dropwise simultaneously at 40 °C temperature and the reaction mixture stirred for next 1 h. Then the reaction mixture was shifted to ice bath and stirred for another 1 h. The obtained white solid was filtered and washed with cold water to obtain the titled compound (**206**) (5.27 g, 62 %), m.p. 113-116 °C.

Analytical data

TLC : R_f 0.53 (n-Hexane-Ethyl acetate 16:4);

IR (KBr, cm^{-1}) : 3342, 3283, 1640, 1590, 1401 and 750.

5.1.2.2.7.2j. 4-(4-Nitrophenyl)morpholin-3-one (207)

To a stirring solution of 4-phenylmorpholin-3-one (**206**) (3.0 g, 0.02 mol) in conc. sulphuric acid (6.9 mL, 0.06 mol), conc. nitric acid (9 mL, 0.14 mol) was added dropwise over 30 min at -10 °C to 0 °C and the reaction mixture stirred for 45 min. Progress of the reaction was monitored by TLC. After the completion of the reaction, water was added to the reaction mixture and neutralized with NH_4OH. White precipitates so obtained were filtered, washed with water and dried to obtain the titled compound (**207**) (2.44 g, 65 %), m.p. 147-149 °C (lit.[112] 148-152 °C).

Analytical data

TLC : R_f 0.67 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3064, 1673, 1593, 1000 and 855.

5.1.2.2.7.2k. 4-(4-Aminophenyl)morpholin-3-one (208)

The title compound (**208**) was synthesized from 4-(4-nitrophenyl) morpholin-3-one (**207**) (1.0 g, 4.50 mmol) as described in **Method J**. Compound (**208**) was obtained as a white solid (0.60 g, 70 %), m.p. 127-129 °C (lit.[115] 115-128 °C).

Analytical data

TLC : R_f 0.60 (Chloroform-Methanol 18:2);

IR (KBr, cm^{-1}) : 3459, 3340, 2886, 1639, 1517, 1120 and 829.

5.1.2.2.7.2l. 1-(4-Amino-3-fluorophenyl)pyridin-2(1*H*)-one (210)

The title compound (**210**) was synthesized from 2-fluoro-4-iodoaniline (**209**) (1.0 g, 4.22 mmol) and 2-hydoxypyridine (0.48 g, 5.06 mmol) as described in **Method I**. Compound (**210**) was obtained as a brown solid (0.54 g, 63 %), m.p. 192-194 °C (lit.[115] 196-198 °C).

Analytical data

TLC : R_f 0.30 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3465, 3302, 1654, 1574, 1518, 1325 and 763.

5.1.2.2.7.3. General method for the synthesis of substituted *N*-phenylacetamides (214-226)

Method K: To a stirring solution of substituted aniline (0.30 g, 1 equiv.) and K_2CO_3 (3 equiv.) in dry acetone, 2-bromoacetyl bromide or 2-chloroacetyl chloride (1.2 equiv.) was added drop-wise maintaining the temperature at 0-10 °C. The reaction mixture was stirred at room temperature for 2-3 h. After completion of the reaction, the reaction mixture was concentrated under reduced pressure. The concentrated reaction mixture was poured into crushed ice (100 mL). The solid so precipitated was filtered, washed with sodium bicarbonate solution (10 %) and water, and dried to obtain the crude product.

5.1.2.2.7.3a. 2-Bromo-*N*-(4-chlorophenyl)acetamide (214)

The title compound (**214**) was synthesized as per **Method K** using 4-chloroaniline (**197**) (0.30 g, 2.35 mmol) and 2-bromoacetyl bromide (0.23 mL, 2.82 mmol). Compound (**214**) was obtained as white solid (0.38 g, 66 %), m.p. 150-152 °C (lit.[116] m.p. 153-155 °C).

Analytical data

TLC : R_f 0.48 (*n*-Hexane-Ethyl acetate 12:8);

IR (KBr, cm^{-1}) : 3291, 3068, 2956, 1660, 1546, 1248 and 830.

5.1.2.2.7.3b. 2-Bromo-*N*-(4-fluorophenyl)acetamide (215)

The title compound (**215**) was synthesized as per **Method K** using 4-fluoroaniline (**198**) (0.30 g, 2.69 mmol) and 2-bromoacetyl bromide (0.27 mL, 3.22 mmol). Compound (**215**) was obtained as white solid (0.39 g, 62 %), m.p. 138-140 °C (lit.[116] m.p. 137-139 °C).

Analytical data

TLC : R_f 0.40 (*n*-Hexane-Ethyl acetate 12:8);

IR (KBr, cm^{-1}) : 3273, 3102, 2836, 1653, 1506, 1210 and 837.

5.1.2.2.7.3c. 2-Bromo-*N*-(4-methoxyphenyl)acetamide (216)

The title compound (**216**) was synthesized as per **Method K** using 4-methoxyaniline (**199**) (0.30 g, 2.43 mmol) and 2-bromoacetyl bromide (0.24 mL, 2.92 mmol). Compound (**216**) was obtained as white solid (0.38 g, 65 %), m.p. 127-129 °C (lit.[116] m.p. 128-130 °C).

Analytical data

TLC : R_f 0.35 (*n*-Hexane-Ethyl acetate 12:8);

IR (KBr, cm^{-1}) : 3297, 3024, 2972, 1652, 1554, 1485 and 798.

5.1.2.2.7.3d. 2-Chloro-*N*-(4-(pyrrolidin-1-yl)phenyl)acetamide (217)

The title compound (**217**) was synthesized as per **Method K** using 4-(pyrrolidin-1-yl)aniline (**200**) (0.30 g, 1.85 mmol) and 2-chloroacetyl chloride (0.18 mL, 2.22 mmol). Compound (**217**) was obtained as white solid (0.27 g, 62 %), m.p. 196-198 °C.

Analytical data

TLC : R_f 0.51 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3329, 2956, 1662, 1519, 1326 and 827.

5.1.2.2.7.3e. 2-Chloro-*N*-(4-(piperidin-1-yl)phenyl)acetamide (218)

The title compound (**218**) was synthesized as per **Method K** using 4-(piperidin-1-yl)aniline (**201**) (0.30 g, 1.70 mmol) and 2-chloroacetyl chloride

(0.16 mL, 2.04 mmol). Compound (**218**) was obtained as white solid (0.30 g, 62 %), m.p. 210-212 °C.

Analytical data

TLC : R_f 0.52 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3185, 3058, 2936, 1663, 1572 and 804.

5.1.2.2.7.3f. 2-Chloro-*N*-(4-morpholinophenyl)acetamide (219)

The title compound (**219**) was synthesized as per **Method K** using 4-morpholinoaniline (**202**) (0.3 g, 1.68 mmol) and 2-chloroacetyl chloride (0.16 mL, 2.04 mmol). Compound (**219**) was obtained as white solid (0.26 g, 62 %), m.p. 167-169 °C (lit.[117] m.p. 170-172 °C).

Analytical data

TLC : R_f 0.50 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3317, 2963, 2818, 1658, 1521, 1119 and 820.

5.1.2.2.7.3g. 2-Chloro-*N*-(4-(4-methylpiperazin-1-yl)phenyl)acetamide (220)

The title compound (**220**) was synthesized as per **Method K** using 4-(4-methylpiperazin-1-yl)aniline (**203**) (0.3 g, 1.57 mmol) and 2-chloroacetyl chloride (0.15 mL, 1.88 mmol). Compound (**220**) was obtained as white solid (0.26 g, 64 %), m.p. 249-251 °C.

Analytical data

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

IR (KBr, cm^{-1}) : 3265, 3038, 2843, 1675, 1515, 1251 and 922;

MS (m/z) : 268.0 $(M)^+$, 270.0 $(M+2)^+$.

5.1.2.2.7.3h. 2-Chloro-*N*-(4-(3-oxopyrrolidin-1-yl)phenyl)acetamide (221)

The title compound (**221**) was synthesized as per **Method K** using 1-(4-aminophenyl)pyrrolidin-2-one (**204**) (0.3 g, 1.7 mmol) and 2-chloroacetyl chloride (0.16 mL, 2.04 mmol). Compound (**221**) was obtained as white solid (0.26 g, 61 %), m.p. 203-205 °C.

Analytical data

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

IR (KBr, cm^{-1}) : 3315, 3194, 2960, 1707, 1669, 1328 and 846;

MS (m/z) : 253.48 $(M)^+$, 255.12 $(M+2)^+$.

5.1.2.2.7.3i. 2-Chloro-*N*-(4-(2-oxopiperidin-1-yl)phenyl)acetamide (222)

The title compound (**222**) was synthesized as per **Method K** using 1-(4-aminophenyl)piperidin-2-one (**205**) (0.3 g, 1.57 mmol) and 2-chloroacetyl chloride (0.15 mL, 1.88 mmol). Compound (**222**) was obtained as white solid (0.27 g, 63 %), m.p. 207-209 °C.

Analytical data

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

IR (KBr, cm^{-1}) : 3258, 3123, 3011, 1699, 1610, 1334 and 839;

MS (m/z) : 267.53 $(M)^+$, 269.17 $(M+2)^+$.

5.1.2.2.7.3j. 2-Chloro-*N*-(4-(2-oxoazepan-1-yl)phenyl)acetamide (223)

The title compound (**223**) was synthesized as per **Method K** using 1-(4-aminophenyl)azepan-2-one (**206**) (0.3 g, 1.47 mmol) and 2-chloroacetyl chloride (0.14 mL, 1.76 mmol). Compound (**223**) was obtained as white solid (0.27 g, 65 %), m.p. 165-167 °C.

Analytical data

TLC : R_f 0.50 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3267, 3125, 2930, 1692, 1629, 1327 and 838;

MS (m/z) : 280.84 $(M)^+$, 283.19 $(M+2)^+$.

5.1.2.2.7.3k. 2-Chloro-*N*-(4-(2-oxopyridin-1(2*H*)-yl)phenyl)acetamide (224)

The title compound (**224**) was synthesized as per **Method K** using 1-(4-aminophenyl)pyridin-2(1*H*)-one (**207**) (0.3 g, 1.73 mmol) and 2-chloro acetyl chloride (0.17 mL, 2.07 mmol). Compound (**224**) was obtained as white solid (0.27 g, 60 %), m.p. >250 °C.

Analytical data

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

IR (KBr, cm^{-1}) : 3275, 3129, 3084, 1704, 1660, 1332 and 839;

MS (m/z) : 263.47 $(M)^+$, 265.10 $(M+2)^+$.

5.1.2.2.7.3l. 2-Chloro-*N*-(4-(3-oxomorpholino)phenyl)acetamide (225)

The title compound (**225**) was synthesized as per **Method K** using 4-(4-aminophenyl)morpholin-3-one (**208**) (0.3 g, 1.56 mmol) and 2-chloroacetyl chloride (0.15 mL, 1.87 mmol). Compound (**225**) was obtained as white solid (0.26 g, 62 %), m.p. 216-218 °C.

Analytical data

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

IR (KBr, cm^{-1}) : 3274, 3134, 2984, 1712, 1635, 1006 and 846;

MS (m/z) : 269.48 $(M)^+$, 271.13 $(M+2)^+$.

5.1.2.2.7.3m. 2-Chloro-*N*-(2-fluoro-4-(2-oxopyridin-1(2*H*)-yl)phenyl) acetamide (226)

The title compound (**226**) was synthesized as per **Method K** using 1-(4-amino-3-fluorophenyl)pyridin-2(1*H*)-one (**209**) (0.3 g, 1.47 mmol) and 2-chloroacetyl chloride (0.14 mL, 1.76 mmol). Compound (**226**) was obtained as white solid (0.26 g, 64 %), m.p. > 250 °C.

Analytical data

TLC : R_f 0.57 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3265, 3020, 1711, 1658, 1535, 1427 and 769;

MS (m/z) : 281.2 $(M)^+$, 283.2 $(M+2)^+$.

5.1.2.2.8. 2-((5-((4-chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)thio)-*N*-phenylacetamides (227-239)

5.1.2.2.8a. *N*-(4-Chlorophenyl)-2-((5-((4-chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)thio)acetamide (227)

To a solution of 5-((4-chlorophenyl)amino)-1,3,4-thiadiazole-2-thiol (**145**) (0.5 g, 2.05 mmol) in DMF (6 mL), potassium carbonate (0.85 g, 6.15 mmol) and 2-bromo-*N*-(4-chlorophenyl)acetamide (**214**) (0.51 g, 2.05 mmol) were added. The reaction mixture was stirred at room temperature for 3 to 4 hrs and progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was poured into ice-cold water with continuous stirring. The precipitate so obtained was filtered and dried. The obtained crude product was purified by column chromatography using silica gel as stationary

phase and chloroform-methanol as an eluent to afford the titled compound (**227**) as off-white solid (0.50 g, 60 %), m.p. 203-205 °C.

Analytical data

TLC	: R_f 0.46 (Chloroform-Methanol 19:1);
IR (KBr, cm^{-1})	: 3299, 3046, 2826, 1671, 1528, 1494 and 822;
^{1}H-NMR (DMSO-d_6)	: δ 10.49 (bs, 1H, -N*H*), 10.43 (bs, 1H, -N*H*), 7.61 (d, *J* = 8.0 Hz, 2H, Ar*H*), 7.59 (d, *J* = 8.0 Hz, 2H, Ar*H*), 7.38 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.37 (d, *J* = 8.8 Hz, 2H, Ar*H*) 4.12 (s, 2H, -SCH_2);
MS (m/z)	: 411.43 $(M)^+$, 413.41 $(M+2)^+$.
RP-HPLC	Purity: 96.5 %, t_R = 4.47 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60)

5.1.2.2.8b. 2-((5-((4-Chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(4-fluorophenyl) acetamide (228)

The title compound (**228**) was prepared as per the method described for compound (**227**) using 5-((4-chlorophenyl)amino)-1,3,4-thiadiazole-2-thiol (**145**) (0.5 g, 2.05 mmol) and 2-bromo-*N*-(4-fluorophenyl)acetamide (**215**) (0.48 g, 2.05 mmol). Compound (**228**) was obtained as off-white solid (0.53 g, 65 %), m.p. 201-203 °C.

Analytical data

TLC	: R_f 0.49 (Chloroform-Methanol 19:1);
IR (KBr, cm^{-1})	: 3310, 3055, 2829, 1668, 1416, 1215 and 829;
^{1}H-NMR (DMSO-d_6)	: δ 10.36 (bs, 1H, -N*H*), 7.58-7.60 (m, 4H, Ar*H*), 7.36 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.13-7.18 (m, 2H, Ar*H*), 4.10 (s, 2H, -SCH_2);
MS (m/z)	: 395.45 $(M)^+$, 397.45 $(M+2)^+$;
RP-HPLC	Purity: 99.1 %, t_R = 3.80 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60)

5.1.2.2.8c. 2-((5-((4-Chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(4-methoxy-phenyl)acetamide (229)

The title compound (**229**) was prepared as per the method described for compound (**227**) using 5-((4-chlorophenyl)amino)-1,3,4-thiadiazole-2-thiol (**145**) (0.5 g, 2.05 mmol) and 2-bromo-*N*-(4-methoxyphenyl)acetamide (**216**) (0.5 g, 2.05 mmol). Compound (**229**) was obtained as off-white solid (0.53 g, 63 %), m.p. 241-243 °C.

Analytical data

TLC : R_f 0.46 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3324, 3018, 2837, 1601, 1409, 1248 and 826;

^{1}H-NMR (DMSO-d_6) : δ 10.50 (bs, 1H, -N*H*), 10.15 (bs, 1H, -N*H*), 7.59 (d, *J* = 8.4 Hz, 2H, Ar*H*), 7.48 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.37 (d, *J* = 8.4 Hz, 2H, Ar*H*), 6.89 (d, *J* = 8.8 Hz, 2H, Ar*H*), 4.08 (s, 2H, -SCH_2) and 3.71 (s, 3H, -OCH_3);

MS (m/z) : 407.52 $(M)^+$, 409.52 $(M+2)^+$;

RP-HPLC Purity: 98.4 %, t_R = 3.62 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60)

5.1.2.2.8d. 2-((5-((4-Chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(4-(pyrrolidin-1-yl)phenyl)acetamide (230)

The title compound (**230**) was prepared as per the method described for compound (**227**) using 5-((4-chlorophenyl)amino)-1,3,4-thiadiazole-2-thiol (**145**) (0.5 g, 2.05 mmol) and 2-chloro-*N*-(4-(pyrrolidin-1-yl)phenyl)acetamide (**217**) (0.60 mg, 2.05 mmol). Compound (**230**) was obtained as off-white solid (0.52 g, 57 %), m.p. 226-228 °C.

Analytical data

TLC : R_f 0.50 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3312, 3056, 2839, 1650, 1543, 1403 and 817;

^{1}H-NMR (DMSO-d_6) : δ 9.94 (bs, 1H, N*H*), 7.59 (d, *J* = 8 Hz, 2H, Ar*H*), 7.38 (d, *J* = 8 Hz, 2H, Ar*H*), 7.37 (d, *J* = 8 Hz, 2H, Ar*H*), 6.48 (d, *J* = 8 Hz, 2H,

Ar*H*), 4.02 (s, 2H, SCH_2), 3.16-3.19 (m, 5H, NCH_2 and N*H*) and 1.93 (t, 4H, CH_2);

MS (m/z) : 446.0 $(M)^+$, 448.0 $(M+2)^+$;

RP-HPLC Purity: 98.8 %, t_R = 4.86 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60)

5.1.2.2.8e. 2-((5-((4-Chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(4-(piperidn-1-yl)phenyl)acetamide (231)

The title compound (**231**) was prepared as per the method described for compound (**227**) using 5-((4-chlorophenyl)amino)-1,3,4-thiadiazole-2-thiol (**145**) (0.5 g, 2.05 mmol) and 2-chloro-*N*-(4-(piperidn-1-yl)phenyl)acetamide (**218**) (0.52 g, 2.05 mmol). Compound (**231**) was obtained as off-white solid (0.56 g, 59 %), m.p. 229-231 °C.

Analytical data

TLC : R_f 0.52 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3313, 3056, 2854, 1651, 1541, 1402 and 817;

^{1}H-NMR (DMSO-d_6) : δ 10.10 (bs, 1H, N*H*), 7.59 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.40 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.38 (d, *J* = 8.8 Hz, 2H, Ar*H*), 6.87 (d, *J* = 8.8 Hz, 2H, Ar*H*), 4.06 (s, 2H, SCH_2), 3.03-3.05 (m, 4H, NCH_2), 1.49-1.59 (m, 6H, CH_2);

MS (m/z) : 460.69 $(M)^+$, 462.68 $(M+2)^+$;

RP-HPLC : Purity: 97.1 %, t_R = 4.99 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60)

5.1.2.2.8f. 2-((5-((4-Chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(4-morpholi-nophenyl)acetamide (232)

The title compound (**232**) was prepared as per the method described for compound (**227**) using 5-((4-chlorophenyl)amino)-1,3,4-thiadiazole-2-thiol (**145**) (0.5 g, 2.05 mmol) and 2-chloro-*N*-(4-morpholinophenyl)acetamide

(**219**) (0.52 g, 2.05 mmol). Compound (**232**) was obtained as off-white solid (0.57 g, 60 %), m.p. 238-240 °C.

Analytical data

TLC : R_f 0.43 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3319, 3053, 1649, 1510, 1406 and 819;

^{1}H-NMR ($CDCl_3$) : δ 10.52 (bs, 1H, N*H*), 10.11 (bs, 1H, N*H*), 7.60 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.45 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.38 (d, *J* = 8.8 Hz, 2H, Ar*H*), 6.90 (d, *J* = 8.8 Hz, 2H, Ar*H*), 4.08 (s, 2H, SCH_2), 3.72-3.74 (m, 4H, OCH_2), 3.03-3.05 (m, 4H, NCH_2);

MS (m/z) : 462.64 $(M)^+$, 464.61 $(M+2)^+$;

RP-HPLC : Purity: 96.6 %, t_R = 3.50 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60)

5.1.2.2.8g. 2-((5-((4-Chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(4-(4-methylpiperazin-1-yl)phenyl)acetamide (233)

The title compound (**233**) was prepared as per the method described for compound (**227**) using 5-((4-chlorophenyl)amino)-1,3,4-thiadiazole-2-thiol (**145**) (0.5 g, 2.05 mmol) and 2-chloro-*N*-(4-(4-methylpiperazin-1-yl)phenyl) acetamide (**220**) (0.55 g, 2.05 mmol). Compound (**233**) was obtained as off-white solid (0.63 g, 67 %), m.p. 240-242 °C.

Analytical data

TLC : R_f 0.44 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3314, 3184, 3059, 2936, 1652, 1620, 1402 and 816;

^{1}H-NMR (DMSO-d_6) : δ 10.52 (bs, 1H, N*H*), 10.08 (bs, 1H, N*H*), 7.61 (d, *J* = 8 Hz, 2H, Ar*H*), 7.44 (d, *J* = 8 Hz, 2H, Ar*H*), 7.39 (d, *J* = 8 Hz, 2H, Ar*H*), 6.90 (d, *J* = 8 Hz, 2H, Ar*H*), 4.08 (s, 2H, SCH_2), 3.08 (t, 2H, NCH_2), 2.45 (t, 2H, CH_2),

2.23 (s, 3H, C*H*$_3$);

MS (m/z) : 475.00 $(M)^+$, 477.00 $(M+2)^+$;

RP-HPLC : Purity: 99.2 %, t_R = 3.56 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60)

5.1.2.2.8h. 2-((5-((4-Chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(4-(2-oxo-pyrrolidin-1-yl)phenyl)acetamide (234)

The title compound (**234**) was prepared as per the method described for compound (**227**) using 5-((4-chlorophenyl)amino)-1,3,4-thiadiazole-2-thiol (**145**) (0.5 g, 2.05 mmol) and 2-chloro-*N*-(4-(2-oxopyrrolidin-1-yl)phenyl) acetamide (**221**) (0.52 g, 2.05 mmol). Compound (**234**) was obtained as off-white solid (0.68 g, 72 %), m.p. >250 °C.

Analytical data

TLC : R_f 0.41 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3296, 3188, 3071, 1654, 1605, 1405 and 827;

^{1}H-NMR (DMSO-d_6) : δ 10.52 (bs, 1H, -N*H*), 10.32 (bs, 1H, -N*H*), 7.56-7.62 (m, 6H, Ar*H*), 7.38 (d, *J* = 8.8 Hz, 2H, Ar*H*), 4.12 (s, 2H, -SC*H*$_2$), 3.80 (t, 2H, -NC*H*$_2$), 2.47 (t, 2H, -COC*H*$_2$) and 2.02-2.08 (m, 2H, -C*H*$_2$);

^{13}C-NMR (DMSO-d_6) : δ 173.99, 165.84, 165.21, 153.26, 139.72, 135.83, 135.18, 129.39, 125.87, 120.29, 119.81, 119.39, 48.56, 38.84, 32.67 and 17.84;

MS (m/z) : 458.48 $(M)^+$, 460.21 $(M+2)^+$;

RP-HPLC : Purity: 98.9 %, t_R = 3.26 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60)

5.1.2.2.8i. 2-((5-((4-Chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(4-(2-oxopiperidin-1-yl)phenyl)acetamide (235)

The title compound (**235**) was prepared as per the method described for compound (**227**) using 5-((4-chlorophenyl)amino)-1,3,4-thiadiazole-2-thiol

(**145**) (0.5 g, 2.05 mmol) and 2-chloro-*N*-(4-(2-oxopiperidin-1-yl)phenyl) acetamide (**222**) (0.55 g, 2.05 mmol). Compound (**235**) was obtained as off-white solid (0.75 g, 77 %), m.p. >250 °C.

Analytical data

TLC : R_f 0.57 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3272, 3123, 3059, 2868, 1683, 1620, 1408 and 831;

^{1}H-NMR (DMSO-d_6) : δ 10.53 (bs, 1H, N*H*), 10.37 (bs, 1H, N*H*), 7.60 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.57 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.38 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.21 (d, *J* = 8.8 Hz, 2H, Ar*H*), 4.12 (s, 2H, SCH_2), 3.56 (t, 2H, NCH_2), 2.37 (t, 2H, COCH_2), 1.83-1.84 (m, 4H, CH_2);

^{13}C-NMR (DMSO-d_6) : δ 168.68, 165.44, 164.66, 152.61, 139.11, 139.03, 138.45, 128.82, 126.52, 125.30, 119.30, 118.82, 50.79, 32.45, 22.90, 20.81.

MS (m/z) : 474.22 $(M)^+$, 476.22 $(M+2)^+$

RP-HPLC : Purity: 98.4 %, t_R = 3.35 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60)

5.1.2.2.8j. 2-((5-((4-Chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(4-(2-oxoazepan-1-yl)phenyl)acetamide (236)

The title compound (**236**) was prepared as per the method described for compound (**227**) using 5-((4-chlorophenyl)amino)-1,3,4-thiadiazole-2-thiol (**145**) (0.5 g, 2.05 mmol) and 2-chloro-*N*-(4-(2-oxoazepan-1-yl)phenyl) acetamide (**223**) (0.58 g, 2.05 mmol). Compound (**236**) was obtained as off-white solid (0.69 g, 69 %), m.p. 227-229 °C.

Analytical data

TLC : R_f 0.42 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3275, 3123, 2949, 1686, 1620, 1603, 1516, 1406 and 827;

^{1}H-NMR (DMSO-d_6) : δ 10.50 (bs, 1H, -N*H*), 10.33 (bs, 1H, -N*H*), 7.59 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.57 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.37 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.14 (d, *J* = 8.8 Hz, 2H, Ar*H*), 4.11 (s, 2H, -SCH_2), 3.68-3.69 (m, 2H, -NCH_2), 2.56-2.59 (m, 2H, -COCH_2) and 1.69-1.72 (m, 6H, -CH_2);

MS (m/z) : 488.32 $(M)^+$, 490.31 $(M+2)^+$;

RP-HPLC : Purity: 95.9 %, t_R = 4.04 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60)

5.1.2.2.8k. 2-((5-((4-Chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(4-(2-oxopyridin-1(2*H*)-yl)phenyl)acetamide (237)

The title compound (**237**) was prepared as per the method described for compound (**227**) using 5-((4-chlorophenyl)amino)-1,3,4-thiadiazole-2-thiol (**145**) (0.5 g, 2.05 mmol) and 2-chloro-*N*-(4-(2-oxopyridin-1(2*H*)-yl)phenyl) acetamide (**224**) (0.54 g, 2.05 mmol). Compound (**237**) was obtained as off-white solid (0.67 g, 70 %), m.p. >250 °C.

Analytical data

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

IR (KBr, cm^{-1}) : 3318, 1659, 1530, 1620, 1409 and 832;

^{1}H-NMR (DMSO-d_6) : δ 10.51 (bs, 1H, N*H*), 10.50 (bs, 1H, N*H*), 7.68-7.70 (m, 2H, Ar*H*), 7.59-7.61 (m, 3H, Ar*H*), 7.47-7.51 (m, 1H, Ar*H*), 7.34-7.39 (m, 4H, Ar*H*), 6.45-6.47 (m, 1H, Ar*H*), 6.28-6.31 (m, 1H, Ar*H*), 4.15 (s, 2H, SCH_2)

^{13}C-NMR (DMSO-d_6) δ 165.74, 164.66, 161.14, 152.54, 140.37, 139.09, 138.98, 138.31, 135.98, 128.80, 127.08, 125.29, 120.33, 119.30, 118.81, 105.39 and 38.05;

MS (m/z) : 470.0 $(M)^+$, 472.0 $(M+2)^+$;

RP-HPLC : Purity: 99.1 %, t_R = 3.20 min (Mobile phase:

Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60)

5.1.2.2.8l. 2-((5-((4-Chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(4-(3-oxomorpholino)phenyl)acetamide (238)

The title compound (**238**) was prepared as per the method described for compound (**227**) using 5-((4-chlorophenyl)amino)-1,3,4-thiadiazole-2-thiol (**145**) (0.5 g, 2.05 mmol) and 2-chloro-*N*-(4-(3-oxo-morpholino)phenyl) acetamide (**225**) (0.55 g, 2.05 mmol). Compound (**238**) was obtained as off-white solid (0.75 g, 77 %), m.p. 224-226 °C.

Analytical data

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

IR (KBr, cm^{-1}) : 3318, 1659, 1530, 1620, 1409 and 832;

^{1}H-NMR (DMSO-d_6) : δ 10.53 (bs, 1H, N*H*), 10.41 (bs, 1H, N*H*), 7.60 (d, *J* = 8 Hz, 2H, Ar*H*), 7.59 (d, *J* = 8 Hz, 2H, Ar*H*), 7.37 (d, *J* = 8 Hz, 2H, Ar*H*), 7.33 (d, *J* = 8 Hz, 2H, Ar*H*), 4.18 (s, 2H, COCH_2), 4.12 (s, 2H, SCH_2), 3.95 (t, 2H, NCH_2), 3.69 (t, 2H, OCH_2);

MS (m/z) : 476.69 $(M)^+$, 478.68 $(M+2)^+$;

RP-HPLC : Purity: 97.4 %, t_R = 3.04 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60)

5.1.2.2.8m. 2-((5-((4-Chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(2-fluoro-4-(2-oxopyridin-1(2*H*)-yl)phenyl)acetamide (239)

The title compound (**239**) was prepared as per the method described for compound (**227**) using 5-((4-chlorophenyl)amino)-1,3,4-thiadiazole-2-thiol (**145**) (0.5 g, 2.05 mmol) and 2-chloro-*N*-(2-fluoro-4-(2-oxopyridin-1(2*H*)-yl) phenyl)acetamide (**226**) (0.58 g, 2.05 mmol). Compound (**239**) was obtained as off-white solid (0.72 g, 70 %), m.p. >250 °C.

Analytical data

TLC : R_f 0.56 (Chloroform-Methanol 18:2);

IR (KBr, cm^{-1})	: 3269, 3069, 1662, 1533, 1620, 1400 and 822;
^{1}H-NMR (DMSO-d_6)	: δ 10.49 (bs, 1H, N*H*), 10.33 (bs, 1H, N*H*), 8.04-8.08 (m, 1H, Ar*H*), 7.60-7.67 (m, 3H, Ar*H*), 7.50-7.54 (m, 2H, Ar*H*), 7.38-7.41 (m, 2H, Ar*H*), 7.24-7.26 (m, 1H, Ar*H*), 6.48-6.51 (m, 1H, Ar*H*), 6.31-6.35 (m, 1H, Ar*H*), 4.15 (s, 2H, SCH_2);
^{13}C-NMR (DMSO-d_6)	δ 166.39, 164.78, 160.97, 153.50, 152.49, 151.54, 140.61, 139.15, 138.77, 137.07, 136.98, 128.83, 125.70, 125.61, 125.32, 123.34, 122.80, 122.78, 120.42, 118.85, 114.75, 114.57, 105.58 and 37.93.
MS (m/z)	: 488.0 $(M)^+$, 490.0 $(M+2)^+$;
RP-HPLC	: Purity: 99.5 %, t_R = 3.10 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60)

5.1.2.2.9. Synthesis of 2-((5-(substituted phenylamino or benzylamino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(4-(2-oxopyridin-1(2*H*)-yl)phenyl)acetamides (240-253)

Method L: To a solution of 5-(substituted phenyl/benzylamino)-1,3,4-thiadiazole-2-thiol (0.5 g, 1 equiv.) in DMF (6 mL), potassium carbonate (3 equiv.) and 2-chloro-*N*-(4-(2-oxopyridin-1(2*H*)-yl)phenyl)acetamide (1 equiv.) were added. The reaction mixture was stirred at room temperature for 3 to 4 h and progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was poured into ice-cold water with continuous stirring. The precipitates so obtained was filtered and dried. The obtained crude product was purified by column chromatography using silica gel as stationary phase and chloroform-methanol as an eluent to afford off-white solid.

5.1.2.2.9a. 2-(5-(Phenylamino)-1,3,4-thiadiazol-2-ylthio)-*N*-(4-(2-oxo pyridin-1(2*H*)-yl)phenyl)acetamide (240)

The title compound (**240**) was prepared as per the method described for compound (**227**) using 5-(phenylamino)-1,3,4-thiadiazole-2-thiol (**144**) (0.5 g, 2.39 mmol) and 2-chloro-*N*-(4-(2-oxopyridin-1(2*H*)-yl)phenyl) acetamide (**224**) (0.54 g, 2.39 mmol). Compound (**240**) was obtained as off-white solid (0.77 g, 74 %), m.p. >250 °C.

Analytical data

TLC : R_f 0.63 (Chloroform-Methanol 18:2);

IR (KBr, cm^{-1}) : 3315, 3061, 1660, 1532, 1411 and 835;

^{1}H-NMR ($CDCl_3$) : δ 10.48 (bs, 1H, N*H*), 10.36 (s, 1H, N*H*), 7.67-7.69 (m, 2H, Ar*H*), 7.58-7.60 (m, 1H, Ar*H*), 7.53-7.56 (m, 2H, Ar*H*), 7.46-7.50 (m, 1H, Ar*H*), 7.30-7.36 (m, 4H, Ar*H*), 6.96-7.00 (m, 1H, Ar*H*), 6.45 (d, 1H, Ar*H*), 6.26-6.30 (m, 1H, Ar*H*), 4.13 (s, 2H, SCH_2);

MS (m/z) : 436.0 $(M)^+$;

RP-HPLC : Purity: 99.1 %, t_R = 2.83 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60)

5.1.2.2.9b. *N*-(4-(2-Oxopyridin-1(2*H*)-yl)phenyl)-2-((5-(*p*-tolylamino)-1,3,4-thiadiazol-2-yl)thio)acetamide (241)

The title compound (**241**) was prepared as per the method described for compound (**227**) using 5-(*p*-tolylamino)-1,3,4-thiadiazole-2-thiol (**142**) (0.5 g, 2.20 mmol) and 2-chloro-*N*-(4-(2-oxopyridin-1(2*H*)-yl)phenyl) acetamide (**224**) (0.59 g, 2.20 mmol). Compound (**241**) was obtained as off-white solid (0.72 g, 72 %), m.p. 240-242 °C.

Analytical data

TLC : R_f 0.52 (Chloroform-Methanol 18:2);

IR (KBr, cm^{-1}) : 3328, 3032, 2855, 1662, 1536, 1409 and 833;

^{1}H-NMR ($CDCl_3$) : δ 10.52 (bs, 1H, N*H*), 10.28 (bs, 1H, N*H*),

7.70-7.72 (m, 2H, Ar*H*), 7.61-7.63 (m, 1H, Ar*H*), 7.44-7.51 (m, 3H, Ar*H*), 7.36-7.38 (m, 2H, Ar*H*), 7.14-7.16 (m, 2H, Ar*H*), 6.47-6.49 (m, 1H, Ar*H*), 6.29-6.33 (m, 1H, Ar*H*), 4.14 (s, 2H, SCH_2), 2.26 (s, 3H, CH_3);

MS (m/z) : 450.36 $(M)^+$;

RP-HPLC : Purity: 99.7 %, t_R = 3.04 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60)

5.1.2.2.9c. 2-(5-(4-Methoxyphenylamino)-1,3,4-thiadiazol-2-ylthio)-*N*-(4-(2-oxopyridin-1(2*H*)-yl)phenyl)acetamide (242)

The title compound (**242**) was prepared as per the method described for compound (**227**) using 5-(4-methoxyphenylamino)-1,3,4-thiadiazole-2-thiol (**143**) (0.5 g, 2.09 mmol) and 2-chloro-*N*-(4-(2-oxopyridin-1(2*H*)-yl) phenyl)acetamide (**224**) (0.55 g, 2.09 mmol). Compound (**242**) was obtained as off-white solid (0.69 g, 71 %), m.p. 228-230 °C.

Analytical data

TLC : R_f 0.61 (Chloroform-Methanol 18:2);

IR (KBr, cm^{-1}) : 3332, 2946, 1662, 1540, 1411 and 830;

^{1}H-NMR ($CDCl_3$) : δ 10.49 (bs, 1H, N*H*), 10.19 (bs, 1H, N*H*), 7.69-7.72 (m, 2H, Ar*H*), 7.61-7.63 (m, 1H, Ar*H*), 7.46-7.53 (m, 3H, Ar*H*), 7.35-7.39 (m, 2H, Ar*H*), 6.91-6.95 (m, 2H, Ar*H*), 6.46-6.49 (m, 1H, Ar*H*), 6.29-6.33 (m, 1H, Ar*H*), 4.12 (s, 2H, SCH_2), 3.74 (s, 3H, OCH_3);

MS (m/z) : 466.02 $(M)^+$;

RP-HPLC : Purity: 99.8 %, t_R = 2.76 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60)

5.1.2.2.9d. 2-((5-((4-Fluorophenyl)amino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(4-(2-oxopyridin-1(2*H*)-yl)phenyl)acetamide (243)

The title compound (**243**) was prepared as per the method described for compound (**227**) using 5-(4-fluorophenylamino)-1,3,4-thiadiazole-2-thiol (**144**) (0.5 g, 2.20 mmol) and 2-chloro-*N*-(4-(2-oxopyridin-1(2*H*)-yl)phenyl) acetamide (**224**) (0.58 g, 2.20 mmol). Compound (**243**) was obtained as off-white solid (0.72 g, 72 %), m.p. >250 °C.

Analytical data

TLC : R_f 0.52 (Chloroform-Methanol 18:2);

IR (KBr, cm^{-1}) : 3321, 2944, 1661, 1538, 1411 and 836;

^{1}H-NMR ($CDCl_3$) : δ 10.51 (bs, 1H, N*H*), 10.40 (s, 1H, N*H*), 7.69-7.73 (m, 2H, Ar*H*), 7.57-7.69 (m, 3H, Ar*H*), 7.49-7.53 (m, 1H, Ar*H*), 7.35-7.39 (m, 2H, Ar*H*), 7.15-7.22 (m, 2H, Ar*H*), 6.46-6.49 (m, 1H, Ar*H*), 6.29-6.33 (m, 1H, Ar*H*) and 4.15 (s, 2H, SCH_2);

MS (m/z) : 454.32 $(M)^+$;

RP-HPLC : Purity: 99.5 %, t_R = 2.82 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60).

5.1.2.2.9e. 2-((5-((2,4-Dichlorophenyl)amino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(4-(2-oxopyridin-1(2*H*)-yl)phenyl)acetamide (244)

The title compound (**244**) was prepared as per the method described for compound (**227**) using 5-(2,4-dichlorophenylamino)-1,3,4-thiadiazole-2-thiol (**146**) (0.5 g, 1.80 mmol) and 2-chloro-*N*-(4-(2-oxopyridin-1(2*H*)-yl) phenyl)acetamide (**224**) (0.47 g, 1.80 mmol). Compound (**244**) was obtained as off-white solid (0.64 g, 71 %), m.p. 248-250 °C.

Analytical data

TLC : R_f 0.63 (Chloroform-Methanol 18:2);

IR (KBr, cm^{-1}) : 3315, 3061, 1660, 1532, 1411 and 835;

^{1}H-NMR ($CDCl_3$) : δ 10.51 (bs, 1H, N*H*), 9.97 (s, 1H, N*H*), 8.34-8.37 (m, 1H, Ar*H*), 7.69-7.73 (m, 2H, Ar*H*),

7.63-7.65 (m, 1H, Ar*H*), 7.61-7.63 (m, 1H, Ar*H*), 7.48-7.53 (m, 1H, Ar*H*), 7.43-7.46 (m, 1H, Ar*H*), 7.36-7.39 (m, 2H, Ar*H*), 6.46-6.49 (m, 1H, Ar*H*), 6.29-6.33 (m, 1H, Ar*H*) and 4.17 (s, 2H, SCH_2);

MS (m/z) : 504.29 $(M)^+$, 506.26 $(M+2)^+$;

RP-HPLC : Purity: 97.7 %, t_R = 3.67 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60).

5.1.2.2.9f. *N*-(4-(2-Oxopyridin-1(2*H*)-yl)phenyl)-2-((5-(pyridin-2-ylamino)-1,3,4-thiadiazol-2-yl)thio)acetamide (245)

The title compound (**245**) was prepared as per the method described for compound (**227**) using 5-(pyridin-2-ylamino)-1,3,4-thiadiazole-2-thiol (**147**) (0.5 g, 2.38 mmol) and 2-chloro-*N*-(4-(2-oxopyridin-1(2*H*)-yl)phenyl) acetamide (**224**) (0.63 g, 2.39 mmol). Compound (**245**) was obtained as off-white solid (0.79 g, 76 %), m.p. >250 °C.

Analytical data

TLC : R_f 0.38 (Chloroform-Methanol 18:2);

IR (KBr, cm^{-1}) : 3189, 2769, 1665, 1531, 1412 and 845;

^{1}H-NMR (DMSO-d_6) : δ 11.75 (bs, 1H, N*H*), 10.50 (s, 1H, N*H*), 8.27-8.29 (m, 1H, Ar*H*), 7.75-7.79 (m, 1H, Ar*H*), 7.68-7.72 (m, 2H, Ar*H*), 7.60-7.63 (m, 1H, Ar*H*), 7.48-7.50 (m, 1H, Ar*H*), 7.34-7.38 (m, 2H, Ar*H*), 7.07-7.10 (m, 1H, Ar*H*), 6.99-7.03 (m, 1H, Ar*H*), 6.46-6.49 (m, 1H, Ar*H*), 6.29-6.33 (m, 1H, Ar*H*) and 4.18 (s, 2H, SCH_2);

MS (m/z) : 437.0 $(M)^+$;

RP-HPLC : Purity: 99.0 %, t_R = 2.85 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60)

5.1.2.2.9g. 2-((5-((5-Methylpyridin-2-yl)amino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(4-(2-oxopyridin-1(2*H*)-yl)phenyl)acetamide (246)

The title compound (**246**) was prepared as per the method described for compound (**227**) using 5-((5-methylpyridin-2-yl)amino)-1,3,4-thiadiazole-2-thiol (**148**) (0.5 g, 2.38 mmol) and 2-chloro-*N*-(4-(2-oxopyridin-1(2*H*)-yl) phenyl)acetamide (**224**) (0.63 g, 2.39 mmol). Compound (**246**) was obtained as off-white solid (0.69 g, 69 %), m.p. >250 °C.

Analytical data

TLC : R_f 0.32 (Chloroform-Methanol 18:2);

IR (KBr, cm^{-1}) : 3258, 3041, 2829, 1680, 1654 and 829;

^{1}H-NMR (DMSO-d_6) : δ 11.63 (bs, 1H, N*H*), 10.49 (s, 1H, N*H*), 8.10-8.11 (m, 1H, Ar*H*), 7.67-7.71 (m, 2H, Ar*H*), 7.60-7.63 (m, 2H, Ar*H*), 7.48-7.53 (m, 1H, Ar*H*), 7.34-7.38 (m, 2H, Ar*H*), 6.99-7.02 (m, 1H, Ar*H*), 6.46-6.49 (m, 1H, Ar*H*), 6.29-6.33 (m, 1H, Ar*H*), 4.16 (s, 2H, SCH_2) and 2.24 (s, 2H, CH_3);

MS (m/z) : 451.0 $(M)^+$;

RP-HPLC : Purity: 99.4 %, t_R = 3.21 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60)

5.1.2.2.9h. 2-((5-((5-Chloropyridin-2-yl)amino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(4-(2-oxopyridin-1(2*H*)-yl)phenyl)acetamide (247)

The title compound (**247**) was prepared as per the method described for compound (**227**) using 5-((5-chloropyridin-2-yl)amino)-1,3,4-thiadiazole-2-thiol (**149**) (0.5 g, 2.38 mmol) and 2-chloro-*N*-(4-(2-oxopyridin-1(2*H*)-yl) phenyl)acetamide (**224**) (0.54 g, 2.05 mmol). Compound (**247**) was obtained as off-white solid (0.67 g, 70 %), m.p. >250 °C.

Analytical data

TLC : R_f 0.65 (Chloroform-Methanol 18:2);

IR (KBr, cm^{-1}) : 3263, 3069, 2923, 1662, 1537, 1413 and 843;

^{1}H-NMR (DMSO-d_6) : δ 11.92 (bs, 1H, N*H*), 10.50 (s, 1H, N*H*), 8.32-8.33 (m, 1H, Ar*H*), 7.86-7.89 (m, 1H, Ar*H*), 7.67-7.71 (m, 2H, Ar*H*), 7.60-7.62 (m, 1H, Ar*H*), 7.48-7.52 (m, 1H, Ar*H*), 7.34-7.38 (m, 2H, Ar*H*), 7.10-7.13 (m, 1H, Ar*H*), 6.46-6.49 (m, 1H, Ar*H*), 6.29-6.33 (m, 1H, Ar*H*) and 4.18 (s, 2H, SCH_2);

^{13}C-NMR (DMSO-d_6) : δ 165.83, 162.11, 160.63, 154.77, 149.07, 144.47, 140.34, 138.96, 138.42, 138.28, 135.95, 127.03, 122.92, 120.33, 119.33, 112.42, 105.37 and 38.50;

MS (m/z) : 471.0 $(M)^+$, 473.0 $(M+2)^+$;

RP-HPLC : Purity: 96.8 %, t_R = 3.38 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60).

5.1.2.2.9i. 2-(5-(Benzylamino)-1,3,4-thiadiazol-2-ylthio)-*N*-(4-(2-oxo pyridin-1(2*H*)-yl)phenyl)acetamide (248)

The title compound (**248**) was prepared as per the method described for compound (**227**) using 5-(4-benzylamino)-1,3,4-thiadiazole-2-thiol (**150**) (0.5 g, 2.42 mmol) and 2-chloro-*N*-(4-(2-oxopyridin-1(2*H*)-yl)phenyl) acetamide (**224**) (0.55 g, 2.42 mmol). Compound (**248**) was obtained as off-white solid (0.71 g, 73 %), m.p. 206-208 °C.

Analytical data

TLC : R_f 0.33 (Chloroform-Methanol 18:2);

IR (KBr, cm^{-1}) : 3262, 3062, 1682, 1650, 1508 and 850;

^{1}H-NMR (DMSO-d_6) : δ 10.48 (bs, 1H, N*H*), 8.33 (t, 1H, N*H*), 7.66-7.68 (m, 2H, Ar*H*), 7.60-7.62 (m, 1H, Ar*H*), 7.48-7.52 (m, 1H, Ar*H*), 7.34-7.38 (m, 6H, Ar*H*), 7.26-7.28 (m, 1H, Ar*H*), 6.47-6.49 (m, 1H, Ar*H*), 6.29-6.33 (m, 1H, Ar*H*), 4.45 (d, 2H, NHCH_2) and 4.01 (s, 2H, SCH_2);

MS (m/z) : 450.0 $(M)^+$;

RP-HPLC : Purity: 96.1 %, t_R = 2.59 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60)

5.1.2.2.9j. 2-((5-((4-Methylbenzyl)amino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(4-(2-oxopyridin-1(2*H*)-yl)phenyl)acetamide (249)

The title compound (**249**) was prepared as per the method described for compound (**227**) using 5-(4-methylbenzylamino)-1,3,4-thiadiazole-2-thiol (**151**) (0.5 g, 2.11 mmol) and 2-chloro-*N*-(4-(2-oxopyridin-1(2*H*)-yl)phenyl) acetamide (**224**) (0.55 g, 2.11 mmol). Compound (**249**) was obtained as off-white solid (0.68 g, 70 %), m.p. 231-233 °C.

Analytical data

TLC : R_f 0.42 (Chloroform-Methanol 18:2);

IR (KBr, cm^{-1}) : 3272, 3081, 2874, 1657, 1513 and 836;

^{1}H-NMR (DMSO-d_6) : δ 10.44 (bs, 1H, N*H*), 8.27 (t, 1H, N*H*), 7.66-7.70 (m, 2H, Ar*H*), 7.61-7.63 (m, 1H, Ar*H*), 7.49-7.53 (m, 1H, Ar*H*), 7.34-7.38 (m, 2H, Ar*H*), 7.22-7.24 (m, 2H, Ar*H*), 7.14-7.16 (m, 2H, Ar*H*), 6.47-6.49 (m, 1H, Ar*H*), 6.29-6.33 (m, 1H, Ar*H*), 4.42 (d, 2H, NHCH_2), 4.03 (s, 2H, SCH_2) and 2.29 (s, 2H, CH_3);

MS (m/z) : 464.0 $(M)^+$;

RP-HPLC : Purity: 99.3 %, t_R = 2.90 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60)

5.1.2.2.9k. 2-((5-((4-Methoxybenzyl)amino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(4-(2-oxopyridin-1(2*H*)-yl)phenyl)acetamide (250)

The title compound (**250**) was prepared as per the method described for compound (**227**) using 5-(4-methoxybenzylamino)-1,3,4-thiadiazole-2-thiol (**152**) (0.5 g, 1.97 mmol) and 2-chloro-*N*-(4-(2-oxopyridin-1(2*H*)-yl)

phenyl)acetamide (**224**) (0.52 g, 1.97 mmol). Compound (**250**) was obtained as off-white solid (0.65 g, 69 %), m.p. 194-196 °C.

Analytical data

TLC : R_f 0.42 (Chloroform-Methanol 18:2);

IR (KBr, cm^{-1}) : 3261, 3076, 2835, 1657, 1413, 1286, 1032 and 835;

^{1}H-NMR (DMSO-d_6) : δ 10.44 (bs, 1H, N*H*), 8.24 (t, 1H, N*H*), 7.68-7.71 (m, 2H, Ar*H*), 7.61-7.63 (m, 1H, Ar*H*), 7.49-7.53 (m, 1H, Ar*H*), 7.34-7.38 (m, 2H, Ar*H*), 7.26-7.30 (m, 2H, Ar*H*), 6.89-6.93 (m, 2H, Ar*H*), 6.47-6.49 (m, 1H, Ar*H*), 6.29-6.33 (m, 1H, Ar*H*), 4.39 (d, 2H, NHCH_2), 4.03 (s, 2H, SCH_2) and 3.74 (s, 2H, OCH_3);

MS (m/z) : 480.0 $(M)^+$;

RP-HPLC : Purity: 97.8 %, t_R = 2.71 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60).

5.1.2.2.9l. 2-((5-((4-Fluorobenzyl)amino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(4-(2-oxopyridin-1(2*H*)-yl)phenyl)acetamide (251)

The title compound (**251**) was prepared as per the method described for compound (**227**) using 5-(4-fluorobenzylamino)-1,3,4-thiadiazole-2-thiol (**153**) (0.5 g, 2.07 mmol) and 2-chloro-*N*-(4-(2-oxopyridin-1(2*H*)-yl)phenyl) acetamide (**224**) (0.54 g, 2.07 mmol). Compound (**251**) was obtained as off-white solid (0.68 g, 70 %), m.p. 211-213 °C.

Analytical data

TLC : R_f 0.48 (Chloroform-Methanol 18:2);

IR (KBr, cm^{-1}) : 3262, 3130, 3075, 1658, 1509, 1411 and 833;

^{1}H-NMR (DMSO-d_6) : δ 10.44 (bs, 1H, N*H*), 8.31 (t, 1H, N*H*), 7.66-7.70 (m, 2H, Ar*H*), 7.61-7.63 (m, 1H, Ar*H*), 7.49-7.53 (m, 1H, Ar*H*), 7.34-7.41 (m, 4H, Ar*H*), 7.15-7.20 (m, 2H, Ar*H*), 6.47-6.49 (m, 1H, Ar*H*), 6.29-6.33 (m, 1H, Ar*H*), 4.45 (d,

2H, NHCH_2) and 4.03 (s, 2H, SCH_2);

MS (m/z) : 468.0 $(M)^+$;

RP-HPLC : Purity: ~100 %, t_R = 2.72 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60).

5.1.2.2.9m. 2-((5-((4-Chlorobenzyl)amino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(4-(2-oxopyridin-1(2*H*)-yl)phenyl)acetamide (252)

The title compound (**252**) was prepared as per the method described for compound (**227**) using 5-(4-chlorobenzylamino)-1,3,4-thiadiazole-2-thiol (**154**) (0.5 g, 1.94 mmol) and 2-chloro-*N*-(4-(2-oxopyridin-1(2*H*)-yl)phenyl) acetamide (**224**) (0.51 g, 1.94 mmol). Compound (**252**) was obtained as off-white solid (0.68 g, 72 %), m.p. 204-206 °C.

Analytical data

TLC : R_f 0.32 (Chloroform-Methanol 18:2);

IR (KBr, cm^{-1}) : 3264, 3120, 2869, 1656, 1573 and 839;

^{1}H-NMR (DMSO-d_6) : δ 10.44 (bs, 1H, N*H*), 8.34 (t, 1H, N*H*), 7.66-7.70 (m, 2H, Ar*H*), 7.61-7.63 (m, 1H, Ar*H*), 7.49-7.53 (m, 1H, Ar*H*), 7.34-7.43 (m, 6H, Ar*H*), 6.47-6.49 (m, 1H, Ar*H*), 6.29-6.33 (m, 1H, Ar*H*), 4.47 (d, 2H, NHCH_2) and 4.04 (s, 2H, SCH_2);

^{13}C-NMR (DMSO-d_6) : δ 169.43, 165.86, 161.11, 149.48, 140.33, 138.95, 138.27, 137.43, 135.93, 131.53, 129.21, 128.17, 127.02, 120.31, 119.27, 105.35, 46.87 and 38.50;

MS (m/z) : 484.0 $(M)^+$, 486.0 $(M+2)^+$;

RP-HPLC : Purity: 99.7 %, t_R = 2.91 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60).

5.1.2.2.9n. 2-((5-((2,4-Dichlorobenzyl)amino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(4-(2-oxopyridin-1(2*H*)-yl)phenyl)acetamide (253)

The title compound (**253**) was prepared as per the method described for compound (**227**) using 5-(2,4-dichlorobenzylamino)-1,3,4-thiadiazole-2-thiol (**155**) (0.5 g, 1.71 mmol) and 2-chloro-*N*-(4-(2-oxopyridin-1(2*H*)-yl)phenyl)acetamide (**224**) (0.45 g, 1.71 mmol). Compound (**253**) was obtained as off-white solid (0.60 g, 68 %), m.p. 176-178 °C.

Analytical data

TLC : R_f 0.35 (Chloroform-Methanol 18:2);

IR (KBr, cm^{-1}) : 3264, 3070, 1657, 1529, 1413 and 833;

^{1}H-NMR (DMSO-d_6) : δ 10.48 (bs, 1H, N*H*), 8.38 (t, 1H, N*H*), 7.60-7.68 (m, 4H, Ar*H*), 7.48-7.52 (m, 1H, Ar*H*), 7.41-7.43 (m, 2H, Ar*H*), 7.33-7.35 (m, 2H, Ar*H*), 6.46-6.48 (m, 1H, Ar*H*), 6.29-6.32 (m, 1H, Ar*H*), 4.52 (d, 2H, NHCH_2) and 4.03 (s, 2H, SCH_2);

MS (m/z) : 518.0 $(M)^+$, 520 $(M+2)^+$;

RP-HPLC : Purity: 99.3 %, t_R = 3.25 min (Mobile phase: Buffer of *p*H = 3.5: acetonitrile: methanol 12:28:60)

5.1.3. Carbazole derivatives as FXa inhibitors

5.1.3.1. Synthesis of the designed carbazole derivatives

5.1.3.1.1. 9-(4-Chlorobenzyl)-9*H*-carbazole (261)

To a rapidly stirring solution of carbazole (**260**) (1.67 g, 10 mmol) in DMSO (10 mL), a few drops of aqueous sodium hydroxide (0.8 g, 20 mmol) was added and the reaction mixture stirred for 5 min. 4-Chlorobenzyl chloride (0.96 mL, 15 mmol) was added slowly to it and the reaction mixture was stirred for further 2-3 h to complete the reaction. After completion of the reaction, the reaction mixture was poured into crushed ice to afford solid precipitates, which were collected and washed with water to remove residual

solvent and dried to obtain the compound (**261**) as pale-yellow solid (2.14 g, 74 %), m.p. 165-167 °C.

Analytical data

TLC : R_f 0.49 (*n*-Hexane-Ethyl acetate 18:2);

IR (KBr, cm^{-1}) : 3049, 2865, 1620, 1485, 1451, 1197, 839 and 747;

^{1}H-NMR ($CDCl_3$) : δ 8.20 (d, 2H, *J* = 7.6 Hz, Ar*H*), 7.65 (d, 2H, *J* = 8.0 Hz, Ar*H*), 7.45 (t, 2H, *J* = 7.6 Hz, Ar*H*), 7.35 (d, 2H, *J* = 8.0 Hz, Ar*H*), 7.23 (t, 2H, Ar*H*), 7.19 (d, 2H, *J* = 8.0 Hz, Ar*H*) and 5.68 (s, 2H, -CH_2).

MS (m/z) : 292.0 $(M)^+$, 294.1 $(M+2)^+$.

5.1.3.1.2. 9-(4-Chlorobenzyl)-9*H*-carbazole-3-carbaldehyde (262)

Phosphorus oxychloride (2.65 mL, 34.28 mmol) was added, over a period of 10 min, to an ice cooled stirred solution of 9-(4-chlorobenzyl)-9*H*-carbazole (**261**) (2 g, 6.86 mmol) and dimethylformamide (3.2 mL, 34.28 mmol) in chloroform (30 mL). The resulting reaction mixture was refluxed overnight. After completion of the reaction, the reaction mixture was poured into crushed ice and neutralized with saturated sodium carbonate solution and extracted with chloroform. The organic phase was washed with water and brine, dried over magnesium sulphate and evaporated at reduced pressure. The residue obtained was purified by column chromatography on silica gel using petroleum ether and ethyl acetate as an eluent in different proportion to obtain the compound (**262**) as white solid (1.42 g, 65 %), m.p. 95-97 °C.

Analytical data

TLC : R_f 0.46 (*n*-Hexane-Ethyl acetate 18:2);

IR (KBr, cm^{-1}) : 3369, 3055, 2924, 1703, 1484 and 754;

^{1}H-NMR ($CDCl_3$) : δ 10.07 (s, 1H, -C*H*O), 8.80 (s, 1H, Ar*H*), 8.32 (d, 1H, *J* = 7.6 Hz, Ar*H*), 7.98 (d, 1H, *J* = 8.0 Hz, Ar*H*), 7.82 (d, 1H, *J* = 8.0 Hz, Ar*H*), 7.7 (d, 1H, *J* = 8.4 Hz, Ar*H*), 7.50 (t, 1H, *J* = 7.6

Hz, Ar*H*), 7.31 (m, 3H, Ar*H*), 7.18 (d, 2H, *J* = 8.4 Hz, Ar*H*) and 5.76 (s, 2H, -NCH_2);

MS (m/z) : 320.22 $(M)^+$, 322.23 $(M+2)^+$.

5.1.3.1.3. 9-(4-Chlorobenzyl)-9*H*-carbazole-3-carboxylic acid (263)

9-(4-Chlorobenzyl)-9*H*-carbazole-3-carbaldehyde (**262**) (1.5 g, 4.7 mmol) was dissolved in DMSO to which TBHP (2.7 mL, 28.2 mmol) was added. The reaction mixture was stirred overnight at 110 °C. After completion of reaction, the reaction mixture was poured into crushed ice, the obtained solid precipitates which were collected, washed with water to remove residual solvent and dried. The obtained residue was purified by column chromatography on silica gel using chloroform, methanol in different proportions to obtain the titled compound (**263**) as white solid (0.86 g, 55 %), m.p. 250-252 °C.

Analytical data

TLC : R_f 0.65 (Chloroform-Methanol 18:2);

IR (KBr, cm^{-1}) : 3053, 2833, 1200, 1489, 1419, 1682 and 762;

^{1}H-NMR ($CDCl_3$) : δ 12.69 (s, 1H, COO*H*), 8.83 (s, 1H, Ar*H*), 8.33 (d, 1H, *J* = 7.6 Hz, Ar*H*), 8.06 (d, 1H, *J* = 8.4 Hz, Ar*H*), 7.73-7.69 (m, 2H, Ar*H*), 7.51 (t, 1H, *J* = 7.6 Hz, Ar*H*), 7.35 (d, 2H, *J* = 8.4 Hz, Ar*H*), 7.28, (t, 1H, *J* = 7.6 Hz, Ar*H*), 7.19 (d, 2H, *J* = 8.4 Hz, Ar*H*) and 5.76 (s, 2H, NCH_2);

MS (m/z) : 334.19 $(M)^+$.

5.1.3.1.4. Synthesis of necessary aromatic amines (197-204, 208, 264, 265, 268)

Experimental data of compounds (**197-204** and **208**) has been given in the previous **Section 5.2**.

5.1.3.1.4a. 1-(4-Nitrophenyl)-1*H*-pyrrole (264)

The title compound (**264**) was prepared from 1-fluoro-4-nitrobenzene (**187**) (3.0 mL, 29 mmol) and 1*H*-pyrrole (2 mL, 42 mmol) following

Method-H. Compound (**264**) was obtained as a light yellow solid (4.50 g, 55 %), m.p. 163-165 °C (lit.[128] 160-162 °C).

Analytical data:

TLC : R_f 0.53 (*n*-Hexane-Ethyl acetate 19:1);

IR (KBr, cm^{-1}) : 3087, 2920, 1595, 1506, 1316 and 845.

5.1.3.1.4b. 1-(4-Nitrophenyl)-1*H*-imidazole (265)

The title compound (**265**) was prepared from 1-fluoro-4-nitrobenzene (**187**) (3.0 mL, 29 mmol) and 1*H*-imidazole (2.89 g, 42 mmol) following **Method-H**. Compound (**265**) was obtained as a light yellow solid (3.64 g, 68 %), m.p. 200-202 °C (lit.[106] 201-203 °C).

Analytical data:

TLC : R_f 0.43 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3138, 1600, 1512, 1339 and 849.

5.1.3.1.4c. 4-(1*H*-Pyrrol-1-yl)benzenamine (266)

The title compound (**266**) was synthesized from 1-(4-nitrophenyl)-1*H*-pyrrole (**264**) (1.0 g, 5.30 mmol) as described in **Method J**. Compound (**266**) was obtained as a brown semisolid (0.51 g, 60 %), m.p. 78-80 °C (lit.[128] 77-79 °C).

Analytical data:

TLC : R_f 0.57 (*n*-Hexane-Ethyl acetate 12:8);

IR (KBr, cm^{-1}) : 3419, 3324, 3141, 1626, 1523 and 824.

5.1.3.1.4d. 4-(1*H*-Imidazol-1-yl)benzenamine (267)

The title compound (**267**) was synthesized from 1-(4-nitrophenyl)-1*H*-imidazole (**265**) (1.0 g, 5.20 mmol) as described in **Method J**. Compound (**267**) was obtained as a brown semisolid (0.55 g, 66 %).

Analytical data:

TLC : R_f 0.57 (*n*-Hexane-Ethyl acetate 12:8);

IR (KBr, cm^{-1}) : 3419,3324, 3141, 1626, 1523 and 824.

5.1.3.1.4e. 4,5,6,7-Tetrahydrobenzo[*d*]thiazol-2-amine (270)

Thiourea (3.8 g, 50 mmol), iodine (6.35 g, 25 mmol) and cyclohexanone (2.6 mL, 25 mmol) were taken in methanol (50 mL) and heated under microwave irradiation conditions at 195 W for 10 min. After completion of the reaction, hot water (30 mL) was added into the reaction mixture and the resulting hot solution was filtered. The filtrate was treated with saturated Na_2CO_3 solution, and extracted with chloroform (3 × 20 mL). The collected chloroform layer was dried over anhydrous magnesium sulphate, filtered and concentrated under reduced pressure to obtain the crude product, which was further purified by column chromatography to get a yellow solid of compound (**270**) (2.64 g, 68 %), m.p. 85-87 °C.

Analytical data:

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

IR (KBr, cm^{-1}) : 3374, 3283, 3092, 2932, 1637, 1523 and 892.

5.1.3.1.5. General method for the synthesis of 9-(4-chlorobenzyl)-*N*-substituted-9*H*-carbazole-3-carboxamides (271-282)

Method M: A stirring solution of compound (**263**) (0.5 g, 1.49 mmol) in DMF (10 mL) was added EDCI (0.34 g, 1.79 mmol) and HOBT (0.24 g, 1.79 mmol). The resulting reaction mixture was stirred at RT for 30 min. The corresponding amine (1.49 mmol) and DMAP (0.36 g, 2.98 mmol) were added to the reaction mixture and it was allowed to stir overnight. After completion of the reaction, it was poured into crushed ice. The obtained precipitates were filtered and washed with cold water to obtain the crude product which was further purified by column chromatography using chloroform, methanol in different proportion.

5.1.3.1.5a. 9-(4-Chlorobenzyl)-*N*-(4-(pyrrolidin-1-yl)phenyl)-9*H*-carbazole -3-carboxamide (271)

The title compound (**271**) was synthesized as per **Method M** using 4-(pyrrolidin-1-yl)benzenamine (0.24 g, 1.49 mmol). The crude product thus obtained was purified to get off-white solid of compound (**271**) (0.36 g, 51 %) m.p. 259–261 °C.

Analytical data

TLC	: R_f 0.47 (*n*-Hexane-Ethyl acetate 18:2);
IR (KBr, cm^{-1})	: 3289, 3046, 2965, 1631, 1522, 1327, 805 and 751;
^{1}H-NMR (DMSO-d_6)	: δ 9.95 (s, 1H, -CON*H*), 8.84 (s, 1H, Ar*H*), 8.28 (d, *J* = 7.6 Hz, 1H, Ar*H*), 8.08 (d, *J* = 8.6 Hz, 1H, Ar*H*), 7.75 (d, *J* = 8.6 Hz, 1H, Ar*H*), 7.69 (d, *J* = 8.4 Hz, 1H, Ar*H*), 7.61 (d, *J* = 8.8 Hz, 1H, Ar*H*), 7.47-7.52 (m, 1*H*, Ar*H*), 7.37 (d, *J* = 8.4 Hz, 2H, Ar*H*), 7.30 (m, 1H, Ar*H*), 7.20 (d, *J* = 8.4 Hz, 2H, Ar*H*), 6.57 (d, *J* = 8.8 Hz, 2H, Ar*H*), 5.74 (s, 2H, -NCH_2), 3.20-3.24 (m, 4H, -CH_2) and 1.96-1.98 (m, 4H, -CH_2);
MS (m/z)	: 480.0 $(M)^+$;
RP-HPLC	: Purity: 99.7 %, t_R = 6.70 min (Mobile phase: acetonitrile-water 50:50 (0.01 % TFA)).

5.1.3.1.5b. 9-(4-Chlorobenzyl)-*N*-(4-(piperidin-1-yl)phenyl)-9*H*-carbazole-3-carbox-amide (272)

The title compound (**272**) was synthesized as per **Method M** using 4-(piperidin-1-yl)benzenamine (0.26 g, 1.49 mmol). The crude product (**272**) thus obtained was purified to get off-white solid (0.36 g, 51 %), m.p. 240-242 °C.

Analytical data

TLC	: R_f 0.40 (*n*-Hexane-Ethyl acetate 18:2);
IR (KBr, cm^{-1})	: 3282, 3048, 2852, 1632, 1129, 817 and 750;
^{1}H-NMR (DMSO-d_6)	: δ 10.04 (s, 1H, -CON*H*), 8.84 (s, 1H, Ar*H*), 8.28 (d, 1H, Ar*H*), 8.08 (d, *J* = 8.8 Hz, 1H, Ar*H*), 7.76 (d, *J* = 8.8 Hz, 1H, Ar*H*), 7.63-7.69 (m, 3H, Ar*H*), 7.50 (t, 1H, Ar*H*), 7.37 (d, *J* = 8.4 Hz, 2H, Ar*H*), 7.30 (t, 1H, Ar*H*), 7.20 (d, *J* = 8.4 Hz, 2H, Ar*H*), 6.95 (d, 2H, Ar*H*), 5.75 (s, 2H, -NCH_2), 3.09-3.11 (m, 4H, -CH_2),

1.61-1.67 (m, 4H, -CH_2) and 1.51-1.56 (m, 4H, -CH_2);

MS (m/z) : 494.2 $(M)^+$;

RP-HPLC : Purity: 98.5 %, t_R = 3.70 min (Mobile phase: acetonitrile-water 50:50 (0.01 % TFA)).

5.1.3.1.5c. 9-(4-Chlorobenzyl)-*N*-(4-morpholinophenyl)-9*H*-carbazole-3-carboxamide (273)

The title compound (**273**) was synthesized as per **Method M** using 4-morpholinobenzenamine (0.27 g, 1.49 mmol). The crude product (**273**) thus obtained was purified to get off-white solid (0.43 g, 58 %), m.p. 234-236 °C.

Analytical data

TLC : R_f 0.37 (*n*-Hexane-Ethyl acetate 12:8);

IR (KBr, cm^{-1}) : 3269, 3010, 2854, 1632, 1116, 816 and 752;

^{1}H-NMR (DMSO-d_6) : δ 10.09 (s, 1H, -CON*H*), 8.85 (s, 1H, Ar*H*), 8.29 (d, 1H, Ar*H*), 8.09 (d, *J* = 8.8 Hz, 1H, Ar*H*), 7.77 (d, *J* = 8.8 Hz, 1H, Ar*H*), 7.65-7.71 (m, 3H, Ar*H*), 7.50 (t, 1H, Ar*H*), 7.36 (d, *J* = 8.4 Hz, 2H, Ar*H*), 7.31 (t, 1H, Ar*H*), 7.20 (d, *J* = 8.4 Hz, 2H, Ar*H*), 6.97 (d, 2H, Ar*H*), 5.75 (s, 2H, -NCH_2), 3.75 (t, 4H, -CH_2) and 3.08 (t, *J* = 4.8 Hz, 4H, -CH_2);

MS (m/z) : 496.0 $(M)^+$;

RP-HPLC : Purity: 99.2 %, t_R = 6.55 min (Mobile phase: acetonitrile-water 50:50 (0.01 % TFA)).

5.1.3.1.5d. 9-(4-Chlorobenzyl)-*N*-(4-(4-methylpiperazin-1-yl)phenyl)-9*H*-carbazole-3-carboxamide (274)

The title compound (**274**) was synthesized as per **Method M** using 4-(4-methylpiperazin-1-yl)benzenamine (0.29 g, 1.49 mmol). The crude product (**274**) thus obtained was purified to get off-white solid (0.43 g, 59 %), m.p. 254-256 °C.

Analytical data

TLC : R_f 0.40 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3284, 3050, 2932, 1628, 1520, 1329, 816 and 750;

^{1}H-NMR (DMSO-d_6) : δ 10.03 (s, 1H, -CON*H*), 8.84 (s, 1H, Ar*H*), 8.27 (d, 1H, Ar*H*), 8.08 (d, *J* = 8.8 Hz, 1H, Ar*H*), 7.75 (d, *J* = 8.8 Hz, 1H, Ar*H*), 7.65-7.68 (m, 3H, Ar*H*), 7.51 (t, 1H, Ar*H*), 7.28-7.36 (m, 3H, Ar*H*), 7.20 (d, 2H, Ar*H*), 6.95 (d, 2H, Ar*H*), 5.76 (s, 2H, -NCH_2), 3.09-3.12 (m, 4H, -NCH_2), 2.42-2.48 (m, 4H, -NCH_2) and 2.23 (s, 3H, -NCH_3);

MS (m/z) : 509.0 $(M)^+$;

RP-HPLC : Purity: 99.2 %, t_R = 4.40 min (Mobile phase: acetonitrile-water 50:50 (0.01 % TFA)).

5.1.3.1.5e. 9-(4-Chlorobenzyl)-*N*-(4-(2-oxopyrrolidin-1-yl)phenyl)-9*H*-carbazole-3-carboxamide (275)

The title compound (**275**) was synthesized as per **Method M** using 1-(4-aminophenyl)pyrrolidin-2-one (0.25 g, 1.49 mmol). The crude product (**275**) thus obtained was purified to get off-white solid (0.39 g, 53 %), m.p. 252-254 °C.

Analytical data

TLC : R_f 0.50 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3297, 1672, 1524, 1408, 1239, 832 and 755;

^{1}H-NMR (DMSO-d_6) : δ 10.26 (s, 1H, -CON*H*), 8.87 (s, 1H, Ar*H*), 8.30 (d, *J* = 8.0 Hz, 1H, Ar*H*), 8.10 (d, *J* = 8.4 Hz, 1H, Ar*H*), 7.84 (d, *J* = 8.4 Hz, 1H, Ar*H*), 7.78 (d, *J* = 8.4 Hz, 1H, Ar*H*), 7.65-7.67 (m, 3H, Ar*H*), 7.51 (t, *J* = 8.0 Hz, 1H, Ar*H*), 7.37 (d, 2H, Ar*H*), 7.31 (t, *J* = 8.0 Hz, 1H, Ar*H*), 7.21 (d, 2H, *J* = 8.4 Hz, 1H, Ar*H*), 5.75 (s, 2H, -NCH_2), 3.85 (t, 2H, -CH_2), 2.48-2.52 (m, 2H,

-C*H*$_2$) and 2.08 (t, 2H, C*H*$_2$);

MS (m/z) : 494.33 $(M)^+$, 496.32 $(M+2)^+$;

RP-HPLC : Purity: 99.3 %, t_R = 6.20 min (Mobile phase: acetonitrile-water 50:50 (0.01 % TFA)).

5.1.3.1.5f. 9-(4-Chlorobenzyl)-*N*-(4-(2-oxopiperidin-1-yl)phenyl)-9*H*-carbazole-3-carboxamide (276)

The title compound (**276**) was synthesized as per **Method M** using 1-(4-aminophenyl)piperidin-2-one (0.28 g, 1.49 mmol). The crude product (**276**) thus obtained was purified to get off-white solid (0.37 g, 49 %), m.p. 266-268 °C.

Analytical data

TLC : R_f 0.40 (Chloroform-Methanol 18:2);

IR (KBr, cm^{-1}) : 3295, 3053, 2931, 1655, 1620, 1013, 834 and 749;

^{1}H-NMR (DMSO-d_6) : δ 10.29 (s, 1H, -CON*H*), 8.87 (s, 1H, Ar*H*), 8.30 (d, *J* = 7.6 Hz, 1H, Ar*H*), 8.10 (d, *J* = 8.4 Hz, 1H, Ar*H*), 7.83 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.79 (d, *J* = 8.4 Hz, 1H, Ar*H*), 7.70 (d, *J* = 8.4 Hz, 1H, Ar*H*), 7.48-7.52 (m, 1H, Ar*H*), 7.37 (d, *J* = 8.4 Hz, 2H, Ar*H*), 7.26-7.34 (m, 1H, Ar*H*), 7.27 (d, 2H, *J* = 8.4 Hz, 1H, Ar*H*), 7.21 (d, *J* = 8.8 Hz, 2H, Ar*H*), 5.76 (s, 2H, -NC*H*$_2$), 3.60-3.62 (m, 2H, C*H*$_2$), 2.40 (t, 2H, -C*H*$_2$) and 1.84-1.88 (m, 4H, C*H*$_2$);

MS (m/z) : 508.35 $(M)^+$, 510.29 $(M+2)^+$;

RP-HPLC : Purity: 98.1 %, t_R = 5.81 min (Mobile phase: acetonitrile-water 50:50 (0.01 % TFA)).

5.1.3.1.5g. 9-(4-Chlorobenzyl)-*N*-(4-(2-oxoazepan-1-yl)phenyl)-9*H*-carbazole-3-carboxamide (277)

The title compound (**277**) was synthesized as per **Method M** using 1-(4-aminophenyl)azepan-2-one (0.30 g, 1.49 mmol). The crude product (**277**) thus obtained was purified to get off-white solid (0.44 g, 56 %), m.p. 243-245 °C.

Analytical data

TLC : R_f 0.45 (Chloroform-Methanol 18:2);

IR (KBr, cm^{-1}) : 3304, 3050, 2928, 1633, 1516, 1324, 827 and 752;

^{1}H-NMR (DMSO-d_6) : δ 10.26 (s, 1H, -CON*H*), 8.86 (s, 1H, Ar*H*), 8.29 (d, 1H, Ar*H*), 8.10 (d, 1H, Ar*H*), 7.76-7.81 (m, 3H, Ar*H*), 7.69 (d, 1H, Ar*H*), 7.50 (t, 1H, Ar*H*), 7.29-7.36 (m, 3H, Ar*H*), 7.18-7.20 (m, 4H, Ar*H*), 5.75 (s, 2H, -NCH_2), 3.69-3.77 (m, 2H, -NCH_2), 2.59-2.61 (m, 2H, -CH_2) and 1.65-1.81 (m, 6H, -CH_2);

MS (m/z) : 521.20 $(M)^+$, 523.22 $(M+2)^+$;

RP-HPLC : Purity: 98.7 %, t_R = 6.99 min (Mobile phase: acetonitrile-water 50:50 (0.01 % TFA)).

5.1.3.1.5h. 9-(4-Chlorobenzyl)-*N*-(4-(2-oxopyridin-1(2*H*)-yl)phenyl)-9*H*-carbazole-3-carboxamide (278)

The title compound (**278**) was synthesized as per **Method M** using 1-(4-aminophenyl)pyridin-2(1*H*)-one (0.28 g, 1.49 mmol). The crude product (**278**) thus obtained was purified to get off-white solid (0.41 g, 54 %), m.p. 256-258 °C.

Analytical data

TLC : R_f 0.67 (Chloroform-Methanol 18:2);

IR (KBr, cm^{-1}) : 3305, 3046, 1660, 1515, 1322 and 801;

^{1}H-NMR (DMSO-d_6) : δ 10.42 (s, 1H, -CON*H*), 8.88 (s, 1H, Ar*H*), 8.31 (d, 1H, Ar*H*), 8.12 (d, *J* = 8.8 Hz, 1H, Ar*H*), 7.93-7.97 (m, 2H, Ar*H*), 7.80 (d, 1H,

Ar*H*), 7.70 (d, 1H, Ar*H*), 7.66-7.68 (m, 1H, Ar*H*), 7.49-7.53 (m, 2H, Ar*H*), 7.31-7.41 (m, 5H, Ar*H*), 7.20 (d, 2H, Ar*H*), 6.50 (d, 1H, Ar*H*), 6.30-6.34 (m, 1H, Ar*H*) and 5.76 (s, 2H, -NCH_2);

MS (m/z) : 504.35 $(M)^+$, 506.34 $(M+2)^+$;

RP-HPLC : Purity: 98.9 %, t_R = 5.38 min (Mobile phase: acetonitrile-water 50:50 (0.01 % TFA)).

5.1.3.1.5i. 9-(4-Chlorobenzyl)-*N*-(4-(3-oxomorpholino)phenyl)-9*H*-carbazole-3-carboxamide (279)

The title compound (**279**) was synthesized as per **Method M** using 4-(4-aminophenyl)morpholin-3-one (0.29 g, 1.49 mmol). The crude product (**279**) thus obtained was purified to get off-white solid (0.34 g, 45 %), m.p. 243-245 °C.

Analytical data

TLC : R_f 0.63 (Chloroform-Methanol 18:2);

IR (KBr, cm^{-1}) : 3327, 3054, 2865, 1643, 1600, 1238, 1100 and 810;

^{1}H-NMR (DMSO-d_6) : δ 10.35 (s, 1H, -CON*H*), 8.87 (s, 1H, Ar*H*), 8.30 (d, *J* = 8.0 Hz, 1H, Ar*H*), 8.10 (d, *J* = 8.0 Hz, 1H, Ar*H*), 7.87 (d, 1H, Ar*H*) 7.80 (d, 1H, Ar*H*), 7.70 (d, *J* = 8.0 Hz, 2H, Ar*H*), 7.50 (t, *J* = 8.0 Hz, 1H, Ar*H*), 7.29-7.40 (m, 5H, Ar*H*), 7.20 (d, 2H, Ar*H*), 5.75 (s, 2H, -NCH_2), 4.21 (s, 2H, -CH_2), 3.98 (t, *J* = 4.8 Hz, 2H, -CH_2) and 3.74 (t, *J* = 4.8 Hz, 2H, -CH_2);

MS (m/z) : 510.40 $(M)^+$, 512.36 $(M+2)^+$;

RP-HPLC : Purity: 99.1 %, t_R = 5.17 min (Mobile phase: acetonitrile-water 50:50 (0.01 % TFA)).

5.1.3.1.5j. *N*-(4-(1*H*-Pyrrol-1-yl)phenyl)-9-(4-chlorobenzyl)-9*H*-carbazole-3-carboxamide (280)

The title compound (**280**) was synthesized as per **Method M** using 4-(1*H*-pyrrol-1-yl)benzenamine (0.24 g, 1.49 mmol). The crude product (**280**) thus obtained was purified to get off-white solid (0.44 g, 62 %), m.p. 229-231 °C.

Analytical data

TLC : R_f 0.71 (Chloroform-Methanol 18:2);

IR (KBr, cm^{-1}) : 3302, 1648, 1527, 1325, 824 and 750;

^{1}H-NMR (DMSO-d_6) : δ 10.35 (s, 1H, -CON*H*), 8.88 (s, 1H, Ar*H*), 8.31 (d, *J* = 8.0 Hz, 1H, Ar*H*), 8.11 (d, *J* = 8.8 Hz, 1H, Ar*H*), 7.94 (d, *J* = 8.8 Hz, 1H, Ar*H*) 7.80 (d, *J* = 8.8 Hz, 1H, Ar*H*), 7.71 (d, 1H, Ar*H*), 7.60 (t, 1H, Ar*H*), 7.53 (t, *J* = 8.0 Hz, 1H, Ar*H*), 7.30-7.37 (m, 5H, Ar*H*), 7.21 (d, *J* = 8.8 Hz, 2H, Ar*H*), 6.24-6.28 (m, 1H, Ar*H*) and 5.76 (s, 2H, -NCH_2);

MS (m/z) : 476.39 $(M)^+$;

RP-HPLC : Purity: 95.5 %, t_R = 15.33 min (Mobile phase: acetonitrile-water 50:50 (0.01 % TFA)).

5.1.3.1.5k. *N*-(4-(1*H*-Imidazol-1-yl)phenyl)-9-(4-chlorobenzyl)-9*H*-carbazole-3-carboxamide (281)

The title compound (**281**) was synthesized as per **Method M** using 4-(1*H*-imidazol-1-yl)benzenamine (0.24 g, 1.49 mmol). The crude product (**281**) thus obtained was purified to get off-white solid, (0.37 g, 52 %), m.p. >260 °C.

Analytical data

TLC : R_f 0.68 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3219, 3116, 3000, 1671, 1521, 1329, 823 and 746;

^{1}H-NMR (DMSO-d_6) : δ 10.40 (s, 1H, -CON*H*), 8.88 (s, 1H, Ar*H*), 8.30 (d, 1H, Ar*H*), 8.22 (s, 1H, Ar*H*), 8.11 (s,

J = 8.8 Hz, 1H, Ar*H*), 7.99 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.79 (d, *J* = 8.8 Hz, 1H, Ar*H*), 7.64-7.73 (m, 4H, Ar*H*), 7.51 (t, 1H, Ar*H*), 7.31-7.37 (m, 3H, Ar*H*), 7.21 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.10-7.12 (m, 1H, Ar*H*) and 5.76 (s, 2H, -NCH_2);

MS (m/z) : 476.1 $(M)^+$, 478.2 $(M+2)^+$;

RP-HPLC : Purity: 99.2 %, t_R = 4.60 min (Mobile phase: acetonitrile-water 50:50 (0.01 % TFA)).

5.1.3.1.5l. 9-(4-Chlorobenzyl)-*N*-(4,5,6,7-tetrahydrobenzo[*d*]thiazol-2-yl)-9*H*-carbaz-ole-3-carboxamide (282)

The title compound (**282**) was synthesized as per **Method M** using 4,5,6,7-tetrahydrobenzo[*d*]thiazol-2-amine (0.23 g, 1.49 mmol,). The crude product (**282**) thus obtained was purified to get off-white solid (0.36 g, 51%), m.p. 220-222 °C.

Analytical data

TLC : R_f 0.47 (Chloroform-Methanol 19:1);

IR (KBr, cm^{-1}) : 3138, 3048, 2928, 1662, 1550, 1323, 801 and 748;

^{1}H-NMR (DMSO-d_6) : δ 12.34 (s, 1H, -CON*H*), 9.07 (s, 1H, Ar*H*), 8.20-8.33 (m, 2H, Ar*H*), 7.78 (d, 1H, Ar*H*), 7.67-7.73 (m, 1H, Ar*H*), 7.52 (t, 1H, Ar*H*), 7.29-7.37 (m, 3H, Ar*H*), 7.20 (d, 2H, Ar*H*), 5.75 (s, 2H, -NCH_2), 2.63-2.69 (m, 4H, -CH_2), and 1.40-1.80 (m, 4H, -CH_2);

MS (m/z) : 472.1 $(M)^+$, 474.2 $(M+2)^+$;

RP-HPLC : Purity: 99.0 %, t_R = 5.72 min (Mobile phase: acetonitrile-water 50:50 (0.01 % TFA)).

5.2. Biological evaluation

5.2.1. *In vitro* FXa and thrombin inhibition assays

For the measurement of direct FXa inhibition, a chromogenic substrate assay was performed on a microplate reader (FlexStation III, Molecular Devices).[82] After an initial screening at a concentration of 100 μM, compounds that caused >50 % reproducible inhibition of the coagulation enzyme IC_{50} values of such compounds were determined. The relative residual enzyme activity at individual concentration of the inhibitor was determined from the ratio of the enzyme activity with and without the inhibitor. For IC_{50} determination, stocks of the compounds were serially diluted to get 24 different aliquots in the wells with final concentrations ranging from 500 – 0.34 μM.

FXa inhibition studies were performed using a *p*H 7.4 buffer containing 20 mM Tris-HCl, 2.5 mM $CaCl_2$, 100 mM NaCl, 0.1% polyethylene glycol (PEG) 8000 and 0.02 % Tween 80. 186 μL of *p*H 7.4 buffer was added to the wells, and 5 μL of potential FXa inhibitor (test compounds or solvent reference) and 5 μL of FXa (1 nM final concentration) were consecutively added. After incubating at 37 °C for 10 mins, 5 μL of FXa substrate (125 μM) was rapidly added and the residual FXa activity was measured from the initial rate of increase in absorbance at 405 nm. Relative residual FXa activity at each concentration of the inhibitor was calculated from the ratio of FXa activity in the presence and absence of inhibitor. Logistic eq 1 was used to fit the dose dependence of residual protease activity to obtain the potency (IC_{50}) and efficacy (ΔY) of inhibition.

$$Y = Y_0 + \frac{Y_m - Y_0}{1+10^{(\log[f]_0 - \log IC50)\,(HS)}}$$

In this equation, Y is the ratio of residual FXa activity in the presence of inhibitor to that in its absence (fractional residual activity), Y_m and Y_0 are the maximum and minimum possible values of the fractional residual proteinase activity respectively, IC_{50} is the concentration of the inhibitor that results in 50 % inhibition of the enzyme activity, and HS is the Hill slope. The

experimental protocol was validated using apixaban as a standard drug. betrixaban could not be utilized due to its non-availability.

5.2.2. Thrombin inhibition studies

For thrombin inhibition studies, a mixture of 20 mM Tris-HCl buffer, *p*H 7.4, containing 100 mM sodium chloride, 2.5 mM calcium chloride, and 0.1% polyethylene glycol (PEG) 8000 was used as a buffer solution. Generally, 189 μL of *p*H 7.4 buffer was added to the wells, and 5 μL of potential thrombin inhibitor (or DMSO) and 4 μL of thrombin (6 nM final concentration) were sequentially added. After 10 min incubation, 2 μL of thrombin substrate (125 μM) was rapidly added and the residual thrombin activity was measured from the initial rate of increase in absorbance at 405 nm. Relative residual thrombin activity was calculated as given above with FXa.

5.2.3. *Ex vivo* PT prolongation[129]

Male rats weighing about 250-300 g were used for performing PT prolongation and clotting time experiments. Test compounds (formulated in 0.5% sodium carboxymethylcellulose), at a dose of 30 mg/kg and 5 mL/kg dosing volume was administered orally to animals using a gastric tube. After 2 h of oral administration of the test compound, citrated blood was collected from retro-orbital plexus. To measure PT, platelet-poor plasma was prepared by centrifugation. The same procedure was followed with the control group and standard drug. The protocol for *ex vivo* and *in vivo* studies was reviewed and approved by IAEC (Institutional Animal Ethics Committee) and experiments were performed as per CPCSEA (Committee for the Purpose of Supervision of Experiments on Animals) guidelines. (Approval No. MSU/IAEC/2016-17/1641).

5.2.4. Whole blood clotting time

Male rats weighing about 250-300 g were used for the measurement of whole blood clotting time using capillary tube method. Test compounds (formulated in 0.5% sodium carboxymethylcellulose), at a dose of 30 mg/kg

and 5 mL/kg dosing volume was administered orally to animals using a gastric tube. After 2 h of oral administration of the test compound, blood was collected into capillary tube from retro-orbital plexus. The timing was noted when blood starts to enter the tube. A piece of 1 cm glass tube was broken off from one end, at intervals of 15 seconds. Formation of fibrin threads between the broken ends was considered as the end-point of the experiment and the time was noted. The same procedure was followed with the control group and standard drug.

5.2.5. *In vitro* anticoagulant activity

Clotting time was determined by standard one-stage recalcification assay with a BBL Fibrosystem fibrometer (BectoneDickinson, Sparles, MD). For PT assay thromboplastin was reconstituted and warmed at 37 ^{0}C as per the manufacturer's directions. 2.5 µL of FXa inhibitor (test compound), with the desired concentration (1 mM) made up to 25 µL using citrated human plasma, was incubated for 30 sec at 37 ^{0}C followed by addition of 50 µL of pre-warmed thromboplastin. For the aPTT assay, 25 µL of pre-warmed aPTT reagent (0.2% ellagic acid) was mixed with 2.5 µL of inhibitor and 22.5 µL of citrated human plasma and kept for incubation at 37 ^{0}C for 4 min. Thereafter, immediate addition of 25 µL of pre-warmed 25 mM calcium chloride caused clotting and the clotting time was noted. Similar procedure was followed in the absence of FXa inhibitor using 2.5 µL of organic vehicle or saline.

5.2.6. *In vivo* $FeCl_3$ induced arterial thrombosis[129]

Male rats weighing between 300 and 350 g (n = 3 for each group) were anaesthetized with ketamine (100 mg/kg) and a polyethylene catheter (PE-205) was inserted into the trachea via tracheotomy to facilitate breathing. The right carotid artery was isolated and a small piece of Parafilm"M" was placed under the vessel to isolate it from surrounding tissues throughout the experiment. The test sample was administered orally before 2 h of initiation of thrombus formation. Thrombus formation was induced by the application of Whatman filter paper (2 × 5 mm) saturated with $FeCl_3$ solution, to the carotid artery for 10 min. The specific concentration of $FeCl_3$ solution was optimized

to 40 % w/v for uniform thrombus formation at our optimized experimental conditions. After removal of filter paper, the experiment was continued as such for 60 min. Then, the carotid artery was excised; thrombus was removed from the artery, blotted dry and immediately weighed.

5.3. Computational studies

5.3.1. Docking studies of compounds (107 and 237) with FXa enzyme

Docking studies were performed with Glide module of Schrodinger Suite 2009. Glide is intended 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 favorable interactions between ligand molecules and a receptor molecule, typically a protein. The 3D 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 using OPLS_2005 force field at physiological *p*H condition using LigPrep module of Schrödinger.

The 3D crystallographic structure for FXa was obtained from RCSB Protein Data Bank (PDB Code: **4A7I** for compound **107** and **2P16** for compound **237**) and prepared for docking with protein preparation wizard within Schrödinger suite. The grid was generated over the active site considering the ligand. The generated grid was validated by the co-crystallized ligand molecule in the 3D structure of FXa (PDB Code: **4A7I** and **2P16**). (Glide version 5.5, Schrödinger, LLC, New York, NY, 2009). After knocking out the co-crystallized ligand molecule, it was reconstructed followed by energy minimization, and the ligand was redocked into the active site of the enzyme. The interactions observed between the redocked ligand molecule and the enzyme were very similar to that obtained for the original co-crystallized structure. A few of the significant similarities included almost similar orientations of the groups in S1 and S4 binding pockets along with similar binding interactions with the amino acid residues TYR228, GLN192, and GLY216. The root mean-square deviation between the predicted conformation and the original conformation of the co-crystallized ligand was found to be

0.26 Å. Docking calculations for the minimized 3D ligand structures were performed in Extra Precision (XP) mode within the active site of the receptor structure. The shown interaction diagrams were generated in Chimera.[130]

5.3.2. Molecular dynamics simulations

Maestro-Desmond (based on OPLS-2005 force field) was used to achieve the simulations of the active molecules (**107** and **237**).[131,132] Initially, the system was solvated with TIP3P water model in orthorhombic box of 10 $Å^3$, and neutralized by Na^+ ions. The long-range electrostatic interactions were evaluated by Smooth particle-mesh Ewald (PME) approximation and non-bonded interactions were exploited by MSHAKE algorithm with the cut-off of 9 Å. To relax the system default six step relaxation protocol was followed, which consisted of restrained and unrestrained minimizations (2 steps) followed by the equilibration processes (4 steps). The production MD was performed for 10 ns with NPT ensemble at 300 K and 1 atm pressure using Nose-Hoover thermostat and Martyna–Tuckerman–Klein barostat. The energies and coordinates were recorded at every 1.2 and 10 ps respectively. The stability of ligand protein complexes was evaluated by calculating root-mean-square deviation of the protein (RMSD-P), root-mean-square fluctuation of the protein (RMSF-P) and root-mean-square deviation of the ligand (RMSD-L), molecular surface area (MolSA), solvent-accessible surface area (SASA), radius-of-gyration (rGyr), intramolecular hydrogen bonds (intraHB), and polar surface area (PSA).