SECTION-III

CHAPTER 4.
EXPERIMENTAL

4. EXPERIMENTAL

The experimental section has been divided into two parts- **chemical studies** involving the syntheses with analytical evaluations of the synthesized compounds and **biological studies** for biological evaluations.

4.1. Chemical studies

Melting points were measured using VEEGO Multi-programmable melting point apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on Bruker Avance II 400 MHz FT-NMR spectrometer. Chemical shifts are expressed in δ units relative to tetramethylsilane (TMS) signal as internal reference and DMSO-d_6 as common solvent. IR spectra were recorded on FT-IR-system-2000 Bruker spectrometer on KBr pellets. Mass spectra were recorded on Thermo Scientific DSQ-II Mass analyzer. Elemental analyses were recorded on ThermoFisher FLASH 2000 Organic elemental analyzer.

4.1.1. General procedure for the synthesis of 2-aminobenzamides (2)

In an oven dried round-bottom flask, substituted isatoic anhydride (**1**) (**R_1 = a-d**) (10 mmol), suitable amine (**R_2 = f-t**) (10 mmol) and K_2CO_3 (2g, 15 mmol) were stirred in DMF (20 ml) at 45^0 C for 45 min. Progress of the reaction was monitored by TLC. After completion, the reaction mixture was allowed to cool at room temperature and diluted with ice cold water (100 ml). The solid (**2**) that separated out, was filtered and washed with water (100ml X 2). The solid so obtained was dissolved in minimum amount of ethanol (10-20 ml) with gentle heating and diluted with ice cold water (100

ml). The precipitate was filtered, washed with water (100 ml) and dried yielding compound (**2**) with sufficient purity.

4.1.1.1. 2-Ethylamino-N-isobutylbenzamide (2bj)

Compound (**2bj**) was obtained as off-white solid (1.7 g, 79%), m. p.: 65-67 ^{0}C from 1-ethyl-*1H*-benzo[*d*]-1,3-oxazine-2,4-dione (**1b**) (1.9 g, 10 mmol).

Anal.:

IR **:** 3359, 3303, 1628, 1583, 1548, 744

^{1}H-NMR **:** 8.19-8.22 (m, 1H), 7.57-7.59 (m, 1H), 7.52-7.54 (d, 1H, *J* = 8.0 Hz), 7.21-7.25 (m, 1H), 6.60-6.62 (m, 1H), 6.50-6.54 (t, 1H, *J* = 8.0 Hz), 3.09-3.14 (m, 2H), 3.0-3.07 (m, 2H), 1.82-1.89 (m, 1H), 1.20-1.24 (t, 3H, *J* = 8.0 Hz), 0.92-0.96 (d, *J* = 8.0, 3H), 0.89-0.91(d, 3H, *J* = 8.0 Hz)

ESI-MS (m/z) **:** 221 (M^++1)

$C_{13}H_{17}N_3O$ requires C, 70.56; H, 9.25; N, 12.68. Found C, 70.21; H, 9.62; N, 12.37%

4.1.1.2. N-(4-Chlorobenzyl)-2-ethylaminobenzamide (2bq)

Compound (**2bq**) was obtained as off-white solid (2.3 g, 82%), m. p.: 94-96 ^{0}C from 1-ethyl-*1H*-benzo[*d*]-1,3-oxazine-2,4-dione (**1b**) (1.9 g, 10 mmol).

Anal.:

IR **:** 3394, 3314, 1627, 1580, 1517, 747

^{1}H-NMR **:** 7.70 (s, 1H), 7.59-7.61(d, 1H, *J* =8.0 Hz)`, 7.23-7.30 (m, 6H), 6.62-6.64 (d, 1H, *J* = 8.0 Hz), 6.51-6.55 (t, 1H, *J* = 8.0 Hz), 4.43 (s, 2H), 2.97-3.14 (m, 2H), 1.23-1.26 (m, 3H)

ESI-MS (m/z) **:** 289 (M^++1)

$C_{16}H_{17}ClN_2O$ requires C, 66.55; H, 5.93; N, 9.70. Found: C, 66.85; H, 5.67; N, 9.62%

4.1.1.3. N-(4-Fluorobenzyl)-2-ethylaminobenzamide (2br)

Compound (**2br**) was obtained as white solid (2.3 g, 85%), m. p.: 102-104 ^{0}C from 1-ethyl-*1H*-benzo[*d*]-1,3-oxazine-2,4-dione (**1b**) (1.9 g, 10 mmol).

Anal.:

IR **:** 3392, 3315, 1627, 1581, 1509, 748

^{1}H-NMR **:** 8.76-8.79 (t, 1H, *J* = 6.0 Hz), 7.70-7.73 (t, 1H, *J* = 6.0 Hz), 7.59-7.61(d, 1H, *J* = 6.0 Hz), 7.32-7.35 (m, 2H), 7.22-7.27 (m, 1H), 7.02-7.08 (m, 2H), 6.61-6.63 (m, 1H), 6.51-6.55 (t, 1H, *J* = 8.0 Hz), 4.41-4.43 (d, 2H, *J* = 8.0 Hz), 3.09-3.16 (m, 2H), 1.21-1.25 (t, 3H, *J* = 8.0 Hz)

ESI-MS (m/z) **:** 273 (M^++1)

$C_{16}H_{17}FN_2O$ requires C, 70.57; H, 6.29; N, 10.29. Found: C, 70.85; H, 6.67; N, 10.17%

4.1.1.4. N-(4-Chlorobenzyl)-2-propylaminobenzamide (2cq)

Compound (**2cq**) was obtained as off-white solid (2.1 g, 72%), m. p.: 69-71 ^{0}C from 1-propyl-*1H*-benzo[*d*]-1,3-oxazine-2,4-dione (**1c**) (2.0 g, 10 mmol).

Anal.:

IR **:** 3374, 3315, 1629, 1581, 1514, 749

^{1}H-NMR **:** 8.80-8.82 (m, 1H), 7.82-7.84 (m, 1H), 7.59-7.61(d, 1H, *J* = 8.0 Hz), 7.28-7.31 (m, 4H), 7.24-7.26 (t, 1H, *J* = 8.0 Hz), 6.62-6.64 (d, 1H, *J* = 8.0 Hz), 6.51-6.54 (m, 1H), 4.41-4.43 (m, 2H), 3.03-3.08 (m, 2H), 1.59-1.64 (m, 2H), 0.95-0.99 (t, 3H *J* = 8.0 Hz)

ESI-MS (m/z) **:** 303 (M^++1)

$C_{17}H_{19}ClN_2O$ requires C, 67.43; H, 6.32; N, 9.25. Found: C, 67.56; H, 6.22; N, 9.28%

4.1.1.5. 2-Allylamino-N-cyclohexylbenzamide (2dl)

Compound (**2dl**) was obtained as white solid (2.3 g, 89%), m. p.: 117-119 ^{0}C from 1-allyl-*1H*-benzo[*d*]-1,3-oxazine-2,4-dione (**1d**) (2.0 g, 10 mmol).

Anal.:

IR **:** 3359, 3266, 1623, 1583, 1515, 744

^{1}H-NMR **:** 7.98-8.12 (m, 2H), 7.50-7.52 (d, 1H, *J* =8.0 Hz), 7.18-7.21 (m, 1H), 6.59-6.61 (d, 1H, *J* = 8.0 Hz), 6.51-6.56 (t, 1H, *J* = 8.0 Hz), 5.95-5.97 (m, 1H), 5.25-5.27 (m, 1H), 5.11-5.14(m, 1H), 3.75-3.77 (m, 3H), 1.83-1.84 (m, 2H), 1.72-1.75 (m, 3H), 1.29-1.34 (m, 5H)

ESI-MS (m/z) **:** 259 (M^++1)

$C_{16}H_{22}N_2O$ requires C, 74.38; H, 8.58; N, 10.84. Found: C, 74.65; H, 8.68; N, 10.82%

4.1.1.6. 2-Allylamino-N-benzylbenzamide (2dm)

Compound (**2dm**) was obtained as off-white solid (2.1 g, 82%), m. p.: 50-51 ^{0}C from 1-allyl-*1H*-benzo[*d*]-1,3-oxazine-2,4-dione (**1d**) (2.0 g, 10 mmol).

Anal.:

IR **:** 3351, 3161, 1623, 1576, 1534, 750

^{1}H-NMR **:** 7.61-7.63 (m, 1H), 7.21-7.34 (m, 8H), 6.61-6.63 (d, 1H, *J* = 8.0 Hz), 6.54-6.58 (t, 1H, *J* = 8.0 Hz), 5.90-5.92 (m, 1H), 5.25-5.27 (m, 1H), 5.14-5.15 (m, 1H), 4.47-4.49 (m, 2H), 3.78-3.81 (m, 2H)

ESI-MS (m/z) **:** 267 (M^++1)

$C_{17}H_{18}N_2O$ requires C, 76.66; H, 6.81; N, 10.52. Found: C, 76.87; H, 6.63; N, 10.46%

4.1.1.7. 2-Allylamino-N-phenethylbenzamide (2ds)

Compound (**2ds**) was obtained as off-white solid (2.4 g, 87%), m. p.: 43-44 ^{0}C from 1-allyl-*1H*-benzo[*d*]-1,3-oxazine-2,4-dione (**1d**) (2.0 g, 10 mmol).

Anal.:

IR **:** 3394, 3346, 1624, 1582, 1516, 746

^{1}H-NMR **:** 7.85-7.89 (m, 1H), 7.46-7.48 (d, *J* = 8.0, 1H), 7.16-7.29 (m, 7H), 6.59-6.61 (d, 1H, *J* =8.0 Hz), 6.50-6.55 (t, 1H, *J* = 8.0 Hz), 5.95-5.97 (m, 1H), 5.25-5.27 (m, 1H), 5.14-5.15(m, 1H), 3.77-3.78 (m, 2H), 3.45-3.50 (m, 2H), 2.83-2.88 (m, 2H)

ESI-MS (m/z) **:** 281 (M^++1)

$C_{18}H_{20}N_2O$ requires C, 77.11; H, 7.19; N, 9.99. Found: C, 77.43; H, 7.12; N, 9.82%

4.1.1.8. 2-Allylamino-N-isopropylbenzamide (2di)

Compound (**2di**) was obtained as off-white solid (2.0 g, 92%), m. p.: 60-61 ^{0}C from 1-allyl-*1H*-benzo[*d*]-1,3-oxazine-2,4-dione (**1d**) (2.0 g, 10 mmol).

Anal.:

IR **:** 3356, 3308, 1624, 1583, 1540, 745

^{1}H-NMR **:** 7.94-7.96 (d, 1H, *J* = 8.0 Hz), 7.83-7.85 (m, 1H), 7.51-7.53 (d, 1H, *J* = 8.0 Hz), 7.19-7.23 (t, 1H, *J* = 8.0 Hz), 6.59-6.61 (d, 1H, *J* = 8.0 Hz), 6.51-6.55 (t, 1H, *J* = 8.0 Hz), 5.91-5.94 (m, 1H), 5.24-5.26 (m, 1H), 5.11-5.14 (m, 1H), 4.09-4.11(m, 1H), 3.76-3.79 (m, 2H), 1.15-1.18 (m, 6H)

ESI-MS (m/z) **:** 219 (M^++1)

$C_{13}H_{18}N_2O$ requires C, 71.53; H, 8.31; N, 12.83. Found: C, 71.87; H, 8.22; N, 12.75%

4.1.1.9. 2-Allylamino-N-butylbenzamide (2dh)

Compound (**2dh**) was obtained as off-white solid (1.8 g, 89%), m. p.: 42-43 ^{0}C from 1-allyl-*1H*-benzo[*d*]-1,3-oxazine-2,4-dione (**1d**) (2.0 g, 10 mmol).

Anal.:

IR **:** 3398, 3293, 1627, 1583, 1540, 749

^{1}H-NMR **:** 7.90-8.12 (m, 2H), 7.21-7.52 (m, 2H), 6.54-6.60 (m, 2H), 5.90-5.92 (m, 1H), 5.22-5.26 (m, 1H), 5.13-5.14 (m, 1H), 3.24-3.78 (m, 4H), 1.35-1.52 (m, 4H), 0.89-0.92 (m, 3H)

ESI-MS (m/z) **:** 233 (M^++1)

$C_{14}H_{20}N_2O$ requires C, 72.38; H, 8.68; N, 12.06. Found: C, 72.59; H, 8.56; N, 12.16%

4.1.1.10. 2-Allylamino-N-cyclopropylbenzamide (2dk)

Compound (**2dk**) was obtained as off-white solid (1.7 g, 82%), m. p.: 64-66 ^{0}C from 1-allyl-*1H*-benzo[*d*]-1,3-oxazine-2,4-dione (**1d**) (2.0 g, 10 mmol).

Anal.:

IR **:** 3397, 3269, 1626, 1583, 1517, 746

^{1}H-NMR **:** 7.97-8.23 (m, 2H), 7.47-7.49 (d, 1H, *J* = 8.0 Hz), 7.19-7.23 (t, 1H *J* = 8.0 Hz), 6.59-6.61 (d, 1H, *J* = 8.0 Hz), 6.49-6.53 (t, 1H *J* = 8.0 Hz) 5.95-5.97 (m, 1H), 5.22-5.26 (m, 1H), 5.12-5.15 (m, 1H), 3.34-3.78(m, 3H), 0.68-0.70 (m, 2H), 0.57-0.60 (m, 2H)

ESI-MS (m/z) **:** 217 (M^++1)

$C_{13}H_{16}N_2O$ requires C, 72.19; H, 7.46; N, 12.95. Found: C, 72.45; H, 7.36; N, 12.88%

4.1.1.11. N-(2-Chlorobenzyl)-2-allylaminobenzamide (2do)

Compound (**2do**) was obtained as off-white solid (2.5 g, 85%), m. p.: 66-68 ^{0}C from 1-allyl-*1H*-benzo[*d*]-1,3-oxazine-2,4-dione (**1d**) (2.0 g, 10 mmol).

Anal.:

IR **:** 3372, 3338, 1629, 1577, 1522, 749

^{1}H-NMR **:** 7.23-7.41 (m, 8H), 6.63-6.65 (d, 1H, *J* = 8.0 Hz), 6.57-6.60 (m, 1H), 5.93-5.95 (m, 1H), 5.21-5.26 (m, 1H), 5.11-5.14 (m, 1H), 3.79-4.53 (m, 4H)

ESI-MS (m/z) **:** 301 (M^++1)

$C_{17}H_{17}ClN_2O$ requires C, 67.88; H, 5.70; N, 9.31. Found: C, 67.68; H, 5.65; N, 9.26%

4.1.1.12. N-(3-Chlorobenzyl)-2-allylaminobenzamide (2dp)

Compound (**2dp**) was obtained as off-white solid (2.3 g, 81%), m. p.: 68-69 ^{0}C from 1-allyl-*1H*-benzo[*d*]-1,3-oxazine-2,4-dione (**1d**) (2.0 g, 10 mmol).

Anal.:

IR **:** 3368, 3341, 1628, 1580, 1519, 751

^{1}H-NMR **:** 7.60-7.63 (m, 2H), 7.24-7.33 (m, 6H), 6.62-6.64 (d, 1H *J* = 8.0 Hz), 6.55-6.58 (m, 1H), 5.90-5.92 (m, 1H), 5.21-5.26 (m, 1H), 5.12-5.14 (d, 1H *J* = 8.0 Hz), 4.43-4.45 (m, 2H), 3.77-3.79 (m, 2H)

ESI-MS (m/z) **:** 301 (M^++1)

$C_{17}H_{17}ClN_2O$ requires C, 67.88; H, 5.70; N, 9.31. Found: C, 67.67; H, 5.65; N, 9.25%

4.1.1.13. N-(4-Chlorobenzyl)-2-allylaminobenzamide (2dq)

Compound (**2dq**) was obtained as off-white solid (2.4 g, 83%), m. p.: 65-66 ^{0}C from 1-allyl-*1H*-benzo[*d*]-1,3-oxazine-2,4-dione (**1d**) (2.0 g, 10 mmol).

Anal.:

IR **:** 3397, 3347, 1627, 1579, 1515, 745

^{1}H-NMR **:** 7.58-7.61 (m, 2H), 7.22-7.31 (m, 6H), 6.61-6.63 (d, 1H, *J* = 8.0 Hz), 6.55-6.58 (m, 1H), 5.85-5.85 (m, 1H), 5.22-5.27 (m, 1H), 5.12-5.14 (d, 1H, *J* = 8.0 Hz), 3.79-4.45 (m, 4H)

ESI-MS (m/z) **:** 301 (M^++1)

$C_{17}H_{17}ClN_2O$ requires C, 67.88; H, 5.70; N, 9.31. Found: C, 67.76; H, 5.65; N, 9.27%

4.1.1.14. N-(4-Fluorobenzyl)-2-allylaminobenzamide (2dr)

Compound (**2dr**) was obtained as off-white solid (2.4 g, 85%), m. p.: 66-68 ^{0}C from 1-allyl-*1H*-benzo[*d*]-1,3-oxazine-2,4-dione (**1d**) (2.0 g, 10 mmol).

Anal.:

IR **:** 3299, 3067, 1626, 1582, 1513, 749

^{1}H-NMR **:** 8.83-8.85 (m, 1H), 7.99-8.02 (m, 1H), 7.60-7.62 (d, 1H, *J* = 8.0 Hz), 7.33-7.36 (m, 2H), 7.22-7.26 (t, 1H, *J* = 8.0 Hz), 7.06-7.10 (t, 2H, *J* = 8.0 Hz), 6.62-6.64 (d, 1H *J* =8.0 Hz), 6.53-6.57 (t, 1H, *J* = 8.0 Hz), 5.87-5.97 (m, 1H), 5.20-5.25 (m, 1H), 5.11-5.14 (m, 1H), 3.79-4.43 (m, 4H)

ESI-MS (m/z) **:** 285 (M^++1)

$C_{17}H_{17}FN_2O$ requires C, 71.81; H, 6.03; N, 9.85. Found: C, 71.68; H, 6.11; N, 9.82%

4.1.1.15. 2-Allylamino-N-isobutylbenzamide (2dj)

Compound (**2dj**) was obtained as off-white solid (2.1 g, 91%), m. p.: 47-48 ^{0}C from 1-allyl-*1H*-benzo[*d*]-1,3-oxazine-2,4-dione (**1d**) (2.0 g, 10 mmol).

Anal.:

IR **:** 3388, 3333, 1626, 1583, 1516, 748

^{1}H-NMR **:** 8.19-8.22 (m, 1H), 7.83-7.86 (m, 1H), 7.53-7.55 (d, 1H, *J* = 8.0 Hz), 7.19-7.23 (t, 1H, *J* = 8.0 Hz), 6.59-6.61 (d, 1H, *J* = 8.0 Hz), 6.52-6.56 (t, 1H, *J* = 8.0 Hz), 5.88-5.97 (m, 1H), 5.21-5.27 (m, 1H), 5.11-5.14 (m, 1H), 3.76-3.79 (m, 2H), 3.05-3.08 (m, 2H), 1.83-1.90 (m, 1H), 0.90-0.92 (d, 6H, *J* = 8.0 Hz)

ESI-MS (m/z) **:** 233 (M^++1)

$C_{14}H_{20}N_2O$ requires C, 72.38; H, 8.68; N, 12.06. Found: C, 72.67; H, 8.62; N, 12.12%

4.1.1.16. 2-Benzylamino-N-phenethylbenzamide (2es)

Compound (**2es**) was obtained as white solid (2.5 g, 76%), m. p.: 65-67 ^{0}C from 1-benzyl-*1H*-benzo[*d*]-1,3-oxazine-2,4-dione (**1e**) (2.5 g, 10 mmol).

Anal.:

IR **:** 3425, 3347, 1628, 1580, 1516, 747

^{1}H-NMR **:** 7.47-7.49(d, 2H, *J* = 8.0 Hz), 7.17-7.33 (m, 12H), 6.57-6.59 (d, 1H, *J* =8.0 Hz), 6.52-6.54 (d, 1H, *J* = 8.0 Hz), 4.34-4.36 (m, 2H), 3.49-3.50 (m, 2H), 2.85-2.90 (m, 2H)

ESI-MS (m/z) **:** 331 (M^++1)

$C_{22}H_{22}N_2O$ requires C, 79.97; H, 6.71; N, 8.48. Found: C, 79.43; H, 6.58; N, 8.56%

4.1.1.17. 2-(Benzylamino)-N-cyclohexylbenzamide (2el)

Compound (**2el**) was obtained as white solid (2.8 g, 92%), m. p.: 132-134 ^{0}C from 1-benzyl-*1H*-benzo[*d*]-1,3-oxazine-2,4-dione (**1e**) (2.5 g, 10 mmol).

Anal.:

IR **:** 3330, 3159, 1621, 1580, 1532, 749

^{1}H-NMR **:** 7.95-7.96 (m, 2H), 7.51-7.53 (d, 1H, *J* = 8.0 Hz), 7.28-7.35 (m, 4H), 7.15-7.24 (m, 2H), 6.57-6.59 (d, 1H, *J* = 8.0 Hz), 6.53-6.55 (d, 1H, *J* = 8.0 Hz), 4.33-4.35 (m, 2H), 3.77-3.78 (m, 1H), 1.17-1.88 (m, 10H)

ESI-MS (m/z) **:** 309 (M^++1)

$C_{20}H_{24}N_2O$ requires C, 77.89; H, 7.84; N, 9.08. Found: C, 77.98; H, 7.64; N, 9.12%

4.1.1.18. 2-Benzylamino-N-cyclopropylbenzamide (2ek)

Compound (**2ek**) was obtained as white solid (2.1 g, 82%), m. p.: 112-114 ^{0}C from 1-benzyl-*1H*-benzo[*d*]-1,3-oxazine-2,4-dione (**1e**) (2.5 g, 10 mmol).

Anal.:

IR **:** 3307, 3082, 1623, 1579, 1526, 749

^{1}H-NMR **:** 8.22-8.27 (m, 1H), 7.49-7.51 (d, 2H, *J* = 8.0 Hz), 7.29-7.36 (m, 4H), 7.15-7.24 (m, 2H), 6.58-6.60 (d, 1H, *J* = 8.0 Hz), 6.50-6.54 (t, 1H, *J* = 8.0 Hz), 4.42-4.44 (m, 2H), 3.32-3.34 (d, 2H, *J* = 8.0 Hz), 2.79-2.81 (m, 1H), 0.69-0.72 (m, 2H), 0.61-0.65 (m, 2H)

ESI-MS (m/z) **:** 267 (M^++1)

$C_{17}H_{18}N_2O$ requires C, 76.66; H, 6.81; N, 10.52. Found: C, 76.87; H, 6.67; N, 10.48%

4.1.1.19. N-(4-Methoxybenzyl)-2-benzylaminobenzamide (2en)

Compound (**2en**) was obtained as white solid (3.0 g, 88%), m. p.: 96-98 ^{0}C from 1-benzyl-*1H*-benzo[*d*]-1,3-oxazine-2,4-dione (**1e**) (2.5 g, 10 mmol).

Anal.:

IR **:** 3345, 3056, 1626, 1581, 1514, 751

^{1}H-NMR **:** 7.67-7.69 (d, 1H, *J* = 8.0 Hz), 7.59-7.61(d, 1H, *J* = 8.0 Hz), 7.16-7.32 (m, 8H), 6.91-6.93 (d, 1H, *J* = 8.0 Hz), 6.82-6.84 (d, 2H, *J* = 8.0 Hz), 6.58-6.60 (d, 1H, *J* = 8.0 Hz), 6.52-6.56 (t, 1H, *J* = 8.0 Hz), 4.38-4.40 (m, 2H), 3.74-4.35 (m, 5H)

ESI-MS (m/z) **:** 347 (M^++1)

$C_{22}H_{22}N_2O_2$ requires C, 76.28; H, 6.40; N, 8.09. Found: C, 76.10; H, 6.56; N, 8.13%

4.1.1.20. N-(2-Chlorobenzyl)-2-benzylaminobenzamide (2eo)

Compound (**2eo**) was obtained as white solid (2.8 g, 82%), m. p.: 92-94 ^{0}C from 1-benzyl-*1H*-benzo[*d*]-1,3-oxazine-2,4-dione (**1e**) (2.5 g, 10 mmol).

Anal.:

IR **:** 3343, 3279, 1625, 1583, 1544, 743

^{1}H-NMR **:** 7.72-7.74(d, 1H, *J* = 8.0 Hz), 7.20-7.46 (m, 12H), 6.61-6.63 (d, 1H, *J* = 8.0 Hz), 6.56-6.60 (t, 1H, *J* = 8.0 Hz), 4.36-4.54 (m, 4H)

ESI-MS (m/z) **:** 351 (M^++1)

$C_{21}H_{19}ClN_2O$ requires C, 71.89; H, 5.46; N, 7.98. Found: C, 71.76; H, 5.52; N, 7.82%

4.1.1.21. N-(4-Chlorobenzyl)-2-benzylaminobenzamide (2eq)

Compound (**2eq**) was obtained as white solid (3.1 g, 89%), m. p.: 112-114 ^{0}C from 1-benzyl-*1H*-benzo[*d*]-1,3-oxazine-2,4-dione (**1e**) (2.5 g, 10 mmol).

Anal.:

IR **:** 3410, 3361, 1631, 1580, 1515, 747

^{1}H-NMR **:** 7.62-7.64 (d, 1H, *J* = 8.0 Hz), 7.18-7.39 (m, 12H), 6.60-6.62 (d, 1H, *J* = 8.0 Hz), 6.53-6.57 (t, 1H, *J* = 8.0 Hz), 4.36-4.43 (m, 4H)

ESI-MS (m/z) **:** 351 (M^++1)

$C_{21}H_{19}ClN_2O$ requires C, 71.89; H, 5.46; N, 7.98. Found: C, 71.56; H, 5.58; N, 7.86%

4.1.1.22. N-(4-Fluorobenzyl)-2-benzylaminobenzamide (2er)

Compound (**2er**) was obtained as white solid (2.8 g, 84%), m. p.: 92-94 ^{0}C from 1-benzyl-*1H*-benzo[*d*]-1,3-oxazine-2,4-dione (**1e**) (2.5 g, 10 mmol).

Anal.:

IR **:** 3352, 3270, 1625, 1579, 1546, 752

^{1}H-NMR **:** 8.28-8.79 (m, 2H), 7.61-7.63 (d, 1H, *J* = 8.0 Hz), 7.28-7.34 (m, 6H), 7.13-7.23 (m, 2H), 7.01-7.05 (m, 2H), 6.59-6.61 (d,

1H, *J* = 8.0 Hz), 6.53-6.57 (t, 1H, *J* = 8.0 Hz), 4.36-4.44 (m, 4H)

ESI-MS (m/z) **:** 335 (M^++1)

$C_{21}H_{19}FN_2O$ requires C, 75.43; H, 5.73; N, 8.38. Found: C, 75.56; H, 5.85; N, 8.42%

4.1.1.23. 2-Benzylamino-N-isobutylbenzamide (2ej)

Compound (**2ej**) was obtained as white solid (2.6 g, 94%), m. p.: 82-84 ^{0}C from 1-benzyl-*1H*-benzo[*d*]1,3-oxazine-2,4-dione (**1e**) (2.5 g, 10 mmol).

Anal.:

IR **:** 3409, 3266, 1624, 1578, 1549, 752

^{1}H-NMR **:** 8.28-8.32 (m, 2H), 7.54-7.56 (d, 1H, *J* = 8.0 Hz), 7.28-7.35 (m, 4H), 7.15-7.23(m, 2H), 6.53-6.59 (m, 2H), 4.32-4.35 (m, 2H), 3.08-3.10 (d, 2H, *J* = 8.0 Hz), 1.83-1.91 (m, 1H), 0.86-0.93 (m, 6H)

ESI-MS (m/z) **:** 283 (M^++1)

$C_{18}H_{22}N_2O$ requires C, 76.56; H, 7.85; N, 9.92. Found: C, 76.43; H, 7.65; N, 9.86%

4.1.2. General Procedures for the synthesis of 1,3-disubstituted-2-imino-2,3-dihydroquinazolin-4(*1H*)-ones (3)

4.1.2.1. Method 1: In an oven dried round bottom flask compound (**2**) (10 mmol), cyanogen bromide (2.6 g, 25 mmol) and ethanol were mixed and refluxed for eight hr.

Progress of the reaction was monitored by TLC. After completion of reaction, the reaction volume was reduced under vacuum and diluted with ice cold water (100 ml). The solid (**3**) that separated out, was filtered and washed with water (100ml X 2). The solid so obtained was dissolved in minimum amount of ethanol (10-20 ml) with gentle heating and diluted with ice cold water (100 ml). The precipitate was filtered, washed with water (100 ml) and dried yielding compound (**3**) with sufficient purity.

4.1.2.2. Method 2: In an oven dried round bottom flask compound (**2**) (10 mmol), cyanogen bromide (2.6 g, 25 mmol) and ethanol (30 ml) were mixed and stirred at RT for eighteen hr. Progress of the reaction was monitored by TLC. After completion of reaction, the reaction volume was reduced under vacuum and diluted with ice cold water (100 ml). The solid that separated out, was filtered and washed with water (100ml X 2). The solid so obtained was dissolved in minimum amount of ethanol (10-20 ml) with gentle heating and diluted with ice cold water (100 ml), precipitate was filtered, washed with water (100 ml) and dried yielding compound (**3**) with sufficient purity.

4.1.2.3. Method 3: In an oven dried round bottom flask compound (**2**) (10 mmol), cyanogen bromide (2.6 g, 25 mmol) and methanol (30 ml) were mixed and stirred at RT for ten hr followed by refluxing for eight hr. The reaction mixture was cooled to RT and poured into ice cold water (100 ml). The product could not be isolated.

4.1.2.4. Method 4: In an oven dried round bottom flask compound (**2**) (10 mmol), cyanogen bromide (2.6 g, 25 mmol) and toluene (30 ml) were mixed and refluxed for

twelve hr. The reaction mixture was cooled at RT and poured into ice cold water (100 ml). The product could not be isolated.

4.1.2.5. Method 5: In an oven dried round bottom flask compound (**2**) (10 mmol), cyanogen bromide (2.6 g, 25 mmol) and DMSO (20 ml) were mixed and stirred at RT for eighteen hr followed by heating at 110^0 C for ten hr. Progress of the reaction was monitored by TLC. After completion of reaction, the reaction mixture was diluted with ice cold water (100 ml). The solid that separated out, was filtered and washed with water (100ml X 2). The solid so obtained was dissolved in minimum amount of ethanol (10-20 ml) with gentle heating and diluted with ice cold water (100 ml). The precipitate was filtered, washed with water (100 ml) and dried yielding compound (**3**) with sufficient purity.

4.1.2.6. Method 6: In an oven dried round bottom flask compound (**2**) (10 mmol), cyanogen bromide (2.6 g, 25 mmol) and IL (15 ml) were mixed and stirred at RT for eight hr followed by heating at 90^0 C for ten hr. Progress of the reaction was monitored by TLC. After completion of reaction, the reaction mixture was diluted with ice cold water (100 ml). The solid that separated out, was filtered and washed with water (100ml X 2). The solid so obtained was dissolved in minimum amount of ethanol (10-20 ml) with gentle heating and diluted with ice cold water (100 ml). The precipitate was filtered, washed with water (100 ml) and dried yielding compound (**3**) with sufficient purity.

4.1.2.7. Method 7: In an oven dried round bottom flask compound (**2**) (10 mmol), cyanogen bromide (2.6 g, 25 mmol) and a mixture of DMSO:IL (10:1) (20 ml) were mixed and stirred at RT for 25 min. Progress of the reaction was monitored by TLC. After completion of reaction, the reaction mixture was diluted with ice cold water (100 ml). The solid that separated out, was filtered and washed with water (100ml X 2). The solid so obtained was dissolved in minimum amount of ethanol (10-20 ml) with gentle heating and diluted with ice cold water (100 ml). The precipitate was filtered, washed with water (100 ml) and dried yielding compound (**3**) with sufficient purity.

4.1.2.8. Method 8: In an oven dried round bottom flask compound (**2**) (10 mmol), cyanogen bromide (2.6 g, 25 mmol) and a mixture of DMSO:IL (10:1) (20 ml) were mixed and stirred at 90^0 C for 8 min. Progress of the reaction was monitored by TLC. After completion of reaction, the reaction mixture was diluted with ice cold water (100 ml). The solid that separated out, was filtered and washed with water (100ml X 2). The solid so obtained was dissolved in minimum amount of ethanol (10-20 ml) with gentle heating and diluted with ice cold water (100 ml). The precipitate was filtered, washed with water (100 ml) and dried yielding compound (**3**) with sufficient purity.

4.1.2.8.1. 1,3-Dimethyl-2,3-dihydroquinazolin-4(*1H*)-one (3af)

Compound (**3af**) was obtained as off-white amorphous solid (1.6 g, 89%), m. p.: 240-243 ^{0}C from compound (**2af**) (1.6 g, 10 mmol).

Anal.:

IR **:** 3122, 1698, 1663, 1610, 1402, 1344

ESI-MS (m/z) **:** 190 (M^++1)

$C_{10}H_{11}N_3O$ requires C, 63.48; H, 5.86; N, 22.21. Found: C, 63.27; H, 5.99; N, 22.12%

4.1.2.8.2. 3-Butyl-2-imino-1-methyl-2,3-dihydroquinazolin-4(*1H*)-one (3ah)

Compound (**3ah**) was obtained as off-white amorphous solid (2.0 g, 90%), m. p.: 219-221 ^{0}C from compound (**2ah**) (1.9 g, 10 mmol).

Anal.:

IR **:** 3128, 1697, 1655, 1612, 1400

^{1}H-NMR **:** 10.57 (bs, 1H), 8.56-8.58 (d, 1H, *J* = 8.0 Hz), 7.92-7.94 (t, 1H, *J* = 8.0 Hz), 7.53-7.55 (d, 1H, *J* = 8.0 Hz), 7.41-7.45 (t, 1H, *J* = 8.0 Hz), 3.67-3.71 (q, 2H, *J* = 8.0 Hz), 3.60 (s, 3H), 1.65-1.70 (m, 2H), 1.40-1.45 (m, 2H), 0.94-0.98 (t, 3H, *J* = 8.0 Hz)

ESI-MS (m/z) **:** 232 (M^++1)

$C_{13}H_{17}N_3O$ requires C, 67.51; H, 7.41; N, 18.17. Found: C, 67.25; H, 7.81; N, 18.33%

4.1.2.8.3. 3-Isopropyl-2-imino-1-methyl-2,3-dihydroquinazolin-4(*1H*)-one (3ai)

Compound (**3ai**) was obtained as off-white amorphous solid (1.7 g, 82%), m. p.: 278-280 ^{0}C from compound (**2ai**) (1.9 g, 10 mmol).

Anal.:

IR **:** 3123, 1719, 1634, 1616, 1400

ESI-MS (m/z) **:** 218 (M^++1)

$C_{12}H_{15}N_3O$ requires C, 66.34; H, 6.96; N, 19.34. Found: C, 66.05; H, 6.49; N, 19.52%

4.1.2.8.4. 3-Cyclohexyl-2-imino-1-methyl-2,3-dihydroquinazolin-4(*1H*)-one (3al)

Compound (**3al**) was obtained as off-white amorphous solid (2.3 g, 92%), m. p.: 279-281 ^{0}C from compound (**2al**) (2.3 g, 10 mmol).

Anal.:

IR **:** 3128, 1719, 1657, 1617, 1400

^{1}H-NMR **:** 9.89-9.91 (bs, 1H), 8.79-8.61 (d, 1H, *J* = 8.0 Hz), 7.89-7.93 (t, 1H *J* = 8.0 Hz), 7.52-7.54 (d, 1H, *J* = 8.0 Hz), 7.40-7.44 (t, 1H, *J* = 8.0 Hz), 4.15-4.23 (m, 1H), 3.59 (s, 3H), 1.94-1.96 (d, 2H, *J* = 8.0 Hz), 1.81-1.84 (d, 2H, *J* = 12.0 Hz), 1.68-1.71 (d, 1H, *J* = 12.0 Hz), 1.55-1.64 (q, 2H, *J* = 12.0 Hz), 1.36-1.45 (q, 2H, *J* = 12.0 Hz), 1.24 (m, 1H)

ESI-MS (m/z) **:** 258 (M^++1)

$C_{15}H_{19}N_3O$ requires C, 70.01; H, 7.44; N, 16.33. Found: C, 70.41; H, 7.64; N, 16.69%

4.1.2.8.5. 3-Benzyl-2-imino-1-methyl-2, 3-dihydroquinazolin-4(*1H*)-one (3am)

Compound (**3am**) was obtained as off-white amorphous solid (2.1 g, 82%), m. p.: 241-244 ^{0}C from compound (**2am**) (2.4 g, 10 mmol).

Anal.:

IR **:** 3129, 1694, 1638, 1614, 1400

ESI-MS (m/z) **:** 266 (M^++1)

$C_{16}H_{15}N_3O$ requires C, 72.43; H, 5.70; N, 15.84. Found: C, 72.15; H, 5.61; N, 15.99%

4.1.2.8.6. 3-(4-Methoxybenzyl)-2-imino-1-methyl-2,3-dihydroquinazolin-4(*1H*)-one (3an)

Compound (**3an**) was obtained as off-white amorphous solid (2.3 g, 81%), m. p.: 241-244 ^{0}C from compound (**2an**) (2.7 g, 10 mmol).

Anal.:

IR **:** 3129, 1691, 1649, 1612, 1400

ESI-MS (m/z) **:** 296 (M^++1)

$C_{17}H_{18}N_2O_2$ requires C, 72.32; H, 6.43; N, 9.92. Found: C, 72.09; H, 6.11; N, 9.79%

4.1.2.8.7. 1-Ethyl-2-imino-3-propyl-2,3-dihydroquinazolin-4(*1H*)-one (3bg)

Compound (**3bg**) was obtained as white amorphous solid (1.9 g, 84%), m. p.: 245-247 ^{0}C from compound (**2bg**) (2.0 g, 10 mmol).

Anal.:

IR **:** 3129, 1699, 1657, 1615, 1400

ESI-MS (m/z) **:** 232 (M^++1)

$C_{13}H_{17}N_3O$ requires C, 67.51; H, 7.41; N, 18.17. Found: C, 67.15; H, 7.89; N, 18.43%

4.1.2.8.8. 3-Butyl-1-ethyl-2-imino-2,3-dihydroquinazolin-4(*1H*)-one (3bh)

Compound (**3bh**) was obtained as white amorphous solid (2.2 g, 92%), m. p.: 204-206 ^{0}C from compound (**2bh**) (2.0 g, 10 mmol).

Anal.:

IR **:** 3126, 1694, 1660, 1613, 1399

^{1}H-NMR **:** 10.56 (bs, 1H), 8.58-8.60 (d, 1H, *J* = 8.0 Hz), 7.90-7.94 (t, 1H, *J* = 8.0 Hz), 7.58-7.60 (d, 1H, *J* = 8.0 Hz), 7.40-7.44 (t, 1H, *J* = 8.0 Hz), 4.19-4.25 (q, 2H, *J* = 8.0 Hz), 3.65-3.71 (q, 2H, *J* = 8.0 Hz), 1.64-1.72 (m, 2H), 1.37-1.47 (m, 2H), 1.28-1.32 (t, 3H, *J* = 8.0 Hz), 0.94-0.98 (t, 3H, *J* = 8.0 Hz)

ESI-MS (m/z) **:** 246 (M^++1)

$C_{14}H_{19}N_3O$ requires C, 68.54; H, 7.81; N, 17.13. Found: C, 68.78; H, 7.97; N, 17.33%

4.1.2.8.9. 1-Ethyl-2-imino-3-isopropyl-2,3-dihydroquinazolin-4(*1H*)-one (3bi)

Compound (**3bi**) was obtained as white amorphous solid (2.1 g, 94%), m. p.: 261-263 ^{0}C from compound (**2bi**) (2.0 g, 10 mmol).

Anal.:

IR **:** 3117, 1694, 1650, 1614, 1400

^{1}H-NMR **:** 10.05 (bs, 1H), 8.62-8.65 (m, 1H), 7.90-7.94 (t, 1H, *J* = 8.0 Hz), 7.53-7.55 (d, 1H, *J* = 8.0 Hz), 7.41-7.43 (t, 1H, *J* = 8.0 Hz), 4.50-4.58 (m, 1H), 4.20-4.26 (q, 2H, *J* = 8.0 Hz), 1.39-1.41 (d, 6H, *J* = 8.0 Hz), 1.31-1.35 (t, 3H, *J* = 8.0 Hz)

ESI-MS (m/z) **:** 232 (M^++1)

$C_{13}H_{17}N_3O$ requires C, 67.51; H, 7.41; N, 18.17. Found: C, 67.35; H, 7.85; N, 18.33%

4.1.2.8.10. 3-Cyclopropyl-1-ethyl-2-imino-2,3-dihydroquinazolin-4(*1H*)-one (3bk)

Compound (**3bk**) was obtained as white amorphous solid (1.8 g, 79%), m. p.: 248-250 ^{0}C from compound (**2bk**) (2.0 g, 10 mmol).

Anal.:

IR **:** 3375, 1689, 1638, 1620, 1400

ESI-MS (m/z) **:** 230 (M^++1)

$C_{13}H_{15}N_3O$ requires C, 68.10; H, 6.59; N, 18.33. Found: C, 68.49; H, 6.78; N, 18.12%

4.1.2.8.11. 3-Cyclohexyl-1-ethyl-2-imino-2,3-dihydroquinazolin-4(*1H*)-one (3bl)

Compound (**3bl**) was obtained as white amorphous solid (2.2 g, 83%), m. p.: 244-246 ^{0}C from compound (**2bl**) (2.2 g, 10 mmol).

Anal.:

IR **:** 3129, 1616, 1597, 1536, 1401

ESI-MS (m/z) **:** 272 (M^++1)

$C_{16}H_{21}N_3O$ requires C, 70.82; H, 7.80; N, 15.49. Found: C, 70.43; H, 7.98; N, 15.16%

4.1.2.8.12. 3-Benzyl-1-ethyl-2-imino-2,3-dihydroquinazolin-4(*1H*)-one (3bm)

Compound (**3bm**) was obtained as white amorphous solid (2.4 g, 88%), m. p.: 241-243 ^{0}C from compound (**2bm**) (2.3 g, 10 mmol).

Anal.:

IR **:** 3129, 1688, 1642, 1614, 1397

^{1}H-NMR **:** 11.40 (bs, 1H), 8.86-8.88 (d, 1H, *J* = 8.0 Hz), 7.83-7.87 (t, 1H, *J* = 8.0 Hz), 7.36-7.39 (m, 4H), 7.27-7.33 (m, 3H), 4.98-5.00 (d, 2H, *J* = 8.0 Hz), 4.13-4.19 (q, 2H, *J* = 8.0 Hz), 1.28-1.32 (t, 3H, *J* = 8.0 Hz)

ESI-MS (m/z) **:** 280 (M^++1)

$C_{17}H_{17}N_3O$ requires C, 73.10; H, 6.13; N, 15.0. Found: C, 73.54; H, 6.29; N, 15.44%

4.1.2.8.13. 3-(2-Chlorobenzyl)-1-ethyl-2-imino-2,3-dihydroquinazolin-4(*1H*)-one (3bo)

Compound (**3bo**) was obtained as white amorphous solid (2.5 g, 82%), m. p.: 222-224 ^{0}C from compound (**2bo**) (2.5 g, 10 mmol).

Anal.:

IR **:** 3129, 1689, 1645, 1616, 1400

ESI-MS (m/z) **:** 314 (M^++1)

$C_{17}H_{17}N_3O$ requires C, 65.07; H, 5.14; N, 13.39. Found: C, 65.36; H, 5.52; N, 13.17%

4.1.2.8.14. 1-Ethyl-2-imino-3-phenethyl-2,3-dihydroquinazolin-4(*1H*)-one (3bs)

Compound (**3bs**) was obtained as white amorphous solid (2.4 g, 83%), m. p.: 234-236 ^{0}C from compound (**2bs**) (2.7 g, 10 mmol).

Anal.:

IR **:** 3111, 1689, 1641, 1618**,** 1401

ESI-MS (m/z) **:** 294 (M^++1)

$C_{18}H_{19}N_3O$ requires C, 73.69; H, 6.53; N, 14.32. Found: C, 73.45; H, 6.12; N, 14.67%

4.1.2.8.15. 2-Imino-3-methyl-1-propyl-2,3-dihydroquinazolin-4(*1H*)-one (3cf)

Compound (**3cf**) was obtained as off-white amorphous solid (1.7 g, 81%), m. p.: 248-250 ^{0}C from compound (**2cf**) (1.9 g, 10 mmol).

Anal.:

IR **:** 3451.41, 3420, 3127, 1721, 1689, 1661, 1614, 1401

ESI-MS (m/z) **:** 218 (M^++1)

$C_{12}H_{15}N_3O$ requires C, 66.34; H, 6.96; N, 19.34. Found: C, 66.18; H, 6.59; N, 19.62%

4.1.2.8.16. 3-Butyl-2-imino-1-propyl-2,3-dihydroquinazolin-4(*1H*)-one (3ch)

Compound (**3ch**) was obtained as off-white amorphous solid (2.4 g, 95%), m. p.: 232-234 ^{0}C from compound (**2ch**) (2.0 g, 10 mmol).

Anal.:

IR **:** 3127, 1699, 1621, 1596, 1400

^{1}H-NMR **:** 10.63 (bs, 1H), 8.65-8.67 (d, 1H, *J* = 8.0 Hz), 7.89-7.93 (t, 1H *J* = 8.0 Hz), 7-51-7.53 (d, 1H, *J* = 8.0 Hz), 7.39-7.43 (t, 1H, *J* = 8.0 Hz), 4.10-4.14 (t, 2H, *J* = 8.0 Hz), 3.67-3.73 (q, 2H, *J* = 8.0 Hz), 1.67-1.78 (m, 4H), 1.39-1.48 (m, 2H), 1.04-1.06 (t, 3H, *J* = 8.0 Hz), 0.95-0.99 (t, 3H, *J* = 8.0 Hz)

ESI-MS (m/z) **:** 260 (M^++1)

$C_{15}H_{21}N_3O$ requires C, 69.47; H, 8.16; N, 16.20. Found: C, 69.27; H, 8.32; N, 16.44%

4.1.2.8.17. 2-Imino-3-isopropyl-1-propyl-2,3-dihydroquinazolin-4(*1H*)-one (3ci)

Compound (**3ci**) was obtained as off-white amorphous solid (1.9 g, 79%), m. p.: 270-272 ^{0}C from compound (**2ci**) (1.9 g, 10 mmol).

Anal.:

IR **:** 3130, 1686, 1648, 1615, 1400

ESI-MS (m/z) **:** 246 (M^++1)

$C_{14}H_{19}N_3O$ requires C, 68.54; H, 7.81; N, 17.13. Found: C, 68.13; H, 7.65; N, 17.39%

4.1.2.8.18. 3-Cyclohexyl-2-imino-1-propyl-2,3-dihydroquinazolin-4(*1H*)-one (3cl)

Compound (**3cl**) was obtained as off-white amorphous solid (2.3 g, 82%), m. p.: 257-259 ^{0}C from compound (**2cl**) (2.2 g, 10 mmol).

Anal.:

IR **:** 3130, 1685, 1617, 1597, 1400

^{1}H-NMR **:** 8.37- 8.39 (bs, 1H), 8.31-8.33(d, 1H, *J* = 8.0 Hz), 7.67-7.71 (t, 1H, *J* = 8.0 Hz), 7.31-7.33 (d, 1H, *J* = 8.0 Hz), 7.18-7.22 (t, 1H, *J* = 8.0 Hz), 4.21-4.29 (m, 1H), 4.04-4.08 (t, 2H, *J* = 8.0 Hz), 1.92-1.95 (m, 2H), 1.77-1.80 (m, 2H), 1.66-1.74 (m, 3H), 1.30-1.50 (m, 4H), 1.12-1.21 (m, 1H), 0.98-1.02 (t, 3H, *J* = 8.0 Hz)

ESI-MS (m/z) **:** 286 (M^++1)

$C_{17}H_{23}N_3O$ requires C, 71.55; H, 8.12; N, 14.72. Found: C, 71.11; H, 8.24; N, 14.86%

4.1.2.8.19. 3-(4-Methoxybenzyl)-2-imino-1-propyl-2,3-dihydroquinazolin-4(*1H*)-one (3cn)

Compound (**3cn**) was obtained as off-white amorphous solid (2.6 g, 82%), m. p.: 203-205 ^{0}C from compound (**2cn**) (2.7 g, 10 mmol).

Anal.:

IR **:** 3128, 1690, 1646, 1611, 1400

ESI-MS (m/z) **:** 324 (M^++1)

$C_{19}H_{21}N_3O_2$ requires C, 70.57; H, 6.55; N, 12.99. Found: C, 70.12; H, 6.86; N 12.81%

4.1.2.8.20. 1-Allyl-3-butyl-2-imino-2,3-dihydroquinazolin-4(*1H*)-one (3dh)

Compound (**3dh**) was obtained as white amorphous solid (2.4 g, 94%), m. p.: 240-242 ^{0}C from compound (**2dh**) (2.2 g, 10 mmol).

Anal.:

IR **:** 3129, 1699, 1662, 1614, 1400

^{1}H-NMR **:** 10.45 (bs, 1H), 8.55-8.57 (d, 1H, *J* = 8.0 Hz), 7.84-7.88 (m, 1H), 7.38-7.45 (m, 2H), 5.89-5.98 (m, 1H), 5.21-5.25 (m, 2H), 4.80-4.81 (m, 2H), 3.64-3.70 (q, 2H, *J* = 8.0 Hz), 1.65-1.73(m, 2H), 1.42-1.47 (m, 2H), 0.94-0.98 (t, 3H, *J* = 8.0 Hz)

ESI-MS (m/z) **:** 258 (M^++1)

$C_{15}H_{19}N_3O$ requires C, 70.01; H, 7.44; N, 16.33. Found: C, 70.25; H, 7.64;

N, 16.67%

4.1.2.8.21. 3-(4-Methoxybenzyl)-1-allyl-2-imino-2,3-dihydroquinazolin-4(*1H*)-one (3dn)

Compound (**3dn**) was obtained as white amorphous solid (2.6 g, 82%), m. p.: 210-212 ^{0}C from compound (**2dn**) (2.7 g, 10 mmol).

Anal.:

IR **:** 3128, 1693, 1648, 1611, 1399

ESI-MS (m/z) **:** 322 (M^++1)

$C_{19}H_{19}N_3O_2$ requires C, 71.01; H, 5.96; N, 13.08. Found: C, 71.42; H, 5.51; N, 13.25%

4.1.2.8.22. 1-Benzyl-3-butyl-2-imino-2,3-dihydroquinazolin-4(*1H*)-one (3eh)

Compound (**3eh**) was obtained as off-white amorphous solid (2.7 g, 90%), m. p.: 250-252 ^{0}C from compound (**2eh**) (2.7 g, 10 mmol).

Anal.:

IR **:** 3143, 1638, 1620, 1604, 1544, 1399

^{1}H-NMR **:** 8.36-8.39 (m, 1H), 8.14-8.16 (d, 1H, *J* = 8.0 Hz), 7.48-7.52 (t, 1H, *J* = 8.0 Hz), 7.18-7.30 (m, 5H), 7.10-7.14 (m, 2H), 5.36 (s, 1H), 3.54-3.59 (m, 2H), 1.63-1.71 (m, 2H), 1.36-1.46 (m, 2H), 0.93-0.97 (t, 3H, *J* = 8.0 Hz)

ESI-MS (m/z) **:** 308 (M^++1)

$C_{19}H_{21}N_3O$ requires C, 74.24; H, 6.89; N, 13.67. Found: C, 74.44; H, 6.91; N, 13.91%

4.1.2.8.23. 1-Benzyl-2-imino-3-isopropyl-2,3-dihydroquinazolin-4(*1H*)-one (3ei)

Compound (**3ei**) was obtained as off-white amorphous solid (2.7 g, 92%), m. p.: 270-272 ^{0}C from compound (**2ei**) (2.6 g, 10 mmol).

Anal.:

IR **:** 3130, 1690, 1654, 1616, 1400

^{1}H-NMR **:** 10.23 (bs 1H), 8.76-8.78(d, 1H, *J* = 8.0 Hz), 7.75-7.79 (t, 1H, *J* = 8.0 Hz), 7.26-7.39 (m, 7H), 5.43 (s, 2H), 4.57-4.65 (m, 1H), 1.45-1.47 (d, 6H, *J* = 8.0 Hz)

ESI-MS (m/z) **:** 294 (M^++1)

$C_{18}H_{19}N_3O$ requires C, 73.69; H, 6.53; N, 14.32. Found: C, 73.89; H, 6.65; N, 14.12%

4.1.2.8.24. 1-Benzyl-3-cyclopropyl-2-imino-2,3-dihydroquinazolin-4(*1H*)-one (3ek)

Compound (**3ek**) was obtained as off-white amorphous solid (2.4 g, 83%), m. p.: 238-240 ^{0}C from compound (**2ek**) (2.6 g, 10 mmol).

Anal.:

IR **:** 3144, 1695, 1639, 1615, 1400

ESI-MS (m/z) **:** 299 (M^++1)

$C_{18}H_{17}N_3O$ requires C, 74.20; H, 5.88; N, 14.42. Found: C, 74.58; H, 5.51; N, 14.65%

4.1.2.8.25. 1-Benzyl-3-cyclohexyl-2-imino-2,3-dihydroquinazolin-4(*1H*)-one (3el)

Compound (**3el**) was obtained as off-white amorphous solid (3.1 g, 95%), m. p.: 270-272 ^{0}C from compound (**2el**) (1.9 g, 10 mmol).

Anal.:

IR **:** 3155, 1696, 1638, 1616, 1400

^{1}H-NMR **:** 8.90 (bs, 1H), 8.39-8.41(d, 1H, *J* = 8.0 Hz), 7.60-7.64 (t, 1H, *J* = 8.0 Hz), 7.21-7.32 (m, 7H), 5.38 (s, 2H), 4.31 (bs, 1H), 1.96-1.98 (d, 2H, *J* = 8.0 Hz), 1.79-1.83 (m, 2H), 1.66-1.70 (m, 1H), 1.44-1.56 (m, 2H), 1.31-1.43 (m, 2H), 1.19-1.23 (m, 1H)

ESI-MS (m/z) **:** 334 (M^++1)

$C_{21}H_{23}N_3O$ requires C, 75.65; H, 6.95; N, 12.60. Found: C, 75.73; H, 6.91; N, 12.36%

4.1.2.8.26. 3-(4-Methoxybenzyl)-1-benzyl-2-imino-2,3-dihydroquinazolin-4(*1H*) -one (3en)

Compound (**3en**) was obtained as off-white amorphous solid (3.0 g, 83%), m. p.: 236-238 ^{0}C from compound (**2en**) (3.4 g, 10 mmol).

Anal.:

IR **:** 3422.03, 3135.63, 1693.55, 1611, 1400

ESI-MS (m/z) **:** 372 (M^++1)

$C_{23}H_{21}N_3O_2$ requires C, 74.37; H, 5.70; N, 11.31. Found: C, 74.19; H, 5.98; N, 11.12%

4.1.2.8.27. 1-Benzyl-2-imino-3-phenethyl-2,3-dihydroquinazolin-4(*1H*)-one (3es)

Compound (**3es**) was obtained as off-white amorphous solid (3.2 g, 92%), m. p.: 243-245 ^{0}C from compound (**2es**) (3.4 g, 10 mmol).

Anal.:

IR **:** 3123, 1703, 1639, 1615, 1401

^{1}H-NMR **:** 10.10 (bs, 1H), 8.40-8.42 (d,1H, *J* = 8.0 Hz), 7.70-7.74 (t, 1H, *J* = 8.0 Hz), 7.21-7.34 (m, 12H), 5.40 (s, 2H), 3.90-3.96 (q, 2H, *J* = 8.0 Hz), 3.03-3.07 (t, 2H, *J* = 8.0 Hz)

ESI-MS (m/z) **:** 356 (M^++1)

$C_{23}H_{21}N_3O$ requires C, 77.72; H, 5.96; N, 11.82. Found: C, 77.92; H, 5.80; N, 11.58%

4.1.2.9. Method 9: In an oven dried round bottom flask compound (**2**) (10 mmol), cyanogen bromide (2.6 g, 25 mmol) and a mixture of DMSO:IL (1:1) (20 ml) were mixed and stirred at RT for 5 hr. Progress of the reaction was monitored by TLC. After completion of reaction, the reaction mixture was diluted with ice cold water (100 ml). The solid that separated out, was filtered and washed with water (100ml X 2). The solid so obtained was dissolved in minimum amount of ethanol (10-20 ml) with gentle heating and diluted with ice cold water (100 ml), precipitate was filtered, washed with water (100 ml) and dried yielding compound (**3**) with sufficient purity.

4.1.2.10. Method 10: In an oven dried round bottom flask compound (**2**) (10 mmol), cyanogen bromide (2.6 g, 25 mmol), potassium bromide (2.9 g, 25 mmol) and DMSO (20 ml) were mixed and stirred at RT for 45 min. Progress of the reaction was monitored by TLC. After completion of reaction, the reaction mixture was diluted

with ice cold water (100 ml). The solid that separated out, was filtered and washed with water (100ml X 2). The solid so obtained was dissolved in minimum amount of ethanol (10-20 ml) with gentle heating and diluted with ice cold water (100 ml), precipitate was filtered, washed with water (100 ml) and dried yielding compound (**3**) with sufficient purity.

4.1.2.11. Method 11: In an oven dried round bottom flask compound (**2**) (10 mmol), cyanogen bromide (2.6 g, 25 mmol), cesium bromide (5 g, 25 mmol) and DMSO (20 ml) were mixed and stirred at RT for 4 hr. Progress of the reaction was monitored by TLC. After completion of reaction, the reaction mixture was diluted with ice cold water (100 ml). The solid that separated out, was filtered and washed with water (100ml X 2). The solid so obtained was dissolved in minimum amount of ethanol (10-20 ml) with gentle heating and diluted with ice cold water (100 ml), precipitate was filtered, washed with water (100 ml) and dried yielding compound (**3**) with sufficient purity.

4.1.2.12. Method 12: In an oven dried round bottom flask compound (**2**) (10 mmol), cyanogen bromide (2.6 g, 25 mmol), sodium bromide (3.2 g, 25 mmol) and DMSO (20 ml) were mixed and stirred at RT for 6 hr. Progress of the reaction was monitored by TLC. After completion of reaction, the reaction mixture was diluted with ice cold water (100 ml). The solid that separated out, was filtered and washed with water (100ml X 2). The solid so obtained was dissolved in minimum amount of ethanol (10-20 ml) with gentle heating and diluted with ice cold water (100 ml), precipitate was

filtered, washed with water (100 ml) and dried yielding compound (**3**) with sufficient purity.

4.1.2.13. Method 13: In an oven dried round bottom flask compound (**2**) (10 mmol), cyanogen bromide (2.6 g, 25 mmol), lithium bromide (2.1 g, 25 mmol) and DMSO (20 ml) were mixed and stirred at RT for 12 hr. Progress of the reaction was monitored by TLC. After completion of reaction, the reaction mixture was diluted with ice cold water (100 ml). The solid that separated out, was filtered and washed with water (100ml X 2). The solid so obtained was dissolved in minimum amount of ethanol (10-20 ml) with gentle heating and diluted with ice cold water (100 ml), precipitate was filtered, washed with water (100 ml) and dried yielding compound (**3**) with sufficient purity.

4.1.2.14. Method 14: In an oven dried round bottom flask compound (**2**) (10 mmol), cyanogen bromide (2.6 g, 25 mmol), potassium chloride (1.8 g, 25 mmol) and DMSO (20 ml) were mixed and stirred at RT for 1 hr. Progress of the reaction was monitored by TLC. After completion of reaction, the reaction mixture was diluted with ice cold water (100 ml). The solid that separated out, was filtered and washed with water (100ml X 2). The solid so obtained was dissolved in minimum amount of ethanol (10-20 ml) with gentle heating and diluted with ice cold water (100 ml), precipitate was filtered, washed with water (100 ml) and dried yielding compound (**3**) with sufficient purity.

4.1.2.15. Method 15: In an oven dried round bottom flask compound (**2**) (10 mmol), cyanogen bromide (2.6 g, 25 mmol), potassium iodide (4.1 g, 25 mmol) and DMSO (20 ml) were mixed and stirred at RT for 16 hr. Progress of the reaction was monitored by TLC. After completion of reaction, the reaction mixture was diluted with ice cold water (100 ml). The solid that separated out, was filtered and washed with water (100ml X 2). The solid so obtained was dissolved in minimum amount of ethanol (10-20 ml) with gentle heating and diluted with ice cold water (100 ml), precipitate was filtered, washed with water (100 ml) and dried yielding compound (**3**) with sufficient purity.

4.1.2.16. Method 16: In an oven dried round bottom flask compound (**2**) (10 mmol), cyanogen bromide (2.6 g, 25 mmol), cesium carbonate (8.1 g, 25 mmol) and DMSO (20 ml) were mixed and stirred at RT for 18 hr. The reaction mixture was diluted with ice cold water (100 ml). No product was formed as the isolated product was identified to be the starting material (**2**).

4.1.2.17. Method 17: In an oven dried round bottom flask compound (**2**) (10 mmol), cyanogen bromide (2.6 g, 25 mmol), copper iodide (4.7 g, 25 mmol) and DMSO (20 ml) were mixed and stirred at RT for 18 hr. Reaction mixture was diluted with ice cold water (100 ml). The starting material only was isolated.

4.1.2.18. Method 18: In an oven dried round bottom flask compound (**2**) (10 mmol), cyanogen bromide (2.6 g, 25 mmol), potassium bromide (2.9 g, 25 mmol) and IL (10 ml) were mixed and stirred at RT for 18 hr. Progress of the reaction was monitored by

TLC. After completion of reaction, the reaction mixture was diluted with ice cold water (100 ml). The solid that separated out, was filtered and washed with water (100ml X 2). The solid so obtained was dissolved in minimum amount of ethanol (10-20 ml) with gentle heating and diluted with ice cold water (100 ml), precipitate was filtered, washed with water (100 ml) and dried yielding compound (**3**) with sufficient purity.

4.1.2.19. Method 19: In an oven dried round bottom flask compound (**2**) (10 mmol), cyanogen bromide (2.6 g, 25 mmol) and ethanol (30 ml) were mixed and exposed to microwave radiation at 100 W, 70^0 C for 5 min. Progress of the reaction was monitored by TLC. After completion of reaction, the reaction mixture was diluted with ice cold water (100 ml). The solid that separated out, was filtered and washed with water (100ml X 2). The solid so obtained was dissolved in minimum amount of ethanol (10-20 ml) with gentle heating and diluted with ice cold water (100 ml), precipitate was filtered, washed with water (100 ml) and dried yielding compound (**3**) with sufficient purity.

4.1.3. General Procedure for the synthesis of 4-(substitutedamino)methyl 3-aminobenzoates (5)

In an oven dried round bottom flask methyl 4-fluoro-3-nitrobenzoate (**4**) (1.9 g, 10 mmol), diisopropylethylamine (4.3 ml, 25 mmol), substituted amine ($\mathbf{R_2}$ = **n/o/s**) (15 mmol) and dimethylformamide (20 ml) were mixed and stirred at RT for 8 hr. Progress of reaction was monitored by TLC. The reaction mixture was diluted with cold water (100 ml) and the precipitate was filtered. The residue was washed with

water (100 ml) and dissolved in methanol (50 ml). Palladium carbon (1 g) was added and fitted with a hydrogen balloon. The reaction mixture was stirred at RT for 8 hr with gradual addition of palladium carbon (1 g X 2). Progress of the reaction was monitored by TLC. The reaction mixture was filtered through a celite bed to remove solid inorganic impurities. The filtrate was concentrated under vacuum and the residue (**5**) was utilized for next step without further purification.

4.1.4. General Procedure for the synthesis of 1-substituted-2-aminoimidazoles (6)

The procedure used for the synthesis of compound (**6**) is similar to **method 8**. In an oven dried round bottom flask compound (**5**) (10 mmol), cyanogen bromide (2.6 g, 25 mmol) and a mixture of DMSO:IL (10:1) (20 ml) were mixed and stirred at 90^0 C for 8 min. Progress of the reaction was monitored by TLC. After completion of reaction, the reaction mixture was diluted with ice cold water (100 ml). The solid that separated out, was filtered, washed with water (2 X 100ml) and dried. The dried solid was re-crystallized with absolute ethanol offering compound (**6**) with sufficient purity.

4.1.4.1. Methyl 1-(4-methoxybenzyl)-2-amino-1*H*-benzo[*d*]imidazole-5-carboxylate (6n)

Compound (**6n**) was obtained as yellow solid (2.9 g, 95%), m. p.: 234-236 ^{0}C from compound (**5n**) (2.8 g, 10 mmol).

Anal.:

IR **:** 3300, 3094, 1698, 1674, 1604, 1287, 1220, 1250, 1032

^{1}H-NMR **:** 9.10 (bs, 2H), 7.98 (s, 1H), 7.86-7.88 (d, 1H, *J* = 8.0 Hz), 7.48-7.50 (d, 1H, *J* = 8.0 Hz), 7.28-7.30 (d, 2H, *J* = 8.0 Hz),

6.87-6.89 (d, 2H, *J* = 8.0 Hz), 5.43 (s, 2H), 3.88 (s, 3H), 3.75 (s, 3H)

ESI-MS (m/z) **:** 312 (M^++1)

$C_{17}H_{17}N_3O_3$ requires C, 65.58; H, 5.50; N, 13.50. Found: C, 65.82; H, 5.10; N, 13.82%

4.1.4.2. Methyl 1-(2-chlorobenzyl)-2-amino-1*H*-benzo[*d*]imidazole-5-carboxylate (6o)

Compound (**6o**) was obtained as yellow solid (2.9 g, 94%), m. p.: 170-172 ^{0}C from compound (**5o**) (2.8 g, 10 mmol).

Anal.:

IR **:** 3417, 3223, 3079, 1720, 1676, 1638, 1300, 1225

^{1}H-NMR **:** 9.10 (bs, 2H), 8.08 (s, 1H), 7.87-7.89 (d, 1H, *J* = 8.0 Hz), 7.50-7.52 (d, 1H, *J* = 8.0 Hz), 7.33-7.37 (t, 1H, *J* = 8.0 Hz), 7.19-7.27 (m, 1H), 7.15-7.17 (d, 1H, *J* = 8.0 Hz), 6.82-6.84 (d, *J* = 8.0 Hz), 5.54 (s, 2H), 3.91 (s, 3H)

ESI-MS (m/z) **:** 316 (M^++1)

$C_{16}H_{14}ClN_3O_2$ requires C, 60.86; H, 4.47; N, 13.31. Found: C, 60.38; H, 4.87; N, 13.55%

4.1.4.3. Methyl 2-amino-1-phenethyl-1*H*-benzo[*d*]imidazole-5-carboxylate (6s)

Compound (**6s**) was obtained as yellow solid (2.6 g, 89%), m. p.: 180-182 ^{0}C from compound (**5s**) (2.8 g, 10 mmol).

Anal.:

IR **:** 3428, 3195, 1710, 1658, 1619, 1324, 1210, 1015

ESI-MS (m/z) **:** 296 (M^++1)

$C_{17}H_{17}N_3O_2$ requires C, 69.14; H, 5.80; N, 14.23. Found: C, 69.52; H, 5.48; N, 14.56%

4.1.5. General Procedure for synthesis of substituted 2-aminobenzohydrazides (8)

In an oven dried round-bottom flask, substituted isatoic anhydride (**1**) (**R_1 = e, c**) (10 mmol), suitable hydrazine (**R_2 = s, t**) (10 mmol) and K_2CO_3 (2g, 15 mmol) were stirred in DMF (20 ml) at 45^0 C for 45 min. Progress of the reaction was monitored by TLC. After completion, the reaction mixture was allowed to cool at room temperature and diluted with ice cold water (100 ml). The solid (**8**) that separated out, was filtered, washed, dried and used for next step without further purification.

4.1.6. General Procedure for the synthesis of 1,4-disubstituted 2-amino1,2,3,4-tetrahydrobenzo[*e*]-1,2,4-triazepin-5-ones (9)

The procedure used for the synthesis of compound (**9**) is similar to **method 8**. In an oven dried round bottom flask compound (**8**) (10 mmol), cyanogen bromide (2.6 g, 25 mmol) and a mixture of DMSO:IL (10:1) (20 ml) were mixed and stirred at 90^0 C for 8 min. Progress of the reaction was monitored by TLC. After completion of reaction, the reaction mixture was diluted with ice cold water (100 ml). The solid (**9**) that separated out, was filtered and washed with water (2 X 100ml). The solid so obtained was dissolved in minimum amount of ethanol (10-20 ml) with gentle heating and diluted with ice cold water (100 ml). The precipitate was filtered, washed with water (100 ml) and dried offering compound (**9**) with sufficient purity.

4.1.6.1. 2-Imino-4-phenyl-1-propyl-1,2,3,4-tetrahydrobenzo[*e*]-1,2,4-triazepin-5-one (9ct)

Compound (**9ct**) was obtained as white amorphous solid (2.6 g, 90%), m. p.: 240-242 ^{0}C from compound (**8ct**) (2.7 g, 10 mmol).

Anal.:

IR **:** 3415, 3095, 1738, 1620, 1535, 1400

ESI-MS (m/z) **:** 295 (M^++1)

$C_{17}H_{18}N_4O$ requires C, 69.37; H, 6.16; N, 19.03. Found: C, 69.12; H, 6.51; N, 19.25%

4.1.6.2. 1-Benzyl-2-imino-4-phenethyl-1,2,3,4-tetrahydrobenzo[*e*]-1,2,4-triazepin-5-one (9es)

Compound (**9es**) was obtained as light green amorphous solid (3.4 g, 92%), m. p.: 226-228 ^{0}C from compound (**8es**) (3.4 g, 10 mmol).

Anal.:

IR **:** 3447, 3128, 1701, 1618, 1567, 1400

^{1}H-NMR **:** 10.5 (bs, 2H), 7.54-7.56 (d, 1H, *J* = 8.0 Hz), 7.24-7.38 (m, 12H), 6.78-6.81 (d, 1H, *J* = 8.0 Hz), 6.71-6.74 (m, 2H), 5.28 (s, 2H), 4.42-4.46 (m, 2H)

ESI-MS (m/z) **:** 371 (M^++1)

$C_{23}H_{22}N_4O$ requires C, 74.57; H, 5.99; N, 15.12. Found: C, 74.97; H, 6.23; N, 15.48%

4.2. Biological studies

Biological studies are divided into two parts: **part A** includes screening of compounds (**3, 6** and **9**) for CNS-activity, and **part B** includes screening of intermediate compounds (**2**) for anti-thrombotic activity.

4.2.1. Part A-Anti-Alzheimer's activity

4.2.1.1. Cholinesterase inhibition assay

The quality of the test compounds to inhibit human AChE (product number C1682, Sigma-Aldrich) and equine serum BuChE (product number C1057, Sigma-Aldrich) was determined using Ellman's method[97] (IC_{50} values, μM) using appropriate reference agent tacrine hydrochloride hydrate (item number A79922 Sigma-Aldrich). Stock solutions of test compounds were dissolved in a minimum volume of DMSO (1%) and were diluted using the buffer solution (50 mM Tris–HCl, *p*H 8.0, 0.1 M NaCl, 0.02 M $MgCl_2.6H_2O$). In 96-well plates, 160 μl 5,50-dithiobis(2-nitrobenzoic acid) (0.15 mM DTNB), 50 μl of AChE (0.022 U/ml prepared in 50 mM Tris–HCl, *p*H 8.0, 0.1% w/v bovine serum albumin, BSA) or 50 μl of BuChE (0.06 U/ml prepared in 50 mM Tris–HCl, *p*H 8.0, 0.1% w/v BSA) were incubated with 10 μl of various concentrations of test compounds (0.001– 100 μM) at room temperature for 30 min followed by the addition of the substrates (30 μl) acetylthiocholine iodide (1.5 mM ATCl) or *S*-butyrylthiocholine iodide (1.5 mM BTCI) and the absorbance was measured at a wavelength of 415 nm BIORAD microplate reader 680XR. Percent inhibition was calculated by the comparison of compounds treated to various control incubations that included 1% DMSO. The concentration of the test compound causing

50% inhibition (IC50, μM) was calculated from the concentration–inhibition response curve on logarithmic scale (quadruplicate determinations).[98]

4.2.1.2. *h*AChE-induced Aβ aggregation inhibition studies

Thioflavin T (ThT) a benzothiazole is commonly used in the detection of amyloid plaque formation. As Aβ peptides begin to aggregate into oligomers and fibrils, ThT binds to the beta sheets formed as a result of the aggregation and the detectable change in its emission spectrum is used to measure the degree of aggregation.[99-101] $A\beta_{1-40}$ was purchased from Sigma Aldrich; Human recombinant AChE lyophilized powder was purchased from Sigma Aldrich (product number C1682) and ThT was purchased from Sigma-Aldrich. $A\beta_{1-40}$ was prepared as previously described. Lyophilized $A\beta_{1-40}$ was dissolved in DMSO to obtain a 2 mM solution and then diluted into 500 μM solution with 0.215 M sodium phosphate buffer (*p*H 8.0). Aliquots (2 μl) of $A\beta_{1-40}$ were incubated with 16 μl of *h*AChE, which was dissolved in 0.215 M sodium phosphate buffer (*p*H 8.0), to give a final concentration of 50 μM of $A\beta_{1-40}$ and 230 μM of *h*AChE. For co-incubation experiments, 2 μl of the test compounds in 0.215 M sodium phosphate buffer *p*H 8.0 solution (final concentration 100 μM) were added into aliquots (2 μl) of $A\beta_{1-40}$ (final concentration 50 μM) along with 16 μl of *h*AChE (final concentration 230 μM). The reaction mixtures were incubated at room temperature for 24 h and 100 μl of ThT (20 μM) in 50 mM glycine-NaOH buffer (*p*H 8.5) was added. Fluorescence was monitored at excitation wavelength of 442 nm and emission at 490 nm using a Shimadzu spectrofluorophotometer RF-5301 PC. Each assay was run in triplicates along with tacrine hydrochloride hydrate (item number A79922 Sigma-Aldrich) as reference

agents. The fluorescence intensities in the presence and absence of inhibitors were compared using appropriate controls containing 1% DMSO and the percentage of inhibition was calculated using the equation: 100 - (IFi/IFo X 100) where IFi and IFo are the fluorescence intensities obtained for $A\beta_{1-40}$ + *h*AChE in the presence and absence of inhibitor, respectively.

4.2.1.3. SH-SY5Y neuroblastoma cell toxicity studies

The cellular reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, MTT (Product No. 30-1010K, from American Type Culture Collection, Manassas, VA) was measured by detecting a purple formazan intermediate at 570 nm. By incubating the cells with varying concentrations of test compounds, their toxicity can be calculated directly by determining the % reduction of MTT or indirectly by reporting the % viability of the cell line used.[102,103] The SH-SY5Y neuroblastoma cells were plated at a density of 10,000 cells per well in 100 µl of complete media consisting of a 1:1 mixture of Dulbecco's Modified Eagle Medium (DMEM) and Ham's F12, supplemented with 10% Fetal Bovine Serum (FBS). The cells were incubated overnight before treatment with 100 µl of test sample solutions and select controls (tacrine hydrochloride hydrate, A79922 Sigma-Aldrich) at various concentrations (0–160 µM) in 2% DMSO for 24 h at 37 ^{0}C (n = 3). A 20 µl of the MTT reagent solution (0.5 mg/ml) was added and the cells were incubated for an additional 3h. The formed formazan crystals were solubilized with 80 µl of DMSO and absorbance was taken in BIORAD microplate reader 680XR (Molecular Devices Spectramax) at 570 nm. The % cell viability was determined by taking the absorbance of control (untreated cells) as 100%.

4.2.1.4. Molecular modeling (docking) studies

Docking studies were performed with Glide 5.5 (Schrödinger 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. For docking purpose Glide offers three different levels of docking precision- HTVS (high-throughput virtual screening), SP (standard precision), and XP (extra precision). The 3D structures of 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 Schrödinger2009, all the parameters were kept to standard value. The 3D crystallographic structures for *h*AChE and *h*BuChE were obtained from RCSB Protein Data Bank (PDB Code: 1B41 and 1P0I respectively). Protein structures were prepared and hydrogens were added to these enzyme structures and Grid was generated around active site which was mapped by site map tool within Schrödinger suit. All the parameters were kept to standard values for grid generation and the generated grid was cross-checked with the results accounted in the reported article for justification. Docking calculations for minimized 3D ligand structures were performed in extra precision (XP) mode with the active site of receptor structures.

4.2.2. Part B-Anti-thrombotic activity

4.2.2.1. Anti-thrombotic activity (Mouse thrombosis model)

Pulmonary thromboembolism was induced by a method described by DiMinno and Silver (1983).[91] The compounds to be tested (30 μmol/kg), standard drug or the

vehicle were administered by oral route 60 min prior to the thrombotic challenge. Ten mice were used for evaluating the effect of each test compound, while a group of five mice was used to evaluate the effect of standard drug or vehicle. A mixture of collagen (1.5 mg/kg) and adrenaline (0.5 mg/kg) was injected into the tail vein to induce hind limb paralysis or death. Results have been reported as % protection, which represents the protection against hind limb paralysis or death. The test compounds exhibiting protection equivalent to standard drug (30 μmol/kg) were considered as active molecules.

4.2.2.2. Bleeding time (BT)

Bleeding time in mice was evaluated by the method of Dejana et al. (1979)[92] to assess the increase in bleeding time in comparison to aspirin at its optimal antithrombotic dose. The tail (2 mm from tip) of mice was incised and the oozed blood was soaked on a filter paper, which was monitored at an interval of 10–15 s till the bleeding stopped. The time elapsed from the tail tip incision to the stoppage of bleeding was recorded as the bleeding time. The tested compounds (30 μmol/kg), standard drug (30 μmol/kg) or vehicle was given orally 60 min prior to the tail incision to a group of five mice each.

4.2.2.3. Coagulation parameters

Sprague-Dawley rats (200–250 g) of either sex were used for these studies. Animals were anaesthetized under anesthetic ether and blood was drawn from the heart into a plastic syringe containing 2.5% trisodium citrate. The blood was centrifuged at 2500 g for 20 min at 20 ^{0}C to plasma. The assay for PT was performed

in plasma samples of all the groups within 2 h of sample collection. Assay was performed using commercial kits as per manufacturer's instructions and measured by using a Coagulometer (Start4 Semi automated, Young Instruments, Stago, France)

4.2.2.4. Acute toxicity study

The procedure was trailed according to the OECD guidelines 423 (Acute toxic class method).[104] The acute toxic class method is a stepwise procedure with 3 animals of single sex per group. After 2-4 steps a testing substance can be predicted for acute toxicity based on the mortality and/or moribund status of the animals. According to this method the number of animals should be minimized for acceptable data and scientific conclusion. The procedure employs defined doses (5, 50, 300, 2000 mg/kg body weight) and the results assign a substance to be ranked and classified according to the globally harmonized system (GHS) for the classification of chemicals which cause acute toxicity.

Animals and treatment

Nulliparous and non-pregnant female Swiss albino mice, 12 weeks old, were obtained from Torrent Research Center, Ahmedabad. Twelve animals out of the available seventeen animals were randomly selected at the start of the study according to their body weights. The animals were specific pathogen free and were clinically examined upon arrival and identified by tail marking. The animals were allowed an acclimatization period of 6 days after delivery and before they entered the study. The animals were housed in polypropylene cages under controlled temperature and 12 hr. light and dark cycle conditions (22 ± 3 ^{0}C, 40–70% relative humidity, 12-h light phase with daylight) with food and water *ad libitum,* (food was temporarily withdrawn for 4

h prior to oral gavage on Day 0). Single animal per cage were housed during the first 48 h after dosing. After the 48-h observation period, animals were returned to their original groups and these groups of three animals were housed for the remainder of the 14-day observation period. The animals were monitored for two hours after dosing and twice daily. Observations were made once daily thereafter for the remainder of the 14-day observation period. Observation parameters included changes in skin, fur, eyes, mucous membranes, respiratory and central nervous systems. Motor activity and behavioural pattern of animals were also observed. Compound (**2en, 2eo, 2er** and **2dr)** were administered by oral gavage, using a stainless steel gavage tube. The animals were dosed with 10 ml/kg body weight of the supplied suspension, corresponding to a dose of 2000 mg/kg body weight. Further, the new animals were repeated at a dose of 2000 mg/kg (**2dr**), since one of the animals in a group of 2000 mg/kg (**2dr**) dose had shown toxicity sign. The day of dosing was designated as Day 0 and animals were weighed at Days 0, 7, and 14 (termination). The animals were sacrificed by overdose of anaesthesia at the end of the observation period. The major organs viz. heart, liver, kidney, stomach and brain were removed and stored in 10% buffered-formalin for histopathological examinations.[105]

4.2.2.5. Molecular modeling (docking) studies

Docking studies were performed using Glide tool of Schrödinger 2009. It performs grid-based ligand docking and searches for favorable interactions between one or more typically small ligand molecules and a typically larger receptor molecule, usually a protein. The molecules for docking 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 2009 by keeping all the parameters to standard values. The 3D structure of serine protease factor Xa (fXa) was obtained from RCSB Protein Data Bank (PDB Code: 4A7I). The Grid was generated on this receptor with van der Waals radius scaling factor of 1 and partial charges cutoff at 0.25. Docking calculations were performed in extra precision (XP) mode with all docking parameters at standard values.