SECTION-II

CHAPTER 4.
EXPERIMENTAL

4. EXPERIMENTAL

The experiment section has been divided into two parts: **chemical studies**; for the syntheses with characterization 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 $CDCl_3$ as 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 performed on ThermoFisher FLASH 2000 Organic elemental analyzer.

4.1.1. General procedure for selective alkylation (2)

An oven-dried flask was charged with 1,3,4,5-tetrahydrobenzo[*d*]azepin-2-one (**1**) (1 g, 6.21 mmol), evacuated and backfilled with nitrogen. Tetrahydrofuran (15 ml) was added to dissolve the solid. The reaction mixture was cooled down to -78 ^{0}C, *n*-BuLi solution (19.8 ml, 1.6 M solu., 12.42 mmol) was added drop wise and the resulting mixture was stirred for 15 min. Reaction mixture was allowed to warm up to 0 ^{0}C for another 15 min and corresponding alkyl halide (6.21 mmol) was added drop wise. Again the reaction mixture was stirred for 2 hr and progress of the reaction was monitored by TLC (hexane/ether 2:1). After completion, volatiles were evaporated. To the resulting mixture, ether (20 ml) and water (40 ml) were added. The aqueous

layer was separated and extracted with ether (20 ml). Organic fractions were combined, dried on magnesium sulphate, volatiles were removed and the residue was purified by column chromatography using silica (100-200 mesh) as stationary phase and hexane-ethyl acetate as eluent.

4.1.1.1. 1-Allyl-4,5-dihydro-*1H*-benzo[*d*]azepin-2(*3H*)-one (2a)

Compound (**2a**) was obtained as white solid (0.98 g, 82%), m. p.: 134-136 ^{0}C.

Anal.:

IR **:** 3290, 3079, 1653, 1492, 1411, 1326, 922, 827, 757

^{1}H-NMR **:** 7.05-7.18 (m, 4H), 5.77-5.88 (m, 2H; 1H exchanged with D_2O), 4.95-5.07 (m, 2H), 3.97-4.01 (t, 1H, J = 8.0 Hz), 3.61-3.69 (m, 1H), 3.32-3.40 (m, 1H), 3.19-3.26 (m, 1H), 2.97- 3.03 (m, 1H) 2.88-2.96 (m, 1H), 2.57-2.64 (m, 1H)

ESI-MS (m/z) **:** 202 (M^++1)

$C_{13}H_{15}NO$ requires C, 77.58; H, 7.51; N, 6.96. Found: C, 77.38; H, 7.75; N, 6.72%

4.1.2. General procedure for symmetrical alkylation (3)

An oven-dried flask was charged with 1,3,4,5-tetrahydro-benzo[*d*]azepin-2-one (**1**) (1 g, 6.21 mmol), evacuated and backfilled with nitrogen. Tetrahedrofuran (15 ml) was added to dissolve the solid. The reaction mixture was cooled down to -78 ^{0}C, *n*-BuLi solution (19.8 ml, 1.6 M solu., 12.42 mmol) was added drop wise and the resulting mixture was stirred for 15 min. The reaction mixture was allowed to warm up to 0 ^{0}C for another 15 min and alkyl halide (R_1) (6.21 mmol) was added. It was

allowed to stir for 1.5 hr and progress of the reaction was monitored by TLC (mobile phase- hexane/ether 2:1). The reaction mixture was cooled at 0 ^{0}C and corresponding alkyl halide (R_2) (6.21 mmol) was added. The reaction mixture was allowed to warm up to room temperature in 1.5 hr and allowed to stir for another 0.5 hr. When the reaction was complete, volatiles were evaporated. To the resulting mixture ether (20 ml) and water (40 ml) were added. The aqueous layer was separated and additionally extracted with ether (20 ml). Organic fractions were combined, dried on magnesium sulphate, volatiles were removed and the residue was purified by column chromatography using silica (100-200 mesh) as stationary phase and hexane-ethyl acetate as eluent.

4.1.2.1. 1,3-Diethyl-4,5-dihydro-*1H*-benzo[*d*]azepin-2(*3H*)-one (3b)

Compound (**3b**) was obtained as pale white solid (1.0 g, 65%) m. p.: 136-138 ^{0}C from compound (**1**) (1 g, 6.21 mmol).

Anal.:

IR **:** 1646, 1485, 1447, 1372, 750

^{1}H-NMR **:** 7.00-7.11 (m, 4H), 3.83-3.92 (m, 2H), 3.44-3.51 (m, 1H), 3.35-3.40 (q, 2H, *J* = 8.0 Hz), 3.13-3.20 (m, 1H), 3.00-3.08 (m, 1H), 2.10-2.19 (m, 1H), 1.81-1.90 (m, 1H), 1.00-1.04 (t, 3H, *J* = 8.0 Hz), 0.95-0.98 (t, 3H, *J* = 8.0 Hz)

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

$C_{14}H_{19}NO$ requires C, 77.38; H, 8.81; N, 6.45. Found: C, 77.14; H, 8.65; N, 6.85%

4.1.2.2. 1,3-Dibenzyl-4,5-dihydro-*1H*-benzo[*d*]azepin-2(*3H*)-one (3c)

Compound (**3c**) was obtained as a white solid (1.4 g, 68%) m. p. 81-83 ^{0}C from compound (**1**) (1 g, 6.21 mmol).

Anal.:

IR **:** 3021, 1652, 1486, 1444, 753, 695

^{1}H-NMR **:** 7.07-7.26 (m, 11H), 6.93-6.95 (m, 1H), 6.86-6.89 (m, 2H), 4.42-4.55 (m, 3H), 3.83-3.90 (m, 1H), 3.59-3.65 (q, 1H, *J* = 8.0 Hz), 3.05-3.34 (m, 3H), 2.79-2.88 (m, 1H)

ESI-MS (m/z) **:** 342 (M^++1)

$C_{24}H_{23}NO$ requires C, 84.42; H, 6.79; N, 4.10. Found: C, 84.26; H, 6.35; N, 4.48%

4.1.2.3. 1,3-Diallyl-4,5-dihydro-*1H*-benzo[*d*]azepin-2(*3H*)-one (3d)

Compound (**3d**) was obtained as yellow liquid (1.0 g, 72%) from compound (**1**) (1 g, 6.21 mmol).

Anal.:

IR **:** 3130, 3017, 1651, 1488, 1399, 1336, 796, 745

^{1}H-NMR **:** 7.03-7.19 (m, 4H), 5.80-5.88 (m, 1H), 4.95-5.09 (m, 2H), 4.11-4.15 (t, 1H, *J* = 8.0 Hz) 3.90-3.97 (m, 1H), 3.31-3.89 (m, 1H), 3.19-3.27 (m, 1H), 3.01-3.07 (m, 1H) 2.89-2.99 (m, 6H), 2.60-2.63 (m, 1H)

ESI-MS (m/z) **:** 242 (M^++1)

4.1.3. General procedure for reduction of amide (3)

Compound (**3**) (4.0 mmol) was taken in dry tetrahydrofuran (20 ml) in a reaction flask and cooled to 0 ^{0}C, lithium aluminum hydride (0.45 g, 12 mmol) was added in portions and the temperature of the reaction mixture was allowed to reach to RT. Progress of the reaction was monitored by TLC. The reaction mixture was quenched with ethyl acetate and filtered. Volatiles were removed and the residue was purified by column chromatography using silica (100-200 mesh) as stationary phase and hexane-ethyl acetate as eluent.

4.1.3.1. 1-Ethyl-3-methyl-2,3,4,5-tetrahydro-*1H*-benzo[*d*]azepine (4a)

Compound (**4a**) was obtained as yellow liquid (0.54 g, 72%) from 1-ethyl-3-methyl-4,5-dihydro-*1H*-benzo[*d*]azepin-2(*3H*)-one (**3a**) (0.65 g, 4.0 mmol).

Anal.:

IR **:** 3018, 1492, 1454, 1382, 751

^{1}H-NMR **:** 7.06-7.17 (m, 4H), 3.05-3.07 (m, 1H), 2.91 (bs, 1H), 2.75 (bs, 1H), 2.52-2.65 (m, 3H), 2.37 (s, 2H), 1.75-1.90 (m, 2H), 1.20-1.28 (m, 2H), 0.92-0.98 (t, 3H, J = 8.0 Hz)

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

4.1.3.2. 1,3-Diethyl-2,3,4,5-tetrahydro-*1H*-benzo[*d*]azepine (4b)

Compound (**4b**) was obtained as yellow liquid (0.52 g, 62%) from compound (**3b**) (0.87 g, 4.0 mmol).

Anal.:

IR **:** 3018, 1493, 1453, 1381, 750

^{1}H-NMR **:** 6.99-7.10 (m, 4H), 2.96-2.98 (m, 1H), 2.85 (bs, 1H), 2.71 (bs, 1H), 2.51-2.53 (m, 5H), 1.67-1.79 (m, 3H), 0.99-1.05 (t, 3H, *J* = 8.0 Hz), 0.86-0.92 (t, 3H, *J* = 8.0 Hz)

ESI-MS (m/z) **:** 204 (M^++1)

4.1.3.3. 1,3-Dibenzyl-2,3,4,5-tetrahydro-*1H*-benzo[*d*]azepine (4c)

Compound (**4c**) was obtained as dark yellow liquid (0.98 g, 75%) from compound (**3c**) (1.2 g, 4.0 mmol).

Anal.:

IR **:** 3025, 1491, 1449, 743, 700

^{1}H-NMR **:** 6.98-7.38 (m, 14H), 3.56-3.60 (d, 1H, *J* = 12.0 Hz), 3.47-3.51 (d, 1H, *J* = 12.0 Hz), 3.19-3.23 (m, 2H), 3.00-3.06 (t, 2H, *J* = 12.0 Hz), 2.74-2.87 (m, 3H), 2.50-2.53 (d, 1H, *J* = 12.0 Hz), 2.35 (bs, 1H)

ESI-MS (m/z) **:** 328 (M^++1)

4.1.4. General procedure for selective alkylation of amidic nitrogen (5)

An oven-dried flask was charged with 1,3,4,5-tetrahydrobenzo[*d*]azepin-2-one (1 g, 6.21 mmol), evacuated and backfilled with nitrogen. Tetrahydrofuran (15 ml) was added to dissolve the solid. The reaction mixture was cooled down to -78 ^{0}C, *n*-BuLi solution (9.9 ml, 1.6 M solu., 6.21 mmol) was added drop wise and the resulting mixture was stirred for 15 min. The reaction mixture was allowed to warm up to -20 ^{0}C for another 15 min and corresponding alkyl halide (6.21 mmol) was added. The reaction mixture was stirred for 8 hr and progress of the reaction was monitored by

TLC (hexane/ether 2:1). After completion, volatiles were evaporated. To the resulting mixture ether (20 ml) and water (40 ml) were added. The aqueous layer was separated and additionally extracted with ether (20 ml). Organic fractions were combined, dried on magnesium sulphate, volatiles were removed and the residue so obtained was purified by column chromatography using silica (100-200 mesh) as stationary phase and hexane-ethyl acetate as eluent.

4.1.4.1. 3-Allyl-4,5-dihydro-*1H*-benzo[*d*]azepin-2(*3H*)-one (5a)

Compound (**5a**) was obtained as dark yellow liquid (0.97 g, 78%) from compound (**1**) (1 g, 6.21 mmol).

Anal.:

IR **:** 3128, 3016, 1653, 1484, 1481, 1405, 798, 748

^{1}H-NMR **:** 7.01-7.12 (m, 4H), 5.69-5.76 (m, 1H), 5.09-5.14 (m, 2H), 3.98-4.00 (d, 2H, J = 8.0 Hz), 3.86 (s, 2H), 3.59-3.63 (t, 2H, J = 8.0 Hz), 3.00-3.06 (t, 2H, J = 8.0 Hz)

ESI-MS (m/z) **:** 202 (M^++1)

4.1.4.2. 3-Benzyl-4,5-dihydro-*1H*-benzo[*d*]azepin-2(*3H*)-one (5b)

Compound (**5b**) was obtained as white solid (1.1 g, 75%), m. p.: 132-134 ^{0}C from compound (**1**) (1 g, 6.21 mmol).

Anal.:

IR **:** 3127, 3055, 1652, 1486, 1452, 1425, 756, 701

^{1}H-NMR **:** 7.17-7.26 (m, 5H), 7.07-7.12 (m, 3H), 6.96 (bs, 1H) 4.57 (s, 2H), 3.91 (s, 2H), 3.56-3.60 (t, 2H, *J* = 8.0 Hz), 2.88-2.92 (t, 2H, *J* = 8.0 Hz)

ESI-MS (m/z) **:** 252 (M^++1)

$C_{17}H_{17}NO$ requires C, 81.24; H, 6.82; N, 5.57. Found: C, 81.64; H, 6.46; N, 5.33%

4.1.5. General procedure for differential tri-alkylation (7/8)

An oven-dried flask was charged with dry sodium hydride (450 mg, 18.6 mmol), evacuated and backfilled with nitrogen. Tetrahydrofuran (20 mL) was added at 0 ^{0}C to suspend the solid. 1,3,4,5-Tetrahydrobenzo[*d*]azepin-2-one (**1**) (1 g, 6.21 mmol) was added and allowed to stir for 15 min. The reaction mixture was allowed to stir at 0 ^{0}C for another 15 min and benzyl bromide (1.45 ml, 12.42 mmol) was added. The reaction mixture was stirred for 1.5 hr and progress of the reaction was monitored by TLC (hexane/ether 2:1). The reaction mixture was cooled at 0 ^{0}C, sodium hydride (130 mg, 6.21 mmol) and corresponding alkyl halide (R_2) (6.21 mmol) was added. The reaction mixture was allowed to warm up to room temperature in 1.5 hr and stirred for another 6 hr. After completion the reaction mixture was poured on crushed ice (100 g) and extracted with ether (20 ml). The aqueous layer was separated and additionally extracted with ether (20 ml). Organic fractions were combined, dried over magnesium sulphate, volatiles removed and the residue was purified by column chromatography using silica (100-200 mesh) as stationary phase and hexane-ethyl acetate as eluent.

4.1.5.1. 1,1,3-Tribenzyl-4,5-dihydro-*1H*-benzo[*d*]azepin-2(*3H*)-one (7a)

Compound (**7a**) was obtained as white solid (1.6 g, 62%), m. p.: 159-161 ^{0}C from compound (**1**) (1 g, 6.21 mmol).

Anal.:

IR **:** 1613, 1487, 1445, 1351, 747, 700

^{1}H-NMR **:** 6.72-7.34 (m, 19H), 4.32 (s, 2H), 3.88-3.90 (m, 2H), 3.27-3.35 (m, 2H), 2.36-2.38 (m, 2H), 1.77-1.81 (t, 2H, *J* = 8.0 Hz)

ESI-MS (m/z) **:** 432 (M^++1)

$C_{31}H_{29}NO$ requires C, 86.27; H, 6.77; N, 3.25. Found: C, 86.69; H, 6.37; N, 3.53%

4.1.5.2. 1,3-Diallyl-1-benzyl-4,5-dihydro-*1H*-benzo[*d*]azepin-2(*3H*)-one (7b)

Compound (**7b**) was obtained as yellow liquid (1.4 g, 71%) from compound (**1**) (1 g, 6.21 mmol).

Anal.:

IR **:** 3066, 3027, 1669, 1490, 1446, 1411, 752, 700

^{1}H-NMR (DMSO-d_6): 7.63-7.65 (d, 1H, *J* = 8.0 Hz), 7.17-7.38 (m, 3H), 6.97-7.14 (m, 3H), 6.84-6.86 (d, 1H, *J* = 8.0 Hz), 6.60-6.62 (d, 1H, *J* = 8.0 Hz), 5.67-5.81 (m, 1H), 5.38-5.49 (m, 1H), 5.09-5.22 (m, 2H), 4.79-4.94 (m, 2H), 3.99-4.01 (d, 1H, *J* = 8.0 Hz), 3.91-3.94 (m, 1H), 3.62-3.64 (d, 1H, *J* = 12.0 Hz), 3.40-3.42 (m, 1H), 3.09-3.28 (m, 1H), 2.97-3.03 (m, 1H), 2.82-2.93 (m, 1H), 2.62-2.67 (m, 1H), 2.12-2.16 (t, 1H, *J* = 8.0 Hz)

ESI-MS (m/z) **:** 332 (M^++1)

4.1.6. General procedure for reduction of amide (7)

Compound (**7**) (3.4 mmol) was taken in dry tetrahydrofuran (20 ml) in a reaction flask and cooled to 0 ^{0}C. Lithium aluminum hydride (0.51 g, 13.6 mmol) was added in portions and temperature of the reaction mixture was allowed to reach to RT. Progress of the reaction was monitored by TLC. The reaction mixture was quenched with ethyl acetate and filtered. Volatiles were removed and the residue was purified by column chromatography using silica (100-200 mesh) as stationary phase and hexane-ethyl acetate as eluent.

4.1.6.1. 1,1,3-Tribenzyl-2,3,4,5-tetrahydro-*1H*-benzo[*d*]azepine (8a)

Compound (**8a**) was obtained as yellow liquid (0.89 g, 63%) from compound (**7a**) (1.5 g, 3.4 mmol).

Anal.:

IR **:** 3025, 1486, 1457, 1384, 750, 698

^{1}H-NMR **:** 7.04-7.41 (m, 19H), 4.02 (s, 2H), 3.28 (bs, 2H), 3.15 (bs, 2H), 2.75 (bs, 2H), 1.81 (s, 2H), 1.25 (s, 2H)

ESI-MS (m/z) **:** 418 (M^++1)

4.1.7. General procedure for mono-/di-alkylation of N-methyl-3-benzazepin-2-one (9/10)

An oven-dried flask was charged with dry sodium hydride (450 mg, 18.6 mmol), evacuated and backfilled with nitrogen. Tetrahydrofuran (20 ml) was added at 0 ^{0}C to suspend the solid. 3-Methyl-1,3,4,5-tetrahydrobenzo[*d*]azepin-2-one (**5c**) (1 g, 5.71mmol) was added to the above suspension and the reaction mixture allowed to stir

for 15 min. The reaction mixture was further stirred at 0 ^{0}C for another 15 min and alkyl halide (5.71 mmol) (**9a**) / (11.42 mmol) (**10a, 10b**) was added. The reaction mixture was stirred for 1.5 hr and progress of the reaction was monitored by TLC (hexane/ether 2:1). The reaction mixture was allowed to warm up to RT in 1.5 hr and stirred for 6 hr. After completion, the reaction mixture was poured on crushed ice (100 g) and extracted with ether (20 ml X 3). Organic fractions were combined, dried over magnesium sulphate, volatiles removed and the residue was purified by column chromatography using silica (100-200 mesh) as stationary phase and hexane-ethyl acetate as eluent.

4.1.7.1. 1-Allyl-3-methyl-4,5-dihydro-*1H*-benzo[*d*]azepin-2(*3H*)-one (9a)

Compound (**9a**) was obtained as yellow liquid (0.84 g, 69%) from 3-methyl-1,3,4,5-tetrahydrobenzo[*d*]azepin-2-one (**5c**) (1 g, 5.71mmol).

Anal.:

IR **:** 3070, 1655, 1488, 1442, 1398, 753

^{1}H-NMR **:** 7.04-7.13 (m, 4H) 5.80-5.89 (m, 1H), 5.03-5.09 (m,1H), 4.95-4.99 (m, 1H), 4.11-4.15 (t, 1H, J = 8.0 Hz), 3.90-3.97 (m, 1H), 3.32-3.38 (m, 1H), 3.20-3.27 (m, 1H), 3.01-3.07 (m, 1H), 2.90-2.96 (m, 1H) 2.89 (s, 3H), 2.58-2.63 (m, 1H)

ESI-MS (m/z) **:** 216 (M^++1)

4.1.7.2. 1,1-Diethyl-3-methyl-4,5-dihydro-*1H*-benzo[*d*]azepin-2(*3H*)-one (10a)

Compound (**10a**) was obtained as yellow liquid (0.85 g, 65%) from 3-methyl-1,3,4,5-tetrahydrobenzo[*d*]azepin-2-one (**5c**) (1 g, 5.71mmol).

Anal.:

IR **:** 1625. 1488, 1458, 1385, 754

^{1}H-NMR **:** 7.31-7.33 (d, 1H, *J* = 8.1 Hz), 7.13-7.17 (t, 1H, *J* = 8.0 Hz), 7.02-7.06 (t, 1H, *J* = 8.0 Hz), 6.94-6.96 (d, 1H, *J* = 8.4 Hz), 3.42- 3.46 (t, 2H, *J* = 4.0 Hz), 3.02 (s, 3H), 2.90- 2.94 (t, 2H, *J* = 8.0 Hz), 2.26-2.35 (m, 2H), 1.77-1.86 (m, 2H), 0.58-0.62 (t, 6H, *J* = 8.0 Hz)

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

4.1.7.3. 1,1-Dibenzyl-3-methyl-4,5-dihydro-*1H*-benzo[*d*]azepin-2(*3H*)-one (10b)

Compound (**10b**) was obtained as white solid (1.3 g, 68%), m.p.: 102-104 ^{0}C from 3-methyl-1,3,4,5-tetrahydrobenzo[*d*]azepin-2-one (**5c**) (1 g, 5.71mmol).

Anal.:

IR **:** 3060, 3027, 1613, 1491, 1445, 1393, 746, 699

^{1}H-NMR **:** 7.81-7.83 (d, 1H, *J* = 8.0 Hz), 7.28-7.32 (t, 1H, *J* = 8.0 Hz), 6.91-7.04 (m, 7H), 6.68-6.70 (d, 4H, *J* = 4.0 Hz), 6.62-6.64 (d, 1H, *J* = 8.0 Hz), 3.83-3.85 (d, 2H, *J* = 12.8 Hz), 3.25-3.27 (m, 2H), 2.76 (s, 3H), 2.41-2.43 (m, 2H), 1.97-1.99 (m, 2H)

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

$C_{25}H_{25}NO$ requires C, 84.47; H, 7.09; N, 3.94. Found: C, 84.15; H, 7.45; N, 3.62%

4.1.8. General procedure for reductive alkylation to obtain (11-14)

Compound (**9**/**10**) (4.0 mmol) was taken in dry tetrahydrofuran (15 ml) in a reaction flask and cooled to 0 ^{0}C; lithium aluminum hydride (0.62 g, 16.0 mmol) was added in portions and the temperature of the reaction mixture was allowed to reach to RT. Progress of the reaction was monitored by TLC. The reaction mixture was quenched with ethyl acetate and filtered. Volatiles were removed and the residue was purified by column chromatography using silica (100-200 mesh) as stationary phase and hexane-ethyl acetate as eluent yielding compound (**11**).

To the purified compound (**11**) excess of ethyl chloroformate (10 ml), N,N-diisopropylethylamine (12.0 ml, 8.0 mmol) and chloroform (20 ml) were added and the reaction mixture was refluxed for 8 hr. Progress of the reaction was monitored by TLC. The reaction mixture was concentrated and purified by column chromatography yielding compound (**12**).

The purified compound (**12**) was refluxed in HCl (10 ml, 10 N) with methanol (30 ml) for 12 hr. The reaction mixture was concentrated and then diluted with distilled water (30 ml), basified with sodium hydroxide solution (10% w/v) and the organic layer was extracted with ethyl acetate (30 ml X 3). The organic layer was to removed, yielding compound (**13**).

Compound (**13**) was taken in dry methanol (40 ml) and corresponding substituted benzaldehyde (7.45 mmol) was added and stirred for 1 hr. Sodium borohydride (0.46 g, 12.0 mmol) was added in portions and the reaction mixture was stirred at RT for 12 hr. Progress of the reaction was monitored by TLC. Volatiles were removed from reaction mixture and the crude product was purified by column chromatography yielding compound (**14**).

4.1.8.1. 1,1-Diethyl-3-methyl-2,3,4,5-tetrahydro-*1H*-benzo[*d*]azepine (11a)

Compound (**11a**) was obtained as dark yellow liquid (0.54 g, 62%) from compound (**10a**) (0.92 g, 4.0 mmol).

Anal.:

IR **:** 3060, 1456, 1380, 750

^{1}H-NMR **:** 7.06-7.22 (m, 4H), 3.02-3.04 (m, 2H), 2.62 (bs, 2H), 2.54 (s, 2H), 2.39 (s, 3H), 1.76-1.87 (m, 4H), 076-0.82 (m, 6H)

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

4.1.8.2. Ethyl 1-methyl-1,2,4,5-tetrahydrobenzo[*d*]azepine-3-carboxylate (12a)

Compound (**12a**) was obtained as pale yellow liquid (0.67 g, 72%) from 1,3-dimethyl-2,3,4,5-tetrahydro-*1H*-benzo[*d*]azepine[63] (0.7 g, 4.0 mmol).

Anal.:

IR **:** 3062, 1697, 1461, 1429, 1383, 1237, 761

^{1}H-NMR **:** 7.08-7.16 (m, 4H), 4.12-4.20 (m, 2H), 3.89 (bs, 1H), 3.70 (bs, 1H), 3.35-3.56 (m, 2H), 3.08-3.10 (m, 2H), 2.79-2.87 (m, 1H), 1.20-1.32 (m, 6H)

ESI-MS (m/z) **:** 234 (M^++1)

4.1.8.3. Ethyl 1-ethyl-1,2,4,5-tetrahydrobenzo[*d*]azepine-3-carboxylate (12b)

Compound (**12b**) was obtained as pale yellow liquid (0.68 g, 68%) from compound (**4a**) (0.76 g, 4.0 mmol).

Anal.:

IR **:** 1696, 1461, 1429, 1384, 1236, 758

^{1}H-NMR **:** 7.09-7.18 (m, 4H), 4.12-4.20 (m, 2H), 3.96-4.03 (m, 1H), 3.34-3.37 (m, 1H), 3.08-3.17 (m, 2H), 2.75-2.78 (m, 2H), 1.62-1.77 (m, 2H), 1.28-1.32 (t, 3H, *J* = 8.0 Hz), 0.87-0.91 (t, 3H, *J* = 8.0 Hz)

ESI-MS (m/z) **:** 248 (M^++1)

4.1.8.4. Ethyl 1,1-diethyl-1,2,4,5-tetrahydrobenzo[*d*]azepine-3-carboxylate (12c)

Compound (**12c**) was obtained as pale yellow liquid (0.83 g, 76%) from compound (**11a**) (0.87 g, 4.0 mmol).

Anal.:

IR **:** 1696, 1467, 1422, 1327, 1281, 767

^{1}H-NMR **:** 7.06-7.25 (m, 4H), 4.10-4.20 (m, 2H), 3.61-3.78 (m, 4H), 3.01-3.07 (q, 2H, *J* = 8.0 Hz), 1.68-1.81 (m, 4H), 1.24-1.35 (m, 3H), 0.74-0.78 (m, 6H)

ESI-MS (m/z) **:** 276 (M^++1)

4.1.8.5. 1,1-Dibenzyl-2,3,4,5-tetrahydro-*1H*-benzo[*d*]azepine (13a)

Compound (**13a**) was obtained as bright yellow liquid (0.86 g, 66%) from compound (**12d**) (1.6 g, 4.0 mmol).

Anal.:

IR **:** 3412, 3026, 1695, 1491, 1401, 743, 701

^{1}H-NMR **:** 7.36-7.38 (m, 2H), 7.09-7.16 (m, 8H), 6.87-6.88 (m, 2H), 4.04-4.13 (m, 2H), 3.90 (bs, 1H), 3.24-3.34 (m, 2H), 2.83-2.98 (m, 2H), 1.12-1.27 (m, 4H)

ESI-MS (m/z) **:** 328 (M^++1)

4.1.8.6. 4-((1-Ethyl-1,2,4,5-tetrahydrobenzo[*d*]azepin-3-yl)methyl)-*N*,*N*-dimethyl--benzenamine (14a)

Compound (**14a**) was obtained as yellow liquid (0.76 g, 62%) from 1-benzyl-2,3,4,5-tetrahydro-*1H*-benzo[*d*]azepine[64] (0.94 g, 4.0 mmol).

Anal.:

IR **:** 3130, 1697, 1459, 1430, 1343, 758

^{1}H-NMR **:** 7.02-7.09 (m, 8H), 4.14 (s, 2H), 3.89-4.08 (m, 2H), 3.27-3.31 (m, 2H), 3.01-3.06 (m, 2H), 2.87 (s, 6H), 2.68-2.71 (m, 1H), 1.57-1.70 (m, 2H), 0.82-0.86 (t, 3H, *J* = 8.0 Hz)

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

4.2. Biological studies

Sprague-Dawley rats (200–250 g) or Swiss albino mice (25–35 g) of either sex were used for these studies. Animals were housed in a temperature and humidity-controlled facility with a 12 h light/dark cycle and free access to food and water throughout the study. All animal procedures were reviewed and approved by Institutional Animal Ethical Committee, Pharmacy Department, The M. S. University of Baroda. Samples of test compounds were prepared using DMSO and distilled water in such a way that the final concentration of DMSO did not exceed 2%.

4.2.1. *In vitro* 5HT assay using isolated rat fundus preparation

Male Sprague Dawley rats (200-250 g) after fasting for 48 hrs were sacrificed by over dose of anaesthetic agent. The abdomen was opened, fundus taken out and washed. The fundus was slit along the greater curvature and spread out. Some of the strips were obtained by cutting the fundus horizontally while certain others were obtained by cutting it longitudinally. The cut strip about 4 cm long was suspended in a 20 ml aerated bath containing Tyrode solution at 37 ^{0}C. Assay was started 30 min later. At least 6 longitudinal and 6 horizontal strip preparations were used for each test substance. Contractile responses were recorded on a 2-channel recorder with an isotonic transducer (UGO Basile, Italy). A 3 min cycle was followed, including a 60 sec exposure time to test the substance. Solutions of varying concentrations of 5-hydroxytryptamine creatinine sulphate complex (Sigma) were used as standared.[65]

4.2.2. *In vitro* 5HT assay using isolated rat thoracic aorta strip

Male Sprague Dawley rats (200-250 g) were used for the study. The animals were sacrificed by cervical dislocation; descending thoracic aortas were removed immediately and placed in ice-cold Kreb's bicarbonate solution of the following composition (mM): NaCl (112), $NaHCO_3$ (12), glucose (11.1), KCl (5.0), $MgSO_4$ (1.2), KH_2PO_4 (1.0) and $CaCl_2$ (2.5). The tissue was aerated with 95% O_2 and 5% CO_2. Peri-adventitious tissue was removed taking care not to stretch the tissue. A needle was inserted in the tissue and rotated gently to denude the endothelium. Following this, the tissue was cut spirally into a helical strip (20 mm × 3 mm) using a surgical blade. The strip was tied at both ends using a cotton thread and suspended in a 25 ml organ tube under an initial resting tension of 2 g. The *p*H of the Kreb's solution was 7.4 and maintained at 37 ^{0}C using a thermostat. The Kreb's solution in

the organ tube was changed every 10 mins during an equilibration period of about 90 mins. Isometric contractions were recorded using a force transducer (UGO BASILE, Italy) coupled to a Gemini 7070 recorder (UGO BASILE, Italy).[66]

4.2.3. Tail Suspension Test (TST) in mice

The tail-suspension test[67] in mice is a corroboration of the forced-swim test, and more sensitive to a broader range of antidepressants. Pre-treatment with fluoxetine (10 mg/kg) as a positive control, compounds (**3b, 4a, 4b, 4c, 7a, 7b, 8a, 10b, 11a, 12a, 12b, 12c, 13a, 14a**), or saline were given to the animals 30 min before testing. Mice were then individually suspended by their tails, 35 cm above the tabletop with the use of an adhesive tape placed 1 cm from the tip of the tail. Behaviour was scored throughout 5 min test as either mobile or immobile. Mice were considered immobile only when hanging passively and completely motionless.[68]

4.2.4. Elevated Plus-Maze (EPM) test

The plus-maze apparatus consisted of two open arms (15×5 cm) and two closed arms (15×5×5 cm), extending from a central platform (5×5 cm) and raised 50 cm above floor level. Mice were randomly assigned to drug treatment groups. 30 Min after the administration of the dose, each mouse was individually placed at the centre of the EPM with its head facing the open arms and allowed for free exploration for 5min The behaviour of the mouse was recorded as the number of entries into the open or closed arms (forelimbs on open or closed arms) and the time spent on open and closed arms.[69]

4.2.5. Hypophagic response in rats

Rats were individually housed on day 1 and deprived of food. Twenty three hours later they were dosed with ***m*CPP**, compounds (**4c, 7a, 10b, 12c** and **13a**), **SB206553**[70] and vehicle, and then returned to their home cages. Fifteen minutes later the **SB206553**-treated group animals were given compounds (**4c, 7a, 10b, 12c** and **13a**) by i.p. and again returned to their home cages. After a period of 20 min, weighed aliquots of their normal food pellets were placed in their food hoppers and the amount remaining after 2 h was measured.[71]

4.2.6. Rat model of penile erection

All experiments were performed between 08:30 and 14:30 h. Groups of five rats, comprising one control and four drug-treated animals were observed simultaneously. Group data are composed of several replication runs over a two-day period. After the agonist injection the rats were placed in individual transparent perspex cages (7.5×18×30 cm). A mirror was placed behind the row of observation cages to facilitate observation of the animals. The number of penile erections and yawns were counted for 1 h following the last injection. A penile erection was considered to occur when the following behaviours were present- repeated pelvic thrusts immediately followed by an upright position, an emerging engorged penis which the rat proceeds to lick while eating the ejaculate.[72] For studies on antagonism of penile erections or other behaviours the antagonist was given 30 min before the agonist.