**Supplementary data**

**2. Materials and Methods**

**2.1. Chemistry**

Melting points of the synthesized compounds were recorded in open capillary tubes and are uncorrected. NMR spectra were recorded on a Bruker 500 MHz instrument in CDCl3 and IR spectra were recorded on a Perkin Elmer system 2000 FT-IR instrument (KBr). Mass spectra were recorded on a DART-ToF-MS mass spectrometer. Perkin Elmer 2400 Series II Elemental CHNS analyzer was used to perform elemental analyses.

**2.1.1. General procedure for the synthesis of cage-like heterocyclic hybrids 4(a-l)**

An equimolar mixture of bisarylmethylidenetetrahydropyridinone **1**, acenaphthenequinone **2** and Isoquinoline-3-carboxylic acid **3** in 100 mg of [bmim]Br was irradiated in a CEM microwave synthesizer at 100 oC for 9-12 min. When the reaction was complete, as judged by TLC analysis, ethyl acetate (10 mL) was added, followed by stirring at room temperature for 15 min. The ethyl acetate layer was washed with water (50 mL) and was evaporated under reduced pressure. The residue was dried *in vacuo* and purified by column chromatography, eluting with a 6:4 petroleum ether–ethyl acetate mixture.

**2.1.1.1. Cage-like heterocyclic hybrid, 4a**

Obtained as pale brown solid, (89%); mp = 176–178 °C; IR (KBr): 1594 (-C=C stretching), 1691 (-C=O stretching), 3421 (-OH) cm-1; 1H NMR (500 MHz, CDCl3): δH 2.37 (s, 3H, CH3), 2.60-2.64 (m, 1H, H-18), 2.81 (s, 3H, CH3), 2.96-3.00 (m, 1H, H-25), 3.21-3.41 (m, 3H, H-18, H-13 and H-24), 3.51 (d, 1H, *J* = 14.5 Hz, H-13), 3.60-3.70 (m, 1H, H-24), 4.18–4.32 (m, 2H, H-20 and H-25), 4.49-4.59 (m, 1H, H-19), 6.50 (s, 1H, H-26), 6.66 (d, 1H, *J* = 7.5 Hz, ArH), 6.80-7.02 (m, 5H, ArH), 7.09-7.48 (m, 8H, ArH), 7.56-7.64 (m, 2H, ArH), 7.67 (d, 1H, *J* = 7.5 Hz, ArH), 7.74 (d, 1H, *J* = 8.0 Hz, ArH). 13C NMR (125 MHz, CDCl3): δC 20.06, 20.71, 33.35, 47.98, 50.85, 52.36, 56.92, 62.94, 72.51, 82.98, 97.34, 121.34, 124.48, 124.97, 125.54, 125.62, 125.83, 125.93, 126.31, 126.40, 127.38, 127.49, 127.83, 127.91, 128.40, 128.98, 129.24, 129.63, 130.37, 131.23, 132.58, 133.02, 134.13, 134.65, 134.79, 135.07, 135.50, 135.65, 137.07, 138.83, 196.23. Mass: 601 [M+]. Anal.calcd for C42H36N2O2: C, 83.97; H, 6.04; N, 4.66%; found: C, 83.84; H, 6.14; N, 4.57%.

**2.1.1.2. Cage-like heterocyclic hybrid, 4b**

Obtained as pale brown solid, (88%); mp = 189–191 °C; IR (KBr): 1597 (-C=C stretching), 1685 (-C=O stretching), 3424 (-OH) cm-1; 1H NMR (500 MHz, CDCl3): δH 2.21 (s, 3H, CH3), 2.39 (s, 3H, CH3), 2.86 (dd, 1H, *J* = 14.5, 9.0 Hz, H-18), 3.06 (d, 1H, *J* = 12.0 Hz, H-25), 3.08-3.11 (m, 1H, H-18), 3.22 (d, 1H, *J* = 13.5 Hz, H-13), 3.42 (d, 1H, *J* = 17.5 Hz, H-24), 3.62-3.69 (m, 2H, H-13 and H-24), 4.21-4.33 (m, 2H, H-20 and H-25), 4.43-4.48 (m, 1H, H-19), 6.16 (s, 1H, H-26), 6.23 (d, 2H, *J* = 7.5 Hz, ArH), 6.27 (s, 1H, ArH), 6.74 (d, 1H, *J* = 7.0 Hz, ArH), 6.92-7.00 (m, 2H, ArH), 7.09-7.14 (m, 2H, ArH), 7.18-7.39 (m, 6H, ArH), 7.49 (d, 1H, *J* = 7.5 Hz, ArH), 7.57 (d, 1H, *J* = 8.0 Hz, ArH), 7.63 (d, 1H, *J* = 7.0 Hz, ArH), 7.73 (d, 1H, *J* = 8.0 Hz, ArH). 13C NMR (125 MHz, CDCl3): δC21.21, 21.58, 34.57, 47.98, 52.42, 52.83, 56.45, 63.23, 72.65, 94.42, 121.15, 124.33, 125.64, 125.71, 125.87, 126.34, 126.39, 126.42, 127.29, 127.50, 127.64, 128.14, 128.43, 128.78, 129.20, 130.21, 130.28, 131.75, 132.03, 133.21, 134.32, 135.42, 135.60, 135.84, 136.81, 136.97, 137.23, 138.17, 196.39. Mass: 601 [M+]. Anal.calcd for C42H36N2O2: C, 83.97; H, 6.04; N, 4.66%; found: C, 83.81; H, 6.17; N, 2.78%.

**2.1.1.3. Cage-like heterocyclic hybrid, 4c**

Obtained as pale brown solid, (92%); mp = 193–195 °C; IR (KBr): 1596 (-C=C stretching), 1687 (-C=O stretching), 3422 (-OH) cm-1; 1H NMR (500 MHz, CDCl3): δH 2.23 (s, 3H, CH3), 2.41 (s, 3H, CH3), 2.85 (dd, 1H, *J* = 14.5, 8.5 Hz, H-18), 3.02 (d, 1H, *J* = 11.0 Hz, H-25), 3.07 (dd, 1H, *J* = 15.0, 4.5 Hz, H-18), 3.18 (d, 1H, *J* = 14.0 Hz, H-13), 3.40 (d, 1H, *J* = 18.0 Hz, H-24), 3.61-3.69 (m, 2H, H-13 and H-24), 4.25-4.28 (m, 2H, H-20 and H-25), 4.40-4.45 (m, 1H, H-19), 6.11 (s, 1H, H-26), 6.35 (d, 2H, *J* = 8.0 Hz, ArH), 6.73 (d, 1H, *J* = 7.5 Hz, ArH), 6.88 (d, 2H, *J* = 8.0 Hz, ArH), 7.07-7.34 (m, 8H, ArH), 7.41 (d, 2H, *J* = 8.0 Hz, ArH), 7.56 (d, 1H, *J* = 7.5 Hz, ArH), 7.62 (d, 1H, *J* = 7.0 Hz, ArH), 7.69 (d, 1H, *J* = 8.0 Hz, ArH). 13C NMR (125 MHz, CDCl3): δC21.03, 21.28, 34.55, 48.00, 52.10, 52.94, 56.37, 63.18, 72.61, 94.39, 121.09, 124.33, 125.80, 126.33, 126.41, 127.27, 127.43, 127.49, 128.56, 128.85, 129.28, 129.31, 129.68, 129.74, 130.61, 130.80, 131.17, 132.07, 132.41, 133.71, 135.36, 135.90, 136.00, 136.96, 137.01, 138.69, 196.64. Mass: 601 [M+]. Anal.calcd for C42H36N2O2: C, 83.97; H, 6.04; N, 4.66%; found: C, 83.75; H, 6.21; N, 4.49%.

**2.1.1.4. Cage-like heterocyclic hybrid, 4d**

Obtained as pale brown solid, (82%); mp = 232–234 °C; IR (KBr): 1596 (-C=C stretching), 1687 (-C=O stretching), 3421 (-OH) cm-1; 1H NMR (500 MHz, CDCl3): δH 2.84 (dd, 1H, *J* = 14.5, 8.5 Hz, H-18), 2.95 (dd, 1H, *J* = 15.0, 4.5 Hz, H-18), 3.05 (d, 1H, *J* = 16.5 Hz, H-25), 3.16 (d, 1H, *J* = 13.5 Hz, H-13), 3.35 (d, 1H, *J* = 18.0 Hz, H-24), 3.58-3.67 (m, 2H, H-13 and H-24), 3.84 (s, 3H, OCH3), 3.96 (s, 3H, OCH3), 4.36-4.39 (m, 1H, H-19), 4.53 (d, 1H, *J* = 12.0 Hz, H-25), 4.73 (d, 1H, *J* = 11.0 Hz, H-20), 6.01 (d, 1H, *J* = 6.5 Hz, ArH), 6.30 (s, 1H, H-26), 6.48-6.62 (m, 4H, ArH), 6.70 (d, 1H, *J* = 7.5 Hz, ArH), 6.88-7.22 (m, 6H, ArH), 7.28-7.60 (m, 5H, ArH), 7.71 (d, 1H, *J* = 8.5 Hz, ArH). 13C NMR (125 MHz, CDCl3): δC34.19, 48.06, 52.43, 52.58, 55.13, 55.82, 56.90, 62.79, 72.29, 82.81, 94.16, 109.85, 110.63, 111.52, 119.34, 120.05, 120.48, 120.96, 121.25, 123.12, 124.34, 125.61, 126.17, 126.26, 127.10, 127.36, 128.30, 129.49, 129.86, 130.12, 130.58, 131.36, 132.82, 133.41, 135.31, 136.10, 137.07, 138.65, 158.30, 158.49, 196.41. Mass: 633 [M+]. Anal.calcd for C42H36N2O4: C, 79.72; H, 5.73; N, 4.43%; found: C, 79.85; H, 5.82; N, 4.24%.

**2.1.1.5. Cage-like heterocyclic hybrid, 4e**

Obtained as pale brown solid, (85%); mp = 241–243 °C; IR (KBr): 1595 (-C=C stretching), 1689 (-C=O stretching), 3424 (-OH) cm-1; 1H NMR (500 MHz, CDCl3): δH 2.85 (dd, 1H, *J* = 14.5, 8.0 Hz, H-18), 3.04 (d, 1H, *J* = 12.5 Hz, H-25), 3.06-3.12 (m, 1H, H-18), 3.20 (d, 1H, *J* = 14.5 Hz, H-13), 3.40 (d, 1H, *J* = 19.0 Hz, H-24), 3.59-3.68 (m, 2H, H-13 and H-24), 3.69 (s, 3H, OCH3), 3.83 (s, 3H, OCH3), 4.25-4.28 (m, 2H, H-19 and H-25), 4.40-4.45 (m, 1H, H-20), 5.93 (s, 1H, ArH), 5.99 (d, 1H, *J* = 7.0 Hz, ArH), 6.24 (s, 1H, H-26), 6.66 (d, 1H, *J* = 7.0 Hz, ArH), 6.72 (d, 1H, *J* = 6.5 Hz, ArH), 6.82 (d, 1H, *J* = 7.0 Hz, ArH), 6.97-7.50 (m, 10H, ArH), 7.56 (d, 1H, *J* = 8.0 Hz, ArH), 7.60 (d, 1H, *J* = 7.0 Hz, ArH), 7.72 (d, 1H, *J* = 8.0 Hz, ArH). 13C NMR (125 MHz, CDCl3): δC34.53, 47.96, 52.41, 52.89, 55.14, 55.22, 55.48, 62.08, 72.91, 86.20, 93.78, 108.77, 114.27, 114.81, 120.92, 121.18, 122.59, 121.99, 124.45, 125.80, 126.34, 127.27, 127.47, 127.64, 128.22, 128.76, 129.51, 129.80, 130.81, 133.03, 133.44, 133.91, 135.32, 135.77, 137.12, 138.73, 155.63, 158.73, 159.66, 196.47. Mass: 633 [M+]. Anal.calcd for C42H36N2O4: C, 79.72; H, 5.73; N, 4.43%; found: C, 79.91; H, 5.59; N, 4.55%.

**2.1.1.6. Cage-like heterocyclic hybrid, 4f**

Obtained as pale brown solid, (84%); mp = 226–228 °C; IR (KBr): 1591 (-C=C stretching), 1683 (-C=O stretching), 3421 (-OH) cm-1; 1H NMR (500 MHz, CDCl3): δH 2.84 (dd, 1H, *J* = 14.0, 8.5 Hz, H-18), 3.05 (d, 1H, *J* = 11.5 Hz, H-25), 3.12 (dd, 1H, *J* = 14.5, 5.0 Hz, H-18), 3.21 (d, 1H, *J* = 14.0 Hz, H-13), 3.42 (d, 1H, *J* = 16.5 Hz, H-24), 3.57-3.67 (m, 2H, H-13 and H-24), 3.71 (s, 3H, OCH3), 3.85 (s, 3H, OCH3), 4.24-4.30 (m, 2H, H-20 and H-25), 4.42-4.48 (m, 1H, H-19), 6.14 (s, 1H, H-26), 6.37 (d, 2H, *J* = 8.0 Hz, ArH), 6.75 (d, 1H, *J* = 7.5 Hz, ArH), 6.91 (d, 2H, *J* = 8.0 Hz, ArH), 7.10-7.39 (m, 8H, ArH), 7.44 (d, 2H, *J* = 8.5 Hz, ArH), 7.52 (d, 1H, *J* = 8.0 Hz, ArH), 7.65 (d, 1H, *J* = 7.5 Hz, ArH), 7.72 (d, 1H, *J* = 7.5 Hz, ArH). 13C NMR (125 MHz, CDCl3): δC34.21, 48.05, 51.89, 52.90, 56.42, 62.74, 72.29, 94.26, 112.35, 114.15, 115.60, 121.22, 121.95, 125.83, 126.31, 126.59, 127.47, 127.83, 127.98, 128.49, 128.80, 129.21, 129.71, 130.28, 131.18, 132.05, 133.60, 135.31, 135.78, 136.96, 138.69, 155.551, 158.84, 159.52, 196.23. Mass: 633 [M+]. Anal.calcd for C42H36N2O4: C, 79.72; H, 5.73; N, 4.43%; found: C, 79.81; H, 5.94; N, 4.65%.

**2.1.1.7. Cage-like heterocyclic hybrid, 4g**

Obtained as pale brown solid, (87%); mp = 203–205 °C; IR (KBr): 1593 (-C=C stretching), 1689 (-C=O stretching), 3420 (-OH) cm-1; 1H NMR (500 MHz, CDCl3): δH 2.93-2.96 (m, 1H, H-18), 3.13-3.41 (m, 4H, H-25, H-18, H-13 and H-24), 3.54-3.68 (m, 2H, H-13 and H-24), 4.16–4.23 (m, 1H, H-25), 4.45–4.54 (m, 1H, H-19), 4.97 (d, 1H, *J* = 11.0 Hz, H-20), 6.32 (s, 1H, H-26), 6.66 (d, 1H, *J* = 7.5 Hz, ArH), 6.91-7.12 (m, 7H, ArH), 7.20-7.62 (m, 8H, ArH), 7.69 (d, 1H, *J* = 8.0 Hz, ArH), 7.76 (d, 1H, *J* = 8.5 Hz, ArH). 13C NMR (125 MHz, CDCl3): δC33.30, 47.98, 50.44, 52.19, 56.72, 62.80, 72.60, 82.85, 96.45, 121.09, 124.60, 125.79, 126.28, 126.35, 126.42, 126.77, 127.34, 127.54, 127.71, 128.39, 129.21, 129.27, 129.49, 129.91, 130.10, 130.62, 130.91, 132.37, 133.19, 133.42, 133.85, 133.96, 134.07, 134.96, 135.41, 136.23, 136.84, 137.66, 138.17, 195.56. Mass: 641 [M+]. Anal.calcd for C40H30Cl2N2O2: C, 74.88; H, 4.71; N, 4.37%; found: C, 74.72; H, 4.82; N, 4.20%.

**2.1.1.8. Cage-like heterocyclic hybrid, 4h**

Obtained as pale brown solid, (85%); mp = 209–211 °C; IR (KBr): 1594 (-C=C stretching), 1686 (-C=O stretching), 3422 (-OH) cm-1; 1H NMR (500 MHz, CDCl3): δH 2.86-2.94 (m, 1H, H-18), 3.16-3.36 (m, 4H, H-25, H-18, H-13 and H-24), 3.50-3.63 (m, 2H, H-13 and H-24), 4.14–4.22 (m, 1H, H-25), 4.44–4.50 (m, 1H, H-19), 4.88 (d, 1H, *J* = 11.0 Hz, H-20), 6.22 (s, 1H, H-26), 6.41 (s, 1H, ArH), 6.65 (d, 1H, *J* = 6.5 Hz, ArH), 6.86-7.00 (m, 3H, ArH), 7.08-7.31 (m, 6H, ArH), 7.08-7.31 (m, 5H, ArH). 13C NMR (125 MHz, CDCl3): δC33.27, 47.91, 50.44, 52.32, 56.61, 62.72, 72.72, 82.90, 96.53, 121.27, 124.72, 126.32, 126.39, 126.43, 126.56, 127.55, 127.69, 127.88, 128.60, 129.22, 129.29, 130.02, 130.07, 130.41, 130.49, 130.97, 131.595, 132.01, 133.23, 133.66, 133.73, 134.43, 134.63, 134.87, 135.22, 136.84, 137.08, 141.02, 195.52. Mass: 711 [M+]. Anal.calcd for C40H28Cl4N2O2: C, 67.62; H, 3.97; N, 3.94%; found: C, 67.80; H, 3.74; N, 3.85%.

**2.1.1.9. Cage-like heterocyclic hybrid, 4i**

Obtained as pale brown solid, (83%); mp = 192–194 °C; IR (KBr): 1595 (-C=C stretching), 1685 (-C=O stretching), 3417 (-OH) cm-1; 1H NMR (500 MHz, CDCl3): δH 2.94-2.98 (m, 1H, H-18), 3.14-3.45 (m, 4H, H-25, H-18, H-13 and H-24), 3.53-3.66 (m, 2H, H-13 and H-24), 4.15–4.21 (m, 1H, H-25), 4.51–4.54 (m, 1H, H-19), 4.99 (d, 1H, *J* = 11.0 Hz, H-20), 6.52 (s, 1H, H-26), 6.67 (d, 1H, *J* = 7.5 Hz, ArH), 6.91-6.98 (m, 3H, ArH), 7.08-7.34 (m, 8H, ArH), 7.45-7.80 (m, 6H, ArH). 13C NMR (125 MHz, CDCl3): δC33.25, 47.97, 51.14, 51.91, 56.61, 65.23, 73.40, 82.82, 96.40, 121.15, 123.79, 124.62, 126.25, 126.35, 126.44, 126.48, 127.32, 127.37, 127.43, 127.68, 127.77, 127.81, 128.71, 129.18, 129.57, 131.01, 132.40, 132.48, 133.71, 134.05, 134.37, 134.575, 134.79, 135.45, 136.68, 136.82, 137.73, 138.32, 195.32. Mass: 825 [M+]. Anal.calcd for C40H30Br2N2O2: C, 65.77; H, 4.14; N, 3.83%; found: C, 65.91; H, 4.26; N, 3.70%.

**2.1.1.10. Cage-like heterocyclic hybrid, 4j**

Obtained as pale brown solid, (82%); mp = 214–216 °C; IR (KBr): 1591 (-C=C stretching), 1684 (-C=O stretching), 3424 (-OH) cm-1; 1H NMR (500 MHz, CDCl3): δH 2.83-2.87 (m, 1H, H-18), 2.99-3.03 (m, 1H, H-25,), 3.20-3.30 (m, 3H, H-18, H-13 and H-24), 3.58-3.73 (m, 2H, H-13 and H-24), 3.98–4.03 (m, 1H, H-25), 4.40–4.53 (m, 1H, H-19), 4.98 (d, 1H, *J* = 11.0 Hz, H-20), 6.18 (t, 1H, ArH), 6.22 (s, 1H, H-26), 6.70 (d, 1H, *J* = 7.5 Hz, ArH), 6.77-6.84 (m, 3H, ArH), 7.04-7.50 (m, 11H, ArH), 7.57 (d, 1H, *J* = 8.0 Hz, ArH), 7.76 (d, 1H, *J* = 8.0 Hz, ArH). 13C NMR (125 MHz, CDCl3): δC34.14, 47.13, 50.67, 52.38, 56.08, 62.75, 72.80, 82.87, 96.41, 115.97, 116.41, 121.08, 123.04, 123.15, 123.80, 123.91, 124.14, 124.41, 126.16, 126.35, 126.44, 127.38, 127.46, 127.57, 128.76, 128.97, 130.05, 130.23, 130.34, 130.77, 130.90, 133.42, 134.82, 135.31, 135.45, 136.95, 138.36, 158.02, 164.90, 196.12. Mass: 609 [M+]. Anal.calcd for C40H30F2N2O2: C, 78.93; H, 4.97; N, 4.60%; found: C, 78.79; H, 4.85; N, 4.71%.

**2.1.1.11. Cage-like heterocyclic hybrid, 4k**

Obtained as pale brown solid, (87%); mp = 223–225 °C; IR (KBr): 1594 (-C=C stretching), 1686 (-C=O stretching), 3428 (-OH) cm-1; 1H NMR (500 MHz, CDCl3): δH 2.78-2.86 (m, 1H, H-18), 2.98 (d, 1H, *J* = 12.0 Hz, H-25), 3.06 (dd, 1H, *J* = 14.5, 5.0 Hz, H-18), 3.21 (d, 1H, *J* = 13.5 Hz, H-13), 3.32 (d, 1H, *J* = 16.5 Hz, H-24), 3.59 (d, 1H, *J* = 14.0 Hz, H-13), 3.65-3.70 (m, 1H, H-24), 4.22-4.26 (m, 2H, H-20 and H-25), 4.38-4.43 (m, 1H, H-19), 6.18 (s, 1H, H-26), 6.39-6.41 (m, 1H, ArH), 6.71-6.79 (m, 2H, ArH), 6.98-7.38 (m, 8H, ArH), 7.46-7.85 (m, 5H, ArH), 7.95 (d, 2H, *J* = 8.5 Hz, ArH). 13C NMR (125 MHz, CDCl3): δC34.34, 47.90, 51.69, 52.52, 56.40, 62.62, 72.28, 94.40, 115.66, 115.83, 121.20, 122.53, 124.26, 125.75, 126.37, 126.46, 127.28, 127.46, 127.55, 127.98, 130.41, 130.58, 131.26, 132.38, 132.45, 133.43, 134.34, 134.60, 134.96, 135.32, 136.63, 139.62, 161.05, 162.41, 196.46. Mass: 609 [M+]. Anal.calcd for C40H30F2N2O2: C, 78.93; H, 4.97; N, 4.60%; found: C, 79.11; H, 4.81; N, 4.47%.

**2.1.1.12. Cage-like heterocyclic hybrid, 4l**

Obtained as pale brown solid, (89%); mp = 201–203 °C; IR (KBr): 1596 (-C=C stretching), 1687 (-C=O stretching), 3421 (-OH) cm-1; 1H NMR (500 MHz, CDCl3): δH 2.82 (dd, 1H, *J* = 14.5, 8.5 Hz, H-18), 2.97 (d, 1H, *J* = 12.0 Hz, H-25), 3.08 (dd, 1H, *J* = 14.0, 5.0 Hz, H-18), 3.28 (d, 1H, *J* = 13.5 Hz, H-13), 3.36 (d, 1H, *J* = 17.5 Hz, H-24), 3.58 (d, 1H, *J* = 13.5 Hz, H-13), 3.65 (dd, 1H, *J* = 17.5, 3.0 Hz, H-24), 4.23–4.28 (m, 2H, H-20 and H-25), 4.42–4.49 (m, 1H, H-19), 6.24 (s, 1H, H-26), 6.63 (1H, d, *J*=7.5 Hz, ArH), 6.71 (1H, d, *J*=7.0 Hz, ArH), 7.05 (d, 1H, *J* = 7.0 Hz, ArH), 7.09 (1H, s, ArH), 7.14-7.22 (m, 8H, ArH), 7.49-7.61 (5H, m, ArH), 7.75 (1H, d, *J*=8.0 Hz, ArH). 13C NMR (125 MHz, CDCl3): δC34.21, 47.96, 51.75, 52.84, 56.27, 62.32, 72.68, 94.45, 121.20, 121.47, 122.77, 124.41, 125.91, 125.98, 126.43, 126.56, 127.41, 127.59, 127.84, 130.63, 130.74, 130.98, 131.05, 131.69, 131.94, 132.12, 132.66, 133.74, 134.31, 135.20, 135.39, 135.86, 136.69, 138.17, 138.62, 140.35, 147.61, 148.13, 196.12. Mass: 663 [M+]. Anal.calcd for C40H30N4O6: C, 72.50; H, 4.56; N, 8.45%; found: C, 72.63; H, 4.75; N, 8.31%.

**2.2. Biology**

Cell lines (MCF7, NCI-H460) were obtained from ATCC (American Type Culture Collection) situated in Virginia, USA. D-MEM (cat no. AL111), Fetal Bovine Serum (cat no. RM10432), D-PBS (cat no. TL1006) was purchased from Himedia, Camptothecin (Cat No: C9911) was procured from Sigma Aldrich, India, Propidium Iodide (cat 556463); APO-DIRECT™ Kit (cat no. 556381); FITC Rabbit Anti- Active Caspase-3 (cat no. 560901); FITC Annexin V Apoptosis Detection Kit I (cat no. 556547); MitoScreen Kit-JC-1 (cat No. 551302) were collectively purchased from BD biosciences.

**2.2.1. Cell line and cell culture**

The MCF-7 and NCI-H460 cell lines were procured from ATCC (American Type Culture Collection) situated in Virginia, USA. The cell lines were grown in Dulbecco’s Modified Eagle’s Medium (DMEM) and for enhancement for growth addition with 10% of fetal bovine serum, 100 µg·mL−1 of streptomycin, 100 UI·mL−1 of penicillin and the cultures were being maintained at 37 °C within a humidified air atmosphere with 5% (V/V) CO2.

**2.2.2. Maintenance of cell line**

The vial containing the MCF-7 and NCI-H460 cell lines acquired from ATCC was removed from liquid nitrogen freezer and immediately placed in a 37 °C water bath. It was whirled until a small ice crystal remained. The entire content was transferred into a 15 mL centrifuge tube. Later it was centrifuged for 10 min at 200 × g, room temperature. Then the supernatant was disposed and cells were washed with fresh medium to remove residual DMSO. The cells were maintained in a T25 flask at recommended density and once 80 % confluency was reached, the cells were sub-cultivated in 1:4 ratio.

**2.2.3. Apoptosis Assay**

Annexin V/PI (BD Biosciences, Catalogue no. 556547) were used to determine the apoptosis inducing nature of compound 4b. In brief, MCF-7 cells (1 × 106 cells/well) were seeded in a 6-well plate and cultured for overnight in a Co2 incubator. After adherence of cells to the culture flasks, the spent medium was replaced with medium containing compounds at IC50 value and further cultured for 48 h. The cells were collected by trypsinization and washed twice with D-PBS. 100 μL of annexinV-FITC binding buffer (1×) and 5 μL annexinV-FITC were added to the cells and incubated in the dark for 10 min at room temperature. The cells were then washed with D-PBS by centrifugation at 2000 rpm for 5 min, and resuspended in 500 μL annexinV-FITC binding buffer (1×) and 5 μL PI. The fluorescence intensities of annexin V-FITC and PI were determined by flow cytometry. CellQuest software (BD Biosciences) was used to analyze the data (Kundu et al., 2014).

**2.2.4. Assessment of Mitochondrial Membrane Potential (ΔΨ m)**

MCF-7 cells (1 × 106 cells/well) were seeded in a 6-well plate and cultured for overnight in a Co2 incubator. After adherence of cells to the culture flasks, the spent medium was replaced with medium containing compounds at IC50 value and further cultured for 48 h. The cells were collected by trypsinization and washed twice with D-PBS. 70% ice-cold ethanol was used to fix and permeabilize the cells by incubating them at -20oC for 30 min. Cells were washed twice with D-PBS and incubated with 0.5 ml of freshly prepared JC-1 solution for 10 – 15 min at 37°C in a CO2 incubator. 1 ml of assay buffer provided in the kit was used to wash the cells and the cells were resuspended in 0.5 ml of assay buffer. The mitochondrial membrane potential (ΔΨ m) was determined by BD FACS Calibur (Feldkamp et al., 2005).

**2.2.5. Caspase 3 Expression studies**

MCF-7 cells (1 × 106 cells/well) were seeded in a 6-well plate and cultured for overnight in a Co2 incubator. After adherence of cells to the culture flasks, the spent medium was replaced with medium containing compounds at IC50 value and further cultured for 48 h. The cells were collected by trypsinization and washed twice with D-PBS. 70% ice-cold ethanol was used to fix and permeabilize the cells by incubating them at -20oC for 30 min. The cells were washed twice with D-PBS and treated with 20µl of Caspase 3 - FITC antibody for 60 min in dark at room temperature. The cells were washed with 1 ml of PBS and resuspended in 0.5 ml of PBS. The Caspase3 protein expression in MCF 7 cells was analyzed by BD FACS Calibur [Sandri et al., 2001).

**2.2.6. Cell Cycle analysis**

Initially MCF-7 cells (1 × 106 cells/well) were seeded and cultured in a 6-well plate for 12 h and then exposed to 14 uM of camptothecin, positive control and IC50 value of test sample, compound 4b and the negative control, culture medium respectively. Post incubation of 48 h, the cells were washed twice with 1xD-PBSand then fixed and permeabilized with 70% ethanol at -20 °C for 30 minutes. These Fixed cells were washed twice with 1xD-PBS and 50μl of RNase A solution and400μl of Propidium Iodide solution per million cells were added directly, vortexed and incubated for 5 to 10 minutes in dark at room temperature. The cell cycle distribution in MCF 7 cells was determined by BD FACS Calibur and CellQuest software (BD Biosciences) [Gray et al., 1979].

# **2.2.7. Analysis of DNA fragmentation using TUNEL Assay**

DNA fragmentation was assessed by Terminal deoxynucleotidyl transferase-mediated dUTP end labeling (TUNEL) assay method. MCF-7 cells were treated with the test drug and controls for 48 hrs. After incubation, ice-cold ethanol (70%) was used to fix and permeabilize the cells at -20oC and washed with wash buffer. Then, cells were incubated with DNA labeling solution for 1 hr. at 37oC. The cells were rinsed with D-PBS and centrifuged at 2000 RPM. After centrifugation, cells were collected and resuspended in PI/RNAse buffer (0.5 ml) and incubated for 30 min at room temperature. DNA fragmentation was evaluated by using flow cytometry.

**2.2.8. Statistical Analysis**

The data are given as the mean ± standard deviation (SD). The data analysis was performed by using SPSS software version 21.0.

**References**

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