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Supporting Information-I

Nature of the Charged Head Group Dictates the Anticancer Potential of Lithocholic Acid-Tamoxifen Conjugates For Breast Cancer Therapy

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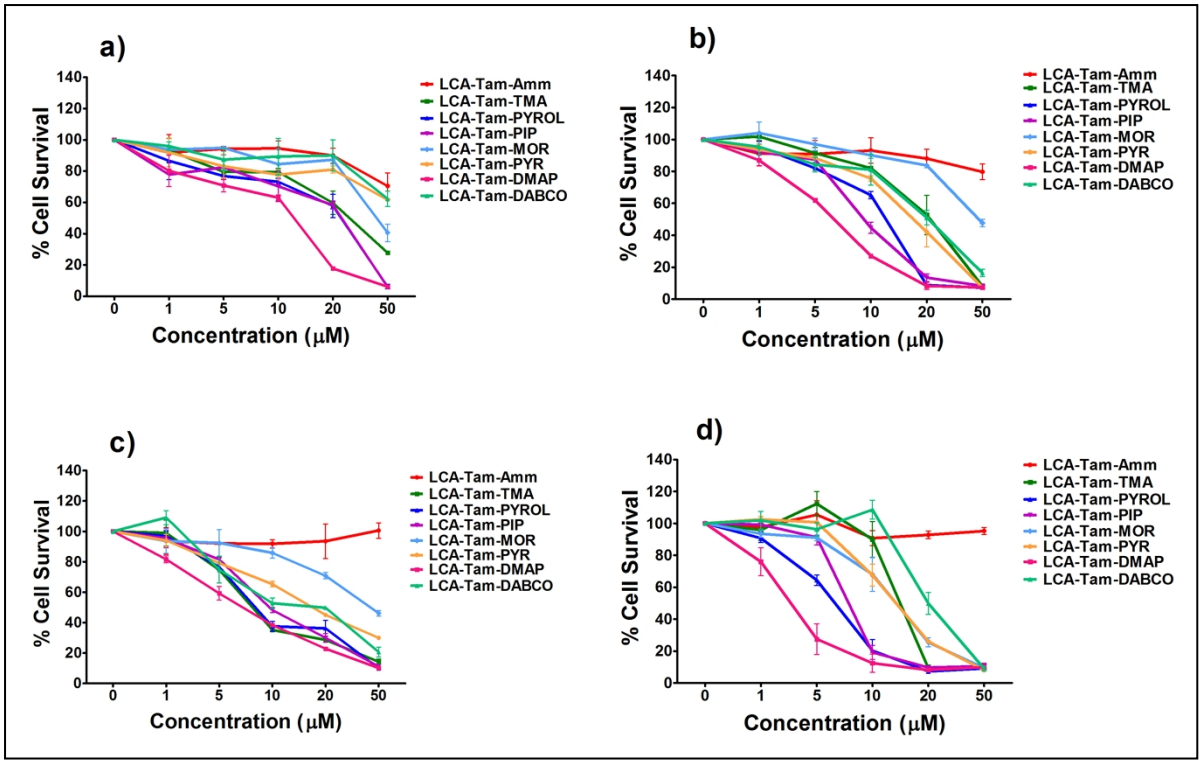


Figure S1. Cellular toxicities of lithocholic acid-tamoxifen amphiphiles possessing different charged head groups using MTT assay against four breast cancer cell lines a) MCF-7, b) MDA-MB-231, c) T47D, d) MDA-MB-468 unraveling the structure-activity relationship.

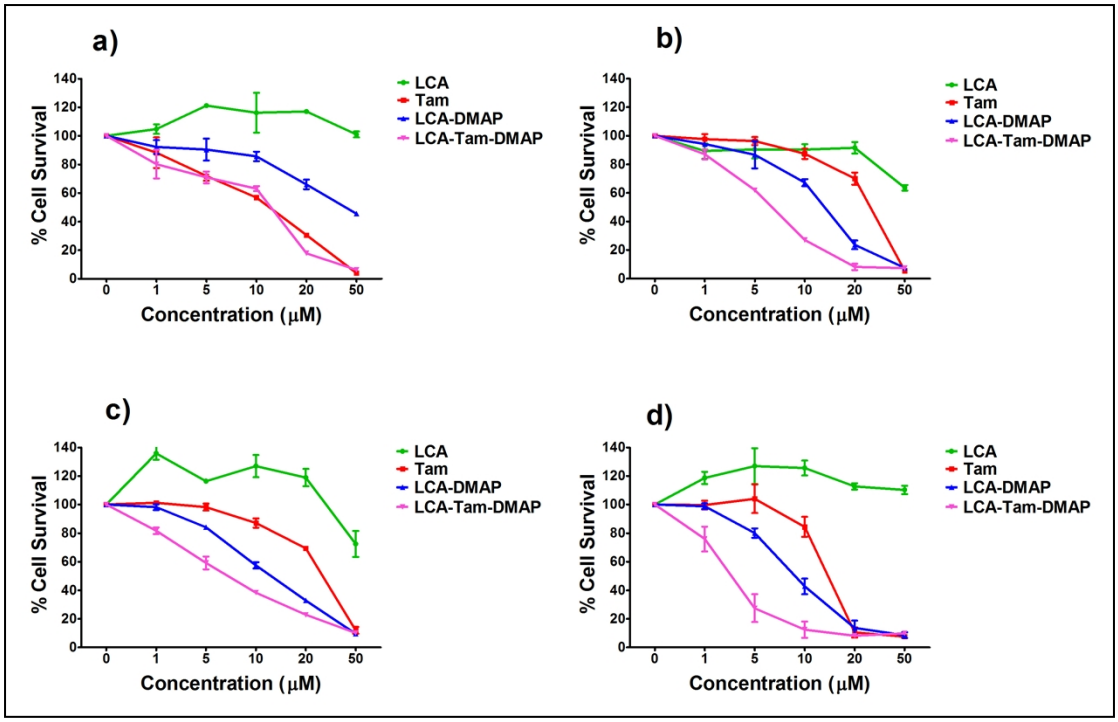


Figure S2. Cellular toxicities showing comparative activities of LCA, Tam, LCA-DMAP, and LCA-Tam-DMAP amphiphiles using MTT assay in four breast cancer cell lines a) MCF-7, b) MDA-MB-231, c) T47D, d) MDA-MB-468 unraveling the high potency of LCA-DMAP-Tam amphiphile.

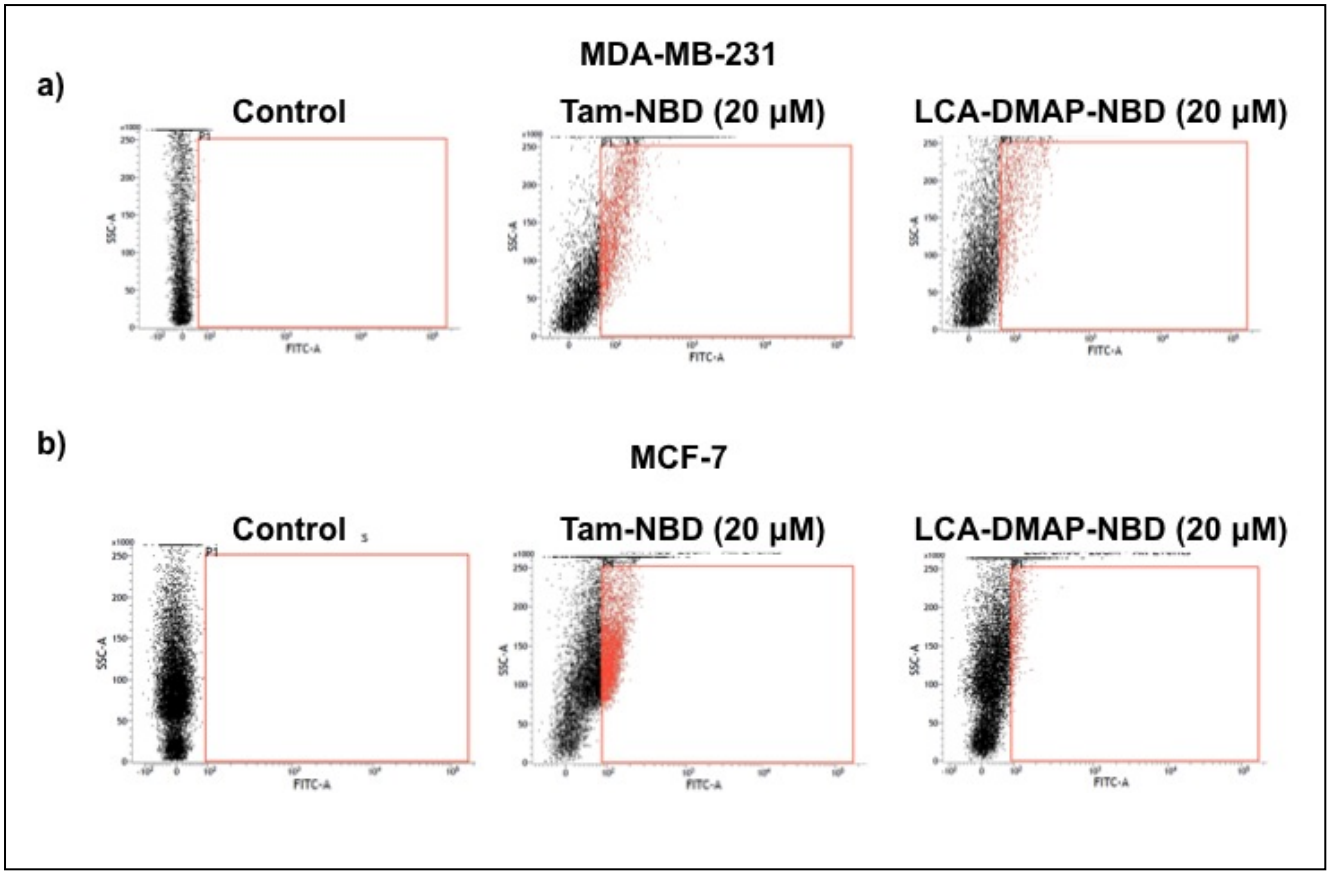
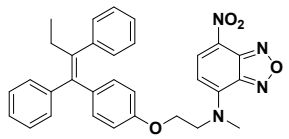


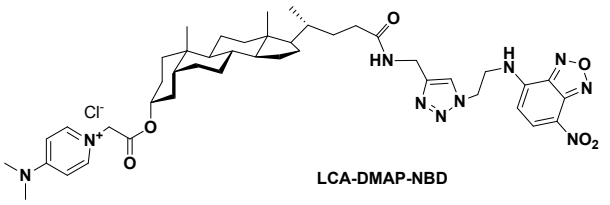
Figure S3. Comparison of cellular uptake of Tam-NBD and LCA-DMAP-NBD amphiphiles after 24 h in MDA-MB-231 (a) and MCF-7 (b) by flow cytometry showing enhanced cellular uptake of Tam-NBD in both the cell lines.

Synthesis of Tam-NBD and LCA-DMAP-NBD conjugates

a)

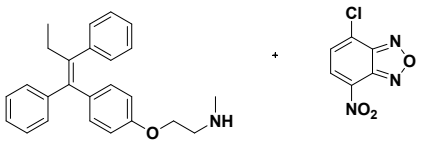


Tam-NBD

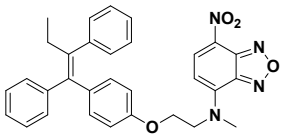


LCA-DMAP-NBD

b)

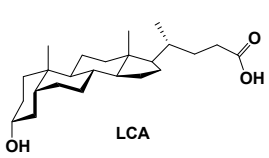


Tam-NHMe

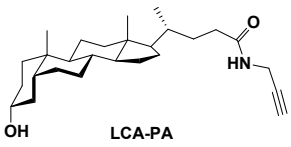


Tam-NBD

12

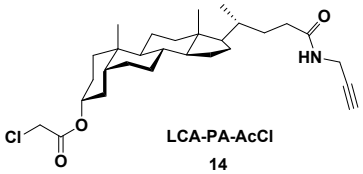


LCA



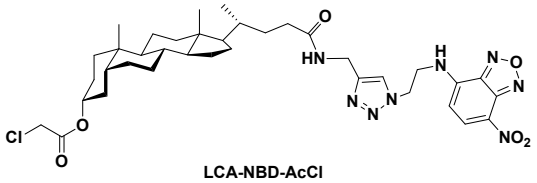
LCA-PA

13



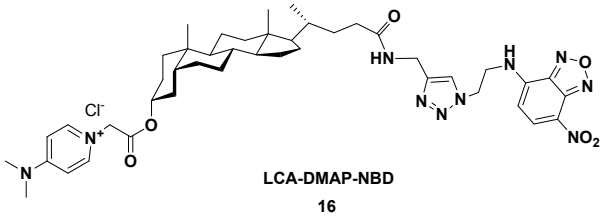
LCA-PA-AcCl

14



LCA-NBD-AcCl

15



LCA-DMAP-NBD

16

Reagents, reaction conditions and yields: i) TEA, DCM, RT, 6h, 82%. **ii**) Propargyl Amine, HBTU, DIPEA, DCM, 0 ˚C-RT, 14h , 64% **iii**) Chloroacetic anhydride, Pyridine, DCM 0 ˚C-RT, 5 h, 91.5% **iv**) NBD-N_3, $CuSO_4$, Sodium Ascorbate, EtOH-DCM-Water 2:2:1, rt, 15h, 60% **v**) DMAP, DMF, 50 ˚C, 48h, 51 %.

Synthesis of Tam-NBD (12): Desmethylated tamoxifen (500 mg, 1.4 mmol) was taken in dry DCM in 25 mL round bottomed flask, followed by addition of triethylamine (0.25mL). Then 4-Chloro-7-nitrobenzofurazan (335 mg, 1.68 mmol) was added and the reaction was continued at room temperature for 6h. Then the reaction mixture was extracted with DCM (2 X 50 mL) and washed with water (2 X 10 mL) and brine (2 X 10 mL) and dried over anhydrous sodium sulphate. Pure product (yellow solid) was obtained by combi flash silica gel column chromatography using EtOAc:Pet Ether 25:75. Yield 82%. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.910 (t, 3H, J = 7.2Hz, -C*H*$_3$), 2.43 (q, 2H, J = 7.6Hz, -C*H*$_2$-), 3.49 (s, 3H, -N-C*H*$_3$), 4.20 (t, 2H, J = 4.8Hz, -C*H*$_2$-), 4.74 (brs, 2H, -O-C*H*$_2$), 6.12 (d, 1H, J = 9.2Hz, Ar*H*), 6.48 (d, 2H, J = 8.8Hz, Ar*H*), 6.77 (d, 2H, J = 8.8Hz, Ar*H*), 7.0-7.52 (m, 10H, Ar*H*), 8.43 (d, 1H, J = 9.2Hz, Ar*H*). ^{13}C-NMR ($CDCl_3$, 400 MHz) δ: 155.87, 145.26, 144.6, 143.57, 142.34, 141.83, 137.94, 136.49, 135.18, 132.01, 129.67, 129.39, 128.14, 127.87, 126.61, 126.07, 113.22, 101.64, 55.06, 42.65, 29.05, 13.5. MS (ESI) *m*/ *z* calculated for $C_{31}H_{28}N_4O_4$ (520.21), found (521.27) $[M+H]^+$, (543.25) $[M+Na]^+$.

Synthesis of LCA-PA (13): LCA (5.31 mmol) and HBTU (10.62 mmol) were Dissolved in dry Dichloromethane. Then added DIPEA (26.5 mmol) to reaction mixture and stirred for 15 minutes at 0 °C. Solution of propargyl amine (7.96 mmol) in Dichloromethane added to reaction mixture dropwise and

stirred at room temperature for 14h. After completion, reaction mixture diluted with Dichloromethane and washed with saturated solution of NaCl twice and 1N aq. HCl twice and concentrated under reduced pressure. The organic phase was dried over anhydrous Na_2SO_4 and the solvent was evaporated to dryness. The crude product was purified by silica gel combi-flash column chromatography at 2% MeOH in DCM as eluent to obtain a white solid. Yield (1.40 g, 64%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.63 (s, 3H, -CH_3), 0.91-0.92 (m, 6H, -CH_3 x 2), 0.96-1.96 (m, 30H, steroid), 2.04-2.12 (m, 1H), 2.23-2.29 (m, 2H), 3.59-3.65 (m, 1H, C_3-CH), 4.04 (s, 2H, -CH_2), 5.63 (s, 1H, -NH), ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 173.2, 79.7, 71.9, 71.6, 56.5, 55.9, 42.8, 42.1, 40.4, 40.2, 38.7, 36.4, 35.8, 35.5, 35.3, 34.6, 33.3, 31.6, 30.5, 29.2, 28.3, 27.2, 26.4, 24.2, 23.4, 20.4, 20.8, 18.6, 18.4, 17.3, 12.1.

Synthesis of LCA-PA-AcCl (14): LCA-PA (**13**, 3.14 mmol) and chloroacetic anhydride (3.77 mmol) were dissolved in dry DCM and stirred at 0 ˚C for 5 minutes. Then added pyridine (3.77 mmol) dropwise to reaction mixture and allowed reaction to stir at room temperature for 5h. After completion, reaction mixture diluted with Ethyl acetate and washed with saturated solution of NaCl and $NaHCO_3$ (2 X 50 mL) twice. The crude product was purified by silica gel combi-flash column chromatography as white solid. Yield (1.08 g, 70 %). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.64 (s, 3H, -CH_3), 0.86-0.93 (m, 6H, -CH_3 x 2), 1.00-2.31 (m, 32 H, steroid), 4.04-4.05 (m, 4H, NH-CH_2, -CH_2-O), 4.77-4.85 (m, 1H, C_3-H), 6.16 (s, 1H, -NH).

Synthesis of LCA-NBD-AcCl (15): LCA-PA-AcCl (**14**, 2 mmol), NBD-NH-CH_2-CH_2-N_3, (2.2 mmol) $CuSO_4.5H_2O$ (0.02 mmol), and sodium ascorbate (1 mmol) in 5 ml solution of DCM:Ethanol:water (2:2:1). Reaction mixture stirred at room temperature for 15h. After completion, reaction mixture directly subjected to column chromatography using DCM and methanol as eluent gave yellow coloured solid. Yield (900 mg, 60%). ^{1}H-NMR (DMSO-d_6, 400 MHz): δ: 0.58 (s, 3H, -CH_3). 0.83 (d, 3H, J = 6.2Hz, -CH_3), 0.89 (s, 3H, -CH_3), 0.99- 2.08 (m, steroid), 3.96 (s, 2H, -CH_2), 4.21 (d, 2H, J = 5.5Hz,), 4.34 (s, 2H, -CH_2), 4.66-4.73 (m, 3H, -CH, -CH_2), 6.34 (d, 1H, J = 8.4Hz, -CH-Ar), 7.93 (s, 1H, -CH-Ar), 8.22-8.27 (m, 1H), 8.47 (d, 1H, J = 8.8Hz, -CH-Ar), 9.43 (s, 1H, -NH).^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 172.9,

167.2, 147.7, 123.8,76.1, 56.2, 55.9, 42.6, 41.7, 41.5, 35.7, 35.3 34.8, 34.5, 34.4, 32.5, 32.1, 31.9, 29.1, 28.1, 26.9, 26.5, 26.3, 24.2, 23.3, 20.8, 18.7, 12.2. MS (ESI) *m/ z* calculated for $C_{37}H_{51}ClN_8O_6$ (738.3620), found 738.1932 $[M]^+$. 739.3520 $[M+H]^+$.

Synthesis of LCA-DMAP-NBD (16): LCA-NBD-AcCl (**15**, 0.27 mmol) and DMAP (0.325 mmol) were dissolved in Dry DMF (3 ml) and stirred at 50 °C for 48h. Then yellow powder product was precipitated from reaction crude by diluting with ethyl acetate and washed with ethyl acetate and dried under high vacuum. Yield (120 mg, 51.5%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.57 (s, 3H, -CH_3), 0.83 (d, 3H, J = 6.2Hz), 0.89 (s, 3H), 1.03-2.05 (m, steroid), 3.21 (s, 6H, -CH_3), 3.95 (s, 1H), 4.21 (d, 2H, J = 8.4Hz), 4.66-4.74 (m, 3H), 5.14 (s, 1H), 6.32 (d, 1H, J = 8.2Hz), 7.07 (d, 1H, J = 7.3Hz), 7.94 (s, 1H), 8.21-8.27 (m, 3H), 8.46 (d, 1H, J = 8.8Hz), 9.47 (s, 1H). MS (ESI) *m/ z* calculated for $C_{44}H_{61}ClN_{10}O_6$ (825.4770), found 825.4596 $[M]^+$. 825.4617 $[M+H]^+$.