Supporting Information

Exploring apoptotic induction of Malabaricone-A in triple-negative breast cancer cells: An acylphenol phyto-entity isolated from the fruit rind of *Myristica malabarica Lam.*

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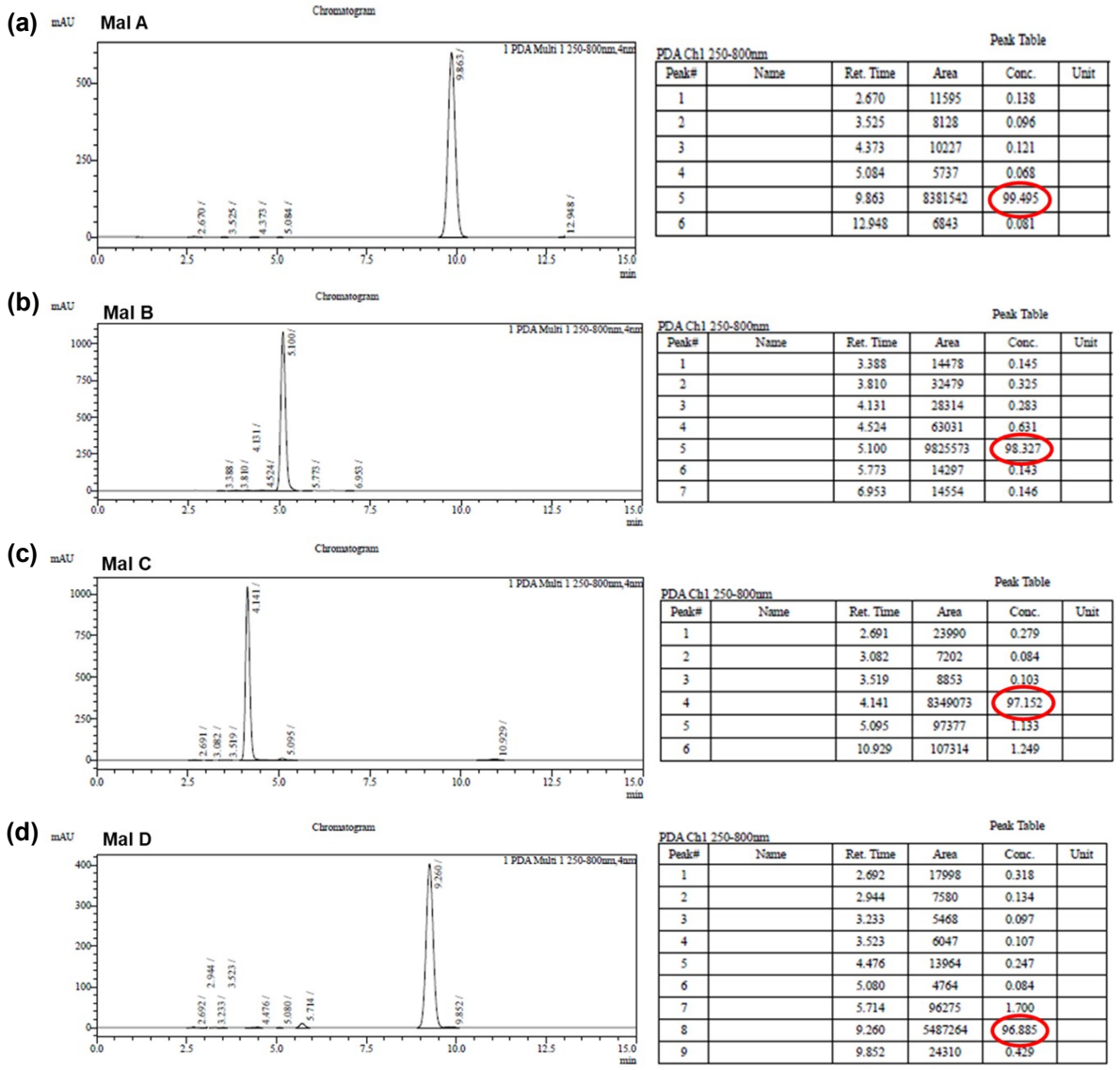


Figure S1: Malabaricones purity check. Chromatogram and peak table of (a) Mal-A, (b) Mal-B, (c) Mal-C, and (d) Mal-D.

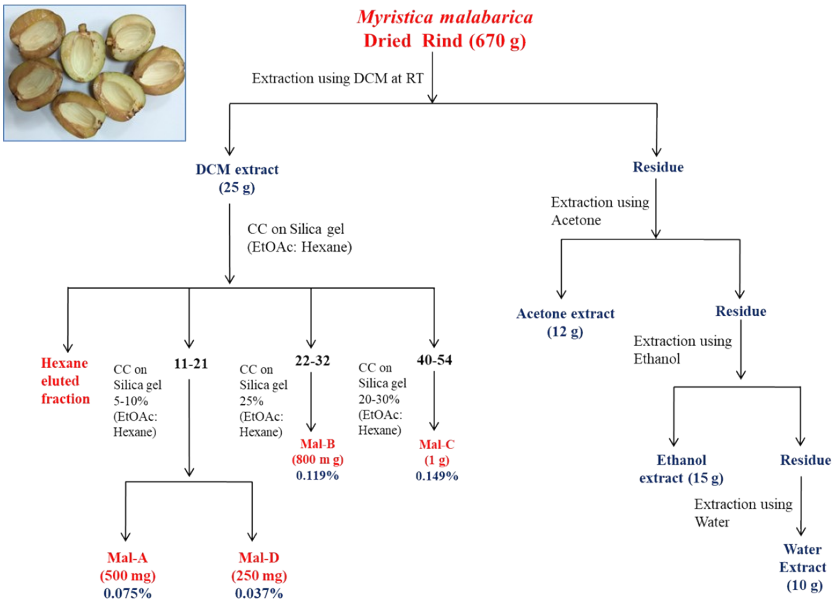
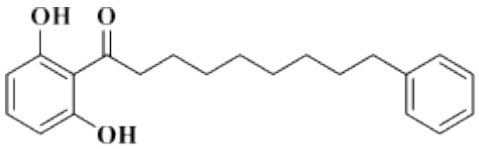
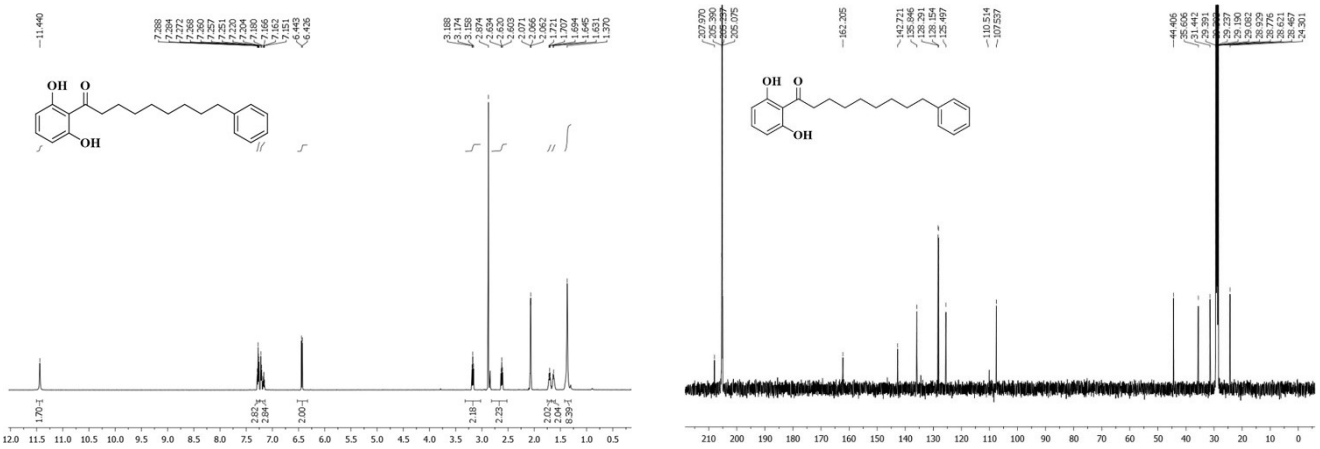


Figure S2: Schematic representation of extraction and isolation procedure from *M. malabarica* rind

Malabaricone A (Mal-A)



Mal-A was obtained from the fraction pool 11-21 after silica gel column chromatography (CC) using Hexane/EtOAc (9:1, v/v) eluent as yellow crystals; mp: 80-82 °C; FT-IR (neat, *v*max, cm-1): 3254 (-OH), 2925, 2850, 1635 (C=O), 1600, 1471, 1246, 1038, 966, 876.; 1H NMR (500MHz, CD_3COCD_3): δ 11.44 (s, 2H, 2-OH), 7.28 (dd, *J*1=2Hz, *J*2=8Hz, 2H), 7.26 (t, *J*=3Hz, 1H), 7.21 (d, *J*=8Hz, 2H), 7.16 (dd, *J*1=1.5Hz, *J*2=7Hz, 1H), 6.43(d, *J*=8.5Hz, 2H, H), 3.17 (t, *J*=7Hz, 2H), 2.62 (t, *J*=7Hz, 2H), 1.74-1.67 (m, 2H), 1.66-1.62 (m, 2H), 1.37 (s, 8H, 4 CH_2) ppm; 13C NMR (125MHz, CD_3COCD_3); δ 208.0 (C=O), 162.2, 142.7, 135.9, 128.3, 128.1, 125.5, 110.2, 107.5, 44.4, 35.6, 31.4, 24.3 ppm; HR-ESIMS *m/z* 349.17833 [M+Na]+ (calcd



for $C_{21}H_{26}O_3Na$, 349.1882).

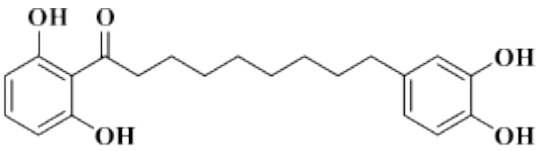
Figure S3: ^{1}H NMR (500 MHz, CD_3COCD_3) and ^{13}C (125MHz, CD_3COCD_3) NMR spectrum of Mal-A

Malabaricone B (Mal-B)

Figure S4: ^{1}H NMR (500 MHz, CD_3COCD_3) spectrum and ^{13}C NMR spectrum (125 MHz, CD_3COCD_3) of Mal-B

Mal-B was afforded from the fraction pool 22-32 after silica gel column chromatography (CC) using Hexane/EtOAc (7.5:2.5, v/v) eluent as pale yellow solid; mp: 110-112 ^{o}C; FT-IR (neat, *v*max, cm-1): 3260 (OH), 2920, 2850, 1635 (C=O), 1600, 1471, 1246, 1038, 966, 876; 1H NMR (500MHz, CD_3COCD_3): δ 11.37 (s, 2H, 2- OH), 8.00 (s, 1H), 7.11 (t, *J*=8Hz, 1H), 6.87 (d, *J*=8.5Hz, 2H), 6.60 (d, *J*=8.5Hz, 2H), 6.29 (d, *J*=8.5Hz, 2H), 3.02 (t, *J*=7Hz, 2H), 2.37(t, *J*=7.5Hz, 2H), 1.56-1.53 (m, 2H), 1.46-1.40 (m, 2H, H-8)1.21 (s, 8H, 4 CH_2) ppm; 13C NMR (125MHz, CD_3COCD_3); δ 208.0 (C=O), 162.2, 155.4, 135.8, 133.3, 129.1, 115.0, 110.1, 107.5, 44.4, 34.7, 31.7, 24.3 ppm; HR-ESIMS *m/z* 365.17291 [M+Na]+ (calcd for $C_{21}H_{26}O_4Na$, 365.1831).

Malabaricone C (Mal-C)



Fraction pool 40–54 was purified by column chromatography by eluting Hexane/ EtOAc (6.5:3.5, v/v) as eluent followed by crystallization using DCM/hexane afforded Mal-C as yellow crystalline solid; mp: 122- 124 ^{o}C; FT-IR (neat, *v*max, cm-1): 3347, 2962, 2849, 2349, 1865, 1623, 1592, 1517, 1432, 1340, 1246, 1120; 1H NMR (500MHz, CD_3COCD_3): δ 11.45 (s, 2H, -OH), 7.63 (s, 2H, -OH), 7.24 (t, *J*=8Hz, 1H), 6.70 (d, *J*=8Hz, 1H), 6.67 (d, *J*=2.5Hz, 1H), 6.50 (dd, *J*1=2Hz, *J*2=8Hz, 1H), 6.42 (d, *J*=8Hz, 2H), 3.15 (t, *J*=8Hz, 2H), 2.44 (t, *J*=7.5Hz, 2H), 1.67-1.55 (m, 2H), 1.54-1.29 (m, 2H), 1.28 (s, 8H, 4 CH_2) ppm; 13C NMR (125MHz, CD_3COCD_3); δ 208.1 (C=O), 162.3, 144.9, 142.9,

135.9, 134.3, 119.4, 115.3, 115.0, 110.1, 107.4, 34.9, 31.7, 24.3 ppm; HR-ESIMS *m/z* 381.16666 [M+Na]+ (calcd for $C_{21}H_{26}O_5Na$, 381.1780).

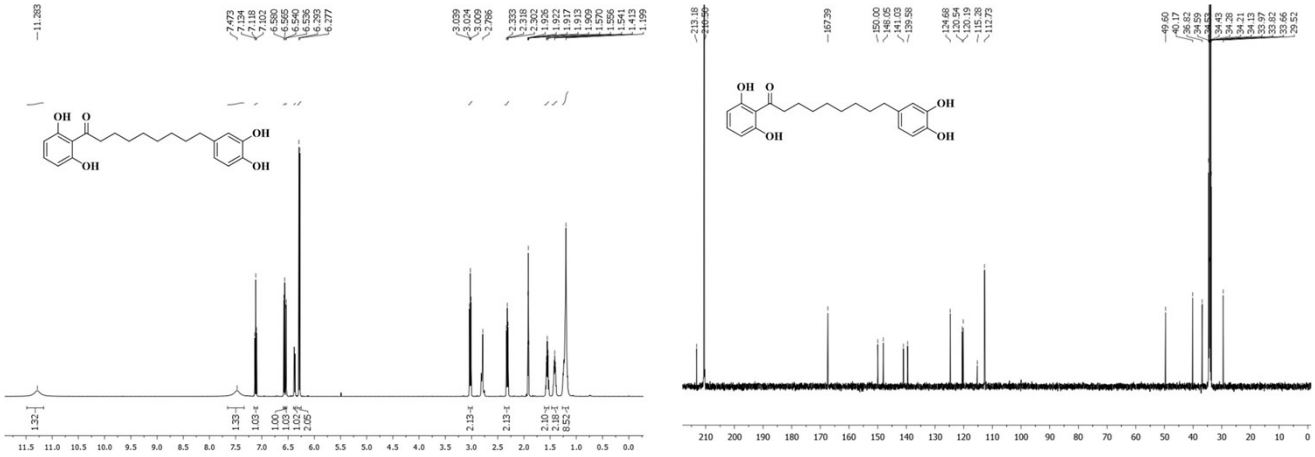
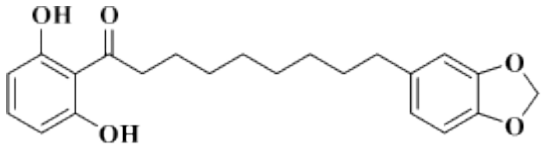
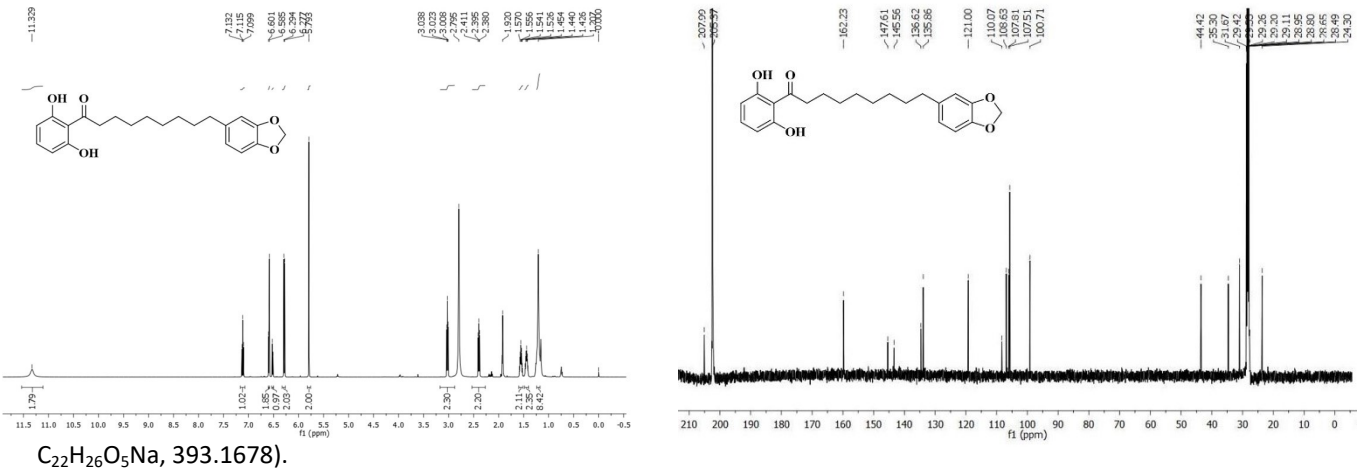


Figure S5: ^{1}H NMR (500MHz, CD_3COCD_3) and ^{13}C NMR (125 MHz, CD_3COCD_3) spectrum of Mal-C

Malabaricone D (Mal-D)



Mal-D was also isolated as pale yellow crystalline solid from the CC separation of the fraction pool 11-21 using gradient elution of Hexane/EtOAc (9.5:0.5 to 9:1, v/v); mp: 68-70 °C; FT-IR (NaCl, *v*max, cm-1): 2924, 2843, 1622 (C=O), 1588, 1492, 1449, 1380, 1362, 1329, 1293, 1249, 1176, 1124, 1091, 1037, 961,938,917, 872, 840, 790, 749, 717, 657; 1H NMR (500MHz, CD_3COCD_3): δ 11.33 (s, 2H, -OH), 7.12 (t, *J*=8.5Hz, 1H), 6.59 (d, *J*=8 Hz, 1H), 6.52 (d, *J*=7.5Hz, 1H), 6.29 (d, *J*=8.5Hz, 2H), 5.79 (s, 2H, -O-CH_2-O-), 3.02 (t, *J*=7.5Hz, 2H), 2.40 (t, *J*=8Hz, 2H), 1.58-1.52 (m, 2H), 1.45-1.41 (m, 2H), 1.21 (s, 8H, 4 CH_2) ppm; 13C NMR (125MHz, CD_3COCD_3); δ 207.9 (C=O), 162.3, 147.6, 145.5, 136.6, 135.9, 121.0, 110.1, 108.6, 107.6, 107.5, 100.7(-O-CH_2- O), 44.4, 35.3, 31.7, 29.4, 29.3, 24.3 ppm; HR-ESIMS *m/z* 393.16763 [M+Na]+ (calcd for



$C_{22}H_{26}O_5Na$, 393.1678).

Figure S6: ^{1}H NMR (500MHz, CD_3COCD_3) and ^{13}C (500MHz, CD_3COCD_3) NMR spectrum of Mal-D

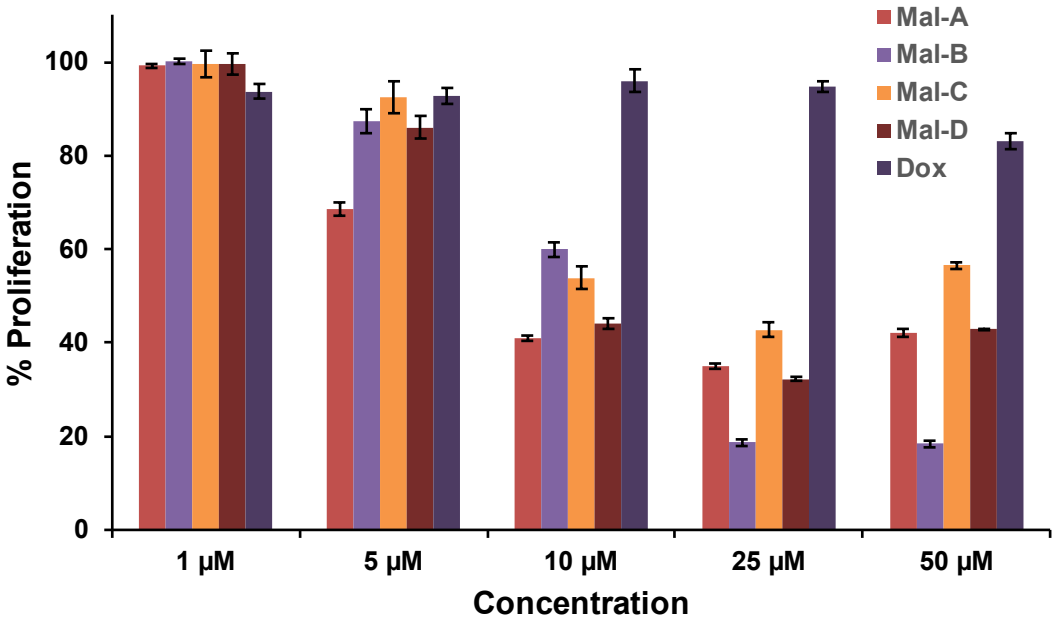


Figure S7: Effect of Mal-A treatment on the proliferation of SK-BR-3 cells

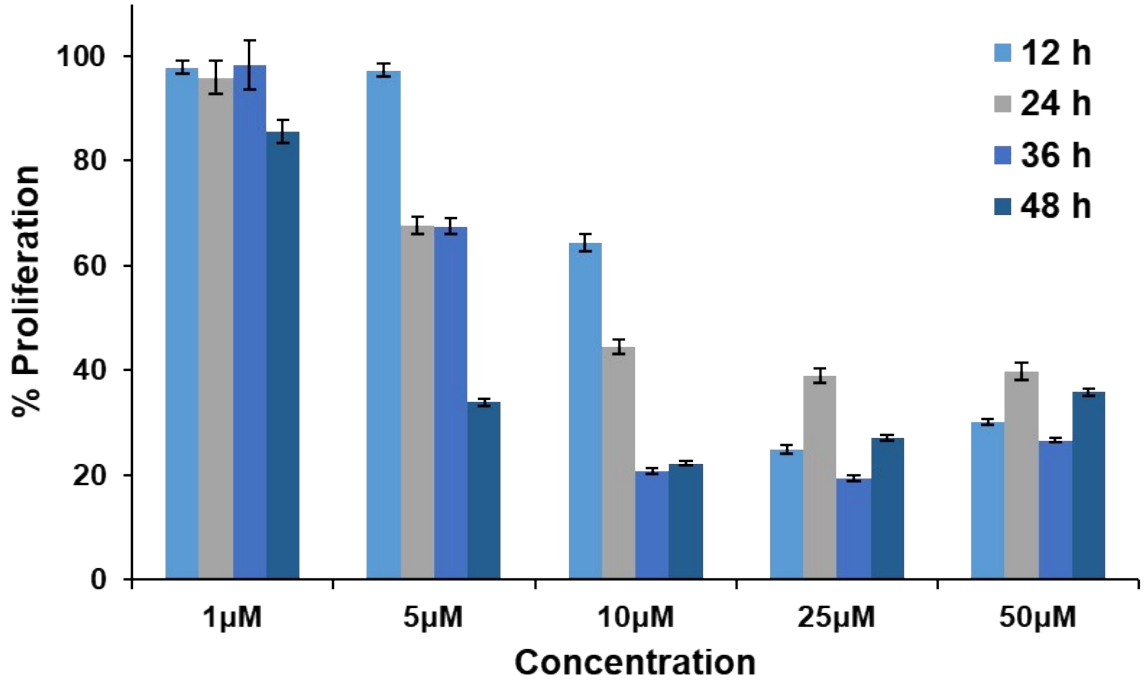


Figure S8: MTT assay: Time and concentration dependent effect of Mal-A in MDA-MB-231 cells

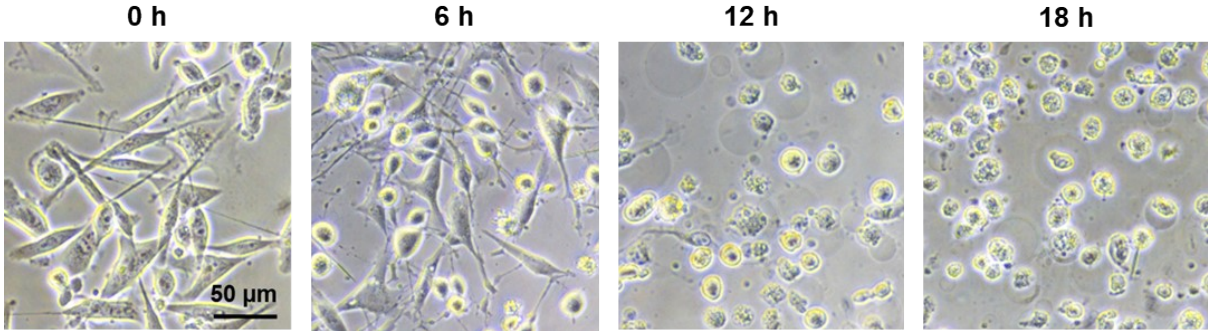


Figure S9: Morphological changes on treatment with Mal-A. Bright field images of MDA-MB-231 cells treated with compound (15 µM), Magnification 200X

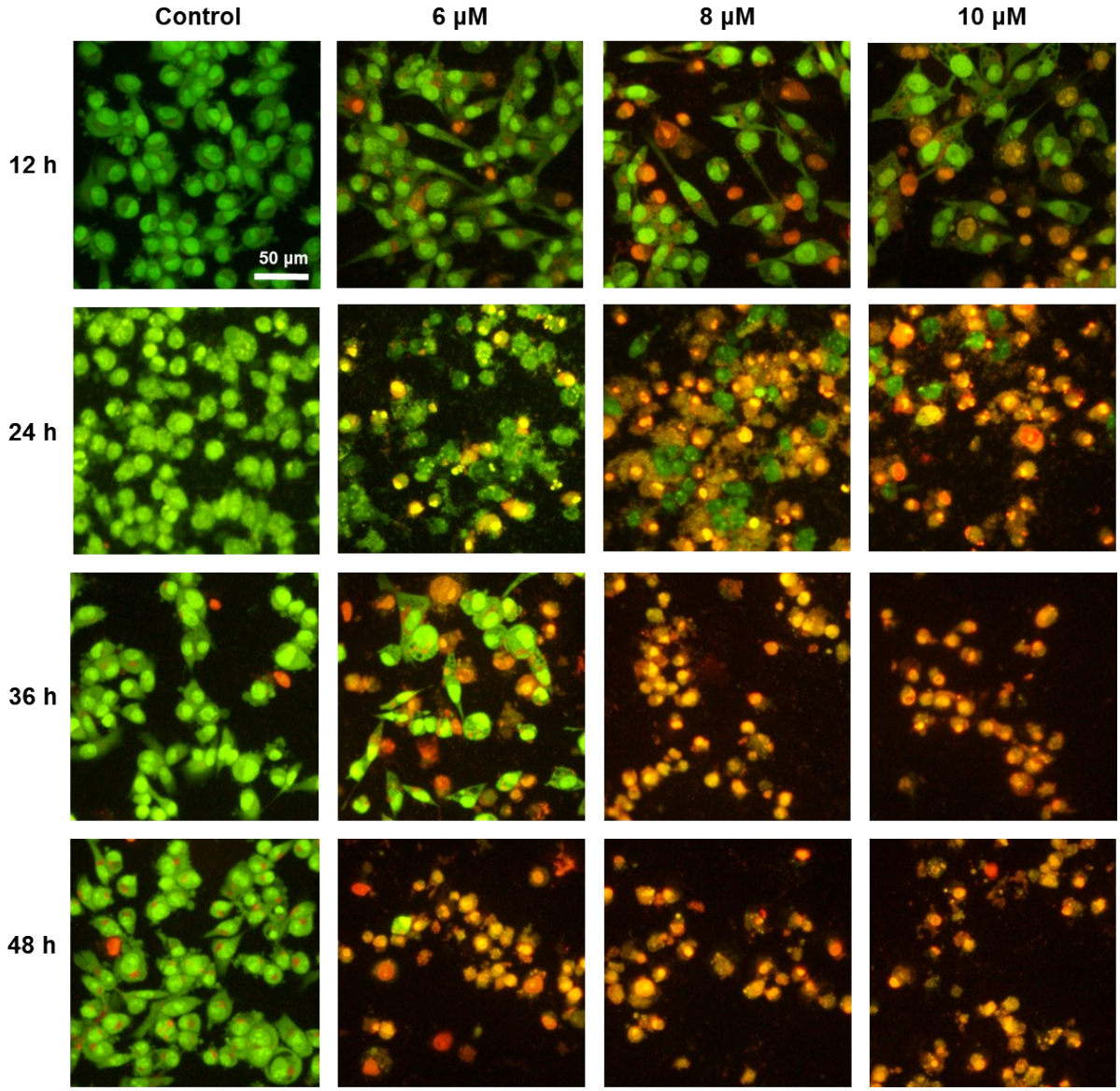


Figure S10: EtBr-AO Live dead Assay - Time and concentration dependent effect of Mal-A in MDA-MB-231 cells. Magnification 200X.

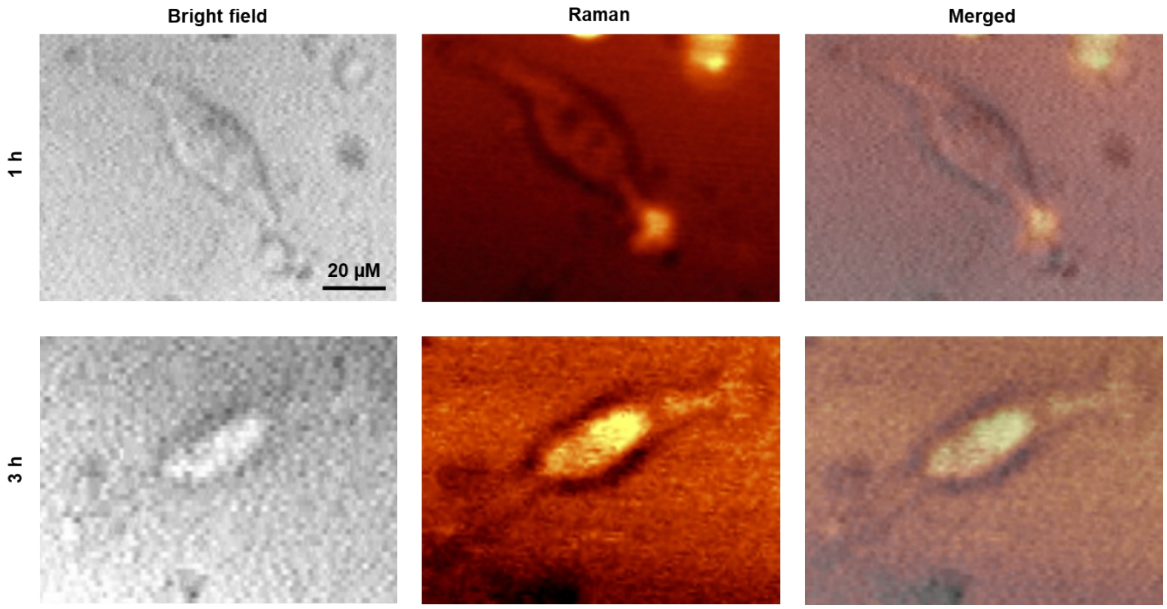


Figure S11: Time dependent internalisation of Mal A by SERS analysis – bright field, Raman field and overlay images of 1 and 3 h

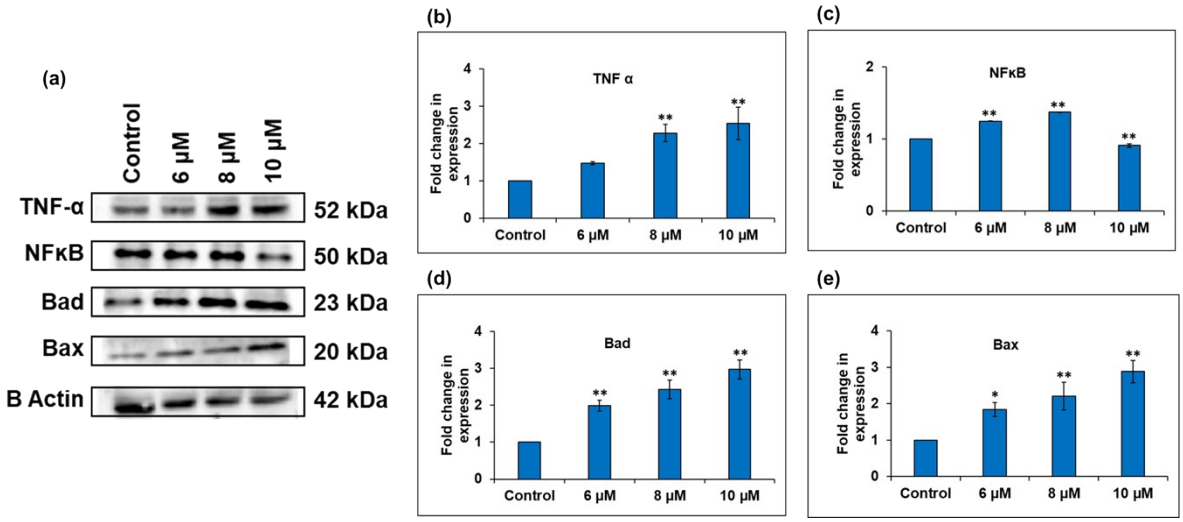


Figure S12: Expression profiles of selected apoptotic proteins in MDA-MB-231 cells treated with Mal A(a) Blotting result of proteins, assessing the expression of (b) TNF-α, (c) NFκB, (d) Bad, and (e) Bax. Triplicate assays were executed, and the results are presented as mean ± SD. Statistical significance was determined with $^{**}p < 0.01$, $^{*}p < 0.05$ were considered to be significant as compared to the control. No asterisk (*) represents a nonsignificant p value.

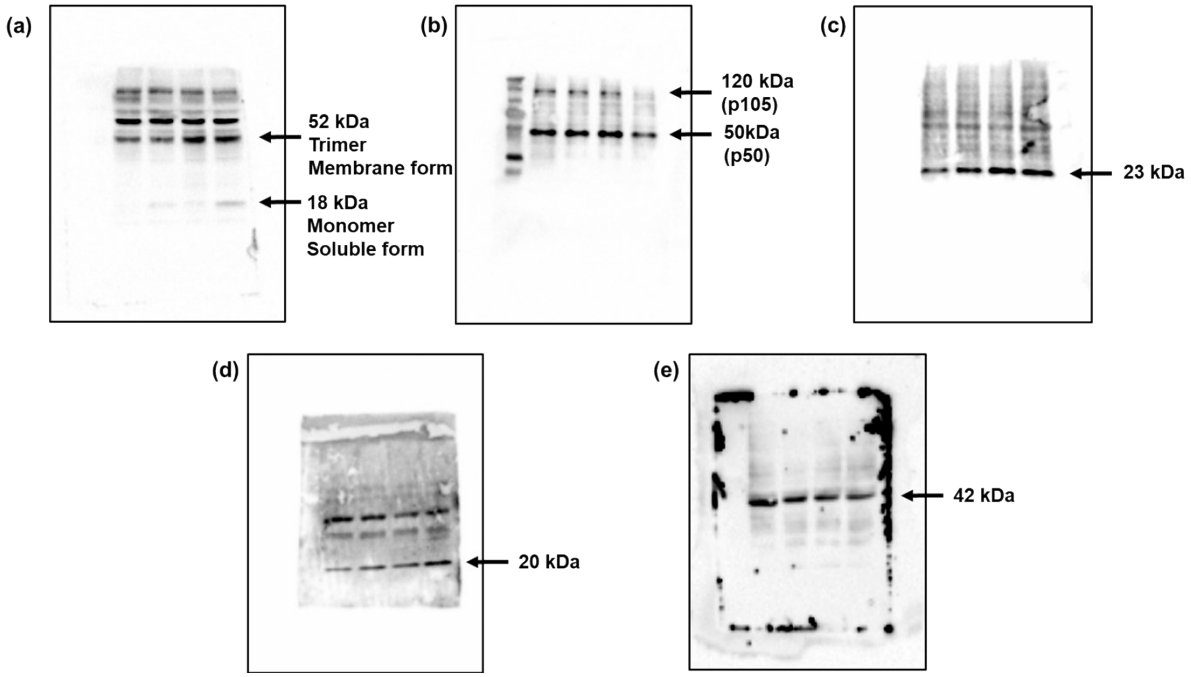


Figure S13: Full blot images of (a) TNF-α, (b) NFκB, (c) Bad, (d) Bax, and (e) β- Actin in the order of control (lane 1), 6 µM (lane 2), 8 µM (lane 3), and 10 µM (lane 4)

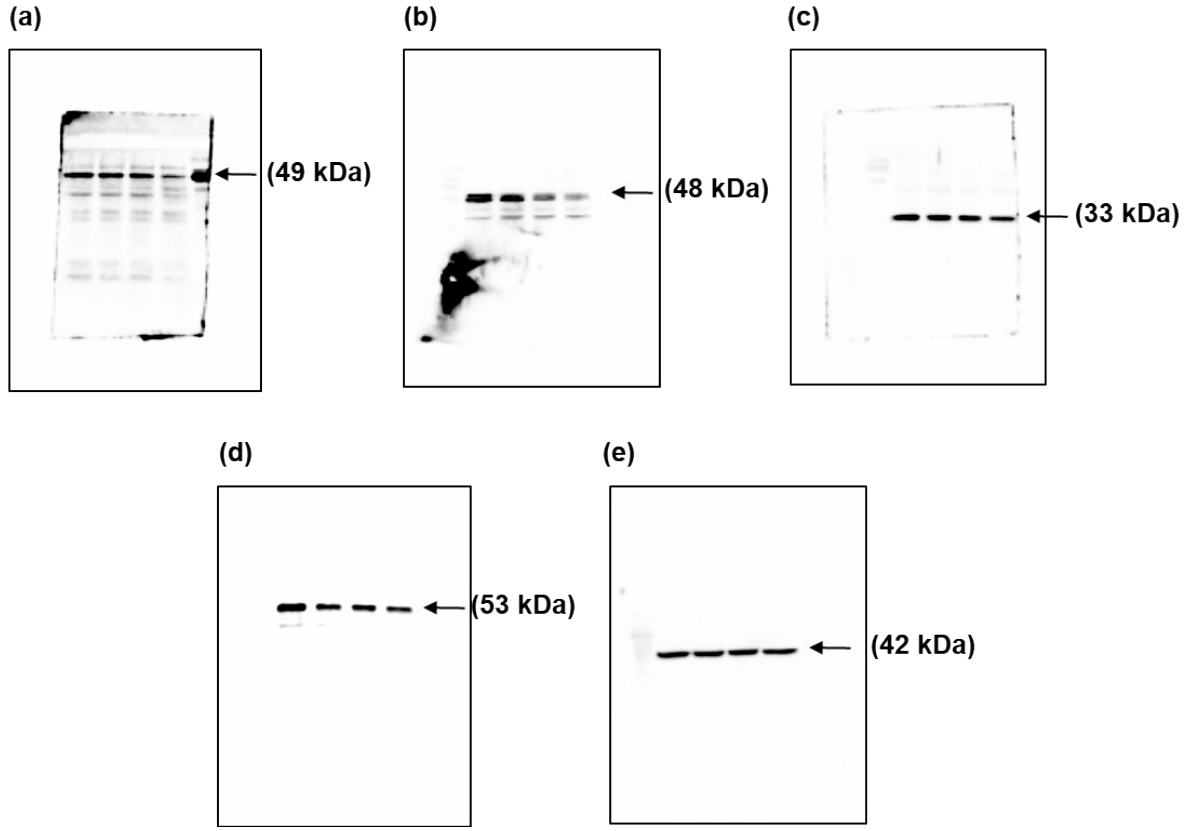


Figure S14: Full blot images of cell cycle proteins (a) cyclin A2, (b) cyclin B1, (c) CDK 2, (d) cdc 25, and (e) β-Actin in the order of control (lane 1), 6 µM (lane 2), 8 µM (lane 3), and 10 µM (lane 4)

Entry	PDB ID	Docking Score	Interacting Residues
1	1TNF	-6.1	ARG 6, PRO 8, VAL 13, ALA 14, HIS 15, ASN 34, LEU 36, LEU 57, TYR 59, TYR 119, TYR 151, GLY 153, ILE 154, ILE155
2	1TNR	-6.1	HIS 32, ILE 34, ASP 50, ALA 52, PHE 74, TYR 76, TYR 134, PRO 161, SER 162, PHE 165, PHE 169
3	1NFK	-5.8	LYS 49, ARG 51, GLY 52, PHE 53, HIS 64, GLY 65, GLY 66, LEU 67, PRO 68, GLY 69, SER 72, GLU 73, LYS 74, LYS 77, SER 78, TYR 79, PRO 80, ALA 135, ASN 136
4	1G5J	-5.1	ALA 304, ALA 305, GLN 306, TYR 308, GLY 309, ARG 310, LEU 312, ARG 313, MET 315, SER 316, PHE 319
5	2BID	-5.1	ASP 15, ILE 18, LEU 22, GLN 81, ILE 84, ILE 85, ILE 88, ARG 189, SER 190, ALA 192, ARG 193
6	6EB6	-5.3	ARG 89, GLU 90, PHE 92, PHE 93, GLY 138, ALA 139, THR 140, ASP 142, PHE 143, GLU 146
7	1KY9	-7.2	LEU 265, GLY 266, ILE 267, MET 268, GLY 269, THR 270, GLU 271, ALA 288, PHE 289, VAL 290, GLN 292, SER 297, SER 298, ALA 299, ALA 302, ILE 304, ILE 310, PHE 321, ALA 322, LEU 324, ARG 325, VAL 328, LEU 339, LEU 341, VAL 350
8	1FEW	-5.5	SER 109, GLU 110, ALA 113, GLU 114, TYR 117, ASP 122, SER 125, ILE 126, ARG 129, ILE 132, GLN 133, LYS 136
9	5OQW	-6.3	LEU 292, LYS 297, VAL 298, LYS 299, GLY 306, LEU 307, THR 308, ASP 309, TRP 310, LYS 311, GLU 314, GLN 319, TRP 323, TYR 324
10	4B4S	-6.6	VAL 37, SER 40, ALA 41, ALA 42, ARG 44, LEU 45, ILE 48, GLY 84, VAL 87, THR 88, PHE 158, PHE 159, ARG 160, THR 161, PHE 163
11	3THM	-5.1	HIS 38, ASP 39, THR 76, ASP 77, LYS 78, ALA 79, HID 80, SER 82, SER 83, LYS 84, CYS 85, ARG 86,
12	3ALQ	-6.4	PRO 20, GLU 23, GLY 24, SER 65, GLY 66, GLN 67, SER 76, ARG 77, CYS 78, SER 79, GLN 82, LYS 108, ARG 113, LEU 114, TYR 115, ASP 140, TYR 141, LEU 142, ASP 143, PHE 144, ALA 145, GLN 149

Table S1: Docking scores and interactive residues of Mal-A upon interaction with 12 selected proteins.