

Supporting Information

Mycophenolic acid-amides derivatives as human Inosine-5'-Monophosphate dehydrogenase 2 inhibitors (*h*IMPDH2)

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Table 1S. Summary of the physicochemical and calculated molecular properties of the synthesized molecules

Code	logS	logS @ pH 7.4	logP	logD	2C9 pKi	hERG pIC50	BBB log ([brain] :[blood])	MW	HBD	HBA	TPSA	Flexibility	Rotatable Bonds
1	2.066	3.741	2.679	-0.3857	4.817	3.674	-0.7722	320.3	2	6	93.06	0.25	6
14	0.5091	0.5091	4.099	4.099	5.635	4.624	-0.3468	423.5	2	6	84.86	0.2727	9
15	0.5091	0.5091	4.099	4.099	5.635	4.624	-0.3468	423.5	2	6	84.86	0.2727	9
16	0.4051	0.4051	4.447	4.447	5.829	4.539	-0.3546	443.9	2	6	84.86	0.2727	9
17	1.439	1.439	3.216	3.216	5.394	4.324	-0.4136	399.4	2	7	98	0.2903	9
18	1.053	1.053	3.548	3.548	5.627	4.635	-0.4211	439.5	2	7	94.09	0.2941	10
19	0.556	0.556	4.162	4.162	5.698	4.647	-0.3585	423.5	2	6	84.86	0.2727	9
20	0.4051	0.4051	4.447	4.447	5.831	4.679	-0.3626	443.9	2	6	84.86	0.2727	9
21	0.9787	0.9787	2.68	2.68	5.474	4.245	-0.4472	410.5	2	7	97.75	0.2812	9
22	0.9787	0.9787	2.68	2.68	5.494	4.261	-0.4472	410.5	2	7	97.75	0.2812	9
23	0.556	0.556	4.162	4.162	5.698	4.501	-0.3504	423.5	2	6	84.86	0.2727	9
24	1.003	1.003	4.647	4.647	5.697	4.965	-0.3445	451.6	2	6	84.86	0.3429	12
25	0.8509	0.8509	3.081	3.081	5.946	4.465	-0.4748	453.5	2	8	103.3	0.25	9
26	3.678	2.429	1.999	1.316	4.805	4.724	-0.4472	432.5	2	8	97.33	0.303	10
27	0.8339	0.8339	3.757	3.757	5.637	4.666	-0.3753	425.5	2	7	94.09	0.2727	9
28	0.7507	0.7507	3.717	3.717	5.651	4.884	-0.3766	425.5	2	7	94.09	0.2727	9

logS: logarithm of the intrinsic aqueous solubility, S in μM , for neutral compounds
logS@pH7.4: logarithm of the apparent solubility at pH 7.4, S in μM , for ionised compounds
logP: logarithm of the octanol/water partition coefficient for neutral compounds
logD: logarithm of the octanol/water partition coefficient for ionised compounds at a fixed pH of 7.4
2C9 pKi: pKi values for affinity with CYP2C9
hERG pIC50: pIC50 values for inhibition of hERG K⁺ channels expressed in mammalian cells
BBB log([brain]:[blood]): logarithm of the Brain/Blood ratio
MW: Molecular weight
HBD: No. of H-bond donors
HBA: No. of H-bond acceptors
TPSA: Topological polar surface area (\AA^2)
Flexibility: ratio of rotatable bonds to total bonds

Experimental Section

General.

All the chemicals were purchased from the producers vendors such as Acros Organics (Geel, Belgium), Alfa Aesar (Karlsruhe, Germany), Sigma Aldrich (St. Louis, USA) or Merck (Darmstadt, Germany) and used without further purification. Solvents were used directly, unless specified. All the reactions were carried out under anhydrous conditions under N₂ atmosphere and the progress of the reaction was monitored by thin- layer chromatography (TLC) using silica gel 60 F₂₅₄ (Merck Millipore, Billerica, MA, USA) coated aluminium plates. All the title compounds were purified by column chromatography packed with silica gel of #230-400. Melting points of the compounds were determined on Veego VMP DS (General Trading Co., India) and were uncorrected. FT-IR spectra were recorded on a Perkin Elmer RX1 instrument (Waltham, MA). The purity of all the compounds was determined to be > 95% by HPLC. HPLC analysis was performed on Agilent 1220 Infinity system (Santa Clara, CA, USA). An isocratic mobile phase system consisting of (A) Acetonitrile and (B) Water (70:30, v/v) was used with a C₁₈ Kromasil® column (15 cm × 4.6 mm, 5 μm particle size, 100 Å pore size) at a flow rate of 1mL/min. Deionised water was obtained from EMD Millipore Elix™ Essential 3 water purification system. ¹H-NMR spectra were recorded on Bruker Advance 400 MHz (Billerica, MA) instrument in DMSO-*d*₆ (D₂O exchange), tetramethyl silane (TMS) as internal standard and the chemical shifts were expressed in δ(ppm) values. Mass spectra (MS) were recorded on Shimadzu 8040 LC-MS/MS system (Kyoto, Japan) using electrospray ionization (ESI) mode. Enzyme inhibition and MTT assay readings were recorded on EPOCH 2 Biotek microplate reader (Winooski, VT, USA). β-Nicotinamide adenine dinucleotide (NAD), inosine-5'-monophosphate (IMP), DL-dithiothreitol (DTT), potassium chloride (KCl), 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), Tris-HCl were obtained from SRL (Mumbai, India), foetal bovine serum (FBS), phosphate-buffered saline

(PBS), Dulbecco's modified eagle's medium (DMEM) and trypsin-EDTA were obtained from CellClone (Delhi, India) and the antibiotics from Hi-Media Laboratories Ltd. (Mumbai, India).

(R)(E)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methyl-N-(1-phenylethyl)hex-4-enamide (15). It was synthesized using **1** (0.1 g, 0.31 mmol), *(R)*-(+)- α -methylbenzylamine **13b** (0.045 mL, 0.35 mmol), DMAP (0.044 g, 0.35 mmol) and EDCI.HCl (0.066 g, 0.34 mmol) as per Method A to yield **15** as off-white solid. Yield: 58%; TLC: R_f = 0.72 (DCM:EtOAc, 8:2); purity (HPLC): 96.67%; mp: 128-130 °C; IR (KBr) cm^{-1} 3443 (OH, str), 3284 (NH, str), 1745 (O-C=O, str), 1638 (NH-C=O, str), 1549 (C=C, str), 1134 (C-O, str); $^1\text{H-NMR}$ (DMSO- d_6 , D₂O exchange, 400 MHz) δ 7.29 – 7.12 (m, 5H), 5.20 (s, 2H), 5.08 (t, 1H), 4.79 (q, J = 7.1 Hz, 1H), 3.63 (s, 3H), 3.24 (d, J = 6.8 Hz, 2H), 2.18 – 2.06 (m, 4H), 2.03 (s, 3H), 1.69 (s, 3H), 1.22 (d, J = 7.0 Hz, 3H); MS (ESI) m/z : 422 [M-H]⁻.

(E)-N-(2-chlorobenzyl)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enamide (16). It was synthesized using **1** (0.1 g, 0.31 mmol), 2-chlorobenzylamine **13c** (0.042 mL, 0.35 mmol), DMAP (0.044 g, 0.35 mmol) and EDCI.HCl (0.066 g, 0.34 mmol) as Method A to yield **16** as off-white solid. Yield: 70%; TLC: R_f = 0.65 (DCM:EtOAc, 8:2); purity (HPLC): 98.69%; mp: 150-152 °C; IR (KBr) cm^{-1} 3423 (OH, str), 3284 (NH, str), 1750 (O-C=O, str), 1636 (NH-C=O, str), 1536 (C=C, str), 1136 (C-O, str); $^1\text{H-NMR}$ (DMSO- d_6 , D₂O exchange, 400 MHz) δ 7.37 (d, J = 3.5, 5.4 Hz, 1H), 7.27 – 7.17 (m, 3H), 5.20 (s, 2H), 5.08 – 5.12 (t, J = 7.2 Hz, 1H), 4.21 (s, 2H), 3.64 (s, 3H), 3.26 (d, J = 6.9 Hz, 2H), 2.19 (dd, J = 8.4, 15.1 Hz, 4H), 2.03 (s, 3H), 1.71 (s, 3H); MS (ESI) m/z : 442 [M-H]⁻.

(E)-N-(furan-2-ylmethyl)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enamide (17). It was synthesized using **1** (0.1 g, 0.31 mmol),

furfurylamine **13d** (0.031 mL, 0.35 mmol), DMAP (0.044 g, 0.35 mmol) and EDCI.HCl (0.066 g, 0.34 mmol) as per Method A to yield **17** as off-white solid. Yield: 59%; TLC: $R_f = 0.59$ (DCM:EtOAc, 8:2); purity (HPLC): 99.99%; mp: 118-120 °C; IR (KBr) cm^{-1} 3426 (OH, str), 3289 (NH, str), 1745 (O-C=O, str), 1641 (NH-C=O, str), 1548 (C=C, str), 1137 (C-O, str); $^1\text{H-NMR}$ (DMSO- d_6 , D₂O exchange, 400 MHz) δ 7.47 (d, $J = 1.7$ Hz, 1H), 6.32 (t, $J = 1.8, 3.2$ Hz, 1H), 6.14 (d, $J = 3.1$ Hz, 1H), 5.10 – 5.07 (m, 3H), 4.14 (s, 2H), 3.59 (s, 3H), 3.19 (d, $J = 6.8$ Hz, 2H), 2.14 – 2.08 (m, 4H), 1.96 (s, 3H), 1.67 (s, 3H); MS (ESI) m/z : 398 [M-H]⁻.

(E)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-*N*-(4-methoxybenzyl)-4-methylhex-4-enamide (**18**). It was synthesized using **1** (0.1 g, 0.31 mmol), 4-methoxybenzylamine **13e** (0.046 mL, 0.35 mmol), DMAP (0.044 g, 0.35 mmol) and EDCI.HCl (0.066 g, 0.34 mmol) as per Method A to yield **18** as off-white solid. Yield: 54%; TLC: $R_f = 0.63$ (DCM:EtOAc, 8:2); purity (HPLC): 99.99%; mp: 144-146 °C; IR (KBr) cm^{-1} 3463 (OH, str), 3302 (NH, str), 1738 (O-C=O, str), 1641 (NH-C=O, str), 1550 (C=C, str), 1136 (C-O, str); $^1\text{H-NMR}$ (DMSO- d_6 , D₂O exchange, 400 MHz) δ 7.08 (d, $J = 8.3$ Hz, 2H), 6.80 (d, $J = 8.4$ Hz, 2H), 5.20 (s, 2H), 5.10 – 5.07 (t, $J = 7.1$ Hz, 1H), 4.09 (s, 2H), 3.67 (s, 3H), 3.64 (s, 3H), 3.26 (d, $J = 6.8$ Hz, 2H), 2.14 (s, 4H), 2.04 (s, 3H), 1.70 (s, 3H); MS (ESI) m/z : 438 [M-H]⁻.

(E)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methyl-*N*-(4-methylbenzyl)hex-4-enamide (**19**). It was synthesized using **1** (0.1 g, 0.31 mmol), 4-methylbenzylamine **13f** (0.045 mL, 0.35 mmol), DMAP (0.044 g, 0.35 mmol) and EDCI.HCl (0.066 g, 0.34 mmol) as per Method A to yield **19** as off-white solid. Yield: 58%; TLC: $R_f = 0.67$ (DCM:EtOAc, 8:2); purity (HPLC): 99.67%; mp: 154-156 °C; IR (KBr) cm^{-1} 3444 (OH, str), 3268 (NH, str), 1750 (O-C=O, str), 1645 (NH-C=O, str), 1556 (C=C, str), 1132 (C-O, str); $^1\text{H-NMR}$ (DMSO- d_6 , D₂O exchange, 400 MHz) δ 7.20 (d, $J = 7.7$ Hz, 2H), 7.09 (d, $J = 7.7$ Hz,

2H), 5.14 – 5.10 (m, 3H), 4.11 (s, 2H), 3.61(s, 3H), 3.23 (d, $J = 6.8$ Hz, 2H), 2.22 (d, $J = 9.4$ Hz, 4H), 2.14 (s, 3H), 2.01 (s, 3H), 1.69 (s, 3H); MS (ESI) m/z : 422 [M-H]⁻.

(*E*)-*N*-(4-chlorobenzyl)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enamide (**20**). It was synthesized using **1** (0.1 g, 0.31 mmol), 4-chlorobenzylamine **13g** (0.043 mL, 0.35 mmol), DMAP (0.044 g, 0.35 mmol) and EDCl.HCl (0.066 g, 0.34 mmol) as per Method A to yield **20** as off-white solid. Yield: 75%; TLC: $R_f = 0.64$ (DCM:EtOAc, 8:2); purity (HPLC): 99.99%; mp: 142-144 °C; IR (KBr) cm^{-1} 3422 (OH, str), 3287 (NH, str), 1729 (O-C=O, str), 1641 (NH-C=O, str), 1549 (C=C, str), 1141 (C-O, str); ¹H-NMR (DMSO-*d*₆, D₂O exchange, 400 MHz) δ 7.27 (dd, $J = 1.8, 8.3$ Hz, 2H), 7.16 (dd, 2H), 5.20 (s, 2H), 5.10 – 5.07 (t, $J = 7.1$ Hz, 1H), 4.14 (s, 2H), 3.63 (d, $J = 1.7$ Hz, 3H), 3.25 (d, $J = 6.8$ Hz, 2H), 2.18 – 2.15 (m, 4H), 2.04 (s, 3H), 1.70 (s, 3H); MS (ESI) m/z : 442 [M-H]⁻.

(*E*)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methyl-*N*-(pyridin-4-ylmethyl)hex-4-enamide (**21**). It was synthesized using **1** (0.1 g, 0.31 mmol), 4-(aminomethyl)pyridine **13h** (0.036 mL, 0.35 mmol), DMAP (0.044 g, 0.35 mmol) and EDCl.HCl (0.066 g, 0.34 mmol) as per Method A to yield **21** as light-brown solid. Yield: 45%; TLC: $R_f = 0.43$ (DCM:EtOAc, 8:2); purity (HPLC): 99.21%; mp: 120-122 °C; IR (KBr) cm^{-1} 3303 (NH, str), 1767 (O-C=O, str), 1643 (NH-C=O, str), 1560 (C=C, str), 1132 (C-O, str); ¹H-NMR (DMSO-*d*₆, D₂O exchange, 400 MHz) δ 8.40 (d, $J = 5.0$ Hz, 2H), 7.17 (d, $J = 5.0$ Hz, 2H), 5.20 (s, 2H), 5.11 (t, 1H), 4.20 (s, 2H), 3.64 (s, 3H), 3.26 (d, $J = 6.9$ Hz, 2H), 2.26 – 2.11 (m, 4H), 2.03 (s, 3H), 1.71 (s, 3H); MS (ESI) m/z : 409 [M-H]⁻.

(*E*)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methyl-*N*-(pyridin-2-ylmethyl)hex-4-enamide (**22**). It was synthesized using **1** (0.1 g, 0.31 mmol), 2-(aminomethyl)pyridine **13i** (0.036 mL, 0.35 mmol), DMAP (0.044 g, 0.35 mmol) and EDCl.HCl (0.066 g, 0.34 mmol) as per Method A to yield **22** as light-brown solid. Yield: 40%;

TLC R_f 0.41 (DCM:EtOAc, 8:2); purity (HPLC): 99.17%; mp: 158-160 °C; IR (KBr) cm^{-1} 3416 (OH, str), 3284 (NH, str), 1761 (O-C=O, str), 1688 (NH-C=O, str), 1563 (C=C, str), 1149 (C-O, str); $^1\text{H-NMR}$ (DMSO- d_6 , D_2O exchange, 400 MHz) δ 8.41 (s, 1H), 7.69 (t, $J = 8.3$ Hz, 1H), 7.20 (dd, $J = 7.4, 8.3$ Hz, 2H), 5.12 (s, 3H), 4.25 (s, 2H), 3.58 (s, 3H), 3.18 (s, 2H), 2.26 – 2.03 (m, 4H), 1.95 (s, 3H), 1.67 (s, 3H); MS (ESI) m/z : 409 [M-H] $^-$.

(E)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methyl-*N*-(2-methylbenzyl)hex-4-enamide (**23**). It was synthesized using **1** (0.1 g, 0.31 mmol), 2-methylbenzylamine **13j** (0.043 mL, 0.35 mmol), DMAP (0.044 g, 0.35 mmol) and EDCl.HCl (0.066 g, 0.34 mmol) as per Method A to yield **23** as off-white solid. Yield: 54%; TLC: $R_f = 0.68$ (DCM:EtOAc, 8:2); purity (HPLC): 99%; mp: 130-132 °C; IR (KBr) cm^{-1} 3433 (OH, str), 3291 (NH, str), 1742 (O-C=O, str), 1639 (NH-C=O, str), 1548 (C=C, str), 1135 (C-O, str); $^1\text{H-NMR}$ (DMSO- d_6 , D_2O exchange, 400 MHz) δ 7.13 – 7.04 (m, 4H), 5.20 (s, 2H), 5.09 (t, 1H), 4.12 (s, 2H), 3.64 (s, 3H), 3.25 (d, $J = 6.8$ Hz, 2H), 2.23 – 2.11 (m, 7H), 2.03 (s, 3H), 1.70 (s, 3H); MS (ESI) m/z : 422 [M-H] $^-$.

(E)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methyl-*N*-(4-phenylbutyl)hex-4-enamide (**24**). It was synthesized using **1** (0.1 g, 0.31 mmol), 4-phenylbutylamine **13k** (0.055 mL, 0.35 mmol), DMAP (0.044 g, 0.35 mmol) and EDCl.HCl (0.066 g, 0.34 mmol) as per Method A to yield **24** as off-white solid. Yield: 60%; TLC: $R_f = 0.71$ (DCM:EtOAc, 8:2); purity (HPLC): 99.83%; mp: 140-142 °C; IR (KBr) cm^{-1} 3449 (OH, str), 3310 (NH, str), 1745 (O-C=O, str), 1639 (NH-C=O, str), 1561 (C=C, str), 1134 (C-O, str); $^1\text{H-NMR}$ (DMSO- d_6 , D_2O exchange, 400 MHz) δ 7.31 – 7.03 (m, 5H), 5.18 (s, 2H), 5.06 (t, 1H), 3.80 (s, 3H), 3.23 (d, $J = 6.9$ Hz, 2H), 2.94 (t, $J = 7.0$ Hz, 2H), 2.47 (t, 2H), 2.08 (s, 4H), 2.03 (s, 3H), 1.68 (s, 3H), 1.45 (p, $J = 7.7$ Hz, 2H), 1.28 (p, $J = 7.1$ Hz, 2H); MS (ESI) m/z : 450 [M-H] $^-$.

(E)-*N*-(benzo[*d*][1,3]dioxol-5-ylmethyl)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enamide (**25**). It was synthesized using **1** (0.1 g, 0.31 mmol), 3,4-(methylenedioxy)benzylamine **13i** (0.044 mL, 0.35 mmol), DMAP (0.044 g, 0.35 mmol) and EDCI.HCl (0.066 g, 0.34 mmol) as per Method A to yield **25** as off-white solid. Yield: 56%; TLC: $R_f = 0.62$ (DCM:EtOAc, 8:2); purity (HPLC): 99.31%; mp: 100-102 °C; IR (KBr) cm^{-1} 3425 (OH, str), 3283 (NH, str), 1739 (O-C=O, str), 1640 (NH-C=O, str), 1551 (C=C, str), 1135 (C-O, str); $^1\text{H-NMR}$ (DMSO- d_6 , D₂O exchange, 400 MHz) δ 6.76 (d, $J = 1.9$, 8.0 Hz, 1H), 6.71 (s, 1H), 6.64 (d, $J = 8.0$ Hz, 1H), 5.91 (s, 2H), 5.20 (s, 2H), 5.07 – 5.10 (t, $J = 6.9$ Hz, 1H), 4.07 (s, 2H), 3.65 (s, 3H), 3.26 (d, $J = 6.8$ Hz, 2H), 2.14 (s, 4H), 2.04 (d, $J = 1.8$ Hz, 3H), 1.70 (s, 3H); MS (ESI) m/z : 452 [M-H]⁻.

(E)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methyl-*N*-(2-morpholinoethyl)hex-4-enamide (**26**). It was synthesized using **1** (0.1 g, 0.31 mmol), 4-(2-aminoethyl)morpholine **13m** (0.046 mL, 0.35 mmol), DMAP (0.044 g, 0.35 mmol) and EDCI.HCl (0.066 g, 0.34 mmol) as per Method A to yield **26** as off-white solid. Yield: 55%; TLC: $R_f = 0.57$ (DCM:EtOAc, 8:2); purity (HPLC): 99.99%; mp: 124-126 °C; IR (KBr) cm^{-1} 3428 (OH, str), 3292 (NH, str), 1739 (O-C=O, str), 1642 (NH-C=O, str), 1562 (C=C, str), 1117 (C-O, str); $^1\text{H-NMR}$ (DMSO- d_6 , D₂O exchange, 400 MHz) δ 5.20 (s, 2H), 5.04 – 5.08 (t, $J = 7.0$ Hz, 1H), 3.65 (s, 3H), 3.50 (t, $J = 4.6$ Hz, 4H), 3.25 (d, $J = 6.8$ Hz, 2H), 3.05 (t, $J = 6.9$ Hz, 2H), 2.31 – 2.23 (m, 4H), 2.20 (t, $J = 7.0$ Hz, 2H), 2.09 (s, 4H), 2.04 (s, 3H), 1.69 (s, 3H); MS (ESI) m/z : 431 [M-H]⁻.

(E)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-*N*-(4-methoxyphenyl)-4-methylhex-4-enamide (**28**). It was synthesized using **1** (0.1 g, 0.31 mmol), 4-methoxyaniline **13o** (0.026 g, 0.21 mmol), HATU (0.158 g, 0.41 mmol) and DIPEA (0.185 mL, 1.06 mmol) as per Method B to yield **28** as off-white solid. Yield: 48%; TLC: $R_f = 0.60$

(DCM:EtOAc, 8:2); purity (HPLC): 99.99%; mp: 166-168 °C; IR (KBr) cm^{-1} 3302 (OH, str), 1753 (O-C=O, str), 1657 (NH-C=O, str), 1613 (C=C, str), 1132 (C-O, str); $^1\text{H-NMR}$ (DMSO- d_6 , D_2O exchange, 400 MHz) δ 7.31 (d, $J = 8.5$ Hz, 2H), 6.77 (d, $J = 8.5$ Hz, 2H), 5.17 (s, 2H), 5.14 – 5.12 (t, $J = 7.0$ Hz, 1H), 3.66 (s, 3H), 3.60 (s, 3H), 3.25 (d, $J = 6.8$ Hz, 2H), 2.28 (t, $J = 7.4$ Hz, 2H), 2.19 (t, $J = 7.5$ Hz, 2H), 2.00 (s, 3H), 1.73 (s, 3H); MS (ESI) m/z : 424 $[\text{M-H}]^-$.

Protocol for human peripheral blood mononuclear cells (hPBMC) assay

hPBMCs were separated from heparinized blood by density gradient centrifugation in Ficoll-Urotropine. Cells were placed in 96-well round-bottom plates (0.1 million cells/well) and Dynabeads® human T-activator CD3/CD28 for cell expansion and activation (culture medium - RPMI containing 10% heat inactivated FBS and 100 units/ml of penicillin and streptomycin). The compound were dissolved in DMSO, the final concentration of which was 1% and 1% DMSO treated cells considered as a control. Compound **24** and MPA(**1**) were tested at 10 μM concentration in triplicate and incubated with the compounds for 48 h (37°C, atmosphere 5% CO_2). MTT solution (20 μL ; 5 mg/mL) was added to each well and incubated for 4 h. After this time, the supernatant was removed and 0.4N HCl/ isopropanol was added in order to dissolve the formazan crystals. The absorbance was measured at 570 nm (Perkin Elmer, Victor X4).

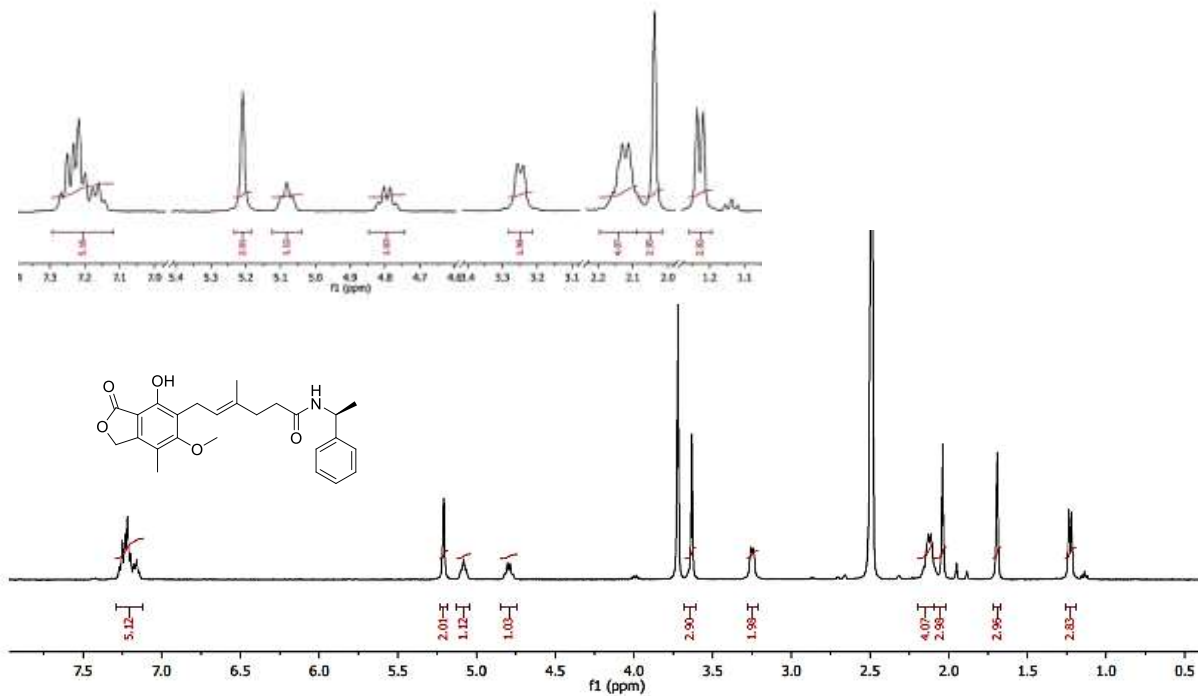


Figure 1S. $^1\text{H-NMR}$ spectrum (D_2O exchg.) of compound **14**

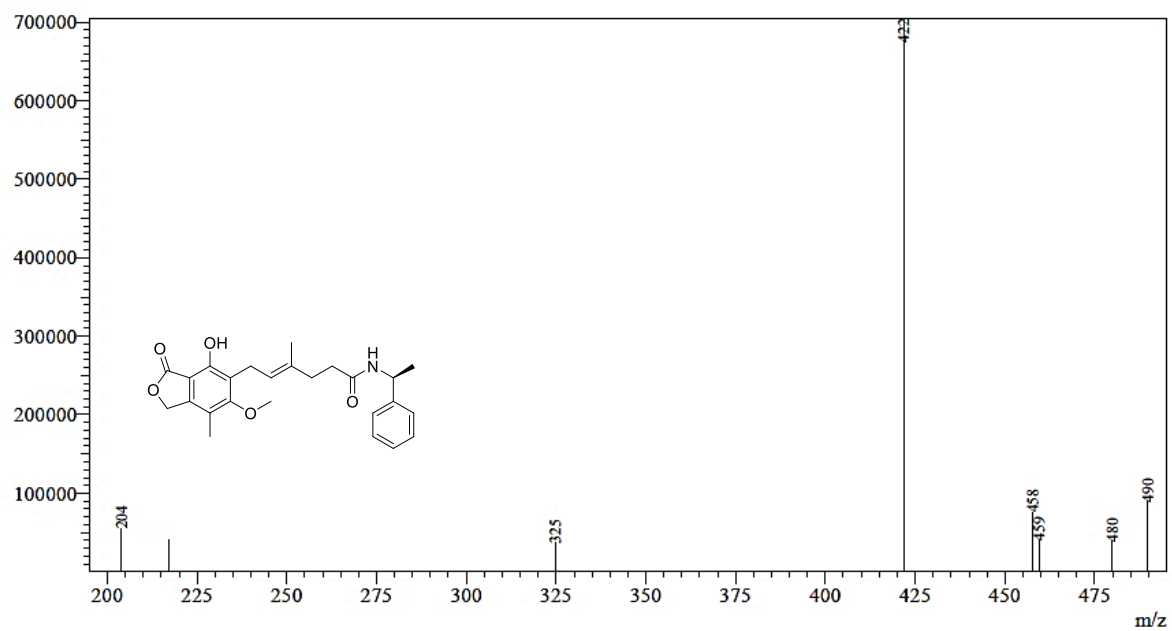


Figure 2S. Mass spectrum of compound **14**

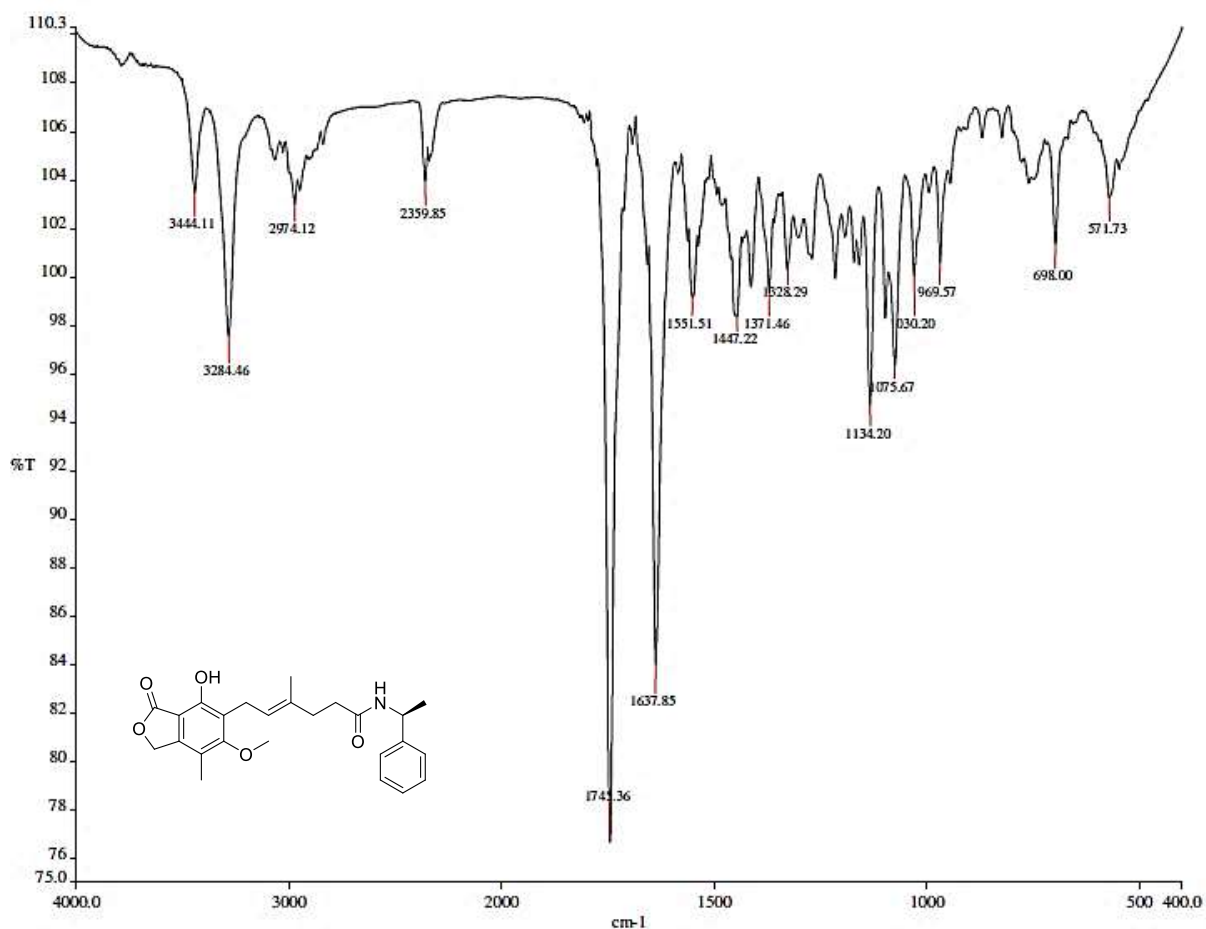
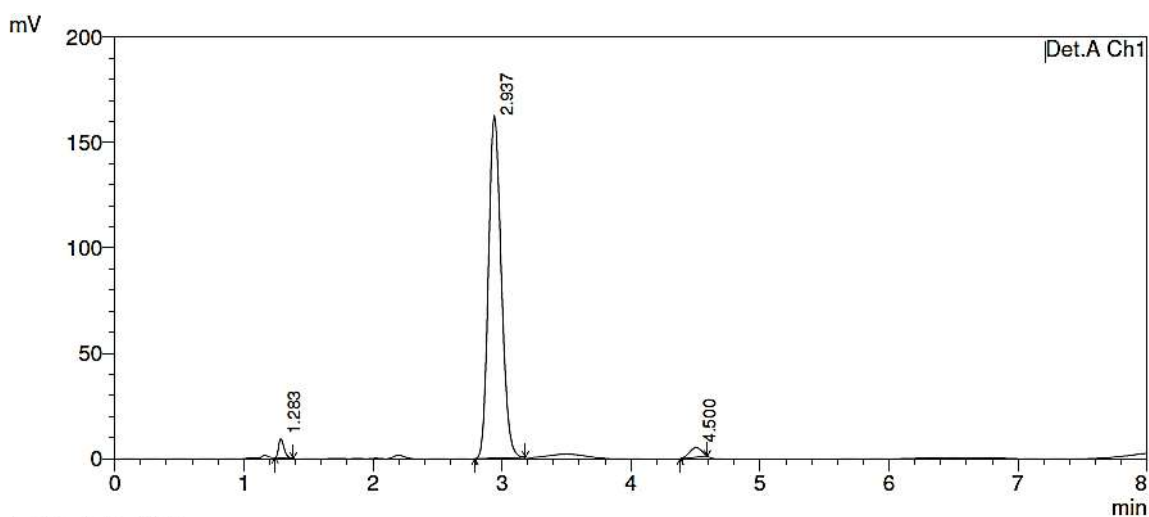


Figure 3S. FT-IR spectrum of compound 14



1 Det.A Ch1/254nm

PeakTable

Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.283	26474	8996	2.251	5.111
2	2.937	1120522	162487	95.284	92.328
3	4.500	28990	4507	2.465	2.561
Total		1175986	175989	100.000	100.000

Figure 4S. HPLC chromatogram of compound 14

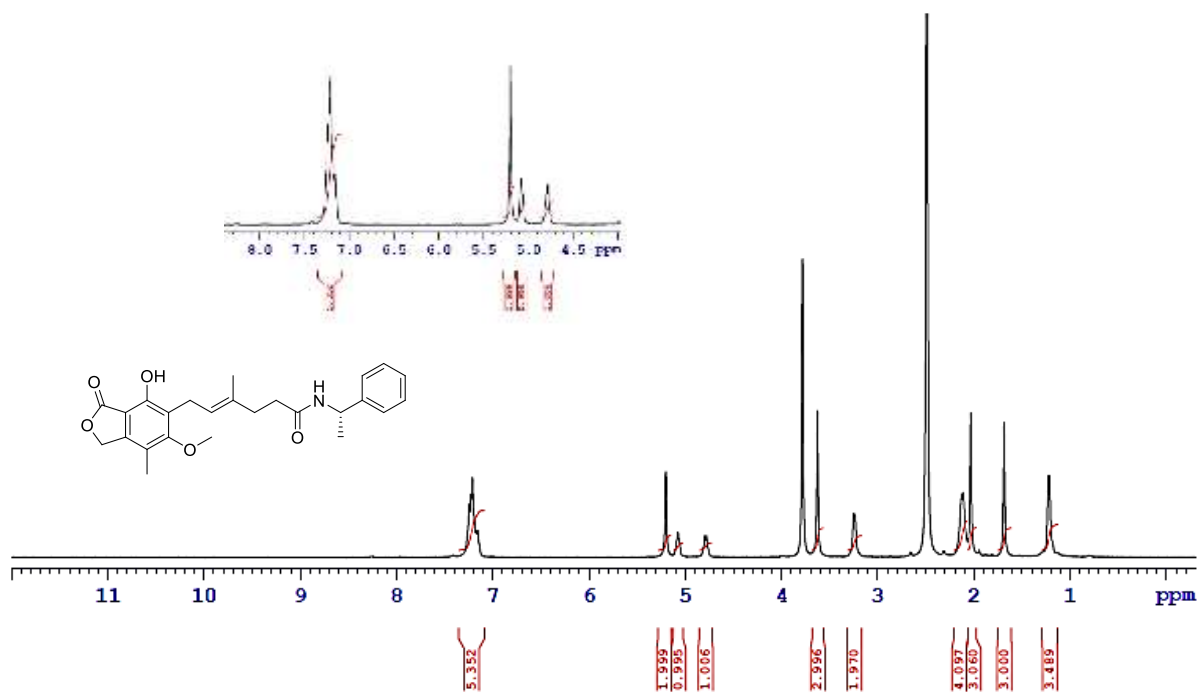


Figure 5S. ¹H-NMR spectrum (D₂O exchg.) of compound **15**

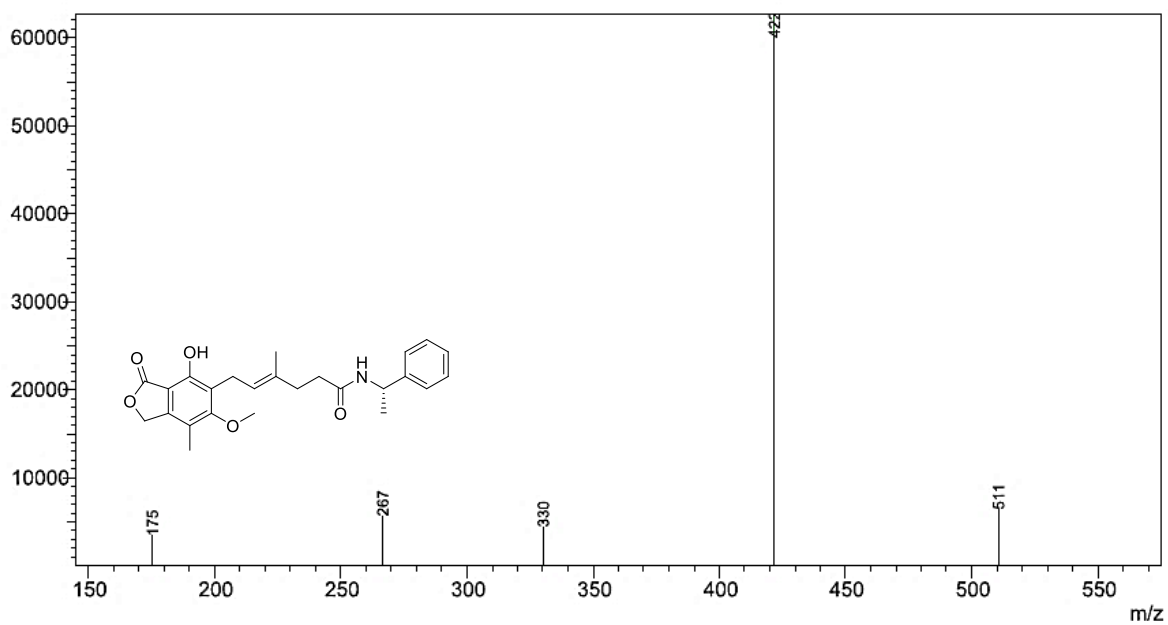


Figure 6S. Mass spectrum of compound **15**

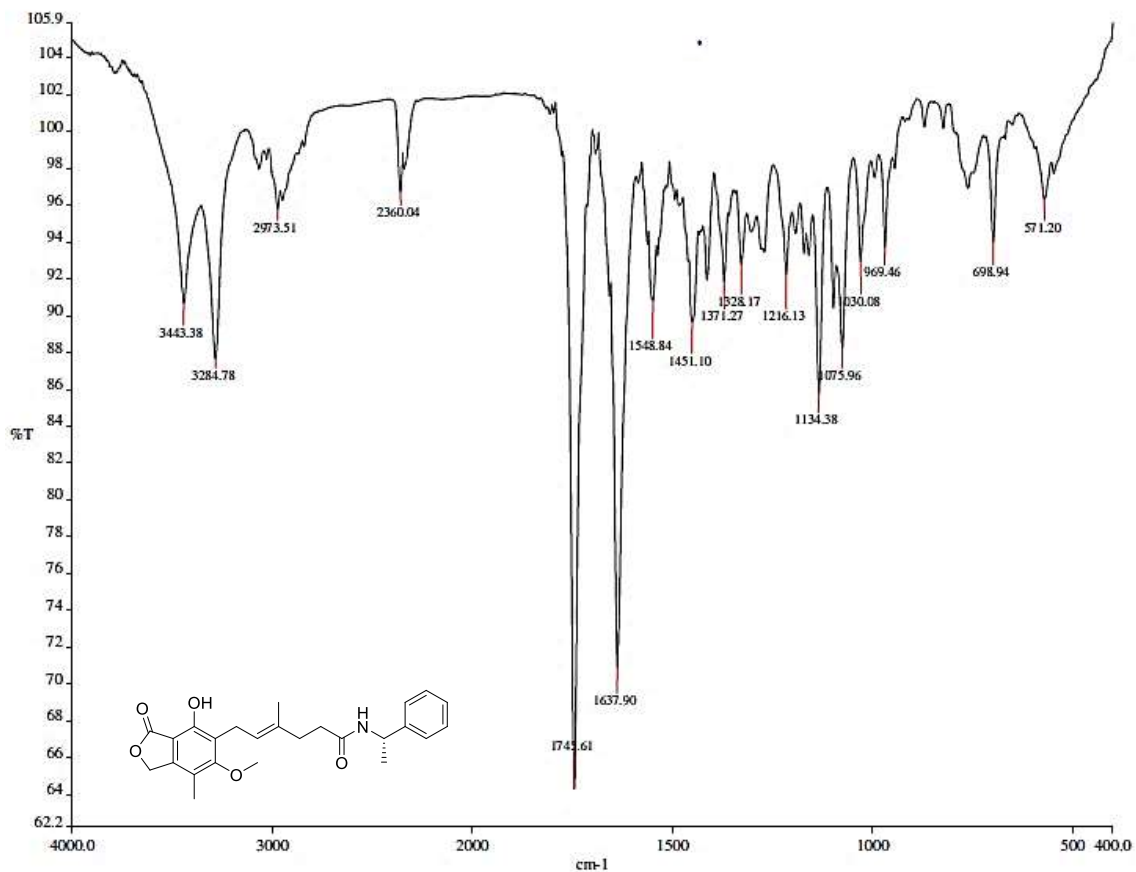
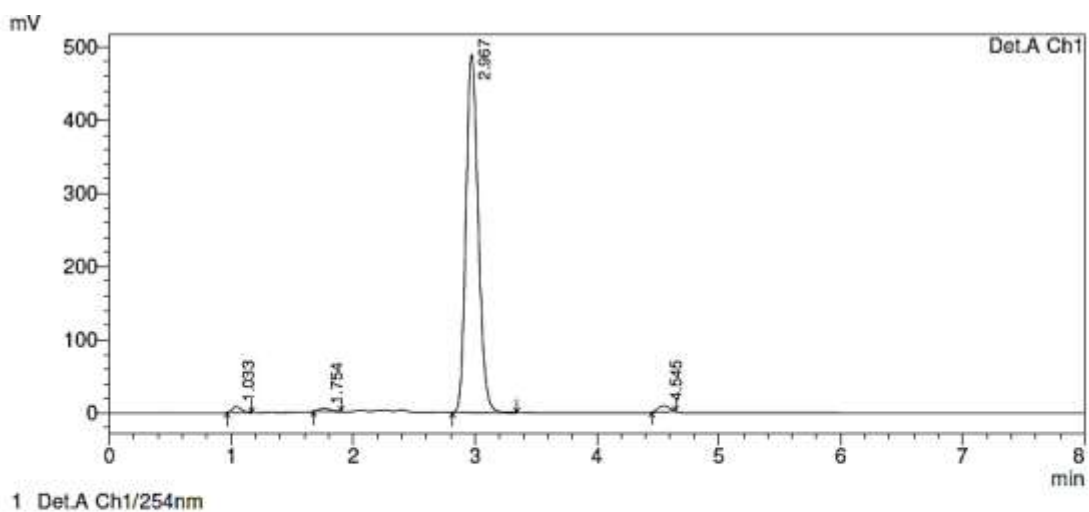


Figure 7S. FT-IR spectrum of compound 15

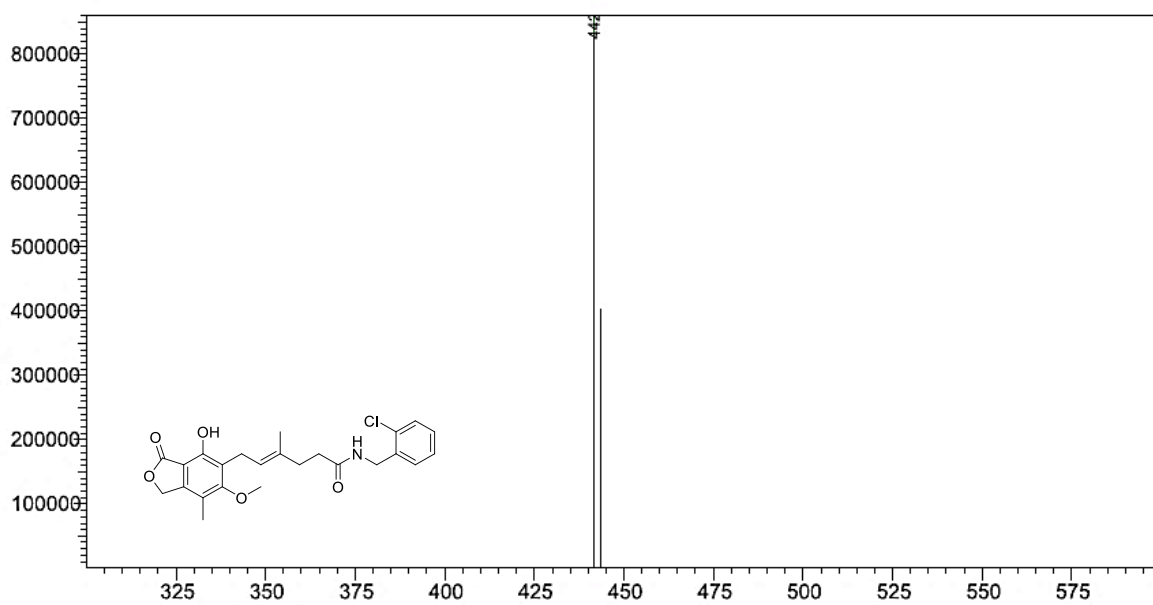
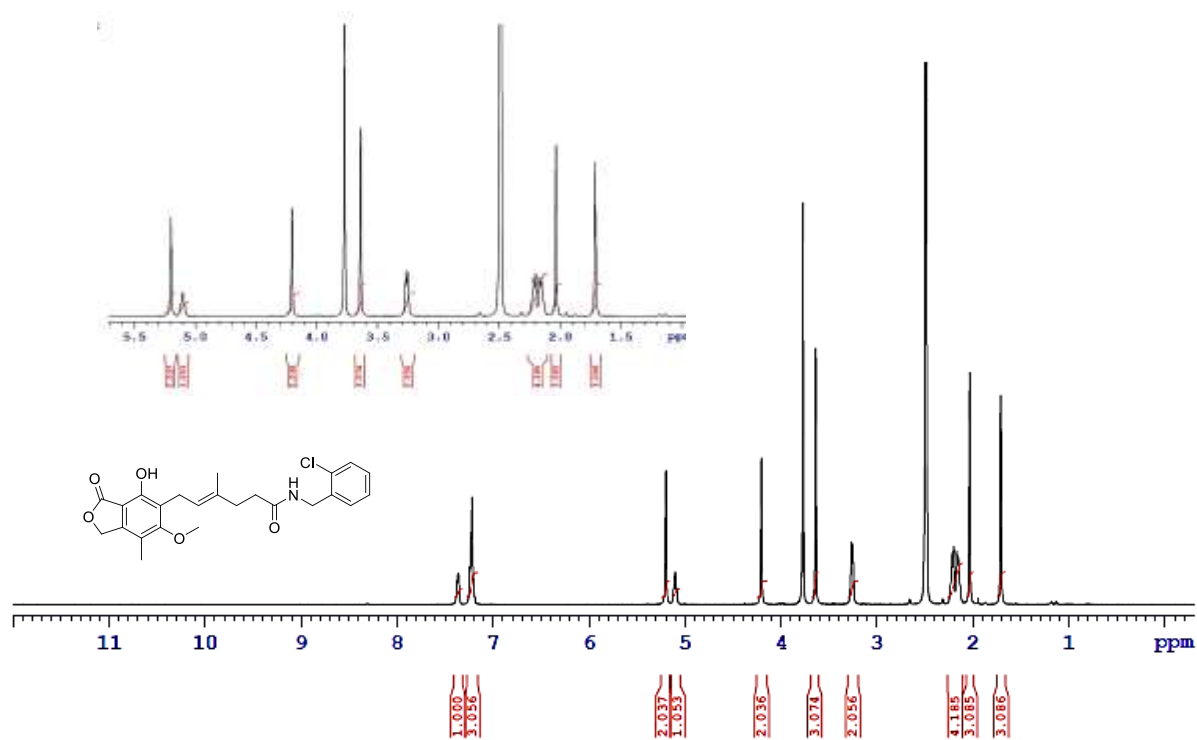


1 Det.A Ch1/254nm

PeakTable

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.033	41631	7940	1.174	1.559
2	1.754	25898	4694	0.731	0.922
3	2.967	3427192	488553	96.672	95.939
4	4.545	50466	8044	1.423	1.580
Total		3545187	509231	100.000	100.000

Figure 8S. HPLC chromatogram of compound 15



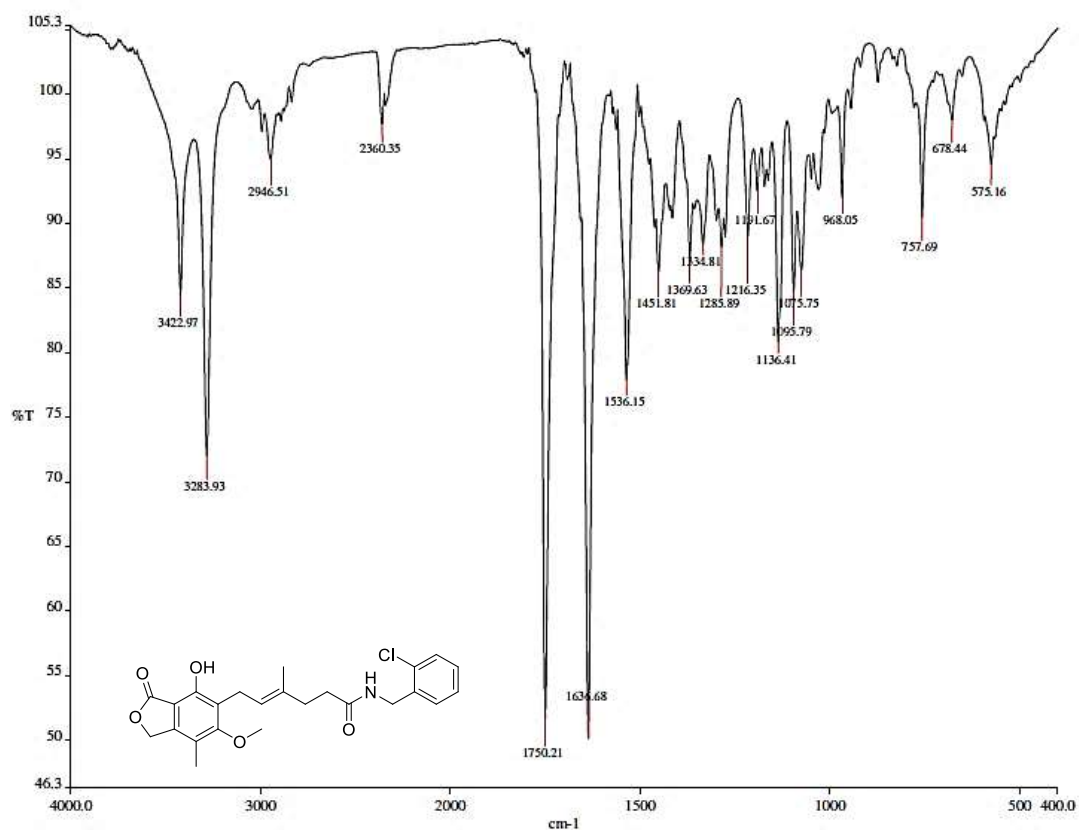
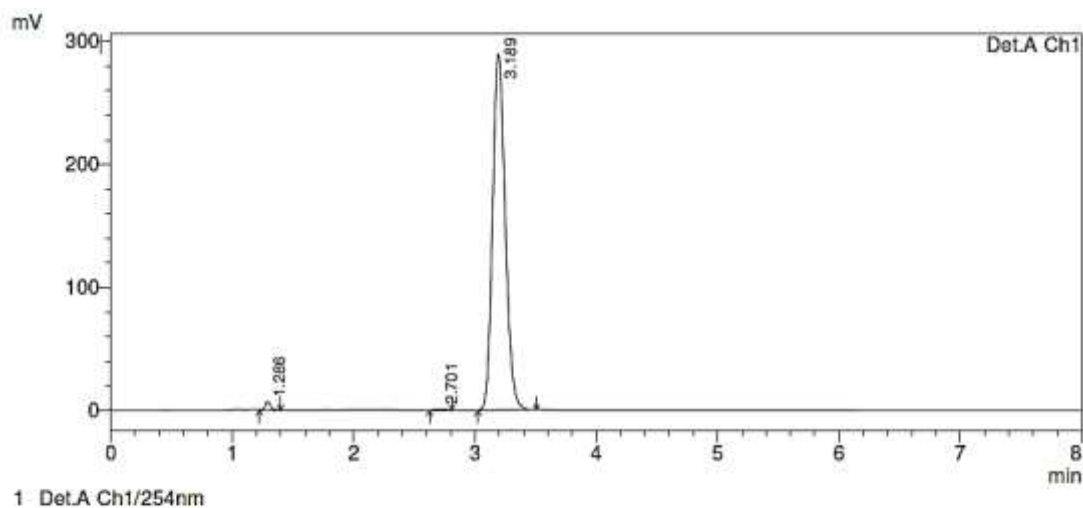


Figure 11S. FT-IR spectrum of compound 16



1 Det.A Ch1/254nm

PeakTable

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.286	21232	7096	1.000	2.382
2	2.701	6544	1150	0.308	0.386
3	3.189	2094609	289709	98.691	97.232
Total		2122384	297956	100.000	100.000

Figure 12S. HPLC chromatogram of compound 16

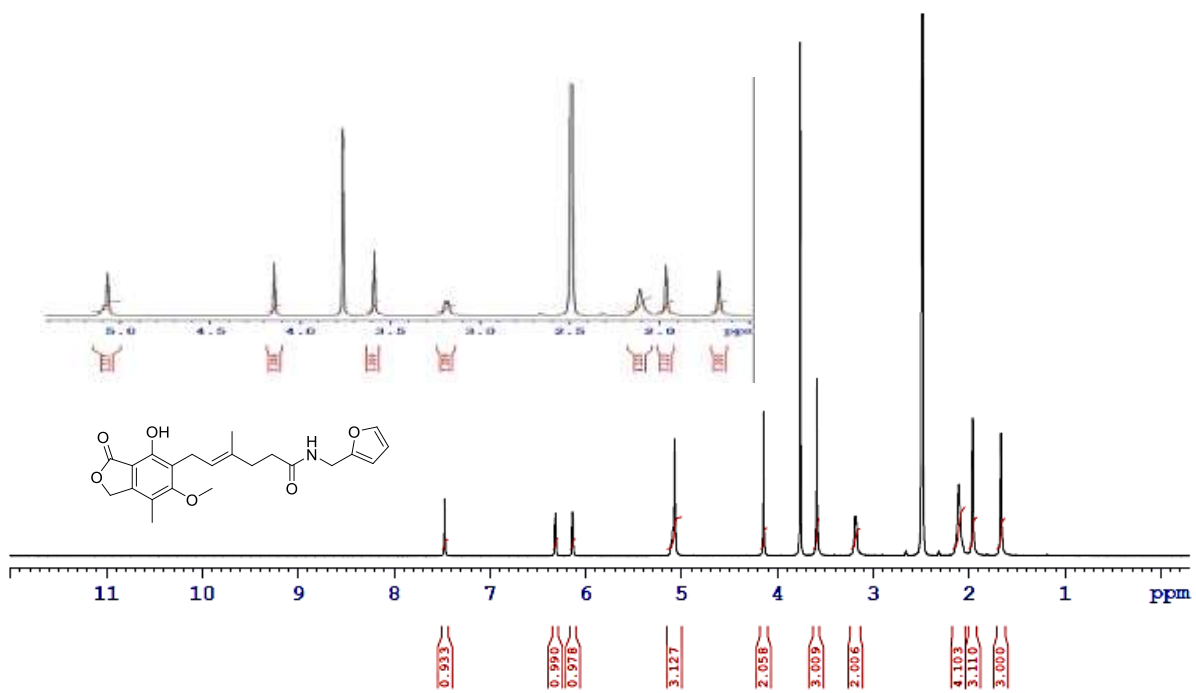


Figure 13S. $^1\text{H-NMR}$ spectrum (D_2O exchg.) of compound **17**

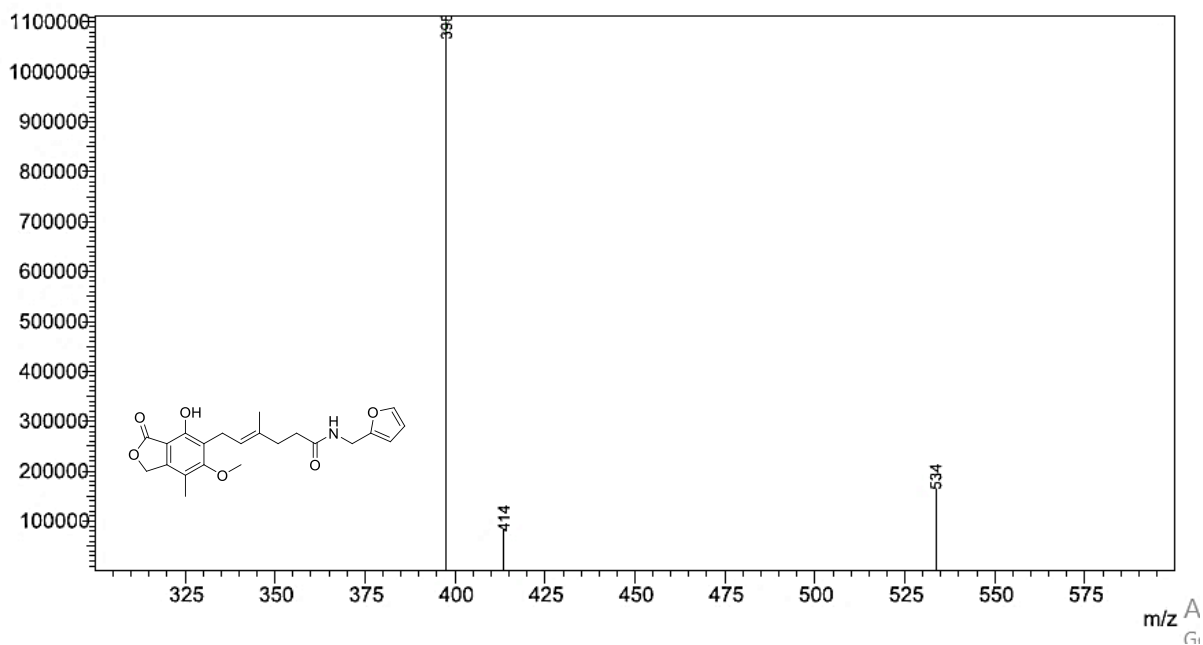


Figure 14S. Mass spectrum of compound **17**

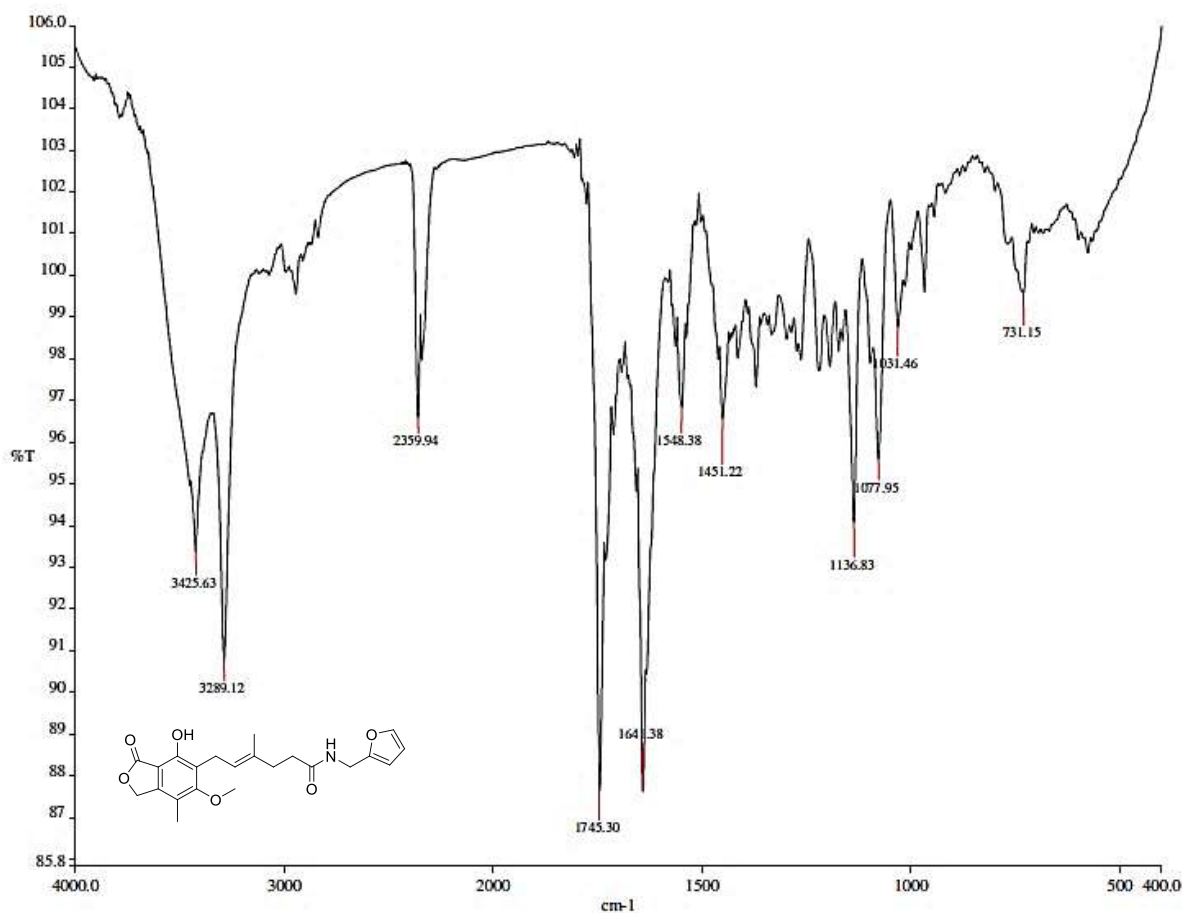
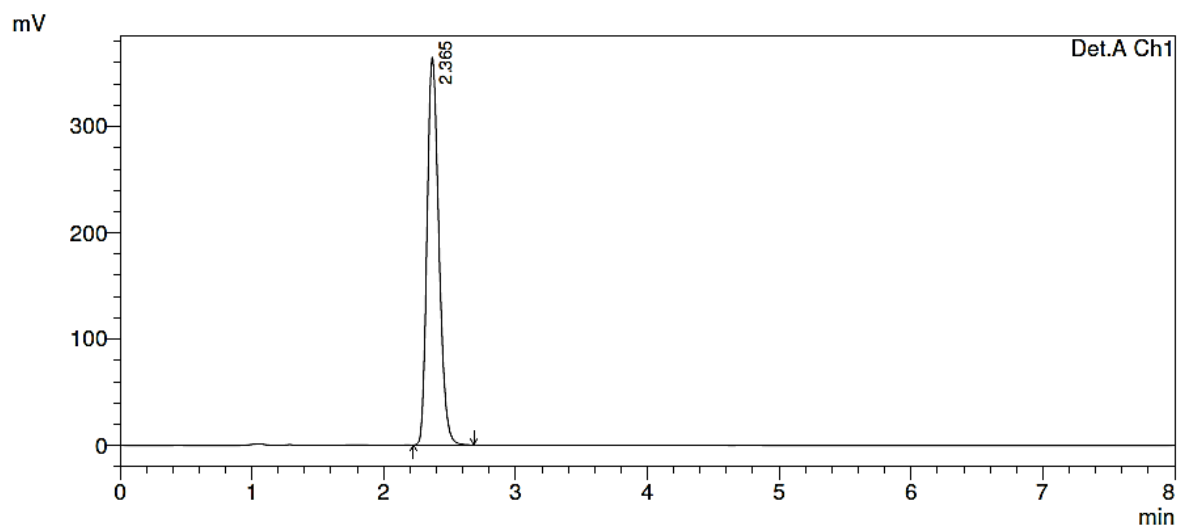


Figure 15S. FT-IR spectrum of compound 17



1 Det.A Ch1/254nm

PeakTable

Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.365	2264238	364544	100.000	100.000
Total		2264238	364544	100.000	100.000

Act

Figure 16S. HPLC chromatogram of compound 17

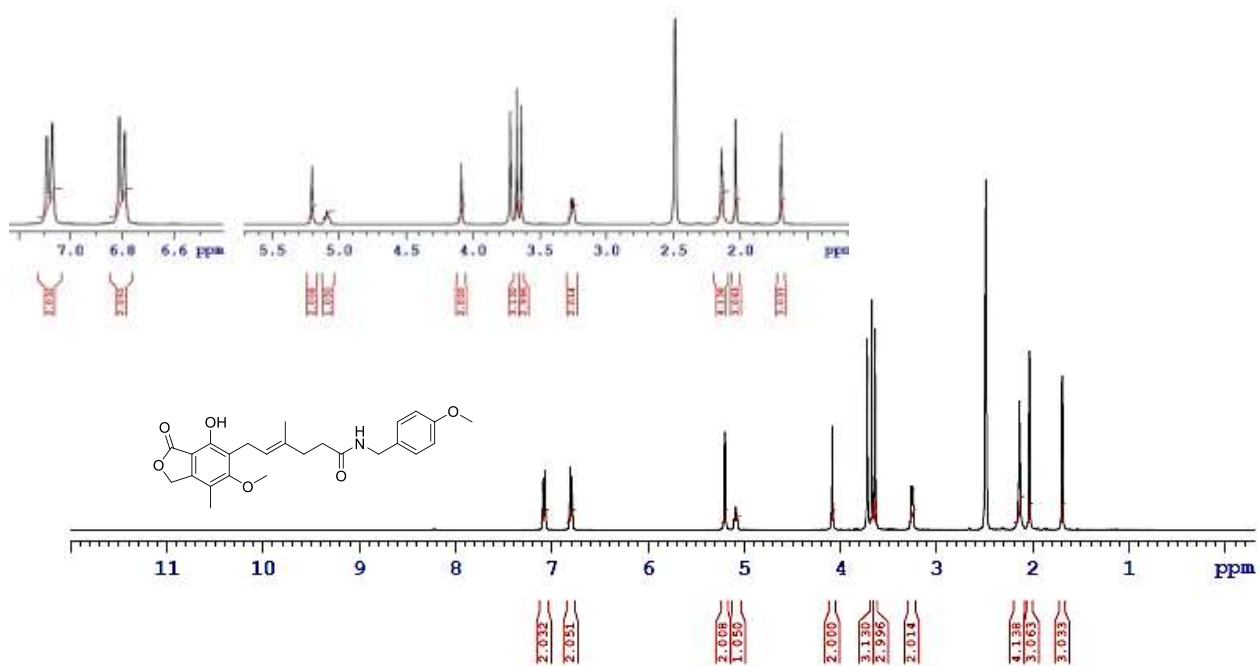


Figure 17S. ¹H-NMR spectrum (D₂O exchg.) of compound 18

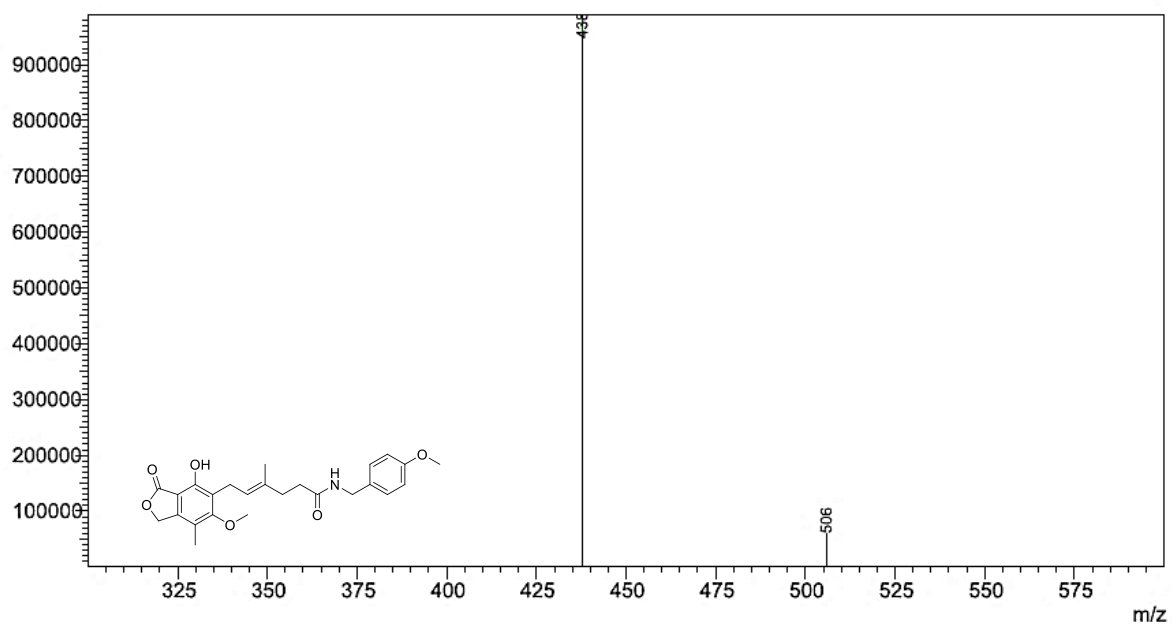


Figure 18S. Mass spectrum of compound 18

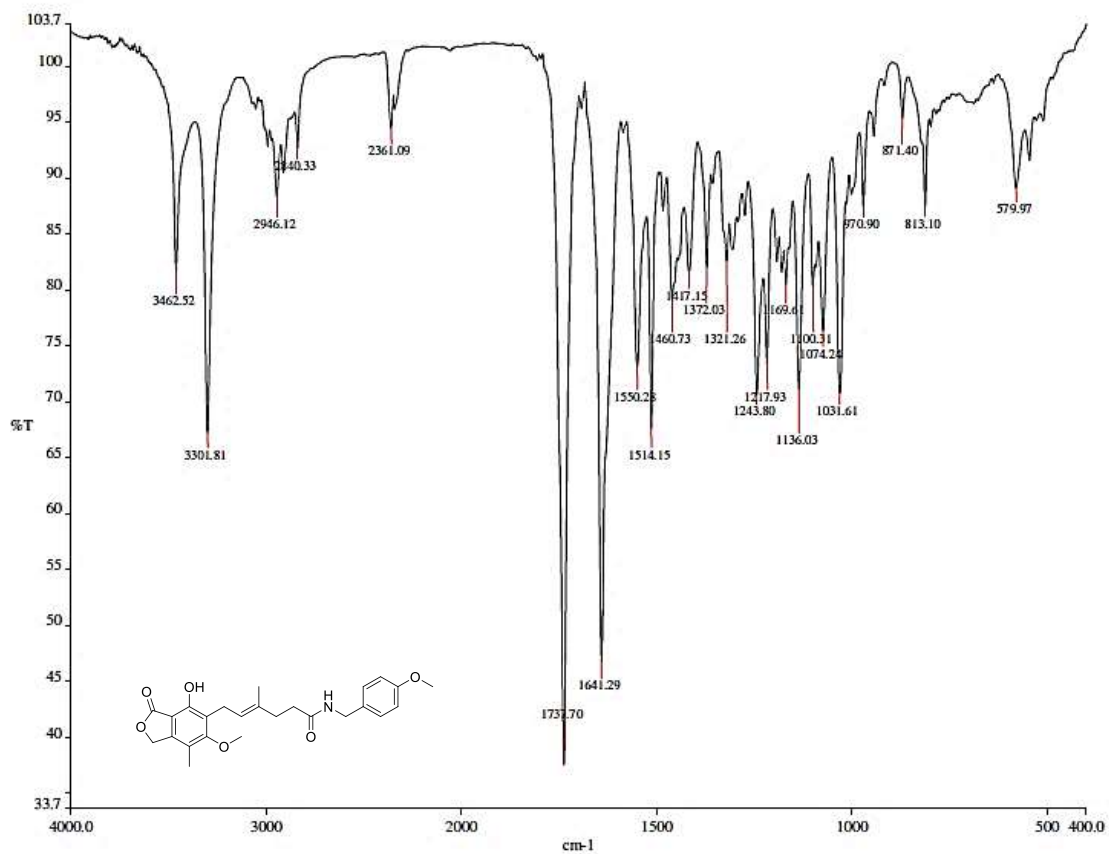
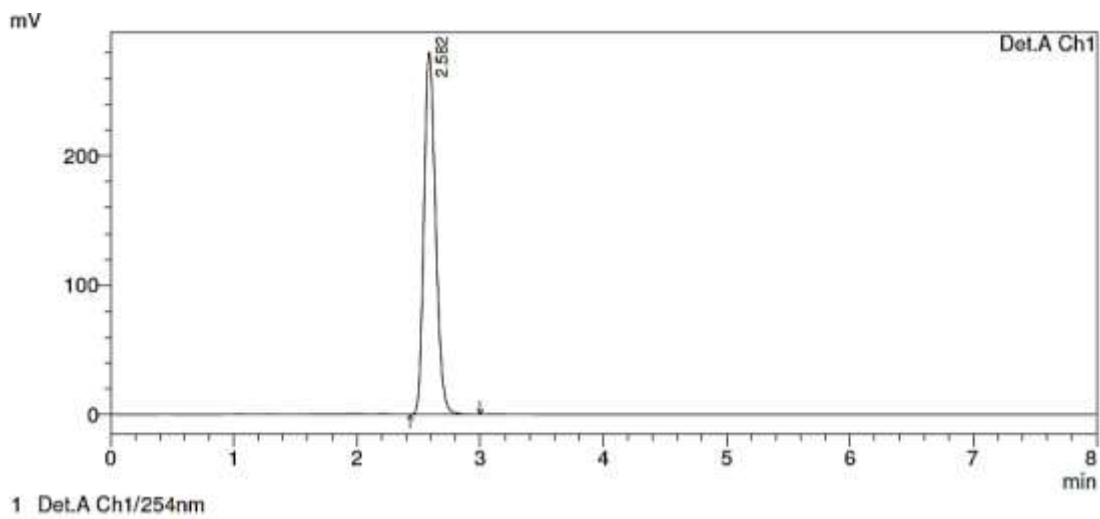


Figure 19S. FT-IR spectrum of compound 18



1 Det.A Ch1/254nm

PeakTable

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.582	1837216	280386	100.000	100.000
Total		1837216	280386	100.000	100.000

Figure 20S. HPLC chromatogram of compound 18

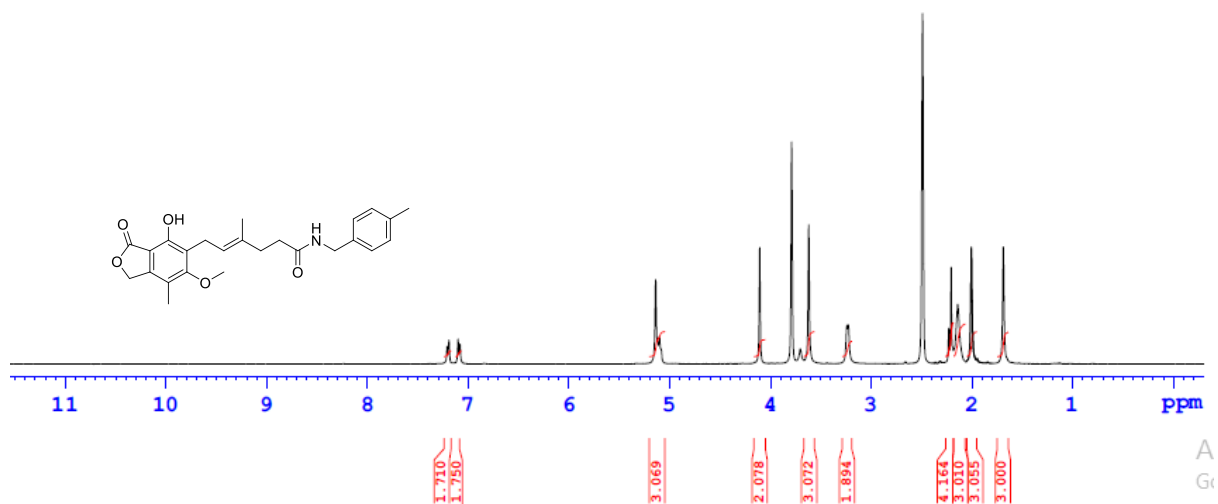


Figure 21S. $^1\text{H-NMR}$ spectrum (D_2O exchg.) of compound **19**

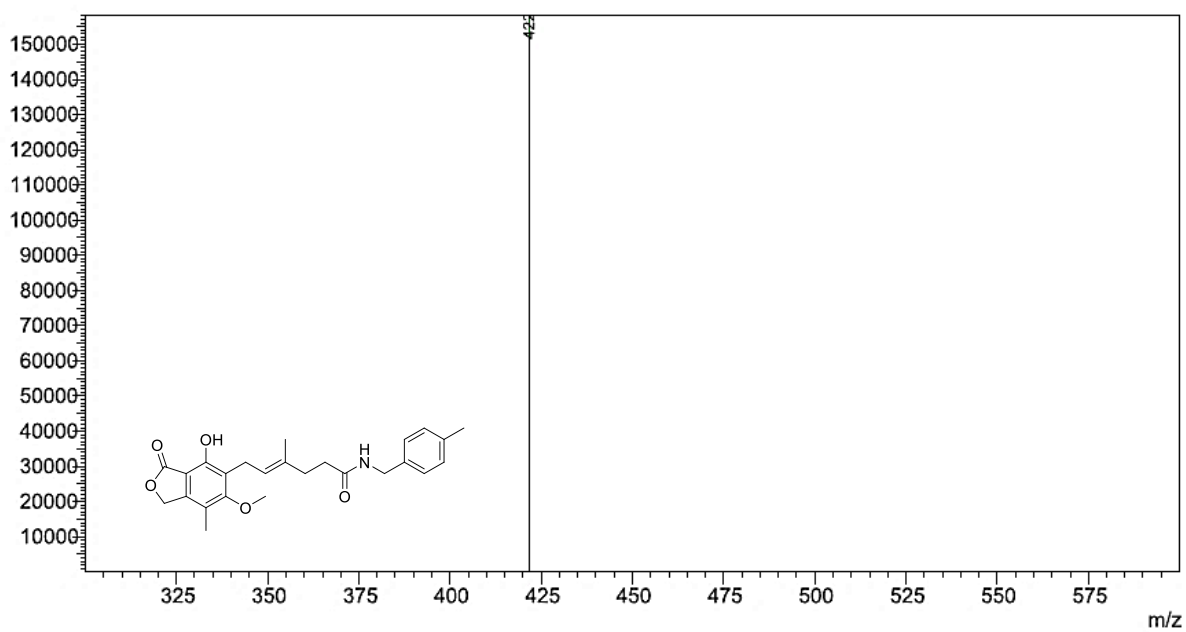


Figure 22S. Mass spectrum of compound **19**

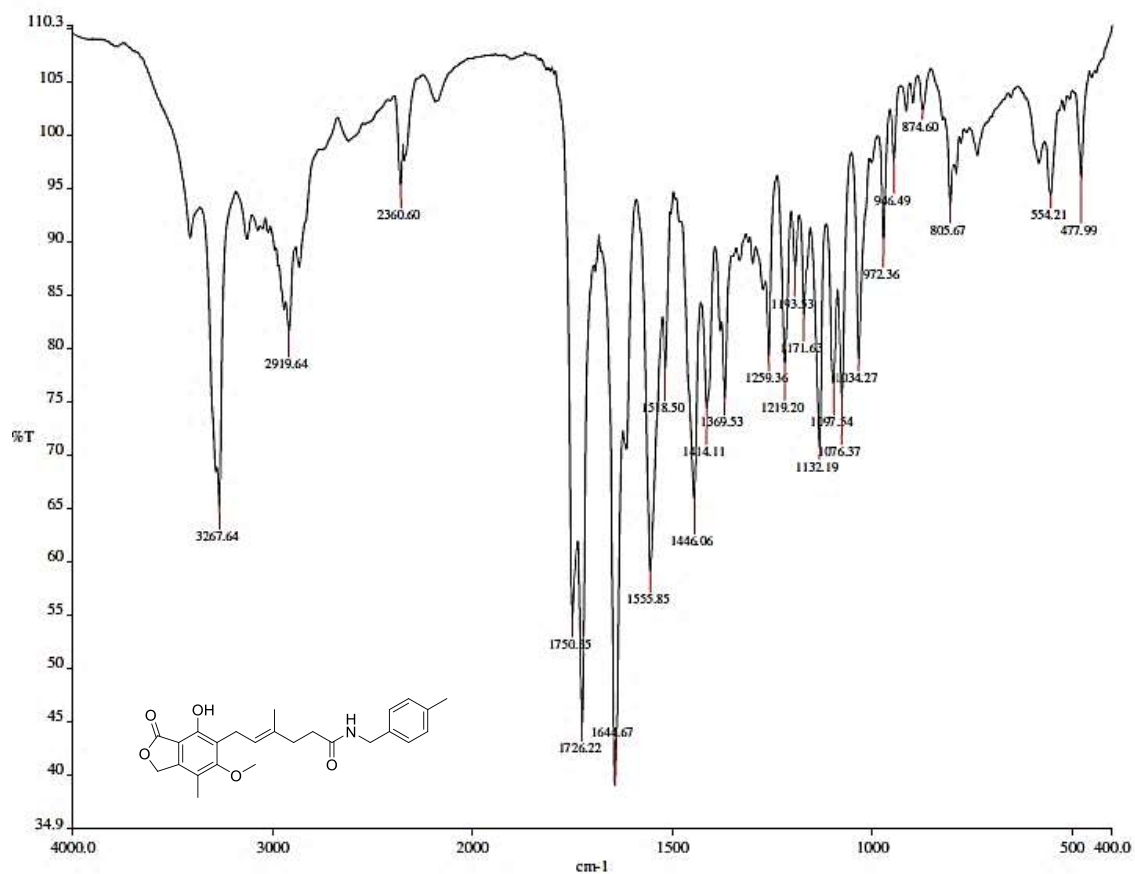
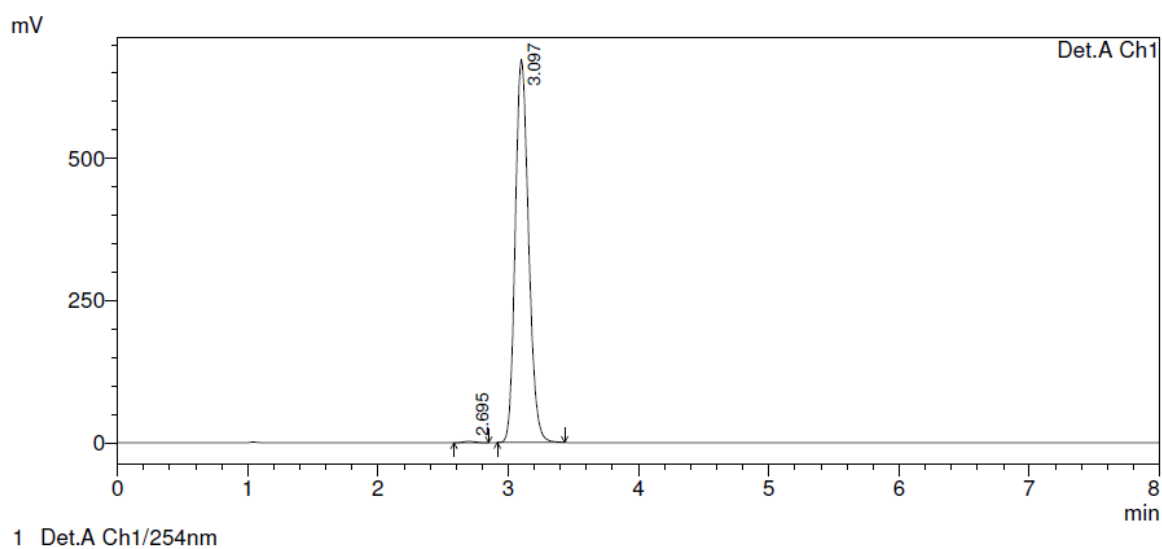


Figure 23S. FT-IR spectrum of compound **19**



1 Det.A Ch1/254nm

PeakTable

Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.695	15677	2233	0.325	0.330
2	3.097	4810232	673700	99.675	99.670
Total		4825909	675934	100.000	100.000

Figure 24S. HPLC chromatogram of compound **19**

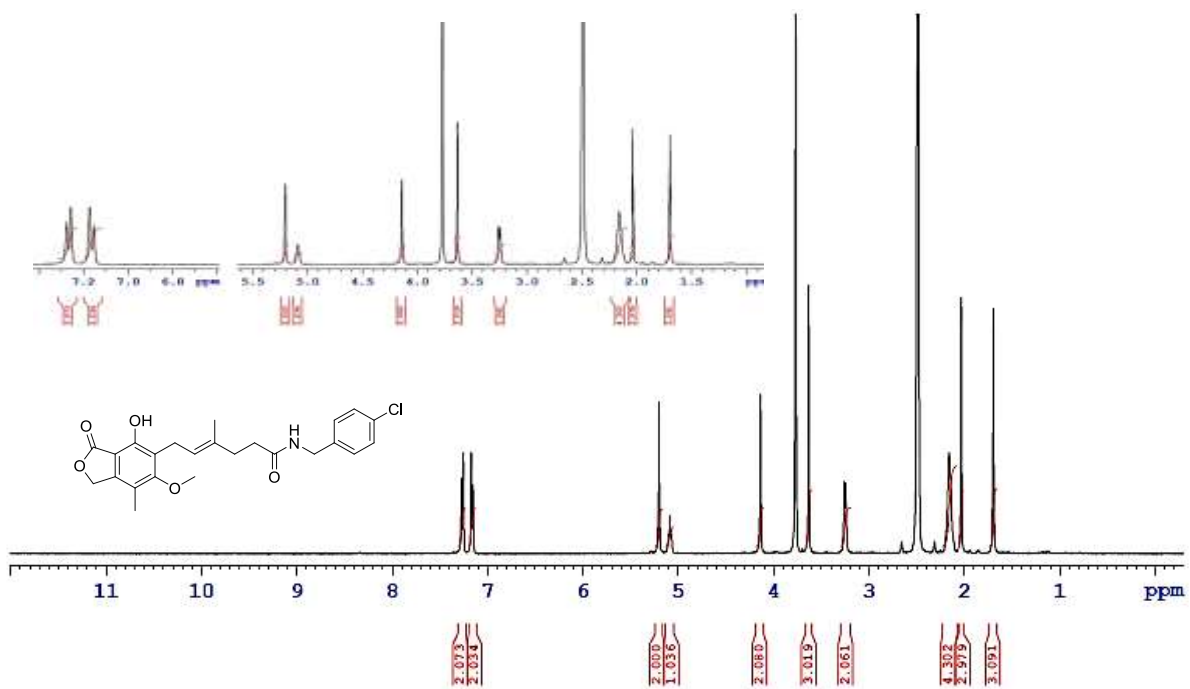


Figure 25S. ¹H-NMR spectrum (D₂O exchg.) of compound **20**

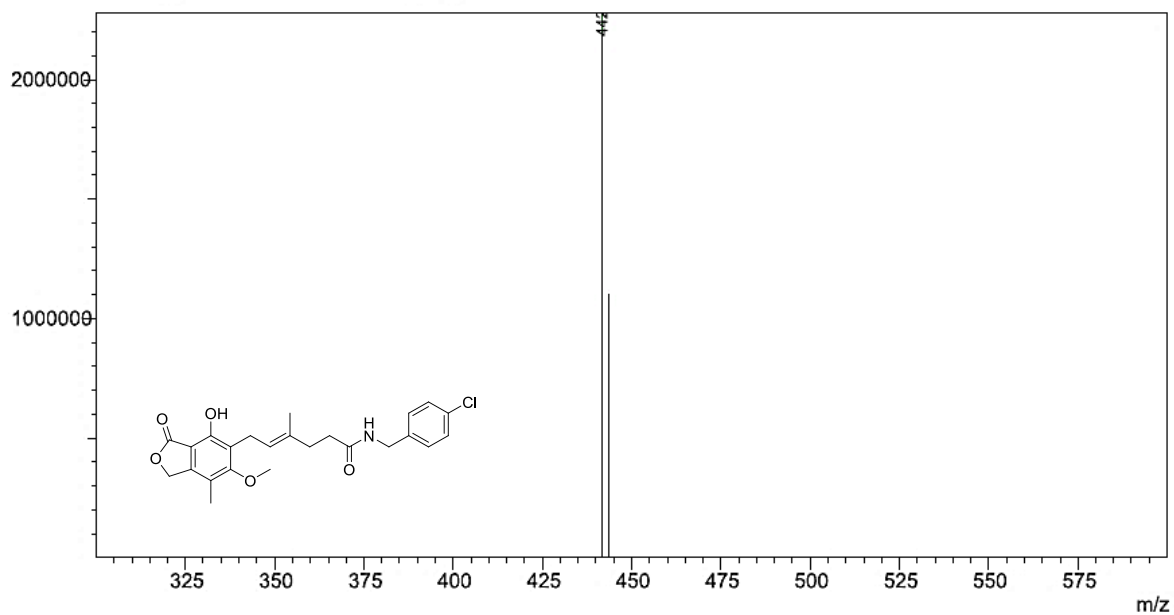


Figure 26S. Mass spectrum of compound **20**

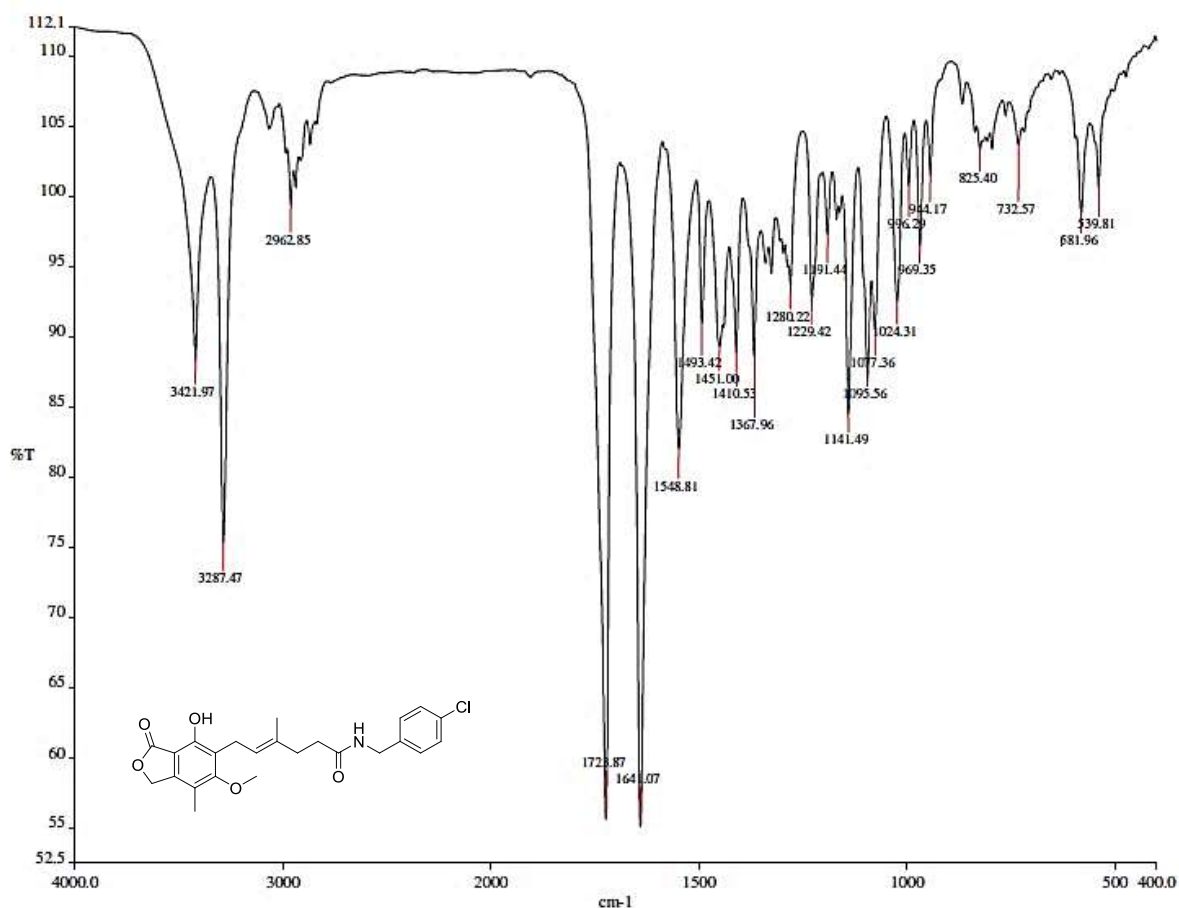
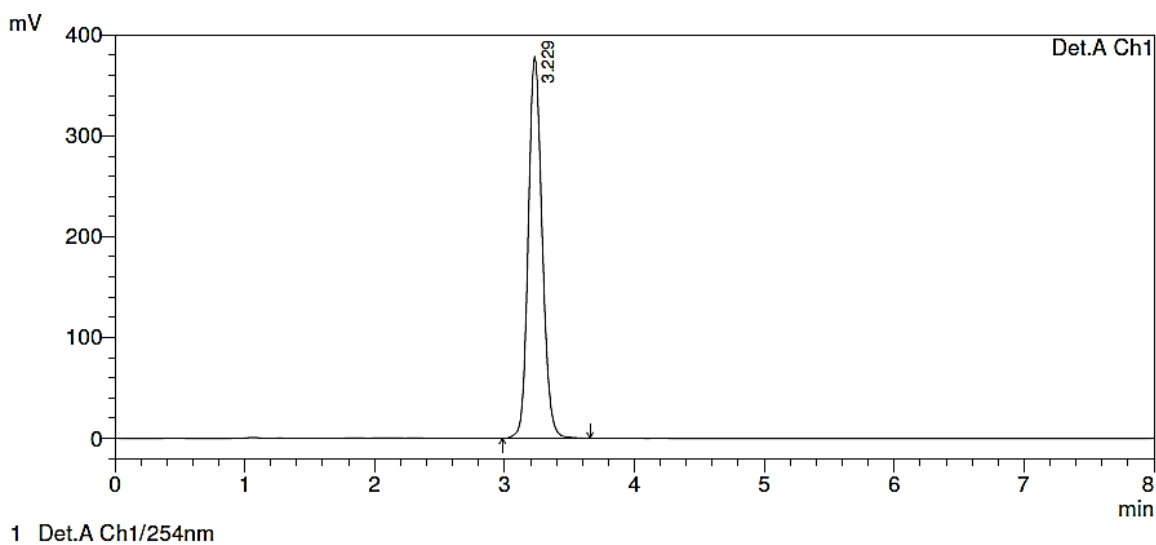


Figure 27S. FT-IR spectrum of compound 20



PeakTable

Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.229	2803722	378678	100.000	100.000
Total		2803722	378678	100.000	100.000

Ac

Figure 28S. HPLC chromatogram of compound 20

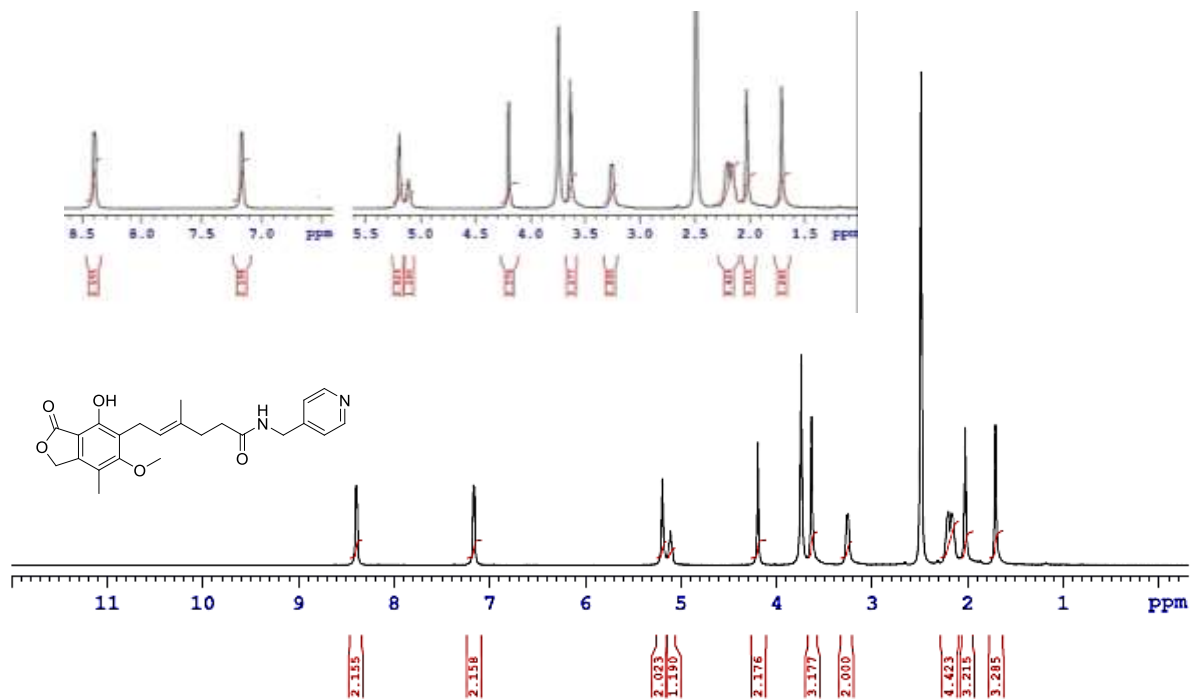


Figure 29S. $^1\text{H-NMR}$ spectrum (D_2O exchg.) of compound **21**

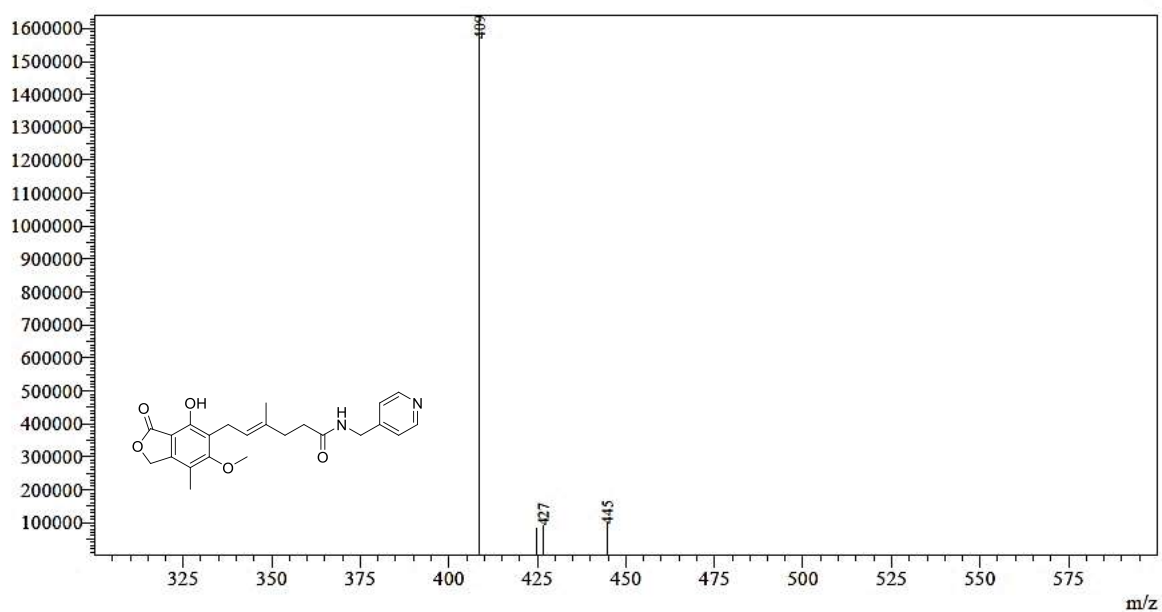


Figure 30S. Mass spectrum of compound **21**

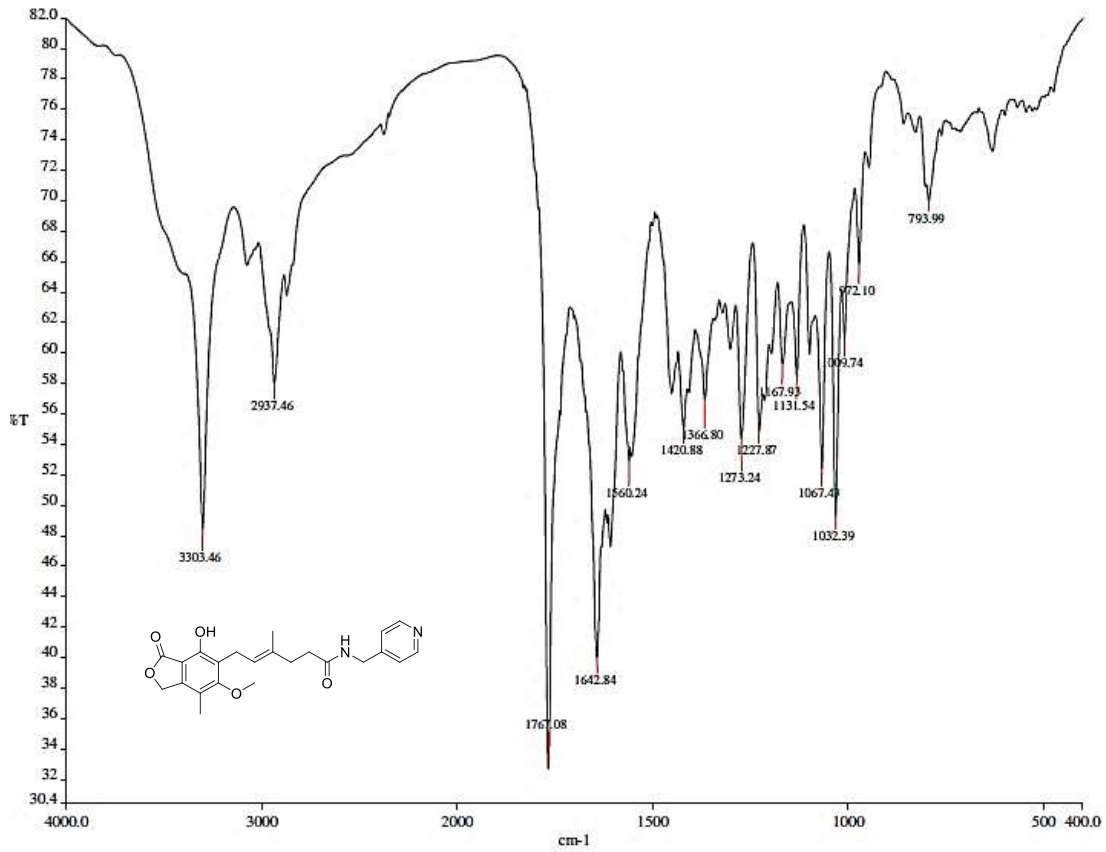
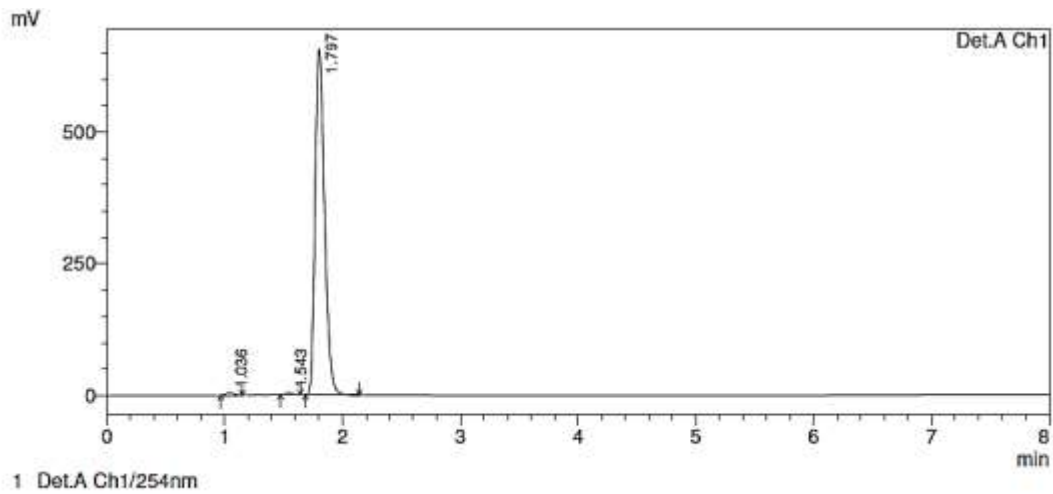


Figure 31S. FT-IR spectrum of compound 21



PeakTable

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.036	19538	4140	0.530	0.625
2	1.543	9541	1753	0.259	0.264
3	1.797	3659242	656943	99.212	99.111
Total		3688321	662835	100.000	100.000

Figure 32S. HPLC chromatogram of compound 21

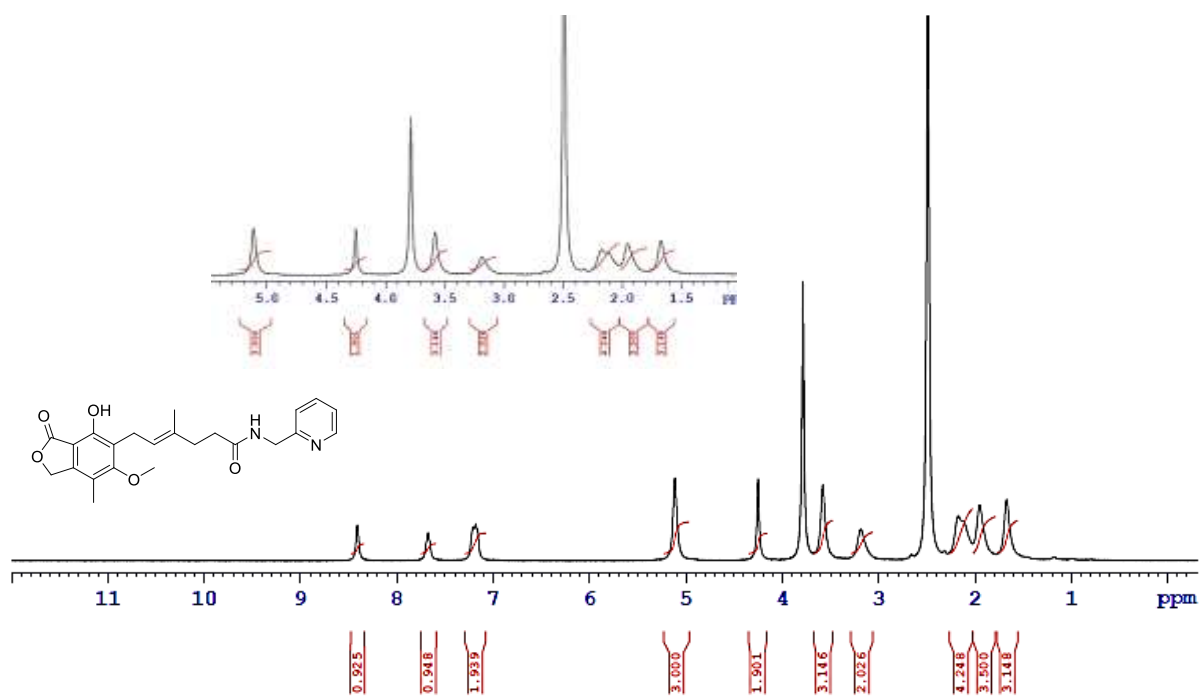


Figure 33S. ¹H-NMR spectrum (D₂O exch.) of compound 22

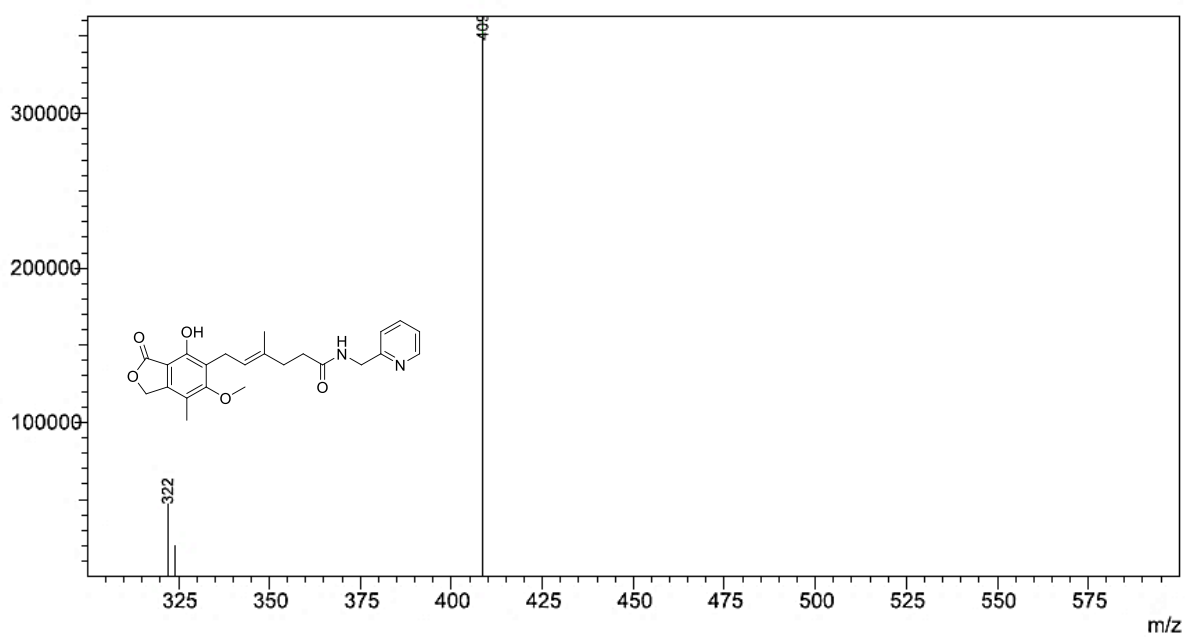


Figure 34S. Mass spectrum of compound 22

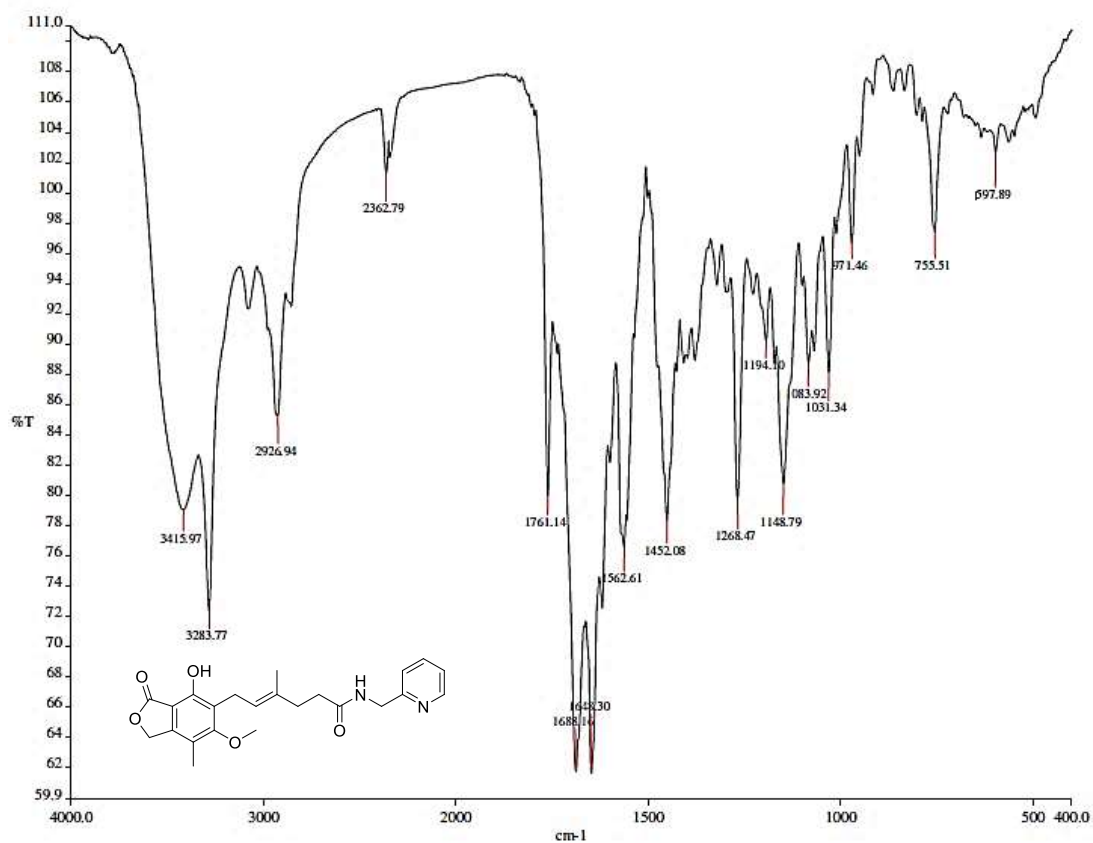
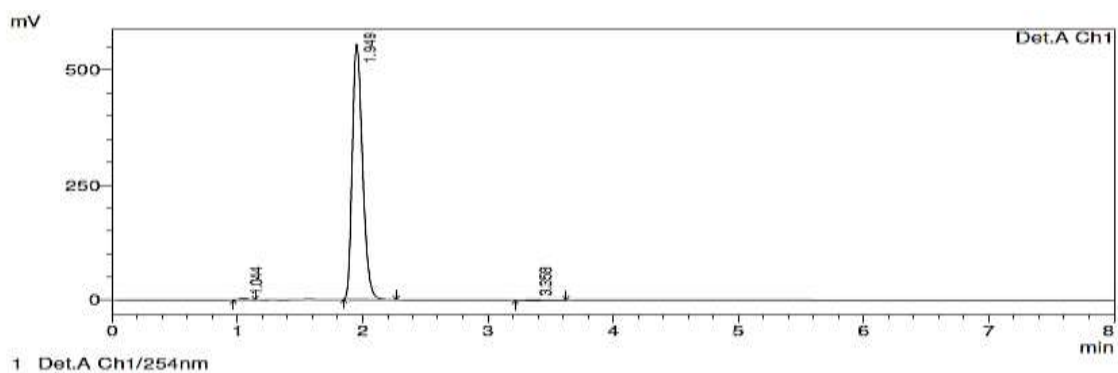


Figure 35S. FT-IR spectrum of compound 22



Detector A Ch1 254nm

PeakTable

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.044	16970	3568	0.532	0.637
2	1.949	3165941	555397	99.174	99.195
3	3.358	9385	941	0.294	0.168
Total		3192296	559906	100.000	100.000

Figure 36S. HPLC chromatogram of compound 22

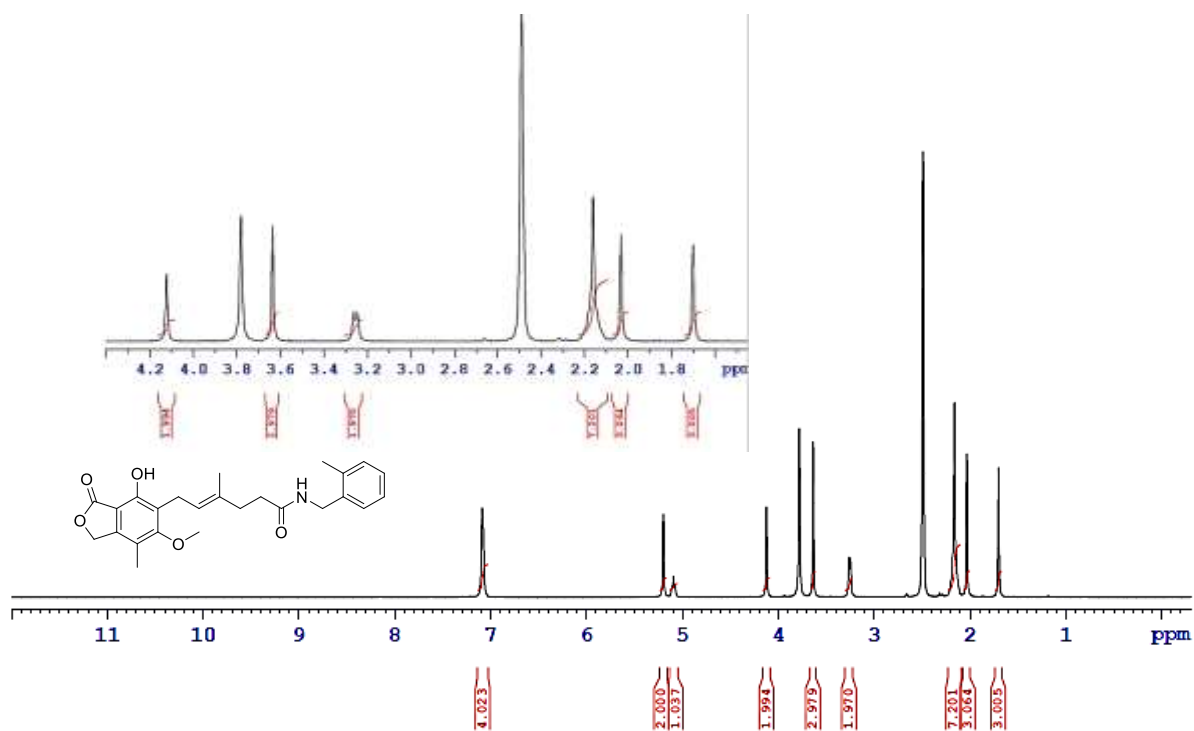


Figure 37S. ¹H-NMR spectrum (D₂O exchg.) of compound **23**

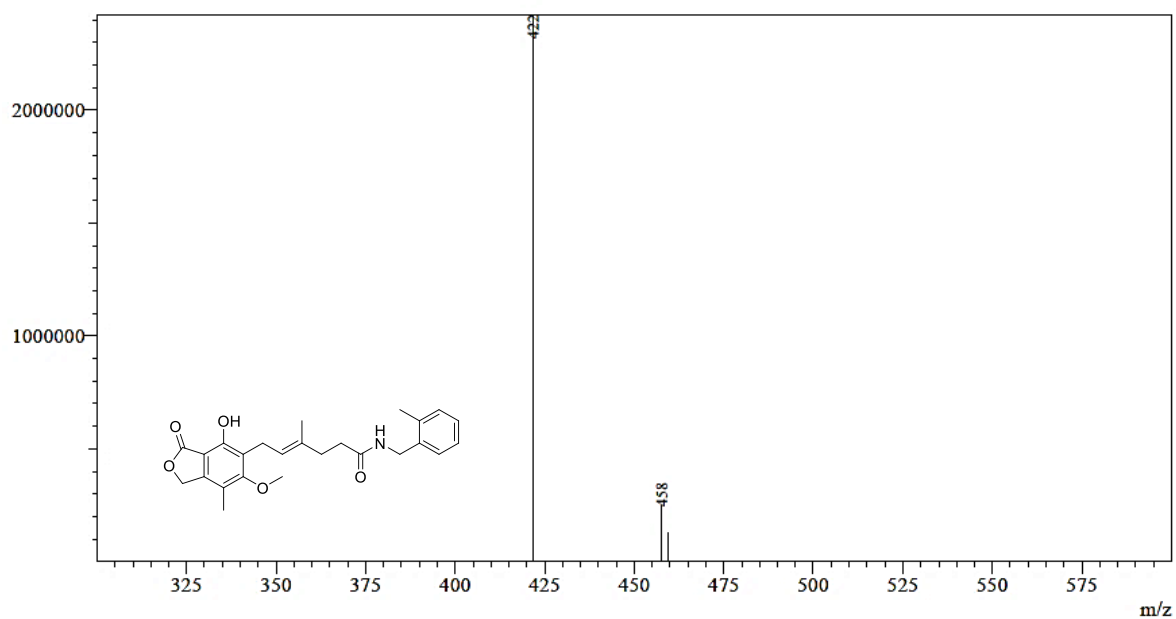


Figure 38S. Mass spectrum of compound **23**

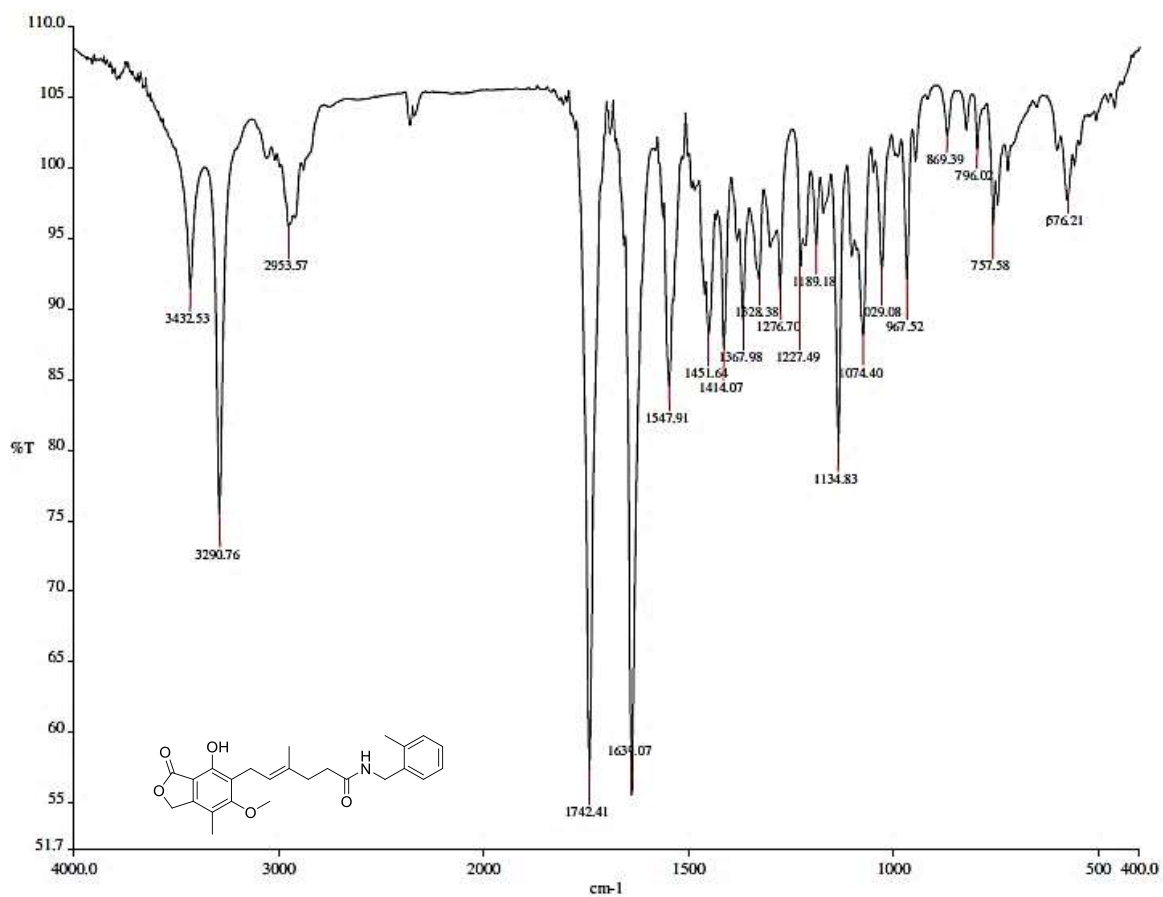
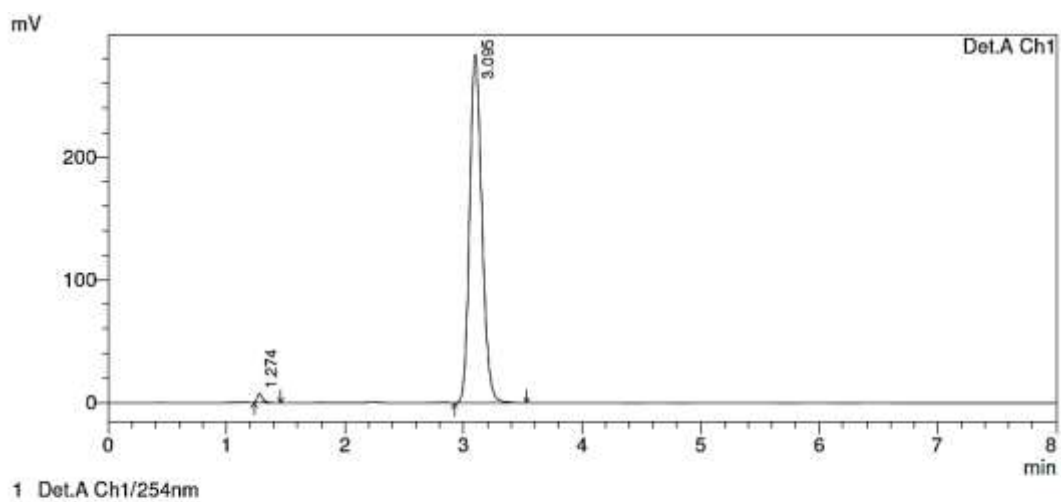


Figure 39S. FT-IR spectrum of compound 23



Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.274	20388	6994	0.989	2.405
2	3.095	2041335	283799	99.011	97.595
Total		2061723	290793	100.000	100.000

Act
Got

Figure 40S. HPLC chromatogram of compound 23

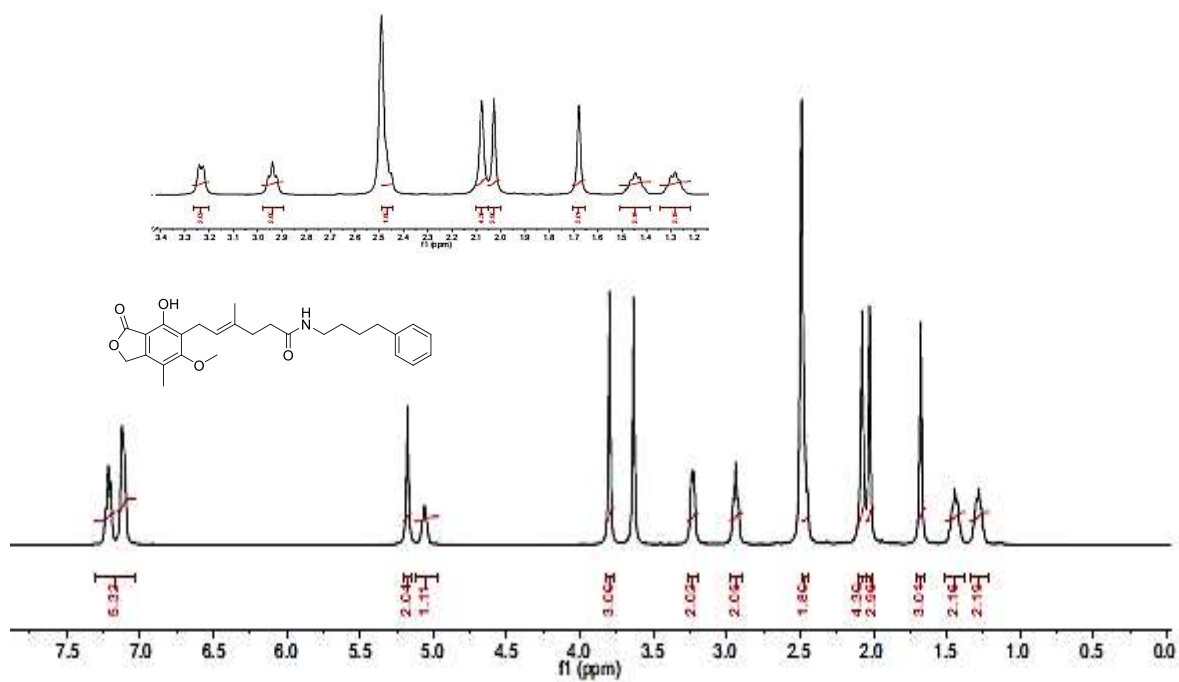


Figure 41S. ¹H-NMR spectrum (D₂O exchg.) of compound 24

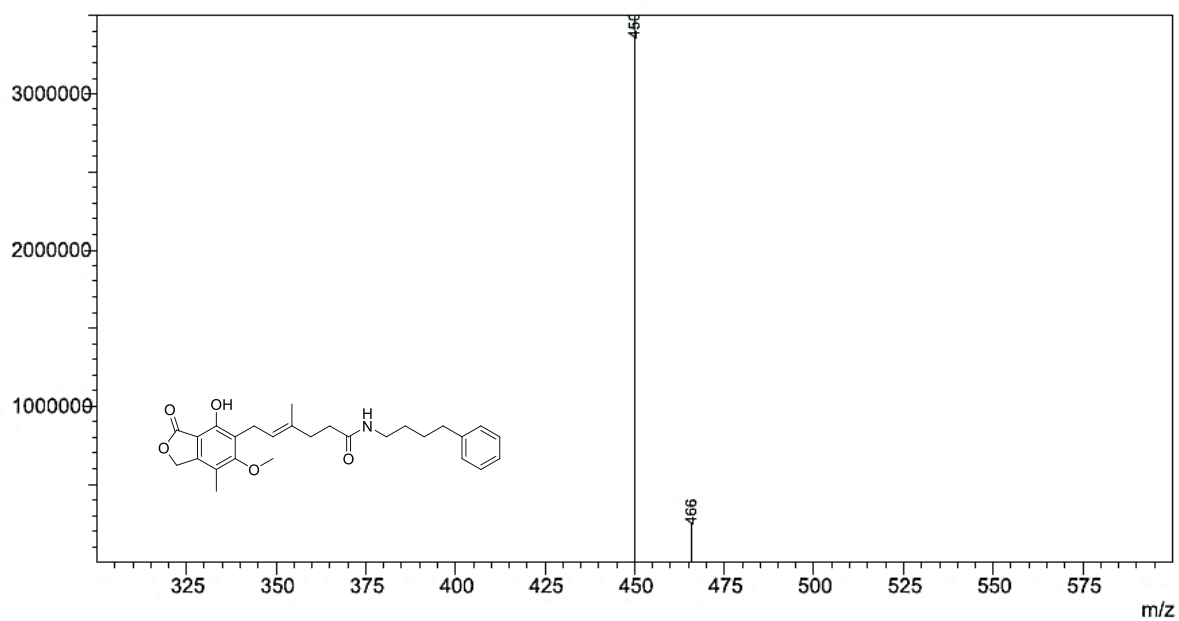


Figure 42S. Mass spectrum of compound 24

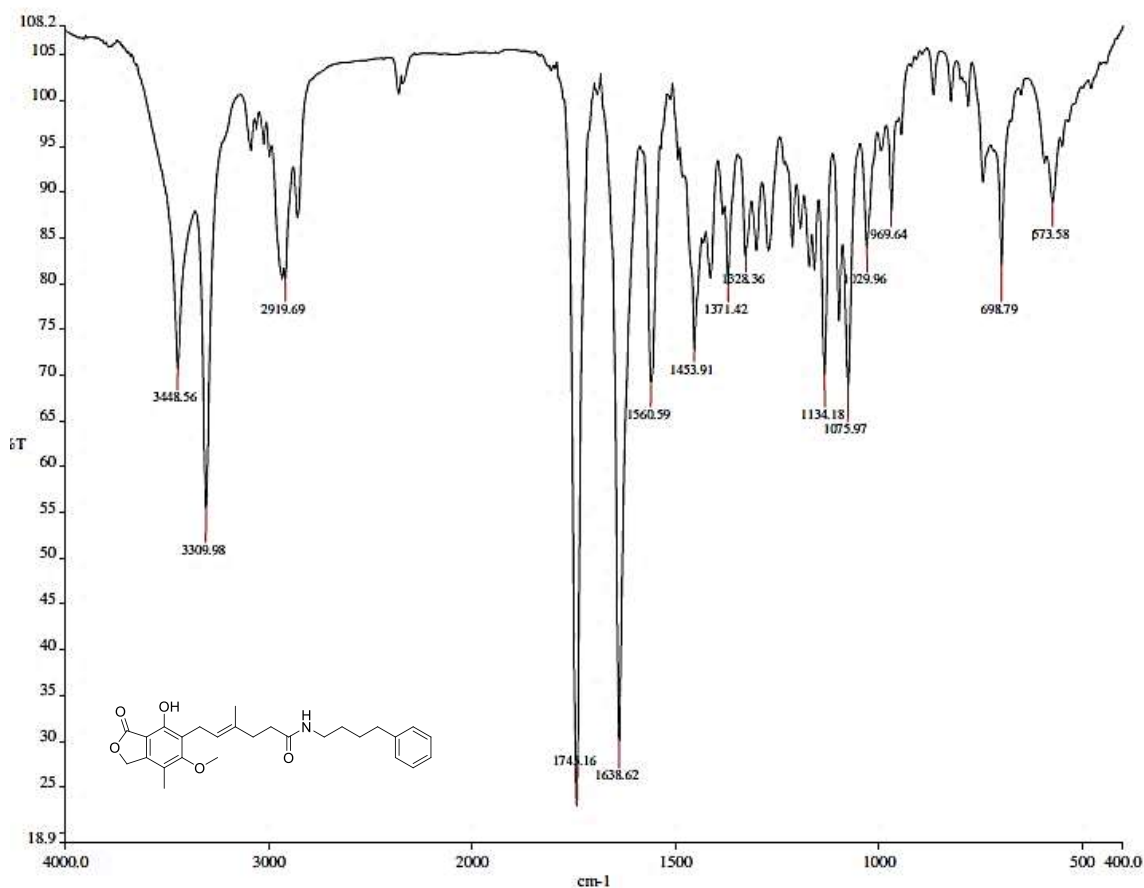
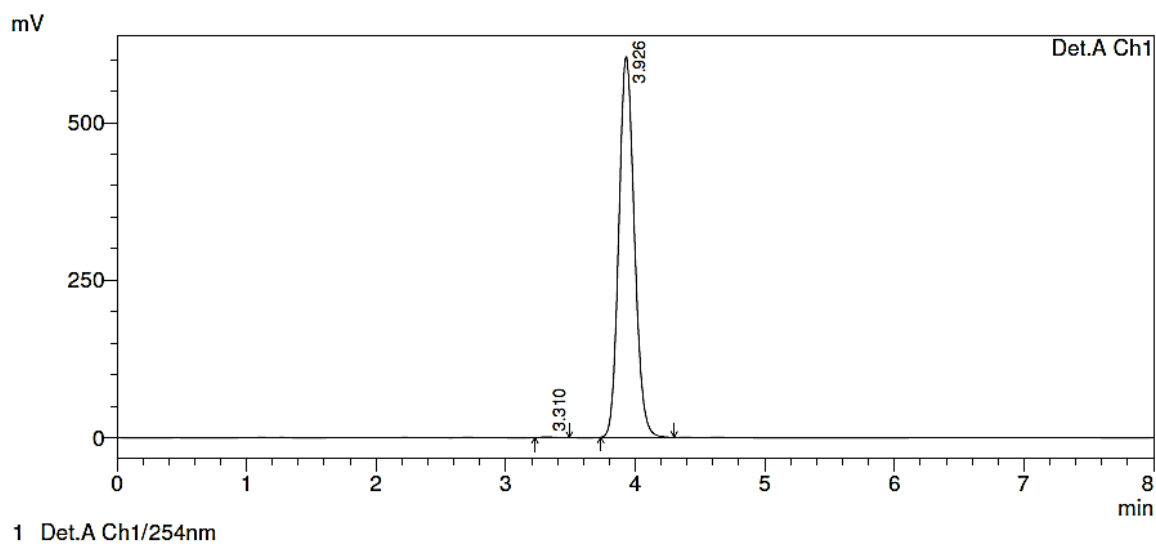


Figure 43S. FT-IR spectrum of compound **24**



1 Det.A Ch1/254nm

PeakTable

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.310	8229	1215	0.163	0.201
2	3.926	5033605	604121	99.837	99.799
Total		5041834	605336	100.000	100.000

Figure 44S. HPLC chromatogram of compound **24**

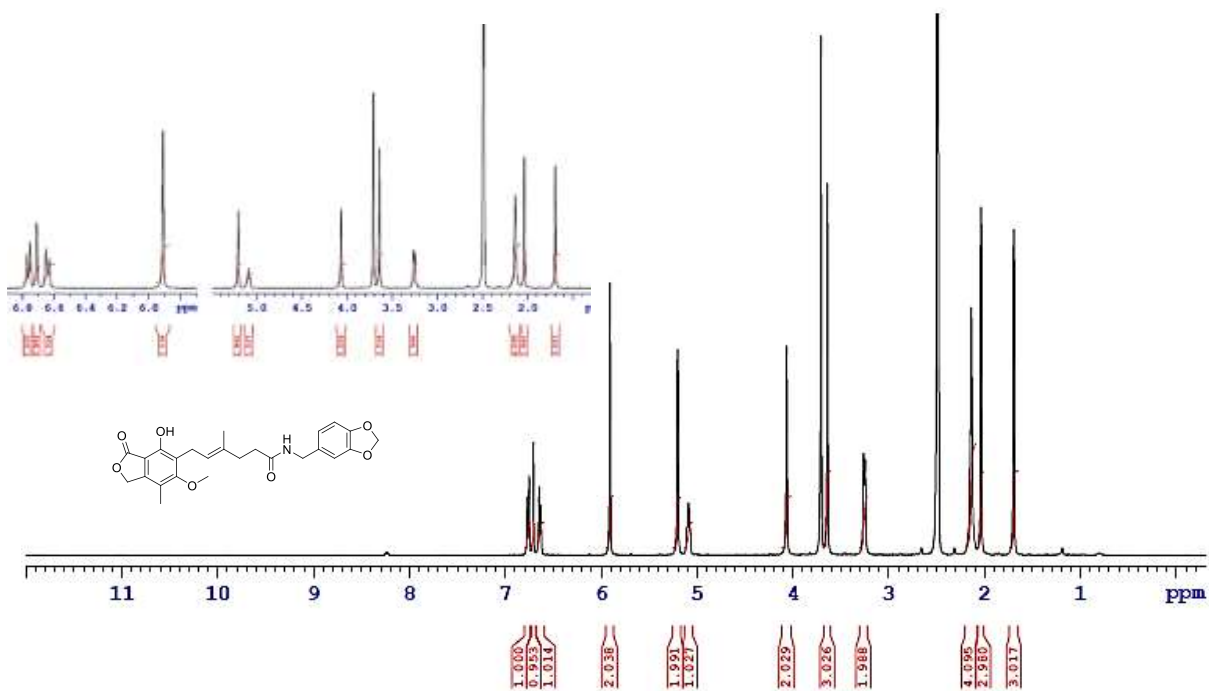


Figure 45S. $^1\text{H-NMR}$ spectrum (D_2O exchg.) of compound **25**

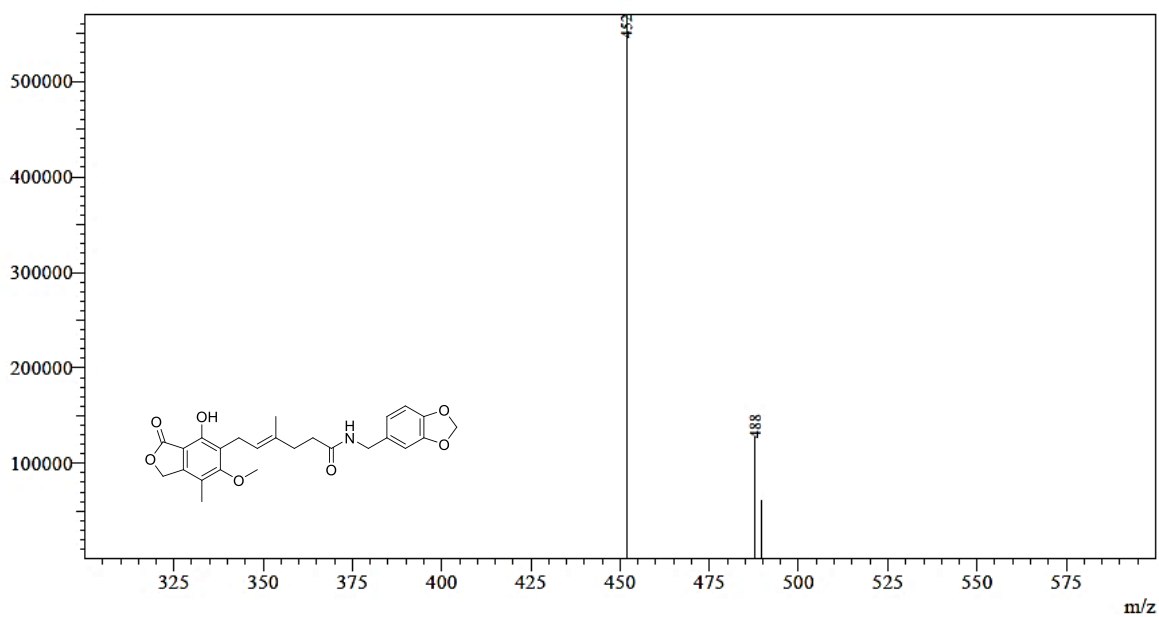


Figure 46S. Mass spectrum of compound **25**

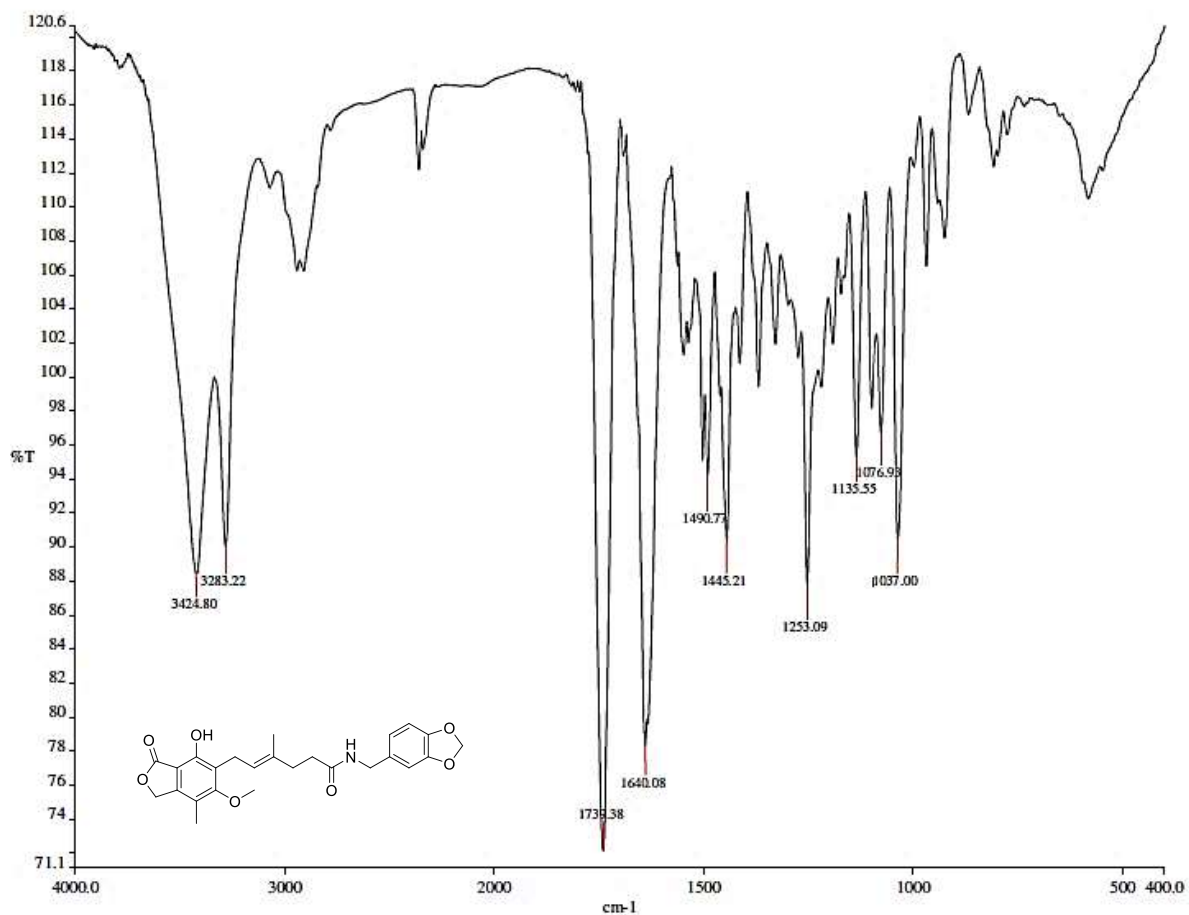
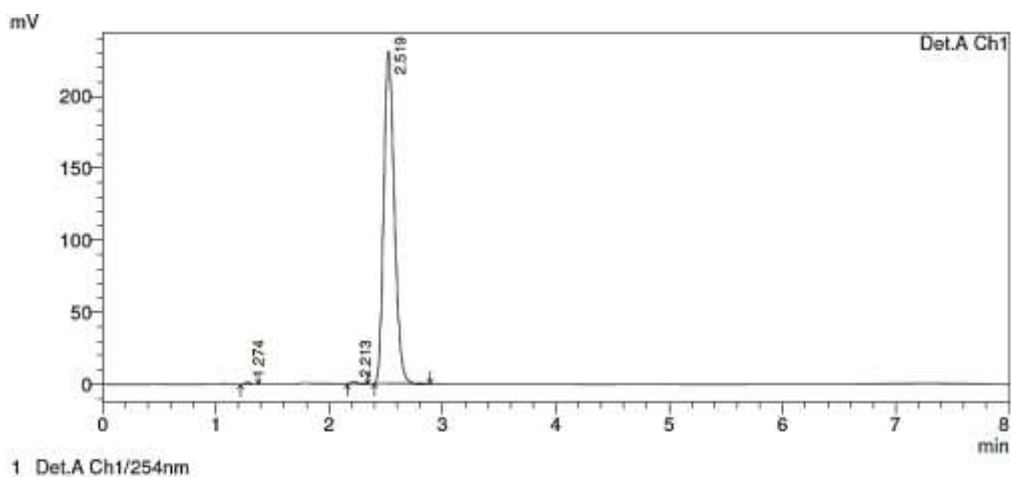


Figure 47S. FT-IR spectrum of compound 25



1 Det.A Ch1/254nm

PeakTable

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.274	4442	1435	0.295	0.615
2	2.213	5945	1165	0.394	0.499
3	2.519	1497425	230875	99.311	98.887
Total		1507812	233474	100.000	100.000

Figure 48S. HPLC chromatogram of compound 25

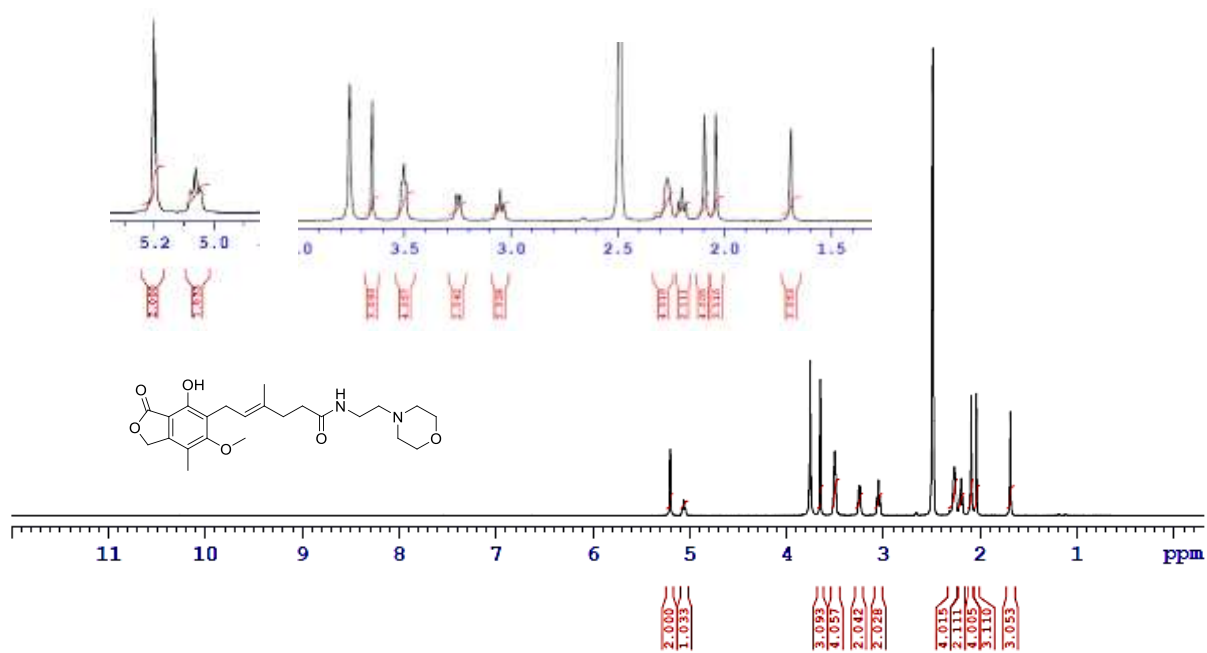


Figure 49S. ¹H-NMR spectrum (D₂O exchg.) of compound 26

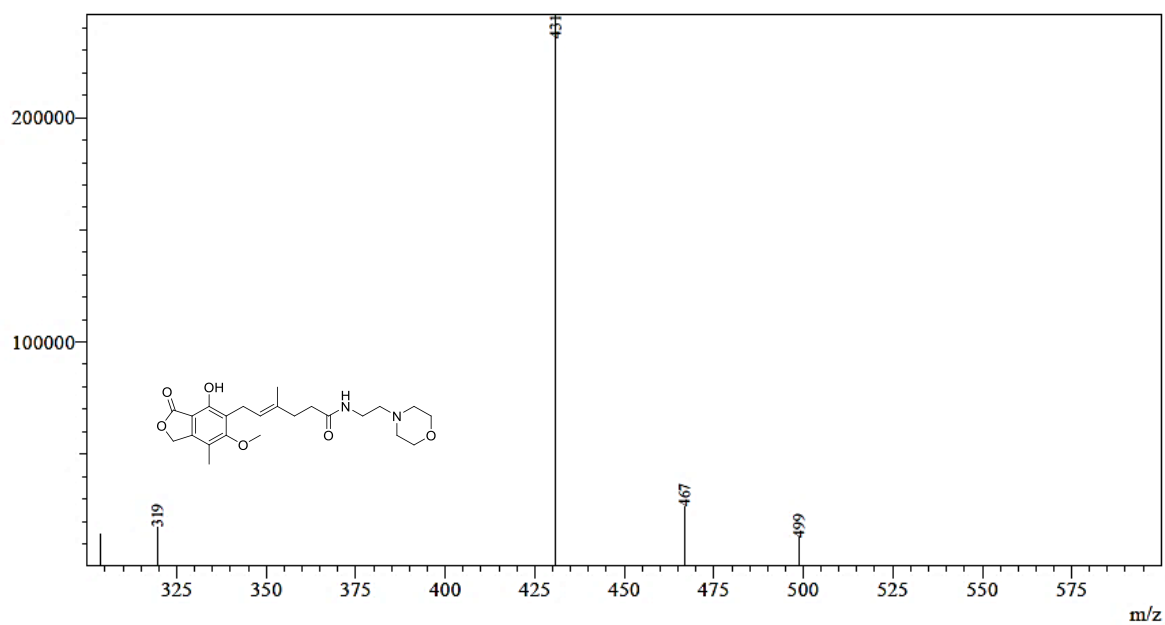


Figure 50S. Mass spectrum of compound 26

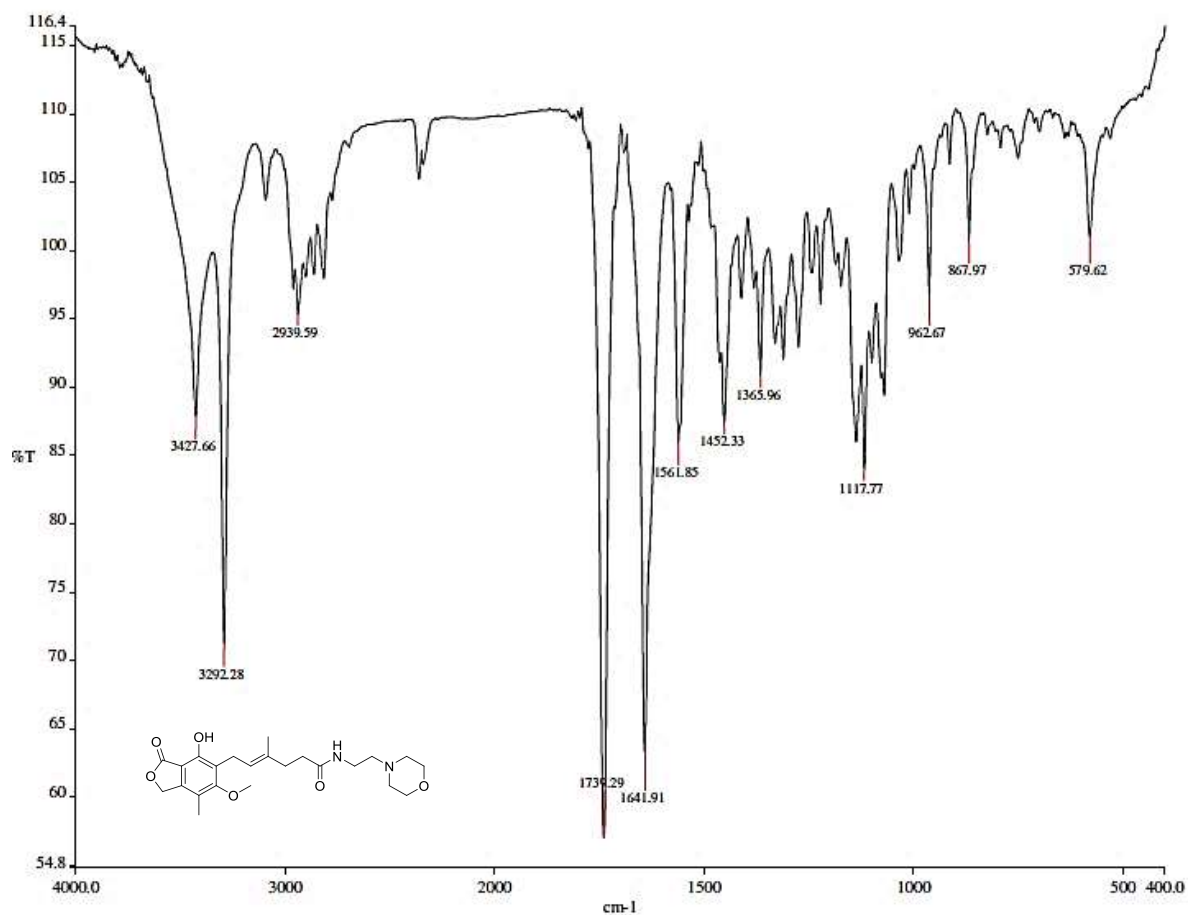
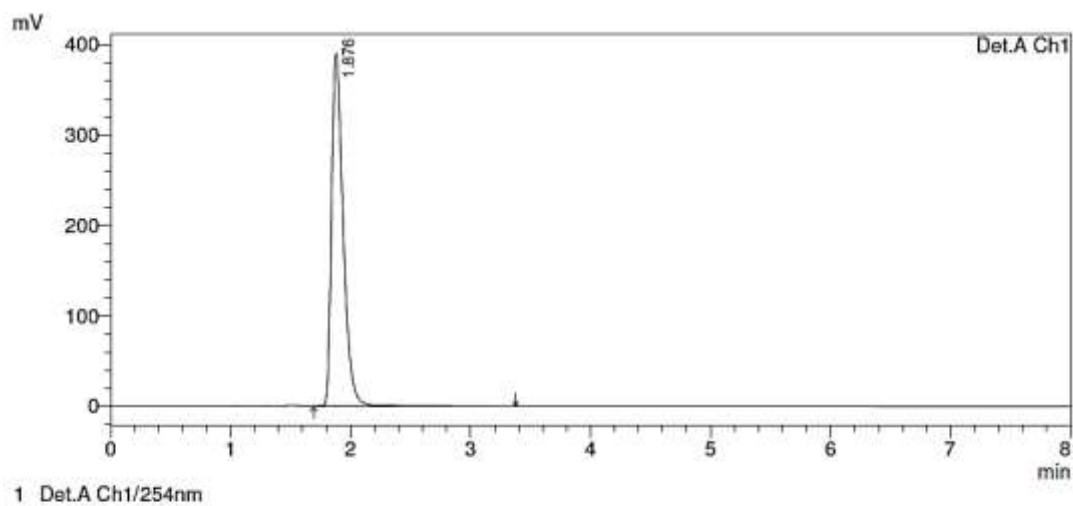


Figure 51S. FT-IR spectrum of compound 26



1 Det.A Ch1/254nm

PeakTable

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.876	2623697	390010	100.000	100.000
Total		2623697	390010	100.000	100.000

L,

A
C

Figure 52S. HPLC chromatogram of compound 26

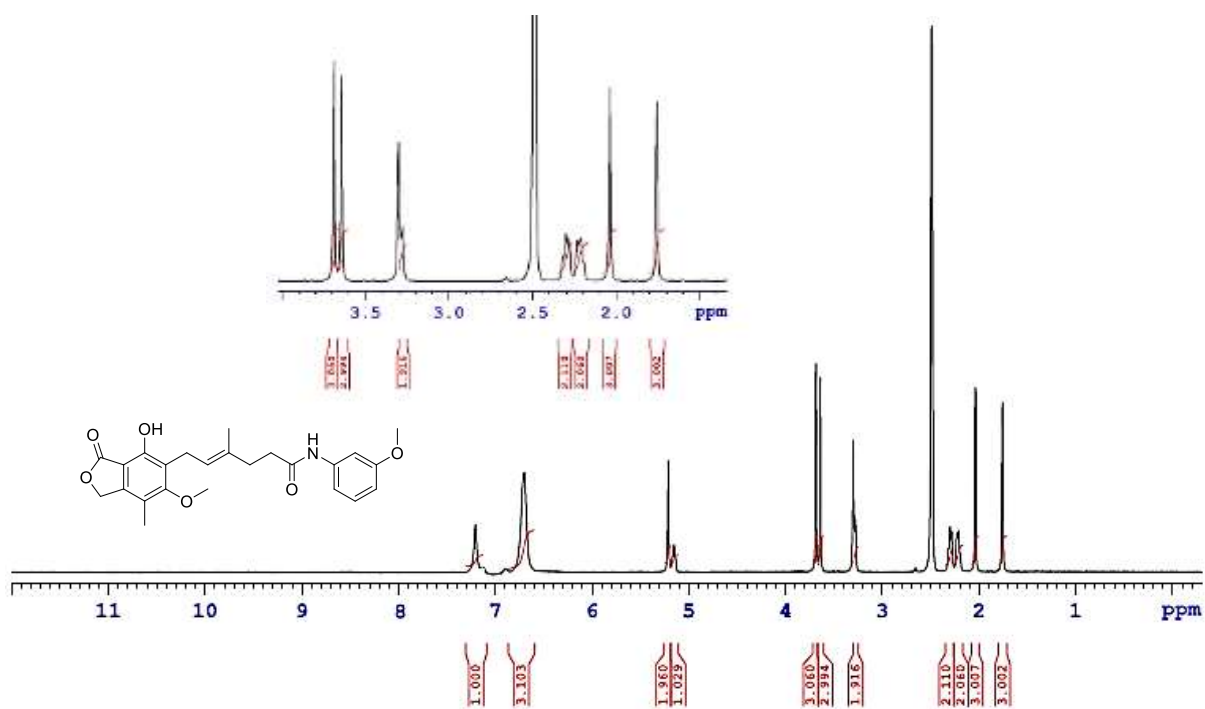


Figure 53S. ¹H-NMR spectrum (D₂O exchg.) of compound 27

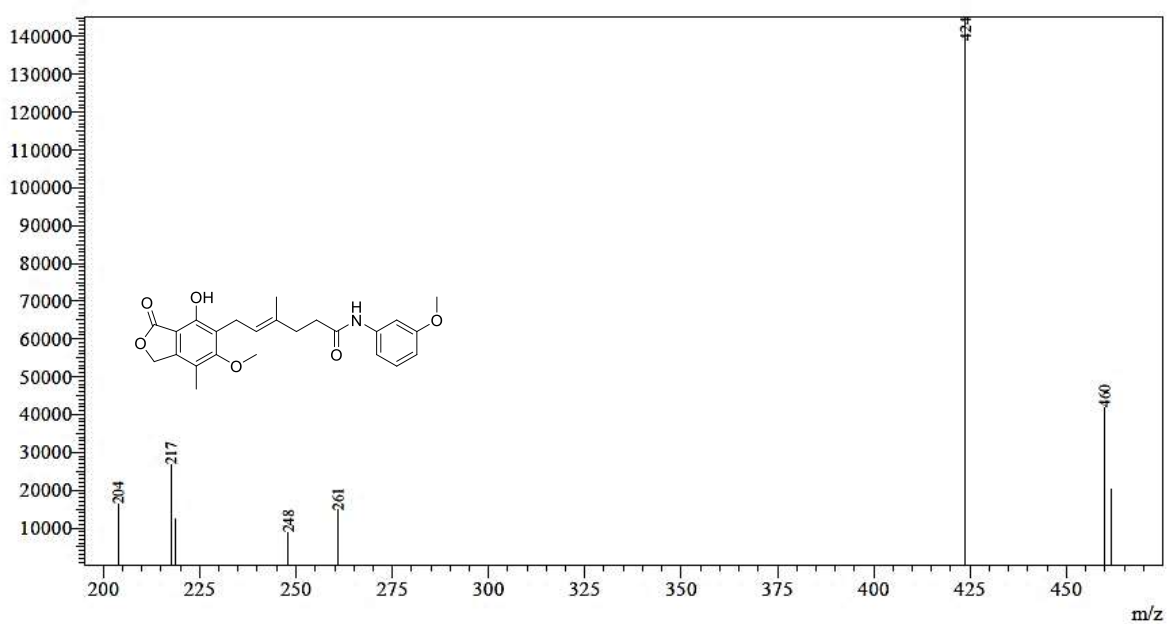


Figure 54S. Mass spectrum of compound 27

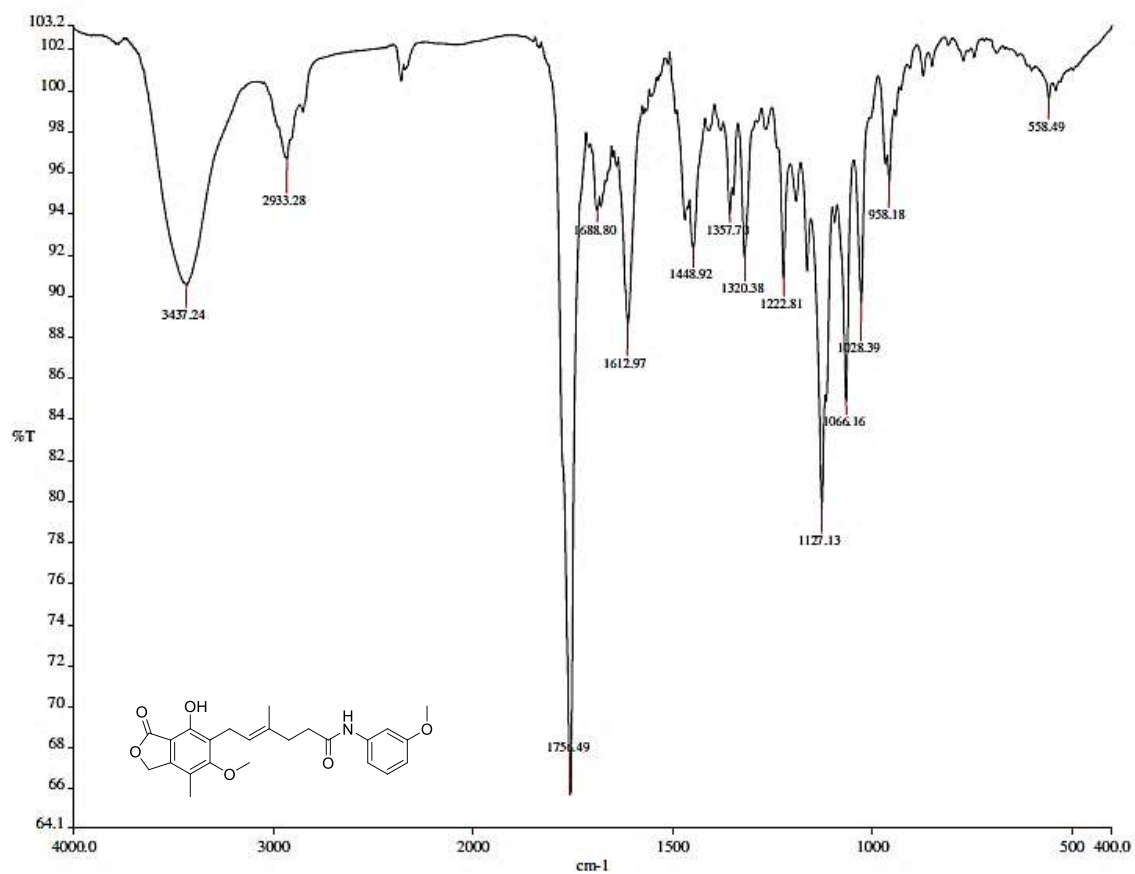
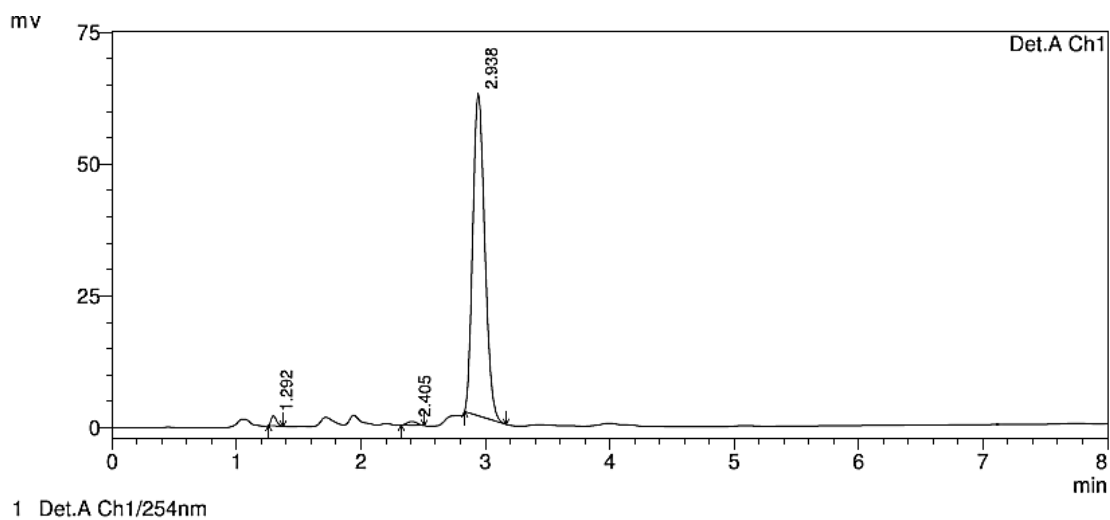


Figure 55S. FT-IR spectrum of compound 27



1 Det.A Ch1/254nm

PeakTable

Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.292	5572	1867	1.335	2.933
2	2.405	4287	724	1.027	1.137
3	2.938	407628	61064	97.638	95.930
Total		417488	63655	100.000	100.000

Figure 56S. HPLC chromatogram of compound 27

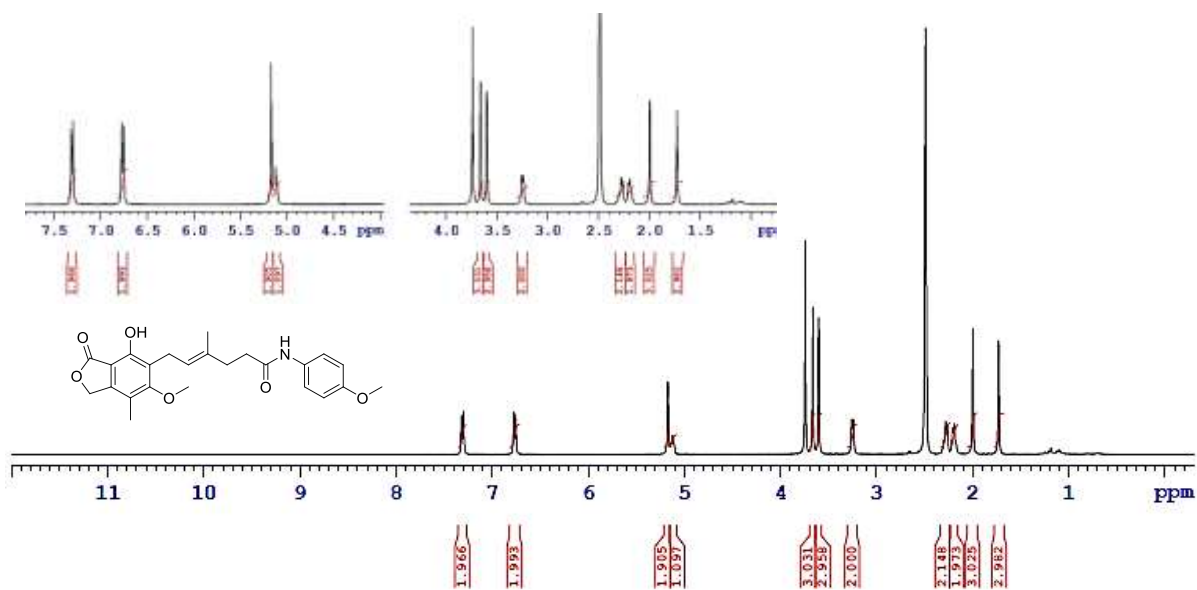


Figure 57S. ¹H-NMR spectrum (D₂O exchg.) of compound **28**

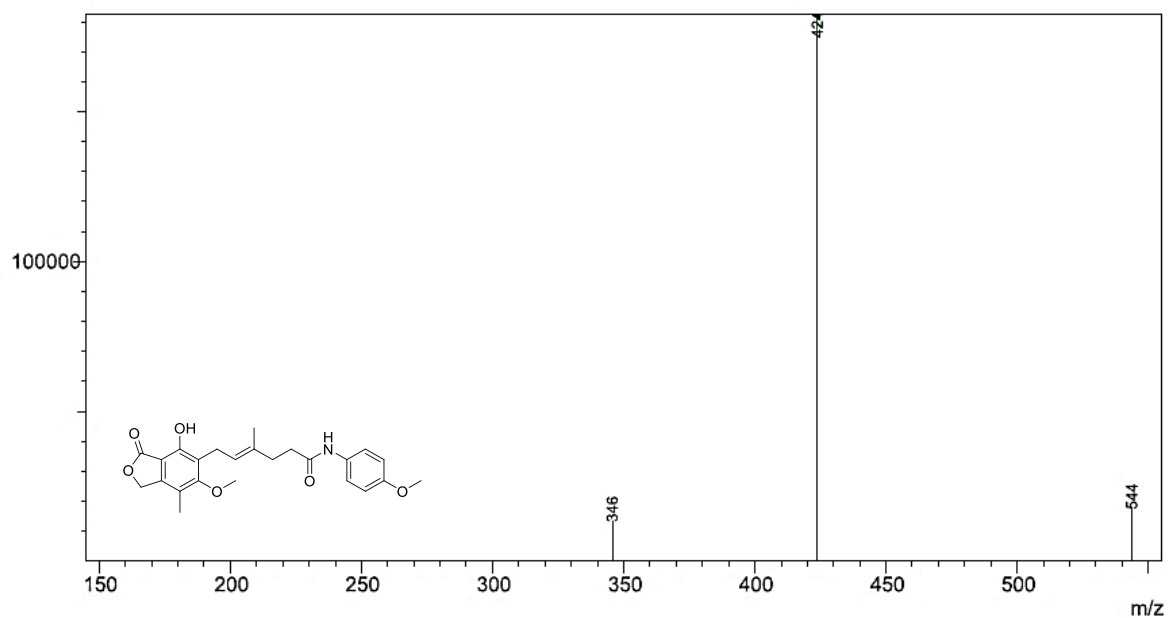


Figure 58S. Mass spectrum of compound **28**

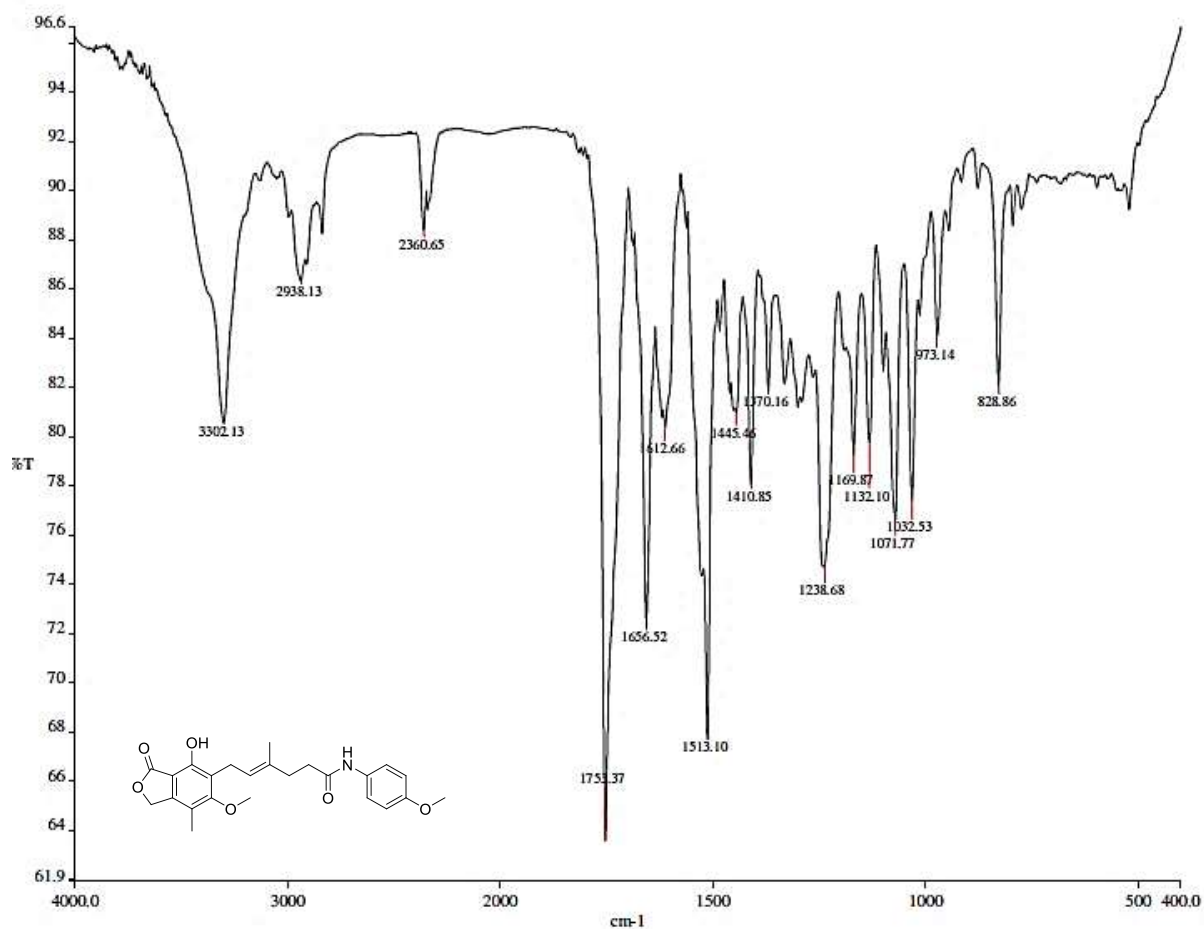
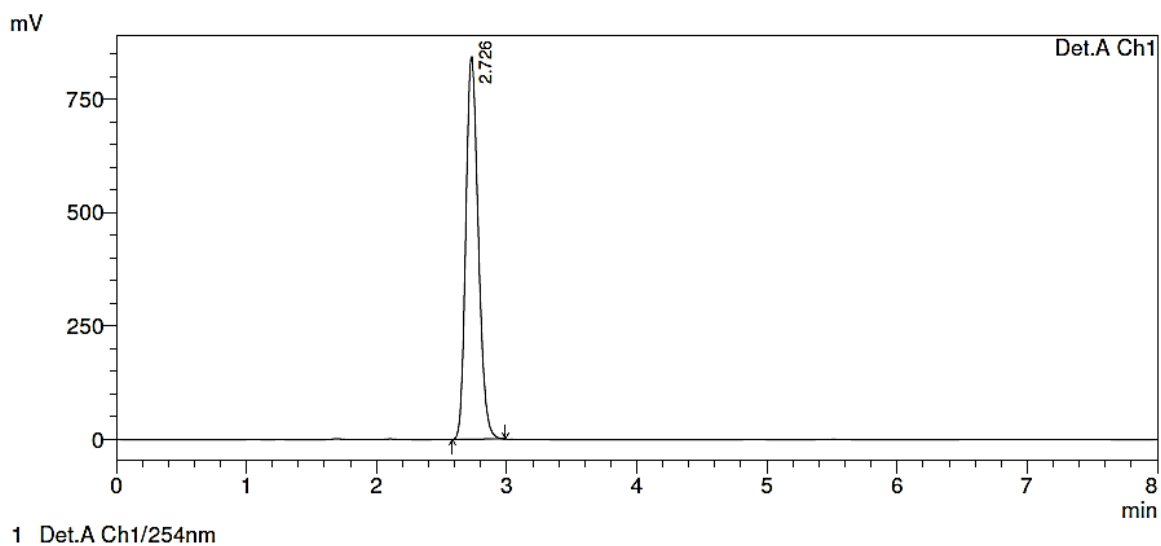


Figure 59S. FT-IR spectrum of compound 28



1 Det.A Ch1/254nm

PeakTable

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.726	5610588	842404	100.000	100.000
Total		5610588	842404	100.000	100.000

Ac

Figure 60S. HPLC chromatogram of compound 28