Supplementary information

Bioactive Secondary Metabolites from Fungal Endophytes, *Penicillium oxalicum* and *Phoma herbarum*, Associated with *Morus nigra* and *Ficus sycomorus*: Supported with *In Silico* Study

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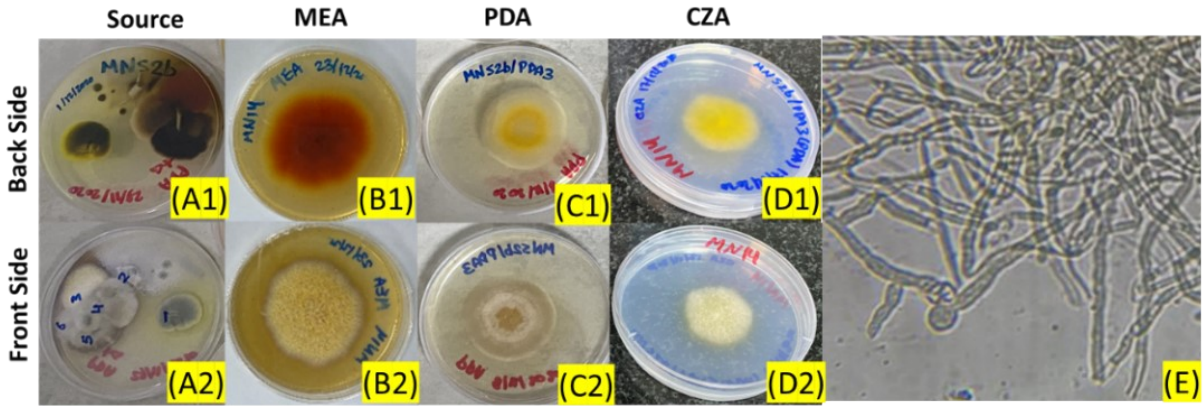


Fig. S1: Macro and micromorphological features of the endophytic fungi MN14 isolate associated with *Morus nigra*.

The isolated fungal strain MN14 macromorphological features as front and back side of petri dish: **(A1-A2):** stem part inoculated in petri dish containing nutrient agar with gentamycin as a source of isolated fungal strain; **(B1-B2):** isolated pure fungal strain on malt extract agar (MAE). **(C1-C2):** isolated pure fungal strain MN14 on potato dextrose agar (PAD); (D1-D2)= isolated fungal strain MN14 on Czapek-dox agar (CZA); **(E)**: Micromorphological features using microscopic examination of the isolated fungal strain MN14 showed *Phoma* sp. microscopic features.

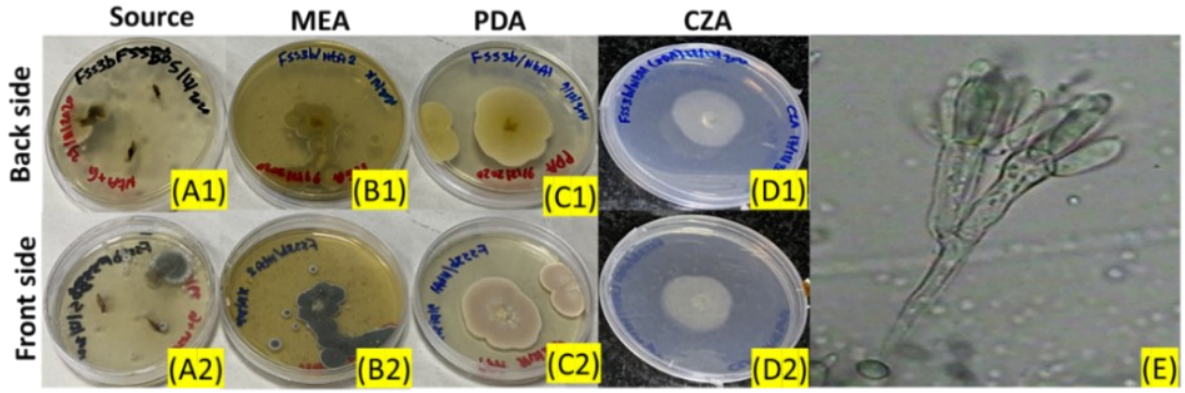


Fig. S2: Macro and micromorphological features of the endophytic fungi FS12 isolate associated with *Ficus sycomorus*.

The isolated fungal strain FS12 macromorphological features as front and back side of petri dish: **(A1-A2)**: stem part inoculated in petri dish containing nutrient agar with gentamycin as a source of isolated fungal strain; **(B1-B2):** isolated pure fungal strain on malt extract agar (MAE). **(C1-C2)**: isolated pure fungal strain FS12 on potato dextrose agar (PAD); **(D1-D2):** isolated fungal strain FS12 on Czapek-dox agar (CZA); **(E):** Micromorphological features using microscopic examination of the isolated fungal strain FS12 showed *Penicillium* sp. hyphae and spores in *Penicillium* type conidia.

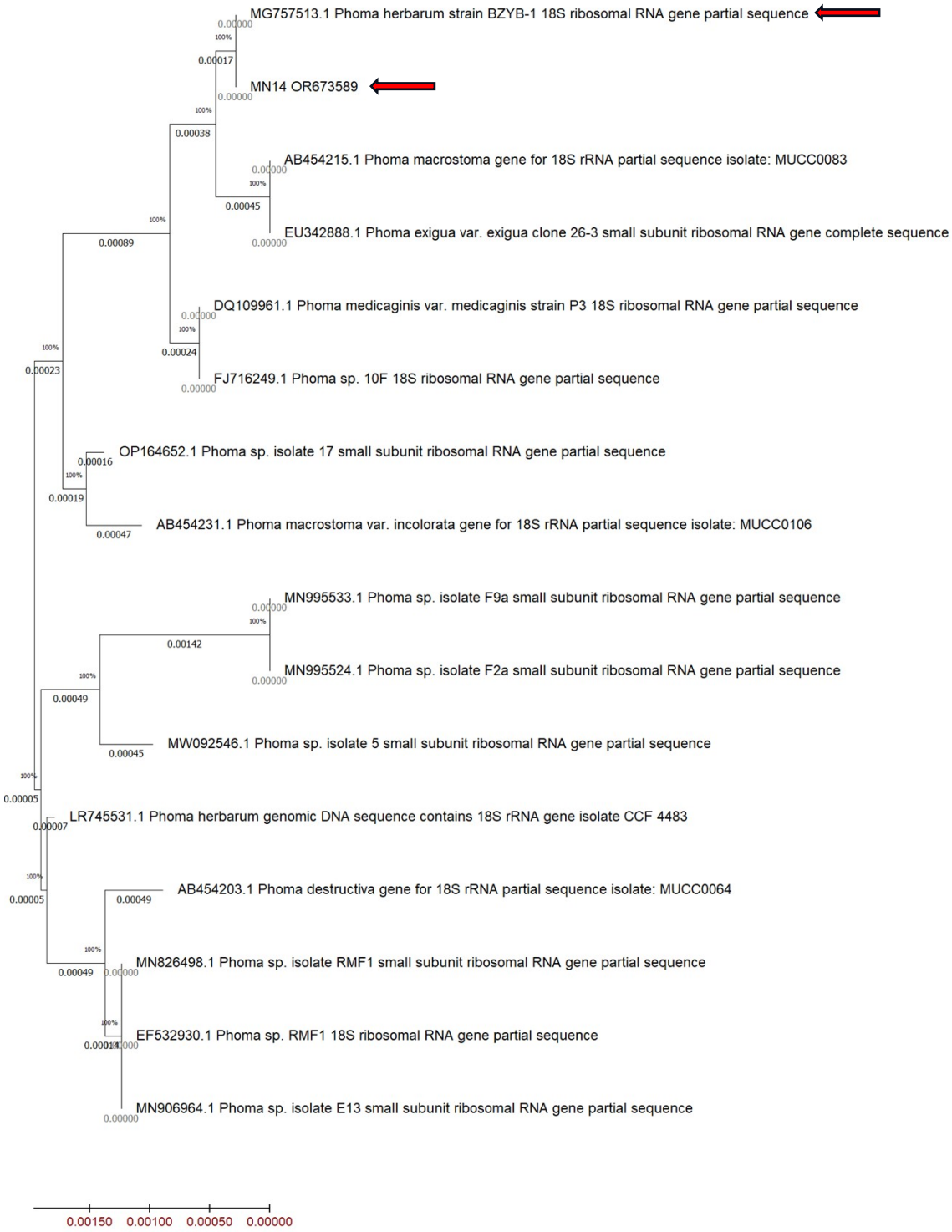


Fig. S3: Evolutionary relationships of taxa phylogenetic tree of *P. herbarum* MN14 associated with *Morus nigra*

Fig. S4: Evolutionary relationships of taxa phylogenetic tree of *P. oxalicum* FS12 associated with *Ficus sycomorus*

Table S1: DPPH and FRAP assays results for *P. herbarum* and *P. oxalicum* total extracts and their fractions.

Sample	DPPH IC_{50} (µg/ml)	FRAP (µM Trolox equivalent /ml)
PHT	**109.5 ± 6.44**	**438.611 ± 1.434**
PH1	>500	126.296 ± 19.950
PH2	**5.649 ± 0.47**	**2926.48 ± 142.654**
PH3	**38.95 ± 1.79**	**1275.93 ± 17.599**
POT	307.2 ± 20.90	154.861 ± 1.145
PO1	>500	169.259 ± 42.375
PO2	**198 ± 7.76**	**633.241 ± 65.5**
PO3	486.6 ± 30.44	195.648 ± 40.307
Trolox	7.217 ± 0.309	-

DPPH: 2,2-Diphenyl-1-picrylhydrazyl**, FRAP:** Ferrous reducing activity power, **PHT**: *Phoma herbarum* total extract, **PH1**: *P. herbarum n*-hexane fraction, **PH2**: *P. herbarum* chloroform fraction, **PH3**: *P. herbarum* ethyl acetate fraction, **POT**: *Penicillium* oxalicum total extract, **PO1**: *P. oxalicum n*-hexane fraction, **PO2**: *P. oxalicum* chloroform fraction, **PO3**: *P. oxalicum* ethyl acetate fraction. **Trolox** was used as a positive control.

Table S2: IC_{50} values of SRB viability assay of *P. herbarum* and *P. oxalicum* total extracts and their fractions.

sample	HepG2 (IC_{50} µg/ml)
PHT	528.9
PH1	198.53
PH2	**77.8**
PH3	188.29
POT	340.4
PO1	343.4
PO2	**7.695**
PO3	499.8
Dox.	0.87

HepG2: Hepatocellular carcinoma cell line, **Dox**.: Doxorubicin as positive control drug, **PHT**: *Phoma herbarum* total extract, **PH1**: *P. herbarum n*-hexane fraction, **PH2**: *P. herbarum* chloroform fraction, **PH3**: *P. herbarum* ethyl acetate fraction, **POT**: *Penicillium* oxalicum total extract, **PO1**: *P. oxalicum n*-hexane fraction, **PO2**: *P. oxalicum* chloroform fraction, **PO3**: *P. oxalicum* ethyl acetate fraction.

Table S3: Nitric oxide inhibition for the anti-inflammatory activity of *P. herbarum* and *P. oxalicum* total extracts and their fractions.

sample	IC_{50} µg/ml
PHT	>1000
PH1	**17.82**
PH2	**26.51**
PH3	76.09
POT	390.2
PO1	65.99
PO2	**14.68**
PO3	317.0
L-NAME	7.316

PHT: *Phoma herbarum* total extract, **PH1**: *P. herbarum n*-hexane fraction, **PH2**: *P. herbarum* chloroform fraction, **PH3**: *P. herbarum* ethyl acetate fraction, **POT**: *Penicillium* oxalicum total extract, **PO1**: *P. oxalicum n*-hexane fraction, **PO2**: *P. oxalicum* chloroform fraction, **PO3**: *P. oxalicum* ethyl acetate fraction, Nitro-L-arginine methyl ester hydrochloride (L-NAME) positive control.

Table S4: Antimicrobial and antibiofilm screening of *P. herbarum* and *P. oxalicum* total extracts and their fractions.

Sample	Inhibition zone in mm			MIC mg/ml			MBIC
	PA	***SA***	***CA***	***PA***	***SA***	***CA***	***PA***
PHT	**25**	**23**	**24**	**3**	**3**	**2**	10
PH1	12	15	15	8	**3**	**3**	**1.25**
PH2	**35**	**31**	**32**	**4**	**3**	**2**	**5**
PH3	**22**	**26**	**23**	**3**	**3**	**2**	10
POT	14	**18**	14	6	**3**	4	20
PO1	10	**17**	11	12	**3**	4	**2.5**
PO2	10	**19**	10	12	**4**	**3**	20
PO3	16	**29**	11	12	6	4	**5**
-ve control	10	10	10	16	6	4	20
Gentamycin	34	40	36	0.0125	0.00625	0.003125	-

MIC: Minimum Inhibitory Concentration, **MBIC**: Minimum Biofilm Inhibitory Concentration**, *SA*:** *Staphylococcus aureus* ATCC-6538**, *PA*:** *Pseudomonas aeruginosa* ATCC-9027, ***CA*:** *Candida albicans* ATCC-10231, **PHT**: *Phoma herbarum* total extract, **PH1**: *P. herbarum n*-hexane fraction, **PH2**: *P. herbarum* chloroform fraction, **PH3**: *P. herbarum* ethyl acetate fraction, **POT**: *Penicillium* oxalicum total extract, **PO1**: *P. oxalicum n*-hexane fraction, **PO2**: *P. oxalicum* chloroform fraction, **PO3**: *P. oxalicum* ethyl acetate fraction, Gentamycin was used as a positive control. The zone diameter for the agar well diffusion method is 10 mm.

Table S5: In vitro α-glucosidase inhibitory activity of *P. herbarum* and *P. oxalicum* total extracts and their fractions.

Sample	IC50 (µg/ml)	Inhibition 1000 (µg/ml)
PHT	**79.17**	**95.43 ± 1.47**
PH1	>1000	0.40 ± 0.26
PH2	**14.91**	**100 ± 0.06**
PH3	**148.5**	**95.43 ± 1.47**
PO	>1000	36.51 ± 0.99
PO1	>1000	27.07 ± 1.09
PO2	>1000	**46.16 ± 1.32**
PO3	>1000	37.15 ± 1.55
Acarbose	224.0	77.16 ± 2.98

PHT: *Phoma herbarum* total extract, **PH1**: *P. herbarum n*-hexane fraction, **PH2**: *P. herbarum* chloroform fraction, **PH3**: *P. herbarum* ethyl acetate fraction, **POT**: *Penicillium* oxalicum total extract, **PO1**: *P. oxalicum n*-hexane fraction, **PO2**: *P. oxalicum* chloroform fraction, **PO3**: *P. oxalicum* ethyl acetate fraction., Acarbose reference as a positive control.

Table S6: Identified compounds of *P. herbarum* chloroform fraction (PH2) using LC-MS.

SN	Compound Name	Rt	M+H (*m/z*)	MF	Exact Mass	Reported Source	Class	Ref.
1	Mevalonolactone **(1)**	1.05	131.0782	$C_6H_{10}O_3$	130.06	*Pestalotiopsis sp. & Penicillium solitum*	Lactone	[1,2]
2	Phomasparapyrone A	2.11	213.1650	$C_{10}H_{12}O_5$	212.06	*Phomopsis asparagi*	pyrone derivative	[3]
3	8-hydroxy-Pregaliellalactone B	3.13	197.1197	$C_{11}H_{16}O_3$	196.11	*Phoma* sp.	polyketide	[4]
4	Phomactin B	6.6	321.1858	$C_{19}H_{28}O_4$	320.2	*Phoma* sp.	Diterpenes	[5–7]
5	Tersone F	7.2	344.1830	$C_{19}H_{21}NO_5$	343.14	*Phomopsis* tersa	pyridine alkaloid	[8]
6	Phomaether A	8.2	423.2958	$C_{21}H_{26}O_9$	422.16	*Phoma* sp.	diphenyl ether	[9]
7	Barcelonyl acetate **(2)**	8.4	335.0775	$C_{17}H_{18}O_7$	334.33	*New*	diphenyl	-
8	Barceloneic acid C	8.5	303.1201	$C_{16}H_{14}O_6$	302.08	*Phoma* sp.	polyketide	[10]
9	Terezine N	9.2	291.1367	$C_{15}H_{18}N_2O_4$	290.12	*P. herbarum*	terezine derivative	[11]
10	Terezine L	10.5	451.3011	$C_{21}H_{26}N_2O_9$	450.16	*P. herbarum*	Pyrazine derivative	[12]
11	Alterporriol S	14.10	613.3747	$C_{31}H_{32}O_{13}$	612.18	*Alternaria* sp.	anthraquinone derivatives	[13]
12	Phomalide	15.4	558.4356	$C_{30}H_{43}N_3O_7$	557.31	*Phoma* sp.	Cyclic polypeptide derivatives	[14]
13	Glycerol monolinoleate **(3)**	22.78	355.3488	$C_{21}H_{38}O_4$	354.28	Saposhnikovia divaricate plant root	glycerides	[15]
14	Ergosterol **(7)**	27.4	397.4515	$C_{28}H_{44}O$	396.34	*P. oxalicum*	Sterol	[16]

Rt: Retention time, **MF**: Molecular Formula

Table S7: Identified compounds of *P. oxalicum* chloroform fraction (PO2) using LC-MS.

SN	Compound Name	Rt	M+H (*m/z*)	MF	Exact Mass	Reported Source	Class	Ref.
1	Mevalonolactone **(1)**	1.0	131.0062	$C_6H_{10}O_3$	130.06	*Pestalotiopsis sp., P. solitum*	Pyrone derivative	[1,2]
2	4-Hydroxyphenyl acetic acid **(4)**	1.0	153.0181	$C_8H_8O_3$	152.05	*Gaeumannomyces sp.*	Phenolic acid	[17]
3	1,3-Dihydroxypropan-2-yl 2,4-dihydroxy-6-methylbenzoate	4.5	243.1353	$C_{11}H_{14}O_6$	242.08	*P. chrysogenum*	Phenolic acid	[18]
4	2-(4-Hydroxybenzyl) quinazolin-4(3H)-one	6.5	253.1020	$C_{15}H_{12}N_2O_2$	252.09	*P. oxalicum*	Alkaloid	[19]
5	Penipanoid A	6.6	296.1747	$C_{16}H_{13}N_3O_3$	295.1	*P. oxalicum*	Alkaloid	[20]
6	2,5-dimethyl-7-Hydroxychromone	7.0	191.0760	$C_{11}H_{10}O_3$	190.06	*P. oxalicum*	Chromanone derivatives	[21]
7	Coniochaetone J	7.5	263.0691	$C_{14}H_{14}O_5$	262.08	*P. oxalicum*	Chromone derivatives	[22]
8	Meleagrin	7.9	434.2026	$C_{23}H_{23}N_5O_4$	433.17	*P. oxalicum*	Alkaloid	[23]
9	Meleagrin A; Me ether	8.4	448.2773	$C_{24}H_{25}N_5O_4$	447.19	*P. oxalicum*	Alkaloid	[20]
10	Penioxamide A	10.3	418.3284	$C_{27}H_{35}N_3O$	417.28	*P. oxalicum*	Alkaloid	[20]
11	Altersolanol A **(6)**	12.9	337.3809	$C_{16}H_{16}O_8$	336.29	*Stemphylium globuliferum*	Anthraquinone derivative	[24,25]
12	Secalonic acid D **(5)**	13.3	639.2608	$C_{32}H_{30}O_{14}$	638.16	*P. oxalicum*	Xanthone derivative	[26,27]
13	Ergosterol **(7)**	27.4	397.4515	$C_{28}H_{44}O$	396.34	*P. oxalicum*	Sterol	[16]

Rt: Retention time, **MF**: Molecular Formula

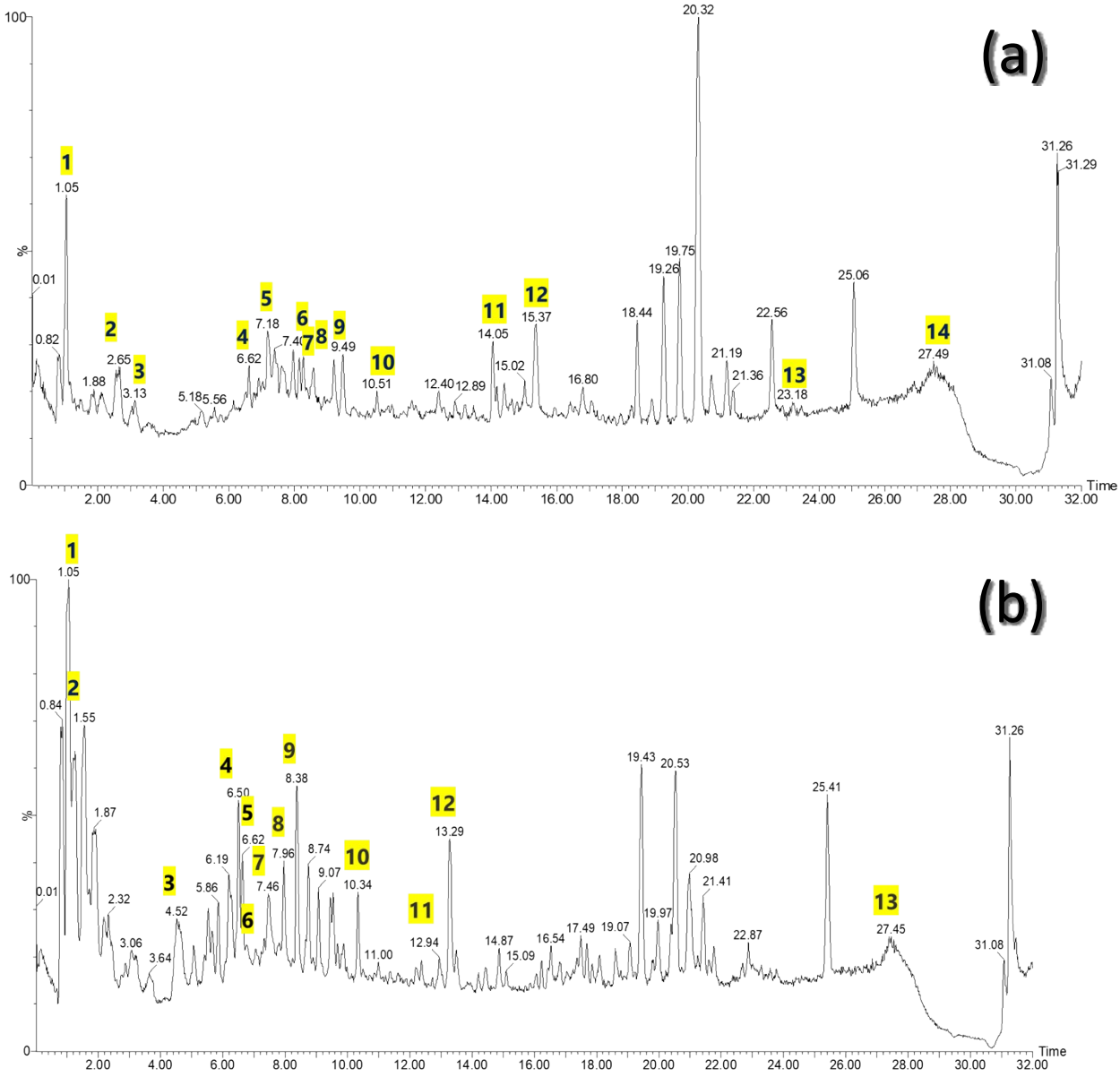


Fig. S5: LC/MS Positive-ESI spectra of *P. herbarum* chloroform fraction (a) and *P. oxalicum* chloroform fraction (b)

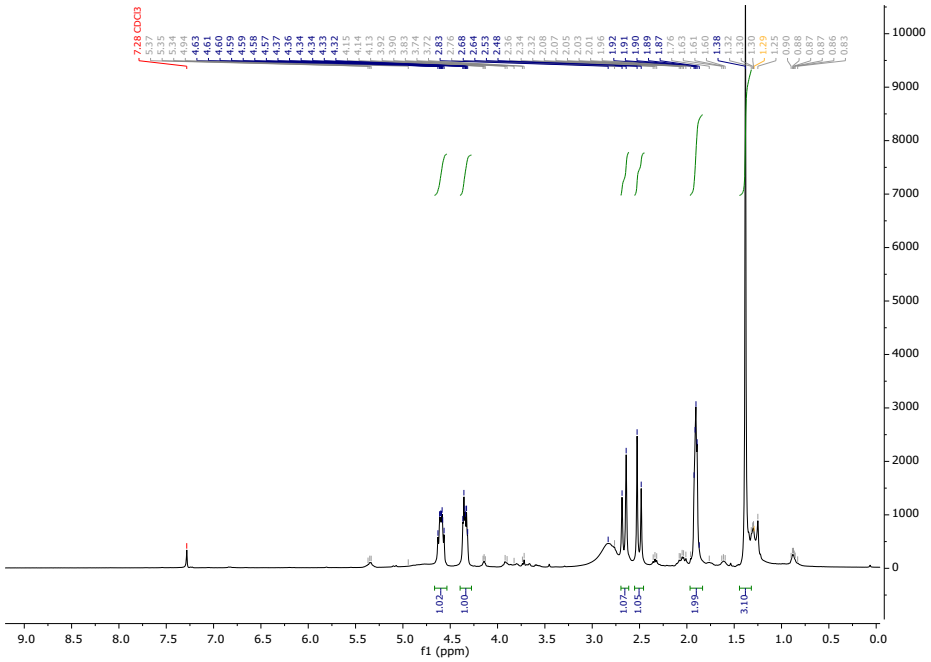


Fig. S6: ^{1}H-NMR spectrum of mevalonolactone (1)

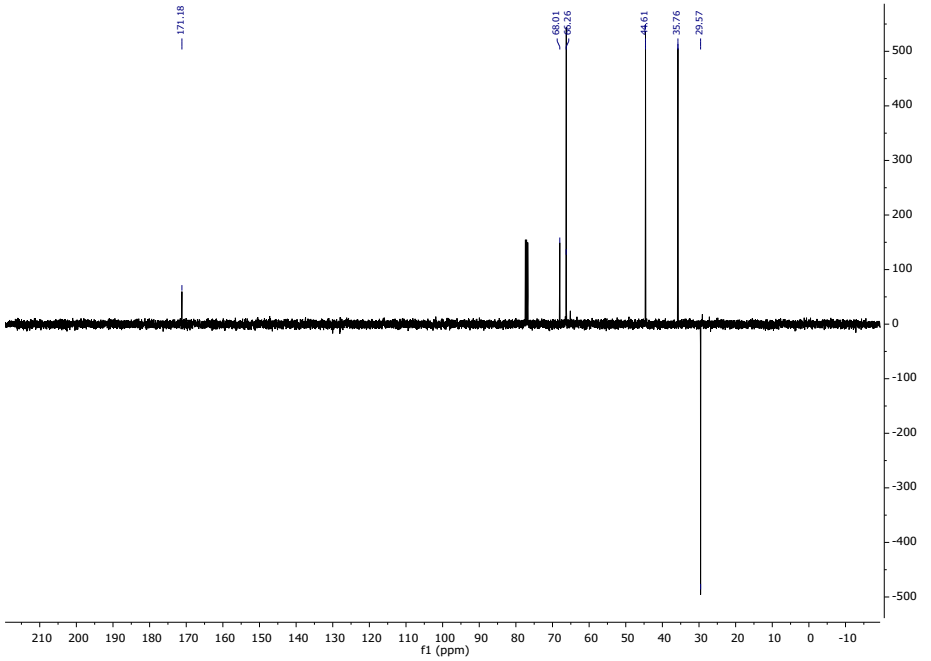


Fig. S7: APT NMR spectrum of mevalonolactone (1)

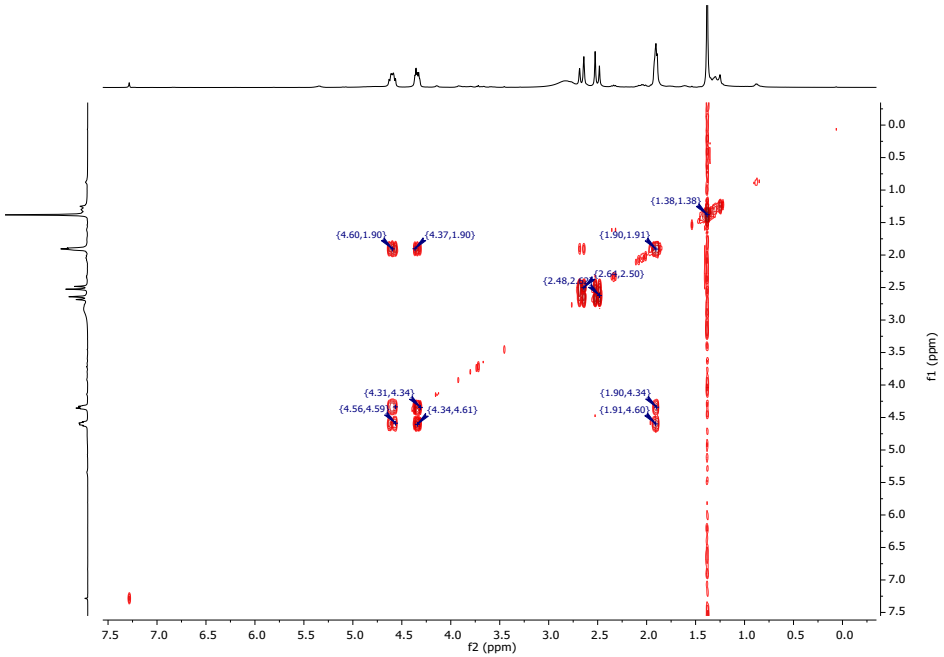


Fig. S8: H-H COSY spectrum of mevalonolactone (1)

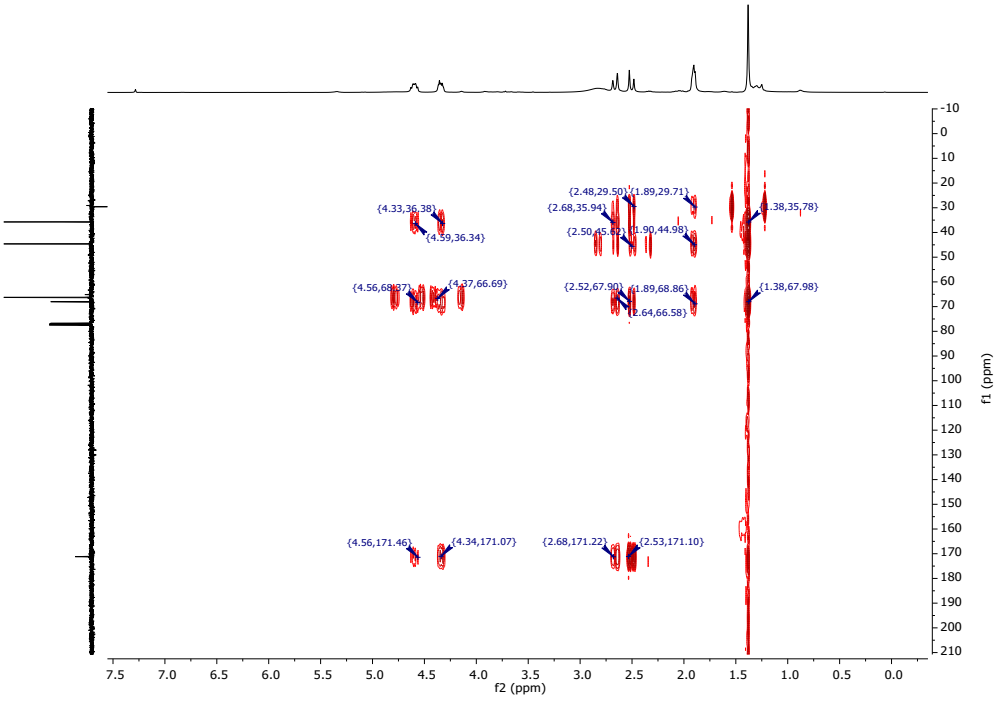


Fig. S9: HMBC spectrum of mevalonolactone (1)

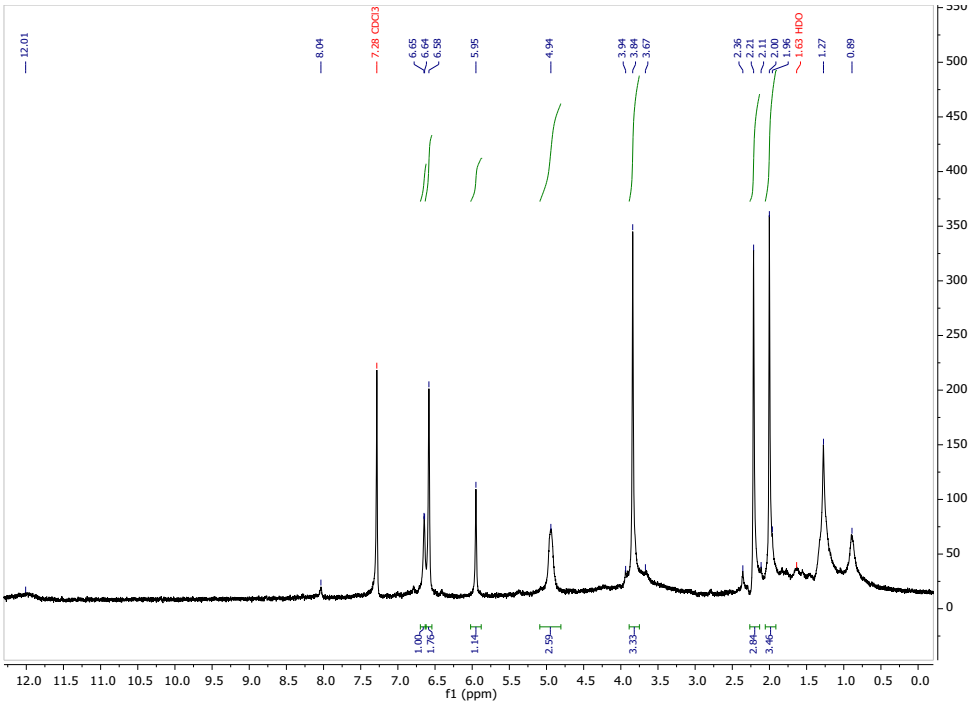


Fig. S10: ^{1}H-NMR spectrum of barcelonyl acetate (2)

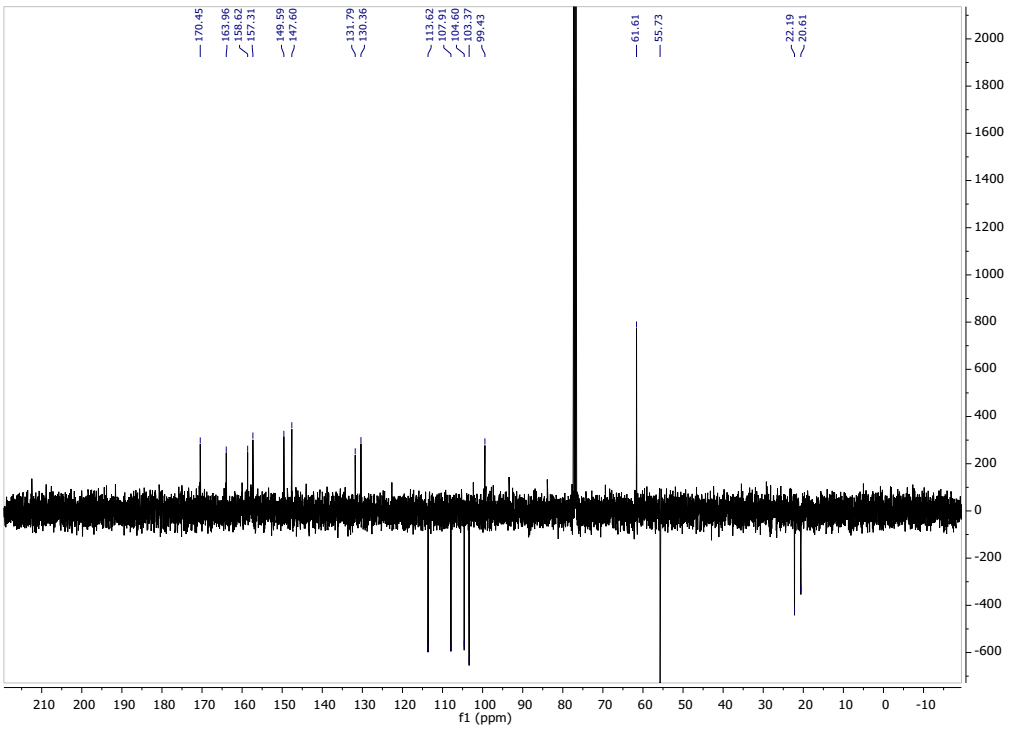


Fig. S11: APT spectrum of barcelonyl acetate (2)

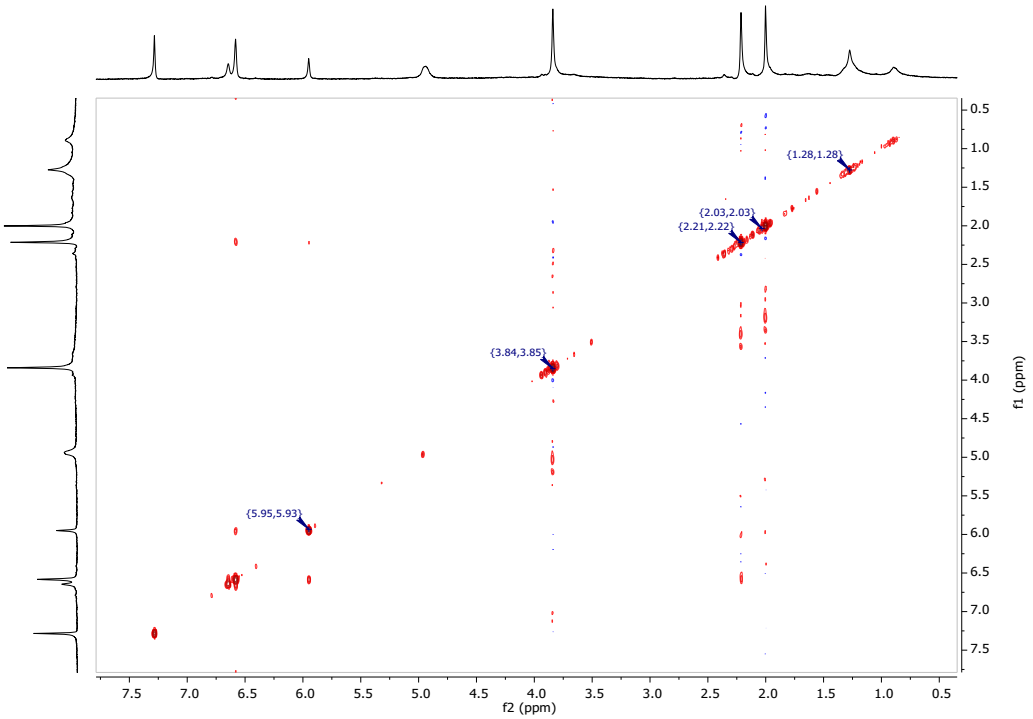


Fig. S12: COSY spectrum of barcelonyl acetate (2)

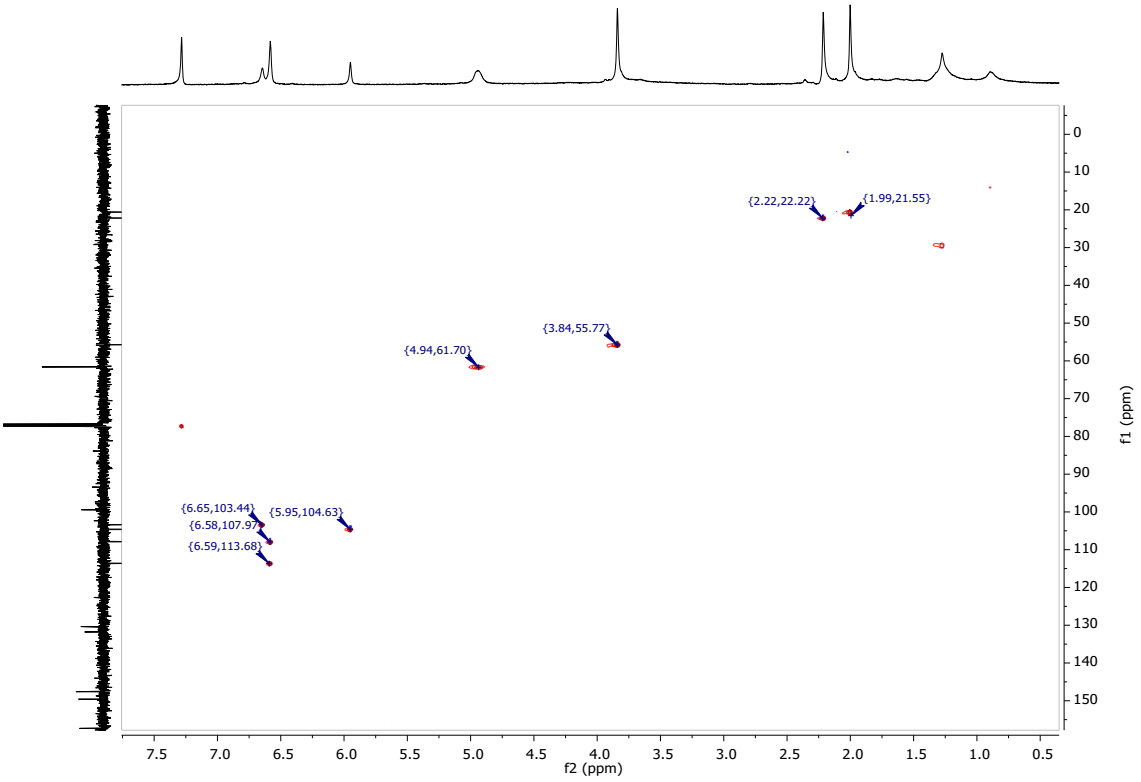


Fig. S13: HSQC spectrum of barcelonyl acetate (2)

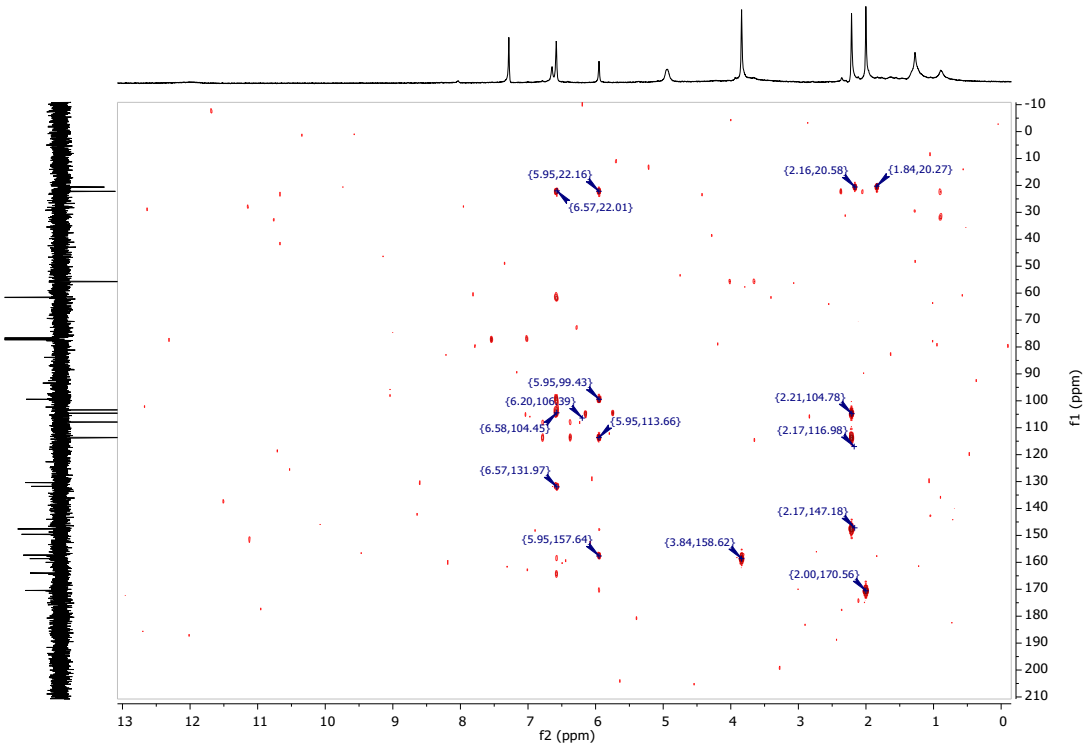


Fig. S14: HMBC spectrum of barcelonyl acetate (2)

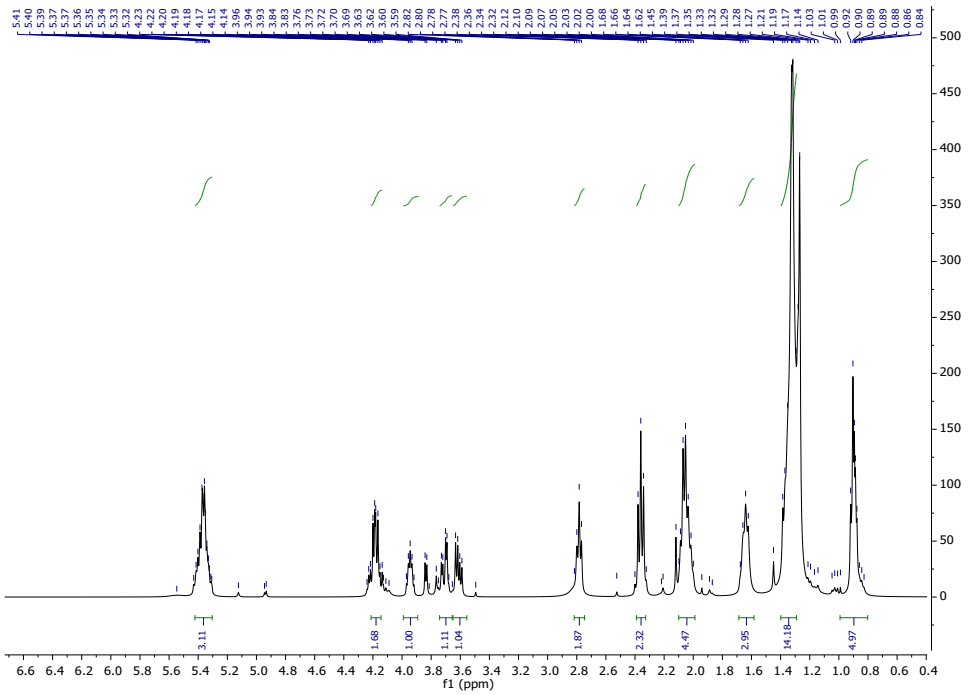


Fig. S15: ^{1}H-NMR spectrum of glycerol monolinoleate (3)

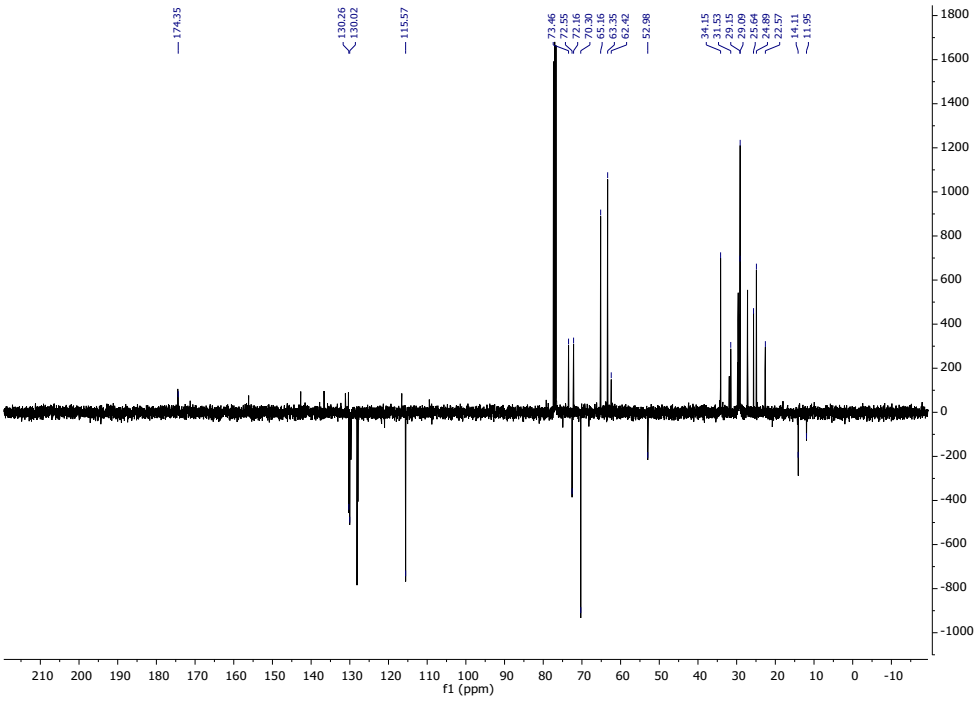


Fig. S16: APT spectrum of glycerol monolinoleate (3)

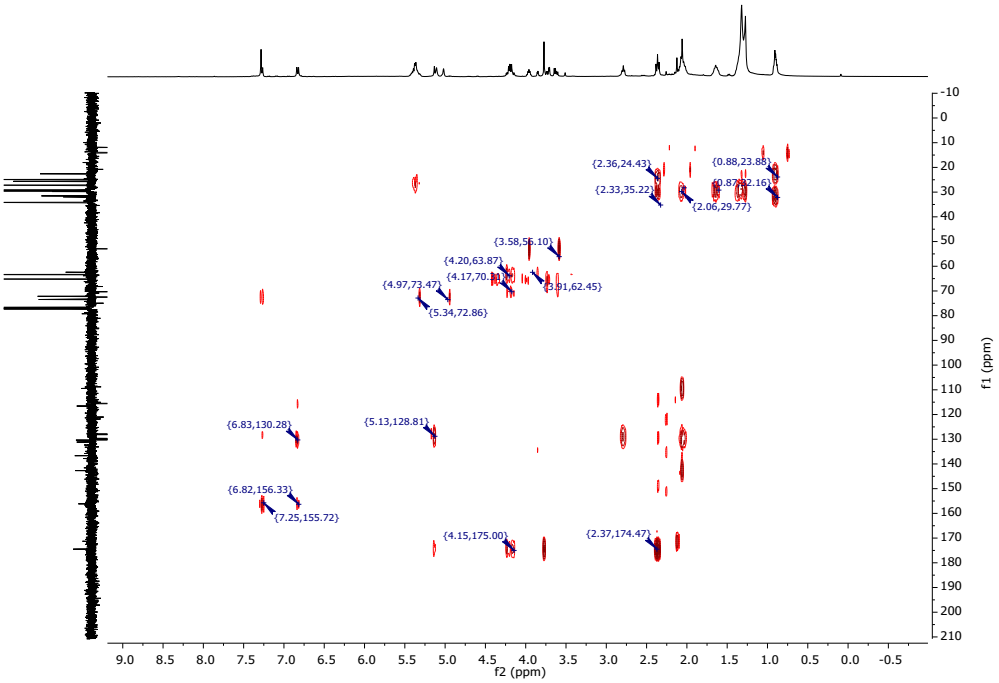


Fig. S17: HMBC spectrum of glycerol monolinoleate (3)

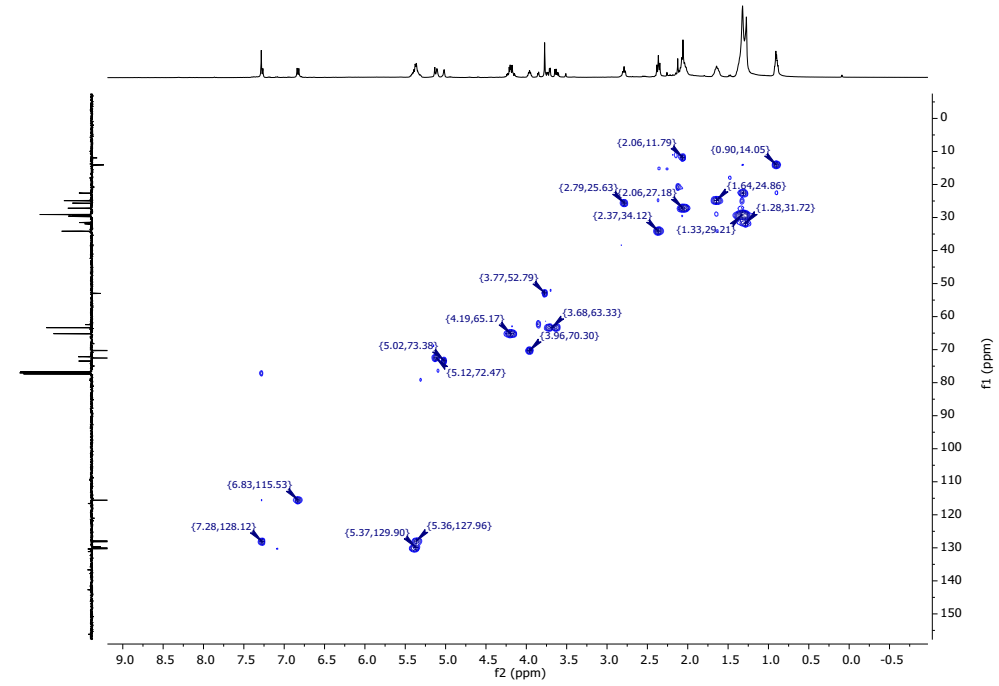


Fig. S18: HSQC spectrum of glycerol monolinoleate (3)

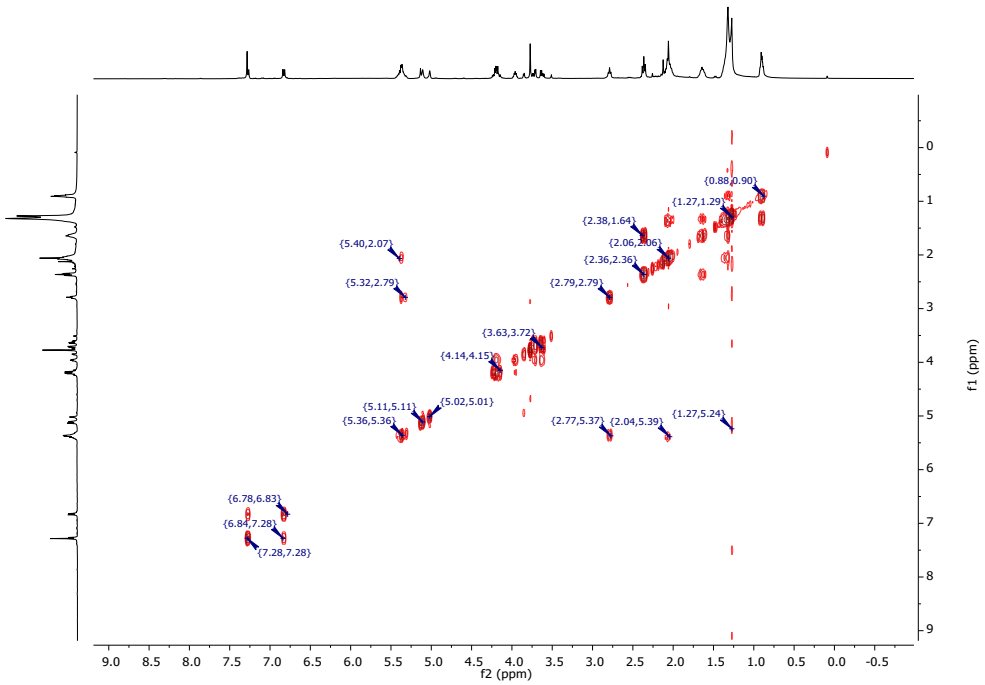


Fig. S19: H-H COSY spectrum of glycerol monolinoleate (3)

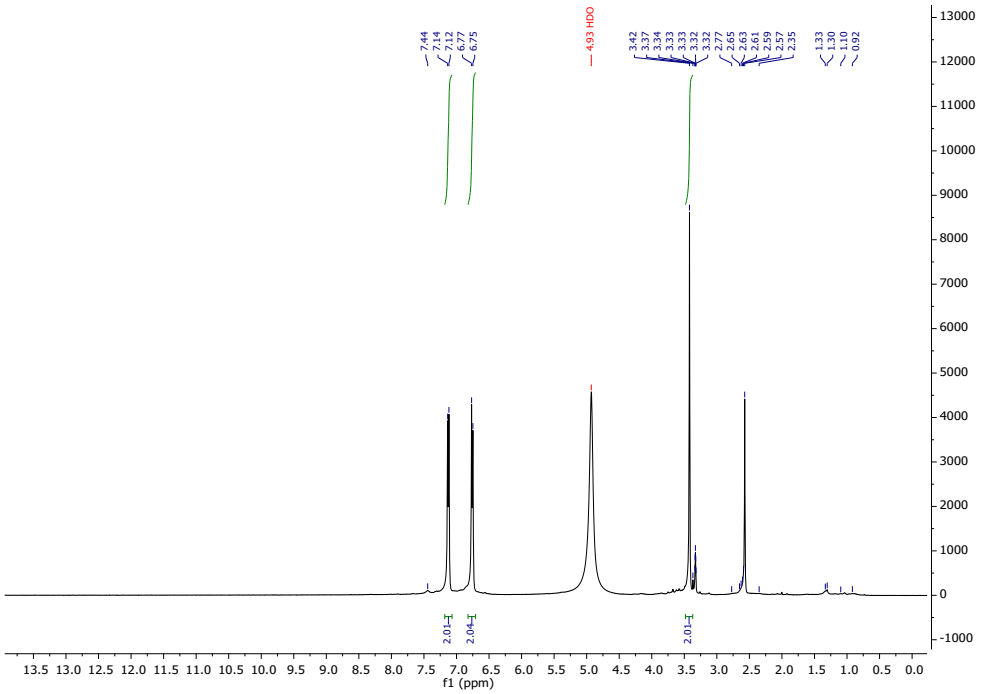


Fig. S20: ^{1}H-NMR spectrum of 4-hydroxyphenyl acetic acid (4)

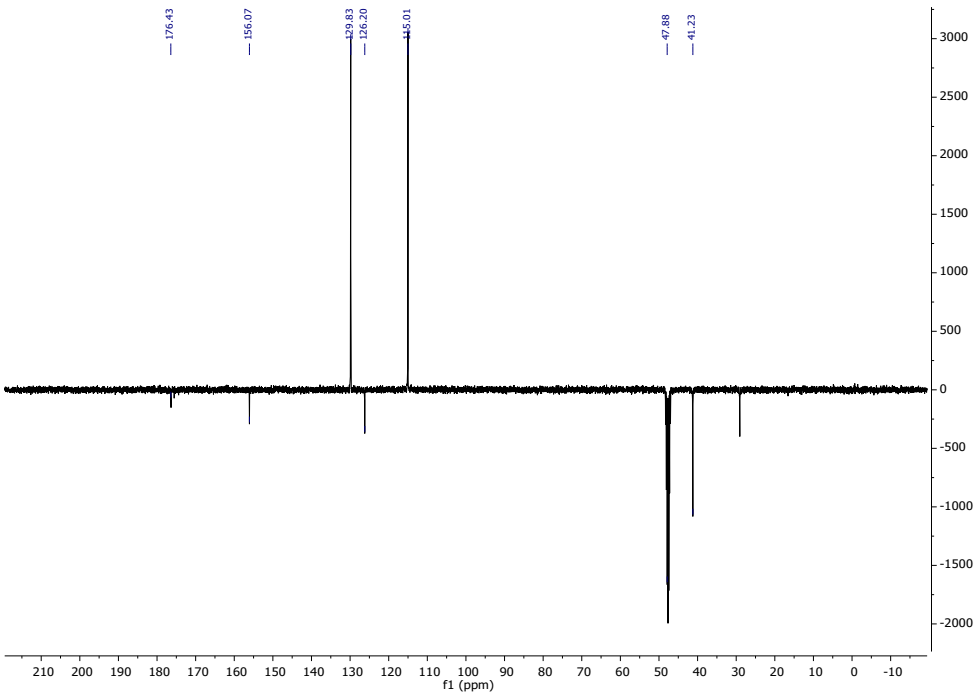


Fig. S21: APT spectrum of 4-hydroxyphenyl acetic acid (4)

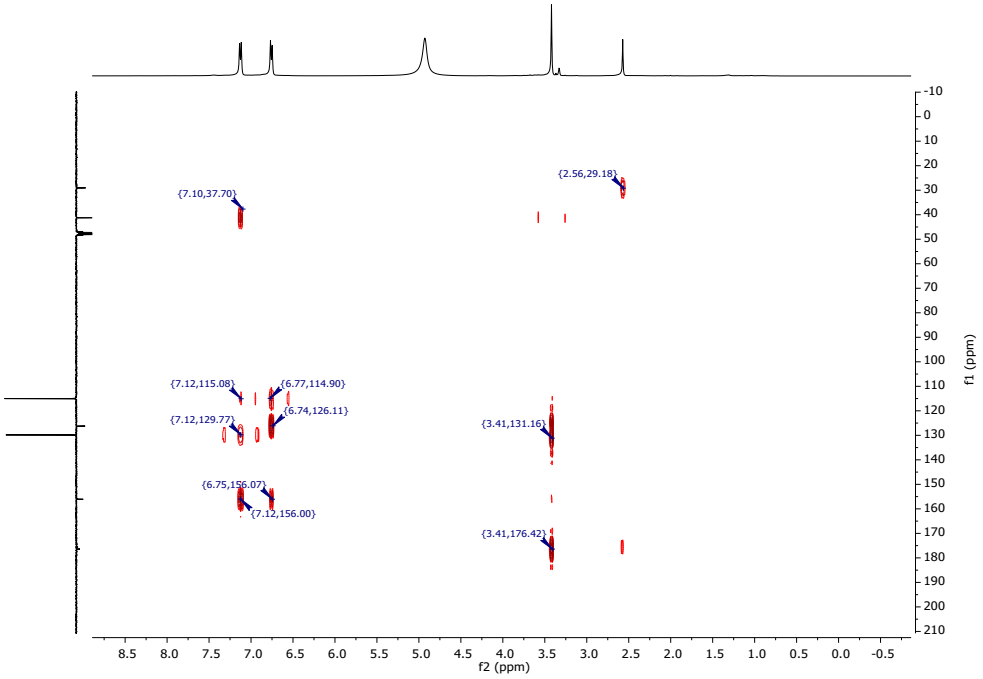


Fig. S22: HMBC spectrum of 4-hydroxyphenyl acetic acid (4)

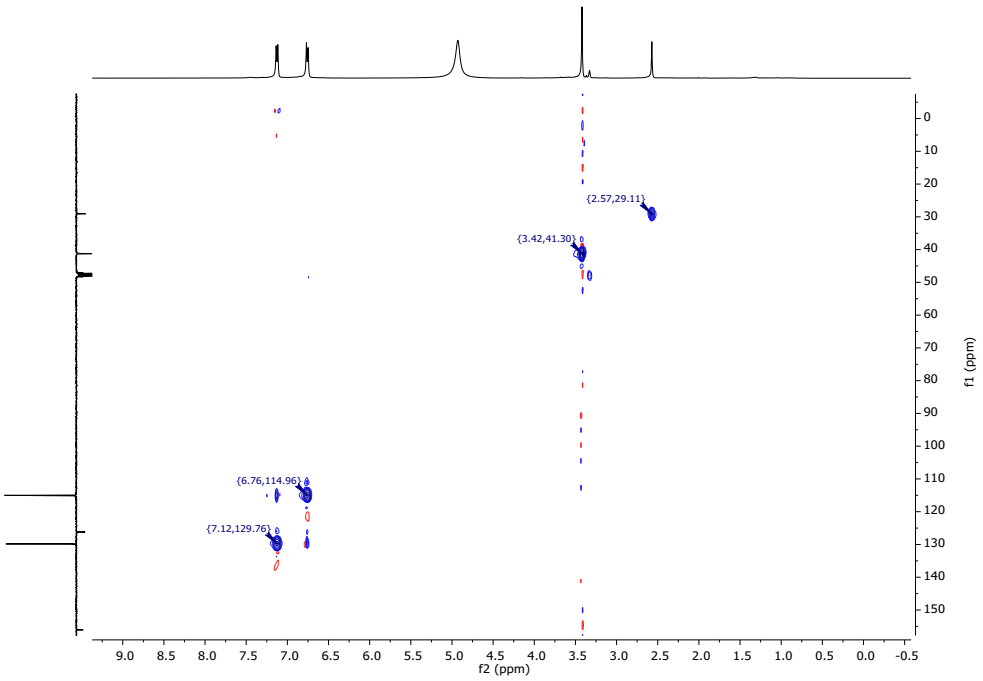


Fig. S23: HSQC spectrum of 4-hydroxyphenyl acetic acid (4)

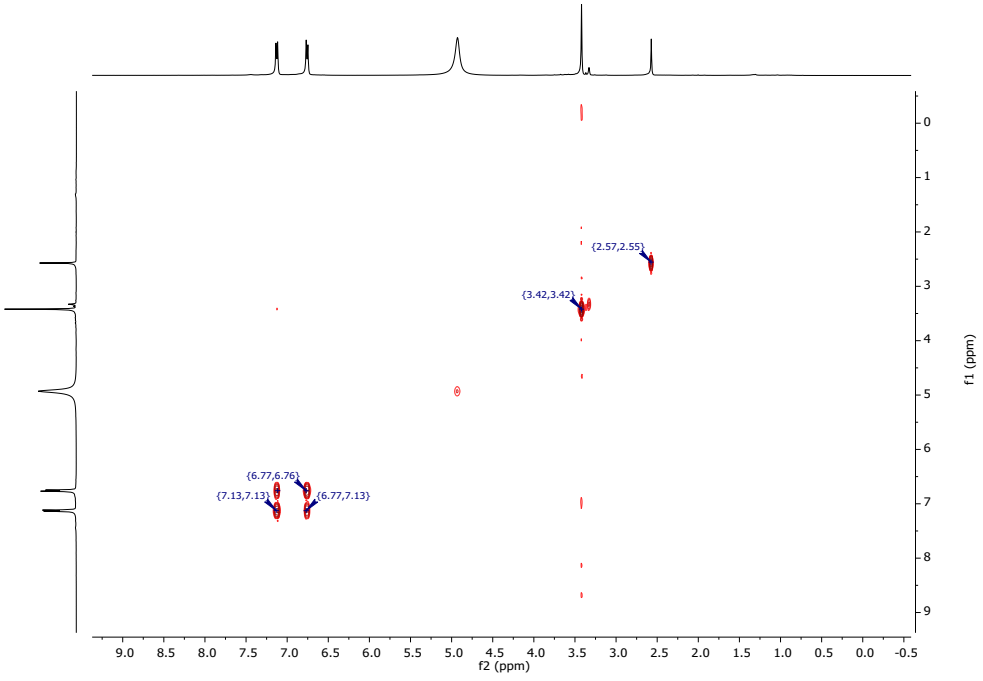


Fig. S24: H-H COSY spectrum of 4-hydroxyphenyl acetic acid (4)

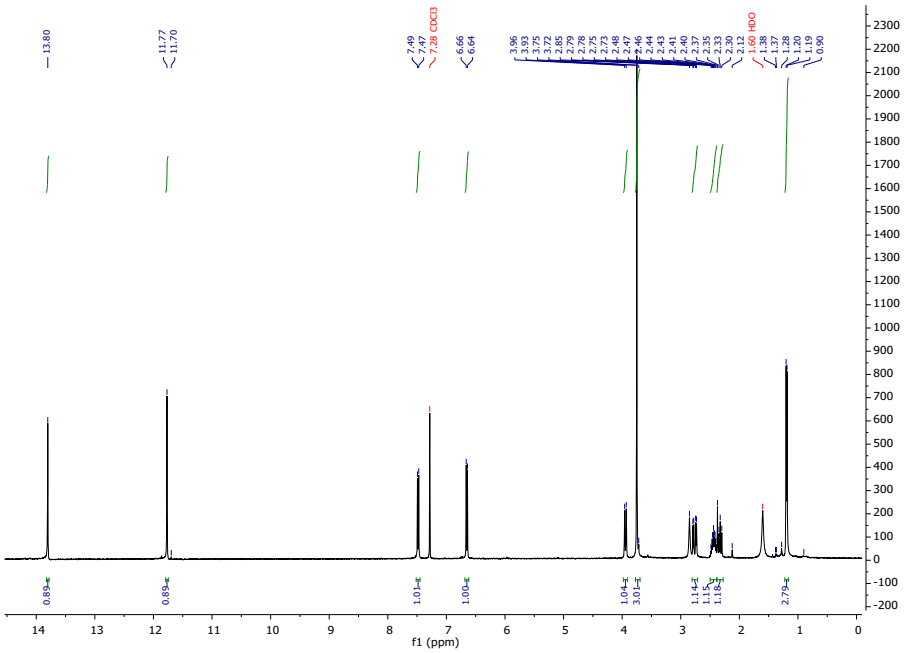


Fig. S25: ^{1}H-NMR spectrum of secalonic acid D (5)

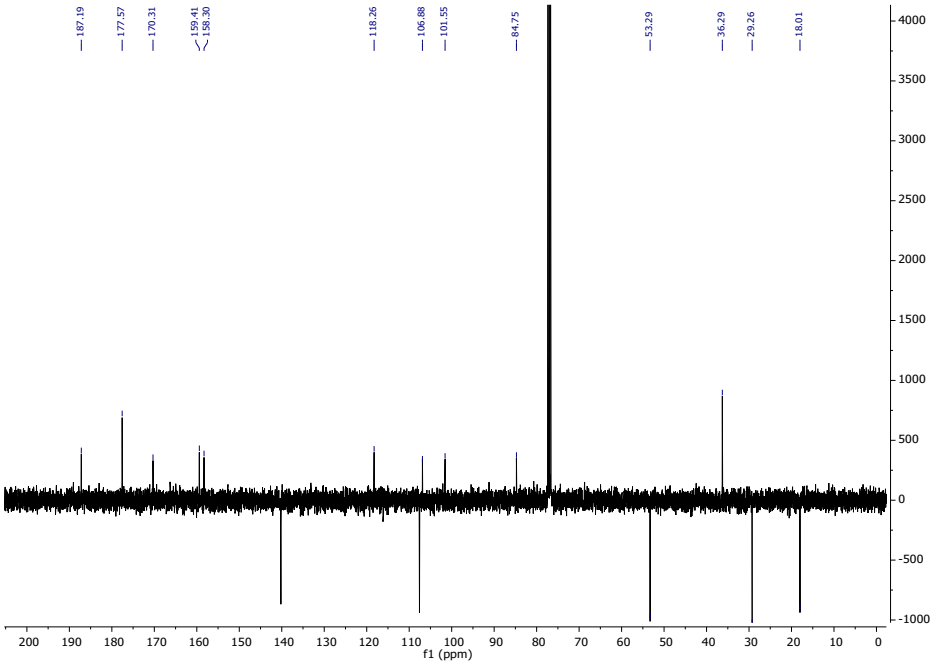


Fig. S26: APT spectrum of secalonic acid D (5)

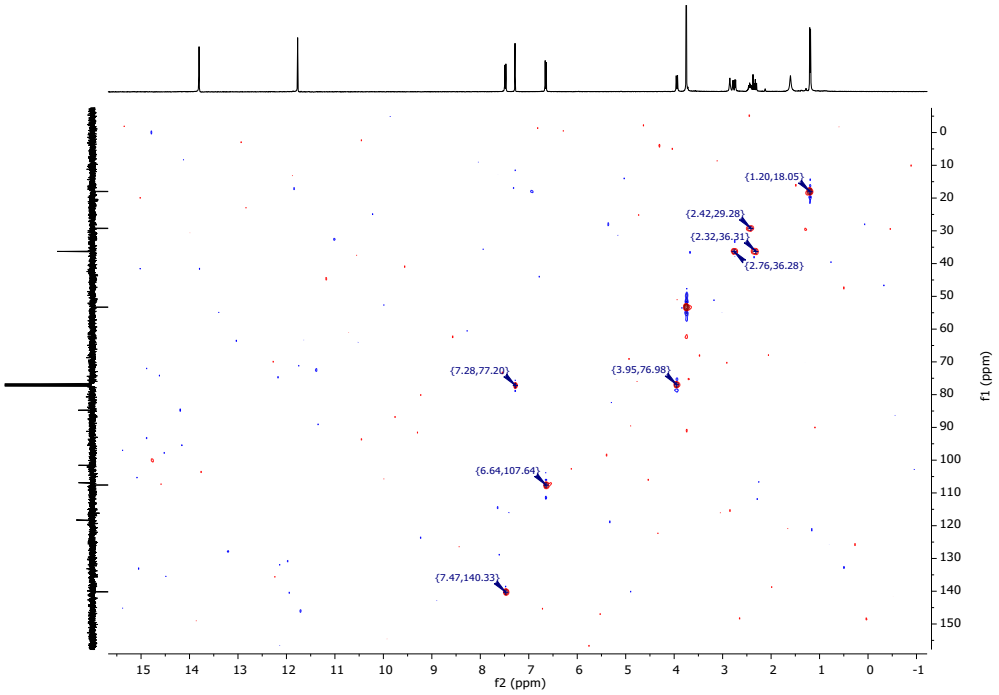


Fig. S27: HSQC spectrum of secalonic acid D (5)

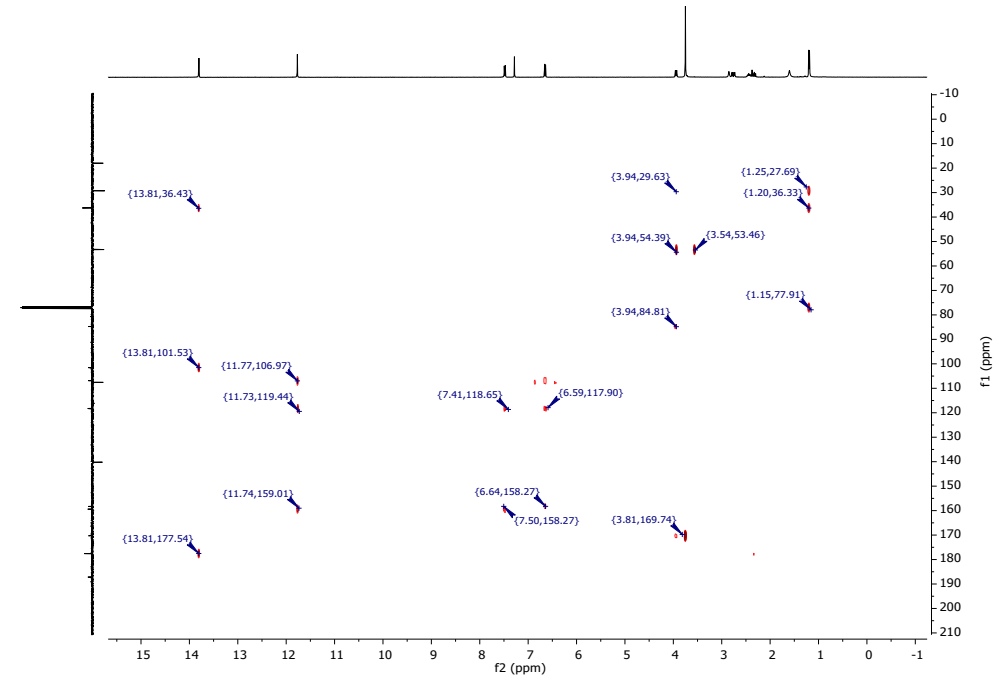


Fig. S28: HMBC spectrum of secalonic acid D (5)

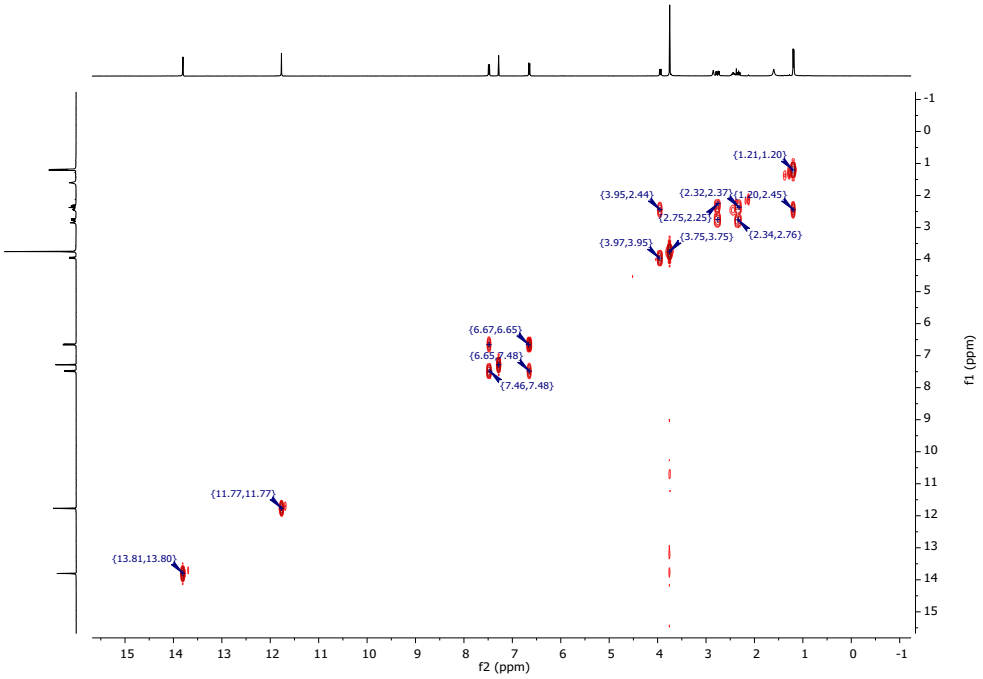


Fig. S29: H-H COSY spectrum of secalonic acid D (5)

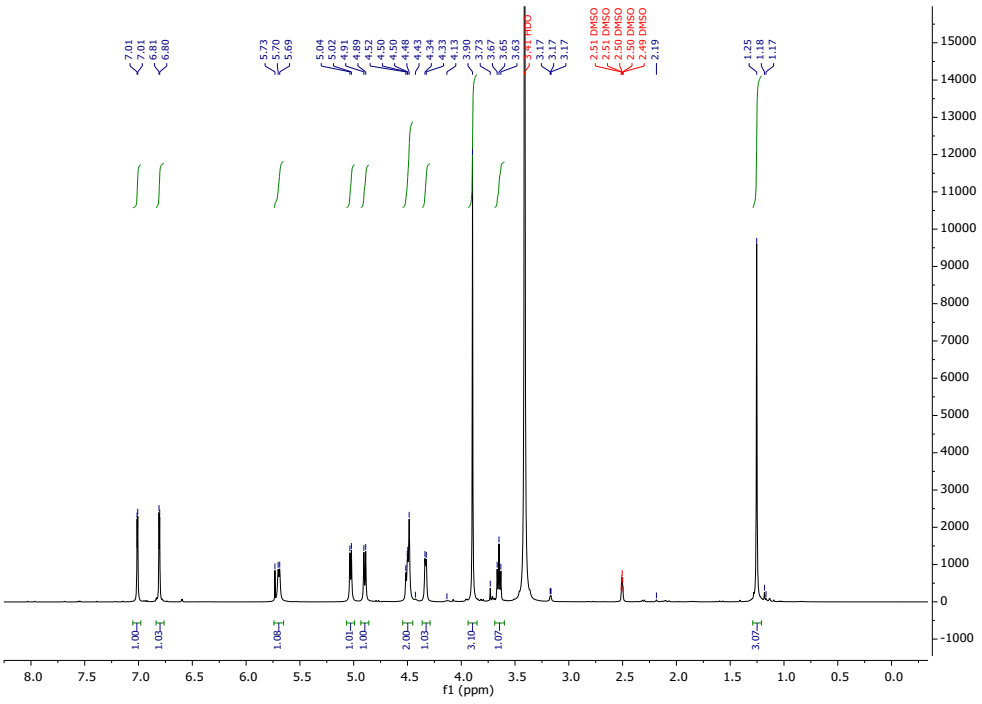


Fig. S30: ^{1}H-NMR spectrum of altersolanol A (6)

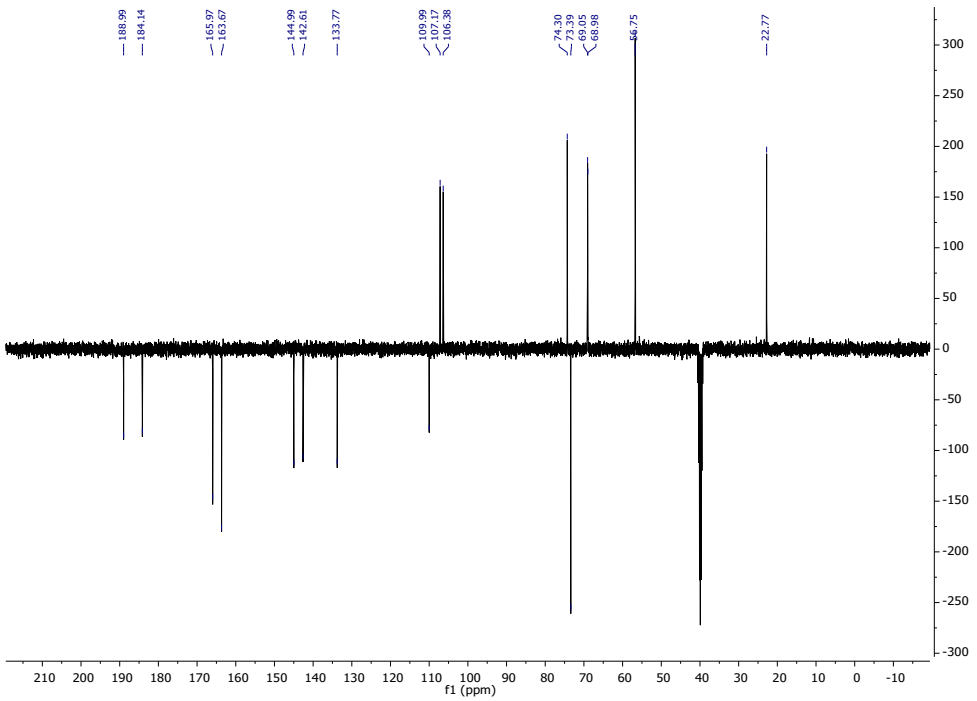


Fig. S31: APT spectrum of altersolanol A (6)

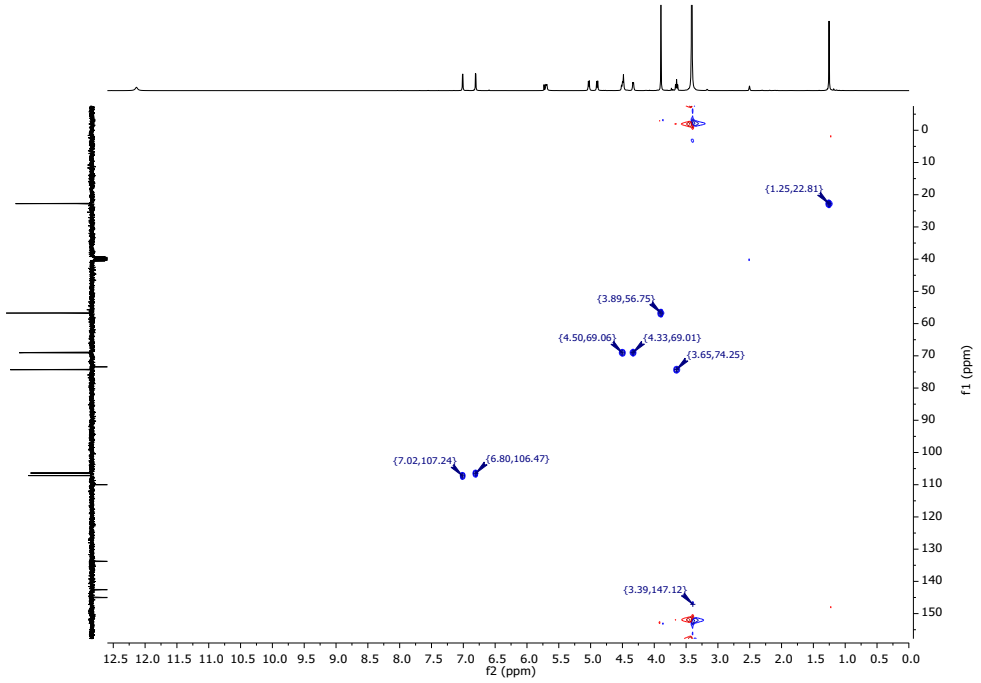


Fig. S32: HSQC spectrum of altersolanol A (6)

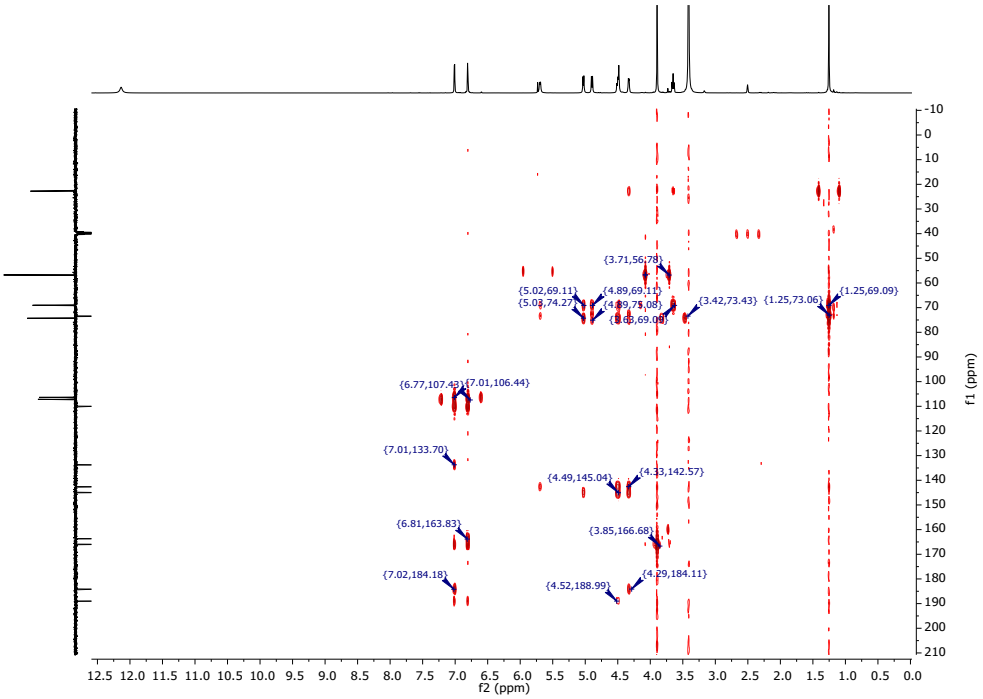


Fig. S33: HMBC spectrum of altersolanol A (6)

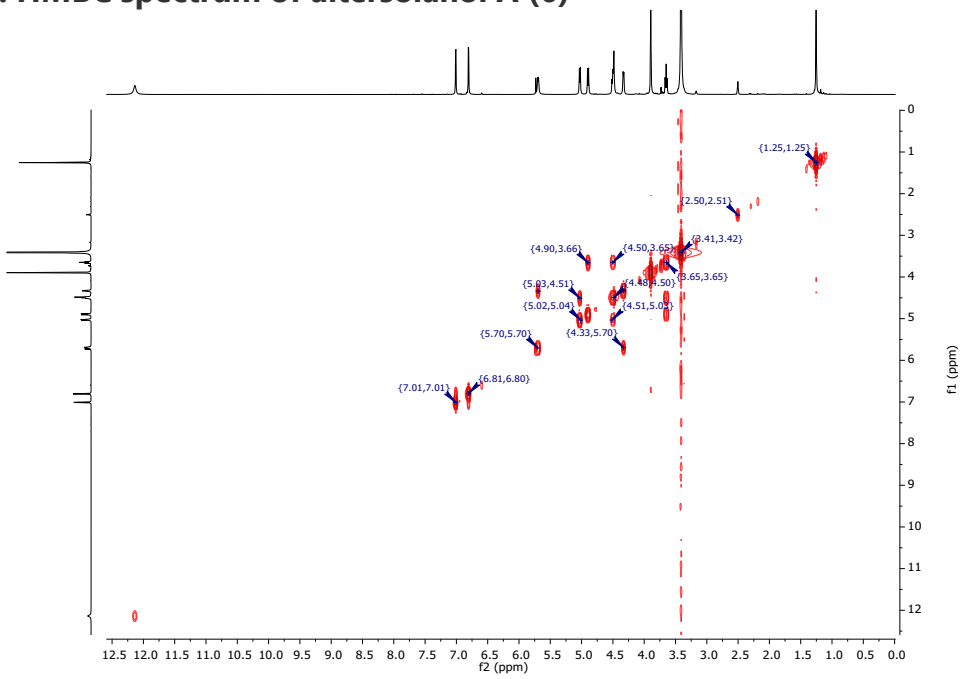


Fig. S34: H-H COSY spectrum of altersolanol A (6)

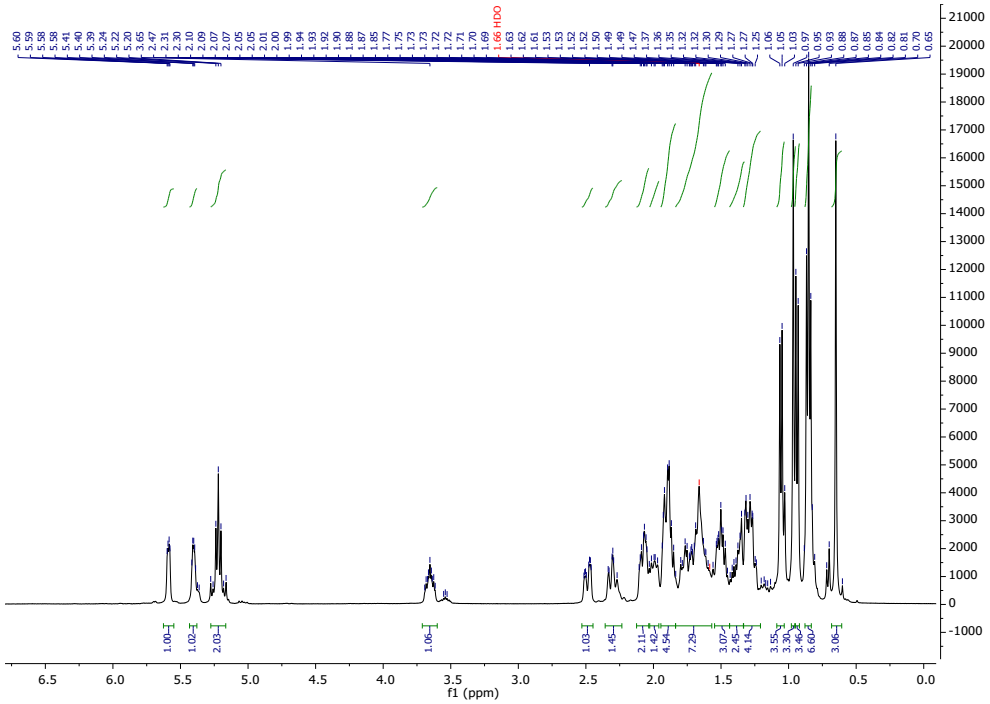


Fig. S35: ^{1}H-NMR spectrum of ergosterol (7)

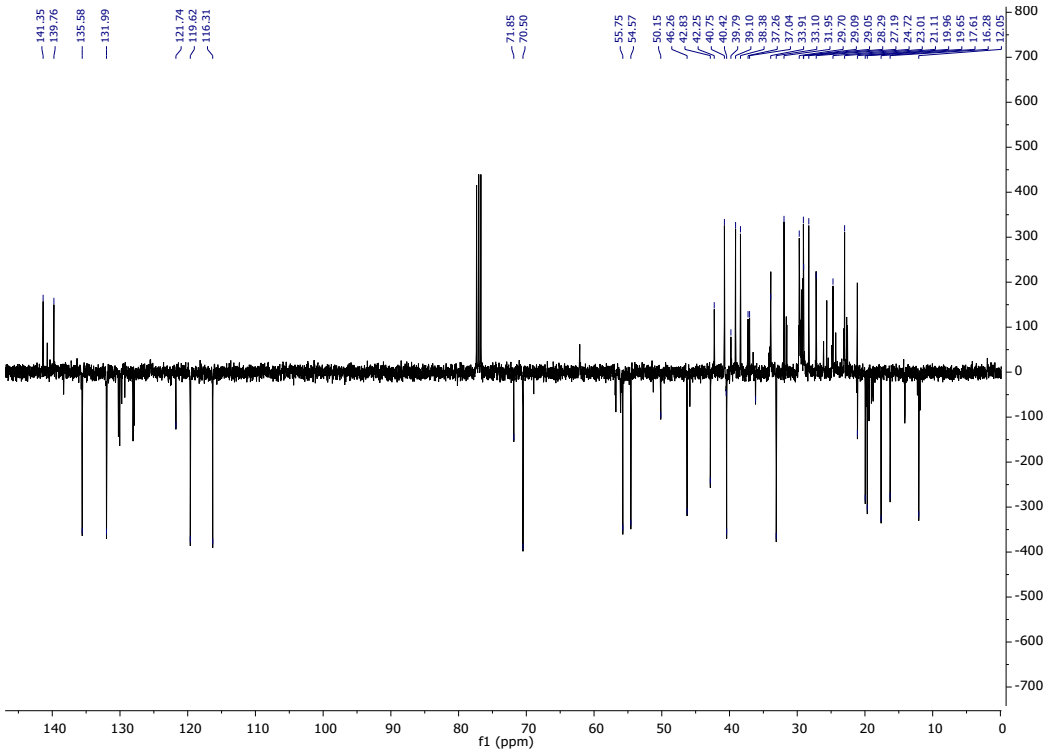


Fig. S36: APT spectrum of ergosterol (7)

Table S8: *In silico* 2D and 3D interaction of the isolated compounds

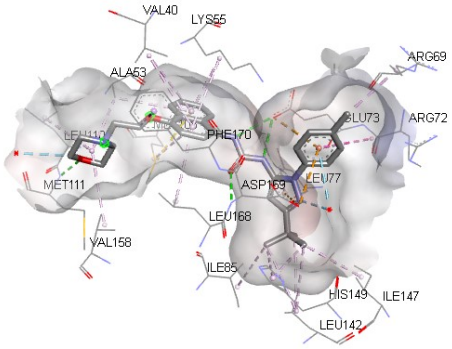
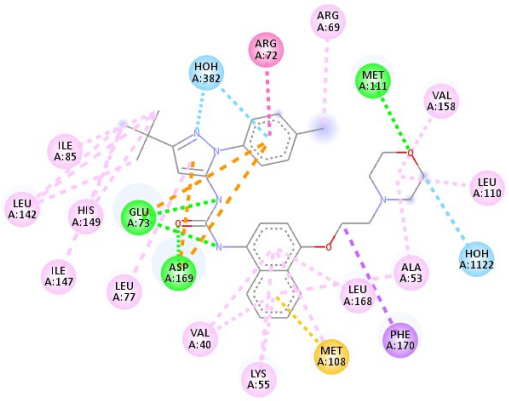
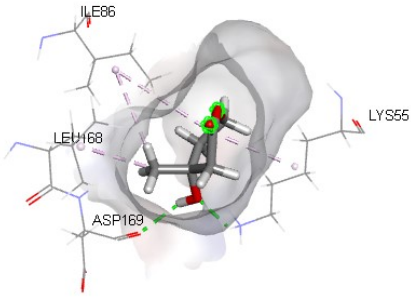
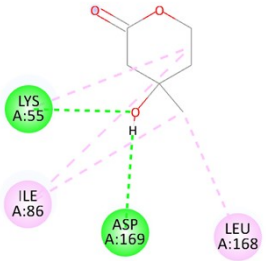
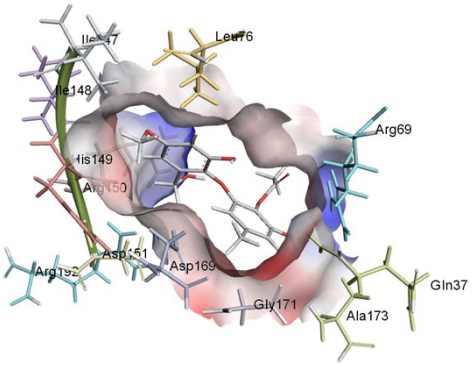
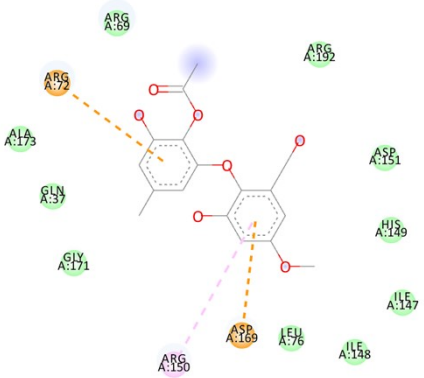
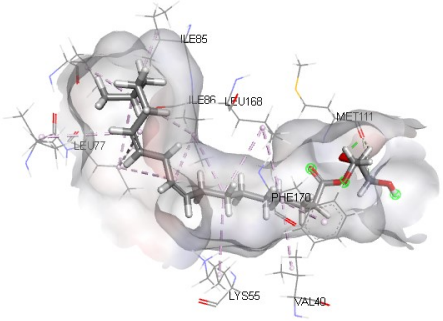
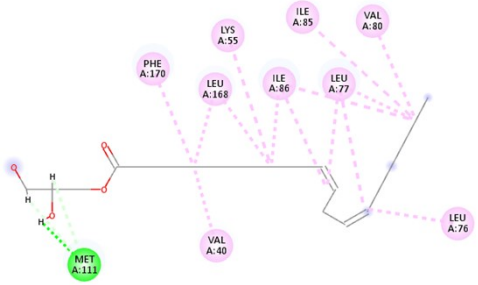
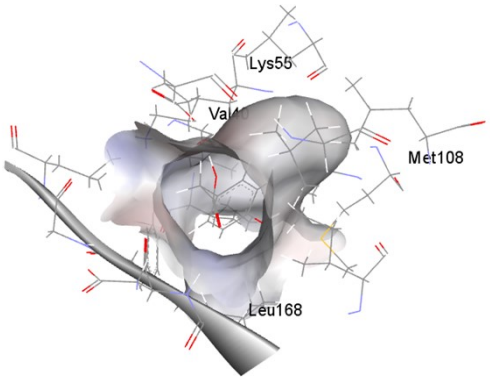
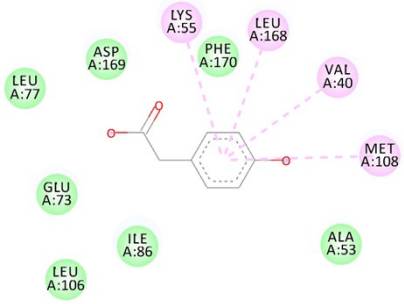
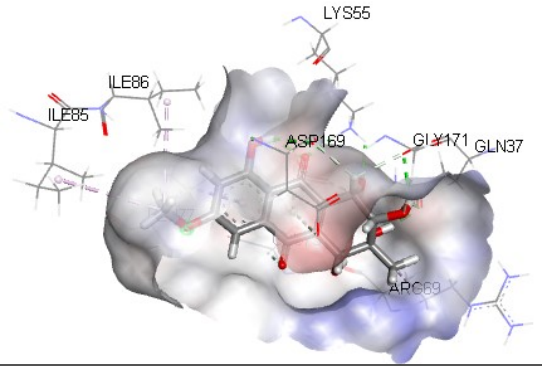
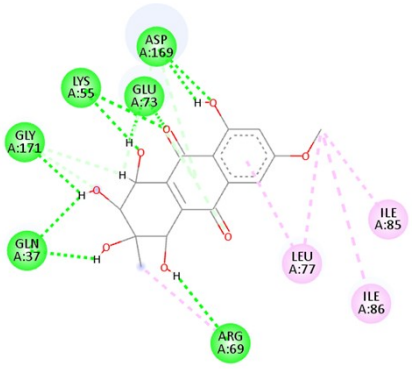
3D interaction	2D interaction
Co crystal (BIRB796)	
	
Mevalonolactone (1)	
	
Barcelonyl acetate (2)	
	
Glycerol monolinoleate (3)	
	

Table S8: (Cont.)

3D interaction	2D interaction
4-Hydroxyphenyl acetic acid (4)	
	
Altersolanol A (6)	
	

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