Supporting Information

Agarperoxinols A and B: Two Unprecedented Tricyclic 6/6/7 Rearranged Humulene-Type Sesquiterpenoids and Attenuated the Neuro-inflammatory in LPS-stimulated Microglial Models

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Experimental Procedures

1. Plant Material

The agarwood chips of *A. malaccensis* was purchased from Industrial Plantation Co. (Vientiane, Laos) in January 2010. A voucher specimen (AM-2010-01) was authenticated by Professor Jeong Hill Park (Natural Products Research Institute, Seoul National University) and deposited at the Herbarium of the Natural Product Research Institute, Seoul National University, Korea.

2. Extraction and Isolation

The agarwood chips of *A. malaccencis* (9.0 kg) was ground and extracted with MeOH 70% under reflux (20 L X 3h, 3 times). The extraction was evaporated under reduced pressure to obtain a crude extract (864 g), which was suspended in water and successively partitioned with diethyl ether, EtOAc, n-BuOH, achieving 225, 155, and 289 g of residue, respectively.

The ether fraction (30 g) was fractionated by a silica gel column (230-400 mesh, 300 g) and eluted with *n*-hexane/EtOAc (gradient, 40:1 → 1:1, v/v) to obtain 7 fractions (Et1-Et7). Fraction Et3 (3.36 g) was separated by silica gel column chromatography (230-400 mesh, 100g), eluting with *n*-hexane/EtOAc (gradient, 95:5 → 7:3, v/v) to achieve 9 sub-fractions (Et3a-Et3i). Sub-fraction Et3b (250 mg) was separated by semi-preparative RP-HPLC (MeOH-H_2O, 65:35, v/v) to obtain two fractions Et3b1 and Et3b2. Compound 1 (3.44 mg) was isolated from fraction Et3b1 by semi-preparative RP-HPLC (t_R = 11.0 min, CH_3CN-H2O, 55:45, v/v) and fraction Et3b2 was further purified by semi-preparative RP-HPLC to yield compound 2 (8.26 mg) (t_R = 9.95 min, CH_3CN-H_2O, 50:50, v/v).

3. Crystallographic data

Table S1. Crystal data and structure refinement for 1

Empirical formula	$C_{15}H_{24}O_4$
Formula weight	268.34
Temperature/K	291(6)
Crystal system	monoclinic
Space group	$P2_1/n$
a/Å	8.9468(3)
b/Å	16.8211(6)
c/Å	10.2017(4)
α/°	90
β/°	109.839(4)
γ/°	90
Volume/Å^3	1444.19(9)
Z	4
ρ_{calc}g/cm^3	1.234
μ/mm^{-1}	0.715
F(000)	584.0
Crystal size/mm^3	0.3 × 0.1 × 0.02
Radiation	CuKα (λ = 1.54184)
2Θ range for data collection/°	10.518 to 153.188
Index ranges	$-10 \leq h \leq 11$, $-20 \leq k \leq 21$, $-12 \leq l \leq 12$
Reflections collected	15045
Independent reflections	2999 [R_{int} = 0.0527, R_{sigma} = 0.0259]
Data/restraints/parameters	2999/0/180
Goodness-of-fit on F^2	1.056
Final R indexes [I>=2σ (I)]	R_1 = 0.0447, wR_2 = 0.1297
Final R indexes [all data]	R_1 = 0.0540, wR_2 = 0.1393
Largest diff. peak/hole / e Å^{-3}	0.18/-0.14

Table S2. Fractional Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Parameters ($Å^2 \times 10^3$) for 1. U_{eq} is defined as 1/3 of of the trace of the orthogonalised U_{IJ} tensor

Atom	*x*	*y*	*z*	U(eq)
O1	10439.5(13)	5570.2(9)	3144.1(13)	74.8(4)
O2	8137.5(11)	4723.5(7)	3940.3(10)	53.3(3)
O3	4276.7(13)	5247.2(8)	3323.6(13)	65.7(3)
O4	3222.5(16)	5850.5(9)	2447.7(17)	81.0(4)
C1	4884.0(18)	3017.1(11)	1475.2(18)	63.3(4)
C2	6489.8(19)	3179.7(11)	1376.2(18)	62.1(4)
C3	7598.1(16)	3621.9(10)	2275.3(16)	55.9(4)
C4	9128.2(19)	3833.3(13)	2053(2)	69.1(5)
C5	9032(2)	4634.3(13)	1333.9(18)	71.4(5)
C6	8903.2(18)	5370.0(12)	2160.7(17)	64.5(4)
C7	7662.0(17)	5344.4(10)	2899.2(15)	55.5(4)
C8	5979.2(17)	5193.6(10)	1857.6(15)	55.4(4)
C9	4853.8(15)	4761.4(9)	2447.5(15)	52.2(3)
C10	5602.9(15)	4042.3(9)	3380.9(14)	48.9(3)
C11	4746.5(16)	3239.5(10)	2880.0(17)	55.9(4)
C12	7355.1(15)	3971.2(9)	3541.6(14)	49.6(3)
C13	7684(2)	6103.9(12)	3702(2)	75.8(5)
C14	2991.7(19)	3293.4(13)	2743(2)	73.7(5)
C15	5510(2)	2592.8(12)	3961(2)	73.8(5)

Table S3. Anisotropic Displacement Parameters ($Å^2×10^3$) for 1. The Anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2}U_{11}+2hka^*b^*U_{12}+\ldots]$.

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
O1	52.2(6)	103.9(10)	57.0(6)	16.1(6)	3.8(5)	-21.7(6)
O2	43.3(5)	68.3(6)	37.7(5)	4.2(4)	-0.1(4)	-8.5(4)
O3	57.3(6)	74.7(7)	61.8(7)	-0.9(5)	15.7(5)	16.3(5)
O4	67.9(7)	75.5(8)	101(1)	19.6(7)	30.8(7)	21.8(6)
C1	48.1(7)	69.3(10)	64.5(9)	-14.3(8)	8.5(6)	-4.7(7)
C2	54.1(8)	70(1)	60.6(8)	-10.8(7)	17.2(7)	2.7(7)
C3	41.8(6)	66.1(9)	56.6(8)	0.4(7)	12.4(6)	5.9(6)
C4	45.7(7)	93.3(12)	69.7(10)	-6.1(9)	21.6(7)	1.7(8)
C5	51.2(8)	111.8(15)	51.3(8)	2.3(9)	17.7(7)	-10.1(8)
C6	50.0(8)	87.1(12)	47.4(8)	13.7(7)	4.6(6)	-11.4(7)
C7	50.5(7)	64.9(9)	42.8(7)	5.6(6)	5.1(6)	-6.5(6)
C8	47.5(7)	65.1(9)	44.0(7)	5.1(6)	3.1(6)	3.7(6)
C9	41.8(6)	61.9(8)	44.8(7)	-2.9(6)	4.1(5)	4.5(6)
C10	39.5(6)	60.4(8)	42.1(6)	-0.1(6)	7.6(5)	1.4(5)
C11	39.9(6)	63.1(9)	59.2(8)	-1.7(7)	9.7(6)	-2.5(6)
C12	37.3(6)	59.8(8)	43.9(6)	4.6(6)	3.5(5)	-1.1(5)
C13	78.7(11)	69.0(11)	70.4(10)	-6.6(9)	13.4(9)	-13.5(9)
C14	45.0(8)	90.3(12)	83.3(12)	-7.3(10)	18.4(8)	-9.0(8)
C15	63.5(9)	69.3(11)	82.5(12)	12.5(9)	16.7(8)	-4.3(8)

Table S4. Bond Lengths for 1.

Atom	Atom	Length/Å	Atom	Atom	Length/Å
O1	C6	1.4398(17)	C5	C6	1.524(3)
O2	C7	1.4468(18)	C6	C7	1.540(2)
O2	C12	1.4365(18)	C7	C8	1.5409(19)
O3	O4	1.4649(17)	C7	C13	1.514(3)
O3	C9	1.4305(19)	C8	C9	1.522(2)
C1	C2	1.499(2)	C9	C10	1.545(2)
C1	C11	1.526(2)	C10	C11	1.551(2)
C2	C3	1.327(2)	C10	C12	1.5246(17)
C3	C4	1.504(2)	C11	C14	1.531(2)
C3	C12	1.501(2)	C11	C15	1.534(2)
C4	C5	1.523(3)			

Table S5. Bond Angles for 1.

Atom	Atom	Atom	Angle/°	Atom	Atom	Atom	Angle/°
C12	O2	C7	115.75(10)	C13	C7	C8	110.01(14)
C9	O3	O4	107.99(12)	C9	C8	C7	115.39(12)
C2	C1	C11	113.77(13)	O3	C9	C8	113.69(13)
C3	C2	C1	124.34(15)	O3	C9	C10	103.61(12)
C2	C3	C4	122.23(16)	C8	C9	C10	114.27(11)
C2	C3	C12	121.47(14)	C9	C10	C11	114.60(11)
C12	C3	C4	116.25(14)	C12	C10	C9	110.05(12)
C3	C4	C5	112.10(14)	C12	C10	C11	110.01(12)
C4	C5	C6	117.03(15)	C1	C11	C10	109.54(13)
O1	C6	C5	110.01(15)	C1	C11	C14	109.62(13)
O1	C6	C7	110.00(13)	C1	C11	C15	110.06(15)
C5	C6	C7	116.78(14)	C14	C11	C10	110.80(13)
O2	C7	C6	107.35(13)	C14	C11	C15	107.47(15)
O2	C7	C8	111.25(12)	C15	C11	C10	109.32(12)
O2	C7	C13	105.40(13)	O2	C12	C3	112.93(12)
C6	C7	C8	111.43(12)	O2	C12	C10	110.23(12)
C13	C7	C6	111.22(14)	C3	C12	C10	112.47(11)

Table S6. Hydrogen Atom Coordinates (Å×10^4) and Isotropic Displacement Parameters (Å2×10^3) for 1.

Atom	*x*	*y*	*z*	U(eq)
H1	10603	5309	3857	112
H4	2200(40)	5740(20)	2610(40)	139(11)
H1A	4647	2456	1307	76
H1B	4093	3312	748	76
H2	6725	2956	635	75
H4A	9382	3425	1491	83
H4B	9979	3848	2947	83
H5A	8118	4625	483	86
H5B	9969	4691	1067	86
H6	8597	5810	1492	77
H8A	5509	5701	1489	66
H8B	6071	4886	1085	66
H9	3941	4573	1667	63
H10	5548	4150	4308	59
H12	7836	3602	4314	60
H13A	6889	6076	4138	114
H13B	7466	6548	3074	114
H13C	8711	6170	4402	114

H14A	2908	3427	3630	111
H14B	2486	2791	2437	111
H14C	2481	3697	2075	111
H15A	6553	2474	3944	111
H15B	4867	2122	3742	111
H15C	5587	2778	4871	111

Figure S1. ^{1}H NMR spectrum of agarperoxinol A (1) in $CDCl_3$ (500 MHz)

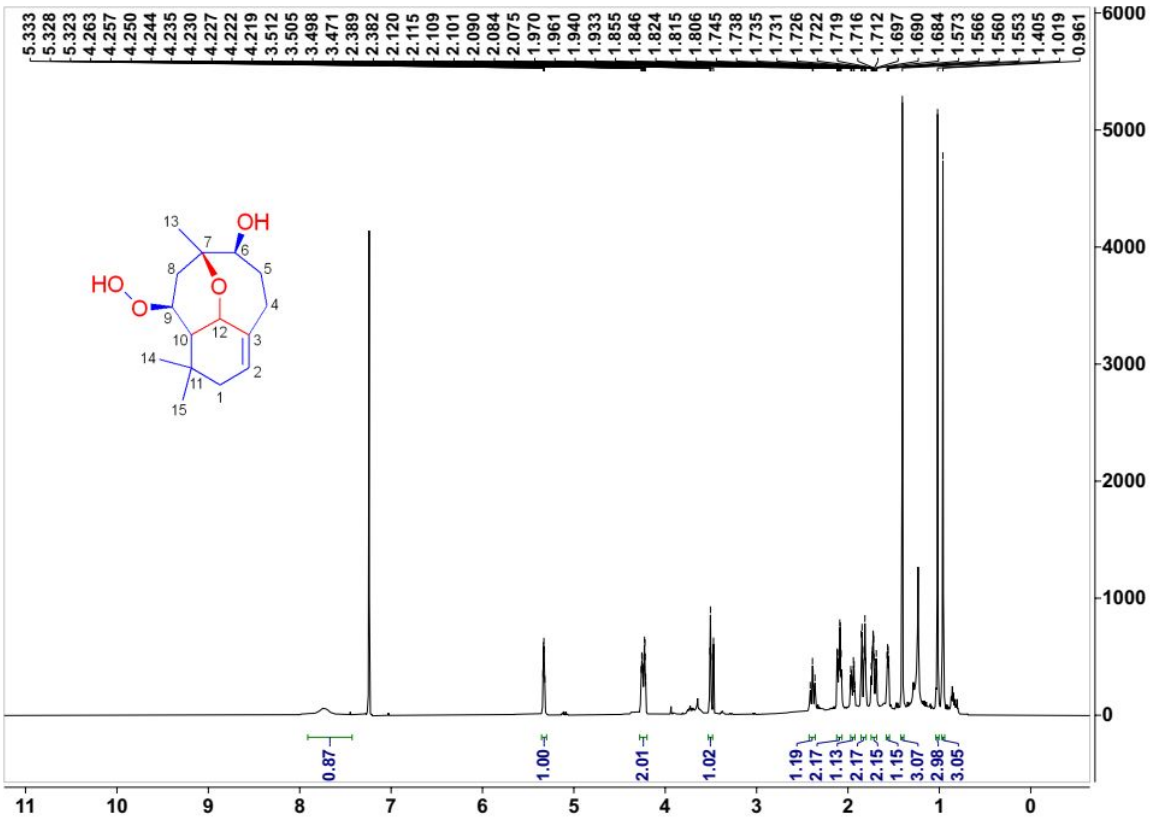


Figure S2. ^{13}C NMR spectrum of agarperoxinol A (1) in $CDCl_3$ (500 MHz)

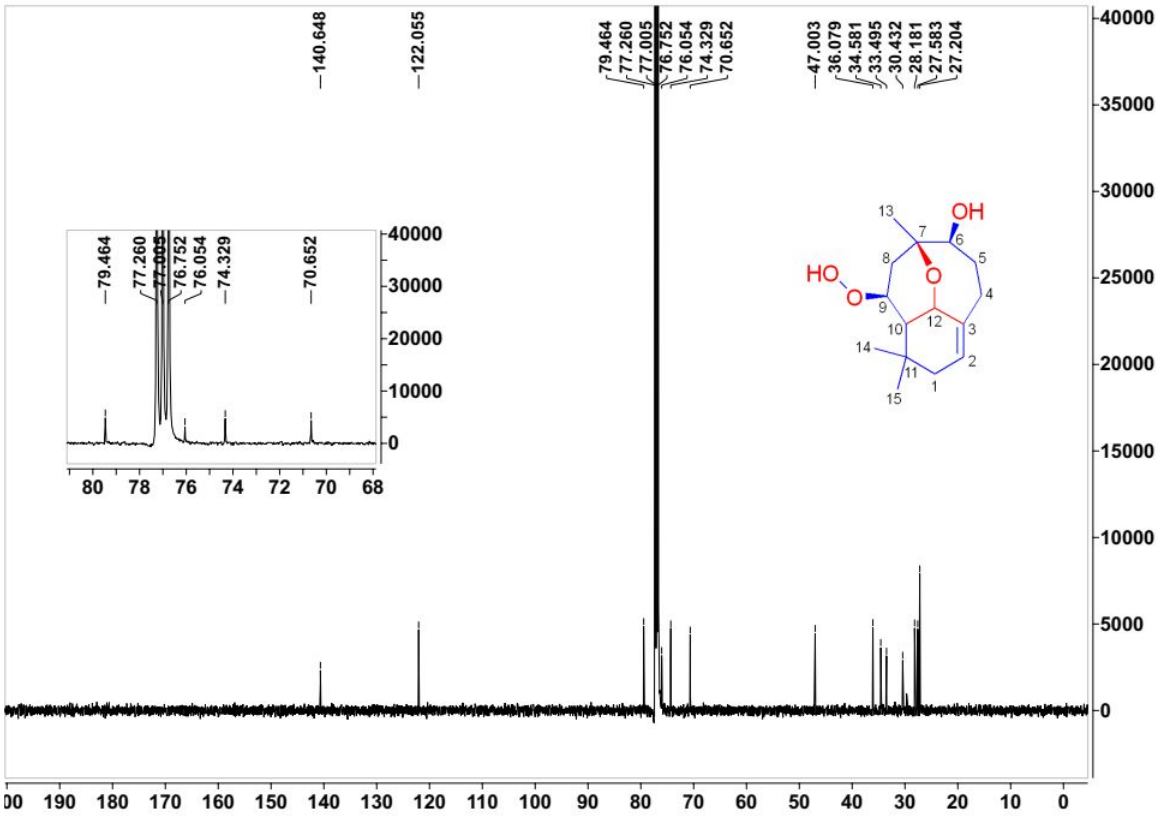


Figure S3. HSQC spectrum of agarperoxinol A (1) in $CDCl_3$ (500 MHz)

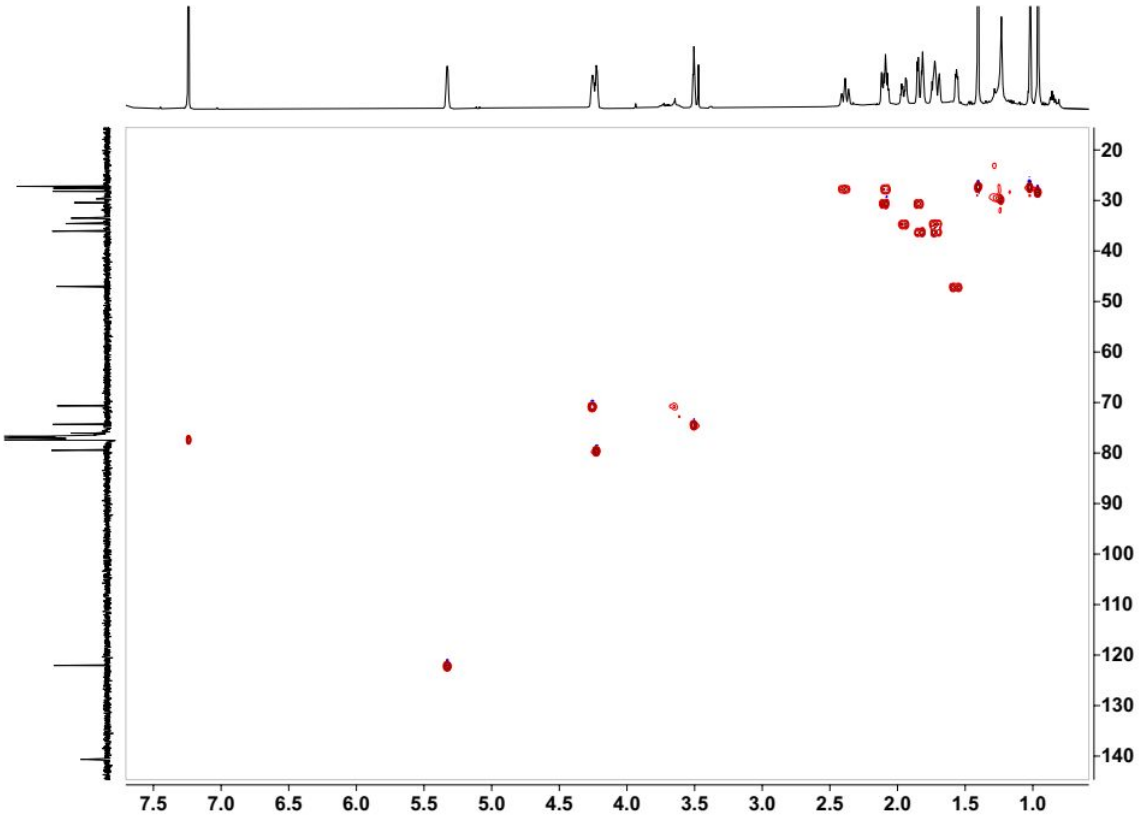


Figure S4. HMBC spectrum of agarperoxinol A (1) in $CDCl_3$ (500 MHz)

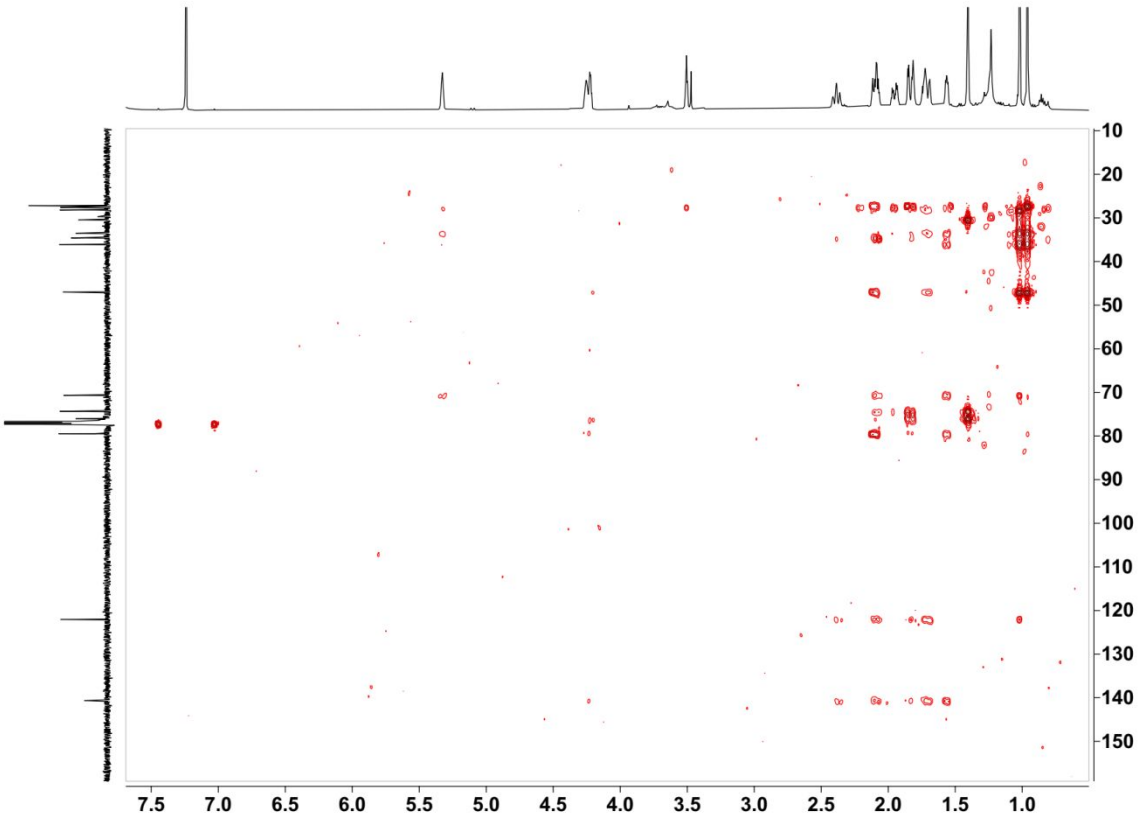


Figure S5. COSY spectrum of agarperoxinol A (1) in $CDCl_3$ (500 MHz)

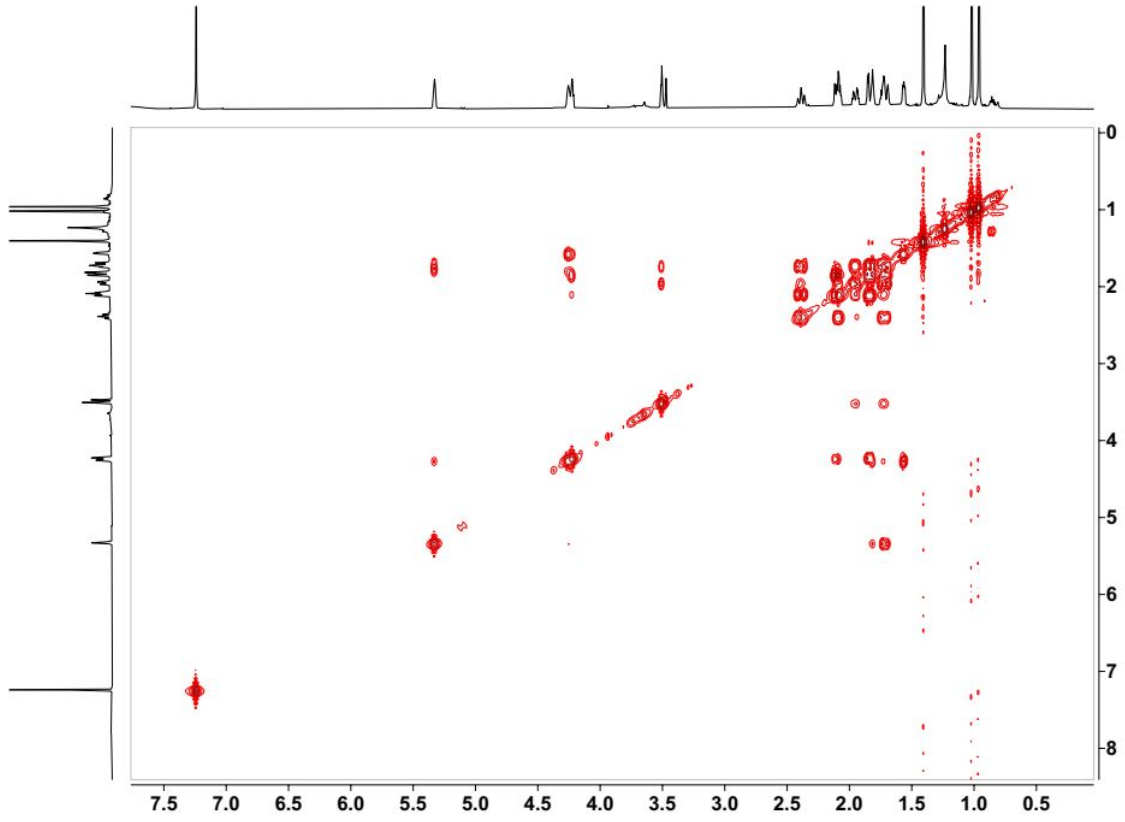


Figure S6. NOESY spectrum of agarperoxinol A (1) in $CDCl_3$ (500 MHz)

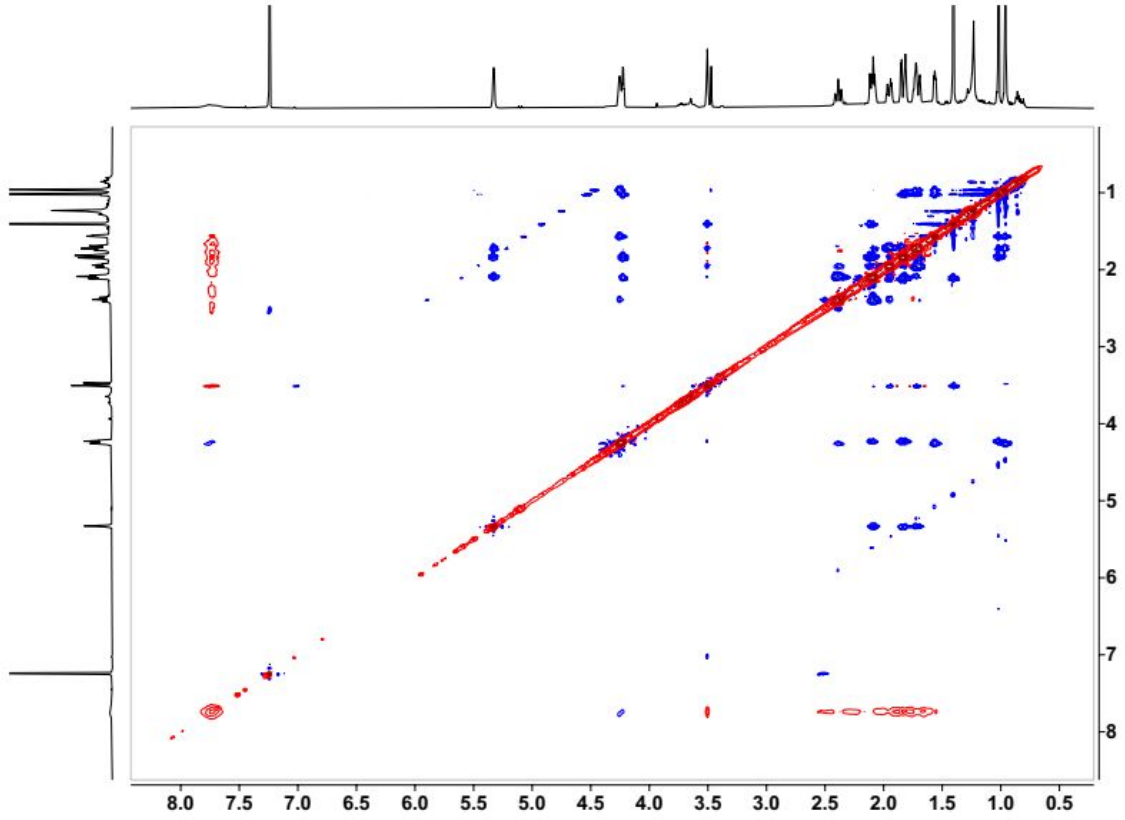


Figure S7. UV spectrum of agarperoxinol A (1)

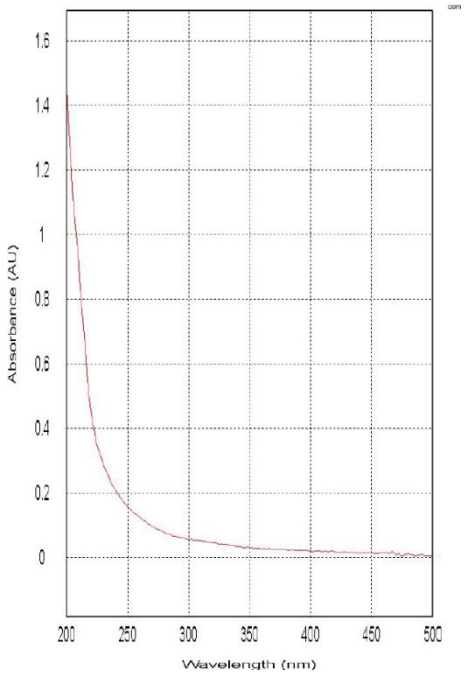


Figure S8. Experimental ECD spectrum of agarperoxinol B (2)

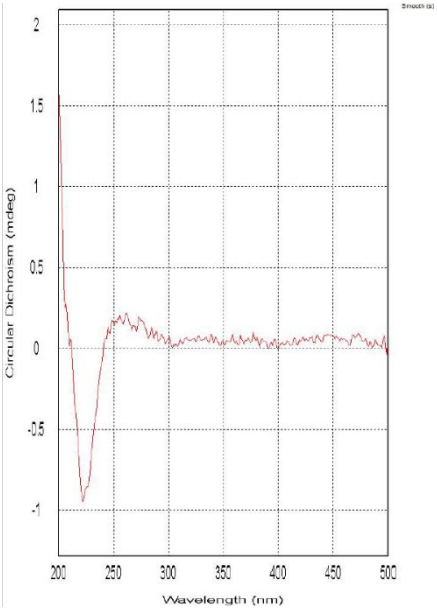


Figure S9. Optical rotation of agarperoxinol A (1)

No.	Sample Name	Optical Rotation Monitor	Specific O.R.	Path Length[mm]	Concentration[w/v%]	Water content[%]	S.D.	C.V.	Comment
1	compound 40	-0.0041	-28.0690	10	0.1450	0	3.3480	11.9276	
2	compound 40-1	-0.0039	-26.8966						
3	compound 40-2	-0.0046	-31.7241						
4	compound 40-3	-0.0037	-25.5172						
5	compound 40-4	-0.0048	-33.1034						
6	compound 40-5	-0.0038	-26.2069						
7	compound 40-6	-0.0031	-21.3793						
8	compound 40-7	-0.0042	-28.9655						
9	compound 40-8	-0.0040	-27.5862						
10	compound 40-9	-0.0043	-29.6552						
11	compound 40-10	-0.0043	-29.6552						

Figure S10. MS spectrum of agarperoxinol A (1)

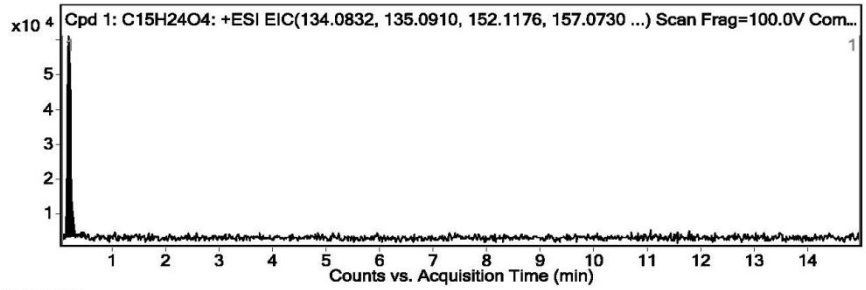
Qualitative Compound Report

Data File	Compound40.d	**Sample Name**	Compound40
Sample Type	Sample	**Position**	P1-A7
Instrument Name	Instrument 1	**User Name**	
Acq Method	DIP method-0.m	**Acquired Time**	4/20/2017 12:55:23 PM
IRM Calibration Status	Success	**DA Method**	Default.m
Comment			
Sample Group		**Info.**	
Stream Name	LC 1		

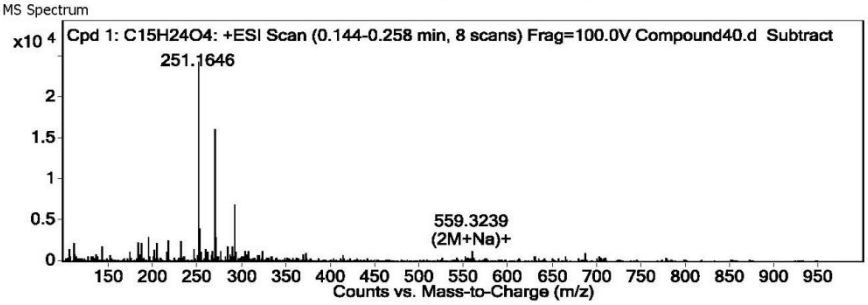
Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)
Cpd 1: C15H24O4	0.177	268.1673	16175	C15H24O4	268.1675	-0.73

Compound Label	RT	Algorithm	Mass
Cpd 1: C15H24O4	0.177	Find By Formula	268.1673

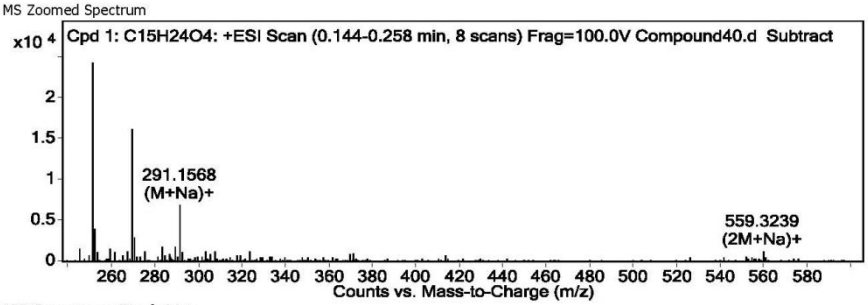


MS Spectrum



Qualitative Compound Report

MS Zoomed Spectrum



MS Spectrum Peak List

m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
251.1646				24294		
268.1619	268.1669	-18.77	1	118	C15 H24 O4	M*+
269.1745	269.1747	-0.85	1	16175	C15 H25 O4	(M+H)+
286.2005	286.2013	-2.78	1	947	C15 H28 N O4	(M+NH4)+
291.1568	291.1567	0.52	1	6963	C15 H24 Na O4	(M+Na)+
307.1347	307.1306	13.29	1	1280	C15 H24 K O4	(M+K)+
537.3259	537.3422	-30.37	1	204	C30 H49 O8	(2M+H)+
554.3707	554.3687	3.49	1	51	C30 H52 N O8	(2M+NH4)+
559.3239	559.3241	-0.42	1	1332	C30 H48 Na O8	(2M+Na)+
575.3091	575.2981	19.1	1	202	C30 H48 K O8	(2M+K)+

--- End Of Report ---

Figure S11. ^{1}H NMR spectrum of agarperoxinol B (2) in $CDCl_3$ (800 MHz)

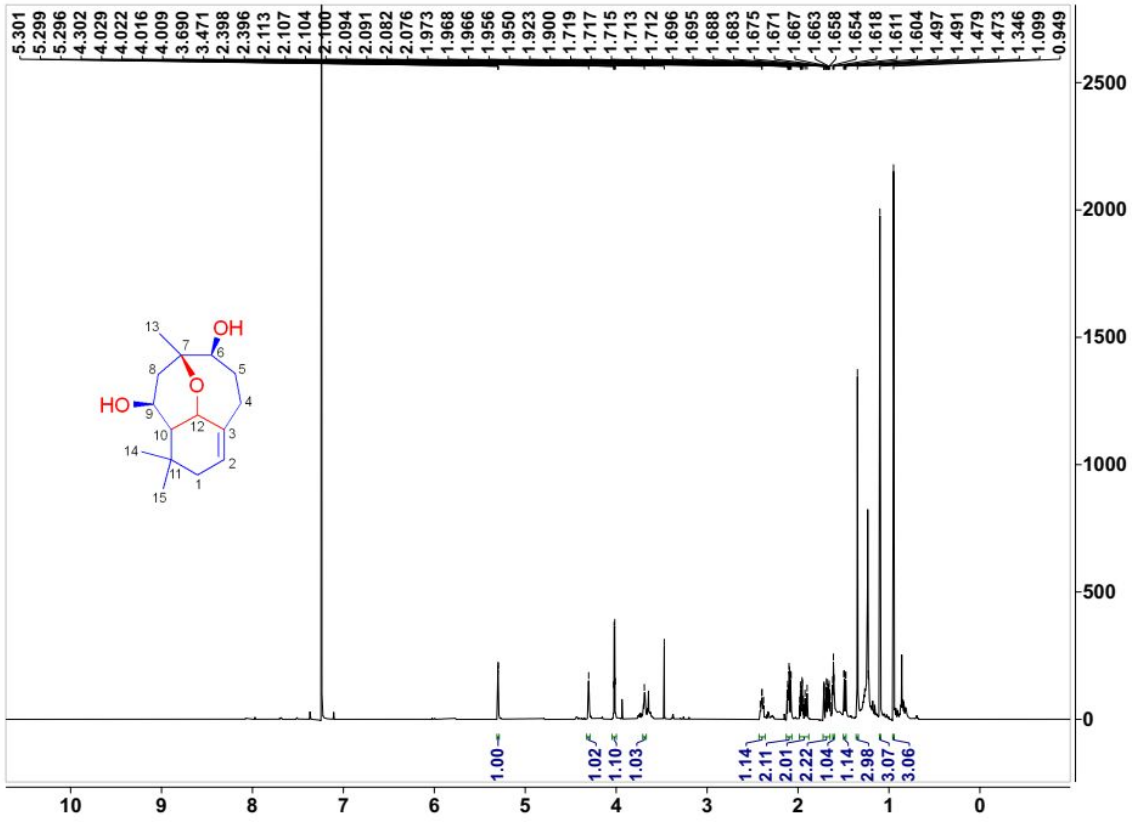


Figure S12. ^{13}C NMR spectrum of agarperoxinol B (2) in $CDCl_3$ (800 MHz)

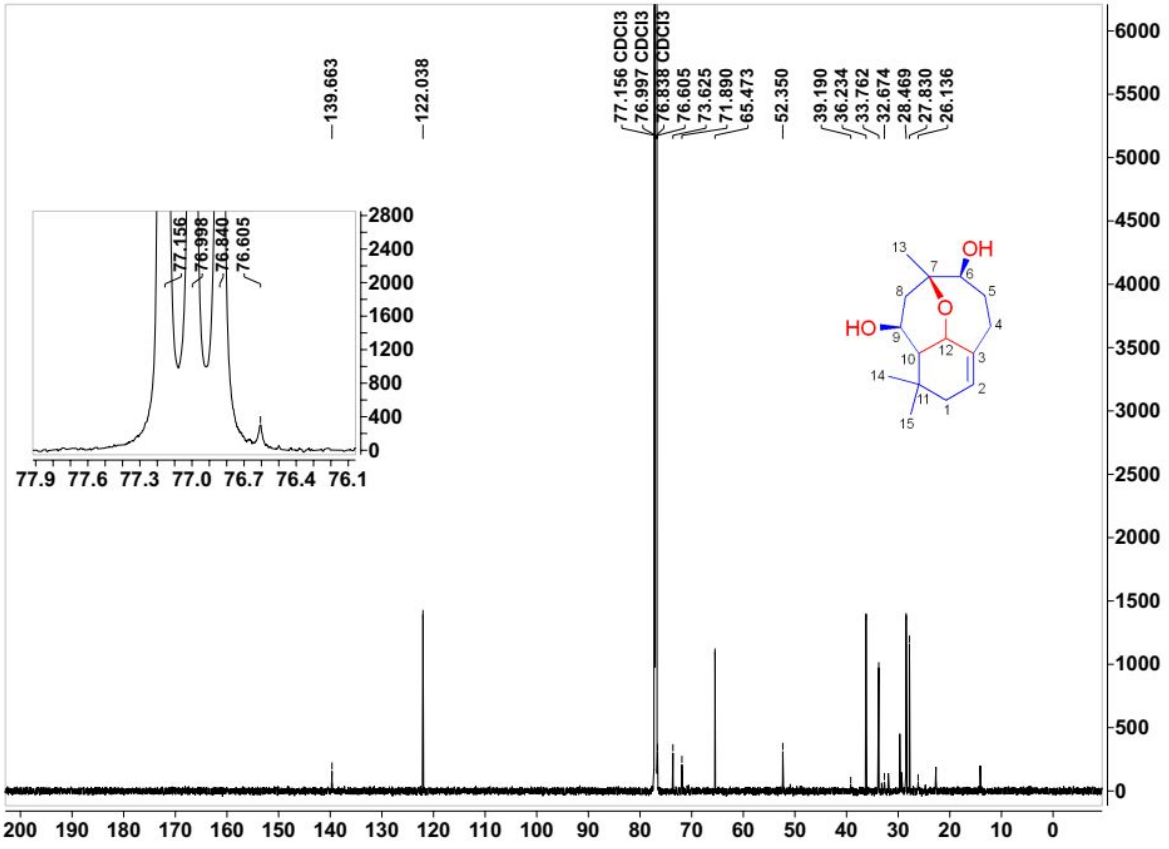


Figure S13. HSQC spectrum of agarperoxinol B (2) in $CDCl_3$ (800 MHz)

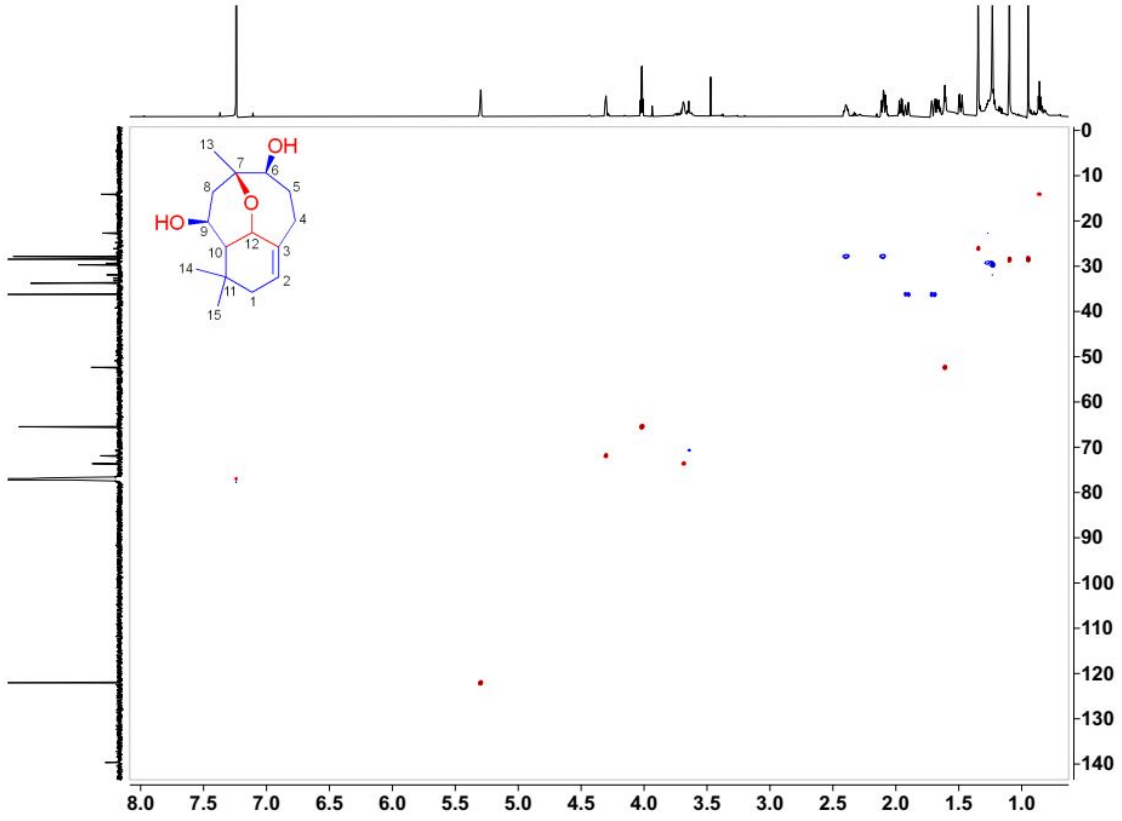


Figure S14. HMBC spectrum of agarperoxinol B (2) in $CDCl_3$ (800 MHz)

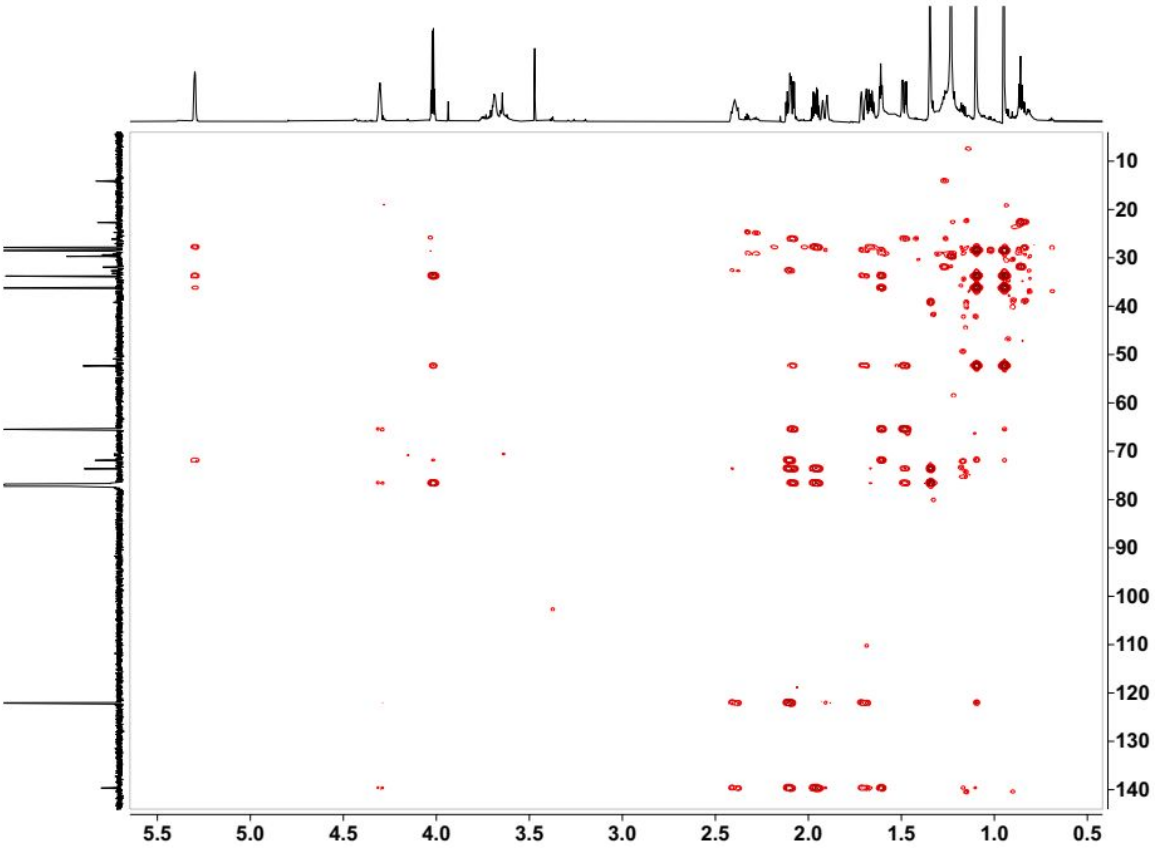


Figure S15. COSY spectrum of agarperoxinol B (2) in $CDCl_3$ (800 MHz)

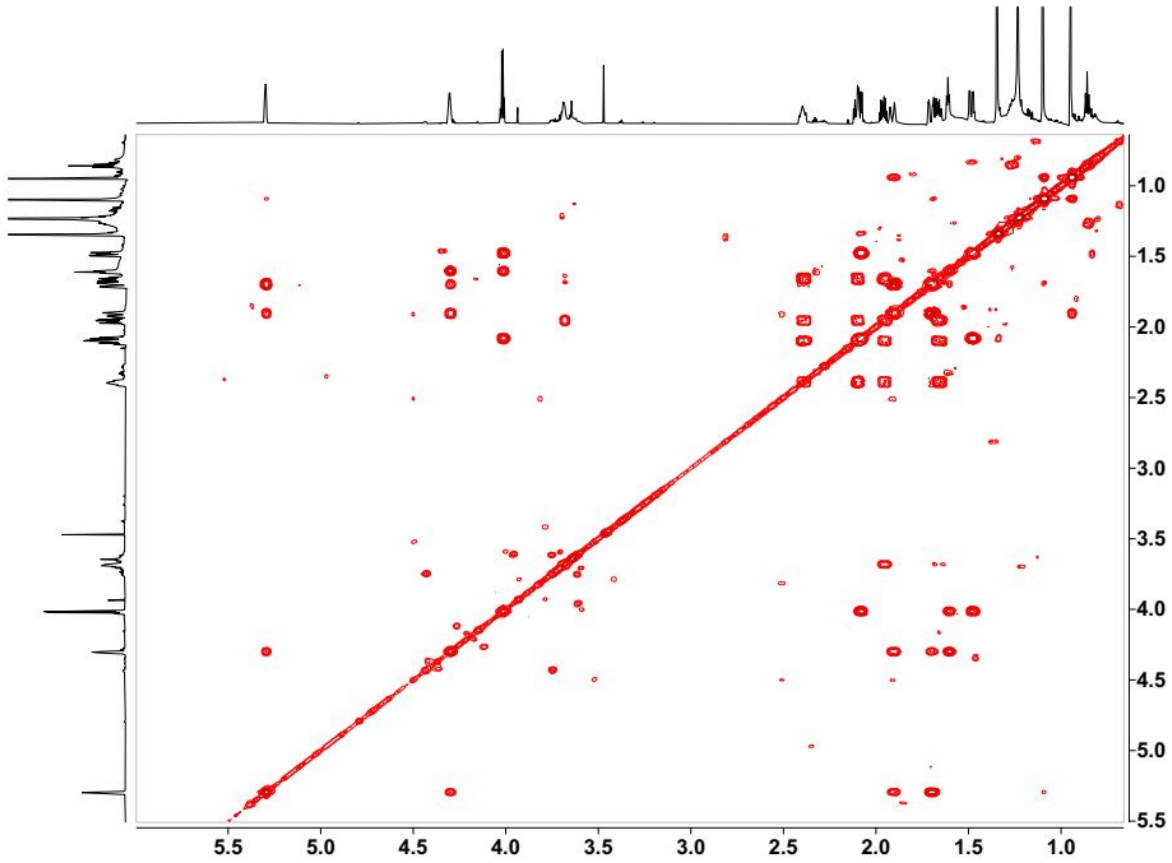


Figure S16. NOESY spectrum of agarperoxinol B (2) in $CDCl_3$ (800 MHz)

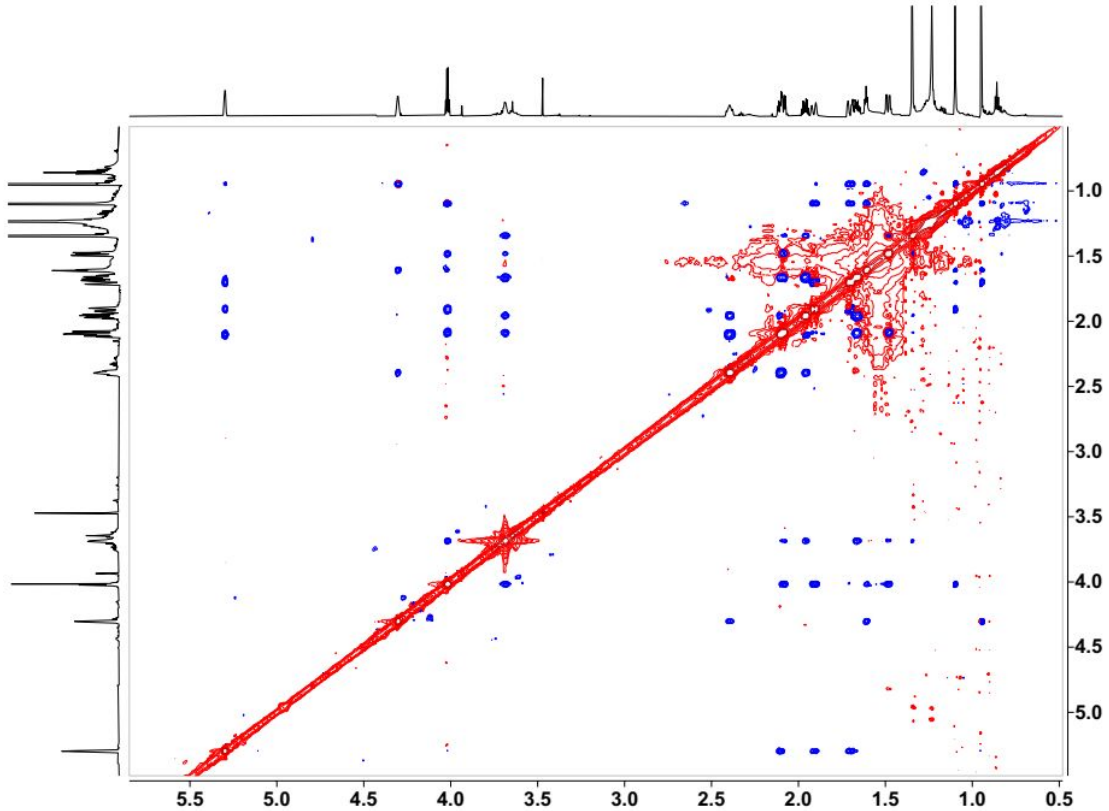


Figure S17. MS spectrum of agarperoxinol B (2)

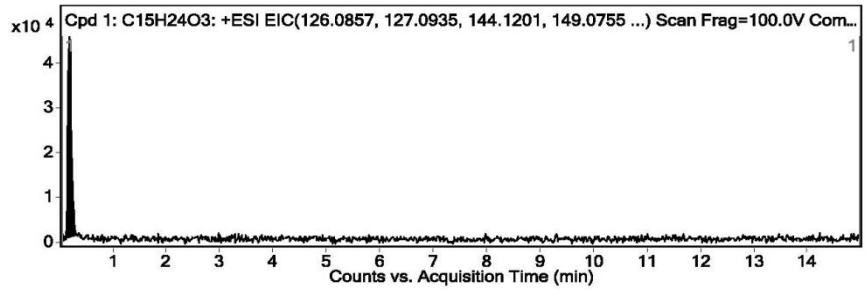
Qualitative Compound Report

Data File	Compound47.d	**Sample Name**	Compound47
Sample Type	Sample	**Position**	P1-B4
Instrument Name	Instrument 1	**User Name**	
Acq Method	DIP method-0.m	**Acquired Time**	4/20/2017 2:29:41 PM
IRM Calibration Status	Success	**DA Method**	Default.m
Comment			
Sample Group		**Info.**	
Stream Name	LC 1		

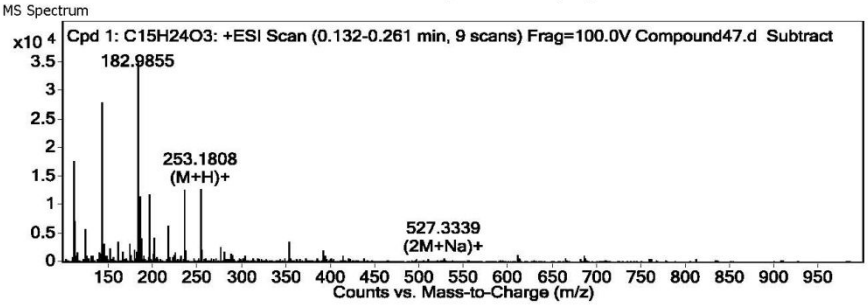
Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)
Cpd 1: C15H24O3	0.18	252.1735	12909	C15H24O3	252.1725	3.68

Compound Label	RT	Algorithm	Mass
Cpd 1: C15H24O3	0.18	Find By Formula	252.1735

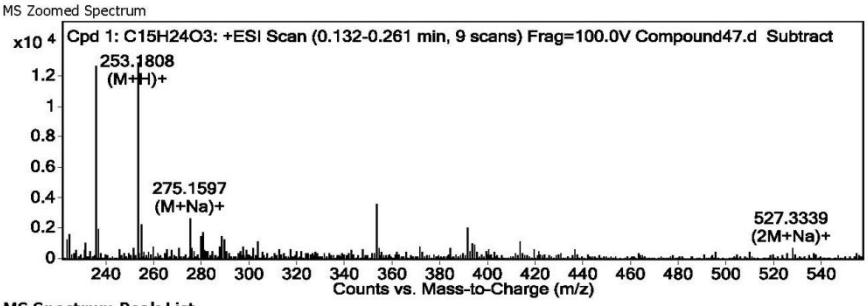


MS Spectrum



Qualitative Compound Report

MS Zoomed Spectrum



MS Spectrum Peak List

m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
182.9855				34867		
184.9862				11442		
252.1733	252.172	5.28	1	292	C15 H24 O3	M*+
253.1808	253.1798	3.8	1	12909	C15 H25 O3	(M+H)+
270.2055	270.2064	-3.17	1	778	C15 H28 N O3	(M+NH4)+
275.1597	275.1618	-7.64	1	2727	C15 H24 Na O3	(M+Na)+
291.1356	291.1357	-0.23	1	411	C15 H24 K O3	(M+K)+
504.3426	504.3445	-3.94	1	158	C30 H48 O6	2M*+
522.3749	522.3789	-7.63	1	89	C30 H52 N O6	(2M+NH4)+
527.3339	527.3343	-0.74	1	774	C30 H48 Na O6	(2M+Na)+

--- End Of Report ---

Figure S18. UV spectrum of agarperoxinol B (2)

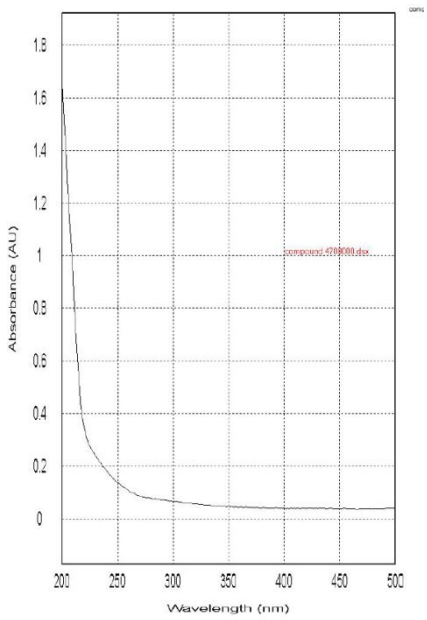


Figure S19. Experimental ECD spectrum of agarperoxinol B (2)

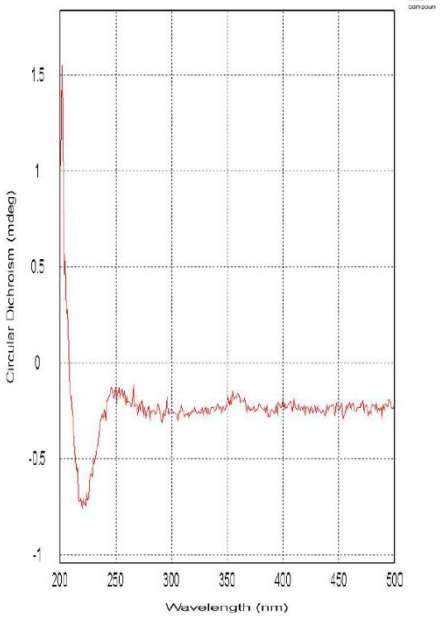


Figure S20. Optical rotation of agarperoxinol B (2)

No.	Sample Name	Optical Rotation Monitor	Specific O.R.	Path Length[mm]	Concentration[w/v%]	Water content[%]	S.D.	C.V.	Comment
1	compound 47	0.0129	108.2744	10	0.1200	1.1	10.8725	10.0416	
2	compound 47-1	0.0138	116.2791						
3	compound 47-2	0.0129	108.6957						
4	compound 47-3	0.0106	89.3158						
5	compound 47-4	0.0132	111.2235						
6	compound 47-5	0.0137	115.4365						
7	compound 47-6	0.0140	117.9643						
8	compound 47-7	0.0131	110.3809						
9	compound 47-8	0.0104	87.6306						
10	compound 47-9	0.0137	115.4365						
11	compound 47-10	0.0131	110.3809						

Figure S21. The Inhibition of p-p38 and p38 phosphorylation protein expressed by agarperoxinol B on LPS-activated microglial cells.

Protein levels were determined by using Western blot analysis. The relative intensity of p-p38/p38 bands were calculated by densitometry. Values are mean±SEM (n=5); ***p<0.001 compared to control group.