SUPPLEMENTARY DATA

In vitro evaluation of anti-inflammatory properties of selected South African medicinal plants: A bio-guided purification of anti-inflammatory compounds from *Conyza scabrida*

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1. **Compound C1:**

Table S1: ^{1}H, ^{13}C, HMBC and COSY-NMR

Compound C1 (5,3',4'-trihydroxy-3,6,7,8-tetramethoxyflavone)				
Position	**δ_C**	**δ_H (J)**	**HMBC**	**COSY**
2	158.6, C	-	-	-
3	139.7, C	-	-	-
4	180.7, C	-	-	-
5	150.5, C	-	-	-
6	137.4, C	-	-	-
7	154.4, C	-	-	-
8	134.5, C	-	-	-
9	146.5, C	-	-	-
10	108.5, C	-	-	-
1'	123.0, C	-	-	-
2'	116.6, CH	7.70, d (2.03)	C2', C1', C3', C4'	-
3'	146.8, C	-	-	-
4'	150.0, C	-	-	-
5'	116.7, CH	6.92, d (8.46)	C2', C1', C3', C4'	H6'
6'	122.7, CH	7.62, dd (2.03, 8.51)	C2, C2', C4'	-
3-OCH3	61.6, OCH_3	3.89, s	C3	H2'
6-OCH3	60.6, OCH_3	3.82, s	C6	-
7-OCH3	62.4, OCH_3	4.08, s	C7	-
8-OCH3	62.8, OCH_3	3.94, s	C8	-

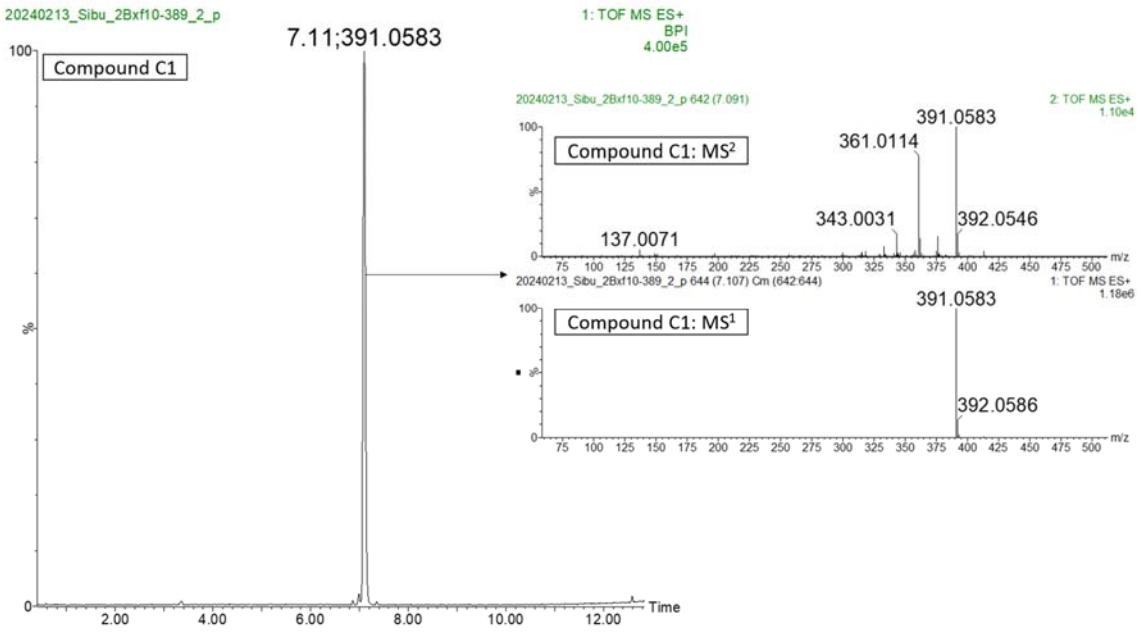

Figure S1: Positive mode ESI-MS *m/z* of compound C1 (5,3',4'-trihydroxy-3,6,7,8-tetramethoxyflavone)

2. Compound C1: Single Crystal X-ray Diffraction report.

Explanation: The molecular formula of compound C1 is C19H18O9 corresponding to molecilar weight 390.34. however, the compound co-crystallised with methanol (32.03)

Table S2: Single Crystal X-ray Diffraction report for compound C1 (5,3',4'-trihydroxy-3,6,7,8-tetramethoxyflavone).

Datablock: CompoundC1

Bond precision:	C-C = 0.0031 A		Wavelength=0.71073
Cell:	a=19.581(3)	b=6.5811(11)	c=15.248(2)
	alpha=90	beta=101.825(5)	gamma=90
Temperature: 173 K			
	Calculated		Reported
Volume	1923.2(5)		1923.2(5)
Space group	P 21/c		P 1 21/c 1
Hall group	-P 2ybc		-P 2ybc
Moiety formula	C19 H18 O9, C H4 O		C19 H18 O9, C H4 O
Sum formula	C20 H22 O10		C20 H22 O10
Mr	422.38		421.38
Dx,g cm-3	1.459		1.455
Z	4		4
Mu (mm-1)	0.118		0.118
F000	888.0		884.7
F000'	888.60		
h,k,lmax	26,8,20		26,8,20
Nref	4857		4812
Tmin,Tmax	0.972,0.980		
Tmin'	0.952		
Correction method= Not given			
Data completeness= 0.991		Theta(max)= 28.450	
R(reflections)= 0.0594(3851)			wR2(reflections)= 0.2072(4812)
S = 1.058	Npar= 284		

3. Compound C2: Hautriwaic acid

Table S3: ^{1}H, ^{13}C, HMBC and COSY-NMR

Compound C2 (Hautriwaic acid)				
Position	**δ_C**	**δ_H (J)**	**HMBC**	**COSY**
1	18.2, CH_2	1.71, m	C3, C9, C20	
2	27.8, CH_2	2.37 α, m		
		2.26 β, m		
3	139.5, CH	6.66, t (3.4)	C1, C2, C5, C18	H2
4	142,7 C	-		
5	43.4, C	-		
6	33.0, CH_2	2.41 α, dt (3.2)	C5, C7, C8, C10	H7
		1.13 β, td (4.5)		
7	28.2, CH_2	1.474, m	C5	H6a
8	37.7, CH	1.68, m	C11, C20	
9	38,7 C			
10	48.0, CH	1.562, m	C2 C5, C11, C20	
11	40.3, CH_2	1.69, m	C13	
		1.59, m		
12	19.3, CH_2	2.36, m	C11, C14, C13, C16	
		2.21, t (5.5)		
13	126,3 C	-		
14	112.0, CH	6.29, dd (0.9)	C13, C15, C16	
15	144.1, CH	7.38, d (1.7)	C14, C16	
16	139.9, CH	7.26, dd (0.9)	C13, C14, C15	
17	16.4, CH_3	0.87, d (6.8)	C7, C8, C11	H11
18	174.1, C	-		
19	66.3, CH_2	3.75, d (10.6)	C4, C5, C6, C10	H19a
		4.15, d (10.6)		
20	19.2, CH_2	0.80, s	C12, C8, C11, C10	

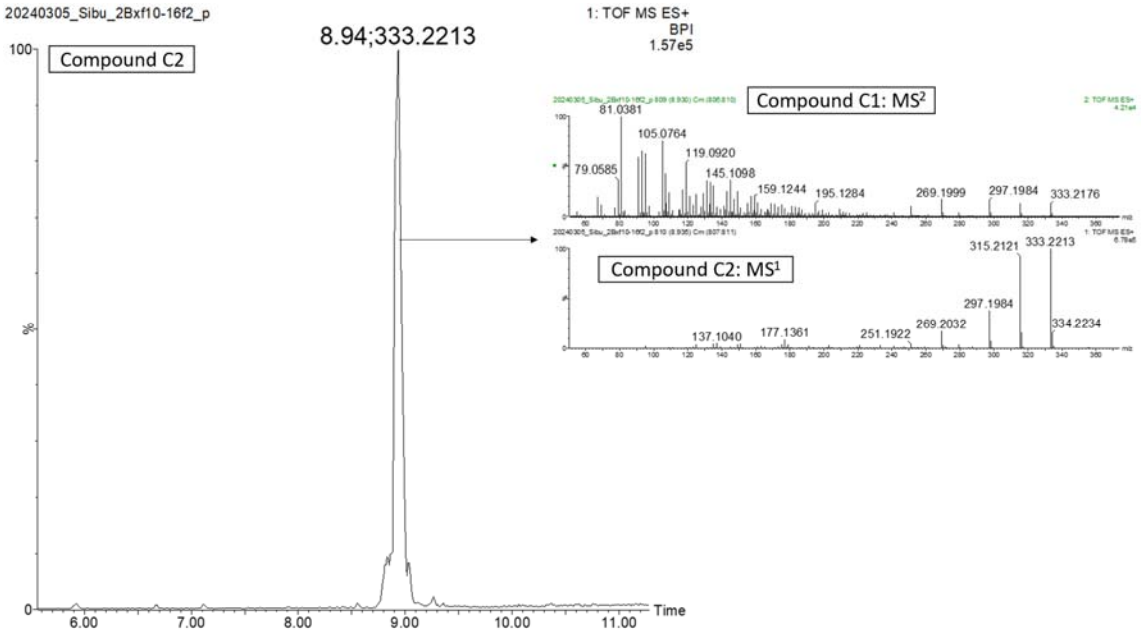

Figure S2: Positive mode ESI-MS *m/z* of compound C2 (Hautriwaic acid)

4. Compound C2

Table S4: Single Crystal X-ray Diffraction report for compound C2 (Hautriwaic acid).

Datablock: compound2final

Bond precision:	C-C = 0.0020 A		Wavelength=0.71073
Cell:	a=10.2101(3)	b=10.5191(4)	c=16.3089(6)
	alpha=90	beta=90	gamma=90
Temperature: 173 K			

	Calculated	Reported
Volume	1751.59(11)	1751.59(11)
Space group	P 21 21 21	P 21 21 21
Hall group	P 2ac 2ab	P 2ac 2ab
Moiety formula	C20 H28 O4	C20 H28 O4
Sum formula	C20 H28 O4	C20 H28 O4
Mr	332.42	332.42
Dx,g cm-3	1.261	1.261
Z	4	4
Mu (mm-1)	0.086	0.086
F000	720.0	720.0
F000'	720.35	
h,k,lmax	13,14,21	13,14,21
Nref	4435[2518]	4390
Tmin,Tmax	0.981,0.989	
Tmin'	0.981	

Correction method= Not given

Data completeness= 1.74/0.99 Theta(max)= 28.483

R(reflections)= 0.0315(4260) wR2(reflections)= 0.0878(4390)

S = 1.075 Npar= 221

5. Std curve

Table S5: Standard curve

	1	2	3	4			*average*	*stdev*
A	0,058	0,057	0,058	0,059	540	0,3125	0,058	0,000816
B	0,059	0,06	0,062	0,064	540	0,625	0,061	0,002217
C	0,074	0,073	0,076	0,08	540	1,25	0,076	0,003096
D	0,101	0,104	0,09	0,099	540	2,5	0,099	0,006028
E	0,17	0,171	0,164	0,179	540	5	0,171	0,006164
F	0,284	0,275	0,275	0,284	540	10	0,280	0,005196
G	0,411	0,404	0,385	0,399	540	15	0,400	0,010996
H	0,509	0,511	0,518	0,508	540	20	0,512	0,004509

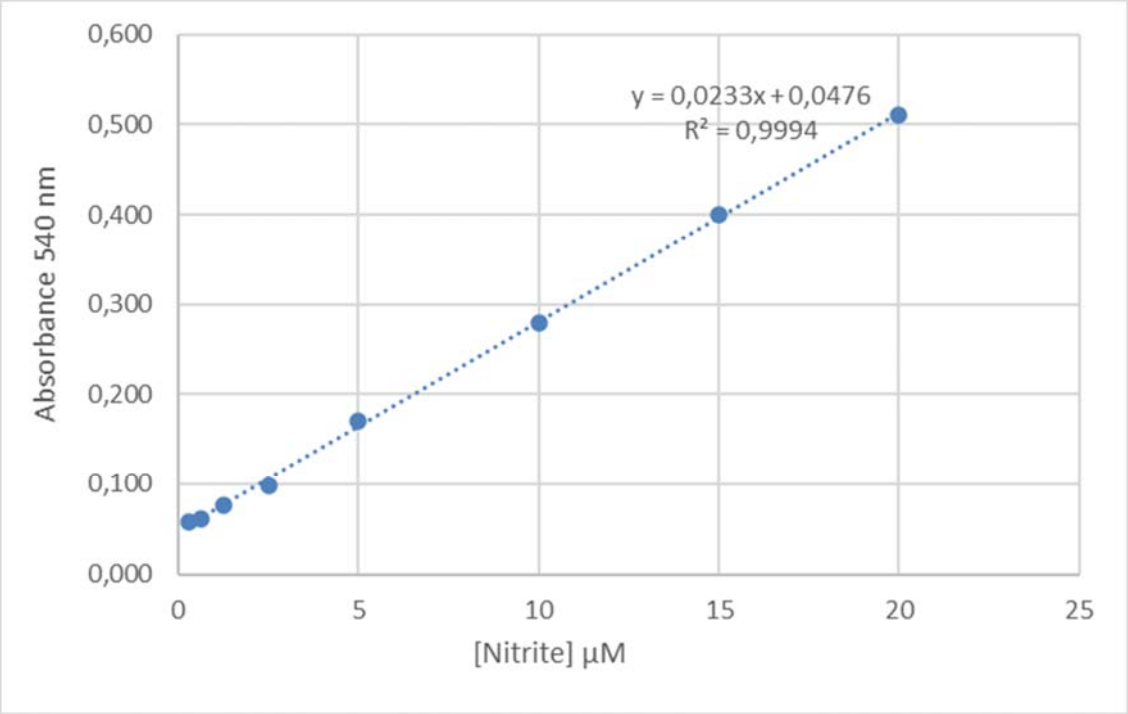

Figure S3: Standard curve

6. Compound C1 and C2: NO production on LPS-activated macrophages

Table S6: Inhibition of NO production of LPS-activated macrophages

		C1	C1	C1	C1	C1	C1	C1	C1		
Untreated	LPS	0,1	1,56	3,13	6,25	12,5	25	50	100	AG control	AG control
0,40	7,40	8,26	8,13	8,17	8,04	7,57	7,91	6,58	3,24	3,32	3,62
0,02	8,69	8,82	8,52	8,77	8,73	7,91	8,64	7,53	3,92	3,54	3,97
0,02	8,77	9,16	8,90	9,12	8,77	9,07	8,60	8,00	4,09	3,67	4,52
0,23	9,12	8,86	8,99	8,77	8,86	8,99	8,64	7,91	4,01	3,54	4,22
0,36	8,82	9,03	9,12	8,94	8,90	8,69	8,60	5,85	0,49	2,38	7,91
0,15	9,03	9,12	9,16	9,12	9,03	8,73	9,24	5,64	0,53	2,33	
0,62	8,77	9,03	8,86	8,90	8,43	8,82	9,16	6,37	0,58	2,46	8,94
0,27	8,73	9,29	9,12	8,86	8,30	8,77	9,03	5,77	0,58	2,29	9,72
		C2	C2	C2	C2	C2	C2	C2	C2		

Average

Concentration	*0,1*	*1,56*	*3,13*	*6,25*	*12,5*	*25*	*50*	*100*
Untreated	0,26							
LPS	8,72							
AG control	4,08	3,52	2,37					
C1	8,77	8,63	8,71	8,60	8,39	8,45	7,51	3,82
C2	9,12	9,06	8,95	8,67	8,75	9,01	5,91	0,54

Stdev

Concentration	*0,1*	*1,56*	*3,13*	*6,25*	*12,5*	*25*	*50*	*100*
Untreated	0,20							
LPS control	0,61							
AG control	0,38	0,14	0,07					
C1	0,38	0,39	0,39	0,38	0,76	0,36	0,65	0,39
C2	0,12	0,14	0,11	0,35	0,06	0,29	0,32	0,04

7. Compound C1 and C2: MTT assay

Table S7: % cell viability of LPS-activated macrophages after 24 hours of exposure to compound C1 (5,3',4'-trihydroxy-3,6,7,8-tetramethoxyflavone) and C2 (Hautriwaic acid)

Untreated	LPS	0,1	1,56	3,13	6,25	12,5	25	50	100	AG	AG
81,26	95,34	94,19	102,73	105,43	108,89	94,88	112,97	101,19	98,73	101,12	110,20
105,35	99,12	98,42	100,65	104,66	105,50	101,27	106,66	102,96	109,04	92,88	86,88
107,27	103,73	95,65	94,96	106,12	106,81	102,35	96,81	99,96	123,82	101,58	95,11
105,04	100,42	94,42	104,73	107,89	101,96	99,19	97,81	100,50	106,73	100,58	95,04
105,66	100,81	103,35	101,81	107,12	101,27	93,57	78,57	82,11	86,26	91,11	93,19
105,35	94,81	96,04	106,66	108,12	102,96	92,80	79,42	86,19	83,57	94,19	73,49
107,27	101,27	97,73	106,89	109,20	113,12	92,11	80,11	94,73	84,65	103,42	103,35
113,81	104,50	97,11	104,58	99,27	104,89	92,88	78,03	93,11	83,80	98,58	98,35

Average

Concentration	***0,1***	***1,56***	***3,13***	***6,25***	***12,5***	***25***	***50***	***100***
Untreated	103,88							
LPS control	97,36							
AG control	96,81	99,04	96,83					
C1	95,67	100,77	106,02	105,79	99,42	103,56	101,15	109,58
C2	98,56	104,98	105,93	105,56	92,84	79,03	89,03	84,57

Stdev

Concentration	***0,1***	***1,56***	***3,13***	***6,25***	***12,5***	***25***	***50***	***100***
Untreated cells	9,58							
LPS control	8,34							
AG control	9,73	4,12	5,36					
C1	1,94	4,22	1,38	2,91	3,30	7,68	1,31	10,47
C2	3,27	2,36	4,52	5,25	0,60	0,92	5,92	1,22

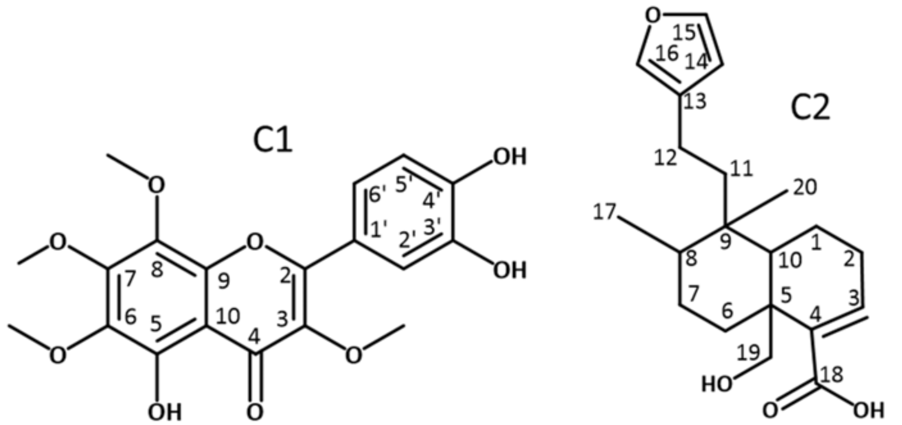

Figure S4: Isolated compounds from the active fraction and extract of *C. scabrida* 5,3',4'-trihydroxy-3,6,7,8-tetramethoxyflavone (C1) and Hautriwaic acid (C2)

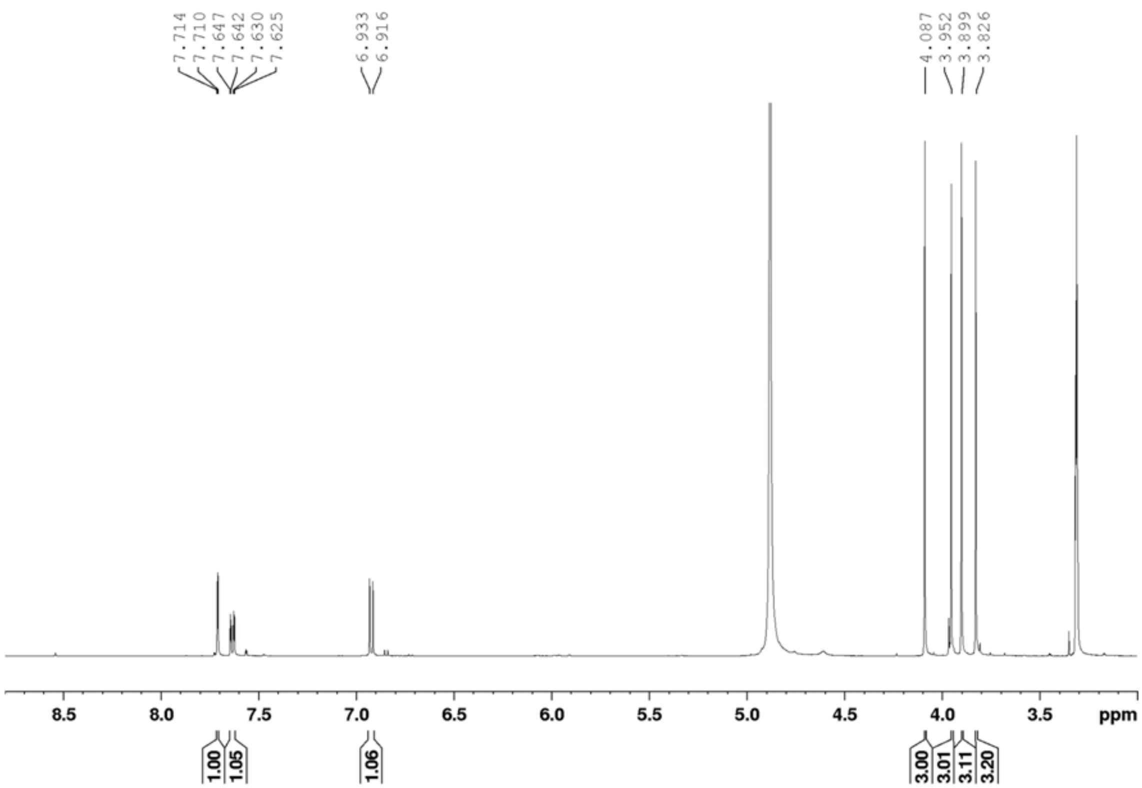

Figure S5: ^{1}H-NMR (500 MHz, CD_3OD) spectrum of compound C1(5,3',4'-trihydroxy-3,6,7,8-tetramethoxyflavone)

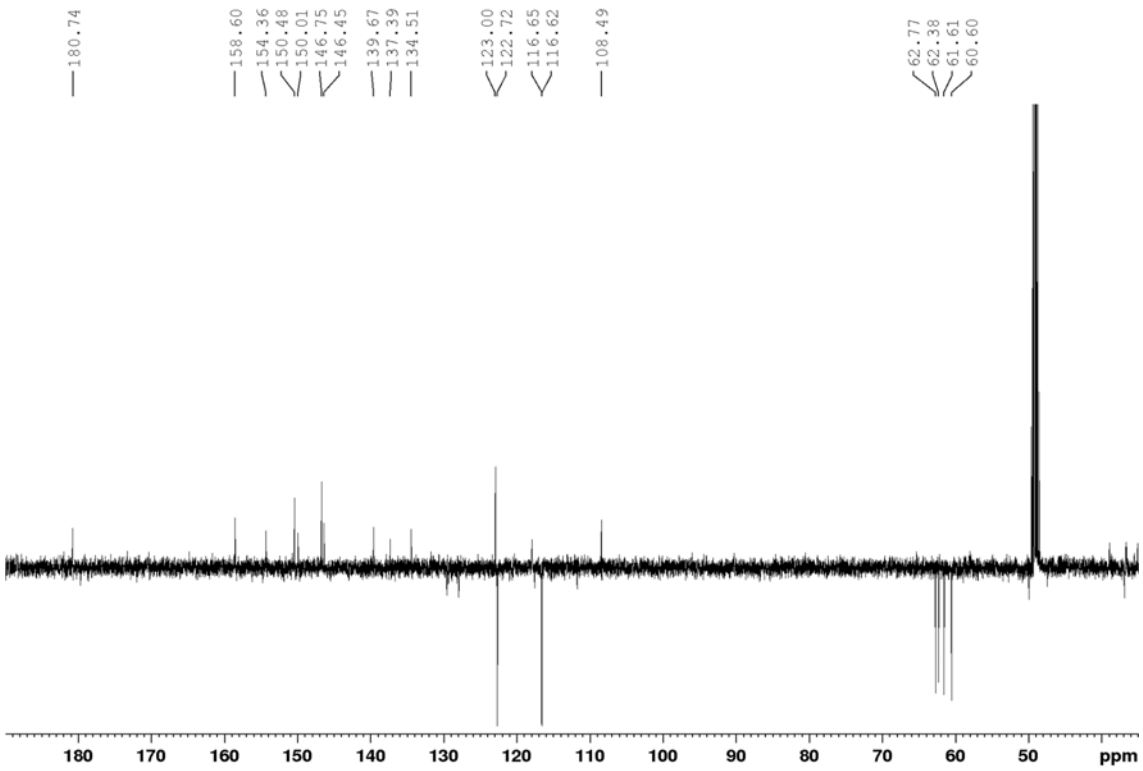

Figure S6: ATP NMR (500 MHz, CD3OD) spectrum of compound C1(5,3',4'-trihydroxy-3,6,7,8-tetramethoxyflavone)

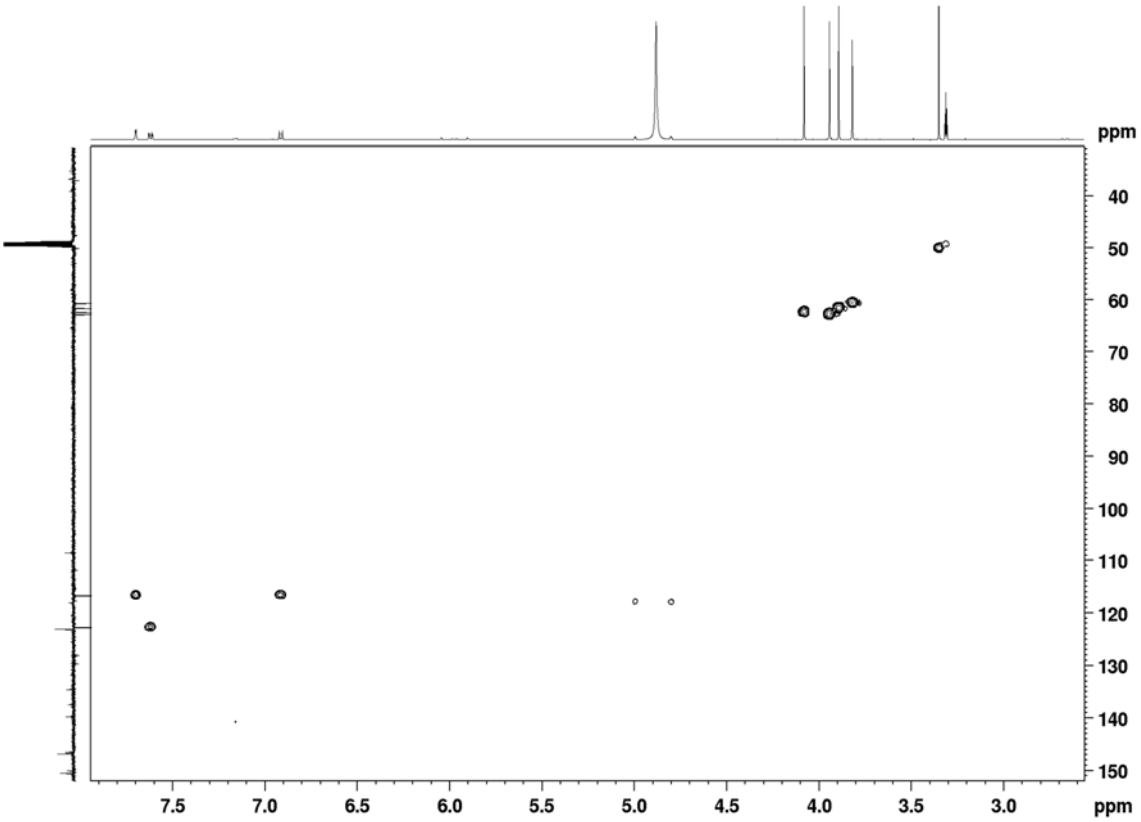

Figure S7: HSQC NMR (500 MHz, CD3OD) spectrum of compound C1(5,3',4'-trihydroxy-3,6,7,8-tetramethoxyflavone)

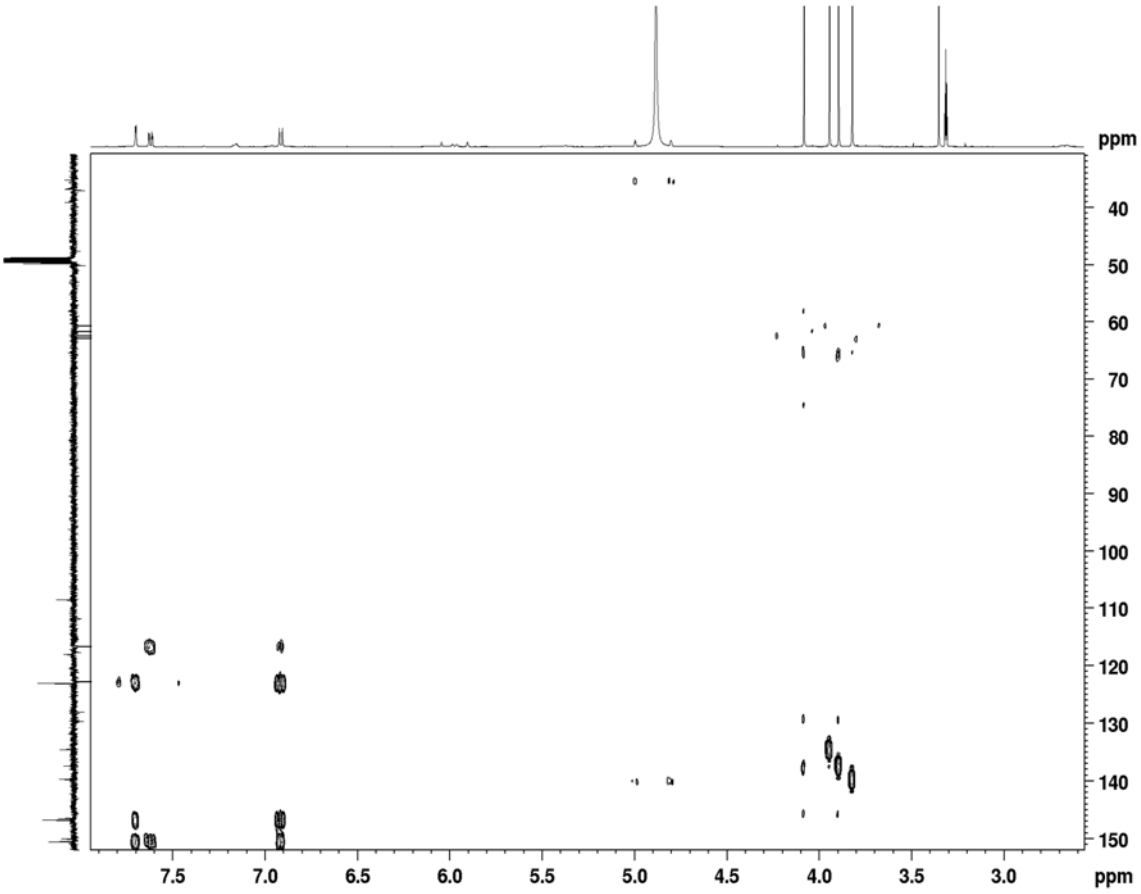

Figure S8: HMBC NMR (500 MHz, CD3OD) spectrum of compound C1(5,3',4'-trihydroxy-3,6,7,8-tetramethoxyflavone)

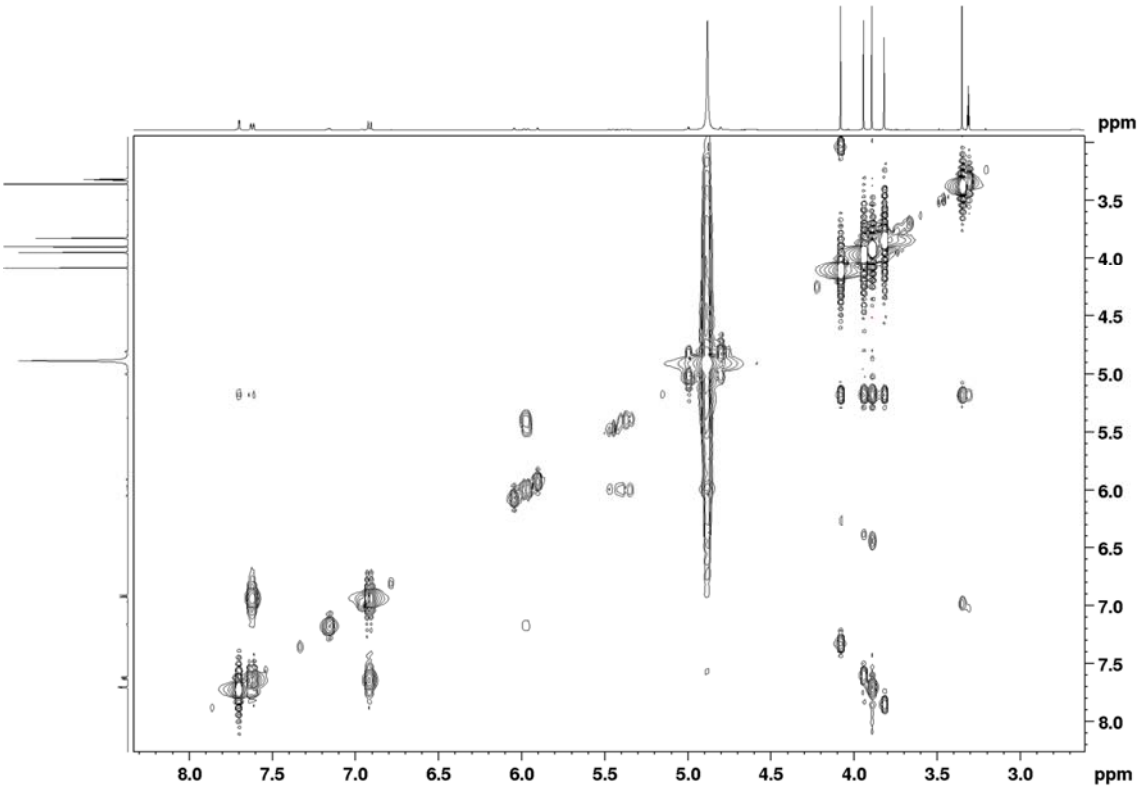

Figure S9: COSY NMR (500 MHz, CD3OD) spectrum of compound C1(5,3',4'-trihydroxy-3,6,7,8-tetramethoxyflavone)

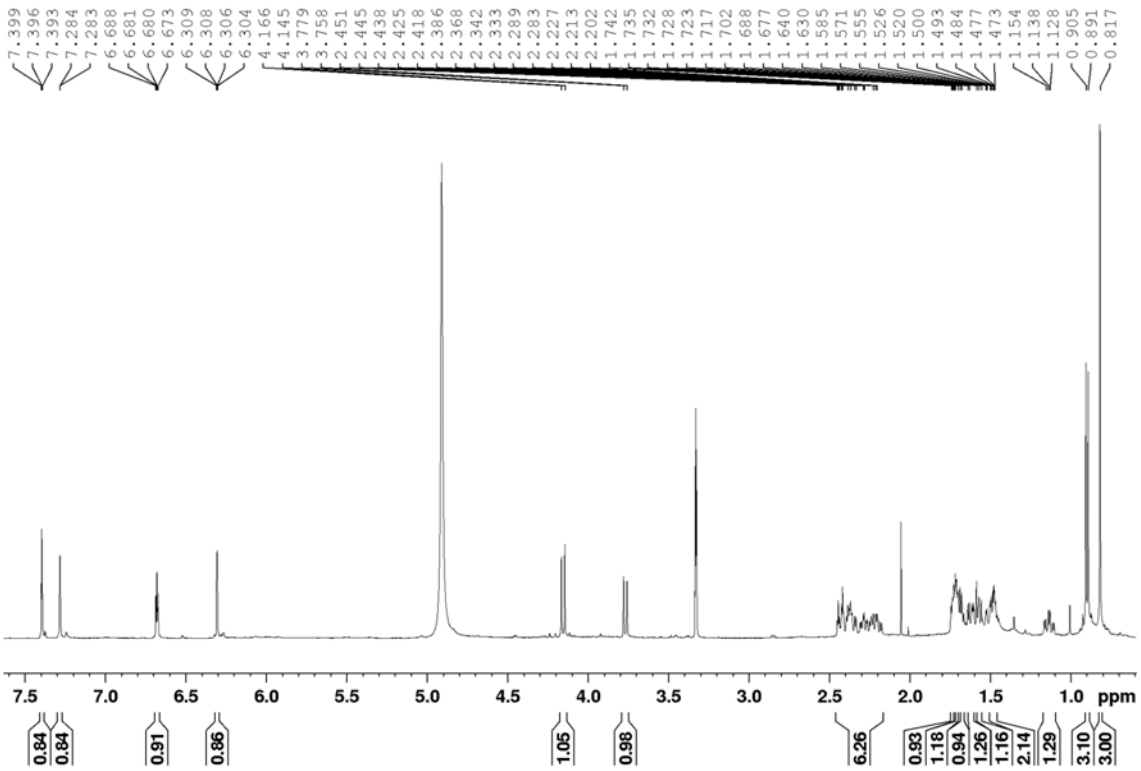

Figure S10: ^{1}H NMR (500 MHz, CD3OD) spectrum of compound C1(Hautriwaic acid)

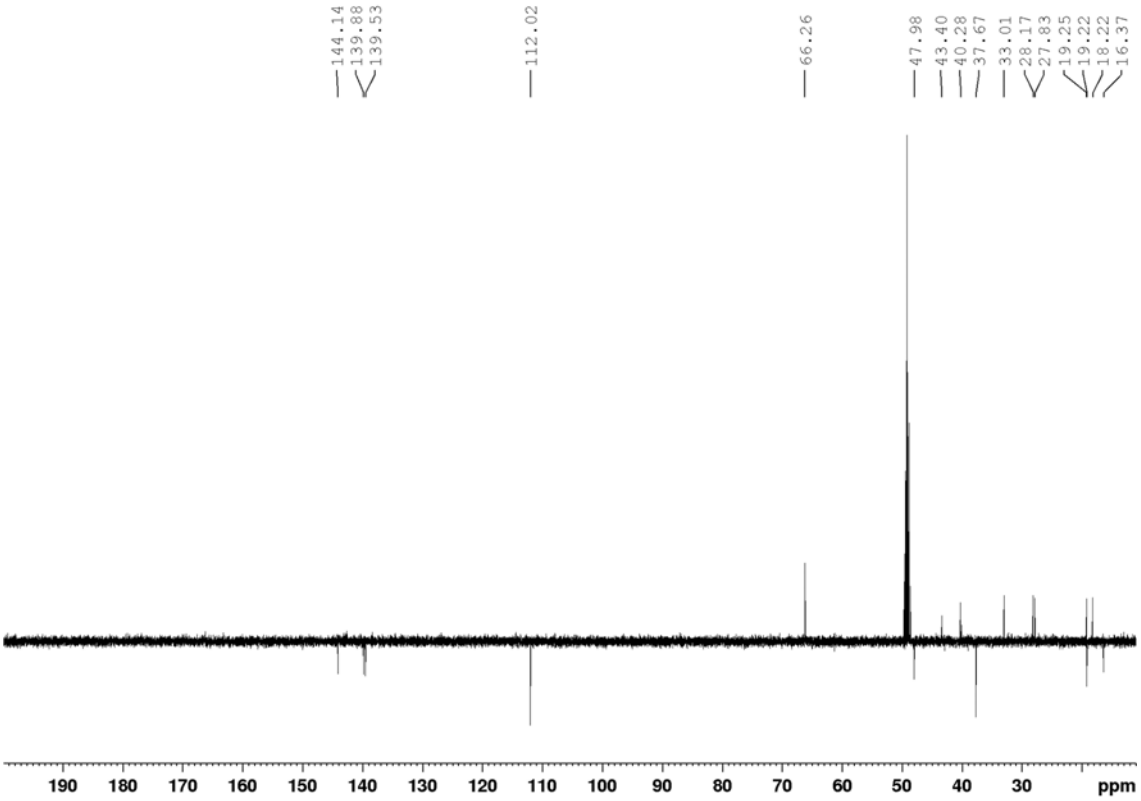

Figure S11: ATP NMR (500 MHz, CD3OD) spectrum of compound C2 (Hautriwaic acid)

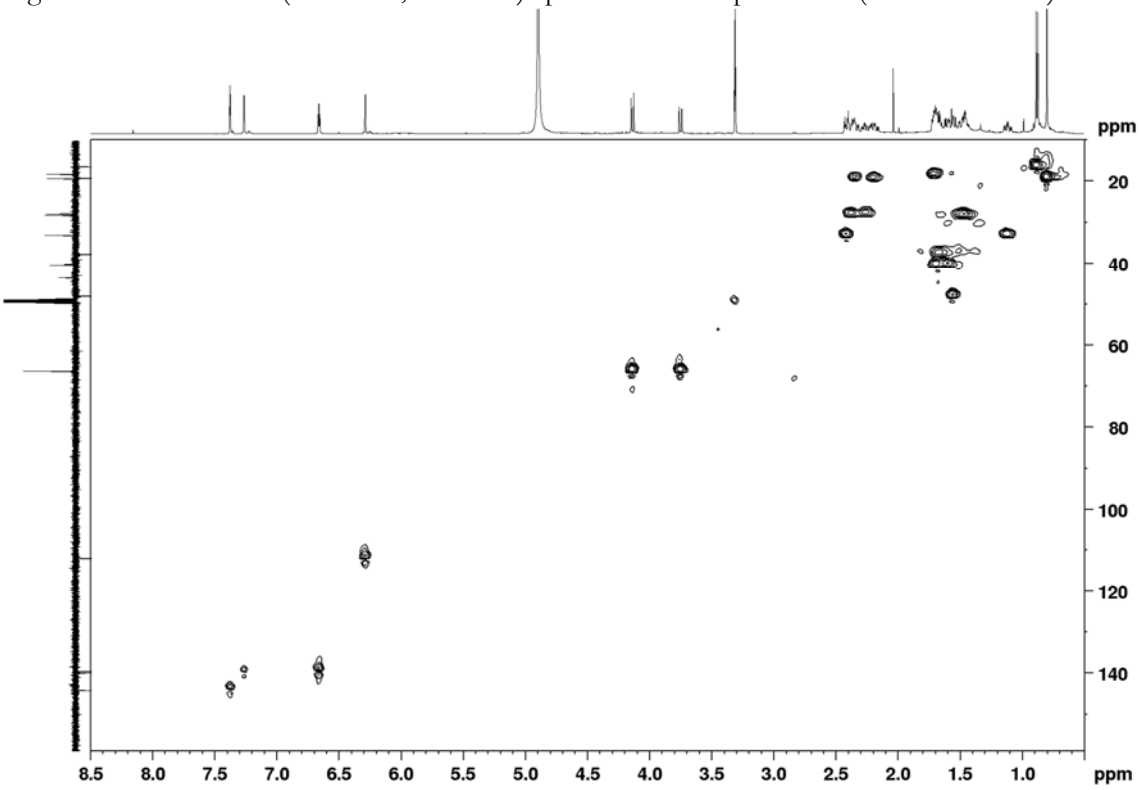

Figure S12: HSQC NMR (500 MHz, CD3OD) spectrum of compound C2 (Hautriwaic acid)

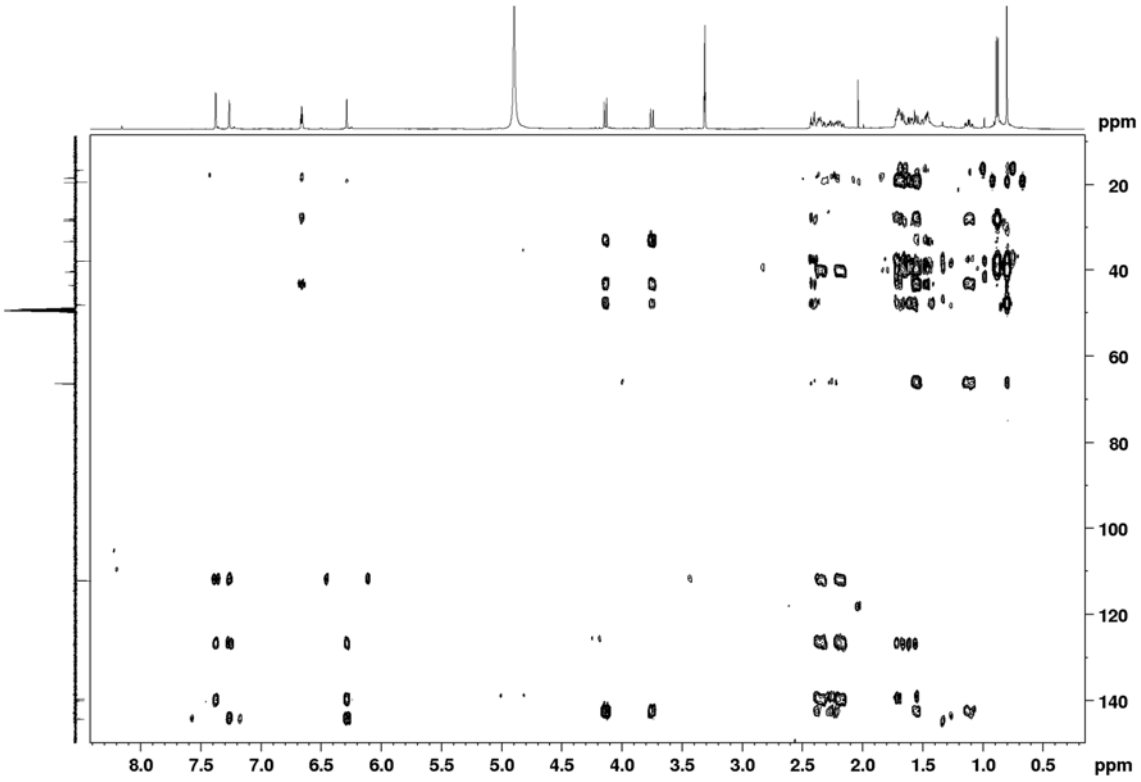

Figure S13: HMBC NMR (500 MHz, CD3OD) spectrum of compound C2 (Hautriwaic acid)

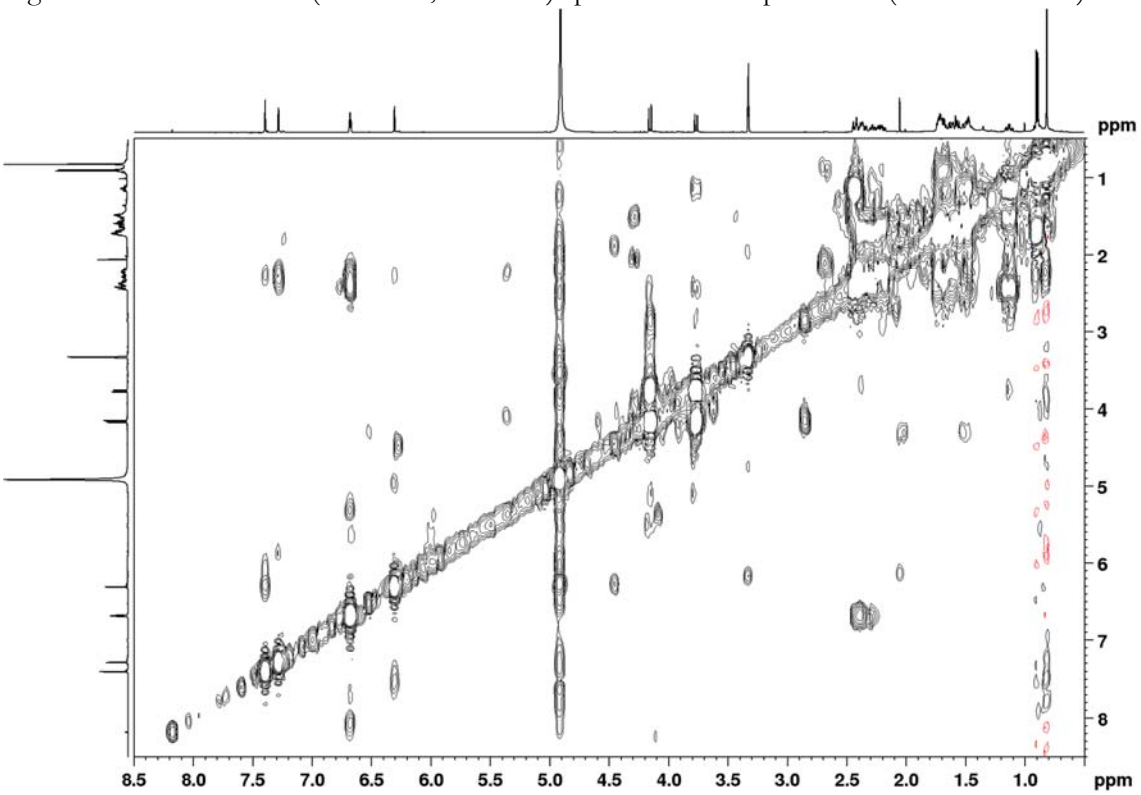

Figure S14: COSY NMR (500 MHz, CD3OD) spectrum of compound C2 (Hautriwaic acid)