Electronic Supplementary Information

Desmoschinensisflavones A and B, two rare flavones having a hybrid benzyl benzoate ester-flavone structural framework from *Desmos chinensis* Lour.

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Abstract: Two rare flavones having a hybrid benzyl benzoate ester-flavone structural framework, desmoschinensisflavones A and B (**1** and **2**), together with 12 known compounds (**3**-**14**) were isolated from the fruit, leaf, and twig extracts of *Desmos chinensis* (red flower). The new structures were characterized by UV, IR, NMR, and HRESITOFMS data. Desmoschinensisflavones A and B have a distinctive skeleton of benzoate ester-flavones with a C-4´´and C-6 and C-8 connection via a methylene group, respectively. Plausible biosynthesis pathways to compounds **1** and **2** are proposed based on an intermolecular nucleophilic 1,4-addition to *ortho*-quinone intermediates. Compounds **6**-**8** and **12** showed weakly antioxidant inhibition with IC_{50} values in the range of 65.4-74.6 µM.

Keywords: *Desmos chinensis*; Hybrid benzoate ester-flavones; Antioxidant; *α*-Glucosidase inhibition

Contents

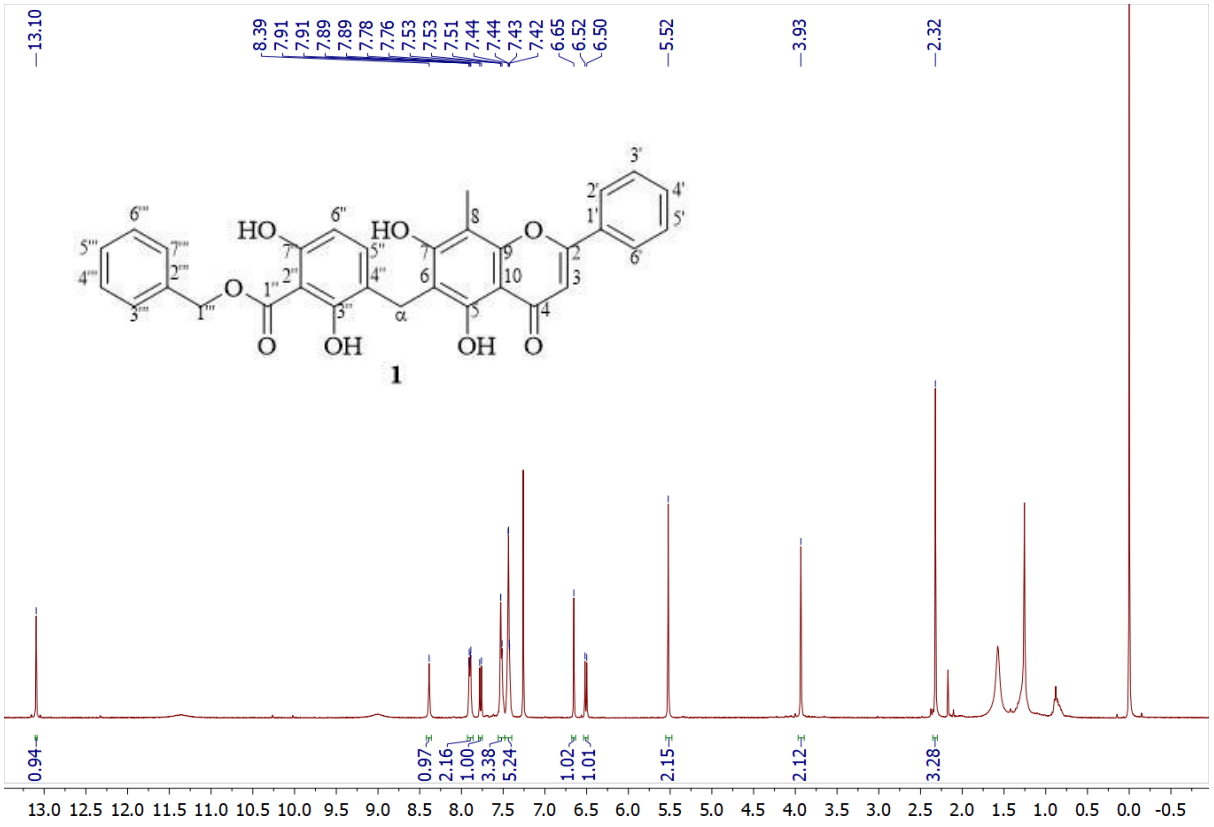


Figure S1 ^{1}H NMR spectrum of **1** recorded in $CDCl_3$

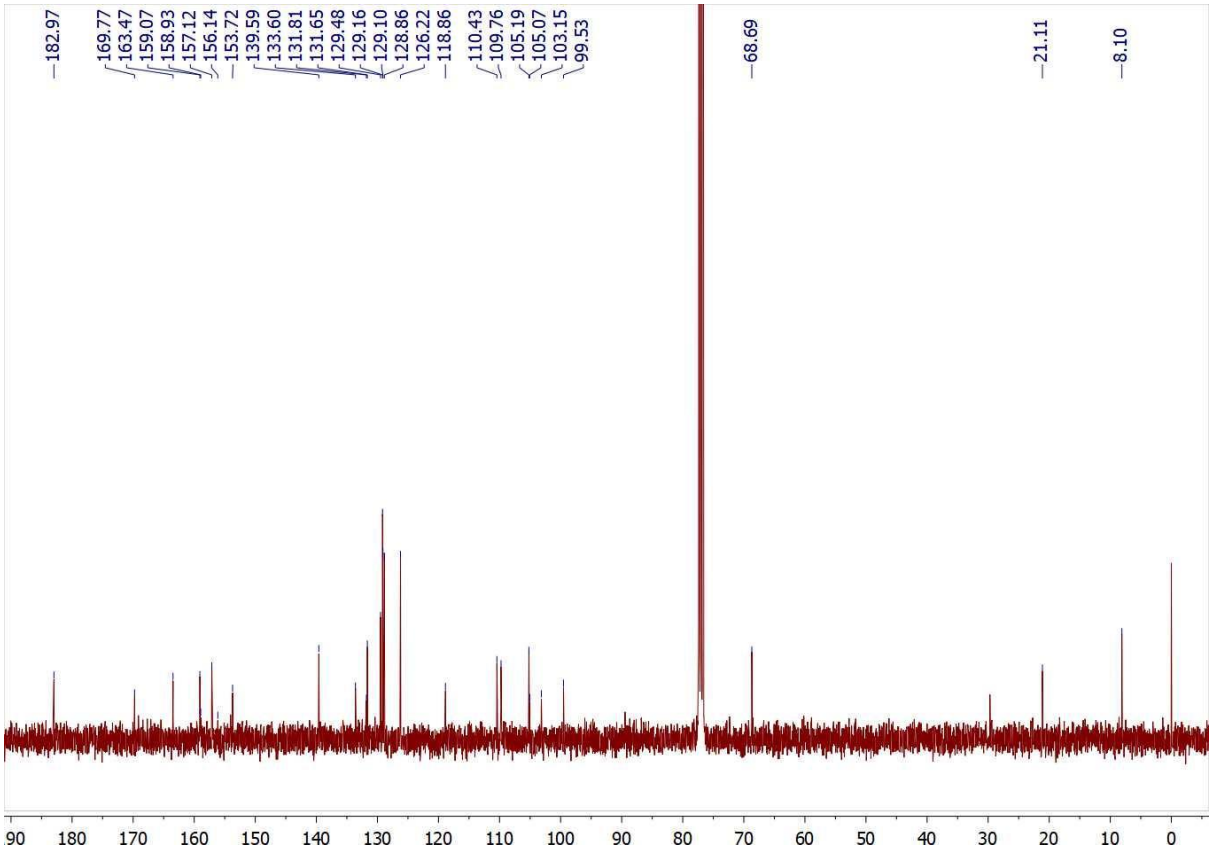


Figure S2 ^{13}C NMR spectrum of **1** recorded in $CDCl_3$

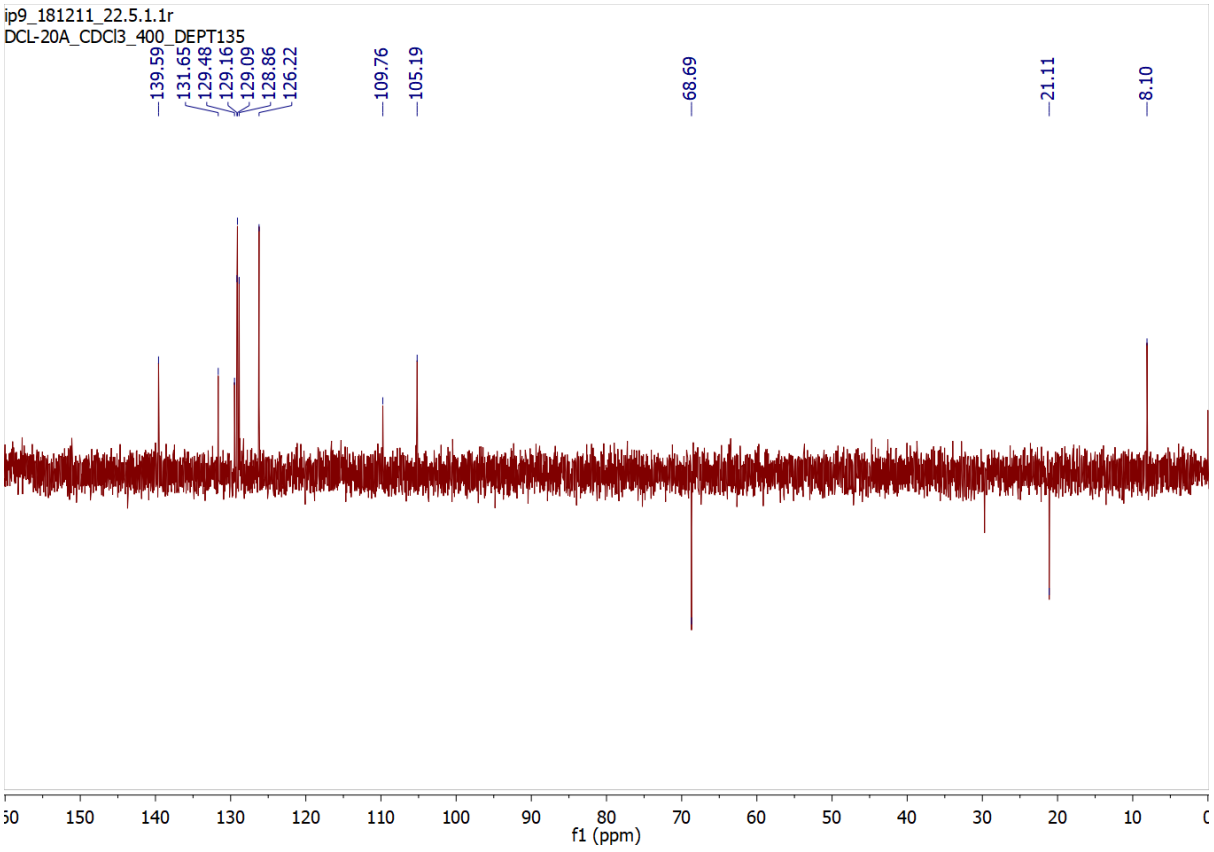


Figure S3 DEPT 135 spectrum of **1** recorded in $CDCl_3$

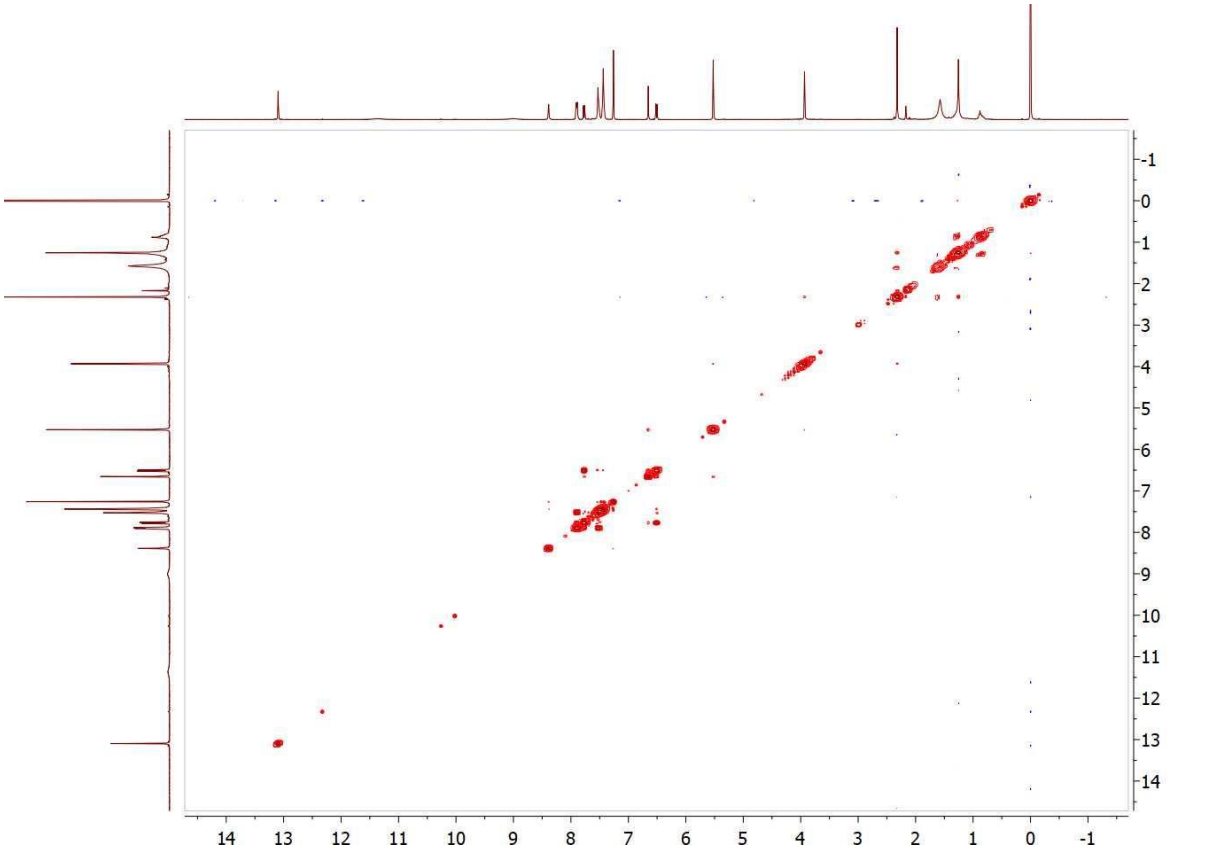


Figure S4 COSY spectrum of **1** recorded in $CDCl_3$

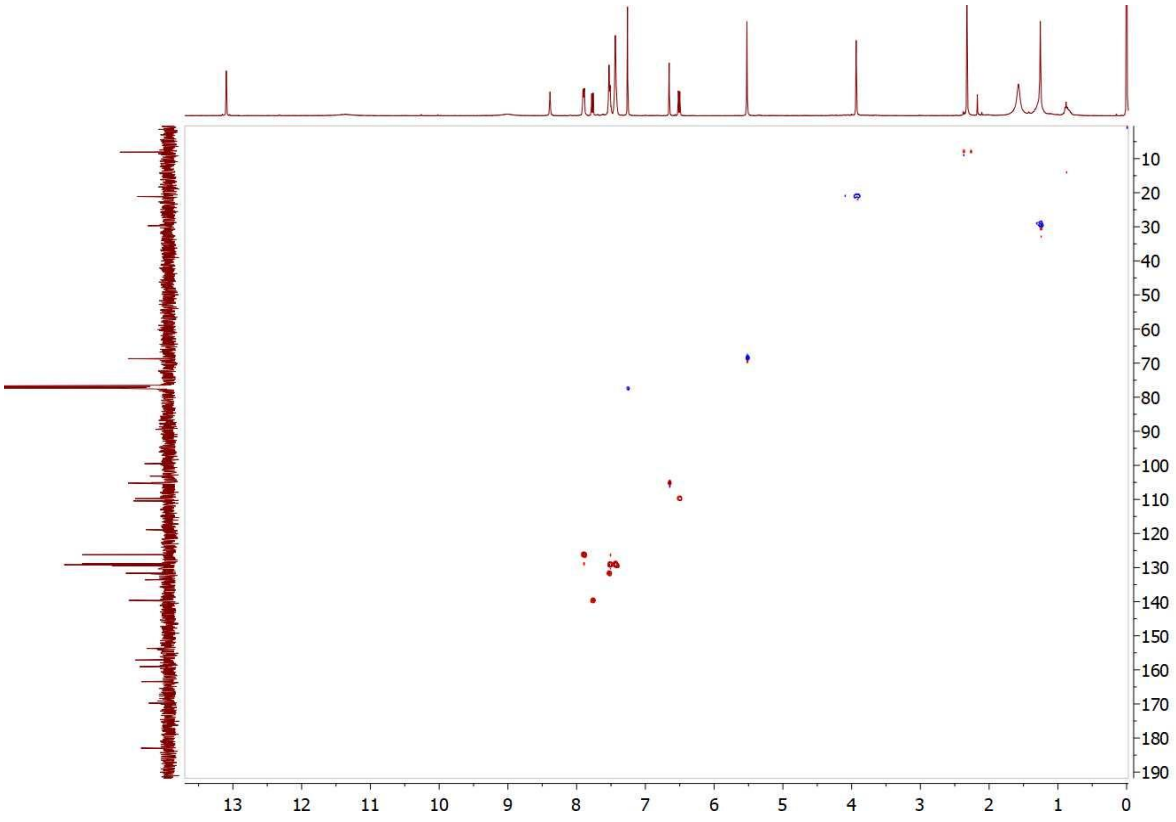


Figure S5 HSQC spectrum of **1** recorded in $CDCl_3$

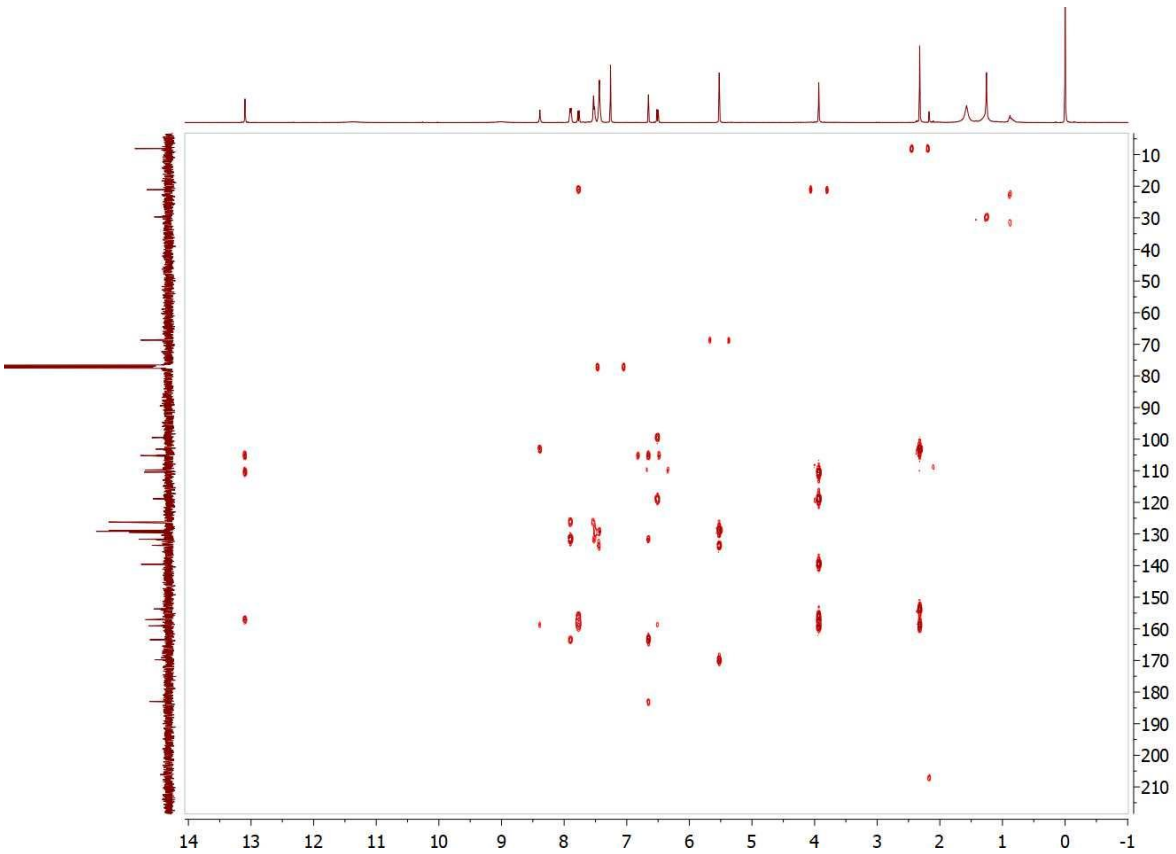


Figure S6 HMBC spectrum of **1** recorded in $CDCl_3$

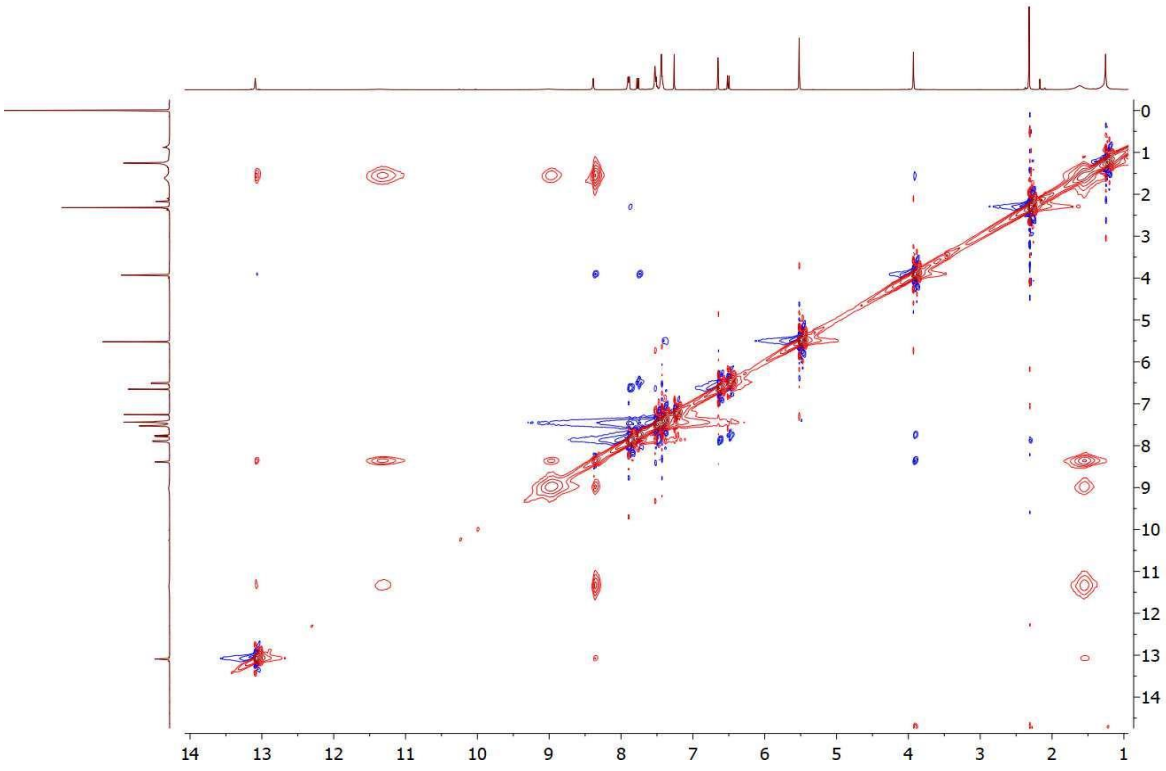


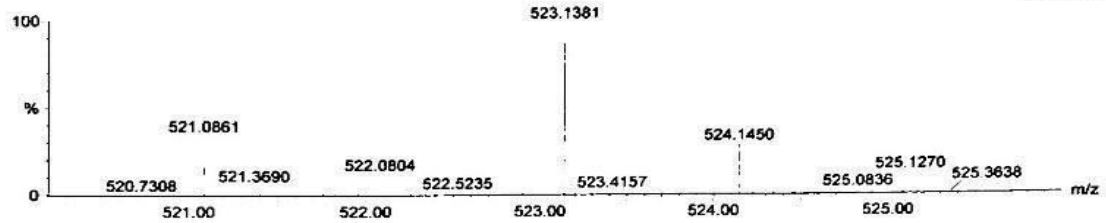
Figure S7 NOESY spectrum of **1** recorded in $CDCl_3$

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 120.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
1144 formula(e) evaluated with 9 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 9-33 H: 3-70 N: 0-10 O: 0-11 Na: 0-1
DCL-20A
SP I Polbuppha DCL-20A 41 (0.835) Cm (40:42)

1: TOF MS ES-
4.85e+002



Minimum:				-1.5			
Maximum:		55.0	5.0	120.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
523.1381	523.1382	-0.1	-0.2	22.5	92.5	2.2	C30 H20 N4 O4 Na
	523.1379	0.2	0.4	26.5	92.5	2.3	C28 H15 N10 O2
	523.1393	**-1.2**	**-2.3**	**20.5**	**92.2**	**1.9**	**C31 H23 O8**
	523.1369	1.2	2.3	17.5	92.0	1.8	C29 H24 O8 Na
	523.1366	1.5	2.9	21.5	92.2	2.0	C27 H19 N6 O6
	523.1396	-1.5	-2.9	27.5	93.0	2.7	C31 H16 N8 Na
	523.1401	-2.0	-3.8	9.5	92.7	2.4	C18 H24 N6 O11 Na
	523.1406	-2.5	-4.8	25.5	92.9	2.6	C32 H19 N4 O4
	523.1355	2.6	5.0	23.5	92.6	2.3	C26 H16 N10 O2 Na

Figure S8 ESITOFMS spectrum of compound **1**.

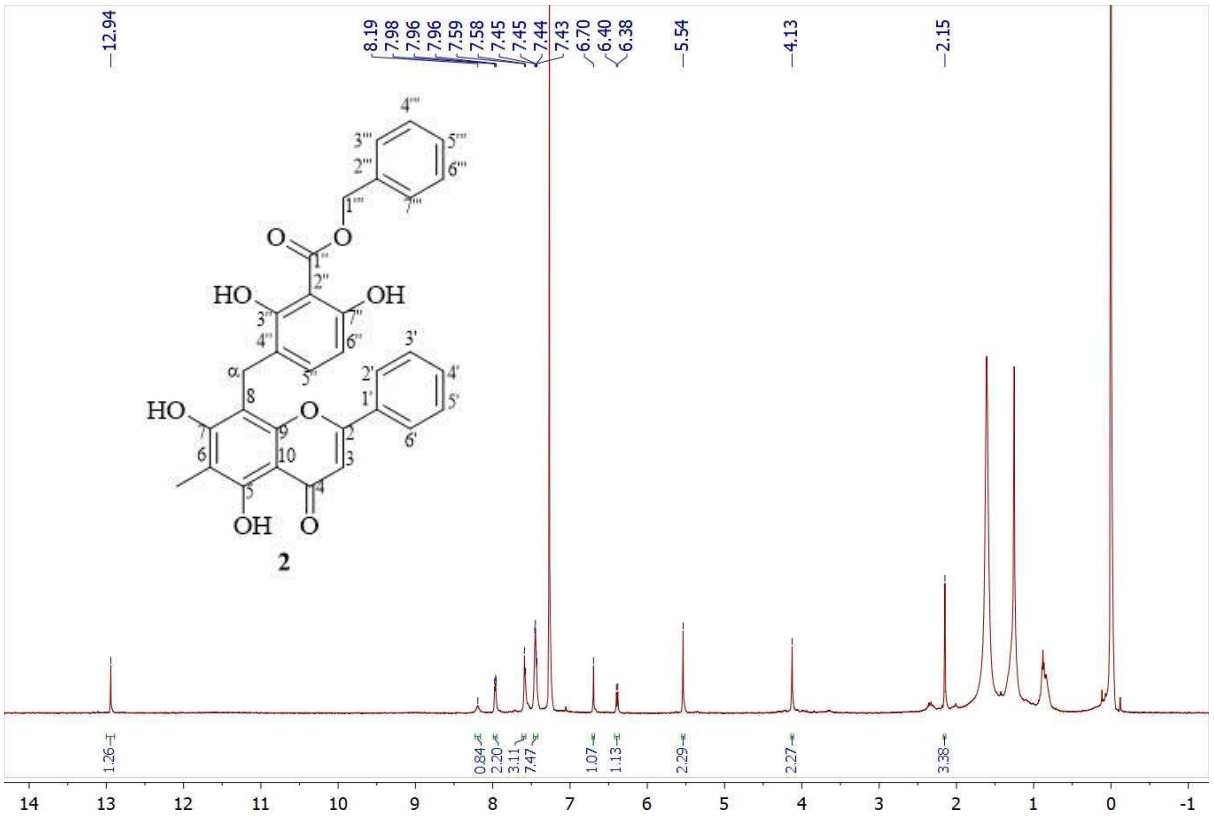


Figure S9 ^{1}H NMR spectrum of **2** recorded in $CDCl_3$

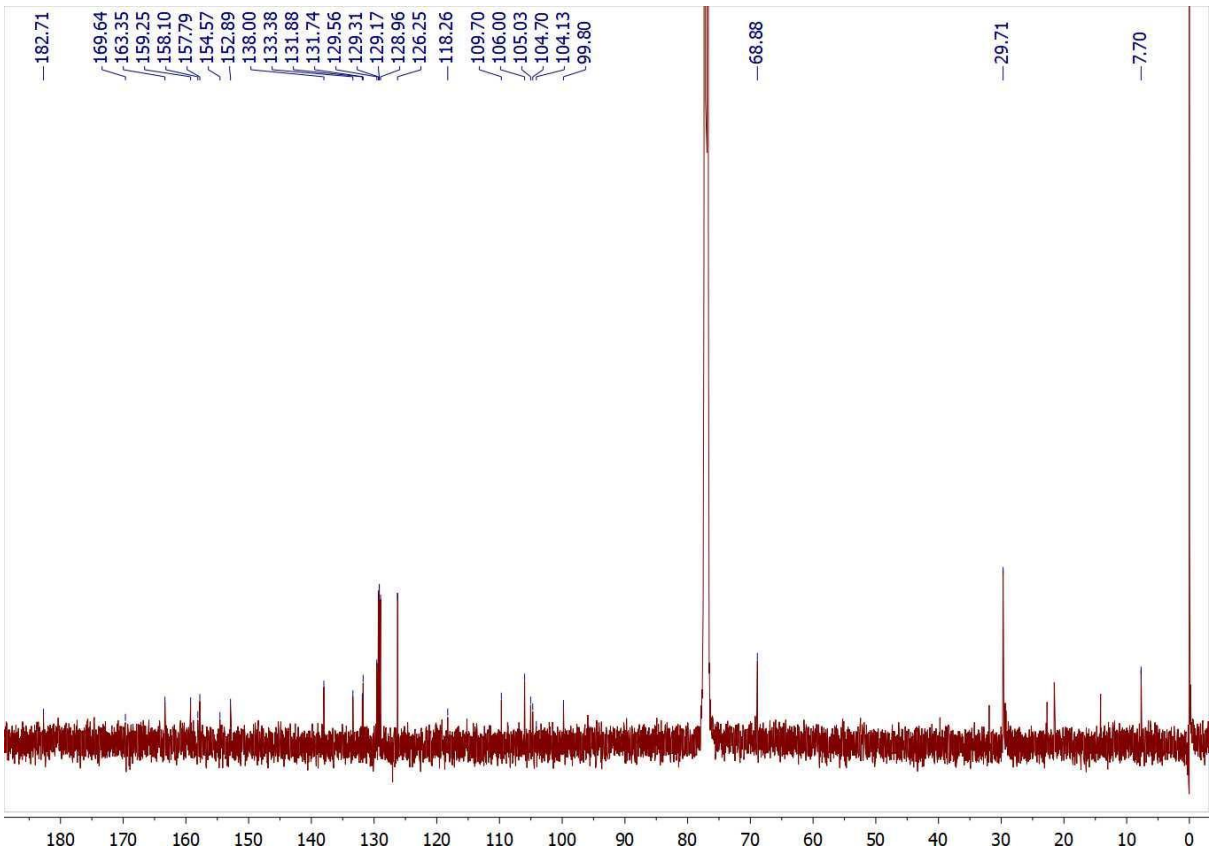


Figure S10 ^{13}C NMR spectrum of **2** recorded in $CDCl_3$

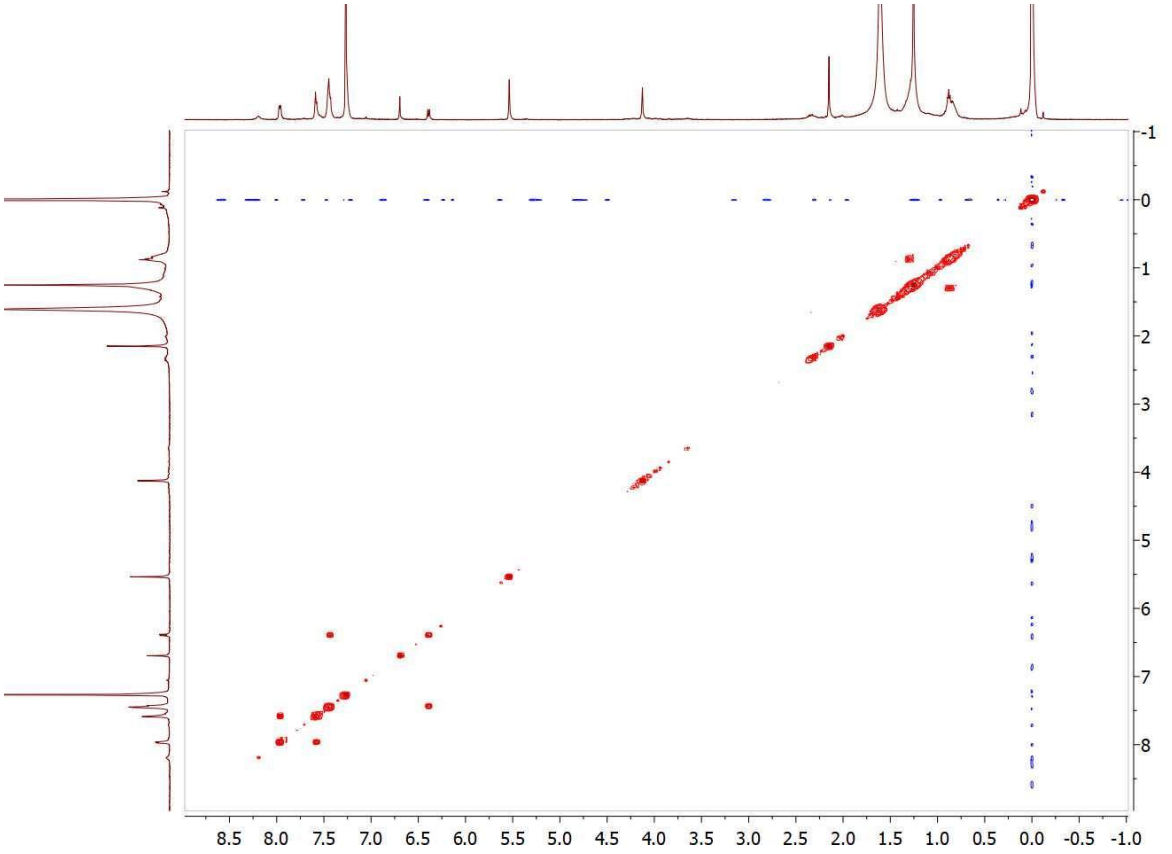


Figure S11 COSY spectrum of **2** recorded in $CDCl_3$

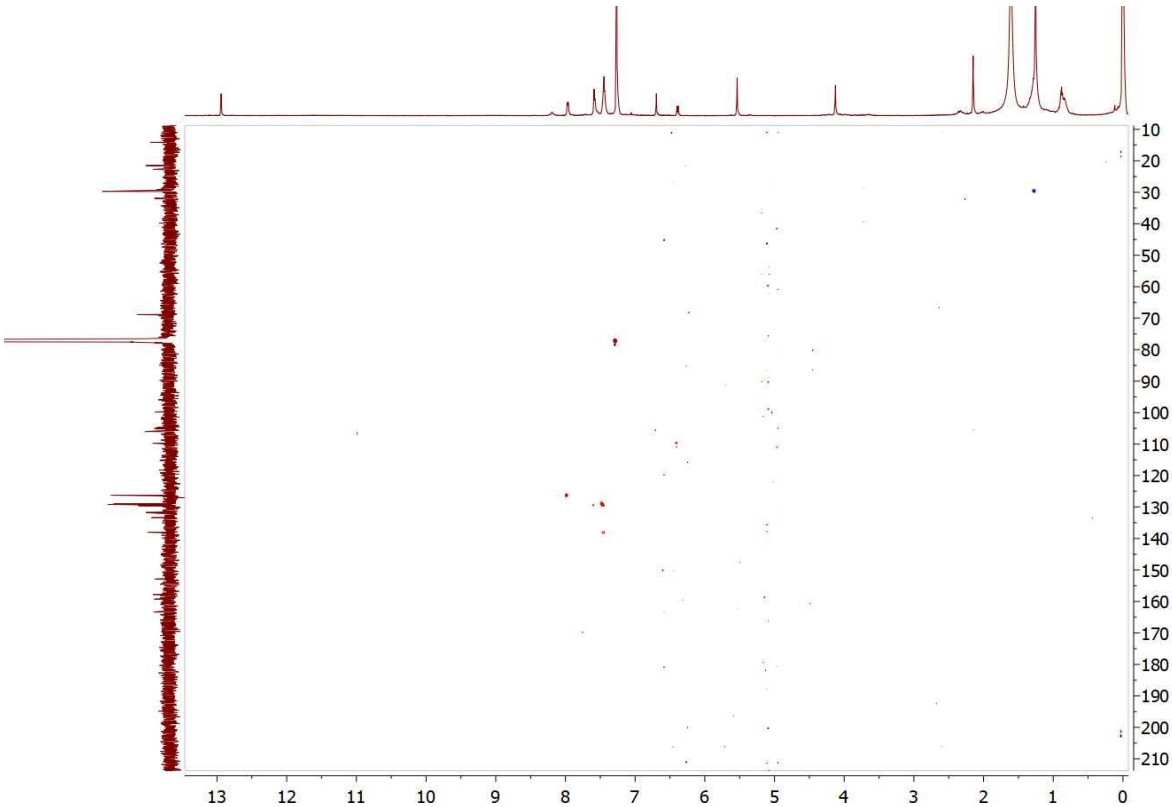


Figure S12 HSQC spectrum of **2** recorded in $CDCl_3$

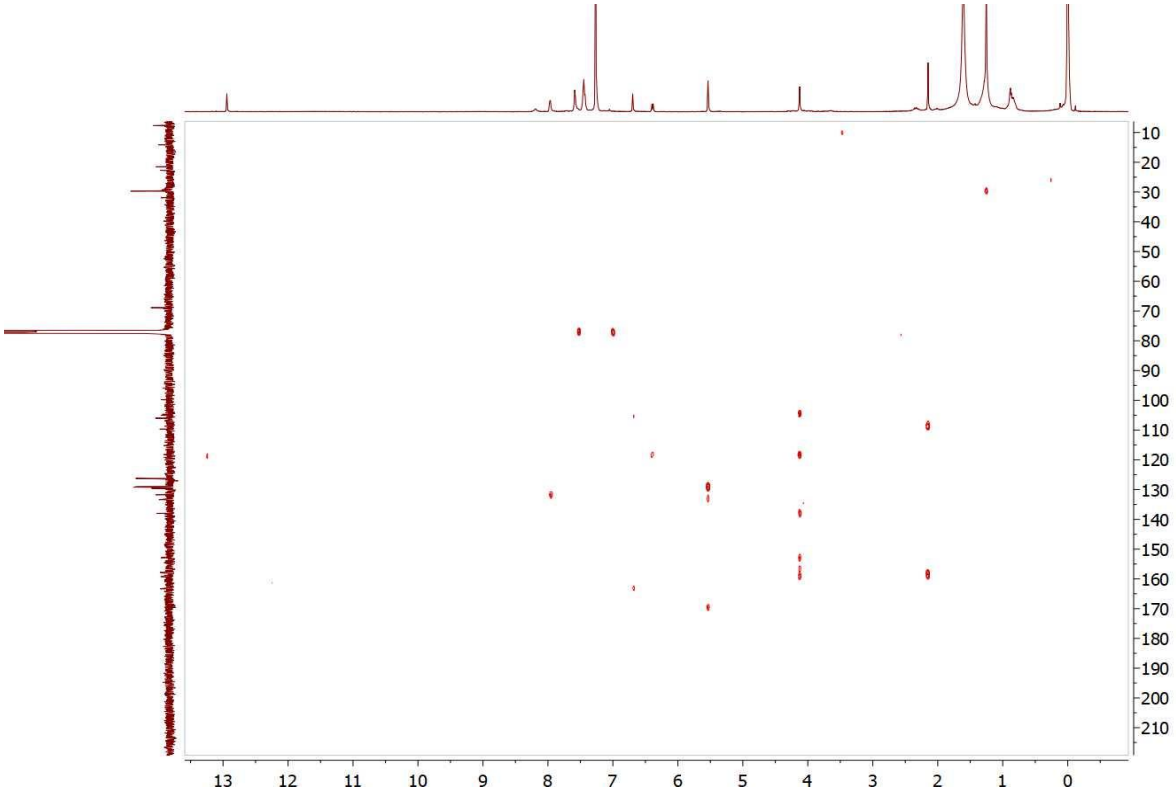


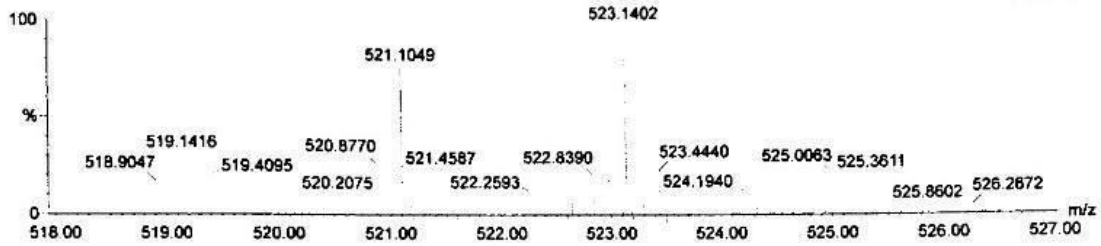
Figure S13 HMBC spectrum of **2** recorded in $CDCl_3$

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 120.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
685 formula(e) evaluated with 5 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 15-41 H: 3-70 N: 0-10 O: 0-11
DCL-22B
SP IPolbuppha DCL-22B 107 (2.119) Cm (107:109)

1: TOF MS ES-
8.62e+001



Minimum:				-1.5			
Maximum:		55.0	5.0	120.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
523.1402	523.1406	-0.4	-0.8	25.5	129.2	2.0	C32 H19 N4 O4
	523.1393	**0.9**	**1.7**	**20.5**	**129.0**	**1.8**	**C31 H23 O8**
	523.1420	-1.8	-3.4	30.5	129.4	2.3	C33 H15 N8
	523.1425	-2.3	-4.4	12.5	127.9	0.8	C20 H23 N6 O11
	523.1379	2.3	4.4	26.5	129.2	2.1	C28 H15 N10 O2

Figure S14 ESITOFMS spectrum of compound **2**

Bioactivities

***DPPH Radical Scavenging Assay*.** The DPPH scavenging activity assay was modified from a previous paper.[1,2] Briefly, 6×10^{-5} M DPPH was prepared in absolute DMSO, and then 100 μL of this solution was mixed with 100 μL of the 1,000 μg/mL sample solution in DMSO in a 96-well microplate. After 30 min of reaction, the absorbance was measured using a microplate reader (PerkinElmer, Inc., USA) at 517 nm in the dark at room temperature. All experiments were performed in triplicate, with ascorbic acid was used as a positive control ($r^2 = 0.9982$). The half--maximal inhibitory concentration (IC_{50}) of DPPH scavenging activity was calculated by plotting inhibition percentages against concentrations of the sample ($IC_{50} = 15.9 \pm 0.3$ μM). The percent inhibition was calculated with the following equation:

Percent inhibition (%) = [((A - B) – (C – D)) / (A – B)] × 100

where,
A is the absorbance of blank reaction containing DPPH solution and DMSO,
B is the absorbance of control reaction containing only DMSO,
C is the absorbance of sample reaction containing sample solution and DPPH solution,
D is the absorbance of control sample containing sample solution and DMSO.

***$ABTS^{\bullet+}$ Scavenging Assay*.** The determination of $ABTS^{\bullet+}$ scavenging activity was carried out using a modified literature procedure.[3,4] $ABTS^{\bullet+}$ were produced by reacting a 7 mM stock solution of ABTS in DI water with 2.45 mM potassium persulfate ($K_2S_2O_8$) and allowing the mixture to stand in the dark at room temperature for 16 hr before use. The $ABTS^{\bullet+}$ solution was diluted with water to an absorbance of 0.7 ± 0.02 at 734 nm. Samples (1,000 μg/mL) in DMSO (100 μL) and $ABTS^{\bullet+}$ solution (160 μL) was added to each well of the 96-well microplate. The absorbance at 750 nm was determined after 5 min of mixture solution. The measurements were performed in triplicate, and the positive control was as an ascorbic acid ($r^2 = 0.9999$, $IC_{50} = 8.2 \pm 0.1$ μM). The percent inhibition was calculated with the following equation:

Percent inhibition (%) = [((A - B) – (C – D)) / (A – B)] × 100

where,
A is the absorbance of blank reaction containing ABTS solution and DMSO,
B is the absorbance of control reaction containing only DMSO,
C is the absorbance of sample reaction containing sample solution and ABTS solution,
D is the absorbance of control sample containing sample solution and DMSO.

***α-Glucosidase Inhibitory Activity*.** A colorimetric *α*-glucosidase assay was performed by previous method.[5,6] Briefly, sample solutions were dissolved at different

concentrations (0.1-1000 µg/mL) with 5% dimethyl sulfoxide (DMSO) in phosphate buffer (pH≈6.8), and 50 µL of each sample was pipetted and mixed with 50 µL of *α*-glucosidase enzyme (0.35 U/mL) in a 96-well microplate and incubated at 37 °C for 10 min. After that, added 50 µL of 1.5 mM *p*-NPG and incubated again at 37 °C for 20 min. Finally, added 100 µL of 1 M Na_2CO_3 to terminate the reaction. The absorbance was measured at 405 nm with a microplate reader (PerkinElmer, Inc., USA). Acarbose was used as a positive control (r^2 = 0.9960, IC_{50} = 93.6 ± 2.1 µM, final conc. = 100, 400, 800, 1200, 1600, and 2000 µg/mL). The process was repeated in triplicate, and the percent inhibition was calculated with the following equation:

Percent inhibition (%) = [((A - B) – (C – D)) / (A – B)] × 100

where,
A is the absorbance of blank reaction containing only 5% DMSO in phosphate buffer,
B is the absorbance of control reaction containing 5% DMSO in phosphate buffer and *α*-glucosidase enzyme,
C is the absorbance of sample reaction containing sample solution and *α*-glucosidase enzyme,
D is the absorbance of control sample containing only sample solution.
The concentration of samples that inhibited *α*-glucosidase activity by 50% was defined as the IC_{50}value.

References

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