Supplementary data

Sophora interrupta Bedd root derived flavonoids as prominent antiviral agents against Newcastle Disease Virus

Cherukupalle Bhuvaneswar[a,d], Aluru Rammohan[b,e*], Baki Vijaya Bhaskar,[g] Pappithi Ramesh Babu[a], Gujjar Naveen[c], Duvvuru Gunasekar[b], Subbiah Madhuri[c*], Wudayagiri Rajendra[a*], Pallu Reddanna[f]

[a]Department of Zoology, Sri Venkateswara University, Tirupati-517502, AP, India.

[b]Department of Chemistry, Sri Venkateswara University, Tirupati-517502, AP, India.

[c]National Institute of Animal Biotechnology (NIAB), Hyderabad-500049, AP, India.

[d]Department of Microbiology, Sri Venkateswara University, Tirupati-517502, AP, India.

[e]Department of Organic and Biomolecular Chemistry, Ural Federal University, Yekaterinburg 620002, Russia.

[f]School of Life Sciences, University of Hyderabad (UOH), Hyderabad-500046, Telangana, India.

[g]Department of Pathophysiology, Shantou University Medical College, Shantou, Guangdong, China-515031

*__*Corresponding Author*__ (All the authors have equal priority)

Email: rwudayagiri16@gmail.com; madhuri@niab.org.in; rammohan4ever@gmail.com

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Table S1. Primers designed for reverse transcription PCR and quantitative PCR

S. No	Primer Name	Sequence 5′ to 3′
		Primers for PCR (To amplify the complete NP gene)
1	**PNP-F**	CAGTGATGACCCAGAAGATAGGTG
2	**PNP-R**	CGCAAAGCTCATCTGGTCACTATC
		Primers for Reverse transcription PCR (Specific for g RNA, cRNA and mRNA)
3	**Pla-G**	ACGATAAAAGGCGAAGGAGCA
4	**Pla-R**	CATCCACACCCGAGCAAGCGAC
5	**Pla-18T**	TTTTTTTTTTTTTTTTTA/G/C
		Primers for Real-Time PCR
6	**Pla-rt 13 fwd**	CAACAATAGGAGTGGAGTGTCTGA
7	**Pla-rt 14 rev**	CAGGGTATCGGTGATGTCTTCT

Table S2. CT values and quantification of NP gene by Real-Time PCR

1	Chemistry/Reporter	SYBR Green
2	Instrument	SDS 7500
3	Passive Reference	ROX
4	Efficiency	106.3356
5	Slope	-3.1789
6	Ct Threshold	0.081355

S.No.		CT Mean	CT SD	Qty Mean	Qty SD	Tm1
1	Standard	5.728245	1.393882	10	0	81.7884
	Standard	9.119411	0.392759	1	0	81.72597
	Standard	11.57143	0.204798	0.1	0	81.6011
	Standard	14.83463	0.298342	0.01	0	81.16409
	Standard	18.60887	0.24044	0.001	0	81.72597
	Standard	21.6346	0.045041	0.0001	0	82.03812
	NTC	35.53358	1.554124	0	0	81.4317
2	g RNA VC	10.07856	0.383878	0.443679	0.06039	82.53757
	g RNA SR-1	11.07814	0.362926	0.198623	0.05325	82.97459
	g RNA SR-2	10.87497	0.245144	0.227114	0.03875	82.72486
	g RNA RB	10.55816	0.252325	0.29869	0.050298	82.72486
	NTC	34.00959	0.606506	0	0	82.58522
3	c RNA VC	9.343909	0.315714	0.613888	0.030032	81.97569
	c RNA SR-1	11.3634	0.091579	0.158048	0.010513	83.03702
	c RNA SR-2	10.76076	0.012903	0.244197	0.002276	82.78729
	c RNA RB	10.44855	0.062672	0.330648	0.026276	82.84972
	NTC	34.4333	0.843918	0	0	82.76646
4	m RNA VC	8.78234	0.190169	0.973482	0.048965	82.6
	m RNA SR-1	10.46209	0.15531	0.304457	0.034672	80.91437
	m RNA SR-2	10.53943	0.245842	0.289601	0.049166	81.53867
	m RNA RB	10.11638	0.231068	0.412246	0.033364	81.47624
	NTC	34.77775	1.788658	0	0	81.30898

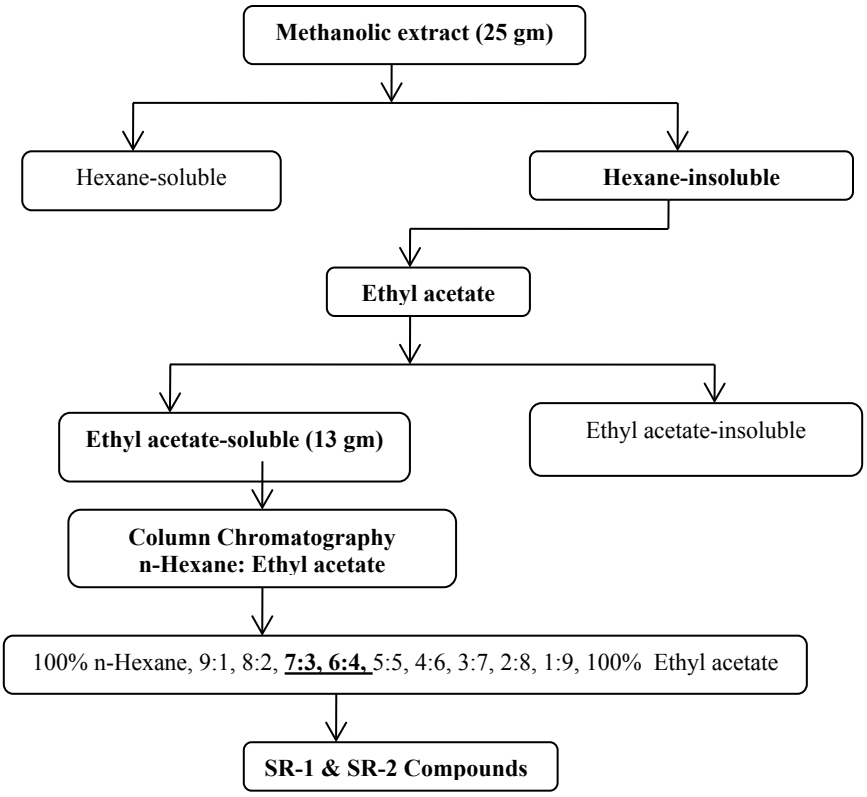


Figure S1 Isolation of compounds from *Sophora interrupta* Bedd root extract by Column chromatography

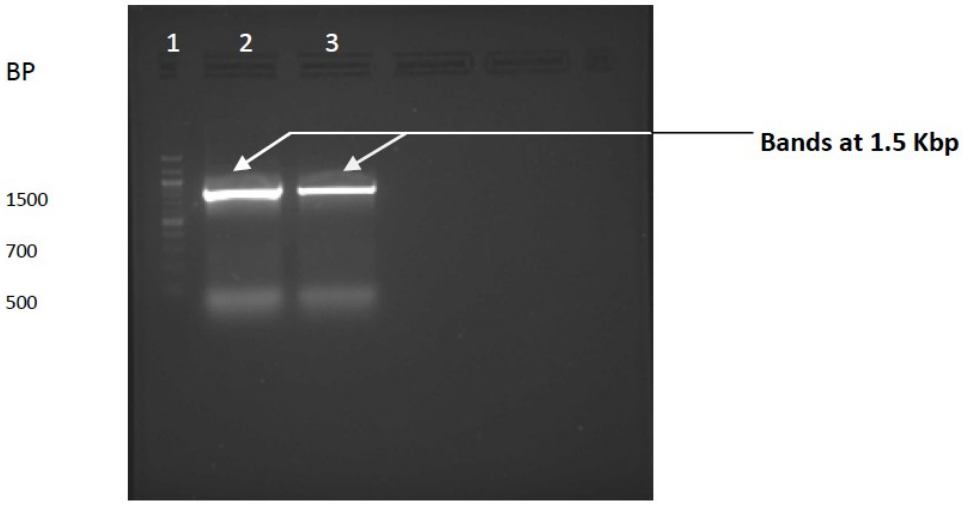


Figure S2 Lane 1 depicts Ladder; 2 & 3 lanes represents amplified NP gene.

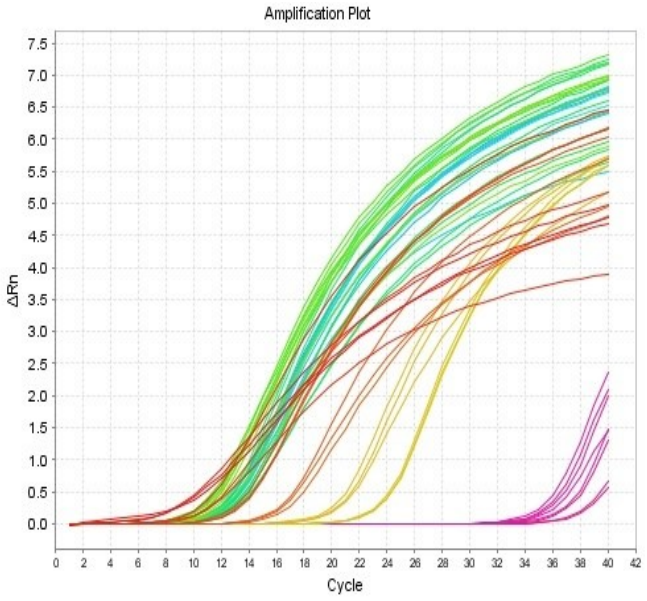


Figure S3 Amplification plot of NP plasmid and viral RNAs

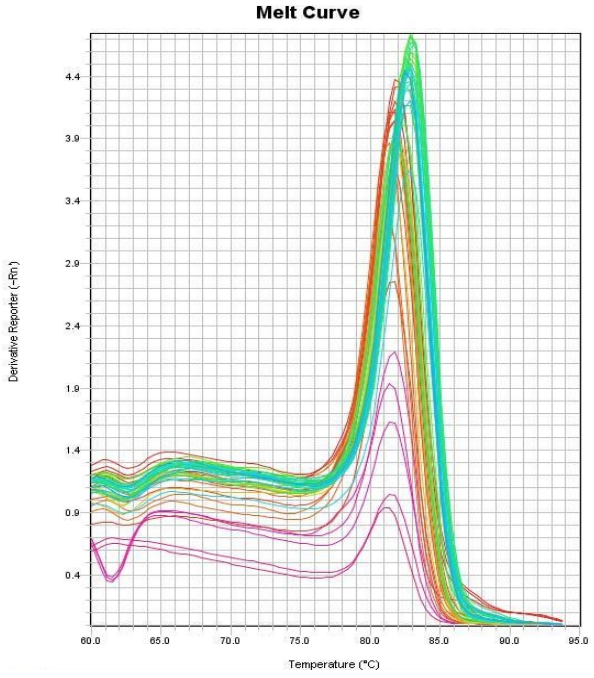


Figure S4 Melting curve of NP plasmid and viral RNAs

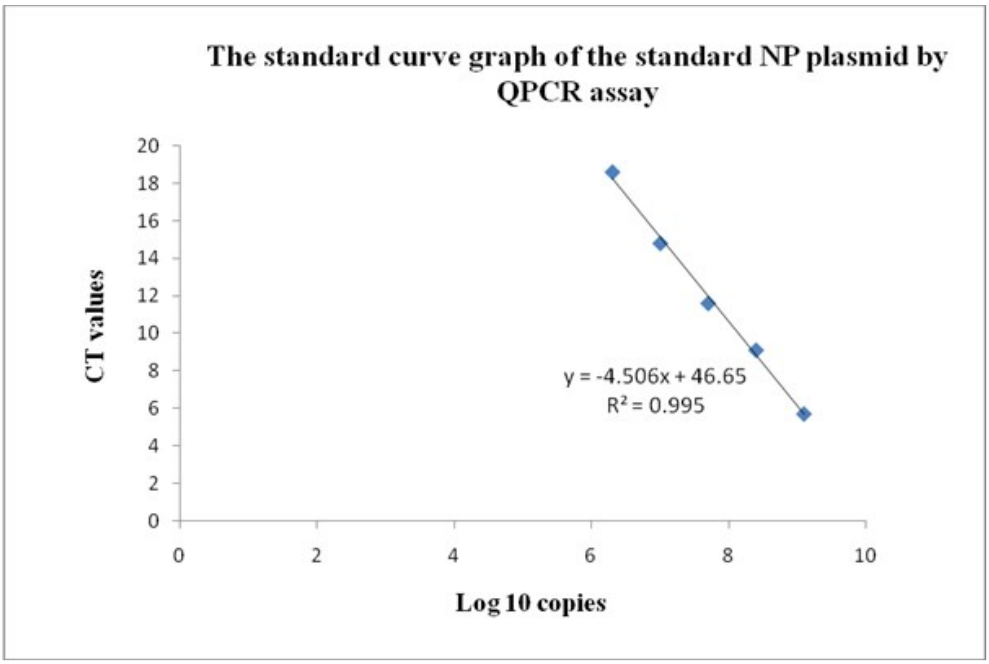


Figure S5 The standard curve graph of the standard NP plasmid by QPCR assay for quantification. The linear regression equation obtained was y=-4.5455X + 46.973 and R^2=0.9975

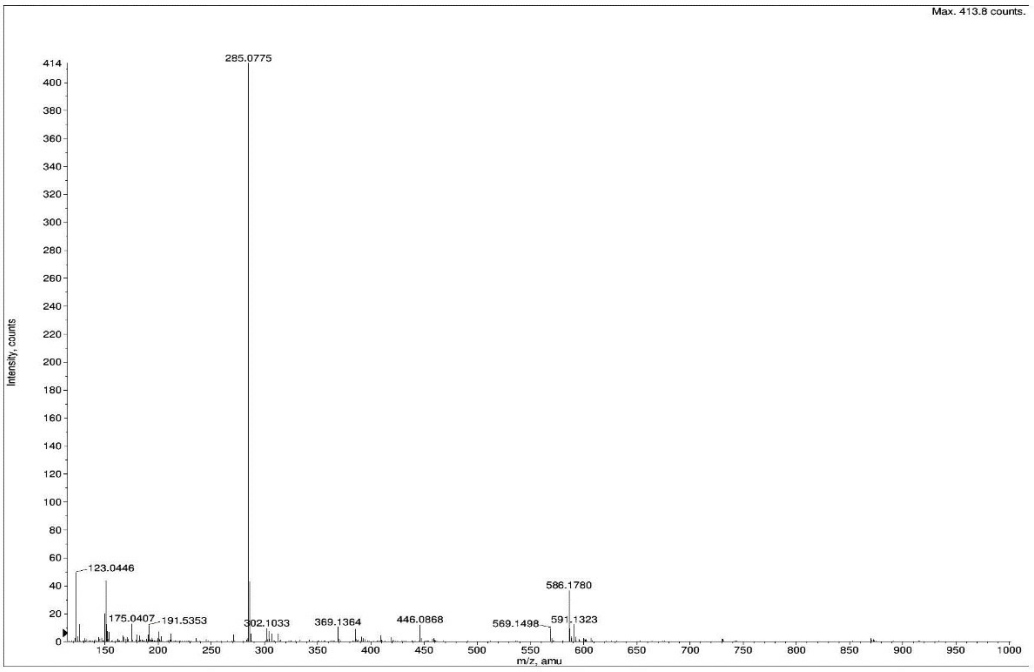


Figure S6: ESITOF Mass Spectrum of SR-1

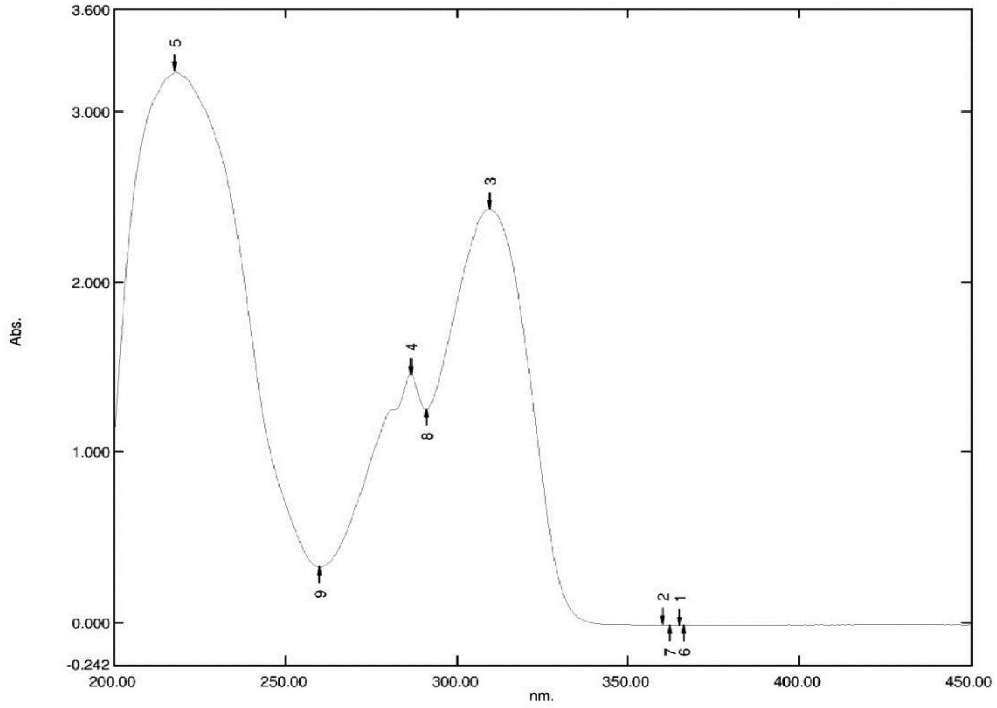


Figure S7: UV Spectrum (MeOH) of SR-1

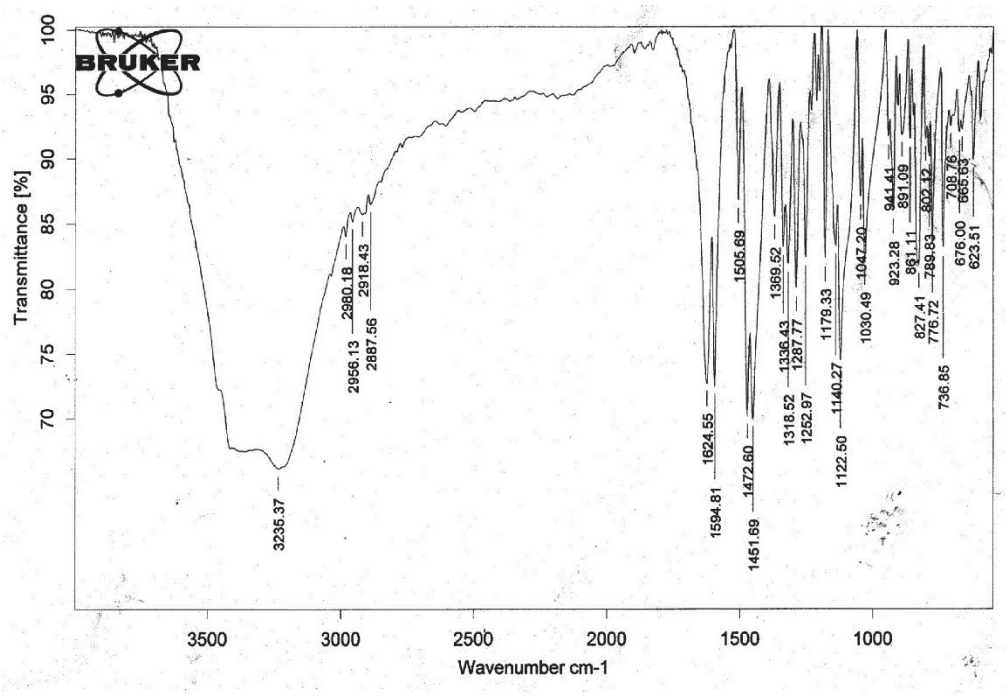


Figure S8: FTIR Spectrum of SR-1

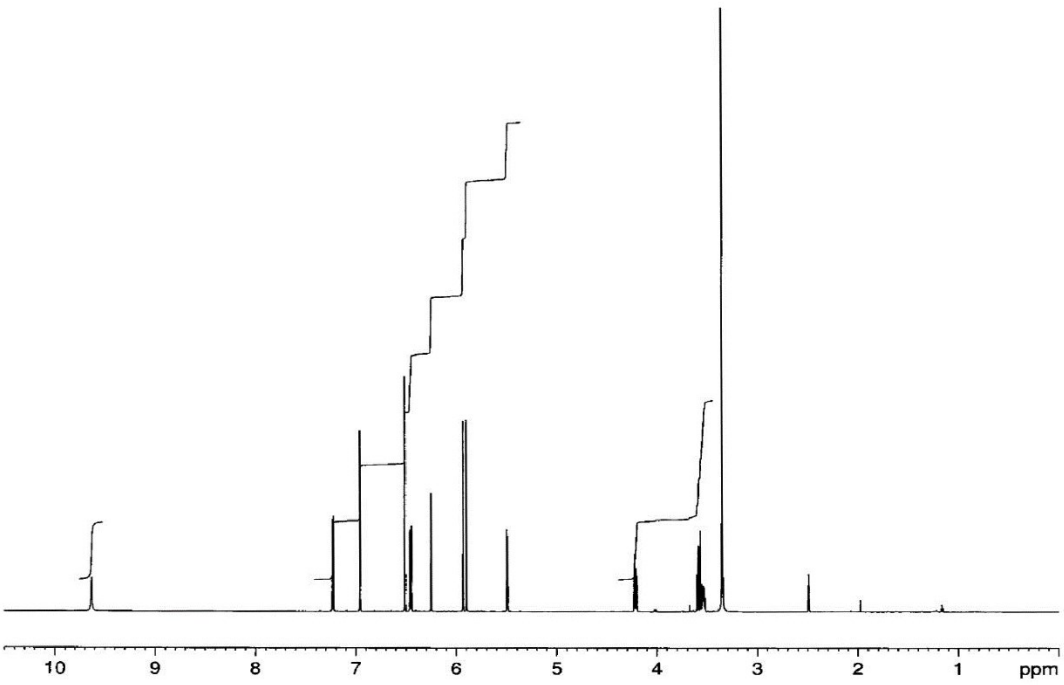


Figure S9: ^{1}H NMR Spectrum (600 MHz, DMSO-d_6) of SR-1

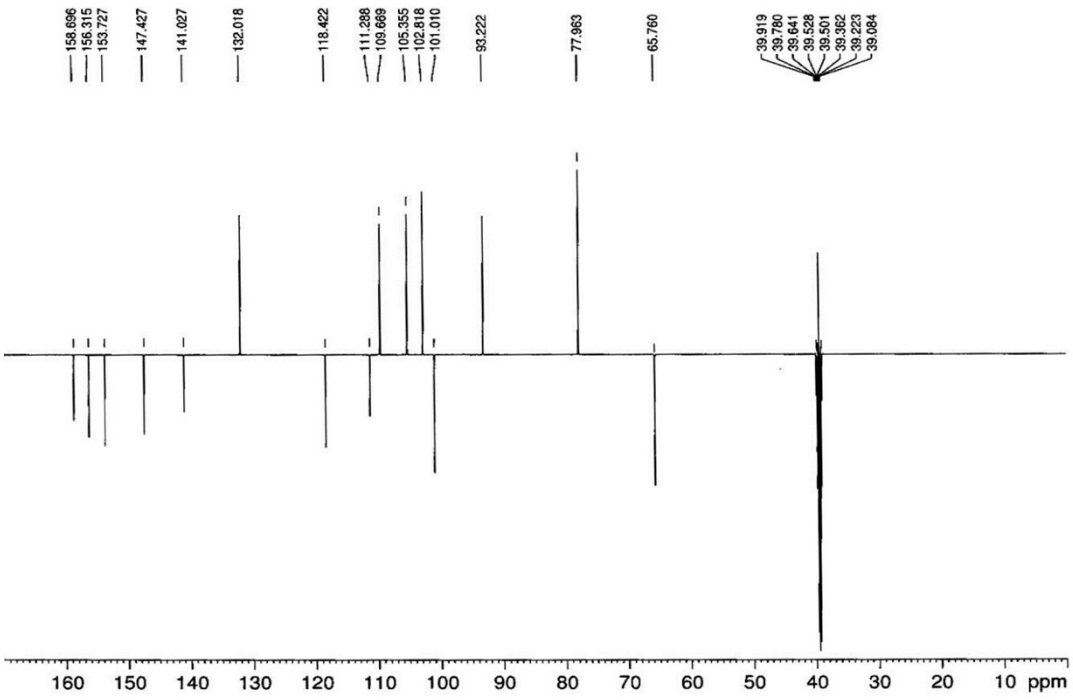


Figure S10: ^{13}C NMR Spectrum (150 MHz, DMSO-d_6) of SR-1

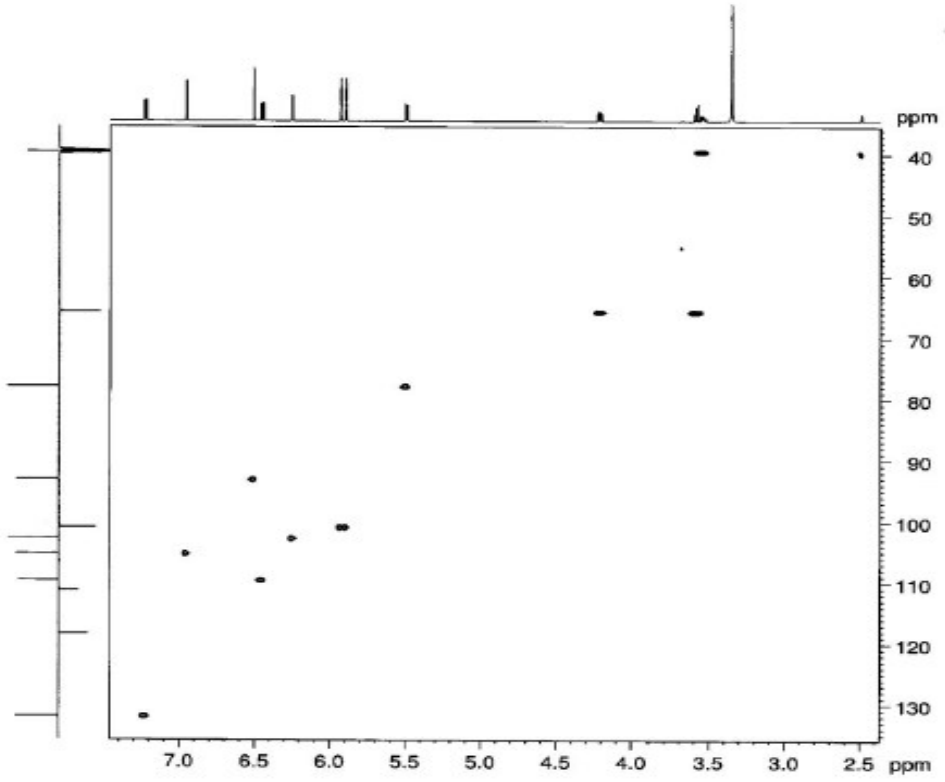


Figure S11: HSQC Spectrum of SR-1

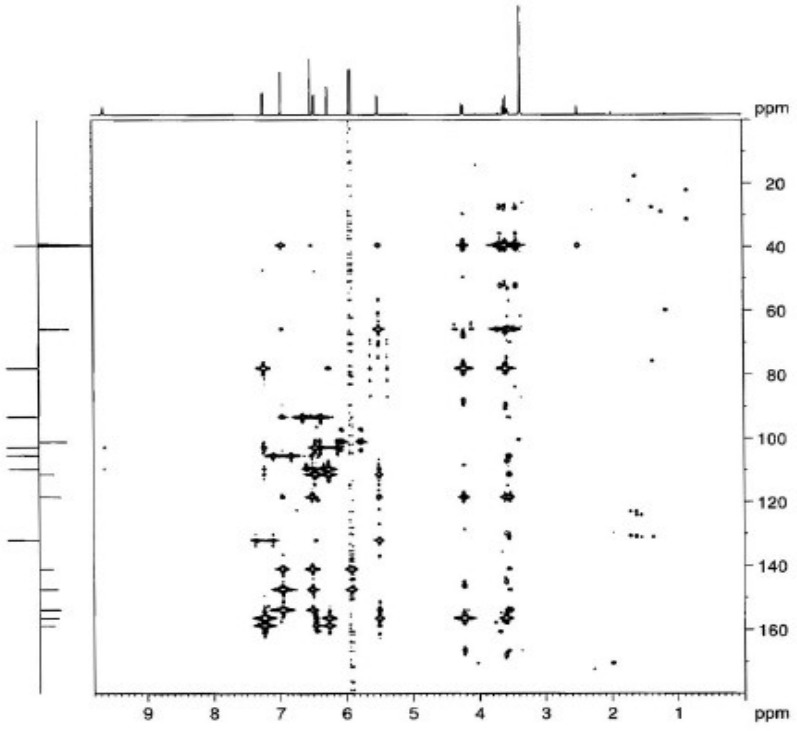


Figure S12: HMBC Spectrum of SR-1

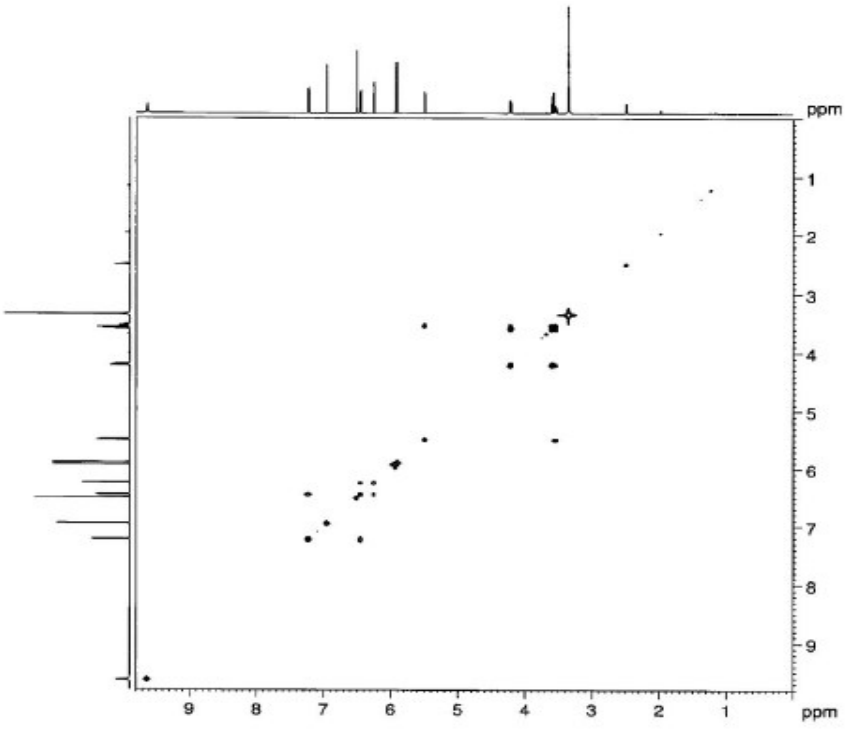


Figure S13: COSY Spectrum of SR-1

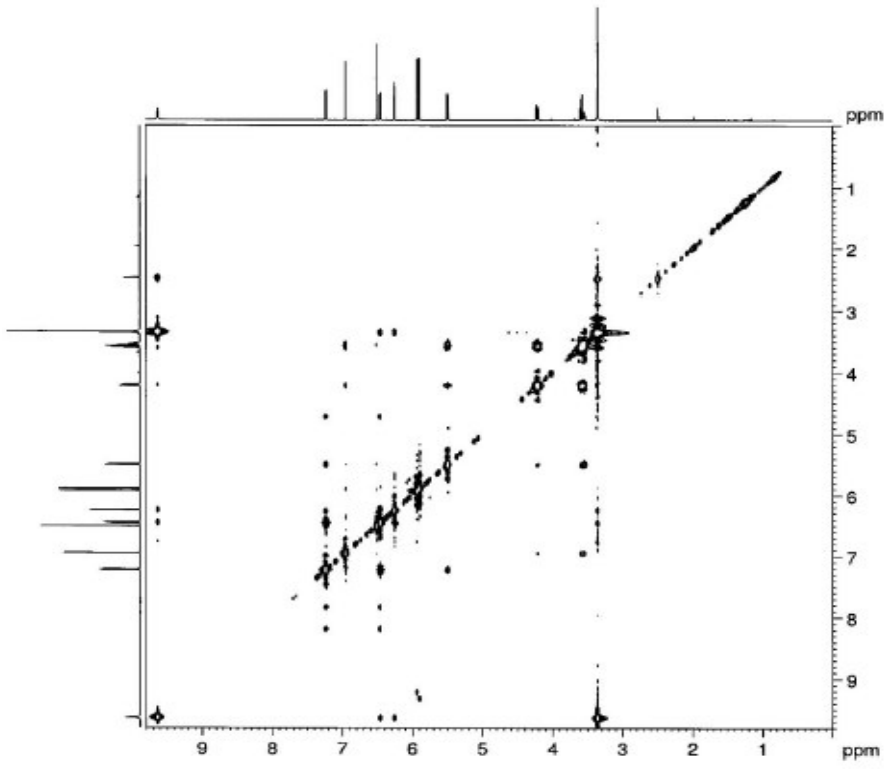


Figure S14: NOESY Spectrum of SR-1

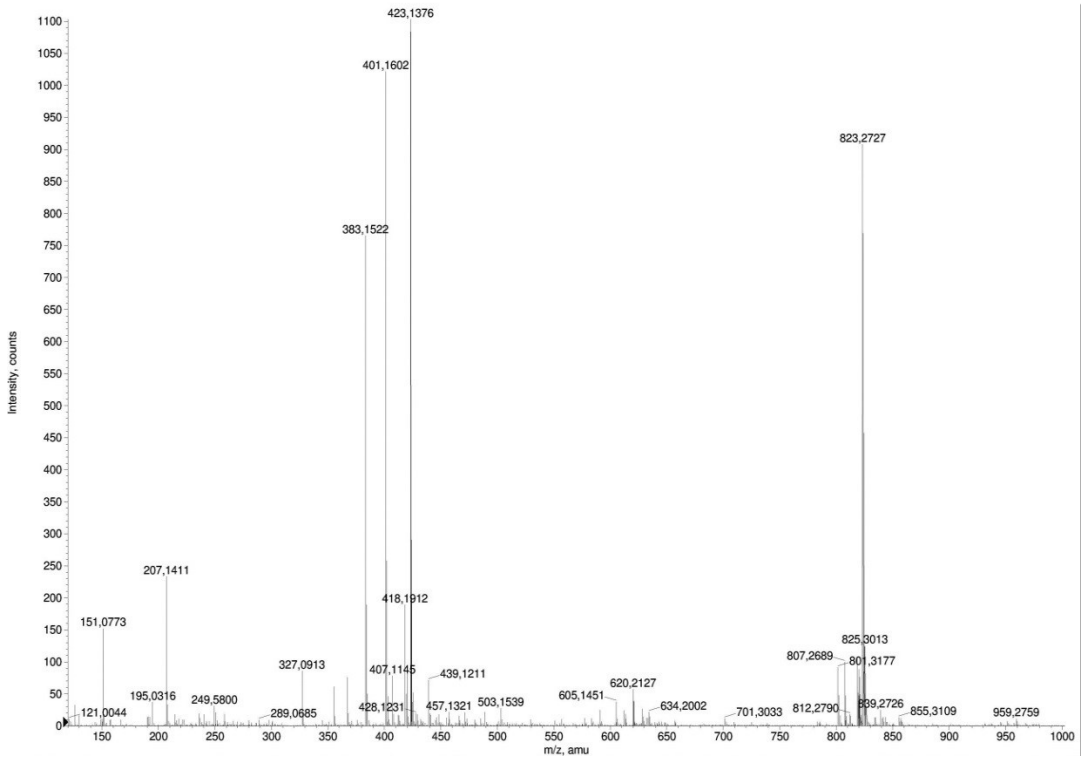


Figure S15: ESITOF MASS Spectrum of SR-2

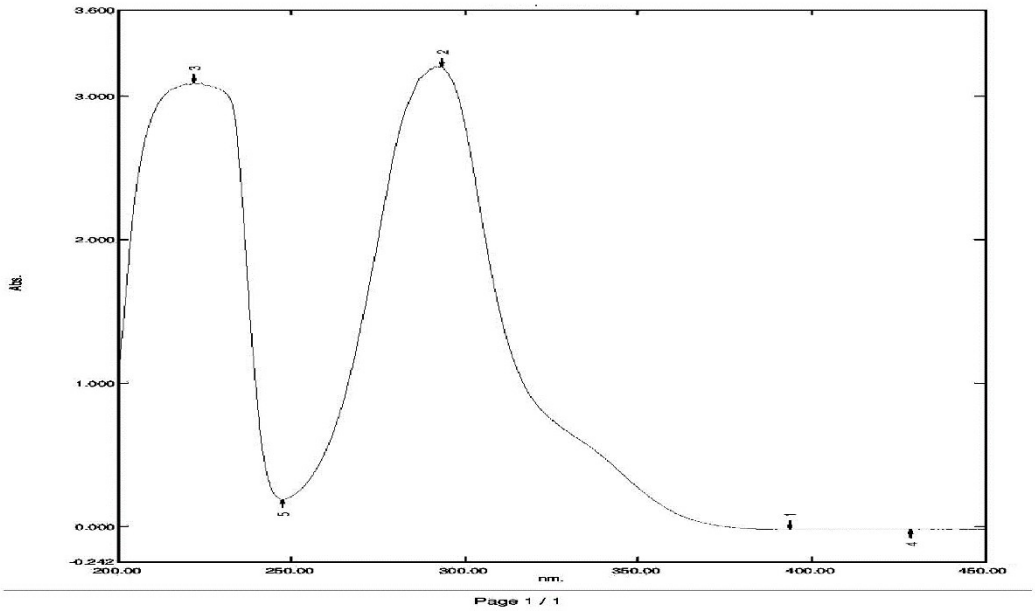


Figure S16: UV Spectrum (MeOH) of SR-2

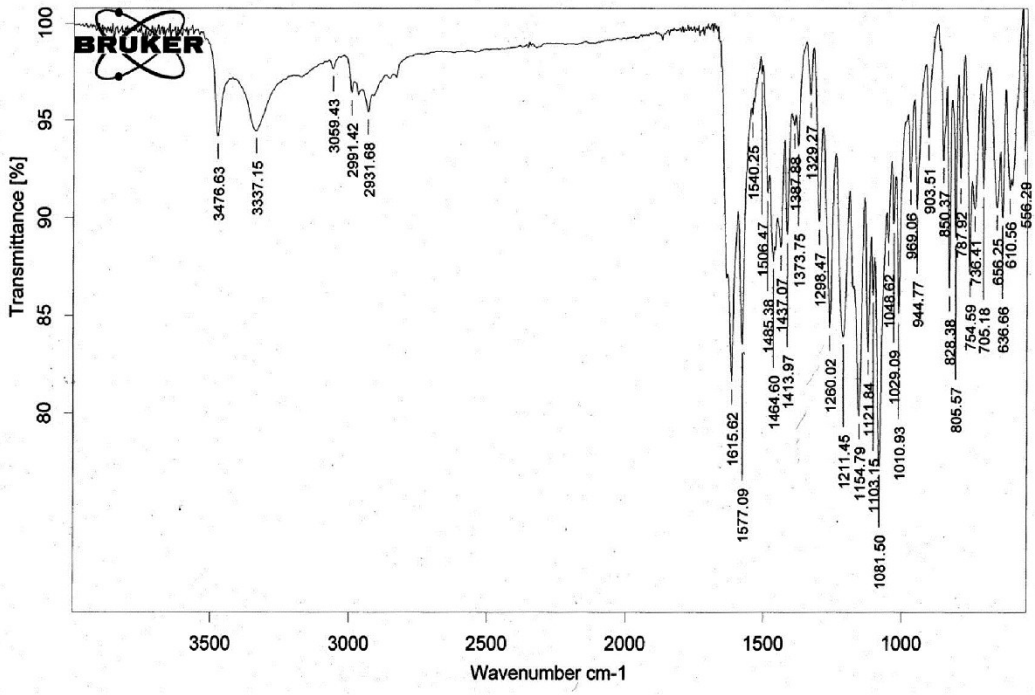


Figure S17: FTIR Spectrum of SR-2

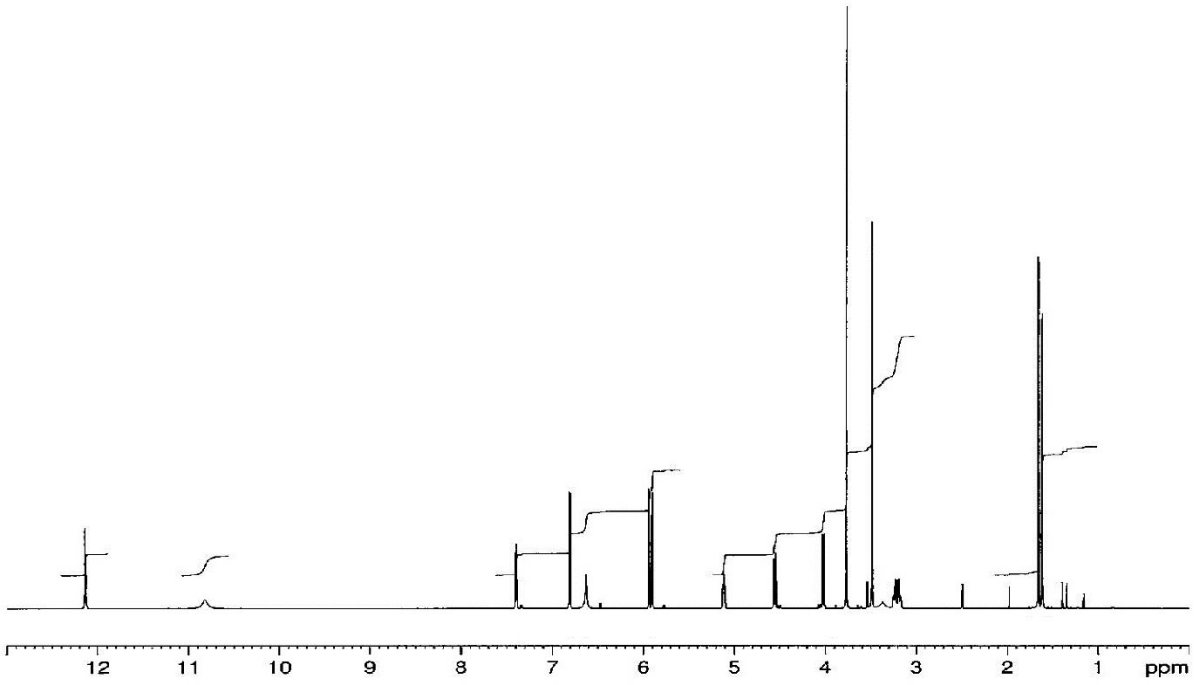


Figure S18: ^{1}H NMR Spectrum (600 MHz, DMSO-d_6) of SR-2

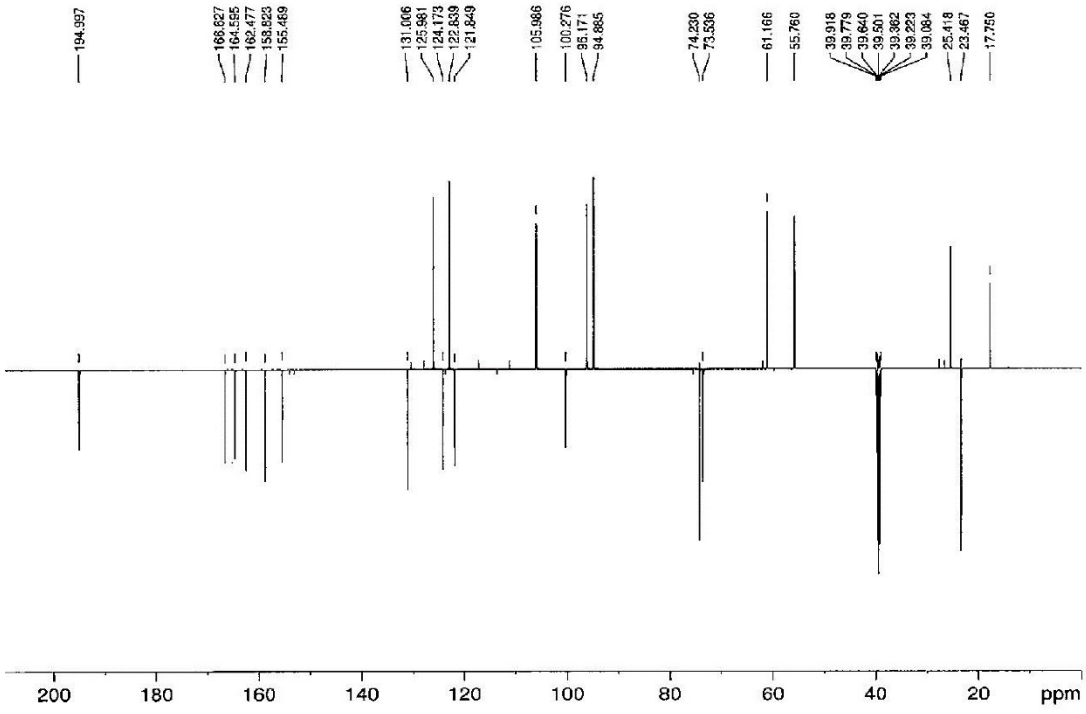


Figure S19: ^{13}C NMR Spectrum (150 MHz, DMSO-d_6) of SR-2

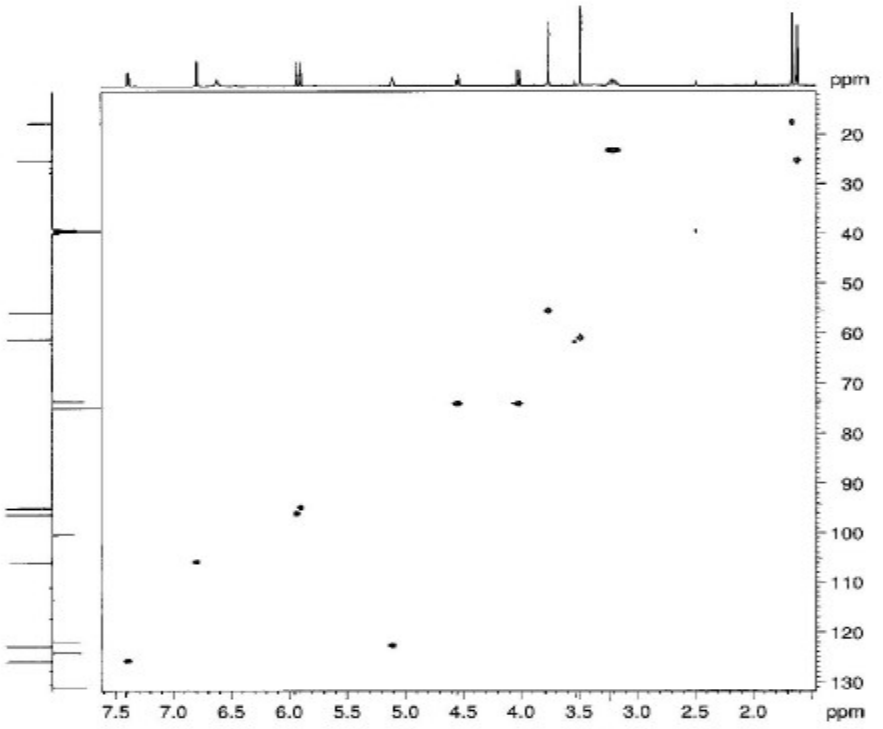


Figure S20: HSQC Spectrum of SR-2

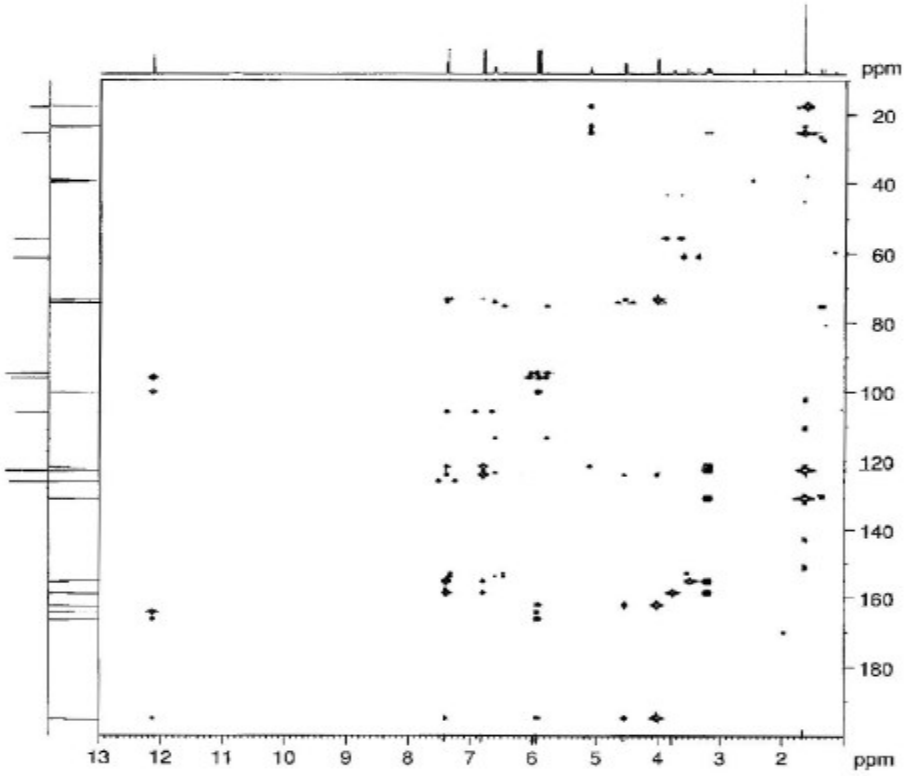


Figure S21: HMBC Spectrum of SR-2

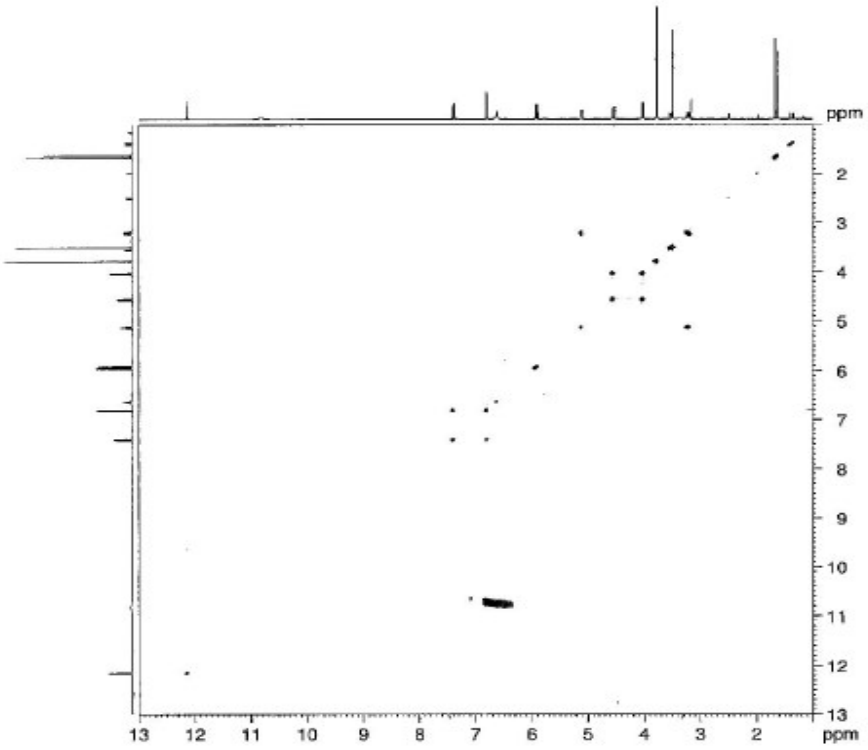


Figure S22: COSY Spectrum of SR-2

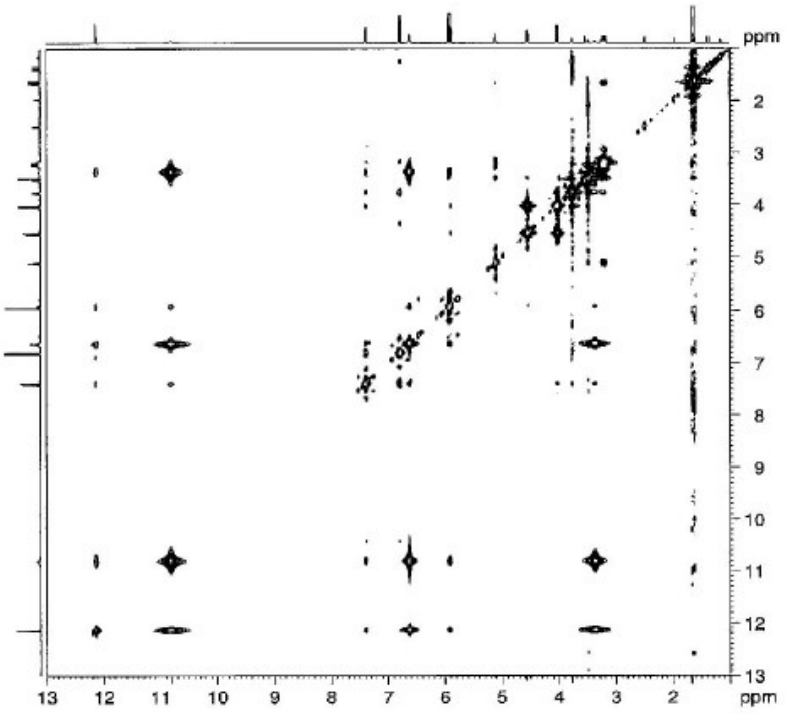


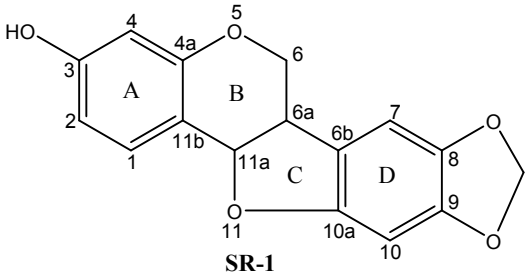
Figure S23: NOESY Spectrum of SR-2

Structural studies of isolated compounds:

3-Hydroxy-8, 9-methylenedioxypterocarpan or Maackiain (SR-1):

The compound SR-1 was isolated as colorless prisms and it was analyses for $C_{16}H_{12}O_5$ which is consistent with the presence of $[M+H]^+$ ion peak at *m/z* 285.0775 in its ESI-TOF mass spectrum (Fig. S6). This was corroborated by the ^{13}C NMR spectrum (Fig. S10), which showed the signals for all the sixteen carbons of the compounds SR-1. The UV spectrum (Fig. S7), showed absorption maxima at 287 and 310 nm, and the IR absorption bands (Fig. S8) indicates the presences of hydroxyl (3235 cm^{-1}), ether (1200-1100 cm^{-1}), furan (1047 cm^{-1}) and methylenedioxy (1030, 941 cm^{-1}) functions, respectively.

The ^1H NMR spectrum (Fig. S9) showed four characteristic signals at δ 4.21 (1H, dd, *J* = 10.5 & 4.3 Hz, H-6_{ax}), 3.59 (1H, dd, *J* = 10.5 & 9.5 Hz, H-6_{eq}), 3.53 (1H, ddd, *J* = 9.8, 6.5 & 4.3 Hz, H-6a) and 5.49 (1H, d, *J* = 6.8 Hz, H-11a) corresponding to the compound SR-1 should be a pterocarpan skeleton. Further, two mutually coupled doublets at δ 5.93 (1H, d, *J* = 1.0) and 5.90 (1H, d, *J* = 1.0) indicating the presence of methylenedioxy group was positioned at C-8/C-9 as these protons showed HMBC correlations with C-8 (141.0) and C-9 (147.4 ppm), which further showed cross correlations with two para related protons of ring D at δ 6.95 (H-7) and 6.51 (H-10) in its HMBC spectrum (Fig. S12). In addition, a typical ABX system at δ 7.23 (1H, *J* = 8.4 Hz, H-1), 6.45 (1H, *J* = 8.4 & 2.4 Hz, H-2) and 6.25 (1H, *J* = 2.4 Hz, H-4), and a downfield signal at δ 9.62 indicates the presence of resorcinol nature A-ring. Further, this downfield signal at δ 9.62 was due to the phenolic hydroxyl group and it was placed at C-3 as it showed ^2J correlation with the carbon C-3 (158.7 ppm) and which showed cross correlations with carbons H-2 (δ 6.45) and C-4 (δ 6.25) in its HMBC spectrum. All the ^1H and ^{13}C NMR chemical shifts of compound SR-1 were confirmed and correlated with the HSQC (Fig. S11), HMBC (Fig. S12), COSY (Fig. S13)and NOSEY (Fig. S14) studies.



SR-1

Thus, the chemical structure of the isolated compound SR-1, was characterized as 3-Hydroxy-8, 9-methylenedioxypterocarpan or Maackiain, as it spectral data was good agreement with the published data.

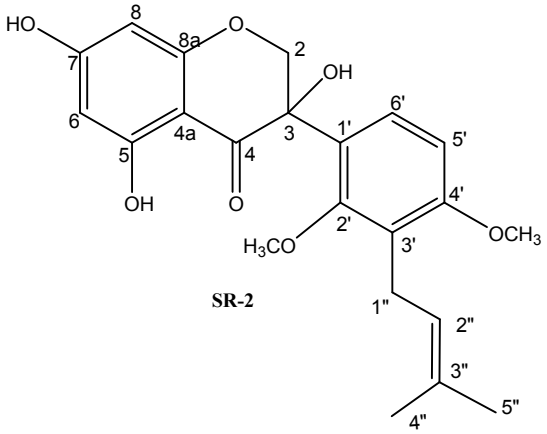
2′,4′-Dimethoxy-3′-(γ,γ-dimethylallyl)-3,5,7-trihydroxy-isoflavanone or Echinoisoflavanone (SR-2):

The compound SR-2 was isolated as yellow crystalline solid and the positive ESI-TOF mass spectrum (Fig. S15) showed the presence of $[M+H]^+$ ion peak at *m/z* 401.1602, consistent with molecular formula $C_{22}H_{24}O_7$. This was further supported by the presence of twenty two carbon signals in its ^{13}C NMR spectrum (Fig. S19). The UV (Fig. S16)

absorption maxima at 222, 293 and 338 (sh) and the two strong IR absorption bands (Fig. S17) at 3476 and 1615 cm^{-1} were due to the presence of hydroxyl and carbonyl functions, respectively.

The ^{1}H NMR spectrum (Fig. S18) showed two mutually coupled doublets at δ 4.02 (1H, d, *J* = 11.7 Hz) and 4.55 (1H, d, *J* = 11.7 Hz), is a characteristic of oxymethylene protons showing HSQC (Fig. S20) correlations with C-2 (δ 74.2) of an isoflavanone moiety. The ^{13}C NMR spectrum showed an oxygenated quaternary carbon at δ 73.5 (C-3) and a carbonyl carbon at δ 195.0 (C-4) along with a aliphatic hydroxyl ^{1}H NMR singlet at δ 6.61 which supports the compound SR-2 should be a 3-hydroxyisoflavanone skeleton. Further, the NMR spectrum of SR-2 consists two meta coupled aromatic signals at δ 5.94 (1H, d, *J* = 2.1 Hz) and 5.90 (1H, d, *J* = 2.1 Hz) of H-6 and H-8 protons of 5,7-dioxygentaed A-ring. It also showed signals corresponding to two methoxyl groups at δ 3.49 (3H, s) and 3.77 (3H, s), a γ,γ-dimethylallyl group [δ 1.66 (3H, s, CH_3), 1.62 (3H, s, CH_3), 3.24 (2H, brd, *J* = 6.5 Hz) and 5.12 (1H, brt, J = 6.5 Hz)] besides a C-5 chelated hydroxyl signal at δ 12.14 and a non-chelated hydroxyl at δ 10.80. The non-chelated hydroxyl δ 10.80 was positioned at C-7, as this carbon showed cross correlations with H-8 (δ 5.90) and H-6 (δ 5.94) in its HMBC spectrum (Fig. S21).

The presence of two ortho-coupled aromatic doublets at δ 6.80 (1H, d, *J* = 8.6 Hz) and 7.39 (1H, d, *J* = 8.6 Hz) were H-5′ and H-6′, respectively of a tri-substituted ring B of an isoflavavnone based on HSQC studies. The three proton singlet δ 3.49 was assigned to the methoxyl group at C-2′ (δ 155.5), is a characteristic signal of a di-ortho substituted environment system. The another methoxyl group at δ 3.77 to be placed at C-4′ as it showed cross correlations with H-5′ (δ 6.80) and H-6′ (δ 7.39) in its HMBC spectrum. This fixes the attachment of the γ,γ-dimethylallyl moiety at C-3′ which is confirmed by HMBC correlations Methylene protons at H-1″ position with C-3′. All the ^{1}H and ^{13}C NMR chemical shifts of compound SR-2 were confirmed and correlated with the HSQC (Fig. S20), HMBC (Fig. S21), COSY (Fig. S22)and NOSEY (Fig. S23) studies.



SR-2

Thus, from the foregoing spectral studies the structure of the isolated compound SR-2 was confirmed as 2′,4′-Dimethoxy-3′-(γ,γ-dimethylallyl)-3,5,7-trihydroxy-isoflavanone or Echinoisoflavanone, as its spectral data is good agreement with the literature values.