

Maca (*Lepidium meyenii* Walp.) inhibits HIV-1 infection through the activity of thiadiazole alkaloids in viral integration

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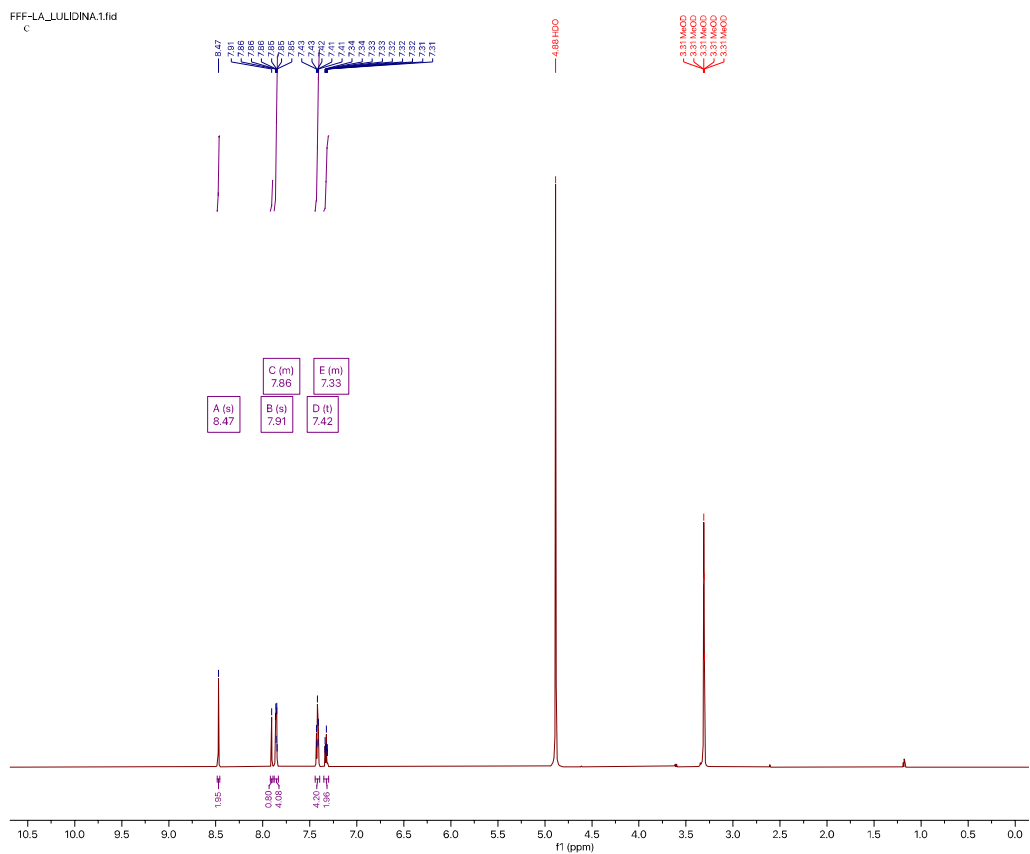
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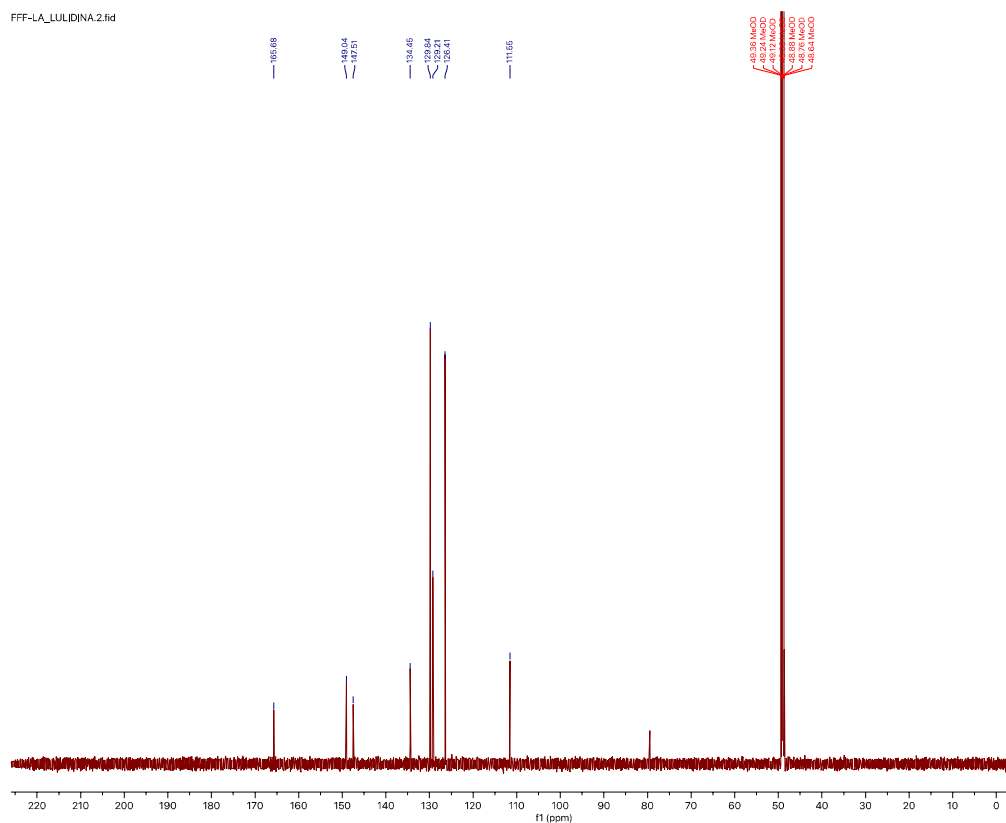
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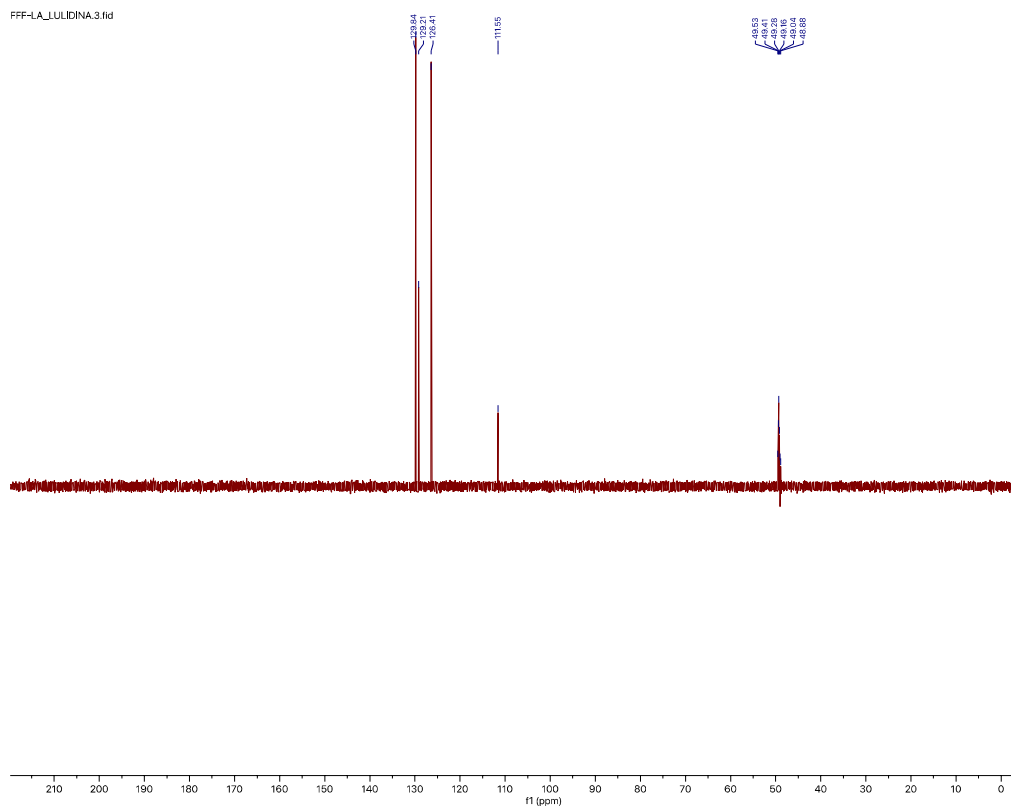
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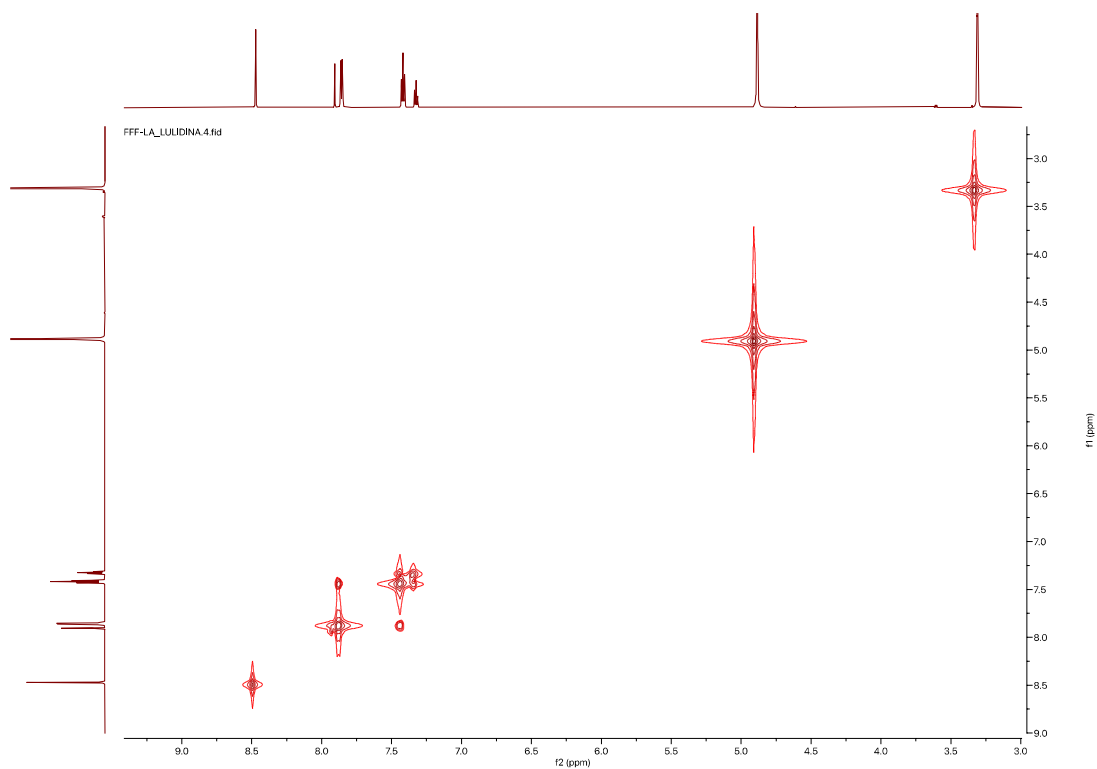
- Figure 5S. ^1H NMR spectrum of compound **1** in MeOD 700 MHz.



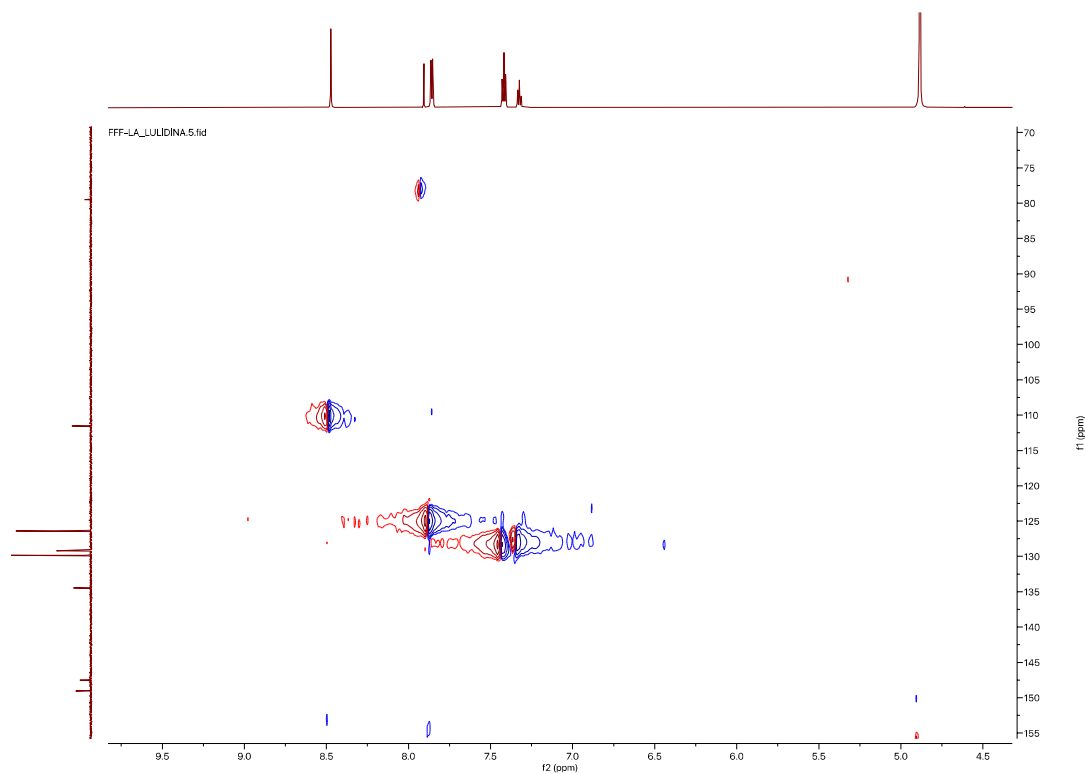
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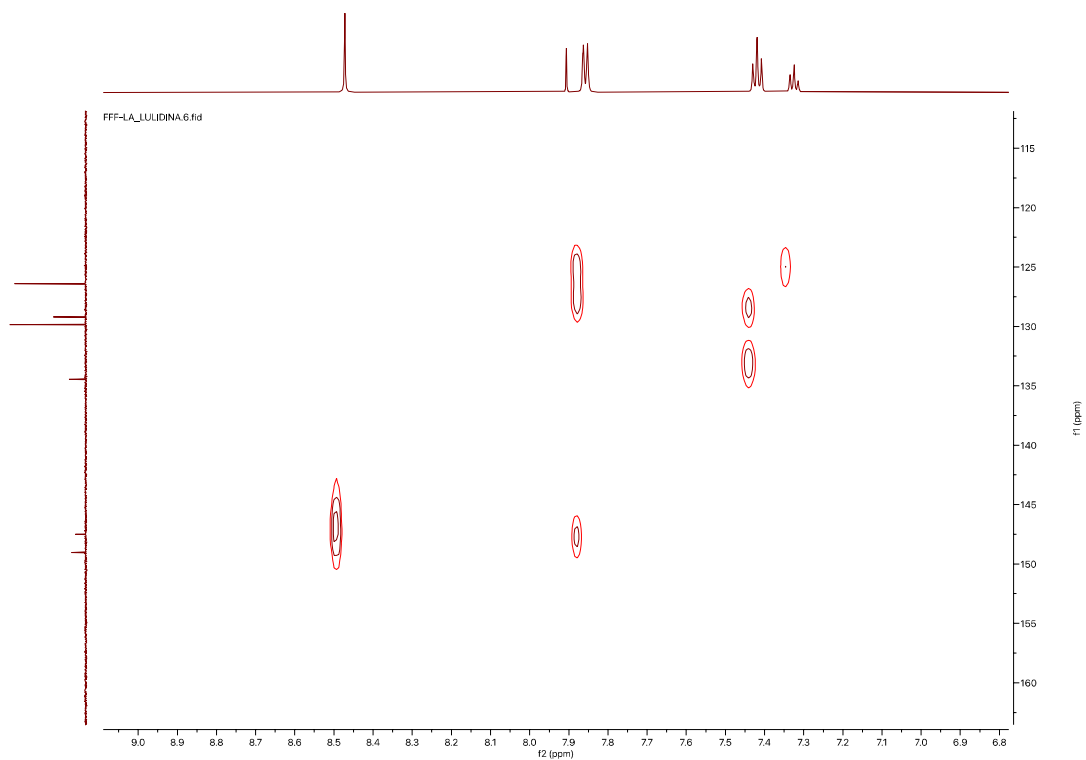
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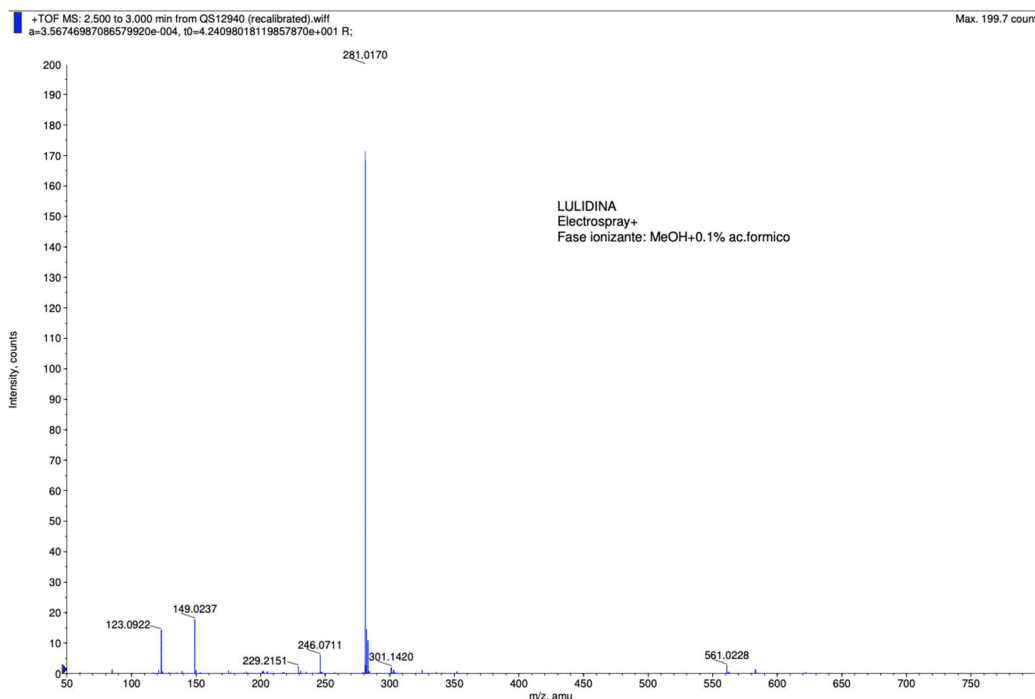
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- Figure 11S. HRESIMS spectrum of compound 1.

	HOJA DE RESULTADOS (2) DE C, H, N y S	Nº DE HOJA DE RUTA: 189/2011
		Fecha de realización: 13 / 12 / 2011
		Página 1 de 1

NOMBRE: Paulina Bermejo Benito

INVESTIGADOR PRINCIPAL: Paulina Bermejo Benito

Teléfono: 91 394 1871

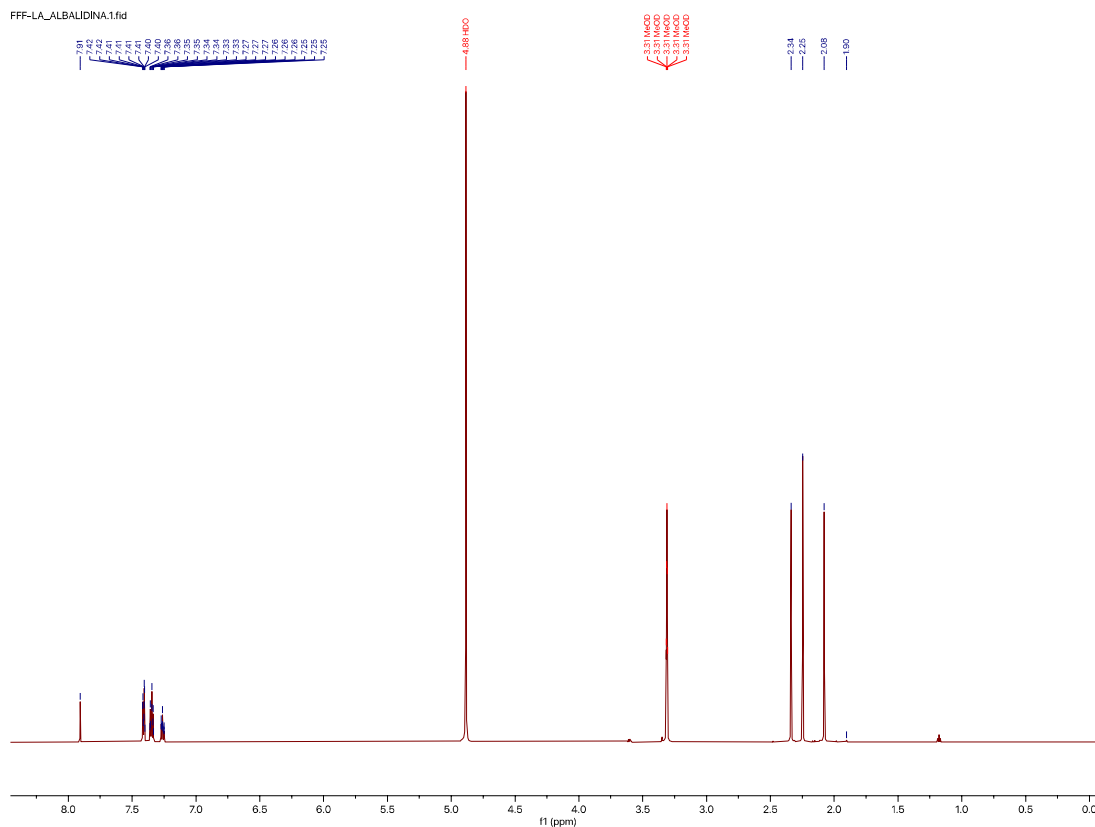
Estos son los resultados de la otra muestra que trajiste para analizar correspondiente a la solicitud de ensayo con **NE 210-01650**. Revisa los datos, como siempre, y me avisas para cerrar la solicitud.

Muestra: LULIDINA

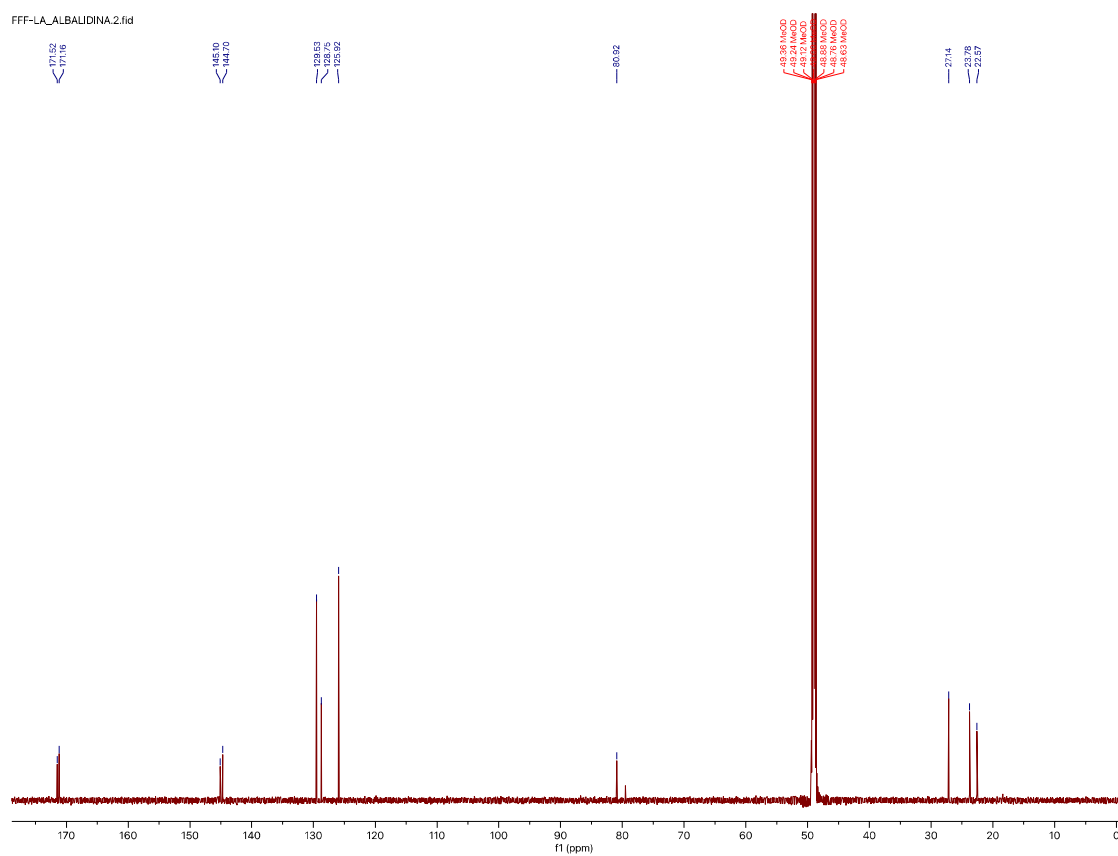
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Pascual Torres Redondo
 Responsable Técnico del Laboratorio

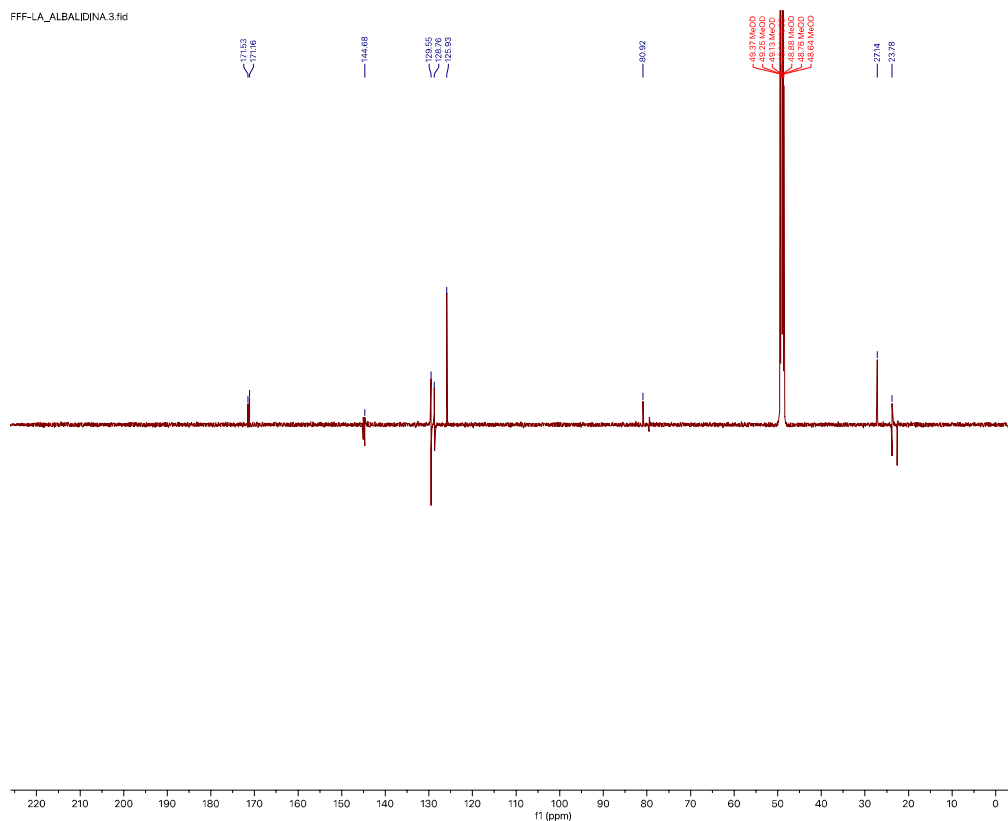
- Figure 12S. Elemental chemical analysis of compound 1.



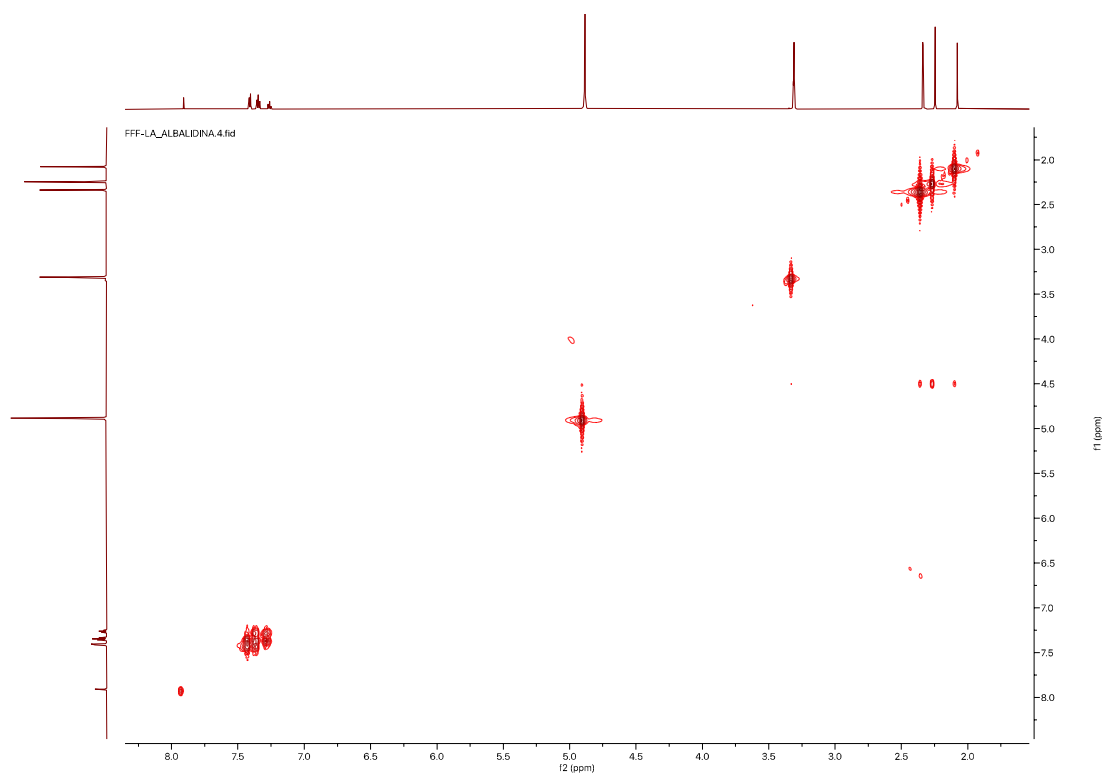
- Figure 13S. ^1H NMR spectrum of compound **2** in MeOD 700 MHz.



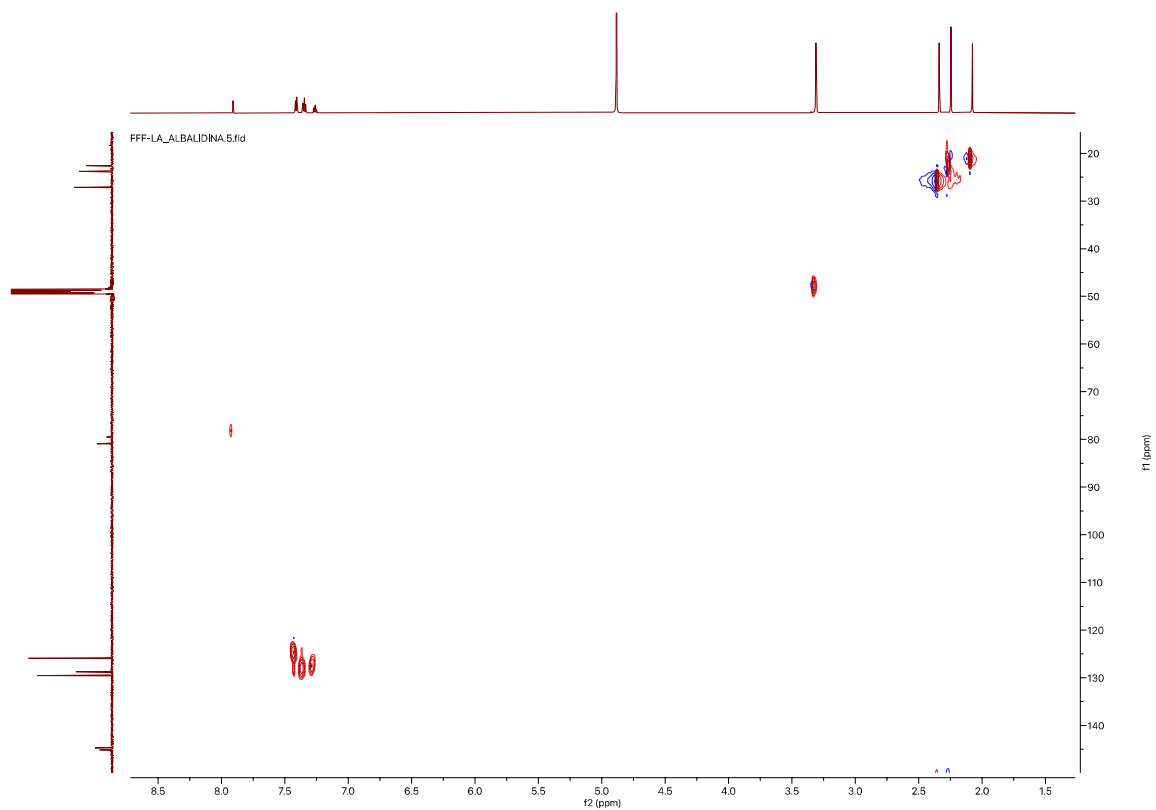
- Figure 14S. ^{13}C NMR spectrum of compound **2** in MeOD 176 MHz.



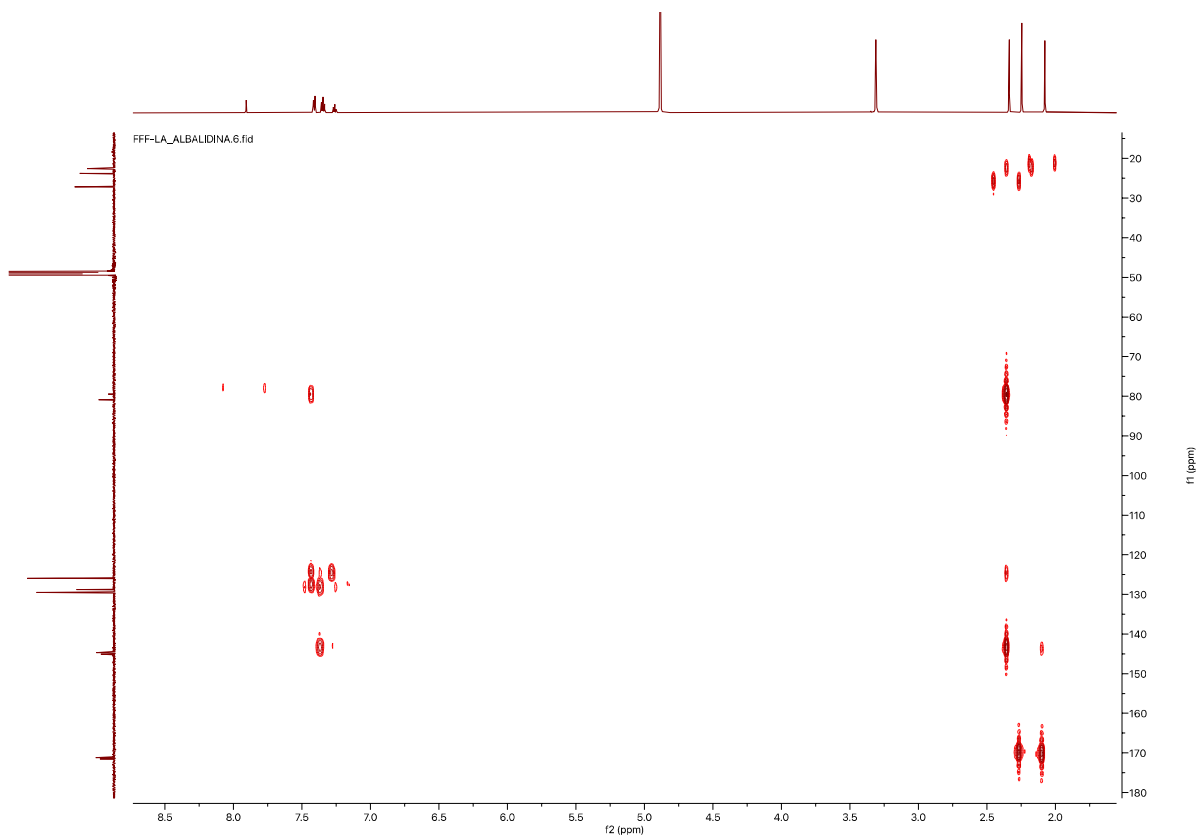
- Figure 15S. DEPT-135 spectrum of compound **2** in MeOD 176 MHz.



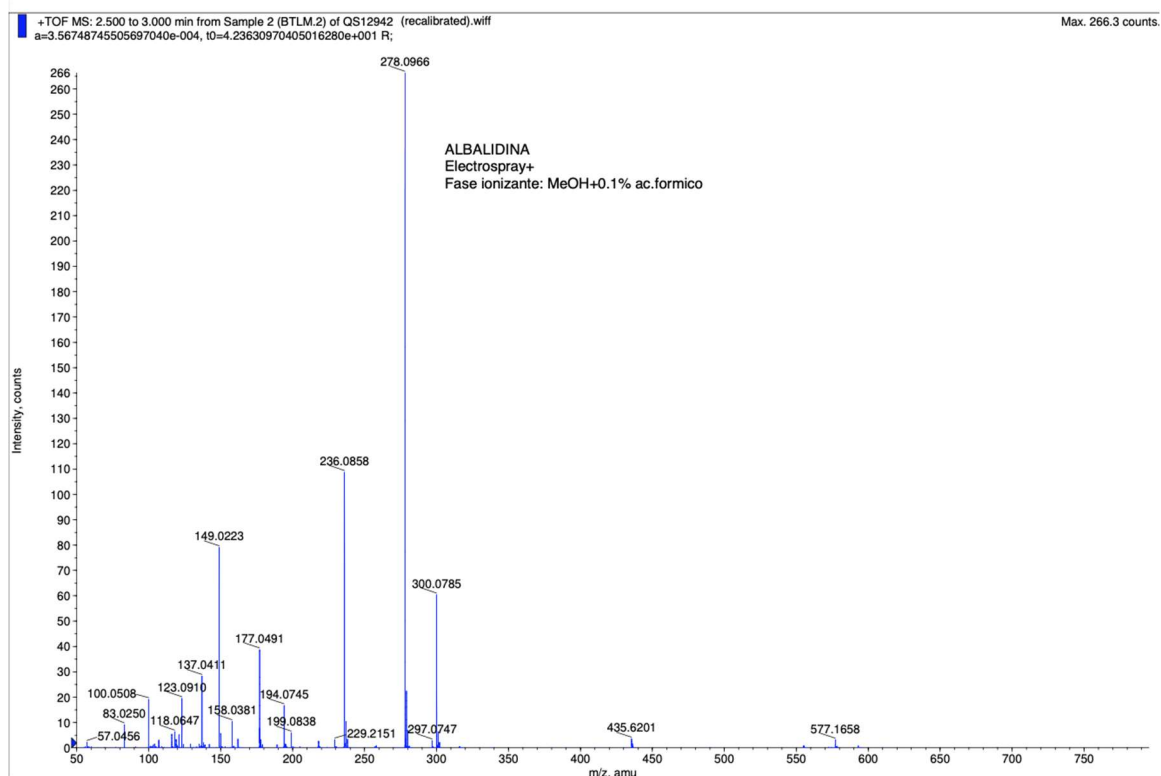
- Figure 16S. ^1H - ^1H COSY spectrum of compound **2** in MeOD.



- Figure 17S. HSQC spectrum of compound **2** in MeOD.



- Figure 18S. HMBC spectrum of compound **2** in MeOD.



- Figure 19S. HRESIMS spectrum of compound 2.

	HOJA DE RESULTADOS (1) DE C, H, N y S	Nº DE HOJA DE RUTA: 189/2011
		Fecha de realización: 13 / 12 / 2011
		Página 1 de 1

NOMBRE: Paulina Bermejo Benito

INVESTIGADOR PRINCIPAL: Paulina Bermejo Benito

Teléfono: 91 394 1871

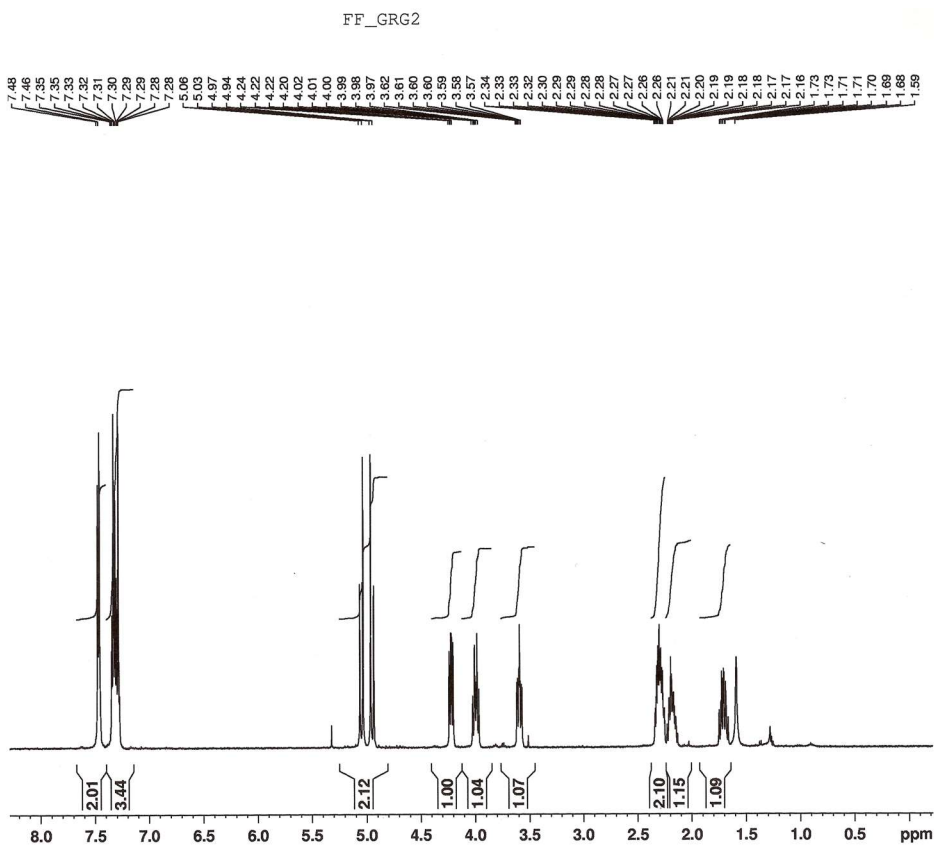
Estos son los resultados de una de las muestras que trajiste para analizar correspondiente a la solicitud de ensayo con **NE 210-01650**. Revisa los datos, como siempre, y me avisas para cerrar la solicitud.

Muestra: ALBALIDINA

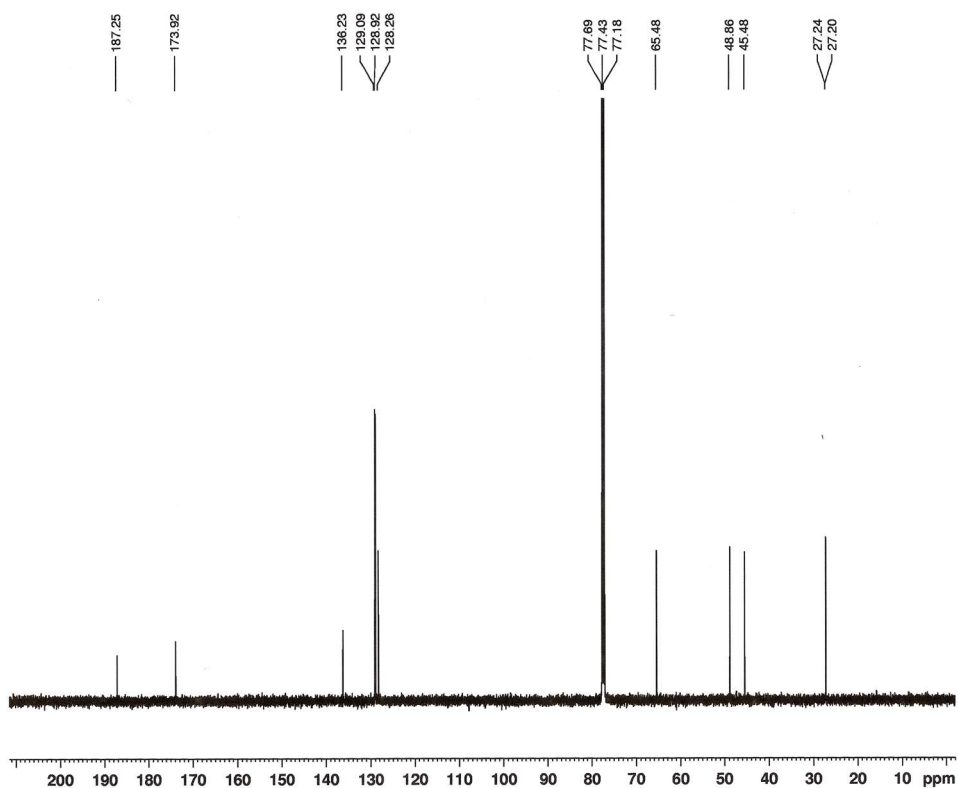
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Pascual Torres Redondo
Responsable Técnico del Laboratorio

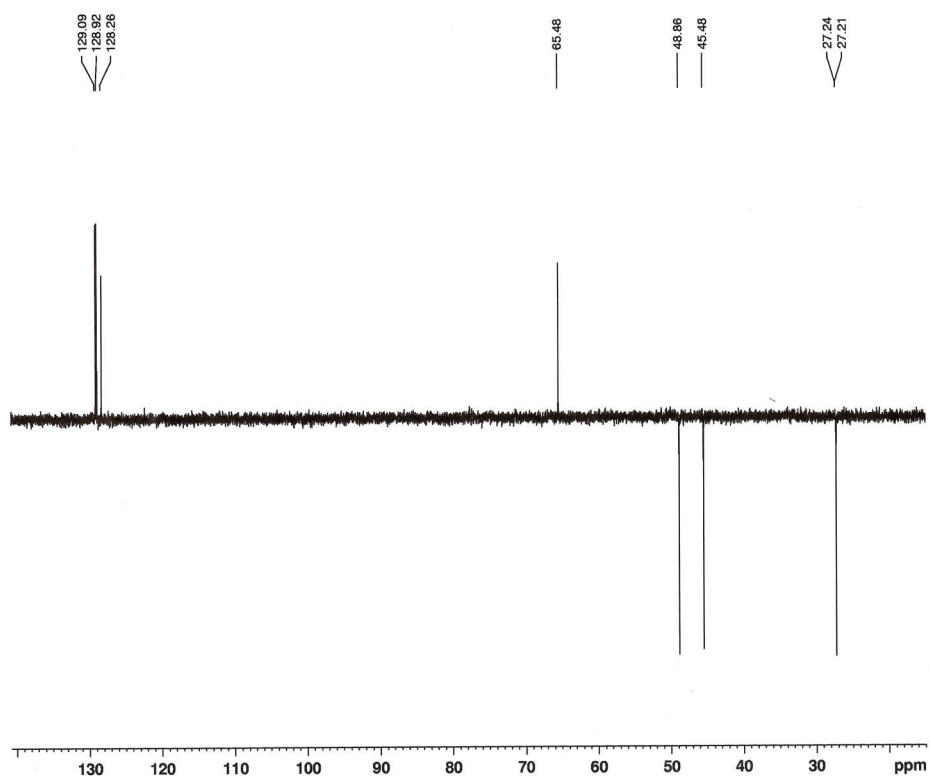
- Figure 20S. Elemental chemical analysis of compound 2.



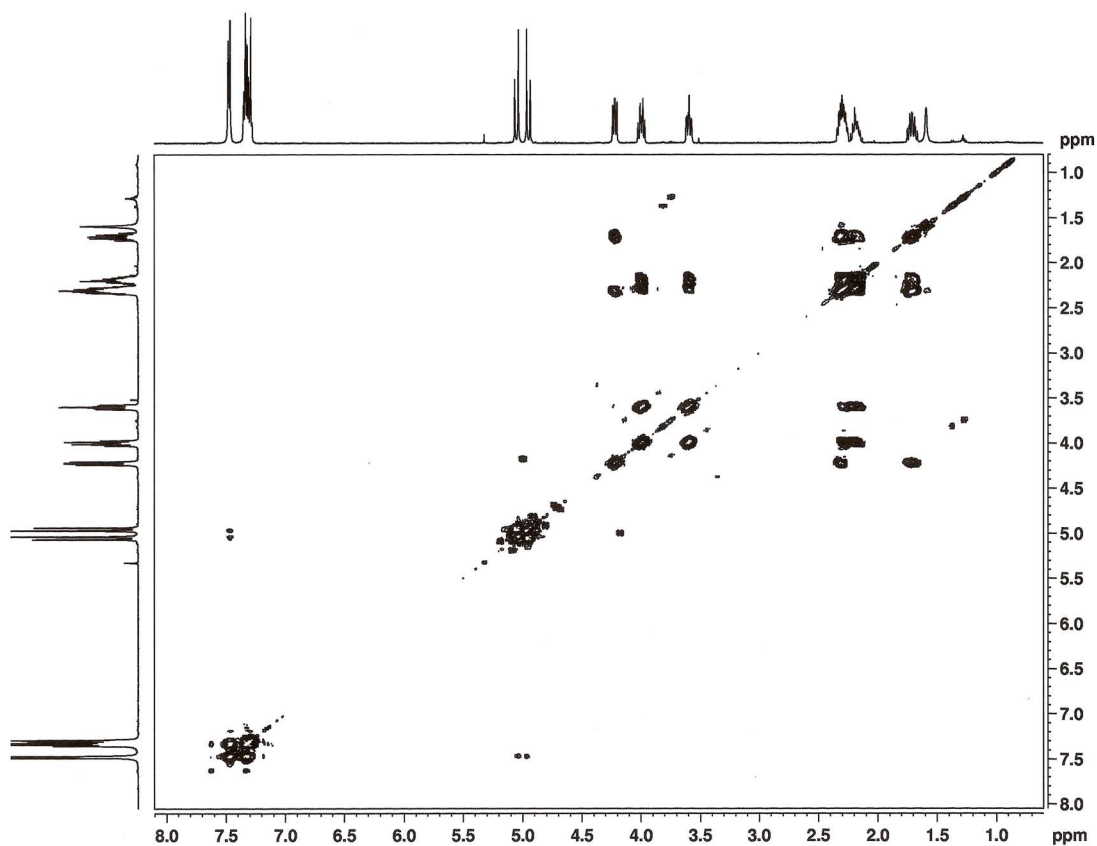
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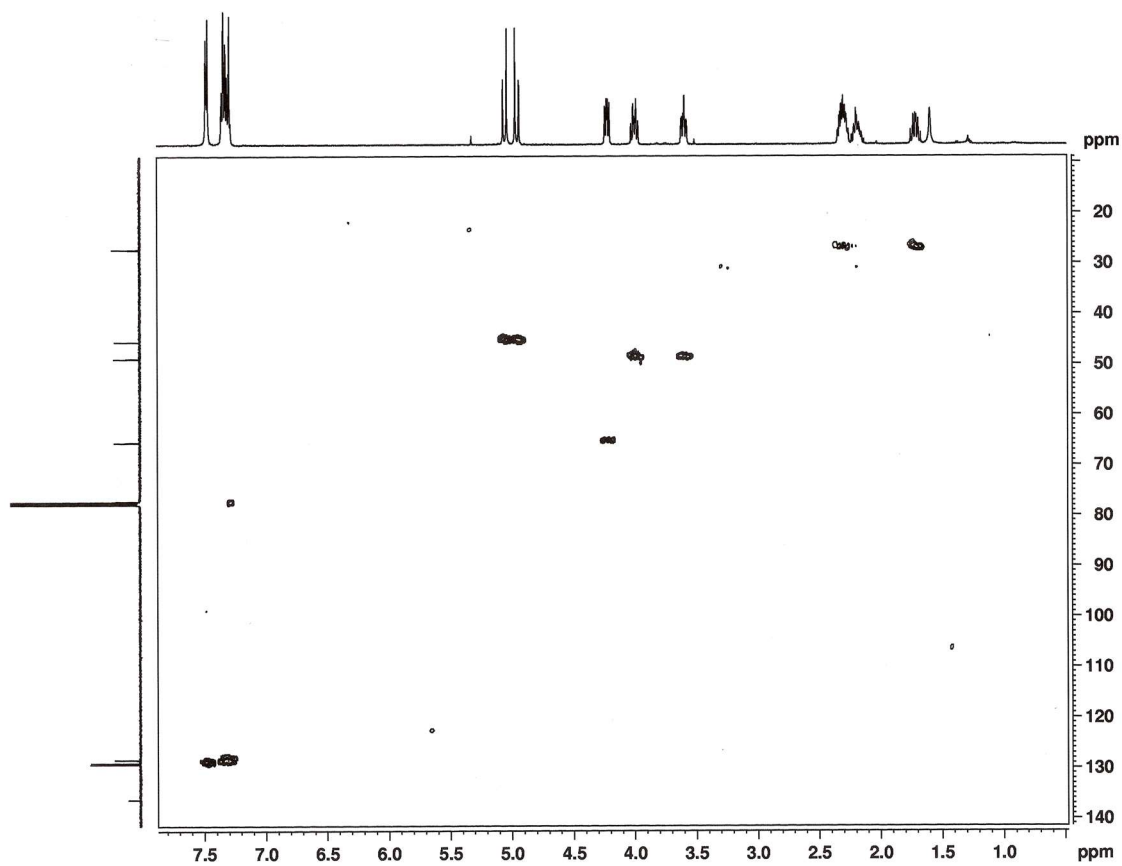
- Figure 22S. ^{13}C NMR spectrum of compound **3** in CDCl_3 60 MHz.



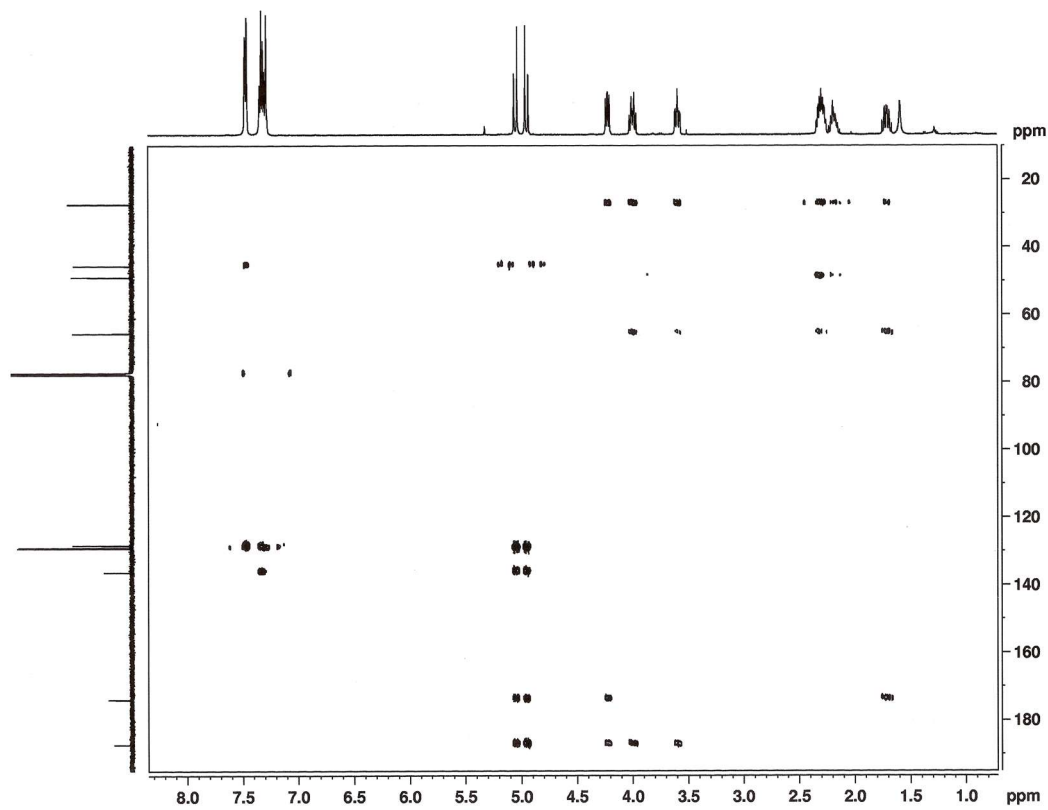
- Figure 23S. DEPT-135 spectrum of compound **3** in CDCl₃ 60 MHz.



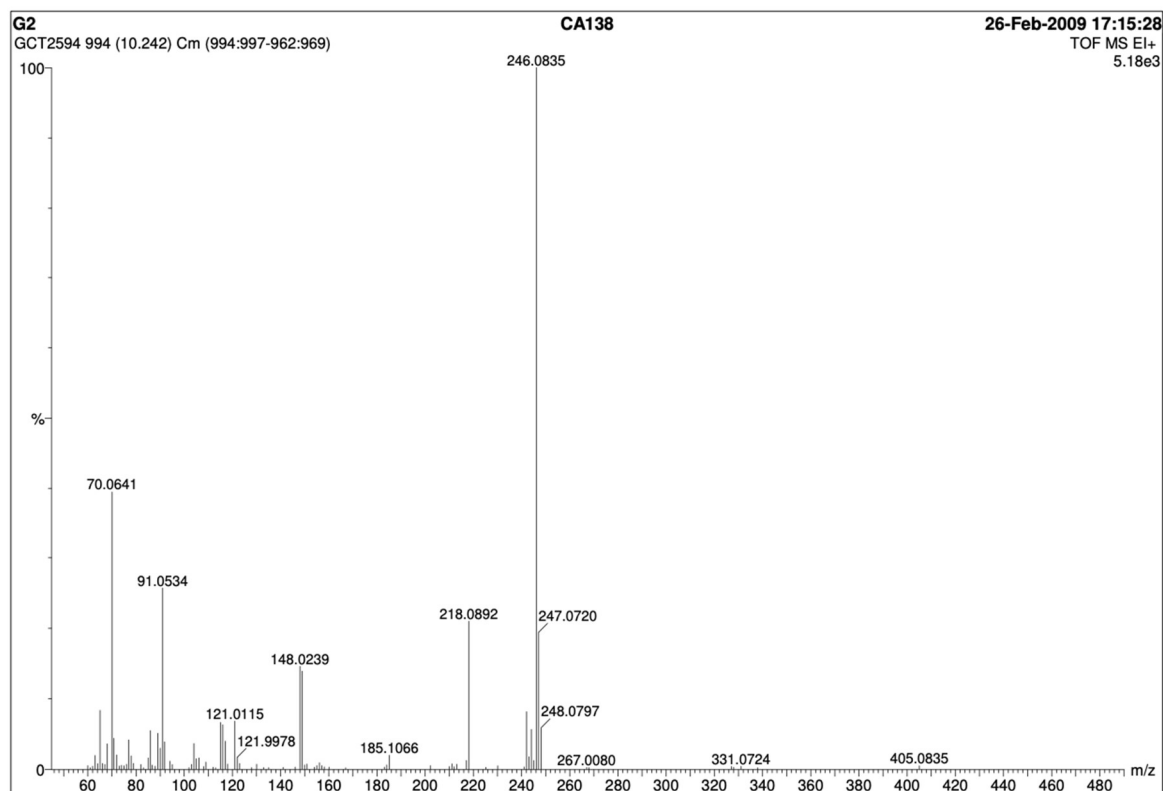
- Figure 24S. ¹H-¹H COSY spectrum of compound **3** in CDCl₃.



- Figure 25S. HSQC spectrum of compound **3** in CDCl_3 .



- Figure 26S. HMBC spectrum of compound **3** in CDCl_3 .



- Figure 27S. HRESIMS spectrum of compound 3.



TIPO DE ENSAYO: CHN , CHNS

Informe de ensayo nº F02-09-039

Usuario CARMEN AVENDAÑO LÓPEZ Código Cliente CAL
 Centro UNIVERSIDAD COMPLUTENSE. FACULTAD DE FARMACIA
 Departamento DPTO. Q. ORGÁNICA Y FARMACÉUTICA
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 Fecha/Hora Entrada 24/02/09 12:50 Fecha/Hora Análisis 03/05/09 11:28
 Observaciones Sólido beis
 Método utilizado PNT01 Equipo LECO CHNS-932. Código 3178

Datos obtenidos por el analizador

Código CAI	C. Muestra	%C	%H	%N	%S
F02-09-039	Sri-G	63,66	5,75	11,18	12,58

RESULTADO DEL ANÁLISIS:

Código CAI	C. Muestra	%C	%H	%N	%S
F02-09-039	Sri-G	DM	DM	DM	DM

Alcance validado %C 0,50-94,7 %H 0,50-7,6 %N 0,50-23 %S 0,50-30,6
 Desviaciones del método Peso inferior al limite



V^oR^o Resp. Técnico
 José Carlos Menéndez Ramos
 Fecha de emisión: 03/05/09
 Fecha de edición: 07/03/08

INFORME DE ENSAYO Nº: F02-09-039 página 1 de 1
 Los resultados aquí expuestos se refieren únicamente a la muestra a la que se hace referencia.
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- Figure 28S. Elemental chemical analysis of compound 3.

Reverse transcription		
HIV-1 Reverse transcription (early)	MA pr-243	GTGCCCGTCTGTTGTGTGAC
	MA pr-244	GCGCCACTGCTAGAGATTT
	MA pr-275 probe	CTAGAGATCCCTCAGACCCCTTTTAGTCAGTGTGG (FAM)
HIV-1 Reverse transcription (late)	MH 531	TGTGTGCCCGTCTGTTGTGT
	MH 532	GAGTCCTGCGTCGAGAGATC
	Probe LRT-P	CAGTGGCGCCCGAACAGGGA (FAM)
CCR5 gene	CCR5_R	CTCACAGCCCTGTGCCTCTTCTTC
	CCR5_F	GCTGTGTTTGCGTCTCTCCAGGA
	PROBE CCR5	AGCAGCGGCAGGACCAGCCCAAG (FAM)
Integration		
1st PCR	Primer Alu 1	TCCCAGCTACTGGGGAGGCTGAGG
	Primer Alu 2	GCCTCCCAAAGTGCTGGGATTACAG
	Primer L-M667	ATGCCACGTAAGCGAAACTCTGGCTAACTAGGGAACCCACTG
2nd PCR	Primer Lambda T	ATGCCACGTAAGCGAAACT
	Primer AA55M	GCTAGAGATTTTCCACACTGACTAA
	Probe MH603	ACACTACTTGAAGCACTCAAGGCAAGCTTT (FAM)
CCR5 gene	CCR5_R	CTCACAGCCCTGTGCCTCTTCTTC
	CCR5_F	GCTGTGTTTGCGTCTCTCCAGGA
	PROBE CCR5	AGCAGCGGCAGGACCAGCCCAAG (FAM)
All PCRs except Integration First PCR		
50 °C	2:00	
95 °C	10:00	Initial denaturation
95 °C	0:15	50 cycles
60°C	1:00	
Integration First PCR		
95 °C	8:00	Initial denaturation
95 °C	1:00	12 cycles
60 °C	1:00	
72 °C	10:00	
72 °C	15:00	Final extension

- Table 1S. PCR thermal cycling conditions, primers and probes used in qPCR experiments to quantify reverse transcripts and integrated provirus.

MATERIAL AND METHODS

- A. Extraction and isolation complete procedure.

Hypocotyls of *L. meyenii* were dried in a hot air oven at 50 °C for 48 h. The resulting product was pulverised until obtaining a fine powder (250 g). Subsequently, the sample was extracted three times with 500 mL of solvents of increasing polarity, n-hexane (LM0), ethanol (LM2), and water (LM3) at room temperature (25 ± 5 °C) for 72 h. The extracts were then concentrated in vacuo, obtaining 20.40 g (LM0), 90.12 g (LM2) and 36.36 g (LM3).

LM0 extract was selected for further study and, following the same procedure as above, black, purple and yellow hypocotyls (250 g for each) were extracted three times with 500 mL of n-hexane (LM0). All the extracts were filtered, and the respective solvents were removed by vacuum rotary evaporation at room temperature (25°C). As a result, three n-hexane extracts were obtained, of the black hypocotyls (29.65 g), purple hypocotyls (23.63 g) and yellow hypocotyls (17.92 g).

The n-hexane extract of purple maca hypocotyls was fractionated using a chromatographic column (2x50 cm), employing Si-60 silica gel (40-63 µm, Merck) as the stationary phase, and using a gradual gradient of HEX/Ethyl acetate (AcOEt) (3:1-1:1 v/v) as eluent. Twelve fractions were obtained: F1 (3.12 g), F2 (2.44 g), F3 (1.43 g), F4 (2.44 g), F5 (1.41 g), F6 (1.52 g), F7 (1.53 g), F8 (2.55 g), F9 (1.50 g), F10 (1.25 g), F11 (1.27 g) and F12 (1.46 g).

Subsequently, based on the antiviral and cell viability data (Table 1), separations of **F4**, **F7** and **F8** fractions were carried out. Fraction **F4** (2.44 g) was separated employing a chromatography column (2x50 cm) with Si-60 Silica gel (40-63 µm, Merck) as a stationary phase and a gradual gradient of HEX/Diethyl ether (Et₂O) (8:2-1:2 v/v) as eluent. Four sub-fractions were obtained: F4a (52.30 mg), F4b (56.70 mg), F4c (12.30 mg) and F4d (27.20 mg). Subsequently, the **F4d** sub-fraction was purified using a microcolumn, obtaining compound **1** (14.80 mg).

Fraction **F7** (1.53 g) was columned employing a chromatography column (2x50 cm) with Si-60 Silica gel (40-63 µm, Merck) as a stationary phase and a gradual gradient of HEX/Et₂O (6:4-1:4 v/v) as eluent. It led to

obtaining ten sub-fractions: F7a (90.23 mg), F7b (40.65 mg), F7c (40.75 mg), F7d (31.23 mg), F7e (24.78 mg), F7f (70.12 mg), F7g (94.34 mg), F7h (48.53 mg), F7i (70.89 mg) and F7j (63.12 mg). The **F7d** sub-fraction was purified employing a microcolumn, obtaining compound **2** (13.93 mg).

Finally, sixteen sub-fractions (F8a 16.70 mg; F8b 11.20 mg; F8c 3.90 mg; F8d 12.06 mg; F8e 19.56 mg; F8f 25.66 mg; F8g 34.97 mg; F8h 23.41 mg; F8i 13.51 mg; F8j 12.44 mg; F8k 36.52 mg; F8l 68.30 mg; F8m 48.32 mg; F8n 76.60 mg; F8o 80.40 mg and F8p 22.60 mg) were obtained from the separation of **F8** (2.55 g) employing a chromatography column (2x50 cm) with Si-60 Silica gel (40-63 μm , Merck) as a stationary phase and a gradual gradient of HEX/AcOEt/Butanol (ButOH) (8:1:1 v/v/v) as eluent. The **F8m** sub-fraction was purified by microcolumn chromatography, obtaining compound **3** (40.10 mg).

- B. STRUCTURAL DATA. Structural data for the three compounds isolated

Compound 1: 6-Phenylimidazo[2,1-b][1,3,4]thiadiazole-2-sulfonamide (**1**), white powder; ^1H NMR (700 MHz, MeOD): δ_{H} 8.47 (1H, s), 7.88 (2H, m), 7.42 (2H, d, $J=7.8$ Hz), 7.33 (1H, m); ^{13}C NMR (176 MHz, MeOD): δ_{C} 165.68 (C-2), 149.04 (C-8), 147.51 (C-6), 134.45 (C-1'), 129.84 (C-3'/C-5'), 129.21 (C-4'), 126.41 (C-2'/C-6'), 111.55 (C-5); Elemental chemical analysis C=42.72%, H=3.18%, N=19.77%, S=22.44%; HRESIMS m/z 281.0167 $[\text{M}]^+$ (calcd for $\text{C}_9\text{H}_7\text{N}_5\text{O}_2\text{S}_2$, 281.0041) (Figures 5S to 11S).

Compound 2: *N*-(4-Acetyl-5-methyl-5-phenyl-4,5-dihydro-1,3,4-thiadiazol-2-yl) acetamide (**2**), white powder; ^1H NMR (700 MHz, MeOD): δ_{H} 7.41 (2H, dd, $J=7.5$; 1.5 Hz), 7.34 (2H, t, $J=7.5$ Hz), 7.26 (1H, tt, $J=7.5$; 1.5 Hz), 2.34 (3H, s), 2.25 (3H, s), 2.08 (3H, s); ^{13}C NMR (176 MHz, MeOD): δ_{C} 171.52 (C-8), 171.16 (C-10), 145.10 (C-2), 144.70 (C-1'), 129.53 (C-3'/C-5'), 128.75 (C-4'), 125.92 (C-2'/C-6'), 80.92 (C-5), 27.14 (C-6), 23.78 (C-11), 22.57 (C-9); Elemental chemical analysis C=56.04%, H=5.58%, N=15.04%, S=11.57%; HRESIMS m/z 278.0966 $[\text{M}+\text{H}]^+$ and 300.0785 $[\text{M}+\text{Na}]^+$ (calcd for $\text{C}_{13}\text{H}_{16}\text{N}_3\text{O}_2\text{S}$, 278.0963 and $\text{C}_{13}\text{H}_{15}\text{N}_3\text{O}_2\text{SNa}$, 300.0783) (Figures 13S to 18S).

Compound 3: 2-Benzyl-3-thioxohexahydropyrrolo[1,2-c]imidazol-1-one (**3**), beige solid; ^1H NMR (250 MHz, CDCl_3): δ_{H} 7.47 (2H, d, $J=7.3$ Hz), 7.32 (2H, dd, $J=7.1$; 7.3 Hz), 7.28 (1H, t, $J=7.1$ Hz), 5.05 (1H, d, $J=14.6$ Hz, H-1'), 4.96 (1H, d, $J=14.6$ Hz, H-1'), 4.22 (1H, dd, $J=10.3$; 7.1 Hz), 4.02 (1H, m, H-5), 3.62 (1H, m, H-5), 2.29 (2H, m, H-6/H-7), 2.18 (1H, m, H-6); ^{13}C NMR (60 MHz, CDCl_3): δ_{C} 187.25 (C-3), 173.92 (C-1), 136.23 (C-2'), 129.09 (C-3'/C-7'), 128.92 (C-4'/C-6'), 128.26 (C-5'), 65.48 (C-8), 48.86 (C-5), 45.48 (C-1'), 27.24 (C-6), 27.20 (C-7); Elemental chemical analysis C=63.66%, H=5.75%, N=11.18%, S=12.58%; HRESIMS m/z 247.0720 $[\text{M}+\text{H}]^+$ (calcd for $\text{C}_{13}\text{H}_{15}\text{N}_2\text{OS}$, 247.0905) (Figures 21S to 27S).

C. Experimental design of the fractionation process and the bioassays used.

