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Article

Chemical Constituents from the Roots of *Spathelia excelsa* and their Antiprotozoal Activity

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A investigação fitoquímica das raízes de *Spathelia excelsa* levou ao isolamento das cromonas 10(2,3-epóxi-3-metilbutanil)spatheliacromeno e 10(2,3-diidroxi-3-metilbutanil) metoxispatheliacromeno (5-metoxispatheliabiscromeno); o limonóide desacetilspathelina e epímero C-21 do protolimonóide 3β-angeloiloxi-7α,24,25-triidroxi-21,23-óxido-14,18-cicloapotirucal-21-hemiacetal; os alcalóides 7,8-dimetoxiflindersina, casimiroina e N-methyl-4,7,8-trimetoxiquinolino-2(1*H*)-ona, além da mistura de β-sitosterol e stigmasterol. Nos ensaios biológicos sobre a forma promastigosta de *Leishmania braziliensis*, desacetilspathelina apresentou atividade moderada e sobre a forma epimastigota de *Trypanossoma cruzi*, 10(2,3-epóxi-3-metilbutanil)spatheliacromeno apresentou atividade forte (IC_{50} = 11 μg mL^{-1}).

Phytochemical investigation from roots of *Spathelia excelsa* yielded the chromones 10(2,3-epoxy-3-methylbutanyl)spatheliachromen and 10(2,3-dihydroxy-3-methylbutanyl) methoxyspatheliacromen (5-methoxyspatheliabischromen); limonoid deacetylspathelin and protolimonoid C-21-epimers 3β-angeloyloxy-7α,24,25-trihydroxy-21,23-oxide-14,18-cycloapotirucall-21-hemiacetal; the alkaloids 7,8-dimethoxyflindersin, casimiroin and N-methyl-4,7,8-trimethoxyquinolin-2(1*H*)-one, besides a mixture of β-sitosterol and stigmasterol. Assays on promastigote forms of *Leishmania braziliensis*, deacetylspathelin showed moderate activity; and on epimastigote forms of *Trypanossoma cruzi*, 10(2,3-epoxy-3-methylbutanyl)spatheliachromen exhibited strong activity (IC_{50} = 11 μg mL^{-1}).

Keywords: Rutaceae, *Spathelia*, *Trypanossoma cruzi*, *Leishmania braziliensis*

Introduction

Spathelia L is a monotypic genus in the subfamily Spatheloideae (Rutaceae), that comprises about 15 species distributed in the Bahamas, Cuba, Jamaica and northern South America.[1] Species of *Spathelia* resembles palm tree reaching up to 20 m, what distinguished them from other genus of Rutaceae. *Spathelia excelsa* (Krause) Cowan & Brizicky (sin. *Sohnreyia excelsa K.*), a hapaxant tree,[2] is found only in the Brazilian Amazon. Quinolone alkaloids, limonoids, flavonoid and coumarin have been reported as constituents of *S. excelsa* leaves.[3,4]

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In this paper we describe the isolation and structural identification of eight compounds from roots of this plant, and evaluate the trypanocidal and leishmanicidal activities of compounds 10(2,3-epoxy-3-methylbutanyl) spatheliachromen, deacetylspathelin, 3β-angeloyloxy-7α,24,25-trihydroxy-21,23-oxide-14,18-cycloapotirucall-21-hemiacetal and casimiroin.

Results and Discussion

Chemical composition of the extracts

Investigation of the *n*-hexane and dichromethane combined extracts from *S. excelsa* roots yielded a mixture of

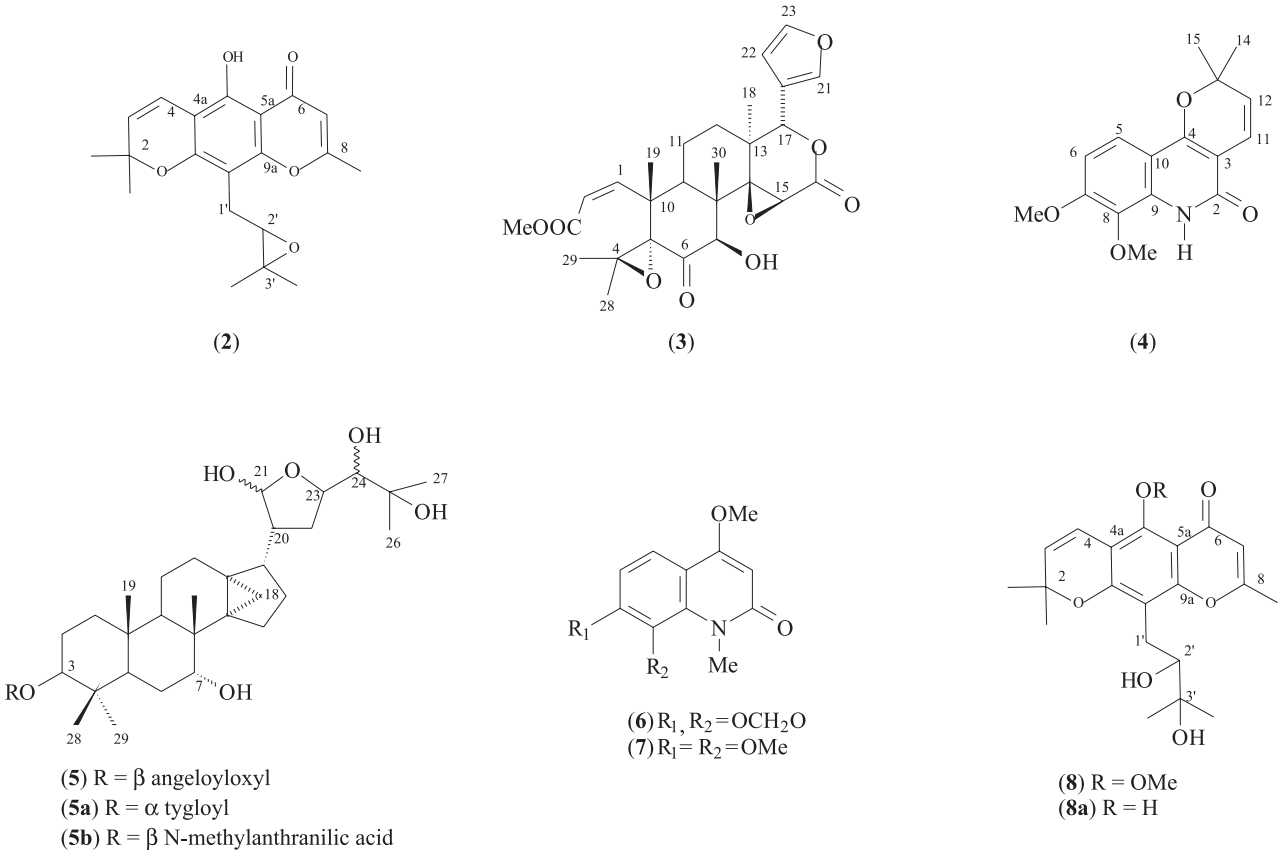

Figure 1. Compounds isolated from *Spathelia excelsa*.

steroids (β-sitosterol and stigmasterol, **1**), pyranochromones (**2**, **8**), limonoid (**3**), protolimonoid (**5**), and alkaloids, pyranoquinolin-2(1*H*)-one (**4**), and quinolin-2(1*H*)-one (**6**, **7**).

The ^{1}H NMR spectrum (Table 1) of compounds **2** and **8** showed signals consistent with the presence of system 2,2-dimethylpyran, singlets for geminal methyls at δ 1.46 and 1.48 of **2,** δ 1.47 and 1.50 of **8**; a pair of doublets (*J* 10 Hz) assigned to the olefinic hydrogen [δ 5.61 (H-3), 6.72 (H-4) **2**] and [δ 5.72 (H-3), 6.72 (H-4) **8**]. The signals of methyl doublet at δ 2.36 (**2**) and 2.29 (**8**), associated with hydrogen quartet at δ 6.02 (*J* 0.72 Hz) **2**, 5.98 (*J* 0.8 Hz) **8**, characterized the 2-methyl-g-pyrone moiety of the chromone.

Chemical shifts of carbon in the PENDANT of **2** and ^{13}C NMR of **8** (Table 1) were compared to those of spatheliabischromen (**8a**).[5] In chromone **2,** the group 2',3'-epoxy-3'-methylbutanyl attached to C-10 was determined by the presence of epoxy, which was confirmed by shifts at d 59.1 (C) and 63.2 (CH). The HSQC experiment showed a correlation of this latter signal with hydrogen at δ 2.95. In the HBMC experiment (Table 2), we observed correlations between methylene hydrogen (δ 2.83 and 3.08) and carbons at δ 63.2 (^2J), 59.1, 155.1, 157.1 (^3J) and 103.3 (^4J). Chromone **2** is thus 10 (2,3-epoxy-3-methylbutanyl) spatheliachromen, reported for first time in the literature.

Spectral data (^{1}H and ^{13}C NMR) comparison established that **8** was distinguished from **8a**[5] by the presence of methoxy group (δ 3.85→ 62.8, by HSQC) attached to C-5. Correlations observed in the HMBC experiment between methoxyl hydrogen and carbon at δ 153.8 (C-5), olefinic hydrogen δ 6.72 (H-4) with 153.8 (C-5), confirmed the position of this methoxy group at C-5, also showed the linearity of the pyranochromone **8** (5-methoxyspatheliabischromen).

The ^{1}H NMR spectrum of **3** showed signals indicative of furan ring at δ 7.64 (dt), 6.51 (ddd) and 7.57 (m); five singlets in region δ 1.27-0.70 relative to the methyl groups. The singlets at δ 5.52 and 4.08 assigned to the H-17 and H-15 protons are typical for limonoids with ring 14,15-epoxy *D* lactone. Data ^{13}C NMR (Table 3) **s**howed similarities with those reported for deacetylspathelin.[3,6]

The ^{1}H NMR of compound **5** presented signals for oxymethine hydrogens at δ 4.84 (dd, H-3), 3.94 (sl, H-7), 4.52 (sl, H-23) and 3.62 (sl, H-24), hydrogen attached to a carbon hemiacetal at δ 5.34 (sl, H-21). ^{13}C NMR spectrum compared with those of **5a**[7] and **5b**[8] indicated that protolimonoid **5** (Table 3) presents a mixture of epimers in C-23 and substitution angeloyl to C-3. The angelate group was evidenced by signals olefinic hydrogen at δ 6.01 (dd, *J* 7.2, 1.4, H-3'), methyls at δ 1.96 (dq, *J* 7.2, 1.4, Me-4') and δ 1.67 (*s*, Me-5'). HSQC

Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) chemical shifts for chromones **2** and **8**, in $CDCl_3$

No.	NMR ^{1}H		PENDANT	NMR ^{13}C
	2	**8**	**2**	**8**
2			78.1	78.2
3	5.61 d (10)	5.72 d (10)	127.8	130.1
4	6.72 d (10)	6.72 d (10)	115.7	116.4
4a			105.2	112.6
5			155.3	153.8
5a			103.3	112.3
6			182.8	177.2
7	6.02 q (0.72)	5.98 q (0.8)	108.6	111.5
8			166.3	163.1
9a			155.1	155.4
10			105.0	110.7
10a			157.1	156.8
1’	2.83 dd (13.83, 7.57) H-a	2.88 dd (10, 13.6) H-a	22.1	25.8
	3.08 dd (13.83, 4.56) H-b	3.03 dd (13.6, 2.4) H-b		
2’	2.95 dd (7.57, 4.56)	3.67 dl	63.2	78.6
3’			59.1	72.9
2,2-diMe	1.48 s, 1.46 s	1.50 s, 1.47 s	28.4, 28.2	28.3, 28.5
3’,3’-diMe	1.44 s, 1.29 s	1.32 s,1.31 s	24.8, 19.2	26.2, 23.6
8-Me	2.36 d (0.72)	2.29 d (0.8)	20.5	19.8
5-OMe		3.85 s		62.8
5-OH	13.03 s			

Chemical shifts (δ, ppm) and coupling constants (J, Hz, in parenthesis).

Table 2. HMBC (400/100 MHz) assignments for chromones **2** and **8**, in $CDCl_3$

H	**2** HMBC			**8** HMBC	
	C ($^{2}J_{C-H}$)	C ($^{3}J_{C-H}$)	C ($^{4}J_{C-H}$)	C ($^{2}J_{C-H}$)	C ($^{3}J_{C-H}$)
3	2	4a, 2a,b-Me		2	4a, 2a,b-Me
4		2,5,10a		4a	2, 5, 10a
7	8	5a, 8-Me		8-Me	5a, 8-Me
1’	2’	3’, 9a, 10a	5a	2’, 10	3’, 10a
2’	1’				
2-Me	2	3, 2a,b-Me		2	3, 2a,b-Me
3’a-Me	3’	2’, 3’b- Me			3’b-Me
3’b-Me	3’	2’, 3’a-Me			3’a-Me
8-Me	8	7		8	7
5-OH	5	4a, 5a			
5-OMe					5

Table 3. ^{13}C (100 MHz) chemical shifts for limonoid **3** and protolimonoid **5**, in $(CD_3)_2CO$

C	**3**	**5**	C	**3**	**5**
1	162.0	38.6	19	22.5	17.3
2	119.5	27.6	20	121.6	49.6
3	167.4	81.0	21	142.4	97.6/ 94.3
4	69.3	37.4	22	112.0	31.5
5	72.5	44.5	23	144.1	78.3
6	208.3	25.5	24		78.2
7	82.8	74.2	25		73.3/ 73.4
8	45.4	38.9	26		28.5
9	46.7	45.6	27		28.1
10	45.9	37.8	28	29.0	30.5
11	21.8	17.0	29	20.2	20.0
12	33.2	24.1	30	13.0	19.8
13	38.6	28.0	3-OMe	52.2	
14	68.0	37.4	1’		167.7
15	52.4	27.2	2’		129.0
16	167.6	27.7	3’		137.3
17	78.6	46.3	4’		15.9
18	20.7	14.6	5’		21.0

showed correlations of hydrogen at δ 4.84 and 3.94 with carbons at δ 81.0 (C-3) and 74.2 (C-7), respectively. In the HMBC experiment, the observed correlations of angelate group were of hydrogen at δ 4.84 (H-3)**,** with carbon at δ 167.7 (C-1’, ^{3}J); hydrogen at δ 6.01 (H-3’), with carbons at δ 15.9 (Me-4’, ^{2}J) and 21.0 (Me-5’, ^{3}J); hydrogen at δ 1.96 (Me-4’), with carbons at δ 129.0 (C-2’, ^{2}J) and 167.7 (C-1’, ^{3}J).

Table 4 shows spectral data of ^{1}H and ^{13}C NMR of alkaloids **4**, **6** and **7**. The ^{1}H NMR spectrum of **4** presented hydrogen assigned to the pyran ring at δ 5.44 and 6.68 (J =10 Hz), two methyls at δ 1.56, and aromatic hydrogens at δ 6.84 and 7.58 (J 8.8 Hz). The carbon chemical shifts of **4** were compared with 8-methoxyflindersine[9] and identified as 7,8-dimethoxyflindersine. Compound **6** was identified as casimiroin by comparing their spectral data (^{1}H and ^{13}C NMR) with those in the literature.[10] The HSQC experiment showed the correlations between methylenedioxy hydrogens (δ 6.06, 2H) and carbon at δ 101.2. The HMBC correlations (Table 5) showed that there is no change in the position of the methylenedioxy group. The ^{1}H and ^{13}C NMR spectra of compound **7** were very similar to those of **6**, except in substitution for C-7 and C-8. In the HSQC, the methyls at δ 3.96 and 3.78 correlated with carbons at δ 56.6 and 61.7, respectively. The HMBC experiment showed correlations which indicated the position of methoxyl groups at C-7 and C-8.

Table 4. ^{1}H (400 MHz) and ^{13}C (100 MHz) chemical shifts for alkaloids **4**, **6** and **7**, in $CDCl_3$

No.	NMR ^{1}H			NMR ^{13}C		
	4	**6**	**7**	**4**	**6**	**7**
2				161.2	164.2	165.5
3		6.08 s	5.92 s	104.3	94.1	94.6
4				157.1	163.2	162.4
5	7.58 d (8.8)	7.56 d (8.5)	7.70 d (8.8)	118.5	118.2	119.1
6	6.84 d (8.8)	6.81 d (8.5)	6.87 d (8.8)	107.4	104.8	107.0
7				153.2	150.1	155,6
OCH_2O		6.06 s			101.2	
8				133.6	133.7	136.8
9				132.0	126.3	135.0
10				110.1	113.1	112.5
11	6.68 d (10)			117.3		
12	5.44 d (10)			125.3		
13				79.1		
14,15-diMe	1.56 s			28.2		
4-OMe		3.92 s	3.91 s		56.1	55.8
N-Me		3.86 s			32.2	33.4
N-H	8.80 s		3.90 s			
7-OMe	3.98 s		3.96 s	56.2		56.6
8-OMe	3.95 s		3.78 s	60.9		61.7

Chemical shifts (δ, ppm) and coupling constants (J, Hz, in parenthesis).

Table 5. HMBC (400/100 MHz) assignments for alkaloids **6** and **7**, in $CDCl_3$

H	**6**		**7**	
	C ($^{2}J_{C-H}$)	C ($^{3}J_{C-H}$)	C ($^{2}J_{C-H}$)	C ($^{3}J_{C-H}$)
3	4	10	4	10
5		4, 7, 9		4, 7, 9
6	7	8, 10	7	8, 10
OCH_2O		7,8		
11				
12				
13a-Me				
4-OMe		4		4
7-OMe				7
8-OMe				8
N-Me		2, 9		2, 9

Antiprotozoal activity evaluation

In this study, the activity of compounds **2**, **3**, **5** and **6** against promastigote forms of *Leishmania braziliensis* was evaluated in different doses. In a concentration of 320 μg mL^{-1}, deacetylspathelin (**3**) and casimiroin (**6**) showed 45.6% and 42.5% inhibition, respectively, activities considered moderate**.** The chromone **2** had inhibition of 14.7% at 320 μg mL^{-1}, and protolimoid (**5**) was not able to inhibit the parasite.

In the examination against epimastigotes forms of *Trypanosoma cruzi*, chromone **2** with IC_{50} = 11 μg mL^{-1} was the most active compound in the present study. The protolimonoid **5** showed moderate activity, 40.5% inhibition at 100 μg mL^{-1} (Table 6).

Table 6. Trypanocidal activity of compounds **2**, **3**, **5** and **6** of *Spathelia excelsa*

Compounds Conc. / (μg mL^{-1})	Inhibition promastigote forms of *T. cruzi* / (%)							IC_{50}
	1000	500	250	100	10	1	0.1	
2				59	49.2	22	16.8	11
3	100	52.5	29.3	25.1	3.17			470
5				40.5	29.2	25.4	12.6	100
6	100	66.0	28.8	23.2	7.65			380

Although this species presented botanical characteristics that differ from other of the Rutaceae, the compounds identified in this study are typical to this family. The compounds **2** and **8** are pyranochromones prenylated at C-10, a metabolite characteristic of *Spathelia*. Deacetylspathelin (**3**) is a characteristic limonoid of Rutaceae, A- and D-ring seco. Protolimonoids**,** as **5**, which undergo *apo*-euphol rearrangement and contain a C-13, C-14 cyclopropane ring (glabretal-type compounds), has been encountered in families of Rutales (Meliaceae, Simaroubaceae and Rutaceae). Alkaloids **4**, **6** and **7** are derivatives of anthranilic acid, a type that occurs widely in Rutaceae.

The results of assays against protozoan parasites showed significant activity in this study, mainly as trypanocidal. Further laboratory and clinical studies of the active compounds are required in order to understand their antiprotozoal principles.

Experimental

General experimental procedures

IR spectra were obtained in a Perkim Elmer Spectrum-2000 apparatus using KBr pellet samples. NMR spectra were measured in a Bruker DRX 400 apparatus;

chemical shifts (δ) were expressed in ppm, and coupling constants (*J*) in Hertz; TMS was used as internal standard. Low resolution EIMS was recorded on 2100, Varian. Column chromatography (CC) was performed with silica gel 60 (Merck, 70-230 and 230-400 mesh), cellulose (Merck), and sephadex LH-20 (Sigma). Analytical thin layer chromatography (TLC) was carried out with Merck Kiesegel 60 F254 (0.25 mm) plates.

Plant material

Spathelia excelsa was collected in the Adolpho Ducke Forest Reserve, Amazonas, Brazil**,** and a voucher (4227) was deposited in the Herbarium of the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, AM.

Extraction and isolation of compounds

Dried and powdered roots of *S. excelsa* (735 g) were macerated at room temperature with *n*-hexane, then with CH_2Cl_2, and finally with MeOH. The hexane and CH_2Cl_2 combined extracts (14.9 g) were fractionated over silica gel (70-230 mesh; h x Φ = 23.0 x 4.5 cm), eluted with hexane, hexane:CH_2Cl_2 (9:1→1:1), CH_2Cl_2 and MeOH, to yield thirty fractions. The combined fractions 15-17 (RSE-8), 24-27 (RSE-9) and 28-30 (RSE-10) were rechromatographed.

Fraction RSE-8 (2.5 g) was fractionated over silica gel (70-230 mesh; h × Φ = 16.0 × 4.5 cm), eluted with CH_2Cl_2:MeOH (1-10%), yielding twenty two subfractions. Subfractions 5-12 yielded a mixture of β-sitosterol, stigmasterol (**1**; 23.7 mg). Through column chromatography over sephadex LH-20 (h × Φ = 27 × 2 cm), eluted with EtOAc from the subfractions 13-20, after column chromatography over silica gel (230-400 mesh; h × Φ = 27 × 2 cm), eluted with CH_2Cl_2, CH_2Cl_2:EtOH (99:1), compound **2** (14 mg) was obtained.

RSE-9 (766 mg) was chromatographed on silica gel (230-400 mesh; h × Φ = 30 × 3 cm), eluted with hexane, hex:EtOAc and EtOH, yielding twenty three subfractions. Subfractions 18-23 in column on silica gel (230-400 mesh; h × Φ = 44.5 × 1.5 cm), eluted with CH_2Cl_2, CH_2Cl_2:acetone (1-50%), and further recrystallized with hexane:acetone, yielded compound **3** (33.3 mg).

Fraction RSE-10 (7 g) was subjected to column chromatography over silica gel (70-230 mesh; h × Φ = 21.0 × 4.5 cm), eluted with hex: EtOAc (5-100%) e EtOAc:EtOH (10-20%), yielding forty one subfractions. The combined subfractions 1-14 (RSE-10A), 20-23 (RSE-10B), 28 (RSE-10C), 29 (RSE-10D), 30-34 (RSE-10E) and 38-41 (RSE-10F) were submitted to a new experimental procedure.

Compound **3** (68.8 mg) was again obtained from RSE-10A after cleaning with hexane and acetone drops. Compound **4** (21 mg) was obtained from RSE-10B, through column chromatography over silica gel (230-400 mesh), eluted with CH_2Cl_2, and CH_2Cl_2:acetone, after purification with hexane:acetone drops. Subfractions RSE-10C and RSE-10D yielded precipitates. These precipitates, which were purified with hexane:acetone drops, yielded compounds **5** (20.5 mg) and **6** (26.7 mg), respectively. Subfraction RSE-10E, subjected to column chromatography over silica gel (230-400 mesh), eluted with CH_2Cl_2:acetone (2-100%), yielded an amorphous solid which was purified with hexane:acetone drops, producing compound **7** (52 mg). RSE-10F was subjected to column chromatography over sephadex LH-20 (h x Φ = 25 x 2 cm), eluted with EtOAc, then subjected to column chromatography over silica gel (230-400 mesh), eluted with CH_2Cl_2, CH_2Cl_2:acetone (2-100%), yielded compound **8** (5 mg).

*10(2,3-Epoxy-3-methylbutanyl)spatheliachromen (**2**)*

Amorphous solid, mp 98.5-100º; IR ν_{max}/cm^{-1}: 1663 (C=O), 1631(C=C), 1588, 1465, 764 (aromatic C=C); $[M]^+$ *m/z* 342 ($C_{20}H_{22}0_5$). ^{1}H NMR(400 MHz, $CDCl_3$): see Table 1; COSY (400 MHz, $CDCl_3$): δ 6.72-5.61, 3.08-2.95, 3.08-2.83, 2.95-2.83; PENDANT (100 MHz,$CDCl_3$): see Table 1; HSQC (400/100 MHz, $CDCl_3$): see Discussion; HMBC (400/100 MHz, $CDCl_3$): see Table 2.

*Deacetylspathelin (**3**)*

Amorphous solid; IR ν_{max}/cm^{-1}: 3458 (OH), 1740 (ester), 1715 (cetone).^{1}H NMR [400 MHz $(CD_3)_2CO$]: δ 7.64 (1H, *dt*, 1.6 e 0.8 Hz, H-21), 7.57 (1H, *m* H-23), 6.51 (1H, *ddd*, 1.8, 0.8, 0.3 Hz, H-22), 6.40 (1H, *d*, 12.4 Hz, H-1), 5.89 (1H, *d*; 12.4 Hz, H-2), 5.52 (1H, *s*, H-17), 4.99 (1H, *d*, 3.95 Hz, H-7), 4.39 (1H, *d*; 3.95 Hz, O<u>H</u>), 4.08 (1H, *s*, H-15), 3.69 (3H, *s*, OMe), 3.13 (1H, *m*, H-9), 1.89-1.79 (2H, *m*, H-11), 1.94 (2H, *m*, H-12), 1.27 (3H, *s*, H-29), 1.26 (3H, *s*, H-19), 1.24 (3H, *s*, H-18), 1.23 (3H, *s*, H-28), 0.70 (3H *s*, H-30). COSY [400 MHz, $(CD_3)_2CO$]: δ 6.40 (H-1) → δ 5.89 (H-2), δ 7.64 (H-21) → δ 6.51 (H-22), δ 7.57 (H-23) → H-22 (δ 6.51). ^{13}C NMR [100 MHz, $(CD_3)_2CO$)]: Table 3.

*7,8-Dimethoxyflindersin (**4**)*

Amorphous solid; ^{1}H NMR (400 MHz, $CDCl_3$): see Table 4; ^{13}C NMR (100MHz, $CDCl_3$): see Table 4.

*3β-Angeloyloxy-7α,24,25-trihydroxy-21,23-oxide-14,18-cycloapotirucall-21-hemiacetal (**5**)*

Amorphous solid, mp 208.9-210.9º; ^{1}H NMR (400 MHz, C_5D_5N): see Discussion; ^{13}C NMR (100 MHz, C_5D_5N): see

Table 3; HSQC (400/100 MHz, C_5D_5N): see Discussion; HMBC (400/100 MHz, C_5D_5N): see Discussion.

*Casimiroin (**6**)*

Amorphous solid; ^{1}H NMR (400 MHz, $CDCl_3$): see Table 4; ^{13}C NMR (100 MHz, $CDCl_3$): see Table 4; HSQC (400/100 MHz, $CDCl_3$): see Discussion; HMBC (400/100 MHz, $CDCl_3$): see Table 5.

*N-methyl-4,7,8-trimethoxyquinolin-2(1H)-one (**7**)*

Amorphous solid; ^{1}H NMR (400 MHz, $CDCl_3$): see Table 4; ^{13}C NMR (100 MHz, $CDCl_3$): see Table 4; HSQC (400/100 MHz, $CDCl_3$): see Discussion; HMBC (400/100 MHz, $CDCl_3$): see Table 5.

*10(2,3-Dihydroxy-3-methylbutanyl)-methoxyspatheliacromen (5-methoxyspatheliabischromen) (**8**)*

^{1}H NMR (400 MHz, $CDCl_3$): see Table 1; ^{13}C NMR (100 MHz, $CDCl_3$): see Table 1; HSQC (400/100 MHz, $CDCl_3$): see Discussion; HMBC (400/100 MHz, $CDCl_3$): see Table 2.

Leishmanicidal activity in vitro

L. braziliensis promastigotes (LTB0016) were grown to a concentration of 4 x 10^6 cells mL^{-1} in Schneider's Drosophila medium, supplemented with 10% bovine fetal serum. The compounds (**2**, **3**, **5** and **6**) were added to the promastigote cultures (4 x 10^6 mL^{-1}) at concentrations of 320 to 0.125 µg mL^{-1}, solubilized in DMSO (the highest concentration used was 1.6%, v/v) and incubated at 25 ºC. After 24 h of incubation, the surviving parasites were counted in a Neubauer's chamber and compared with controls which only had DMSO. All tests were done in triplicate**,** and pentamidine isethionate (Eurofarma) was used as reference drug.[11] The LD_{50}/24 values were determined by linear regression analysis from this inhibition percentage, using statistic error limits up to 10%.

Trypanocidal activity in vitro

Epimastigote forms of *T. cruzi* Y strain were grown in liver infusion tryptose (LIT) medium supplemented with 10% inactivated bovine fetal serum and assayed with different concentrations of compounds (**2**, **3**, **5** and **6**) which were dissolved in DMSO. Next, 1x10^6 protozoa *per* mL was introduced into 24-well plate each well containing 1 mL of diluted compound. Cell growth was determined by counting the parasites with a Neubauer haemocytometer after incubation for 96 h at 28 ºC. Assays were performed in duplicate.

Acknowledgments

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Supplementary Information

Supplementary data is available free of charge at http://jbcs.sbq.org.br, as PDF file.

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Chemical Constituents from the Roots of *Spathelia excelsa* and their Antiprotozoal Activity

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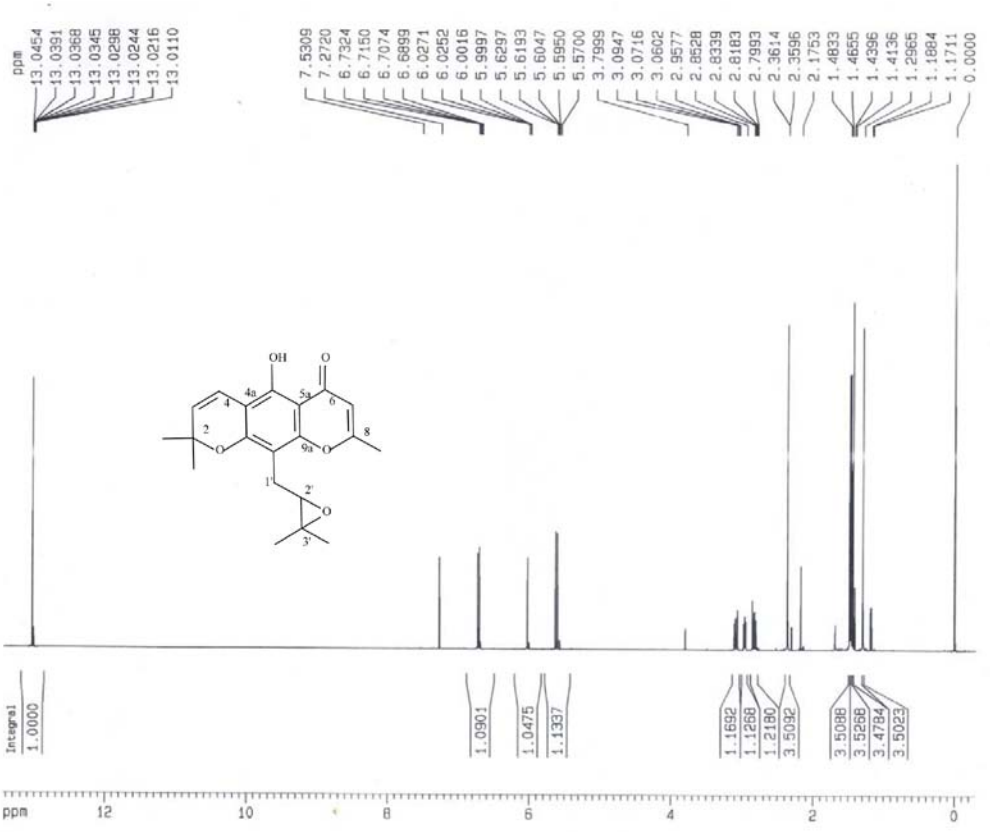

Figure S1. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **2**.

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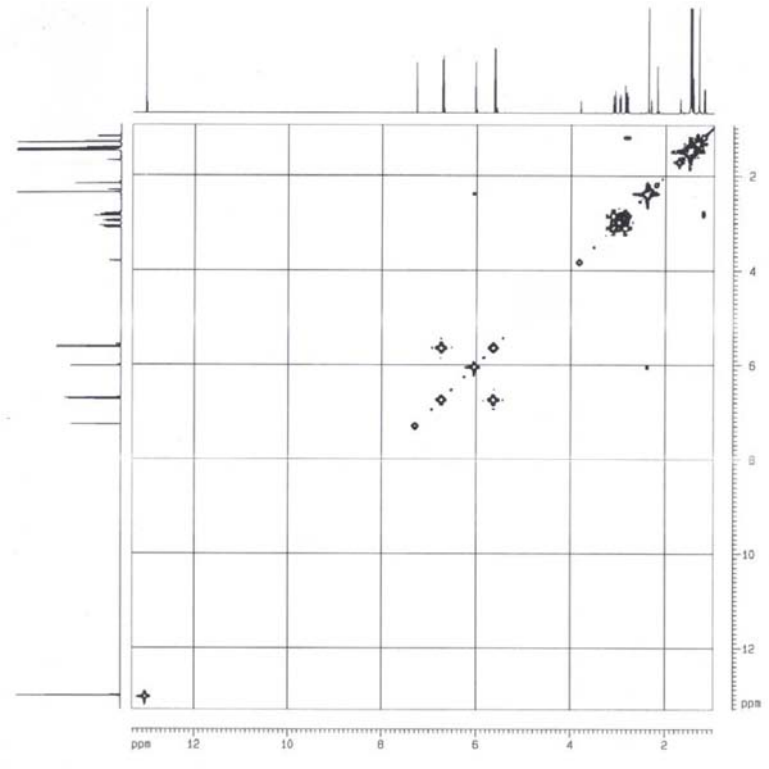

Figure S2. ^{1}H-^{1}H COSY spectrum (400 MHz, $CDCl_3$) of **2**.

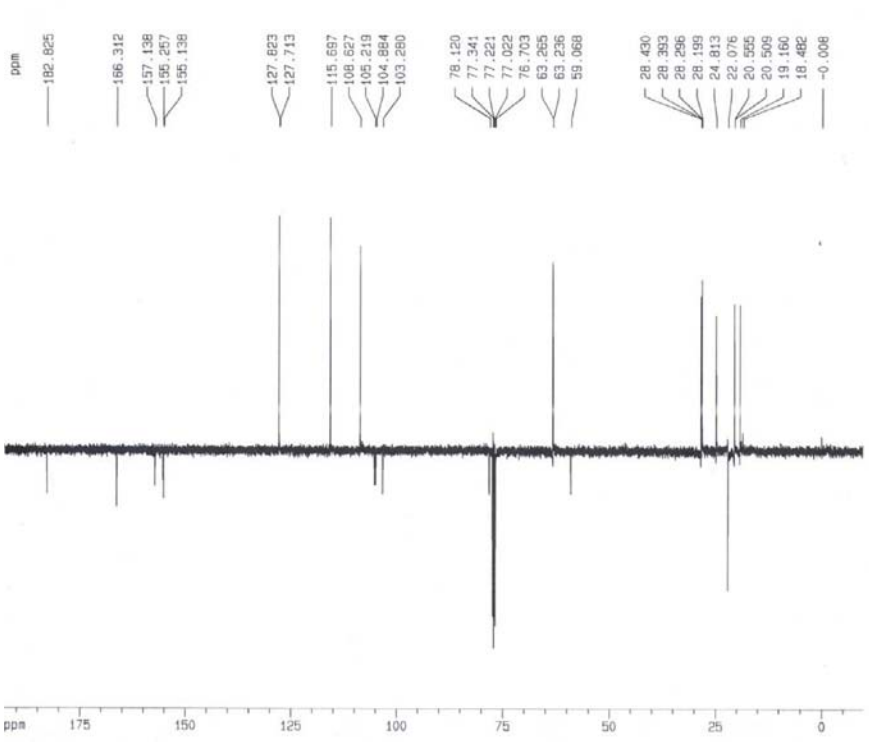

Figure S3. ^{13}C Pendant spectrum (100 MHz, $CDCl_3$) of **2**.

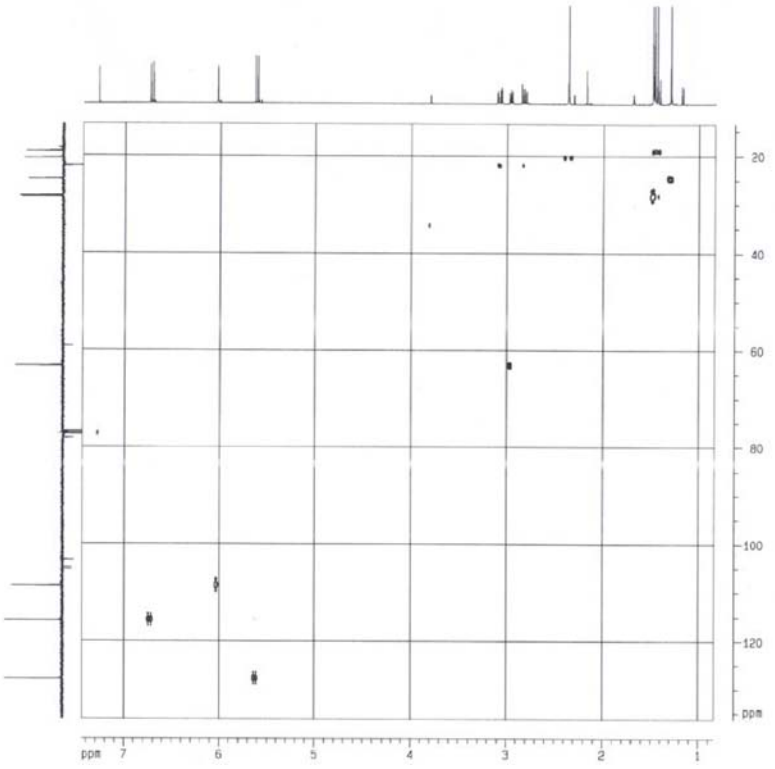

Figure S4. HSQC spectrum (400/100 MHz, $CDCl_3$) of **2**.

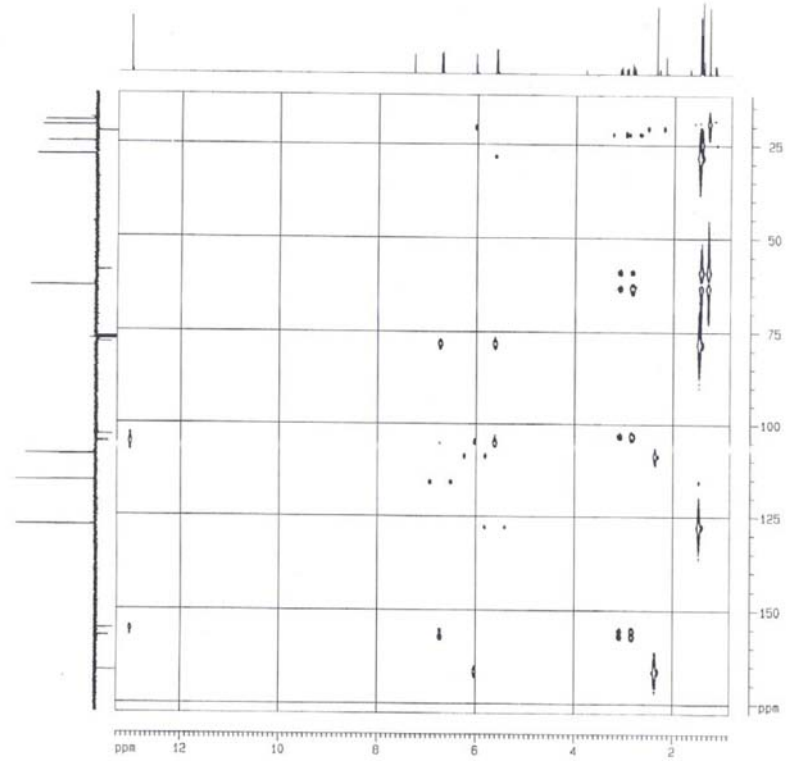

Figure S5. HMBC spectrum (400/100 MHz, $CDCl_3$) of **2**.

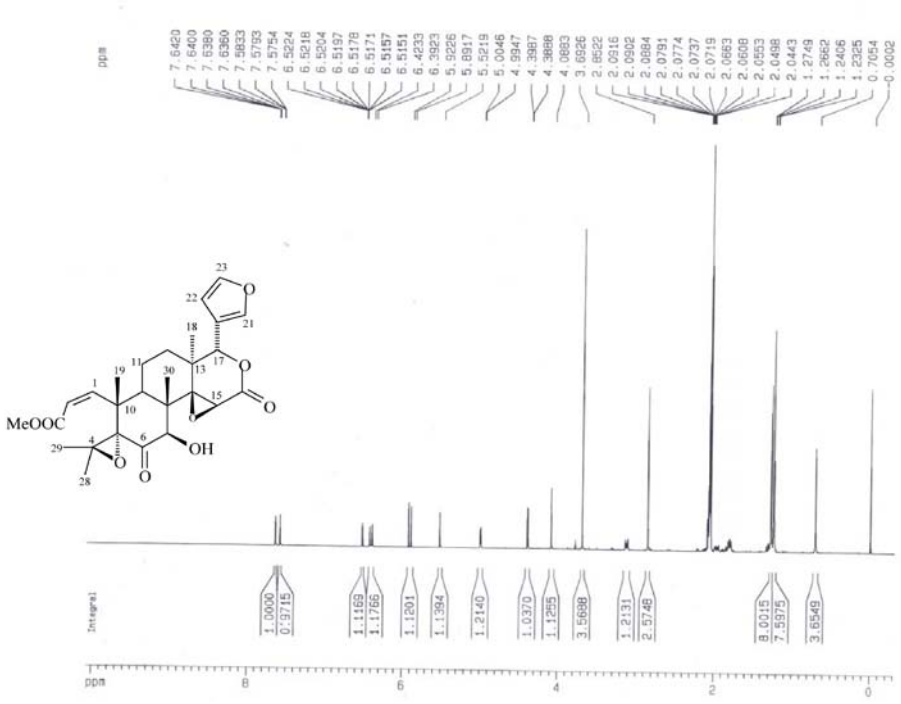

Figure S6. ^{1}H NMR spectrum [400 MHz $(CD3)_2CO$] of **3**.

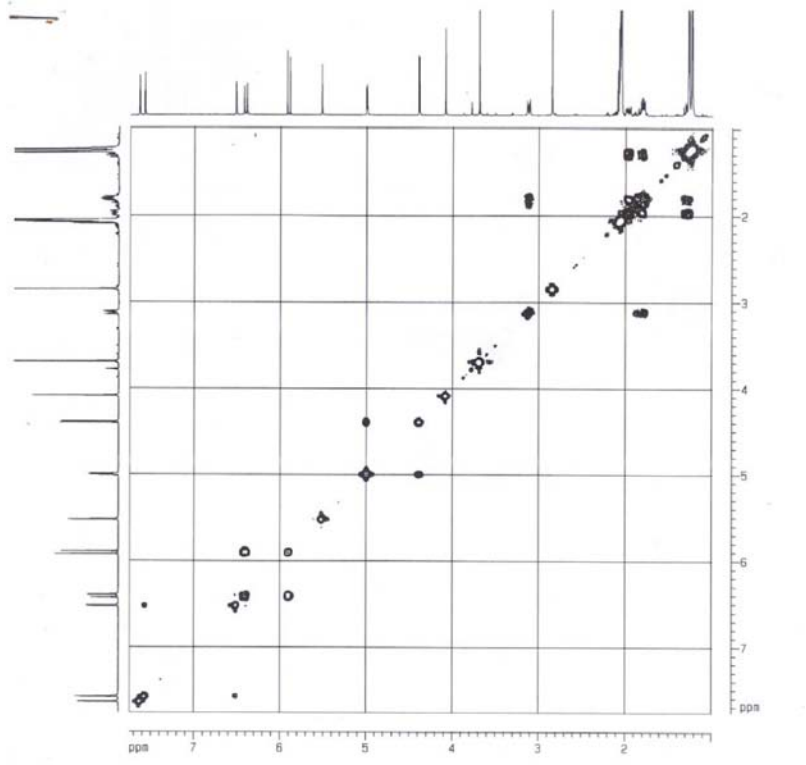

Figure S7. ^{1}H-^{1}H COSY spectrum [400 MHz $(CD3)_2CO$] of **3**.

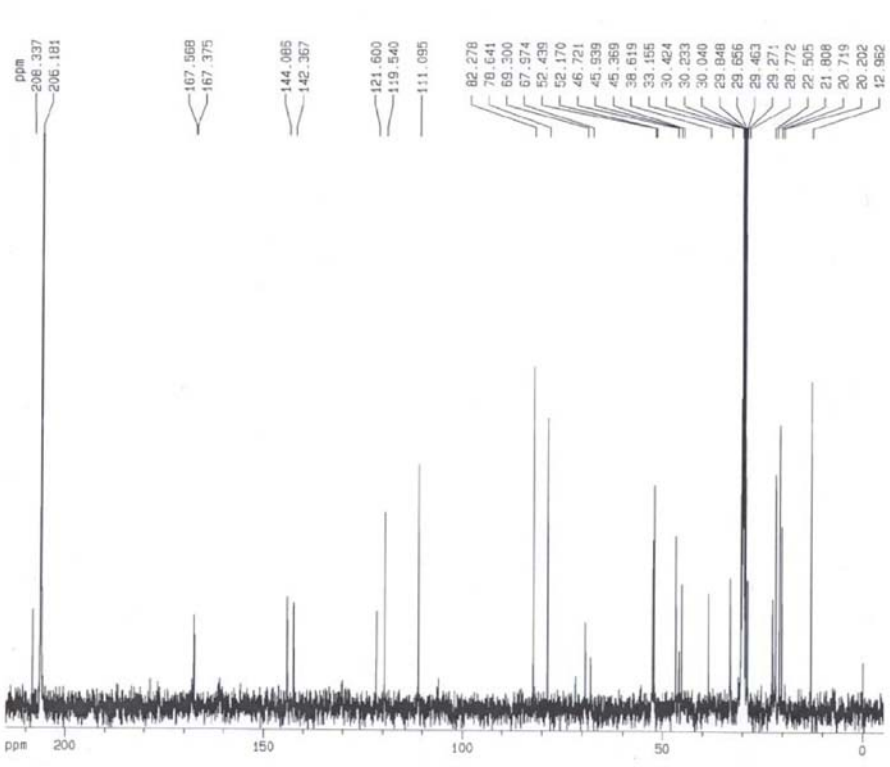

Figure S8. ^{13}C RMN spectrum [100 MHz $(CD3)_2CO$] of **3**.

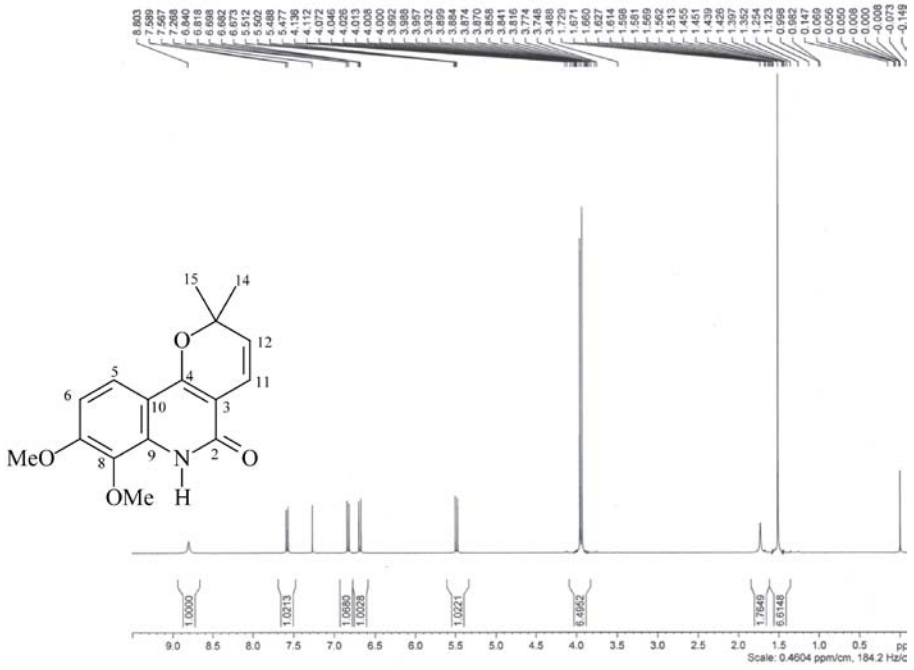

Figure S9. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **4**.

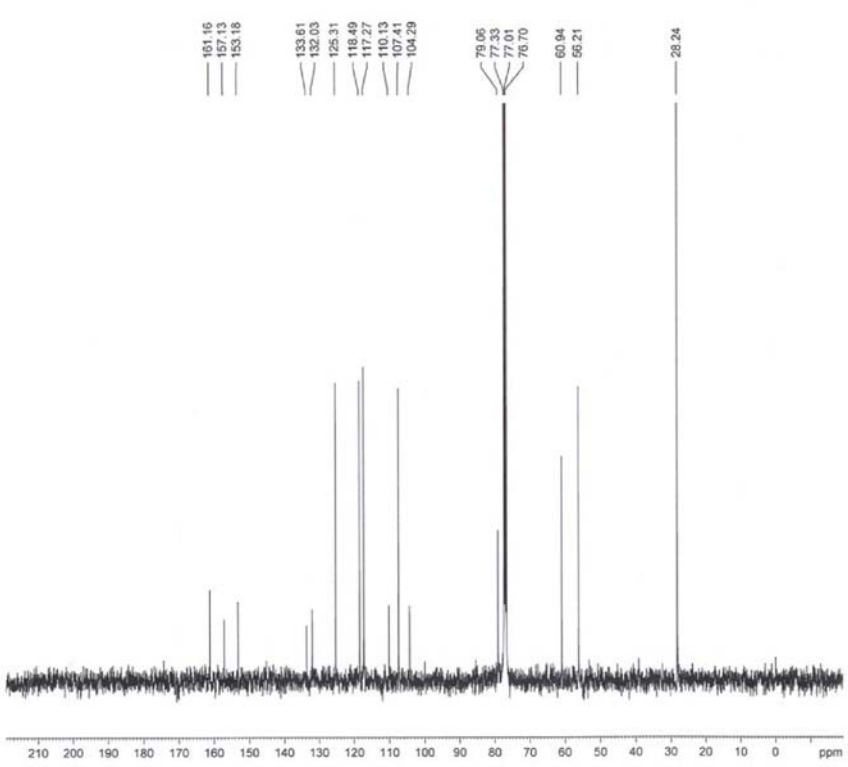

Figure S10. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **4**.

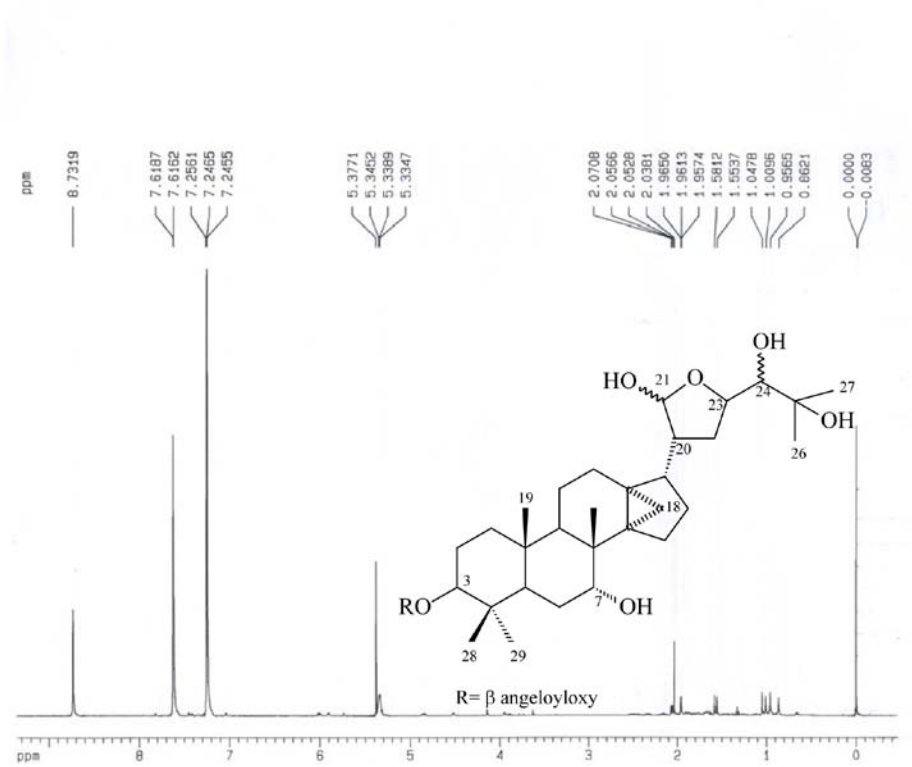

Figure S11. ^{1}H NMR spectrum (400 MHz, C_5D_5N) of **5**.

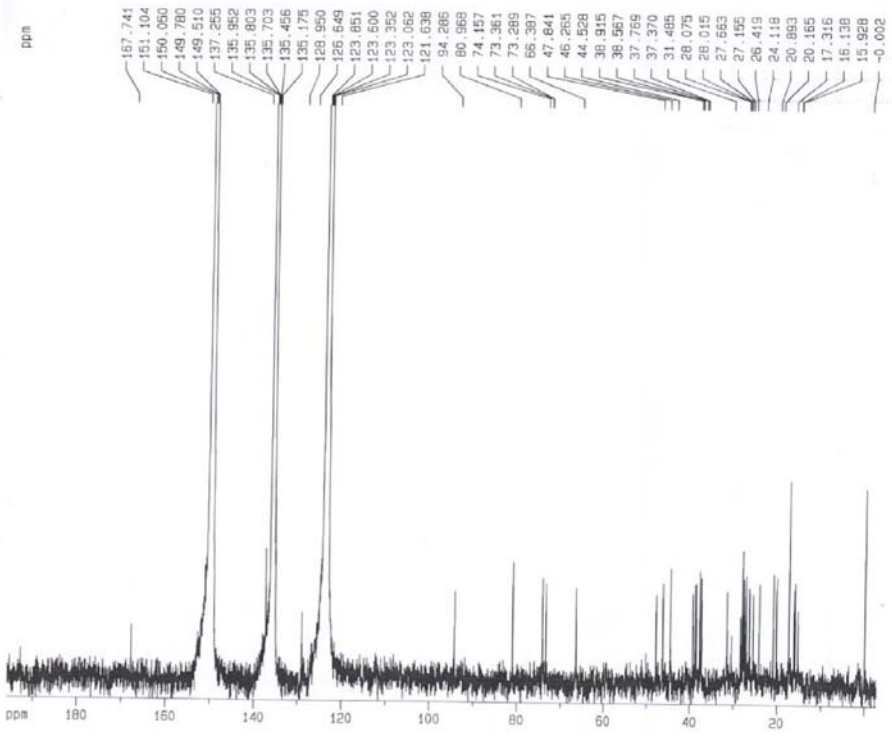

Figure S12. ^{1}H NMR spectrum (100 MHz, C_5D_5N) of **5**.

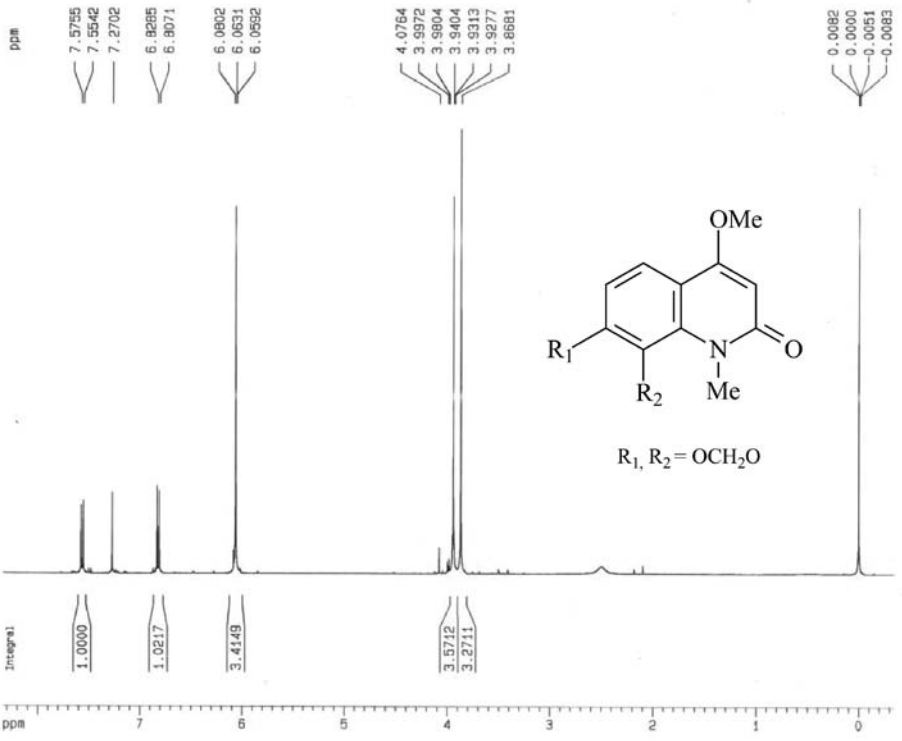

Figure S13. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **6**.

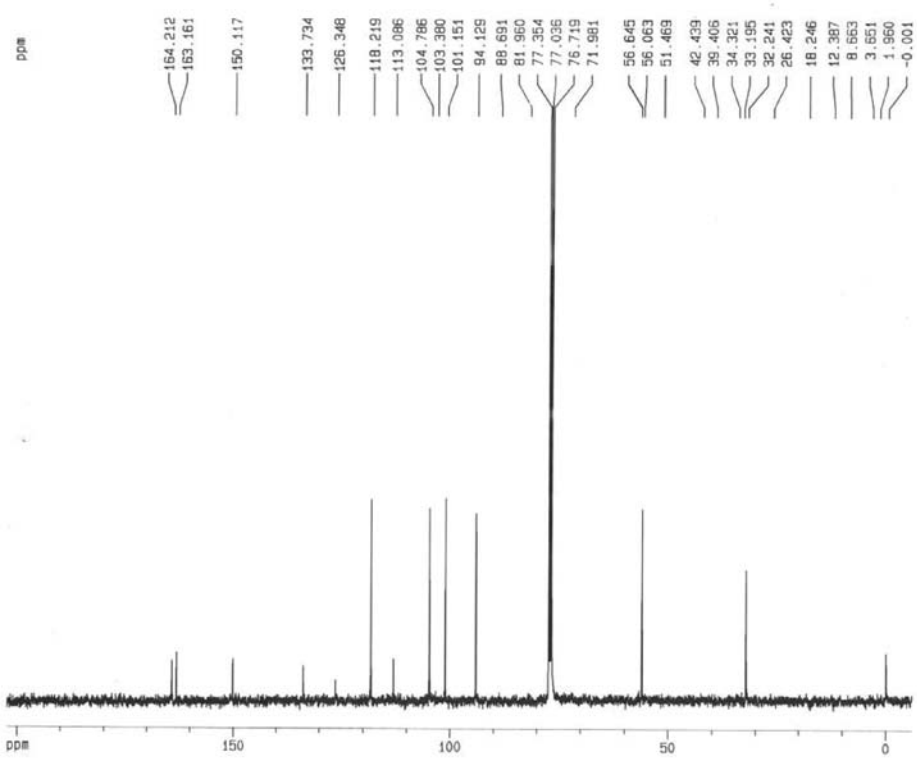

Figure S14. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **6**.

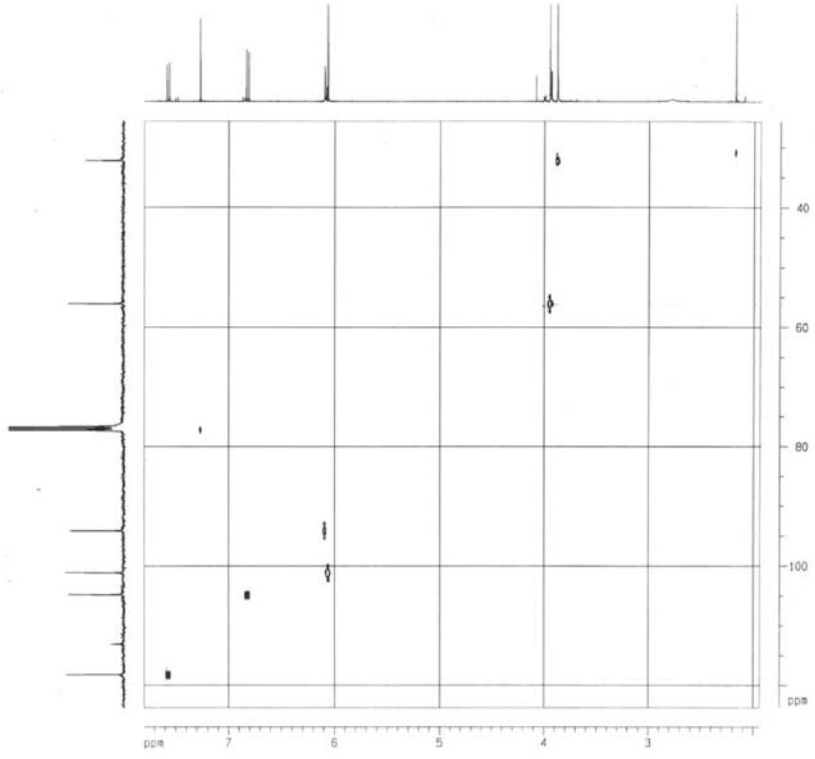

Figure S15. HSQC spectrum (400/100 MHz, $CDCl_3$) of **6**.

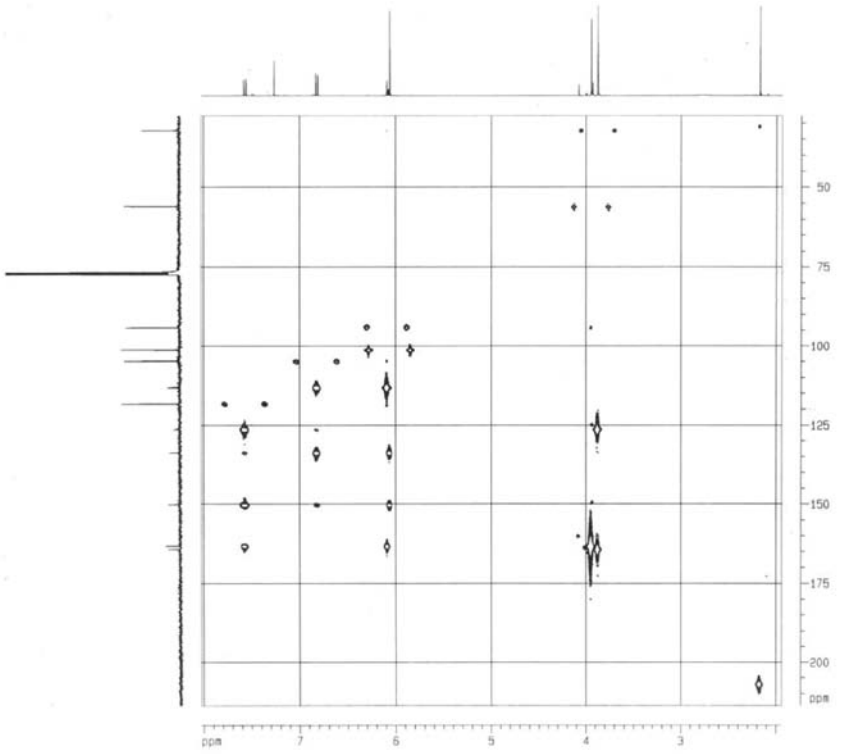

Figure S16. HMBC spectrum (400/100 MHz, $CDCl_3$) of **6**.

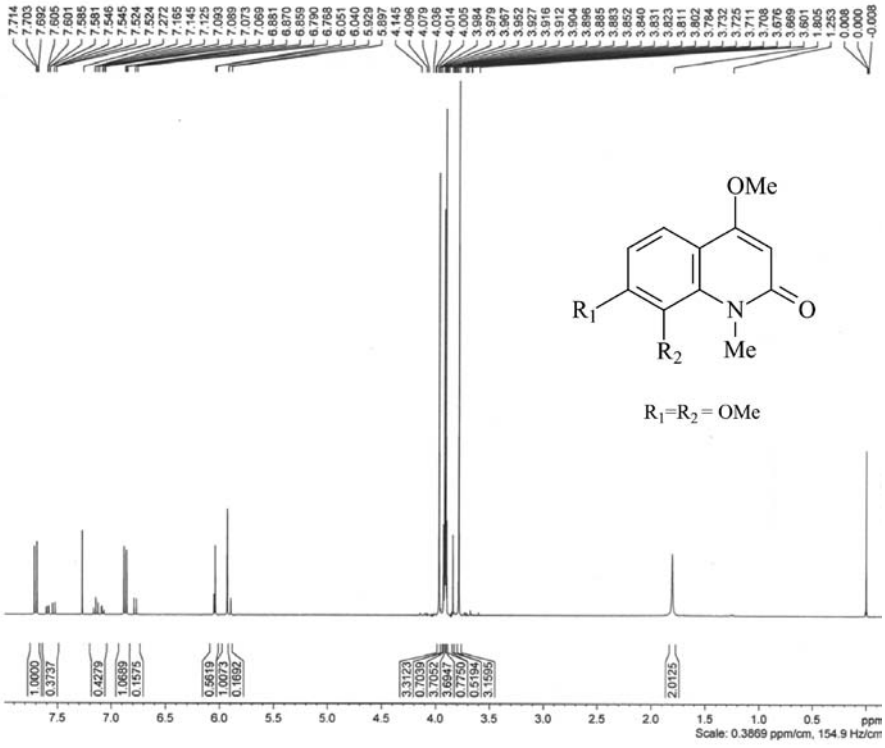

Figure S17. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **7**.

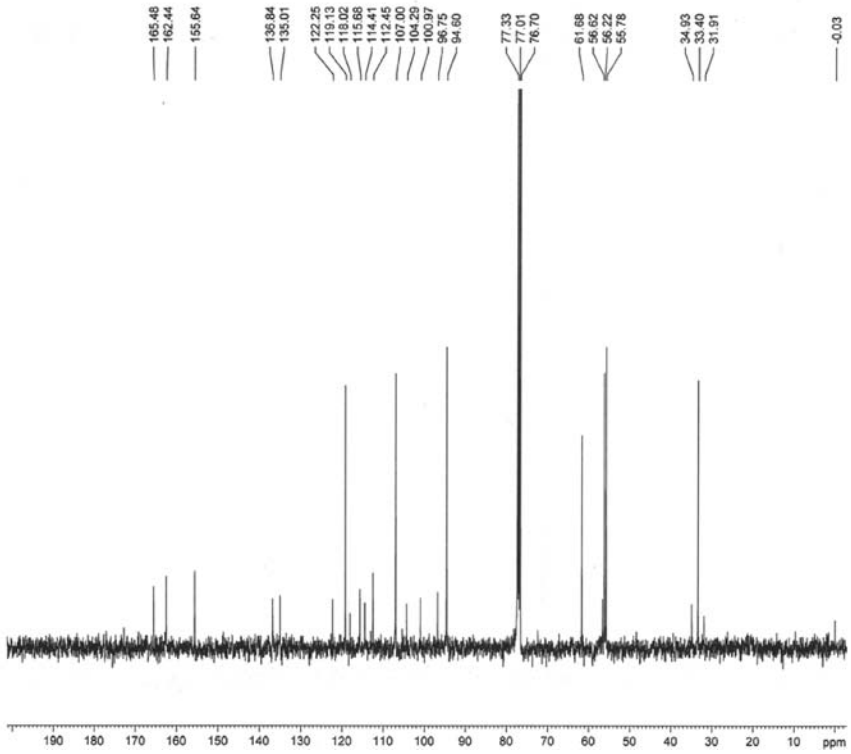

Figure S18. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **7**.

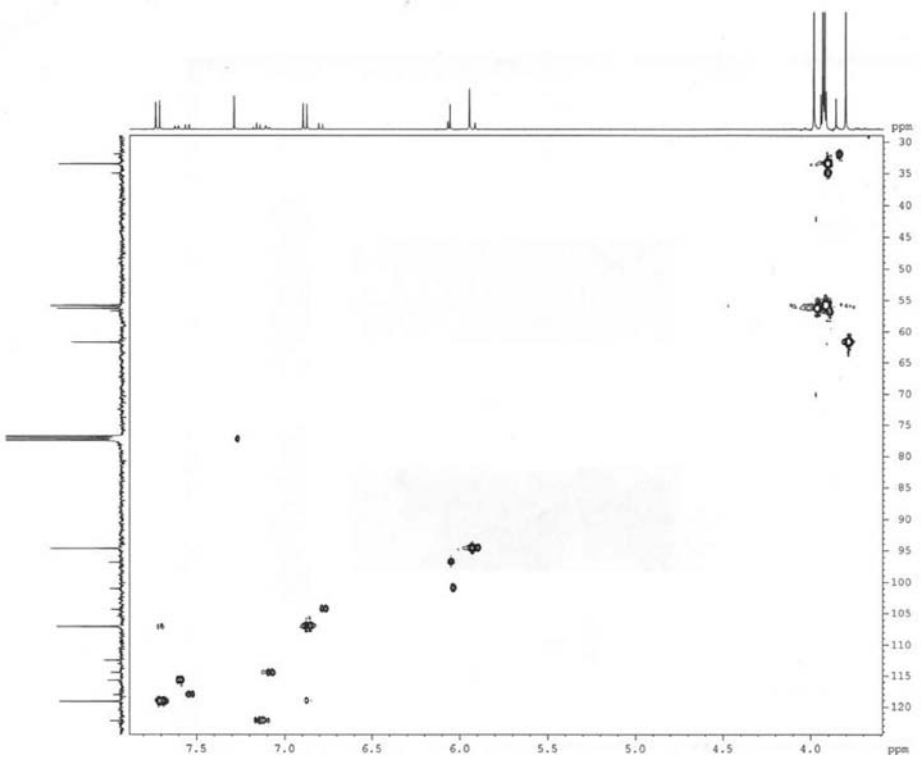

Figure S19. HSQC spectrum (400/100 MHz, $CDCl_3$) of **7**.

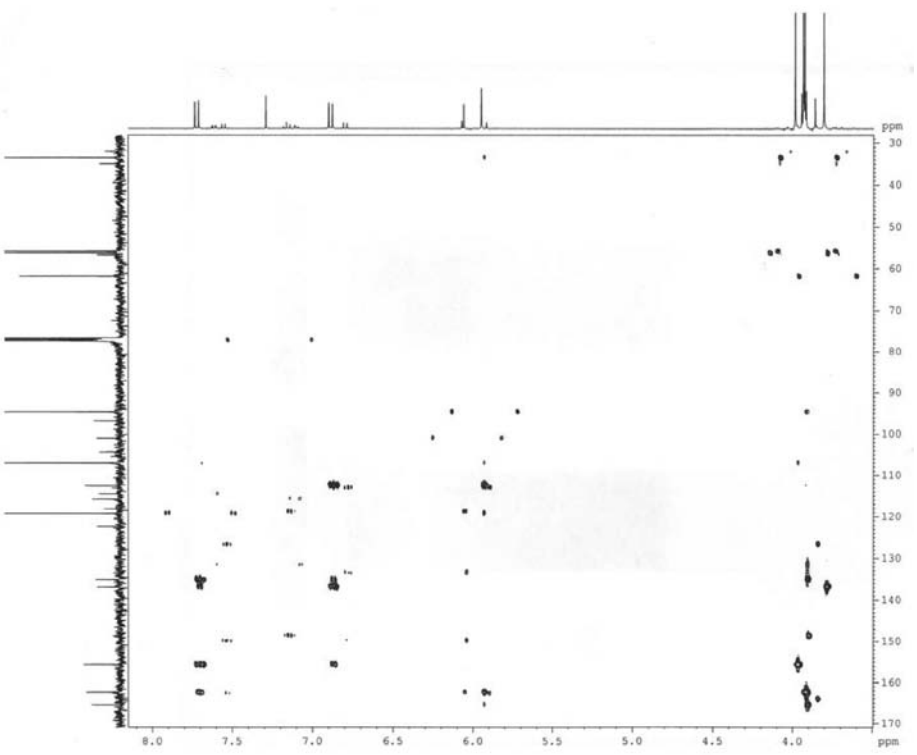

Figure S20. HMBC spectrum (400/100 MHz, $CDCl_3$) of **7**.

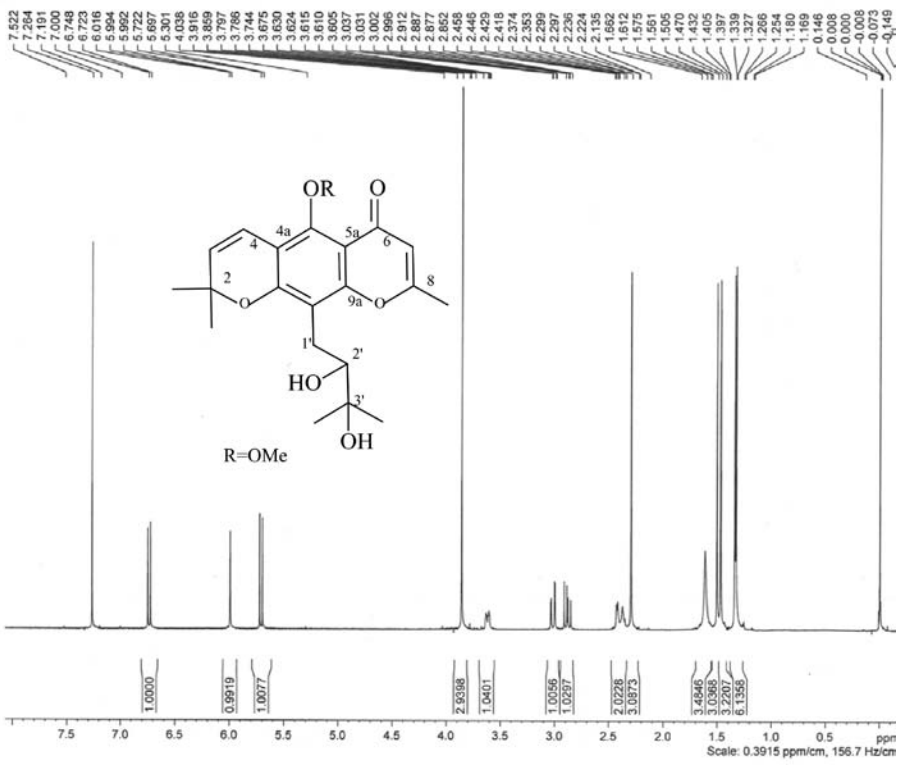

Figure S21. ^1H NMR spectrum (400 MHz, $CDCl_3$) of **8**.

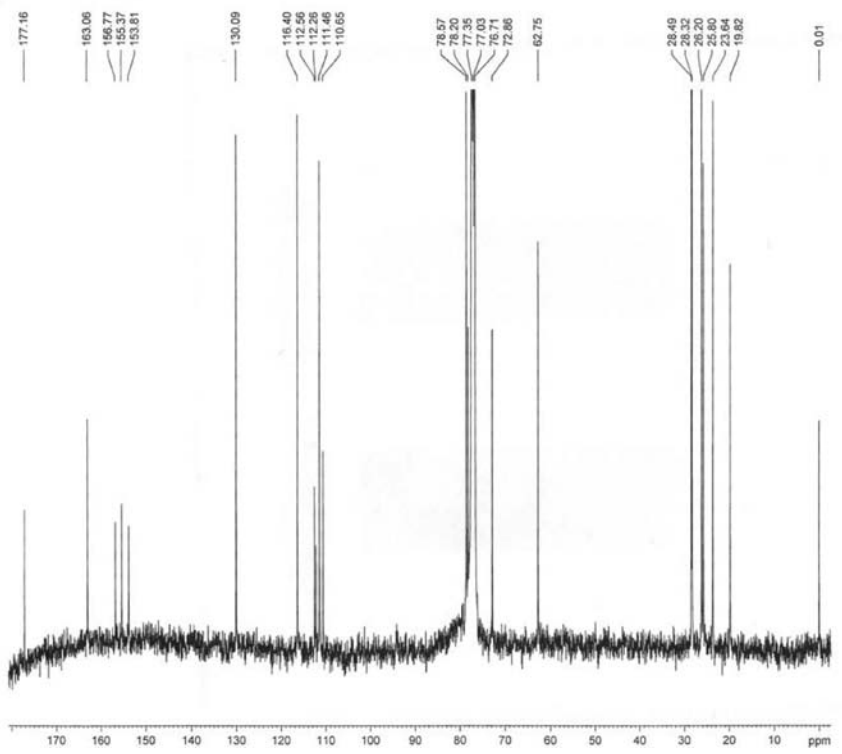

Figure S22. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **8**.

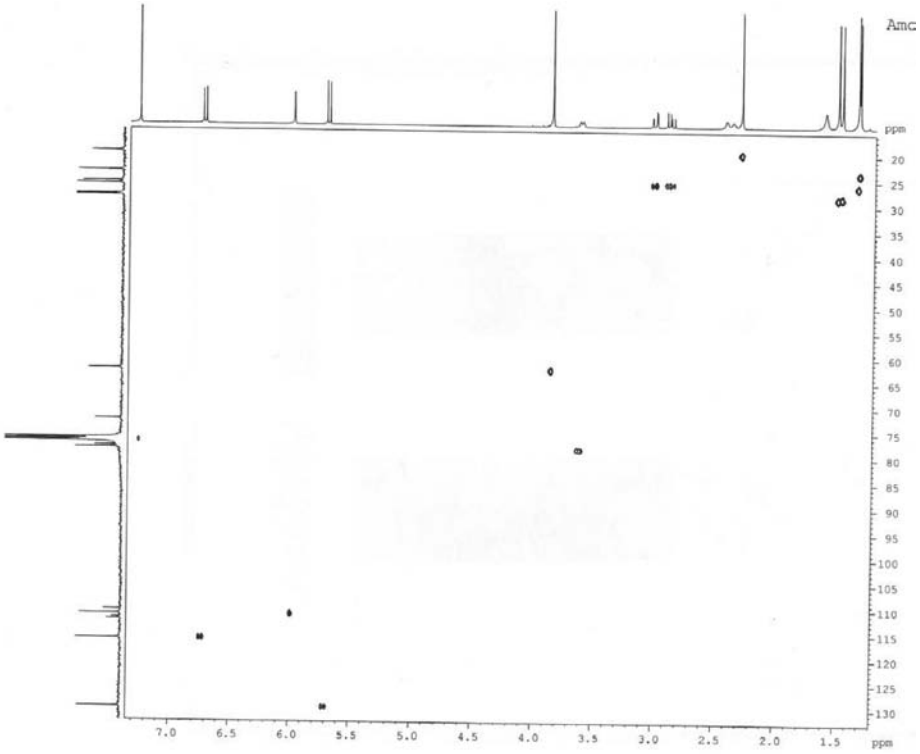

Figure S23. HSQC spectrum (400/100 MHz, $CDCl_3$) of **8**.

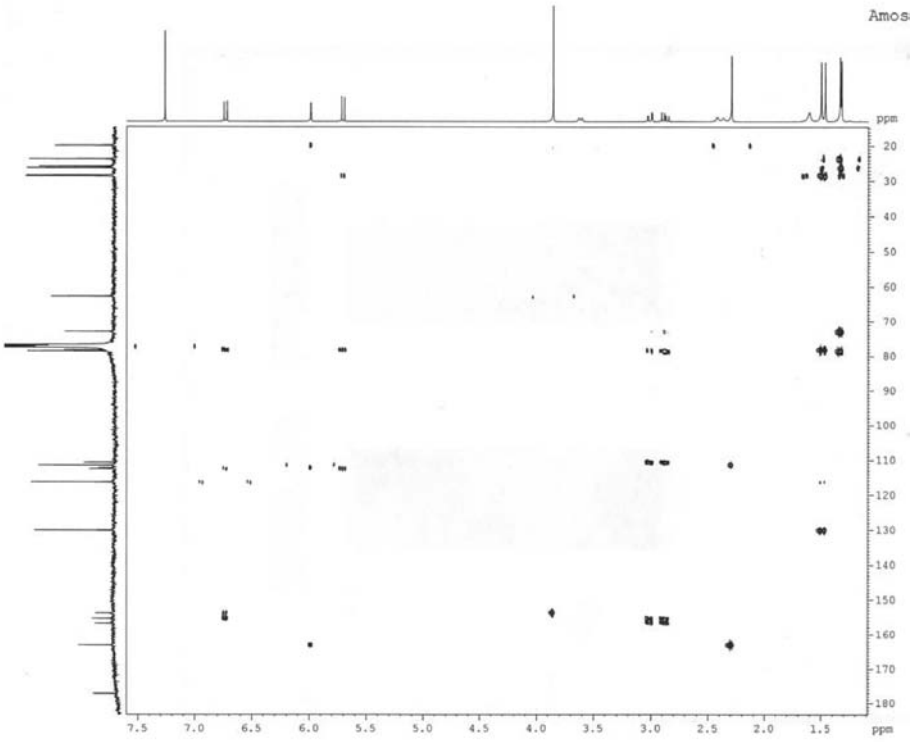

Figure S24. HMBC spectrum (400/100 MHz, $CDCl_3$) of **8**.