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Article

Bioactive Flavonoids and Triterpenes from *Terminalia fagifolia* (Combretaceae)

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Da madeira e das cascas do caule de *Terminalia fagifolia* foram isolados dois 1,3-diarilpropanos, 1-(4'-hidróxi-2'-metóxifenil)-3-(3"-metóxi-4"-hidróxifenil)-propano e 1-(2'-hidróxi-4',6'-dimetóxifenil)-3-(3"-metóxi-4"-hidróxifenil)-propano, sete flavanonas, naringenina, 5-hidróxi-4',7-dimetóxiflavanona, sakuranetina, isosakuranetina, 7,4'-dimetóxiflavanona, 7-hidróxi-4'-metóxiflavanona, 7-metóxi-4'-hidróxiflavanona, duas chalconas, 2',4'-diidroxi-4-metóxichalcona e 2'-4-diidroxi-4'-metóxichalcona, uma flavana, 7,4'-diidróxi-3'-metóxiflavana e nove triterpenos pentacíclicos, ácido arjúnico, arjunetina, arjungenina, arjunglucosídeo I, ácido arjunólico, arjunglucosídeo II, 23-galoilarjunglucosídeo (isolado como seus derivados mono-, di- e trimetilados após metilação com diazometano), ácido betulínico e acetato do ácido ursólico, além de ácido gálico e sitosterol. Os diarilpropanos representam os primeiros membros desta classe em Combretaceae e as flavanonas e chalconas estão sendo descritas pela primeira vez na família. As substâncias isoladas foram avaliadas quanto às atividades citotóxica *in vitro* (células Hep_2 e H_{292}, carcinomas de laringe e mucoepidermóide de pulmão humanos, respectivamente) e antioxidante. As chalconas, o diarilpropano 1-(2'-hidróxi-4',6'-dimetóxifenil)-3-(3"-metóxi-4"-hidróxifenil)-propano e os derivados di- e tri-metilados de 23-galoilarjunglucosídeo foram os mais ativos quanto à atividade citotóxica.

Two 1,3-diarylpropanes, 1-(4'-hydroxy-2'-methoxyphenyl)-3-(3"-methoxy-4"-hydroxyphenyl)-propane and 1-(2'-hydroxy-4',6'-dimethoxyphenyl)-3-(3"-methoxy-4"-hydroxyphenyl)-propane, seven flavanones, naringenin, naringenin-4',7-dimethyl-ether, sakuranetin, isosakuranetin, liquiritigenin-4',7-dimethyl-ether, liquiritigenin-7-methyl-ether and liquiritigenin-4'-methyl-ether, two chalcones, isoliquiritigenin-4-methyl-ether and isoliquiritigenin-4'-methyl-ether, one flavan, 7,4'-dihydroxy-3'-methoxyflavan, nine triterpenes, arjunic acid, arjunetin, arjungenin, arjunglucoside I, arjunolic acid, arjunglucoside II, 23-galloylarjunglucoside II (isolated as its mono-, di- and tri-*O*-methyl derivatives after methylation with diazomethane), betulinic acid and ursolic acid acetate, along with gallic acid and sitosterol were isolated from the heartwood and trunk bark of of *Terminalia fagifolia*. The flavanones and chalcones obtained in the present work are new in the Combretaceae and this is the first report of the occurrence of 1,3-diarylpropanes in this family. The isolated compounds were evaluated for their *in vitro* cytotoxic activity against two human cancer cell lines (Hep_2 larynx carcinoma and H_{292} lung mucoepidermoid carcinoma) and antioxidant properties. Isoliquiritigenin-4-methyl-ether, isoliquiritigenin-4'-methyl-ether, 1-(2'-hydroxy-4',6'-dimethoxyphenyl)-3-(3"-methoxy-4"-hydroxyphenyl)-propane and the di- and tri-*O*-methyl derivatives of 23-galloylarjunglucoside II were the most active in the cytotoxic assay.

Keywords: *Terminalia fagifolia*, Combretaceae, diarylpropanes, cytotoxic activity, antioxidant activity

Introduction

Terminalia fagifolia Mart. & Zucc. (Combretaceae), popularly known as "cachaporra do gentio" and "capitão do seco", is a tree found in the "cerrado" of Mato Grosso do Sul, Brazil and in the Brazilian folk medicine the trunk bark is used for the treatment of tumors and aphthas.[1] To date, no phytochemical or biological studies on this species have been reported.

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In a continuation of our study on constituents of plants of the *Terminalia* genus which occur in the central-western region of Brazil,[2,3] we have examined the composition of the ethanol extracts from the heartwood and the trunk bark of *T. fagifolia*. Herein we describe the isolation and structural identification of twelve flavonoids, comprising seven flavanones (**1**-**7**), one flavan (**8**), two chalcones (**9** and **10**), two diarylpropanes (**11** and **12**) and nine pentacyclic triterpenes (**13**-**21**), in addition to gallic acid and sitosterol. The flavanones and chalcones are new in the Combretaceae. To date, diarylpropanes were mostly reported in members of the Myristicaceae and this is the first report for their occurrence in the Combretaceae family.

The structural elucidation of these isolates was established on the basis of 1D and 2D NMR spectroscopic techniques.

The evaluation of the *in vitro* cytotoxic activities of sixteen compounds against two human cancer cell lines (Hep_2 and H_{292}), as well as the antioxidative properties of eighteen isolated compounds are also reported.

Results and Discussion

After a series of partition procedures and a combination of column chromatography separations of the ethanol extracts from the heartwood and trunk bark, twelve flavonoids (**1**–**12**) and nine triterpenoids (**13**–**21**) were isolated, together with gallic acid (**22**) and sitosterol (**23**).

Compounds **1**–**7** are known flavanones and identified as naringenin-4',7-dimethyl-ether,[4] isosakuranetin (naringenin-4'-methyl-ether),[5] naringenin,[6] liquiritigenin-4',7-dimethyl-ether (7,4'-dimethoxyflavanone),[7,8] liquiritigenin-4'-methyl-ether,[9] liquiritigenin-7-methyl-ether[8,9] and sakuranetin,[10] respectively, which have not been described in the Combretaceae yet. Since the ^{13}C-NMR data of **5** have not been reported, the carbon signals of this compound were assigned and are listed in Table 1.

Compound **8**, identified as 7,4'-dihydroxy-3'-methoxyflavan, was previously isolated from *T. argentea*.[3] Beyond **8**, only two flavans have been obtained from members of this genus.[11]

The isomeric chalcones **9** and **10** differed only by the hydroxyl or methoxyl groups at C-4 and C-4' and were identified as 2',4'-dihydroxy-4-methoxychalcone (isoliquiritigenin-4-methyl-ether)[9] and 2',4-dihydroxy-4'-methoxychalcone (isoliquiritigenin-4'-methyl-ether),[8,12] respectively, which had not been previously isolated from any member of the Combretaceae. The occurrence of flavanones and chalcones in a *Terminalia* species is of particular interest since to date only four flavanones and one chalcone have been isolated from *Terminalia* species.[13]

Compounds **11** and **12** were identified as the diarylpropanes 1-(4'-hydroxy-2'-methoxyphenyl)-3-(3"-methoxy-4"-hydroxyphenyl)-propane, also known as virolane (**11**) and 1-(2'-hydroxy-4',6'-dimethoxyphenyl)-3-(3"-methoxy-4"-hydroxyphenyl)-propane (**12**).[14] Although the presence of diarylpropanes is, with very few exceptions, restricted to members of the Myristicaceae family, this is the first report of the occurrence of this class of compounds in the Combretaceae. With regard to the spectroscopic NMR properties of **11** and **12** only ^{1}H data have been reported and to date no ^{13}C NMR values are available for these compounds. In the present study, several 1D and 2D NMR data, including those from ^{1}H-^{1}H COSY, NOESY, HMQC and HMBC experiments, were assigned accordingly for all carbon resonances of **11** and **12** (Table 1).

Table 1. ^{13}C (75 MHz) NMR spectral data for **5**, **11** and **12** (δ, $CDCl_3$)

C	5	11	12
1	-	134.7	134.6
2	79.9	111.0	110.9
3	44.1	146.2	146.3
4	191.0	143.4	143.5
5	129.4	114.0	114.1
6	110.5	120.9	120.9
7	164.0	-	-
8	103.4	-	-
9	162.5	-	-
10	115.1	-	-
1'	130.7	123.0	108.9
2'	127.7	158.5	154.7
3'	114.2	98.8	93.3
4'	160.0	154.8	159.0
5'	114.2	106.3	91.3
6'	127.7	130.1	159.2
α	-	31.8	30.9
β	-	35.3	35.3
β'	-	29.2	22.2
OMe-2'	-	55.3	
OMe-4'	55.4	-	55.6
OMe-6'	-	-	55.3
OMe-3	-	55.9	55.8

Assignments confirmed by HMQC, HMBC and NOESY experiments.

Compounds **13**-**18** and **20**, known pentacyclic triterpenes whose occurrence is not uncommon in the genus *Terminalia*, were identified as arjunic acid, arjunetin, arjungenin, arjunglucoside I, arjunolic acid, arjunglucoside II and betulinic acid, respectively, and their corresponding NMR spectral data were in full agreement with those reported in the literature.[15,16] 23-Galloylarjunglucoside II (**19**) has been formerly obtained solely from *T. macroptera*[17] and in the present work it was isolated and characterized after methylation with diazomethane as its mono-, di- and tri-*O*-methyl derivatives 23-(4"-*O*-methyl)-galloylarjunglucoside II (**19a**), 23-(3",4"-di-*O*-methyl)-galloylarjun-

glucoside II (**19b**) and 23-(3",4",5"-tri-*O*-methyl)-galloylarjunglucoside II (**19c**). The carbon signals of **19a**-**19c** were very similar to those of **19**, except for the signals attributable to the galloyl moiety, which were consistent with the presence of methoxy groups at C-4" in **19a**, C-3" and C-4" in **19b** and C-3", C-4" and C-5" in **19c**, as shown in Table 2. Compound **21** was identified as ursolic acid acetate[15] and in spite of the wide distribution of this triterpene in other plant genera, no record is available for its presence in *Terminalia*.

Table 2. ^{13}C (75 MHz) NMR spectral data for **19**, **19a**, **19b** and **19c** (δ, Py-d_5)

C	19[17]	19a*	19b*	19c*
1	47.8	47.6	47.7	48.0
2	68.3	68.2	68.2	68.3
3	77.6	77.5	77.3	77.3
4	43.2	43.1	43.0	43.1
5	48.9	48.8	48.7	48.7
6	18.8	18.6	18.5	18.5
7	32.9	32.7	32.7	32.9
8	39.9	39.8	39.8	39.9
9	48.5	48.4	48.4	48.5
10	38.2	38.1	38.1	38.2
11	23.9	23.8	23.7	23.8
12	122.2	122.4	122.4	122.6
13	144.2	144.0	143.9	144.0
14	42.1	42.0	41.9	41.9
15	28.0	27.9	27.8	27.9
16	23.4	23.2	23.1	23.1
17	47.0	46.9	46.8	46.8
18	41.8	41.7	41.5	41.5
19	46.2	46.0	45.9	46.0
20	30.7	30.6	30.5	30.6
21	34.0	33.8	33.7	33.7
22	32.5	32.3	32.2	32.3
23	67.0	67.2	67.6	66.9
24	14.0	13.8	13.7	13.8
25	17.3	17.4[a]	17.3[b]	17.3[c]
26	17.6	17.1[a]	17.1[b]	17.2[c]
27	25.9	25.7	25.7	25.6
28	176.5	176.4	176.4	176.4
29	33.1	32.9	32.8	32.9
30	23.6	23.5	23.4	23.5
Glucose moiety				
1'	95.7	95.6	95.5	95.5
2'	74.0	73.9	73.8	73.9
3'	78.8	78.7	78.6	78.7
4'	71.0	70.9	70.9	70.9
5'	79.3	79.2	79.1	79.1
6'	62.2	62.1	62.0	62.0
Galloyl moiety				
1"	121.5	122.6	126.3	125.9
2"	110.0	109.8	104.9	107.1
3"	147.5	152.3	153.6	153.5
4"	140.7	141.3	141.7	142.8
5"	147.5	152.3	152.0	153.5
6"	110.0	109.8	111.9	107.1
7"	167.1	166.6	166.2	165.9
OMe-3"	-	-	55.7	55.9
OMe-4"	-	60.1	60.3	60.5
OMe-5"	-	-	-	55.9

*Assignments confirmed by HMQC and HMBC experiments. [a, b, c] Interchangeable signals.

Compounds **2**, **3**, **6**, **8**-**16, 19a**-**19c** and **22** were assayed *in vitro* against the Hep_2 (larynx carcinoma) and H_{292} (lung mucoepidermoid carcinoma) human cell lines. As depicted in Table 3, compounds **9**, **10**, **12**, **19b** and **19c** displayed significant cytotoxic activity on both cell lines (IC_{50} values in the range of 9.7-23.2 μg mL^{-1}), while **11** and **13** exhibited weak cytotoxicity in this assay (IC_{50} values in the range of 30.7 - 41.4 μg mL^{-1}). The cytotoxic anti-tumor drug cisplatin was taken as positive control (IC_{50} 5.1 and 7.8 μg mL^{-1}). The detection of cytotoxic compounds in *Terminalia fagifolia* associated to its use in the Brazilian folk medicine for the treatment of tumors requires further investigations.

Table 3. Cytotoxicity of compounds **2**-**3**, **6**, **8**-**16**, **19a**-**19c** and **22** against Hep_2 and H_{292} cell lines

Compound	IC_{50} / (μg mL^{-1})	
	Hep_2	H_{292}
2	> 50	> 50
3	> 50	> 50
6	> 50	> 50
8	> 50	> 50
9	19.81	20.70
10	22.62	18.42
11	32.41	30.66
12	22.02	23.19
13	41.36	38.07
14	> 50	> 50
15	> 50	> 50
16	> 50	> 50
19a	> 50	> 50
19b	15.81	16.23
19c	16.93	9.70
22	> 50	> 50
Cisplatin*	5.10	7.80

*positive control.

In the antioxidant assay, compounds **8**, **11-13** and **19a**-**19c** strongly inhibited bleaching of β-carotene on TLC plates, while **14**-**16** and **22** showed moderate activity. On the other hand, compounds **2**-**6** and **9**-**10** were inactive. Although the antioxidative properties of a number of *ortho*-dioxygenated flavonoid derivatives are well known and several oxygenated pentacyclic triterpenes have also been reported as antioxidant compounds,[18,19] there is no report for the antioxidant activity of diarylpropanes **11** and **12** and triterpenes **19a**-**19c**. Recently arjungenin (**15**) and its glucoside (**16**) were found to show a moderate free radical scavenging activity in the DPPH model, while arjunic acid (**13**) and arjunetin (**14**) exhibited no significant activity in the same assay.[19] However, in the present work **13** and **14** showed strong and moderate activities, respectively, in the autography assay towards β-carotene.

Although a large number of cytotoxic constituents from different flavonoid classes has been described, to the best of our knowledge this is the first report on the cytotoxicity of 1,3-diarylpropanes. In addition, the isolation of this class of compounds from a member of the Combretaceae adds new phytochemical data, which might have chemotaxonomical importance.

Experimental

General experimental procedures

IR spectra were recorded as KBr pellets on a Bomem-Hartmann & Braun FT IR spectrometer. ^{1}H and ^{13}C 1D and 2D NMR spectra were recorded at 300 MHz (^{1}H) and 75 MHz (^{13}C) on a Bruker DPX-300 spectrometer. Standard pulse sequences were used for homo - and heteronuclear correlation experiments. Column chromatography procedures were performed on silica gel 70-230 mesh and 230-400 mesh, RP-18 silica gel 230-400 mesh, and Sephadex LH-20. Preparative TLC was carried out on silica gel PF_{254} plates. Reversed phase semi-preparative HPLC separations were performed with a Shimadzu LC-6AD pump, using a RP-18, 25 × 250 mm, 5µm particle size, Shim-Pack PREP-ODS(H) column, with a flow rate of 10 mL or 14 mL min^{-1} and monitoring at 254 or 280 nm.

Plant material

Heartwood and trunk bark of *Terminalia fagifolia* Mart. were collected in Aquidauana, Mato Grosso do Sul, Brazil, in June 2002. The plant material was identified by MSc. Ubirazilda M. Resende, CGMS Herbarium, Universidade Federal de Mato Grosso do Sul, Brazil, where a voucher specimen (No. 0938) was deposited.

Extraction and isolation of chemical constituents

Air-dried and powdered heartwood (2684 g) was extracted at room temperature with EtOH. After concentration *in vacuo*, the residue was partitioned between hexane/CH_3CN/$CHCl_3$/H_2O (20:34:10:10). The CH_3CN/$CHCl_3$ phase (21.4 g) was applied to a silica gel CC (70-230 mesh) eluted with hexane/EtOAc and EtOAc/MeOH gradient systems. The fractions showing similar spots by TLC were combined to give nineteen fractions (I→XIX). Fraction III (hexane/EtOAc 9:1, 282.0 mg) was further separated by CC over Sephadex LH-20 (hexane/CH_2Cl_2 1:4). Compound **1** was identified as the major component of fractions 10-13 (50.0 mg) whereas **23** (30.0 mg) was obtained from fractions 17-18. Fraction V (hexane/EtOAc 1:1, 259.0 mg) was chromatographed on a Sephadex LH-20 column using hexane/CH_2Cl_2 (1:4) followed by CH_2Cl_2/acetone (3:2) as solvents. Fractions 13-16 (hexane/CH_2Cl_2 1:4) from this column provided **4** (3.0 mg) while fractions 53-56 (CH_2Cl_2/acetone 3:2) yielded **2** (7.2 mg) and **10** (6.2 mg), after semi-preparative HPLC (MeOH/H_2O 17:3 and MeOH/H_2O 8:2, respectively). Fractions 57-69 (CH_2Cl_2/acetone 3:2) contained a mixture of **2**, **5** and **9** and were again separated by CC on Sephadex LH-20 ($CHCl_3$/MeOH 3:2) to give **5** (4.0 mg) and an unresolved mixture of **2** and **9** which was further separated by semi-preparative HPLC (MeOH/H_2O 8:2) to yield **2** (3.6 mg) and **9** (2.3 mg). Separation of fraction VI (hexane/EtOAc 1:1, 311.8 mg) by repeated CC on Sephadex LH-20 ($CHCl_3$/MeOH 3:2) gave two main fractions. The former furnished **6** (5.9 mg) and **12** (7.5 mg) while the latter gave **11** (3.8 mg) and further amounts of **12** (10.5 mg), after semi-preparative HPLC (MeOH/H_2O 7:3 and CH_3CN/H_2O 11:9, respectively). Fraction VII (hexane/EtOAc 1:1, 202.0 mg) was subjected to CC on Sephadex LH-20 ($CHCl_3$/MeOH 3:2). Fractions 12-19 from this column yielded **7** (12.4 mg), after semi-preparative HPLC (MeOH/H_2O 7:3) while fractions 23-25 consisted of **3** (12.8 mg). Fraction IX (EtOAc, 2.88 g) afforded **13** (12.1 mg) and **22** (34.6 mg), fraction XII (EtOAc/MeOH 9.5:0.5, 1.30 g) yielded **15** (186.0 mg) and **17** (6.0 mg) and fraction XIV (EtOAc/MeOH 9:1, 504.6 mg) gave **14** (13.6 mg), after a series of CC on Sephadex LH-20 (MeOH), followed by CC on silica gel 230-400 mesh ($CHCl_3$/MeOH 97:3 and $CHCl_3$/MeOH 9:1). Fraction XV (EtOAc/MeOH 9:1, 1.28 g) was subjected to CC on Sephadex LH-20 (MeOH). Fraction 9 from this column provided **18** (4.0 mg), while fractions 11-12 (171.0 mg) consisted of a complex mixture which was treated with an ethereal solution of diazomethane to give, after successive CC separations on Sephadex LH-20 (MeOH) and silica gel 230-400 mesh ($CHCl_3$/MeOH 95:5), the corresponding three *O*-methyl derivatives of compound **19**: **19a** (7.9 mg), **19b** (8.0 mg) and **19c** (12.4 mg). Compound **16** (29.1 mg) was isolated from fraction XVII (EtOAc/MeOH 9:1, 1.49 g) after CC on Sephadex LH-20 (MeOH) followed by CC on silica gel 230-400 mesh ($CHCl_3$/MeOH 9:1).

Air-dried and powdered trunk bark (1500 g) was extracted at room temperature with EtOH. The residue obtained from the EtOH extract was subsequently partitioned between MeOH/H_2O (9:1) and hexane; and MeOH/H_2O (1:1) and CH_2Cl_2. The CH_2Cl_2 phase (22.2 g) was subjected to CC on silica gel (70-230 mesh) eluted with hexane, CH_2Cl_2, EtOAc and EtOAc/MeOH 20% to

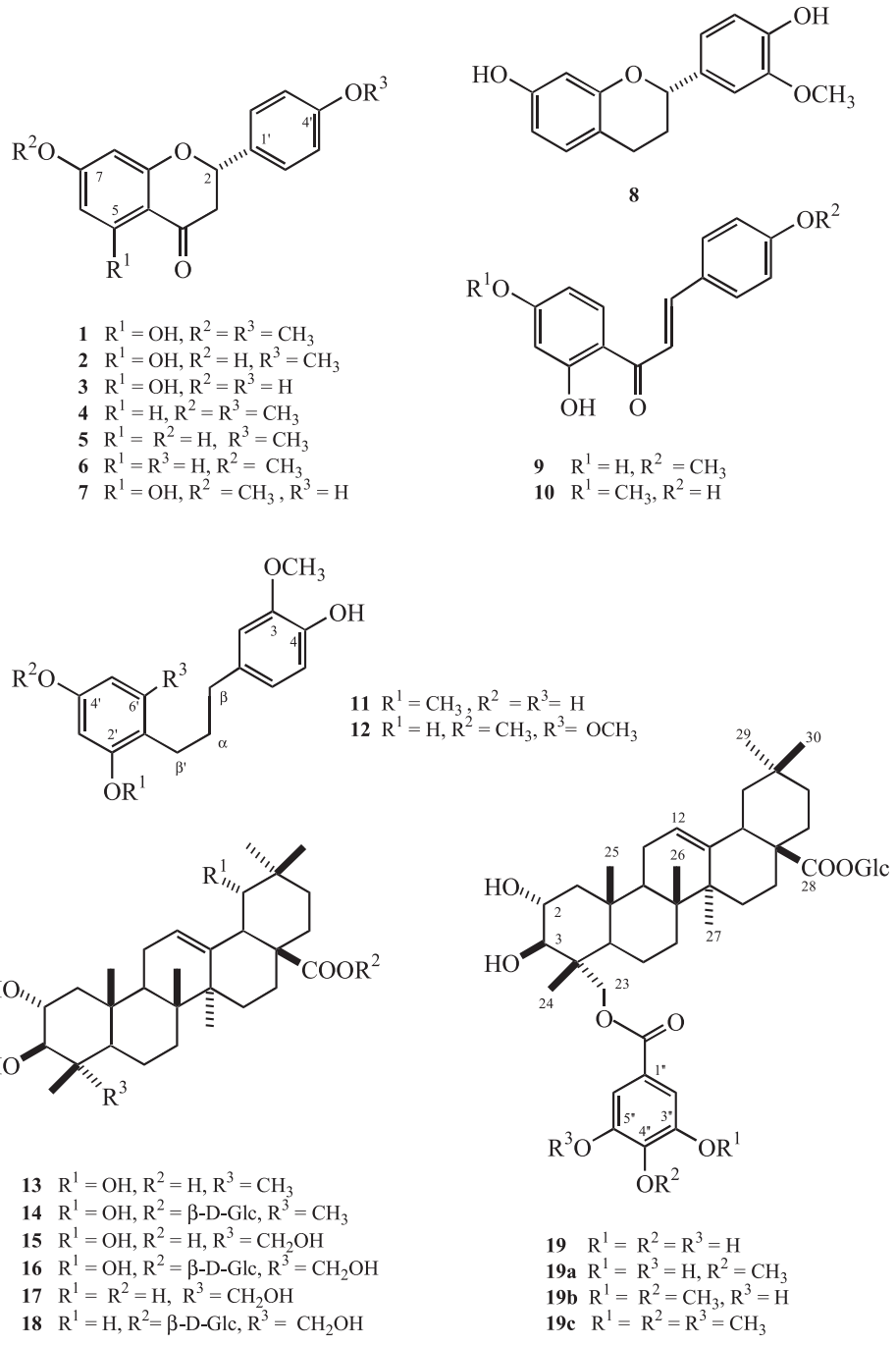

Figure 1. Chemical constituents from *T. fagifolia*.

yield twelve combined fractions (I→XII). Fraction V (CH_2Cl_2, 306.5 mg) was chromatographed on a Sephadex LH-20 column ($CHCl_3$/MeOH 3:2). Fractions 12-13 from this column were again subjected to CC on silica gel 230-400 mesh developed with hexane/acetone (9:1) followed by hexane/acetone (8.5:1.5) to give **21** (4.7 mg). Fraction VI (CH_2Cl_2, 1.16 g) was separated on a Sephadex LH-20 column eluted with $CHCl_3$/MeOH (3:2). The major constituent of fractions 7-8 (130.0 mg) was found to be **20**, which was not further purified. Fractions 15-19 (36.2 mg) yielded a mixture of **2** and **7** (6.0 mg) and of **9** and **10** (5.0 mg), after separation on Sephadex LH-20 ($CHCl_3$/MeOH 3:2) followed by preparative TLC (hexane/acetone/acetic acid 3.5:1.5:0.1). Fraction IX (EtOAc, 101.0 mg) yielded **13** (6.0 mg) after CC on RP-18 silica gel, eluted with MeOH/H_2O (6:4 to pure MeOH). Fraction XI (EtOAc, 3.88 g) afforded **14** (27.3 mg), **15** (8.0 mg) and **16** (95.5 mg) after a series of CC procedures on Sephadex LH-20 (MeOH) and on silica gel 230-400 mesh eluted with $CHCl_3$/MeOH (9.5:0.5).

In vitro cytotoxic assay

In vitro cytotoxic activities were measured against Hep_2, a human larynx carcinoma cell line and H_{292}, a human lung mucoepidermoid carcinoma cell line, obtained from Instituto Adolfo Lutz (São Paulo, SP, Brazil). Cells were cultivated in DMEM medium supplemented with 10% foetal calf serum, 100 μg mL^{-1} streptomycin, 100 U mL^{-1} penicillin and 0.25 μg mL^{-1} anfotericin B, at 37 °C in a humidified incubator with 5% CO_2. Cellular viability was assessed by formazan production from methylthiazolyldiphenyltetrazolium bromide (MTT colorimetric assay) as described previously.[20]

Bleaching experiments on β-carotene

The test was carried out on TLC plates, using a solution of β-carotene as a spraying reagent and α-tocopherol as the reference compound.[21] Sample solutions of **2**-**6**, **8**-**16**, **19a**-**19c** and **22** at similar concentrations were applied on TLC plates. After developing and drying, the plates were sprayed with a 0.02% solution of β-carotene in CH_2Cl_2 and subsequently placed under natural light until discoloration of the background.

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Supplementary Information

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

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Bioactive Flavonoids and Triterpenes from *Terminalia fagifolia* (Combretaceae)

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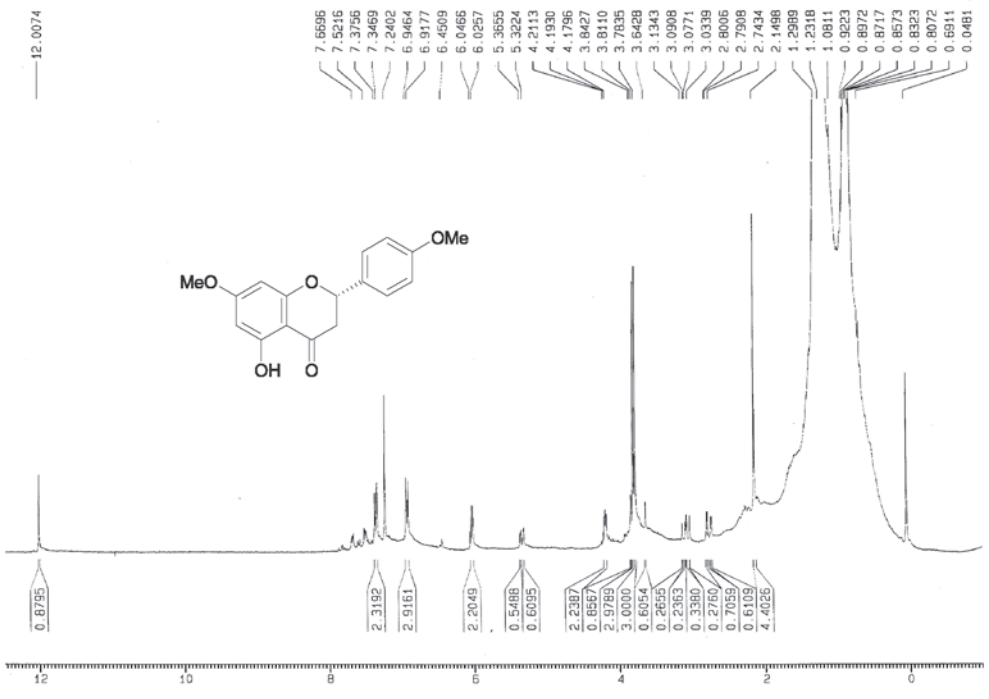

Figure S1. ^{1}H NMR spectrum of compound **1** (300 MHz, $CDCl_3$).

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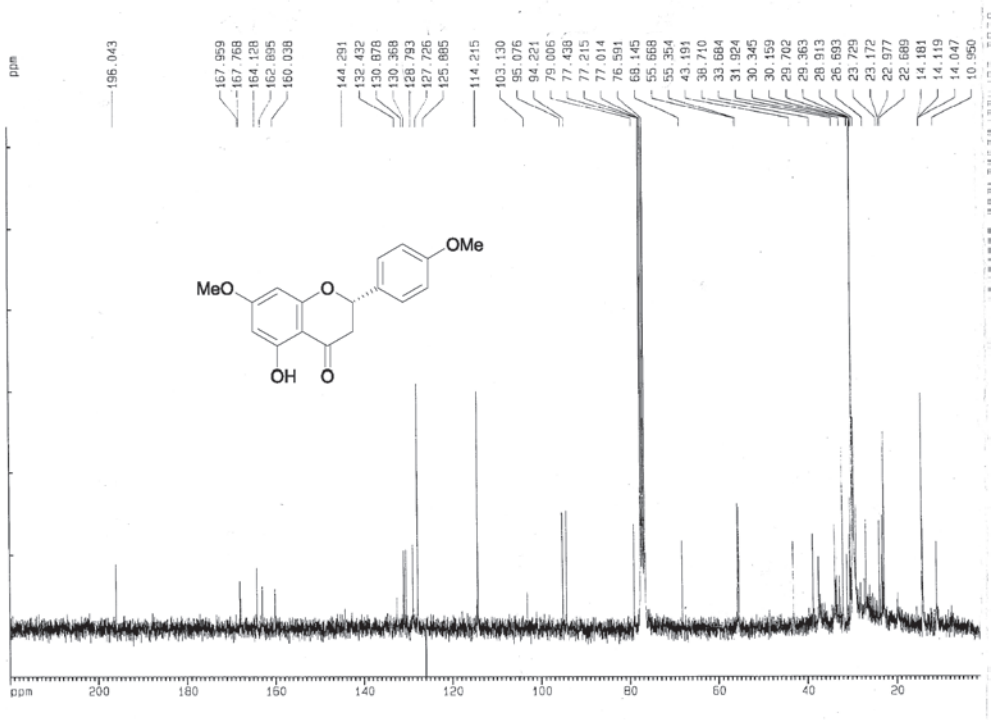

Figure S2. ^{13}C NMR spectrum of compound 1 (75 MHz, $CDCl_3$).

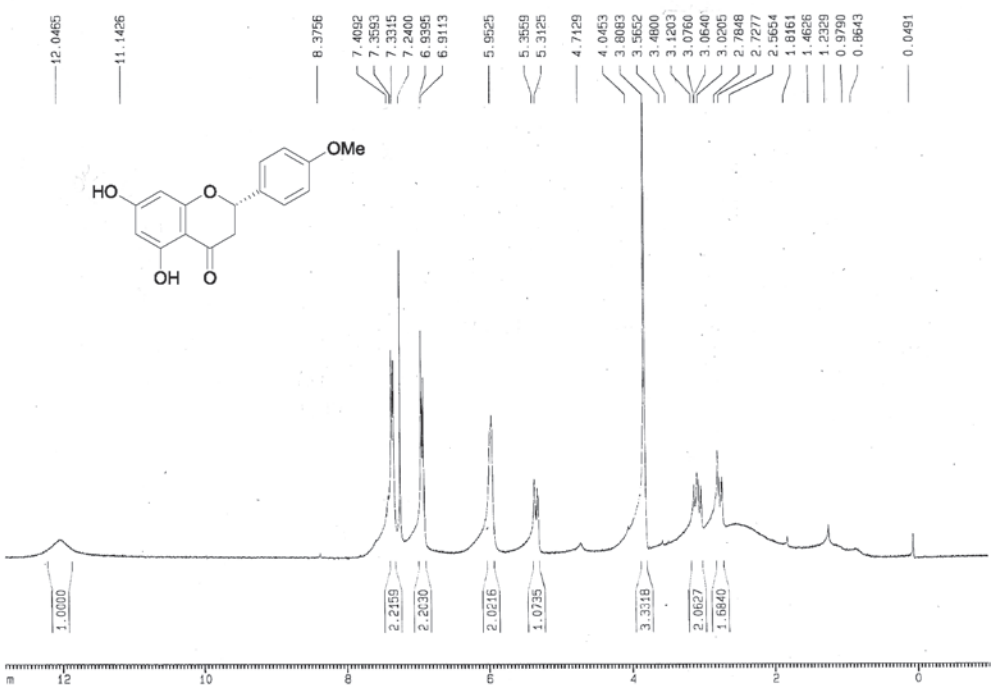

Figure S3. ^{1}H NMR spectrum of compound **2** (300 MHz, $CDCl_3$).

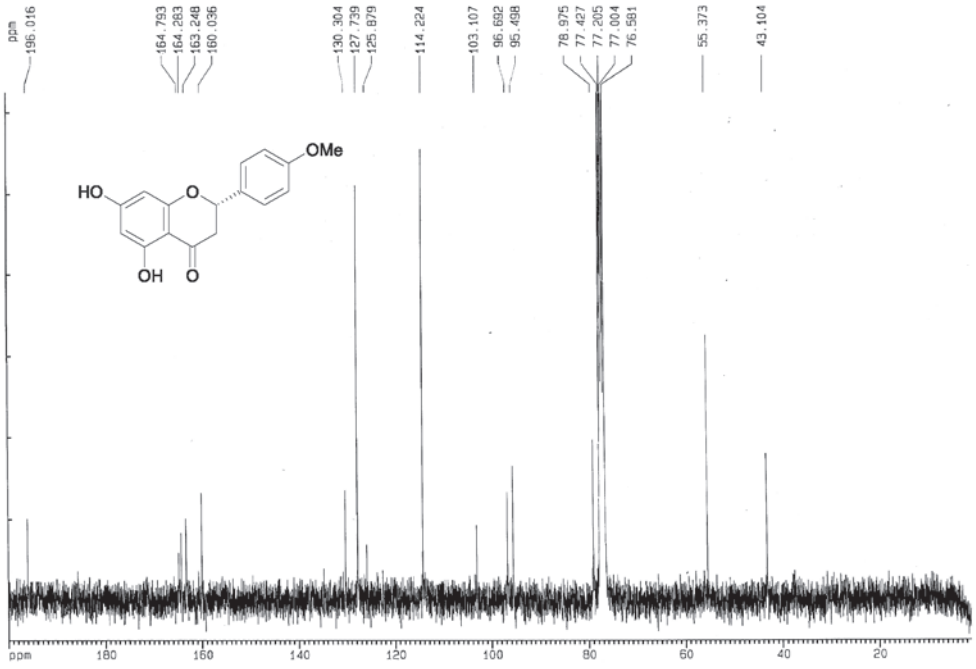

Figure S4. ^{13}C NMR spectrum of compound 2 (75 MHz, $CDCl_3$).

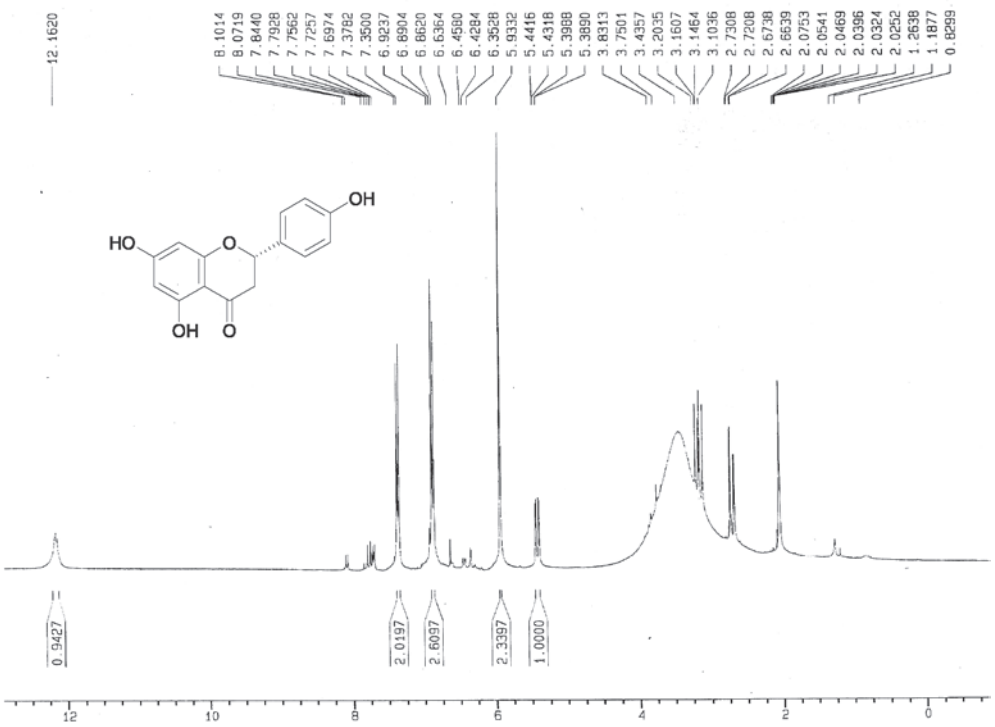

Figure S5. ^{1}H NMR spectrum of compound **3** (300 MHz, acetone-d_6).

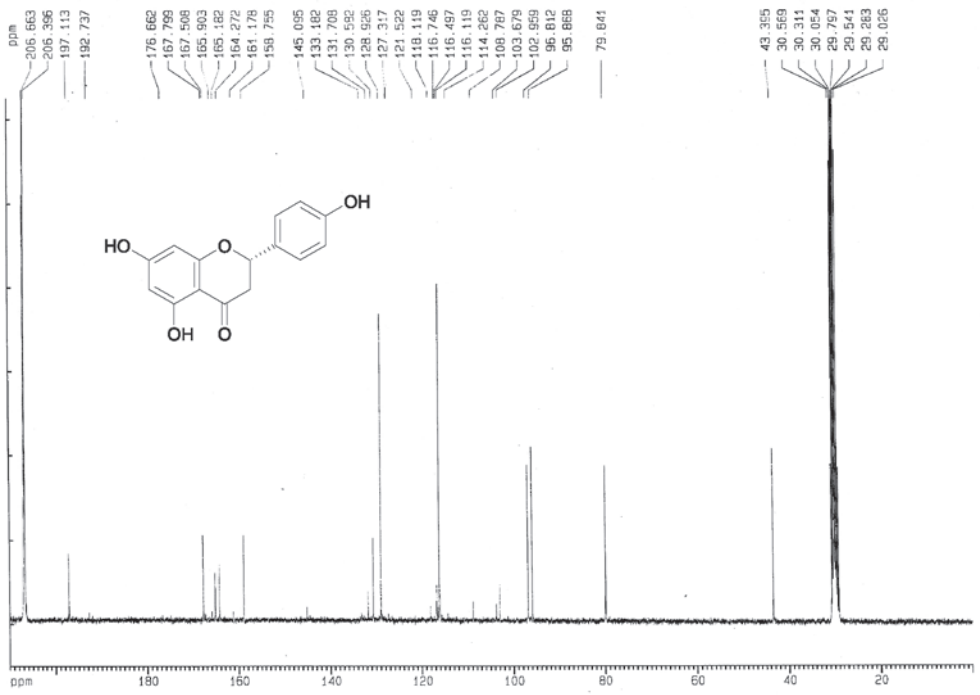

Figure S6. ^{13}C NMR spectrum of compound **3** (75 MHz, acetone-d_6).

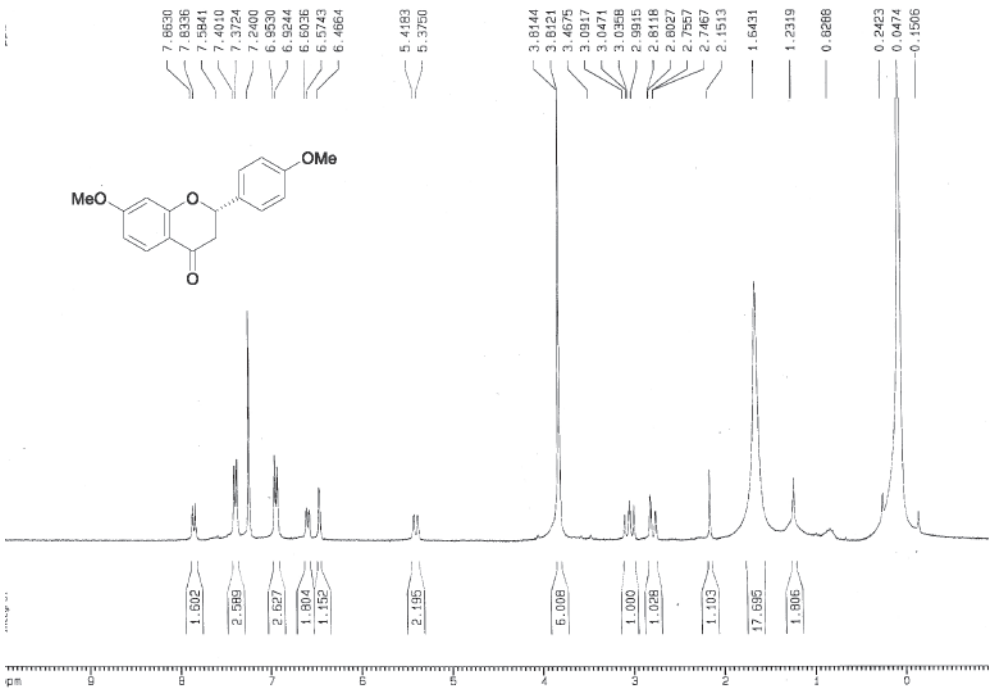

Figure S7. ^{1}H NMR spectrum of compound **4** (300 MHz, $CDCl_3$).

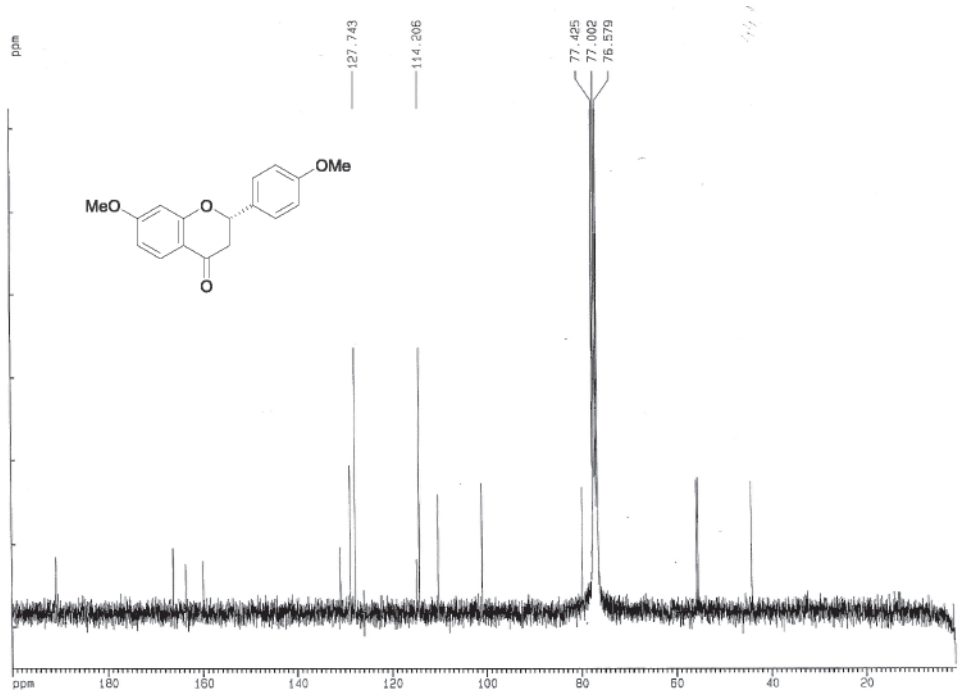

Figure S8. ^{13}C NMRspectrum of compound **4** (75 MHz, $CDCl_3$).

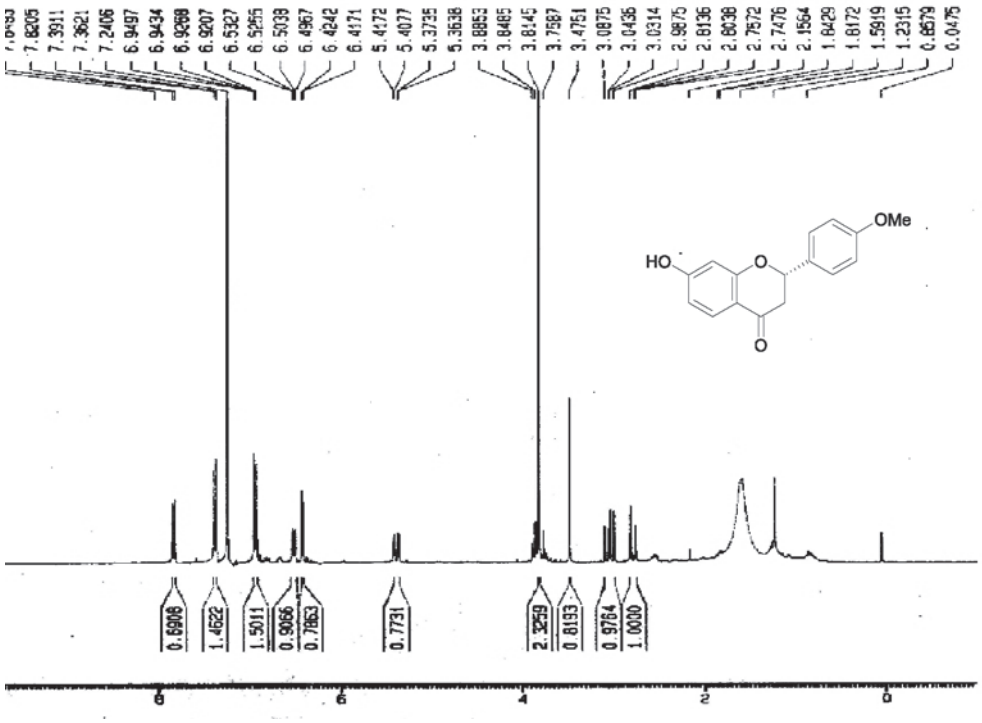

Figure S9. ^{1}H NMR spectrum of compound **5** (300 MHz, $CDCl_3$).

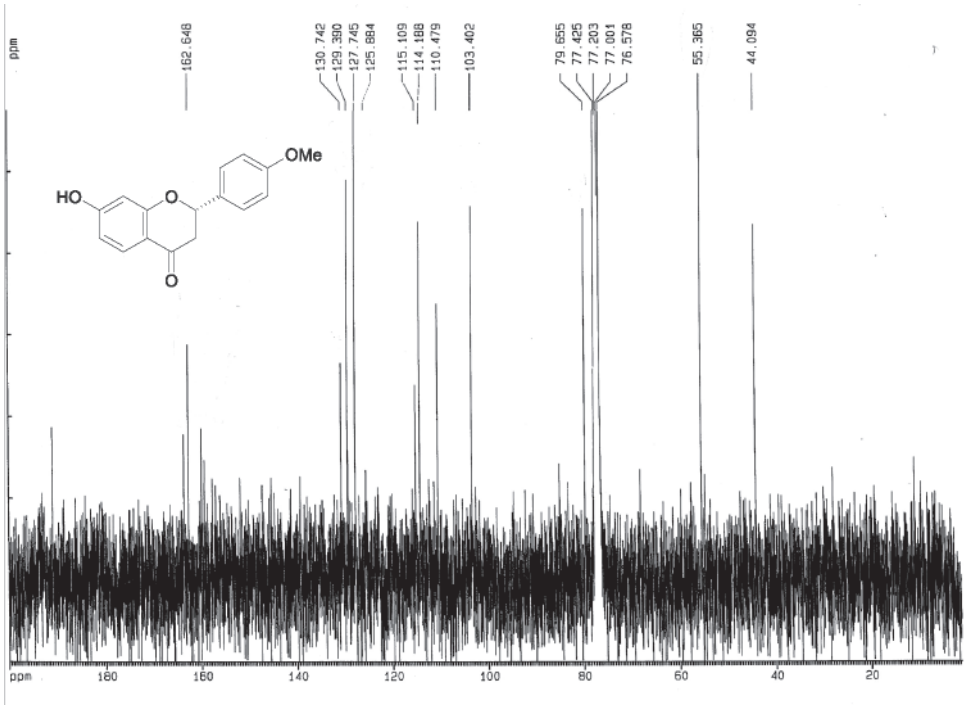

Figure S10. ^{13}C NMR spectrum of compound **5** (75 MHz, $CDCl_3$).

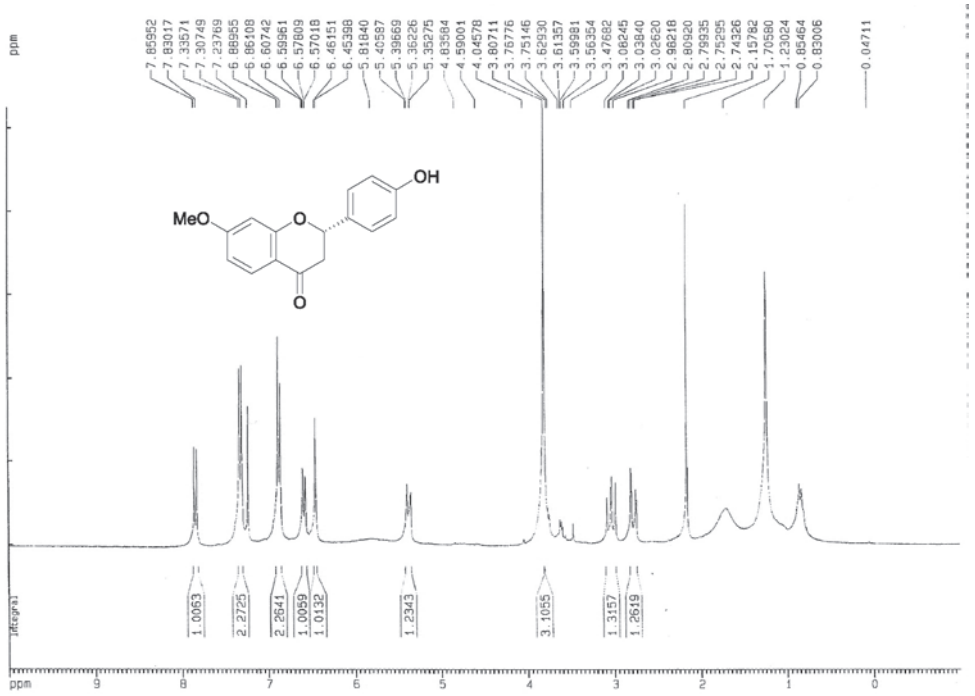

Figure S11. ^{1}H NMR spectrum of compound **6** (300 MHz, $CDCl_3$).

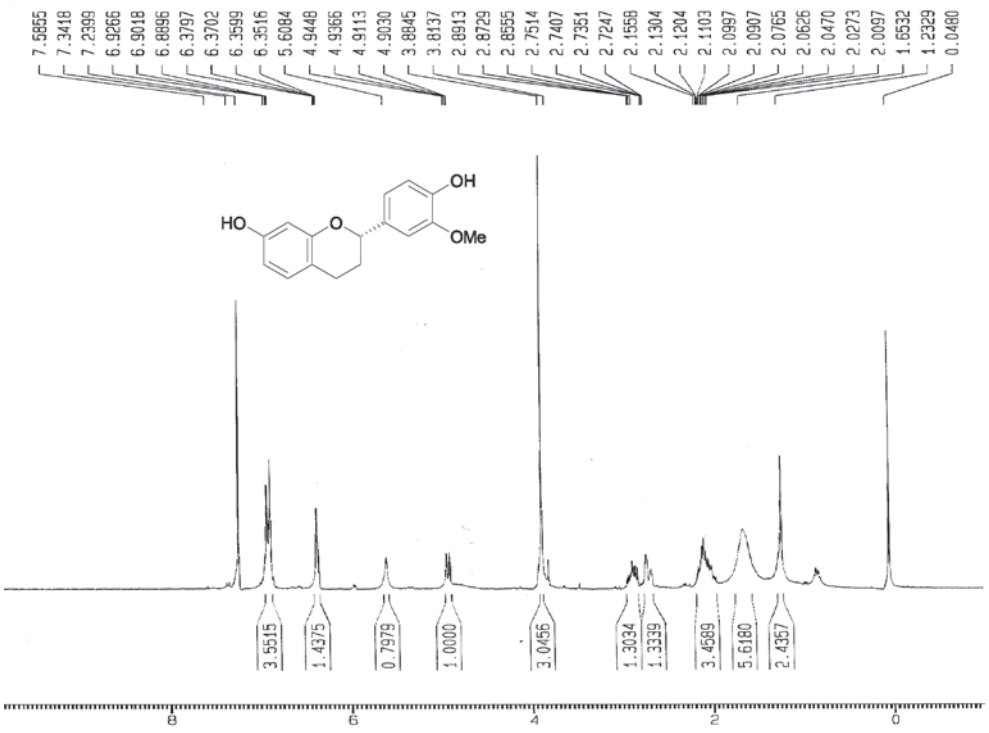

Figure S12. ^{13}C NMR spectrum of compound **6** (75 MHz, $CDCl_3$).

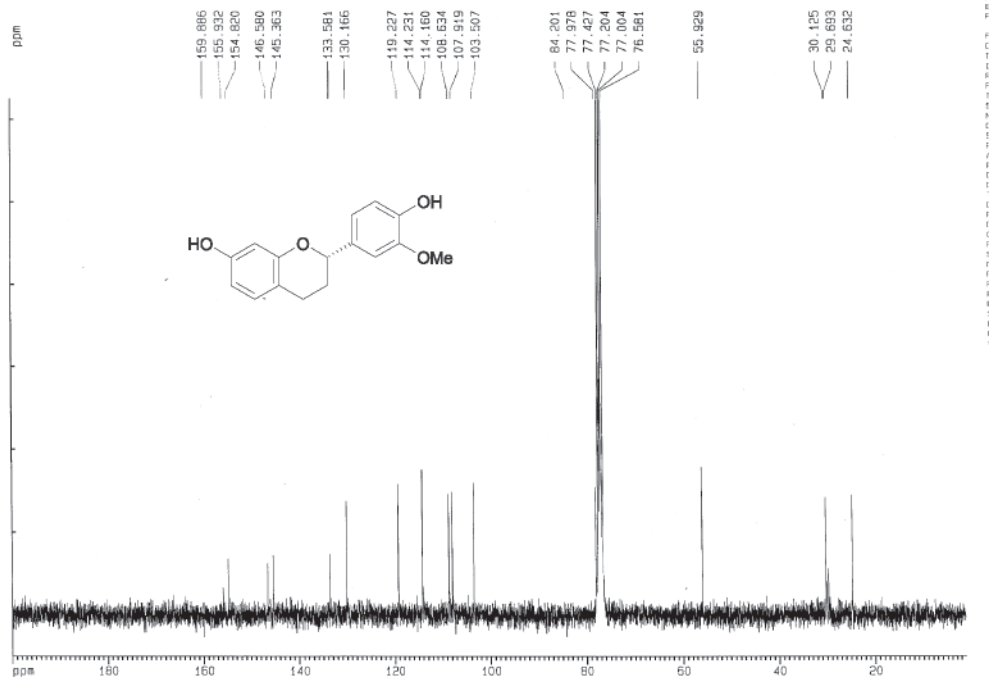

Figure S13. ^{1}H NMR spectrum of compound **8** (300 MHz, $CDCl_3$).

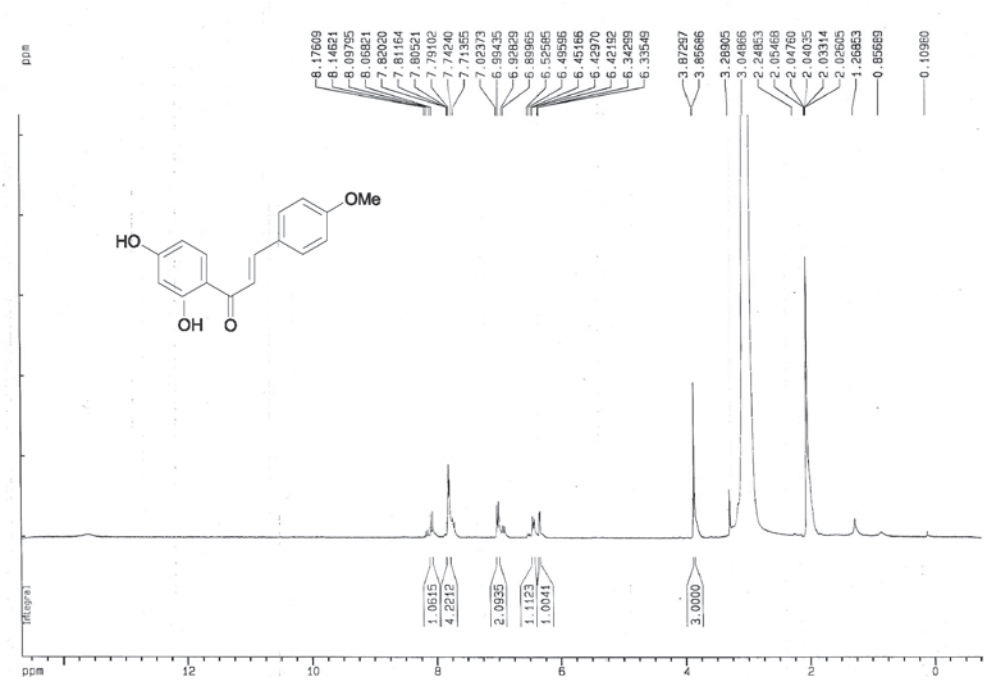

Figure S14. ^{13}C NMR spectrum of compound **8** (75 MHz, $CDCl_3$).

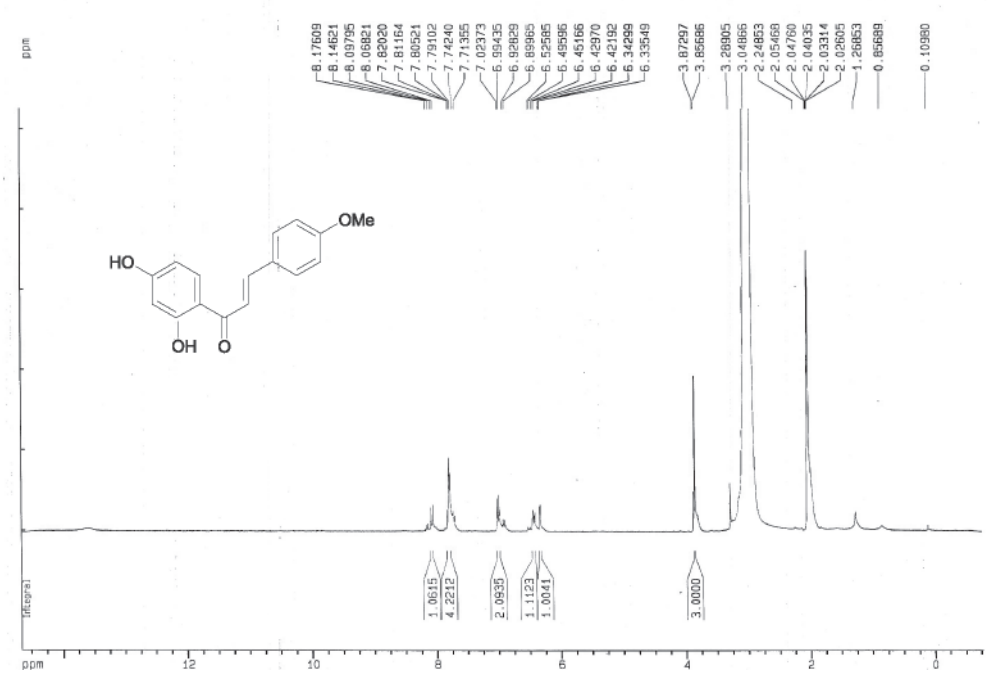

Figure S15. ^{1}H NMR spectrum of compound **9** (300 MHz, acetone-d_6).

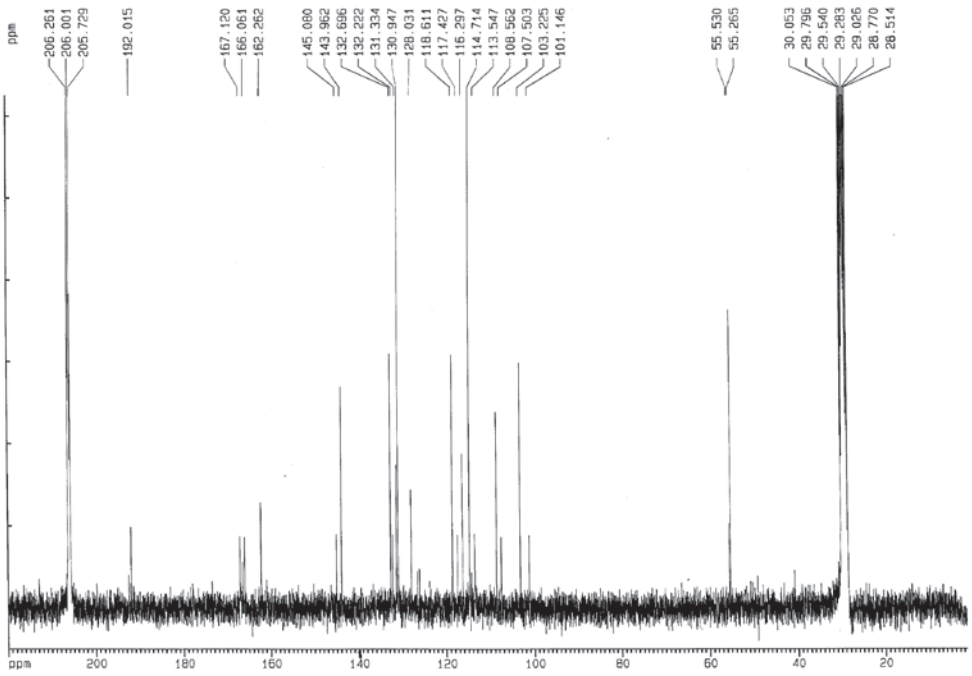

Figure S16. ^{13}C NMR spectrum of compound **9** (75 MHz, acetone-d_6).

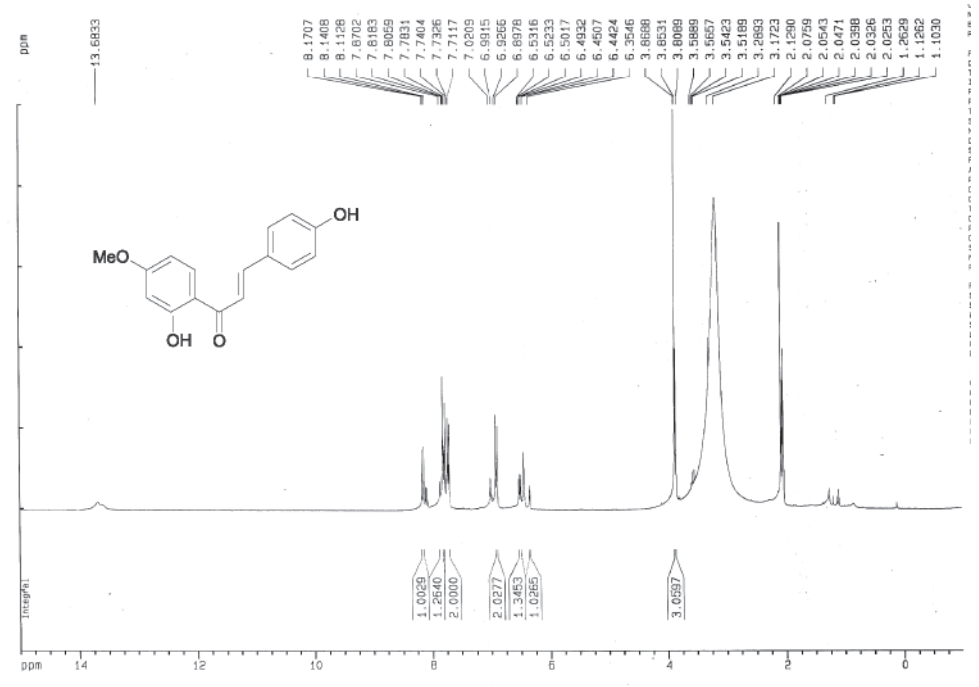

Figure S17. ^{1}H NMR spectrum of compound **10** (300 MHz, acetone-d_6).

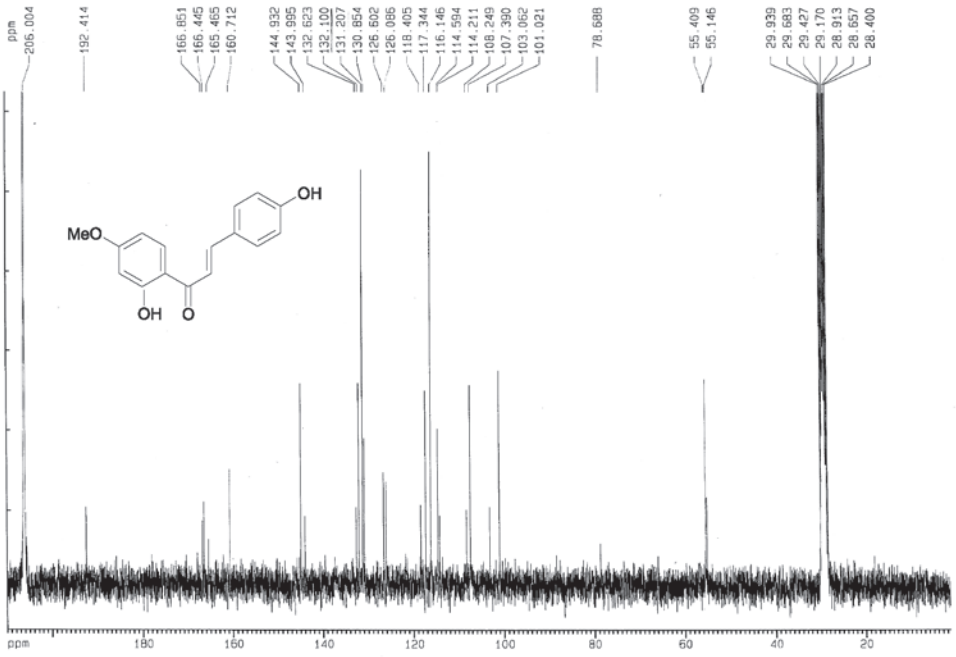

Figure S18. ^{13}C NMR spectrum of compound **10** (75 MHz, acetone-d_6).

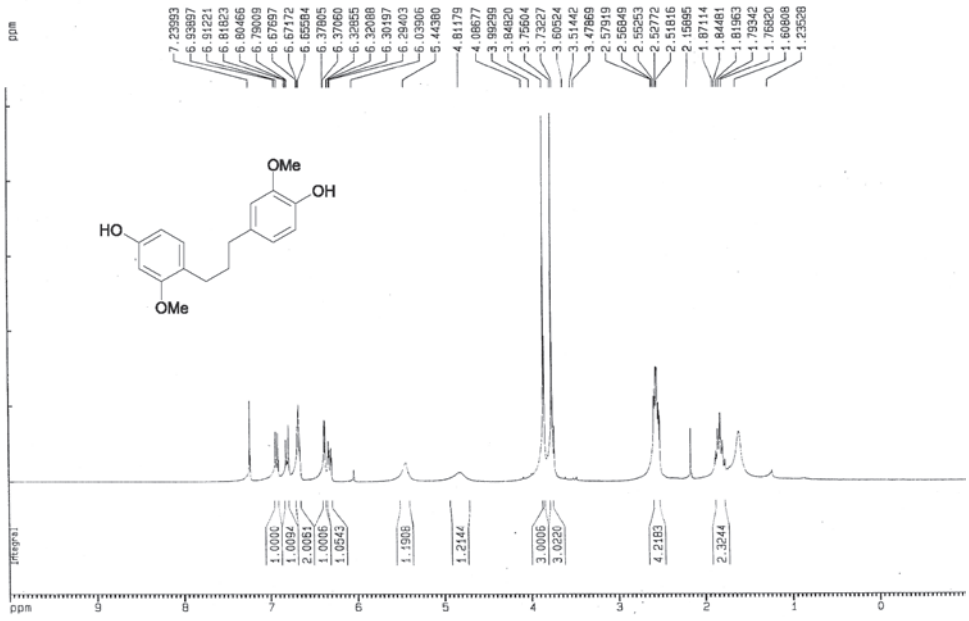

Figure S19. ^{1}H NMR spectrum of compound **11** (300 MHz, $CDCl_3$).

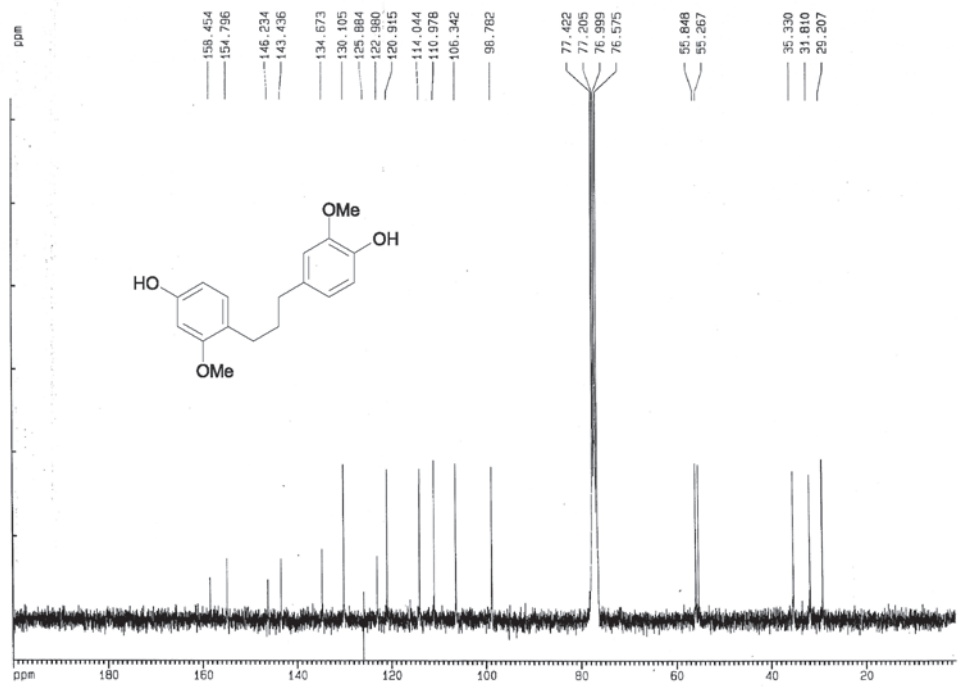

Figure S20. ^{13}C NMR spectrum of compound **11** (75 MHz, $CDCl_3$).

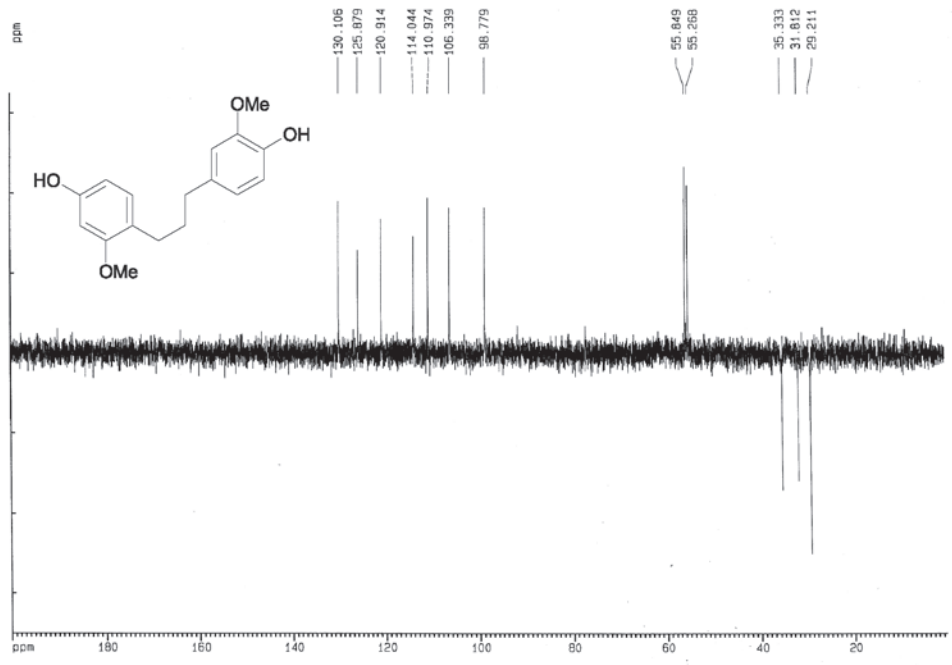

Figure S21. DEPT-135^{O} spectrum of compound **11** (75 MHz, $CDCl_3$).

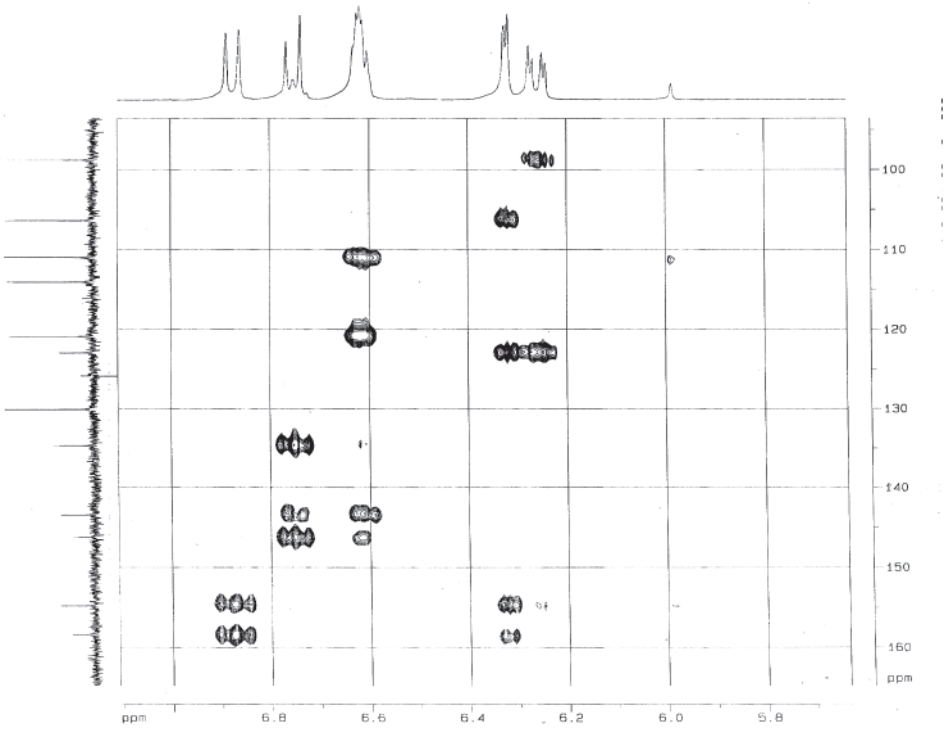

Figure S22. HMBC experiment of compound **11.**

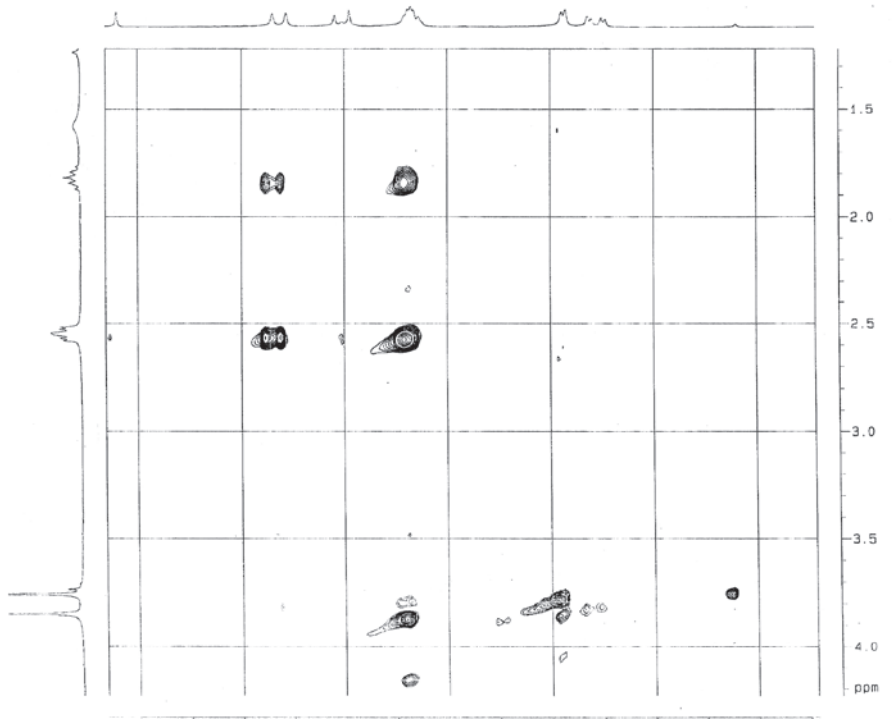

Figure S23. NOESY experiment of compound **11**.

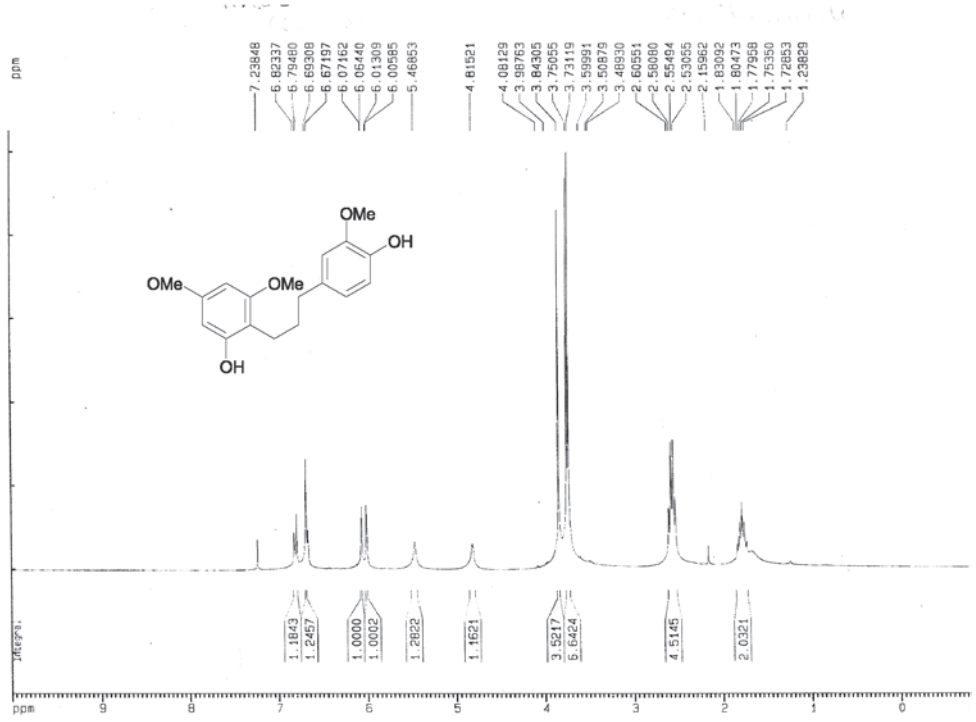

Figure S24. ^{1}H NMR spectrum of compound **12** (300 MHz, $CDCl_3$).

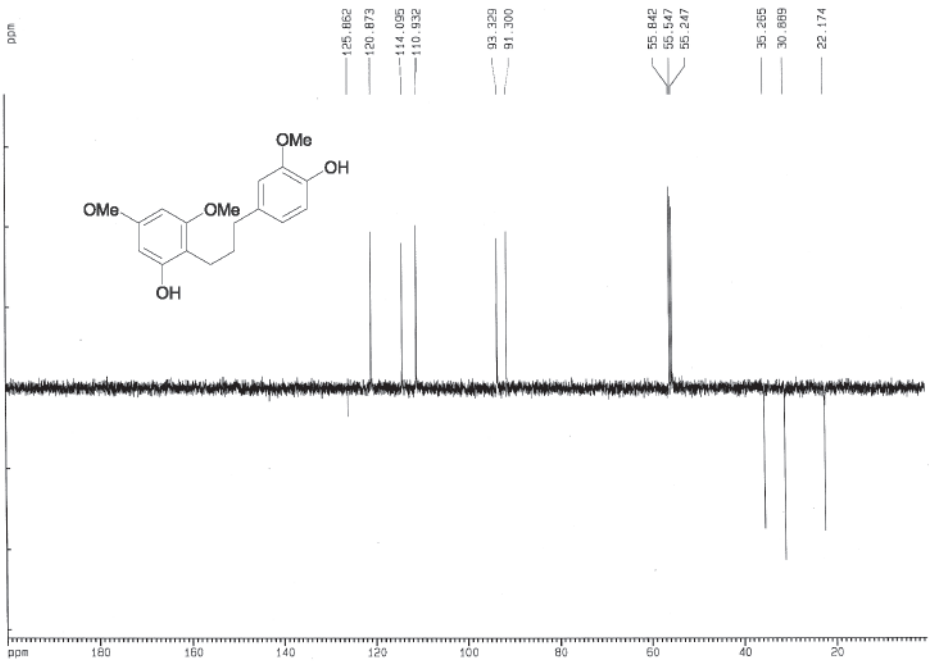

Figure S25. ^{13}C NMR spectrum of compound **12** (75 MHz, $CDCl_3$).

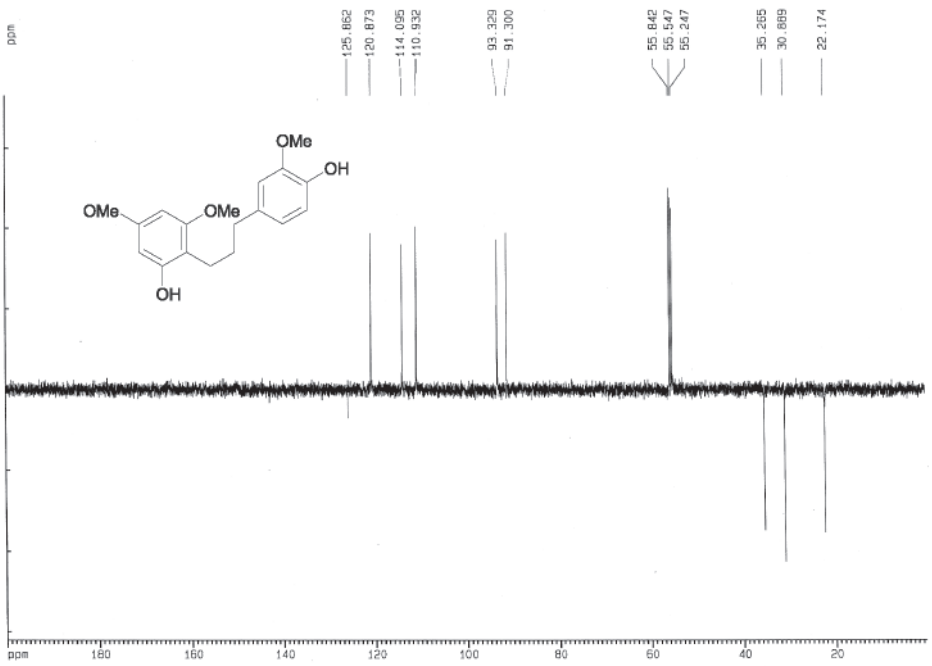

Figure S26. DEPT-135^{O} spectrum of compound **12** (75 MHz, $CDCl_3$).

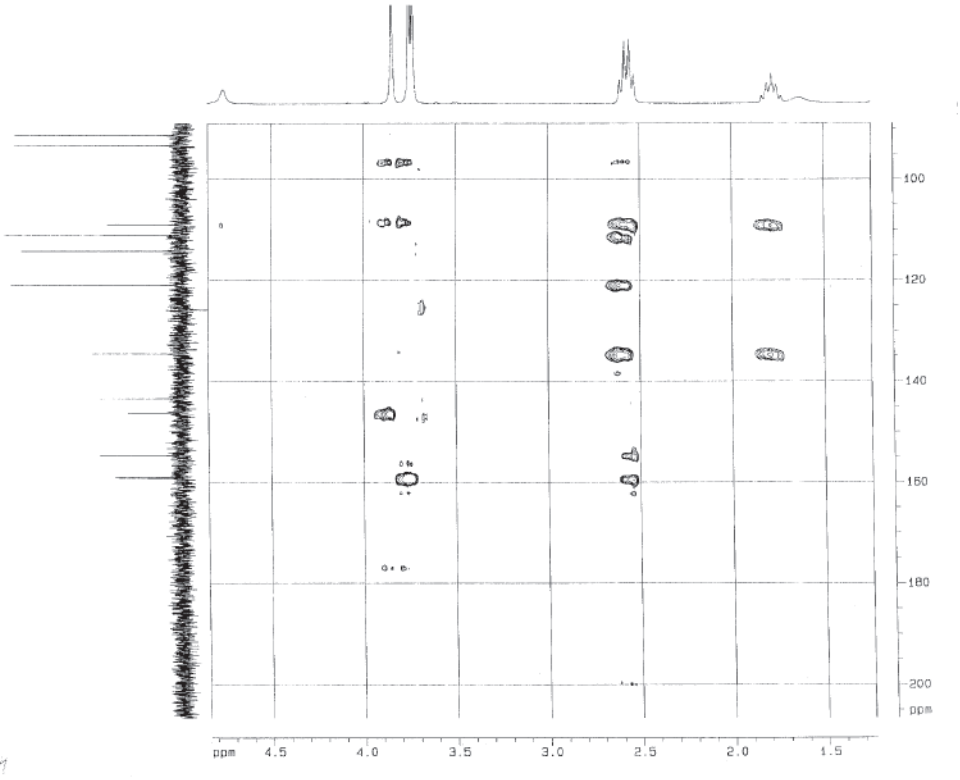

Figure S27. HMBC experiment of compound **12**.

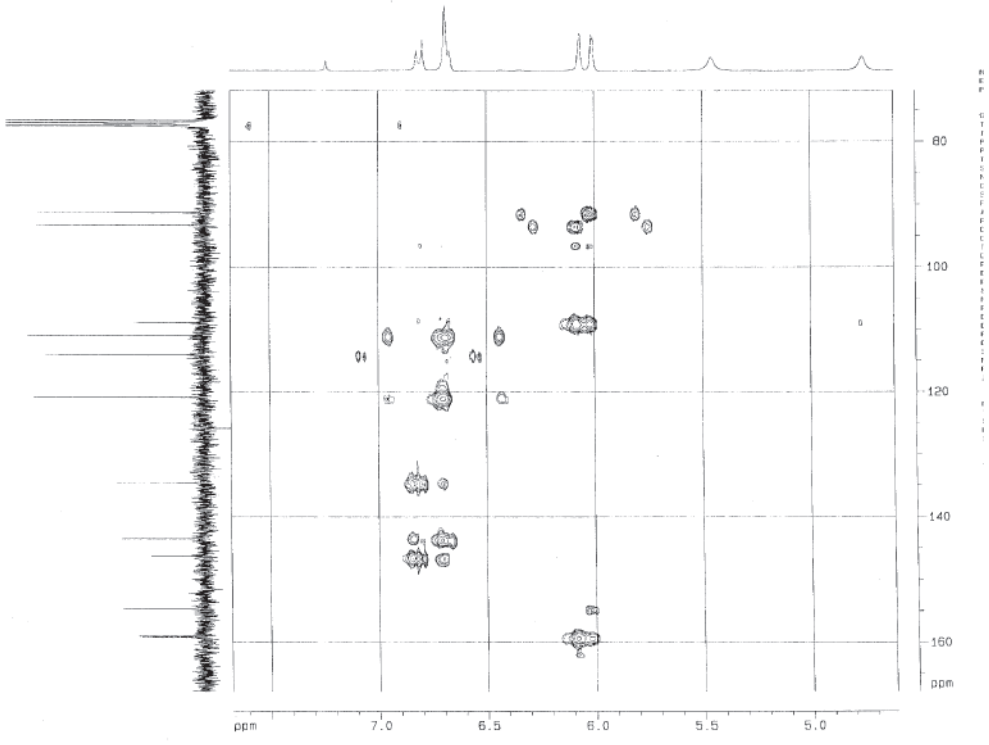

Figure S28. HMBC experiment of compound **12**.

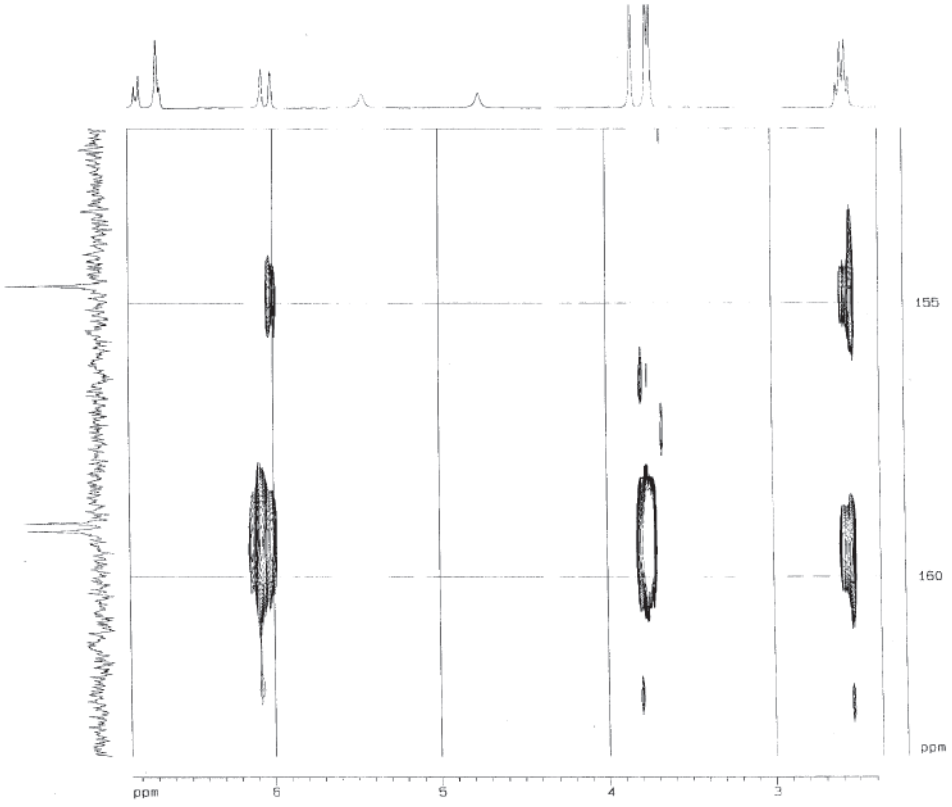

Figure S29. HMBC experiment of compound **12**.

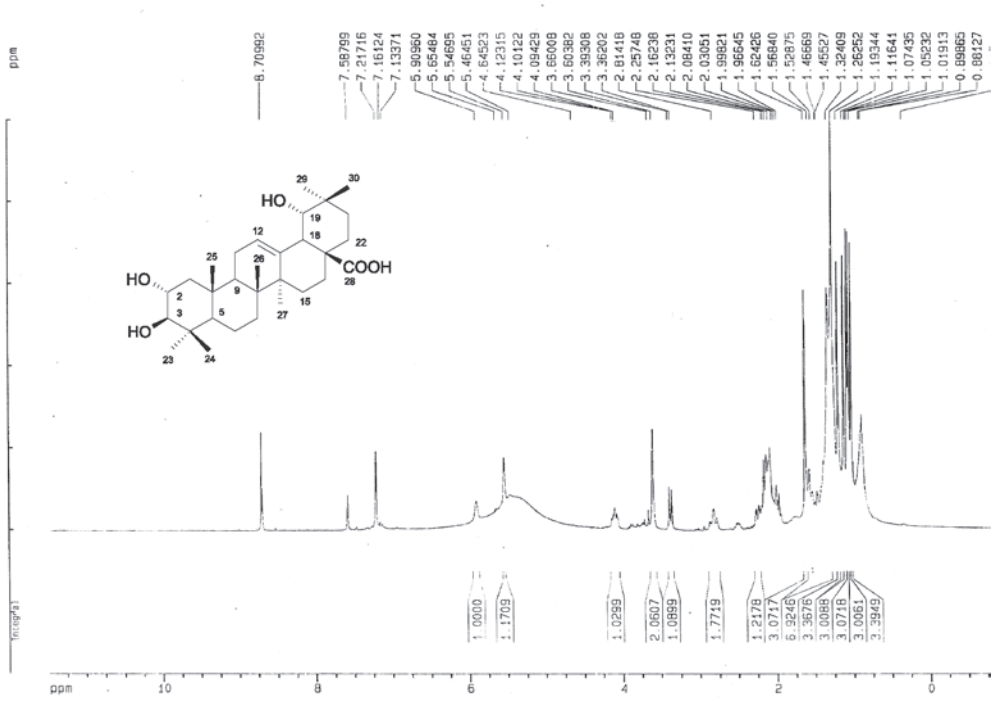

Figure S30. [1]H NMR spectrum of compound **13** (300 MHz, Py-d_5).

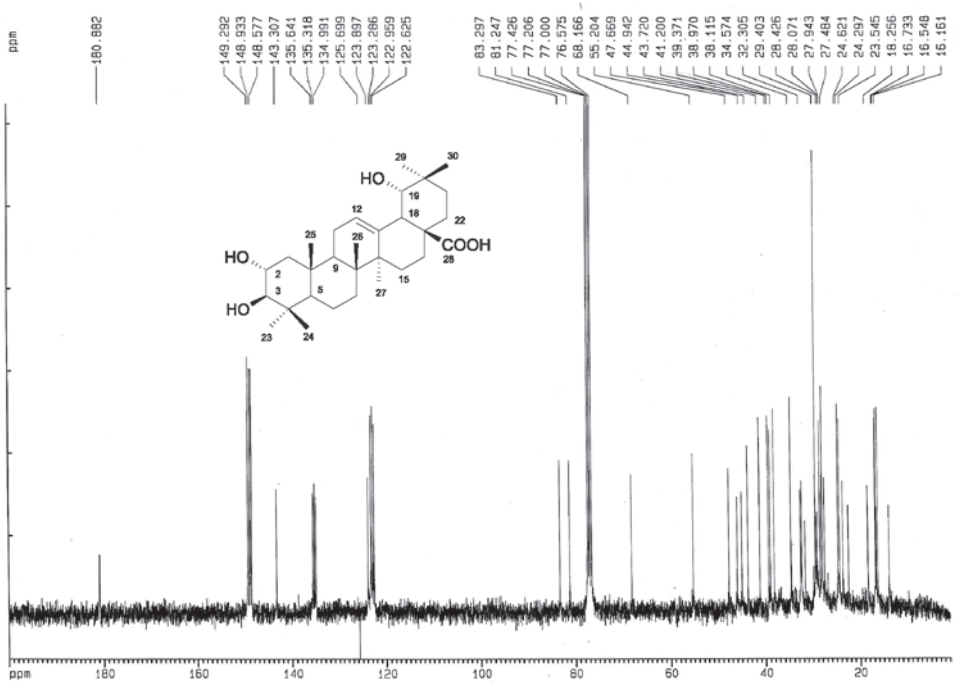

Figure S31. [13]C NMR spectrum of compound **13** (75 MHz, $CDCl_3$ + Py d_5).

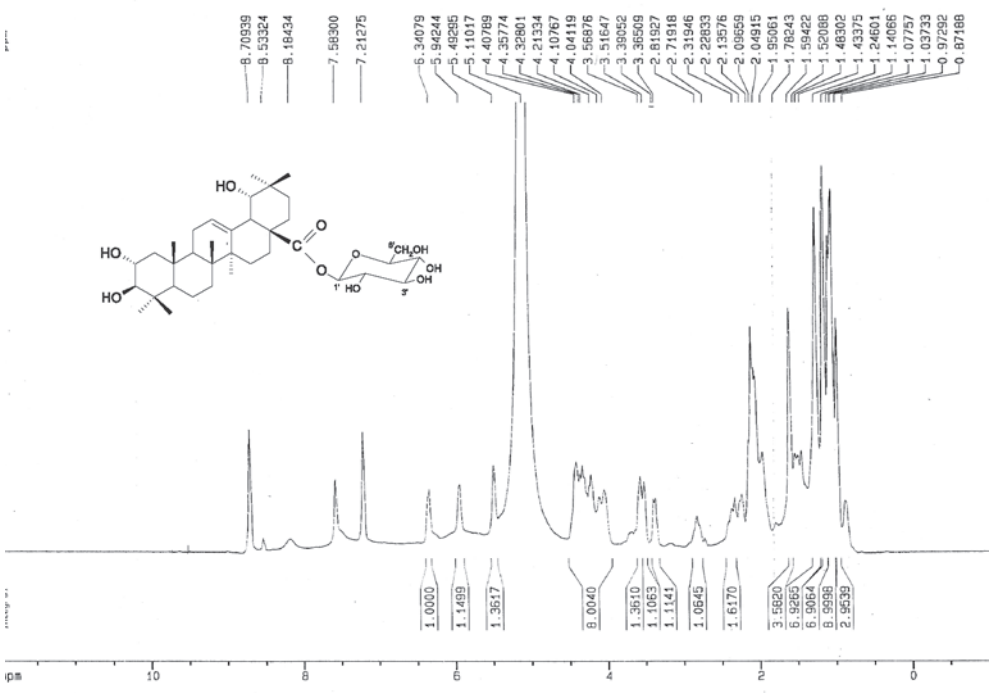

Figure S32. ^{1}H NMR spectrum of compound **14** (300 MHz, Py-d_5).

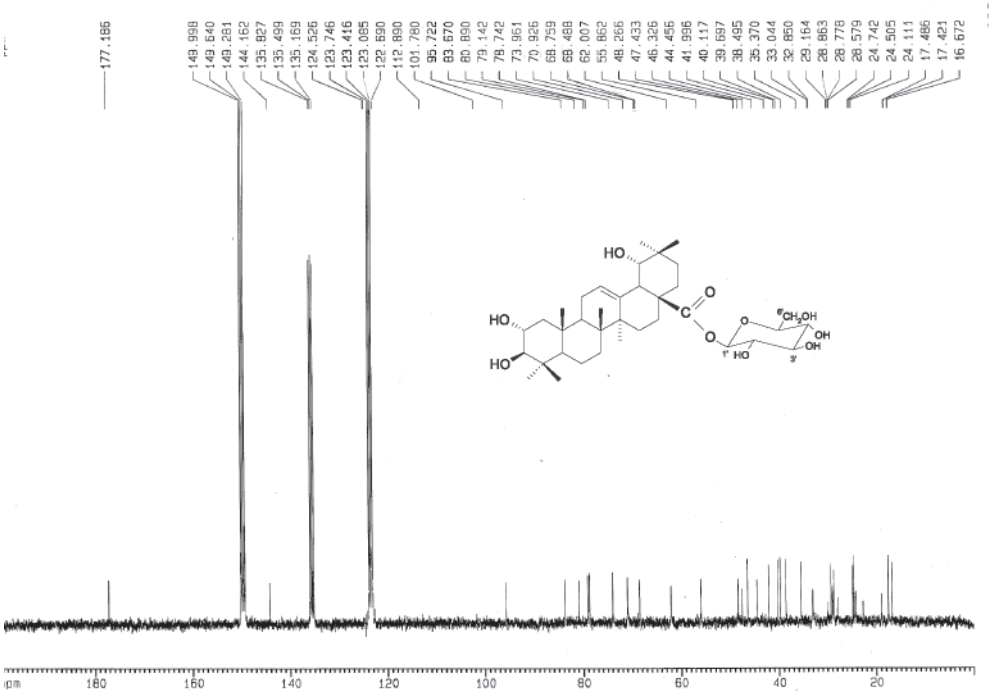

Figure S33. ^{13}C NMR spectrum of compound **14** (75 MHz, Py-d_5).

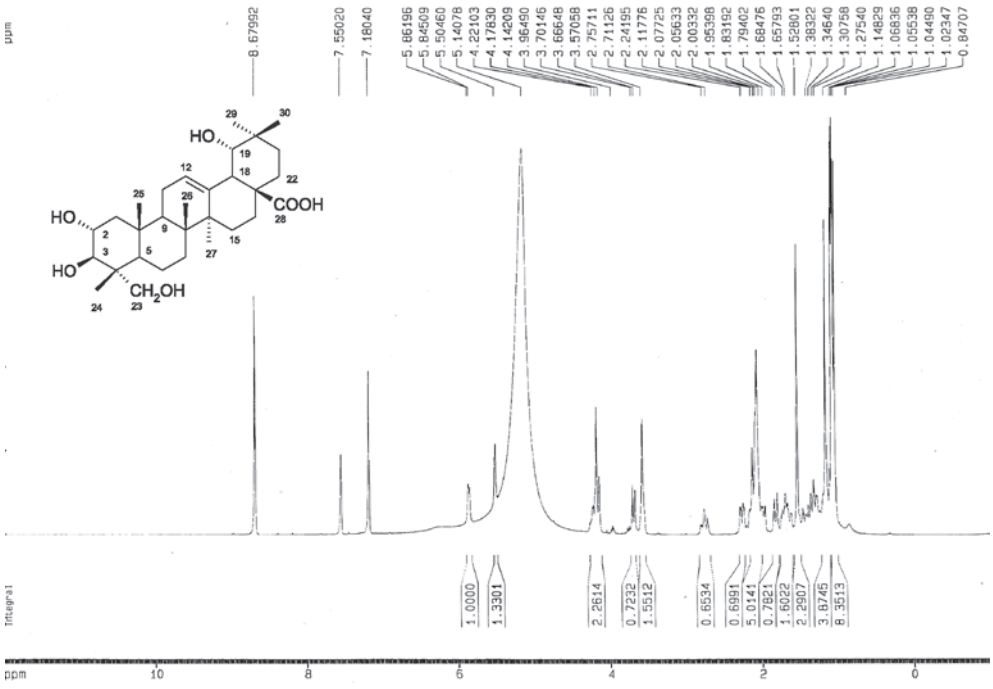

Figure S34. ^{1}H NMR spectrum of compound **15** (300 MHz, Py-d_5).

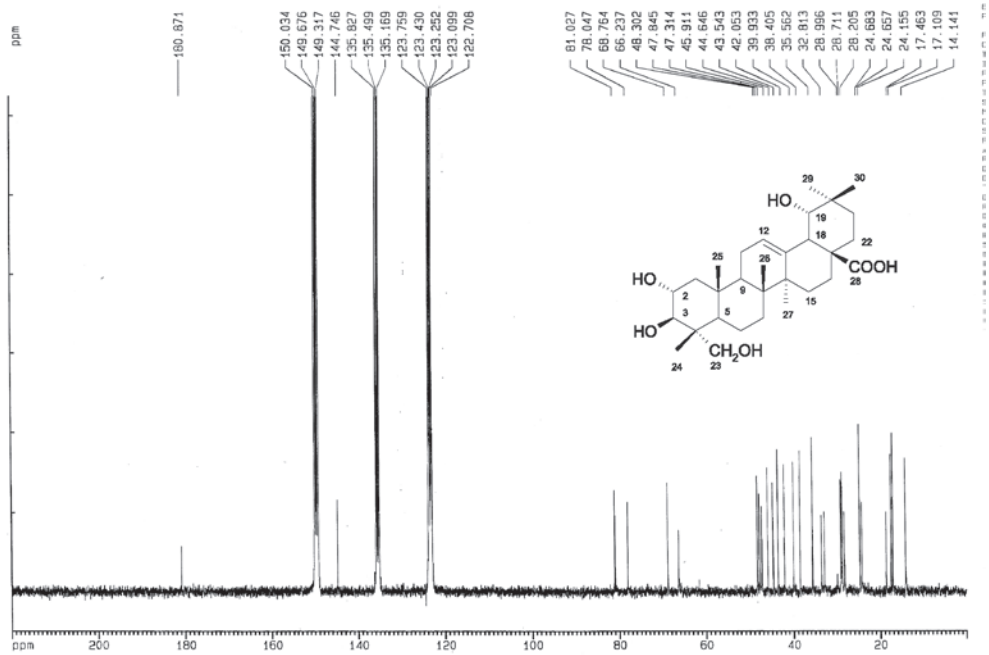

Figure S35. ^{13}C NMR spectrum of compound **15** (75 MHz, Py-d_5).

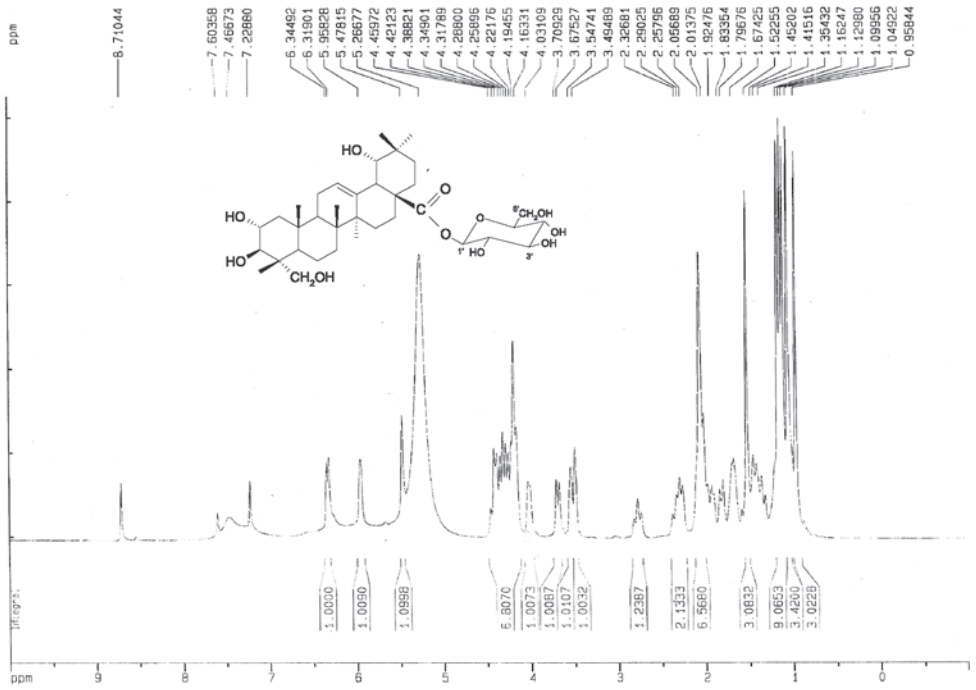

Figure S36. ^{1}H NMR spectrum of compound **16** (300 MHz, Py-d_5).

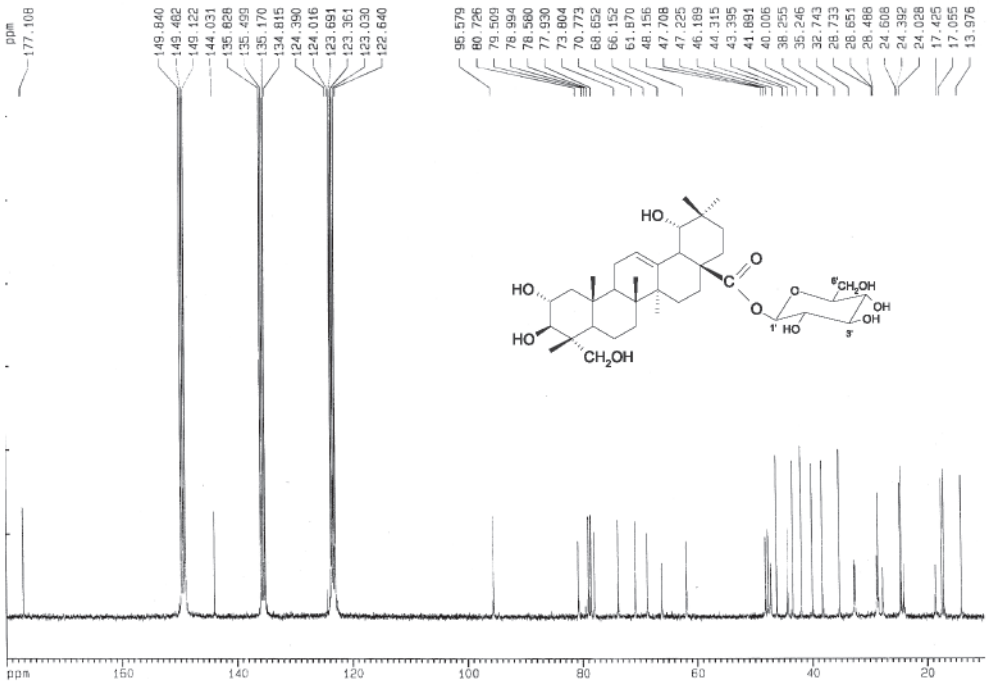

Figure S37. ^{13}C NMR spectrum of compound **16** (75 MHz, Py-d_5).

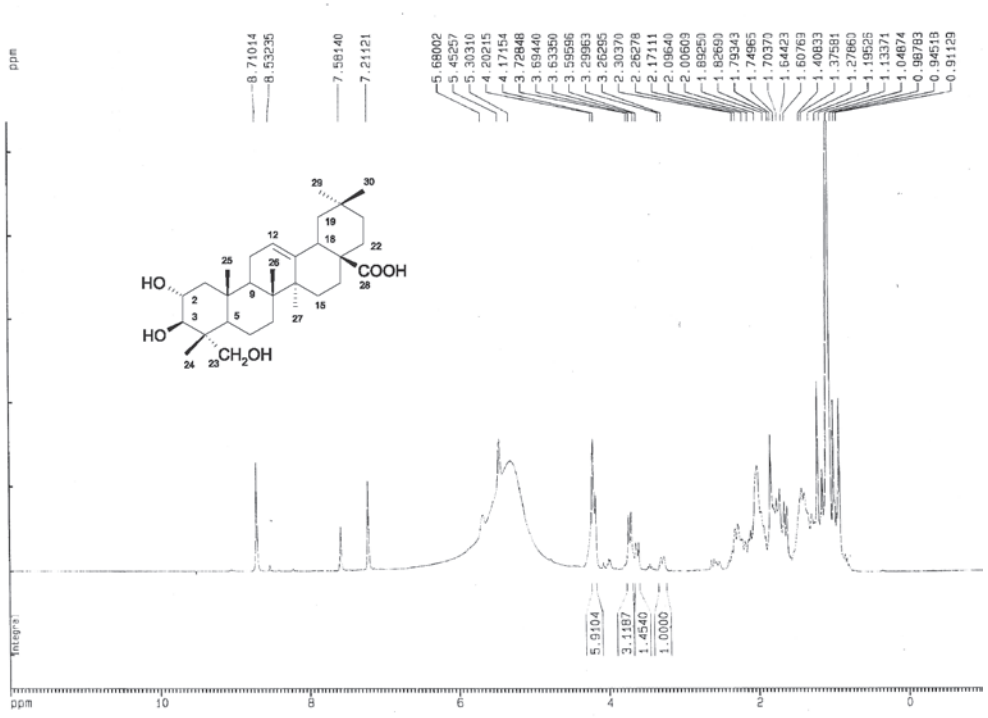

Figure S38. ^{1}H NMR spectrum of compound **17** (300 MHz, Py-d_5).

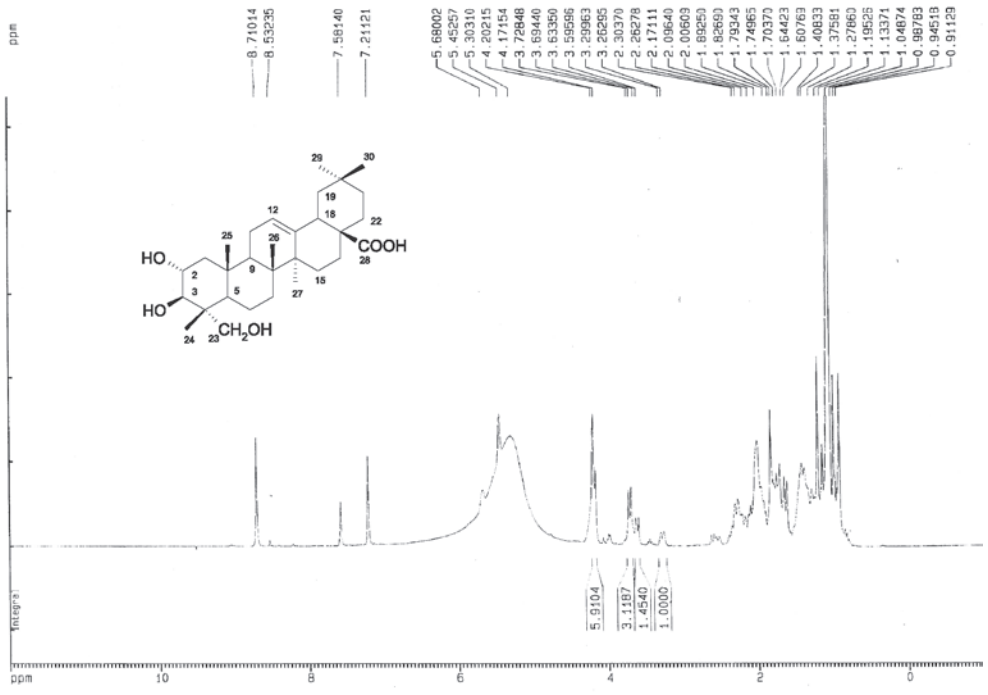

Figure S39. ^{13}C NMR spectrum of compound **17** (75 MHz, Py-d_5).

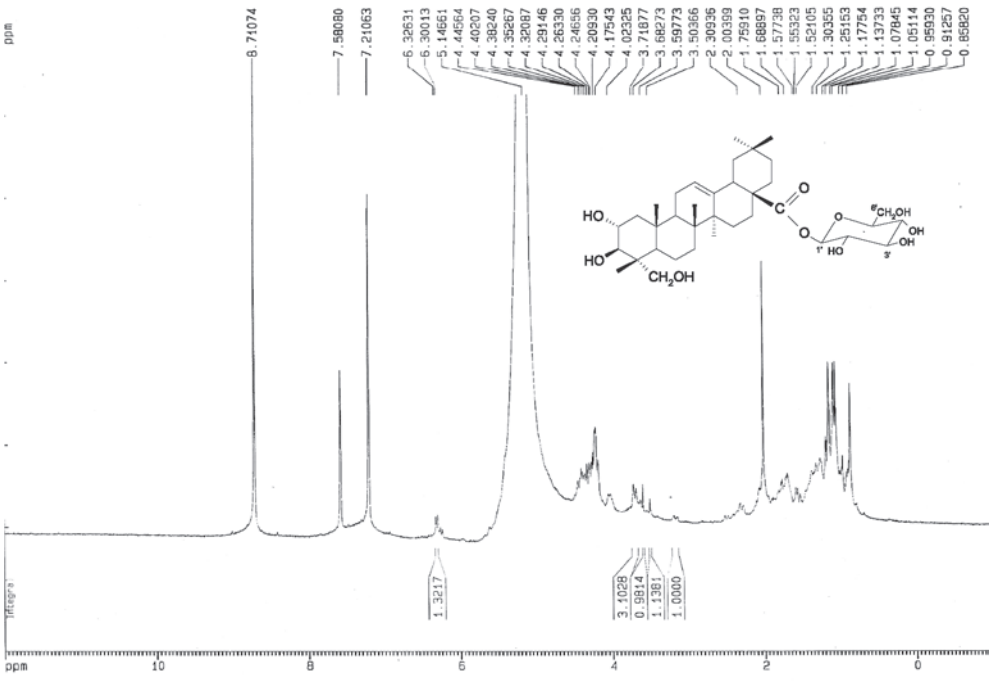

Figure S40. [1]H NMR spectrum of compound **18** (300 MHz, Py-d_5).

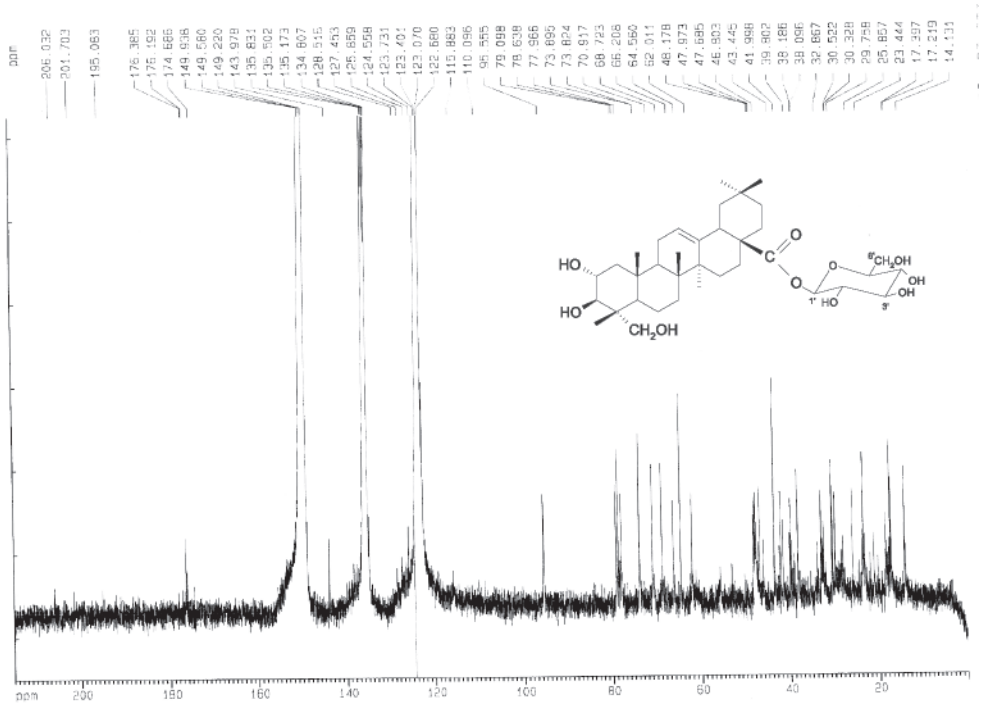

Figure S41. [13]C NMR spectrum of compound **18** (75 MHz, Py-d_5).

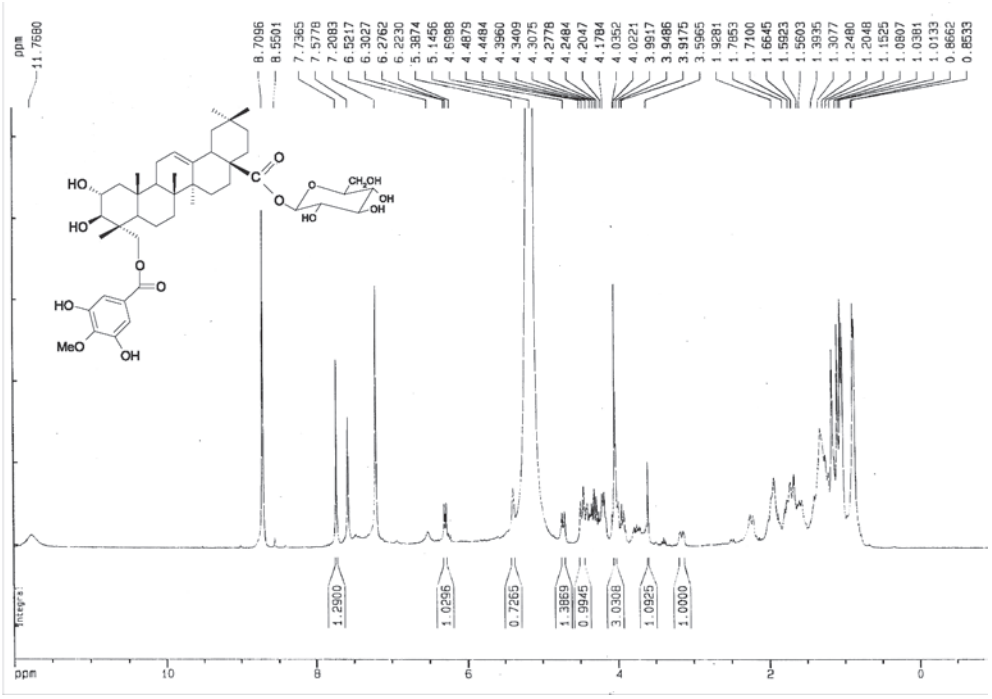

Figure S42. [1]H NMR spectrum of compound **19a** (300 MHz, Py-d_5).

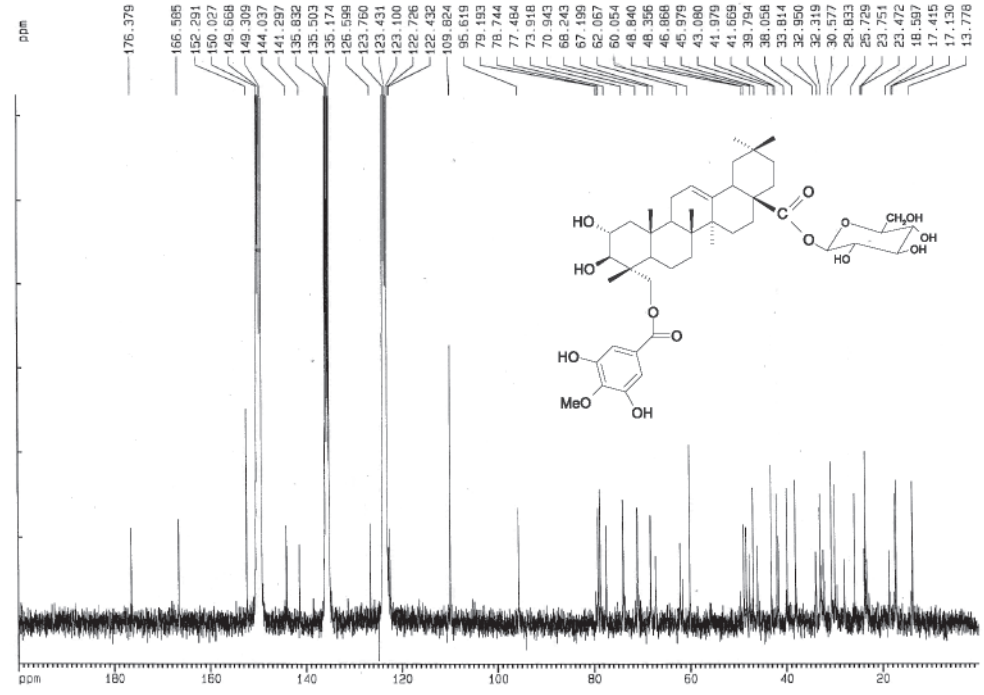

Figure S43. [13]C NMR spectrum of compound **19a** (75 MHz, Py-d_5).

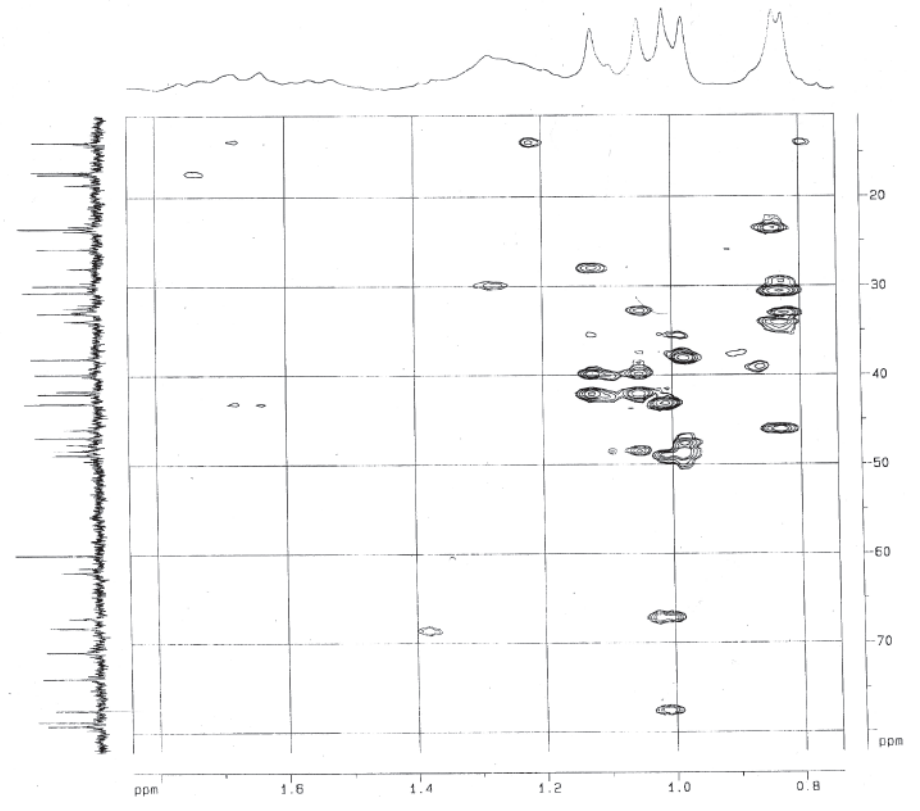

Figure S44. HMBC experiment of compound **19a**.

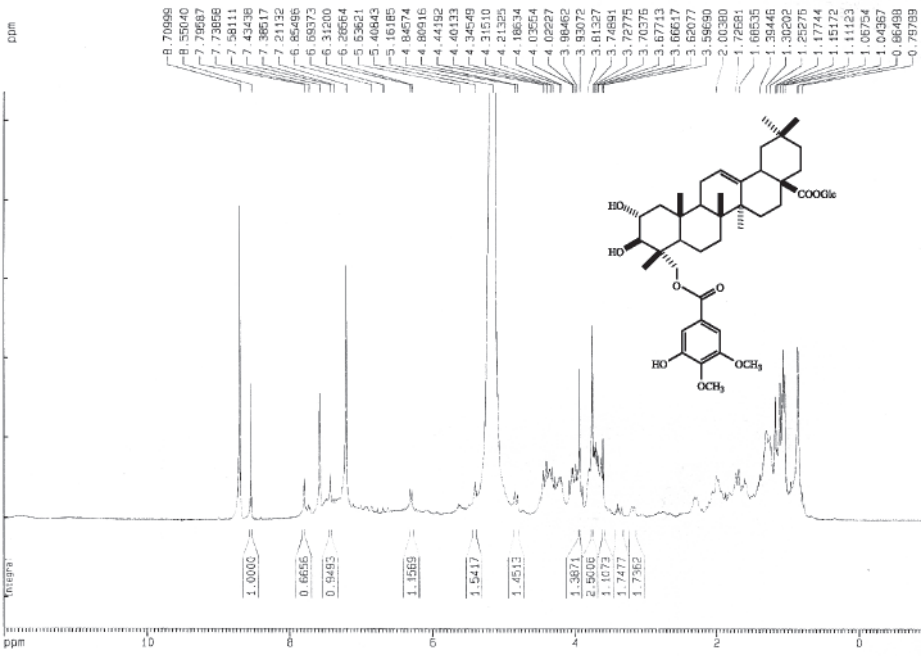

Figure S45. ^{1}H NMR spectrum of compound **19b** (300 MHz, Py-d_5).

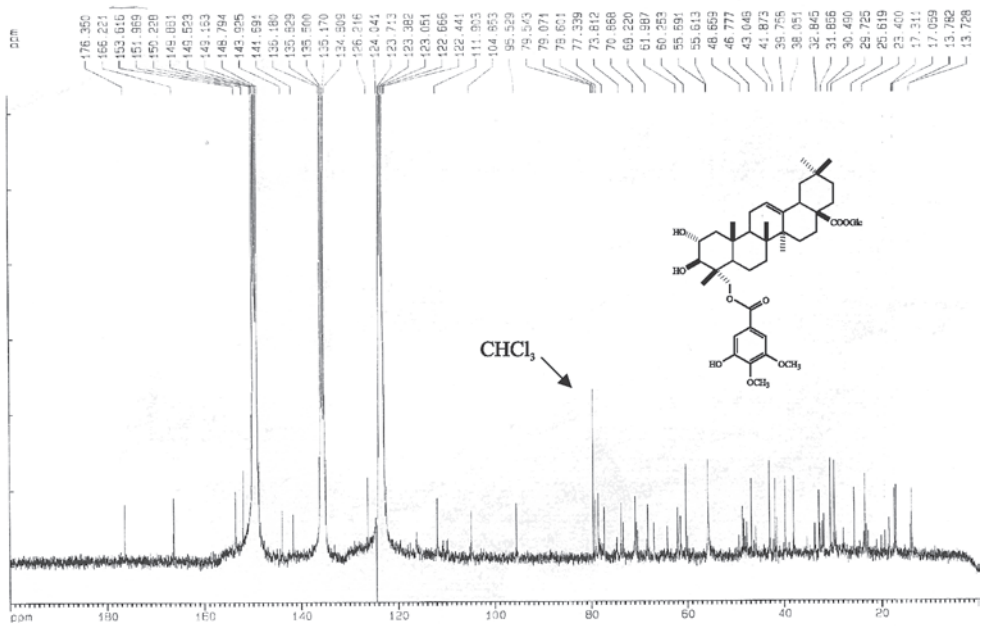

Figure S46. ^{13}C NMR spectrum of compound **19b** (75 MHz, Py-d_5).

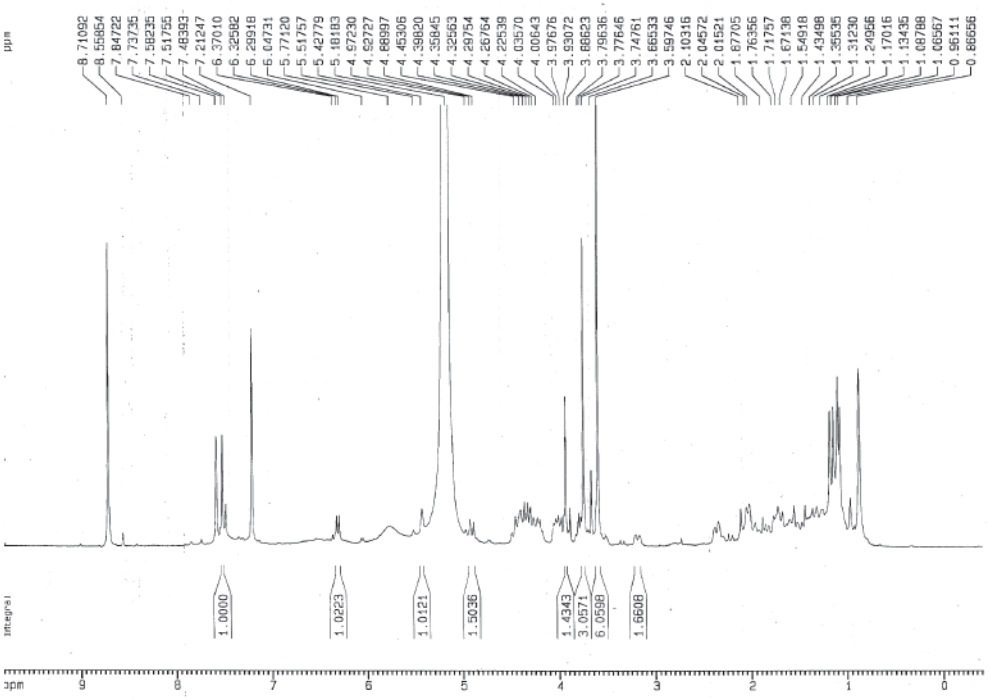

Figure S47. ^{1}H NMR spectrum of compound **19c** (300 MHz, Py-d_5).

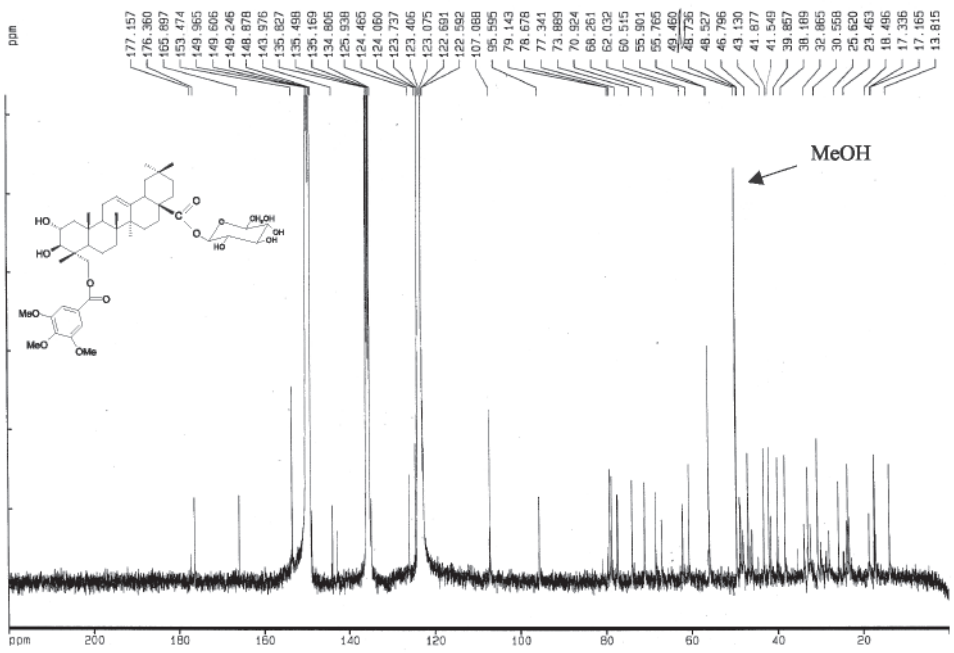

Figure S48. ^{13}C NMR spectrum of compound **19c** (75 MHz, Py-d_5).

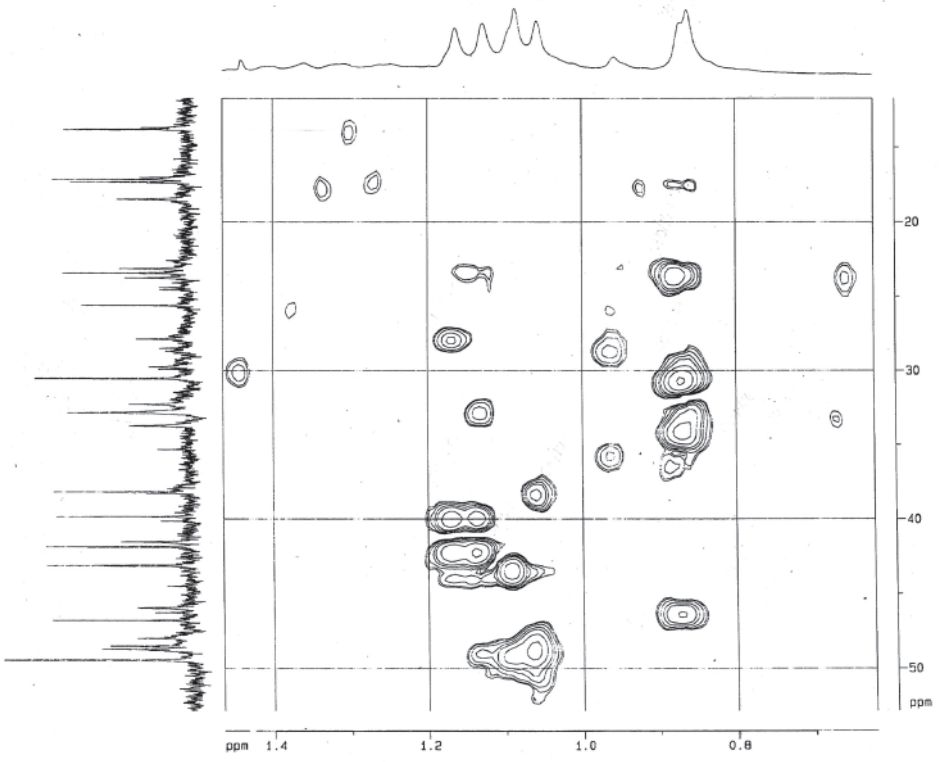

Figure S49. HMBC experiment of compound **19c**.

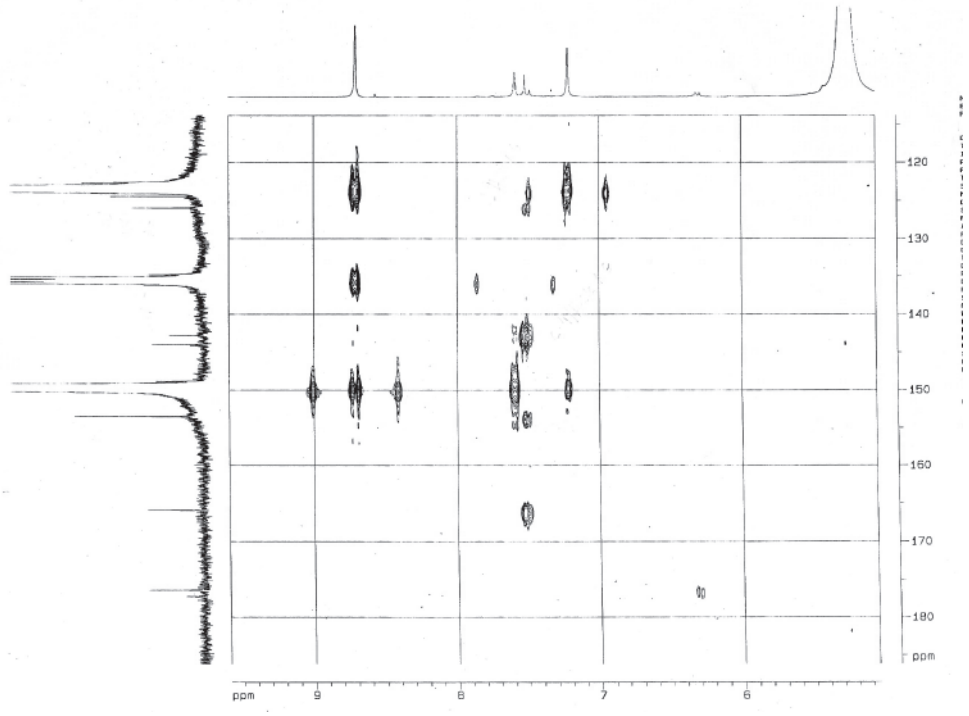

Figure S50. HMBC experiment of compound **19c**.

Table S1. ^{13}C (75 MHz) NMR spectral data for **1**, **2** (d, $CDCl_3$) and **3** (acetone-d_6)

C	**1**	**2**	**3**
2	79,0	79,0	79,8
3	43,2	43,1	43,4
4	196,0	196,0	197,1
5	164,1	164,3	165,2
6	95,1	96,7	96,8
7	167,8	164,8	167,8
8	94,2	95,5	95,9
9	162,9	163,2	164,3
10	103,1	103,1	103,0
1'	130,4	130,3	130,6
2' e 6'	127,7	127,7	128,9
3' e 5'	114,2	114,2	116,1
4'	160,0	160,0	158,8
OMe	55,7; 55,4	55,4	—

Table S2. ^{13}C (75 MHz) NMR spectral data for **4**, **5** and **6** (d, $CDCl_3$)

C	**4**	**5**	**6**
2	79,8	79,9	79,7
3	44,1	44,1	44,1
4	190,8	191,0	191,1
5	128,7	129,4	128,8
6	110,2	110,5	110,3
7	166,2	164,0	166,3
8	100,9	103,4	100,9
9	163,6	162,5	163,7
10	114,8	115,1	114,7
1'	130,8	130,7	130,8
2' e 6'	127,7	127,7	128,0
3' e 5'	114,2	114,2	115,7
4'	160,0	160,0	156,2
OMe	55,7; 55,4	55,4	55,6

Table S3. ^{13}C (75 MHz) NMR spectral data for **8** (d, $CDCl_3$)

C	**8**	**C**	**8**
2	78,0	10	114,2
3	24,6	1’	133,6
4	30,1	2’	108,6
5	130,2	3’	146,6
6	107,9	4’	145,4
7	154,8	5’	114,2
8	103,5	6’	119,2
9	155,9	OMe-3	55,9

Table S4. ^{13}C (75 MHz) NMR spectral data for **9** and **10** (d, acetone-d_6)

C	**9**	**10**
a	118,6	118,4
b	144,0	144,9
b’	192,0	192,4
1	128,0	126,6
2 e 6	130,9	131,2
3 e 5	114,7	116,1
4	162,3	160,7
1’	113,5	114,6
2’	166,1	166,4
3’	103,2	101,0
4’	167,1	166,8
5’	108,6	107,4
6’	132,7	132,1
OMe	55,3	55,4

Table S5. ^{13}C (75 MHz) NMR spectral data for **13** and **14** (d, Py-d_5)

C	**13**	**14**	C	**13**	**14**
1	45,9	47,4	19	81,3	80,9
2	68,2	68,5	20	34,6	35,4
3	83,3	83,7	21	27,5	28,8
4	39,0	38,5	22	32,6	32,8
5	55,2	55,9	23	28,4	29,2
6	18,3	18,9	24	16,7	16,7
7	32,3	33,0	25	16,2	17,4[a]
8	39,4	40,1	26	16,5	17,5[a]
9	47,7	48,3	27	24,3	24,7
10	38,1	39,7	28	180,9	177,2
11	23,5	24,1	29	27,9	28,6
12	123,9	123,5	30	24,6	24,5
13	143,4	144,2	1’	—	95,7
14	41,2	42,0	2’	—	74,0
15	28,1	28,9	3’	—	78,7
16	29,1	27,8	4’	—	70,9
17	44,9	46,3	5’	—	79,1
18	43,7	44,5	6’	—	62,0

Table S6. ^{13}C (75 MHz) NMR spectral data for **15** and **16** (d, Py-d_5)

C	**15**	**16**	C	**15**	**16**
1	47,3	47,2	19	81,0	80,7
2	68,8	68,7	20	35,6	35,2
3	78,0	77,9	21	29,0	28,7
4	43,5	43,4	22	32,8	32,7
5	47,8	48,2	23	66,2	66,2
6	18,5	18,5	24	14,1	14,0
7	33,5	32,6	25	17,5[c]	17,4[c]
8	39,9	40,0	26	17,1[c]	16,9[c]
9	48,3	47,7	27	24,7	24,4
10	38,4	38,3	28	180,9	177,1
11	24,2	24,0	29	28,7	28,5
12	123,3	123,4	30	24,7	24,6
13	144,7	144,0	1’	___	95,6
14	42,1	41,9	2’	___	73,8
15	28,2	27,7	3’	___	78,6
16	29,0	28,7	4’	___	70,8
17	45,9	46,2	5’	___	79,0
18	44,6	44,3	6’	___	61,9

Table S7. ^{13}C (75 MHz) NMR spectral data for **17** and **18** (d, Py-d_5)

C	**17**	**18**	C	**17**	**18**
1	47,5	47,5	19	46,3	45,9
2	68,7	68,7	20	30,8	30,5
3	78,0	78,0	21	34,1	33,7
4	43,5	43,4	22	32,7	32,3
5	48,0	48,0	23	66,3	66,2
6	18,4	18,3	24	14,2	14,1
7	33,1	32,7	25	17,4	17,6
8	39,7	39,8	26	17,2	17,4
9	47,7	47,7	27	26,0	25,9
10	38,2	38,2	28	180,3	176,4
11	23,8	23,6	29	33,1	32,9
12	122,3	123,9	30	23,6	23,4
13	144,8	144,0	1’	___	95,6
14	42,1	42,0	2’	___	73,8
15	28,1	28,0	3’	___	78,6
16	23,8	23,2	4’	___	70,9
17	46,5	46,8	5’	___	79,1
18	41,8	41,5	6’	___	62,0