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Chemical Constituents Isolated from the Bark of *Guatteria blepharophylla* (Annonaceae) and their Antiproliferative and Antimicrobial Activities

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O estudo fitoquímico das cascas de *Guatteria blepharophylla* (Mart.) Mart. forneceu 12 compostos, sendo dois sesquiterpenos, óxido de cariofileno (**1**) e espatulenol (**3**), uma xantona, liquexantona (**2**), uma mistura de esteróides, β-sitosterol (**4**) e estigmasterol (**5**), e sete alcalóides isoquinolínicos, *O*-metilmoschatolina (**6**), lysicamina (**7**), nornuciferina (**8**), liriodenina (**9**), isocoreximina (**10**), subsessilina (**11**) e isomoschatolina (**12**). Suas estruturas foram determinadas através de métodos espectroscópicos. Os compostos **1**-**6**, **11** e **12** são reportados pela primeira vez nesta espécie. Os dados de RMN ^{13}C (ressonância magnética nuclear) para os compostos **11** e **12** são descritos pela primeira vez na literatura. As atividades antiproliferativa em linhagens de células tumorais humanas e antimicrobiana foram investigadas para os compostos majoritários. O composto **9** mostrou significativa atividade contra linhagens de células de mama (MCF-7, Michigan Cancer Foundation-7), superior ao controle positivo doxorrubicina. O composto **12** apresentou atividade antifúngica similar ao controle positivo nistatina contra *Candida albicans*.

Phytochemical study of the bark of *Guatteria blepharophylla* (Mart.) Mart. afforded twelve compounds, namely two sesquiterpenes, caryophyllene oxide (**1**) and spathulenol (**3**), one xanthone, lichexanthone (**2**), a mixture of steroids, β-sitosterol (**4**), and stigmasterol (**5**), and seven isoquinoline alkaloids, *O*-methylmoschatoline (**6**), lysicamine (**7**), nornuciferine (**8**), liriodenine (**9**), isocoreximine (**10**), subsessiline (**11**), and isomoschatoline (**12**). Their structures were established on the basis of spectroscopic methods. Compounds **1**-**6**, **11** and **12** were reported for the first time in this species. The ^{13}C NMR (nuclear magnetic resonance) data for the compounds **11** and **12** are described for the first time in the literature. The antiproliferative activity against human tumour cell lines and antimicrobial activities were investigated for the major compounds. Compound **9** showed significant activity against cell lines of breast (MCF-7, Michigan Cancer Foundation-7), superior to the positive control doxorubicin. Compound **12** presented antifungal activity similar to the positive control nystatin against *Candida albicans*.

Keywords: *Guatteria blepharophylla*, antifungal and antiproliferative activities, alkaloids, terpenes, xanthone

Introduction

The genus *Guatteria* Ruiz & Pav. contains close to 290 species and is the largest genus within the Annonaceae family.[1] Species of *Guatteria* are frequent constituents of Neotropical (lowland) forests, and it is widely distributed throughout Mesoamerica, the Caribbean and tropical South America.[1]

Plants of this family are known for their edible fruits and medicinal properties of many species.[2] Previous chemical

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and pharmacological investigations on some species of this family, including *Guatteria*, have indicated the presence of important bioactive compounds, exhibiting many pharmacological activities, such as, cytotoxicity against human tumor cell lines,[3-5] antimicrobial,[5,6-9] and antiparasitic properties, particularly against *Leishmania* sp.,[3,7,10-12] *Plasmodium falciparum*[5,12,13] and *Trypanosoma* sp.[3,12,14]

Despite the importance of annonaceous members in folk medicine, the number of species that have been chemically investigated is still very small. One of them is *Guatteria blepharophylla* Mart *in* Mart, a small tropical tree that occurs in the Amazonian region (Brazil, Peru, Guyana, Ecuador and Venezuela).[1] In Brazil this species is common in Amazonas and Pará states, where it is known as "envireira".[15] Previous phytochemical studies on this species described the isolation and identification of essential oils and isoquinoline alkaloids.[8,15,16]

In continuation of our research on bioactive compounds from Amazonian annonaceous plants, we report herein the phytochemical study of the bark of *G. blepharophylla* and the evaluation of antiproliferative and antimicrobial properties of the main isolated compounds.

Experimental

General experimental procedures

Melting points were determined on a Quimis Q-340S23 micromelting point apparatus. UV-Vis spectra were obtained in CH_3OH on a Hewlett-Packard HP 8452A diode array spectrophotometer. IR spectra were acquired on a BIORAD FTS-3500 GX spectrophotometer. Mass spectra were recorded on a Varian Saturn 2000 spectrometer operating at 70 eV. NMR data, 1D and 2D, were recorded at 293 K in $CDCl_3$ or $CDCl_3$ + CD_3OD or CD_3OD on a Bruker Avance DRX 400 and Brucker ARX 200 spectrometers. The spectrometers were equipped with a 5 mm multinuclear direct detection probe with *z*-gradient. One-bond and long-range ^1H-^{13}C correlation (HSQC, (heteronuclear single quantum correlation) and HMBC (heteronuclear multiple bond coherence), respectively) experiments were optimized for an average coupling constants $^1J_{(C,H)}$ and $^{LR}J_{(C,H)}$ of 140 and 8 Hz, respectively. All ^1H and ^{13}C NMR chemical shifts (δ) are given in ppm related to the TMS (tetramethylsilane) signal at 0.00 ppm as internal reference and the coupling constants (*J*) in Hz. Silica gel 60 (70-230 mesh) was used for column chromatography, while silica gel 60 F_{254} were used for analytical (0.25 mm), and preparative (1.00 mm) TLC. Compounds were visualized by exposure under $UV_{254/366}$ light, spraying *p*-anisaldeyde reagent followed by heating on a hot plate, or spraying with Dragendorff's reagent.

Plant material

The bark of *G. blepharophylla* was collected in January 2005 on the campus of the Universidade Federal do Amazonas (UFAM), Manaus-AM, Brazil, and identified by the taxonomist Prof. Dr. A. C. Webber from UFAM. A voucher specimen (number 7340) has been deposited at the Herbarium of the UFAM. After identification, *G. blepharophylla* bark was dried at room temperature and finely powdered.

Extraction and isolation of the chemical constituents

The dried powdered bark (1500 g) of *G. blepharophylla* was successively extracted with *n*-hexane followed by MeOH to yield *n*-hexane (28.18 g) and MeOH (212.50 g) extracts.

The *n*-hexane extract (3.50 g) was subjected to silica gel column chromatography eluted with the gradient systems: petroleum ether-CH_2Cl_2 from 100:0 to 10:90 (v/v) followed by CH_2Cl_2-EtOAc from 100:0 to 10:90 (v/v), and EtOAc-MeOH from 100:0 to 80:20 (v/v), affording 295 fractions (each 15 mL). The eluted fractions were evaluated and pooled according to TLC analysis, to afford 24 groups of fractions. Fraction 4 (340.7 mg) was submitted to further silica gel column chromatography eluted with the gradient systems: petroleum ether-CH_2Cl_2 from 100:0 to 10:90 (v/v), and CH_2Cl_2-EtOAc from 100:0 to 10:90 (v/v), yielding 40 subfractions that were pooled in nine subfractions according to TLC analysis. Subfraction 4.4 (154.7 mg) was purified by preparative TLC eluted with petroleum ether-EtOAc (90:10, v/v, two times) to give **1** (56.7 mg) and **2** (20.0 mg). Fraction 5 (187.2 mg) was also submitted to another silica gel column chromatography using the same methodology described for fraction 4 affording 40 new subsfractions that were pooled in nine groups of subfractions, according to TLC analysis. Subfractions 5.3 (14.0 mg) and 5.4 (55.7 mg) were purified by preparative TLC eluted with petroleum ether-EtOAc (90:10, v/v, two times) to give **2** (3.0 mg), and **3** (16.8 mg), respectively. Fraction 6 were also submitted to silica gel column chromatography using the same methodology above yielding 56 subfractions that were pooled in ten groups of subfractions, according to TLC analysis. Subfraction 6.6 was purified by preparative TLC eluted with petroleum ether-EtOAc (80:20, v/v, two times) to yield a mixture of **4** and **5** (36.1 mg).

TLC investigations indicated a high concentration of alkaloids in the MeOH extract. Therefore, an aliquot of the MeOH extract (210.0 g) was initially subjected to an acid-base extraction[10] to give the CH_2Cl_2 alkaloid fraction (1.18 g) and the CH_2Cl_2 neutral fraction (5.24 g). The

alkaloid fraction (1.0 g) was subjected to 10% $NaHCO_3$ treated silica gel column chromatography[10] eluted with the gradient systems: petroleum ether-CH_2Cl_2 from 100:0 to 10:90 (v/v) followed by CH_2Cl_2-EtOAc from 100:0 to 10:90 (v/v), and EtOAc-MeOH from 100:0 to 50:50 (v/v), yielding 97 fractions (each 25 mL). The eluted fractions were evaluated and pooled according to TLC analysis, to afford 13 groups of fractions. Fraction 4 (112.8 mg) was purified by preparative TLC eluted with CH_2Cl_2-MeOH (95:05, v/v, two times) to give **6** (13.0 mg), **7** (7.0 mg), and **8** (8.0 mg). Fraction 5 (87.0 mg) was also purified by preparative TLC eluted with CH_2Cl_2-MeOH (95:05, v/v, two times) to yield **7** (12.6 mg), **8** (2.8 mg) and **9** (2.0 mg). Fraction 6 (282.3 mg) was also submitted to another silica gel column chromatography using the same methodology above affording 27 new subsfractions that were pooled in six groups of subfractions, according to TLC analysis. Subfraction 6.3 (101.5 mg) was purified by preparative TLC eluted with CH_2Cl_2-MeOH (90:10, v/v, two times) to give **10** (9.0 mg). Fraction 8 was purified by preparative TLC eluted with CH_2Cl_2-MeOH (95:05, v/v, three times) to give **11** (12.7 mg). Fraction 10 was also purified by preparative TLC eluted with CH_2Cl_2-MeOH (90:10, v/v, three times) to give **12** (31.2 mg).

*Subsessiline (**11**)*

Orange needles ($CHCl_3$:MeOH 2:1); UV λ_{max}/nm (MeOH) (log ε) 204 (3.89), 238 (3.41), 278 (3.54), 472 (2.95); IR ν_{max}/cm^{-1} (KBr) 3431, 3132, 3097, 2955, 2934, 2855, 1646, 1602, 1582, 1543, 1492, 1466, 1394, 1380, 1326, 1258, 1206, 1087, 1055, 1015, 994, 872; ^1H and ^{13}C NMR data: Table 2.

*Isomoschatoline (**12**)*

Blue needles ($CHCl_3$:MeOH 2:1); UV λ_{max}/nm (MeOH) (log ε) 204 (4.23), 238 (3.84), 282 (4.01), 304 (3.93), 468 (3.15), 595 (3.09); IR ν_{max}/cm^{-1} (KBr) 3325, 2938, 2843, 1660, 1614, 1596, 1578, 1541, 1494, 1410, 1367, 1337, 1300, 1266, 1202, 1042, 996, 801, 691; ^1H and ^{13}C NMR data: Table 2.

In vitro anticancer activity assay

Human tumour cell lines UACC-62 (melanoma), MCF-7 (breast), NCI-H460 (lung, non-small cells), OVCAR-03 (ovarian), PC-3 (prostate), HT-29 (colon), 786-0 (renal), K562 (leukemia) and NCI-ADR/RES (ovarian expressing phenotype multiple drugs resistance) were kindly provided by National Cancer Institute (NCI). Stock cultures were grown in medium containing 5 mL RPMI 1640 (GIBCO® BRL) supplemented with 5% fetal bovine serum. Penicillin:streptomycin (1000 µg mL^{-1}:1000 UI mL^{-1}, 1 mL L^{-1}) was added to experimental cultures. Cells in 96 well plates (100 µL cells well-1) were exposed to sample concentrations in DMSO/RPMI (0.25, 2.5, 25 and 250 µg mL^{-1}) (DMSO, dimethyl sulfoxide) at 37 °C, 5% of CO_2 in air for 48 h. Final DMSO concentration did not affect cell viability. Afterwards, cells were fixed with 50% trichloroacetic acid and cell proliferation determined by spectrophotometric quantification (540 nm) of cellular protein content using sulforhodamine B assay. Using the concentration-response curve for each cell line, TGI (concentration that produces total growth inhibition or cytostatic effect)[17] was determined through non-linear regression analysis (Table 1) using software ORIGIN 7.5 (OriginLab Corporation).

In vitro antimicrobial activity

The growth inhibitory activity of the crude extracts, fractions and isolated compounds was tested against 11 microorganisms (*Bacillus subtilis* ATCC 5061, *Candida albicans* ATCC 10231, *Enterococcus faecium* CCT 5079, *Enterococcus hirae* ATCC 10541, *Escherichia coli* ATCC 11775, *Micrococcus luteus* ATCC 4698, *Pseudomonas aeruginosa* ATCC 13388, *Rhodococcus equi* ATCC 6939, *Salmonella choleraesuis* ATCC 10708, *Staphylococcus aureus* ATCC 6538 and *Staphylococcus epidermidis* ATCC 12228).

The bacteria strains were cultured overnight at 36 °C in Nutrient Agar (Merck), while *C. albicans* was cultured in Saboraud Dextrose Agar. Inoculum for the assays was prepared by diluting a scraped cell mass in 0.85% NaCl solution, adjusted to McFarland scale 0.5 and confirmed by spectrophotometer reading at 580 nm. Cell suspensions were finally diluted to 10^4 CFU mL^{-1} for using in the activity assays. MIC (minimal inhibitory concentration) tests were carried out according to Eloff,[18] using Müller-Hinton broth on a tissue-culture test plate (96 wells). The stock solution crude extracts, fractions and isolated compounds were diluted and transferred into the first well, and serial dilutions were made so that concentrations in the range of 1.0-0.015 mg mL^{-1} were obtained. Chloramphenicol and nystatin (Merck) were used as the reference antibiotic control in the range of 0.25-0.002 mg mL^{-1}. The inoculum was added to all wells, and the plates were incubated at 36 °C for 48 h. Each concentration was screened in triplicate. Antimicrobial activity was detected by adding 20 µL of 0.5% TTC (triphenyl tetrazolium chloride, Merck) aqueous solution. MIC was defined as the lowest concentration of the sample that inhibited visible growth, as indicated by TCC staining (dead cells are not stained by TTC).

Table 1. Antiproliferative activity of extracts, fractions and major compounds against cancer cell lines

Extracts and fractions	TGI (μg mL^{-1})								
	Tumor cell lines								
	UACC-62	MCF-7	NCI-H460	OVCAR-03	PC-3	HT-29	786-0	K562	NCI-ADR/RES
Hexane	21.37	28.16	9.05	16.96	34.32	39.20	16.18	7.23	16.18
MeOH	229.46	152.25	179.35	221.16	169.99	129.73	39.27	71.43	>250
Alkaloid fraction	92.53	51.53	>250	208.77	244.53	185.62	169.56	NT	136.60
Neutral fraction	>250	>250	>250	>250	>250	>250	>250	>250	>250
Compounds	TGI (μmol L^{-1})								
1	260.45	282.54	305.90	333.63	290.27	293.22	330.13	NT	347.50
2	275.07	274.12	554.96	>874.12	>874.12	643.28	271.85	NT	295.31
6	NT	>778.82	>778.82	NT	>778.82	>778.82	>778.82	NT	>778.82
7	NT	289.38	49.52	NT	>859.11	228.93	>859.11	380.62	528.18
8	NT	215.58	201.99	NT	542.38	191.38	615.23	153.88	255.37
9	63.02	37.67	87.41	372.18	>909.09	>909.09	>909.09	NT	>909.09
10	>764.52	>764.52	>764.52	>764.52	>764.52	>764.52	>764.52	NT	131.50
11	NT	>741.84	511.39	NT	>741.84	>741.84	>741.84	135.70	>741.84
12	84.53	73.97	186.74	89.87	351.79	242.02	547.72	NT	348.47
Doxorubicin[a]	0.48	46.04	14.27	10.92	5.06	>46.04	4.77	1.66	14.80

[a] Positive control; NT (Not tested); UACC-62 (melanoma), MCF-7 (breast), NCI-H460 (lung, non-small cells), OVCAR-03 (ovarian), PC-3 (prostate), HT-29 (colon), 786-0 (renal), K562 (leukemia), and NCI-ADR/RES (ovarian expressing phenotype multiple drugs resistance); TGI (Total Growth Inhibition).

Results and Discussion

Once *n*-hexane and MeOH crude extracts were found to have antiproliferative activity (Table 1), these extracts were subjected to successive chromatographic fractionations as described in the Experimental section leading to the isolation of the chemical constituents **1**-**12** (Figure 1).

Compounds **1**-**10** were identified by comparison of their spectroscopic data with those reported in the literature as caryophyllene oxide[19] (**1**), lichexanthone[20] (**2**), spathulenol[19] (**3**), a mixture of β-sitosterol[21] (**4**), and stigmasterol[21] (**5**), *O*-methylmoschatoline[22,23] (**6**), lysicamine[22,23] (**7**), nornuciferine[22] (**8**), liriodenine[6,23] (**9**), and isocoreximine[15] (**10**). Compounds **7**-**10** were recently found on the stem of this species,[15] while compounds **1**-**6**, **11** and **12** were reported for the first time in this specie. Compound **2** is related for the first time in the genus *Guatteria*, and it is the second report in the Annonaceae, previously isolated from the roots of *Rollinia leptopetala*.[24]

Compound **11** was obtained as an orange amorphous powder which was positive to the Dragendorff's test. Its UV-vis spectrum showed absorption peaks at 204, 238,

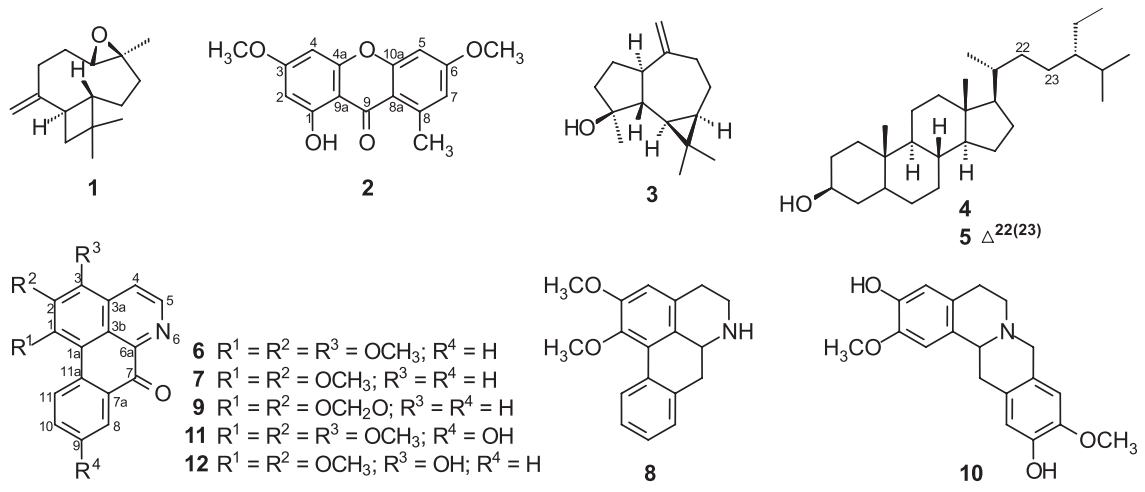

Figure 1. Chemical constituents isolated from the bark of *Guatteria blepharophylla*.

278 and 472 nm, which are typical of molecules with an oxoaporphine skeleton. The IR spectrum showed absorption bands at 3431 and 1646 cm^{-1}, characteristic of phenolic hydroxyl and carbonyl groups. The ^{1}H NMR spectrum indicated a tetrasubstituted-oxoaporphine alkaloid. Its ^{1}H NMR spectrum revealed the presence of a pair of doublets at δ 8.88 and δ 8.24 (H-5 and H-4, *J* 4.7 Hz, respectively), which was consistent with the presence of a pyridine system. Additionally, it was found to have a spin system consisting of three hydrogens with the signals at δ 8.96 (1H, d, *J* 9.0 Hz), δ 7.82 (1H, d, *J* 2.8 Hz), and δ 7.25 (1H, dd, *J* 9.0 and 2.8 Hz), which were attributed to H-11, H-8, and H-10, respectively, and indicated substitution in the D ring. The signals at δ 4.05, δ 4.11 and δ 4.17 (each 3H, *s*) were assigned to three methoxyl groups located in the A ring, according to long-range ^{1}H-^{13}C correlation in the HMBC NMR experiment (Table 2). The presence of a hydroxyl group in the molecule located in the D ring at C-9 was established on the basis of the long-range ^{1}H-^{13}C correlation of the hydrogen H-11 at δ 8.96 with the carbon C-9 at δ 157.3, which showed no correlation with the methoxyl groups.

The ^{13}C NMR experiment along with HSQC and HMBC experiments allowed to verify the presence of 19 carbons, including a carbonyl group at δ 183.1, 15 aromatic carbons between δ 157.3-113.4, and three methoxyl groups at δ 61.9, δ 61.4, and δ 60.9, which was in agreement with structure **11** (Table 2). The hydrogen H-8 at δ 7.82 showed strong long-range ^{1}H-^{13}C correlation with the carbon C-7 at δ 183.1, which confirmed the oxoaporphine skeleton. According with ^{1}H and ^{13}C NMR 1D/2D this compound was identified as the oxoaporphine alkaloid known subsessiline. This compound has been found only in two species of Annonaceae *Guatteria ouregou*[25] and *G. subsessilis*,[26] but the physical data are incomplete. The ^{13}C NMR data are reported for the first time. The complete physical data for this compound are described in this work.

Compound **12** was obtained as a blue amorphous powder. Its UV-Vis spectrum showed absorption peaks at 204, 238, 282, 304, 468 and 595 nm, which are typical of molecules with an oxoaporphine skeleton. The IR spectrum showed absorption bands at 3325 and 1660 cm^{-1}, characteristic of phenolic hydroxyl and carbonyl groups. The ^{1}H and ^{13}C NMR spectra of **12** were very similar to those of **11**, except for the absence of a methoxyl signal, which was replaced by a hydroxyl group, and absence of substitution in the D ring, in structure **12** according to the IR spectrum and NMR data (Table 2). The absence of

Table 2. NMR spectroscopy data (400 MHz) for compounds **11** and **12**

Position	**11**		**12**	
	δ_C [a,c]	δ_H mult. (*J* in Hz)[a,c]	δ_C [b,c]	δ_H mult. (*J* in Hz)[b,c]
1	155.5		162.7	
1a	116.2		102.7	
2	147.9		144.0	
3	147.5		166.0	
3a	131.4		136.1	
3b	122.2		124.7	
4	119.8	8.24 d (4.7)	124.0	8.60 d (5.1)
5	144.0	8.88 d (4.7)	141.9	8.73 d (5.1)
6a	145.1		145.0	
7	183.1		184.5	
7a	132.6		130.6	
8	113.4	7.82 d (2.8)	128.8	8.41 ddd (8.0, 1.6 and 0.5)
9	157.3		125.5	7.33 ddd (8.0, 7.0 and 1.0)
10	123.2	7.25 dd (9.0 and 2.8)	135.1	7.68 ddd (8.6, 7.0 and 1.6)
11	129.9	8.96 d (9.0)	127.3	9.08 ddd (8.6, 1.0 and 0.5)
11a	126.8		138.7	
1-OCH_3	60.9	4.05 s	61.2	4.08 s
2-OCH_3	61.9	4.11 s	60.6	3.97 s
3-OCH_3	61.4	4.17 s		

The experiments were obtained at 293 K and TMS as internal reference (0.00 ppm) in [a] $CDCl_3$ + drops of CD_3OD or [b] CD_3OD. [c] Long-range ^{1}H-^{13}C HMBC correlations, optimized for 8 Hz, is from hydrogens stated for the indicated carbon.

substitution in the D ring was confirmed by the presence of four adjacent aromatic hydrogens at δ 9.08 (1H, ddd, *J* 8.6, 1.0 and 0.5 Hz), δ 8.41 (1H, ddd, *J* 8.0, 1.6 and 0.5 Hz), δ 7.68 (1H, ddd, *J* 8.6, 7.0 and 1.6 Hz), and δ 7.33 (1H, ddd, *J* 8.0, 7,0 and 1.0 Hz) which were attributed to H-11, H-8, H-10, and H-9, respectively (Table 2). The position of the hydroxyl group at C-3 was established on the basis of the long-range ^{1}H-^{13}C correlation of the hydrogen H-4 at δ 8.60 with the carbon C-3 at δ 166.0, which showed no correlation with the methoxyl groups. According with ^{1}H and ^{13}C NMR 1D/2D, this compound was identified as the oxoaporphine alkaloid known isomoschatoline. As well as observed for **11**, the physical data published for this compound are incomplete and presented in this work. The ^{13}C NMR data is reported for the first time. This compound has been found only in three species of Annonaceae *Uvaria mocoli*,[27] *Guatteria melosma* and *Cleistopholis patens*.[28]

The isolated compounds **1-2** and **6-12** were evaluated for *in vitro* antiproliferative activity against nine human tumour cell lines (Table 1), while compounds **6**, **7**, **10** and **12** were also tested for antimicrobial activity. Compound **9** showed the higher antiproliferative activity for breast (MCF-7) with a TGI value of 37.67 μmol L^{-1}, more active than positive control doxorubicin (TGI value of 46.04 μmol L^{-1}). Compound **7** presented significant antiproliferative activity for lung, non-small cells (NCI-H460) with a TGI value of 49.52 μmol L^{-1} (doxorubicin, TGI value of 14.27 μmol L^{-1}), while compound **12** showed significant activity for breast (MCF-7) with TGI value of 73.97 μmol L^{-1}. Compound **10** showed selective activity for ovarian expressing phenotype for multiple drug resistance (NCI-ADR/RES) with a TGI value of 131.50 μmol L^{-1}, but was less active than doxorubicin (TGI value of 14.80 μmol L^{-1} (Table 1). Compounds **9** and **12** also showed significant antiproliferative activity against different tumor cell lines with TGI values below to 100 μmol L^{-1} (Table 1).

It is important to notice that compounds **6**, **7**, **9**, **11** and **12** shared the same basic skeleton with different substitution patterns. This way, our results suggested that a methoxylated substitute at R_3 reduces antiproliferative activity (compound **11**) or even results in an inactive compounds as **6**, but a hydroxyl group at R_3 favored the activity (compound **12**). The best results were obtained for methylenedioxy group (compound **9**) or methoxy groups (compounds **7** and **12**) at R_1 and R_2.

No significant antibacterial activity was observed for the tested compounds. The only significant antimicrobial result was observed for compound **12** that showed antifungal activity against *C. albicans* (MIC value of 50.81 μmol L^{-1}) similar to the positive control nystatin (MIC value of 54.00 μmol L^{-1}).

Conclusion

The chemical investigation of the bark of *G. blepharophylla* has resulted in the isolation of several compounds common in the taxon *Guatteria*, such as, **1**, **3**, **6**, **7** and **9** that could be considered chemotaxonomic markers of this genus. Compounds **1-6**, **11** and **12** are being reported for the first time in this species, and are important for the chemotaxonomy of Annonaceae family. The significant *in vitro* antiproliferative results obtained against several human tumour cell lines and antimicrobial activities demonstrated by the major compounds indicate that this species is a natural source of biologically active compounds. Compound **12** showed significant antifungal and antiproliferative activities against *C. albicans* and human tumor cell lines of breast (MCF-7). Therefore, studies involving mechanisms of action are necessary to fully understand its biological significance.

Supplementary Information

Supplementary data including physical data for compounds **1-10** and ^{1}H, ^{13}C NMR, HSQC and HMBC for compounds **11** and **12** are available free of charge at http://jbcs.sbq.org.br as a PDF file.

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Supplementary Information

Chemical Constituents Isolated from the Bark of *Guatteria blepharophylla* (Annonaceae) and their Antiproliferative and Antimicrobial Activities

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Table S1. Chemical constituents isolated from the bark of *Guatteria blepharophylla* and the respective morphology and data spectra numbering (Figure S_)

Caryophyllene oxide (**1**):	Colorless oil. EI-MS *m/z* 220 $[M]^+$. ^1H NMR (S1). ^{13}C NMR (S2).
Lichexanthone (**2**):	Light yellow needles ($CHCl_3$). Mp 189-190 °C. ^1H NMR (S3). ^{13}C NMR (S4).
Spathulenol (**3**):	Colorless oil. EI-MS *m/z* 220 $[M]^+$. ^1H NMR (S5). ^{13}C NMR (S6).
Mixture of β-sitosterol (**4**) and stigmasterol (**5**):	White needles (Hexane:CH_2Cl_2 2:1). ^1H NMR (S7). ^{13}C NMR (S8).
O-methylmoschatoline (**6**):	Orange needles ($CHCl_3$); mp 182-183 °C. ^1H NMR (S9). ^{13}C NMR (S10).
Lysicamine (**7**):	Yellow needles ($CHCl_3$); mp 186-187 °C. ^1H NMR (S11). ^{13}C NMR (S12).
Nornuciferine (**8**):	Brown amorphous solid. ^1H NMR (S13). ^{13}C NMR (S14).
Liriodenine (**9**):	Yellow needles ($CHCl_3$:MeOH 2:1); mp 279-280 °C. ^1H NMR (S15). HSQC (S16). HMBC (S17).
Isocoreximine (**10**):	Light yellowish prisms ($CHCl_3$:MeOH 2:1); mp 241-242 °C. ^1H NMR (S18). ^{13}C NMR (S19).
Subsessiline (**11**):	Orange needles ($CHCl_3$:MeOH 2:1). ^1H NMR (S20). ^{13}C NMR (S21). HSQC (S22). HMBC (S23).
Isomoschatoline (**12**):	Blue needles ($CHCl_3$:MeOH 2:1). ^1H NMR (S24). ^{13}C NMR (S25). HSQC (S26). HMBC (S27).

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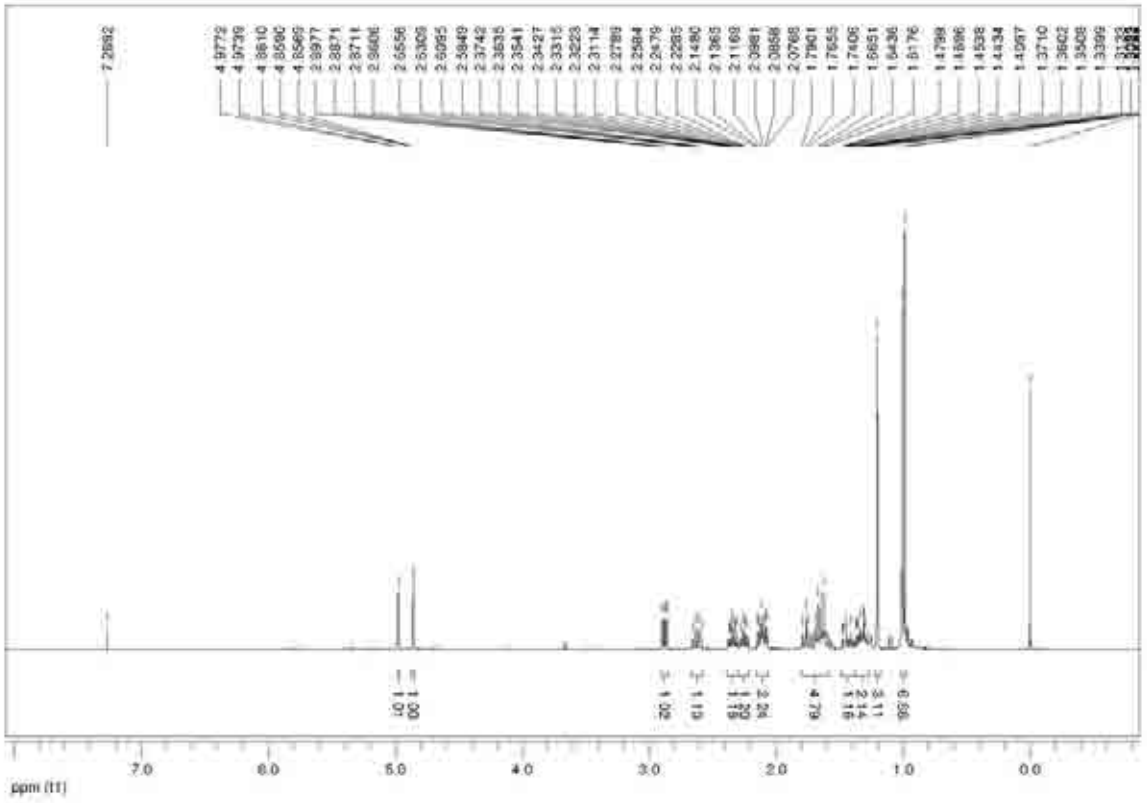

Figure S1. ^{1}H NMR spectrum of compound **1** in $CDCl_3$ at 400 MHz.

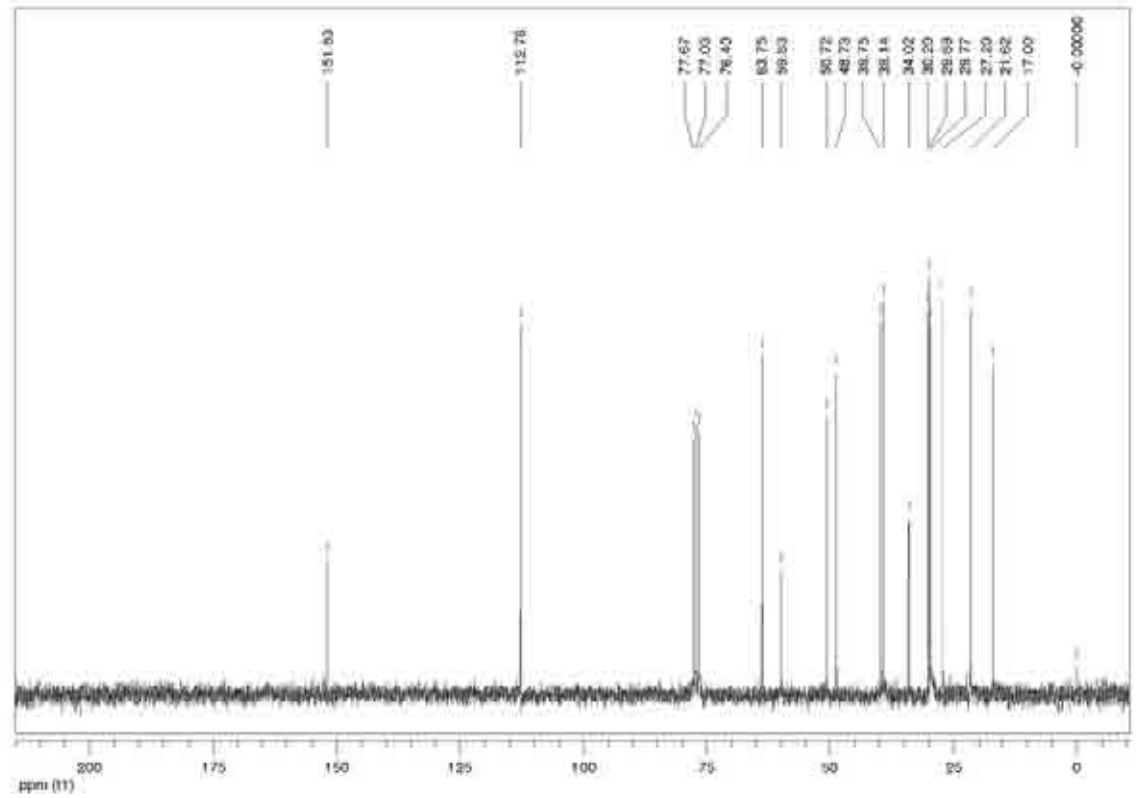

Figure S2. $^{13}C\{^{1}H\}$ NMR spectrum of compound **1** in $CDCl_3$ at 100 MHz.

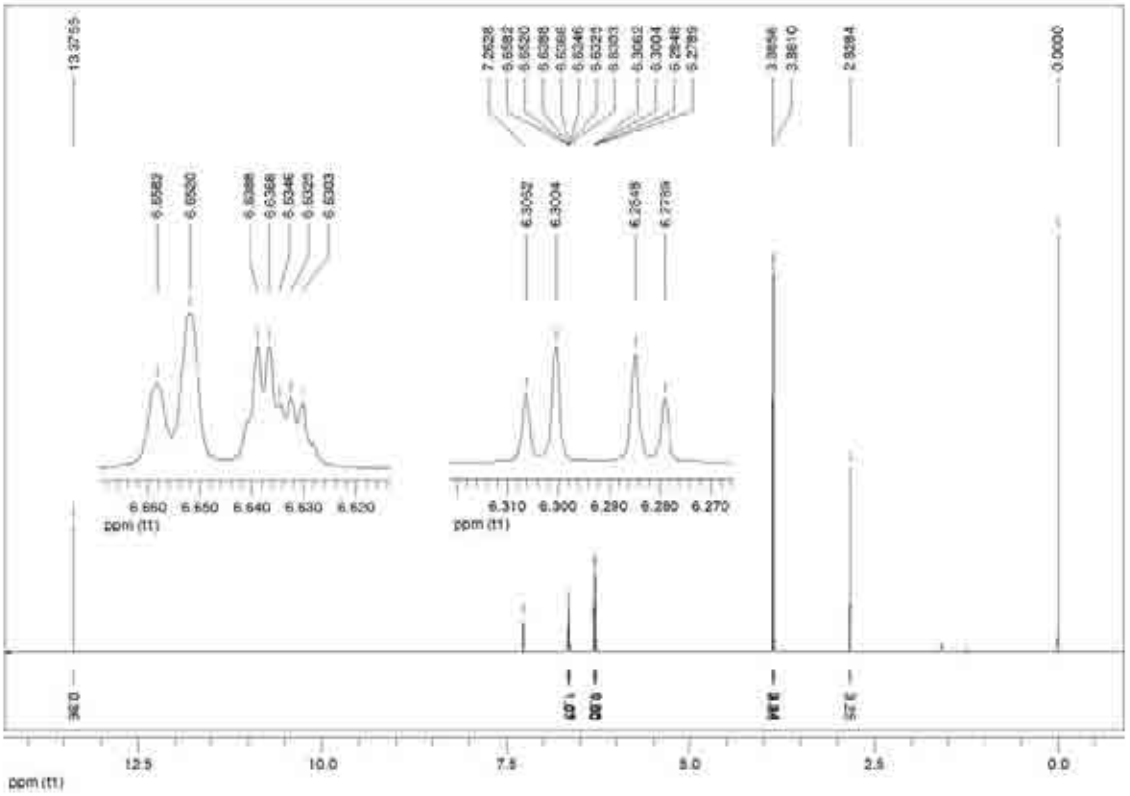

Figure S3. ^{1}H NMR spectrum of compound **2** in $CDCl_3$ at 400 MHz.

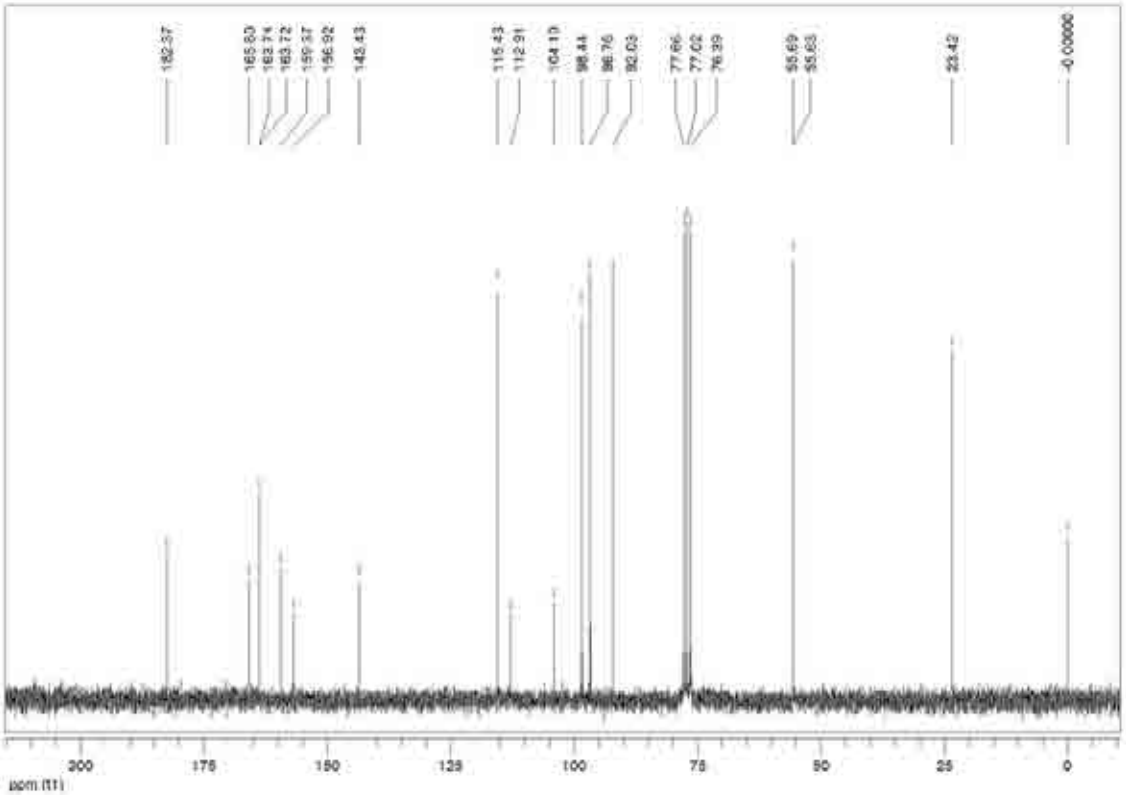

Figure S4. $^{13}C\{^{1}H\}$ NMR spectrum of compound **2** in $CDCl_3$ at 100 MHz.

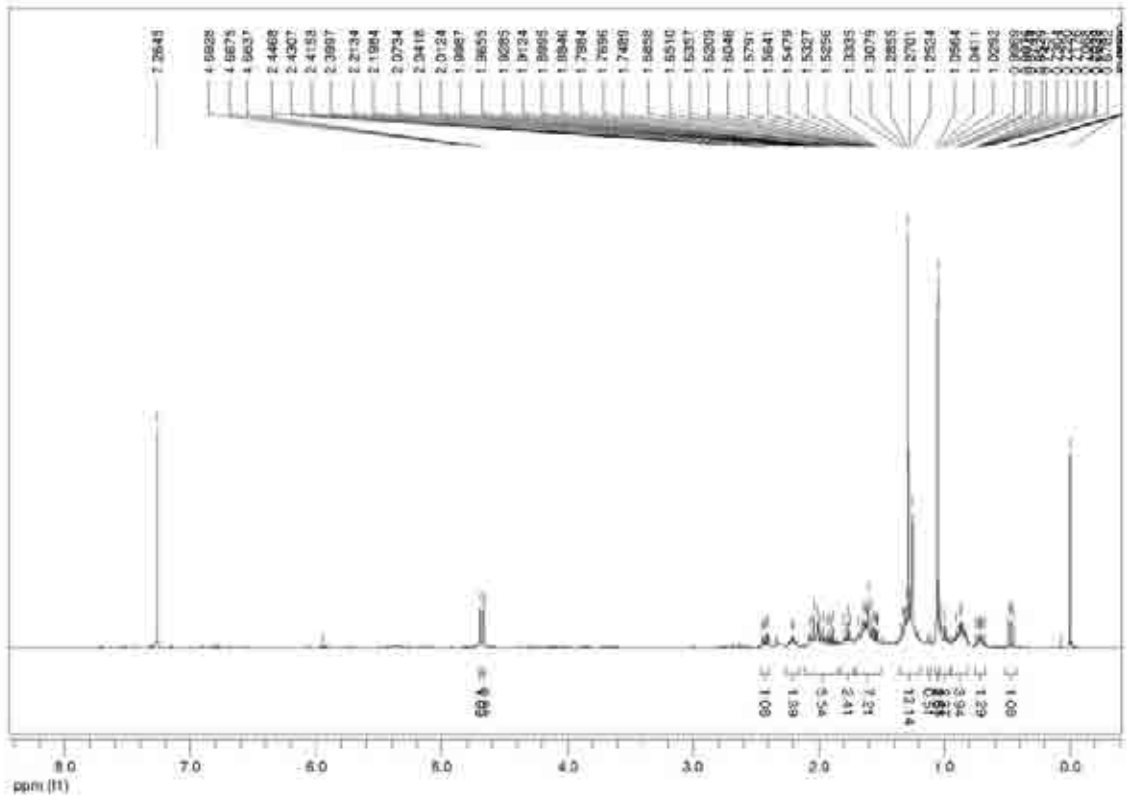

Figure S5. ^{1}H NMR spectrum of compound **3** in $CDCl_3$ at 400 MHz.

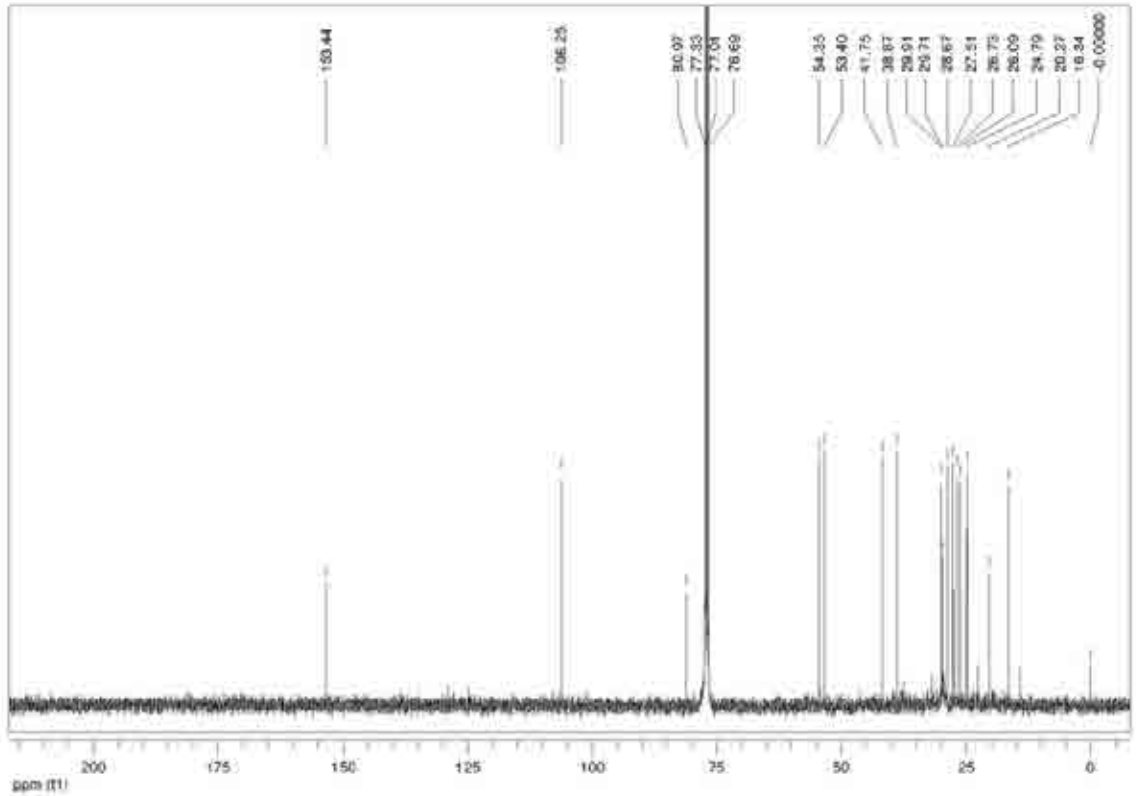

Figure S6. $^{13}C\{^{1}H\}$ NMR spectrum of compound **3** in $CDCl_3$ at 100 MHz.

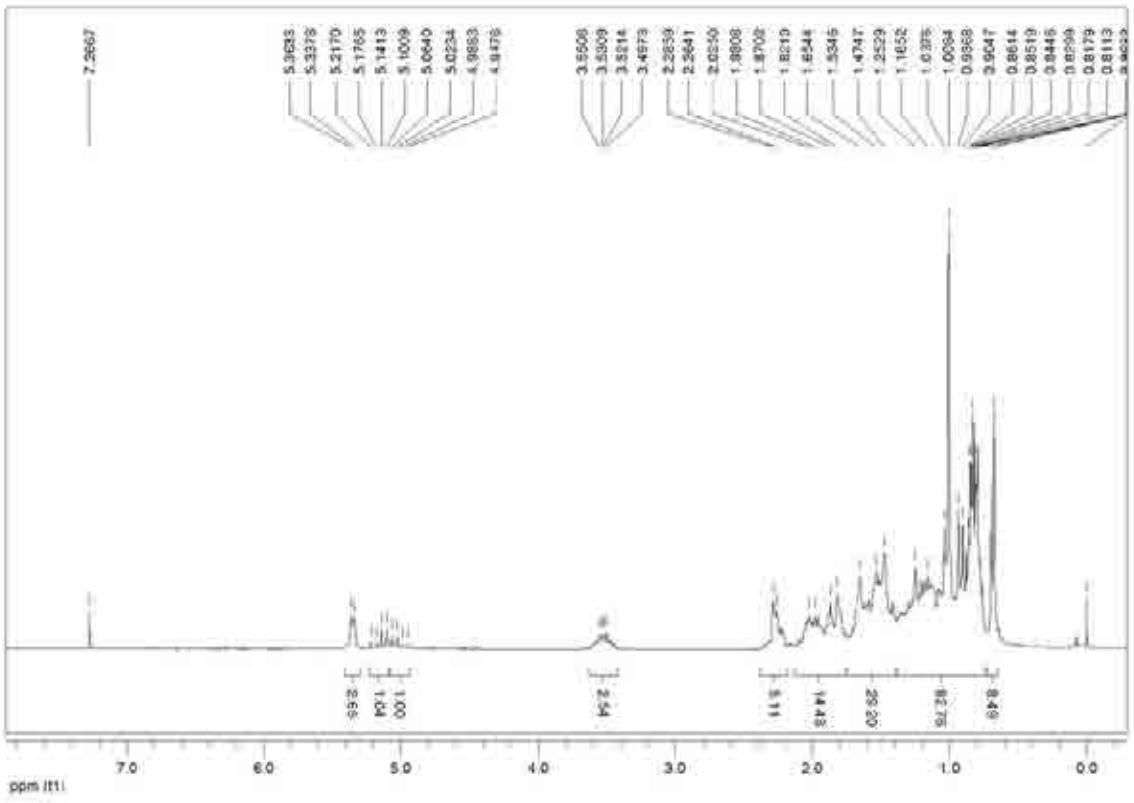

Figure S7. ^{1}H NMR spectrum of the mixture of compounds **4** and **5** in $CDCl_3$ at 200 MHz.

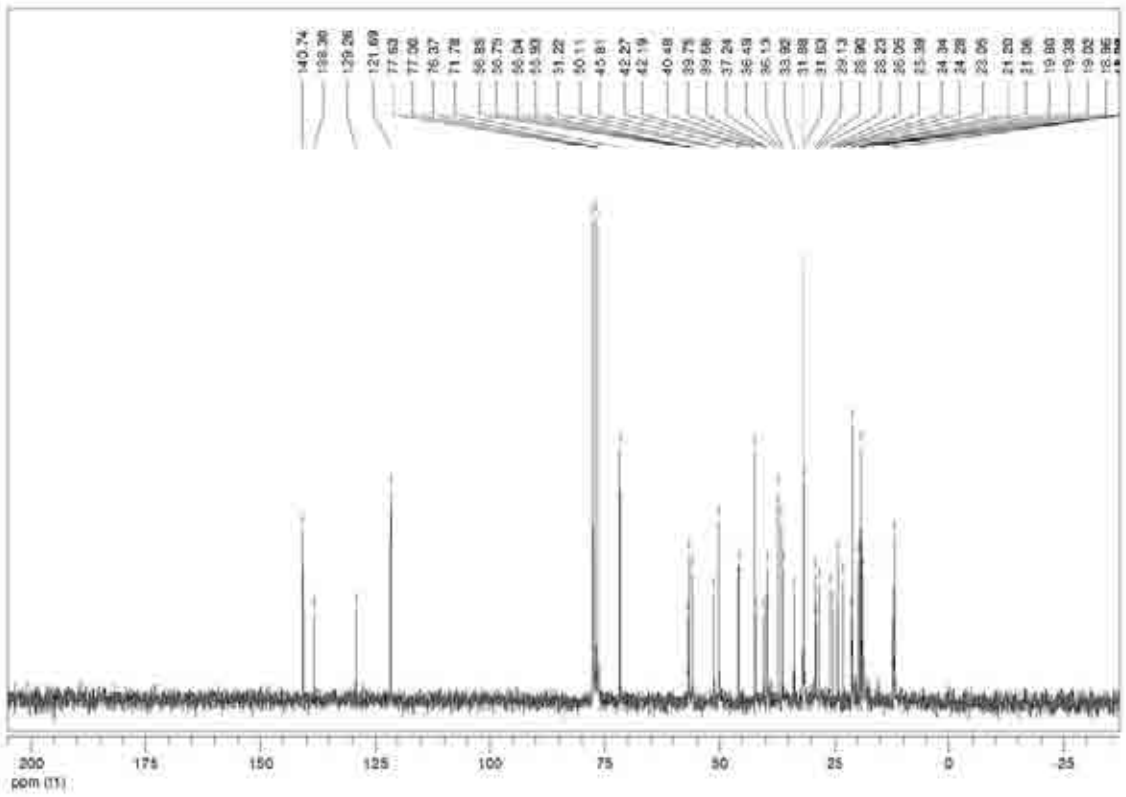

Figure S8. $^{13}C\{^{1}H\}$ NMR spectrum of the mixture of compounds **4** and **5** in $CDCl_3$ at 50 MHz.

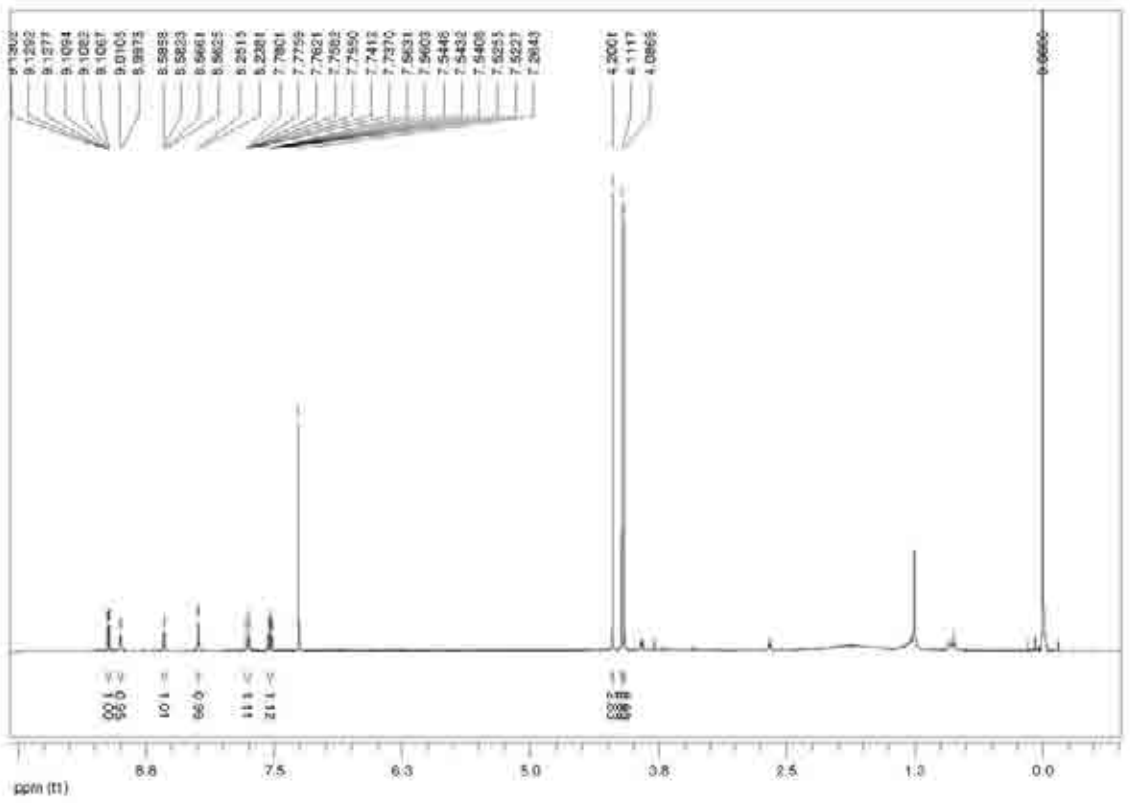

Figure S9. ^{1}H NMR spectrum of compound **6** in $CDCl_3$ at 400 MHz.

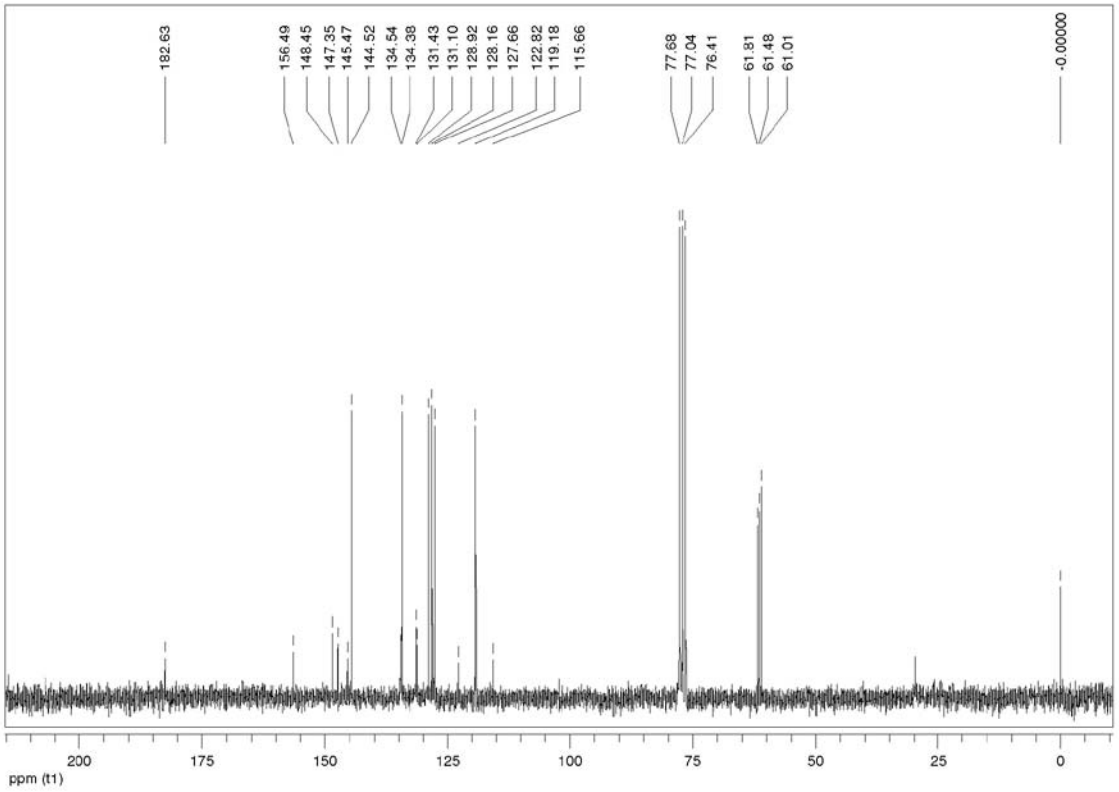

Figure S10. $^{13}C\{^{1}H\}$ NMR spectrum of compound **6** in $CDCl_3$ at 100 MHz.

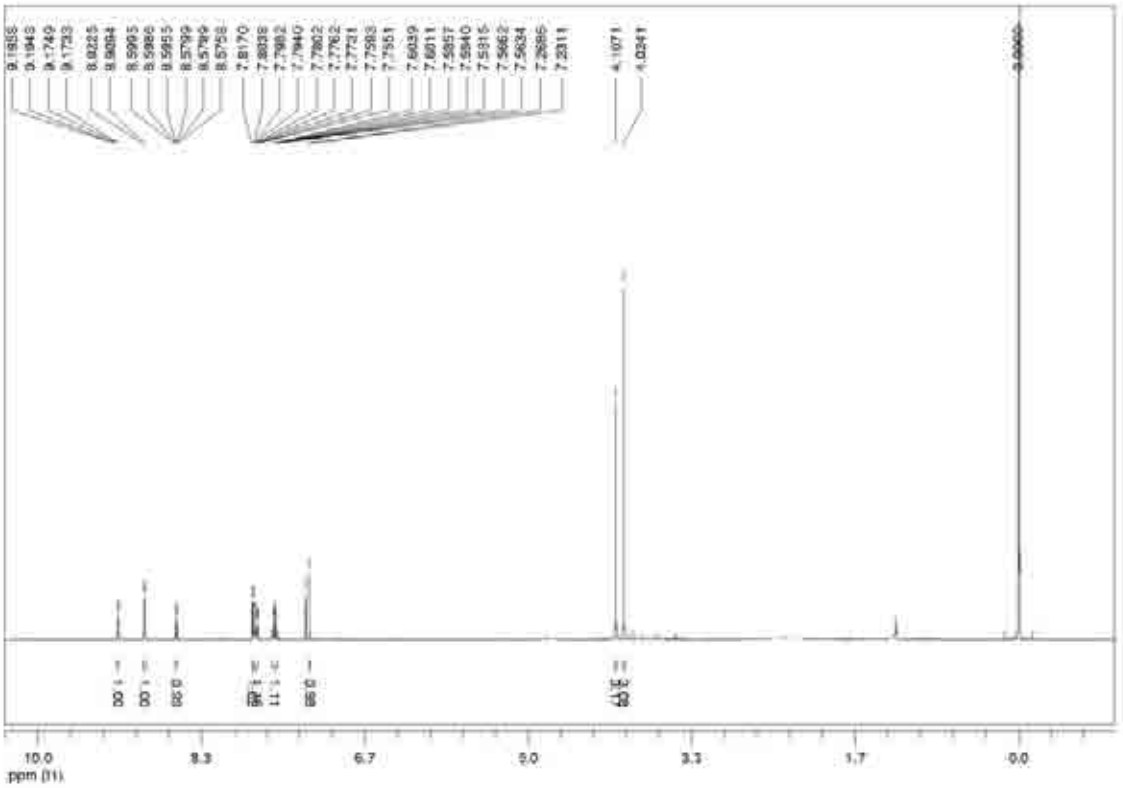

Figure S11. ^{1}H NMR spectrum of compound **7** in $CDCl_3$ at 400 MHz.

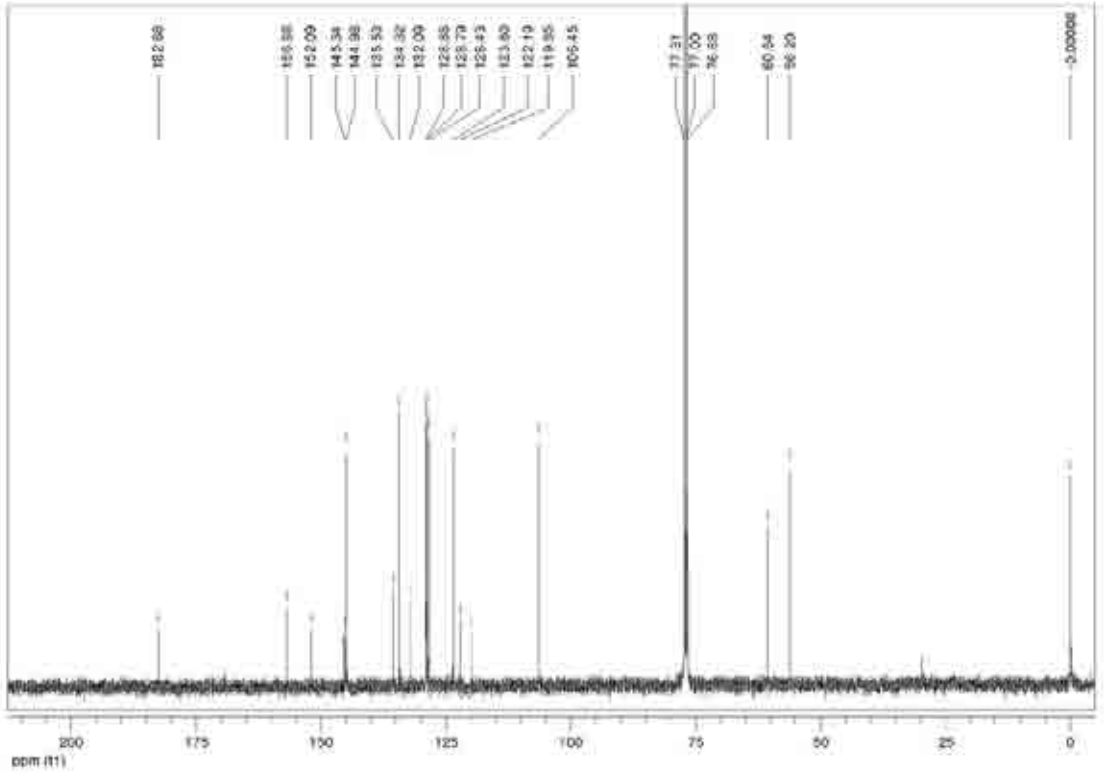

Figure S12. $^{13}C\{^{1}H\}$ NMR spectrum of compound **7** in $CDCl_3$ at 100 MHz.

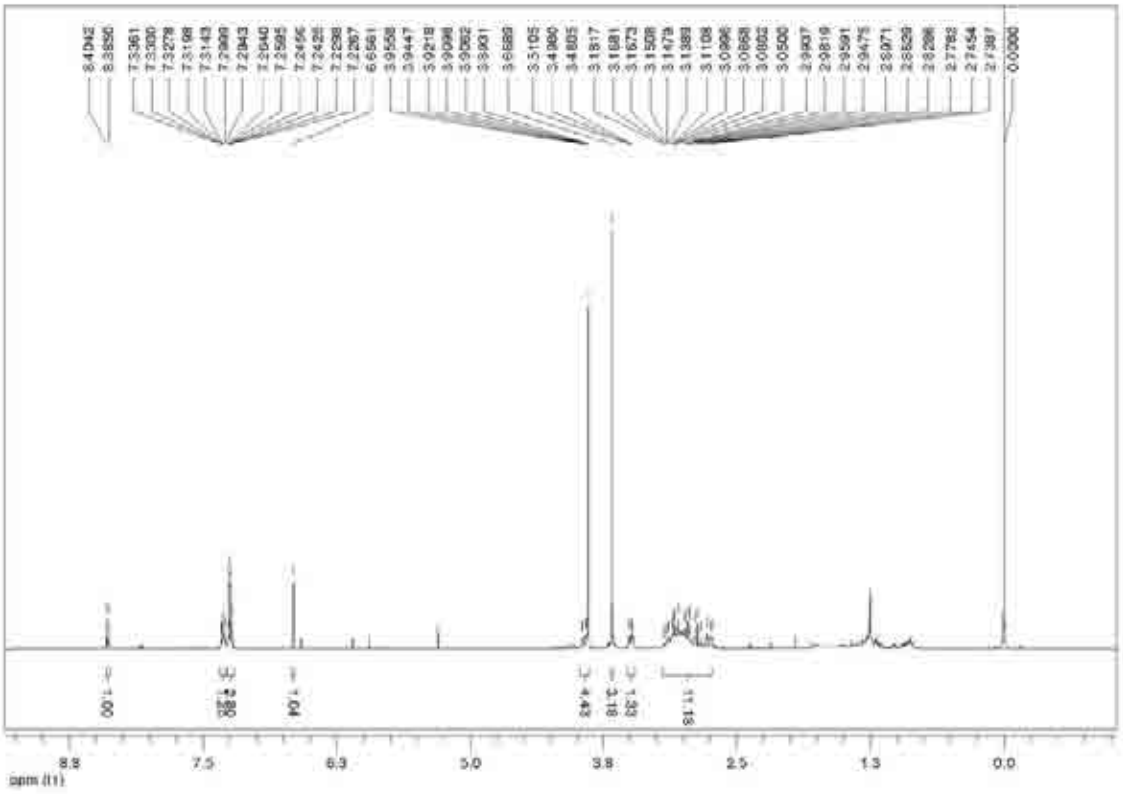

Figure S13. ^{1}H NMR spectrum of compound **8** in $CDCl_3$ at 400 MHz.

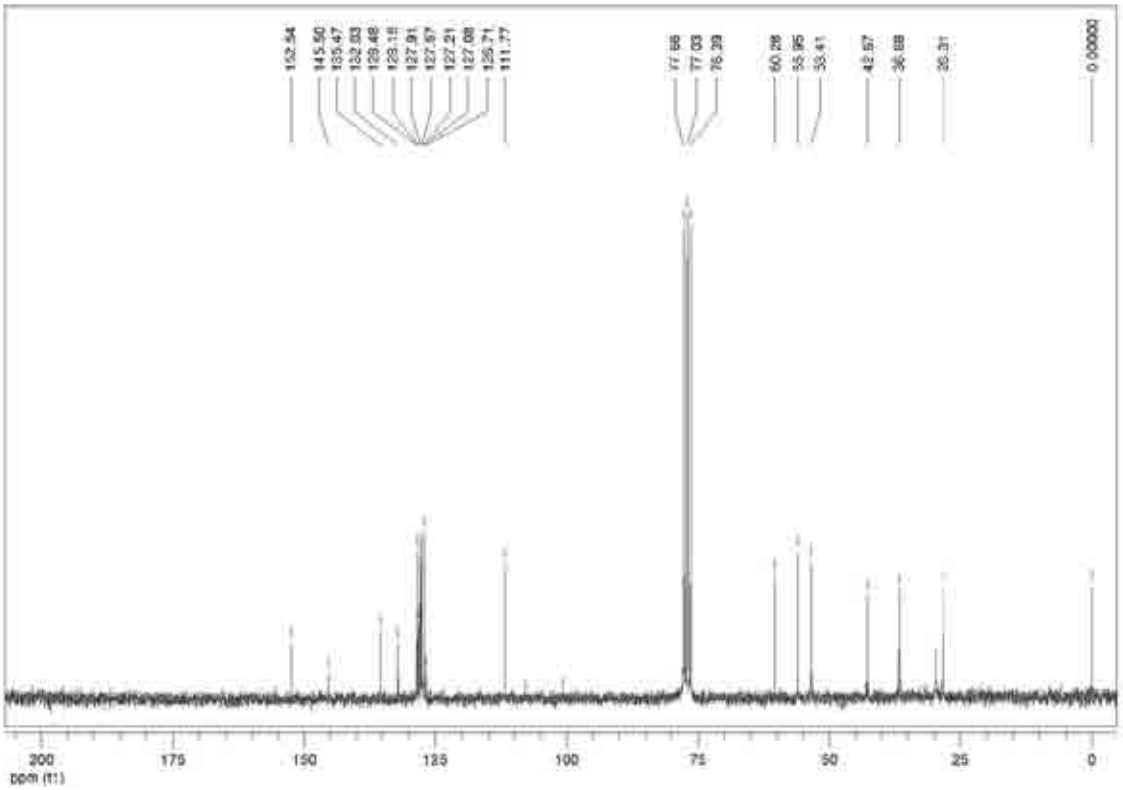

Figure S14. $^{13}C\{^{1}H\}$ NMR spectrum of compound **8** in $CDCl_3$ at 100 MHz.

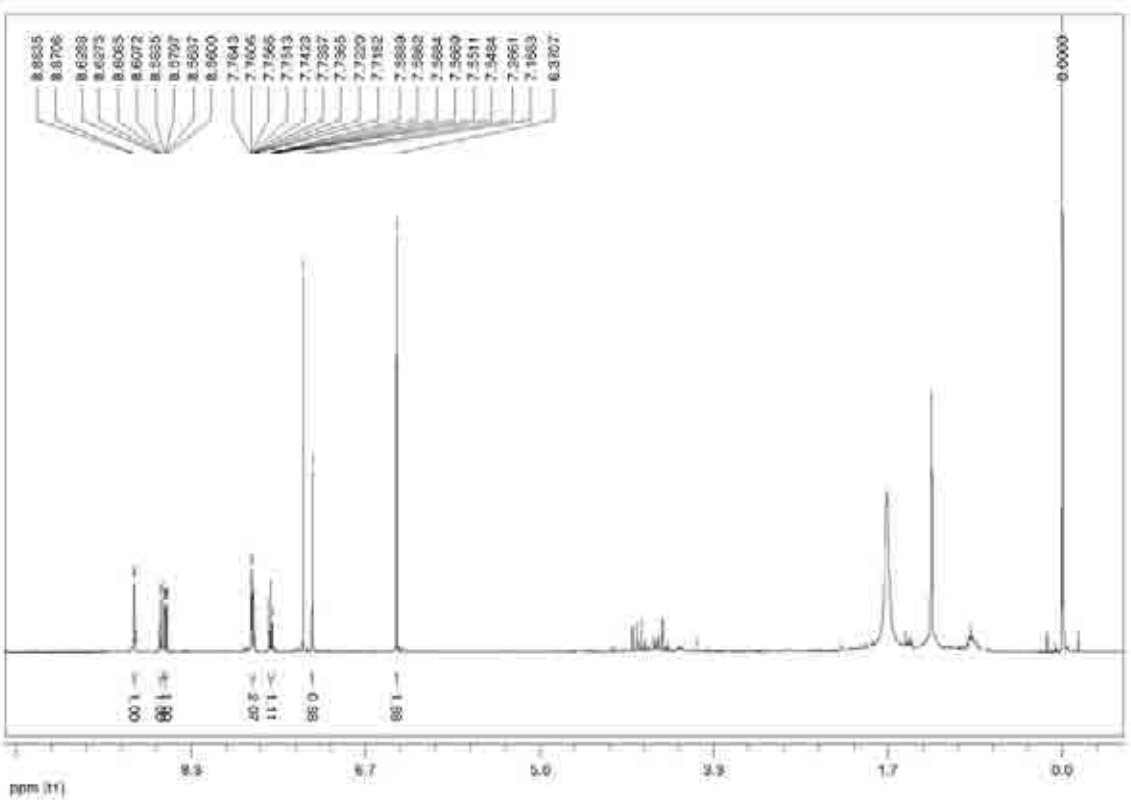

Figure S15. ^{1}H NMR spectrum of compound **9** in $CDCl_3$ at 400 MHz.

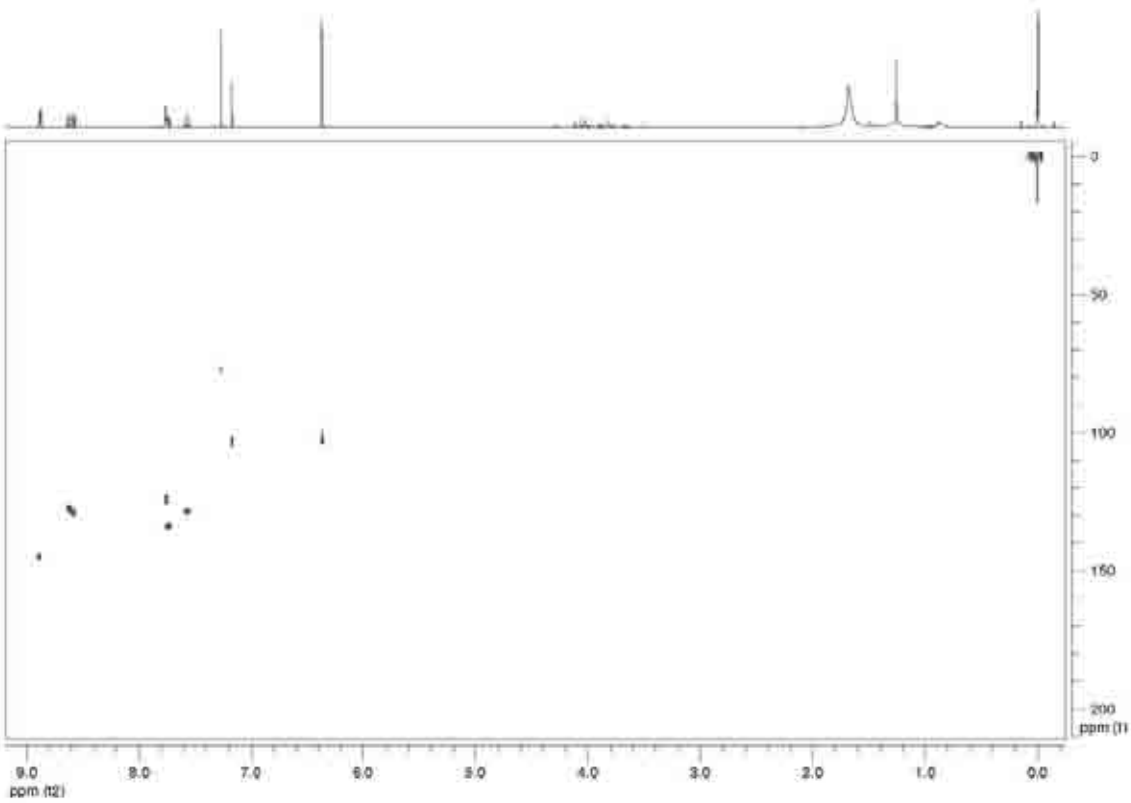

Figure S16. ^{1}H-^{13}C one-bond correlation map from HSQC NMR experiment of compound **9** in $CDCl_3$ at 400 and 100 MHz.

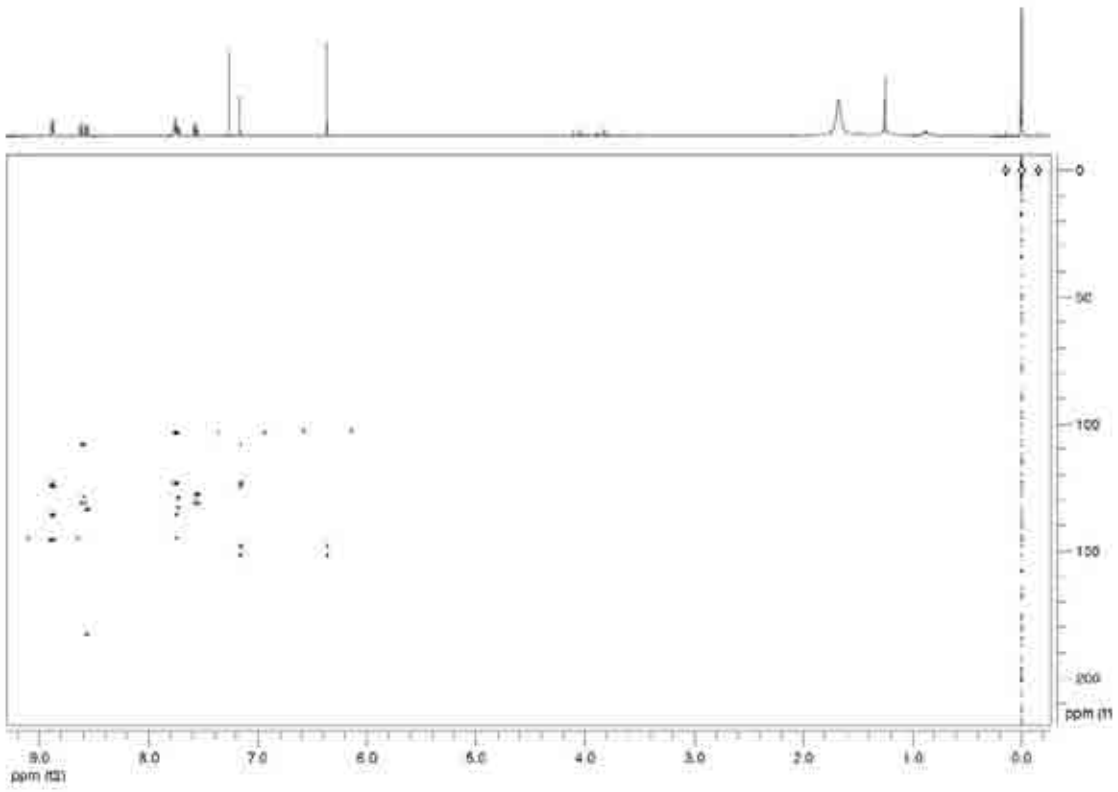

Figure S17. ^{1}H-^{13}C long-range correlation map from HMBC NMR experiment of compound **9** in $CDCl_3$ at 400 and 100 MHz.

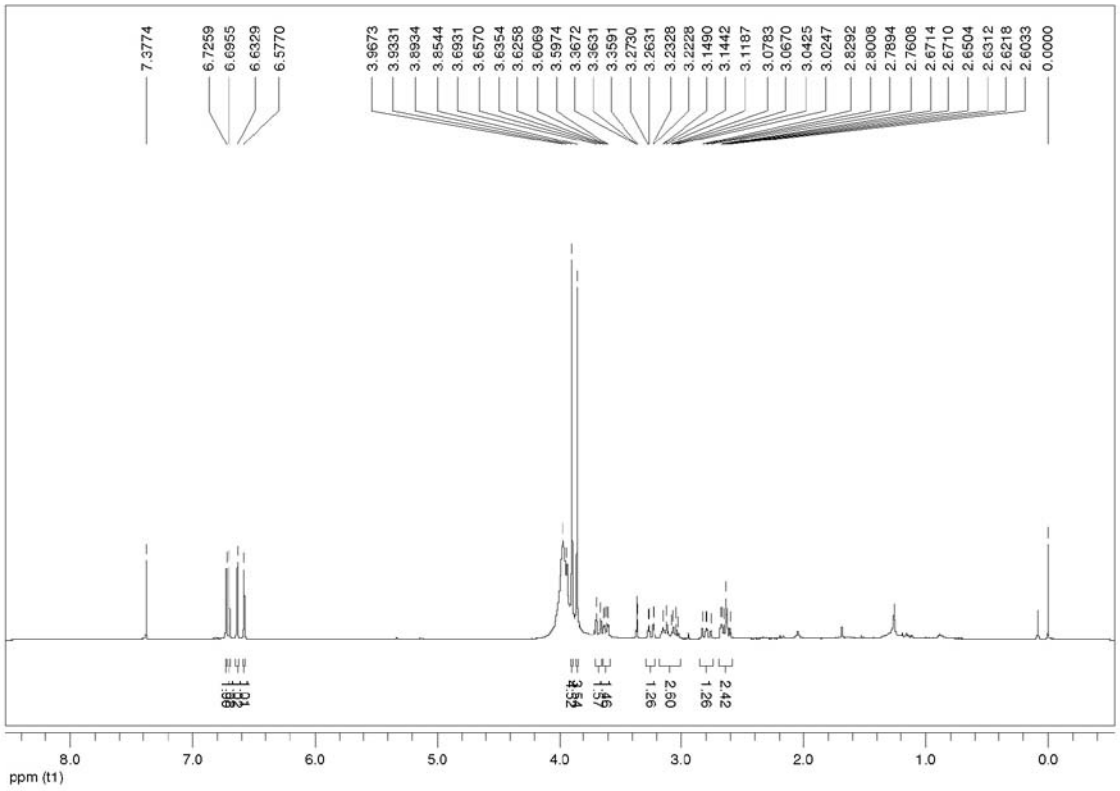

Figure S18. ^{1}H NMR spectrum of compound **10** in $CDCl_3$ + drops of CD_3OD at 400 MHz.

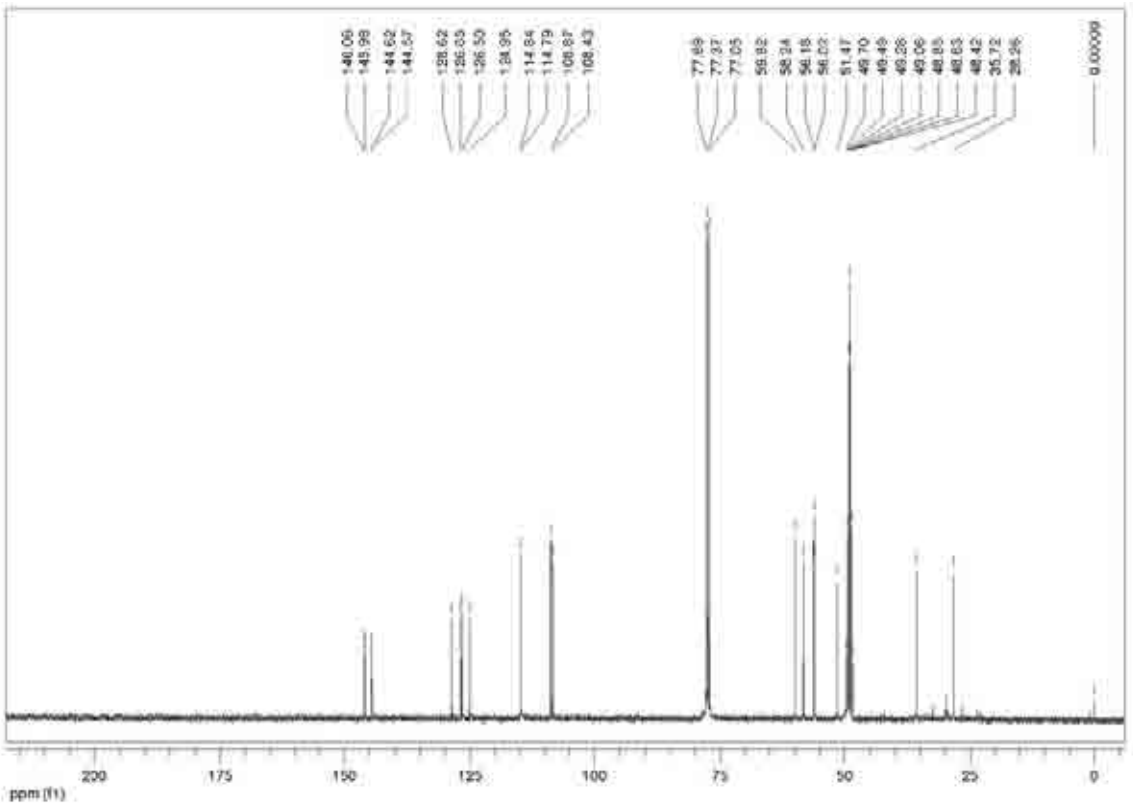

Figure S19. $^{13}C\{^{1}H\}$ NMR spectrum of compound **10** in $CDCl_3$ + drops of CD_3OD at 100 MHz.

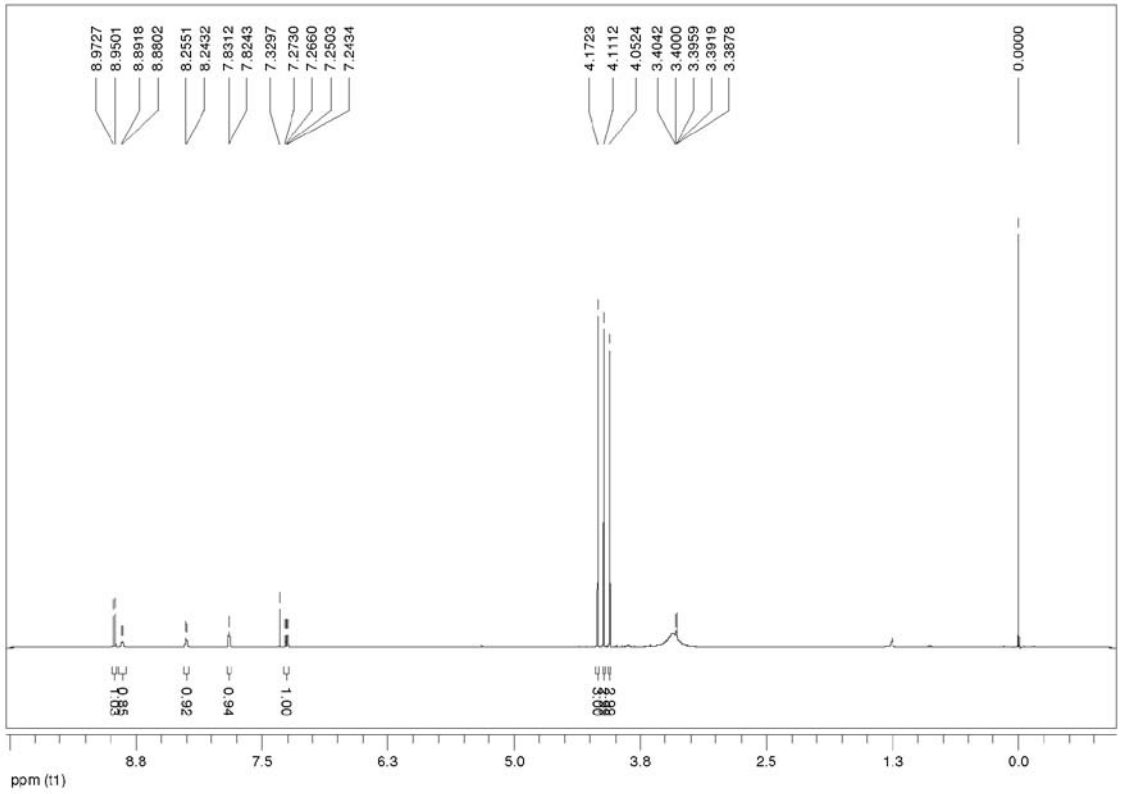

Figure S20. ^{1}H NMR spectrum of compound **11** in $CDCl_3$ + drops of CD_3OD at 400 MHz.

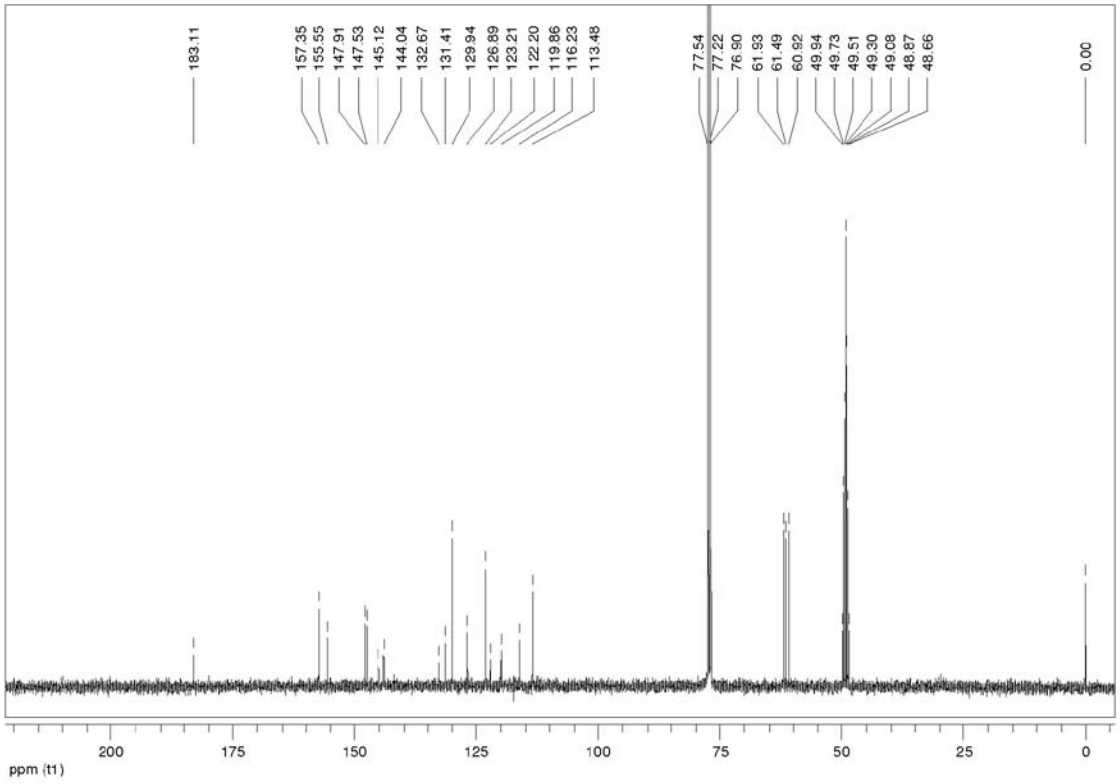

Figure S21. $^{13}C\{^{1}H\}$ NMR spectrum of compound **11** in $CDCl_3$ + drops of CD_3OD at 100 MHz.

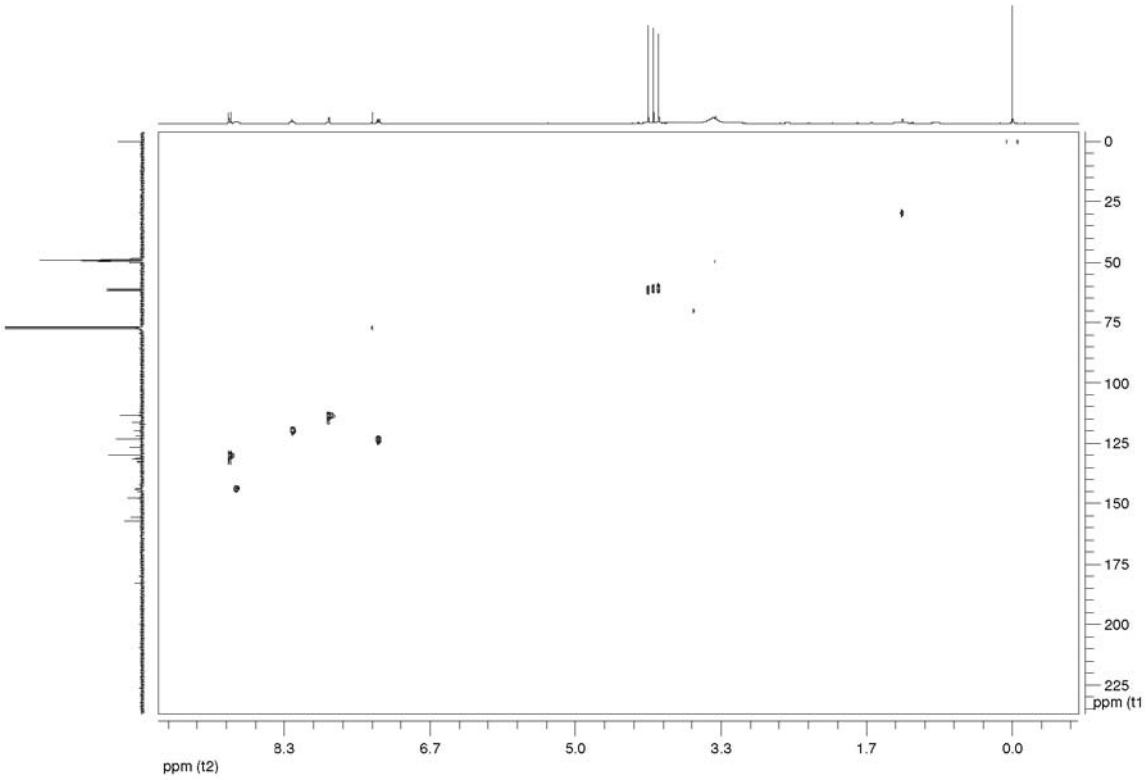

Figure S22. ^{1}H-^{13}C one-bond correlation map from HSQC NMR experiment of compound **11** in $CDCl_3$ + drops of CD_3OD at 400 and 100 MHz.

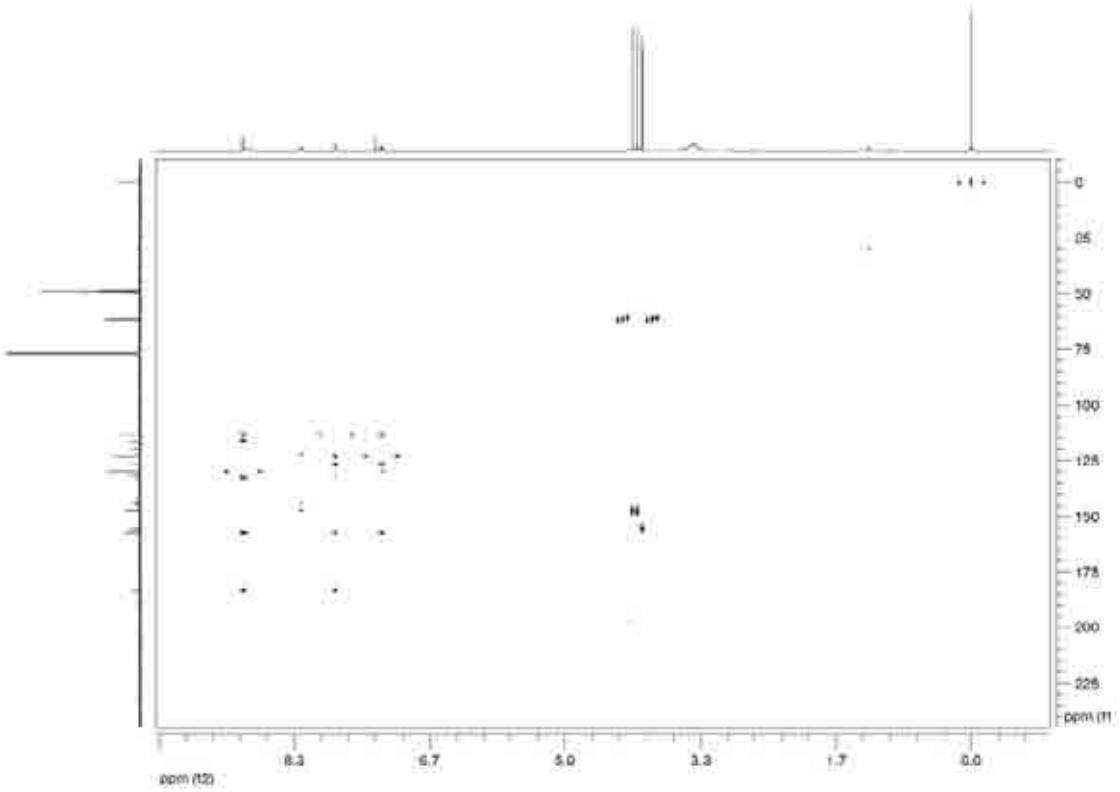

Figure S23. ^{1}H-^{13}C long-range correlation map from HMBC NMR experiment of compound **11** in $CDCl_3$ + drops of CD_3OD at 400 and 100 MHz.

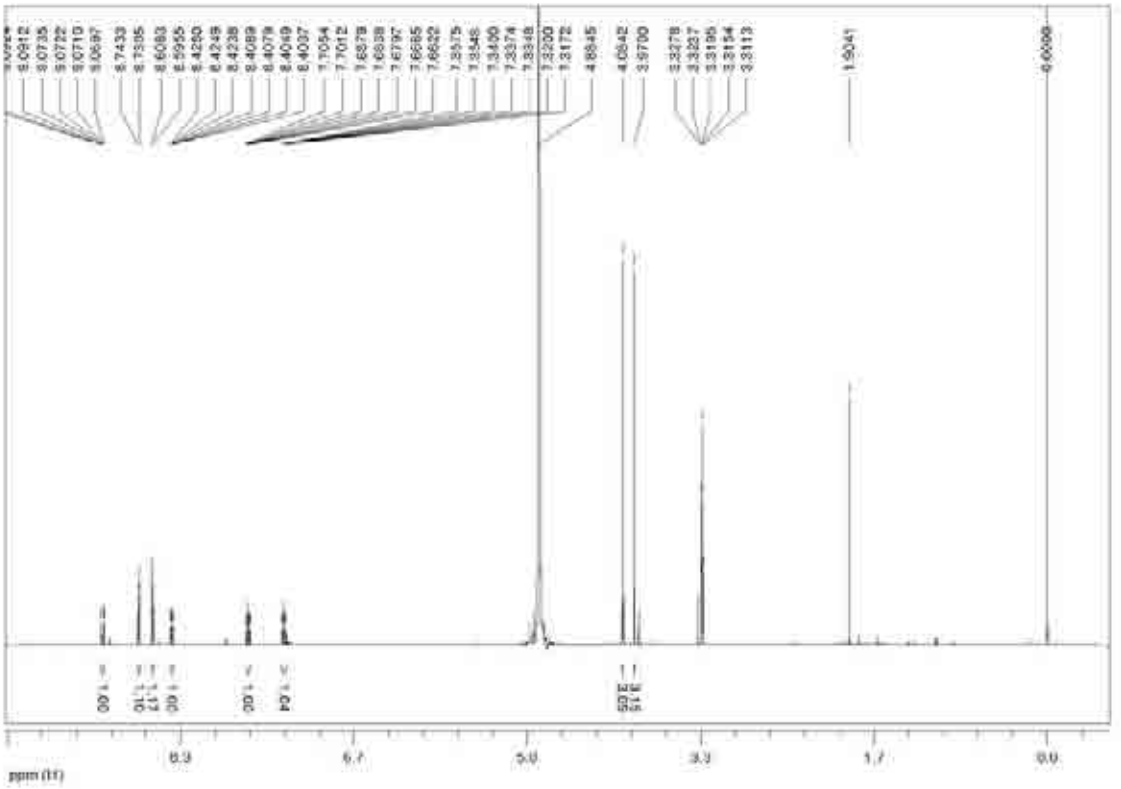

Figure S24. ^{1}H NMR spectrum of compound **12** in CD_3OD at 400 MHz.

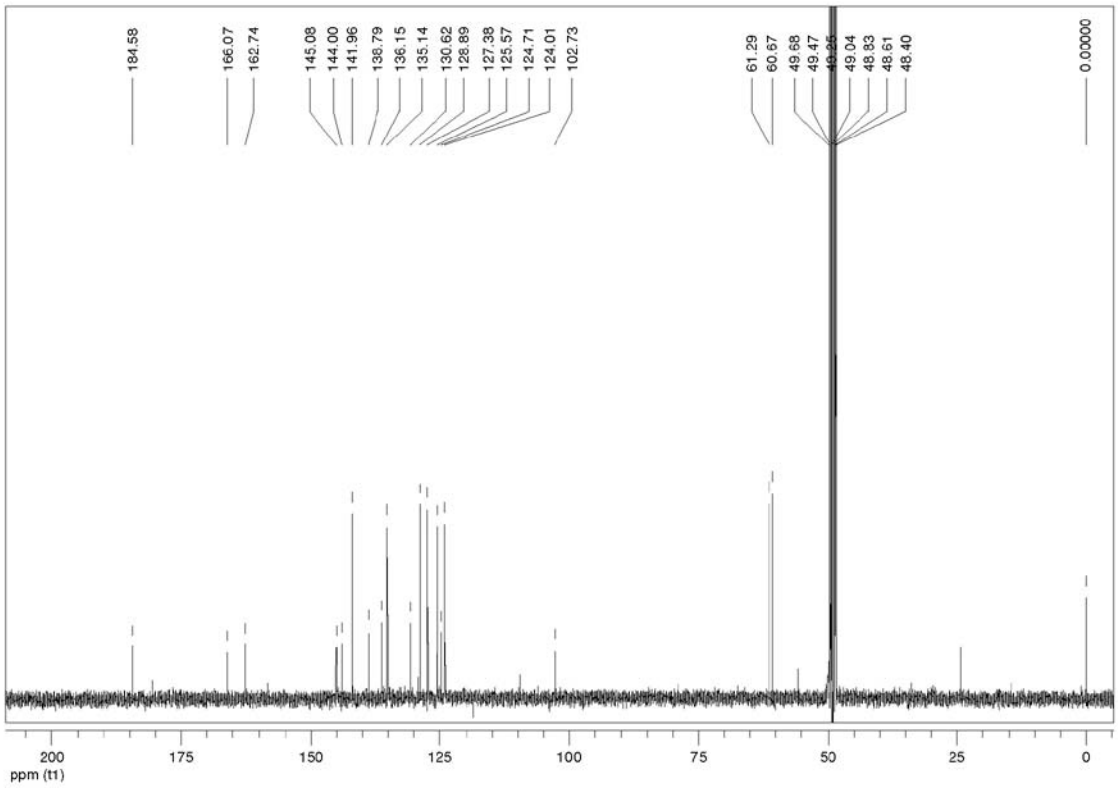

Figure S25. $^{13}C\{^{1}H\}$ NMR spectrum of compound **12** in CD_3OD at 100 MHz.

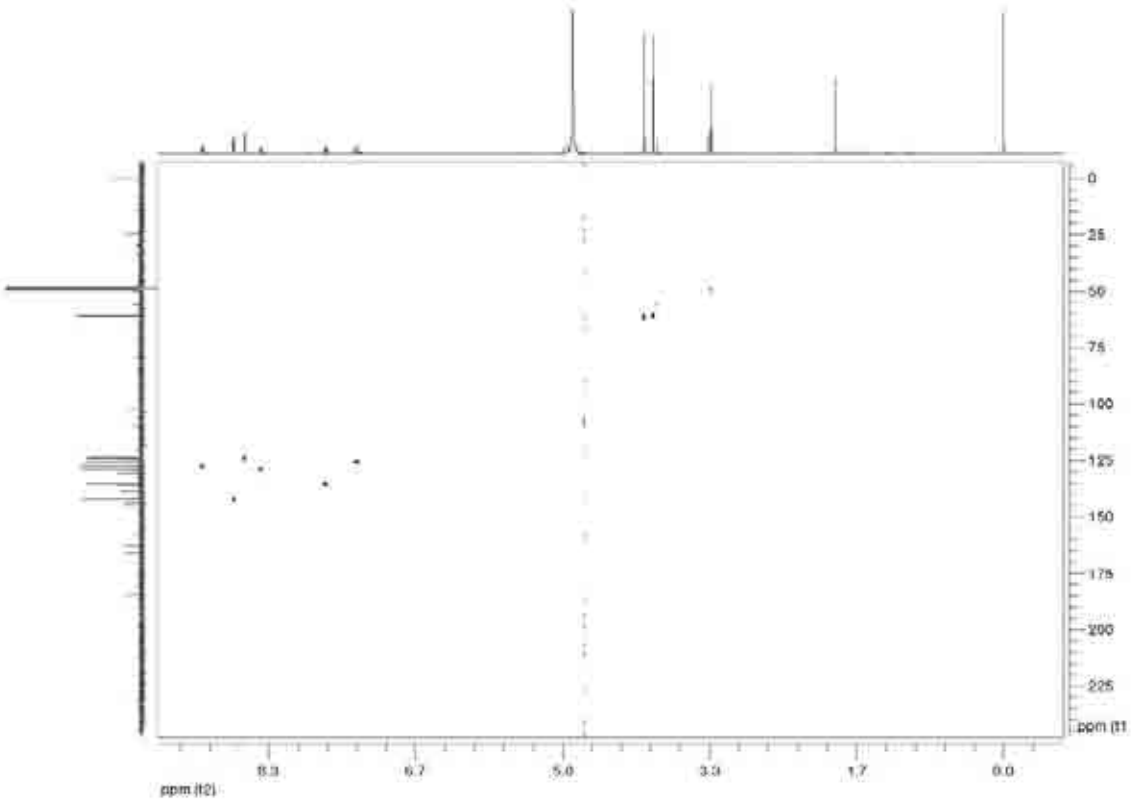

Figure S26. ^{1}H-^{13}C one-bond correlation map from HSQC NMR experiment of compound **12** in CD_3OD at 400 and 100 MHz.

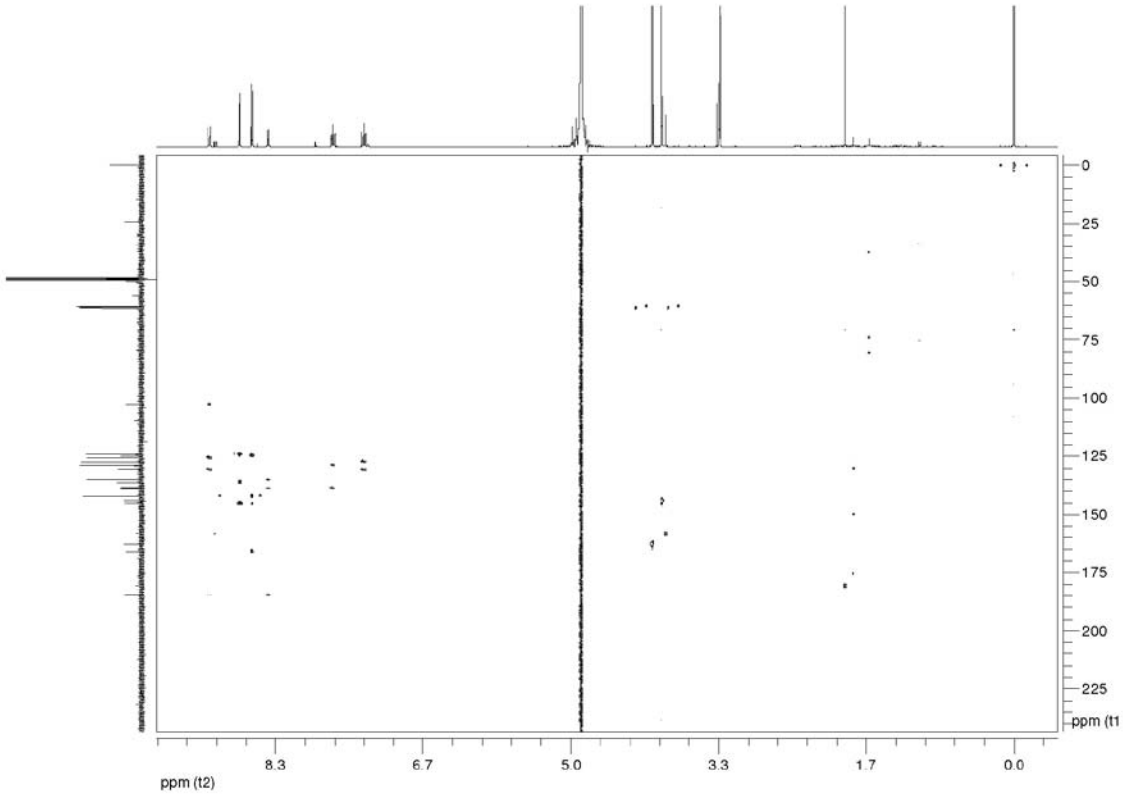

Figure S27. ^{1}H-^{13}C long-range correlation map from HMBC NMR experiment of compound **12** in CD_3OD at 400 and 100 MHz.