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Article

New Coumarins from *Clausena lansium* Twigs

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Duas novas cumarinas, Clausenalansimin A (**5**) e B (**9**), juntamente com sete cumarinas conhecidas (**1-4** e **6-8**), foram isoladas de galhos de *Clausena lansium*. Todos os compostos foram determinados por métodos espectroscópicos. Alguns dos compostos isolados apresentaram citotoxicidade contra linhagens de células humanas cancerígenas (KB, MCF7 e NCI-H187).

Two new coumarins namely Clausenalansimin A (**5**) and B (**9**) together with seven known coumarins (**1**-**4** and **6**-**8**), were isolated from twigs of *Clausena lansium*. All compounds were determined by spectroscopic methods. Some of isolates had cytotoxicity against human cancer cell lines (KB, MCF7 and NCI-H187).

Keywords: *Clausena lansium*, coumarin, clausenalansimin A, clausenalansimin B, cytotoxicity

Introduction

*Clausena lansium (*Lour.) Skeels is a distant relative of citrus fruit belonging to the Rutaceae family. Several parts of this plant have been used as a folk medicine in China and Taiwan. For example, the leaves have been used for the treatment of coughs, asthma and gastro-intestinal diseases, and the seeds for acute and chronic gastro-intestinal inflammation and ulcers.[1] In addition, the fruits are used for influenza, colds and abdominal colic pains in the Philippines.[2] Recently, the seed extract of *C. lansium* was found to exhibit antifungal, antiproliferative, and HIV reverse transcriptase-inhibitory activities.[3] Previous chemical investigations of this plant have revealed a number of alkaloids and coumarins.[2,4-6] As parts of our continuing study on chemical constituents and biological activity of Thai medicinal plants, we report herein the isolation and structure elucidation of two new coumarins (**5** and **9**) along with seven known coumarins (**1**-**4**, **6**-**8**) from the twigs of *C. lansium* as well as the evaluation of cytotoxicity against KB, MCF7 and NCI-H187 cancer cell lines. In addition, the ^{1}H and ^{13}C NMR spectral data of **7** are also reported herein for the first time.

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Results and Discussion

The combination of CH_2Cl_2 and acetone extracts of twigs of *C. lansium* was separated by chromatographic techniques to yield two new coumarins, clausenalansimin A (**5**) and B (**9**), together with seven known compounds (**1**-**4**, **6**-**8**). All structures were elucidated using spectroscopic data and compared with those reported in the literature.

Clausenalansimin A (**5**) was isolated as yellow viscous oil. Its molecular formula was established as $C_{21}H_{22}O_5$ by HRMS. The UV-Vis spectrum showed an absorption band of a conjugated furanocoumarin at 204-300 nm,[7] whereas IR spectroscopic data displayed absorption bands of hydroxyl and carbonyl functionalities at 3442 and 1722 cm^{-1}, respectively. The ^{1}H NMR spectrum (Table 1) of **5** showed the common signals of furanocoumarin similar to that of **1** at δ 6.36 (1H, d, *J* 9.6 Hz, H-3), 7.36 (1H, d, *J* 2.0 Hz, H-2′), 6.81 (1H, s, H-5), 7.68 (1H, d, *J* 2.0 Hz, H-3′) and 7.76 (1H, d, *J* 9.6 Hz, H-4).[8] The main different signals were observed at 5.68 (1H, dt, *J* 7.2 and 1.2 Hz, H-2″), 5.11 (1H, m, H–6″), 5.04 (2H, d, *J* 7.2 Hz, H–1″), 4.41 (1H, m, H-5″), 2.17 (2H, m, H-4″), 1.76 (3H, s, H-9″), 1.68 (3H, s, H-8″) 1.65 (3H, s, H-10″) in the ^{1}H NMR spectral data and could be identified as the 5-hydroxy-

3,7-dimethylocta-2,7-dienyloxy moiety. This moiety was also confirmed by COSY and HMBC correlations (Figure 2) and located at C-8 because the H-1″ (δ 5.04) showed ^{3}J HMBC correlation with the carbon at C-8 (δ 131.5). Therefore, clausenalansimin A was deduced to be **5**.

Clausenalansimin B (**9**) was isolated as yellow viscous oil with a molecular formula of $C_{19}H_{18}O_6$ on the basis of HREIMS. The ^{1}H NMR spectral data of **9** revealed an α,β-unsaturated lactone at δ 6.12 (1H, d, *J* 9.6 Hz, H-3) and 7.96 (1H, d, *J* 9.6 Hz, H-4) and two *meta*-coupling aromatic protons at δ 6.48 (1H, d, *J* 1.6 Hz, H-6) and 6.26 (1H, d, *J* 1.6 Hz, H-8).[9] These results implied that this molecule is a 5,7-oxygenated coumarin nucleus. Moreover, the ^{1}H NMR spectrum also showed signals of $-OCH_2-CH=C-$ unit at δ 4.66 (2H, m, H-1″) and 5.53 (1H, t, *J* 5.6 Hz, H-2″) and an α,β-unsaturated γ-lactone moiety at δ 2.35 (1H, dd, *J* 17.0, 7.2 Hz, H-4a″), 2.61 (1H, dd, *J* 17.0, 5.2 Hz, H-4b″), 5.11 (1H, m, H-5″), 7.09 (1H, br t, H-6″), 1.92 (3H, br s, H-8″).[10] The side chain unit was also supported by COSY and HMBC experiments (Figure 2) and located at C–5 due to the ^2J and ^3J HMBC correlations of the H-4 (δ 7.96), H-6 (δ 6.48) and H-1″ (δ 4.66) with C-5 (δ 156.1). The structure of clausenalansimin B, therefore, was assigned to **9**.

The remaining seven known coumarins included xanthotoxol (**1**),[7] imperatorin (**2**),[11] heraclenin (**3**),[12] heraclenol (**4**),[13] wampetin (**6**),[14] indicolactonediol (**7**),[15] and isoscopoletin (**8**)[16] were determined by the 1D and 2D NMR spectral data and comparison with their reported physical and spectroscopic data. In addition, the complete assignments of ^{1}H and ^{13}C NMR of indicolactonediol (**7**) are also reported herein for the first time (Table 1).

Only the stable compounds and sufficient quantity were evaluated for their cytotoxicity against three human cancer cell lines including oral cavity cancer (KB), breast cancer (MCF7) and small cell lung cancer (NCI-H187). The results of cytotoxicity of the tested compounds (**1**-**3**, **5** and **6**) are summarized in Table 2. All these compounds showed weak activity with cytotoxicity against KB, MCF7 and NCI-H187 cell lines, except coumarins **3** and **6** which were found to be inactive with MCF7 cancer cell line.

Table 1. ^{1}H and ^{13}C NMR spectral data of **5**, **7** and **9** in $CDCl_3$

Position	**5**		**7**		**9**	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1	–	–	–	–	–	–
2	–	160.4	–	160.4	–	162.1
3	6.36 (d, 9.6)	114.7	6.37 (d, 9.6)	114.8	6.12 (d, 9.6)	110.3
4	7.76 (d, 9.6)	144.3	7.78 (d, 9.6)	144.3	7.96 (d, 9.6)	139.2
4a	–	116.5	–	116.5	–	103.8
5	7.36 (s)	113.4	7.42 (s)	114.0	–	156.1
6	–	125.8	–	126.0	6.48 (d, 1.6)	96.4
7	–	148.6	–	148.3	–	161.0
8	–	131.5	–	131.4	6.26 (d, 1.6)	96.9
8a	–	143.8	–	143.5	–	156.6
2′	7.68 (d, 2.0)	146.7	7.71 (d, 2.0)	146.8	–	–
3′	6.81 (d, 2.0)	106.8	6.84 (d, 2.0)	106.8	–	–
1″	5.04 (d, 7.2)	69.8	4.56 (dd, 11.2, 5.6) 4.66 (dd, 11.2, 5.6)	72.0	4.66 (m)	66.0
2″	5.68 (dt, 7.2, 1.2)	122.9	3.52 (t, 5.6)	59.6	5.53 (t, 5.6)	125.1
3″	–	139.6	–	73.9	–	133.4
4″	2.17 (m)	47.8	1.84 (dd, 14.8, 5.2) 2.11 (dd, 14.8, 8.4)	41.5	2.35 (dd, 17.0, 7.2) 2.61 (dd, 17.0, 5.2)	42.6
5″	4.41 (m)	66.2	4.92 (m)	72.0	5.11 (m)	79.6
6″	5.11 (m)	127.2	7.13 (t, 1.6)	148.5	7.09 (br t)	148.9
7″	–	135.1	–	130.2	–	130.4
8″	1.68 (s)	16.9	1.92 (t, 1.6)	10.6	1.92 (br s)	10.6
9″	1.76 (s)	25.7	1.39 (s)	18.1	1.74 (s)	17.6
10″	1.65 (s)	18.1	–	174.2	–	177.0

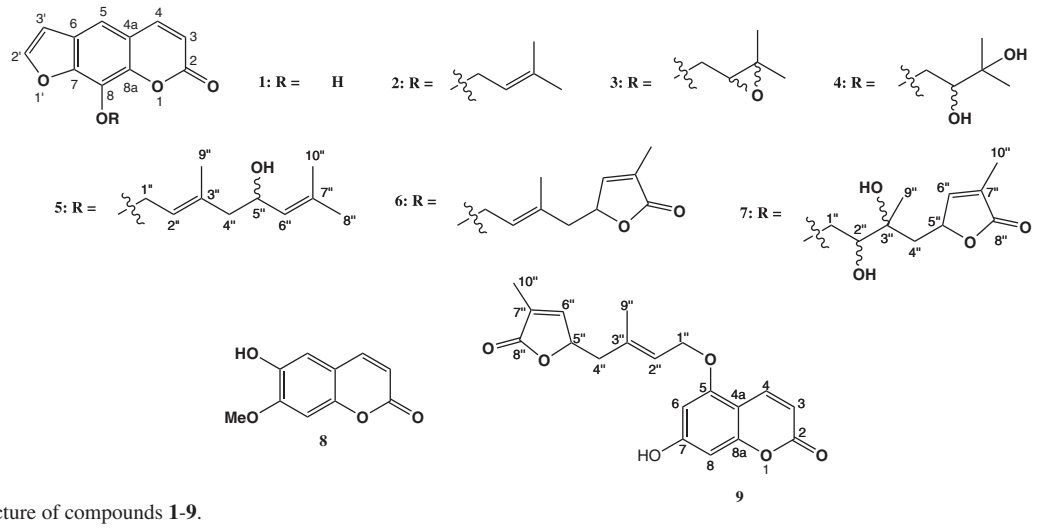

Figure 1. Structure of compounds **1-9**.

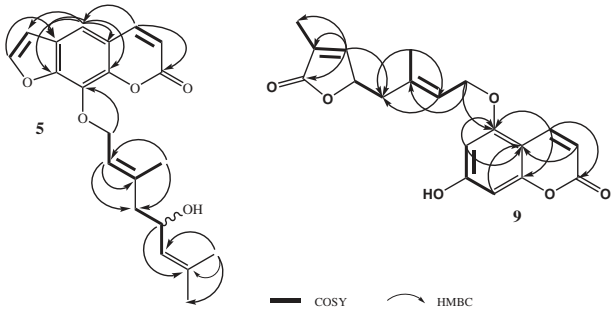

Figure 2. COSY and selective HMBC Correlations of **5** and **9**.

Table 2. Biological activity of furanocoumarins isolated from the stems of *C. lansium*

Compound	Cytotoxicity (IC_{50}, μg mL^{-1})		
	KB[a]	MCF7[b]	NCI–H187[c]
1	26.97	11.92	40.41
2	9.60	37.62	28.58
3	19.03	Inactive	17.40
5	22.09	31.02	28.83
6	17.93	Inactive	26.23
Doxorubicin	0.172	0.976	0.061

[a]KB = Oral cavity cancer; [b]MCF7 = Breast cancer; [c]NCI–H187 = Small cell lung cancer

Experimental

General procedures

The optical rotation $[\alpha]_D$ values were determined with a Bellingham & Stanley ADP440 polarimeter. UV-Vis spectra were recorded with a Perkin-Elmer UV-Vis spectrophotometer. The IR spectra were recorded with a Perkin-Elmer FTS FT-IR spectrophotometer. The NMR spectra were recorded using 400 MHz Bruker spectrometer. Chemical shifts were recorded in parts per million (δ) in $CDCl_3$ with tetramethylsilane (TMS) as an internal reference. The HRMS was obtained from a MicroTOF, Bruker Daltonics or MAT 95 XL mass spectrometers. Quick column chromatography (QCC) and column chromatography (CC) were carried out on silica gel 60 H (Merck, 5-40 μm) and silica gel 100 (Merck, 63-200 μm), respectively. Precoated plates of silica gel 60 F_{254} were used for analytical purposes.

Plant material

The twigs of *C. lansium* were collected in April 2008 from Nan Province, northern part of Thailand. Botanical identification was achieved through comparison with a voucher specimen number QBG 25077 in the herbarium collection of Queen Sirikit Garden, Mae Rim District, Chiang Mai, Thailand.

Extraction and Isolation

Air-dried twigs of *C. lansium* (6.73 Kg) were successively extracted with CH_2Cl_2 and acetone over a period of 3 days each at room temperature. The CH_2Cl_2 and acetone extracts were combined (34.02 g) and subjected to QCC over silica gel eluted with a gradient of hexane-acetone (100% hexane to 100% acetone) to provide seventeen fractions (A-Q). Fraction G (562.9 mg) upon standing at room temperature gave compound **2** (247.1 mg). Fraction I (1.83 g) was separated by CC using 20% EtOAc-hexane to give seven subfractions (I1-I7). Subfraction I7 (546.4 mg) was further purified by CC with 30% hexane-CH_2Cl_2 yielding compound **3** (207.8 mg). Fraction K (4.64 g) was separated by CC eluted with a gradient of CH_2Cl_2-EtOAc (5% EtOAc-CH_2Cl_2 to 100% EtOAc) to give compound **1** (5.6 mg), and nine subfractions (K1-K9). Subfraction K6 (124.2 mg) was subjected to

repeated CC with 2% acetone-CH_2Cl_2 to afford compound **5** (8.7 mg). Purification of fraction M (806.5 mg) was performed by sephadex LH20 with 60% CH_2Cl_2-MeOH, yielding five subfractions (M1-M5). Subfraction M2 (199.9 mg) was further subjected to repeated CC with a gradient of $CHCl_3$-hexane (70% $CHCl_3$-hexane to 100% $CHCl_3$) to afford eleven subfractions (M2a-M2k). Subfraction M2b (49.9 mg) was further subjected to repeated CC with 80% $CHCl_3$-hexane to yield four fractions (M2b1-M2b4). Compound **6** (4.8 mg) was derived from fraction M2b2 (18.5 mg) by repeated CC using 60% $CHCl_3$-hexane whereas compound **7** (1.7 mg) was obtained from fraction M2b3 (21.5 mg) by repeated CC with 5% EtOAc-$CHCl_3$. Subfraction M2d (8.6 mg) was further purified by prep.TLC with 5% EtOAc-$CHCl_3$ to afford **6** (7.3 mg). Fraction N (621.1 mg) was performed by CC with 40% EtOAc-hexane, yielding eleven subfractions (N1–N11). Subfraction N8 (65.5 mg) was separated by CC using a gradient of EtOAc-CH_2Cl_2 (10% EtOAc-CH_2Cl_2 to 25% EtOAc-CH_2Cl_2) to yield compound **9** (4.0 mg) while subfraction N10 (39.4 mg) was purified by prep.TLC with 7% EtOAc-CH_2Cl_2 to give compound **4** (9.8 mg).

*Clausenalansimin A **(5)***

Yellow viscous oil; $[\alpha]_D^{27}$ +48.1° (*c* 0.02, $CHCl_3$); UV λ_{max}/nm (MeOH): 204, 216, 247, 299; IR (neat) ν_{max}/cm^{-1}: 3442, 2924, 2855, 1722, 1626, 1587, 1443, 1401, 1327, 1261, 1150, 1092, 1026, 870, 801, 754; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; HRMS (APCI, –ve) *m/z*: 389.1161 ([M+Cl]$^-$, calc. $C_{21}H_{22}O_5Cl$, 389.1156).

*Clausenalansimin B **(9)***

Yellow viscous oil; $[\alpha]_D^{27}$ +17.24° (*c* 0.02, $CHCl_3$); UV λ_{max}/nm (MeOH): 207 and 329; IR (neat) ν_{max}/cm^{-1}: 2921, 1726, 1609, 1454, 1364, 1237, 1154, 1115, 823, 612; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz): see Table 1; HREIMS *m/z*: 342.1158 ([M]$^+$, calc. $C_{19}H_{18}O_6$, 342.1103).

Cytotoxicity assay

The procedures for cytotoxic assay were performed by sulphorhodamine B (SRB) assay (anti-KB and MCF7) and colorimetric method (anti-NCI-H187) as described by Skehan *et al.*[6] In this study, three cancer cell lines, MCF7 (breast cancer), NCI-H187 (human, small cell lung cancer) and KB (oral human epidermal carcinoma) were used. Doxorubicin was the reference substance in this study.

Supplementary Information

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

Acknowledgments

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References

1. Adebajo, A. C.; Iwalewa, E. O.; Obuotor, E. M.; Ibikunle, G. F.; Omisore, N. O.; Adewunmi, C. O.; Obaparusi, O. O.; Klaes, M.; Adetogun, G. E.; Schmidt, T. J.; Verspohl, E. J.; *J. Ethnopharmacol.* **2009**, *10*, 122.
2. Lin, J. H.; *Phytochemistry* **1989**, *28*, 621.
3. Ng, T. B.; Lam, S. K.; Fong, W. P.; *Biol. Chem.* **2003**, *384*, 289.
4. Yang, M. H.; Chen, Y. Y.; Huang, L.; *Phytochemistry* **1988**, *27*, 445.
5. Kumar, V.; Vallipuram, K.; Adebajo, A. C.; Reisch, J.; *Phytochemistry* **1995**, *40*, 1563.
6. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, R. M.; *J. Natl. Cancer Inst.* **1990**, *82*, 1107.
7. Ito, C.; Katsuno, S.; Furukawa, H.; *Chem. Pharm. Bull.* **1998,** *46*, 341.
8. Gellért, M.; Reisch, J.; Szendrei, K.; Novák, I.; Csedö, K.; *Phytochemistry* **1972**, *11*, 2894.
9. Ngadjui, B. T.; Ayafor, J. F.; Sondengam B. L.; Connolly, J. D.; *Phytochemistry* **1989**, *28*, 585.
10. Nakamura, K.; Takemura, Y.; Ju-ichi, M.; Ito, C.; Furukawa, H.; *Heterocycles* **1998**, *43*, 549.
11. Masuda, T.; Takasugi, M.; Anetai, M.; *Phytochemistry* **1998**, *47*, 13.
12. Adityachaudhury, N.; Ghosh, D.; Choudhuri, A.; *Phytochemistry* **1974**, *13*, 235.
13. Razdan, T. K.; Kachroo, V.; Harkar, S.; Koul, S.; *Phytochemistry* **1982**, *21*, 923.
14. Khan, N. U.; Navqi, S. W. I.; Ishratullah, K.; *Phytochemistry* **1983**, *22*, 2624.
15. Rakash, D.; Raj, K.; Kapil, R. S.; Popli, S. P.; *Phytochemistry* **1978**, *17*, 1194.
16. Al-Barwani, F. M.; Eltayeb, E. A.; *Biochem. Syst. Ecol.* **2004**, *32*, 1097.

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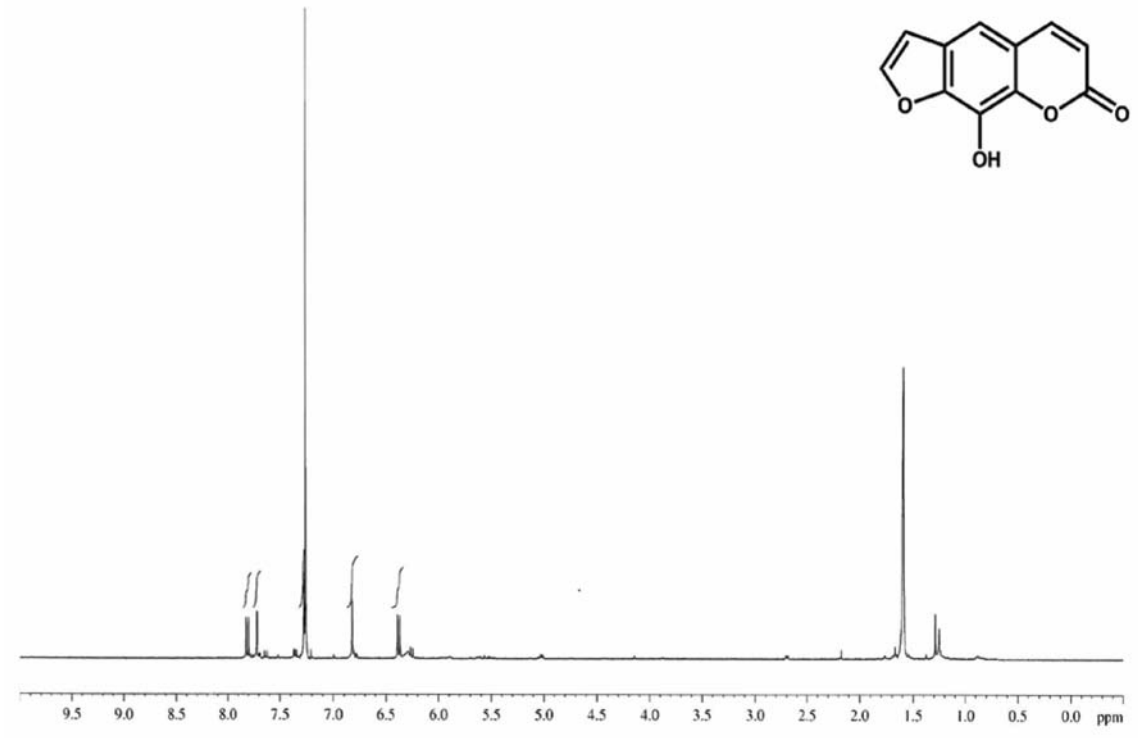

Figure S1. ^{1}H NMR spectrum of xanthotoxol (**1**) (400 MHz, $CDCl_3$).

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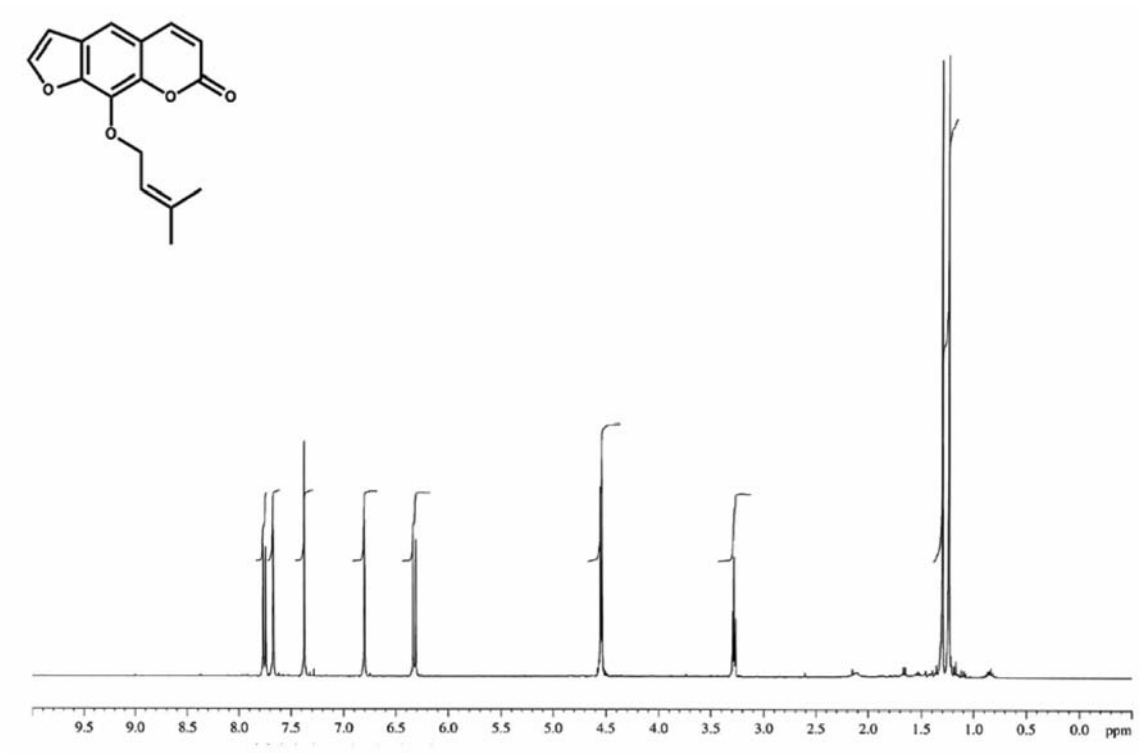

Figure S2. ^{1}H NMR spectrum of imperatorin (**2**) (400 MHz, $CDCl_3$).

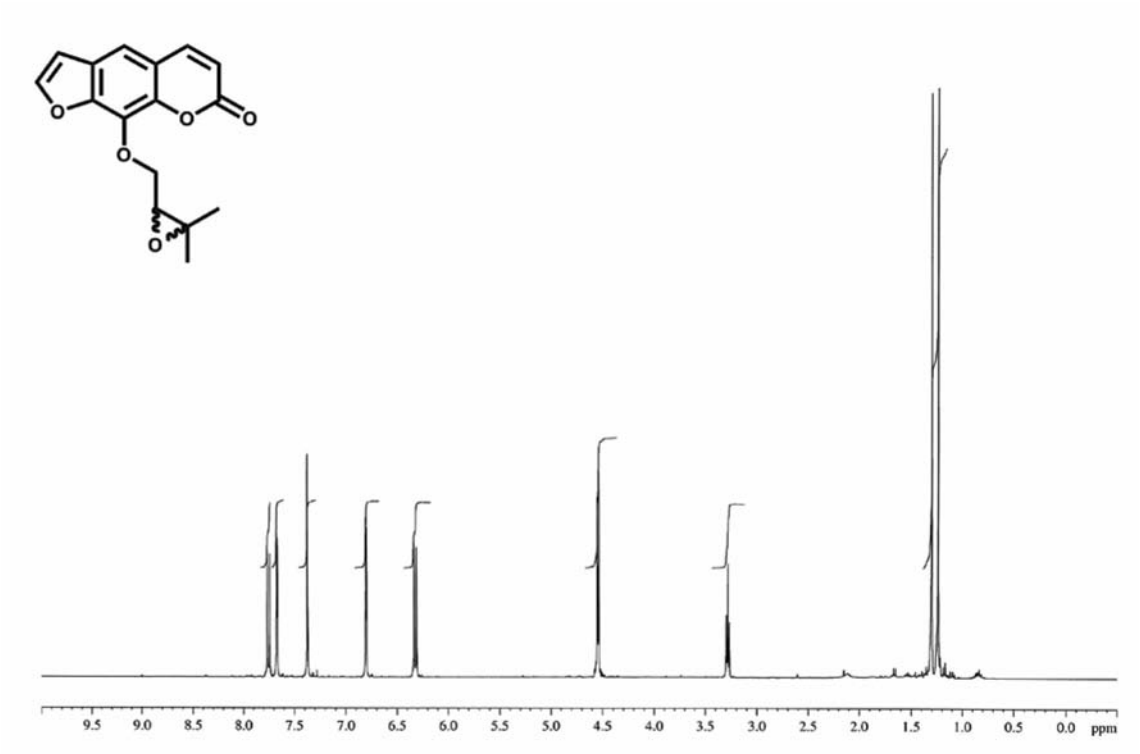

Figure S3. ^{1}H NMR spectrum of heraclenin (**3**) (400 MHz, $CDCl_3$).

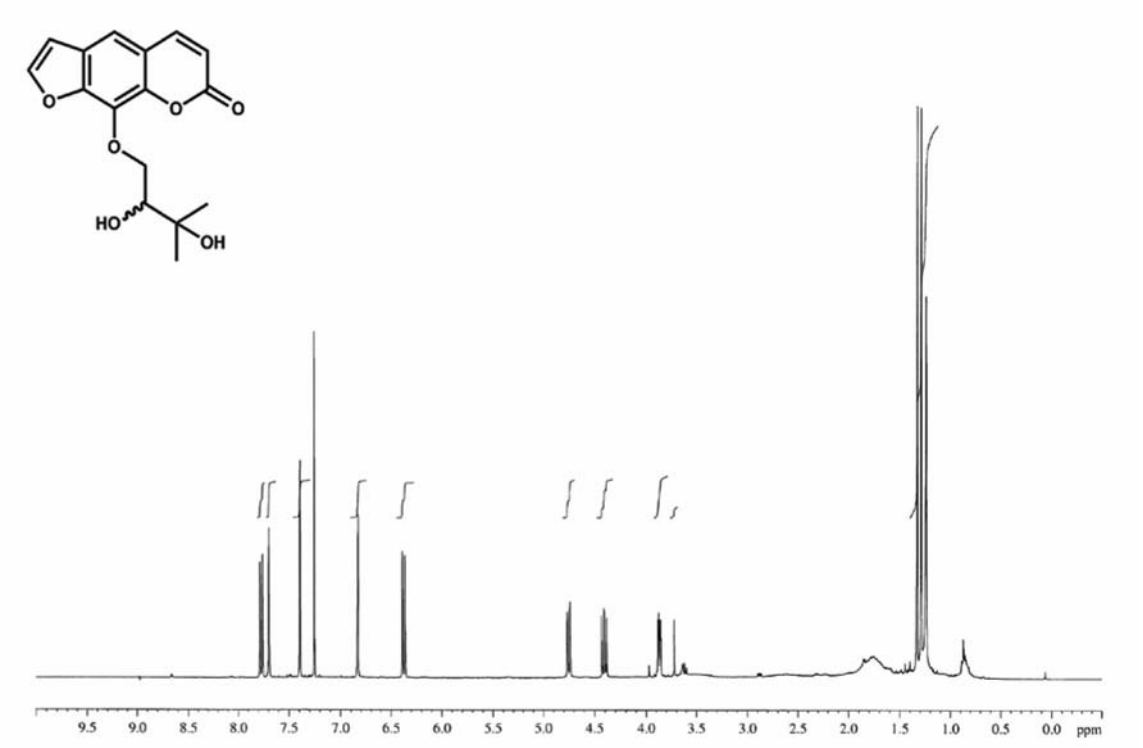

Figure S4. ^{1}H NMR spectrum of heraclenol (**4**) (400 MHz, $CDCl_3$).

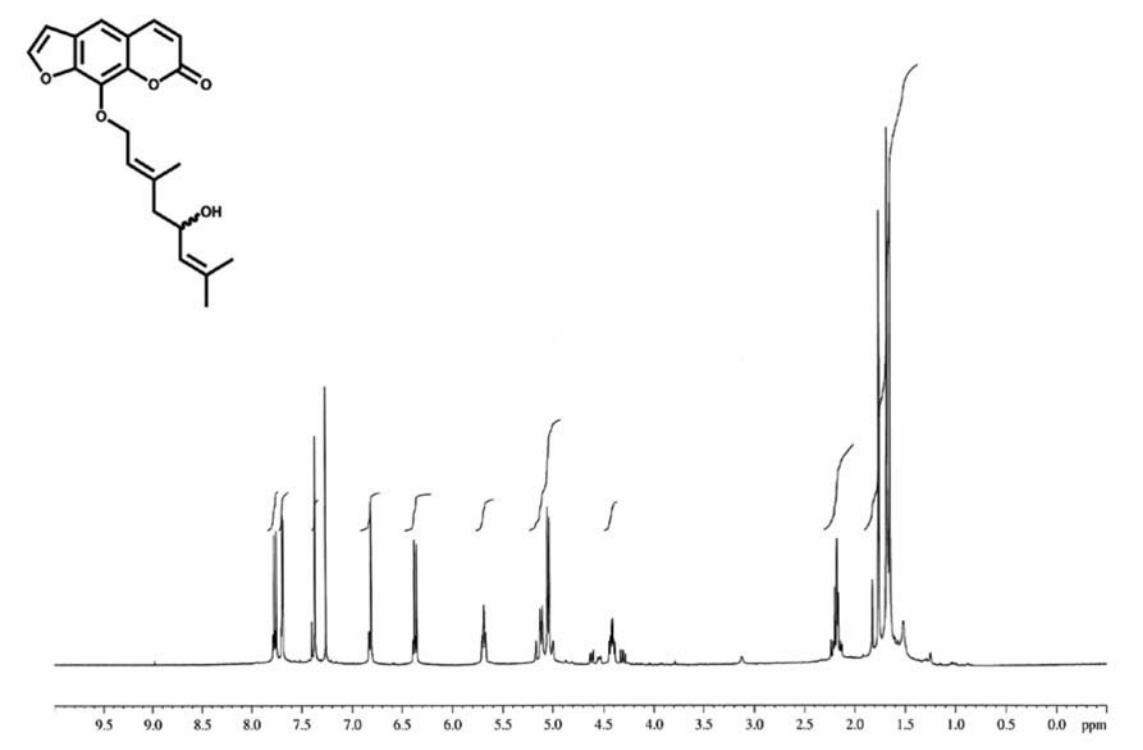

Figure S5. ^{1}H NMR spectrum of clausenalansimin A (**5**) (400 MHz, $CDCl_3$).

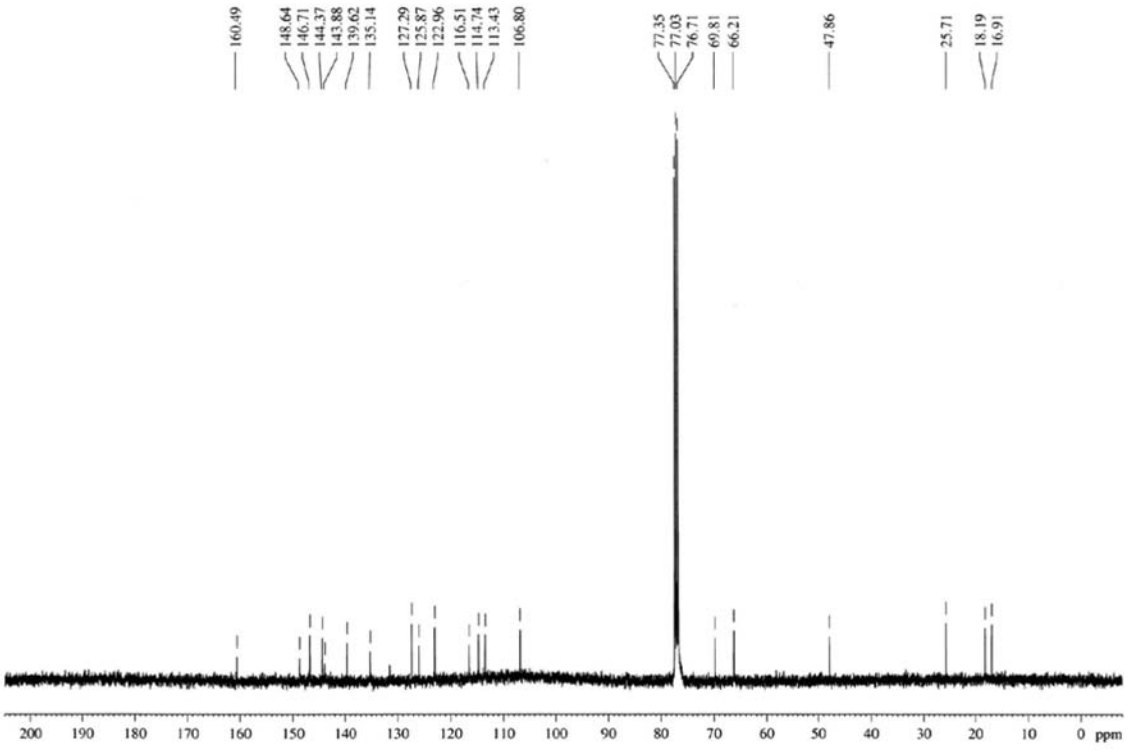

Figure S6. ^{13}C NMR spectrum of clausenalansimin A (**5**) (100 MHz, $CDCl_3$).

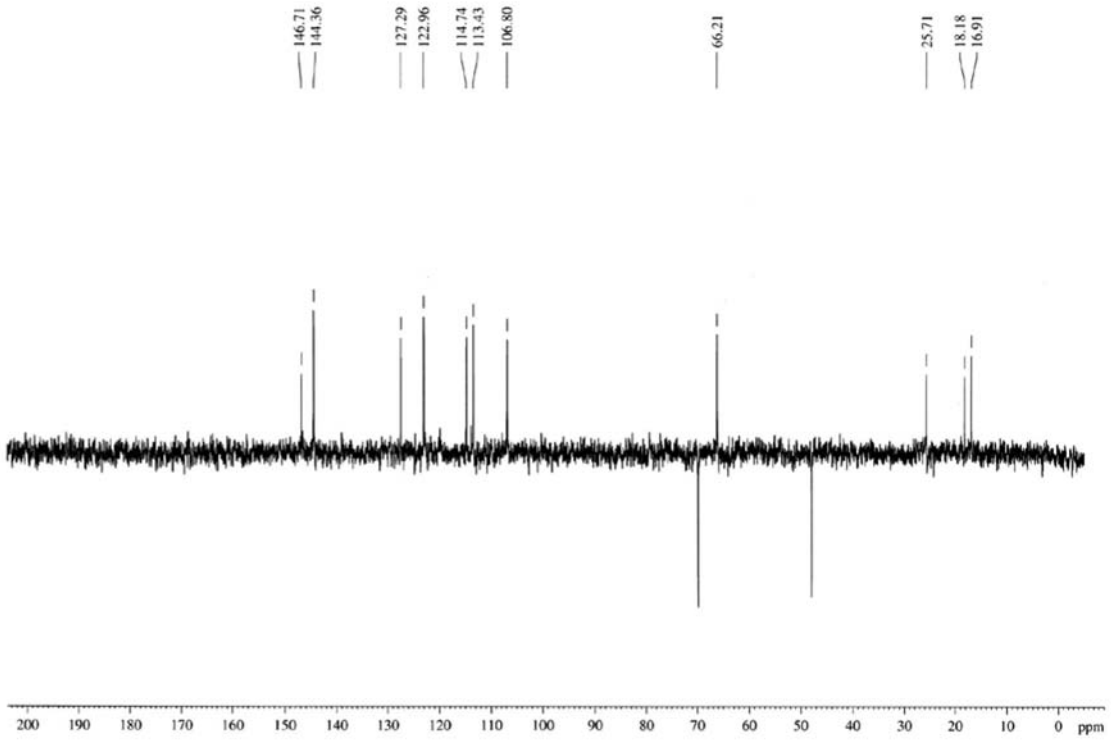

Figure S7. DEPT 135° spectrum of clausenalansimin A (**5**) (100 MHz, $CDCl_3$).

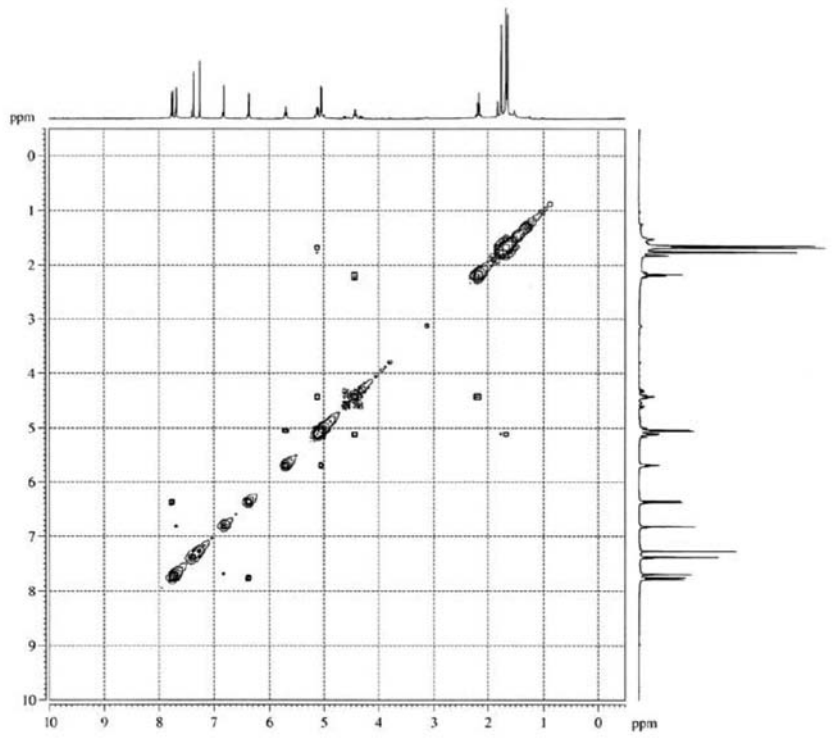

Figure S8. COSY spectrum of clausenalansimin A (**5**) (400 MHz, $CDCl_3$).

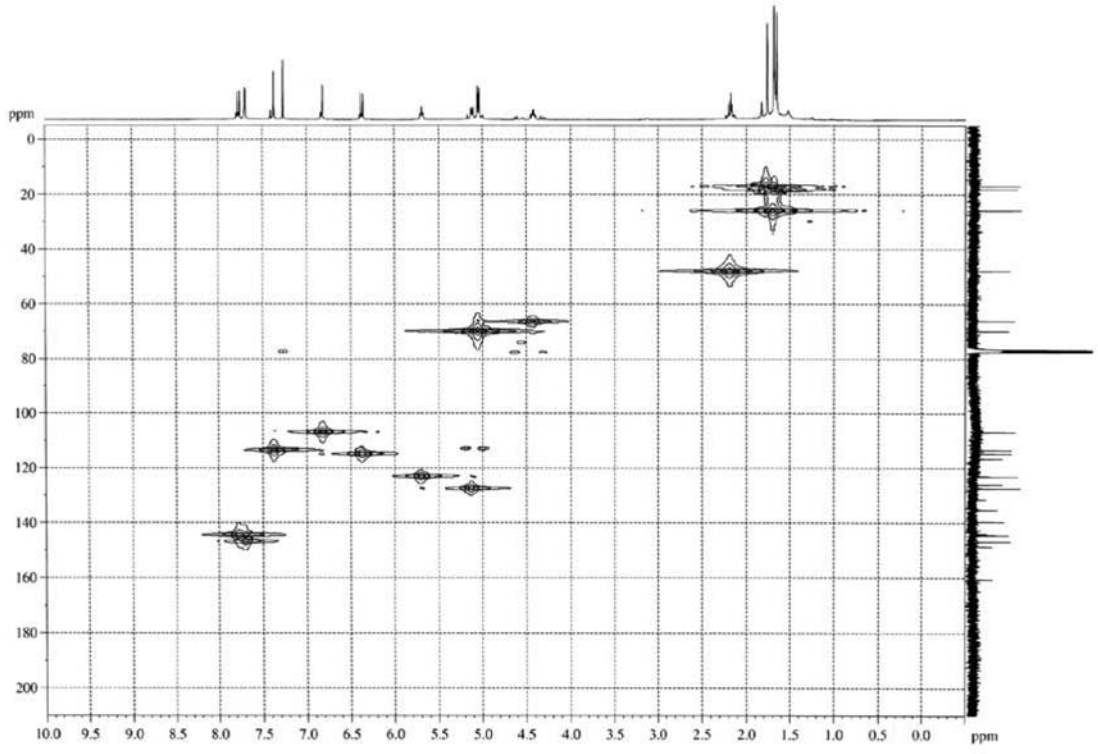

Figure S9. HMQC spectrum of clausenalansimin A (**5**) (400 MHz, $CDCl_3$).

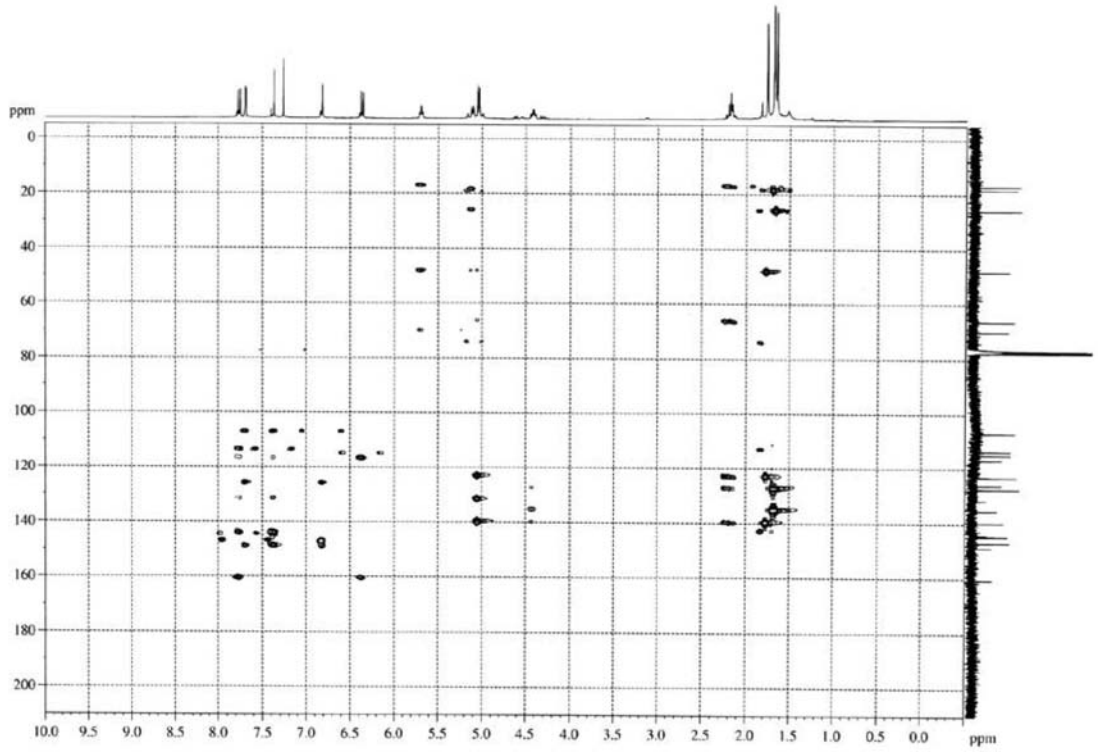

Figure S10. HMBC spectrum of clausenalansimin A (**5**) (400 MHz, $CDCl_3$).

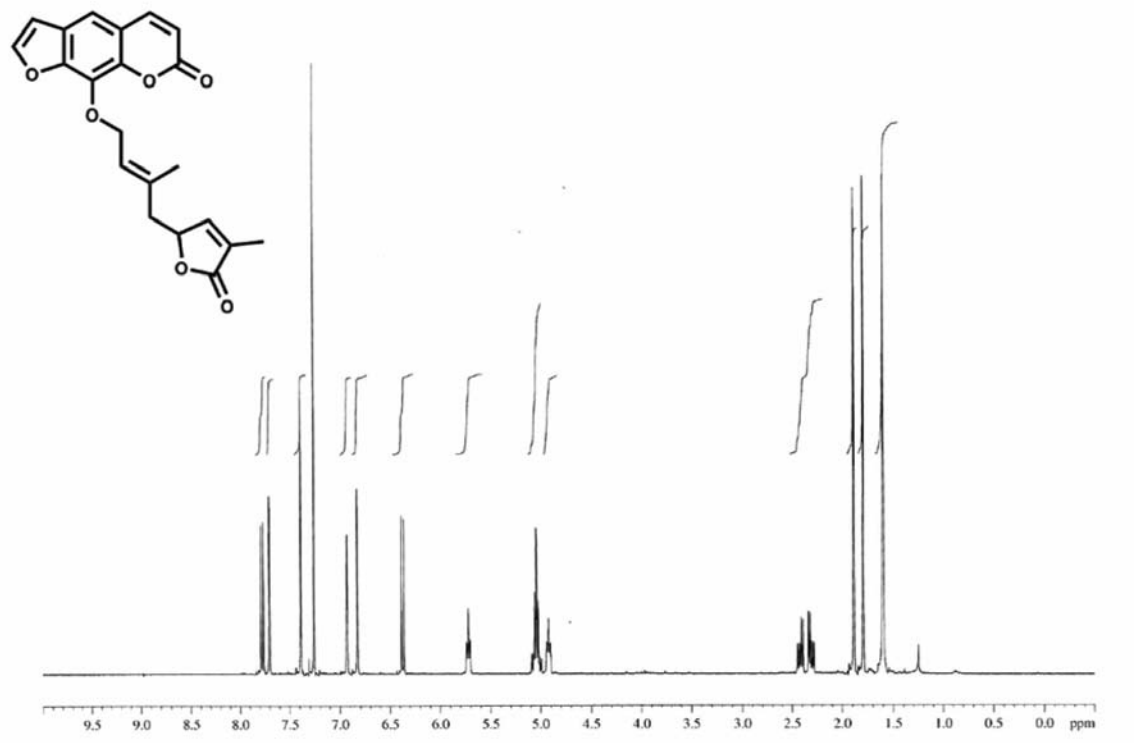

Figure S11. ^1H NMR spectrum of wampetin (**6**) (400 MHz, $CDCl_3$).

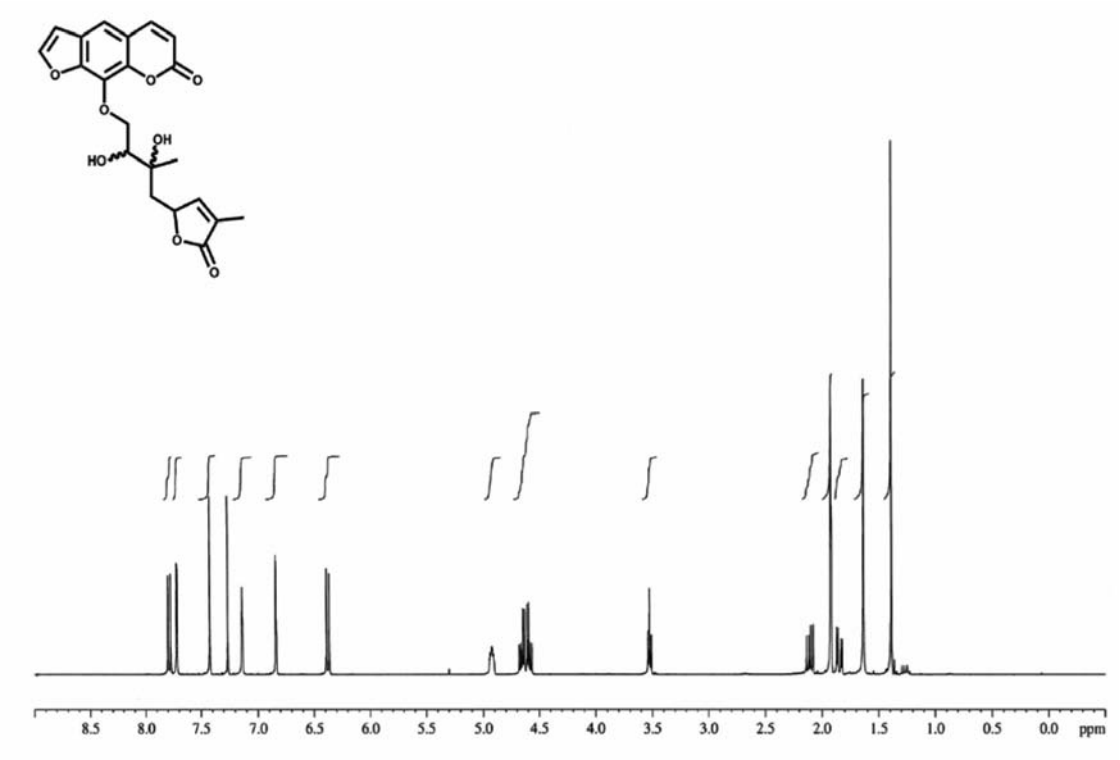

Figure S12. ^{1}H NMR spectrum of indicolactonediol (**7**) (400 MHz, $CDCl_3$).

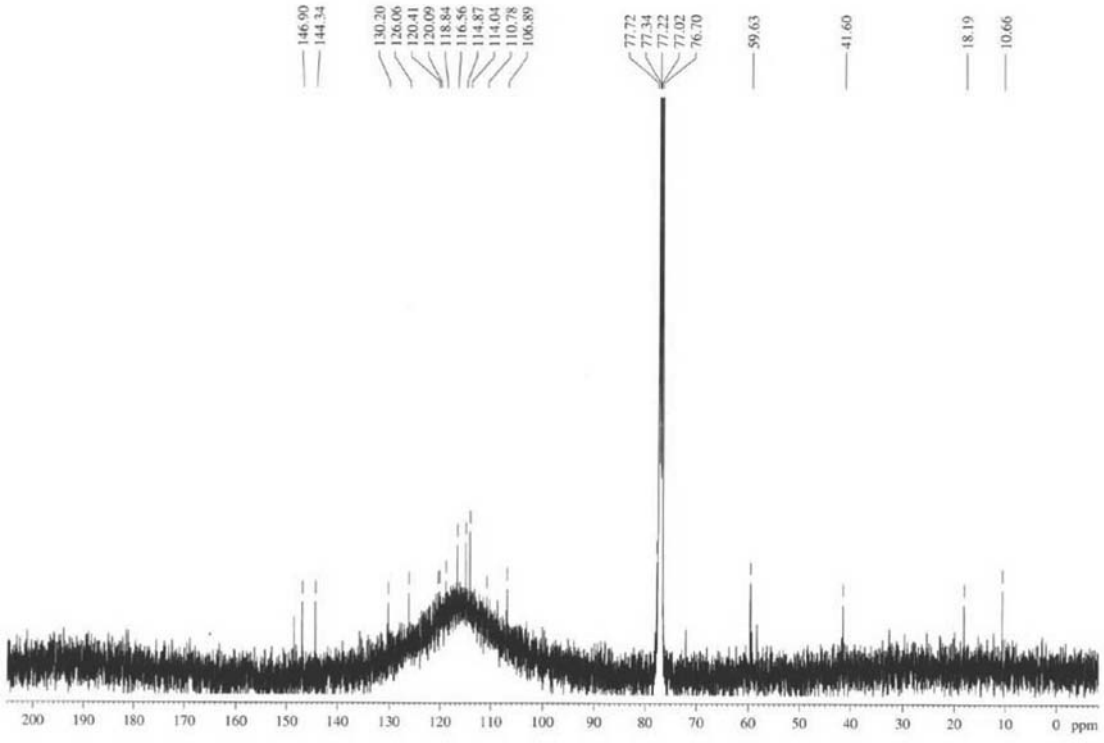

Figure S13. ^{13}C NMR spectrum of indicolactonediol (**7**) (100 MHz, $CDCl_3$).

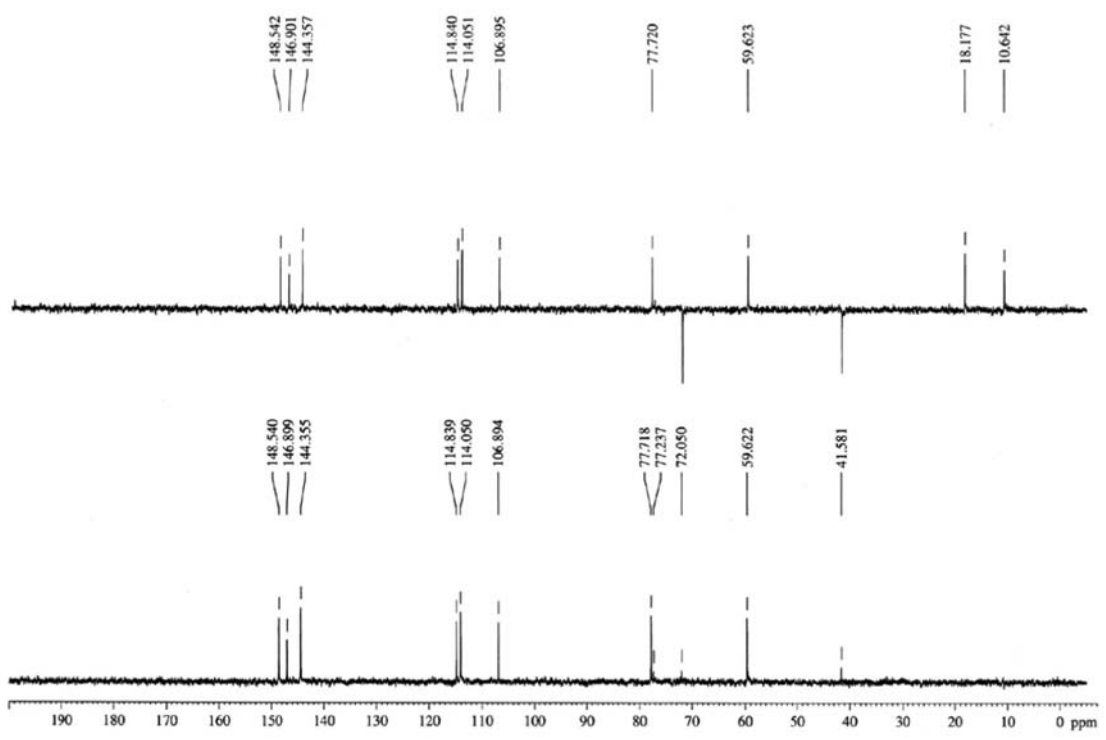

Figure S14. DEPT 135° and 90° spectrum of indicolactonediol (**7**) (100 MHz, $CDCl_3$).

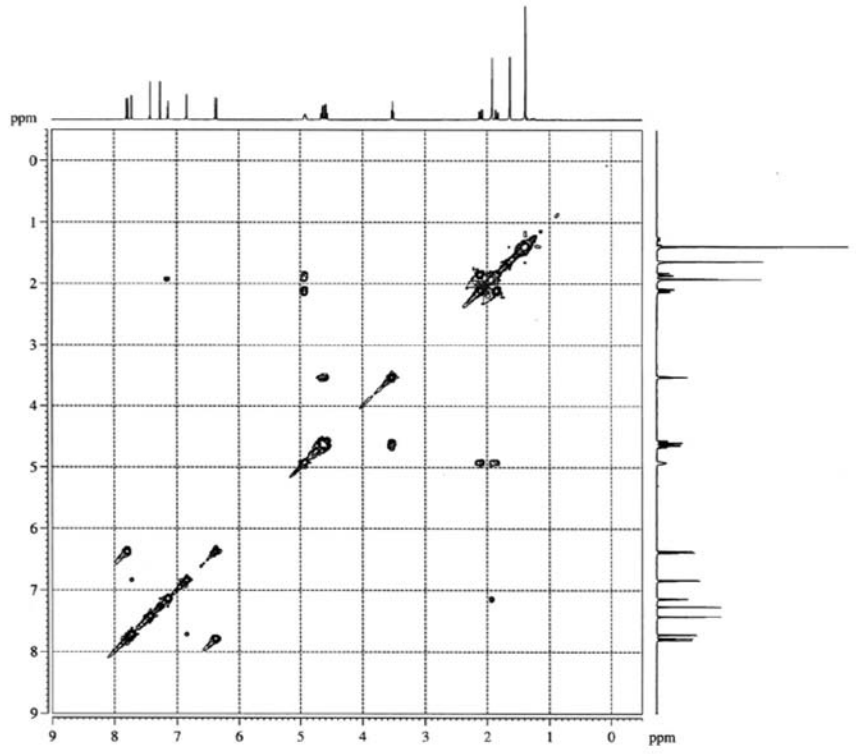

Figure S15. COSY spectrum of indicolactonediol (**7**) (400 MHz, $CDCl_3$).

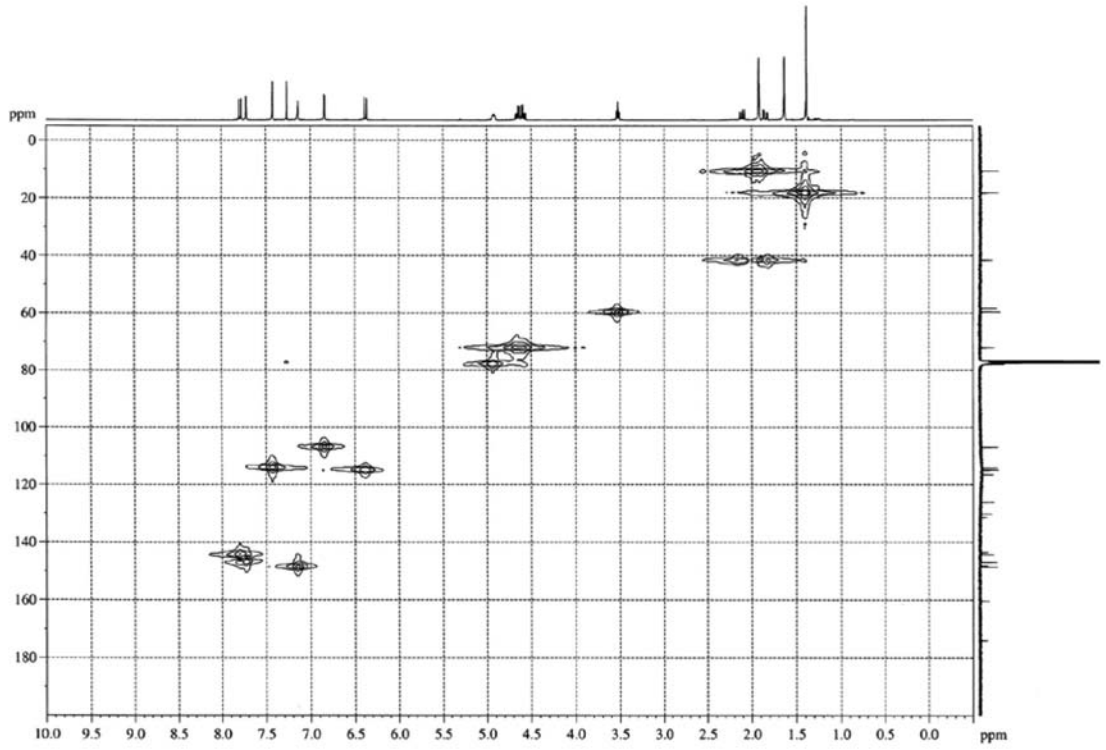

Figure S16. HMQC spectrum of indicolactonediol (**7**) (400 MHz, $CDCl_3$).

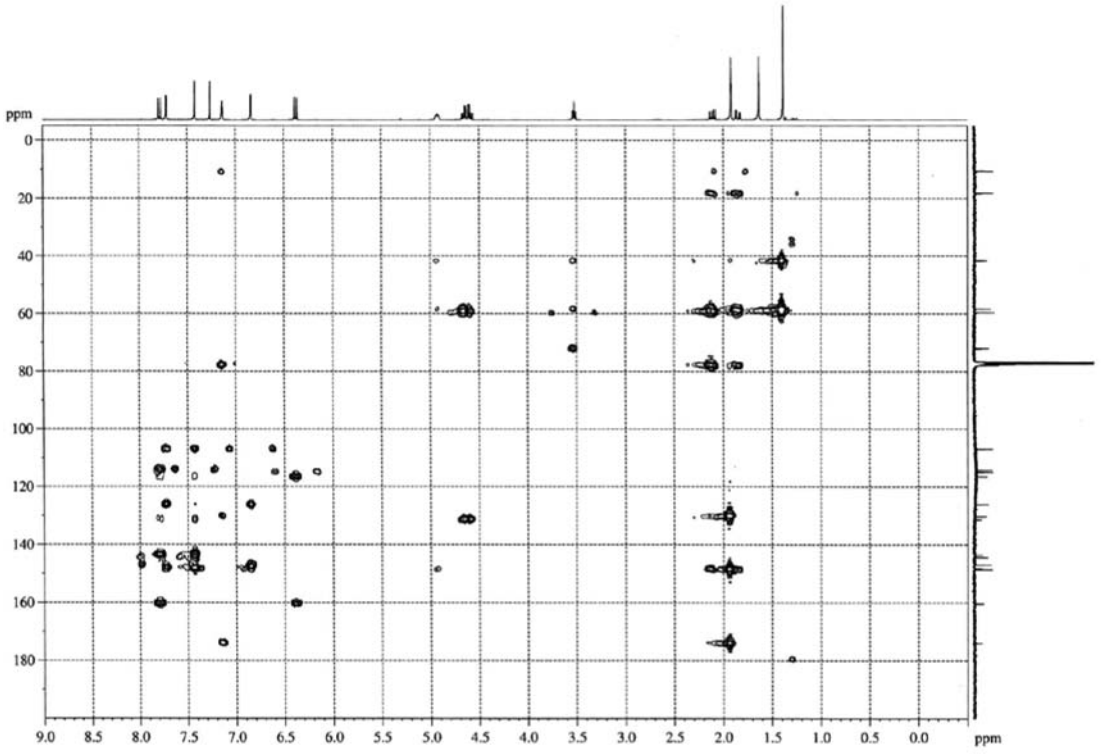

Figure S17. HMBC spectrum of indicolactonediol (**7**) (400 MHz, $CDCl_3$).

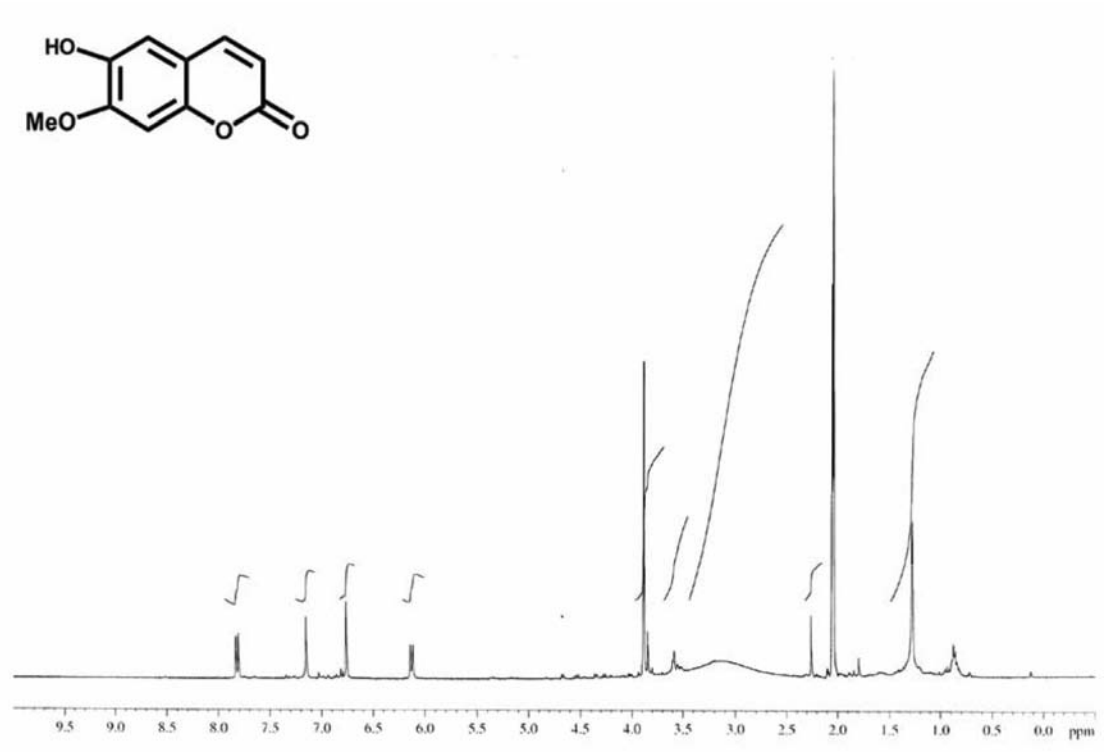

Figure S18. ^{1}H NMR spectrum of indicolactonediol (**8**) (400 MHz, Acetone-d_6).

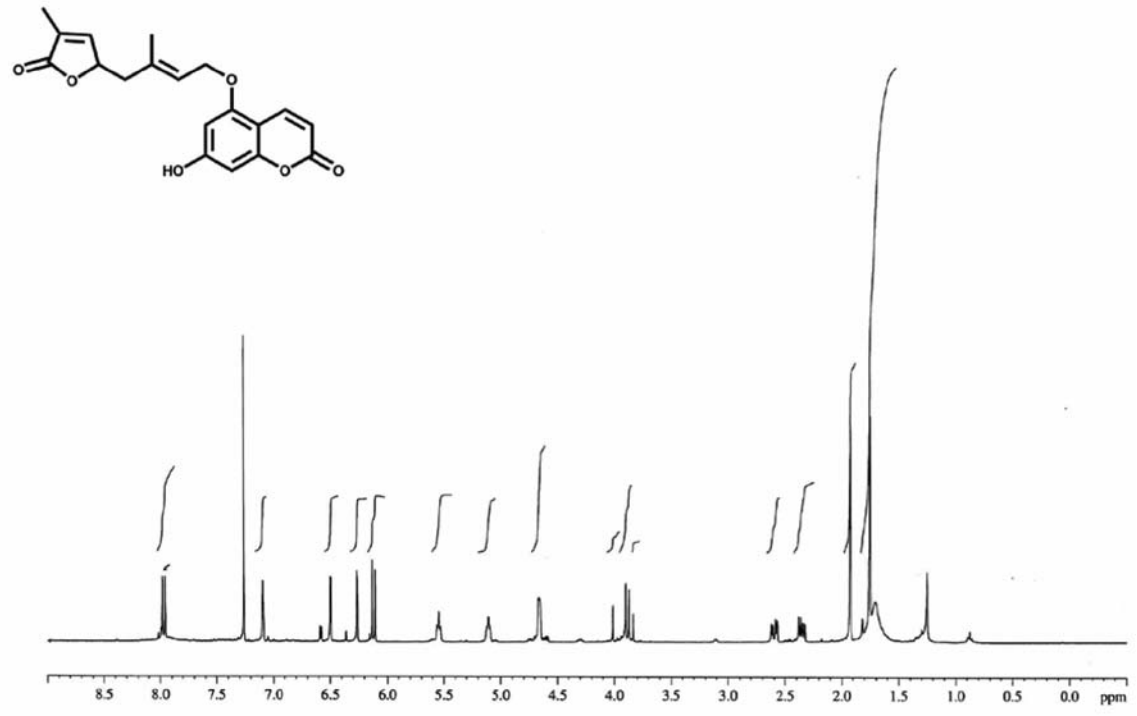

Figure S19. ^{1}H NMR spectrum of clausenalansimin B (**9**) (400 MHz, $CDCl_3$).

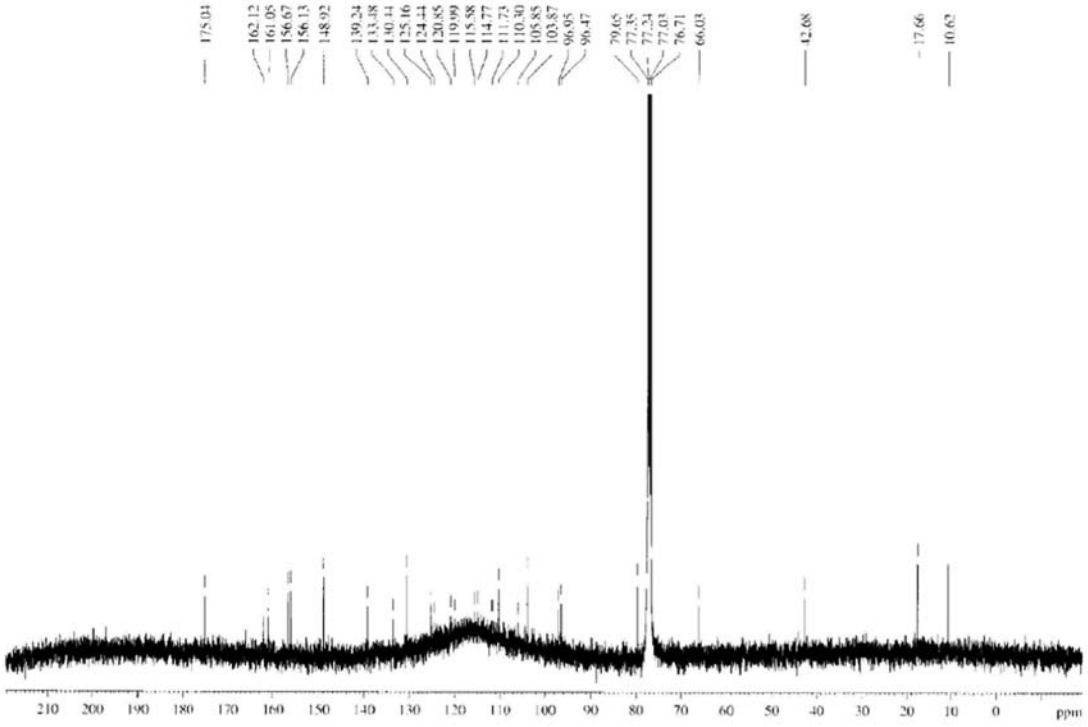

Figure S20. ^{13}C NMR spectrum of clausenalansimin B (**9**) (100 MHz, $CDCl_3$).

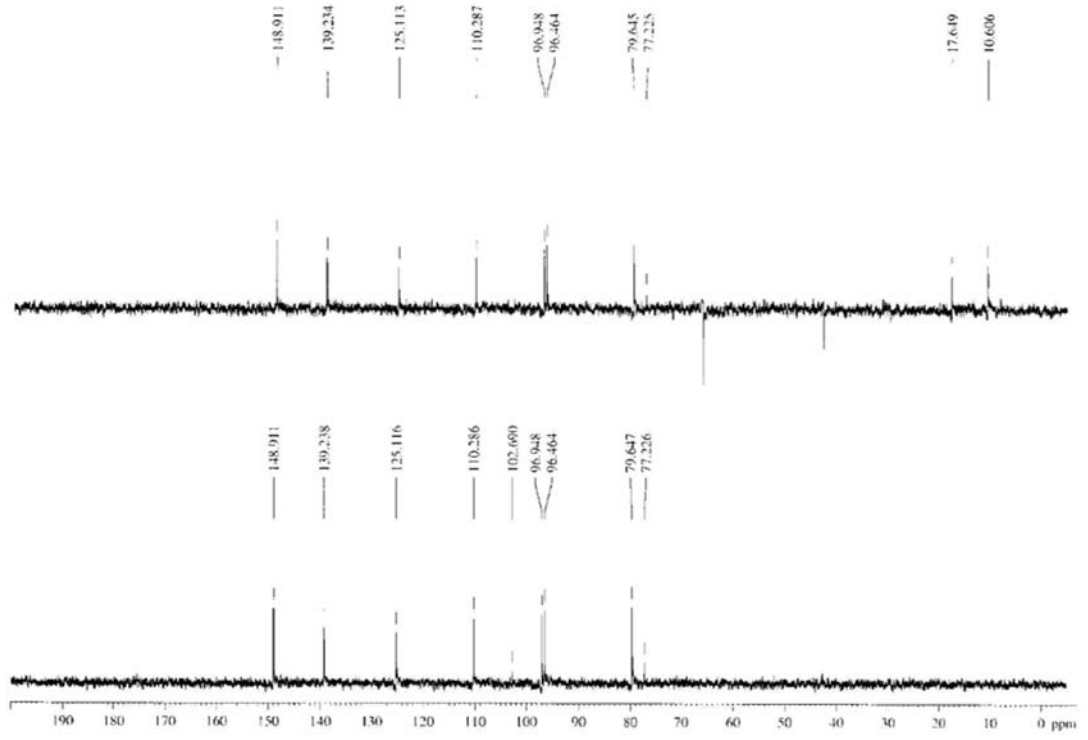

Figure S21. DEPT 135° and 90° spectrum of clausenalansimin B (**9**) (100 MHz, $CDCl_3$).

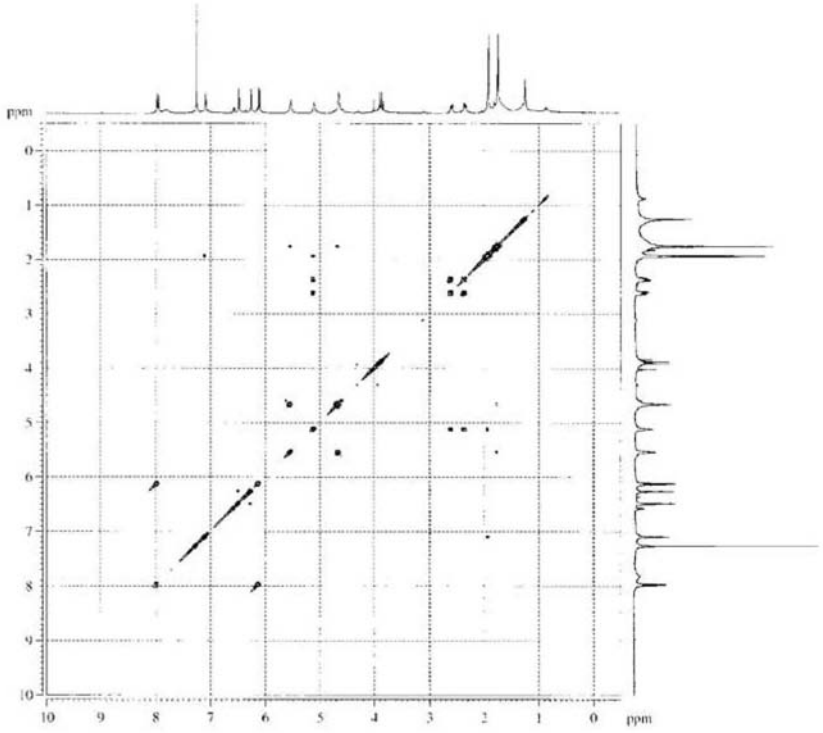

Figure S22. COSY spectrum of clausenalansimin B (**9**) (400 MHz, $CDCl_3$).

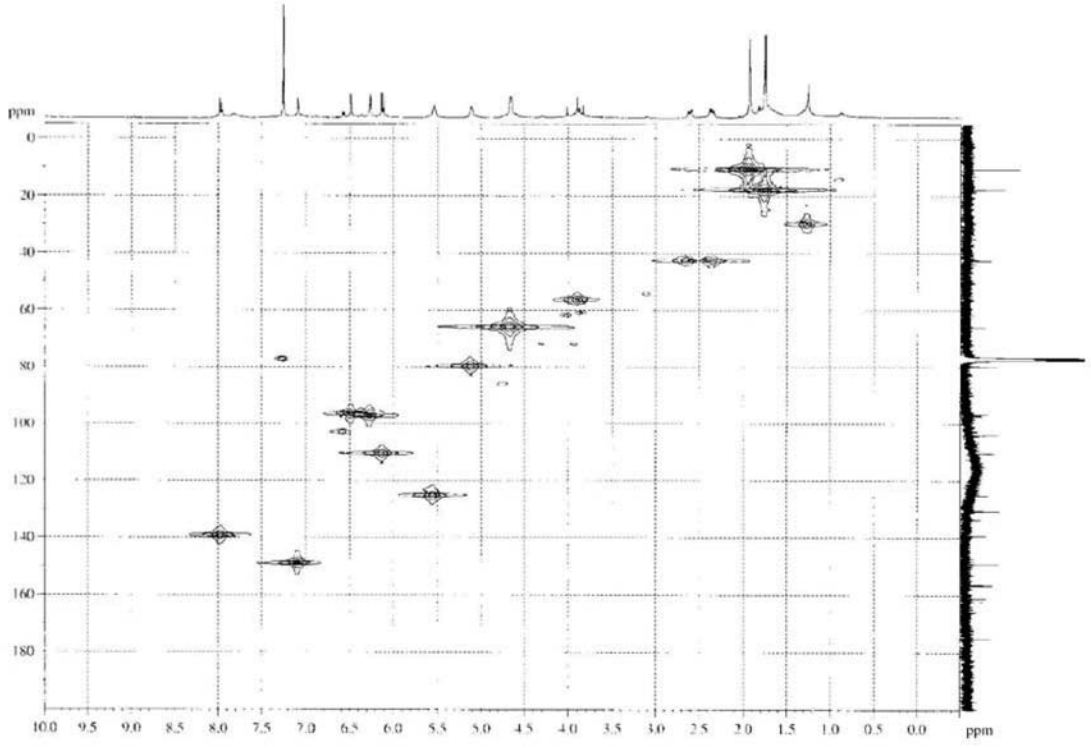

Figure S23. HMQC spectrum of clausenalansimin B (**9**) (400 MHz, $CDCl_3$).

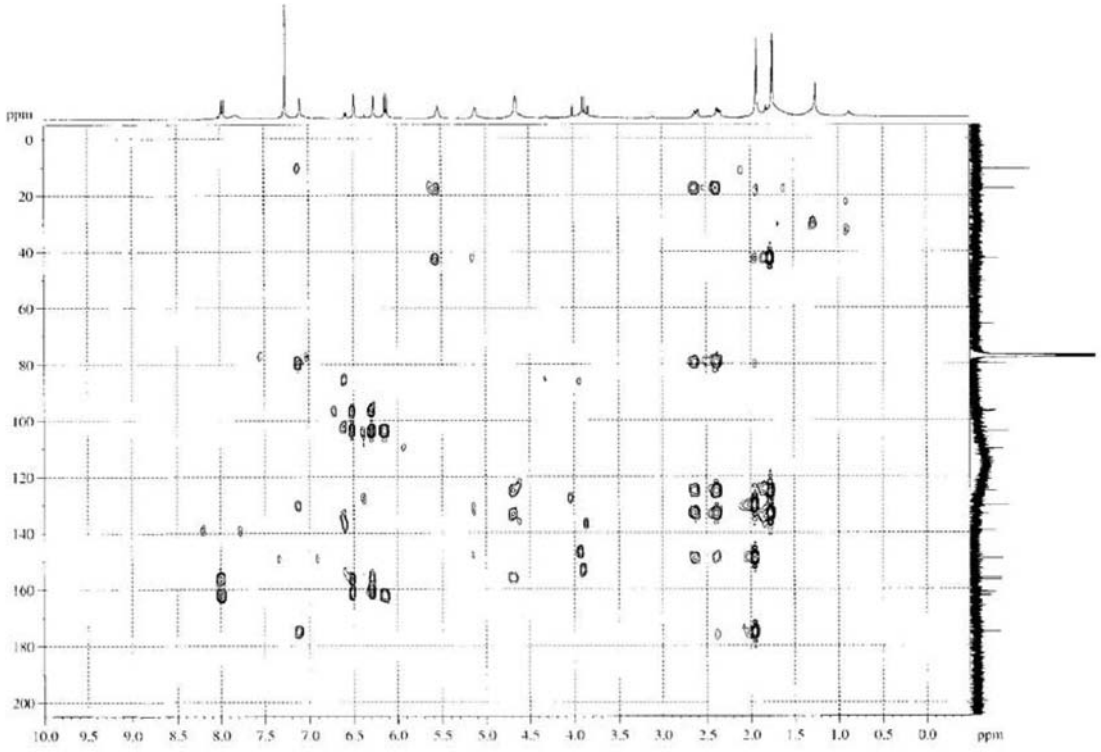

Figure S24. HMBC spectrum of clausenalansimin B (**9**) (400 MHz, $CDCl_3$).