

Chemical Constituents from *Aegle marmelos*

Surat Laphookhieo,* Chalita Phungpanya, Cholpisut Tantapakul, Somsak Techa,
Suphara Tha-in and Wanwasan Narmdorkmai

Natural Products Research Laboratory, School of Science, Mae Fah Luang University, Tasud,
Muang, Chiang Rai 57100, Thailand

Um novo produto natural derivado da oxazolina, chamado aeglemarmelosina (**1**), juntamente com oito compostos conhecidos (**2-9**), foi isolado a partir de raízes e ramos de *Aegle marmelos*. Os compostos **1-6** foram isolados das raízes e **7-9** dos galhos. Os compostos **5** e **6** também foram encontrados nos galhos. Todas as estruturas foram caracterizadas por métodos espectroscópicos de RMN uni e bidimensionais

A new natural product oxazoline derivative named aeglemarmelosine (**1**) along with eight known compounds (**2-9**) were isolated from roots and twigs of *Aegle marmelos*. Compounds **1-6** were isolated from the roots whereas compounds **7-9** were obtained from twigs. Compounds **5** and **6** were also detected from the twigs. All structures were characterized by extensive 1D and 2D NMR spectroscopic methods.

Keywords: *Aegle marmelos*, oxazoline, coumarins, alkaloids and Rutaceae

Introduction

Aegle marmelos, commonly known as “Ma Toom” in Thai, belongs to the Rutaceae family. Several parts of this plant have been used by the local Thai people in folk medicines. For example, the infusion of dried unripe fruits has been used as antidiarrhea and antidyentery agents, the juice from crushed leaves has been used for the treatment of bronchitis, and the decoction of root barks has also been used as anti-malarial drug.¹ In addition, young leaves are used as vegetable. The chemical investigation of the leaves of this plant has revealed the presence of a number of alkaloids²⁻⁷ and coumarins.^{1,8,9} In our continuing study on chemical constituents from Thai medicinal plants, we now report herein the isolation and structure elucidation of a rare natural product oxazoline derivative product (**1**) along with eight known compounds (**2-9**) (Figure 1) from the roots and twigs of *A. marmelos*.

Experimental

General procedure

UV spectra were recorded with a Perkin-Elmer UV-Vis spectrophotometer. The IR spectra were recorded

with a Perkin-Elmer FT-IR spectrophotometer. The ¹H and ¹³C NMR spectra were recorded using 400 MHz Bruker FT-NMR Ultra Shield spectrometer. Chemical shifts were recorded in parts per million (δ) in CDCl₃

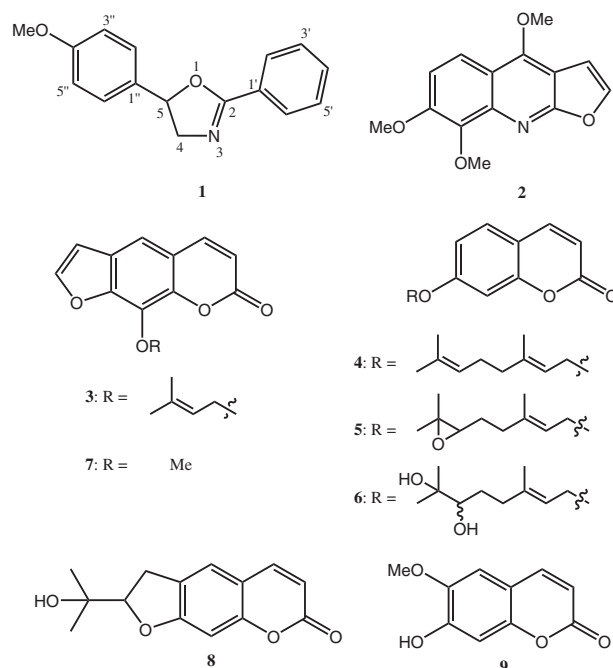


Figure 1. Compounds isolated from *A. marmelos*.

*e-mail: surat@mfu.ac.th, suratlpk@gmail.com

with tetramethylsilane (TMS) as an internal reference. High resolution mass spectra were obtained using Bruker microTOF mass spectrometer. Dried flash column chromatography 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 F₂₅₄ were used for analytical purposes.

Plant material

The roots and twigs of *A. marmelos* were collected from Chiang Rai Province, northern part of Thailand, in December 2008. Botanical identification was achieved through comparison with a voucher specimen number QBG 33029 in the Herbarium Collection of Queen Sirikit Garden, Mae Rim District, Chiang Mai, Thailand.

Extraction and isolation

The roots (160 g) and the twigs (4.5 kg) of *A. marmelos* were extracted with dichloromethane (CH_2Cl_2), over a period of 3 days, at room temperature, and evaporated under reduced pressure, to provide crude CH_2Cl_2 extracts of roots (8.40 g) and twigs (15.34 g).

The CH_2Cl_2 extract from the roots was chromatographed by dried flash column chromatography over silica gel and eluted with a gradient of hexane-EtOAc (10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, 0:10, v/v), to afford compound **2** (504 mg) and twenty-four fractions (MTC_1A-MTC_1Z). Fraction MTC_1D (48 mg) was purified by preparative TLC with acetone-hexane (1:5, v/v) to afford compound **4** (10 mg). Compound **5** (10 mg) was derived from Fraction MTC_1G (40 mg) by preparative TLC with 100% CHCl_3 . Fraction MTC_1J (36 mg) was further purified by preparative TLC with CH_2Cl_2 -hexane (4:1, v/v) to give compounds **1** (9.7 mg) and **3** (5.7 mg). Fractions MTC_1L (1.4 g) was subjected to CC (CH_2Cl_2 -hexane, 1:1, v/v) to give compounds **3** (50 mg) and **6** (165 mg).

The CH_2Cl_2 extract from the twigs was subjected to dried flash column chromatography over silica gel and eluted with a gradient of hexane-EtOAc (10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, 0:10, v/v), to afford ten fractions. Fraction AMD3 (631.5 mg) was repeated dried flash column chromatography with 100% CH_2Cl_2 , to give compounds **8** (25.2 mg) and **9** (25.6 mg). Compounds **5** (5.97 mg) and **7** (8.4 mg) were derived from fraction AMD5 (1.11 g) by repeated dried flash column chromatography with CH_2Cl_2 -hexane (3:7, v/v). Fraction AMD6 (1.37 g) was further purified by CC with MeOH- CH_2Cl_2 (1:99, v/v), to yield compound **6** (79.5 mg).

Aeglemarmelosine (1)

Orange viscous oil; $[\alpha]_{\text{D}}^{27} + 7.89^\circ$ (*c* 0.20, CHCl_3); UV (CHCl_3) $\lambda_{\text{max}}/\text{nm}$: 240; IR (neat) $\nu_{\text{max}}/\text{cm}^{-1}$: 2918, 2489, 1650, 1615, 1515, 1248, 756; $^1\text{H NMR}$ (400 MHz, CDCl_3): δ 8.10 (2H, m, H-2'/H-6'), 7.48 (1H, tt, *J* 8.0, 2.0 Hz, H-4'), 7.42 (2H, m, H-3'/H-5'), 7.28 (2H, d, *J* 8.8 Hz, H-2''/6''), 6.91 (2H, d, *J* 8.8 Hz, 3''/5''), 5.51 (1H, dd, *J* 10.0, 8.0 Hz, H-5), 4.44 (1H, dd, *J* 14.8, 10.0 Hz, H-4a), 3.99 (1H, dd, *J* 14.8, 8.0 Hz, 4b), and 3.81 (3H, s, 4''-OMe); $^{13}\text{C NMR}$ (100 MHz, CDCl_3): δ 164.0 (C-2), 159.7 (C-4''), 132.9 (C-1''), 131.4 (C-4'), 128.6 (C-1'), 128.4 (C-2' and C-6'), 128.3 (C-3' and C-5'), 127.4 (C-2'' and C-6''), 114.2 (C-3'' and C-5''), 81.1 (C-5), 62.9 (C-4) and 55.3 (4''-OMe); HR-MS (APCI, +ve) *m/z* 254.1180 [$\text{M}+\text{H}$]⁺ (calc. for $\text{C}_{16}\text{H}_{16}\text{NO}_2$, 254.1181).

Antimalarial assay

Antimalarial activity was evaluated against the parasite *Plasmodium falciparum* (K₁, mutidrug resistant), using the method of Trager and Jensen.¹⁰ Quantitative assessment of *in vitro* malarial activity was determined by means of the microculture radioisotope technique based on the method described by Desjardins *et al.*¹¹ The inhibitory concentration (IC_{50}) represented the concentration that caused 50% reduction in parasite growth which was indicated by the *in vitro* uptake of [^3H]-hypoxanthine by *P. falciparum*. The standard compound was dihydroartemisinin (IC_{50} 4.1 nmol L⁻¹).

Results and Discussion

Aeglemarmelosine (**1**) [$\alpha]_{\text{D}}^{27} + 7.89^\circ$ (*c* 0.20, CHCl_3) was isolated as an orange viscous oil. A molecular formula, $\text{C}_{16}\text{H}_{15}\text{NO}_2$ was established by HR-MS analysis of its ion peak [$\text{M}+\text{H}$]⁺ at *m/z* 254.1180 (calc. for $\text{C}_{16}\text{H}_{16}\text{NO}_2$ *m/z* 254.1181). The $^1\text{H NMR}$ spectral data of **1** (Table 1) showed the characteristic of oxazoline framework² at δ 5.51 (dd, 10.0, 8.0 Hz), 4.44 (dd, 14.8, 10.0 Hz) and 3.99 (dd, 14.8, 8.0 Hz) which were identified for H-5, and H-4a and H-4b, respectively. The remaining $^1\text{H NMR}$ spectral data could be characterized as being due to a monosubstituted aromatic ring [δ 8.10 (m, 2H, H-2' and H-6'), 7.42 (m, H-3' and H-5') and 7.48 (tt, 8.0, 2.0 Hz, H-4')], and a 4-methoxyphenyl group (1,4-disubstituted aromatic ring) [δ 7.28 (d, 8.8 Hz, 2H, H-2'' and H-6''), 6.91 (d, 8.8 Hz, 2H, H-3'' and H-5'') and 3.81 (s, 4''-OMe)]. The monosubstituted aromatic ring was connected to C-2 of the oxazoline ring because of the 2J and 3J connectivity of H-2' (δ 8.10) and H-3' (δ 7.42), respectively, to C-2 (δ 164.0) in HMBC correlation

(Figure 2), whereas the 4-methoxyphenyl group was placed on C-5 of the oxazoline ring because the H-5 (δ 5.51) and H-4 (δ 4.44 and 3.99) showed HMBC correlation to C-1'' (δ 132.9). The oxazoline derivative **1**, therefore, was identified as being aeglemarmelosine (2-phenyl-5-(4-methoxyphenyl)- Δ^2 -oxazoline) which has been reported as a synthetic compound by Callens *et al.*¹²

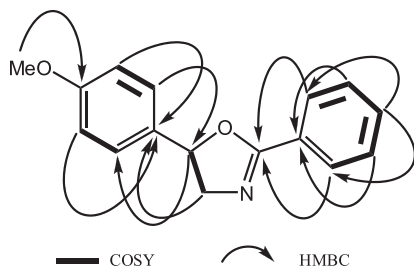


Figure 2. COSY and key HMBC correlations of aeglemarmelosine (**1**).

The remaining compounds were characterized as skimmianine (**2**),¹³ imperatorin (**3**),¹⁴ auraptin (**4**),¹⁵ epoxyauraptin (**5**),¹⁵ marmin (**6**),¹⁵ xanthotoxin (**7**),¹⁴ marmisin (**8**)¹⁶ and scopoletin (**9**)¹⁷ by using extensive 1D and 2D NMR spectroscopy and by comparing of their spectral data with reported values. All compounds were tested for their antimalarial activity but, unfortunately, they were inactive.

Supplementary Information

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

Acknowledgments

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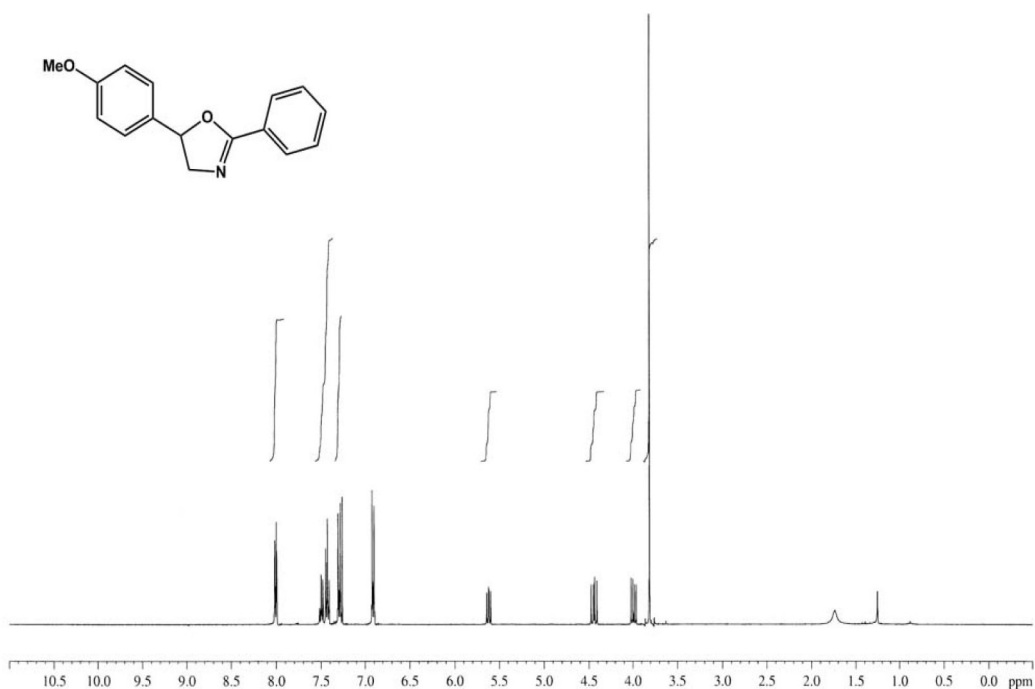


Figure S1. ¹H NMR spectrum of **1** (400 MHz, CDCl₃).

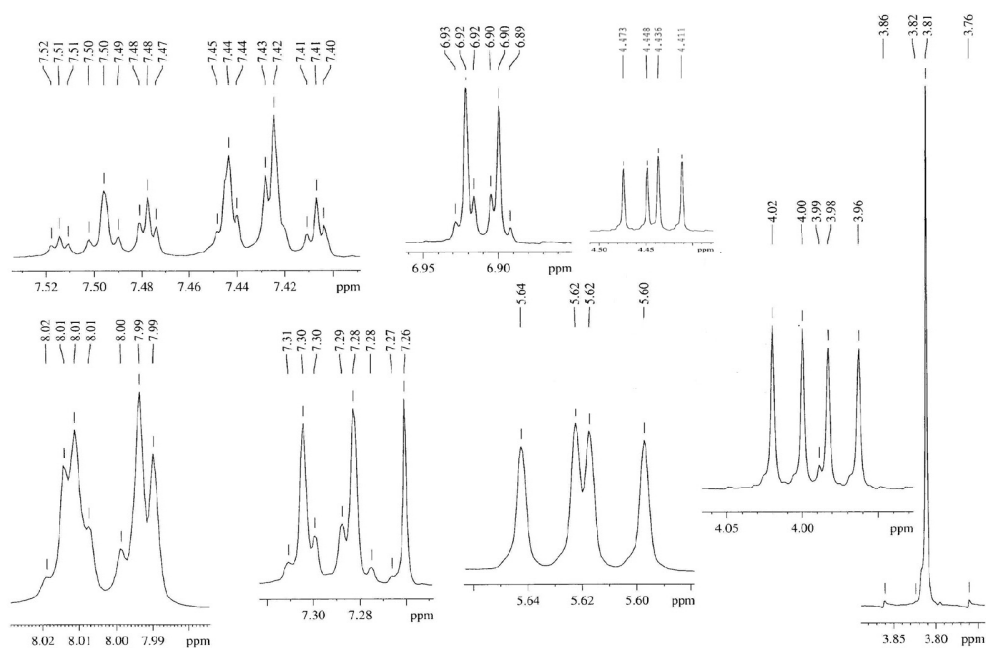


Figure S2. ¹H NMR spectrum of **1** (expanded) (400 MHz, CDCl₃).

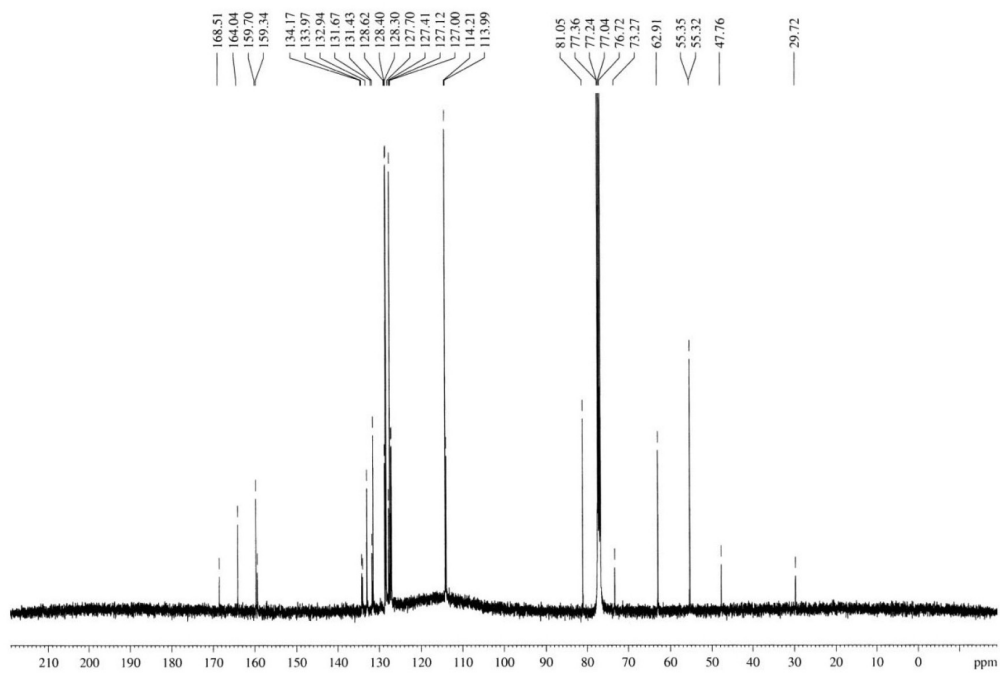


Figure S3. ¹³C NMR spectrum of **1** (100 MHz, CDCl₃).

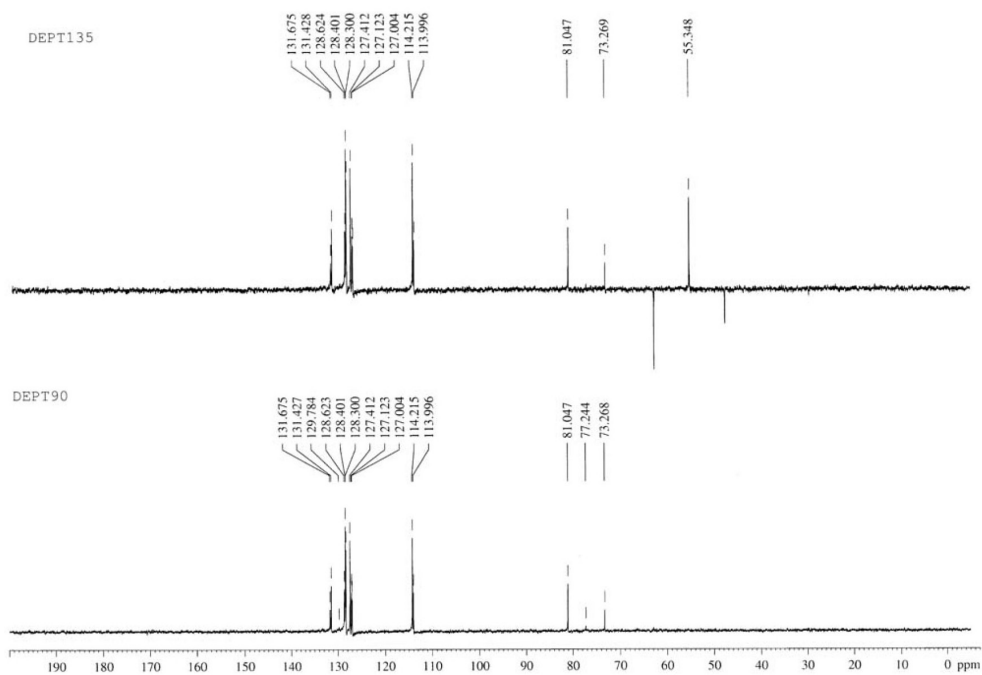


Figure S4. DEPT 135 and 90 spectra of 1.

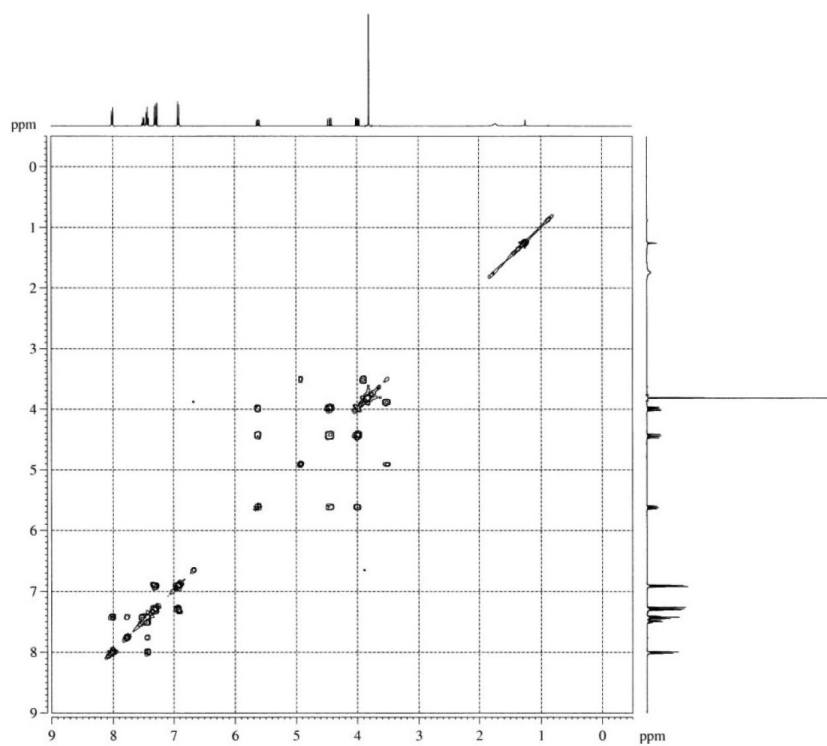


Figure S5. COSY spectrum of 1.

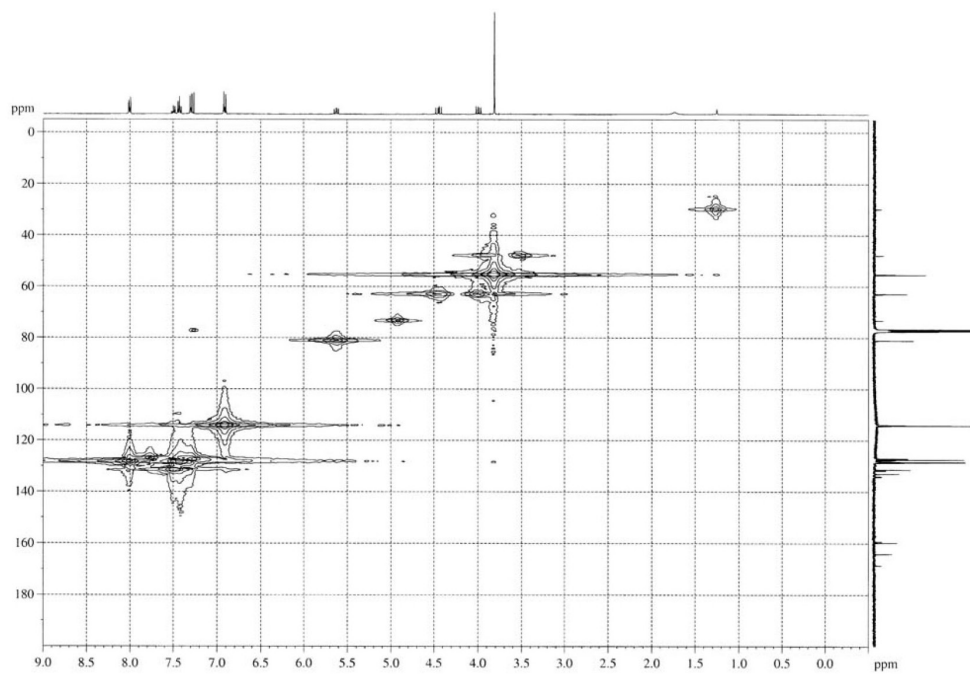


Figure S6. HMQC spectrum of **1**.

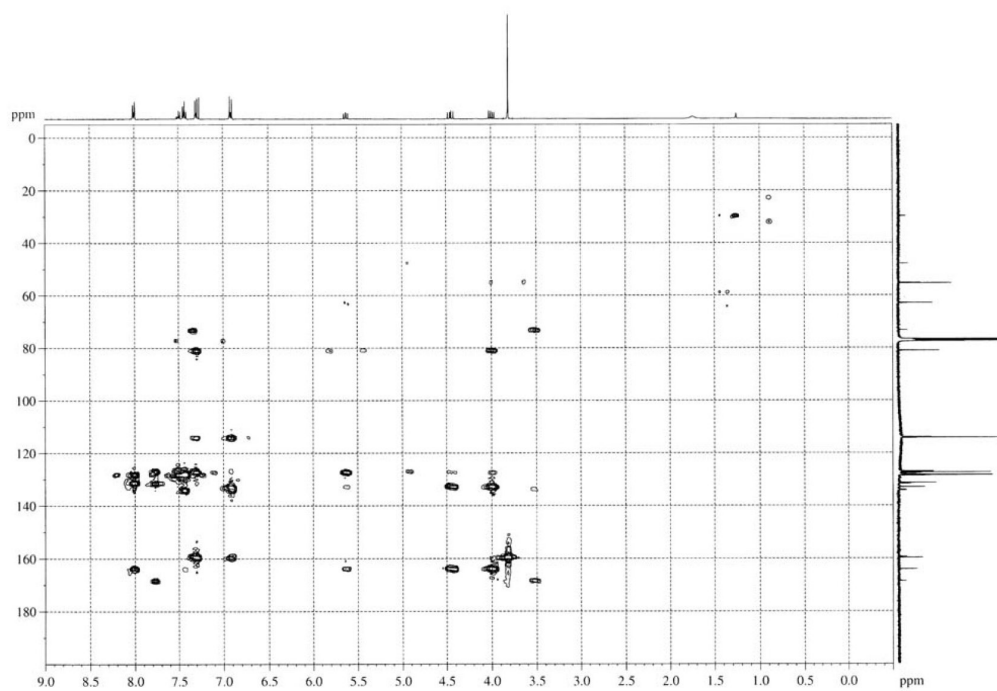


Figure S7. HMBC spectrum of **1**.

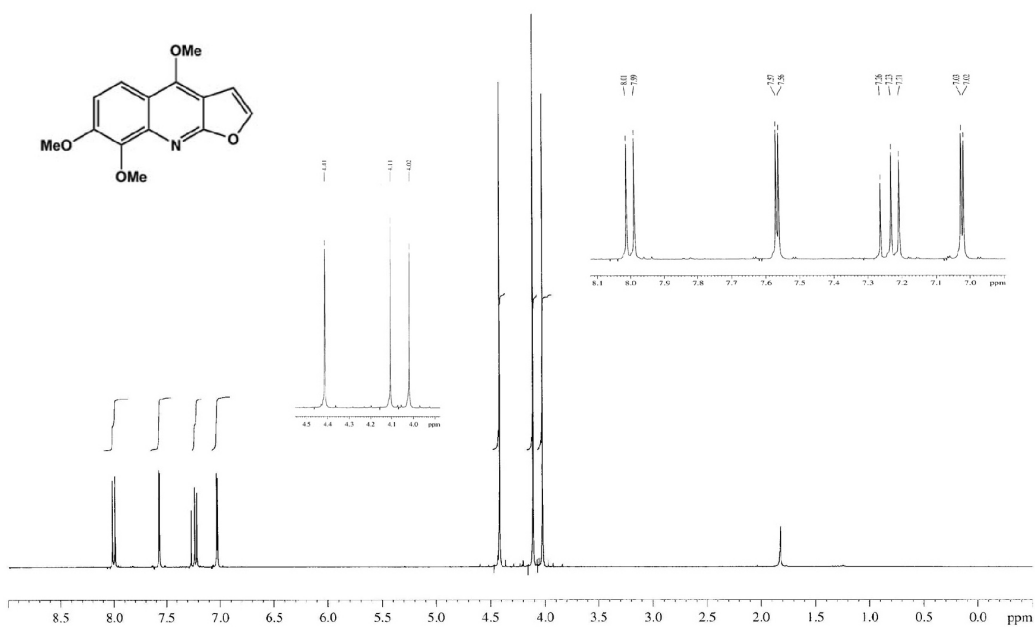


Figure S8. ¹H NMR spectrum of 2 (400 MHz, CDCl₃).

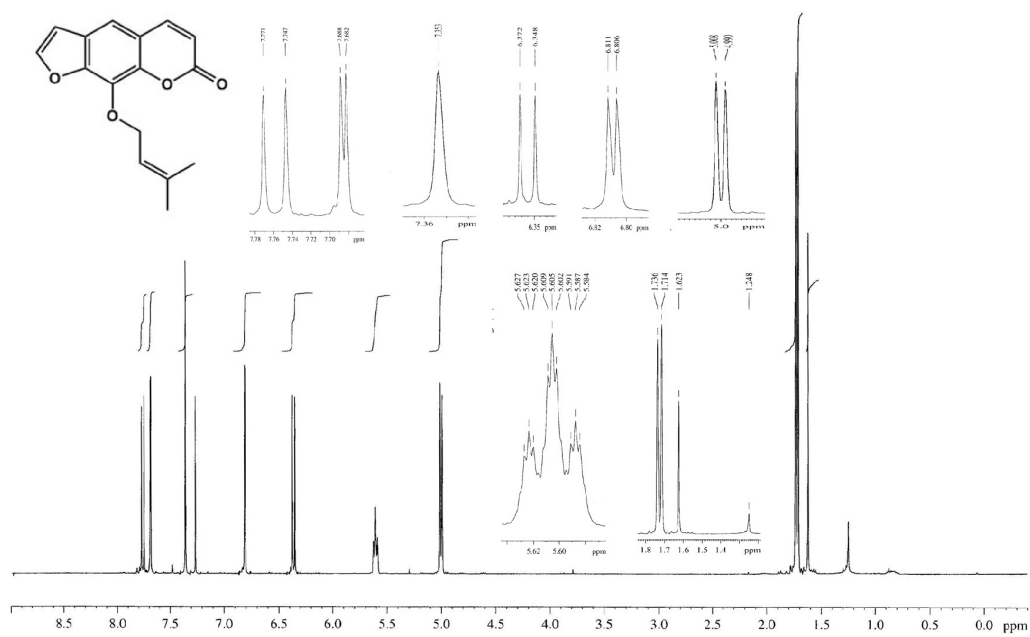


Figure S9. ¹H NMR spectrum of 3 (400 MHz, CDCl₃).

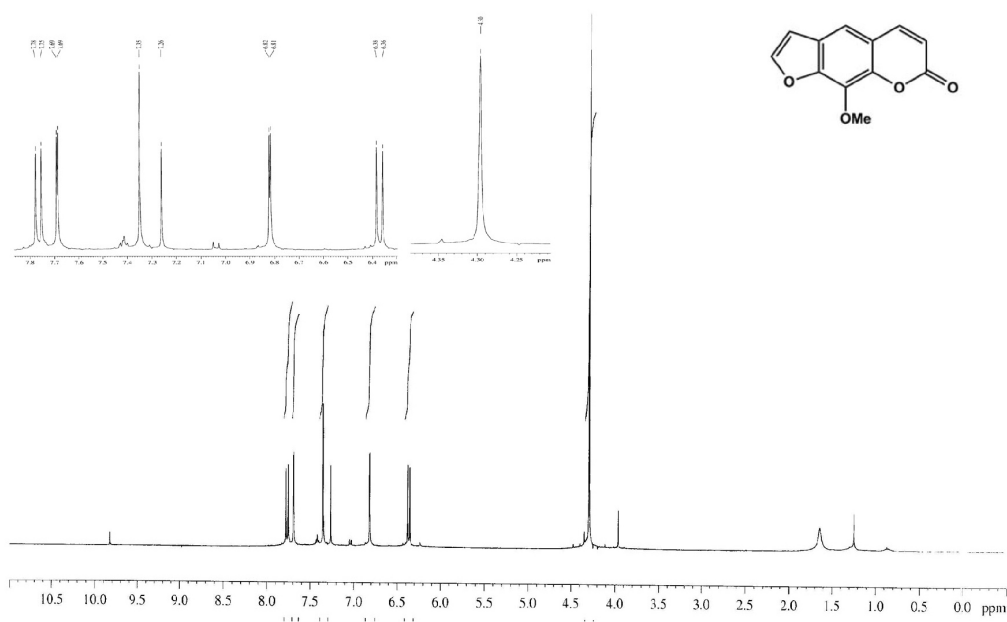


Figure S10. ¹H NMR spectrum of **4** (400 MHz, CDCl₃).

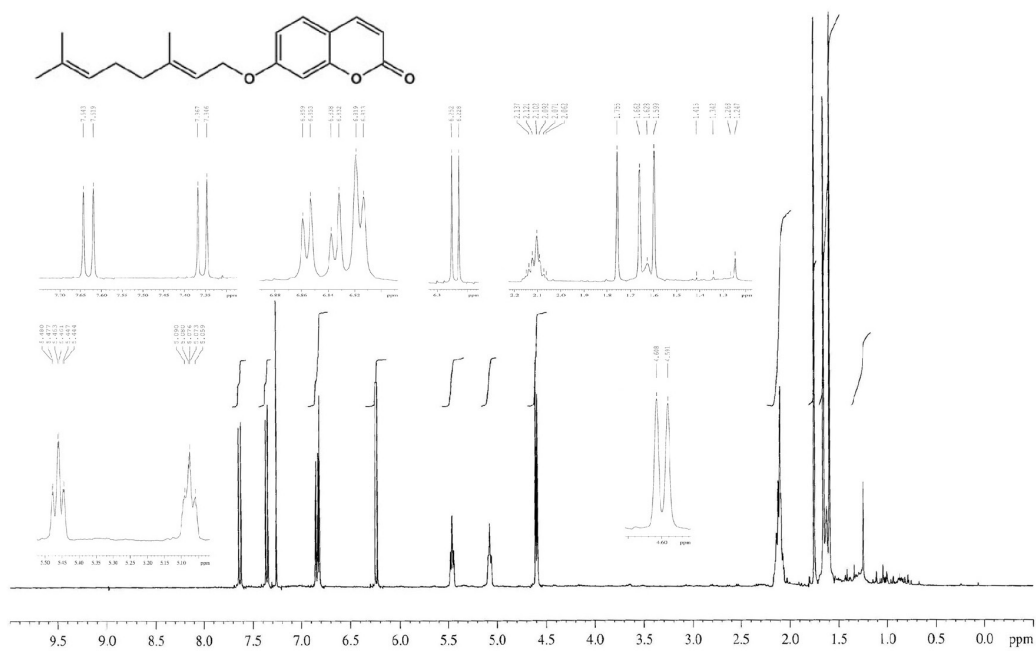


Figure S11. ¹H NMR spectrum of **5** (400 MHz, CDCl₃).

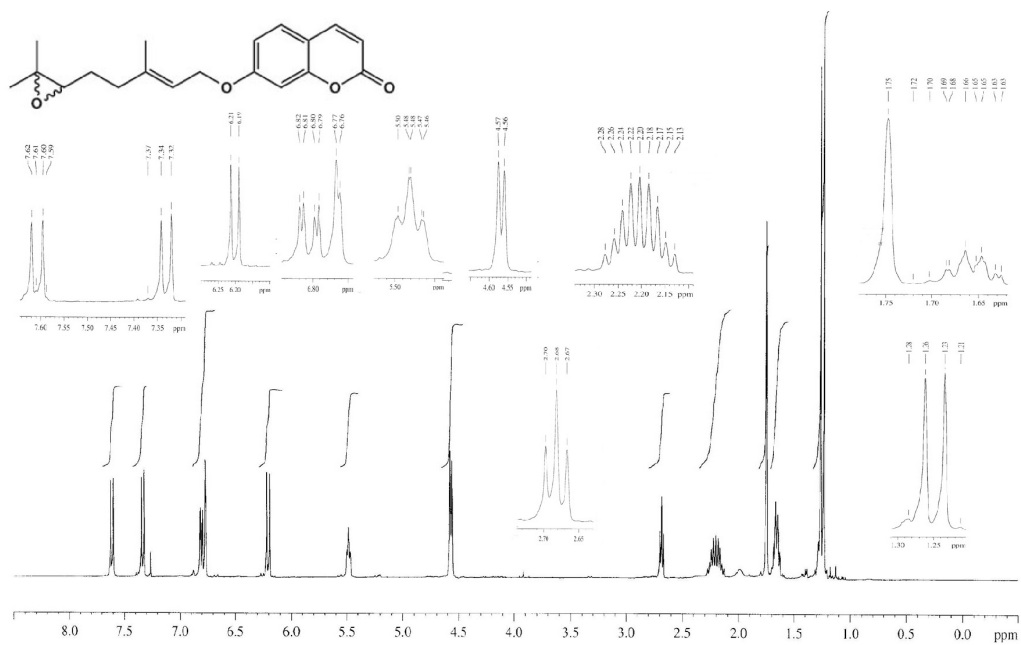


Figure S12. ¹H NMR spectrum of **6** (400 MHz, CDCl₃).

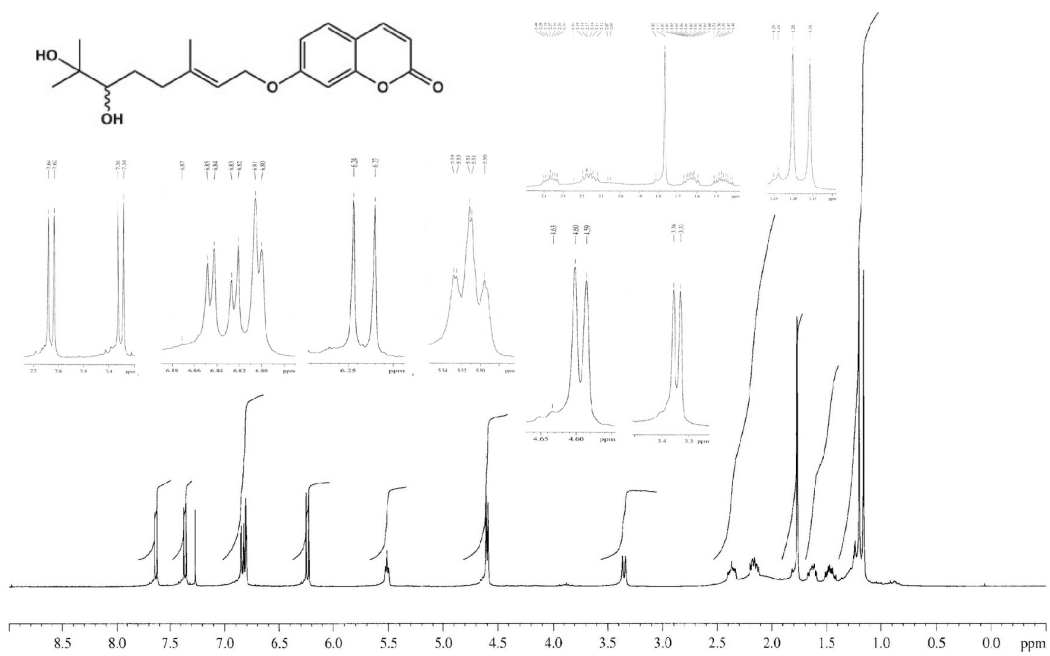


Figure S13. ¹H NMR spectrum of **7** (400 MHz, CDCl₃).

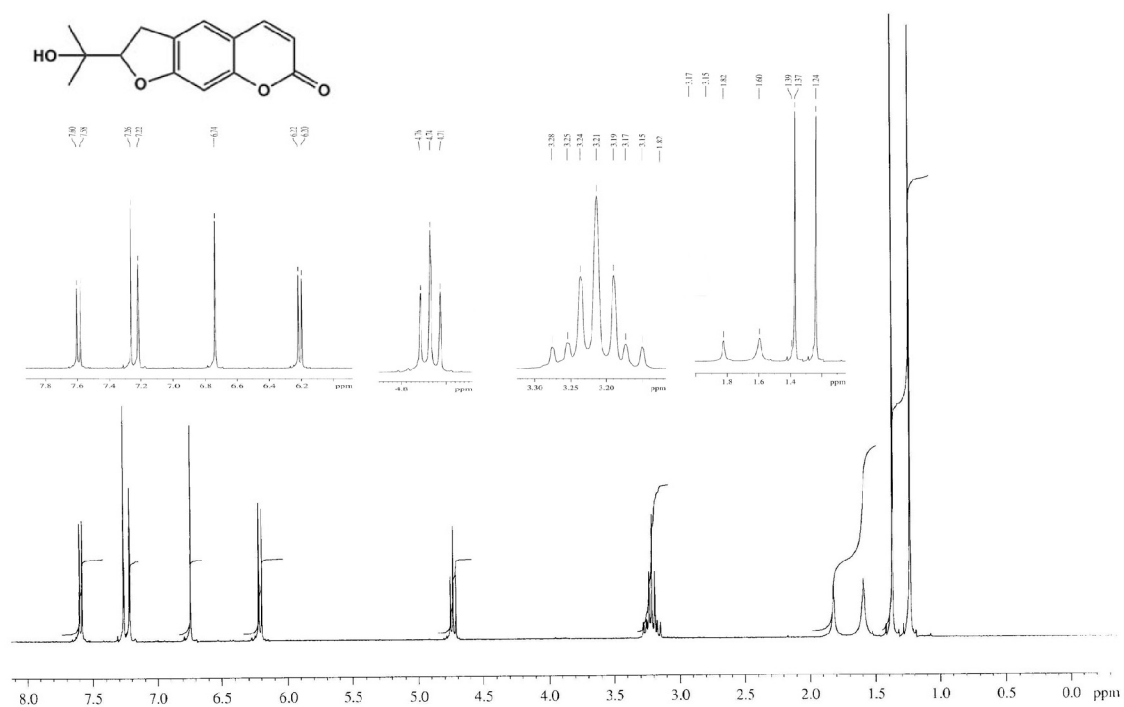


Figure S14. ¹H NMR spectrum of **8** (400 MHz, CDCl₃).

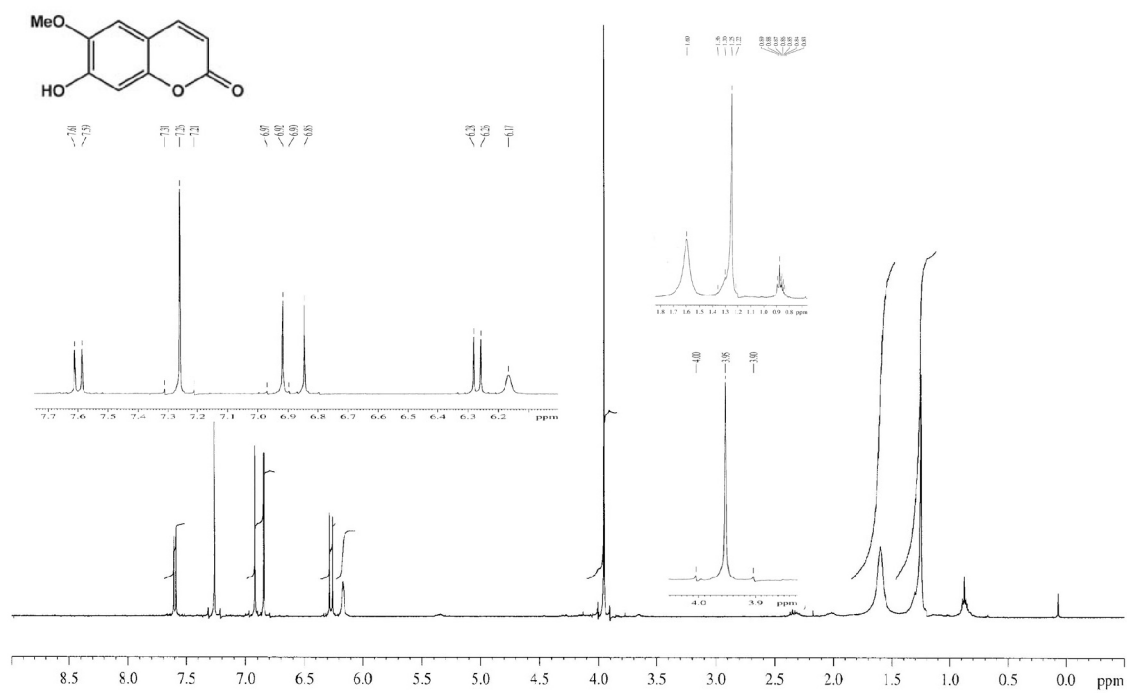


Figure S15. ¹H NMR spectrum of **9** (400 MHz, CDCl₃).