

SUPPORTING INFORMATION

Anti-inflammatory Diterpenoids from *Croton tonkinensis*

Ping-Chung Kuo,[†] Mei-Lin Yang,[‡] Tsong-Long Hwang,^{§,⊥} Yuan-Yu Lai,[†] Yue-Chiun Li,[†] Tran Dinh Thang,^{||} and Tian-Shung Wu ^{*,‡,▽,○}

[†] *Department of Biotechnology, National Formosa University, Yunlin 632, Taiwan, ROC*

[‡] *Department of Chemistry, National Cheng Kung University, Tainan 701, Taiwan, ROC*

[§] *Graduate Institute of Natural Products, Chang Gung University, Taoyuan 333, Taiwan, ROC*

[⊥] *Chinese Herbal Medicine Research Team, Healthy Aging Research Center, Chang Gung University, Taoyuan 333, Taiwan, ROC*

^{||} *Department of Chemistry, Vinh University, Vinh City, Vietnam*

[▽] *Department of Pharmacy, China Medical University, Taichung 404, Taiwan, ROC*

[○] *Chinese Medicinal Research and Development Center, China Medical University Hospital, Taichung 404, Taiwan, ROC*

Supporting Information Contents:

- S1.** Extraction and isolation of all the compounds.
- S2.** Identities and reference materials for the forty-five known compounds.
- S3.** Table S1. Inhibitory effects of crude extract and partial purified fractions of *C. tonkinensis* on superoxide anion generation and elastase release by human neutrophils in response to FMLP/CB.
- S4.** Figure S1. Significant HMBC correlations of **3-10**.
- S5.** The ^1H NMR spectrum of **3** (CDCl_3 , 400 MHz)
- S6.** The ^{13}C and DEPT NMR spectrum of **3** (CDCl_3 , 100 MHz)
- S7.** The ^1H NMR spectrum of **4** (CDCl_3 , 400 MHz)
- S8.** The ^{13}C and DEPT NMR spectrum of **4** (CDCl_3 , 100 MHz)
- S9.** The ^1H NMR spectrum of **5** (CDCl_3 , 400 MHz)
- S10.** The ^{13}C and DEPT NMR spectrum of **5** (CDCl_3 , 100 MHz)
- S11.** The ^1H NMR spectrum of **6** (CDCl_3 , 400 MHz)
- S12.** The ^{13}C and DEPT NMR spectrum of **6** (CDCl_3 , 100 MHz)
- S13.** The ^1H NMR spectrum of **7** (CDCl_3 , 400 MHz)
- S14.** The ^{13}C and DEPT NMR spectrum of **7** (CDCl_3 , 100 MHz)
- S15.** The ^1H NMR spectrum of **8** (CDCl_3 , 400 MHz)
- S16.** The ^{13}C and DEPT NMR spectrum of **8** (CDCl_3 , 100 MHz)
- S17.** The ^1H NMR spectrum of **9** (CDCl_3 , 400 MHz)
- S18.** The ^{13}C and DEPT NMR spectrum of **9** (CDCl_3 , 100 MHz)
- S19.** The ^1H NMR spectrum of **10** (CDCl_3 , 400 MHz)
- S20.** The ^{13}C and DEPT NMR spectrum of **10** (CDCl_3 , 100 MHz)
- S21.** The ^1H NMR spectrum of **11** (CDCl_3 , 300 MHz)

S1. Extraction and isolation of all the compounds.

Air-dried and powdered whole plants of *C. tonkinensis* (10.0 Kg) were extracted with MeOH (10 × 25 L) under reflux for 8 hours and concentrated to give a brown syrup (700 g). The methanol extract was suspended in MeOH-H₂O (90:10) and partitioned with *n*-hexane to afford *n*-hexane-solubles (250 g). The solvent was removed in vacuum from the MeOH-H₂O fraction, and the residue was fractionated between CH₂Cl₂ and H₂O, to afford CH₂Cl₂- (300 g), and H₂O-fractions (150 g), respectively.

The *n*-hexane-soluble fraction (CTH) was subjected to silica gel column chromatography (CC) eluted with a step gradient of *n*-hexane/acetone (100:1 to 50:50) to afford nineteen fractions (H1–H19) based on thin layer chromatography (TLC) profile. Fractions H1, H2, H4, H18, and H19 did not display significant spots so that they were not further purified. Fraction H3 was isolated by the assistance of silica gel CC and eluted with the step gradient of *n*-hexane/acetone (100:1 to 50:50) to afford four subfractions (H3-1~H3-4). Only subfraction H3-3 displayed significant spots and was applied to CC over silica gel with the step gradient eluent of benzene/acetone (100:1 to 1:1) to afford five minor fractions (H3-3-1~H3-3-5). The minor fraction H3-3-2 was recrystallized with chloroform-methanol to yield the mixture of β -sitosterol and stigmasterol (50.0 mg). The minor fraction H3-3-3 was separated by silica gel CC with chloroform/acetone (100:1) and further purified by preparative TLC (pTLC) to afford crotonkinins E (**5**) (10.0 mg) and F (**6**) (24.0 mg), respectively. The minor fraction H3-3-4 was purified by silica gel CC with benzene/acetone (100:1 to 1:1) and further purified by pTLC to afford (16*S*)-*ent*-18-acetoxy-7 β -hydroxykaur-15-one (**12**) (3.3 mg). Fraction H5 was subjected into the silica gel CC and eluted with the step gradient of *n*-hexane/ethyl acetate (300:1 to 1:1) to afford four subfractions (H5-1~H5-4). Subfraction H5-2 was purified by silica gel CC eluted with benzene and further recrystallized with acetone to yield crotonkinins D (**4**) (3.5 mg) and J (**10**) (1.7 mg). Subfraction H5-3 was applied to silica gel CC eluted with *n*-hexane/chloroform (1:1) and further recrystallized with chloroform-methanol to yield the mixture of stigmasta-4-en-3-one and stigmasta-4,22-dien-3-one (2.0 mg), and crotonkinins I (**9**) (1.2 mg), respectively.

Fractions H6-H10 displayed similar spots under TLC monitoring and were combined. The combined samples were applied to CC over silica gel with the step gradient of *n*-hexane/ethyl acetate (20:1 to 1:1) to afford five subfractions (H6-1~H6-5). Only subfraction H6-4 displayed significant spots and was purified with silica gel CC eluted with the step gradient of *n*-hexane/acetone (100:1 to 1:1) to afford three minor fractions (H6-4-1~H6-4-3). The minor fraction H6-4-2 was further purified by pTLC with benzene/ethyl acetate (300:1) to yield crotonkinin H (**8**) (20.0 mg). Fraction 11 was isolated with the aid of silica gel CC and eluted with the step gradient of *n*-hexane/acetone (50:1 to 1:1) to afford five subfractions (H11-1~H11-5). The subfraction H11-3 was further purified by CC over silica gel eluted with *n*-hexane/ethyl acetate (20:1) and one of the minor fraction (H11-3-3) was recrystallized with acetone to yield 3-acetylaleuritolic acid (48.5 mg). Fraction 12 was applied to the silica gel CC and eluted with the step gradient of *n*-hexane/acetone (50:1 to 1:1) to afford four subfractions (H12-1~H12-4). The subfraction H12-3 was recrystallized with acetone to afford mixture of stigmastan-3-one and stigmast-22-en-3-one (5.7 mg).

Fractions H13 and H14 were combined and isolated by silica gel CC eluted with the step gradient of *n*-hexane/acetone (20:1 to 1:1) to afford five subfractions (H13-1~H13-5). Subfraction H13-3 was chromatographed over silica gel by benzene/ethyl acetate (30:1) and further purified by pTLC with benzene/acetone (30:1) to yield crotonkinin A (**1**) (4.6 mg). Subfraction H13-4 was purified by silica gel CC by *n*-hexane/acetone (10:1) and one of the minor fraction was recrystallized with chloroform-methanol to yield *ent*-18-acetoxy-7 α -hydroxykaur-16-en-15-one (**13**) (75.7 mg). Fraction H15 was silica gel column chromatographed by step gradient of benzene/acetone (50:1 to 1:1) to yield four subfractions (H15-1~H15-4). Subfraction H15-2 was isolated by the silica gel CC eluted by step gradient of chloroform/acetone (50:1 to 1:1) to afford five minor fractions (H15-2-1~H15-2-5). The minor fraction H15-2-2 was recrystallized with chloroform-methanol to yield *ent*-1 β -acetoxy-7 α , 14 β -dihydroxykaur-16-en-15-one (**14**) (34.9 mg). H15-2-3 was further purified by pTLC eluted with chloroform/methanol (300:1) to afford *ent*-14 β -acetoxy-7 α -hydroxykaur-16-en-15-one (**15**) (1.0 mg). H15-2-4 and H15-2-5 were also purified by pTLC eluted with *n*-hexane/acetone (5:1) to afford crotonkinin C (**3**) (1.5 mg) and ergosterol peroxide (1.5 mg), respectively. Subfraction H15-3 was subjected to silica gel CC and eluted by step gradient of chloroform/acetone (50:1 to 1:1) and one of the

four minor fractions (H15-3-3) was further purified by pTLC eluted with chloroform/ethyl acetate (10:1) to afford *ent*-7 α ,14 β -dihydroxykaur-16-en-15-one (**16**) (4.3 mg). Subfraction H15-4 was isolated by silica gel CC and eluted with *n*-hexane/ethyl acetate (50:1) to yield four minor fractions (H15-4-1~H15-4-4). The minor fraction H15-4-2 was recrystallized with chloroform-methanol to yield mixture of 3 β -hydroxysitost-5-en-7-one and 3 β -hydroxystigmasta-5,22-dien-7-one (5.1 mg). H15-4-3 was further purified by pTLC eluted with benzene/acetone (8:1) to afford crotonkinensin A (**17**) (2.0 mg). Fraction H16 was applied to silica gel CC and eluted with *n*-hexane/ethyl acetate (10:1) to yield five subfractions (H16-1 ~H16-5). Subfraction H16-2 was further purified by silica gel CC eluted with the step gradient of chloroform/acetone (10:1 to 1:1) and one of the minor fractions (H16-2-3) was recrystallized with acetone to yield *ent*-7 β ,18-dihydroxykaur-16-en-15-one (**18**) (28.6 mg). The minor fraction H16-2-4 was further purified by pTLC eluted with benzene/acetone (20:1) to afford *ent*-15-oxokaur-16-en-18-oic acid (**19**) (5.0 mg). Subfraction H16-4 was purified by silica gel CC eluted with the step gradient of chloroform/acetone (10:1 to 1:1) and minor fraction H16-4-2 was further purified by pTLC eluted with benzene/acetone (6:1) to afford crotonkinin G (**7**) (12.3 mg).

The CH₂Cl₂-solubles (CTD) were subjected to column chromatography over silica gel, eluted using a step gradient of *n*-hexane/acetone (50:1 to 1:1), to obtain nine fractions (D1–D9) based on TLC profile. Fraction D2 gave five subfractions (D2-1~D2-5) after column chromatography over silica gel using step gradient mixtures of benzene/acetone (200:1 to 1:1). Subfraction D2-1 was applied to CC over silica gel with the step gradient eluent of benzene/acetone (100:1 to 1:1) to afford five minor fractions (D2-1-1~D2-1-5). The minor fraction D2-1-2 was purified by pTLC eluted with benzene/ethyl acetate (20:1) to afford *ent*-18-acetoxokaur-16-en-15-one (**20**) (2.0 mg). The minor fraction D2-1-4 was recrystallized with chloroform-methanol to yield the mixture of β -sitosterol and stigmasterol (45.0 mg). Subfraction D2-2 was subjected to column chromatography over silica gel using a stepwise gradient of *n*-hexane/EtOAc (20:1 to 1:1) to afford five minor fractions (D2-2-1~D2-2-5). The minor fraction D2-2-2 was further purified by pTLC eluted with dichloromethane/acetone (200:1) to afford *ent*-7 β -hydroxykaur-16-en-15-one (**21**) (7.2 mg) and methylparaben (1.3 mg), respectively. The minor fraction D2-2-3 was further purified by pTLC eluted with benzene/acetone (8:1) to result in *ent*-14 β -hydroxykaur-

16-en-15-one (**22**) (1.5 mg) and *p*-hydroxybenzoic acid (1.0 mg), respectively. Another minor fraction D2-2-4 was also purified by pTLC eluted with *n*-hexane/acetone (8:1) to yield crotonkinin B (**2**) (3.0 mg) and *ent*-11 α -acetoxykaur-16-en-18-oic acid (**23**) (1.0 mg), respectively. Subfraction D2-3 was applied into the silica gel CC eluted with step gradient of dichloromethane/acetone (200:1 to 1:1) to result in three minor fractions (D2-3-1~D2-3-3). The minor fractions D2-3-1 and D2-3-3 were further purified by pTLC eluted with dichloromethane/acetone (30:1) to afford *ent*-18-hydroxykaur-16-en-15-one (**24**) (2.0 mg) and *ent*-18-acetoxy-7 α -hydroxykaur-16-ene (**25**) (2.5 mg), respectively. Another minor fraction D2-3-2 was recrystallized with acetone to afford crotonkinin A (**1**) (60.0 mg). The last two subfractions D2-4 and D2-5 were combined and subjected to the silica gel CC. Purification by repeated column chromatography followed by pTLC eluted with *n*-hexane/acetone (10:1) to yield (16*S*)-*ent*-18-acetoxy-7 β -hydroxykaur-15-one (**12**) (2.0 mg) and *ent*-7 α ,14 β -dihydroxykaur-16-en-15-one (**16**) (6.9 mg), respectively.

Further purification of fraction D3 by silica gel CC eluted with step gradient of dichloromethane/acetone (200:1 to 1:1) afforded five subfractions (D3-1~D3-5). Subfractions D3-2 and D3-3 were purified by pTLC eluted with *n*-hexane/acetone (10:1) to produce indole-3-carboxylic acid methyl ester (1.0 mg), (*E*)-methyl ferulate (1.0 mg); and (*R*)-*N*-(1'-methoxycarbonyl-2'-phenylethyl)benzamide (**11**) (1.0 mg), respectively. Subfraction D3-4 was recrystallized with chloroform to afford *ent*-18-acetoxy-7 α -hydroxykaur-16-en-15-one (**13**) (10.0 mg). Fractions D4 and D5 were combined and purified by silica gel CC eluted with dichloromethane/acetone (20:1) to result in six subfractions (D4-1~D4-6). Subfraction D4-2 was recrystallized with chloroform to produce *ent*-1 β -acetoxy-7 α ,14 β -dihydroxykaur-16-en-15-one (**14**) (15.0 mg). The subfraction D4-3 was applied to silica gel CC eluted with step gradients of benzene/acetone (100:1 to 1:1) and further purified by pTLC to yield sesamin (1.0 mg) and vanillin (2.5 mg). The subfraction D4-4 was purified by pTLC eluted with chloroform/methanol (100:1) to afford crotonkinin F (**6**) (24.2 mg) and *ent*-11 α ,18-diacetoxy-7 β -hydroxykaur-16-en-15-one (**26**) (1.3 mg). The subfraction D4-5 was subjected to CC over silica gel eluted with the step gradients of chloroform/methanol (100:1 to 1:1) and further purified by pTLC to yield *ent*-18-acetoxy-7 α ,14 β -dihydroxykaur-16-en-15-one (**27**) (1.6 mg).

Fraction D6 was chromatographed over silica gel column with step gradients of benzene/acetone (50:1 to 1:1) to afford 12 subfractions (D6-1~D6-12) based on the TLC monitoring results. Subfractions D6-1 to D6-4 were purified by pTLC eluted with *n*-hexane/acetone (5:1) to afford *p*-hydroxybenzaldehyde (1.5 mg), syringaldehyde (2.0 mg), dehydrovomifoliol (2.5 mg), and 4-isopropyl- benzoic acid (1.0 mg), respectively. Subfraction D6-5 was applied to pTLC eluted with chloroform/acetone (30:1) to afford pinoresinol (1.0 mg). Subfraction D6-6 and D6-7 were pTLC separated eluted with benzene/ethyl acetate (2:1) to yield syringaresinol (1.6 mg) and 3,4-dimethoxybenzoic acid (1.0 mg), respectively. Subfraction D6-8 was isolated by pTLC and eluted with chloroform/ methanol (20:1) to afford vanillic acid (1.6 mg). Subfractions D6-9 to D6-11 were purified by pTLC eluted with *n*-hexane/ethyl acetate (1:1) to result in corydaldine (4.0 mg), (*S*)-2-*cis*-abscisic acid (1.0 mg), and phaseic acid (1.0 mg), respectively. Fraction D7 was chromatographed over silica gel eluted with CH₂Cl₂-MeOH (from 30:1 to 1:1), and then purified by pTLC with *n*-hexane-acetone (3:1) to yield *ent*-18-acetoxy-7 α ,14 β -dihydroxykaur-16-en-15-one (**14**) (2.6 mg), *ent*-7 β ,18-dihydroxykaur-16-en-15-one (**18**) (30.0 mg), and *ent*-18-hydroxykaur-16-en-15-one (**24**) (1.5 mg), respectively.

The H₂O fraction and the precipitates were combined and subjected to reversed-phase Diaion HP-20 CC eluted with the step gradient of methanol/water (0:1, 1:9, 1:5, 1:2, 1:1, 3:1, 9:1, 1:0) and yielded ten fractions (W1–W10). Most of these subfractions (W1–W8) did not display significant spots after first isolated by C-18 CC so that they were not purified furthermore. Fraction W9 was subjected to CC over silica gel eluted with the step gradients of chloroform/methanol (10:1 to 1:1) and afforded 3,4-dimethoxybenzoic acid (1.0 mg) after purified by pTLC with chloroform/methanol (100:1). Fraction W10 was purified by silica gel CC and eluted with the step gradients of chloroform/methanol (50:1 to 1:1) to result in three subfractions (W10-1~W10-3). Subfractions W10-2 and W10-3 were purified by pTLC with chloroform/methanol (100:1) to yield blumenol-A (1.0 mg) and benzoic acid (3.0 mg), respectively.

S2. Identities and reference materials for the forty-five known compounds.

- (16*S*)-*ent*-18-acetoxy-7 β -hydroxykaur-15-one (**12**): Giang, P. M.; Son, P. T.; Lee, J. J.; Otsuka, H. *Chem. Pharm. Bull.* **2004**, *52*, 879–882.
- ent*-18-acetoxy-7 α -hydroxykaur-16-en-15-one (**13**): Son, P. T.; Giang, P. M.; Taylor, W. C. *Aust. J. Chem.* **2000**, *53*, 1003–1005.
- ent*-1 β -acetoxy-7 α ,14 β -dihydroxykaur-16-en-15-one (**14**), *ent*-7 α ,14 β -dihydroxykaur-16-en-15-one (**16**), and *ent*-18-acetoxy-7 α ,14 β -dihydroxykaur-16-en-15-one (**27**): Giang, P. M.; Jin, H. Z.; Son, P. T.; Lee, J. H.; Hong, Y. S.; Lee, J. J. *J. Nat. Prod.* **2003**, *66*, 1217–1220.
- ent*-14 β -acetoxy-7 α -hydroxykaur-16-en-15-one (**15**): Flegel, M.; Becker, H. Z. *Naturforsch.* **1999**, *54C*, 481–487.
- crotonkinensin A (**17**): Thuong, P. T.; Dao, T. T.; Pham, T. H. M.; Nguyen, P. H.; Le, T. V. T.; Lee, K. Y.; Oh, W. K. *J. Nat. Prod.* **2009**, *72*, 2040–2042.
- ent*-7 β ,18-dihydroxykaur-16-en-15-one (**18**): Minh, P. T. H.; Ngoc, P. H.; Taylor, W. C.; Cuong, N. M. *Fitoterapia* **2004**, *75*, 552–556.
- ent*-15-oxokaur-16-en-18-oic acid (**19**): Asakawa, Y.; Takikawa, K.; Toyota, M.; Ueda, A.; Tori, M.; Kumar, S. S. *Phytochemistry* **1987**, *26*, 1019–1022.
- ent*-18-acetoxykaur-16-en-15-one (**20**) and *ent*-18-hydroxykaur-16-en-15-one (**24**): Fraga, B. M.; González, P.; Guillermo, R.; Hernández, M. G. *Tetrahedron* **1996**, *52*, 13767–13782.
- ent*-7 β -hydroxykaur-16-en-15-one (**21**): Buchanan, M. S.; Connolly, J. D.; Kadir, A. A.; Rycroft, D. S. *Phytochemistry* **1996**, *42*, 1641–1646.
- ent*-14 β -hydroxykaur-16-en-15-one (**22**): Perry, N. B.; Burgess, E. J.; Baek, S. H.; Weavers, R. T.; Geis, W.; Mauger, A. B. *Phytochemistry* **1999**, *50*, 423–433.
- ent*-11 α -acetoxykaur-16-en-18-oic acid (**23**) and *ent*-11 α ,18-diacetoxy-7 β -hydroxykaur-16-en-15-one (**26**): Giang, P. M.; Son, P. T.; Hamada, Y.; Otsuka, H. *Chem. Pharm. Bull.* **2005**, *53*, 296–300.
- ent*-18-acetoxy-7 α -hydroxykaur-16-ene (**25**): Dao, T. T.; Lee, K. Y.; Jeong, H. M.; Nguyen, P. H.; Tran, T. L.; Thoun, P. T.; Nguyen, B. T.; Oh, W. K. *J. Nat. Prod.* **2011**, *74*, 2526–2531.
- mixture of β -sitosterol and stigmasterol: Kuo, Y. H.; Li, Y. C. *J. Chin. Chem. Soc.* **1997**, *44*, 321–325.

mixture of stigmasta-4-en-3-one and stigmasta-4,22-dien-3-one: Shu, Y.; Jones, S. R.; Kinney, W. A.; Selinsky, B. S. *Steroids* **2002**, *71*, 647–652.

mixture of stigmastan-3-one and stigmast-22-en-3-one: Shu, Y.; Jones, S.R.; Kinney, W.A.; Selinsky, B.S. *Steroids* **2002**, *67*, 291–304.

ergosterol peroxide: Jinming, G.; Lin, H.; Jikai, L. *Steroids* **2001**, *66*, 771–775.

mixture of 3 β -hydroxysitost-5-en-7-one and 3 β -hydroxystigmasta-5,22-dien-7-one: Zhang, X.; Geoffroy, P.; Miesch, M.; Julien-David, D.; Raul, F.; Aoudé-Werner, D.; Marchioni, E. *Steroids* **2005**, *70*, 886–895. Foley, D. A.; O’Callaghan, Y.; O’Brien, N. M.; McCarthy, F. O.; Maguire, A. R. *J. Agric. Food Chem.* **2010**, *58*, 1165–1173.

3-acetylaleuritolic acid: Mclean, S.; Perpich-Dumont, M.; Reynolds, W. F.; Jacobs, H.; Lachmansing, S. S. *Can. J. Chem.* **1987**, *65*, 2519–2525.

dehydrovomifoliol: Serra, S.; Barakat, A.; Fuganti, C. *Tetrahedron: Asymmetry* **2007**, *18*, 2573–2580.

(S)-2-cis-abscisic acid: Abrams, S. R.; Reaney, M. J. T.; Abrams, G. D.; Mazurek, T.; Shaw, A. C.; Gusta, L. V. *Phytochemistry* **1989**, *28*, 2885–2889.

phaseic acid: Kitahara, T.; Touhara, K.; Watanabe, H.; Mori, K. *Tetrahedron* **1989**, *45*, 6387–6400.

blumenol-A: Ito, N.; Etoh, T.; Hagiwara, H.; Kato, M. *J. Chem. Soc. Perkin Trans. 1* **1997**, 1571–1579.

indole-3-carboxylic acid methyl ester and methylparaben: Wu, T. S.; Leu, Y. L.; Chan, Y. Y. *J. Chin. Chem. Soc.* **2000**, *47*, 221–226.

corydaldine: Bois-Choussy, M.; Paolis, M. D.; Zhu, J. *Tetrahedron Lett.* **2001**, *42*, 3427–3430.

sesamin: Jong, T. T.; Jean, M. Y. *J. Chin. Chem. Soc.* **1993**, *40*, 399–402.

pinoresinol: Xie, L. H.; Akao, T.; Hamasaki, K.; Deyama, T.; Hattori, M. *Chem. Pharm. Bull.* **2003**, *51*, 508–515.

syringaresinol: El-Hassan, A.; El-Sayed, M.; Hamed, A. I.; Rhee, I. K.; Ahmed, A. A.; Zeller, K. P.; Verpoorte, R. *Fitoterapia* **2003**, *74*, 184–187.

p-hydroxybenzoic acid and vanillic acid: Chen, C. Y.; Chang, F. R.; Teng, C. M.; Wu, Y. C. *J. Chin. Chem. Soc.* **1999**, *46*, 77–86.

(E)-methyl ferulate: Galland, S.; Mora, N.; Abert-Vian, M.; Rakotomanomana, N.; Dangles, O. *J. Agric. Food Chem.* **2007**, *55*, 7573–7579.

vanillin: Lee, T. H.; Chiou, J. L.; Lee, C. K.; Kuo, Y. H. *J. Chin. Chem. Soc.* **2005**, *52*, 833–841.

p-hydroxybenzaldehyde: Fujimoto, H.; Satoh, Y.; Yamazaki, M. *Chem. Pharm. Bull.* **1998**, *46*, 211–216.

syringaldehyde: Chiji, H.; Tanaka, S.; Izawa, M. *Agric. Biol. Chem.* **1980**, *44*, 205–207.

4-isopropylbenzoic acid: Jayasinghe, L.; Mallika Kumarihamy, B. M.; Nishantha Jayarathna, K. H. R.; Gayathri Udishani, N. W. M.; Ratnayake Bandara, B. M.; Hara, N.; Fujimoto, Y. *Phytochemistry* **2003**, *62*, 637–641.

3,4-dimethoxybenzoic acid: Bernini, R.; Coratti, A.; Provenzano, G.; Fabrizi, G.; Tofani, D. *Tetrahedron* **2005**, *61*, 1821–1825.

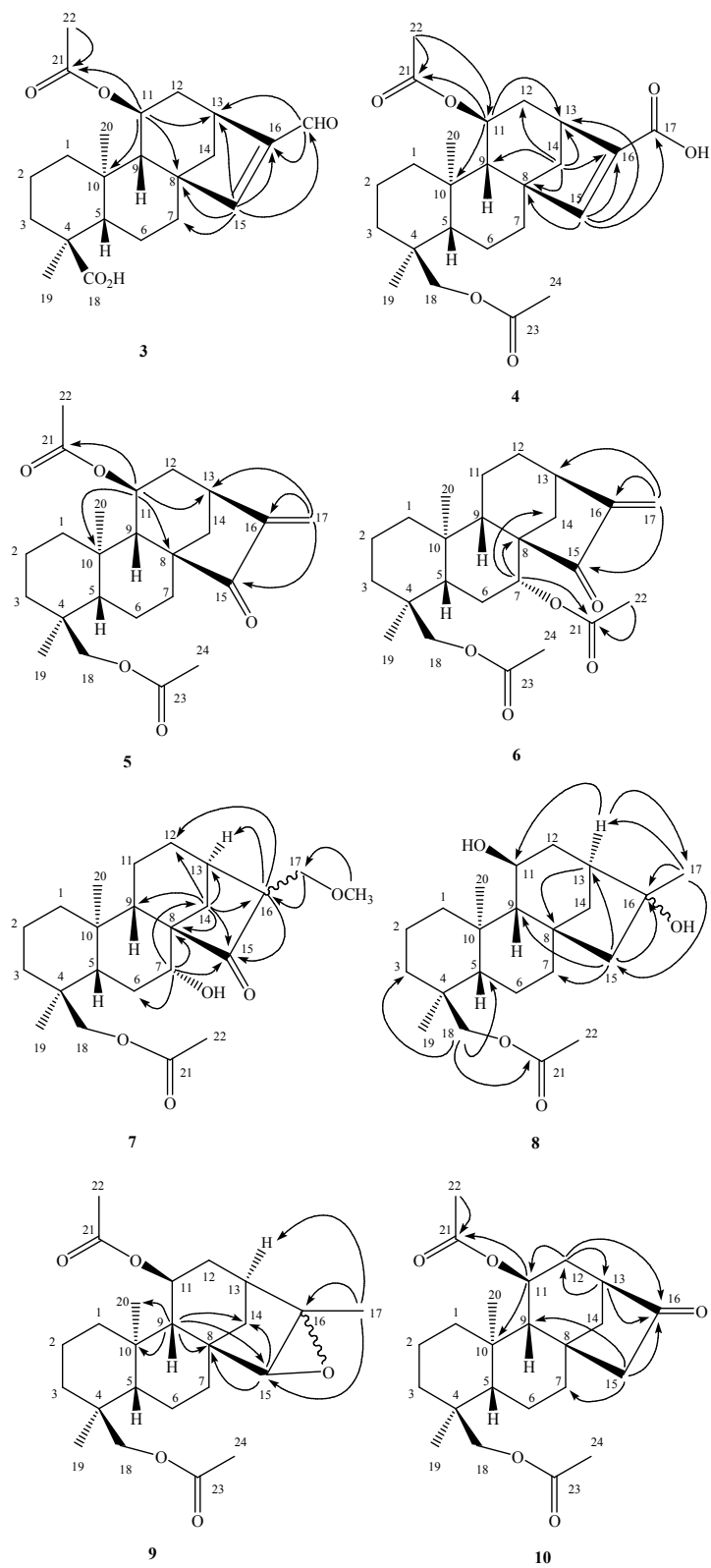
benzoic acid: Laurent, P.; Lebrun, B.; Braekman, J. C.; Daloze, D.; Pasteels, J. M. *Tetrahedron* **2001**, *57*, 3403–3412.

S3. Table S1. Inhibitory effects of crude extract and partial purified fractions of *C. tonkinensis* on superoxide anion generation and elastase release by human neutrophils in response to FMLP/CB.

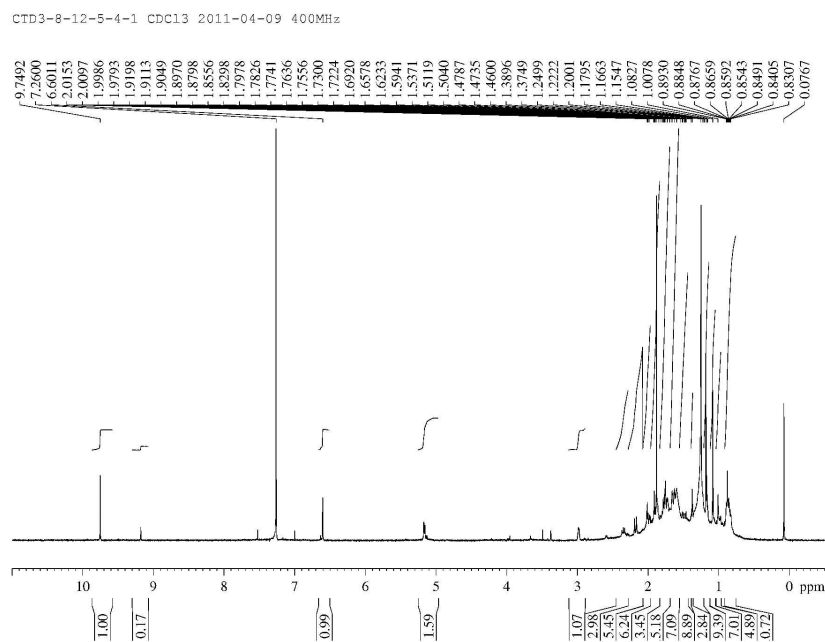
Samples	Inhibition percentage (%) ^a	
	superoxide anion generation	elastase release
methanol extract	37.50 ± 1.95 *	77.58 ± 1.01 ***
<i>n</i> -hexane fraction	62.58 ± 5.41 ***	88.04 ± 2.28 ***
dichloromethane fraction	79.75 ± 2.87 **	107.29 ± 5.11 *
water fraction	15.38 ± 2.25 ***	20.73 ± 5.42 ***

^a Percentage of inhibition (Inh %) at 10 µg/ml concentration. Results are presented as mean ± S.D. (n = 3). * p < 0.05, ** p < 0.01, *** p < 0.001 compared with the control value.

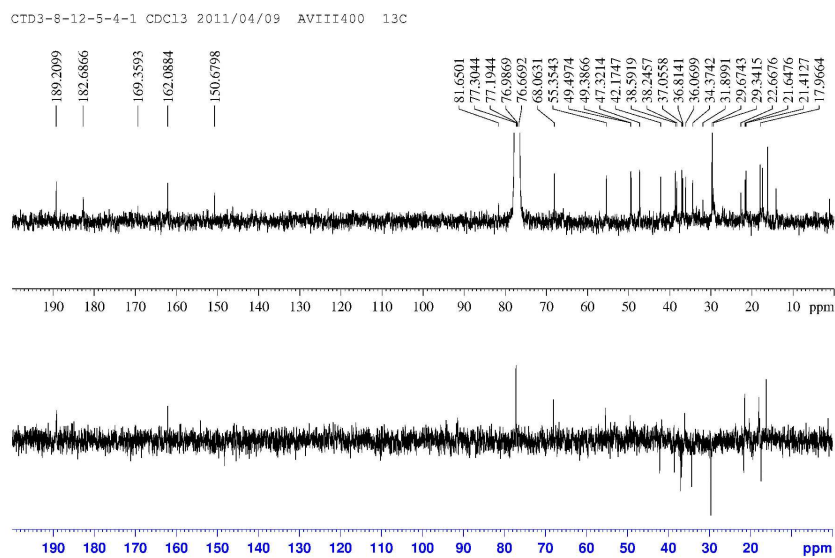
S4. Figure S1. Significant HMBC correlations of **3-10**.



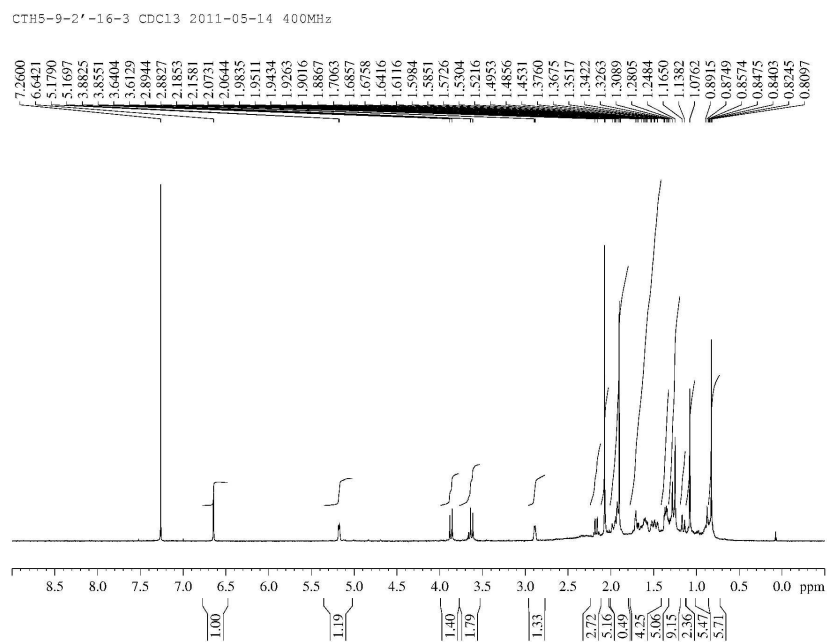
S5. The ^1H NMR spectrum of **3** (CDCl_3 , 400 MHz)



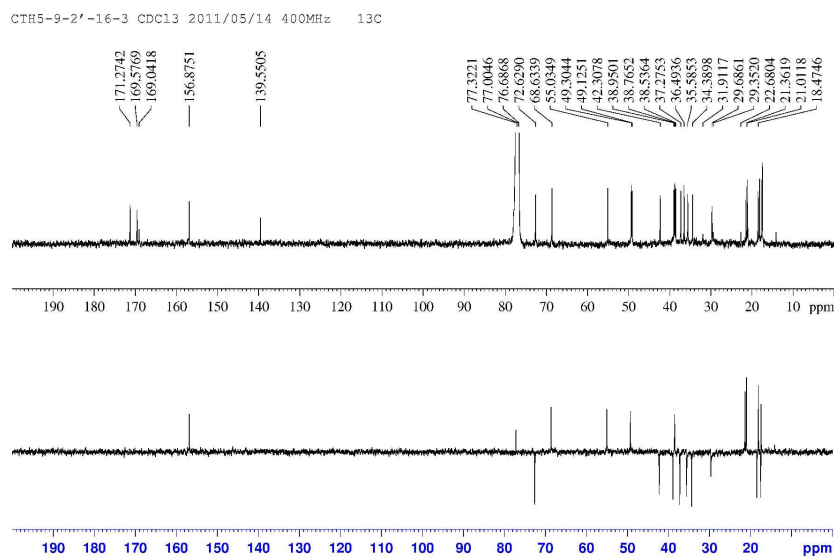
S6. The ^{13}C and DEPT NMR spectrum of **3** (CDCl_3 , 100 MHz)



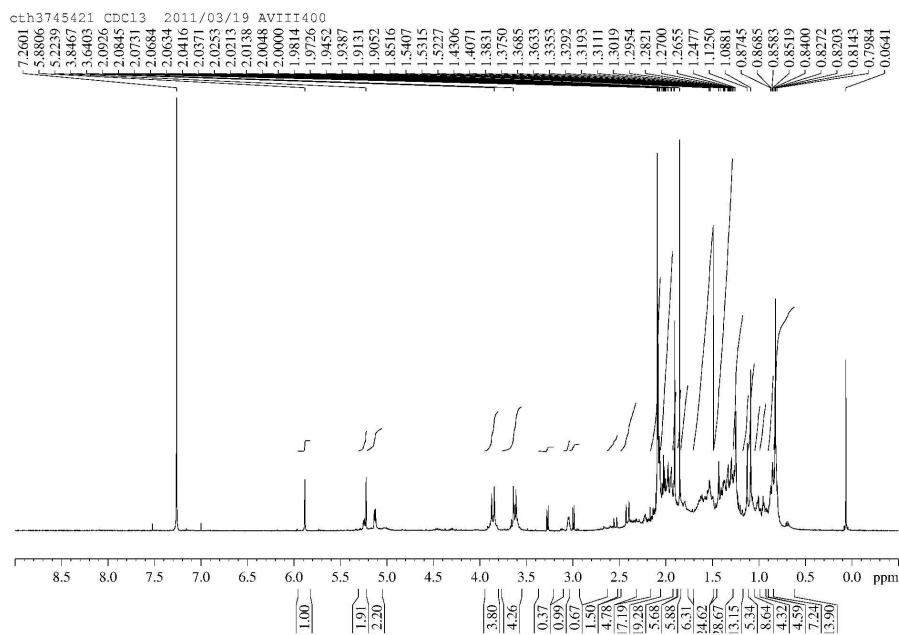
S7. The ^1H NMR spectrum of **4** (CDCl_3 , 400 MHz)



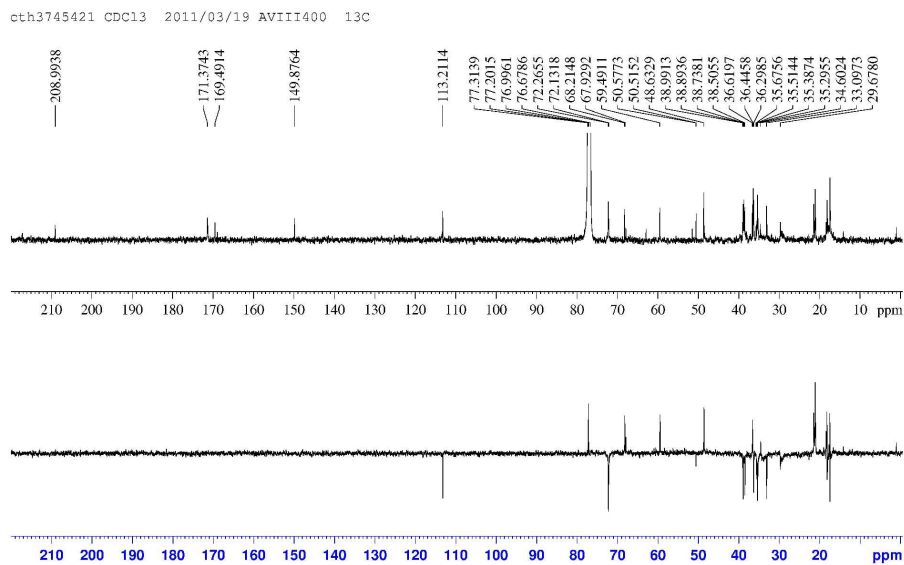
S8. The ^{13}C and DEPT NMR spectrum of **4** (CDCl_3 , 100 MHz)



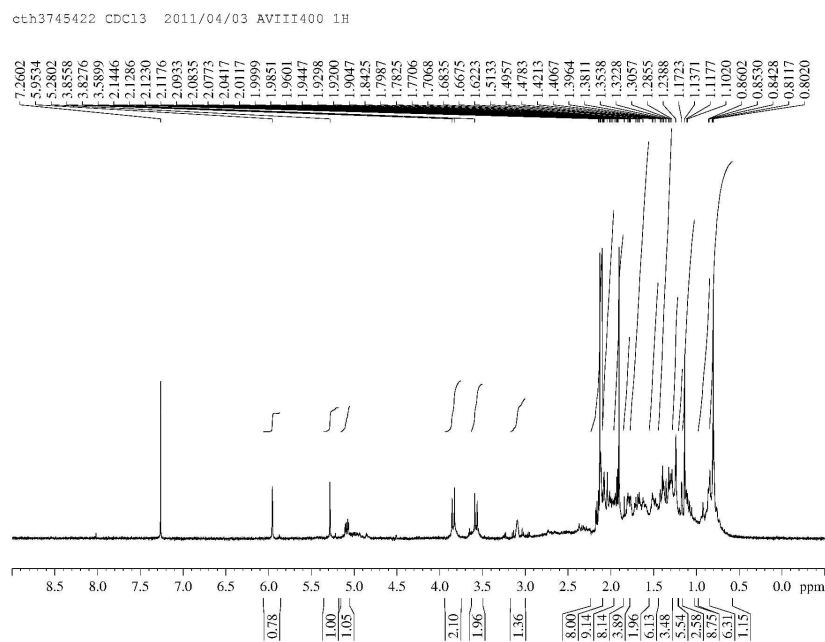
S9. The ^1H NMR spectrum of **5** (CDCl_3 , 400 MHz)



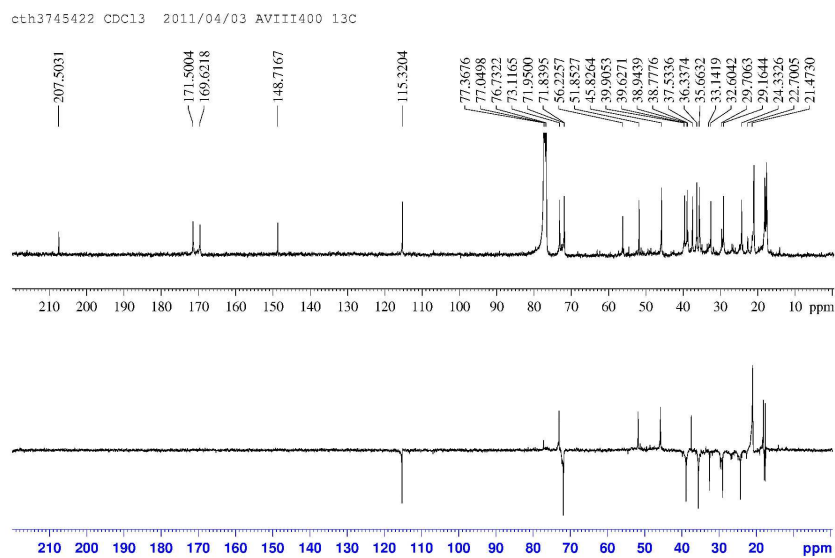
S10. The ^{13}C and DEPT NMR spectrum of **5** (CDCl_3 , 100 MHz)



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

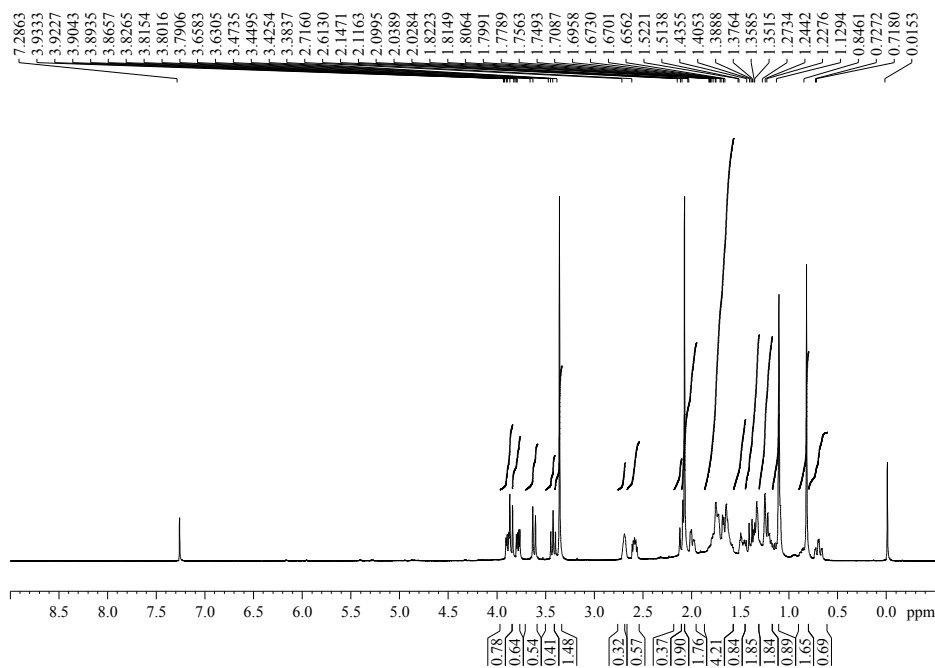


S12. The ^{13}C and DEPT NMR spectrum of **6** (CDCl_3 , 100 MHz)



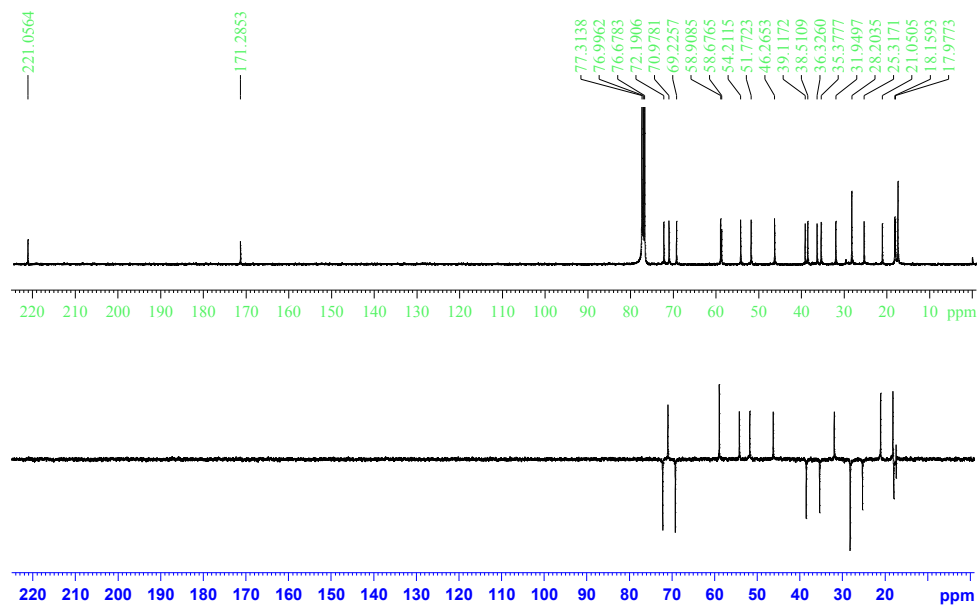
S13. The ^1H NMR spectrum of **7** (CDCl_3 , 400 MHz)

CTD48438641 CDCl_3 2010/06/21 AVIII400

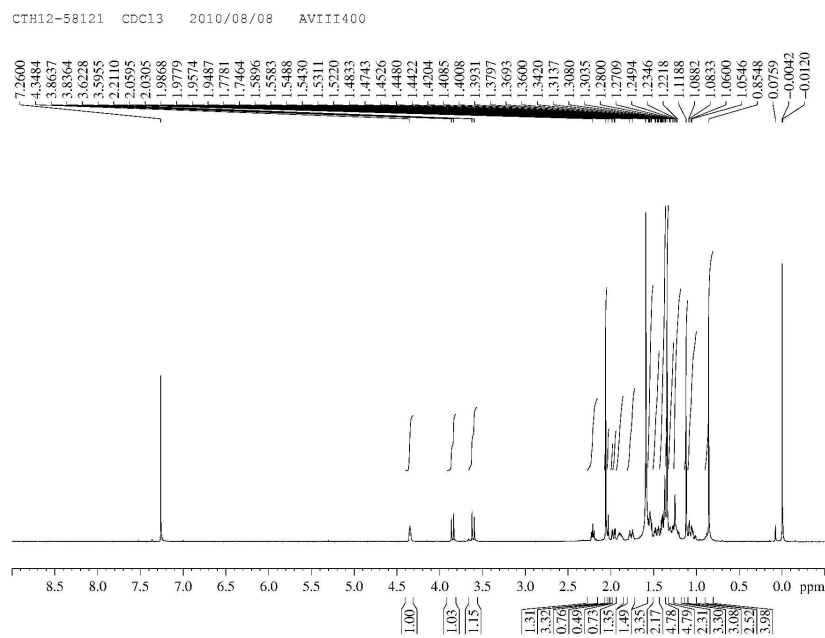


S14. The ^{13}C and DEPT NMR spectrum of **7** (CDCl_3 , 100 MHz)

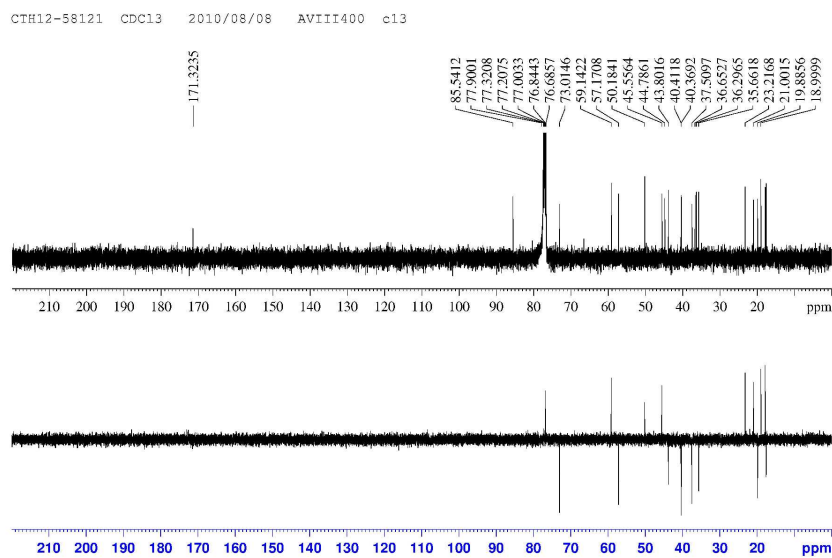
CTD48438641 CDCl_3 2010/06/21 AVIII400 C13



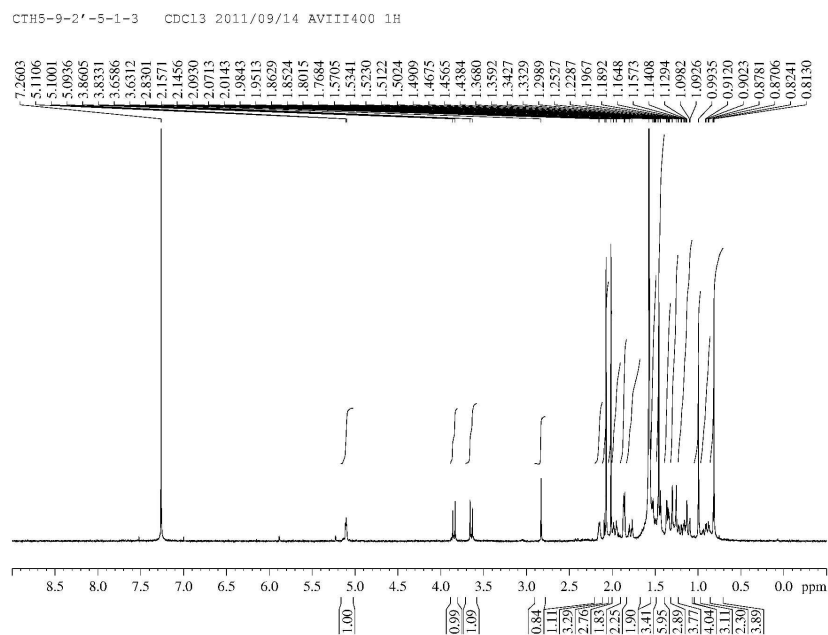
S15. The ^1H NMR spectrum of **8** (CDCl_3 , 400 MHz)



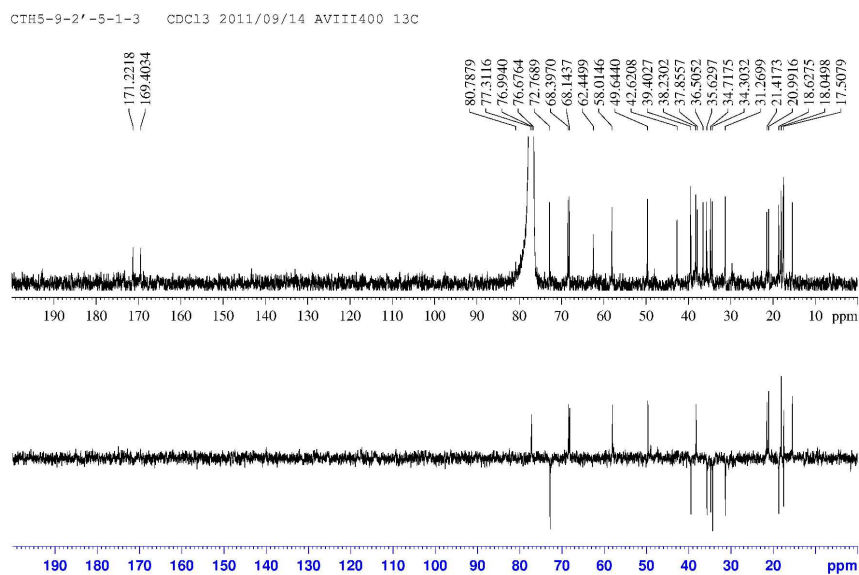
S16. The ^{13}C and DEPT NMR spectrum of **8** (CDCl_3 , 100 MHz)



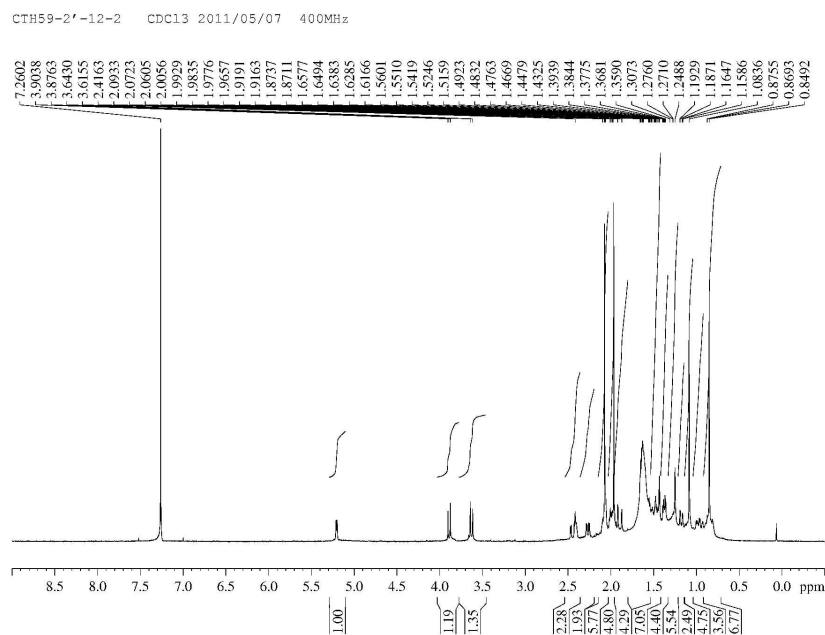
S17. The ^1H NMR spectrum of **9** (CDCl_3 , 400 MHz)



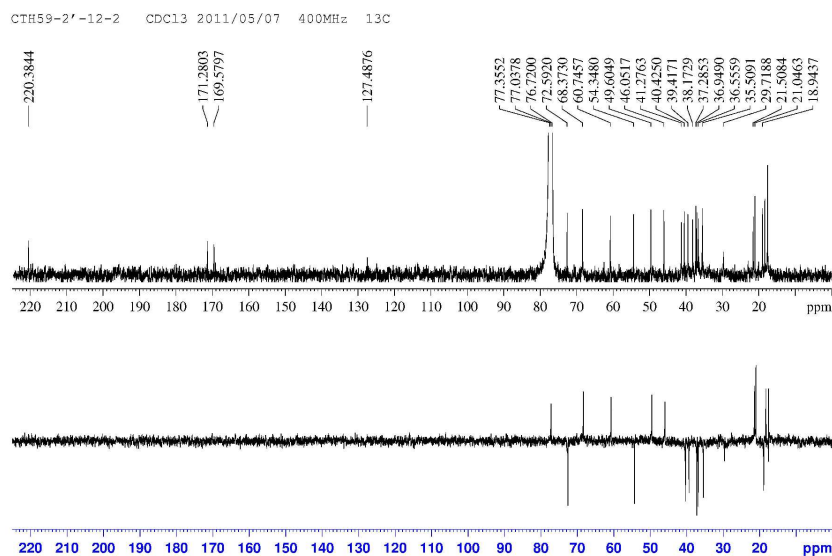
S18. The ^{13}C and DEPT NMR spectrum of **9** (CDCl_3 , 100 MHz)



S19. The ^1H NMR spectrum of **10** (CDCl_3 , 400 MHz)



S20. The ^{13}C and DEPT NMR spectrum of **10** (CDCl_3 , 100 MHz)



S21. The ^1H NMR spectrum of **11** (CDCl_3 , 300 MHz)

