HETEROCYCLES, Vol. 85, No. 11, 2012, pp. 2723 - 2734. © 2012 The Japan Institute of Heterocyclic Chemistry
Received, 16th August, 2012, Accepted, 18th September, 2012, Published online, 26th September, 2012
DOI: 10.3987/COM-12-12568

OXIMES OF 3-HYDROXYPRENYLFLAVANONES AND THEIR CYTOTOXIC ACTIVITIES

Sanit Thongnest,[a] Ranu Sawangsri,[a] Korakot Navakhun,[a] Jantana Yahuafai,[b] Pongpun Siripong,[b] and Somyote Sutthivaiyakit*[a]

[a]Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Ramkhamhaeng University, Ramkhamhaeng road, Bangkapi, Bangkok 10240, Thailand
[b]Natural Products Research Section, Research Division, National Cancer Institute, Rama 6 road, Bangkok 10400, Thailand

E-mail: s_somyote@ru.ac.th

Abstract – Oximination of 3-hydroxyprenylflavanones using hydroxylamine hydrochloride yielded 4-hydroxy-3-oxime and 3-hydroxy-4-oxime derivatives. The 3β-*O*-acetylhydroxyprenylflavanones, under similar reaction condition, yielded corresponding 4-*O*-acetyl-3-oximes. The configurations at C-4 of the 4-hydroxy-3-oximes obtained from both 3α- and 3β-hydroxyprenylflavanones and of 4-*O*-acetyl-3-oxime derivative from 3β-*O*-acetylprenylflavanones were proposed. Cytotoxic activities of the oximes and their parent compounds were evaluated against a panel of human cancer cell lines.

INTRODUCTION

In the course of our search for bioactive compounds from *Eriosema chinense* we were able to obtain a few 3-hydroxyprenylflavanones which showed strong cytotoxic activity[1] and since several oximes of flavonoids showed higher potency than the parent flavonoids[2] and as there has been limited report on the preparation and biological activities of oximes of 3-hydroxyflavanones,[3] we herein report the formation and characterization of the oximes from the reaction between 3-hydroxyprenylflavanones (**1-3**) and $NH_2OH{\cdot}HCl$, in addition to their cytotoxic activity.

RESULTS AND DISCUSSION

In this study we found that reaction of 3β-hydroxyprenylflavanone **1**, khonklonginol A,[1] with

$NH_2OH·HCl$ gave two oxime products, 4β-hydroxy-3-oxime (**4**, in 28% isolated yield) and 3β-hydroxy-4-oxime (**5**, in 6.5% isolated yield), whereas reaction of 3α-hydroxyprenylflavanone (khonklonginol B, **2**) with $NH_2OH·HCl$ gave **4** as the only product (in 62% isolated yield), as detected identical by ^1H and ^{13}C NMR spectra and specific rotations. The ^1H NMR spectrum of **4** showed signals at δ_H 4.97 (s) and 5.27 (d) assignable to H-2 and H-4, respectively, as supported by HMBC correlations of H-2/C-3, C-4, C-1′, C-2′, C-6′, and of H-4/C-2, C-3, C-10 (Figure 2). Additionally, reaction of lupinifolinol (**3**) with $NH_2OH·HCl$ gave oximes **6** and **7** in 35 and 18% isolated yields, respectively.

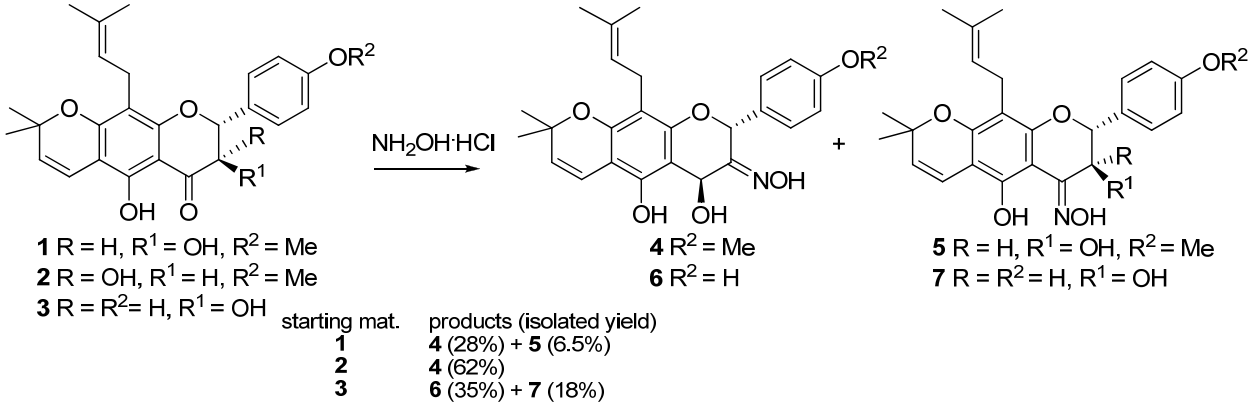

Figure 1. Structures of compounds **1**-**7**

These transformations indicated that the 3-hydroxy-4-keto function in **1**, **2** and **3** could isomerize to 4-hydroxy-3-keto analogs through a ketol rearrangement under the influence of $NH_2OH·HCl$, leading subsequently to the formation of corresponding 4-hydroxy-3-oximes (**4** and **6**). There are a number of reports on the rearrangement reactions of α-hydroxy-keto compounds, some contains a tertiary alcohol group[4] and some with a secondary alcohol group as found in steroids[5-7] and carbohydrates[8] but none of the 3-hydroxyflavanone was reported previously.

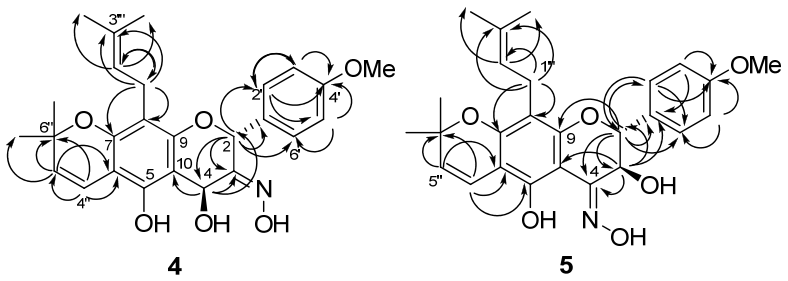

Figure 2. HMBC correlations in **4** and **5**

Since both 3α- and 3β-hydroxyprenylflavanones (**1** and **2**) gave the same 4-hydroxy-3-oxime (**4**), we thus proposed two possible transformation pathways. Pathway a, an acid catalyzed rearrangement of 3-hydroxy-4-keto compound proceeds via an enediol intermediate **4a**, which is protonated preferentially on the α-face to furnish the thermodynamically more stable 4β-hydroxy-3-keto analog (**4b**). The latter could then react further with $NH_2OH{\cdot}HCl$ to yield the corresponding oxime **4**.

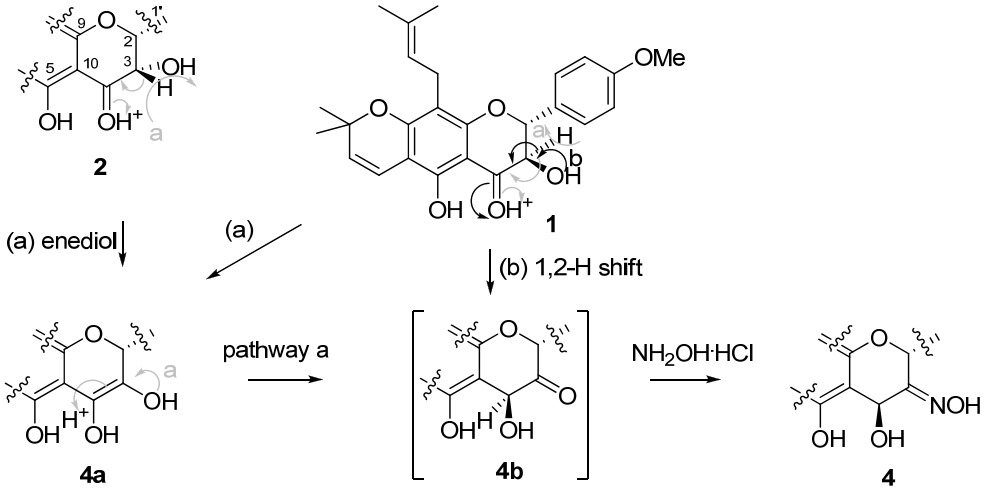

Figure 3. Proposed rearrangement pathways of the hydroxyprenylflavanones **1** and **2**

Pathway b involves a stereoselective 1,2-hydride migration of the 3α-H of **1** leading to the formation of **4b** as a sole intermediate which, with $NH_2OH{\cdot}HCl$, yields **4**. Protonation of the carbonyl oxygen atom facilitates the hydride shift as illustrated in Figure 3. The formation of **4b** from **2**, the 3-epimer of **1**, involves solely an enediol intermediate **4a** (pathway a, Figure 3), since a stereospecific 1,2-hydride migration of the 3β-H of **2** would lead to the 4α-hydroxy-3-keto intermediate and finally to the corresponding 4α-hydroxy-3-oxime, which was not observed in this study. The use of density functional theory (DFT) calculation at the B3LYP/6-31G(d) level of theory showed that **4** is the thermodynamically most favored and is most stable when the oxygen atom of the 3-NOH group is H-bond to the hydrogen atom of the 4-OH group as indicated in Figure 4.

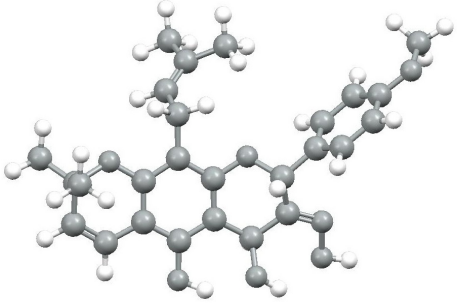

Figure 4. DFT calculated most stable 4β-hydroxy-3-oxime **4**

We also found that both 3*β*-*O*-acetyl derivatives of **1**, compounds **8** and **9**, transformed to 4*β*-*O*-acetyl-3-oxime **10** (in 32 and 18% isolated yield, respectively) after reacting with $NH_2OH{\cdot}HCl$. These transformations are proposed to occur by an acid-catalyzed reaction via a 5-membered ring intermediate (**8a** and **9a**). The ring opening step promotes the stereospecific 1,2-hydride shift of the 3α-H giving **10a** which is transformed into **10** under the influence of $NH_2OH{\cdot}HCl$. Based on the proposed pathway (Figure 5), the configuration at C-4 of **10** could therefore be established as *S*. Under this reaction condition, the more electrophilic 5-*O*-acetyl group in **8** was also hydrolyzed.

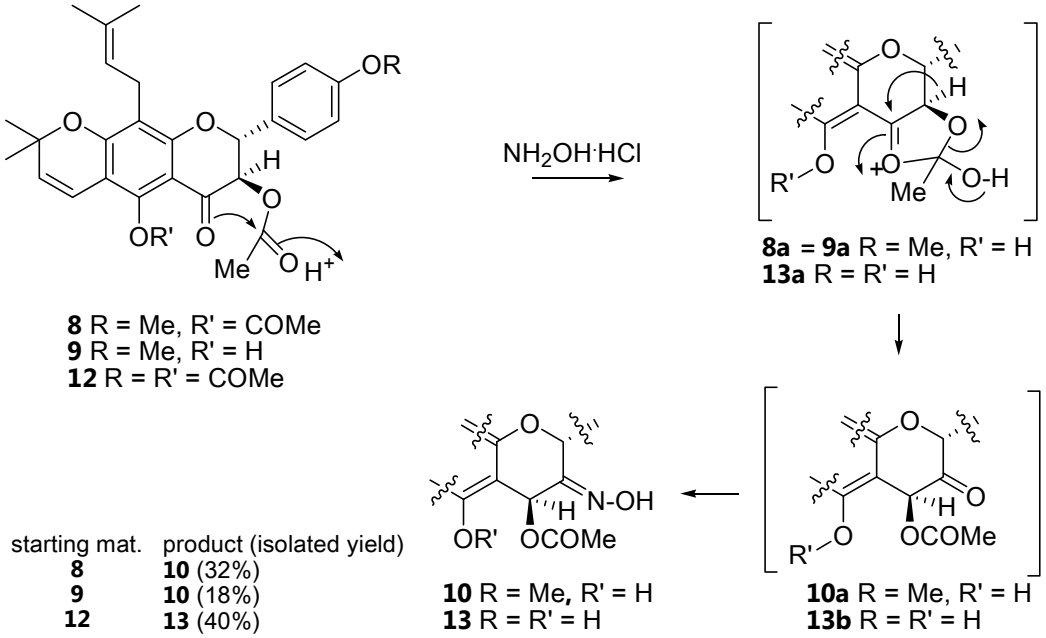

Figure 5. Proposed transformation pathway of **8**, **9** and **12**

Upon reacting either **4** or **10** with Ac_2O/pyridine and DMAP, the triacetate **11** was obtained (Figure 6). Compound **11** which was obtained from **4** showed $[\alpha]_D^{29}$ −75.6 (*c* 1.98, $CHCl_3$), whereas **11** from **10** exhibited $[\alpha]_D^{29}$ −76.2 (*c* 0.70, $CHCl_3$). Based on this data, the configuration at C-4 of oxime **4** obtained from both the 3α- and 3β-*O*-hydroxyflavanones (**1** and **2**) could also be proposed as *S*.

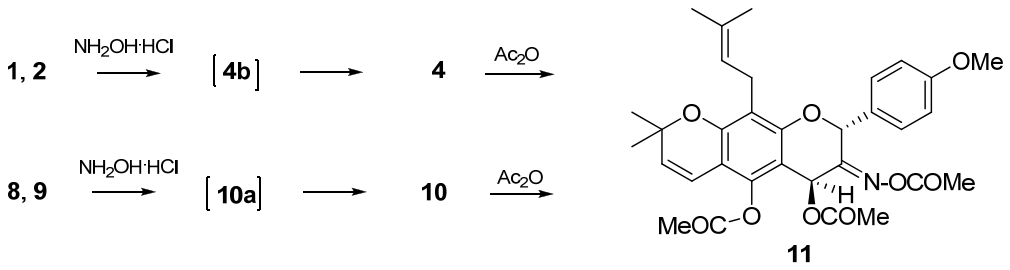

Figure 6. Relationships between compounds

We also found that reaction of 3β,5,4′-tri-*O*-acetylhydroxyflavonane **12** with $NH_2OH·HCl$ yielded 5,4′-dideacetyl- 4β-*O*-acetyl-3-oxime **13** with only one *O*-acetyl group at C-4 as a sole product, showing $[\alpha]_D^{29}$ –64.9 (*c* 0.70, $CHCl_3$). The reaction between **12** and $NH_2OH·HCl$ proceeds with similar rearrangement pathway (Figure 5) and led to the hydrolysis of the more electrophilic 5- and 4′-acetyl groups.

It is also worth noting that the 4-oxime derivatives (**5** and **7**) obtained as a minor product from the reaction of **1** and **3**, respectively with $NH_2OH·HCl$, exhibited ^{1}H NMR chemical shifts of H-2 and H-3 as doublets at ca. δ_H 5.07, and 5.22, respectively. The vicinal coupling constant, $J_{2,3}$ was found to be 6.9 Hz, which is noticeably less than that found (ca. 12 Hz) in the corresponding protons of the starting materials **1** and **3**.[1] The change of the $J_{2,3}$ value in 4-oxime obtained from 3-hydroxyflavanone was documented to be due to the distortion of hetero ring with C=N bond causing a change by ca. 20-30º of the H-2-H-3 dihedral angle as compared with the ketone as found in **1** and **3**.[3] However, the 4-oxime derivative of the 3α-hydroxyflavanone (**2**), which might provide further evidence concerning stereochemistry at C-3, was unfortunately not obtained under similar condition.

Table 1. Cytotoxic activities of 3-hydroxyprenylflavanones and their oximes

Compound	Cytotoxic activities IC_{50}[a]			
	MCF-7[a]	KB[a]	Hela[a]	Vero cell[a]
1	nd[b]	10.78	>230	18.10
4	34.50	31.91	25.28	29.49
5	25.46	42.73	48.07	nd[b]
3	nd[b]	13.03	39.57	33.41
6	14.8	14.51	13.99	27.09
7	44.10	51.24	48.07	48.60
8	nd[b]	11.53	38.27	nd[b]
9	nd[b]	33.47	>209	nd[b]
adriamycin[c]	8.01	3.34	2.48	19.60

[a]IC_{50} in μM. [b]nd = not determined. [c]positive control substance.

Due to the stability and limit quantities of the products obtained, only compounds **1**, **3**-**9** were evaluated for their cytotoxic activities against human breast adenocarcinoma (MCF-7), human oral epidermoid carcinoma (KB) and human cervical carcinoma (Hela) cell lines (Table 1). Results showed that both 3- and 4-oxime derivatives of khonklonginol A (compounds **4** and **5**) were less active than the parent compound (**1**) particularly against KB cell line. The inhibitory activity of the 4-oxime of lupinifolinol (**7**) was less potent than lupinifolinol (**3**) against all cell lines tested, while the 3-oxime of lupinifolinol (**6**) exhibited comparable inhibitory activity against KB cell line as that of **3**, however compound **6** also showed strong inhibitory activity against MCF-7 and Hela cell lines.

This is among a few report on oximination of 3-hydroxyflavanones and their 3-*O*-acetyl derivatives with $NH_2OH{\cdot}HCl$. Based on experimental results, the configuration at C-4 of the 3-oxime derivatives could be proposed as *S*. Cytotoxic activities of some synthetic products were evaluated and some exhibited broader spectrum of activities than the parent flavonoid.

EXPERIMENTAL

General experimental procedures

Solvents and reagents were dried by distillation from the usual drying agents prior to use: dichloromethane was distilled from calcium hydride, and pyridine was distilled from barium oxide. The progression of reactions was monitored by analytical thin layer chromatography using Merck precoated silica gel 60 F_{254} aluminum sheets and analyzed with 254 nm UV light. Flash column chromatography was performed using Merck silica gel 60. The yields referred to the isolated yields of compounds after purification. Melting points were measured on an electrothermal apparatus and are uncorrected. Optical rotations were recorded on a JASCO DIP 1020 polarimeter spectropolarimeter. The IR spectra were run on a Perkin-Elmer 1760x FT-IR spectrophotometer. The ^{1}H, ^{13}C, and 2D NMR data were recorded on a Bruker AVANCE 400 MHz spectrometer. The chemical shifts are in parts per million quoted relative to the residual signals of chloroform (δ_H 7.24 and δ_C 77.0 ppm) as the internal standard. HRESIMS was recorded on a Bruker Daltonics micro TOF mass spectrometer.

Computational Methods

The optimized structures of compounds were computed with the density functional theory (DFT) calculation method. DFT calculations were performed with the Becke's three-parameter exchange functional[9] with the Lee-Yang-Parr correlation functional (B3LYP).[10] All optimized structures were obtained by computation at the B3LYP/6-31G(d) level of theory using the GAUSSIAN09 program.[11]

Method A, Oximination: Preparation of 4β-hydroxy-3-oxime flavanone (4) and 3β-hydroxy-4-oxime flavanone (5) from 1: To a stirred solution containing 97.5 mg (0.229 mmol, 1.0 equiv) of khonklonginol A[1] (**1**) in dried pyridine (2 mL) was added 162.2 mg (2.291 mmol, 10.0 equiv) of $NH_2OH{\cdot}HCl$. The

reaction mixture was stirred under N_2 atmosphere at 65 °C for 60 h. After completion, the mixture was quenched with 5 mL of water and extracted with 2×10 mL of CH_2Cl_2. The combined organic extract was washed with 2×5 mL of an aqueous 1N HCl, followed by 10 mL of water, then dried over anhydrous Na_2SO_4, and the filtrate was concentrated using rotary evaporator under reduced pressure. The crude residue was purified by silica gel column chromatography eluting with CH_2Cl_2 to yield 23.8 mg (24%) of compound **4** together with 8.9 mg (9%) of compound **5**.

3-Oxime of khonklonginol A (4): a yellow amorphous solid; $[\alpha]_D^{29}$–28.6 (*c* 0.44, MeOH); IR (KBr) ν_{max} 3379, 2922, 2851, 1640, 1614, 1516, 1463, 1376, 1304, 1249, 1198, 1173, 1127, 1097, 1031 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ_H: 7.47 (2H, d, *J* = 8.6 Hz, H-2′, H-6′), 6.96 (2H, d, *J* = 8.6 Hz, H-3′, H-5′), 6.65 (1H, d, *J* = 9.9 Hz, H-4″), 5.48 (1H, d, *J* = 9.90 Hz, H-5″), 5.27 (1H, d, *J* = 1.6 Hz, H-4), 5.18 (1H, t, *J* = 7.3 Hz, H-2‴), 4.97 (1H, s, H-2), 3.82 (3H, s, OCH_3-4′), 3.30 (1H, dd, *J* = 13.9, 7.3 Hz, H-1‴), 3.25 (1H, dd, *J* = 13.9, 7.3 Hz, H-1‴), 1.67 (3H, s, CH_3-3‴), 1.62 (3H, s, CH_3-3‴), 1.41 (3H, s, CH_3-6″), 1.40 (3H, s, CH_3-6″); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 159.6 (C, C-4′), 156.0 (C, C-3), 154.6 (C, C-7), 154.4 (C, C-9), 152.4 (C, C-5), 130.6 (C, C-3‴), 128.1 (C, C-1′), 127.9 (CH, C-2′, C-6′), 126.5 (CH, C-5″), 123.1 (CH, C-2‴), 116.5 (CH, C-4″), 113.9 (CH, C-3′, C-5′), 109.6 (C, C-8), 104.1 (C, C-6), 95.9 (C, C-10), 79.3 (CH, C-2), 76.8 (C, C-6″), 61.3 (CH, C-4), 55.3 (CH_3, OCH_3-4′), 28.1 (CH_3, CH_3-6″), 28.0 (CH_3, CH_3-6″), 25.8 (CH_3, CH_3-3‴), 21.7 (CH_2, C-1‴), 17.9 (CH_3, CH_3-3‴); HR-ESI-MS *m/z*: 452.2073 $[M+H]^+$ (calcd for $C_{26}H_{30}NO_6$, 452.2073).

4-Oxime of khonklonginol A (5): a yellow amorphous solid, $[\alpha]_D^{29}$–24.9 (*c* 0.77, $CHCl_3$); IR (KBr) ν_{max} 3367, 2975, 2924, 2855, 1639, 1614, 1518, 1450, 1375, 1240, 1200, 1169, 1126, 1102, 1027, 1013 cm^{-1} ;^1H NMR (400MHz, $CDCl_3$) δ_H 10.50 (br s, OH), 7.93 (br s, OH), 7.31 (2H, d, *J* = 8.6 Hz, H-2′, H-6′), 6.86 (2H, d, *J* = 8.6 Hz, H-3′, H-5′), 6.62 (1H, d, *J* = 9.9 Hz, H-4″), 5.46 (1H, d, *J* = 9.9 Hz, H-5″), 5.23 (1H, d, *J* = 6.9 Hz, H-3), 5.12 (1H, t, *J* = 7.2 Hz, H-2‴), 5.08 (1H, d, *J* = 6.9 Hz, H-2), 3.78 (3H, s, OCH_3-4′), 3.21 (2H, d, *J* = 6.5 Hz, H-1‴), 1.63 (3H, s, CH_3-3‴), 1.61 (3H, s, CH_3-3‴), 1.41 (3H, s, CH_3-6″), 1.38 (3H, s, CH_3-6″); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 159.6 (C, C-4′), 156.4 (C, C-4), 154.6 (C, C-7), 153.9 (C, C-9), 152.1 (C, C-5), 130.7 (C, C-3‴), 129.2 (C, C-1′), 128.2 (C, C-2′, C-6′), 126.4 (CH, C-5″), 122.9 (CH, C-2‴), 116.5 (CH, C-4″), 113.9 (C, C-3′, C-5′), 109.5 (C, C-8), 104.1 (C, C-6), 96.9 (C, C-10), 80.2 (CH, C-2), 76.9 (C, C-6″), 66.1 (CH, C-3), 55.3 (CH_3, OCH_3-4′), 28.2 (CH_3, CH_3-6″), 27.9 (CH_3, CH_3-6″), 25.8 (CH_3, CH_3-3‴), 21.6 (CH_2, C-1‴), 17.8 (CH_3, CH_3-3‴); HR-ESI-MS *m/z*: 452.2075 $[M+H]^+$ (calcd for $C_{26}H_{30}NO_6$, 452.2073).

3-Oxime of khonklonginol B (4) from 2: a yellow amorphous solid, $[\alpha]_D^{29}$–19.7 (*c* 1.23, MeOH); HR-ESI-MS *m/z*: 452.2081 $[M+H]^+$ (calcd for $C_{26}H_{30}NO_6$, 452.2073).

Compounds **6** and **7** were prepared according to method A using 61.7 mg (0.146 mmol, 1.0 equiv) of

lupinifolinol (**3**) in dried pyridine (1 mL) with 103.0 mg (1.420 mmol, 9.7 equiv) of $NH_2OH{\cdot}HCl$ (30 h) to yield 22.2 mg (35%) of **6** and 11.7 mg (18%) of compound **7**.

3-Oxime of lupinifolinol (6): a sticky solid, $[\alpha]_D^{29}$ –24.6 (*c* 2.94, MeOH); IR (KBr) ν_{max} 3400, 2974, 2925, 2855, 1637, 1614, 1518, 1384, 1236, 1169, 1126, 1105 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ_H: 7.39 (2H, d, *J* = 8.4 Hz, H-2′, H-6′), 6.85 (2H, d, *J* = 8.4 Hz, H-3′, H-5′), 6.63 (1H, d, *J* = 9.9 Hz, H-4″), 5.47 (1H, d, *J* = 9.9 Hz, H-5″), 5.25 (1H, br s, H-4), 5.17 (1H, t, *J* = 7.2 Hz, H-2‴), 4.94 (1H, br s, H-2), 3.27 (2H, m, H-1‴), 1.66 (3H, s, CH_3-3‴), 1.61 (3H, s, CH_3-3‴), 1.39 (2H, s, 2×CH_3-6″); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 155.5 (C, C-3, C-4′), 154.5 (C, C-7), 154.4 (C, C-9), 152.2 (C, C-5), 130.8 (C, C-3‴),128.0 (CH, C-2′, C-6′), 126.6 (C, C-5″), 123.1 (CH, C-2‴), 116.4 (CH, C-4″), 115.4 (CH, C-3′, C-5′), 109.3 (C, C-8), 104.1 (C, C-10), 96.0 (C, C-10), 79.2 (CH, C-2), 76.8 (C, C-6″), 61.3 (CH, C-4), 28.1 (CH_3, C-6″), 27.9 (CH_3, C-6″), 25.8 (CH_3, C-3‴), 21.7 (CH_2, C-1‴), 17.9 (CH_3, C-3‴); HR-ESI-MS *m/z*: 438.1914 $[M+H]^+$ (calcd for $C_{25}H_{28}NO_6$ 438.1917).

4-Oxime of lupinifolinol (7): a yellow amorphous solid, $[\alpha]_D^{29}$ –119.5 (*c* 0.88, MeOH); IR (KBr) ν_{max} 3367, 2924, 2855, 1639, 1614, 1518, 1375, 1240, 1200, 1169, 1126, 1102 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ_H 10.50 (1H, br s, OH), 7.93 (1, br s, OH), 7.25 (2H, d, *J* = 8.4 Hz, H-2′, H-6′), 6.77 (2H, d, *J* = 8.4 Hz, H-3′, H-5′), 6.61 (1H, d, *J* = 10.0 Hz, H-4″), 5.47 (1H, d, *J* = 10.0 Hz, H-5″), 5.21 (1H, d, *J* = 6.9 Hz, H-3), 5.06 (1H, d, *J* = 6.9 Hz, H-2), 5.11 (1H, t, *J* = 7.3 Hz, H-2‴), 3.21 (2H, d, *J* = 6.0 Hz, H-1‴), 1.62 (3H, s, H-3‴), 1.61 (3H, s, H-3‴), 1.41 (3H, s, H-6″), 1.38 (3H, s, H-6″); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 156.6 (C, C=N), 154.8 (C, C-4′), 153.8 (C, C-7), 153.6 (C, C-9), 151.6 (C, C-5), 130.5 (C, C-3‴), 128.3 (C, C-1′), 128.1 (CH, C-2′, C-6′), 126.2 (CH, C-5″), 123.0 (CH, C-2‴), 116.4 (C-4″), 115.1 (CH, C-3′, C-5′), 109.1 (C, C-8), 103.7 (C, C-6) , 97.2 (C, C-10), 80.3 (CH, C-2), 76.5 (C, C-6″), 65.1 (CH, C-3), 27.9 (CH_3, C-6″), 27.6 (CH_3, C-6″), 25.5 (CH_3, C-3‴), 21.4 (CH_2, C-1‴), 17.6 (CH_3, C-3‴); HR-ESI-MS *m/z*: 438.1916 $[M+H]^+$ (calcd for $C_{25}H_{28}NO_6$, 438.1917).

Method B, Acetylation reaction: Preparation of 3,5-di-*O*-acetylkhonklonginol A (8) and 3-*O*-acetylkhonklonginol A (9): To a stirred solution containing 52.0 mg (0.119 mmol, 1.0 equiv) of khonklonginol A (**1**) in 0.1 mL of dried pyridine and 0.1 mL of acetic anhydride, was added catalytic amount of DMAP. The reaction mixture was stirred at room temperature under N_2 atmosphere and monitored the progression of the reaction by TLC. Upon completion (5 h), the mixture of EtOAc (10 mL) and water (10 mL) was added, the layers were separated and the aqueous layer was further extracted with EtOAc (10 mL). The combined organic layer was then dried over anhydrous Na_2SO_4 and the filtrate concentrated using rotary evaporator. The crude residue was purified by silica gel column chromatography using hexane-EtOAc (94:6 to 85:15) to afford 48.7 mg (82%) of compound **8** together with 13.2 mg (24%) of compound **9**.

3,5-Di-*O*-acetylkhonklonginol A (8): an amorphous solid, $[\alpha]_D^{29}$ +43.0 (*c* 0.87, MeOH); IR (KBr) ν_{max} 2976, 2922, 1761, 1605, 1517, 1465, 1187, 1123 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ_H: 7.35 (2H, d, *J* = 8.8 Hz, H-2′, H-6′), 6.90 (2H, d, *J* = 8.0 Hz, H-3′, H-5′), 6.35 (1H, d, *J* = 8.0 Hz, H-4″), 5.65 (1H, d, *J* = 12.0 Hz, H-2), 5.63 (1H, d, *J* = 8.0 Hz, H-4″), 5.27 (1H, d, *J* = 12.0 Hz, H-3), 5.10 (1H, tt, *J* = 7.6, 1.2 Hz, H-2‴), 3.81 (3H, s, OCH_3-4′), 3.24 (1H, dd, *J* = 13.8, 7.5 Hz, H-1‴), 3.19 (1H, dd, *J* = 14.2, 8.0 Hz, H-1‴), 2.38 (3H, s, OCO*CH*$_3$-5), 1.98 (3H, s, OCO*CH*$_3$-3), 1.62 (3H, s, CH_3-3‴), 1.55 (3H, s, CH_3-3‴), 1.44 (3H, s, CH_3-6″), 1.42 (3H, s, CH_3-6″); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 185.6 (C, C-4), 169.3 (C, 2 × O*CO*CH_3), 160.2 (C, C-4′, C-7), 157.7 (C, C-9), 144.1 (C, C-5), 131.8 (C, C-3‴), 129.9 CH, C-5″), 128.7 (CH, C-2′, C-6′), 127.8 (C, C-1′), 121.4 (CH, C-2‴), 115.3 (CH, C-4″), 113.9 (CH, C-3′, C-5′), 109.9 (C, C-8), 106.9 (C, C-6), 80.8 (CH, C-2), 78.1 (C, C-6″), 73.4 (CH, C-3), 55.3 (CH_3, OCH_3-4′), 28.4 (2×CH_3, CH_3-6″), 25.8 (CH_3, CH_3-3‴), 21.8 (CH_2, C-1‴), 20.9 (CH_3, OCO*CH*$_3$-5), 20.4 (CH_3, OCO*CH*$_3$-3), 17.8 (CH_3, CH_3-3‴); HR-ESI-MS *m/z*: 543.1994 $[M+Na]^+$ (calcd for $C_{30}H_{32}O_8Na$, 543.1995).

3-*O*-Acetylkhonklonginol A (9): a yellow amorphous solid, IR (KBr) ν_{max}: 2922, 1756, 1586, 1463, 1190, 1123 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ_H 11.68 (br s, OH-5), 7.36 (2H, d, *J* = 8.7 Hz, H-2′, H-6′), 6.91 (2H, d, *J* = 8.7 Hz, H-3′, H-5′), 6.61 (1H, d, *J* = 10.0 Hz, H-4″), 5.75 (1H, d, *J* = 11.8 Hz, H-2), 5.50 (1H, d, *J* = 10.0 Hz, H-5″), 5.25 (1H, d, *J* = 11.8 Hz, H-3), 5.09 (1H, tt, *J* = 7.4, 1.2 Hz, H-2‴), 3.82 (3H, s, OCH_3-4′), 2.01 (3H, s, OCO*CH*$_3$), 3.14 (2H, dd, *J* = 7.4, 2.7 Hz, H-1‴), 1.62 (3H, s, CH_3-3‴), 1.56 (3H, s, CH_3-3‴), 1.43 (3H, s, CH_3-6″), 1.42 (3H, s, CH_3-6″); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 192.2 (C, C-4), 169.3 (C, O*CO*CH_3), 160.4 (C, C-4′), 160.3 (C, C-7), 158.8 (C, C-9), 156.5 (C, C-5), 131.3 (C, C-3‴), 128.7 (CH, C-2′, C-6′), 127.7 (C, C-1′), 126.2 (CH, C-5″), 122.2 (CH, C-2‴), 115.5 (CH, C-4″), 114.0 (CH, C-3′, C-5′), 109.1 (C, C-8), 103.3 (C, C-6), 101.4 (C, C-10), 80.8 (CH, C-2), 78.4 (C, C-6″), 72.6 (CH, C-3), 55.3 (CH_3, OCH_3-4′), 28.4 (2×CH_3, CH_3-6″), 25.8 (CH_3, CH_3-3‴), 21.3 (CH_2, C-1‴), 20.4 (CH_3, OCO*CH*$_3$), 17.8 (CH_3, CH_3-3‴); HR-ESI-MS *m/z*: 501.1889 $[M+Na]^+$ (calcd for $C_{28}H_{30}O_7Na$, 501.1889).

Preparation of 4-*O*-acetyl-3-oxime flavanone 10 from 8: compound **10** was prepared according to method A using 13.0 mg (0.025 mmol, 1.0 equiv) of **8** in 0.5 mL of dried pyridine with 17.1 mg (0.250 mmol, 10.0 equiv) of $NH_2OH{\cdot}HCl$ (30 h) to obtain 4.0 mg (32%) of **10**.

Preparation of 4-*O*-acetyl-3-oxime flavanone 10 from 9: compound **10** was prepared according to method A using 21.0 mg (0.044 mmol, 1.0 equiv) of **9** in 0.5 mL of dried pyridine with 30.5 mg (0.440 mmol, 10.0 equiv) of $NH_2OH{\cdot}HCl$ (32 h) to obtain 3.8 mg (18%) of **10**.

4-*O*-Acetyl-3-oxime flavanone (10): a yellow amorphous solid, $[\alpha]_D^{29}$ –74.7 (*c* 1.12, MeOH); IR (KBr) ν_{max} 3401, 2973, 2925, 2855, 1755, 1733, 1641, 1615, 1587, 1516, 1462, 1374, 1303, 1250, 1219, 1203, 1173, 1128, 1101, 1031 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ_H: 7.37 (2H, d, *J* = 8.7 Hz, H-2′, H-6′), 6.89 (2H, d, *J* = 8.7 Hz, H-3′, H-5′), 6.67 (1H, d, *J* = 9.9 Hz, H-4″), 6.53 (1H, d, *J* = 1.9 Hz, H-4), 5.49 (1H, d, *J* = 9.9

Hz, H-5″), 5.18 (1H, t, *J* = 7.3 Hz, H-2‴), 5.07 (1H, d, *J* = 1.9 Hz, H-2), 3.81 (3H, s, OCH_3-4′′), 3.30 (1H, dd, *J* = 14.0, 7.2 Hz, H-1‴), 3.22 (1H, dd, *J* = 14.0, 7.2 Hz, H-1‴), 1.85 (3H, s, OCO*CH₃*-4), 1.64 (3H, s, CH_3-3‴), 1.62 (3H, s, CH_3-3‴), 1.42 (3H, s, CH_3-6″), 1.40 (3H, s, CH_3-6″); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 168.6 (C, O*CO*CH_3), 159.6 (C, C-4′), 154.6 (C, C-7, C-9), 152.4 (C, C-3), 152.2 (C, C-5), 130.7 (C, C-3‴), 127.8 (CH, C-2′, C-6′), 127.5 (C, C-1′), 126.5 (CH, C-5″), 123.0 (CH, C-2‴), 116.5 (CH, C-4″), 113.7 (CH, C-3′, C-5′), 109.3 (C, C-8), 104.1 (C, C-6), 96.3 (C, C-10), 78.5 (CH, C-2), 76.9 (C, C-6″), 61.1 (CH, C-4), 55.2 (CH_3, OCH_3-4′), 28.1 (CH_3, CH_3-6″), 28.0 (CH_3, CH_3-6″), 25.8 (CH_3, CH_3-3‴), 21.7 (CH_2, C-1‴), 20.4 (CH_3, OCO*CH₃*), 17.9 (CH_3, CH_3-3‴); HR-ESI-MS *m/z*: 516.2009 $[M+Na]^+$ (calcd for $C_{28}H_{31}NO_7Na$, 516.1998).

Preparation of triacetate derivative 11 from 4β-hydroxy-3-oxime flavanone 4: compound **11** was prepared according to method B using 9.3 mg (0.021 mmol, 1.0 equiv) of **4** and catalytic amount of DMAP in 0.5 mL of acetic anhydride and 0.5 mL of dried pyridine (20 h) to afford 4.7 mg (40%) of **11**.

Preparation of triacetate derivative 11 from 4β-*O*-acetyl-3-oxime flavanone 10: compound **11** was prepared according to method B using 11.2 mg (0.023 mmol, 1.0 equiv) of **10** and catalytic amounts of DMAP in 0.05 mL of acetic anhydride and 0.05 mL of pyridine (25 h) to afford 7.0 mg (54%) of **11**.

Compound 11 from 4: a yellow amorphous solid, $[\alpha]_D^{29}$ –75.6 (*c* 1.98, $CHCl_3$); IR (KBr) ν_{max} 2926, 2852, 1755, 1634, 1612, 1516, 1464, 1371, 1251, 1216, 1127, 1030 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.34 (2H, d, *J* = 8.7 Hz, H-2′, H-6′), 6.90 (2H, d, *J* = 8.7 Hz, H-3′, H-5′), 6.45 (1H, br s, H-4), 6.35 (1H, br s, H-4″), 5.63 (1H, d, *J* = 10.0 Hz, H-5″), 5.16 (1H, t, *J* = 7.2 Hz, H-2‴), 5.14 (1H, br s, H-2), 3.80 (3H, s, OCH_3-4′), 3.35 (1H, dd, *J* = 13.7, 7.3 Hz, H-1‴), 3.28 (1H, dd, *J* = 13.9, 7.4 Hz, H-1‴), 2.45 (3H, s, N-O-$COCH_3$-3), 2.08 (3H, s, OCO*CH₃*-5), 1.82 (3H, s, OCO*CH₃*-4), 1.63 (6H, s, CH_3-3‴), 1.44 (3H, s, CH_3-6″), 1.41 (3H, s, CH_3-6″); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 168.1 (C, O*CO*CH_3), 167.0 (C, O*CO*CH_3), 166.98 (C, O*CO*CH_3), 159.8 (C, C-4′), 156.6 (C, C=N), 155.0 (C, C-9), 153.4 (C, C-7), 142.7 (C, C-5), 131.5 (C, C-1′), 130.0 (CH, C-5″), 127.8 (CH, C-2′, C-6′), 127.1 (C, C-3‴), 121.9 (CH, C-2‴), 115.9 (CH, C-4″), 115.7 (C, C-10), 113.7 (CH, C-3′, C-5′), 110.1 (C, C-8), 106.3 (C, C-6), 78.5 (CH, C-2), 77.5 (C, C-6″), 63.3 (CH, C-4), 55.2 (CH_3, OCH_3-4′), 28.3 (CH_3, CH_3-6″), 28.2 (CH_3, CH_3-6″), 25.8 (CH_3, CH_3-3‴), 22.1 (CH_2, C-1‴), 21.2 (CH_3, OCO*CH₃*), 20.3 (CH_3, OCO*CH₃*), 19.5 (CH_3, OCO*CH₃*), 17.9 (CH_3, CH_3-3‴); HR-ESI-MS *m/z*: 600.2210 $[M+Na]^+$ (calcd for $C_{32}H_{35}NO_9Na$, 600.2210).

Compound 11 from 10: a yellow amorphous solid, $[\alpha]_D^{29}$ –76.2 (*c* 0.70, $CHCl_3$).

Preparation of 12: Compound **12** was prepared according to method B using 66.0 mg (0.156 mmol, 1.0 equiv) of lupinifolinol (**3**) and catalytic amount of DMAP in 0.5 mL of acetic anhydride and 0.5 mL of pyridine (12 h) to obtain 89.6 mg (100%) of **12**.

3,5,4′-Tri-*O*-acetyllupinifolinol (12): a yellow amorphous solid, $[\alpha]_D^{29}$ +36.2 (*c* 0.82, MeOH); IR (KBr)

ν_{max} 3503, 2919, 1771, 1760, 1640, 1511, 1466, 1371, 1216, 1166 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ_H 7.45 (2H, d, *J* = 8.5 Hz, H-2′, H-6′), 7.13 (2H, d, *J* = 8.5 Hz, H-3′, H-5′), 6.35 (1H, d, *J* = 10.1 Hz, H-4″), 5.63 (1H, d, *J* = 10.1 Hz, H-5″), 5.62 (1H, d, *J* = 12.1 Hz, H-2), 5.34 (1H, d, *J* = 12.1 Hz, H-3), 5.09 (1H, t, *J* = 7.3 Hz, H-2‴), 3.26 (1H, dd, *J* = 13.8, 7.5 Hz, H-1‴), 3.19 (1H, dd, *J* = 13.8, 7.5 Hz, H-1‴), 2.38 (3H, s, OCO*CH*$_3$), 2.29 (3H, s, OCO*CH*$_3$), 1.99 (3H, s, OCO*CH*$_3$), 1.62 (3H, s, CH_3-3‴), 1.57 (3H, s, CH_3-3‴), 1.44 (3H, s, CH_3-6″), 1.42 (3H, s, CH_3-6″); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 185.2 (C, C-4), 169.2 (C, O*CO*CH_3), 169.1 (C, O*CO*CH_3), 160.0 (C, C-9), 157.8 (C, C-7), 151.3 (C, C-4′), 144.1 (C, C-5), 133.2 (C, C-1′), 131.8 (C, C-3‴), 130.0 (CH, C-5″), 128.4 (CH, C-2′, C-6′), 121.7 (CH, C-3′, C-5′), 121.4 (CH, C-2‴), 115.4 (C, C-10), 115.3 (CH, C-4″), 110.1 (C, C-8), 106.2 (C, C-6), 80.4 (CH, C-2), 78.2 (C, C-6″), 73.4 (CH, C-3), 28.4 (2×CH_3, CH_3-6″), 25.7 (CH_3, CH_3-3‴), 21.8 (CH_2, C-1‴), 21.1 (CH_3, OCO*CH*$_3$), 20.9 (CH_3, OCO*CH*$_3$), 20.4 (CH_3, OCO*CH*$_3$), 17.8 (CH_3, CH_3-3‴); HR-ESI-MS *m/z*: 549.2114 $[M+H]^+$ (calcd for $C_{31}H_{33}O_9$, 549.2125).

Preparation of 4β-*O*-acetyl-3-oxime flavanone 13: compound **13** was prepared according to method A using 20.0 mg (0.037 mmol, 1 .0 equiv) of **12** in 0.5 mL of dried pyridine with 27.0 mg (0.365 mmol, 10.0 equiv) of $NH_2OH{\cdot}HCl$ (for 42 h) to yield 7.0 mg (40%) of **13**.

4β-*O*-Acetyl-3-oxime flavanone 13: a yellow amorphous solid, $[\alpha]_D^{29}$–64.9 (*c* 0.70, $CHCl_3$); IR (KBr) ν_{max} 3368, 2922, 2853, 1730, 1615, 1519, 1240, 1169, 1101 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ_H 7.31 (2H, d, *J* = 8.5 Hz, H-2′, H-6′) , 6.81 (2H, d, *J* = 8.5 Hz, H-3′, H-5′), 6.65 (1H, d, *J* = 9.9 Hz, H-4″), 5.49 (1H, d, *J* = 9.9 Hz, H-5″), 5.18 (1H, t, *J* = 7.4 Hz, H-2‴), 5.06 (1H, d, *J* = 1.5 Hz, H-2), 3.30 (1H, dd, *J* = 13.9, 7.6 Hz, H-1‴), 3.22 (1H, dd, *J* = 13.6 , 7.5 Hz, H-1‴), 1.87 (3H, s, OCO*CH*$_3$), 1.64 (3H, s, CH_3-3‴), 1.62 (3H, s, CH_3-3‴), 1.42 (3H, s, CH_3-6‴), 1.40 (3H, s, CH_3-6″); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 169.3 (C, O*CO*CH_3), 155.9 (C, C-4), 154.6 (C, C-7), 154.5 (C, C-9), 152.3 (C, C-3), 152.1 (C, C-5), 130.7 (C, C-3‴), 127.9 (CH, C-2′, C-6′), 127.4 (C, C-1′), 126.6 (CH, C-5″), 123.0 (CH, C-2‴), 116.4 (CH, C-4″), 115.3 (CH, C-3′, C-5′), 109.3 (C, C-8), 104.1 (C, C-6), 96.3 (C, C-10), 78.5 (CH, C-2), 76.9 (C, C-6″), 61.4 (CH, C-4), 28.1 (CH_3, C-6″), 28.0 (CH_3, C-6″), 25.8 (CH_3, C-3‴), 21.7(CH_3, C-1‴), 20.4 (CH_3, OCO*CH*$_3$), 17.9 (CH_3, C-3‴); HR-ESI-MS *m/z*: 480.2021 $[M+H]^+$ (calcd for $C_{27}H_{31}NO_7$, 480.2022).

Bioassay: cytotoxicity assays were performed using MTT colorimetric assay as previously reported.[12]

ACKNOWLEDGEMENTS

We are grateful to Ramkhamhaeng University for financial support. S.T. and R.S. acknowledge the Center of Excellence for Innovation in Chemistry (PERCH-CIC), the Office of Higher Education, Ministry of Education for a scholarship. We thank Professor R. Grigg, the School of Chemistry, University of Leeds, for his valuable comment. We acknowledge the Chemistry Department of Chiangmai University, and Chulabhorn Research Institute for HRMS measurements.

REFERENCES

1. S. Sutthivaiyakit, O. Thongnak, T. Lhinhatrakool, O. Yodchun, R. Srimark, P. Dowtaisong, and M. Chuankamnerdkarn, *J. Nat. Prod.*, 2009, **72**, 1092.
2. C. Yenjai and S. Wanich, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 2821.
3. G. Janzsó, F. Kállay, I. Koczor, and L. Radics, *Tetrahedron*, 1967, **23**, 3699.
4. L. A. Paquette and J. E. Hofferberth, Chapter 3. The α-hydroxyl ketone (α-ketol) and related rearrangements. In L. E. Overman, *et al.,* (Eds.), *Org. Reactions*, Vol. 62, John Wiley, 2003, pp. 477−567.
5. M. Numazawa and M. Nagaoka, *J. Org. Chem.*, 1982, **47**, 4024.
6. M. Numazawa, M. Nagaoka, and A. Mutsumi, *Chem. Pharm. Bull.*, 1987, **35**, 4763.
7. A. C. Bruttomesso, D. Doller, and E. G. Gros, *Bioorg. Med. Chem.*, 1999, **7**, 943.
8. D. W. Harris and M. S. Feather, *J. Amer. Chem. Soc.*, 1975, **97**, 178.
9. A. D. Becke, *J. Chem. Phys.*, 1993, **98**, 5648.
10. C. Lee, W. Yang, and R. G. Parr, *Phys. Rev.*, 1988, **B37**, 785.
11. M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, Jr., J. A. Montgomery, J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, N. J. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L .Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, Ö. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, and D. J. Fox, *Gaussian09*, *Revision B.01*, Gaussian, Inc., Wallingford CT, 2009.
12. P. Siripong, C. Hahnvajanawong, J. Yahuafai, S. Piyaviriyakul, K. Kanokmedhakul, N. Kongkathip, S. Ruchirawat, and N. Oku, *Biol. Pharm. Bull*., 2009, **32**, 1251.