

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

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Abstract – Oximation of 3-hydroxyprenylflavanones using hydroxylamine hydrochloride yielded 4-hydroxy-3-oxime and 3-hydroxy-4-oxime derivatives. The 3 β -*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 3 α - and 3 β -hydroxyprenylflavanones and of 4-*O*-acetyl-3-oxime derivative from 3 β -*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 activity¹ and since several oximes of flavonoids showed higher potency than the parent flavonoids² 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 NH₂OH·HCl, in addition to their cytotoxic activity.

RESULTS AND DISCUSSION

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

$\text{NH}_2\text{OH}\cdot\text{HCl}$ gave two oxime products, 4 β -hydroxy-3-oxime (**4**, in 28% isolated yield) and 3 β -hydroxy-4-oxime (**5**, in 6.5% isolated yield), whereas reaction of 3 α -hydroxypropenylflavanone (khonklonginol B, **2**) with $\text{NH}_2\text{OH}\cdot\text{HCl}$ 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 $\text{NH}_2\text{OH}\cdot\text{HCl}$ gave oximes **6** and **7** in 35 and 18% isolated yields, respectively.

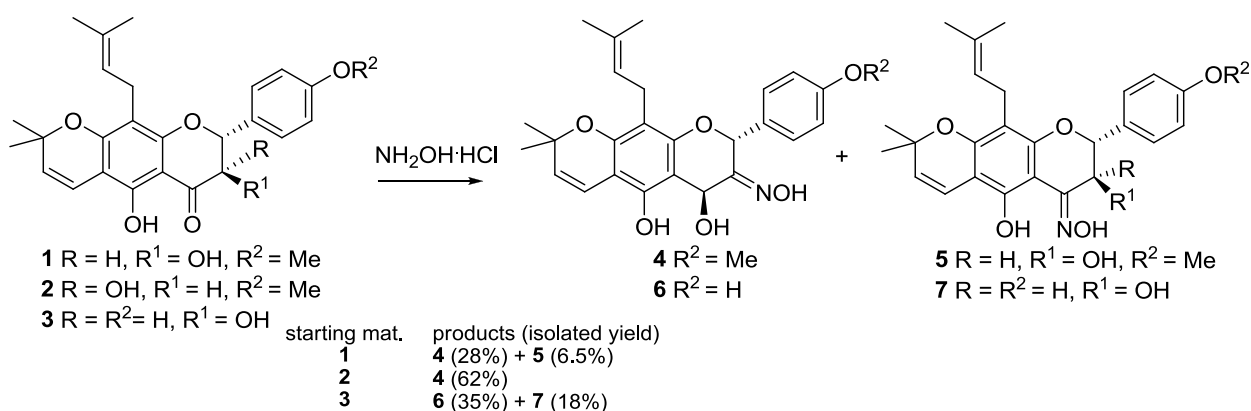


Figure 1. Structures of compounds **1-7**

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 $\text{NH}_2\text{OH}\cdot\text{HCl}$, 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 group⁴ and some with a secondary alcohol group as found in steroids⁵⁻⁷ and carbohydrates⁸ but none of the 3-hydroxyflavanone was reported previously.

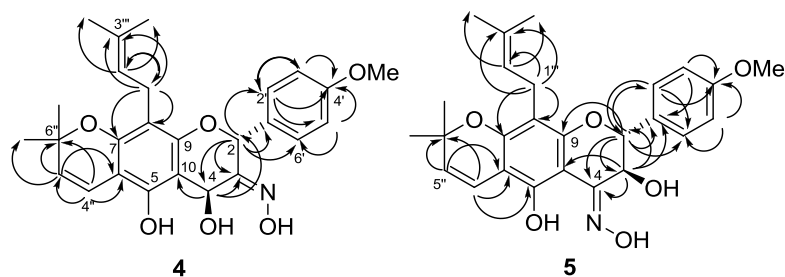


Figure 2. HMBC correlations in **4** and **5**

Since both 3 α - and 3 β -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 4 β -hydroxy-3-keto analog (**4b**). The latter could then react further with $\text{NH}_2\text{OH}\cdot\text{HCl}$ to yield the corresponding oxime **4**.

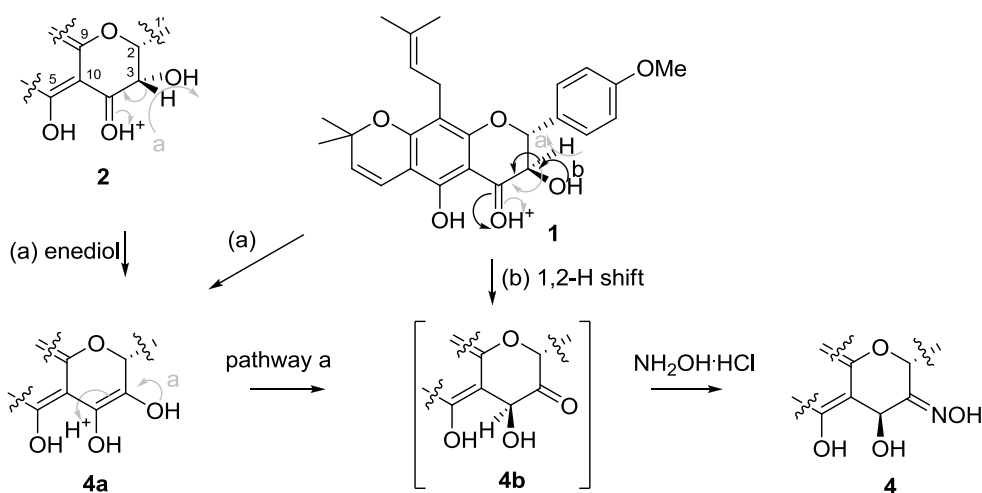


Figure 3. Proposed rearrangement pathways of the hydroxyprenylflavanones **1** and **2**

Pathway b involves a stereoselective 1,2-hydride migration of the 3 α -H of **1** leading to the formation of **4b** as a sole intermediate which, with $\text{NH}_2\text{OH}\cdot\text{HCl}$, 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 3 β -H of **2** would lead to the 4 α -hydroxy-3-keto intermediate and finally to the corresponding 4 α -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.

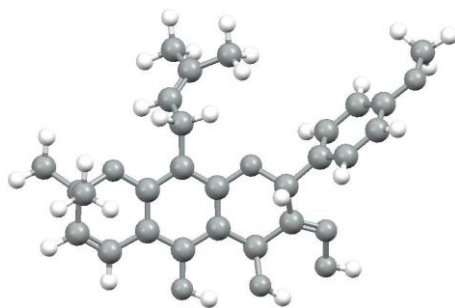


Figure 4. DFT calculated most stable 4 β -hydroxy-3-oxime **4**

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 NH₂OH·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 3 α -H giving **10a** which is transformed into **10** under the influence of NH₂OH·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.

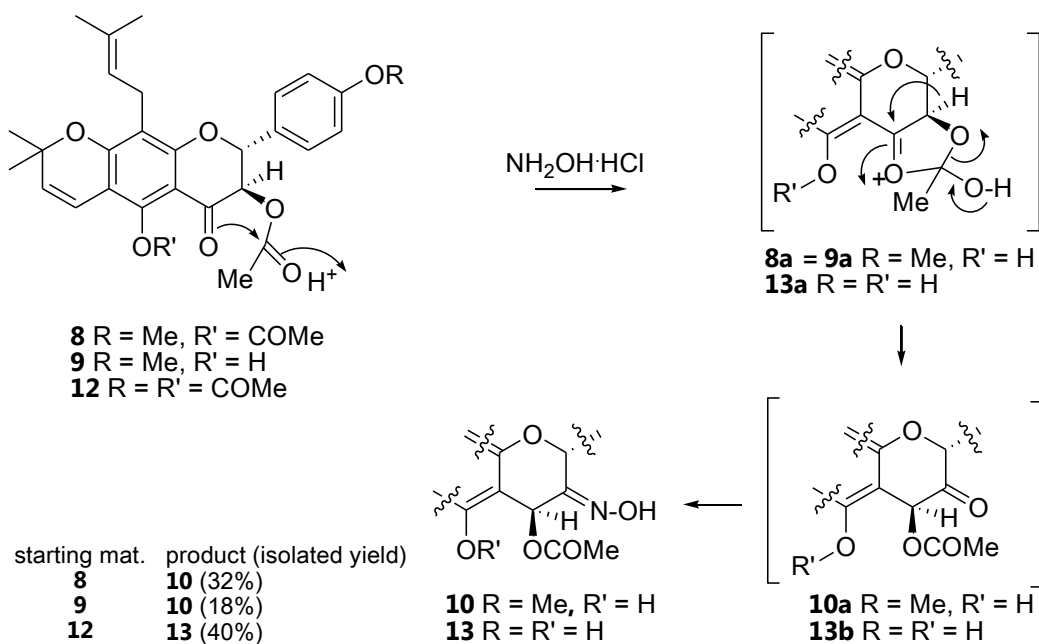


Figure 5. Proposed transformation pathway of **8**, **9** and **12**

Upon reacting either **4** or **10** with Ac₂O/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, CHCl₃), whereas **11** from **10** exhibited $[\alpha]_D^{29} -76.2$ (*c* 0.70, CHCl₃). Based on this data, the configuration at C-4 of oxime **4** obtained from both the 3 α - and 3 β -*O*-hydroxyflavanones (**1** and **2**) could also be proposed as *S*.

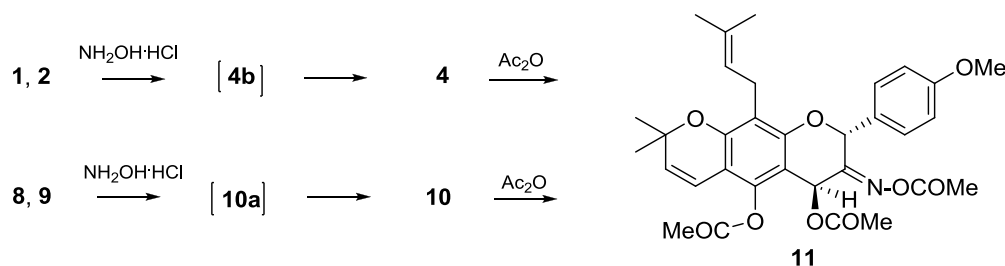


Figure 6. Relationships between compounds

We also found that reaction of 3 β ,5,4'-tri-*O*-acetylhydroxyflavonane **12** with NH₂OH·HCl yielded 5,4'-dideacetyl- 4 β -*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, CHCl₃). The reaction between **12** and NH₂OH·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 NH₂OH·HCl, exhibited ¹H 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. 20-30° 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 3 α -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

^aIC₅₀ in μ M. ^bnd = not determined. ^cpositive 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 khonkloninol 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 oximation of 3-hydroxyflavanones and their 3-*O*-acetyl derivatives with $\text{NH}_2\text{OH}\cdot\text{HCl}$. 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 F₂₅₄ 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 ¹H, ¹³C, 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 functional⁹ 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, Oximation: Preparation of 4 β -hydroxy-3-oxime flavanone (4**) and 3 β -hydroxy-4-oxime flavanone (**5**) from **1**:** To a stirred solution containing 97.5 mg (0.229 mmol, 1.0 equiv) of khonkloninol A¹ (**1**) in dried pyridine (2 mL) was added 162.2 mg (2.291 mmol, 10.0 equiv) of $\text{NH}_2\text{OH}\cdot\text{HCl}$. The

reaction mixture was stirred under N₂ 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 CH₂Cl₂. 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 Na₂SO₄, and the filtrate was concentrated using rotary evaporator under reduced pressure. The crude residue was purified by silica gel column chromatography eluting with CH₂Cl₂ 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, CDCl₃) 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, OCH₃-4'), 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, CH₃-3'''), 1.62 (3H, s, CH₃-3'''), 1.41 (3H, s, CH₃-6''), 1.40 (3H, s, CH₃-6''); ¹³C NMR (100 MHz, CDCl₃) 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 (CH₃, OCH₃-4'), 28.1 (CH₃, CH₃-6''), 28.0 (CH₃, CH₃-6''), 25.8 (CH₃, CH₃-3'''), 21.7 (CH₂, C-1'''), 17.9 (CH₃, CH₃-3'''); HR-ESI-MS *m/z*: 452.2073 [M+H]⁺ (calcd for C₂₆H₃₀NO₆, 452.2073).

4-Oxime of khonklonginol A (5): a yellow amorphous solid, $[\alpha]_D^{29}$ -24.9 (*c* 0.77, CHCl₃); 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, CDCl₃) 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, OCH₃-4'), 3.21 (2H, d, *J* = 6.5 Hz, H-1'''), 1.63 (3H, s, CH₃-3'''), 1.61 (3H, s, CH₃-3'''), 1.41 (3H, s, CH₃-6''), 1.38 (3H, s, CH₃-6''); ¹³C NMR (100 MHz, CDCl₃) 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 (CH₃, OCH₃-4'), 28.2 (CH₃, CH₃-6''), 27.9 (CH₃, CH₃-6''), 25.8 (CH₃, CH₃-3'''), 21.6 (CH₂, C-1'''), 17.8 (CH₃, CH₃-3'''); HR-ESI-MS *m/z*: 452.2075 [M+H]⁺ (calcd for C₂₆H₃₀NO₆, 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 C₂₆H₃₀NO₆, 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 $\text{NH}_2\text{OH}\cdot\text{HCl}$ (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, $[\alpha]_{\text{D}}^{29} -24.6$ (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, $2\times\text{CH}_3$ -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 $[\text{M}+\text{H}]^+$ (calcd for $\text{C}_{25}\text{H}_{28}\text{NO}_6$ 438.1917).

4-Oxime of lupinifolinol (7): a yellow amorphous solid, $[\alpha]_{\text{D}}^{29} -119.5$ (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 $[\text{M}+\text{H}]^+$ (calcd for $\text{C}_{25}\text{H}_{28}\text{NO}_6$, 438.1917).

Method B, Acetylation reaction: Preparation of 3,5-di-O-acetylkhonklonol A (8) and 3-O-acetylkhonklonol A (9): To a stirred solution containing 52.0 mg (0.119 mmol, 1.0 equiv) of khonklonol 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*-acetylkhonkloninol 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, OCOCH_3 -5), 1.98 (3H, s, OCOCH_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, $2 \times \text{OCOCH}_3$), 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 ($2 \times \text{CH}_3$, CH_3 -6''), 25.8 (CH_3 , CH_3 -3'''), 21.8 (CH_2 , C-1'''), 20.9 (CH_3 , OCOCH_3 -5), 20.4 (CH_3 , OCOCH_3 -3), 17.8 (CH_3 , CH_3 -3'''); HR-ESI-MS m/z : 543.1994 $[\text{M}+\text{Na}]^+$ (calcd for $\text{C}_{30}\text{H}_{32}\text{O}_8\text{Na}$, 543.1995).

3-*O*-Acetylkhonkloninol 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, OCOCH_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, OCOCH_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 ($2 \times \text{CH}_3$, CH_3 -6''), 25.8 (CH_3 , CH_3 -3'''), 21.3 (CH_2 , C-1'''), 20.4 (CH_3 , OCOCH_3), 17.8 (CH_3 , CH_3 -3'''); HR-ESI-MS m/z : 501.1889 $[\text{M}+\text{Na}]^+$ (calcd for $\text{C}_{28}\text{H}_{30}\text{O}_7\text{Na}$, 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 $\text{NH}_2\text{OH} \cdot \text{HCl}$ (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 $\text{NH}_2\text{OH} \cdot \text{HCl}$ (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, OCH₃-4''), 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, OCOCH₃-4), 1.64 (3H, s, CH₃-3'''), 1.62 (3H, s, CH₃-3'''), 1.42 (3H, s, CH₃-6''), 1.40 (3H, s, CH₃-6''); ¹³C NMR (100 MHz, CDCl₃) δ_C: 168.6 (C, OCOCH₃), 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 (CH₃, OCH₃-4'), 28.1 (CH₃, CH₃-6''), 28.0 (CH₃, CH₃-6''), 25.8 (CH₃, CH₃-3'''), 21.7 (CH₂, C-1'''), 20.4 (CH₃, OCOCH₃), 17.9 (CH₃, CH₃-3'''); HR-ESI-MS m/z : 516.2009 [M+Na]⁺ (calcd for C₂₈H₃₁NO₇Na, 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, CHCl₃); IR (KBr) ν_{max} 2926, 2852, 1755, 1634, 1612, 1516, 1464, 1371, 1251, 1216, 1127, 1030 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) 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, OCH₃-4'), 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-COCH₃-3), 2.08 (3H, s, OCOCH₃-5), 1.82 (3H, s, OCOCH₃-4), 1.63 (6H, s, CH₃-3'''), 1.44 (3H, s, CH₃-6''), 1.41 (3H, s, CH₃-6''); ¹³C NMR (100 MHz, CDCl₃) δ_C 168.1 (C, OCOCH₃), 167.0 (C, OCOCH₃), 166.98 (C, OCOCH₃), 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 (CH₃, OCH₃-4'), 28.3 (CH₃, CH₃-6''), 28.2 (CH₃, CH₃-6''), 25.8 (CH₃, CH₃-3'''), 22.1 (CH₂, C-1'''), 21.2 (CH₃, OCOCH₃), 20.3 (CH₃, OCOCH₃), 19.5 (CH₃, OCOCH₃), 17.9 (CH₃, CH₃-3'''); HR-ESI-MS m/z : 600.2210 [M+Na]⁺ (calcd for C₃₂H₃₅NO₉Na, 600.2210).

Compound 11 from 10: a yellow amorphous solid, $[\alpha]_D^{29} -76.2$ (c 0.70, CHCl₃).

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-acetyl lupinifolinol (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, OCOCH_3), 2.29 (3H, s, OCOCH_3), 1.99 (3H, s, OCOCH_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, OCOCH_3), 169.1 (C, OCOCH_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 \times CH_3 , CH_3 -6''), 25.7 (CH_3 , CH_3 -3'''), 21.8 (CH_2 , C-1'''), 21.1 (CH_3 , OCOCH_3), 20.9 (CH_3 , OCOCH_3), 20.4 (CH_3 , OCOCH_3), 17.8 (CH_3 , CH_3 -3'''); HR-ESI-MS m/z : 549.2114 [$\text{M}+\text{H}$] $^+$ (calcd for $\text{C}_{31}\text{H}_{33}\text{O}_9$, 549.2125).

Preparation of 4 β -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 $\text{NH}_2\text{OH}\cdot\text{HCl}$ (for 42 h) to yield 7.0 mg (40%) of **13**.

4 β -O-Acetyl-3-oxime flavanone 13: a yellow amorphous solid, $[\alpha]_{\text{D}}^{29} -64.9$ (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, OCOCH_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, OCOCH_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 , OCOCH_3), 17.9 (CH_3 , C-3'''); HR-ESI-MS m/z : 480.2021 [$\text{M}+\text{H}$] $^+$ (calcd for $\text{C}_{27}\text{H}_{31}\text{NO}_7$, 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.

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