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Cytotoxicity against KB and NCI-H187 cell lines of modified flavonoids from *Kaempferia parviflora*

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ABSTRACT

Flavones **1**–**4** isolated from *Kaempferia parviflora* were used for structural modification. Sixteen flavonoid derivatives, including four new derivatives, were synthesized and evaluated for cytotoxicity against KB and NCI-H187 cell lines. Flavanones **2a**–**4a** demonstrated higher cytotoxic activity than the parent compounds. Cytotoxicity against KB cell line of oxime **1c** was about 7 times higher than the ellipticine standard. Interestingly, oximes **1c** and **2c** exhibited highly potent cytotoxicity against NCI-H187 cell line with IC_{50} values of 0.014 and 0.23 μM, respectively. Oximes **4c** and **5c** showed strong cytotoxicity against NCI-H187 cell line with IC_{50} values of 4.04 and 2.32 μM, respectively.

Kaempferia parviflora belongs to the family of Zingiberaceae and is known in Thai as kra-chai-dam. The rhizomes of this plant have been used for treatment of gastrointestinal disorders,[1] as health-promoting herbs and as anti-inflammatory agents.[2] In recent years, there have been many reports of biological activities of this plant, for example, antibacterial, antiplasmodial,[3] anti-peptic ulcer,[1] anti-viral protease effects,[4] anti-allergic activity[5] as well as modulators of multidrug resistance in cancer cells.[6] In addition, it was found that this plant has no testosterone-like effect on reproduction in male rats.[7] In our previous study, we reported the quantitation of 11 flavonoids in *K. parviflora* by gas chromatography. The major components in this plant are 5,7-dimethoxyflavone, 5,7,4′-trimethoxyflavone, 5-hydroxy-3,7-dimethoxyflavone, and 5-hydroxy-7-methoxyflavone.[8] It was reported that 5,7-dimethoxyflavone showed cancer chemopreventive properties in a variety of human cell lines while 5,7-dihydroxyflavone, an unmethylated analog, had potential chemopreventive properties but very poor bioavailability.[9,10] Trifluoromethyl derivatives of 5,7-dimethoxyflavone were synthesized and evaluated for their inhibitory effect on the cell cycle of U2OS cells in vitro and it was found that 4-trifluoromethyl derivative possessed strong cytotoxicity.[11] It has been reported that 6-amino-5,7-dihydroxyflavone demonstrates potent and specific rat intestinal α-glucosidase inhibitory activity.[12] Due to the continuing need for new cancer chemopreventive agents with novel structures and as part of our research program on anti-tumor drugs from natural products, we therefore planned to modify the chemical structure of flavonoids from *K. parviflora* and also screened for cytotoxic activity of the derivatives of those flavonoids. We recently reported that aminoflavonoid derivatives from this plant demonstrated strong cytotoxicity against the KB cell line.[13] Moreover, flavanone oximes exhibited cytotoxicity against HepG2 and T47D cell lines.[14] We report herein the simple synthetic methods and active cytotoxic structures of modified flavonoids.

Catalytic hydrogenation of flavones **1**–**4** was performed under hydrogen gas and using palladium on charcoal as a catalyst to furnish flavanones **1a**–**4a** as the major products (Scheme 1). Flavanone **2a** was obtained as a single diastereomer as cis configuration which was confirmed by the correlation of protons at C2 and C3 in the NOESY experiment (Fig. 1). The ^{1}H NMR spectrum of **2a** showed resonance signals at δ 5.34 (1H, d, J = 2 Hz) and δ 3.73 (1H, d, J = 2 Hz) which were assigned to H-2 and H-3, respectively. Dihydrochalcones **1b, 3b**, and **4b** were detected as minor products which were obtained from benzylic cleavage of flavanones **1a**, **3a**, and **4a**, respectively (Scheme 1). However, there was no product from benzylic cleavage of **2a** in the same condition. This may be due to the methoxy group at C3 position retarding the benzylic cleavage.

The demethylation reaction of flavanone **3a** was carried out using HBr in AcOH to obtain the corresponding 5,7-dihydroxyflavanone **5a** in 48% yield (Scheme 2). After treatment of **1a**–**5a** with hydroxylamine hydrochloride in the presence of KOH, oximes **1c**–**5c** were obtained in high yield (Scheme 3). The ^{13}C NMR spectra of oxime derivatives showed upfield shift signals of carbonyl carbons (δ 189.2–195.7) to the oxime carbons (δ 148.8–157.2). The IR

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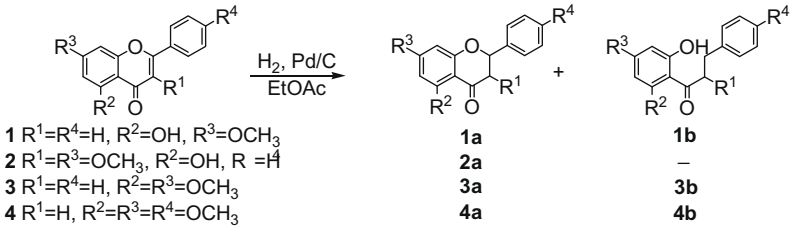


Scheme 1. Reagents and conditions: H_2, Pd/C, EtOAc, rt, 24 h, **1a** (57%), **1b** (27%), **2a** (66%), **3a** (60%), **3b** (17%), **4a** (48%), **4b** (18%).

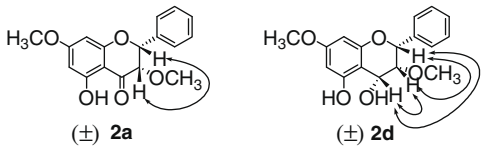
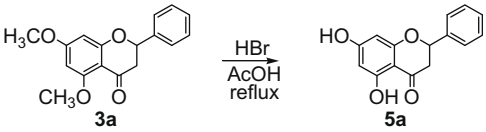
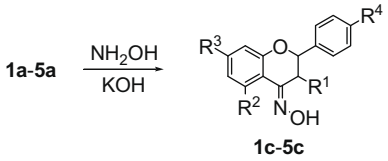


Figure 1. NOESY experiments of **2a** and **2d**.



Scheme 2. Reagents and conditions: HBr, AcOH, reflux, 24 h, 48%.

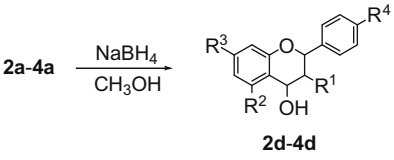


Scheme 3. Reagents and conditions: $NH_2OH{\cdot}HCl$, 20% KOH, EtOH, reflux, 1–3 h, **1c** (96%), **2c** (80%), **3c** (89%), **4c** (82%), **5c** (98%).

spectra showed characteristic absorption bands of OH stretching between ν_{max} of 3445–3345 cm^{-1}.

Flavanones **2a**–**4a** were treated with $NaBH_4$ at room temperature to afford a single diastereomer of flavanols **2d**–**4d** in moderate to high yield (Scheme 4). The NOESY experiment of **2d** showed relatively cis orientation of protons at C2, C3, and C4 (Fig. 1). This means that the insertion of the hydride ion occurred on the less hindered side. The ^{13}C NMR spectra of alcohol derivatives showed signals of C4 upfield shifted from carbonyl carbons (δ 189.2–195.7) to oxycarbons (δ 63.3–67.0). In addition, the ^1H NMR spectra showed signals of H-4 at δ 5.24–5.27. In total, sixteen flavonoid derivatives were synthesized and four of them (**2a**, **2c, 2d**, and **4c**) were new derivatives.

Cytotoxicity assays against human epidermoid carcinoma of oral cavity (KB) and human small cell lung cancer (NCI-H187) cell lines were performed employing Resazurin Microplate Assay (REMA).[15] Cytotoxicity assay against African green monkey kidney cell line (Vero cells) was evaluated using Green Fluorescent Protein (GFP) based assay.[16] Ellipticine was included as a reference substance.



Scheme 4. Reagents and conditions: $NaBH_4$, CH_3OH, rt, 1 h, **2d** (95%), **3d** (92%), **4d** (76%).

The cytotoxicity results are shown in Table 1. Compound **2a** showed cytotoxicity against KB and NCI-H187 with IC_{50} values of 30.37 and 98.23 μM, respectively. Flavanones **3a** and **4a** demonstrated moderate cytotoxicity against NCI-H187 cells with IC_{50} values of 33.06 and 25.10 μM, respectively, while showing weak cytotoxicity against KB cells with IC_{50} values of 39.85 and 78.80 μM, respectively. However, these three compounds exhibited more cytotoxicity than the parent flavones. These results suggest that the flavanone skeleton is essential for cytotoxicity. In contrast, flavanone **1a** did not show cytotoxicity against any cell lines. Unmethylated flavanone **5a** showed weak cytotoxicity ($IC_{50} \approx 95$–100 μM) in comparison with the parent compound (**3a**).

Dihydrochalcones **1b**, **3b**, and **4b** showed no cytotoxicity against any cell lines, except **1b** which exhibited strong cytotoxicity against NCI-H187 cells with an IC_{50} value of 9.70 μM. This may be due to the structure of this compound containing two phenolic groups.

On attempting to find active substances, other functional groups were produced and we anticipate obtaining useful results. From the cytotoxicity of **2a**–**4a**, we found that the flavanone structure showed potent cytotoxicity and therefore we decided to change the carbonyl group to oxime and investigate the activity of a series of oxime derivatives. Fortunately, **1c** showed strong cytotoxic activity against KB cell line with an IC_{50} value of 0.26 μM. Interestingly, oximes **1c** and **2c** showed dramatic improvement in potency against NCI-H187 cells by showing IC_{50} values of 0.014 and 0.23 μM, respectively, which is higher than the ellipticine standard (IC_{50} = 2.77 μM) by nearly 200- and 12-fold. In addition, oximes **4c** (IC_{50} = 4.04 μM) and **5c**

Table 1
Cytotoxicity of modified flavonoids[a]

Compound	Cytotoxicity, IC_{50} (μM)		
	KB	NCI-H187	Vero cells
1–4	Inactive[b]	Inactive[b]	Inactive[b]
1a	Inactive[b]	Inactive[b]	–
2a	30.37	98.23	–
3a	39.85	33.06	–
4a	78.80	25.10	–
5a	99.16	95.53	–
1b	Inactive[b]	9.70	–
3b	Inactive[b]	Inactive[b]	–
4b	Inactive[b]	Inactive[b]	–
1c	0.26	0.014	2.67
2c	23.91	0.23	41.20
3c	Inactive[b]	53.19	–
4c	43.15	4.04	Inactive[b]
5c	84.90	2.32	Inactive[b]
2d	56.79	95.56	–
3d	92.14	Inactive[b]	–
4d	Inactive[b]	Inactive[b]	–
Ellipticine	1.79	2.77	2.29

[a] Data shown are replicate experiments.
[b] Inactive at >100 μM.

(IC_{50} = 2.32 μM) showed strong cytotoxicity against NCI-H187 cell line. The results show convincingly that the oxime group at position 4 of flavanone plays an important role in cytotoxicity. In contrast, **3c** showed weak cytotoxicity against NCI-H187 cell line and was inactive against KB cells. As in the previous report, this compound showed weak cytotoxicity against HepG2 and T47D cell lines.[14] These results suggest that the hydroxyl group at C5 position is crucial for cytotoxicity. In case of **5c**, it showed stronger toxicity against KB and NCI-H187 cell lines (IC_{50} = 84.90 and 2.32 μM, respectively) than the corresponding methylated derivative **3c**. This derivative also showed good activity against HepG2 and T47D cell lines.[14] These results confirm that the hydroxyl group is favorable to the activity. In addition, it is interesting to note that the structure of compounds **1c** and **2c** are 5-hydroxy-7-methoxyflavanone oxime derivatives. These substances are likely to be useful as lead compounds for the development of a novel class of cytotoxic agents. However, cytotoxicity against normal cells should be concerned. Therefore, cytotoxicity of these four compounds (**1c**, **2c**, **4c**, and **5c**) was evaluated against Vero cells. As shown in Table 1, **4c** and **5c** exhibited no cytotoxicity against this cell lines. Compound **1c** showed an IC_{50} value of 2.67 μM against Vero cells which is about 10- and 190-fold lower toxicity than KB and NCI-H187 cell lines, respectively. In case of **2c**, it demonstrated an IC_{50} value of 41.20 μM against Vero cells which is about 180-fold lower toxicity than NCI-H187 cell line. All results show convincingly that these substances are likely to be useful as lead compounds for the development of a novel class of cytotoxic agents, especially against lung cancer. Finally, flavanols **2d**–**4d** showed less potent cytotoxicity against KB and NCI-H187 cell lines in comparison with the parents **2a**–**4a**. These results confirm that the 4-carbonyl groups or carbonyl analogs of flavanone are crucial for the activity.

In summary, we prepared a series of modified flavonoids under simple reactions and evaluated their cytotoxicity. We found that oxime derivatives, especially **1c** and **2c** possess stronger cytotoxicity than the ellipticine standard and may be promising leads for the development of cytotoxic agents. These results encourage the synthesis of flavonoid analogs for improving cytotoxic activity.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.03.054.

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