



# Mannich aminomethylation of flavonoids and anti-proliferative activity against breast cancer cell

T. Kim-Dung Hoang<sup>1,3</sup> · T. Kim-Chi Huynh<sup>1,3</sup> · T. Hong-Tuoi Do<sup>4</sup> · Thanh-Danh Nguyen<sup>2,3</sup>

Received: 13 November 2017 / Accepted: 23 January 2018 / Published online: 30 January 2018  
© Institute of Chemistry, Slovak Academy of Sciences 2018

## Abstract

We herein report Mannich aminomethylation of variously structural flavonoids and their biological evaluation against human breast cancer cell. Mannich reaction showed that substitution at C-6 position depends on amine basicity and C-ring feature of flavonoids. All five flavonoid substrates reacted with strong amine bases to afford the bis(6,8-aminomethyl) derivatives, while with weak amines, the different products were obtained dependently on structural characteristic of flavonoid. 3-OH and 3-*O*-substituted groups on the C-ring exhibited the deactivated aminomethylation at C-6 position, whereas substitution at this position was independent on bond feature at C-2 and C-3 on the C-ring. Screening anti-proliferative activity showed six flavonoids possessed activity against breast cancer cell, MDA-MB-231. Among them, the flavonoids, luteolin (**2**) and 3',4',5,7-tetrahydroxy-6,8-bis(pyrrolidin-1-ylmethyl)-3-rutinosylflavone (**3a**), displayed the highest anti-proliferative activity with the lowest IC<sub>50</sub> values.

**Keywords** Mannich aminomethylation · Flavonoid · Synthesis · Anti-proliferative · MDA-MB-231

## Introduction

Flavonoid structures are based upon a 15-carbon skeleton consisting of two benzene rings linked via a heterocyclic pyran ring. They can be divided into a variety of classes which differ in the level of oxidation and pattern

of substitution of the pyran ring (e.g., quercetin, a flavonol, and luteolin, a flavone, bearing different substitution groups at C-3 position) (Fig. 1). Individual compounds within a class differ in the pattern of substitution of the benzene rings (Kumar and Pandey 2013). Biological activities of flavonoids depend remarkably on their structural characteristics such as degree of hydroxylation and substitution. A small alteration in the chemical structure may lead to significant changes in biological activities. Hence, the flavonoids possess a wide range of bioactivities such as antioxidant (Rice-Evans et al. 1996; Rice-Evans 2001; Pietta 2000), antifungal (Tempesti et al. 2012), antiviral (Ng et al. 1997; Orhana et al. 2010; Kaul et al. 1985), anti-proliferative (Chahar et al. 2011; Kanadaswami et al. 2005; Ravishankar et al. 2013), and anti-inflammatory agent (Pan et al. 2010; Cottiglia et al. 2005; Candiracci et al. 2012).

In recent years, numerous studies have been conducted on the potential antimetastasis activity of natural flavonoids in various tumor cell lines. For instance, quercetin has been shown to reduce the human prostate cancer, PC-3 cells (Vijayababu et al. 2006), and kaempferol, genistin, and daidzein revealed to inhibit human breast cancer, MDA-MB-231 cells (Lee et al. 2008; Phromnoi et al. 2009). Among of the flavonoid derivatives, aminomethylated flavonoids are particularly valued due to potential

**Electronic supplementary material** The online version of this article (<https://doi.org/10.1007/s11696-018-0402-1>) contains supplementary material, which is available to authorized users.

- ✉ T. Kim-Dung Hoang  
htkdung@ict.vast.vn
- ✉ Thanh-Danh Nguyen  
danh5463bd@yahoo.com

- <sup>1</sup> Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam
- <sup>2</sup> Institute of Research and Development, Duy Tan University, Da Nang, Vietnam
- <sup>3</sup> Institute of Chemical Technology, Vietnam Academy of Science and Technology, 1 Mac Dinh Chi Street, District 1, Ho Chi Minh, Vietnam
- <sup>4</sup> Department of Pharmacology, Faculty of Pharmacy, University of Medicine and Pharmacy, Ho Chi Minh City, 41 Dinh Tien Hoang Street, District 1, Ho Chi Minh, Vietnam

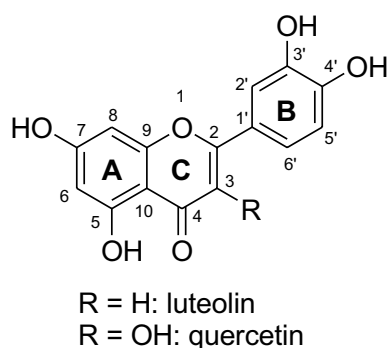


Fig. 1 Structures of flavonoids

activity against human cancer cells (Ha et al. 2016; Nguyen et al. 2017; Chen et al. 2015; Tugrak et al. 2015). Although Mannich aminomethylation of flavonoids has been known, most of the reported studies used C-6-substituted flavonoids as substrates (Zhang et al. 2008; Babu et al. 2008; Yusakul et al. 2016) or cyclic imines were used as the electrophilic reagents (Nguyen et al. 2011). Recently, there are several studies on the aminomethylation of natural flavonoids using in situ generated iminium ions that proceeded with formation of C-6 (Frasinyuk et al. 2015; Kukhareva et al. 2004) or C-8-monosubstituted (Buravlev et al. 2017; Gorbunov et al. 2016) or disubstituted flavonoids (Nguyen et al. 2015b; Zhurakulov et al. 2015; Nifant'ev et al. 2013). Helgen et al. (2015) have aminomethylated quercetin to afford only a 8-monoaminomethyl derivative when using an imine salt made from 1-methylpiperazine while only a 6,8-diaminomethyl derivative was produced with the imine salts of piperidine. All cases focused on the investigation of bioactivity and no systematic studies were carried out for investigating the influence of amine and iminium ions reactivity and features of flavonoid skeleton driving the Mannich aminomethylation.

In connection with our ongoing research program involving the effect of natural product structures on the synthesis (Dang et al. 2014, 2017; Nguyen et al. 2015a, 2016; Hoang et al. 2015), we have showed that alkylation and acylation of 5-hydroxyflavonoids significantly depended on reagents as well as structural characteristic of ring C of flavonoids, and these derivatives also exhibited especial bioactivities (Hoang et al. 2015). The previously reported results prompted us to investigate this effect on the aminomethylation of flavonoids as well as their bioactive evaluation. Herein, we report that the Mannich reaction of various flavonoid substrates with iminium salts generated in situ from secondary cyclic amines and aqueous formaldehyde can provide either 8-monosubstituted or 6,8-disubstituted derivatives depending on amine reagents and substrate features.

## Results and discussion

### Chemistry

The general synthetic routes are illustrated in Scheme 1. Our strategy for the synthesis of the aminomethylated derivatives at C-6 and C-8 of A-ring of flavonoids relied upon electrophilic substitution via the Mannich reaction carried out between flavonoids with formaldehyde and secondary cyclic amines at room temperature in ethanol. Three cyclic amines with different basicity, pyrrolidine ( $pK_a = 11.27$ ), 1-methylpiperazine ( $pK_a = 9.14$ ), and morpholine ( $pK_a = 8.36$ ) were converted in situ into the respective electrophilic iminium ions. To get an insight into influence of flavonoid structures on the substitution at various positions, five natural flavonoids with structural variations were used as the substrates.

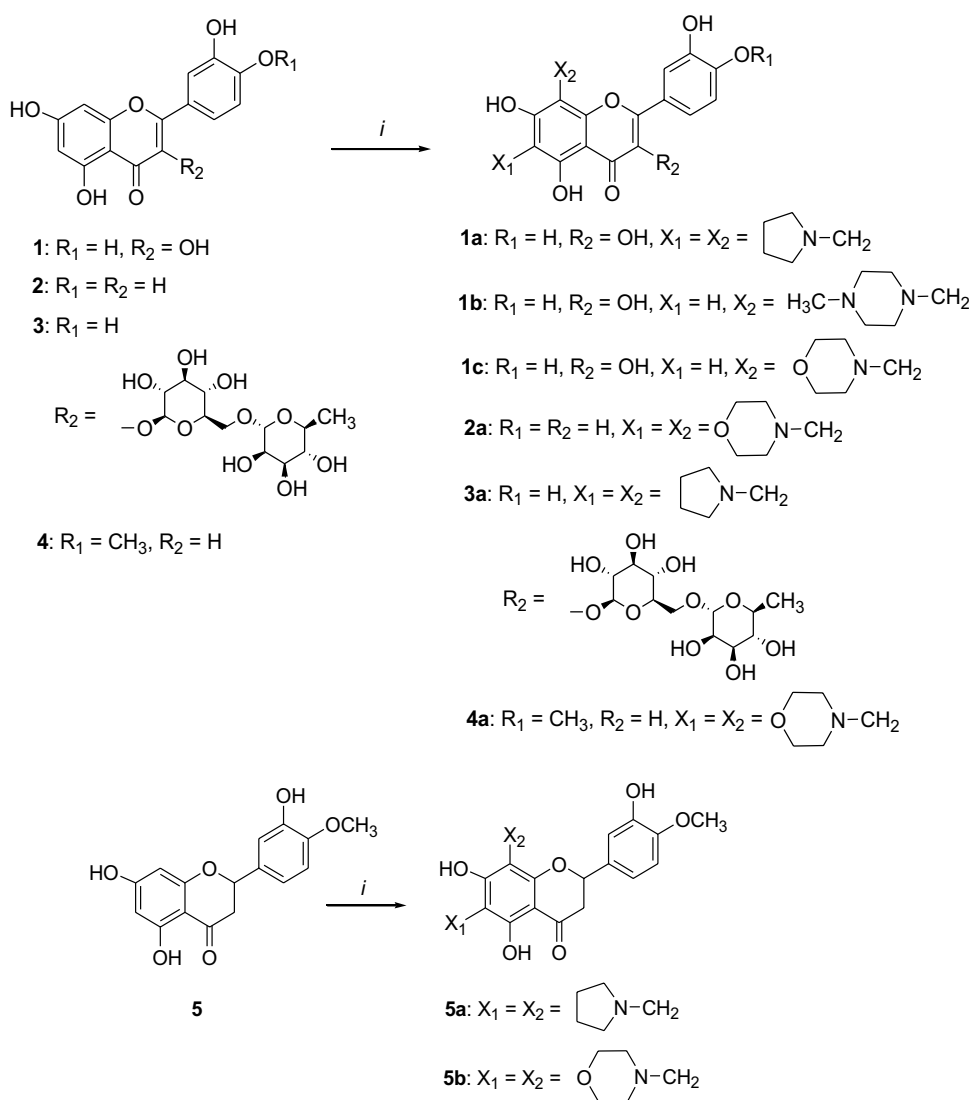
The aminomethylation of quercetin with these bases and aqueous formaldehyde afforded products **1a–1c** in over 60% yields. The result showed that aminomethylation of flavonoids was strongly affected by basicity of amines. The substitution with pyrrolidine occurred at both C-6 and C-8 positions. This is evident from  $^1\text{H NMR}$  of **1a** which protons at C-6 ( $\delta$  6.20 ppm) and C-8 ( $\delta$  6.40 ppm) positions of quercetin disappeared. Interestingly, only C-8 monosubstituted products were obtained when flavonoids were treated with an excess of 1-methylpiperazine and morpholine to give **1b** and **1c**, respectively. This is attributed to the lower basicity of these amines. Similar results were reported by Lis and Marisca (1987) for the other substrates under the same condition.

The position of the aminomethyl groups in products **1b** and **1c** was unambiguously determined by HMBC NMR spectroscopy. The aminomethylene protons of **1b** and **1c** ( $\delta$  3.87 and 3.82 ppm, respectively), which were well resolved, were coupled long-range to respective C-9 carbons at  $\delta$  153.63 and 153.94 ppm. This allows for the assignment of the aminomethyl group to the C-8 position of the quercetin derivatives.

To determine influence of 3-OH group in flavonoids, luteolin (**2**) was used in comparison with quercetin. As expected, the aminomethylation of **2** with the weakest base afforded the bis(6,8-morpholin) product (**2a**) in 63% yield. In comparison with formation of **1c**, this result reflected that 3-OH group obstructed to electrophilic substitution at C-6 in the flavonoid skeleton. Although reason of the effect has not been described previously, it may be because the electron pair of oxygen at C-3 position stabilized the non-covalent six-membered ring (Hoang et al. 2015) which is attributed to reduction of electron density at C-6 position of quercetin.

In a similar fashion, rutin (**3**) was treated with all bases. Unfortunately, conversion occurred in very poor yields and

**Scheme 1** Synthetic routes of flavonoids. Reagents. (i) amines (10 equiv.), HCHO (37%, 10 equiv.), ethanol, rt



no pure aminomethylation products were obtained when treating with either 1-methylpiperazine or morpholine under the same reaction conditions. However, the aminomethylation of **3** with pyrrolidine was observed more easily to afford bis(6,8-pyrrolidine) product (**3a**) in 54% yield, indicating that 3-*O*-substituted group is also an important factor in this transformation.

Finally, to understand the influence of the double bond of C-ring on the flavonoid aminomethylation, hesperetin (**4**) was employed in comparison with substitution of diosmetin (**5**). Similar to luteolin, the reaction of **4** with morpholine was converted into bis(6,8-morpholine) derivative (**4a**) in a good yield (81%). In addition, conversion of **5** with both pyrrolidine and morpholine gave diaminomethylated derivatives **5a** and **5b**, respectively in over 60% yields. It showed that bond feature at C-2 and C-3 was independent on the Mannich aminomethylation at the C-6 position. All the synthesized flavonoids, including new derivatives **1a**, **2a**, **3a**, **4a**,

**5a**, and **5b**, were determined physical properties and identified the structure by HRMS and NMR spectra.

### Anti-proliferative activity

The anti-proliferative activity of all flavonoid derivatives was tested in the breast cancer cell line MDA-MB-231 (Denizot and Lang 1986). A screening program revealed that several flavonoids exhibited anti-proliferative activity at 100  $\mu\text{M}$ , as depicted in Table 1. Six compounds **1**, **1c**, **2a**, **3a**, and **5a** showed good anti-proliferative activity (inhibition more than 50%) which would be further used to test  $\text{IC}_{50}$  values. It is noteworthy that the disubstituted derivatives of rutin (**3a**) and hesperetin (**5c**) possessed the activity greater than the parent flavonoids, while all derivatives of quercetin exhibited the lower activity than the parent compounds.

$\text{IC}_{50}$  values against MDA-MB-231 cell are shown in Table 2. Although the  $\text{IC}_{50}$  values of the flavonoids are

**Table 1** Percent inhibition of MDA-MB-231 cell by all flavonoids

Sub.	Inhibition at 100 $\mu$ M (%)
<b>1</b>	78.4 $\pm$ 7.4
<b>1a</b>	0 $\pm$ 0.5
<b>1b</b>	12.9 $\pm$ 0.2
<b>1c</b>	59.5 $\pm$ 2.3
<b>2</b>	82.5 $\pm$ 10.2
<b>2a</b>	77.6 $\pm$ 5.7
<b>3</b>	6.1 $\pm$ 0.1
<b>3a</b>	88.6 $\pm$ 6.9
<b>4</b>	8.2 $\pm$ 0.2
<b>4a</b>	9.2 $\pm$ 0.3
<b>5</b>	31 $\pm$ 0.6
<b>5a</b>	77.4 $\pm$ 9.1
<b>5b</b>	11.6 $\pm$ 0.6

**Table 2** IC<sub>50</sub> value of selected flavonoids against MDA-MB-231 cell

Sub.	IC <sub>50</sub> ( $\mu$ mol/mL)
<b>1</b>	25.30 $\pm$ 0.66
<b>1c</b>	38.67 $\pm$ 4.52
<b>2</b>	7.29 $\pm$ 0.08
<b>2a</b>	44.74 $\pm$ 5.01
<b>3a</b>	7.57 $\pm$ 0.40
<b>5a</b>	58.61 $\pm$ 4.02
<b>Paclitaxel</b>	0.12 $\pm$ 0.00

higher than that of the standard drug (paclitaxel), most of the compounds displayed a significant anti-proliferative activity against MDA-MB-231 cell. Among them, flavonoids **2** and **3a** exhibit the highest anti-proliferative activity with IC<sub>50</sub> of 7.29 and 7.57  $\mu$ mol/mL, respectively.

## Conclusions

Our studies on Mannich aminomethylation of flavonoids were suggested as follows: (1) amine basicity was critical to design the aminomethylated products and iminium ions prepared from strong bases attracted to both C-6 and C-8 positions of flavonoid. (2) With weak bases, 3-OH and 3-O-substituted flavonoids could obstruct electrophilic substitution at C-6 of flavonoid. (3) Bond feature at C-2 and C-3 did not almost affect the substitution at C-6 even the weak bases.

All the compounds were evaluated for cytotoxicity of breast cancer cell, MDA-MB-231. Six compounds **1**, **1c**, **2**, **2a**, **3a**, and **5a** showed good anti-proliferative activity. Among them, the flavonoids **2** and **3a** displayed the lowest IC<sub>50</sub> values.

## Experimental

### Chemistry

All the materials were purchased from Merck (Germany) or Aldrich. The other solvents were purchased from Fluka and used without further purification. Melting points were measured with an Electrothermal Model 9200 (England). UV–Vis spectra were measured using a Shimadzu, Model UV-1650PC spectrophotometer, and reported as  $\lambda_{\max}$  in nm (abs.). FTIR spectra were obtained with an Equinox 55 IR—Bruker (Germany) spectrometer; absorption bands are recorded in wave number ( $\text{cm}^{-1}$ ). The ESI–MS were performed on a VG Zab Spec (70 eV) instrument. <sup>1</sup>H (500 MHz) and <sup>13</sup>C (125 MHz) NMR were recorded on a BRUKER AVANCE 500 NMR spectrometer using DMSO-*d*<sub>6</sub> as solvent and tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported in  $\delta$  relative to TMS.

### General procedure for aminomethylation of flavonoids

To a stirred solution of flavonoid (3.31 mmol) in ethanol (50 mL) was slowly added amines (33.3 mmol) and a 37% formaldehyde solution (2.48 g, 35 mmol). The mixture was stirred at room temperature for 4 h, monitoring by TLC. The residue was filtered off, washed with 50 mL of ethanol, and then dried in the vacuum.

### 2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-6,8-bis(pyrrolidin-1-ylmethyl)-4H-chromen-4-one (**1a**)

Yellow powder, m.p. 223–225 °C. Yield 68%. FTIR (KBr)  $\nu/\text{cm}^{-1}$  3376, 2968, 1651, 1560, 1260, 1069. UV–Vis  $\lambda_{\max}$  (nm) (abs.) 238 (0.15), 246 (0.16), 287 (0.09), 321 (0.11), 341 (0.10), 268 (0.12). <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>,  $\delta$ , ppm): 7.69 (1H, d, J 2.0 Hz, H-2'), 7.44 (1H, dd, J 8.5, 2.0 Hz, H-6'), 6.85 (1H, d, J 8.5 Hz, H-5'), 3.92 (2H, s, C-8-CH<sub>2</sub>), 3.88 (2H, s, C-6-CH<sub>2</sub>), 2.72–2.62 (8H, m, N(CH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>), 1.76–1.67 (8H, m, N(CH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>). <sup>13</sup>C NMR (125 MHz, DMSO-*d*<sub>6</sub>,  $\delta$ , ppm): 175.35 (C-4), 168.00 (C-7), 156.92 (C-5), 153.52 (C-9), 147.61 (C-4'), 145.50 (C-2), 145.14 (C-3'), 135.26 (C-3), 122.42 (C-1'), 119.67 (C-6'), 115.56 (C-5'), 114.89 (C-2'), 103.83 (C-10), 100.85 (C-6), 100.30 (C-8), 52.82 (C-8-CH<sub>2</sub>), 52.73 (C-6-CH<sub>2</sub>), 48.68 (N(CH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>), 46.90 (N(CH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>), 23.12 (N(CH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>), 23.05 (N(CH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>). HRMS (*m/z*): 469.1965 [M+H]<sup>+</sup>; 469.1975 calcd [M+H]<sup>+</sup> for C<sub>25</sub>H<sub>29</sub>N<sub>2</sub>O<sub>7</sub>.

**2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-8-((4-methylpiperazin-1-yl)methyl)-4H-chromen-4-one (1b)**

Yellow powder, m.p. 233–235 °C (233–235 °C, Helgen et al. 2015). Yield 66%. FTIR (KBr)  $\nu/\text{cm}^{-1}$  3296, 2975, 1655, 1555, 1201, 1043. UV–Vis  $\lambda_{\text{max}}$  (nm) (abs.) 229 (0.78), 246 (1.09), 277 (0.36), 363 (1.16).  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ ,  $\delta$ , ppm) (Zhang et al. 2008; Helgen et al. 2015): 12.52 (1H, brs, OH) 7.70 (1H, d, J 2.0 Hz, H-2'), 7.57 (1H, dd, J 8.5, 2.0 Hz, H-6'), 6.89 (1H, d, J 8.5 Hz, H-5'), 6.17 (1H, s, H-6), 3.87 (2H, s, C-8- $\text{CH}_2$ ), 2.63–2.58 (4H, m,  $\text{CH}_3\text{-N}(\text{CH}_2\text{CH}_2)_2$ ), 2.38–2.36 (4H, m,  $\text{CH}_3\text{-N}(\text{CH}_2\text{CH}_2)_2$ ), 2.16 (3H, s, N- $\text{CH}_3$ ).  $^{13}\text{C}$  NMR (125 MHz, DMSO- $d_6$ ,  $\delta$ , ppm): 175.90 (C-4), 164.15 (C-7), 159.54 (C-5), 153.63 (C-9), 147.66 (C-4'), 146.45 (C-2), 145.08 (C-3'), 135.62 (C-3), 122.17 (C-1'), 119.89 (C-6'), 115.61 (C-5'), 114.97 (C-2'), 102.73 (C-8), 99.74 (C-10), 98.12 (C-6), 54.43 ( $\text{CH}_3\text{-N}(\text{CH}_2\text{CH}_2)_2$ ), 51.95 ( $\text{CH}_3\text{-N}(\text{CH}_2\text{CH}_2)_2$ ), 51.18 (C-8- $\text{CH}_2$ ), 45.45 (N- $\text{CH}_3$ ). HRMS ( $m/z$ ): 415.1471  $[\text{M}+\text{H}]^+$ ; 415.1505 calcd  $[\text{M}+\text{H}]^+$  for  $\text{C}_{21}\text{H}_{23}\text{N}_2\text{O}_7$ .

**2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-8-(morpholinomethyl)-4H-chromen-4-one (1c)**

Yellow powder, m.p. 253–257 °C, yield 65%. FTIR (KBr)  $\nu/\text{cm}^{-1}$  3320, 2970, 1654, 1552, 1201, 1072. UV–Vis  $\lambda_{\text{max}}$  (nm) (abs.) 229 (0.20), 246 (0.28), 276 (0.09), 363 (0.30).  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ ,  $\delta$ , ppm) (Zhang et al. 2008): 7.73 (1H, d, J 2.0 Hz, H-2'), 7.61 (1H, dd, J 8.5, 2.0 Hz, H-6'), 6.91 (1H, d, J 8.5 Hz, H-5'), 6.22 (1H, s, H-6), 3.82 (2H, C-8- $\text{CH}_2$ ), 3.59 (4H, m, N-( $\text{CH}_2\text{CH}_2$ ) $_2$ O), 2.53 (4H, m, N-( $\text{CH}_2\text{CH}_2$ ) $_2$ O).  $^{13}\text{C}$  NMR (125 MHz, DMSO- $d_6$ ,  $\delta$ , ppm): 175.95 (C-4), 163.48 (C-7), 159.54 (C-5), 153.94 (C-9), 147.68 (C-2), 146.58 (C-4'), 145.08 (C-3'), 135.64 (C-3), 122.21 (C-1'), 119.93 (C-6'), 115.60 (C-5'), 115.05 (C-2'), 102.87 (C-10), 100.17 (C-8), 97.94 (C-6), 66.08 (N-( $\text{CH}_2\text{CH}_2$ ) $_2$ O), 52.70 (N-( $\text{CH}_2\text{CH}_2$ ) $_2$ O), 50.96 (C-8- $\text{CH}_2$ ). HRMS ( $m/z$ ): 402.1189  $[\text{M}+\text{H}]^+$ ; 402.1189 calcd  $[\text{M}+\text{H}]^+$  for  $\text{C}_{20}\text{H}_{20}\text{NO}_8$ .

**2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-6,8-bis(morpholinomethyl)-4H-chromen-4-one (2a)**

Yellow powder, m.p. 230–233 °C, yield 63%. FTIR (KBr)  $\nu/\text{cm}^{-1}$  3260, 2963, 1649, 1583, 1213, 1072. UV–Vis  $\lambda_{\text{max}}$  (nm) (abs.) 236 (0.65), 247 (0.68), 252 (0.68), 289 (0.38).  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ ,  $\delta$ , ppm): 7.44 (1H, dd, J 9.0, 2.0 Hz, H-6'), 7.42 (1H, d, J 2.0 Hz, H-2'), 6.91 (1H, d, J 9.0 Hz, H-5'), 6.68 (1H, s, H-3), 3.78 (2H, s, C-8- $\text{CH}_2$ ), 3.73 (2H, s, C-6- $\text{CH}_2$ ), 3.60–3.56 (8H, m, N-( $\text{CH}_2\text{CH}_2$ ) $_2$ O), 2.54–2.49 (8H, m, N-( $\text{CH}_2\text{CH}_2$ ) $_2$ O).  $^{13}\text{C}$  NMR (125 MHz, DMSO- $d_6$ ,  $\delta$ , ppm): 181.95 (C-4), 164.37 (C-7), 163.61 (C-2), 158.16 (C-5), 154.43 (C-9), 149.75 (C-4'), 145.79

(C-3'), 121.75 (C-1'), 118.97 (C-6'), 113.43 (C-5'), 116.06 (C-2'), 103.71 (C-10), 102.76 (C-6), 102.55 (C-3), 100.90 (C-8), 66.15 (N-( $\text{CH}_2\text{CH}_2$ ) $_2$ O), 65.97 (N-( $\text{CH}_2\text{CH}_2$ ) $_2$ O), 52.78 (N-( $\text{CH}_2\text{CH}_2$ ) $_2$ O), 52.36 (N-( $\text{CH}_2\text{CH}_2$ ) $_2$ O), 51.43 (C-6- $\text{CH}_2$ ), 50.36 (C-8- $\text{CH}_2$ ). HRMS ( $m/z$ ): 485.1920  $[\text{M}+\text{H}]^+$ ; 485.1924 calcd  $[\text{M}+\text{H}]^+$  for  $\text{C}_{25}\text{H}_{29}\text{N}_2\text{O}_8$ .

**2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-6,8-bis(pyrrolidin-1-ylmethyl)-3-(((2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(((2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)methyl)tetrahydro-2H-pyran-2-yl)oxy)-4H-chromen-4-one (3a)**

Orange powder, m.p. 154–156 °C, yield 57%. FTIR (KBr)  $\nu/\text{cm}^{-1}$  3479, 2983, 1754, 1629, 1503, 1218, 1073. UV–Vis  $\lambda_{\text{max}}$  (nm) (abs.) 229 (0.56), 243 (0.64), 269 (0.31), 299 (0.53).  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ ,  $\delta$ , ppm): 7.62–7.60 (2H, m, H-5',6'), 6.83 (1H, d, J 9.0 Hz, H-2'), 5.30 (1H, d, J 7.5 Hz, H-1-glucose), 4.33 (1H, s, H-1-rhamnose), 3.94 (2H, s, C-8- $\text{CH}_2$ ), 3.84 (2H, s, C-6- $\text{CH}_2$ ), 3.70 (2H, d, J 10.5 Hz, H-6-glucose), 3.30–3.19 (5H, m, H-2,3,4,5-glucose, H-5-rhamnose), 3.09–3.02 (3H, m, H-2,3,4-rhamnose), 2.81 (2H, m, N( $\text{CH}_2\text{CH}_2$ ) $_2$ ), 2.69 (2H, m, N( $\text{CH}_2\text{CH}_2$ ) $_2$ ), 1.81 (2H, m, N( $\text{CH}_2\text{CH}_2$ ) $_2$ ), 1.74 (2H, m, N( $\text{CH}_2\text{CH}_2$ ) $_2$ ), 1.00 (3H, d, J 6.5 Hz, H-6-rhamnose).  $^{13}\text{C}$  NMR (125 MHz, DMSO- $d_6$ ,  $\delta$ , ppm): 176.64 (C-4), 162.30 (C-7), 157.50 (C-2), 155.34 (C-9), 153.98 (C-5), 148.79 (C-3), 148.65 (C-4'), 144.85 (C-3'), 132.75 (C-1'), 121.44 (C-6'), 116.15 (C-5'), 115.15 (C-2'), 103.88 (C-10), 103.19 (C-6), 101.46 (C-1-glucose), 100.94 (C-1-rhamnose), 100.73 (C-8), 76.57 (C-3-glucose), 75.93 (C-5-glucose), 74.05 (C-2-glucose), 71.90 (C-2-rhamnose), 70.52 (C-4-rhamnose), 70.25 (C-4-glucose, C-3-rhamnose), 68.21 (C-5-rhamnose), 66.90 (C-6-glucose), 53.25 (N( $\text{CH}_2\text{CH}_2$ ) $_2$ ), 52.70 (N( $\text{CH}_2\text{CH}_2$ ) $_2$ ), 48.93 (C-8- $\text{CH}_2$ ), 47.03 (C-6- $\text{CH}_2$ ), 23.10 (N( $\text{CH}_2\text{CH}_2$ ) $_2$ ), 23.04 (N( $\text{CH}_2\text{CH}_2$ ) $_2$ ), 17.72 (C-6-rhamnose). HRMS ( $m/z$ ): 777.3099  $[\text{M}+\text{H}]^+$ ; 777.3082 calcd  $[\text{M}+\text{H}]^+$  for  $\text{C}_{37}\text{H}_{49}\text{N}_2\text{O}_{16}$ .

**5,7-Dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-6,8-bis(morpholinomethyl)-4H-chromen-4-one (4a)**

Pale yellow powder, m.p. 278–280 °C, yield 81%. FTIR (KBr)  $\nu/\text{cm}^{-1}$  3420, 2961, 1649, 1513, 1217, 1069. UV–Vis  $\lambda_{\text{max}}$  (nm) (abs.) 250 (0.29), 262 (0.34), 294 (0.18), 343 (0.31).  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ ,  $\delta$ , ppm): 7.57 (1H, dd, J 8.5, 2.5 Hz, H-6'), 7.47 (1H, d, J 2.5 Hz, H-2'), 7.12 (1H, d, J 8.5 Hz, H-5'), 6.76 (1H, s, H-3), 3.87 (3H, s,  $\text{OCH}_3$ ), 3.79 (2H, s, C-6- $\text{CH}_2$ ), 3.75 (2H, s, C-8- $\text{CH}_2$ ), 3.62–3.57 (8H, m, N-( $\text{CH}_2\text{CH}_2$ ) $_2$ O), 2.54–2.50 (8H, m, N-( $\text{CH}_2\text{CH}_2$ ) $_2$ O).  $^{13}\text{C}$  NMR (125 MHz, DMSO- $d_6$ ,  $\delta$ , ppm): 181.97 (C-4), 164.45 (C-7), 163.21 (C-2), 158.12

(C-5), 154.46 (C-9), 151.17 (C-4'), 146.84 (C-3'), 123.22 (C-1'), 118.64 (C-6'), 112.99 (C-2'), 112.22 (C-5'), 103.68 (C-6), 103.19 (C-10), 102.80 (C-3), 100.99 (C-8), 66.14 (N-(CH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>O), 65.94 (N-(CH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>O), 55.77 (OCH<sub>3</sub>), 52.81 (N-(CH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>O), 52.33 (N-(CH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>O), 51.47 (C-8-CH<sub>2</sub>), 50.33 (C-8-CH<sub>2</sub>). HRMS (*m/z*): 499.2077 [M+H]<sup>+</sup>; 499.2080 calcd [M+H]<sup>+</sup> for C<sub>26</sub>H<sub>31</sub>N<sub>2</sub>O<sub>8</sub>.

#### 5,7-Dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-6,8-bis(pyrrolidin-1-ylmethyl)chroman-4-one (5a)

Pale yellow powder, m.p. 178–180 °C, yield 64%. FTIR (KBr)  $\nu/\text{cm}^{-1}$  3418, 2958, 1643, 1537, 1257, 1060. UV–Vis  $\lambda_{\text{max}}$  (nm) (abs.) 239 (0.20), 248 (0.21), 259 (0.19), 283 (0.27). <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>,  $\delta$ , ppm): 6.97 (1H, d, J 2.0 Hz, H-2'), 6.89 (1H, dd, J 8.0, 2.0 Hz, H-6'), 6.94 (1H, d, J 8.0 Hz, H-5'), 5.38 (1H, dd, J 12.0, 3.0 Hz, H-2), 4.01 (2H, s, C-8-CH<sub>2</sub>), 3.98 (2H, s, C-6-CH<sub>2</sub>), 3.77 (3H, s, OCH<sub>3</sub>), 3.17–3.12 (8H, m, N-(CH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>), 3.01 (2H, dd, J 17.0, 12.0 Hz, H-3a), 2.65 (1H, dd, J 17.0, 3.0 Hz, H-3b), 1.90–1.87 (8H, m, N-(CH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>). <sup>13</sup>C NMR (125 MHz, DMSO-*d*<sub>6</sub>,  $\delta$ , ppm): 191.65 (C-4), 177.57 (C-7), 163.11 (C-5), 160.91 (C-9), 147.68 (C-4'), 146.54 (C-3'), 131.85 (C-1'), 117.20 (C-6'), 113.88 (C-2'), 112.03 (C-5'), 98.88 (C-6), 98.64 (C-8), 96.03 (C-10), 77.64 (C-2), 55.67 (OCH<sub>3</sub>), 52.50 (N-(CH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>), 52.31 (N-(CH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>), 47.83 (C-8-CH<sub>2</sub>), 47.69 (C-6-CH<sub>2</sub>), 41.37 (C-3), 22.68 (N-(CH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>). HRMS (*m/z*): 469.2345 [M+H]<sup>+</sup>; 469.2339 calcd [M+H]<sup>+</sup> for C<sub>26</sub>H<sub>33</sub>N<sub>2</sub>O<sub>6</sub>.

#### 5,7-Dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-6,8-bis(morpholinomethyl)chroman-4-one (5b)

Pale yellow powder, m.p. 175–180 °C, yield 62%. FTIR (KBr)  $\nu/\text{cm}^{-1}$  3450, 2948, 1622, 1535, 1259, 1027. UV–Vis  $\lambda_{\text{max}}$  (nm) (abs.) 244 (0.21), 279 (0.86). <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>,  $\delta$ , ppm): 13.26 (1H, br.s, OH), 9.15 (1H, br.s, OH), 6.96 (1H, m, H-2'), 6.94 (1H, m, H-5'), 6.91 (1H, dd, J 8.0, 2.0 Hz, H-6'), 5.46 (1H, dd, J 12.0, 3.0 Hz, H-2), 4.04 (2H, s, C-8-CH<sub>2</sub>), 4.00 (2H, s, C-6-CH<sub>2</sub>), 3.78 (3H, s, OCH<sub>3</sub>), 3.78 (8H, m, N-(CH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>O), 3.14 (8H, dd, J 17.0, 12.0 Hz, H-3a), 3.07 (8H, m, N-(CH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>O), 2.75 (1H, dd, J 17.0, 3.5 Hz, H-3b). <sup>13</sup>C NMR (125 MHz, DMSO-*d*<sub>6</sub>,  $\delta$ , ppm): 193.91 (C-4), 162.76 (C-5), 161.08 (C-9), 147.79 (C-4'), 146.53 (C-3'), 131.28 (C-1'), 117.37 (C-6'), 113.95 (C-2'), 112.04 (C-5'), 98.21 (C-6), 97.47 (C-8), 89.16 (C-10), 78.05 (C-2), 63.81–63.13 (N-(CH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>O), 55.64 (OCH<sub>3</sub>), 50.96–50.85 (N-(CH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>O), 50.62 (C-8-CH<sub>2</sub>), 50.16 (C-6-CH<sub>2</sub>), 41.37 (C-3). HRMS (*m/z*): 501.2234 [M+H]<sup>+</sup>; 501.2237 calcd [M+H]<sup>+</sup> for C<sub>26</sub>H<sub>33</sub>N<sub>2</sub>O<sub>8</sub>.

## Cell Culture

Human breast adenocarcinoma (MDA-MB-231) cell line (ATCC<sup>®</sup> HTB-26<sup>™</sup>) was provided by School of Medicine, Sungkyunkwan University, Korea. MDA-MB-231 cells were cultured in DMEM (Gibco, USA), containing 10% FCS (Gibco, USA), 2 mM L-glutamine, 100 IU/mL penicillin, and 100  $\mu\text{g}/\text{mL}$  streptomycin (Gibco, USA) in a humidified atmosphere of 5% CO<sub>2</sub> at 37 °C to attain confluency.

## Anti-proliferative activity assay

The samples were dissolved in DMSO at 10 mM and then diluted with culture medium to get tested concentrations. Paclitaxel (Anzatax<sup>®</sup>, Mayne Pharma, New Zealand) was used as the reference compound. DMSO at different concentrations was used as a blank control. The cells were then trypsinized, harvested, and counted using trypan blue (Sigma-Aldrich, USA), and seeded in 96-well plates at 10<sup>4</sup> cells/well. After 24 h incubation at 37 °C, 5% CO<sub>2</sub>, cells were treated with culture medium containing tested compounds at 100  $\mu\text{M}$  for 72 h. After 72 h treatment, cell viability was evaluated as mitochondrial succinate dehydrogenase (SDH) activity, a marker of viable cells using MTT test as described by Denizot and Lang (1986).

Briefly, SDH activity was detected after 3 h incubation in culture medium without serum containing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), which was converted into formazan crystals dissolved in acidified isopropanol. The produced purple solution was spectrophotometrically measured at 570 nm on Multiskan<sup>™</sup> microplate reader. Each tested concentration was performed in triplicate. The percentage of proliferation inhibition was calculated as follows:

$$\% \text{ Proliferation inhibition} = 100 - [(A_{\text{sample}}/A_{\text{control}}) \times 100].$$

The compounds with significant potential against MDA-MB-231 cells (percent of proliferation inhibition at 100  $\mu\text{M}$  more than 50%) were then tested for their toxicity at five different concentrations (2–100  $\mu\text{M}$ ). Concentrations inducing a 50% inhibition of cell growth (IC<sub>50</sub>) were deduced by exponential regression of the inhibitory percentage – tested concentration. Values were mean  $\pm$  SD from three independent experiments.

## References

- Babu TH, Rao VRS, Tiwari AK, Babu KS, Srinivas PV, Ali AZ, Rao JM (2008) Synthesis and biological evaluation of novel 8-aminomethylated oroxylin A analogues as  $\alpha$ -glucosidase inhibitors.

- Bioorg Med Chem Lett 18:1659–6662. <https://doi.org/10.1016/j.bmcl.2008.01.055>
- Buravlev EV, Shevchenko OG, Chukicheva IY, Kutchin AV (2017) Synthesis and membrane-protective properties of aminomethyl derivatives of quercetin at the C-8 position. Chem Pap. <https://doi.org/10.1007/s11696-017-0272-y>
- Candiracci M, Piatti E, Dominguez-Barragan M, Garcia-Antras D, Morgado B, Ruano D, Gutierrez JF, Parrado J, Castano A (2012) Anti-inflammatory activity of a honey flavonoid extract on lipopolysaccharide-activated N13 microglial cells. J Agric Food Chem 60:12304–12311. <https://doi.org/10.1021/jf302468h>
- Chahar MK, Sharma N, Dobhal MP, Joshi YC (2011) Flavonoids: a versatile source of anticancer drugs. Pharmacogn Rev 5:1–12. <https://doi.org/10.4103/0973-7847.79093>
- Chen Y, Cass SL, Kuty SK, Yee EMH, Chan DSH, Gardner CR, Vittorio O, Pasquier E, Black DS, Kumar N (2015) Synthesis, biological evaluation and structure–activity relationship studies of isoflavene based Mannich bases with potent anti-cancer activity. Bioorg Med Chem Lett 25:5377–5383. <https://doi.org/10.1016/j.bmcl.2015.09.027>
- Cottiglia F, Casu L, Bonsignore L, Casu M, Floris C, Sosa S, Altinier G, Loggia RD (2005) Topical anti-inflammatory activity of flavonoids and a new xanthone from *Santolina insularis*. Z Naturforsch 60c:63–66. <https://doi.org/10.1515/znc-2005-1-212>
- Dang CH, Nguyen CH, Nguyen TD, Im C (2014) Synthesis and characterization of *N*-acyl-tetra-*O*-acyl glucosamine derivatives. RSC Adv 4:6239–6245. <https://doi.org/10.1039/C3RA46007J>
- Dang CH, Le VD, Nguyen CH, Nguyen TD (2017) A facile synthesis of aggregation pheromones of Rhinoceros beetle and Rhycho-phorus weevil. ARKIVOC 2017; 2017(v):187–195. <https://doi.org/10.24820/ark.5550190.p010.271>
- Denizot F, Lang R (1986) Rapid colorimetric assay for cell growth and survival: modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. J Immunol Methods 89:271–277. [https://doi.org/10.1016/0022-1759\(86\)90368-6](https://doi.org/10.1016/0022-1759(86)90368-6)
- Frasiyuk MS, Mrug GP, Bondarenko SP, Sviripa VM, Zhang W, Cai X, Fiandalo MV, Mohler JL, Liu C, Watt DS (2015) Application of Mannich bases to the synthesis of hydroxymethylated isoflavonoids as potential antineoplastic agents. Org Biomol Chem 13:11292–11301. <https://doi.org/10.1039/c5ob01828e>
- Gorbunov EB, Rusinov GL, Ulomskii EN, Eltsov OS, Rusinov VL, Kartsev VG, Charushin VN, Khalymbadza IA, Chupakhin ON (2016) Direct modification of quercetin by 6-nitroazolo[1,5-*a*]pyrimidines. Chem Nat Compd 52:708–710. <https://doi.org/10.1007/s10600-016-1749-6>
- Ha L, Qian Y, Zhang S, Ju X, Sun S, Guo H, Wang Q, Li K, Fan Q, Zheng Y, Li H (2016) Synthesis and biological evaluation of scutellaria flavone cyclaneaminol Mannich base derivatives as novel CDK1 inhibitors. Anticancer Agents Med Chem 16:914–924. <https://doi.org/10.2174/1871520615666150928114425>
- Helgen TR, Sciotti RJ, Lee P, Duffy S, Avery VM, Igbino O, Akoto M, Hagen TJ (2015) The synthesis, antimalarial activity and CoMFA analysis of novel aminoalkylated quercetin analogs. Bioorg Med Chem Lett 25:327–332. <https://doi.org/10.1016/j.bmcl.2014.11.039>
- Hoang TKD, Huynh TKC, Nguyen TD (2015) Synthesis, characterization, anti-inflammatory and anti-proliferative activity against MCF-7 cells of *O*-alkyl and *O*-acyl flavonoid derivatives. Bioorg Chem 63:45–52. <https://doi.org/10.1016/j.bioorg.2015.09.005>
- Kanadaswami C, Lee LT, Lee PP, Hwang JJ, Ke FC, Huang YT, Lee MT (2005) The antitumor activities of flavonoids. In Vivo 19:895–910
- Kaul TN, Middleton JMDE, Ogra PL (1985) Antiviral effect of flavonoids on human viruses. J Med Virol 15:71–79. <https://doi.org/10.1002/jmv.1890150110>
- Kukhareva TS, Krasnova VA, Koroteev MP, Kaziev GZ, Kuleshova LN, Korlyukov AA, Antipin MY, Nifant'ev EE (2004) Electrophilic substitution in the dihydroquercetin system. Aminomethylation. Russ J Org Chem 40(8):1190–1193. <https://doi.org/10.1023/B:RUJO.0000045904.63368.06>
- Kumar S, Pandey AK (2013) Chemistry and biological activities of flavonoids: an overview. Sci World J. <https://doi.org/10.1155/2013/162750> article ID 162750
- Lee WJ, Chen WK, Wang CJ, Lin WL, Tseng TH (2008) Apigenin inhibits HGF promoted invasive growth and metastasis involving blocking PI3K/Akt pathway and beta 4 integrin function in MDA-MB-231 breast cancer cells. Toxicol Appl Pharmacol 226:178–191. <https://doi.org/10.1016/j.taap.2007.09.013>
- Lis R, Marisca AJ (1987) Methanesulfonanilides and the Mannich reaction. J Org Chem 52:4377–4379. <https://doi.org/10.1021/jo00228a041>
- Ng TB, Huang B, Fong WP, Yeung HW (1997) Anti-human immunodeficiency virus (anti-HIV) natural products with special emphasis on HIV reverse transcriptase inhibitors. Life Sci 61:933–949. [https://doi.org/10.1016/S0024-3205\(97\)00245-2](https://doi.org/10.1016/S0024-3205(97)00245-2)
- Nguyen TB, Wang Q, Gueritte F (2011) An efficient one-step synthesis of piperidin-2-yl and pyrrolidin-2-yl flavonoid alkaloids through phenolic Mannich reactions. Eur J Org Chem 2011:7076–7079. <https://doi.org/10.1002/ejoc.201101312>
- Nguyen TD, Nguyen CH, Im C, Dang CH (2015a) Synthesis of corn rootworm pheromones from commercial diols. Chem Pap 69(2):380–384. <https://doi.org/10.1515/chempap-2015-0027>
- Nguyen VS, Shi L, Luan FQ, Wang QA (2015b) Synthesis of kaempferide Mannich base derivatives and their antiproliferative activity on three human cancer cell lines. Acta Biochim Pol 6:547–552. [https://doi.org/10.18388/abp.2015\\_992](https://doi.org/10.18388/abp.2015_992)
- Nguyen TD, Nguyen CH, Im C, Dang CH (2016) A facile synthesis of sex pheromone of the Cabbage Looper, *Trichoplusia ni*. Chem Nat Compd 52(5):877–879. <https://doi.org/10.1007/s10600-0-016-1800-7>
- Nguyen VS, Shi L, Wang SC, Wang QA (2017) Synthesis of icaritin and  $\beta$ -anhydroicaritin Mannich base derivatives and their cytotoxic activities on three human cancer cell lines. Anticancer Agents Med Chem 17:137–142. <https://doi.org/10.2174/1871520616666160404111210>
- Nifant'ev EE, Mosyurov SE, Kukhareva TS, Vasyanina LK (2013) *N*, *N'*-diethyl-1,3-propanediamine in the dihydroquercetin aminomethylation reaction. Dokl Chem 451(2):197–200. <https://doi.org/10.1134/S0012500813080016>
- Orhana DD, Ozelik B, Ozgen S, Ergun F (2010) Antibacterial, antifungal, and antiviral activities of some flavonoids. Microbiol Res 165:496–504. <https://doi.org/10.1016/j.micres.2009.09.002>
- Pan MH, Lai CS, Ho CT (2010) Anti-inflammatory activity of natural dietary flavonoids. Food Funct 1:15–31. <https://doi.org/10.1039/C0FO00103A>
- Phromnoi K, Yodkeeree S, Anuchapreeda S, Limtrakul P (2009) Inhibition of MMP-3 activity and invasion of the MDAMB-231 human invasive breast carcinoma cell line by bioflavonoids. Acta Pharmacol Sin 30:1169–1176. <https://doi.org/10.1038/aps.2009.107>
- Pietta PG (2000) Flavonoids as antioxidants. J Nat Prod 63:1035–1042. <https://doi.org/10.1021/np9904509>
- Ravishankar D, Rajora AK, Greco F, Osborn HMI (2013) Flavonoids as prospective compounds for anti-cancer therapy. Int J Biochem Cell Biol 45:2821–2831. <https://doi.org/10.1016/j.biocel.2013.10.004>
- Rice-Evans CA (2001) Flavonoid antioxidants. Curr Med Chem 8:797–807. <https://doi.org/10.2174/0929867013373011>
- Rice-Evans CA, Miller NJ, Paganga G (1996) Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med 20:933–956. [https://doi.org/10.1016/0891-5849\(95\)02227-9](https://doi.org/10.1016/0891-5849(95)02227-9)

- Tempesti TC, Alvarez MG, Araujo MFD, Junior FEAC, de Carvalho MG, Durantini EN (2012) Antifungal activity of a novel quercetin derivative bearing a trifluoromethyl group on *Candida albicans*. *Med Chem Res* 21:2217–2222. <https://doi.org/10.1007/s00044-011-9750-x>
- Tugrak M, Yamali C, Sakagami H, Gul HI (2015) Synthesis of mono Mannich bases of 2-(4-hydroxybenzylidene)-2,3-dihydroinden-1-one and evaluation of their cytotoxicities. *J Enzyme Inhib Med Chem* 31:818–823. <https://doi.org/10.3109/14756366.2015.1070263>
- Vijayababu MR, Arunkumar A, Kanagaraj P, Venkataraman P, Krishnamoorthy G, Arunakaran J (2006) Quercetin downregulates matrix metalloproteinases 2 and 9 proteins expression in prostate cancer cells (PC-3). *Mol Cell Biochem* 287:109–116. <https://doi.org/10.1007/s11010-005-9085-3>
- Yusakul G, Sakamoto S, Juengwatanatrakul T, Putalun W, Tanaka H, Morimoto S (2016) Preparation and application of a monoclonal antibody against the isoflavone glycoside daidzin using a Mannich reaction-derived hapten conjugate. *Phytochem Anal* 27:81–88. <https://doi.org/10.1002/pca.2604>
- Zhang S, Ma J, Bao Y, Yang P, Zou L, Li K, Sun X (2008) Nitrogen-containing flavonoid analogues as CDK1/cyclin B inhibitors: synthesis, SAR analysis, and biological activity. *Bioorg Med Chem* 16:7127–7132. <https://doi.org/10.1016/j.bmc.2008.06.055>
- Zhurakulov SN, Babkin VA, Chernyak EI, Morozov SV, Grigor'ev IA, Levkovich MG, Vinogradova VI (2015) Aminomethylation of 1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolines by dihydroquercetin. *Chem Nat Compd* 51(1):57–61. <https://doi.org/10.1007/s10600-051-1203-1>