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Discovery of xanthine oxidase inhibitors and/or α-glucosidase inhibitors by carboxyalkyl derivatization based on the flavonoid of apigenin

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ABSTRACT

Three series of apigenin derivatives have been prepared by coupling the carboxyl alkyl group to 4′-, 5- or 7-hydroxyl groups of apigenin respectively. Preliminary biological evaluation in vitro revealed that xanthine oxidase inhibitory activity was improved by modifications at 4′-position and decreased by similar modifications at 5-, 7-positions while α-glucosidase inhibitory activity was maintained by modifications at 5-, 7-positions but lost by modifications at 4′-position. Administration (ip) of **7e** markedly lowered serum uric acid levels in potassium oxonate induced hyperuricemic mouse model and administration (p.o.) of **11d** or **11e** effectively suppressed the elevation of serum glucose in the oral sucrose tolerance test in mice, while apigenin were not significantly effective in both tests.

Apigenin, 5,7-dihydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one, is one of the most investigated flavonoids. It belongs to a less-toxic and non-mutagenic flavone subclass of flavonoids and is abundantly present in human diet including in a variety of fruits and vegetables.[1]

Various beneficial effects of apigenin, such as anti-inflammatory activity, antioxidative activity, as well as anticancer activity, have been well documented.[2–4] Apigenin has been reported as a potent competitive inhibitor of xanthine oxidase (XO) and showed inhibitory activity comparable to that of allopurinol.[5] Structure-based molecular modeling of six flavonoids with xanthine oxidase inhibitory activity revealed that apigenin was the most potent inhibitor which displayed the most favorable interaction in the reactive site of xanthine oxidase.[6] Oral administration of apigenin or its 4′-methyl ether derivative acacetin (Fig. 1) was able to elicit hypouricemic actions in hyperuricemic mice or rat induced by potassium oxonate.[7,8]

Apigenin was found to be a strong α-glucosidase (AG) inhibitor as well.[9] In streptozotocin (STZ)—induced diabetic rats, apigenin not only significantly lowered the blood glucose level and improved glucose tolerance, but also effectively protected the liver and kidney against STZ-induced damage.[10]

However, as is the case with most flavonoids, the clinical utility of apigenin is limited due to its low solubility in both water and lipid, which results in relatively low oral bioavailability.[11–13] Various derivatives of flavonoids have been prepared to improve their biological activity or bioavailability.[14–19]

Carboxyl group is one of the most common chemical groups that is presented in a variety of drugs, such as xanthine oxidase inhibitors (Febuxostat), non-steroidal anti-inflammatory drugs (Indomethacin), cholesterol-lowering drug (Gemfibrozil). As an amphipathic group, the introduction of carboxyl group in molecules can improve the solubility in both water and lipid.

In the present study, three series of apigenin derivatives have been prepared by coupling the carboxyl alkyl groups to 4′-, 5- or 7-hydroxyl groups of apigenin, respectively, in attempt to obtain novel derivatives of apigenin with improved phamocokinetic properties or biological activities. The purpose of introducing the sole carboxyalkyl group into apigenin is to retain the skeletal structures of flavonoids in a maximum extent to maintain the antioxidant activity, and to estimate the respective effect of each hydroxyl group over XO and AG.

There are 3 hydroxyl groups with different reactivity in apigenin. As the 7-OH is the most reactive in the electrophilic substitution reaction, 7-subsituted derivatives of apigenin were conveniently prepared by reaction of bromoalkyl carboxylates with apigenin followed by hydrolysis of the carboxylates. The 4′-subsituted derivatives were prepared by 4 steps: reaction of apigenin with chloromethyl methyl ether gave the 7-OH protected

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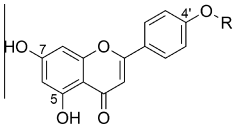


R = H, Apigenin

R = CH_3, Acacetin

Figure 1. Chemical structures of apigenin and acacetin.

derivative **4**, bromoalkyl carboxylates were reacted with the 4′-OH of **4** selectively to give **5a–f**, deprotection of **5a–f** in HCl gave **6a–f**, **7a–f** were obtained by hydrolysis. The 5-subsituted derivatives were prepared as follows: protection of both 7- and 4′-OH was realized by reaction of apigenin with 2 equiv of bromomethyl methyl ether, the 7- and 4′-OH protected derivative **8** was then coupled with bromoalkyl carboxylates to give 5-substitued alkyl carboxylates **9a–f**, deprotection of **9a–f** in HCl product **10a–f**, **11a–f** were prepared by hydrolysis (Scheme 1).[20]

Both xanthine oxidase and α-glucosidase inhibitory activity were evaluated. XO and AG inhibition assays were performed spectrophotometrically according to those described in the literature.[9,21] The results are summarized in Table 1.

The activities of the apigenin derivatives are highly related to the site of the substitution. XO inhibitory activity was improved three- to thirty-fold (IC_{50} = 0.098–0.82 μM) modifications at 4′-position but lost by similar modifications at 5-, 7-positions (IC_{50} >100 μM) while AG inhibitory activity was maintained (IC_{50} = 18.31–112.7 μM) by modifications at 5-, 7-positions but lost by modifications at 4′-position (IC_{50} >500 μM).

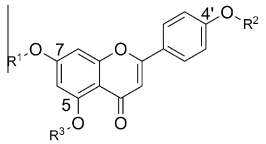
Introduction of a carboxyl alkyl group into apigenin resulted in the elevated inhibitory activity against XO in vitro. Structure–activity relationship study revealed the carboxylate group in febuxostat and Y-700 is the most tightly bound part of the inhibitor molecules, suggesting that the carboxylate group is necessary for the non-purine XO inhibitors.[22,23] Thus, the introduction of a carboxyl alkyl group into 4′-OH of apigenin may also enhance its binding with XO and increase the XO inhibitory activity.

7e was further evaluated for its hypouricemic effects in vivo. Intraperitoneal (ip) injection of potassium oxonate to mice led to a marked elevation of serum uric acid level which was maintained throughout the experiment. As shown in Figure 2, at doses of 10 mg/kg (ip), compound **7e** and allopurinol significantly decreased the serum uric acid levels in hyperuricemic mice while the effects of apigenin was not significant.

Compounds **3f** and **11c–e** were tested using acute hypoglycemic as well as oral glucose and sucrose tolerance tests (OGTT and OSTT, respectively). In both tests, blood glucose levels increased after oral loading of glucose or sucrose. In the oral sucrose tolerance test, the treatments orally of **11d** or **11e** provoked significant decrease of the elevation of the postprandial blood glucose levels of the mice, while **3f**, **11c** and apigenin showed no significant effects (Fig. 3). In contrast, all of the tested compounds exhibited little effect on the blood glucose levels of the mice in OGTT (data not shown). These results revealed that compounds **11d** and **11e** lowed the postprandial blood glucose levels by inhibition of the hydrolysis of disaccharide rather than glucose absorption in the small intestine.

Though compound **7e** showed much more potent XO inhibitory activity than allopurinol and compounds **11d–e** displayed more potent AG inhibitory activity than acarbose in vitro, but the hypouricemic effects of **7e** in vivo was lower than that of allopurinol and the hypoglycemic effects of **11d–e** in vivo was weaker than that of acarbose. It might be due to the poor bioavailability or short half-life of the target compounds in vivo.

Table 1
Inhibitory activity of apigenin derivatives against XO and AG in vitro



Compound	R^1	R^2	R^3	IC_{50}[a,b] (μM)	
				XO	AG
3a	$-CH_2COOH$	–H	–H	>100	61.21
3b	$-(CH_2)_3COOH$	–H	–H	>100	44.46
3c	$-(CH_2)_4COOH$	–H	–H	>100	33.7
3d	$-(CH_2)_5COOH$	–H	–H	>100	101.2
3e	$-(CH_2)_6COOH$	–H	–H	>100	90.39
3f	$-(CH_2)_7COOH$	–H	–H	>100	18.31
7a	–H	$-CH_2COOH$	–H	0.82	>500
7b	–H	$-(CH_2)_3COOH$	–H	0.60	>500
7c	–H	$-(CH_2)_4COOH$	–H	0.22	>500
7d	–H	$-(CH_2)_5COOH$	–H	0.32	>500
7e	–H	$-(CH_2)_6COOH$	–H	0.098	>500
7f	–H	$-(CH_2)_7COOH$	–H	0.25	>500
11a	–H	–H	$-CH_2COOH$	>100	112.7
11b	–H	–H	$-(CH_2)_3COOH$	>100	35.86
11c	–H	–H	$-(CH_2)_4COOH$	>100	39.04
11d	–H	–H	$-(CH_2)_5COOH$	>100	26.15
11e	–H	–H	$-(CH_2)_6COOH$	>100	51.43
11f	–H	–H	$-(CH_2)_7COOH$	>100	>500
Apigenin	–H	–H	–H	3.2	24.3
Allopurinol	–	–	–	2.9	–
Acarbose	–	–	–	–	222.3

[a] Concentration required to inhibit 50% of enzyme activity under the assay conditions.
[b] Values represent the mean of three experiments.