Synthesis and immunosuppressive activity of L-rhamnopyranosyl flavonoids

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Astilbin, a flavonoid isolated from different plants, shows diverse biological activities. This paper reports the synthesis and immunosuppressive activity of seven analogues of astilbin, which may shed light on the structure–activity relationship of the compounds. The following glycosyl flavonoids, 6-α-L-rhamnopyranosyloxyflavanone (**20**), 3-α-L-rhamnopyranosyloxyflavone (**22**), 3-α-L-rhamnopyranosyloxyflavanone (**24**), 3-α-L-rhamnopyranosyloxychromanone (**26**), 4-α-L-rhamnopyranosyloxychromanol (**27**), 7-hydroxy-3-α-L-rhamnopyranosyloxyflavanone (**30**) and 4′-hydroxy-3-α-L-rhamnopyranosyloxyflavanone (**32**) were prepared respectively by glycosylation of 6-hydroxyflavanone (**1**), 3-hydroxyflavone (**2**), 3-hydroxyflavanone (**5**), 3-hydroxychromanone (**8**), 4-chromanol (**9**), 7-benzyloxy-3-hydroxyflavanone (**12**), 4′-benzyloxy-3-hydroxyflavanone (**15**). Among them, compounds **5**, **8**, **12** and **15** were synthesized from flavanone (**3**), 4-chromanone (**6**), 7-hydroxyflavanone (**10**) and 4′-hydroxyflavanone (**13**) respectively. Similar to astilbin (**4**), compounds **22**, **24**, **26**, **30** and **32** significantly inhibited the single mixed lymphocytes reaction (sMLR) and enhanced the apoptosis of spleen cells isolated from mice with sheep red blood cell-induced delayed-type hypersensitivity respectively. However, compound **20** only showed a slight tendency to inhibit sMLR at higher concentration. Both compounds **20** and **27** did not influence the cell apoptosis. These data suggest that the following factors play essential roles in determining the biological activity of the flavonoids: the position at which the sugar is linked to the flavone, the presence of carbonyl on C-4 and phenol hydroxyl group in A or B ring. However, the presence of a B ring is unfavorable for the biological activity and the double bond at C(2)–C(3) in C-ring shows little effect on the activity.

Introduction

There is now overwhelming evidence from diverse studies that flavonoid glycosides, as well as their aglycones, exhibit significant biological activities such as antitumor and antimicrobial activity, radical-scavenging properties and immunoactivity coupled with low toxicity.[1–5] Therefore, their use as potential therapeutic compounds against a variety of diseases is of great interest.[6] More than one thousand flavonol *O*-glycosides have so far been isolated. And there are many variations in both the nature of the sugar moiety and its position of attachment to the aglycone. About 80% of flavonol *O*-glycosides have a sugar linkage at the 3-OH.

Astilbin, a flavanoid with a rhamnose, representing a typical structure of a flavonol 3-*O*-glycoside, has been isolated from the root of *Astilbe Odontophylla Miquel*,[7] the bark of *Hymenaea martiana*[8] and from the rhizome of *Smilax glabra Roxb.*[9] This compound has recently been proven to exhibit some important bioactivities including antioxidant and aldose reductase inhibitory effects.[10] Although the mechanism of action has yet to be determined at the molecular level, both astilbin and dihydroquercetin have been applied to the assay of hepatotoxicity caused by nonparenchymal cells, where astilbin was shown to prevent the hepatocyte damage from nonparenchymal cells by inducing the dysfunction of liver-infiltrating cells contained, and the rhamnose may be an essential requirement for the dysfunction.[9] It has been confirmed that the activity of astilbin is mainly targeted to the activated T lymphocytes.[11,12]

Despite the biological importance of flavonol glycosides, the synthesis of these compounds appears to be rare.[13–24] Major advances in the synthesis of flavonoid glycosides have been made by the development of the Koenigs–Knorr method,[25] in which the key-step of most syntheses involves the condensation of a flavonoid aglycon with a per-*O*-acetylglycosyl halide. However, due to the presence of a high concentration of silver salts, the final workup for this method is complicated.[26] The Zemplen–Farkas synthesis,[27] which involves the use of a per-*O*-acetyl glycosyl bromide in a homogeneous alkaline medium, is more convenient from this point of view, but the yields generally remain low probably owing to the partial hydrolysis of per-*O*-acetylglycosyl bromide during the course of the reaction. With the development of various glycosylation procedures and sophisticated protecting group strategies, other methods are reported in the synthesis of 7-*O*-glucosides[28] and astilbin.[13,29]

The aim of the present study is to develop an alternative strategy for the efficient synthesis of glycosyl flavonoids starting from readily available 6-hydroxyflavanone, 3-hydroxyflavone, 3-hydroxyflavanone, chromanone, 4-chromanol, 7-hydroxyflavanone, 4′-hydroxyflavanone and rhamnose, and to investigate the biological activity of the compounds synthesized on T lymphocytes. This work may be important for the understanding of the structure–activity relationship of astilbin-related species.

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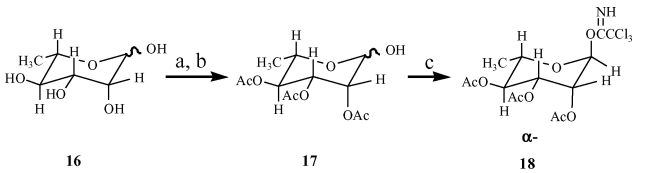
Results and discussion

Chemistry

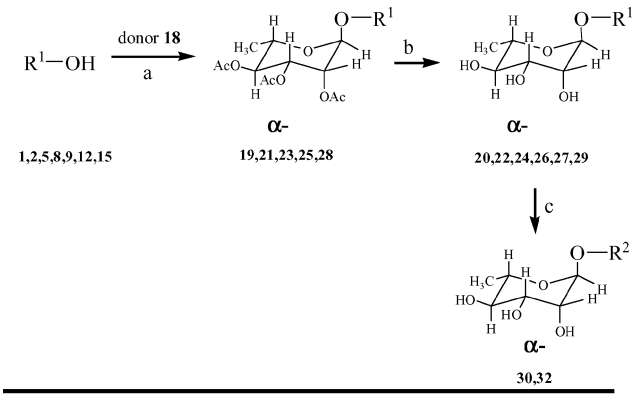
With the commercially available glycosyl acceptors **1**, **2**, **9** and the synthesized glycosyl acceptors **5**,[30] **8**,[31] **12** and **15**,[32] the strategy adopted for the synthesis of the glycosides involves the following steps: first, the synthesis of the glycosyl donors (Scheme 1), and then the glycosylation of 6-hydroxyflavanone, 3-hydroxyflavone, 3-hydroxyflavanone, 3-hydroxychromanone, 4-chromanol, 7-benzyloxy-3-hydroxyflavanone or 4′-benzyloxy-3-hydroxyflavanone, and finally the deprotection of the acetyl of the sugars and the benzyl of the flavanone (Scheme 2).



Scheme 1 *Conditions and reagents*: a: $(Ac)_2O$, pyridine, b: NH_3 (gas), THF and CH_3OH (2 : 1), c: CCl_3CN, DBU, CH_2Cl_2.

In the first step, the 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl trichloroacetimidate (**18**)[33] was directly attained by base-catalyzed reaction of 2,3,4-tri-*O*-acetyl-rhamnopyranose (**17**) with trichloroacetonitrile. The commonly used bases were NaH, K_2CO_3[34] or 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), with NaH[35] being used more often. In this work, DBU was chosen as the catalyst because its boiling point is low and it can be removed easily at the end of the reaction. It was found that compound **18** was unstable in the course of purification and easily decomposed in the moist atmosphere. For example, when compound **18** was purified by column chromatography using EtOAc–petroleum ether (bp, 60–90 °C) as the eluent, only its decomposed product 1-hydroxyrhamnose (**17**) was obtained, Therefore, the corresponding 1-hydroxy sugars were treated with CCl_3CN in the presence of cat. DBU in dry CH_2Cl_2 under N_2 protection, and the α imidate of target compound can be synthesized in excellent yields (>98%). Further experiment showed that compounds **18** synthesized by this method can be used directly without purification.

The glycosylation of acceptors **1**, **2**, **5**, **8** or **12** with rhamnosyl imidates was carried out in dry CH_2Cl_2 with cat. TMSOTf to afford the acetyl protected flavone derivatives **19**, **21**, **23**, **25** and **28** (Scheme 2). This reaction required considerable optimization as summarized in Table 1. The optimal activator is TMSOTf.[36] High yield synthesis of the desired glycoside in a short reaction time was achieved when the reaction was conducted at −20 °C followed by gradual warming up to room temperature (Table 1). Concerning the synthesis of glycosides **19** with acceptor **1**, a higher yield was originally anticipated due to the lack of internal hydrogen bonding between the C(3)-hydroxyl group and C(4) carbonyl in acceptors **2**, **5** and **8**,[13] which would lower the reactivity. However, it turned out not to be the case and both glycosyl acceptors **1** and **2** gave poor results mainly due to the presence of phenolic hydroxyls as well as the reaction temperature rather than the internal hydrogen bond of the glycosyl acceptor. Phenolic hydroxyls are weaker nucleophiles compared to aliphatic hydroxyls, therefore they usually require activation. Thus, the glycosylation product



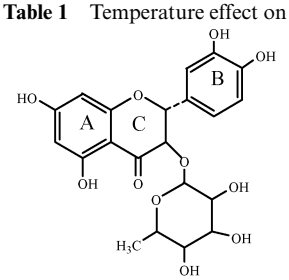
compounds	R^1	R^2
1, 19, 20		-
2, 21, 22		-
5, 23, 24		-
8, 25, 26		-
9, 27		-
12, 28, 29		-
30	-	
15		-
32	-	

Scheme 2 *Conditions and reagents*: a: TMSOTf, CH_2Cl_2, −20–0 °C, b: NH_3 (gas), CH_3OH, c: 10% Pd/C, cyclohexene, EtOH.

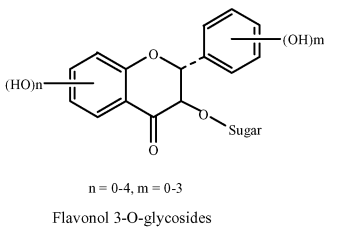
was designed to be obtained by using the "inverse procedure" of the Schmidt reaction,[37] where acceptors **1** and **2** should be activated firstly by a catalytic amount of TMSOTf before the addition of donor **18**. It should be noted that the "inverse procedure" of the Schmidt reaction was developed to improve the glycosylation efficiency by activating the less reactive acceptors or by preventing the decomposition of the highly reactive donors. However, this work demonstrated that it is not an efficient method for the glycosylation of phenolic hydroxyls.

The conversion of acetyl protected flavone derivatives **19**, **21**, **23** or **25** and **28** to final target molecules was initially attempted by

Table 1 Temperature effect on glycosidation

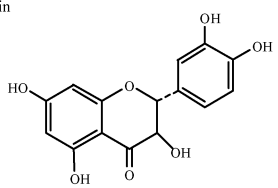


4 Astilbin



n = 0-4, m = 0-3

Flavonol 3-O-glycosides



5 dihydroquercetin

Entry	Acceptor	Donor	Glycosidation product (yield, %)[a]	
			−78 °C–rt	−20 °C–rt
1	**1**	**18**	**19** (33.8)	**19** (62.3)
2	**2**	**18**	**21** (24.3)	**21** (50.7)
3	**5**	**18**	—	**23** (76.1)
4	**8**	**18**	—	**25** (84.5)

[a] Isolated yields.

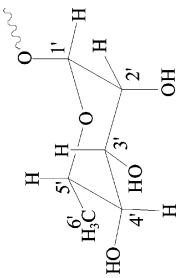
removing the acetyl groups under basic conditions such as with CH_3ONa–CH_3OH.[38] However, the sugar moieties were cleaved under these conditions. For example, in the case of compound **22**, the isolated adduct was 3-hydroxyflavone. The cleavage of sugar was effectively suppressed when NH_3 (gas) was employed in CH_3OH at room temperature. This procedure provided target compound **22** with 83.6% yield. The optimization of reaction time is crucial and the best reaction time was found to be within 2 h. Longer reaction time was found to cause partial cleavage of the sugar moieties.

Seven novel glycosyl flavonoids as well as some precursors were synthesized. The new compounds were fully characterized by ^{1}H NMR, ^{13}C NMR, 2D H–H COSY, 2D C–H COSY, IR, and EA. The selected ^{1}H and ^{13}C NMR data on the novel glycosyl flavonoids are listed in Tables 2 and 3.

The configurations of the glycoside adducts were mainly determined by ^{1}H NMR, and discussed based on the mechanism of glycosylation. According to the mechanism of the glycosylation reaction which has been studied by Kochetkov and coworkers,[39] the normal glycosidic products were mainly derived from the corresponding sugar 1,2-orthoester intermediates, which are classical glycosyl donors used in the construction of 1, 2-*trans*-glycosidic linkages in glycosylation with donors carrying C-(2) acetyl protecting groups.[40] Then the rhamnosides compounds **19**, **21**, **23**, **25** and **28** should have the α configuration.

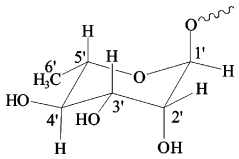
The ^{1}H NMR spectra confirmed that the configuration at the anomeric center in compounds **19**, **21**, **23**, **25** and **28** was α with the coupling constant of the anomeric proton signal being $J = ca.$ 1.2 Hz. These data were in agreement with the mechanism of the glycosylation reaction.[40]

Table 2 Selected ^{1}H NMR data on novel glycosyl flavonoids



	20 DMSO-d_6	**22** DMSO-d_6	**24** MeOH-d_4	**26** MeOH-d_4	**27** $CDCl_3$	**30** MeOH-d_4	**32** $CDCl_3$
H-2	5.64–5.58	—	5.33 (d, 11.3, 0.45H) 5.24 (d, 11.9, 0.55H)	4.61–4.37	4.32–4.21	5.42 (d, 9.4, 0.34H) 5.28 (d, 11.3, 0.66H)	5.31 (d, 11.2, 0.34H) 5.23 (d, 11.7, 0.66H)
H-3	2.84–2.79	—	4.73 (d, 11.9, 0.55H) 4.69 (d, 11.3, 0.45H)	4.61–4.37	2.16–2.03	4.68 (d, 11.3, 0.66H) 4.64 (d, 9.4, 0.34H)	4.77 (d, 11.7, 0.34H) 4.72 (d, 11.2, 0.66H)
H-1′	5.05 (d, 1.1)	5.38 (d, 1.1)	5.14 (d, 1.4, 0.55H) 3.91 (d, 1.3, 0.45H)	5.11 (d, 1.3, 0.66H) 4.97 (d, 1.2, 0.34H)	5.01 (d, 0.9, 0.34 H) 4.98 (d, 0.9, 0.66H)	4.95 (d, 1.0, 0.66H) 3.90–2.94 (0.34H)	5.14 (d, 0.9, 0.66H) 4.03–4.00 (m, 0.34H)
H-2′	3.85 (m)	4.05 (brs)	4.05 (dd, 1.7, 3.2, 0.55H) 3.47 (dd, 1.6, 3.2, 0.45H)	3.93 (dd, 1.7, 3.3, 0.66H) 3.87 (dd, 1.7, 3.4, 0.34H)	3.84–3.81(m)	3.90–2.94	4.03–4.00 (m, 0.66H) 3.53–3.12 (m, 0.34H)
H-3′	3.63 (m)	3.45 (dd, 2.9, 9.2)	3.26–3.28 (0.55H) 3.68 (dd, 3.3, 9.6, 0.45H)	3.75–3.62 (m)	3.75–3.67 (m)		3.70–3.56 (m, 0.34H) 3.53–3.12 (m, 0.66H)
H-4′	3.26 (m)	3.14 (t, 9.4)	3.19 (t, 9.6, 0.55H) 3.28–3.36 (0.45H)	3.43 (t, 9.5, 0.66H) 3.37 (t, 9.6, 0.34H)	3.47 (t, 9.36)		3.53–3.12 (m)
H-5′	3.49 (m)	2.91 (m)	1.99, 4.26	3.75–3.62 (m)	3.75–3.67 (m)	3.90–2.94 (0.34H) 2.01–1.85 (m, 0.66H)	4.22–4.16 (m, 0.34H) 2.28–2.21 (m, 0.66H)
H-6′	1.11 (d, 6.1)	0.75 (d, 6.1)	1.18 (d, 6.2, 1.35H) 0.82 (d, 6.2, 1.65H)	1.32 (d, 6.2, 2H) 1.16 (d, 6.2, 1H)	1.39–1.22 (d, 6.2)	1.01 (d, 5.5, 1H) 0.68 (d, 5.4, 2H)	1.13 (d, 6.2, 1H) 0.88 (d, 6.2, 2H)

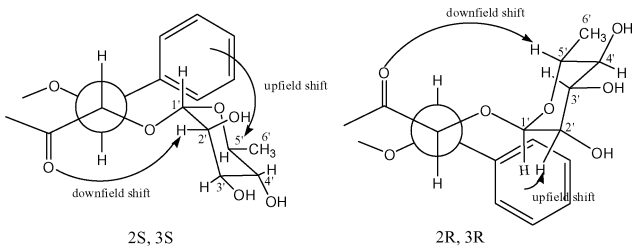
Table 3 Selected ^{13}C NMR data on novel glycosyl flavonoids



	20 DMSO-d_6	**22** MeOH-d_4	**24** MeOH-d_4	**26** MeOH-d_4	**27** DMSO-d_6	**30** DMSO-d_6	**32** Acetone-d_6
C-2	—	—	81.87, 81.46	73.54, 72.46	62.27, 62.18	82.81, 82.66	83.30,82.90
C-3	—	—	83.12, 82.86	70.08, 69.17	29.11, 27.46	78.05, 76.35	78.46, 76.67
C-1′	100.01	102.35	101.48, 101.33	101.00, 99.69	98.11, 97.72	101.70, 101.21	101.22, 101.06
C-2′	70.98	71.84	70.87, 70.67	71.06, 70.84	71.90, 71.59	70.96, 70.88	70.93, 70.71
C-3′	71.27	71.05	71.14, 70.92	71.16, 70.99	68.96	71.24, 71.00	71.54, 71.35
C-4′	72.65	71.33	72.71, 72.16	72.74, 72.70	73.30, 73.25	72.49, 71.97	72.81, 72.43
C-5′	70.41	70.95	69.62, 69.09	69.72, 69.47	71.90, 71.59	69.75, 69.45	69.51, 69.04
C-6′	18.74	18.28	17.06, 16.91	17.07, 16.74	17.94, 17.77	18.60	17.59, 17.51

According to the ^1H NMR spectral data (Table 2), the configuration at C(2)–C(3) in compounds **24**, **29**, **30** and **32** can be determined to be a *trans*-configuration with two diastereomers. For compound **24**, the H-(2) and H-(3) signals appeared in pairs at δ 5.33 (0.45 H, d, $J = 11.30$ Hz), δ 5.24 (0.55 H, d, $J = 11.90$ Hz) and δ 4.69 (0.45 H, d, $J = 11.30$ Hz), δ 4.73 (0.55 H, d, $J = 11.90$ Hz), respectively. For **29**, the H-(2) and H-(3) signals appeared in pairs at δ 5.24 (0.34 H, d, $J = 11.21$ Hz), δ 5.19 (0.67 H, d, $J = 11.73$ Hz) and δ 4.68 (0. 67 H, d, $J = 11.81$ Hz), δ 4.62 (0.34 H, d, $J = 11.24$ Hz) respectively. The coupling constants of the signals ($J > 6$ Hz) suggested that both of them adopt a *trans*-configuration at C(2)–C(3).

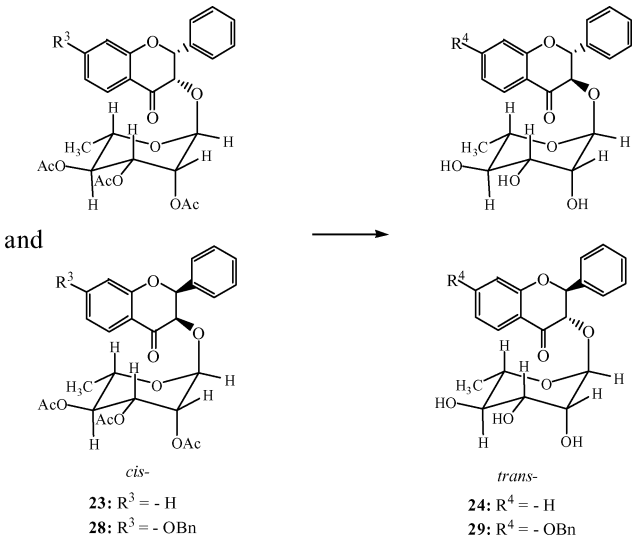
All the diastereomers of astilbin have been isolated and their chirality has been proposed,[41–43] and it is noteworthy that each isomer has diagnostic signals in the ^1H-NMR spectra. When both C-2 and C-3 have an (*S*) configuration, anisotropy of the benzene ring (B-ring) causes upfield shifts of H-5′ and H-6′, and that of carbonyl group at C-4 causes downfield shifts of H-1′ and H-2′. In case of a (2*R*,3*R*) configuration, the contrary effects are expected to produce the signals shown in Scheme 3.



Scheme 3

Comparing the ^1H NMR signals of compounds **24**, **29**, **30**, **32** with that of astilbin, the aglycone chirality of **24** was assigned as a mixture of (2*S*,3*S*) and (2*R*,3*R*) with a ratio of 1.2 : 1, the aglycone chirality of **29**, **30** and **32** was assigned as a mixture of (2*S*,3*S*) and (2*R*,3*R*) with a ratio of 2 : 1 respectively.

From the ^1H NMR data of these compounds, we can conclude that the configuration at the anomeric center was not affected neither during the conversion of acetyl protected flavone derivatives **19**, **21**, **23**, **25** and **28** to the target molecules **20**, **22**, **24**, **26** and **29** using NH_3 (gas) in CH_3OH at room temperature nor during the process of separation by column chromatography. However, the configuration on C(2)–C(3) was changed from *cis*- for **23** and **28** to *trans*- for **24** and **29** (Scheme 4); whether the transformation derived from the reaction under basic conditions or from the separation by column chromatography was unknown.



Scheme 4

Biological activities of the compounds

The biological activity of compounds **20**, **22**, **24**, **26**, **27**, **30** and **32** was evaluated with their effects on single mixed lymphocytes reaction and apoptosis of spleen cells, and compared with that of astilbin. The former assay was to examine the proliferation of the lymphocytes from BALB/c mice under the stimulation of the mitomycin C-treated spleen cells from C57BL/6 mice. In the latter experiment, the spleen cells were isolated from mice with a footpad reaction induced by sheep red blood cells. These two assays were aimed at evaluating the effects of these compounds on the T lymphocyte function since the single mixed lymphocytes reaction is usually used for evaluating lymphocyte proliferation and the main effector cells involved in the footpad reaction are mainly $CD4^+$ T cells.

As shown in Fig. 1, astilbin and compounds **22**, **24**, **26**, **30** and **32** significantly inhibited the single mixed lymphocytes reaction compared with the control. Compound **20** tended to inhibit the single mixed lymphocytes reaction at higher concentrations, but not significantly. Compound **27** did not have the tendency.

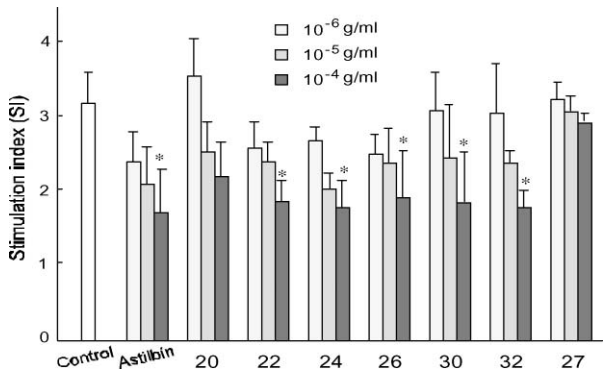


Fig. 1 Effect of compounds on single mixed lymphocytes reaction. The lymphocytes (5×10^5) from BALB/c mice were incubated with the lymphocytes (5×10^5) from C57BL/6 mice, which had been treated with mitomycin C (500 mg mL^{-1}) for 1 h in the presence or absence of the various concentrations of compounds at 37 °C in 5% CO_2 for 72 h. The proliferation of lymphocytes was measured by the MTT method. The experiment was repeated three times. *P < 0.05 *vs.* control (Student's t test).

As shown in Fig. 2, astilbin and compounds **22**, **24**, **26**, **30** and **32** significantly increased the apoptotic cells of spleen cells activated by sheep red blood cell-induced delayed-type hypersensitivity reaction in a dose-dependent manner (Fig. 2). However, compounds **20** and **27** failed to cause the apoptosis.

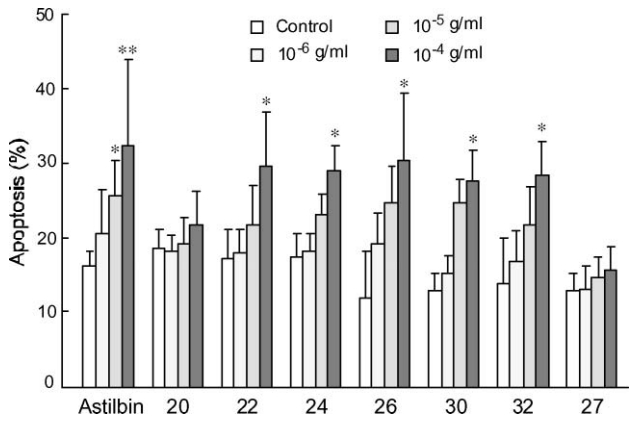


Fig. 2 Effect of compounds on the apoptosis of spleen cells isolated from mice with sheep red blood cell-induced delayed-type hypersensitivity. BALB/c mice were sensitized by s.c. injecting 40 μL of 2.5×10^8 mL^{-1} sheep red blood cells in saline into the left footpad. Five days later, they were challenged by s.c. injecting 40 μL of 2.5×10^9 mL^{-1} of the cells in saline into the right footpad. Six hours later, spleen cells were isolated and incubated with the compounds at 37 °C for 12 h. *P < 0.05, **P < 0.01 *vs.* control (Student's t test).

These results suggest that the compounds having the aglycone of astilbin show an inhibitory activity against T lymphocytes through decreasing the proliferation and inducing the apoptosis of T cells. Among them, compounds **22** and **24**, which are 3-*O*-flavone glycoside and 3-*O*-flavanone glycoside respectively, showed a similar activity. This finding suggests that the double bond at C(2)–C(3) in C-ring is not important for their biological activities. However, compounds **20** and **27**, which are 6-*O*-flavanone glycoside and 4-*O*-chromanol glycoside respectively, did not show a significant effect in both assays. Therefore, the linking position of sugar and the carbonyl on C(4) are more important for the activities. In addition, the phenolic hydroxyls on the flavanone may contribute to the effect of the compounds since the activity of compounds **30** and **32** seems to be more remarkable than of compounds **22** and **24**. Finally, lacking B ring on C(2) seems favorable to the activity since compound **26** showed a significant biological effect in both assays.

Conclusion

In conclusion, seven flavonoids of rhamnose were synthesized and characterized. The biological data showed that compounds **22**, **24**, **26**, **30** and **32** significantly suppressed single mixed lymphocytes reaction and induced the apoptosis of spleen cells isolated from mice with sheep red blood cell-induced delayed-type hypersensitivity. The activity of these compounds is comparable to that of astilbin. However, the activity of compounds **20** and **27** is much less pronounced, suggesting that the linking position of the sugar to the flavone and the carbonyl on C(4) are very important, while the double bond at C(2)–C(3) in the C ring does not have a major impact on the biological activity. The B ring of astilbin appears unnecessary for the activity. These results will be important for the rational design of astilbin-type immunosuppressive agents.

Experimental

Materials and methods

All experiments dealing with air- and moisture-sensitive compounds were conducted under an atmosphere of dry nitrogen. Dichloromethane was distilled from CaH_2 and stored over 4 Å molecular sieves. For thin-layer Chromatography (TLC) analysis, Merck precoated plates (silica gel GF254) were used. Solvents such as acetic anhydride, pyridine, petroleum ether, ethyl acetate, tetrahydrofuran (THF) and methanol are all analytical grade and used as received. The 6-hydroxyflavanone, 3-hydroxyflavone, flavanone, trichloroacetonitrile, TMSOTf and DBU were purchased from Aldrich.

Melting points were determined with a 'Mel-Temp' apparatus. The IR spectra were recorded on a Hitachi 260–01 spectrometer in KBr discs. The ^1H NMR and ^{13}C NMR spectra were recorded with a Bruker DPX 300 spectrometer. The thin-layer chromatography (TLC) was performed on silica gel GF254 by iodo gas or UV detection. Column chromatography was conducted by elution of a column (8 × 100 mm, 16 × 240 mm, 18 × 300 mm) and EtOAc–petroleum ether (bp, 60–90 °C) as the eluent. Optical rotations were measured at 20 ± 2 °C with a Perkin Elmer Model 343 digital polarimeter, using a 10 cm, 10 ml cell.

Preparation of the compounds

Glycosylation

The acceptor **2** (1.12 g, 0.005 mol) and compound **18** (2.39 g, 0.0055 mol) were dried together under high vacuum for 2 h, then dissolved in anhydrous CH_2Cl_2 (60 ml). TMSOTf (50 μl) was added dropwise at −20 °C under N_2 protection. The reaction mixture was stirred for 3 h, during which time the temperature was gradually raised to ambient temperature. Then the mixture was neutralized with Et_3N and concentrated under reduced pressure to give a syrupy residue, which was purified by column chromatography (3 : 2 petroleum–EtOAc) to give compound **21**.

6-(2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl)-oxy-flavanone (19). Yellow powder. Mp 67–69 °C; $[\alpha]_D^{20}$ −7.6 (*c* 1.0, MeOH), ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.63 (t, 1 H, $J = 2.5$ Hz), 7.50–7.40 (m, 5 H), 7.27–7.23 (m, 1 H), 7.03 (d, 1 H, $J = 9.0$ Hz), 5.52–5.44 (m, 4 H), 5.17 (t, 1 H, $J = 9.9$ Hz), 4.02–3.97 (m, 1 H), 3.13–2.86 (m, 2 H), 2.21 (s, 3 H), 2.07 (s, 3 H), 2.04 (s, 3 H), 1.23 (d, 3 H, $J = 6.2$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 191.91, 170.46, 170.40, 170.34, 157.73, 150.65, 150.59, 139.06, 139.04, 129.24, 129.19, 126.52, 121.52, 119.91, 113.02, 112.99, 96.55, 96.53, 80.13, 80.12, 71.29, 69.97, 69.23, 67.69, 44.91, 44.89, 21.26, 21.16, 21.11,17.82. IR (KBr); cm^{-1} 2984.91, 2939.81, 1750.13, 1693.00, 1616.93, 1484.09, 1437.13, 1371.72, 1278.03, 1248.04, 1223.08, 1129.91, 1056.22, 984.37, 905.02, 828.42, 813.72, 762.52, 699.87. Elemental anal. for $C_{27}H_{28}O_{10}$: found (calculated) (%): C, 63.31 (63.28); H, 5.40 (5.47).

3-(2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl)-oxy-flavone (21). White powder. Mp 66–68 °C; $[\alpha]_D^{20}$ −12.1 (*c* 1.0, MeOH), ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.10 (d, 1H, $J = 7.5$ Hz), 7.92 (m, 2 H), 7.83 (t, 1 H, $J = 7.8$, 7.1 Hz), 7.71 (d, 1 H, $J = 8.2$, 7.8 Hz), 7.61 (m, 3 H), 7.51 (dd, 1 H, $J = 7.5$, 7.1 Hz), 5.54 (s, 1 H), 5.11 (dd, 1 H, $J = 3.0$, 10.2 Hz), 4.81 (t, 1 H, $J = 10.0$ Hz) 3.25 (m, 1 H), 2.11 (s, 3 H), 1.98 (s, 3 H), 1.96 (s, 3 H), 0.74 (d, 3 H, $J = 6.1$ Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.22, 170.48, 170.37, 170.27, 157.78, 155.74, 137.21, 135.21, 131.96, 130.86, 129.66, 129.46, 126.20, 125.86, 124.19, 119.33, 98.59, 70.10, 69.35, 69.15, 68.59, 21.35, 21.31, 21.23, 17.57. IR (KBr); cm^{-1} 3063.18, 2985.54, 2938.81, 1751.49, 1644.46, 1619.09, 1568.46, 1467.79, 1393.82, 1370.89, 1223.39, 1201.32, 1135.65, 1055.19, 982.20, 965.61, 905.07, 795.59 763.12, 698.81. Elemental anal. for $C_{27}H_{26}O_{10}$: found (calculated) (%): C, 63.59 (63.53); H, 5.10 (5.10).

3-(2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl)-oxy-flavanone (23). White solid. Mp 68–70 °C; $[\alpha]_D^{20}$ −3.5 (*c* 1.0, MeOH), ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.96–7.92 (m, 1 H), 7.61–7.54 (m, 3 H), 7.50–7.34 (m, 3 H), 7.14–7.08 (m, 2 H), 5.53 (d, 0.34H, $J = 2.0$ Hz, H-2,), 5.42 (d, 0.66 H, $J = 1.7$ Hz, H-2,), 5.15–4.99 (m, 2 H, H-2′ and H-3′), 4.96–4.89 (t, 0.66 H, H-4′), 4.90 (d, 0.34 H, $J = 1.1$ Hz, H-1′), 4.81–4.74 (t, 0.34 H, $J = 9.9$ Hz, H-4′), 4.20–4.17 (m, 1.66 H, H-3 and 0.66 H-1′), 3.73–3.68 (m, 0.66 H, H-5′), 2.13–2.07 (m, 0.34 H, H-5′), 2.05–1.92 (9 H), 1.03 (d, 2 H, $J = 6.2$ Hz, H-6′), 0.78 (d, 1 H, $J = 6.2$ Hz, H-6′); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 189.12, 188.69, 170.27, 170.19, 170.16, 170.12, 170.08, 169.87, 161.71, 161.62, 137.14, 137.03, 136.22, 135.38, 129.28, 129.19, 129.09, 128.81, 128.16, 128.07, 127.20, 127.00, 122.53, 122.43, 119.56, 119.11, 118.69, 118.66, 98.54, 95.35, 81.87, 81.46, 77.80, 73.90, 71.13, 70.77, 69.55, 69.32, 69.20, 69.08, 67.61, 66.75, 21.09, 21.02, 20.99, 17.52, 17.23. IR (KBr); cm^{-1} 2984.81, 1749.66, 1693.84, 1609.02, 1576.71, 1466.32, 1370.97, 1311.21, 1225.54, 1139.16, 1080.47, 1039.88, 982.78, 918.12, 883.86, 839.61, 763.54, 699.29. Elemental anal. for $C_{27}H_{28}O_{10}$: Found (calculated) (%): C, 63.22 (63.28); H, 5.51 (5.47).

3 - (2,3,4 - Tri -*O*-acetyl-α-L-rhamnopyranosyl)-oxy-chromanone (25). White solid. Mp 48–50 °C; $[\alpha]_D^{20}$ −7.9 (*c* 1.0, MeOH), ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.89 (d, 1 H, $J = 7.88$ Hz), 7.48 (t, 1 H, $J = 7.78$ Hz), 7.05 (t, 1 H, $J = 7.50$ Hz), 6.98 (d, 1 H, $J = 8.37$ Hz), 5.33–5.29 (m, 2 H, H-2′ and H-3′), 5.06 (t, 1 H, $J = 9.57$ Hz, H-4′), 4.95 (bs, 1 H, H-1′), 4.49–4.38 (m, 3 H, H-2 and H-3), 4.27–4.21 (m, 1 H, H-5′), 2.14 (s, 3 H), 2.03 (s, 3 H), 1.97 (s, 3 H), 1.10 (d, 3 H, $J = 6.24$ Hz, H-6′); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 189.56, 170.46, 170.39, 170.21, 161.56, 136.70, 127.95, 122.28, 120.31, 118.20, 97.20, 74.13, 71.15, 70.08, 69.35, 69.10, 67.50, 21.16, 21.12, 21.01, 17.47. IR (KBr); cm^{-1} 2986.35, 2938.81, 1749.42, 1702.85, 1607.74, 1579.83, 1479.26, 1466.95, 1371.50, 1325.63, 1288.13, 1222.53, 1146.72, 1095.90, 1048.31, 982.85, 937.69, 909.02, 835.76, 763.80. Elemental anal. for $C_{29}H_{30}O_{12}$, found (calculated) (%), C, 57.72 (57.80); H, 5.72 (5.50).

7-Benzyloxy-3-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-oxy-flavanone (28). White solid. Mp, 56–57 °C; $[\alpha]_D^{20}$ −8.3 (*c* 1.0, MeOH), ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.92–7.87 (m, 1 H), 7.60–7.35 (m, 10 H), 6.78–6.73 (m, 1 H), 6.65 (m, 1 H), 5.51 (s, 0.66 H, $J = 0.91$ Hz, H-2), 5.40 (s, 0.34 H, $J = 1.01$ Hz, H-2), 5.17–4.91 (m, 5 H, CH_2, 0.66 H-1′, H-2′, H-3′ and 0.34 H-4′), 4.78 (t, 0.66 H-4′, $J = 4.97$ Hz), 4.12 (m, 1 H, H-3), 4.07 (d, 0.34 H-1′, $J = 0.93$ Hz), 3.80–3.75 (m, 0.34 H-5′), 2.15–1.94 (9.66 H, 3 Ac, 0.66 H-5′), 1.09 (d, 1 H, $J = 6.22$ Hz), 0.78 (d, 2 H, $J = 6.22$ Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 187.39, 187.21, 170.35, 170.28, 170.24, 170.18, 170.13, 169.88, 166.20, 166.10, 163.77, 163.71, 136.30, 136.19, 136.16, 135.45, 129.92, 129.85, 129.24, 129.20, 129.14, 129.08, 128.78, 128.75, 127.93, 127.90, 127.12, 126.99, 113.56, 113.09, 112.19, 102.29, 102.24, 98.58, 95.31, 82.23, 81.82, 77.50, 73.76, 71.18, 70.86, 70.83, 70.76, 69.53, 69.25, 69.24, 69.13, 67.52, 66.65, 21.13, 21.05, 17.53, 17.30; IR (KBr); cm^{-1} 3096.20, 3062.34, 2984.50, 2850.12, 1750.27, 1684.15, 1609.52, 1575.81, 1498.60, 1445.92, 1371.15, 1244.31, 1224.20, 1173.76, 1137.26, 1038.89, 971.89, 901.62, 837.35, 785.42, 739.74, 698.81, 604.58; Elemental anal. for $C_{34}H_{34}O_{11}$, found (calculated) (%), C, 66.06 (66.02); H, 5.58 (5.50).

Removal of the acetyl groups of the sugars

Compound **21** was dissolved in a saturated solution of NH_3 (gas) in MeOH (20 ml). After 48 h at rt, the reaction mixture was concentrated, and the residue was purified by chromatography (1 : 3 petroleum ether–EtOAc) to afford compound **22**.

6-α-L-Rhamnopyranosyloxyflavanone (20). Yellow powder. Mp 71–73 °C; $[\alpha]_D^{20}$ −10.5 (*c* 1.0, MeOH), ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.54 (d, 2 H, $J = 7.7$ Hz), 7.48–7.41 (m, 4 H), 7.31–7.27 (m, 1 H), 7.06 (d, 1 H, $J = 9.0$ Hz), 5.64–5.58 (m, 1 H, H-2), 5.32 (d, 1 H, $J = 1.2$ Hz, H-1′), 5.05 (d, 1 H, OH), 4.88 (d, 1 H, OH), 4.75 (d, 1 H, OH), 3.85 (m, 1 H, H-2′), 3.66–3.63 (m, 1 H, H-3′), 3.52–3.46 (m, 1 H, H-5′), 3.34–3.17 (m, 1 H, H-4′), 2.84–2.79 (m, 2 H, H-3), 1.11 (d, 3 H, $J = 6.1$ Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 192.30, 157.20, 157.18, 151.21, 151.17, 139.81, 129.39, 127.43, 127.12, 127.07, 121.63, 120.15, 112.83, 100.01, 79.74, 79.71, 72.65,

71.27, 70.98, 70.41, 44.29, 18.74. IR (KBr); cm^{-1} 3406.51, 2931.66, 1689.38, 1617.28, 1484.49, 1437.19, 1280.05, 1219.42, 1197.94, 1123.74, 1060.04, 1020.05, 982.68, 911.92, 808.63, 760.72, 698.22. Elemental anal. for $C_{21}H_{22}O_7$: found (calculated) (%): C, 65.12 (65.28); H, 5.72 (5.70).

3-α-L-Rhamnopyranosyloxyflavone (24). Yellow powder. Mp 96–98 °C; $[a]_D^{20}$ −14.6 (*c* 1.0, MeOH), ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.12 (d, 1 H, J = 7.7 Hz), 7.91 (m, 2 H), 7.83 (t, 1H, J = 8.2, 7.2 Hz), 7.77 (d, 1 H, J = 8.2 Hz), 7.58 (m, 3H), 7.51 (t, 1 H, J = 7.7, 7.2 Hz), 5.38 (d, 1 H, J = 1.1 Hz), 4.05 (brs, 1 H), 3.45 (dd, 1 H, J = 2.9, 9.2 Hz), 3.14 (t, 1 H, J = 9.4 Hz), 2.91 (m, 1 H), 0.75 (d, 3 H, J = 6.1 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.57, 157.55, 155.68, 138.02, 135.05, 131.74, 131.27, 129.69, 129.31, 126.07, 125.87, 124.27, 119.31, 102.35, 71.84, 71.33, 71.05, 70.95, 18.28. IR (KBr); cm^{-1} 3422.65, 3064.11, 2920.93, 2930.99, 1637.47, 1616.67, 1561.11, 1467.37, 1396.97, 1203.44, 1134.14, 1059.28, 945.84, 914.98, 760.15, 695.90; MS: 385.0 (M + 1), 407.1 (M + Na), 790.9 (2M + Na). Elemental anal. For $C_{21}H_{20}O_7$: found (calculated) (%): C, 65.27 (65.63); H, 5.30 (5.21).

3-α-L-Rhamnopyranosyloxyflavanone (24). White solid. Mp 82–84 °C; $[a]_D^{20}$ −11.5 (*c* 1.0, MeOH), ^{1}H-NMR (300 MHz, MeOH-d_4): δ 7.86–7.83 (m, 1 H), 7.59–7.53 (m, 3 H), 7.48–7.40 (m, 3 H), 7.13–7.03 (m, 2 H), 5.33 (d, 0.45 H, J = 11.3 Hz, H-2), 5.24 (d, 0.55 H, J = 11.9 Hz, H-2), 5.14 (d, 0.55 H, J = 1.4 Hz, H-1′), 4.73 (d, 0.55 H, J = 11.9 Hz, H-3), 4.69 (d, 0.45 H, J = 11.3 Hz, H-3), 4.32–4.20 (m, 0.45 H, H-5′), 4.05 (dd, 0.55 H, J = 1.7, 3.2 Hz, H-2′), 3.91 (d, 0.45 H, J = 1.3 Hz, H-1′), 3.68 (dd, 0.45 H, J = 3.3, 9.6 Hz, H-3′), 3.47 (dd, 0.45 H, J = 1.6, 3.2 Hz, H-2′), 3.36–3.28 (m, 1 H, 0.45 H-4′ and 0.55 H-3′), 3.19 (t, 0.55 H, J = 9.6 Hz, H-4′), 2.00 (m, 0.55 H, H-5′), 1.18 (d, 1.35 H, J = 6.2 Hz, H-6′), 0.82 (d, 1.65 H, J = 6.2 Hz, H-6′); ^{13}C-NMR (300 MHz, MeOH-d_4): δ 193.50, 191.66, 161.54, 161.32, 137.69, 136.92, 136.71, 136.55, 129.34, 129.24, 128.75, 128.73, 127.78, 127.68, 127.21, 127.06, 122.14, 120.42, 120.08, 117.99, 117.96, 101.48, 101.33, 83.37, 83.00, 79.25, 76.90, 72.71, 72.16, 71.14, 70.92, 70.87, 70.67, 69.62, 69.09, 17.06, 16.91. IR (KBr); cm^{-1} 3420.98, 2931.80, 1689.90, 1607.95, 1467.08, 1382.48, 1311.80, 1231.90, 1136.59, 1100.63, 1061.22, 1028.71, 980.16, 838.96, 796.89, 761.66, 696.77. Elemental anal. for $C_{21}H_{22}O_7$: Found (calculated) (%): C, 65.37 (65.28); H, 5.85 (5.70).

3-α-L-Rhamnopyranosyloxychromanone (26). White solid. Mp 60–62 °C; $[a]_D^{20}$ −10.4 (*c* 1.0, MeOH-d_4), ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.84 (dd, 1 H, J = 1.57, 7.88 Hz, H-5), 7.55 (t × d, 1 H, J = 1.69, 7.86 Hz, H-7), 7.06 (t, 1 H, J = 7.52 Hz, H-6), 7.01 (dd, 1 H, J = 8.41, 8.40 Hz, H-8), 5.11 (d, 0.66 H, J = 1.34 Hz, H-1′), 4.97 (d, 0.34 H, J = 1.17 Hz, H-1′), 4.61–4.37 (m, 3 H, H-2 and H-3), 3.94–3.92 (dd, J = 1.73, 3.29 Hz, 0.66 H, H-2′), 3.87 (dd, J = 1.69, 3.35 Hz, 0.34 H, H-2′), 3.75–3.62 (m, 2 H, H-3′ and H-5′), 3.43 (t, 0.66 H, J = 9.49 Hz, H-4′), 3.37 (t, 0.34 H, J = 9.58 Hz, H-4′), 1.32 (d, 2 H, J = 6.20 Hz), 1.16 (d, 1 H, J = 6.21 Hz); ^{13}C-NMR (300 MHz, $CDCl_3$); δ 191.20, 190.47, 161.92, 161.75, 136.64, 136.45, 127.28, 127.20, 121.85, 121.72, 120.13, 119.91, 117.89, 117.84, 100.99, 99.69, 73.54, 72.74, 72.70, 72.46, 71.16, 71.06, 70.99, 70.84, 70.08, 69.72, 69.47, 69.17, 17.07, 16.74. IR (KBr); cm^{-1} 2954.59, 2900.47, 1757.31, 1737.02, 1688.30, 1605.37, 1478.19, 1466.47, 1380.48, 1327.47, 1293.77, 1236.39, 1217.27, 1153.05, 1122.09, 1079.61, 1037.58, 954.70, 914.09, 779.16. Elemental anal. Found (calculated) (%), C, 55.80 (55.87); H, 5.40 (5.26).

4-α-L-Rhamnopyranosyoxychromanol (27). White solid. Mp 125–127 °C; $[a]_D^{20}$ −5.8 (*c* 1.0, MeOH), ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.21 (m, 2 H), 6.85 (m, 2 H), 5.01 (d, 1 H, J = 0.86 Hz, H-1′), 4.98 (d, 0.66 H, J = 0.84 Hz, H-1′), 4.70 (m, 0.33 H, H-4), 4.64 (m, 0.66 H, H-4), 4.32–4.21 (m, 2 H, H-2), 3.84–3.81 (m, 1 H, H-2′), 3.75–3.67 (m, 2 H, H-3′ and H-5′), 3.47 (t, 1 H, J = 9.36 Hz, H-4′), 2.16–2.03 (m, 2 H, H-3), 1.39–1.22 (m, 3 H, H-6′); ^{13}C-NMR (300 MHz, $CDCl_3$): δ 155.33, 155.19, 131.37, 130.89, 130.55, 130.19, 121.76, 120.68, 120.31, 120.22, 117.53, 117.40, 98.11 (C-1′), 97.72 (C-1′), 73.30 (C-4′), 73.25 (C-4′), 72.20 (C-5′), 72.02 (C-5′), 71.90 (C-2′), 71.59 (C-2′), 68.96 (C-3′), 68.05 (C-4), 67.99 (C-4), 62.27 (C-2), 62.18 (C-2), 29.11 (C-3), 27.46 (C-3), 17.94 (C-6′), 17.77 (C-6′). IR (KBr); cm^{-1}, 3385.61, 3083.14, 3041.59, 2975.32, 2926.50, 1610.54, 1584.97, 1489.45, 1455.09, 1381.98, 1360.76, 1270.02, 1254.03, 1224.59, 1120.28, 1097.20, 1060.89, 1081.98, 1015.46, 981.51, 912.31, 835.93, 806.56, 754.64. Elemental anal. for $C_{15}H_{20}O_6$, found (calculated) (%): C, 60.83 (60.81); H, 6.78 (6.76).

Removal of the benzyl on the flavanone[44]

Compound **28** (0.067 g, 0.1362 mmol) was dissolved in dry EtOH (10 ml) and cyclohexene (3.4 ml), 0.67 g 10% Pd/C was added and the suspension was stirred under 62 °C for 4 h (TLC monitoring). The catalyst was removed by filtration and the filtrate was processed by evaporation to dryness. The residue was purified by chromatography (1 : 2 petroleum ether–EtOAc) to afford compound **29**.

7-Benzyloxy-3-α-L-rhamnopyranosyloxyflavanone (29). White solid. Mp 74–76 °C; $[a]_D^{20}$ −10 (*c* 1.0, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.83 (d, 1 H, J = 8.79 Hz, 0.34 H), 7.77 (d, 0.66 H, J = 8.82 Hz), 7.54–7.36 (m, 10 H), 6.70–6.67 (m 1 H), 6.53–6.50 (m, 1 H), 5.26 (s, 0.66 H-1′), 5.24 (d, 0.34 H, J = 11.21 Hz, H-2), 5.19 (d, 0.66 H, J = 11.73 Hz, H-2), 5.04 (s, 2 H), 4.66–4.59 (dd, 1 H, J = 11.24, 11.81 Hz, H-3), 4.24–4.18 (m, 0.34 H-5′ and 0.66 H-2′), 3.86–3.60 (m, 0.34 H-1′, 0.34 H-2′ and 0.34 H-3′), 3.41–3.34 (m, 0.66 H-3′ and 0.34 H-4′), 3.25 (t, 0.66 H-4′, J = 9.51 Hz), 1.91–1.82 (m, 0.66 H-5′), 1.22 (d, 1 H, J = 6.08 Hz), 0.83 (d, 2 H, J = 6.00 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 192.12, 190.44, 165.99, 165.87, 163.49, 163.34, 137.46, 136.55, 136.11, 136.08, 129.90, 129.73, 129.47, 129.22, 129.16, 129.11, 128.74, 127.97, 127.86, 127.74, 114.43, 114.03, 111.80, 111.68, 102.14, 102.10, 100.93, 100.40, 83.63, 83.24, 78.17, 76.38, 73.24, 72.59, 71.68, 70.81, 70.75, 69.47, 68.79, 17.89, 17.82. IR (KBr): cm^{-1} 3423.61, 3064.93, 3033.98, 2972.47, 2931.63, 1686.45, 1609.39, 1574.29, 1498.08, 1443.76, 1364.45, 1253.97, 1227.92, 1168.14, 1102.35, 1061.08, 1026.76, 981.19, 836.26, 757.39, 739.42, 697.32; Elemental anal. for $C_{28}H_{28}O_8$, found (calculated) (%), C, 68.30 (68.29); H, 5.75 (5.69).

7-Hydroxy-3-α-L-rhamnopyranosyloxyflavanone (30). White solid. Mp 105–107 °C; $[a]_D^{20}$ −9 (*c* 1.0, MeOH); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.61–7.40 (m, 6 H), 6.50 (d, 1 H, J = 8.34 Hz), 6.28 (d, 1 H, J = 8.13 Hz), 5.42 (d, 0.34 H, J = 9.41 Hz), 5.28 (d, 0.66 H, J = 11 27 Hz), 4.95 (s, 0.66 H-1′), 4.68 (d, 0.66 H, J = 11.32 Hz), 4.64 (d, 0.34 H, J = 9.46 Hz), 3.90–2.94 (m, 0.34 H-1′, H-2′, H-3′, H-4′ and 0.34 H-5′), 2.02–1.85 (m, 0.66

H, H-5′), 1.01 (d, 1 H, J = 5.46 Hz), 0.68 (d, 2 H, J = 5.43 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 191.41, 189.44, 167.63, 167.23, 163.54, 163.22, 138.28, 137.58, 129.68, 129.61, 129.52, 129.46, 129.22, 129.10, 128.57, 128.45, 112.75, 112.67, 112.40, 112.19, 103.29, 103.25, 101.96, 101.70, 101.21, 82.81, 82.66, 78.05, 76.35, 72.49, 71.97, 71.24, 71.00, 70.96, 70.88, 69.75, 69.45, 18.60. IR (KBr); cm^{-1} 3405.80, 2976.13, 2932.18, 2856.87, 1676.63, 1610.19, 1498.95, 1466.61,1453.52, 1360.98, 1262.99, 1166.58, 1098.79, 1061.74, 1036.02, 980.30, 913.18, 841.79, 812.52, 751.82, 698.65; Elemental anal. for $C_{21}H_{22}O_8$, found (calculated) (%), C, 62.66 (62.69); H, 5.62 (5.47).

4′-Hydroxy-3-α-L-rhamnopyranosyloxyflavanone (32). White solid. Mp 89–91 °C; $[\alpha]_D^{20}$ −10 (c 1.0, MeOH); ^{1}H NMR (300 MHz, acetone-d_6): δ 7.85–7.80 (m, 1 H), 7.63–7.56 (m, 1 H), 7.45–7.38 (m, 1 H), 7.16–7.02 (m, 2 H), 6.93–6.86 (m, 2 H), 5.31 (d, 0.34 H, J = 11.22 Hz), 5.23 (d, 0.66 H, J = 11.72 Hz), 5.14 (d, 0.66 H-1′, J = 0.85 Hz), 4.77 (d, 0.66 H, J = 11.72 Hz), 4.72 (d, 0.34 H, J = 11.22 Hz), 4.22–4.16 (m, 0.34 5′-H), 4.03–4.00 (m, 0.34 H-1′, 0.66 H-2′), 3.70–3.56 (m, 0.34 H-3′), 3.53–3.12 (m, 0.34 H-2′, 0.66 H-3′ and H-4′), 2.28–2.21 (m, 0.66 H-5′), 1.13 (d, 1 H, J = 6.20 Hz), 0.88 (d, 2 H, J = 6.16 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 193.51, 191.60, 161.61, 161.43, 158.55, 136.74, 136.55, 129.32, 128.51, 127.75, 127.36, 127.18, 122.13, 122.06, 120.33, 119.47, 118.15, 118.13, 115.66, 101.22, 101.06, 83.30, 82.90, 78.46, 76.67, 72.81, 72.43, 71.54, 71.35, 70.93, 70.71, 69.51, 69.04, 17.59, 17.51. IR (KBr); cm^{-1} 3406.63, 2925.55, 2854.36, 1694.34, 1607.90, 1579.01, 1519.23, 1465.25, 1370.05, 1323.59, 1278.52, 1232.14, 1172.83, 1146.97, 1104.31, 1060.79, 1033.02, 979.28, 912.45, 873.71, 832.64, 813.70, 762.45, 692.17; Elemental anal. for $C_{21}H_{22}O_8$, found (calculated) (%), C, 62.71 (62.69); H, 5.55 (5.47).

Biological assays

Animals

Female BALB/c and C57BL/6 mice (SPF, 18–22 g) were obtained from the Experimental Animal Center of Shanghai (Shanghai, China). They were maintained with free access to pellet food and water in plastic cages at 21 ± 2 °C and kept on a 12 h light–dark cycle. Animal welfare and experimental procedures were carried out strictly in accordance with the guide for the care and use of laboratory animals (National Research Council of USA, 1996) and the related ethical regulations of our university. All efforts were made to minimize animal's suffering and to reduce the number of animals used.

Spleen cell preparation[45]

Spleen was aseptically taken from mice, crushed gently and separated into single cells by squeezing in 5 mL D-Hank's solution (GIBCO BRL). The cells obtained were passed though the gauze of eight-layers and centrifuged at 200g for 5 min at 4 °C. The pellet was added into 10 mL sterile 0.17 M Tris (hydroxymethyl aminomethane) −0.75% NH_4Cl (pH 7.5) followed by centrifugation to remove erythrocytes. After washing twice with RPMI 1640 medium (GIBCO BRL), they were re-suspended in the medium and used for culture. The cells were found to be about 98% viable, as estimated by trypan blue exclusion.

Single mixed lymphocytes reaction[46]

The lymphocytes (5×10^5) from BALB/c mice were incubated with the lymphocytes (5×10^5) from C57BL/6 mice, which had been treated with mitomycin C (500 mg mL^{-1}) for 1 h in the presence or absence of the various concentrations of compounds at 37 °C in 5% CO_2 for 72 h. The proliferation of lymphocytes was measured by MTT method. The OD540 values were determined by an ELISA reader. Stimulation index was calculated as following formula: stimulation index = $(OD_{sample} - OD_{C57BL/6})/OD_{BALB/c}$. The experiment was repeated three times.

Apoptosis measurement

Sheep red blood cell-induced delayed-type hypersensitivity was induced in BALB/c mice. Mice were sensitized by injecting 40 μL of 2.5×10^8 mL^{-1} sheep red blood cells in saline into the left footpad. Five days later, they were challenged by painting 40 μL of 2.5×10^9 mL^{-1} of the cells in saline into the right footpad. Six hours later, spleen cells were isolated and incubated with the compounds at 37 °C for 12 h, then were used for apoptosis assay.

Spleen cell apoptosis was measured by flow cytometry as previously described.[47] Briefly, spleen cells were suspended in 0.1 mol L^{-1} citrate buffer (pH 7.2) containing 0.1% Triton X-100 and incubated at 37 °C for 30 min. The tubes were then vortexed and centrifuged and the resultant pellets were washed and stained with 10 μg mL^{-1} propidium iodide in the citrate buffer at room temperature. The percentage of apoptotic nuclei was analyzed by FACScan cytofluorimeter (Becton Dickinson).

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References

1 K. Y. Park, S.-H. Lee, B.-K. Min, K.-S. Lee, J.-S. Choi, S. R. Chung, K. R. Min and Y. Kim, *Planta Med.*, 1999, **65**, 457–459.
2 Y. Sakamoto, T. Ohmoto, T. Nikaido, K. Koike and T. Tomimori, *Bull. Chem. Soc. Jpn.*, 1989, **62**, 2450–2454.
3 M. Shimizu, T. Ito, S. Terrashhima, T. Hayashi, M. Arisawa, N. Morita, S. Kurokawa, K. Ito and Y. Hashimoto, *Phytochemistry*, 1984, **23**, 1885–1887.
4 (*a*) Y. H. Miura, I. Tomita, T. Watanabe, T. Hirayama and S. Fukui, *Biol. Pharm. Bull.*, 1998, **21**, 93–96; (*b*) M. Yoshino and K. Murakami, *Anal. Biochem.*, 1998, **1**, 40–44.
5 B. Havsteen, *Biochem. Pharmacol.*, 1983, **23**, 1141–1148.
6 B. E. Leibovitz and J. A. Mueller, *J. Optim. Nutr.*, 1993, **2**, 17–35.
7 (*a*) R. Kasai, S. Hirono, W.-H. Chou, O. Tanaka and F.-H. Chen, *Chem. Pharm. Bull.*, 1988, **36**, 4167–4170; (*b*) J. D. Britto, V. S. Manichckam, S. Gopalakrishinam, T. Ushioda and N. Tanaka, *Chem. Pharm. Bull.*, 1995, **43**, 338–339.
8 C. Eliane, C. Joao, D. M. Franco and A. Y. Rosendo, *Int. J. Pharmacogn.*, 1993, **31**, 38–46.
9 T. Chen, J. X. Li and J. S. Cao, *Planta Med.*, 1999, **65**, 56–59.
10 (*a*) L. K. Han, H. Ninomiya, M. Taniguchi, K. Baba, Y. Kimura and H. Okuda, *J. Nat. Prod.*, 1998, **61**, 1006–1011; (*b*) H. Hiraguchi, Y. Mochida, S. Sakai, H. Masuda, Y. Tamura, K. Mizutani, O. Tanaka and W. H. Chou, *Biosci., Biotechnol., Biochem.*, 1996, **60**, 945–948; (*c*) H. Hiraguchi, I. Ohmi, A. Fukuda, Y. Tamura, K. Mizutani, O. Tanaka and W. H. Chou, *Biosci., Biotechnol., Biochem.*, 1997, **61**, 651–654.

11 (*a*) Q. Xu, F. H. Wu, J. S. Cao, T. Chen, J. Y. Jiang, I. Saiki and A. Koda, *Eur. J. Pharmacol.*, 1999, **37**, 93–100; (*b*) R. Yan and Q. Xu, *Pharmacol. Res.*, 2001, **44**(2), 135–139.
12 Y. Cai, T. Chen and Q. Xu, *Inflammation Res.*, 2003, **52**, 334–340.
13 K. Ohmori, H. Ohrui and K. Suzuki, *Tetrahedron Lett.*, 2000, **41**, 5537–5541.
14 M. Li, X. W. Han and B. Yu, *J. Org. Chem.*, 2003, **68**, 6842–6845.
15 Y. G. Du, G. H. Wei and R. J. Linhardt, *J. Org. Chem.*, 2004, **69**, 2206–2209.
16 B. Alluis and O. Dangles, *Helv. Chim. Acta*, 2001, **84**, 1133–1156.
17 B. Vermes, V. M. Chari and H. Wagner, *Helv. Chim. Acta*, 1981, **64**, 1964–1967.
18 M. Bouktaib, A. Atmani and C. Rolando, *Tetrahedron Lett.*, 2002, **43**, 6263–6266.
19 C. Demetzos, A.-L. Skaltsounis, F. Tillequin and M. Koch, *Carbohydr. Res.*, 1990, **207**, 131–137.
20 M. Li, X. Han and B. Yu, *Tetrahedron Lett.*, 2002, **43**, 9467–9470.
21 K. A. O'Leary, A. J. Day, P. W. Needs, W. S. Sly, N. M. O'Brien and G. Williamson, *FEBS Lett.*, 2001, **503**, 103–106.
22 S. T. Caldwell, A. Crozier and R. C. Hartley, *Tetrahedron*, 2000, **56**, 4101–4106.
23 Z. Zhang and B. Yu, *J. Org. Chem.*, 2003, **68**, 6309–6313.
24 G. Capozzi, C. Falciani, S. Menichetti, C. Nativi and B. Raffaelli, *Chem. Eur. J.*, 1999, **5**, 1748–1754.
25 (*a*) H. Wagner, L. Horhammer, R. Dirscherl, L. Farkas and M. Nogradi, *Chem. Ber.*, 1968, **101**, 1186–1189; (*b*) L. Farkas and M. Nogradi, *Chem. Ber.*, 1968, **101**, 3419–3422; (*c*) L. Horhammer, H. Wagner, H.-G. Arndt, R. Dirscherl and L. Farkas, *Chem. Ber.*, 1968, **101**, 450–453; (*d*) L. Farkas, B. Vermes and M. Nogradi, *Chem. Ber.*, 1974, **107**, 1518–1525; (*e*) J. Frechetand and C. Schuerch, *J. Am. Chem. Soc.*, 1972, **94**, 604–609; (*f*) L. Farkas and B. J. Vermes, *J. Indian Chem. Soc.*, 1978, 1192–1197.
26 R. R. Schmidt, *Angew. Chem., Int. Ed. Engl.*, 1986, **25**, 212–235.
27 F. Tillequin and M. Koch, *Carbohydr. Res.*, 1990, **207**, 131–137.
28 (*a*) M. Zh. Liu, B. Yu and Y. Zh Hui, *Tetrahedron Lett.*, 1998, **39**, 415–418; (*b*) M. Li, X. W. Han and B. Yu, *Tetrahedron Lett.*, 2002, **43**, 9467–9470.
29 K. Ohmori, K. Htakeyama, H. Ohrui and K. Suzuki, *Tetrahedron*, 2004, **60**, 1365–1373.
30 R. M. Moriarty, O. Prakash and C. T. Thachet, *Synth. Commun.*, 1984, **14**, 1373–1378.
31 R. M. Moriarty and O. Prakash, *Acc. Chem. Res.*, 1986, **19**, 244–250.
32 X. L. Yang, Q. Xu and Z. J. Guo, unpublished data.
33 (*a*) R. R. Schmidt and M. Stump, *Liebigs Ann. Chem.*, 1983, 1249–1256; (*b*) R. R. Schmidt, M. Stump and J. Michel, *Tetrahedron Lett.*, 1982, **23**, 405–408; (*c*) R. R. Schmidt, J. Michel and M. Roos, *Liebigs Ann. Chem.*, 1984, 1343–1357; (*d*) R. R. Schmidt and J. Michel, *Tetrahedron Lett.*, 1984, **25**, 821–824; (*e*) H. Rathore, T. Igarashi, H. Nukaya and D. S. Fullerton, *Tetrahedron*, 1985, **41**, 5427–5438; (*f*) R. R. Schmidt, *Pure Appl. Chem.*, 1989, **61**, 1257–1270.
34 R. R. Schmidt and J. Michel, *Angew. Chem., Int. Ed. Engl.*, 1980, **19**, 731–732.
35 (*a*) J. R. Pougny and P. Sinay, *Tetrahedron Lett.*, 1976, 4073–4076; (*b*) J. R. Pougny, J. C. Jacquinet, M. Nassr, M. L. Milat and P. Sinay, *J. Am. Chem. Soc.*, 1977, **99**, 6762–6763.
36 (*a*) R. R. Schmidt and G. Grundler, *Angew. Chem., Int. Ed. Engl.*, 1982, **21**, 781–782; (*b*) H. Paulsen, *Angew. Chem., Int. Ed. Engl.*, 1982, **21**, 155–224; (*c*) P. Sinay, *Pure Appl. Chem.*, 1991, **63**, 519–528; (*d*) E. Juaristi and G. Cuevas, *Tetrahedron*, 1992, **48**, 5019–5087.
37 (*a*) R. R. Schmidt and A. Toepfer, *Tetrahedron Lett.*, 1991, **32**, 3353–3356; (*b*) D. Kahne, S. Walker, Y. Cheng and D. V. Engen, *J. Am. Chem. Soc.*, 1989, **111**, 6881–6882.
38 M. G. Bebault, G. G. S. Dutton and K. C. Warfield, *Carbohydr. Res.*, 1974, **34**, 174–179.
39 (*a*) N. K. Kochetkov, A. F. Bochkov, T. A. Sokolovskaya and V. J. Snyatkova, *Carbohydr. Res.*, 1971, **16**, 17–27; (*b*) A. F. Bochkov and N. K. Kochetkov, *Carbohydr. Res.*, 1975, **39**, 355–357; (*c*) V. I. Betaneli, M. V. Ovchinnikov, L. V. Backinowsky and N. K. Kochetkov, *Carbohydr. Res.*, 1979, **76**, 252–256; (*d*) L. V. Backinowsky, Y. E. Tsvetkov, N. F. Balan, N. E. Byramova and N. K. Kochetkov, *Carbohydr. Res.*, 1980, **85**, 209–221; (*e*) N. K. Kochetkov, V. M. Zhulin, E. M. Klimov, N. N. Malysheva, Z. G. Makarova and A. Y. Ott, *Carbohydr. Res.*, 1987, **164**, 241–254; (*f*) N. K. Kochetkov, S. A. Nepogod'ev and L. V. Backinowsky, *Tetrahedron*, 1990, **46**, 139–150.
40 (*a*) P. H. Seeberger, M. Eckhaedt, C. E. Cutteridge and S. J. Danishefsky, *J. Am. Chem. Soc.*, 1997, **119**, 10064–10073; (*b*) W. J. Sanders, D. D. Manning, K. M. Koeller and L. L. Kiessling, *Tetrahedron*, 1997, **53**, 16391–16422; (*c*) J. Gass, M. Strobl, A. Loibner, P. Kosma and U. Zaehringer, *Carbohydr. Res.*, 1993, **244**, 69–84; (*d*) F. J. Urban, B. S. Moore and R. Breitenbach, *Tetrahedron Lett.*, 1990, **31**, 44214424; (*e*) V. Magnus, D. Kikic-Topic, S. Iskric and S. Kveder, *Carbohydr. Res.*, 1983, **114**, 209–224; (*f*) H. Kunz and A. Harreus, *Liebigs Ann. Chem.*, 1982, 41–48.
41 J. D. Britto, V. S. Manichckam, S. Gopalakrishinam, T. Ushioda and N. Tanaka, *Chem. Pharm. Bull.*, 1995, **43**, 338–339.
42 R. Kasai, S. Hirono, W.-H. Chou, O. Tanaka and F.-H. Chen, *Chem. Pharm. Bull.*, 1988, **36**, 4167–4170.
43 G. Nonaka, Y. Goto, J. Kinjo, T. Nohara and I. Nishioka, *Chem. Pharm. Bull.*, 1987, **35**, 1105–1108.
44 (*a*) W. M. Pearlman, *Tetrahedron Lett.*, 1967, **17**, 1663–1664; (*b*) E. I. Amin, *J. Org. Chem.*, 1979, **44**, 3442; (*c*) A. Felix, *J. Org. Chem.*, 1978, **43**, 4194–4196.
45 Y. Sun, T. Chen and Q. Xu, *J. Pharm. Pharmacol.*, 2003, **55**, 839–846.
46 H. Tahara, N. Iwanami, N. Tabata, H. Matsumura, T. Matsuura, T. Takashiand Kurita and M. Miyazawa, *Transplant Immunol.*, 2004, **13**, 25–32.
47 L. Santucci, S. Fiorucci, F. Cammilleri, G. Servillo, B. Federici and A. Morelli, *Hepatology (Hoboken, NJ, U. S.)*, 2000, **31**(2), 399–406.