Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 2528–2532
Bioorganic & Medicinal Chemistry

Exploration of the correlation between the structure, hemolytic activity, and cytotoxicity of steroid saponins

Yibing Wang,[a] Yichun Zhang,[a] Ziyan Zhu,[b] Shilei Zhu,[c] Yingxia Li,[c] Ming Li[a] and Biao Yu[a,*]

[a]*State Key Laboratory of Bio-organic and Natural Products Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 200032, China*
[b]*Shanghai Blood Center, Shanghai 200051, China*
[c]*Key Laboratory of Marine Drugs, The Ministry of Education of China, Marine Drug and Food Institute, Ocean University of China, Qingdao 266003, China*

Received 14 December 2006; revised 31 January 2007; accepted 31 January 2007
Available online 3 February 2007

Abstract—The hemolytic activity of a collection of 63 steroid saponins was determined. The correlations between these structures and their hemolytic and cytotoxic activities are discussed. It has been demonstrated that the hemolytic activity of steroid saponins is highly dependent on their structures, that is, the sugar length, the sugar linkage, the substitutes on the sugar, as well as the aglycone. It has also been disclosed that the hemolytic activity and cytotoxicity of steroid saponins are not correlated. These results suggest that steroid saponins execute hemolysis and cytotoxic activity in different mechanisms, and encourage to develop steroid saponins into potent antitumor agents devoid of the detrimental effect of hemolysis.

1. Introduction

Saponins are anciently classified as any of various plant glycosides that form soapy lathers when mixed and agitated with water; and thus have been used routinely as detergents, foaming agents, and emulsifiers.[1] These natural surfactants are later found to be mainly glycosides of steroids and triterpenes, which exist widely in terrestrial plants, including many significant foods, forage crops, and herbal medicinal plants. So far, thousands of homogeneous saponins have been isolated and characterized. These glycosides display a tremendous structural diversity and a wide spectrum of biological activities.[2] Among those activities, hemolysis is probably the most general one shared by many structurally disparate saponins. It is believed that those saponins can form complexes with sterols of the erythrocyte membrane, thus causing an increase in permeability and the subsequent loss of hemoglobin.[3–5] However, the detailed mechanism of action of this hemolytic cascade remains unclear. On the other hand, the hemolytic property of saponins discourages the development of the various therapeutic potentials of this type of abundant natural product. Thus the correlations between the hemolytic and other biological activities of saponins, such as antitumor, adjuvant, and anti-inflammatory activities, require exploration.

However, the structure–activity relationship (SAR) studies on saponins have been hampered by the limited availability of homogeneous saponins in each laboratory. Those glycosides exist in such a micro-heterogeneous manner in plants that separation of each component in an appreciable amount is always a formidable task.[2] Recently, chemical synthesis has become a realistic alternative to determine the availability of homogeneous saponins, thus affording new opportunities for SAR studies.[6] For example, employing the natural and synthetic compounds, a good SAR has been obtained for the hederagenin diglycosides.[7] However, SAR studies on the hemolytic steroid saponins are few and lack relevance.[8–14] We have been working on the chemical synthesis of steroid saponins, to search for anticancer agents, for more than 10 years.[15–22] A good collection

Keywords: Steroid saponins; Hemolytic activity; Cytotoxicity; Dioscin; Structure–activity relationship.
* Corresponding author. Tel.: +86 21 54925132; fax: +86 21 64166128; e-mail: byu@mail.sioc.ac.cn

0968-0896/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2007.01.058

of the saponins bearing diosgenin and its congeners as the aglycones enabled us to explore a thorough SAR on this type of commonly occurring natural steroid saponin. Herein we report the hemolytic activity of 63 such steroid saponins (Figs. 1–3), and discuss the correlations between the structure, hemolytic activity, and their previously reported cytotoxicity.

2. Results

The hemolytic activity of the 63 steroid saponins, expressed as the concentrations that cause 50% hemolysis of human erythrocytes, is shown in Tables 1 and 2. Listed are also the cytotoxicities previously reported for most of the compounds.

The HD_{50} data listed in Tables 1 and 2 show clearly that the hemolytic activity of steroid saponins is highly sensitive to the change of their structures. We could draw the following structure–activity relationships:

(1) *With a same aglycone, trisaccharides (e.g.,* **10**–**16***) are more hemolytic than disaccharides (e.g.,* **2**–**9***), and disaccharides are more hemolytic than monosaccharides (e.g.,* **1***).* However, trisaccharide **25** was only slightly weaker in hemolysis than its disaccharide congener **24**.

(2) *With a same aglycone and a same length of sugar chain, the sugar linkage determines the hemolytic potency.* This point is clearly demonstrated by the four disaccharide congeners **2**, **5**, **6**, and **8**. Compounds **2**, **6**, and **8** bearing (1 → 2)-, (1 → 4)-, and (1 → 6)-α-L-Rha → β-D-Glc linkage, respectively, showed comparable activity (HD_{50} = 21.6, 27.3, and 28.6 μM, respectively), however, compound **5** with a (1 → 3)-linkage had much lower activity (15% hemolysis at 40 μg/mL).

(3) *Substituents on the hydroxyl groups in the sugar residues could affect significantly the hemolytic activity of the parent compounds.* Thus, acetylation of the 6′-OH of the disaccharide **2** (leading to compounds **3** and **4**) and the 4″-OH of the disaccharide **8** (leading to compound **9**) greatly lowered the parent activity. However, acetylation (and even substitution with a longer acyl group) of the 4″-OH of the disaccharide **6** (leading to compound **7**), the 4‴-OH of the trisaccharide **10** (leading to compounds **11**–**13**), and the 3‴, 5‴-OH of the trisaccharide **15** (leading to compound **16**) changed the hemolytic activity negligibly.

(4) *Changes on the algycone could also change the hemolytic activity.* Comparing the saponins bearing 3-*O*-β-charcotrioside (**10**, **17**–**23**), it shows that small changes on the aglycone (cf. compounds **10**, **17**, and **18**) led to slight changes in activity, and more

Compound	Sugar residue
1 (trillin)	β-D-Glc-(1→
2 (ophiopogonin C')	α-L-Rha-(1→2)-β-D-Glc-(1→
3	α-L-Rha-(1→2)-6-*O*-Ac-β-D-Glc-(1→
4	4-*O*-Ac-α-L-Rha-(1→2)-6-*O*-Ac-β-D-Glc-(1→
5 (polyphillin C)	α-L-Rha-(1→3)-β-D-Glc-(1→
6	α-L-Rha-(1→4)-β-D-Glc-(1→
7	4-*O*-Ac-α-L-Rha-(1→4)-β-D-Glc-(1→
8	α-L-Rha-(1→6)-β-D-Glc-(1→
9	4-*O*-Ac-α-L-Rha-(1→6)-β-D-Glc-(1→
10 (dioscin)	α-L-Rha-(1→4)-[α-L-Rha-(1→2)]-β-D-Glc-(1→
11	4-*O*-Ac-α-L-Rha-(1→4)-[α-L-Rha-(1→2)]-β-D-Glc-(1→
12	4-*O*-$CH_3(CH_2)_2CO$-α-L-Rha-(1→4)-[α-L-Rha-(1→2)]-β-D-Glc-(1→
13	4-*O*-$CH_3(CH_2)_4CO$-α-L-Rha-(1→4)-[α-L-Rha-(1→2)]-β-D-Glc-(1→
14 (taccaoside)	α-L-Rha-(1→3)-[α-L-Rha-(1→2)]-β-D-Glc-(1→
15 (polyphillin D)	α-L-Ara*f*-(1→4)-[α-L-Rha-(1→2)]-β-D-Glc-(1→
16	3,5-di-*O*-Ac-α-L-Ara*f*-(1→4)-[α-L-Rha-(1→2)]-β-D-Glc-(1→

Figure 1. Saponins **1**–**16** bearing diosgenin as the aglycone.

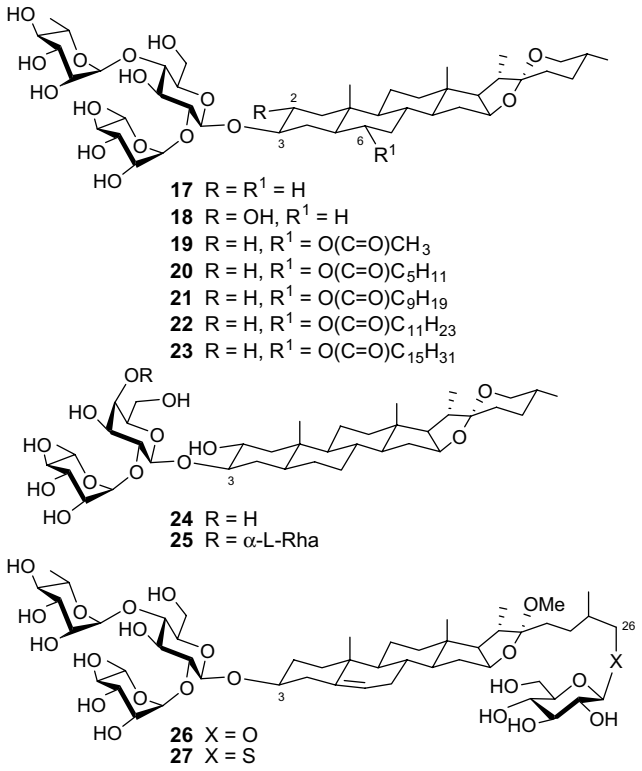
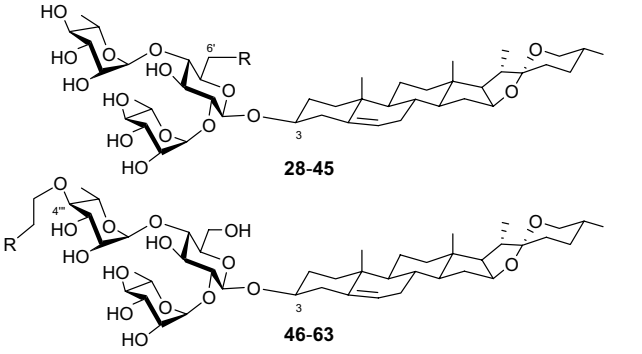


Figure 2. Steroid saponins **17**–**27**.



28/46 R =	$-N_3$	**37/55** R =	-NHCOPh
29/47	$-NH_2$	**38/56**	-NHCOPh(*p*-OMe)
30/48	$-NHCOCH_3$	**39/57**	-NHCOPh(*p*-NO_2)
31/49	$-NHCOCF_3$	**40/58**	-NHCOPh(*o*-I)
32/50	$-NHCO(CH_2)_2CH_2Cl$	**41/59**	$-NHCOCH_2OPh$
33/51	$-NHCOCH=CHCH=CHCH_3$	**42/60**	$-NHCOCH_2OPh$(*p*-Cl)
34/52	$-NHCO(CH_2)_8CH=CH_2$	**43/61**	-NHCOCH=CHPh
35/53	$-NHCO(CH_2)_{12}CH_3$	**44/62**	-NHFmoc
36/54	$-NHCO(CH_2)_{16}CH_3$	**45/63**	-NHdansyl

Figure 3. Saponins **28**–**63** with modified chacotriosyl residues on dioscin.

sizable changes (compounds **22** and **23** bearing long C_{12} and C_{16} fatty acids at C-6) diminished the activity. However, interestingly, substitution of a C_{10} and C_6 fatty acids at C-6 (compounds **20** and **21**) largely maintained the activity (compared to **17**) while the acetyloxy compound **19** was significantly less active.

(5) *The furostanol saponins (***26** *and* **27***), which bear an additional monosaccharide at the other side of the aglycone (compared to the spirostan saponins mentioned above), were not hemolytic at all.*

(6) *Substitution/modification on the various hydroxyl groups in the sugar residues could affect the hemolytic activity differently.* This point has been discussed above and is further proved by the saponins **28**–**63** with modifications at the 6′- and 4‴-OH of the chacotriosyl residue. Compared to the parent compound dioscin (**10**, HD_{50} = 6.8 μM), replacement of the 6′-OH with an azide or amino group (compounds **28** and **29**) significantly lowered the hemolytic activity. However, substitution of the amino group (of **29**, HD_{50} = 24.8 μM) could enhance the activity remarkably, even to the level of dioscin (the HD_{50} values of compounds **33**, **38**, **40**–**42**, **44**, and **45** are below 10 μM). The 4‴-*O*-(2-*N*-acyl)ethyl derivatives (**46**–**63**) were mostly more hemolytic than the parent dioscin; compounds **51**, **58**, **61**, and **63** had the HD_{50} values below 2.0 μM. The long fatty acid substitutions, whether at the 6′ or 4‴ position (compounds **35**, **36**, **53**, and **54**), could abolish the hemolytic activity. However, the same C_{11} substituent at 6′ (compound **34**) abolished the activity, while at 4‴ position (compound **52**) enhanced the activity (HD_{50} = 3.8 μM).

3. Discussion

Saponins with diosgenin and its congeners as the aglycones constitute one of the most abundant types of steroid saponins in Nature. Then the structure diversity lies mainly in their 'glycoforms'.[2] A common structural pattern of the 'glycoforms' starts with a β-D-glucopyranosyl unit at the 3-OH of the steroid aglycones and extends mostly with α-L-rhamnopyranosyl residues. For example, diosgenin 3-*O*-β-D-glucopyranoside (trillin **1**, Fig. 1) is the simplest one in this family; substitution of a α-L-rhamnopyranosyl residue at the 2-OH of the first glucose residue provides the disaccharide saponin ophiopogonin C′ (**2**); and further addition of an α-L-rhamnopyranosyl residue at the 4-OH of the glucose residue produces the trisaccharide saponin dioscin (**10**). Many such saponins show both hemolytic activity (toward erythrocyte) and cytotoxicity against tumor cells. Dioscin, for example, is among the potent members with a cytotoxicity IC_{50} at μM range against various tumor cell lines.[24,25] The previous sporadic data suggest that the hemolysis and cytotoxicity of steroid saponins are dependent on their structures,[8–14] especially on the nature, number, and sequence of the sugars in the saponins.

When comparing the hemolytic activity of the steroid saponins with their cytotoxicity (Table 1), one might first have an impression that these two activities are correlated. Several weakly hemolytic compounds are weakly toxic, such as compounds **1**, **5**, **22**, and **23**. And other highly hemolytic compounds are highly toxic, such as compounds **10**, **11**, **14**–**17**, **24**, and **25**. However, when comparing closely the whole collection of the data, as depicted in Figures 4 and 5, it is very clear that these

Table 1. Hemolytic activity of saponins **1**–**27**

Compound	Hemolytic activity HD_{50} (μM)	Cytotoxicity IC_{50} (μM)
1	≫100 (no activity at 100 μg/mL)	>34.5[a]
2	21.6 ± 2.0	2.5[a]
3	>75 (29% at 75 μg/mL)	9.6[b]
4	>75 (8% at 75 μg/mL)	9.4[b]
5	>40 (15% at 40 μg/mL)	>27.7[a]
6	27.3 ± 1.8	>27.7[a]
7	29.3 ± 0.7	
8	28.6 ± 0.4	
9	>50 (12% at 50 μg/mL)	
10	6.8 ± 0.3	3.8[a]; 0.52[c]; 3.5[d]
11	3.2 ± 0.3	8.2[b]
12	2.8 ± 0.4	
13	2.2 ± 0.2	
14	6.8 ± 0.2	2.3[a]
15	3.9 ± 0.1	0.6[a]
16	2.7 ± 0.2	0.5[b]
17	3.0 ± 0.2	0.81[c]
18	13.1 ± 0.7	2.3[c]
19	>30 (34% at 30 μg/mL)	26[d]
20	7.6 ± 0.2	21[d]
21	6.7 ± 0.2	No activity up to 30 μM[d]
22	>100 (8.5% at 100 μg/mL)	No activity up to 30 μM[d]
23	≫100 (no activity at 100 μg/mL)	No activity up to 30 μM[d]
24	7.5 ± 0.4	2.5[c]
25	9.6 ± 0.6	3.9[c]
26	≫100 (no activity at 100 μg/mL)	
27	≫100 (no activity at 100 μg/mL)	

[a] Data taken from Ref. 23 (where IC_{50} was expressed in μg/mL against HL-60 human promyelocytic leukemia cells).
[b] Data taken from Ref. 17 (against A-549 human pulmonary carcinoma).
[c] Data taken from Ref. 19 (against A-549 cells).
[d] Data taken from Ref. 18 (against HeLa cells).

Table 2. Hemolytic activity of saponins **28**–**63**

Compound	Hemolytic activity HD_{50} (μM)	Cytotoxicity IC_{50}[a] (μM)[a]
28	>50 μg/mL	≫10
29	24.8 ± 0.5	≫10
30	>50 μg/mL	≫10
31	18.5 ± 0.6	≫10
32	20.9 ± 0.0	≫10
33	9.3 ± 0.1	≫10
34	>50 μg/mL	≫10
35	>50 μg/mL	≫10
36	>50 μg/mL	≫10
37	10.7 ± 0.2	≫10
38	9.5 ± 0.1	≫10
39	39.0 ± 0.2	≫10
40	7.4 ± 0.1	≫10
41	9.4 ± 0.1	≫10
42	9.4 ± 0.1	≫10
43	11.8 ± 0.2	≫10
44	9.0 ± 0.2	≫10
45	6.9 ± 0.1	50.0[b]
46	9.7 ± 0.5	9.5
47	2.6 ± 0.2	9.2
48	9.2 ± 0.9	10.3
49	5.7 ± 0.5	3.7
50	3.8 ± 0.5	10.1
51	1.6 ± 0.3	4.9
52	3.8 ± 0.4	2.6
53	>50 μg/mL	≫10
54	>50 μg/mL	≫10
55	49.3 ± 0.4	11.2
56	4.5 ± 0.1	10.3
57	4.9 ± 0.2	15.3
58	1.7 ± 0.2	4.2
59	7.6 ± 0.5	7.7
60	2.9 ± 0.3	7.4
61	1.5 ± 0.3	11.2
62	3.0 ± 1.1	3.6
63	1.3 ± 0.1	11.9[b]

[a] Data taken from Ref. 21 (against A-549 human pulmonary carcinoma; IC_{50} of dioscin **10** as a control is 4.2 μM).
[b] Data taken from Ref. 22.

two activities are indeed not correlated. Not only it is not true that the compounds having stronger hemolytic activity must have stronger cytotoxicity, but also there are compounds showing remarkable disparity in these two activities (only those data from a simultaneous assay are discussed below). Thus, disaccharides **2** and **6** showing similar hemolytic activity (HD_{50} = 21.6, 27.3 μM, respectively) have disparate cytotoxicity (IC_{50} = 1.8, >27.7 μM, respectively). Trisaccharides **19** and **20** showing similar cytotoxicity (IC_{50} = 26, 21 μM, respectively) have disparate hemolytic activity (HD_{50} > 30 and = 7.6 μM, respectively). And the 6′-*N*-acyl-dioscin derivatives possessing hemolysis potency comparable to that of dioscin (compounds **33**, **37**–**44**) show no obvious toxicity at 10 μM, while the IC_{50} of dioscin is 4.2 μM.

The significant discrepancy between the two activities strongly suggests that steroid saponins execute hemolysis and cytotoxic activity in different mechanisms. These mechanisms wait for deciphering. Similar phenomenon was also observed by Voutquenne-Nazabadioko and co-workers for hederagenin diglycosides.[7] On the other hand, the present results demonstrate that it shall be realistic to develop steroid saponins into potent antitumor agents devoid of the detrimental effect of hemolysis.

4. Materials and methods

4.1. Saponins

All the 63 saponins (Figs. 1–3) were synthesized in our laboratory. The synthetic approaches and their characterization have been previously reported.[15–22]

4.2. Hemolysis assay

The human erythrocytes were obtained from Shanghai Blood Center. A slightly modified protocol as that used by Voutquenne-Nazabadioko et al. was employed.[26] Thus, all the tested compounds were dissolved in DMSO (from Sigma–Aldrich) at a concentration of 250 μg/mL, then PBS (Phosphate-buffered saline: NaCl = 8 g/L,

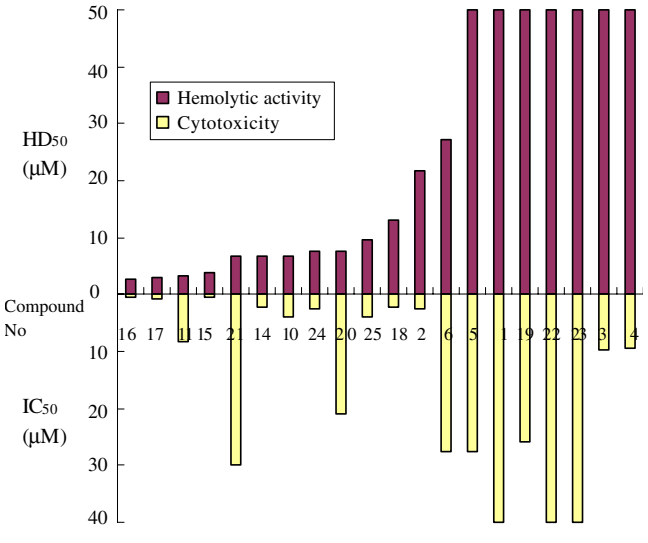


Figure 4. Comparison of the hemolytic and cytotoxic activities of saponins. (The compounds are lined up from the strongest hemolytic compound to the least one, data taken from Table 1.)

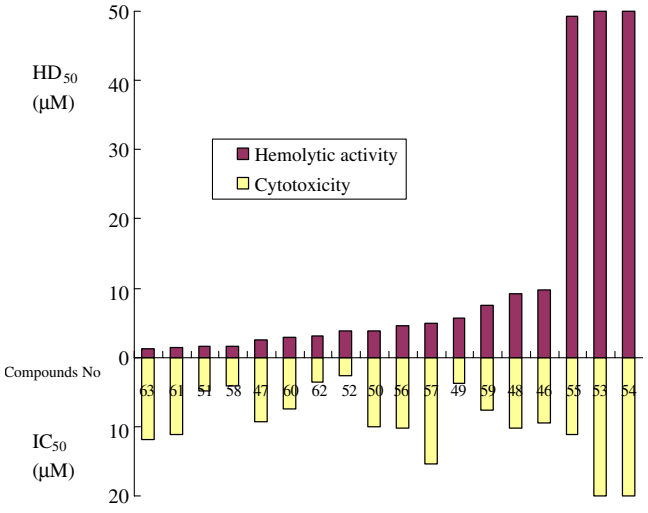


Figure 5. Comparison of the hemolytic and cytotoxic activities of saponins **46**–**63** with modified chacotriosyl residue on dioscin.

KCl = 0.2 g/L, Na_2HPO_4 = 1.44 g/L, and KH_2PO_4 = 0.24 g/L; pH 7.4) was added to dilute the solution to the testing concentrations ranging from 2.5 μg/mL to 100 μg/mL, if not especially mentioned. The final volume of the saponin solutions was 1.0 mL. The erythrocytes were diluted with PBS to obtain a 10% suspension. The erythrocyte suspension (100 μL) was added to 1 mL of the suspension to be tested and the samples were rapidly stirred and incubated at 37 °C with periodic stirring during a 60 min incubation period. The solutions were then centrifuged at 3000 rpm for 5 min. Absorbance of the supernatant was measured at 540 nm using a Bio-Rad model 3550 microplate reader (Bio-Rad, USA). And the hemolysis% was calculated by comparison with the 100% hemolysis caused by dioscin (positive control) with the distilled water as a negative control. All experiments were done in triplicate and the data were processed and HD_{50} (the concentrations that cause 50% hemolysis) was calculated by Origin 6.0 Program. The data were expressed as means ± SD.

Acknowledgments

This work is supported by the Chinese Academy of Sciences (KGCX2- SW-213 and KGCX2 -SW-209) and the National Natural Science Foundation of China (20321202).

References and notes

1. Tschesche, R.; Wulff, G. *Fortschr. Chem. Org. Naturst.* **1973**, *30*, 461.
2. Hostettmann, K.; Marston, A. *Saponins*; Cambridge University Press: Cambridge, 1995.
3. Bangham, A. D.; Horne, R. W.; Glauert, A. M.; Dingle, J. T.; Lucy, J. A. *Nature* **1962**, *196*, 952.
4. Elias, P. M.; Goerke, J.; Friend, D. S. *J. Cell Biol.* **1978**, *78*, 577.
5. Akiyama, T.; Takagi, S.; Sankawa, U.; Inari, S.; Saito, H. *Biochemistry* **1980**, *19*, 1904.
6. Yu, B.; Hui, Y. In *Glycochemistry: Principles Synthesis and Applications*; Wang, P. G., Bertozzi, C. R., Eds.; Marcel Dekker: New York, 2001; p 167.
7. Chwalek, M.; Lalun, N.; Bobichon, H.; Ple, K.; Voutquenne-Nazabadioko, L. *Biochim. Biophys. Acta* **2006**, *1760*, 1418.
8. Takechi, M.; Uno, C.; Tanaka, Y. *Phytochemistry* **1996**, *41*, 121.
9. Takechi, M.; Doi, K.; Wakayama, Y. *Phytother. Res.* **2003**, *17*, 83.
10. Takechi, M.; Uno, C.; Tanaka, Y. *Phytochemistry* **1997**, *44*, 299.
11. Takechi, M.; Uno, C.; Tanaka, Y. *Phytochemistry* **1991**, *30*, 2557.
12. Takechi, M.; Shimada, S.; Tanaka, Y. *Phytochemistry* **1992**, *31*, 3280.
13. Takechi, M.; Tanaka, Y. *Phytochemistry* **1993**, *34*, 1241.
14. Takechi, M.; Uno, C.; Tanaka, Y. *Biol. Pharm. Bull.* **1998**, *21*, 1234.
15. Li, B.; Yu, B.; Hui, Y.; Li, M.; Han, X.; Fung, K.-P. *Carbohydr. Res.* **2001**, *331*, 1.
16. Li, C.; Yu, B.; Hui, Y. *J. Carbohydr. Chem.* **1999**, *18*, 1107.
17. Yu, B.; Xing, G.; Hui, Y.; Han, X. *Tetrahedron Lett.* **2001**, *42*, 5513.
18. Zhang, Y.; Li, Y.; Guo, T.; Guan, H.; Shi, J.; Yu, Q.; Yu, B. *Carbohydr. Res.* **2005**, *340*, 1453.
19. Li, Y.; Zhang, Y.; Guo, T.; Guan, H.; Hao, Y.; Yu, B. *Synthesis* **2006**, 775.
20. Li, M.; Yu, B. *Org. Lett.* **2006**, *8*, 2679.
21. Zhu, S.; Zhang, Y.; Li, M.; Yu, J.; Zhang, L.; Li, Y.; Yu, B. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5629.
22. Wang, Y.; Zhang, Y.; Yu, B. *ChemMedChem* **2007**, in press.
23. Mimaki, Y.; Yokosuka, A.; Kuroda, M.; Sashida, Y. *Biol. Pharm. Bull.* **2001**, *24*, 1286.
24. Chiang, H. C.; Tseng, T. H.; Wang, C. J. *Anticancer Res.* **1991**, *11*, 1911.
25. Hu, K.; Dong, A.; Yao, X.; Kobayashi, H.; Iwasaki, S. *Planta Med.* **1996**, *62*, 573.
26. Chwalek, M.; Ple, K.; Voutquenne-Nazabadioko, L. *Chem. Pharm. Bull.* **2004**, *52*, 965.