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PII: S0960-894X(17)30683-2
DOI: http://dx.doi.org/10.1016/j.bmcl.2017.06.070
Reference: BMCL 25104

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 16 May 2017
Revised Date: 25 June 2017
Accepted Date: 26 June 2017

Please cite this article as: Yamauchi, S., Wukirsari, T., Ochi, Y., Nishiwaki, H., Nishi, K., Sugahara, T., Akiyama, K., Kishida, T., Syntheses of cytotoxic novel arctigenin derivatives bearing halogen and alkyl groups on aromatic rings, *Bioorganic & Medicinal Chemistry Letters* (2017), doi: http://dx.doi.org/10.1016/j.bmcl.2017.06.070

Syntheses of cytotoxic novel arctigenin derivatives bearing halogen and alkyl groups on aromatic rings

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ABSTRACT

The new lignano-9,9'-lactones (α,β-dibenzyl-γ-butyrolactone lignans), which showed the higher cytotoxicity than arctigenin, were synthesized. The well-known cytotoxic arctigenin showed activity against HL-60 cells (EC_{50} = 12 μM), however, it was inactive against HeLa cells (EC_{50} > 100 μM). The synthesized (3,4-dichloro, 2'-butoxy)-derivative **55** and (3,4-dichloro, 4'-butyl)-derivative **66** bearing the lignano-9,9'-lactone structures showed the EC_{50} values of 10 μM and 9.4 μM against HL-60 cells, respectively. Against HeLa cells, the EC_{50} value of the derivative **66** was 27 μM. By comparing the activities with the corresponding 9,9'-epoxy structure (tetrahydrofuran compounds), the importance of the lactone structure of **55** and **66** for the higher activities was shown. The substituents on the aromatic ring of the lignano-9,9'-lactones affected the cytotoxicity level, observing more than 10-fold difference.

Key words: lignan; arctigenin; matairesinol; γ-butyrolactone; lignano-9,9'-lactone; α,β-dibenzyl-γ-butyrolactone

The naturally occurring (8*R*,8'*R*)-arctigenin (**1**) bearing a lignano-9,9'-lactone was isolated from fruits of *Arctium lappa* and phagocytic activity on leulemia cells have been reported.[1] It was recently found that *Jurinea mollis* is a rich source of (8*R*,8'*R*)-arctigenin (**1**).[2] The mechanism of cytotoxicity,[3,4] the activity against multidrug resistant cancer cells,[5] and attenuation of learning and memory deficits[6] have been examined. The research on the arctigenin derivatives have also been continued.[7-9] Matairesinols (**2**, **3**) bearing similar structure to arctigenin are dietary lignano-9,9'-lactones and contained in vegetables (Figure 1).[10] The IgE-suppressive activity[11] and immunomodulatory activity[12] of (-)-matairesinol have been reported.

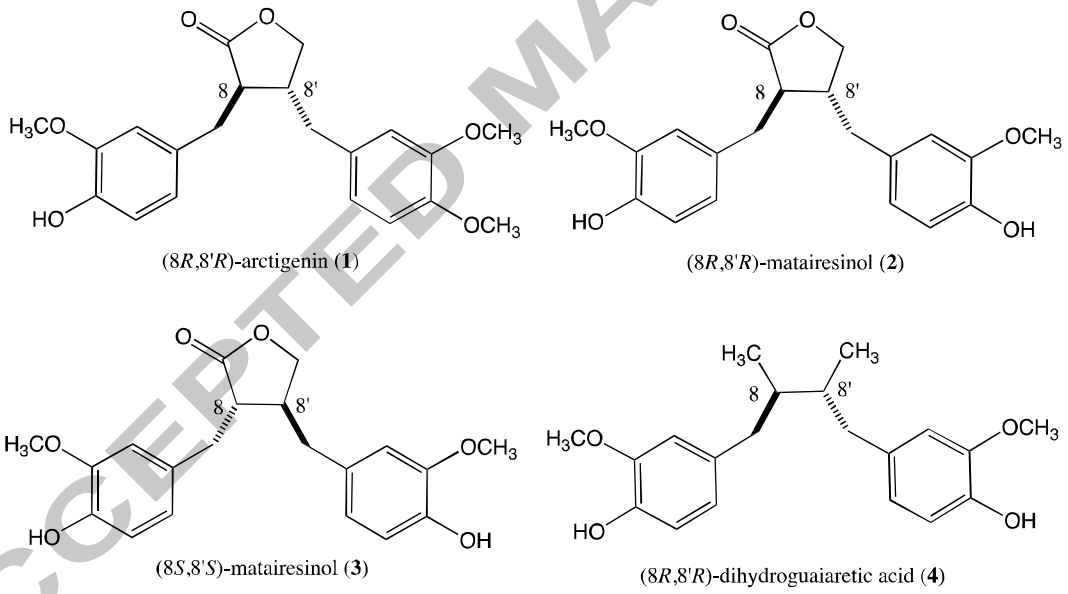


Figure 1. Structures of (8*R*,8'*R*)-arctigenin (**1**), both enantiomers of matairesinol (**2**, **3**), and (8*R*,8'*R*)-dihydroguaiaretic acid (**4**).

(8*R*,8'*R*)-Dihydroguaiaretic acid (**4**), which is a butane type lignan, is also a natural cytotoxic lignan containing butane type structure. We previously clarified the structure

containing stereochemistry-activity relationship of dihydroguaiaretic acid, discovering the compounds showing the higher activity than the natural compound **4** by preparing the benzene ring derivatives.[13] As a first stage for this project, the cytotoxicities of **1-3** were compared to evaluate the effect of the lignano-9,9'-lactone structure on the activity using H-60 and HeLa cells. After this evaluation, the lignano-9,9'-lactones bearing different kinds of substituents on the benzene ring were synthesized, and then the structure-activity relationship was examined to develop the new lignano-9,9'-lactones showing the higher activity than the natural lignano-9,9'-lactones **1-3** against HL-60 and HeLa cells. The results of this project would contribute to the construction of chemical library of lignan and develop a new medicine based on the lignan structure.

We can find the synthetic reports of the optically active arctigenin[14-16] and (*R*)-3-benzyl-4-butanolide,[17,18] which is a synthetic intermediate for the derivatives **5**-**46** (Table 2). In this experiment, all derivatives were synthesized by employing the previously described method[19,20] with modification. The α-benzylation was applied to (*R*)-3-benzyl-4-butanolide to prepare **5**-**46** and (*R*)-3-arylmethyl-4-butanolide to prepare **52**-**75** (Table 3). The synthesis of the racemic compound **5** was reported. The NMR data of our synthetic (8*R*,8'*R*)-**5** agreed with those in the literature.[21,22] The 9,9'-lactone structure were transformed to 9,9'-epoxy structure **76**-**99** (Table 3) by the previously described method.[19] The α,β-unsaturated butyrolactones **49** and **50** were prepared from the corresponding aldol product from (*R*)-3-benzyl-4-butanolide by dehydration employing $KHSO_4$,[23] followed by separation of *E*- and *Z*-form. The *E*- and *Z*-forms were identified by ^{1}H-NMR data.[24] The differential NOE between 7-H and 8'-H

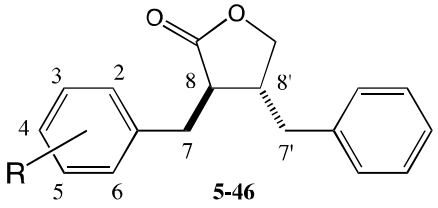
confirmed the *E* and *Z* structures. The benzyl ethers, which are the protective group for the phenolic group in the synthetic process, were cleaved by BBr_3[25] to obtain **52**, **58**, **62**, **76**, **82**, **86** or BCl_3[26] to obtain **68**, **69**, **92**, **93**. The hydrogenolysis using 5% Pd/C and H_2 was performed to get the other phenolic compounds. The synthetic method, NMR, and MS data were descried in the supporting information.

Table 1. Cytotoxicities of **1-4**

Compounds	EC_{50} (μM±SD)	
	HL-60 cells	HeLa Cells
(8*R*,8'*R*)-arctigenin (**1**)	12 ± 1.5	> 100
(8*R*,8'*R*)-matairesinol (**2**)	74 ± 2.2	> 100
(8*S*,8'*S*)-matairesinol (**3**)	> 100	> 100
(8*R*,8'*R*)-dihydroguaiaretic acid (**4**)	26 ± 0.9	22 ± 4.1

The activities of (8*R*,8'*R*)-arctigenin (**1**), (8*R*,8'*R*)-matairesinol (**2**), and (8*S*,8'*S*)-matairesinol (**3**) against HL-60 and HeLa cells were compared in Table 1. Against HL-60 cells, (8*R*,8'*R*)-arctigenin (**1**) exhibited the highest activity to show the EC_{50} value of 12 μM. In the case of matairesinol, (8*R*,8'*R*)-matairesinol (**2**), which was 6-fold less potent than that of (8*R*,8'*R*)-arctigenin (**1**), exhibited the enantiospecific activity. (8*S*,8'*S*)-Matairesinol (**3**) did not show the activity at 100 μM. The cytotoxicities of all lignano-9,9'-lactones **1**-**3** against HeLa cells were not observed at 100 μM. However, (8*R*,8'*R*)-dihydroguaiaretic acid (**4**), which is a butane type of lignan, was effective against both HL-60 and HeLa cells. It could be assumed that the lignano-9,9'-lactone structure is disadvantageous for the cytotoxicity against HeLa cells. We tried to improve the activity of the lignano-9,9'-lactone by syntheses of the

Table 2. Cytotoxicities of lignano-9,9'-lactone derivatives **5**-**46** bearing different kinds of substituents on the 7-atomatic ring against HL-60 and HeLa cells (nt: not tested)



5-46

No.	R	EC_{50} (μM±SD) HL-60	No.	R	EC_{50} (μM±SD) HL-60	HeLa
5	H	77 ± 2.7	**28**	4-OCF_3	27 ± 5.1	nt
6	2-OH	83 ± 1.8	**29**	4-OCH_2OCH_3	71 ± 2.0	nt
7	2-OCH_3	79 ± 6.6	**30**	4- $O(CH_2)_3CH_3$	23 ± 1.5	27 ± 4.7
8	2-OCF_3	39 ± 1.6	**31**	4-$O(CH_2)_6CH_3$	>100	nt
9	2-OCH_2OCH_3	76 ± 7.3			17% inhibition	
10	2-$O(CH_2)_3CH_3$	20 ± 1.1	**32**	4-CH_3	44 ± 1.1	nt
11	2-$O(CH_2)_6CH_3$	75 ± 9.3	**33**	4-CF_3	22 ± 1.0	29 ± 4.5
12	2-CH_3	57 ± 3.0	**34**	4-F	57 ± 2.6	nt
13	2-CF_3	37 ± 1.3	**35**	4-Cl	36 ± 1.7	nt
14	2-F	72 ± 1.8	**36**	3-OCH_3, 4-OH	93 ± 5.2	nt
15	2-Cl	37 ± 0.8	**37**	3-$O(CH_2)_3CH_3$	35 ± 3.5	nt
16	3-OH	>100		4-OH		
		45% inhibition	**38**	3-OCH_3, 4-OCH_3	>100	nt
17	3-OCH_3	66 ± 3.8			38% inhibition	
18	3-OCF_3	34 ± 3.7	**39**	3-$O(CH_2)_3CH_3$	>100	nt
19	3-OCH_2OCH_3	80 ± 9.4		4-$O(CH_2)_3CH_3$	29% inhibition	
20	3-$O(CH_2)_3CH_3$	26 ± 1.7	**40**	3-$O(CH_2)$O-4	57 ± 5.8	nt
21	3-$O(CH_2)_6CH_3$	81 ± 0.4	**41**	3-CH_3, 4-CH_3	35 ± 5.1	nt
22	3-CH_3	53 ± 8.3	**42**	3-F, 4-F	50 ± 3.7	nt
23	3-CF_3	34 ± 1.9	**43**	3-Cl, 4-Cl	19 ± 0.6	26 ± 3.8
24	3-F	75 ± 3.4	**44**	2-Cl, 4-Cl	19 ± 0.5	23 ± 3.8
25	3-Cl	36 ± 1.9	**45**	3-Cl, 5-Cl	23 ± 1.9	17 ± 3.5
26	4-OH	88 ± 4.1	**46**	3-OCH_3, 4-OCH_3	>100	nt
27	4-OCH_3	67 ± 6.0		5-OCH_3	44% inhibition	

derivatives bearing many kinds of the substituents on the aromatic rings. Because of the results of the activity tests of (8*R*,8'*R*)- and (8*S*,8'*S*)-matairesinol (**2** and **3**), we decided to select the (8*R*,8'*R*)-configuration for the following derivatives.

The derivatives **5**-**46** bearing many kinds of the aromatic rings at the 7-position and the phenyl group without any substituent at the 7'-position were synthesized and their cytotoxicities were examined (Table 2). The activity of the derivative **5** against HL-60 cells, whose benzene rings do not have any substituent, was similar to that of (8*R*,8'*R*)-matairesinol (**2**) in Table 1, suggesting that phenolic hydroxy groups and methoxy groups of matairesinol are not necessary to show this level of activity. The results of the tests of the 2-hydroxy **6**, 4-hydroxy **26**, 2-methoxy **7**, 3-methoxy **17**, and 4-methoxy **27** derivatives reflects this fact, showing the same level of the EC_{50} values (66-88 μM) as the derivative **5**. The introduction of a hydroxyl group to the 3-position dramatically reduced the activity (**16**: EC_{50} > 100 μM, against HL-60 cells). The electron-withdrawing trifluoromethoxy derivatives **8**, **18**, **28** showed 2-3-fold higher activities than the methoxy derivatives **7**, **17**, **27** to exhibit the EC_{50} values of 23-40 μM. Though the activities of the methoxymethoxy derivatives **9**, **19**, **29** and heptoxy derivatives **11**, **21**, **31** were similar to those of the methoxy derivatives **7**, **17**, **27**, the butoxy derivatives **10**, **20**, **30** showed 2-4-fold more potent activities than the methoxy derivatives **7**, **17**, **27**, exhibiting the EC_{50} values of 20-26 μM. It is suggested that the suitable length of the alkoxy group for the higher activity would be present. Thus, the derivatives bearing the methoxy group and heptoxy group showing lower activities than the butoxy derivatives. Comparing the trifluoromethyl

derivatives **13**, **23**, **33** with the methyl derivatives **12**, **22**, **32**, the derivatives bearing the higher electron-withdrawing trifluoromethyl group, whose EC_{50} values were 22-37 μM, were about 2-fold more potent than the methyl compounds. As for the comparison of the halogen derivatives, the chloro-derivatives **15**, **25**, **35** were around 2-fold more potent than the fluoro-derivatives **14**, **24**, **34** to show the EC_{50} values of 36-37 μM. The activity levels of the fluoro-derivatives **14**, **24**, **34** (EC_{50} = 57-72 μM) were similar to the phenyl derivative **5**, suggesting that the activity is not affected by the density of electron. The di-substituted derivatives were also tested. The activity of the 3-methoxy-4-hydroxy derivative **36** was almost similar to that of mono-hydroxy derivatives, showing activity (EC_{50} = 93 μM). However, the activity of the phenolic derivative was increased by the introduction of a longer alkoxy group at the 3-position. The 3-butoxy-4-hydroxy derivative **37** (EC_{50} = 35 μM) was 3-fold more potent than the 3-methoxy-4-hydroxy derivative **36**. Though the hydrophobicity seemed to be important for the higher activity, the activities of the 3,4-dimethoxy derivative **38** and 3,4-dibutoxy derivative **39** were very weak (EC_{50} > 100 μM). On the other hand, the EC_{50} value of the 3,4-methylenedioxy derivative **40** was 57 μM, which was almost similar to that of the 3,4-difluoro derivative **42** (EC_{50} = 50 μM). The 3,4-dimethyl derivative showed higher EC_{50} value of 35 μM than **40** and **42**, suggesting that the presence of the oxygen atoms is disadvantageous and some size of hydrophobic groups is necessary for the higher activity. This assumption was confirmed by the tests using the dihalogen derivatives. The dichloro derivatives **43**-**45** (EC_{50} = 19-23 μM) were 2-fold more potent than the 3,4-difluoro derivative **42**. The 3,4,5-trimethoxy

derivative **46** was not effective. The activity level did not depend on the position of the hydrophobic group on the 7-aromatic ring. Some effective derivatives were applied to HeLa cells. The EC_{50} values of the 4-butoxy **30**, 4-trifluoromethyl **33**, and dichloro derivatives **43**-**45** were 17-29 μM against HeLa cells, showing higher activities than natural (8*R*,8'*R*)-arctigenin (**1**). These activities were similar to that of (8*R*,8'*R*)-dihydroguaiaretic acid (**4**), which is a butane type of natural lignan, against both HL-60 and HeLa cells.

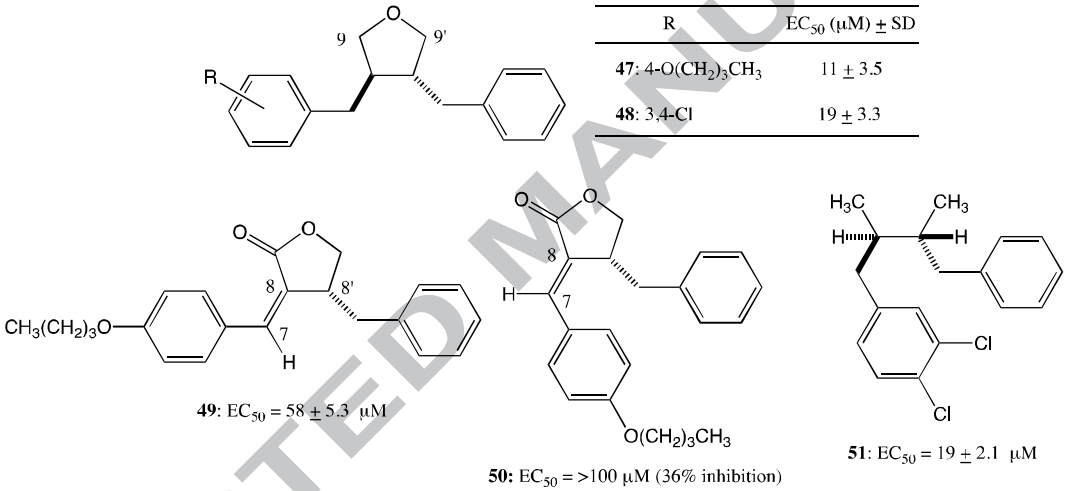


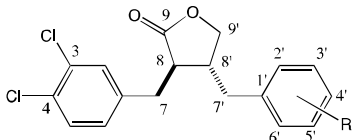
Figure 2. Cytotoxicities of 9,9'-epoxy (**47**, **48**), lign-7-eno-9,9'-lactone (**49**, **50**), and butane **51** type lignans against HL-60 cells

The effect of main lignan structure on the activity was also examined. Figure 2 shows the cytotoxicities of 9,9'-epoxylignans **47**, **48**, lign-7-eno-9,9'-lactone **49**, **50**, and butane type lignan **51** bearing the 3,4-dichlorophenyl group or 4-butoxyphenyl group at the 7-position against HL-60 cells. The activities of the 9,9'-epoxylignan **48** and butane type lignan **51** were similar to that of the corresponding lignano-9,9'-lactone derivative bearing 3,4-dichlorophenyl or 4-butoxy group, however, the butoxy

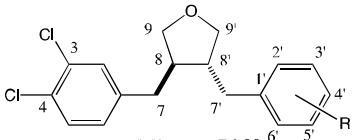
derivative of 9,9'-epoxylignan **47** showed 2-fold more potent than corresponding lignano-9,9'-lactone derivative **30**. In this case, carbonyl group of lactone structure does not play an important role. Comparing *Z*-form **49** with *E*-form **50**, *Z*-form showed the higher activity (EC_{50} = 58 μM), however, the corresponding lignano-9,9'-lactone **30** was 2-fold more potent. It was suggested that the presence of α,β-unsaturated double bond, which is found in the natural lignans,[27] would be disadvantageous. The higher activity of *Z*-form suggests that the same direction of 7-aryl group as the carbonyl group is favorable for the higher activity.

To evaluate the effect of the aromatic ring at the 7'-position, the 3,4-dichlorophenyl group was employed for the 7-position. Table 3 shows the results of the cytotoxic assay. In the 2'-position, the alkoxy derivatives **53**-**55** (EC_{50} = 10-15 μM) were 2-3-fold more effective than the phenolic **52**, methyl **56**, and fluoro **57** derivatives against HL-60 cells. The 2'-butoxy derivative **55** was most effective among the 2'-derivetives against HL-60 cells, showing the EC_{50} value of 10 μM. Among the 3'-derivativs, the 3'-fluoro derivative **61** showed the highest activity against HL-60 cells (EC_{50} = 14 μM). The 3'-hydroxy **58**, 3'-methoxy **59**, and 3'-methyl derivative **60** showed the EC_{50} values of 22-48 μM. These facts suggest that the smaller hydrophobic group would be important for the higher activity at the 3'-position. In the case of the 4'-position, the 4'-methyl derivative **64** was around 2-fold more potent than the 4'-hydroxy **62** and 4'-methoxy derivative **63** to show the EC_{50} value of 19 μM against HL-60 cells. The disadvantage of the presence of the oxygen atom at the 4'-position was suggested. The test of the relationship between the length of the alkyl

Table 3. Cytotoxicities of 3,4-dichlorolignan-9,9'-lactone **52**-**69** and 9,9'-epoxy derivatives **76**-**93** (nt: not tested). Arctigenin (**1**): against HL-60 (EC_{50} = 12 μM), against HeLa (EC_{50} >100 μM)



9,9'-lactone **52-75**



9,9'-epoxy **76-99**

R	No.	9,9'-lactone, EC_{50} (μM±SD)		No.	9,9'-epoxy, EC_{50} (μM±SD)
		HL-60	HeLa		HL-60
2'-OH	**52**	33 ± 3.6	nt	**76**	34 ± 2.7
2'-OCH_3	**53**	14 ± 4.3	24 ± 4.0	**77**	55 ± 12
2'-OCH_2CH_3	**54**	15 ± 4.1	31 ± 6.7	**78**	29 ± 9.6
2'-$O(CH_2)_3CH_3$	**55**	10 ± 2.8	>100	**79**	20 ± 6.9
2'-CH_3	**56**	22 ± 4.9	nt	**80**	21 ± 9.5
2'-F	**57**	28 ± 4.8	nt	**81**	51 ± 7.7
3'-OH	**58**	48 ± 8.4	nt	**82**	31 ± 4.2
3'-OCH_3	**59**	34 ± 1.0	nt	**83**	73 ± 9.0
3'-CH_3	**60**	22 ± 4.3	nt	**84**	28 ± 5.7
3'-F	**61**	14 ± 2.4	20 ± 3.2	**85**	20 ± 2.5
4'-OH	**62**	37 ± 4.2	nt	**86**	31 ± 3.9
4'-OCH_3	**63**	37 ± 3.4	nt	**87**	39 ± 6.8
4'-CH_3	**64**	19 ± 7.8	nt	**88**	21 ± 3.3
4'-CH_2CH_3	**65**	16 ± 2.7	nt	**89**	26 ± 7.9
4'-$(CH_2)_3CH_3$	**66**	9.4 ± 2.3	27 ± 3.3	**90**	23 ± 8.6
4'-F	**67**	14 ± 2.7	19 ± 1.3	**91**	43 ± 6.1
3'-OCH_3, 4'-OH	**68**	36 ± 5.6	nt	**92**	21 ± 4.1
3'-OH, 4'-OCH_3	**69**	32 ± 8.7	nt	**93**	33 ± 4.3
3'-OCH_3, 4'-OCH_3	**70**	24 ± 8.7	nt	**94**	16 ± 3.1
3'-$O(CH_2)$O-4'	**71**	51 ± 7.8	nt	**95**	22 ± 3.7
3'-$O(CH_2)_2$O-4'	**72**	28 ± 12	nt	**96**	38 ± 9.1
3'-CH_3, 4'-CH_3	**73**	16 ± 4.5	nt	**97**	36 ± 6.3
3'-F, 4'-F	**74**	25 ± 6.3	nt	**98**	18 ± 2.1
3'-Cl, 4'-Cl	**75**	20 ± 4.9	nt	**99**	16 ± 7.3

chain and the activity showed the 4'-butyl derivative **66** was more potent (EC_{50} = 9.4μM) than the methyl derivative **64**. The 4'-fluoro derivative **67** showed almost similar activity to the 4'-butyl derivative **66**. It could be assumed that the longer hydrophobic group is advantageous for the higher activity. To evaluate the effects of two substituents, which are found in the natural compounds, activities of derivatives **68**-**72** were also examined. There is a possibility that the effect of one substituent is increased and the bulky effects could be also evaluated. Among the 3',4'-disubstituted derivatives, the 3',4'-dimethyl derivative **73** showed the highest activity, showing the EC_{50} value of 16 μM. The derivatives **68**-**72** and **74** containing the phenolic and alkoxy substituents or two fluorine atoms were 1.5-3-fold less potent than the 3',4'-dimethyl derivative **73**. The activities of the dichloro-derivative **75** was almost similar to that of the 3',4'-dimethyl derivative **73**. Some size of the hydrophobic group would be more potent in the case of the 3',4'-disubstituted derivatives. The almost similar activities to arctigenin **1** were observed in the 2'-butoxy **55** and 4'-butyl derivative **66** against HL-60 cells. The most less potent 9,9'-lactone structure against HL-60 cells were the 3'-OH **58** and 3',4'-methylenedioxy **71** derivatives, which were 5-6-fold less potent than the 4'-butyl derivative **66**. The derivatives **53**-**55**, **61**, **66**, and **67** were applied to the tests against HeLa cells. Except for the 2'-butoxy derivative **55**, the EC_{50} values of 19-31 μM, which were higher than that of arctigenin **1**, were shown. Considering the inactive 2'-butoxy derivative **54** and active 4'-butyl derivative **66** against HeLa cells, the presence of oxygen atom would be disadvantageous for the higher activity against HeLa cells in the case of the derivative bearing longer

hydrophobic group on the 7'-aromatic ring. Since natural arctigenin bearing two methoxy groups on 7'-aromatic ring is inactive against HeLa cells, more hydrophobicity would be effective against HeLa cells. The activities of the corresponding 9,9'-epoxy derivatives **76**-**99** were also evaluated against HL-60 cells. The obvious advantage of the 9,9'-lactone structure was shown in the 2'-methoxy derivative **53**, which was 4-fold more potent than the corresponding 9,9'-epoxy structure **77**. The most effective 9,9'-epoxy structure was the 3',4'-dimethoxy **94** and 3',4'-dichloro derivative **99**, showing the EC_{50} values of 16 μM. However, the obvious advantage of the 9,9'-epoxy structure than the 9,9'-lactone was not observed in most of the cases. The lowest activity of the 9,9'-epoxy structure was shown in the 3'-methoxy derivative **83** (EC_{50} = 73 μM). The most effective 9,9'-epoxy lignan was 4-butoxy derivative **30** (Figure 2, against HL-60 dells, EC_{50} = 11 μM), suggesting that the longer alkoxy substituent on the 7-aryl group and phenyl group without substituent on the 7'-position is advantageous in the case of 9,9'-epoxy structure.

In summary, the novel cytotoxic lignano-9,9'-lactones were developed. Two chlorines on the 7-aromatic ring and 2'-butoxy/4'-butyl group on the 7'-aromatic ring were effective to improve the activity level of arctigenin. In the previous study,[7] the alkoxy derivatives were synthesized and their activities were evaluated. In our research, the chloro and alkyl derivatives were prepared and they showed almost similar activity to arctigenin against HL-60 cells. On the other hand, their activities against HeLa cells were dramatically improved comparing natural arctigenin. It could be assumed that hydrophobic substituents on the aromatic ring are important for the

activity against HeLa sells. We have showed the effects of aromatic substituents in the other lignan structure on the cytotoxicity by our previous study.[13,28-30] It was clarified that the substituents on the aromatic ring of the lignano-9,9'-lactone structure effects on the activity in this study. Since many biological activities of lignano-9,9'-lactone have been reported,[31-35] there is a possibility that the new biological activities of these compounds would be discovered.

Acknowledgements

Part of this study was performed at ADRES (Johoku station) of Ehime University. We are grateful to MARUTOMO Co., LTD for financial support.

Supplementary data

Supplementary data associated with this article can be found in the online version.

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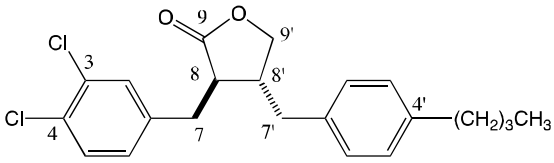
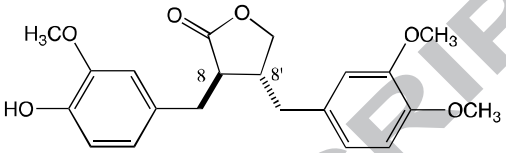
GA Graphic



HL-60 cells: EC_{50} = 9.4 μM
HeLa cells: EC_{50} = 27 μM



(8*R*,8'*R*)-arctigenin (**2**)
HL-60 cells: EC_{50} = 12 μM
HeLa cells: EC_{50} > 100 μM