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Acetoxybenzhydrols as highly active and stable analogues of 1′*S*-1′-acetoxychavicol, a potent antiallergic principal from *Alpinia galanga*

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ABSTRACT

Through SAR studies on 1′*S*-1′-acetoxychavicol acetate (**1**) against Type I antiallergic activity by indexing release of β-hexosaminidase, a marker of antigen-IgE-mediated degranulation in RBL-2H3 cells, more stable and potent analogue, 4-(methoxycarbonyloxyphenylmethyl)phenyl acetate (**16**), has been developed. The compound **16** also strongly inhibited the antigen-IgE-mediated TNF-α and IL-4 production.

We describe here that 4-acylbenzhydrol esters designed as stable analogues of naturally occurring anti-allergic phenylpropanoid, 1′*S*-1′-acetoxychavicol acetate (**1**), showed stronger inhibitory activity against release of β-hexosaminidase than **1**. These esters were also found to show as strong inhibitory activities as **1** against antigen induced production of TNF-α and IL-4.

Despite its simple structure, 1′*S*-1′-acetoxychavicol acetate (**1**) has been reported to bear a variety of important biological activities, such as antitumor,[1–6] anti-inflammatory,[7] antifungal,[8] antioxidative,[9,10] anti-HIV,[11,12] and xanthine oxidase inhibitory activity.[7,13] Intensive structure activity relationship (SAR) studies on **1** as a growth inhibitor of human leukemia HL-60 cells have been reported recently.[14] In the course of our continuing studies on exploring bioactive constituents from medicinal foodstuffs of Thailand, the authors also have revealed notable biological activities, such as gastroprotective,[15] anti nitric oxide production,[16–18] anti-interferon-β production,[19] and antiallergy activities,[20–23] in **1** which was isolated from the rhizomes of *Alpinia* (*A.*) *galanga* SWARTZ WARTZ (syn. *Languas galanga* STUNZ).[24] Among them, the inhibitory activity against Type I allergy was so potent, and found much stronger than that of a synthetic antiallergic medicine, ketotifen fumalate. Inhibitory activity against antigen induced production of TNF-α and IL-4, both of which are known to participate in the late phase of type I allergic reactions, were also found to be potent. However, **1** suffered from instability, especially under acidic conditions, and gradual decomposition was detected on standing for few months even at room temperature, resulting in the complex mixture concomitant with acetic acid. By our previous SAR studies on **1**, structural requirements essential for the activity have been revealed to some extent.[24] In this Letter we describe the results of our further SAR studies on **1**, which enabled us to develop more stable and potent inhibitor (see Fig. 1).

On the basis of our previous SAR studies,[24] two ester groups were proved essential for the activity, and reduction of the vinyl group resulted in the significant loss of the activity. Furthermore, chirality of **1** was found not to be influential to the activity. Thus, the authors designed, as a more stable alternative to **1**, a series of benzhydrol analogue (**2**), in which the vinyl group in **1** was substituted by the phenyl group.

All benzhydrol analogues were synthesized from a common intermediate **3**, which was readily prepared from commercially available 4-hydroxybenzophenone **4** according to Scheme 1. Protection of the hydroxyl of **4** by *tert*-butyldimethylsilyl chloride gave the corresponding silyl ether **5**, which on reduction with sodium borohydride afforded a racemic mixture of benzhydrol **6**.

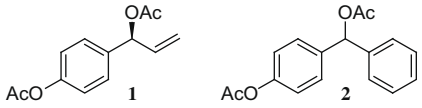

Figure 1. 1′*S*-1′-Acetoxychavicol acetate (**1**) and its phenyl analogue **2**.

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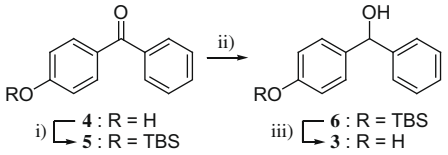
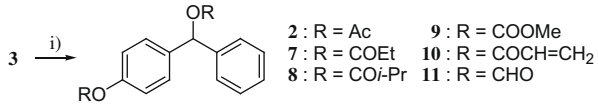


Scheme 1. Reagents and conditions: (i) TBSCl, imidazole, DMF, 0 °C, 1 h, 96%; (ii) $NaBH_4$, THF, 0 °C, 3 h, 60%; (iii) TBAF, THF, 0 °C, 0.5 h, 95%.



Scheme 2. Reagents and conditions: (i) **2**: Ac_2O, Et_3N, DMAP, CH_2Cl_2, 0 °C, 1 h, 97%; **7–9**: EtCOCl, ^iPrCOCl or MeOCOCl, pyridine, 0 °C, 2 h, 90%, 93% and 86%, respectively; **10**: CH_2=CHCOCl, NaH, THF, 0 °C, 0.5 h, 72%; **11**: formic acid, DCC, CH_2Cl_2, acetone, rt, 24 h, 90%.

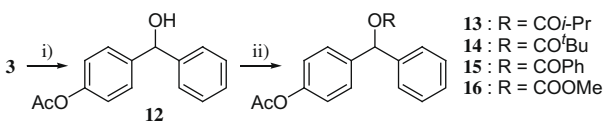
Treatment of **6** with tetrabutylammonium fluoride in THF yielded 4-hydroxybenzhydrol **3** in 55% overall yield from **4**.

Treatment of **3** with acetic anhydride, appropriate acyl chlorides or methyl chlorocarbonate under usual conditions afforded corresponding five esters (**2** and **7–10)** in good yields (Scheme 2). The formate **11** was prepared by condensation reaction of **3** with formic acid activated by dicyclohexylcarbodiimide[25] in 90% yield.

Selective acetylation of the phenolic hydroxyl of **3** leading to the monoacetate **12** was accomplished with acetic anhydride in the presence of triethylamine and dimethylaminopyridine in 97% yield. Acylation of another hydroxyl by acyl chlorides or methyl chlorocarbonate gave the corresponding 2′-acyloxy or 2′-methoxycarbamate derivatives (**13–16**) in 95%, 93%, 88% and 77% yields, respectively (Scheme 3).

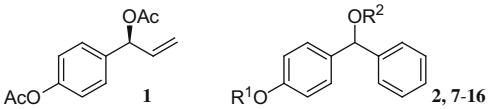
The chemical stability of the most active compound **16** was compared with that of **1**. A solution of 10 mg of racemic mixture of **1** in 0.5 mL $CDCl_3$ had been kept at 50 °C, and the ^1H NMR spectra was taken after 24 h. Complete decomposition of **1** into a complex mixture was observed. In a solution of DMSO-d_6/D_2O (4/1) kept at 40 °C, ca. 50% of compound **1** decomposed in 48 h giving a complex mixture concomitant with acetic acid. On the other hand, no change was detected by ^1H NMR spectroscopic studies on **16** conducted at the same conditions as described above for **1**.

The antiallergic activities of benzhydrol derivatives thus synthesized were evaluated by indexing the inhibition of release of β-hexosaminidase from RBL-2H3 cells.[26] The simplest analogue **2**, in which the vinyl group of **1** was substituted by the phenyl group, showed nearly the same inhibitory activity as **1** (IC_{50} 14 μM (Table 1). However, the activity decreased along with the increased bulk of the ester moieties, activities of propanoate (**7**) and isobutanoate (**8**) being reduced to 38 μM and >100 μM, respectively. Propenate (**10**) maintained the activity to some extent, IC_{50} of which being 28 μM. Methylcarboxylate analogue (**9,** $R^1 = R^2 = COOMe$) have nearly equal activity (18 μM) to **1**. Formate analogue **11** ($R^1 = R^2 = CHO$) showed no inhibitory activity (>100 μM). Analogues **12–16** have common phenyl acetate moiety (R^1 = Ac), and were modified only at R^2 group. Removal of the ester group at R^2 (**12**, R^2 = H) caused considerable decrease of its activity (>100 μM). However, analogues **13–15**, which have large R^2 acyl moieties such as CO^iPr, CO^tBu and COPh, gain the stronger inhibitory activity, while **8** showed no activity. Finally acetoxybenzhydrol methylcarboxylate analogue **16** (R^2 = COOMe) was found more active than **1**.



Scheme 3. Reagents and conditions: (i) Ac_2O, Et_3N, DMAP, CH_2Cl_2, 0 °C, 1 h, 97%; (ii) **13–16**: ^iPrCOCl, ^tBuCOCl, PhCOCl or MeOCOCl, Et_3N, DMAP, CH_2Cl_2, 0 °C, 1 h, 95%, 93%, 88% and 77%, respectively.

Table 1
Effects of benzhydrol analogues on the release of β-hexosaminidase, TNF-α and IL-4 from RBL-2H3 cells



	IC_{50} (μM) for release of		β-Hexosaminidase	TNF-α	IL-4
1	1′*S*-1′-acetoxy chavicol acetate		17	17	12
	ketotifen fumalate		158		
	R^1	R^2			
2	Ac	Ac	14		
7	COEt	COEt	38		
8	CO^iPr	CO^iPr	>100		
9	COOMe	COOMe	18		
10	COCH=CH_2	COCH=CH_2	28		
11	CHO	CHO	>100		
12	Ac	H	>100		
13	Ac	CO^iPr	27		
14	Ac	CO^tBu	36		
15	Ac	COPh	17	28	23
16	Ac	COOMe	6.5	11	12

The immediate responses of the Type I allergy are known mainly due to small molecule chemical mediators (e.g., histamine, serotonin) from mast cells. Mast cells also produce cytokines including TNF-α, IL-4, and IL-5, and these cytokines play an important role in the late phase of the allergic reactions.[20–23] In the present study, effects of analogues **15** and **16**, which exhibited strong inhibitory effects against the release of β-hexosaminidase, on production of TNF-α and IL-4 in RBL-2H3 cells 4 h after challenge, were also examined. As shown in Table 1, **15** and **16** inhibited the production of TNF-α and IL-4 as strong as **1** (12–17 μM) with IC_{50} values of 11–28 μM. These findings suggest that these esters **15** and **16** are effective against not only the immediate phase but the late phase of the Type I allergy reactions.

In conclusion, we developed stable and more potent antiallergic agent **16** by the structural modification of natural inhibitor 1′*S*-1′-acetoxychavicol acetate (**1**). The structural requirement of 1′*S*-1′-acetoxychavicol acetate analogues for the inhibitory activity against release of β-hexosaminidase were clarified as follows: (1) Substitution of the vinyl group in **1** to the phenyl group keeps the activity. (2) Phenyl acetate moiety was essential for the activity. (3) As for the ester moiety on 1′ position smaller one was preferred for higher activity. In addition, benzhydrol derivatives **15** and **16** inhibited the antigen-IgE-mediated TNF-α and IL-4 production as strong as **1**, thus these compounds were revealed to exert their antiallergic activity on both the immediate and late phase of the type I allergic reactions.

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26. The RBL-2H3 cells [Cell No. JCRB0023, obtained from Health Science Research Resources Bank (Osaka, Japan)] cultured in 24-well multiplate were sensitized with anti-DNP IgE antibody. Test sample solution was added to the each well 10 min prior to the challenge with DNP-BSA. The release of β-hexosaminidase into the medium was determined 10 min after the challenge, and the releases of TNF-α and IL-4 were measured 4 h after. Details of bioassay methods of inhibition effects on the release of β-hexosaminidase, TNF-α and IL-4 from RBL-2H3 cells were described in Ref. 23.