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Synthesis of 6-*epi*-tuberiferin and the biological activities of tuberiferin, dehydrobrabrachylaenolide, 6-*epi*-tuberiferin, and their synthetic intermediates

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ABSTRACT

Tuberiferin, 6-*epi*-tuberifelin, dehydrobrachylaenolide and two series of eudesmanolides, eudesmane-12,6 α-lactones and eudesmane-12,6β-lactones, were synthesized for the studies of the structure–activity relationships to explore novel anti-inflammatory, anti-cancer and crop disease prevention agents. The anti-inflammatory activities were tested by the inhibitory on the induction of inter-cellular adhesion molecule (ICAM-1), the permeation of leucocyte into inflammatory air pouch of murine, the killing function of cytotoxic *T*-lymphocytes (CTL), production of IL-1; The anti-cancer activities were established on the cytotoxic activities to six kinds of cell lines (P388, CCRF-CEM, VA-13, HepG2, QG-56, and WI-38). Results showed that Dehydrobrachylaenolide (an *exo*-endo cross conjugated dienone and α-methylene γ-lactone) was the most effective compound inhibiting ICAM-1 (IC_{50} 3.0 μM) and the cell line VA-13 (IC_{50} 0.45 μM); Compound **20** with an α-bromo-ketone moiety embraced the most potent inhibitory activity towards the permeation of leucocyte into inflammatory air pouches of murine in vivo (inhibitory ratio 54% at 10 mg); Compound **25** with an α-bromo-ketone and α-methylene *trans*-γ-lactone) showed the most significant inhibitory activity on the killing function of CTL (IC_{50} 18 μM), as well as the cell lines of CCRF-CEM (IC_{50} 1.1 μM) and P388 (IC_{50} 1.21 μM) ; Tuberiferin (an α,*β*-unsaturated ketone and α-methylene γ-lactone) was on the top effective inhibitory on the production of IL-1; Compounds **19** with an α-bromo-ketone and α-methylene *cis*-γ-lactone exhibited the most potent inhibitory of QG-56 (IC_{50} 12.5 μM); Compound **29** with an α-bromo-α,β-unsaturated ketone and α-methylene γ-lactone) showed significant inhibitory for HepG2 (IC_{50} 1.23 μM) , though potently inhibited WI 38 (IC_{50} 0.31 μM) as well. A conclusion may be reached that the α-Methylene γ-lactone moiety, *exo*-endo cross conjugated dienone moiety, and α-bromo-ketone withα-methylene moiety might be essential for eudesmanolides in the expression of their anti-inflammatory, anti-tumour biological activities. Similarly, the above mentioned key moieties are also responsible for the preventive activity of crop disease controlling.

1. Introduction

Inflammation plays an important role in host defence, it is one of the common events in the majority of acute as well as chronic debilitating diseases and represent a chief cause of morbidity in today's era of modern lifestyle [1].It is the body's response to tissue damage, caused by physical injury, ischemic injury, infection, exposure to toxins, or other types of trauma [2]. The body's inflammatory response causes cellular changes and immune responses that result in repair of the damaged tissue and cellular proliferation at the site of the injured tissue. When these inflammatory responses become chronic, it has been found to mediate a wide variety of diseases, including cardiovascular diseases, cancer, diabetes, arthritis, Alzheimer's disease, pulmonary diseases, and autoimmune diseases [3]. Cell mutation and proliferation can result, often creating an environment that is conducive to the development of cancer. It is only during the past decade that clear evidence has been obtained that inflammation plays a critical role in tumorigenesis [4].The lack of specific therapeutic agents has impaired effective treatment for

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these inflammatory conditions.Therefore, treating the inflammatory causes is always essential, and finding a safe and effective treating approach to control inflammation has been a challenge. In the past few years, plant-derived molecules known as phytochemicals or phytoconstituents or natural products appeared to be a major source of drugs and evaluated as a drug candidate for anti-inflammatory actions [1].

Sesquiterpenene lactones with an α-methylene γ-lactone moiety fused on various skeletons are a rapidly expanding group of natural products, comprising to date>900 varieties [5]. Some of them has been shown to possess considerable biological activities including allergenic agents, cytotoxic and anti-tumour agents, and preventive activity in controlling crop diseases [6]. However, the research on the activities of eudesmanolide type sesquiterpene lactones was few reported. Due to the scarce natural resource of these biological active sesquiterpene lactones, and with the interests in finding novel promising phytotherapeutics anti-inflammation and anti-cancer agents, as well as crop diseases preventive agents, synthesis of these biological compounds are designed and carried out in the present study. Although the synthesis of a naturally occurring eudesmanolide, (+)-tuberiferin (**1**) possessing *trans*-fused α-methylene γ-lactone, has already been reported [5,7,8], the studies of biological activities of **1** and its synthetic intermediates were limited, such as their cytotoxic activity against murine lymphocytic leukemia (P388), plant growth inhibitory activities, and preventive activities of some crop diseases [5]. In the current study, the synthesis of 6-*epi*-tuberiferin (**2**) possessing *cis*-fused α-methylene γ-lactone, and improved synthesis of (+)-tuberiferin(**1**), dehydrobrachyaenolide (**3**)(Fig. 1), and their related compounds were designed and developed for detailed study of their biological activities, as well as the structure–activity relationship.

The immunomodulatory and anti-inflammatory agents screening were based on inhibitory activity on the production of IL-1, inhibitory activity against induction of intercellular adhesion molecule-1 (ICAM-1), and inhibitory activity against the killing function of cytotoxic T lymphocyte (CTL), in *vitro*, respectively, as well as inhibitory activities against the permeation of leucocyte into inflammatory air pouch of murine in *vivo*. Furthermore, the possibility of these synthetic compounds as anti-tumour agents was carried out by screening of the cytotoxic activity of compounds towards six kinds of cell lines (P388, CRF-CEM, VA-13, HepG2, QG-56, and WI-38). Also, the preventive or curative activities of the compounds in controlling crop disease were tested by pots test.

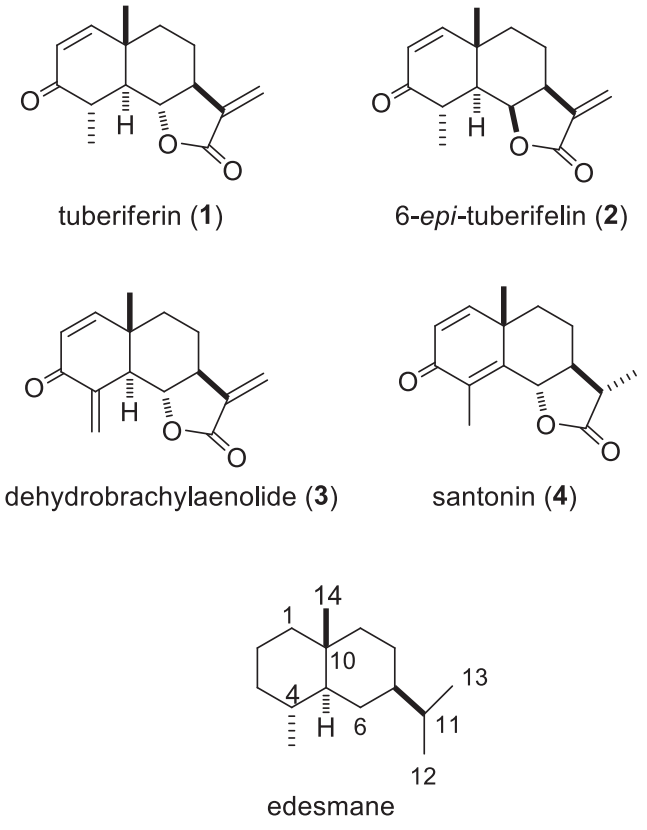


Fig. 1. Chemical structures of edesmane, santonin and the target compounds (**1, 2, 3**).

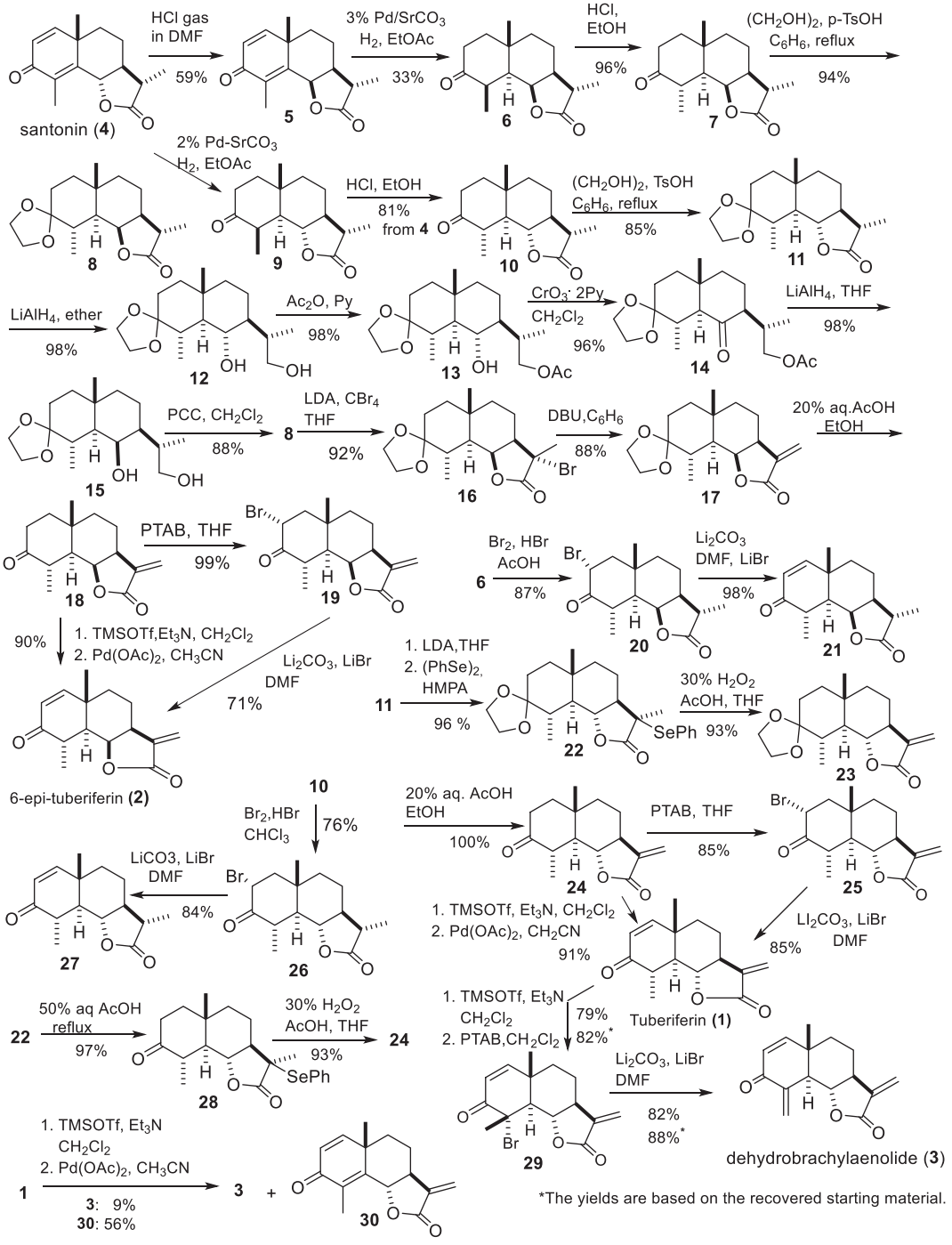
2. Results and discussion

2.1. Synthesis of 6-epi-tuberiferin (2) possessing cis-fused methylene-lactone, and improved synthesis of 1, dehydrobrachyaenolide (3), and their related compounds

The starting material of **2** is commercially available *l*-α-santonin (**4**) (Fig. 1). Epimerization of **4** with 5 wt% HCl gas in anhydrous DMF gave 6-*epi*-santonin (**5**) in 59% yield [9]. By products of this reaction were phenolic compounds produced by dienon-phenol rearrangement [9]. Catalytic hydrogenation of **5** over 3% palladium on strontium carbonate gave **6** in 33% yield. The poor yield of **6** was induced by the hydrogenolysis of β (ax) γ- lactone oxygen at C-6. Epimerization of the resulting **6** possessing β(ax)-methyl group at C-4 by 2 M hydrochloric acid (HCl) in ethanol (EtOH) gave **7** in 96% yield. Acetalization of the carbonyl group at C-3 under standard condition gave acetal **8** in 94% yield (Scheme 1). The overall yield of **8** was poor because of the low yields of **5** and **6**. And compound **8** was also prepared from **4** by a different procedure (Scheme 1). Catalytic hydrogenation of **4** over 2% palladium on strontium carbonate (Pd-$SrCO_3$) in ethyl acetate (EtOAc) and successive epimerization of resulting crude product of **9** by 2 M HCl in EtOH gave **10** in 81% overall yield from **4**. Pure **9** was obtained by the recrystallization of crude **9** from EtOH in 69% yield. Acetalization of the carbonyl group of **10** under standard conditions gave **11** in 85% yield. Reduction of lactone **11** with lithium aluminium hydride ($LiAlH_4$) gave diol **12** in 98% yield. Selective acetylation of the primary hydroxyl group of **12** gave acetate **13** in 98% yield. Oxidation of 6α-hydroxyl group of **13** by Collins reagent (CrO_3Py in CH_2Cl_2) and successive reduction of 6-carbonyl group of **14** with $LiAlH_4$ gave **15** in 94% overall yield. Oxidation of **15** with pyridinium chlorochromate (PCC) gave desired *cis*-lactone **8** in 88% yield. The overall yield of **8** by the eight steps conversion from **4** was 55% (Scheme 1).

Enolization of **8** with lithium diisopropylamide (LDA) and successive bromination of resulting enolate with carbon tetrabromide (CBr_4)[6] gave the bromide **16** in 92% yield. Dehydrobromination [10] of **16** with DBU in benzene at refluxing temperature gave an α-methylene γ-lactone **17** in 88% yield. Deacetalization of **17** was achieved by treatment with 20% aqueous acetic acid (aq AcOH) in ethanol (EtOH) at 85 °C for 4 h to give **18** in a quantitative yield. The selective bromination of **18** at C-2 with phenyltrimethylammonium perbromide (PTAB) in tetrahydrofuran (THF) gave α-bromo ketone **19** in 99% yield. Dehydrobromination of **19** with lithium carbonate (Li_2CO_3) and lithium bromide (LiBr) in N,*N*-dimethyl formamid (DMF) at 125 °C for 90 min gave **2** in 71% yield. Another synthesis of **2** was achieved from **18** in high yield(90%). Enolsilylation of **18** with trimethylsilyl trifluoromethanesulfonate (TMSOTf) in the presence of triethylamine (Et_3N) in CH_2Cl_2 gave corresponding silyl enol ether. Treatment of the silyl enol ether with palladium acetate [$Pd(OAc)_2$] in acetonitrile (CH_3CN) gave **2** in 90% overall yield (Scheme 1).

Reference compounds, α-methyl γ-lactone **20** and **21,** for the comparison of their bioactivity with the corresponding α-methylene γ-lactone **19** and **2,** were prepared by the following procedure. Bromination of **6** with bromine (Br_2) in the presence of hydrobromic acid (HBr) in AcOH gave 2α-bromoketone **20** in 87% yield. Dehydrobromination of **20** with Li_2CO_3 in DMF gave α,β-unsaturated ketone **21** in 98% yield (Scheme 1). Phenylselenenylation of **11** by Grieco's method [11] gave the corresponding phenyl selenide **22** in 96% yield. Treatment of



Scheme 1. The synthetic routes of tuberiferin, 6-*epi*-tuberiferin, dehydrobrachyaenolide, and their related compounds starting from commercially available material santonin.

22 with 30% hydrogen peroxide (H_2O_2) in THF in the presence of AcOH gave α-methylene γ-lactone **23** in 93% yield. Deacetalization of **23** was achieved by treatment with 20% aq AcOH in EtOH at 85 °C for 4 h to give **24** in a quantitative yield. Compound **24** was also prepared by a different procedure. Deacetalization of **22** in boiling 50% aq AcOH gave selenide **28** in 97% yield. Treatment of **28** with 30% H_2O_2 in THF in the presence of AcOH gave **24** in 93% yield. The selective bromination at the C-2 position of **24** with PTAB in THF at –6 °C for 1 h gave α-bromo ketone **25** in 85% yield. Dehydrobromination of **25** with Li_2CO_3 and LiBr in DMF at 123–131 °C for 90 min gave **1** in 85% yield. Compound **1** was also obtained in 87% overall yield from **24** without purification of the crude product of **25**. Another new procedure of synthesis of **1** from **24** was achieved in higher yield. Enolsilylation of **24** with TMSOTf in the presence of Et_3N in CH_2Cl_2 gave the corresponding silyl enol ether. Treatment of this silyl enol ether with $Pd(OAc)_2$ in CH_3CN afforded **1** in 91% yield.

Reference compounds, α-methyl γ-lactones **26** and **27,** for the comparison of their biological activity with the corresponding α-methylene γ-lactones **25** and **1,** were prepared by the following known procedure [5]. Bromination of **10** with Br_2 in the presence of HBr in $CHCl_3$ gave bromo ketone **26** in 76% yield. Dehydrobromination of **26** with LiBr, Li_2CO_3 in DMF gave α,β-unsaturated ketone **27** in 84% yield.

Finally, from the interest in the influence of cross conjugated *exo*-endo dienone moiety to the biological activities of the compound, we

synthesized dehydrobrachylaenolide (**3**) from tuberiferin (**1**). Enolsilylation of **1** with TMSOTf and Et_3N in CH_2Cl_2 and successive treatment of the resulting silyl enol ether with PTAB gave bromide **29** in 79% yield. Dehydrobromination of **29** with Li_2CO_3 and LiBr in DMF afforded **3** in 82% yield. On the contrary, treatment of the silyl enol ether with Pd $(OAc)_2$ gave **3** and **30** in 9% and 56% yields, respectively. The physical constants and spectral data of **3** were in good agreement with those of natural dehydrobrachylaenolide (**3**) in the literature [12] (Scheme 1).

2.2. Immuno-modulating and Anti-inflammatory activities of compounds

2.2.1. Anti-inflammatory Activities of Compounds Based on Inhibitory Activity on Expression of Intercellular Adhesion Molecule (ICAM-1) Induced by Interleukin-1 (IL-1)

Expression of intercellular adhesion molecule-1 (ICAM-1) is induced by interleukin-1 (IL-1) on the surface of endothelial cells of blood vessels. ICAM-1 on the activated endothelial cells interacts with lymphocyte function-associated antigen-1 (LFA-1) on leucocytes in the blood stream, and the leucocytes begin rolling, adhere to the surface of endothelium, and finally migrate from the inside of the blood vessel to the inflammatory portion by chemotaxis. The attack of leucocyte causes serious damage to the inflammatory tissue. Expression of excess amount of ICAM-1 on the surface of endothelial cells of blood vessel plays an important role in the progress on inflammatory reaction [13]. These facts suggest that the inhibitors of induction of ICAM-1 may turn out to yield a new type of anti-inflammatory agent.

In the present study, two series of eudesmanolides, eudesmane-12,6α-lactones (*trans*-lactones, **1**, **3**, **10**, **11**, **22**–**27**) and eudesmane-12,6β-lactones (*cis*-lactones, **2**, **7**, **8**, **16**–**21**), were tested for the inhibitory activity of induction of ICAM-1.

As shown in Table 1, all compounds possessing α-methylene γ-lactone moiety (**1**, **3**, and **23**–**25** in *trans*-lactones and **2**, and **17**–**18** in *cis*-lactones) showed significant inhibitory activity on the induction of ICAM-1. In α-methyl γ-lactones, α-bromoketons **26** with *trans*-lactone structure and **20** with *cis*-lactone structure showed significant activity. α,β-Unsaturated ketones **27** and **21** with *trans*- and *cis*-α-methyl γ-lactone ring showed moderate and weak activity, respectively. Other *trans*-α-methyl γ-lactones, **10**, **11**, and **22** and *cis*-α-methyl γ-lactones, **7**, **8**, and **16** showed no activity. The order of the inhibitory activity of eudesm-12,6α-lactones (*trans*-lactones) on induction of ICAM-1 was **3** > **25** > **1** > **26** > **23** > **24** > **27**≫>>≫**10**, **11**, **22,** and the order of the inhibitory activity of 3-oxoeudesm-12,6β-lactones (*cis*-lactones) on induction of ICAM-1 was **19** > **2** > **18** > **17** > **20** > **21**>>>**7**, **8**, **16.** The most active compound was *trans*-α-methylene γ-lactone with *exo*-end cross conjugated carbonyl moiety (**3**, IC50 3.0 μM) followed by *trans*-α-methylene γ-lactone with α-bromoketone moiety (**25**, IC50 5.2 μM) and it's *cis*- counterpart (19, IC50 6.1 μM), *trans*-α-methylene γ-lactone with α,β-unsatrated carbonyl moiety (**1**, IC50 7.1 μM) and it's *cis*-counterpart (**2**, IC50 7.3 μM). In α-methylene γ-lactones, change of α,β-unsaturated carbonyl moiety of **1** to *exo*-end cross conjugated carbonyl moiety of **3,** and change of 3-carbonyl moiety of **24** to 2α-bromo-3-carbonyl moiety of **25** showed 2.5 and 3.3 folds stronger activity, respectively. In *cis*-α-methylene γ-lactones, change of 3-carbonyl moiety of **18** to α-bromo-3-carbonyl moiety of **19,** and change of α,β-unsatrated 3-carbonyl moiety of **2** to α-bromo-3-carbonyl moiety of **19** showed 3 and 1.2 fold stronger activity, respectively. Change of α-methyl γ-lactone moiety of **20** to α-methylene γ-lactone moiety of **19,** and Change of α-methyl γ-lactone moiety of **27** to α-methylene γ-lactone moiety of **1** showed 3.6 and 7.6 fold stronger activity, respectively.

Therefore, a conclusion could be reached that an α-methylene γ-lactone moiety in the molecule is essential for the activity, moreover, α-bromo-3-carbonyl moiety, *exo*-end cross conjugated carbonyl moiety, and α,β-unsturated carbonyl moiety are responsible for the increment of the activity of corresponding α-methylene γ-lactones (see Table 1). In addition, the activities of eudesmane-12,6α-lactones (*trans*-lactones) are higher than those of the corresponding eudesmane-12,6β-lactones (*cis*-lactones).

The transcription of the ICAM-1 gene induced by IL-1 is largely depended on the transcription factor NF-κB. Upon IL-1 stimulation, NF-κB translocates from the cytoplasm into the nucleus and activates variety of target genes. As reported previously [12,14], *trans*-α-methyl γ-lactone with α-bromoketone moiety (**26**) and the compounds possessing an α-methylene γ-lactone moiety control the signaling pathway upstream of the nuclear transcription of NF-κB, which gives a hint of the potential mechanism of the above tested active compounds, and further research is under consideration.

2.2.2. Inhibitory activity of the test samples against the permeation of leucocyte into inflammatory air pouch of murine in vivo

The leucocytes migrate from the inside of the blood vessel to the inflammatory portion by chemotaxis in the final stage of inflammatory reaction. Since the attack of leucocyte causes serious damage to the inflammatory tissue in this stage, the compounds possessing the inhibitory activity of permeation of leucocyte may be anti-inflammatory agents [15]. Thus, we examined the assay of anti-inflammatory activity of compounds using inflammatory air pouch of murine in vivo. As

Table 1
Inhibitory Activity on Induction of ICAM-1 of trans- and cis-lactones[a]

Compounds	IC_{50} (μM)	SDEV (%)	Compounds	IC_{50}(μM)	SDEV(%)
trans-α-methyl γ-lactone			*cis*-α-methyl γ-lactone		
10	>1000		**7**	>1000	
11	>316		**8**	>1000	
22	>1000		**16**	>1000	
26	11.1	1.9	**20**	22.5	1.2
27	59.0	4.8	**21**	164.7	2.2
***trans*-α-methylene γ-lactone**			***cis*-α-methylene γ-lactone**		
23	7.7	4.2	**17**	19.1	0.9
24	4.3	3.4	**18**	18.3	8.4
25	4.1	2.9	**19**	5.4	3.0
3	1.1	4.6	**2**	7.2	2.4
1	4.8	2.7			

[a] A549 cells (3 × 104 cells/well) were pretreated with various concentrations of compounds for 1 h and then incubated in the presence of IL-1β for 6 h. Absorbance of 415 nm was assayed after treatment of the cells with primary and secondary antibodies and addition of the enzyme substrate as described in the Materials and Methods for Bioassays. The experiments were carried out in triplicate cultures. IC_{50} was caluculated by using the formula in Materials and Method for Bioassay.

Table 2
Inhibitory Activity of Compounds against the Permeation of Leucocyte into Inflammatory Air Pouch in vivo

compound	Additional amounts (mg)	Inhibitory ratio[a] %	
		Quantity of wetability solution (mL)	Number of all leucocyte
26	10	–15.5	35.6
	20	5.4	83.6
27	10	9.1	7.7
20	10	2.5	54
21	10	–7.8	16.4

[a] Inhibitory ratio (%) = (1–*T*/*C*) × 100 where *T* = leucocytes count after wet with compound and carrageenin, C = Leucocytes count after wet with carrageenan.

shown in Table 2, *cis*- and *trans*-lactones 20 and 26 with bromo-ketone structure showed significant inhibitory activity against the permeation of leucocyte into inflammatory air pouch of murine in vivo.

2.2.3. Inhibitory activity of killing function of cytotoxic T Lymphocytes

Cytotoxic T lymphocytes (CTL) plays a vital role in the elimination of virus-infected cells , tumors and graft rejection in the human body. On the other hand, the attack of CTL causes sever damage to the inflammatory tissue; as a result, autoimmune disease are finally induced [16]. It suggests the inhibitors of the killing function of the CTL may turn out to be a new type of immuno-modulating and anti-inflammatory agent. As shown in our previous study [6,15], Dehydrocostus lactone and its related guaiane type α-methylene γ-lactones exerted efficient inhibitory activity toward the killing function of CTL, which prompted us to examine the structure–activity relationships of tuberiferin (**1**), 6-*epi*-tuberifelin (**2**), and their 16 kinds of synthetic intermediates**.** The compounds were screened for inhibitors of cytotoxic activity of CTL clone OE4.

Two series of eudesmanolides, namely, eudesmane-12,6α-lactones (*trans*-lactones, **1**, **10, 11**, **22**–**27**) and eudesmane-12,6β-lactones (*cis*-lactones, **2**, **7**, **8**, **16**–**21**) were tested the inhibitory activity of on the killing function of CTL. As shown in Table 3, all compounds possessing an α-methylene γ-lactone moiety, such as **1** and **23**–**25** in *trans*-lactones and **2**, and **17**–**19** in *cis*-lactones, showed significant inhibitory activity on killing function of CTL. α-bromo ketone **26** with α-methyl *trans*-γ-lactone ring showed moderate activity and α,β-unsaturated ketone **27** with α-methyl *trans*-γ-lactone ring showed weak activity. Other α-methyl *trans*-γ-lactones, **10**, **11**, and **22** and α-methyl *cis*-γ-lactones, **7**, **8**, and **16** showed no activity. The order of the inhibitory activity of 3-oxoeudesm-12,6α-lactones (*trans*-lactones) on killing function of CTL was **25** > **1** > **23** > **24** > **26** > **27**≫>>≫**10**, **11**, **22** and that of 3-oxoeudesm-12,6β-lactones (*cis*-lactones) was **19** > **2, 17** > **18**>>**21**>>>**7**, **8**, **16.** The most active compounds was *trans*-α-methylene γ-lactone with α-bromo-carbonyl moiety (**25**, IC_{50} 18 μM), followed by it's *cis*- counterpart(**19**, IC_{50} 20 μM), and *trans*-α-Methylene γ-lactone with α,β-unsaturated carbonyl moiety (**1,** IC_{50} 23 μM). In *trans*-α-methylene γ-lactones, change of 3-carbonyl moiety of **24** to 2α-bromo-3-carbonyl moiety of **25** and change of 3-carbonyl moiety of **24** to α,β-unsaturated-carbonyl moiety of **1,** the activity was increased by 0.5 and 0.2 folds, respectively; In *cis*-α-methylene γ-lactones, change of 3-carbonyl moiety of **18** to α-bromo-3-carbonyl moiety of **19** and change of α,β-unsatrated 3-carbonyl moiety of **2** to α-bromo-3-carbonyl moiety of **19** showed 2.5 and 2.3 folds stronger activity, respectively. In addition, Change of α-methyl γ-lactone moiety of **27** to α-methylene γ-lactone moiety of **1** and change of α-methyl γ-lactone moiety of **21** to α-methylene γ-lactone moiety of **2** showed 15 and 7.6 fold stronger activity, resoectively. Hence, a conclusion could be reached that α-methylene γ-lactone moiety in the molecule must be essential for the inhibitory activity; and 2α-bromo-3-carbonyl moiety and α,β-unsturated carbonyl moiety play a key role in the increment of the activity of corresponding α-methylene γ-lactones.

2.2.4. Inhibitory activity on production of IL-1.

IL-1 is a potent enhancer of immune responses but also very potent inducers of acute- phase responses and inflammation [17]. IL-1 has also played a role in the biology of cancer cells and solid tumors. The biologic effects and function of IL-1 involve synthetic and local effects that have influence immunologic properties including *T*-cell and leucocyte [18]. The attack of IL-1 and leucocyte to own tissue causes serious damage to the inflammatory tissue and autoimmune disease are finally induced. Thus, the compounds with inhibitory activity on over-production of IL-1 may be useful agents for allergenic diseases. As shown in Table 4, Tuberiferin (**1**) showed the highest inhibitory activity and other compounds **2**, **17**–**19**, **25**, **27** with α-methylene γ-lactone structure, showed

Table 4
Inhibitory Activity of Compounds on Production of IL-1

Compound	IC_{50}(μM)	SDEV(%)
***Trans*-lactone**		
4	NE[a]	
27	10.6	4.7
25	2.29	2.8
1	0.75	3.1
***Cis*-lactone**		
17	4.22	1.2
18	7.79	2.3
19	1.60	6.6
2	2.97	6.2

[a] Not effective.

Table 3
Inhibitory Activity on Killing Function of CTL of trans- and *cis*-lactones[a]

Compounds	IC_{50} (μM)	SDEV (%)	Compound	IC_{50} (μM)	SDEV (%)
trans-α-methyl γ-lactone			*cis*-α-methyl γ-lactone		
10	724	2.7	**7**	>1000	
11	>1000		**8**	>1000	
22	>1000		**16**	>1000	
26	55	2.5	**20**	>1000	
27	165	2.4	**21**	630	6.9
***trans*-α-methylene γ-lactone**			***cis*-α-methylene γ-lactone**		
23	23	4.8	**17**	46	3.9
24	28.9	1.7	**18**	53	1
25	18	8.7	**19**	29	3.7
1	21	3.6	**2**	46	3.9

[a] To examine the dose response of the compounds, OE4 cells (1×10^4 cells/well) were preincubated with various concentrations of the compounds for 2 h, mixed with P815 (1×103 cells/well) labeled with thymidine, and incubated for 4 h. The experiments were carried out in triplicate cultures. IC_{50} was deduced from the 50% inhibition of the specific release, which was calculated by using the formula descrived in materials and Methods for Bioassay.

Table 5
Cell Growth Inhibitory Activity of trans- and *cis*-Lactone[a,b]

Compound	WI-38		VA-13			HepG2			QG-56			CCRF-CEM			P388	
	IC_{50} (μM)	SDEV (%)	IC_{50} (μM)	SDEV (%)	$\frac{WI-38}{VA-13}$ (fold)[c]	IC_{50} (μM)	SDEV (%)	$\frac{WI-38}{HepG2}$ (fold)[c]	IC_{50} (μM)	SDEV (%)	$\frac{WI-38}{QG-56}$ (fold)[c]	IC_{50} (μM)	SDEV (%)	$\frac{WI-38}{CCRF-CEM}$ (fold)[c]	IC_{50} (μM)	SDEV (%)
trans-lactone																
26	19.8	3.3	186	2.2	0.12	27.1	3.0	0.73	19.1	0.77	1.04	11.1	2.0	1.78	15.8	2.1
23	22.5	2.0	21.2	1.2	1.06	12.7	1.7	1.77	59.5	4.7	0.37	14.2	0.5	1.58	12.4	0.3
24	16.6	2.4	19.4	2.1	0.85	21.4	3.3	0.78	51.4	2.3	0.32	12.4	0.3	1.34	14.0	3.8
25	1.78	7.0	2.1	3.2	0.85	8.8	5.3	0.2	13.7	1.8	0.13	1.1	4.6	1.62	1.06	3.4
1	6.11	6.1	2.8	7.8	2.18	12.4	0.3	0.49	15.8	0.3	0.39	1.8	3.8	3.39	1.50	2.6
3	2.29	2.6	0.45	2.1	5.09	2.5	1.2	0.96								
29	0.33	1.1	0.79	1.6	0.42	1.33	3.0	0.25								
cis-lactone																
20									55.4	2.35		12.4	0.69		12.7	1.7
17	19.1	0.87	22.5	2.0	0.84	20.7	3.7	0.92	64.3	2.2	0.3	17.8	2.5	1.07	18.3	9.3
18									54.9	2.1		15.3	2.5		17.8	5.2
19	14.9	4.7	17.8	4.8	0.83	13.4	4.2	1.11	12.4	0.32	1.2	1.6	3.0	9.3	1.70	3.4
2	7.2	2.4	2.8	7.9	2.57	22.5	2.0	0.32	18.3	8.6	0.39	2.1	2.4	3.42	1.70	3.0
control adoriamycin	0.78	1.4	0.45	2.1	1.73	1.06	3.4	0.74	0.23	2.1	3.39	0.36	3.2	2.17	0.33	1.3

[a] QG-56: QG-56 human lung carcinoma cells, CCRF-CEM: CCRF-CEM human lymphocytic leukemia cells,P388: P388 murine lymphocytic leukemia cells, WI-38: WI-38 human fibroblast lung cells as the model of human normal cells, VA-13: VA-13 malignant lung tumor cells induced fromWI-38 by infection of SV-40 virus, HepG2: HepG2 human hepatocellular carcinoma cells. [b]IC_{50} represent of triplicate determination. [c]WI-38/ cell line name (fold): the times of the IC50 value for WI-38 to the IC50 value for each kind of cell line, the amount of the fold > 1 means the cytotoxicity to each cancer cell is greater than the cytotoxicity to normal cells, which indicates this compound is the ideal compound. Otherwise is denoted as an undesirable compound.

significant inhibitory activity on production of IL-1 (Table 4). Therefore, α-methylene γ-lactone structure in the molecule was the key for the inhibitory activity; and 2α-bromo-3-carbonyl moiety and α,β-unsturated carbonyl moiety play a vital role in the increment of the activity of corresponding α-methylene γ-lactones.

2.3. Cell growth inhibitory activity of the test samples to QG-56, CCRF-CEM, P388, WI-38, VA-13, and HepG2 cells in vitro

Two series of eudesmanolides, eudesmane-12,6α-lactones (*trans*-lactones, **1**, **3**, **9**–**11**, **22**–**27**, **29**) and eudesmane-12,6β-lactones (*cis*-lactones, **2**, **6**–**8**, **16**–**21**), were tested the cell growth inhibitory activities against six cell lines, results were shown in Table 5. Those compounds with a value of WI-38/name of a cell line(fold) > 1 are with excellent safety and are within the expectation of becoming candidate leading drugs. Otherwise, they are excluded due to low security.

As we can see from Table 5, almost all compounds exhibited moderate to significant inhibitory activity towards CCRF-CEM, as well as the P388 cell line, and with excellent safety. Among those compounds, trans- α-bromoketone with α-methylene γ-lactone(**25**, IC_{50}, 1.1 μM)) and cis- α-bromoketone with α-methylene γ-lactone(**19**, IC_{50}, 1.6 μM)) showed top inhibitory effect, followed by trans- α,β-unsaturated ketone with α-methylene γ-lactone moiety **(1,** IC_{50}, 1.8 μM**),** and cis-α,β-unsaturated ketone with α-methylene γ-lactone moiety (**2**, IC_{50}, 2.1 μM). Besides, trans- α,β-unsaturated ketone with α-methylene γ-lactone moiety (**3**, IC_{50}, 0.45 μM) exhibited most strongest inhibition against VA-13, followed by followed by trans- α,β-unsaturated ketone with α-methylene γ-lactone moiety **(1,** IC_{50}, 2.8 μM**),** and cis- α,β-unsaturated ketone with α-methylene γ-lactone moiety (**2,** IC_{50}, 2.8 μM). α-bromoketons with α-methylene γ-lactone moiety (**19**, IC_{50}, 12.4 μM) and α-bromoketone with α-methyl γ-lactone moiety (**26,** IC_{50}, 19.1 μM) showed moderate inhibition against QG-56.

Interestingly, trans- α,β-unsaturated ketone with α-methylene γ-lactone moiety (**29**) exhibited most potent inhibition against HepG2 (IC_{50}, 1.33 μM) , but also significantly inhibited WI-38 (IC_{50}, 0.33 μM), which means it is toxic. And the same trend could be found in α-bromoketons (**3**, IC_{50}, 2.5, 2.29 μM) with α-methylene γ-lactone moiety and Exo-endo cross conjugated ketone (**25,** IC_{50}, 8.8, 1.78 μM) with α-methylene γ-lactone; and those exhibited moderate inhibition against WI-38, also showed moderate inhibitory activity towards HepG2, such as α-methylene γ-lactone **23**(IC_{50}, 22.5, 12.7 μM, respectively), and α-Bromoketons with α-methylene γ-lactone moiety(**19**, IC_{50}, 14.9, 13.4 μM).

In conclusion, α-methylene γ-lactone moiety in the molecule are essential for the anti-cancer activity, and α-bromo-3-carbonyl moiety, α,β-unsaturated carbonyl moiety and *exo*-endo cross conjugated carbonyl moiety increase the activity of corresponding α-methylene γ-lactone, significantly, for the inhibitory activity against CCRF-CEM, P388 and VA-13cell lines.

2.4. Control of crop disease

The preventive and curative activities of α-methyl *trans*-γ-lactones **9**–**11**, **26**, **27**, and α-methylene *trans*-γ-lactones, tuberiferin (**1**) and **23**–**25** in controlling crop diseases were examined by pot test. α-Methylene *trans*-γ-lactones **1**, **24**, and **25** showed significant preventive activity in downy mildew of grape and late blight of tomato. α-Methyl *trans*-γ-lactone **10** showed significant preventive activity in leaf rust of wheat and leaf biotech of barley. α-Methyl *trans*-γ-lactone **11** showed moderate preventive activity in sheath blight of rice. α-Methylene *trans*-γ-lactones **23** and **25,** and α-Methyl *trans*-γ-lactones **26** and **27** showed moderate preventive activity in blast of rice, damping off of cucumber, scab of apple and leaf rust of wheat, respectively (see Table 6 in supporting information).

Then, α-methyl *cis*-γ-lactones **5**, **7**, **8**, **16**, α-methylene *cis*-γ-lactones, 6-*epi*-tuberiferin (**2**), **18**, and **21** in controlling crop diseases were

examined by pot test. *epi*-Tuberiferin (**2**) showed excellent preventive activity in leaf rust of wheat and weak preventive activity in glume biotech of wheat. α-methyl *cis*-γ-lactones, **5** and **7**, and α-methylene *cis*-γ-lactones, **18** and **21** showed moderate preventive activity in leaf rust of wheat. α-methylene *cis*-γ-lactones **2** and **18** showed weak preventive activity in glume biotech of wheat (see Table 7 in supporting information).

The pot test procedures of compounds in controlling crop disease are shown in see Table 8 and Table 9 in supporting information.

3. Experimental Section

3.1. General Experimental procedures

All melting points are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded at 500 (200) MHz and at 125 (50) MHz respectively in CDCl3. The assignments of ^{1}H NMR spectra were determined by decoupling and H—H COSY experiments. The assignments of ^{13}C NMR spectra were determined by DEPT, C—H COSY, HMQC, and HMBC experiments. All reactions were under an atmosphere of N2. CHCl3 was dried over CaCl2 and distilled from CaH2. CH2Cl2, benzene, toluene, DMF, pyridine, diisopropylamine, and triethylamine were distilled from CaH2. MeOH was distilled from Mg(OMe)2·THF and ether were distilled from sodium benzophenone ketyl. Water in Ethylene glycol was eliminated as the benzene azeotrope. To describe HPLC conditions, the column, solvent, flow rate (mL/min), and retention time (*t*R min) are designated in this order. The column code are as follows: A, 25- × 0.46-cm i.d. stainless column packed with 10 μm silica gel; B, 25- × 0.8-cm column packed with 10 μm silicagel; C, 15- × 0.46-cm i.d. stainless column packed with 5 μm silicagel; D 25- × 1.0-cm i.d. stainless column packed with 10 μm silica gel. Silica gel (230–400 mesh) was employed for flash chromatography and 70– 230 mesh silica gel was employed for column chromatography. To describe the conditions of column and flash chromatographies, the weight of silicagel, column i.d. and solvent are designated in this order.

Chemistry Section (Detailed please refer to supporting information)

Biological Activity Experimental Section(Detailed please refer supporting information)

4. Conclusion

Tuberiferin, 6-*epi*-tuberifelin, dehydrobrachylaenolide and two series of eudesmanolides, eudesmane-12,6 a-lactones and eudesmane-12,6b-lactones, were successfully synthesized. The structures of the compounds were determined by IR, 1H NMR, 13C NMR, and 2D-NMR. The anti-inflammatory, anti-cancer and crop disease prevention activity were tested, respectively. Moreover, the structure–activity relationship was analyzed. A conclusion could be drawn that the α-methylene γ-lactones moiety, *exo*-endo cross conjugated dienone moiety, and α-bromo-ketone with a-methylene moiety might be essential for eudesmanolides in the expression of their anti-inflammatory, anti-tumour biological activities as well as the preventive activity of crop disease control.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

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