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Original article

Synthesis and innate immunosuppressive effect of 1,2-cyclopentanediol derivatives

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ABSTRACT

Innate immunity is the front line of self-defense against infectious microorganisms. In mammals, innate immunity interacts with adaptive immunity and plays a key role in regulating the immune response. Therefore, innate immunity is a good pharmaceutical target for the development of immune regulators. After searching for natural substances that regulate innate immunity using an *ex vivo Drosophila* culture system, we identified a cyclopentanediol-type compound **1** as an immunosuppressor. In this study, we synthesized and evaluated **1** and its derivatives. Several methylamide- or phenylamide-containing derivatives showed effects that were 20–25 times more potent than those of **1**.

1. Introduction

Innate immunity is the front line of self-defense against infectious microorganisms [1,2], and the basic mechanisms of this process, including pathogen recognition and immune response activation, are evolutionarily conserved [3]. In mammals, innate immunity interacts with adaptive immunity and plays a key role in regulating the immune response [4]. Therefore, innate immunity is a good pharmaceutical target for the development of immune regulators that suppress unwanted immune responses, such the responses that contribute to septic shock, inflammatory diseases, or autoimmunity. For example, eritoran, a lipopolysaccharide (LPS) antagonist [5,6], is in clinical trials for the treatment of severe sepsis.

To screen pharmaceuticals that target innate immunity, we established an *ex vivo* culture system based on the *Drosophila* IMD signaling pathway [7,8]. Because of the striking conservation between the mechanisms that regulate insect immunity and mammalian innate immunity [2,3], *Drosophila* is a model organism for genetic and molecular studies of innate immunity, and our culture system has proven to be useful for identifying immune regulators that act on human innate immunity [7,8]. We used this system to search for natural substances that regulate innate immunity and identified and isolated a cyclopentanediol derivative **1** from *Aspergillus* sp. as an immunosuppressive substance [9]. Compound **1** showed the immunosuppressive effects on the mammalian TNF-α signaling pathway as well as on the *Drosophila* IMD signaling pathway. In this study, we synthesized and evaluated compound **1** and its derivatives to identify more potent compounds.

2. Results and discussion

2.1. Syntheses and evaluation of cyclopentanediol 1 and its derivatives

Cyclopentanediol **1** was synthesized as shown in Scheme 1. In the presence of lithium hydride, diethyl malonate was condensed with *cis*-1,4-dichloro-2-butene to afford the cyclopentene derivative **2** [10]. Dihydroxylation of **2** by osmium tetroxide and subsequent acetonidization produced **3**, which was decarboxylated with the lithium chloride–H_2O–DMSO system to furnish a mixture of *trans*-**4** and *cis*-**4** in a ratio of 1:2. The relative configurations of these compounds were confirmed by an NOE difference experiment (Fig. 1). The primary alcohol **5** was obtained by the reduction of *cis*-**4** using lithium aluminum hydride. Oxidation of **5** by TEMPO

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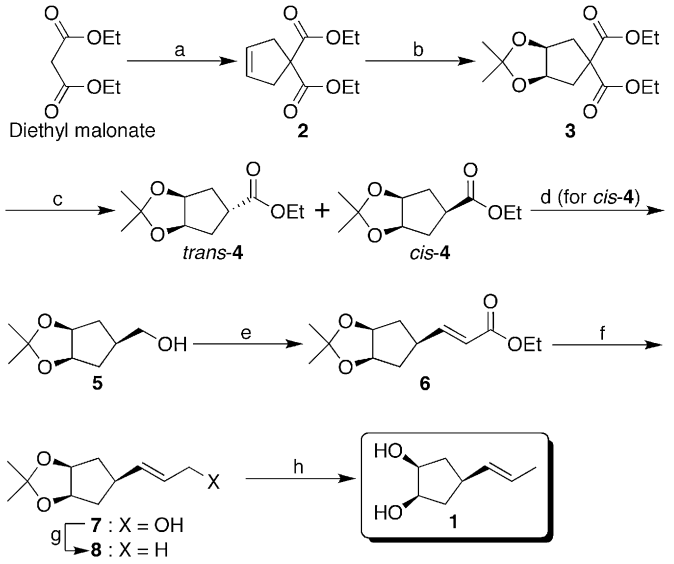


Scheme 1. Synthesis of compound **1**. Reagents and conditions: (a) *cis*-1,4-dichloro-2-butene, LiH, DME, rt; (b) (1) OsO_4, NMO, acetone-acetonitrile-H_2O (1:1:1); (2) 2,2-DMP, *p*TsOH, rt (3 steps, 59%); (c) LiCl, H_2O, DMSO, reflux (18% (for *trans*-**4**) and 39% (for *cis*-**4**)); (d) $LiAlH_4$, THF, 0 °C (71%); (e) (1) TEMPO, NCS, *n*-Bu_4NCl, $NaHCO_3$, K_2CO_3, CH_2Cl_2–H_2O (1:1); (2) $(EtO)_2P(O)CH_2COOEt$, NaH, toluene, rt (64%, 2 steps); (f) DIBAL-H, CH_2Cl_2, 0 °C (89%); (g) SO_3–pyridine, THF, 0 °C, then $LiAlH_4$; (h) 6 M HCl, THF, rt (35%, 2 steps).

gave an aldehyde that was then reacted with ethyl diethylphosphonoacetate to yield the α,β-unsaturated ester **6**, which bears an *E*-olefin. Compound **6** was reduced with DIBAL-H to afford the allyl alcohol **7**. Reaction of **7** with a sulfur trioxide–pyridine complex and reduction of the resulting reaction product with $LiAlH_4$ gave the dehydrated product **8** [11]. Finally, deprotection of an acetonide group in **8** afforded cyclopentanediol **1**.

The use of *trans*-**4** in place of *cis*-**4** in the method used to synthesize **1** led to compound **13**, a stereoisomer of **1** (Scheme 2). Compound **14** was afforded by the catalytic hydrogenation of **1** (Scheme 3). An aldehyde produced by the TEMPO oxidation of **5** was reacted with the pentyl Wittig ylide to yield **15**. Following catalytic hydrogenation of **15**, deprotection of an acetonide group provided **16**, which bears side chain that are longer than the side chain of **1**. An α,β-unsaturated ester derivative **17** was obtained by deacetonidation of **6**.

The immunosuppressive activities of **1**, **13**, **14**, **16**, and **17** on *Drosophila* innate immunity were evaluated (Table 1). Cyclopentanediol **1** showed moderate immunosuppressive activity (EC_{50} 100 μM) but did not show cytotoxicity toward S2 cells. These results indicated that compound **1** selectively suppressed *Drosophila* innate immunity. The activity of **13** was weaker than that of **1**, suggesting that an all-*cis* configuration on the cyclopentane ring was important for activity. The effects of compound **14** were equivalent to those of **1**, indicating that the presence of a double bond in the side chain was not necessary. The longer side chain in

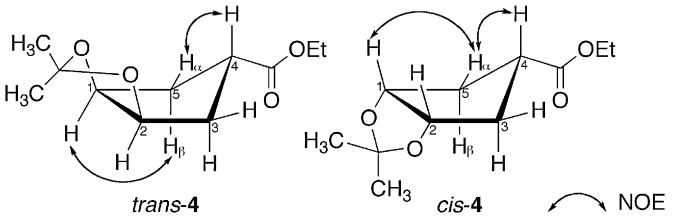
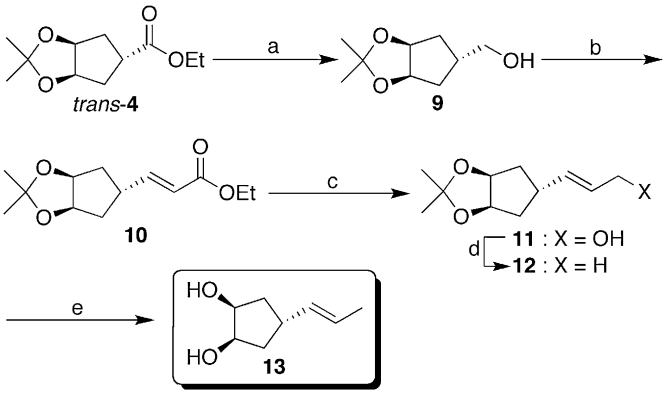


Fig. 1. NOE correlations and stereochemistry of *trans*-**4** and *cis*-**4**.



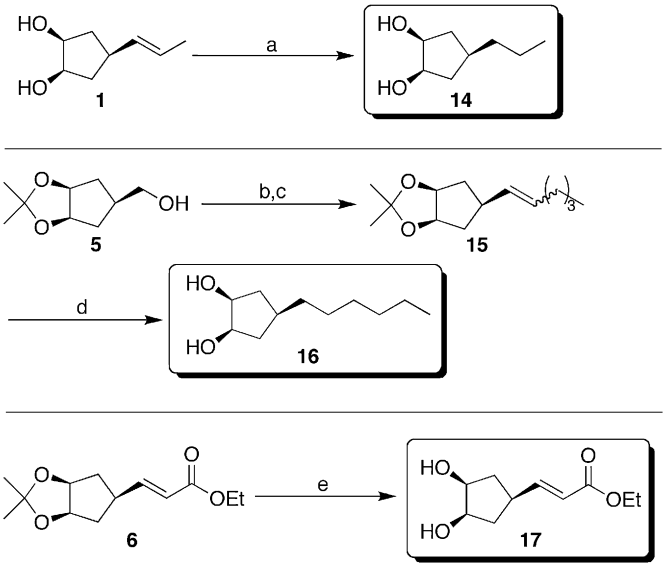
Scheme 2. Synthesis of compound **13**. Reagents and conditions: (a) $LiAlH_4$, THF, 0 °C (89%); (b) (1) TEMPO, NCS, *n*-Bu_4NCl, $NaHCO_3$, K_2CO_3, CH_2Cl_2-H_2O (1:1); (2) $(EtO)_2P(O)CH_2COOEt$, NaH, toluene, rt (65%, 2 steps); (c) DIBAL-H, CH_2Cl_2, 0 °C (84%); (d) SO_3–pyridine, THF, 0 °C, then $LiAlH_4$; (e) 6 M HCl, THF, rt (30%, 2 steps).

16 enhanced only the cytotoxicity. The activity of **17** was equivalent to that of **1**, facilitating the synthesis of additional derivatives because it was easy to alter the α,β-unsaturated ester moiety of **17**.

2.2. α,β-Unsaturated ester and amide derivatives

The α,β-unsaturated ester derivatives were synthesized as shown in Scheme 4. An aldehyde, which was produced by oxidation of **5**, was reacted with Horner–Emmons reagents to afford **18a**–**c**. Deprotection of an acetonide group gave methyl (**19a**), *n*-hexyl (**19b**), and phenyl (**19c**) ester derivatives, respectively.

The α,β-unsaturated carboxylic acid and amide derivatives were also synthesized. *tert*-Butyl ester **22** was obtained in a manner similar to that used to obtain **18a**–**c**. Treatment of **22** with TFA removed the acetonide and *tert*-butyl groups to give the α,β-unsaturated carboxylic acid **23**. After re-acetonication of **23** to afford **24**, condensation with several amines by the mixed anhydride method and subsequent deacetonidation produced the amide (**21c**), methylamide (**21d**), ethylamide (**21e**), *n*-butylamide (**21f**),



Scheme 3. Synthesis of compounds **14**, **16** and **17**. Reagents and conditions: (a) H_2 (ballon), $Pd(OH)_2$/C, MeOH, rt (54%); (b) TEMPO, NCS, *n*-Bu_4NCl, $NaHCO_3$, K_2CO_3, CH_2Cl_2–H_2O (1:1); (c) $Ph_3PC_5H_{11}Br$, KHMDS, THF, −78 °C → rt; (d) H_2 (ballon), Pd$(OH)_2$/C, 10% HCl–MeOH, rt (23%, 3 steps); (e) TFA, CH_2Cl_2, 0 °C (88%).

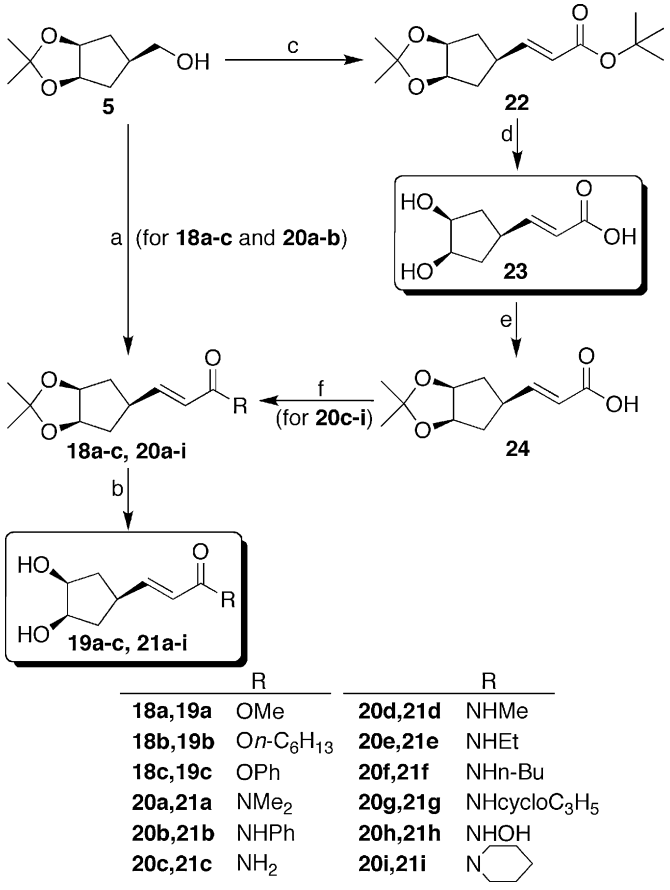
Table 1
The immunosuppressive activity and cytotoxicity of cyclopentanediol **1** and its derivatives.

Compounds	Immunosuppressive effect[a]	Cytotoxicity[b]
	EC_{50} (μM)	EC_{50} (μM)
1	100	>350
13	350	>350
14	100	>350
16	110	110
17	100	>350

[a] On the IMD signaling pathway in *Drosophila* innate immunity.
[b] Against *Drosophila* S2 cells.

cyclopropylamide (**21g**), hydroxyamide (**21h**), and piperidineamide (**21i**). Dimethylamide **21a** and phenylamide **21b** were synthesized from **20a** and **21b**, respectively, which were obtained by Horner–Emmons reaction in a manner similar to that used to obtain the α,β-unsaturated ester derivatives **18a**–**c**.

The immunosuppressive activities and cytotoxicities of these compounds are compiled in Table 2. The ester-type compounds **19a**–**c** and the carboxilic acid **23** showed equivalent or weaker activity than the ethyl ester **17**. Although many amide-type derivatives have shown only moderate activity, the activities of compounds **21b**, **21c**, **21d**, and **21h** were remarkably enhanced (EC_{50} 4–19 μM). In particular, the phenylamide **21b** and



Scheme 4. Synthesis of compounds **19a**–**c**, **21a**–**i** and **23**. Reagents and conditions: (a) (1) TEMPO, NCS, *n*-Bu_4NCl, $NaHCO_3$, K_2CO_3, CH_2Cl_2–H_2O (1:1); (2) corresponding HWE reagents, NaH, toluene, rt (58–76%, 2 steps); (b) TFA, CH_2Cl_2, rt (for **19a**–**c**, 46–75%) or 10% HCl–MeOH, rt (for **21a**–**i**, 51–95%); (c) (1) TEMPO, NCS, *n*-Bu_4NCl, $NaHCO_3$, K_2CO_3, CH_2Cl_2–H_2O (1:1); (2) $(EtO)_2P(O)CH_2COOt$-Bu, NaH, toluene, rt (90%, 2 steps); (d) TFA, CH_2Cl_2, 0 °C (89%); (e) 2,2-DMP, *p*TsOH, rt (75%); (f) *t*-BuOCOCl, NMM, corresponding amines, 0 °C (78–94%).

Table 2
The immunosuppressive activity and cytotoxicity of α,β-unsaturated ester and amide derivatives.

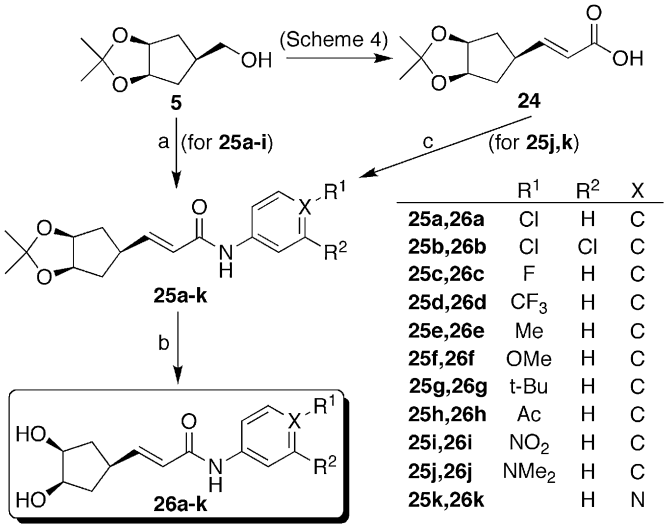
Compounds	Immunosuppressive effect[a]	Cytotoxicity[b]
	EC_{50} (μM)	EC_{50} (μM)
17	100	>350
19a	>350	>350
19b	150	>350
19c	100	>200
23	110	>350
21a	120	>250
21b	4.5	>200
21c	19	>250
21d	5.4	>250
21e	250	>250
21f	>250	>250
21g	240	>250
21h	13	>250
21i	180	>250

[a] On the IMD signaling pathway in *Drosophila* innate immunity.
[b] Against *Drosophila* S2 cells.

methylamide **21d** displayed 20 times stronger activities than the mother compound **1**. We plan to synthesize additional modified compounds based on **21b** and **21d**.

2.3. The derivatives based on phenylamide *21b*

To evaluate the effects of the substituents on the benzene ring, substituted phenylamide derivatives **26a**–**k** were synthesized as shown in Scheme 5. In a manner similar to that employed in the synthesis of **21b**, compounds **25a**–**i** were afforded by Horner–Emmons reaction between an aldehyde produced from **5** and its corresponding phosphonoacetamide. Acid treatment of **25a**–**i** gave the 4-chloro (**26a**), 3,4-dichloro (**26b**), 4-fluoro (**26c**), 4-trifluoromethyl (**26d**), 4-methyl (**26e**), 4-methoxy (**26f**), 4-*tert*-butyl (**26g**), 4-acetyl (**26h**), and 4-nitro (**26i**) derivatives, respectively. Because of the difficulty associated with synthesizing the corresponding phosphonoacetamide, 4-(dimethylamino)phenylamide **26j** and 4-pyridylamide **26k** were produced via carboxylic acid **24**.



Scheme 5. Synthesis of compounds **26a**–**k**. Reagents and conditions: (a) (1) TEMPO, NCS, *n*-Bu_4NCl, $NaHCO_3$, K_2CO_3, CH_2Cl_2–H_2O (1:1); (2) corresponding HWE reagents, NaH, toluene, rt (41–83%, 2 steps); (b) 10% HCl–MeOH, rt (34–92%); (c) *N*,*N*-dimethyl-1,4-phenylenediamine or 4-aminopyridine, MNBA, Et_3N, DMAP, CH_2Cl_2, rt (65% (for **25j**) and 67% (for **25k**)).

Table 3 shows the immunosuppressive activities of the compounds synthesized as described above. The immunosuppressive activities of 4-methoxyphenylamide **26f** and 4-*tert*-butylphenylamide **26g** (EC_{50} 3.1 and 4.4 μM, respectively) were equivalent to the immunosuppressive activity of phenylamide **21b**. Compound **26j** also displayed moderate activity (EC_{50} 19 μM). The other derivatives synthesized showed weak or no activity. Thus, the substituents on the benzene ring greatly affected the immunosuppressive activity, although the relationship between the structures of the substituents and the activity remains uncertain.

2.4. The derivatives based on methylamide *21d*

The ring-opened analog **27** was synthesized as shown in Scheme 6. Oxidative cleavage of the methylamide **21d** by sodium metaperiodate and subsequent reduction by sodium borohydride produced **27**. The saturated side chain-bearing compound **28** was also afforded by catalytic hydrogenation of **21d**.

Scheme 7 shows the synthetic procedure for compounds **32**, **37**, and **40**, which bear shorter or longer side chains and were designed in an effort to optimize the chain length for immunosuppressive activity. Tosylation of **5** and subsequent treatment with sodium cyanide produced compound **30**, which was hydrolyzed to give the carboxylic acid **31**. Condensation with methylamine and deacetonidation afforded **32**, which is a one-carbon shortened derivative of **28**. In a similar manner, the one-carbon lengthened derivative **37** was synthesized from the primary alcohol **33**, which was obtained by reduction and hydrogenation of compound **6**.

To synthesize the two-carbon lengthened derivative **40**, compound **5** was oxidized to the aldehyde, which was then reacted with triethyl 4-phosphonocrotonate to produce the α,β,γ,δ-unsaturated ester **38**. Catalytic hydrogenation of **38** and subsequent hydrolysis afforded the carboxylic acid **39**. Finally, condensation with methylamine and deacetonidation yielded **40**.

Compound **43**, in which the carboxyamide group in **21d** is replaced by a sulfonamide group, was also synthesized (Scheme 8). After oxidation of **5**, Horner–Emmons reaction with ethyl diethylphosphorylmethanesulphonate [12] gave **41**. Conversion of **41** into the tetrabutylammonium salt and chlorination produced the sulfonyl chloride **42** [13]. Treatment of **42** with methylamine afforded a methylsulfonamide, which was deprotected under acidic conditions to yield **43**.

Table 4 shows the immunosuppressive activities of methylamide derivatives. The ring-opened compound **27** showed no activity, suggesting that the cyclopentane moiety was crucial to activity. The moderate activity of **28** indicated that the double bond in the side chain was not crucial, but was important for the immunosuppressive activity. Among compounds **32**, **37**, and **40**, which feature varying chain lengths, only **37** displayed activity equivalent to that of **21d**. This fact implied that the optimal chain length was four carbons. In addition, the sulfonamide **43** was slightly more potent than the carboxyamide **21d**.

Table 3
The immunosuppressive activity and cytotoxicity of phenylamide derivatives.

Compounds	Immunosuppressive effect[a]	Cytotoxicity[b]
	EC_{50} (μM)	EC_{50} (μM)
21b	4.5	>200
26a	>180	>180
26b	56	>160
26c	>180	>180
26d	>160	>160
26e	190	>190
26f	3.1	>180
26g	4.4	>180
26h	>180	>180
26i	>170	>170
26j	19	>170
26k	>200	>200

[a] On the IMD signaling pathway in *Drosophila* innate immunity.
[b] Against *Drosophila* S2 cells.

2.5. Suppressive effect on TNF-α-stimulated production of IL-8 in HUVECs

The *Drosophila* IMD signaling pathway is similar to the mammalian TNF-α signaling pathway [2,14]. The TNF-α signaling pathway plays a critical role in the inflammatory response, in sepsis, and in rheumatoid arthritis by producing costimulatory molecules, cytokines, chemokines, and adhesion molecules, through activation of NF-κB [15]. We investigated the effects of compounds **21b**, **21d**, **26f**, **26g**, **37** and **43**, EC_{50} for *Drosophila* innate immunity of which were less than 10 μM, on TNF-α-stimulated production of IL-8, a neutrophil chemotactic factor, in human umbilical vein endothelial cells (HUVECs). As shown in Fig. 2, the substituted phenylamide derivatives **26f** and **26g** showed toxicity toward HUVECs at 50 μg/mL. In contrast, compounds **21b**, **21d**, **37**, and **43** suppressed the production levels of IL-8 to 70–90% of the control levels without toxicity. Although the effects are not so potent, these cyclopentanediol derivatives were found to suppress both the mammalian TNF-α signaling pathway and *Drosophila* innate immunity, and, therefore, present a new type of immunosuppressive drug or research reagent.

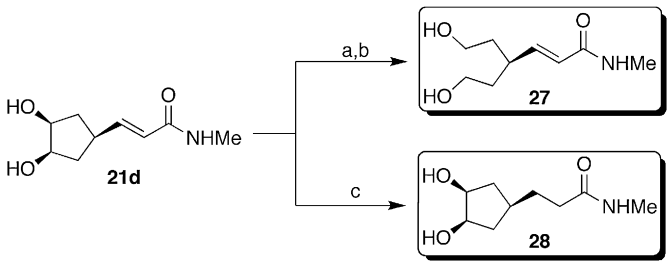
3. Experimental section

3.1. General methods

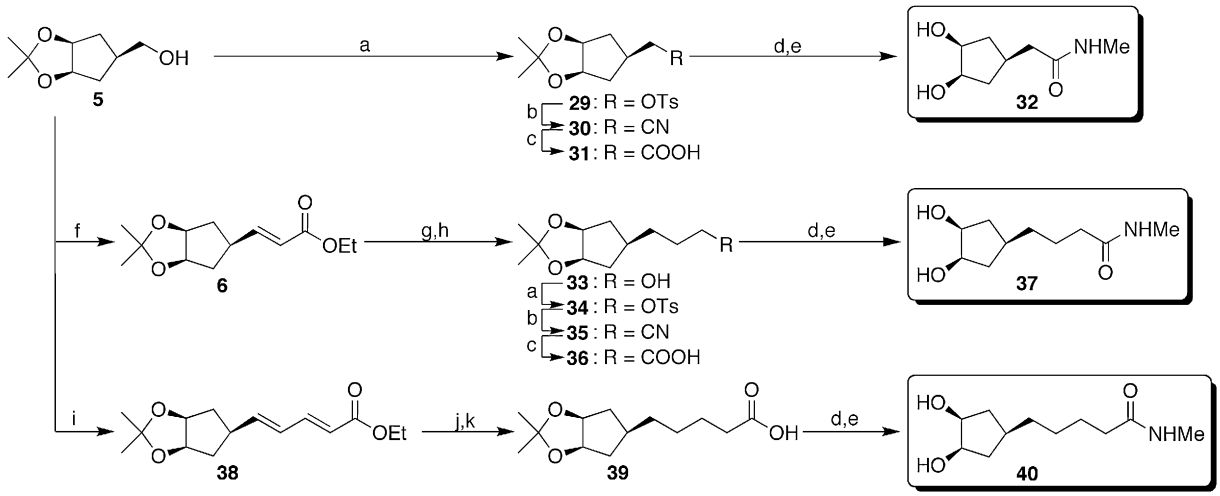
Analytical TLC was performed on silica gel 60 F_{254} (Merck). Column chromatography was carried out on silica gel 60 (70–230 mesh, Merck). NMR spectra were recorded on JEOL ECA-600, ECP-500 and AL-400. Mass spectra were measured on JEOL JMS-700 and JMS-DX303. Starting materials were either commercially available or prepared as reported previously in the literature. Analytical data of compounds except evaluated compounds (**1**, **13**, **14**, **16**, **17**, **19a–c**, **21a–i**, **23**, **26a–k**, **27**, **28**, **32**, **37**, **40** and **43**) are shown in Supplementary data.

3.1.1. Diethyl 3,4-(dimethylmethylenedioxy)cyclopentane-1,1-dicarboxylate (*3*)

To a solution of diethyl malonate (14.1 g, 88.0 mmol) in dimethoxyethane (150 mL) was added lithium hydride (2.46 g, 309 mmol) at 0 °C. After 3 h, *cis*-1,4-dichloro-2-butene (11.2 mL, 106 mmol) was added to the solution. After being stirred for 72 h at room temperature, the reaction mixture was poured into water, and extracted with Et_2O–hexane (1:4) three times. The combined



Scheme 6. Synthesis of compounds **27** and **28**. Reagents and conditions: (a) $NaIO_4$, THF–H_2O (1:1), 0 °C; (b) $NaBH_4$, MeOH, 0 °C (57%, 2 steps); (c) H_2 (ballon), $Pd(OH)_2$–C, MeOH, rt (75%).



Scheme 7. Synthesis of compounds **32**, **37**, and **40**. Reagents and conditions: (a) *p*TsCl, pyridine rt (83% (for **29**) and 77% (for **34**)); (b) NaCN, DMF, 80 °C (89% (for **30**) and 83% (for **34**)); (c) (1) 5 M NaOH, THF, reflux; (2) 2,2-DMP, *p*TsOH, rt (68% (for **31**) and 68% (for **36**)); (d) t-BuOCOCl, NMM, 40% $MeNH_2$ aq, 0 °C; (e) 10% HCl–MeOH, rt (65% (for **32**, 2 steps), 78% (for **37**, 2 steps) and 65% (for **40**, 2 steps)); (f) (1) TEMPO, NCS, *n*-Bu_4NCl, $NaHCO_3$, K_2CO_3, CH_2Cl_2–H_2O (1:1); (2) $(EtO)_2P(O)CH_2COOEt$, NaH, toluene, rt (56%, 2 steps); (g) (1) H_2 (ballon), $Pd(OH)_2$–C, MeOH, rt; (2) 2,2-DMP, *p*TsOH, rt; (h) $LiAlH_4$, THF, 0 °C (44%, 3 steps); (i) (1) TEMPO, NCS, *n*-Bu_4NCl, $NaHCO_3$, K_2CO_3, CH_2Cl_2–H_2O (1:1); (2) $(EtO)_2P(O)CH_2CH$=CHCOOEt, NaH, toluene, rt (57%, 2 steps); (j) (1) H_2 (ballon), $Pd(OH)_2$–C, MeOH, rt; (k) (1) 5 M NaOH, THF, reflux; (2) 2,2-DMP, *p*TsOH, rt (42%, 3 steps).

organic layer was washed with brine, dried over sodium sulfate, and evaporated to give a crude of diethyl cyclopent-3-ene-1,1-dicarboxylate (**2**) [10].

This crude was dissolved in water–acetonitrile–acetone (1:1:1) (180 mL), and 4-methylmorpholine *N*-oxide (17.1 g, 146 mmol) and osmium tetroxide (2% solution in water) (1.8 mL, 0.14 mmol) were added to the solution at room temperature. After being stirred for 5 h, the reaction mixture was poured into 10% sodium sulfite solution, and extracted with EtOAc three times. The combined organic layer was washed with brine, dried over sodium sulfate, and evaporated.

The residue and *p*TsOH (3.30 g, 19.3 mmol) were dissolved in 2,2-dimethoxypropane (400 mL). After being stirred for 2 h at room temperature, the reaction mixture was poured into saturated sodium bicarbonate solution, and extracted with EtOAc three times. The combined organic layer was washed with brine, dried over sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by hexane–EtOAc (19:1) to give **3** (15.2 g, 53.0 mmol, 59% (3 steps)).

3.1.2. Ethyl c-3,c-4-(dimethylmethylenedioxy)-r-1-cyclopentanecarboxylate (cis-4) and ethyl tert-3,tert-4-(dimethylmethylenedioxy)-r-1-cyclopentanecarboxylate (trans-4)

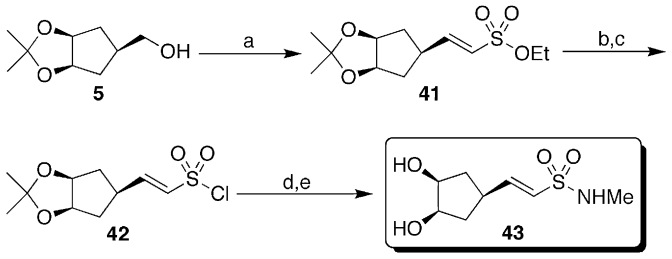
To a solution of **3** (15.2 g, 53.0 mmol) in DMSO (80 mL) were added lithium chloride (9.40 g, 222 mmol) and water (1.4 mL, 79.4 mmol). After being stirred for 10 h at 170 °C, the reaction mixture was poured into water, and extracted with EtOAc three times. The combined organic layer was washed with brine, dried over sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by hexane–EtOAc (33:1) and hexane–EtOAc (19:1) to give *trans*-**4** (2.02 g, 9.43 mmol, 18%) and *cis*-**4** (4.43 g, 20.7 mmol, 39%), respectively.

*3.1.3. c-3,c-4-(Dimethylmethylenedioxy)-r-1-cyclopentylmethanol (**5**)*

A solution of **4** (4.41 g, 20.4 mmol) in THF (10 mL) was added to a suspension of lithium aluminum hydride (877 mg, 23.1 mmol) at 0 °C. After being stirred for 1 h, acetone and 5 M sodium hydroxide solution were added to the reaction mixture. Then, the mixture was filtered through a Celite pad, and the filter cake was washed with EtOAc. The filtrate was evaporated, and the residue was chromatographed over silica gel eluted by hexane–EtOAc (2:1) to give **5** (2.49 g, 14.5 mmol, 71%).

*3.1.4. Ethyl (E)-3-[c-3,c-4-(dimethylmethylenedioxy)-r-1-cyclopentyl]propenoate (**6**)*

To a solution of **5** (502 mg, 2.92 mmol) in water–dichloromethane (1:1) (30 mL) were added sodium bicarbonate (640 mg, 7.62 mmol), potassium carbonate (108 mg, 0.78 mmol), TEMPO (61 mg, 0.38 mmol) and *N*-chlorosuccinimide (507 mg, 3.80 mmol). After being stirred for 4 h at room temperature, the reaction



Scheme 8. Synthesis of compound **43**. Reagents and conditions: (a) (1) TEMPO, NCS, *n*-Bu_4NCl, $NaHCO_3$, K_2CO_3, CH_2Cl_2–H_2O (1:1); (2) $(EtO)_2P(O)CH_2SO_3Et$, NaH, THF, 0 °C (58%, 2 steps); (b) *n*-Bu_4NI, acetone, reflux; (c) SO_2Cl_2, PPh_3, CH_2Cl_2, rt; (d) 40% $MeNH_2$ aq, dioxane, rt; (e) 10% HCl–MeOH, rt (19%, 4 steps).

Table 4
The immunosuppressive activity and cytotoxicity of methylamide derivatives.

Compounds	Immunosuppressive effect[a]	Cytotoxicity[b]
	EC_{50} (μM)	EC_{50} (μM)
21d	5.4	>250
27	>250	>250
28	15	>250
32	55	>250
37	8.4	>250
40	>250	>250
43	4.0	>250

[a] On the IMD signaling pathway in *Drosophila* innate immunity.
[b] Against *Drosophila* S2 cells.

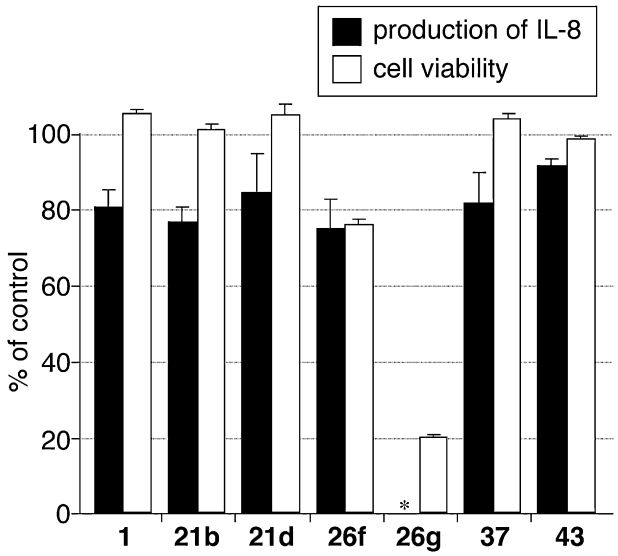


Fig. 2. Effects of synthesized cyclopentanediol derivatives on IL-8 production induced by TNF-α and cell viability in HUVECs. HUVECs were treated with 50 μg/mL of each compound for 3 h prior to stimulation with TNF-α (1 ng/mL). The bars indicate the standard errors of three independent measurements. The asterisk (*) indicates an untested compound.

mixture was poured into water, and extracted with EtOAc three times. The combined organic layer was washed with brine, dried over sodium sulfate, and evaporated to give a crude aldehyde.

To a solution of ethyl diethyphosphonoacetate (0.69 mL, 3.50 mmol) in toluene (12 mL) was added sodium hydride (60% mineral oil suspension) (175 mg, 4.37 mmol) at 0 °C. After 30 min, the crude aldehyde in toluene (2.0 mL) was added to the mixture. After being stirred for additional 1 h at room temperature, the mixture was poured into 0.5 M hydrochloric acid, and extracted with EtOAc three times. The combined organic layer was washed with saturated sodium bicarbonate solution and brine, dried over sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by hexane–EtOAc (19:1) to give **6** (451 mg, 1.88 mmol, 64% (2 steps)).

3.1.5. (E)-3-[c-3,c-4-(Dimethylmethylenedioxy)-r-1-cyclopentyl]-2-propene-1-ol (7)

To a solution of 6 (417 mg, 1.74 mmol) in dichloromethane (5.0 mL) was added 1.0 M diisobutylaluminum hydride solution in toluene (3.7 mL, 3.70 mmol) at 0 °C. After being stirred for 1 h, a saturated Rochelle salt solution was added. After being stirred for additional 30 min, the mixture was poured into water and extracted three times with diethyl ether. The combined organic layer was washed with water and brine, dried over sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by hexane–EtOAc (4:1) to give **7** (306 mg, 1.55 mmol, 89%).

*3.1.6. c-4-[(E)-1-Propenyl]cyclopentane-r-1,c-2-diol (**1**)*

To a solution of **7** (238 mg, 1.20 mmol) in THF (15 mL) was added sulfur pyridine complex (471 mg, 2.96 mmol) at 0 °C. After being stirred for 1 h at room temperature, the mixture was treated with lithium aluminum hydride (393 mg, 10.4 mmol), and further stirred for 15 h. The reaction was quenched by methanol (5.0 mL), and the mixture was filtered by a Celite pad. The filter cake was washed with diethyl ether, and the filtrate was concentrated to give a crude of **8**.

This crude was dissolved in THF (4.0 mL), and 6 M hydrochloric acid (4.0 mL) was added at room temperature. After being stirred 2 h, the mixture was poured into 2 M sodium hydroxide solution, and extracted with EtOAc three times. The combined organic layer was washed with brine, dried over sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by hexane–EtOAc (2:1) to give **1** (57.7 mg, 0.41 mmol, 35% (2 steps)). Data for **1**: colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.33–5.48 (2H, m), 3.97–4.04 (2H, m), 2.76 (2H, br.s), 2.33–2.42 (1H, m), 2.06–2.14 (2H, m), 1.63 (3H, d, J = 4.8 Hz), 1.47 (2H, ddd, J = 13.8, 9.6, 6.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.5, 123.5, 73.1 (2C), 38.8 (2C), 36.8, 17.8; EIMS m/z 142 $[M]^+$, 124 (base), 109, 97, 83; HREIMS m/z 142.0985 $[M]^+$ (142.0993 calcd. for $C_8H_{14}O_2$)

*3.1.7. tert-4-[(E)-1-Propenyl]cyclopentane-r-1,c-2-diol (**13**)*

In the similar way from *cis*-4 to 1, compound 13 was synthesized from *trans*-**4**

*3.1.7.1. Data for tert-4-[(E)-1-propenyl]cyclopentane-r-1,c-2-diol (**13**).* Colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.57–1.64 (2H, m), 1.63 (3H, d, J = 5.4 Hz), 1.88 (2H, ddd, J = 13.9, 6.2, 3.9 Hz), 2.84–2.95 (1H, m), 3.01 (2H, br.s), 4.11–4.15 (2H, m), 5.28–5.44 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.4, 123.4, 73.5 (2C), 38.8 (2C), 37.7, 17.8; EIMS m/z 124 $[M - H_2O]^+$ (base), 109, 95, 83; HREIMS m/z 124.0850 $[M - H_2O]^+$ (124.0888 calcd. for $C_8H_{12}O$).

*3.1.8. c-4-Propylcyclopentane-r-1,c-2-diol (**14**)*

Compound **1** (15.0 mg, 0.106 mmol) and 20% $Pd(OH)_2$ on carbon (3.0 mg) in MeOH (2.0 mL) was stirred at room temperature for 1 h under hydrogen atmosphere. After filtration, the filtrate was evaporated. The residue was chromatographed over silica gel eluted by hexane–EtOAc (2:1) to give **14** (8.3 mg, 0.054 mmol, 54%). Data for **14**: colorless powder; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.97–4.01 (2H, m), 2.26 (2H, br.s), 2.06–2.14 (2H, m), 1.72–1.84 (1H, m), 1.21–1.43 (6H, m), 0.88 (3H, t, J = 7.2 Hz); ^{13}C NMR (100 MHz, CDCl3) 73.4 (2C), 39.6 (2C), 38.5, 33.5, 21.5, 14.2; EIMS m/z 144 $[M]^+$, 126 (base), 99, 83; HREIMS: m/z 144.1140 $[M]^+$ (144.1149 calcd. for $C_8H_{16}O_2$).

*3.1.9. c-4-Hexylcyclopentane-r-1,c-2-diol (**16**)*

TEMPO oxidation of **5** (48.8 mg, 0.283 mmol) gave the crude aldehyde by the same way in the synthesis of **6**. To a solution of pentyltriphenylphosphonium bromide (470 mg, 1.14 mmol) was added 0.5 M KHMDS solution in toluene (2.3 mL, 1.15 mmol) at −78 °C. After being stirred for 30 min, the crude aldehyde in THF (2.0 mL) was added to the mixture. After being stirred for additional 6 h at room temperature, the mixture was poured into 0.5 M hydrochloric acid, and extracted with EtOAc three times. The combined organic layer was washed with saturated sodium bicarbonate solution and brine, dried over sodium sulfate, and evaporated to give a crude of **15**.

This crude and 20% $Pd(OH)_2$ on carbon (3.0 mg) in 10% hydrogen chloride solution in MeOH (2.0 mL) was stirred at room temperature for 1 h under hydrogen atmosphere. After filtration, the filtrate was evaporated. The residue was chromatographed over silica gel eluted by hexane–EtOAc (2:1) to give **16** (12.0 mg, 0.064 mmol, 23% (3 steps)). Data for **16**: colorless amorphous solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (3H, t, J = 7.1 Hz), 1.22–1.34 (8H, m), 1.31 (2H, dt, J = 7.3, 5.8 Hz), 1.34–1.41 (2H, m), 1.70–1.79 (1H, m), 2.05–2.13 (2H, m), 2.42 (2H, br.s), 3.96–4.02 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 73.4 (2C), 38.5 (2C), 37.3, 33.8, 31.9, 29.4, 28.3, 22.7, 14.2; EIMS m/z 186 $[M]^+$, 168, 150, 141, 123, 101, 83 (base); HREIMS m/z 186.1590 $[M]^+$ (186.1619 calcd. for $C_{11}H_{22}O_2$).

*3.1.10. Ethyl (E)-3-(c-3,c-4-dihydroxycyclopent-r-1-yl)propenoate (**17**)*

To a solution of **6** (15.8 mg, 0.066 mmol) in dichloromethane (1.0 mL) was added TFA (1.0 mL) at 0 °C. After being stirred for 1 h,

the reaction mixture was evaporated. The residue was chromatographed over silica gel eluted by hexane–EtOAc (1:1) to give **17** (11.6 mg, 0.058 mmol, 88%). Data for **17**: colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.98 (1H, dd, J = 15.5, 8.0 Hz), 5.75 (1H, d, J = 15.5 Hz), 4.18 (2H, q, J = 7.2 Hz), 4.02–4.28 (4H, m), 2.60–2.71 (1H, m), 2.14–2.23 (2H, m), 1.66–1.78 (2H, m), 1.28 (3H, t, J = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.2, 152.9, 119.6, 73.6 (2C), 60.6, 37.5 (2C), 36.7, 14.2; EIMS m/z 182 $[M]^+$, 156 (base), 137, 127, 110, 99, 81; HREIMS m/z 182.0932 $[M - H_2O]^+$ (182.0942 calcd. for $C_{10}H_{14}O_3$).

3.1.11. Methyl (E)-3-[c-3,c-4-(dimethylmethylenedioxy)-r-1-cyclopentyl]propenoate (**18a**)

TEMPO oxidation of **5** (79.3 mg, 0.460 mmol) gave the crude aldehyde by the same way in the synthesis of **6**. To a solution of methyl diethyphosphonoacetate (116 mg, 0.552 mmol) in toluene (1.0 mL) was added sodium hydride (60% mineral oil suspension) (27.7 mg, 0.693 mmol) at 0 °C. After 30 min, the crude aldehyde in toluene (1.0 mL) was added to the mixture. After being stirred for additional 1 h at room temperature, the mixture was poured into 0.5 M hydrochloric acid, and extracted with EtOAc three times. The combined organic layer was washed with saturated sodium bicarbonate solution and brine, dried over sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by hexane–EtOAc (9:1) to give **18a** (78.4 mg, 0.346 mmol, 76% (2 steps)).

In the similar procedure, compounds **18b** (yield 58%), **18c** (58%), **22** (76%), **20a** (58%), **20b** (71%), **25a** (51%), **25b** (47%), **25c** (72%), **25d** (83%), **25e** (41%), **25f** (44%), **25g** (73%), **25h** (78%) and **25i** (73%) were synthesized by using the corresponding HWE reagents.

3.1.12. (E)-3-(c-3,c-4-Dihydroxycyclopent-r-1-yl)propenoic acid (**23**)

To a solution of **22** (320 mg, 1.19 mmol) in dichloromethane (3.0 mL) was added TFA (3.0 mL) at 0 °C. After being stirred for 1 h, the reaction mixture was evaporated. The residue was chromatographed over silica gel eluted by chloroform–MeOH (9:1) to give **23** (183 mg, 1.06 mmol, 89%). Data for **23**: colorless amorphous solid; ^{1}H NMR (500 MHz, CD_3OD) δ 6.90 (1H, dd, J = 15.6, 8.5 Hz), 5.69 (1H, d, J = 15.6 Hz), 3.91–4.00 (2H, m), 2.58–2.67 (1H, m), 2.00–2.12 (2H, m), 1.49–1.58 (2H, m); ^{13}C NMR (125 MHz, CD_3OD) δ 168.8, 155.1, 120.0, 74.4 (2C), 39.3, 37.6 (2C); EIMS m/z 154 $[M]^+$, 128, 110, 99, 81, 69, 45 (base); HREIMS m/z 154.0626 $[M - H_2O]^+$ (154.0629 calcd. for $C_8H_{10}O_3$).

3.1.13. (E)-3-[c-3,c-4-(Dimethylmethylenedioxy)-r-1-cyclopentyl] propenoic acid (**24**)

Compound **23** (202 mg, 1.17 mmol) and *p*TsOH (40.4 mg, 0.235 mmol) were dissolved in 2,2-dimethoxypropane (3.5 mL). After being stirred for 2 h at room temperature, the reaction mixture was poured into water, and extracted with EtOAc three times. The combined organic layer was washed with brine, dried over sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by chloroform–MeOH (24:1) to give **24** (185 mg, 0.874 mmol, 75%).

3.1.14. (E)-3-[c-3,c-4-(Dimethylmethylenedioxy)-r-1-cyclopentyl] propenamide (**20c**)

To a solution of **24** (98 mg, 0.461 mmol) in THF (2.5 mL) were added isobutyl chloroformate (70 μL, 0.539 mmol) and *N*-methylmorpholine (60 μL, 0.546 mmol) at 0 °C. After 30 min, 28% ammonia solution in water (90 μL) was added to the reaction mixture. After being stirred for additional 3 h, the mixture was poured into water, and extracted with EtOAc three times. The combined organic layer was washed with 0.5 M hydrochloric acid and brine, dried over sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by chloroform–MeOH (49:1) to give **20c** (69 mg, 0.327 mmol, 71%).

In the similar procedure, compounds **20d** (yield 95%), **20e** (98%), **20f** (95%), **20g** (95%), **20h** (98%) and **20i** (69%) were synthesized by using the corresponding amines.

3.1.15. (E)-N-[4-(dimethylamino)phenyl]-3-[c-3,c-4-(dimethylmethylenedioxy)-r-1-cyclo- pentyl]propenamide (25j)

To a solution of **24** (34.0 mg, 0.160 mmol) in dichloromethane (1.5 mL) were added triethylamine (50 μL, 0.363 mmol), DMAP (2.2 mg, 0.018 mmol) and 2-methyl-6-nitrobenzoic anhydride [16] (68.0 mg, 0.198 mmol) at 0 °C. After 20 min, *N*,*N*-dimethyl-1,4-phenylenediamine (26.0 mg, 0.191 mmol) was added to the reaction mixture. After being stirred for additional 2 h, the reaction mixture was poured into water, and extracted with EtOAc three times. The combined organic layer was washed with brine, dried over sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by chloroform–MeOH (19:1) to give **25j** (34.4 mg, 0.104 mmol, 65%).

In the similar procedure, compounds **25k** (67%) was synthesized by using 4-aminopyridine.

3.1.16. Methyl (E)-3-(c-3,c-4-dihydroxycyclopent-r-1-yl) propenoate (19a)

To a solution of **18a** (19.5 mg, 0.086 mmol) in dichloromethane (1.0 mL) was added TFA (1.0 mL) at 0 °C. After being stirred for 1 h, the reaction mixture was evaporated. The residue was chromatographed over silica gel eluted by hexane–EtOAc (1:1) to give **19a** (8.8 mg, 0.047 mmol, 55%). Data for **19a**: colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.99 (1H, dd, J = 15.6, 8.5 Hz), 5.77 (1H, d, J = 15.5 Hz), 4.08–4.16 (2H, m), 3.72 (3H, s), 2.81 (2H, br.s), 2.60–2.69 (1H, m), 2.16–2.24 (2H, m), 1.61–1.71 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.1, 152.7, 119.4, 73.2 (2C), 51.6, 37.6 (2C), 36.6; EIMS m/z 168 $[M]^+$, 142 (base), 137, 113, 81; HREIMS m/z 168.0799 $[M - H_2O]^+$ (168.0786 calcd. for $C_9H_{12}O_3$).

In the similar procedure, compounds **19b** (yield 46%) and **19c** (75%) were synthesized from **18b** and **18c**, respectively.

3.1.16.1. Data for hexyl (E)-3-(c-3,c-4-dihydroxycyclopent-r-1-yl) propenoate (**19b**).

Colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.95 (1H, dd, J = 14.7, 8.5 Hz), 5.77 (1H, d, J = 14.7 Hz), 4.04–4.18 (2H, m), 4.11 (2H, t, J = 6.7 Hz), 2.70 (2H, br.s), 2.57–2.67 (1H, m), 2.13–2.23 (2H, m), 1.59–1.73 (4H, m), 1.24–1.46 (6H, m), 0.89 (3H, t, J = 6.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.8, 152.3, 119.8, 73.2 (2C), 64.6, 37.6 (2C), 36.5, 31.5, 28.6, 25.6, 22.6, 14.1; EIMS m/z 256 $[M]^+$, 238, 212 (base), 186, 154, 137, 128, 110, 81; HREIMS m/z 256.1665 $[M]^+$ (256.1673 calcd. for $C_{14}H_{24}O_4$).

3.1.16.2. Data for phenyl (E)-3-(c-3,c-4-dihydroxycyclopent-r-1-yl) propenoate (**19c**).

Colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (2H, t, J = 7.6 Hz), 7.25 (1H, t, J = 7.6 Hz), 7.13 (1H, dd, J = 15,4, 8.3 Hz), 7.08 (2H, d, J = 7.6 Hz), 5.94 (1H, d, J = 15.4 Hz), 4.07–4.24 (4H, m), 2.63–2.75 (1H, m), 2.13–2.24 (2H, m), 1.67–1.78 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.2, 154.8, 150.5, 129.3 (2C), 125.7, 121.5 (2C), 119.0, 73.4 (2C), 37.4, 36.8 (2C); EIMS m/z 248 $[M]^+$, 230, 204, 155 (base), 137, 119, 109, 94, 81; HREIMS m/z 248.1028 $[M]^+$ (248.1048 calcd. for $C_{14}H_{16}O_4$).

3.1.17. (E)-N,N-Dimethyl-3-(c-3,c-4-dihydroxycyclopent-r-1-yl) propenamide (**21a**)

Compound **20a** (46.2 mg, 0.193 mmol) was dissolved into 10% hydrogen chloride solution in MeOH (2.0 mL). After being stirred for 1 h at room temperature, the reaction mixture was evaporated. The residue was chromatographed over silica gel eluted by

chloroform–MeOH (9:1) to give **21a** (22.7 mg, 0.114 mmol, 59%). Data for **21a**: colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.86 (1H, dd, J = 14.9, 8.3 Hz), 6.17 (1H, d, J = 14.9 Hz), 4.48 (2H, br.s), 4.09–4.16 (2H, m), 3.06 (3H, s), 2.98 (3H, s), 2.59–2.67 (1H, m), 2.10–2.20 (2H, m), 1.64–1.75 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.0, 150.7, 118.1, 73.4 (2C), 37.8, 37.5, 37.0 (2C), 35.9; EIMS m/z 199 $[M]^+$, 155, 136, 126 (base); HREIMS m/z 199.1212 $[M]^+$ (199.1207 calcd. for $C_{10}H_{17}NO_3$).

In the similar procedure, compounds **21b** (yield 51%), **21c** (91%), **21d** (86%), **21e** (70%), **21f** (69%), **21g** (94%), **21h** (95%) and **21i** (71%) were synthesized from **20b**–**i**, respectively. Compounds **26a** (yield 77%), **26b** (78%), **26c** (79%), **26d** (54%), **26e** (92%), **26f** (66%), **26g** (75%), **26h** (78%), **26i** (66%), **26j** (65%) and **26k** (34%) were also synthesized from **25a**–**k**, respectively.

*3.1.17.1. Data for (E)-N-phenyl-3-(c-3,c-4-dihydroxycyclopent-r-1-yl) propenamide (**21b**).* Colorless amorphous solid; ^{1}H NMR (400 MHz, CD_3OD) δ 7.58 (2H, d, J = 7.2 Hz), 7.30 (2H, t, J = 7.2 Hz), 7.08 (1H, t, J = 7.2 Hz), 6.94 (1H, dd, J = 15.1, 8.5 Hz), 6.05 (1H, d, J = 15.1 Hz), 3.97–4.09 (2H, m), 2.66–2.76 (1H, m), 2.11–2.22 (2H, m), 1.59–1.70 (2H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 166.9, 151.1, 140.0, 129.8 (2C), 125.2, 123.4 (2C), 121.4, 74.4 (2C), 38.5, 37.7 (2C); EIMS m/z 247 $[M]^+$ (base), 204, 146, 137, 93; HREIMS m/z 247.1196 $[M]^+$ (247.1207 calcd. for $C_{14}H_{17}NO_3$).

*3.1.17.2. Data for (E)-3-(c-3,c-4-dihydroxycyclopent-r-1-yl)propenamide (**21c**).* Colorless oil; ^{1}H NMR (400 MHz, CD_3OD) δ 6.80 (1H, dd, J = 15.4, 8.5 Hz), 5.88 (1H, dd, J = 15.4, 1.1 Hz), 3.94–3.99 (2H, m), 2.59–2.68 (1H, m), 2.07–2.16 (2H, m), 1.53–1.62 (2H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 172.1, 154.0, 120.5, 74.3 (2C), 38.2, 37.8 (2C); EIMS m/z 153 $[M]^+$, 137, 119 (base), 72; HREIMS m/z 153.0788 $[M]^+$ (153.0789 calcd. for $C_8H_{11}NO_2$).

*3.1.17.3. Data for (E)-N-methyl-3-(c-3,c-4-dihydroxycyclopent-r-1-yl)propenamide (**21d**).* Colorless oil; ^{1}H NMR (400 MHz, CD_3OD) δ 6.66 (1H, dd, J = 15.4, 8.3 Hz), 5.73 (1H, dd, J = 15.4, 1.2 Hz), 3.84–3.92 (2H, m), 2.67 (3H, s), 1.96–2.06 (2H, m), 1.43–1.52 (2H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 169.9, 151.0, 121.6, 74.4 (2C), 38.4, 37.7 (2C), 26.7; EIMS m/z 185 $[M]^+$, 141, 112 (base); HREIMS m/z 185.1042 $[M]^+$ (185.1051 calcd. for $C_9H_{15}NO_3$).

*3.1.17.4. Data for (E)-N-ethyl-3-(c-3,c-4-dihydroxycyclopent-r-1-yl) propenamide (**21e**).* Colorless oil; ^{1}H NMR (400 MHz, CD_3OD) δ 6.86 (1H, dd, J = 15.4, 8.5 Hz), 5.89 (1H, d, J = 15.4 Hz), 3.98–4.07 (2H, m), 3.34 (2H, q, J = 7.3 Hz), 2.62–2.73 (1H, m), 2.09–2.21 (2H, m), 1.55–1.67 (2H, m), 1.15 (3H, t, J = 7.2 Hz),; ^{13}C NMR (100 MHz, CD_3OD) δ 169.2, 151.5, 121.5, 74.4 (2C), 38.4, 37.8 (2C), 35.9, 14.5; EIMS m/z 199 $[M]^+$, 155, 126 (base); HREIMS m/z 199.1193 $[M]^+$ (199.1207 calcd. for $C_{10}H_{17}NO_3$).

*3.1.17.5. Data for (E)-N-butyl-3-(c-3,c-4-dihydroxycyclopent-r-1-yl) propenamide (**21f**).* Colorless oil; ^{1}H NMR (400 MHz, CD_3OD) δ 6.84 (1H, dd, J = 15.5, 8.3 Hz), 5.90 (1H, d, J = 15.5 Hz), 3.95–4.03 (2H, m), 3.27 (2H, t, J = 7.2 Hz), 2.62–2.71 (1H, m), 2.07–2.18 (2H, m), 1.52–1.67 (4H, m), 1.37 (2H, sextet, J = 7.2 Hz), 0.94 (3H, t, J = 7.2 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 169.3, 151.4, 121.6, 74.4 (2C), 40.7, 39.1, 37.8 (2C), 32.2, 21.1, 14.0; EIMS m/z 227 $[M]^+$, 212, 183, 154, 137 (base); HREIMS m/z 227.1505 $[M]^+$ (227.1520 calcd. for $C_{12}H_{21}NO_3$).

*3.1.17.6. Data for (E)-N-cyclopropyl-3-(c-3,c-4-dihydroxycyclopent-r-1-yl)propenamide (**21g**).* Colorless oil; ^{1}H NMR (400 MHz, CD_3OD) δ 0.60–0.67 (2H, m), 0.79–0.84 (2H, m), 1.57–1.66 (2H, m), 2.08–2.17 (2H, m), 2.63–2.74 (1H, m), 2.79–2.85 (1H, m), 3.96–4.05 (2H, m), 5.90 (1H, d, J = 14.8 Hz), 6.90 (1H, dd, J = 14.8, 8.5 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 170.8, 151.9, 121.1, 74.3 (2C), 38.3, 37.7 (2C), 24.0, 6.6 (2C); EIMS m/z 211 $[M]^+$, 183, 167, 155, 137 (base), 119, 109, 95, 81; HREIMS m/z 211.1230 $[M]^+$ (211.1207 calcd. for $C_{11}H_{17}NO_3$)

*3.1.17.7. Data for (E)-3-(c-3,c-4-dihydroxycyclopent-r-1-yl)propenohydroxamic acid (**21h**).* Colorless oil; ^{1}H NMR (400 MHz, CD_3OD) δ 1.55–1.63 (2H, m), 2.07–2.18 (2H, m), 2.63–2.73 (1H, m), 3.97–4.04 (2H, m), 5.80 (1H, d, J = 15.5 Hz), 6.91 (1H, dd, J = 15.5, 8.2 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 166.2, 151.7, 117.8, 74.4 (2C), 38.3, 37.8 (2C); EIMS m/z 187 $[M]^+$, 155, 137 (base); HREIMS m/z 187.0822 $[M]^+$ (187.0844 calcd. for $C_8H_{13}NO_4$).

*3.1.17.8. Data for (3-[(E)-c-3,c-4-Dihydroxycyclopent-r-1-yl]propenoyl)piperidine (**21i**).* Colorless oil; ^{1}H NMR (400 MHz, CD_3OD) δ 6.75 (1H, dd, J = 15.1, 8.5 Hz), 6.35 (1H, dd, J = 15.1, 1.0 Hz), 3.95–4.00 (2H, m), 3.55–3.61 (4H, m), 2.63–2.74 (1H, m), 2.08–2.17 (2H, m), 1.67–1.73 (2H, m), 1.53–1.65 (6H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 167.8, 152.0, 119.8, 74.7 (2C), 48.2, 44.6, 38.7 (2C), 38.2, 28.0, 27.1, 25.8; EIMS m/z 239 $[M]^+$, 195, 166, 138 (base); HREIMS m/z 239.1508 $[M]^+$ (239.1520 calcd. for $C_{13}H_{21}NO_3$).

*3.1.17.9. Data for (E)-N-(4-chlorophenyl)-3-(c-3,c-4-dihydroxycyclopent-r-1-yl)propenamide (**26a**).* Colorless amorphous solid; ^{1}H NMR (400 MHz, CD_3OD) δ 7.60 (2H, d, J = 8.7 Hz), 7.29 (2H, d, J = 8.7 Hz), 6.94 (1H, dd, J = 15.3, 8.5 Hz), 6.05 (1H, dd, J = 15.3, 1.1 Hz), 3.97–4.03 (2H, m), 2.63–2.75 (1H, m), 2.09–2.19 (2H, m), 1.61 (2H, ddd, J = 13.3, 8.8, 5.6 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 166.8, 151.6, 138.8, 130.0, 129.8 (2C), 123.1, 121.4 (2C), 74.4 (2C), 38.4 (2C), 37.7; EIMS m/z 283 $[M+2]^+$, 281 $[M]^+$, 238, 169, 137, 127 (base); HREIMS m/z 281.0821 $[M]^+$ (281.0818 calcd. for $C_{14}H_{16}NO_3Cl$).

*3.1.17.10. Data for (E)-N-(3,4-dichlorophenyl)-3-(c-3,c-4-dihydroxycyclopent-r-1-yl)propenamide (**26b**).* Colorless amorphous solid; ^{1}H NMR (400 MHz, CD_3OD) δ 7.95 (1H, d, J = 2.3 Hz), 7.46 (1H, dd, J = 8.8, 2.3 Hz), 7.42 (1H, d, J = 8.8 Hz), 6.96 (1H, dd, J = 15.2, 8.2 Hz), 6.00 (1H, dd, J = 15.2, 1.1 Hz), 3.96–4.02 (2H, m), 2.63–2.75 (1H, m), 2.08–2.17 (2H, m), 1.55–1.65 (2H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 166.8, 152.1, 140.1, 133.3, 131.5, 127.7, 122.9, 122.5, 120.6, 74.4 (2C), 38.4 (2C), 37.7; EIMS m/z 319 $[M+4]^+$, 317 $[M+2]^+$, 315 $[M]^+$, 272, 203, 161 (base), 137; HREIMS m/z 315.0412 $[M]^+$ (315.0428 calcd. for $C_{14}H_{15}NO_3Cl_2$).

*3.1.17.11. Data for (E)-N-(4-fluorophenyl)-3-(c-3,c-4-dihydroxycyclopent-r-1-yl)propenamide (**26c**).* Colorless amorphous solid; ^{1}H NMR (400 MHz, CD_3OD) δ 7.55–7.61 (2H, m), 6.99–7.04 (2H, m), 6.94 (1H, dd, J = 15.3, 7.3 Hz), 6.02 (1H, dd, J = 15.4, 1.4 Hz), 3.98–4.04 (2H, m), 2.62–2.74 (1H, m), 2.12–2.20 (2H, m), 1.58–1.67 (2H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 166.5, 160.3 (d, J = 242 Hz), 150.7, 134.3 (d, J = 3 Hz), 123.0, 122.8 (2C, d, J = 8 Hz), 116.0 (2C, d, J = 23 Hz), 74.0 (2C), 38.2 (2C), 37.3; EIMS m/z 265 $[M]^+$, 222, 167, 91 (base); HREIMS m/z 265.1090 $[M]^+$ (265.1113 calcd. for $C_{14}H_{16}NO_3F$).

*3.1.17.12. Data for (E)-N-[4-(trifluoromethyl)phenyl]-3-(c-3,c-4-dihydroxycyclopent-r-1-yl)propenamide (**26d**).* Colorless amorphous solid; ^{1}H NMR (400 MHz, CD_3OD) δ 7.80 (2H, d, J = 8.5 Hz), 7.59 (2H, d, J = 8.5 Hz), 6.99 (1H, dd, J = 15.3, 7.7 Hz), 6.06 (1H, dd, J = 15.3, 1.4 Hz), 3.97–4.03 (2H, m), 2.64–2.76 (1H, m), 2.10–2.19 (2H, m), 1.57–1.67 (2H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 167.0, 152.1, 143.6, 127.0 (2C, q, J = 4 Hz), 126.5 (q, J = 33 Hz), 125.7 (q, J = 270 Hz), 123.0 (2C), 120.9, 74.4 (2C), 38.3 (2C), 37.7; EIMS m/z 315 $[M]^+$, 296, 279 (base), 272, 203, 188, 116, 137, 109; HREIMS m/z 315.1070 $[M]^+$ (315.1081 calcd. for $C_{15}H_{16}NO_3F_3$).

*3.1.17.13. Data for (E)-N-(4-methylphenyl)-3-(c-3,c-4-dihydroxycyclopent-r-1-yl)propenamide (**26e**).* Colorless amorphous solid; ^{1}H NMR (400 MHz, CD_3OD) δ 7.46 (2H, d, J = 8.3 Hz), 7.11 (2H, d, J = 8.3 Hz), 6.92 (1H, dd, J = 15.4, 8.2 Hz), 6.03 (1H, dd, J = 15.4, 1.1 Hz), 3.96–4.02 (2H, m), 2.65–2.76 (1H, m), 2.29 (3H, s), 2.08–2.18 (2H, m), 1.57–1.66 (2H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 166.6, 150.7, 137.2, 134.8, 130.1 (2C), 123.2 (2C), 121.3, 74.4 (2C), 38.4 (2C), 37.6, 20.9; EIMS m/z 261 $[M]^+$, 243, 218, 107 (base); HREIMS m/z 261.1339 $[M]^+$ (261.1364 calcd. for $C_{15}H_{19}NO_3$).

*3.1.17.14. Data for (E)-N-(4-methoxyphenyl)-3-(c-3,c-4-dihydroxycyclopent-r-1-yl)propenamide (**26f**).* Colorless amorphous solid; ^{1}H NMR (400 MHz, CD_3OD) δ 7.48 (2H, d, J = 9.2 Hz), 6.90 (1H, dd, J = 15.2, 8.3 Hz), 6.87 (1H, dd, J = 9.2 Hz), 6.03 (1H, dd, J = 15.2, 1.1 Hz), 3.96–4.02 (2H, m), 3.77 (3H, s), 2.62–2.74 (1H, m), 2.09–2.18 (2H, m), 1.56–1.65 (2H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 166.5, 157.8, 150.5, 132.8, 123.2, 122.9 (2C), 114.9 (2C), 74.4 (2C), 55.8, 38.5 (2C), 37.6; EIMS m/z 277 $[M]^+$, 259, 204, 176, 123 (base); HREIMS m/z 277.1313 $[M]^+$ (277.1313 calcd. for $C_{15}H_{19}NO_4$).

*3.1.17.15. Data for (E)-N-[4-(1,1-dimethylethyl)phenyl]-3-(c-3,c-4-dihydroxycyclopent-r-1-yl)propenamide (**26g**).* Colorless amorphous solid; ^{1}H NMR (400 MHz, CD_3OD) δ 1.30 (9H, s), 1.56–1.66 (2H, m), 2.09–2.18 (2H, m), 2.64–2.74 (1H, m), 3.95–4.02 (2H, m), 6.03 (1H, dd, J = 15.3, 1.2 Hz), 6.92 (1H, dd, J = 15.3, 8.1 Hz), 7.34 (2H, d, J = 8.7 Hz), 7.49 (2H, d, J = 8.7 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 166.8, 150.9, 148.3, 137.3, 126.6 (2C), 123.4, 121.1 (2C), 74.4 (2C), 38.5 (2C), 37.6, 35.2, 31.8 (3C); EIMS m/z 303 $[M]^+$, 288, 260, 202, 149, 134 (base); HREIMS m/z 303.1823 $[M]^+$ (303.1833 calcd. for $C_{18}H_{25}NO_3$).

*3.1.17.16. Data for (E)-N-(4-acetylphenyl)-3-(c-3,c-4-dihydroxycyclopent-r-1-yl)propenamide (**26h**).* Colorless amorphous solid; ^{1}H NMR (400 MHz, CD_3OD) δ 7.92 (2H, d, J = 9.0 Hz), 7.74 (2H, d, J = 9.0 Hz), 6.99 (1H, dd, J = 15.2, 7.8 Hz), 6.03 (1H, dd, J = 15.2, 1.2 Hz), 3.99–4.06 (2H, m), 2.61–2.73 (1H, m), 2.13–2.22 (2H, m), 1.59–1.69 (2H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 197.9, 165.2, 149.9, 143.1, 131.9, 129.3 (2C), 121.8, 118.8 (2C), 72.7 (2C), 36.9 (2C), 36.1, 26.0; EIMS m/z 289 $[M]^+$, 253, 246, 177, 162, 135, 120 (base); HREIMS m/z 289.1292 $[M]^+$ (289.1313 calcd. for $C_{16}H_{19}NO_4$).

*3.1.17.17. Data for (E)-N-(4-nitrophenyl)-3-(c-3,c-4-dihydroxycyclopent-r-1-yl)propenamide (**26i**).* Colorless amorphous solid; ^{1}H NMR (400 MHz, CD_3OD) δ 8.19 (2H, d, J = 8.9 Hz), 7.84 (2H, d, J = 8.9 Hz), 7.03 (1H, dd, J = 15.4, 8.3 Hz), 6.07 (1H, dd, J = 15.3, 1.2 Hz), 3.99–4.06 (2H, m), 2.65–2.77 (1H, m), 2.13–2.22 (2H, m), 1.60–1.70 (2H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 166.7, 152.3, 146.0, 144.1, 125.5 (2C), 122.6, 120.2 (2C), 73.9 (2C), 38.0 (2C), 37.4; EIMS m/z 292 $[M]^+$, 249, 180, 137 (base); HREIMS m/z 292.1053 $[M]^+$ (292.1058 calcd. for $C_{14}H_{16}N_2O_5$).

*3.1.17.18. Data for (E)-N-[4-(dimethylamino) phenyl]-3-(c-3,c-4-dihydroxycyclopent-r-1-yl)propenamide (**26j**).* Colorless amorphous solid; ^{1}H NMR (400 MHz, CD_3OD) δ 7.42 (2H, d, J = 9.1 Hz), 6.88 (1H, dd, J = 15.2, 8.2 Hz), 6.76 (2H, d, J = 9.1 Hz), 6.02 (1H, dd, J = 15.2, 1.0 Hz), 3.94–4.06 (2H, m), 2.89 (6H, s), 2.61–2.73 (1H, m), 2.09–2.18 (2H, m), 1.56–1.65 (2H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 166.5, 150.2, 149.5, 130.1, 123.5, 122.8 (2C), 114.5 (2C), 74.4 (2C), 41.4 (2C), 38.6 (2C), 37.6; EIMS m/z 290 $[M]^+$, 256, 136 (base); HREIMS m/z 290.1612 $[M]^+$ (290.1629 calcd. for $C_{16}H_{22}N_2O_3$).

*3.1.17.19. Data for (E)-N-(4-pyridinyl)-3-(c-3,c-4-dihydroxycyclopent-r-1-yl)propenamide (**26k**).* Colorless amorphous solid; ^{1}H NMR (400 MHz, CD_3OD) δ 8.38 (2H, d, J = 4.9 Hz), 7.68 (2H, d, J = 4.9 Hz), 7.03 (2H, dtd J = 15.3, 8.2 Hz), 6.05 (1H, dd, J = 15.3, 1.1 Hz), 3.96–4.06 (2H, m), 2.66–2.76 (1H, m), 2.10–2.21 (2H, m), 1.56–1.68 (2H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 167.3, 153.0, 150.7 (2C), 148.2, 122.7, 115.2 (2C), 74.3 (2C), 38.3 (2C), 37.6; EIMS m/z 248 $[M]^+$, 212, 205, 136, 121, 95 (base); HREIMS m/z 248.1161 $[M]^+$ (248.1160 calcd. for $C_{13}H_{16}N_2O_3$).

*3.1.18. (E)-N-Methyl-6-hydroxy-4-(2-hydroxyethyl)-2-hexeneamide (**27**)*

To a solution of **21d** (9.0 mg, 0.049 mmol) in THF (1.0 mL) were added sodium metaperiodate (11.4 mg, 0.053 mmol) and water (50 μL) at 0 °C. After being stirred for 1 h, the reaction mixture was evaporated. The residue was dissolved in MeOH (1.0 mL), and sodium tetrahydroborate (9.3 mg, 0.245 mmol) was added to the solution. After being stirred for 2 h at 0 °C, the reaction mixture was evaporated. The residue was chromatographed over silica gel eluted by chloroform–MeOH (4:1) to give **27** (5.1 mg, 0.028 mmol, 57%). Data for **27**: colorless oil; ^{1}H NMR (400 MHz, CD_3OD) δ 6.52 (1H, dd, J = 15.4, 9.5 Hz), 5.91 (1H, dd, J = 15.4, 0.9 Hz), 3.57 (2H, ddd, J = 10.8, 7.2, 5.4 Hz), 3.48 (2H, dt, J = 10.8, 7.1 Hz), 3.31 (3H, s), 2.44–2.54 (1H, m), 1.66–1.76 (2H, m), 1.51–1.60 (2H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 169.1, 148.3, 125.1, 60.6 (2C), 38.4 (2C), 36.7, 26.3; EIMS m/z 169 $[M - H_2O]^+$, 157, 144, 128, 115, 102, 73 (base); HREIMS m/z 169.1081 $[M - H_2O]^+$ (169.1102 calcd. for $C_9H_{15}NO_2$).

3.1.19. N-Methyl-3-(c-3,c-4-dihydroxycyclopent-r-1-yl) propanamide (28)

Under hydrogen atmosphere, compound **21d** (10.2 mg, 0.055 mmol) and 20% $Pd(OH)_2$ on carbon (2.0 mg) in MeOH (2.0 mL) was stirred at room temperature for 1 h. After filtration, the filtrate was evaporated. The residue was chromatographed over silica gel eluted by hexane–EtOAc (1:2) to give **28** (7.7 mg, 0.041 mmol, 75%). Data for **28**: colorless oil; ^{1}H NMR (400 MHz, CD_3OD) δ 3.87–3.95 (2H, m), 2.70 (3H, s), 2.17 (2H, t, J = 7.3 Hz), 2.01–2.09 (2H, m), 1.72–1.83 (1H, m), 1.66–1.74 (2H, m), 1.31–1.39 (2H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 176.8, 74.5 (2C), 38.6 (2C), 35.9, 34.7, 34.6, 26.3; EIMS m/z 188 $[M + H]^+$, 169 $[M - H_2O]^+$, 159, 139, 114, 86, 73 (base); HREIMS m/z 169.1111 $[M - H_2O]^+$ (169.1102 calcd. for $C_9H_{15}NO_2$).

*3.1.20. [c-3,c-4-(Dimethylmethylenedioxy)-r-1-cyclopentyl]methyl p-toluenesulfonate (**29**)*

To a solution of **5** (152 mg, 0.883 mmol) in pyridine (3.0 mL) was added p-toluenesulfonyl chloride (675 mg, 3.54 mmol) at room temperature. After being stirred for 1 h, the reaction mixture was poured into saturated ammonium chloride solution, and extracted with EtOAc three times. The combined organic layer was washed with saturated sodium bicarbonate solution and brine, dried over sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by hexane–EtOAc (4:1) to give **29** (240 mg, 0.735 mmol, 83%).

In the similar procedure, compounds **34** (77%) was synthesized from **33**.

*3.1.21. 2-[c-3,c-4-(Dimethylmethylenedioxy)-r-1-cyclopentyl] acetonitrile (**30**)*

To a solution of **29** (240 mg, 0.735 mmol) in DMF (2.0 mL) was added sodium cyanide (108 mg, 2.21 mmol). After being stirred for 6 h at 80 °C, the reaction mixture was poured into water, and extracted with EtOAc three times. The combined organic layer was washed with brine, dried over sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by hexane–EtOAc (4:1) to give **30** (118 mg, 0.651 mmol, 89%).

In the similar procedure, compounds **35** (83%) was synthesized from **34**.

*3.1.22. 2-[c-3,c-4-(Dimethylmethylenedioxy)-r-1-cyclopentyl]acetic acid (**31**)*

To a solution of **30** (63.1 mg, 0.348 mmol) in THF (5.0 mL) was added 5 M sodium hydroxide solution (5.0 mL). After being refluxed for 48 h, the reaction mixture was poured into 3 M hydrochloric acid, and extracted with EtOAc three times. The combined organic layer was washed with brine, dried over sodium sulfate, and evaporated. The residue was dissolved in 2,2-dimethoxypropane (2.0 mL), and *p*TsOH (6.0 mg, 0.032 mmol) was added to this solution. After being stirred for 1 h at room temperature, the reaction mixture was poured into water, and extracted with EtOAc three times. The combined organic layer was washed with brine, dried over sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by chloroform–MeOH (9:1) to give **31** (47.0 mg, 0.235 mmol, 68%).

In the similar procedure, compounds **36** (68%) was synthesized from **35**.

*3.1.23. N-Methyl-2-(c-3,c-4-dihydroxycyclopent-r-1-yl)acetamide (**32**)*

To a solution of **31** (22.4 mg, 0.112 mmol) in THF (1.0 mL) were added isobutyl chloroformate (16 μL, 0.123 mmol) and *N*-methylmorpholine (14 μL, 0.127 mmol) at 0 °C. After 30 min, 40% methylamine solution in water (15 μL) was added to the reaction mixture. After being stirred for additional 2 h at room temperature, the mixture was poured into water, and extracted with EtOAc three times. The combined organic layer was washed with 0.5 M hydrochloric acid and brine, dried over sodium sulfate, and evaporated to give the crude of *N*-Methyl-2-[*c*-3,*c*-4-(dimethylmethylenedioxy)-*r*-1-cyclopentyl]acetamide. This crude was dissolved into 10% hydrogen chloride solution in MeOH (2.0 mL). After being stirred for 1 h at room temperature, the reaction mixture was evaporated. The residue was chromatographed over silica gel eluted by chloroform–MeOH (4:1) to give **32** (15.5 mg, 0.073 mmol, 65% (2 steps)). Data for **32**: colorless oil; ^{1}H NMR (400 MHz, CD_3OD) δ 3.91–3.97 (2H, m), 2.70 (3H, s), 2.19–2.28 (3H, m), 2.02–2.08 (2H, m), 1.34–1.42 (2H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 175.7, 74.5 (2C), 44.2, 38.2 (2C), 32.0, 26.2; EIMS *m*/*z* 173$[M]^+$, 155, 138, 129, 100, 73 (base); HREIMS *m*/*z* 173.1027 $[M]^+$ (173.1051 calcd. for $C_8H_{15}NO_3$).

In the similar procedure, compounds **37** (78% (2 steps)) and **40** (65% (2 steps)) were synthesized from **36** and **39**, respectively.

*3.1.23.1. Data for N-Methyl-4-(c-3,c-4-dihydroxycyclopent-r-1-yl) butanamide (**37**).* Colorless oil; ^{1}H NMR (400 MHz, CD_3OD) δ 1.30–1.47 (4H, m), 1.56–1.65 (2H, m), 1.76–1.84 (1H, m), 2.00–2.09 (2H, m), 2.18 (2H, t, J = 7.2 Hz), 2.72 (3H, s), 3.89–3.97 (2H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 176.7, 74.4 (2C), 38.8, 38.1 (2C), 37.0, 34.7, 26.3, 25.8; EIMS *m*/*z* 201 $[M]^+$, 183, 128, 100, 86, 73 (base); HREIMS *m*/*z* 201.1352 $[M]^+$ (201.1364 calcd. for $C_{10}H_{19}NO_3$).

*3.1.23.2. Data for N-Methyl-5-(c-3,c-4-dihydroxycyclopent-r-1-yl) pentanamide (**40**).* Colorless oil; ^{1}H NMR (400 MHz, CD_3OD) δ 3.85–3.91 (2H, m), 2.69 (3H, s), 2.16 (2H, t, J = 7.5 Hz), 1.97–2.07 (2H, m), 1.73–1.82 (1H, m), 1.52–1.62 (2H, m), 1.36–1.43 (2H, m), 1.23–1.35 (4H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 176.7, 74.5 (2C), 38.9 (2C), 38.3, 37.0, 34.8, 29.1, 26.7, 26.3; EIMS *m*/*z* 216 $[M + H]^+$, 197, 142, 128, 115, 100, 86, 73 (base); HREIMS *m*/*z* 216.1606 $[M + H]^+$ (216.1598 calcd. for $C_{11}H_{22}NO_3$).

*3.1.24. 3-[c-3,c-4-(Dimethylmethylenedioxy)-r-1-cyclopentyl]-1-propanol (**33**)*

Compound **6** (291 mg, 1.21 mmol) and 20% $Pd(OH)_2$ on carbon (30.0 mg) in MeOH (24 mL) was stirred at room temperature for 1 h under hydrogen atmosphere. After filtration, the filtrate was evaporated. The residue was dissolved in 2,2-dimethoxypropane (3.0 mL), and *p*TsOH (41.6 mg, 0.241 mmol) was added to this solution. After being stirred for 1 h at room temperature, the reaction mixture was poured into saturated sodium bicarbonate solution, and extracted with EtOAc three times. The combined organic layer was washed with brine, dried over sodium sulfate, and evaporated to give the crude of ethyl 3-[*c*-3,*c*-4-(dimethylmethylenedioxy)-*r*-1-cyclopentyl]propanoate.

The solution of this crude in THF (3.0 mL) was added to a suspension of lithium aluminum hydride (26.4 mg, 0.695 mmol) at 0 °C. After being stirred for 30 min, acetone and 5 M sodium hydroxide solution were added to the reaction mixture. Then, the mixture was filtered through a Celite pad, and the filter cake was washed with EtOAc. The filtrate was evaporated, and the residue was chromatographed over silica gel eluted by hexane–EtOAc (2:1) to give **33** (107 mg, 0.536 mmol, 44% (3 steps)).

*3.1.25. Ethyl (2E,4E)-5-[c-3,c-4-(dimethylmethylenedioxy)-r-1-cyclopentyl]-2,4- pentadienoate (**38**)*

TEMPO oxidation of **5** (201 mg, 1.17 mmol) gave the crude aldehyde by the same way in the synthesis of **6**. To a solution of triethyl 4-phosphonocrotonate (350 mg, 1.40 mmol) in toluene (5.0 mL) was added sodium hydride (60% mineral oil suspension) (70.4 mg, 1.76 mmol) at 0 °C. After 30 min, the crude aldehyde in toluene (1.0 mL) was added to the mixture. After being stirred for additional 1 h at room temperature, the mixture was poured into 0.5 M hydrochloric acid, and extracted with EtOAc three times. The combined organic layer was washed with saturated sodium bicarbonate solution and brine, dried over sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by hexane–EtOAc (19:1) to give **38** (179 mg, 0.672 mmol, 57% (2 steps)).

*3.1.26. 5-[c-3,c-4-(Dimethylmethylenedioxy)-r-1-cyclopentyl] pentanoic acid (**39**)*

Compound **38** (172 mg, 0.646 mmol) and 20% $Pd(OH)_2$ on carbon (8.7 mg) in MeOH (15 mL) was stirred at room temperature for 1 h under hydrogen atmosphere. After filtration, the filtrate was evaporated. The residue was dissolved in THF (10.0 mL) and 5 M sodium hydroxide solution (10.0 mL). After being refluxed for 48 h, the reaction mixture was poured into 3 M hydrochloric acid, and extracted with EtOAc three times. The combined organic layer was washed with brine, dried over sodium sulfate, and evaporated. The residue was dissolved in 2,2-dimethoxypropane (5.0 mL), and *p*TsOH (12.5 mg, 0.066 mmol) was added to this solution. After being stirred for 1 h at room temperature, the reaction mixture was poured into water, and extracted with EtOAc three times. The combined organic layer was washed with brine, dried over sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by hexane–EtOAc (1:1) to give **39** (65.0 mg, 0.268 mmol, 42% (3 steps)).

*3.1.27. Ethyl (E)-2-[c-3,c-4-(dimethylmethylenedioxy)-r-1-cyclopentyl]ethenesulfonate (**41**)*

TEMPO oxidation of **5** (189 mg, 1.10 mmol) gave the crude aldehyde by the same way in the synthesis of **6**. To a solution of ethyl diethyphosphorylmethanesulphonate [12] (572 mg, 2.20 mmol) in THF (6.0 mL) was added sodium hydride (60% mineral oil suspension) (66.0 mg, 1.65 mmol) at 0 °C. After 30 min, the crude aldehyde in THF (1.0 mL) was added to the mixture. After being stirred for additional 1 h at room temperature, the mixture was poured into 0.5 M hydrochloric acid, and extracted with EtOAc three times. The combined organic layer was washed with saturated sodium bicarbonate solution and brine, dried over sodium sulfate, and evaporated. The residue was chromatographed over

silica gel eluted by hexane–EtOAc (4:1) to give **41** (177 mg, 0.641 mmol, 58% (2 steps)).

*3.1.28. (E)-N-Methyl-2-(c-3,c-4-dihydroxycyclopent-r-1-yl) ethenesulfonamide (**43**)*

To a solution of **41** (41.5 mg, 0.150 mmol) in acetone (2.5 mL) was added tetrabutylammonium iodide (61.1 mg, 0.165 mmol). After being refluxed for 24 h, the reaction mixture was poured into water, and extracted with EtOAc three times. The combined organic layer was washed with brine, dried over sodium sulfate, and evaporated to give the crude of tetrabutylammonium salt.

To a solution of triphenylphosphine (86.6 mg, 0.330 mmol) in dichloromethane (1.0 mL) was added sulfuryl chloride (44.5 mg, 0.330 mmol) at 0 °C. After 5 min, a solution of the crude tetrabutylammonium salt in dichloromethane (1.0 mL) was added to the reaction mixture. After being stirred for 1 h at room temperature, this mixture was evaporated to afford the crude of sulfonyl chloride **41**.

This crude was dissolved in 1,4-dioxane (1.5 mL), and 40% methylamine solution in water (60 μL) was added to the solution. After being stirred for 2 h, the reaction mixture was poured into saturated ammonium chloride solution, and extracted with EtOAc three times. The combined organic layer was washed with brine, dried over sodium sulfate, and evaporated. The residue was dissolved into 10% hydrogen chloride solution in MeOH (1.5 mL). After being stirred for 1 h at room temperature, the reaction mixture was evaporated. The residue was chromatographed over silica gel eluted by chloroform–MeOH (9:1) to give **43** (6.2 mg, 0.028 mmol, 19% (4 steps)). Data for **43**: colorless amorphous solid; ^{1}H NMR (400 MHz, CD_3OD) δ 6.65–6.76 (1H, m), 6.17 (1H, d, J = 15.5 Hz), 3.96–4.07 (2H, m), 2.69–2.78 (1H, m), 2.58 (3H, s), 2.12–2.22 (2H, m), 1.57–1.67 (2H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 151.0, 126.3, 74.4 (2C), 38.3, 37.0 (2C), 29.1; EIMS m/z 222 $[M + H]^+$, 203, 177, 148, 126, 117, 83 (base); HREIMS m/z 222.0794 $[M + H]^+$ (222.0799 calcd. for $C_8H_{16}NO_4S$).

3.1.29. ex vivo Drosophila culture assay and cytotoxic assay

The detailed procedure was described previously [8]. Briefly, third-instar larvae were washed with lipopolysaccharide (LPS)-free water and LPS-free saline. The abdominal cavity of the larva was opened using fine pincettes in LPS-free saline. Individual whole larval tissues were cultured in Schneider's *Drosophila* medium (Gibco-BRL, Invitrogen, Carlsbad, CA) containing 20% fetal bovine serum (Valley Biomedical, Winchester, VA) and 1% antibiotics/antimycotics (Gibco-BRL) in each well of a 96-well plate at 25 °C. For each condition, six females were cultured to produce six replicates. The test compounds were dissolved in DMSO and added to the culture medium. To determine the effects of the test compounds on the innate immune response, *Dpt-lacZ* larvae were cultured in the presence of 10 μg/mL LPS (Nacalai Tesque, Kyoto, Japan) and the compound at 25 °C for 12 h. The expression of *Dpt-lacZ* is thought to be induced by diaminopimelic acid-containing peptidoglycans contaminating the LPS fraction [17]. The cultured individual larvae were sonicated with 200 μL reaction buffer (60 mM Na_2HPO_4, 40 mM NaH_2PO_4, 10 mM KCl, and 1 mM $MgCl_2$) using an Ultrasonic Processor (Misonix, New York, NY). After centrifugation (10000 × g) at 4 °C for 10 min, supernatant was harvested, and β-galactosidase activity and total protein amount of supernatant were determined as previously described [7]. β-Galactosidase activity was normalized to total protein amount.

Drosophila S2 cells were cultured in Schneider's medium (Gibco-BRL) supplemented with 20% FBS (Valley Biomedical) and 1% antibiotics/antimycotics (Gibco-BRL) at 25 °C. Cytotoxicity was measured using the colorimetric thiazoyl blue conversion assay as described previously [8].

3.2. Chemokine assay

The detailed procedure was described previously [8]. Briefly, human umbilical vein endothelial cells (HUVECs) were purchased from Lonza (Walkersville, MA), cultured in 25 cm^2 culture flasks, and maintained in EGM-2 medium (Lonza) at 5% CO_2, 37 °C. The cells were incubated in the presence or absence of the compound in a final volume of 100 μL for 3 h at 5% CO_2, 37 °C, and then 11 μL of 10 ng/mL *h*TNF-α was added to each well. After 12 h of incubation at 5% CO_2 and 37 °C, the HUVEC culture supernatants were harvested for enzyme-linked immunosorbent assay. Commercial enzyme-linked immunosorbent assay kits were used for immunologic quantification of *h*IL-8 (Biosource, Invitrogen).

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Supplementary data

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