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Pseurotin A and its analogues as inhibitors of immunoglobuline E production

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ABSTRACT

A natural product, pseurotin A inhibits IgE production in vitro. Wide variety of chemical modification of pseurotin A was performed. Structure–activity relationship studies of pseurotin analogues elucidated that 10-deoxypseurotin A strongly inhibits IgE production with IC_{50} of 0.066 μM. An immunosuppressive activity of another natural product, synerazol was also found.

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Atopy, characterized by raised immunoglobulin E (IgE) levels, underlies allergic diseases such as asthma, rhinitis, and dermatitis. Asthma is defined as a chronic inflammatory disorder of the airway in which many cells play a role, particularly mast cells, eosinophils and T lymphocytes. The resulting inflammation causes episodes of wheezing, breathlessness, chest tightness, and cough. About the medications, inhaled corticosteroids acting in a multifunctional manner are very effective at suppressing allergic symptoms, and remain the mainstay for treating serious asthma.[1] However, corticosteroids are rather non-specific in their action and their use raises concerns regarding side effects and compliance particularly in children and adolescents. Further, a recent clinical study showed that 51% of patients, who were prescribed with inhaled corticosteroids, were classified as having uncontrolled asthma.[2] For the oral treatments, drugs such as histamine and leukotriene antagonists target single effector molecules and provide some relief, but the heterogeneous nature of asthma makes this approach unsuccessful for most patients.[3]

IgE levels are elevated in allergic diseases and correlate well with severity of symptom.[4] Further, humanized anti-IgE monoclonal antibody omalizumab, which binds to free circulating IgE, has clinical efficacy in allergic asthma.[5] However, high cost or insufficient compliance with injection may impact on the active usage of this therapy. On the basis of this evidence, IgE production inhibitors as oral, disease modifying agents may be seen as a major therapeutic advance. So far, among the several classes of small molecule inhibitors of IgE production have been identified,[6–11] none is on the market or in clinical development. Herein, we describe the novel IgE production inhibitor, pseurotin A, and structure–activity relationship of pseurotin analogues.

The inhibitory activity of IgE production was evaluated as previously described manner.[12] Briefly, IgE responses were elicited from B-cells that were isolated from spleens of naive BALB/c mice and cultured 6 days with interleukin-4 and lipopolysaccharide (LPS). IgE levels were determined by enzyme-linked immunosorbent assay (ELISA). As a result of screening, an inhibitor of specific IgE production with IC_{50} of 3.6 μM was isolated from the fermentation broth of *Aspergillus* sp. This compound was identical to pseurotin A. The cytotoxicity, inhibition of phytohemagglutinin (PHA)-induced T-cell proliferation[13] or a B-cell viability of pseurotin A were far from the concentration that inhibited IgE production, which indicates that observed inhibition was not due to cytotoxicity or a general antiproliferative effect. Furthermore, pseurotin A did not inhibit mixed-lymphocyte reaction (MLR) [14] at 10 μM indicated that the observed inhibition was not due to non-specific immunosuppressive activity, but was effected on the B-cell. In contrast, a corticosteroid prednisolone inhibited IgE production with IC_{50} of 5.9 nM. But prednisolone also inhibited PHA-induced T-cell proliferation (84% at 10 μM) and MLR (IC_{50}: 78 nM). Therefore, the basic concept could be verified in relevant target cells.

Pseurotin A (**1**) is a microbial secondary metabolite isolated from the fermentation broth of *Pseudeurotium ovalis* STORK (*Ascomycetes*) in 1976,[15] and possesses a highly substituted 1-oxa-7-

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azaspiro[4,4]non-2-ene-4,6-dione skeleton. The core structure of **1** is found in other natural products, too (Fig. 1). These pseurotin-related natural products also possess interesting biological activities, including apomorphine-antagonistic activity of pseurotin A,[16a] D[16a] and F,[16b] chitin synthase inhibitory activity[17] of pseurotin A and F, monoamine oxidase inhibitory activity of pseurotin A,[18] induction of cell differentiation of pseurotin A,[19] antifungal activity and synergistic activity with azole-type antifungal agents of synerazol (**4**) isolated from the fermentation broth of *Aspergillus fumigatus* SANK 10588 in 1991,[20] and anti-angiogenic activity of azaspirene[21a] and synerazol.[21b] But immunomodulate activity of these natural products has not been reported.

These important biological properties of pseurotins and related natural products have attracted attention to structure–activity relationship (SAR) studies of their analogues. But only limited number of chemical modifications of pseurotin-related natural products obtained from fermentation have been reported as shown in Figure 2, that is, 12,13-dihydroxypseurotin A (**5a**),[22] 12,13-dibromopseurotin A (**5b**),[15a] 10,11-acetonide (**5c**),[15a] aldehyde **5d**,[15a,22b] (10*R*, 11*S*)-diastereomer of **4** (**5f**),[23] triacetylpseurotin A (**6a**),[15a] tetraacetylpseurotin A (**6b**),[15a] 7,9-*N*,*O*-diacetylsynerazol,[24] 17-dihydropseurotin A (**7**).[22a] Synthesis of **1** from **4**,[24] and syntheses of **4** and pseurotin E (**2**) from **1**[23] were also reported. Fluorinated **1** (**8a**, **8b**) and **4** were produced by the precursor-directed biosynthesis using fluorophenylalanine.[21a] Total syntheses of **1**,[25,26] pseurotin F_2 (**3**),[25,26] **4**[27] and azaspirene[28,26] were reported by Hayashi et al. and Tadano et al. But total syntheses of pseurotin analogues[27] were reported few.

The novel pharmacological activity of **1** and limited number of its analogues reported prompted us to modify **1** obtained from fermentation. We envisioned that **5d** obtained via oxidative cleavage of **1** could be used for the modification of the C10–C15 side chain of **1** with structural diversity (Scheme 1). First of all, Wittig reaction of stabilized ylides or Horner–Wadsworth–Emmons (HWE) reaction[29] with **5d** afforded (*E*)-olefin **9a–d**. Consecutive Wittig reaction with **9c** provided (*E*, *E*)-diene **9e** which resembled azaspirene. (*Z*)-olefin **9f** was also synthesized under HWE reaction with the Ando reagent.[30] Reductive amination of **5d** with a variety of amines afforded **10a–f**. Next, the reported olefin cross metathesis[23] method was applied to substrates and gave **11a–f**. Hydrogenation of **11b–e** gave various saturated side chain **12b–e**. As results, we efficiently synthesized pseurotin analogues which possesses variously modified the side chain.

Hydroxyl groups, methoxyl group and lactam moiety were modified as following. Selectively methylated analogues **13**–**15** were synthesized with MeI and K_2CO_3, MeI and Ag_2O,[31] or Me_3OBF_4.[32] Ethyl acetalization of **1** in acidic condition[26] provided **16**. 9-Deoxypseurotin A (**18**) was synthesized by Robins's deoxygenation condition.[33] In detail, treatment of **1** with thioacyl chloride and DMAP in CH_3CN gave 9-*O*-phenoxythiocarbonyl derivative **17a**. While investigating the thiacylation conditions, 10-*O*-thioacylated **17b** was obtained in 46% yield when pyridine as base and DCM as solvent were used. Reductive cleavage of **17a–b** gave **18** and 10-deoxypseurotin A (**19**), respectively.

12, 13-epoxypseurotin A (**20**) and 12,13-cyclopropylpseurotin A (**21**) were synthesized from **1** via epoxidation or modified Sim-

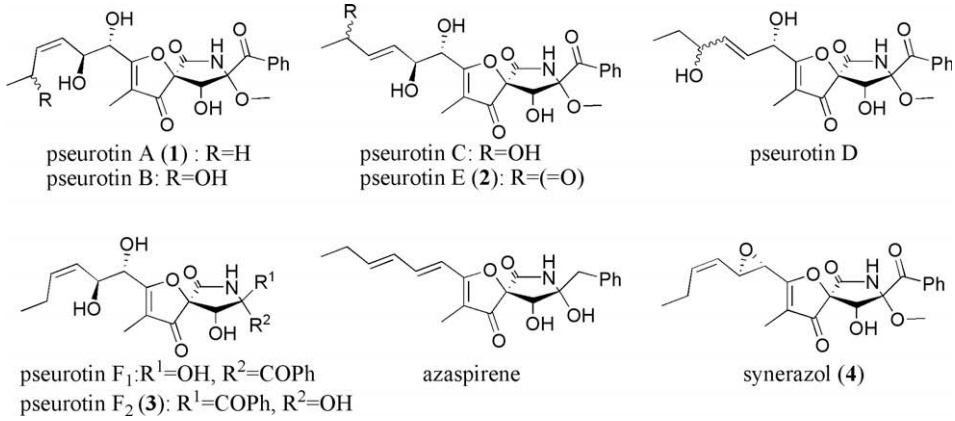


Figure 1. Structure of pseurotin-related natural products.

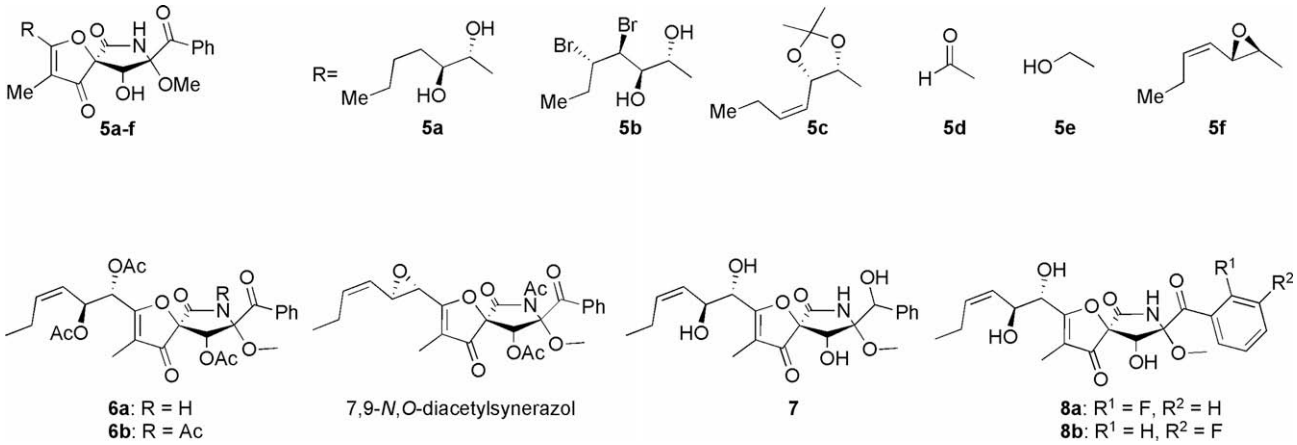
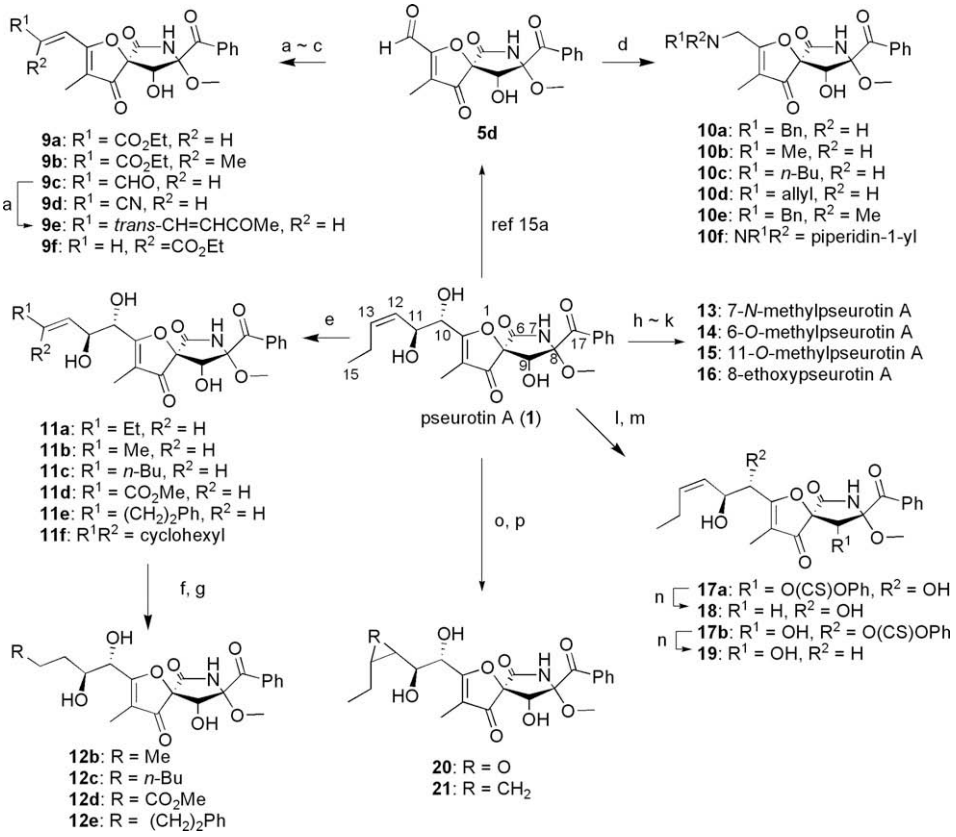


Figure 2. Reported pseurotin analogues.



Scheme 1. Chemical modification of pseurotin A. Reagents and conditions: (a) $Ph_3P=CR^1R^2$, THF, 4 °C, 50% (**9a**), 72% (**9b**), 85% (**9c**), 55% (**9e**); (b) $(EtO)_2POCH_2CN$, *i*-Pr_2EtN, LiCl, CH_3CN, −30 °C, 9% (**9d**); (c) $(PhO)_2POCH_2CO_2Et$, Triton B, THF, −78 °C, 22% (**9f**); (d) R^1R^2 NH, $NaBH_3CN$, AcOH, MeOH, 4 °C–rt, 28% (**10a**), 14% (**10b**), 11% (**10c**), 19% (**10d**), 19% (**10e**), 10% (**10f**); (e) Grubbs second generation, DCM, 40 °C, (*E*)-hex-3-ene, 70% (**11a**), but-2-ene, 64% (**11b**), hex-1-ene, 61% (**11c**), methyl acrylate, 80% (**11d**), but-3-enylbenzene, 91% (**11e**), methylenecyclohexane, 25% (**11f**); (f) $(PPh_3)_3RhCl$, H_2, toluene, EtOH, rt, 53% (**12b**); (g) Pd/C, H_2, EtOH, rt, 53% (**12c**), 30% (**12d**), 70% (**12e**); (h) MeI, K_2CO_3, DMF, 4 °C, 60% (**13**); (i) MeI, Ag_2O, CH_3CN, 100 °C (μW), 3% (**14**); (j) Me_3OBF_4, K_2CO_3, DCM, rt, 32% (**15**); (k) AcCl, EtOH, 30 °C, 28% (**16**); (l) PhO(CS)Cl, DMAP, CH_3CN, rt, 19% (**17a**); (m) PhO(CS)Cl, pyridine, DCM, 7 °C, 46% (**17b**); (n) *n*-Bu_3SnH, AIBN, toluene, 75 °C, 26% (**18**), 46% (**19**) (o) mCPBA, DCM, rt, 55% (**20**); (p) $ZnEt_2$, $ClCH_2I$, DCE, 4 °C, 26% (**21**).

mons–Smith reaction.[34] Stereochemistry of **20** and **21** have not been determined.

Both known and newly synthesized pseurotin analogues were evaluated for the inhibitory activity of IgE production. First of SAR studies, available pseurotin related natural products (**2**,[23] **3**,[35] and **4**[23] in Fig. 1) were evaluated for IgE production inhibition (Table 1). Among them, **4** showed ten times stronger IgE inhibitory activity compared with **1** (IC_{50}: 0.26 μM).

(10*R*, 11*S*)-Diastereomer **5f**[23,27] was inactive, suggesting that stereochemistry of the epoxide is important for the activity. C12–C13 (*E*)-olefin **2** and **11a–e**, trisubstituted olefin **11f**, 12,13-dihydro derivatives **5a** and **12b–e**, 12,13-epoxide **20** and 12,13-cyclopropane **21** did not inhibit IgE production, suggesting that C12–C13 (*Z*)-olefin is essential for the activity. 11-*O*-methyl analog **15** lost the activity indicated that 11-hydroxy group is also important for the activity. 10-Deoxy analog **19** showed fifty times more potent activity than **1** with IC_{50} of 0.066 μM. This result suggested that 10-hydroxy group is unnecessary for the activity. *n*-Butyl amino derivative **10c** was equipotent to **1** (IC_{50}: 3.1 μM). The amino group might be mimic to 11-hydroxy group.

Modification of the spiro skeleton (**3**, **13**, **14**, **16** and **18**) or the benzoyl moiety (**7** and **8a–b**) was almost inactive. These results indicated that the spiro skeleton and the benzoyl moiety are important for the activity.

Potent inhibitors[36] of IgE production were further investigated for the specificity (Table 2). The K562 cytotoxicity, or the B-cell viability of these inhibitors were far from the concentration that inhibited IgE production. In addition, the most potent inhibitor **19** did not inhibit PHA-induced T-cell proliferation (10 μM). Furthermore, the specificity of **19** for other immunoglobulin were investigated. The IC_{50}s of immunoglobulin[37] were IgE selective

Table 1
IgE inhibitory activity of pseurotin analogs

Compound	IgE production IC_{50} (μM)
1	3.6
2	>10
3	>10
4	0.26
5a–f	>10
6a–b	>10
7	>10
8a–b	>10
9a–f	>10
10a	>10
10b	>10
10c	3.1
10d	>10
10e	>10
10f	>10
11a–f	>10
12b–e	>10
13	>10
14	>10
15	>10
16	>10
18	>10
19	0.066
20	>10
21	>10

Table 2
Specificity of IgE production inhibitors

Compound	IgE production IC_{50} (μM)	K562 cytotoxicity IC_{50} (μM)	B-cell viability IC_{50} (μM)	T-cell proliferation % inhibition at 10 μM	MLR IC_{50} (μM)
1	3.6	>10	>10	0%	>10
4	0.26	>10	>10	54%	3.1
10c	3.1	>10	>10	2%	nt[a]
19	0.066	>10	4.4	0%	nt[a]
Prednisolone	0.0059	nt[a]	>10	84%	0.078

[a] Not tested.

(IgM/IgE: 44, IgG2a/IgE: 23, IgG1/IgE: 3.5). In contrast, prednisolone inhibited IgE, IgG and IgM production with no selectivity (IgM/IgE: 0.4, IgG2a/IgE: 0.8, IgG1/IgE: 1.0). These data suggested that **19** might be potent and specific inhibitor of IgE production. On the other hand, surprisingly, **4** showed T cell proliferation-inhibitory activity of 54% inhibition at 10 μM. Furthermore, **4** inhibited MLR with IC_{50} of 3.1 μM, indicating **4** has also immunosuppressive activity.

In summary, we found the inhibitory activity of the IgE production of the natural product, pseurotin A (**1**). Wide variety of chemical modification of **1**, especially the C10–C15 side chain was performed by, that is, Wittig olefination, reductive amination, olefin cross metathesis. Structure–activity relationship of pseurotin analogues revealed the pharmacophore and elucidated that 10-deoxypseurotin A (**19**) inhibits IgE production with IC_{50} of 0.066 μM. We also showed the immunosuppressive activity of the pseurotin A analogue, synerazol (**4**). Other biological activities of synthesized analogues, including reported activities of pseurotin related natural products, are also interesting.

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36. **10c**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.59 (3H, t, J = 7.3 Hz), 1.04 (2H, tq, J = 7.6, 7.3 Hz), 1.24 (2H, tt, J = 7.6, 7.3 Hz), 1.43 (3H, s), 2.39–2.54 (2H, m), 3.07 (3H, s), 3.56 (2H, d, J = 4.1 Hz), 4.37 (1H, s), 7.17 (2H, dd, J = 7.6, 8.2 Hz), 7.33 (1H, br t, J = 7.6 Hz), 8.00 (2H, br d, J = 8.2 Hz); FABMS m/z 403 $(M+H)^+$.**19**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.98 (3H, t, J = 7.6 Hz), 1.57 (3H, s), 2.04–2.18 (2H, m), 2.61 (1H, dd, J = 3.9, 10.2 Hz), 2.91 (1H, dd, J = 5.1, 9.0 Hz), 3.14 (1H, d, J = 4.4 Hz), 3.39 (3H, s), 4.16 (1H, d, J = 12.7 Hz), 4.64 (1H, d, J = 12.7 Hz), 4.92–4.99 (1H, m), 5.44–5.56 (2H, m), 7.37 (1H, br s), 7.50 (2H, br dd, J = 7.3, 8.4 Hz), 7.66 (1H, br t, J = 7.3 Hz), 8.33 (2H, dd, J = 1.2, 8.4 Hz); ESIMS m/z 416 $(M+H)^+$.
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