Polyenylidene Thiazolidine Derivatives with Retinoidal Activities

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Several polyenylidene thiazolidinedione or 2-thioxo-4-thiazolidinone derivatives were synthesized and their retinoidal activities were examined in terms of the differentiation-inducing ability towards human promyelocytic leukemia HL-60 cells and inhibitory effect on interleukin (IL)-1α-induced IL-6 production in MC3T3-E1 cells. Compounds containing a trimethylcyclohexenyl ring induced HL-60 cell differentiation with weaker activity than retinoic acid (1a) by one or two orders of magnitude. The thiazolidinedione derivatives (2, 5, 7) showed stronger activity than the corresponding 2-thioxo-4-thiazolidinone derivatives (3, 6, 8). The effects of a retinoid antagonist (LE540) and synergists (retinoid X receptor (RXR) agonists, HX600 or HX630) on the activities of thiazolidine derivatives indicate that these compounds elicit their activities through the nuclear retinoic acid receptors (RARs). All the thiazolidines examined also inhibited IL-1α-induced IL-6 production with IC_{50} values of 10 nM order. The retinoidal activities of the thiazolidines are significant, considering that replacement of the carboxylic acid in retinoid structures with bioisosteric functional groups is generally ineffective, as seen in the structure-activity relationships of retinoidal benzoic acids.

Key words thiazolidinedione; retinoic acid; retinoid; differentiation; interleukin-6

Retinoic acid (all-*E*, **1a**), an oxidative metabolite of vitamin A (retinol), acts as an internal hormone that regulates a number of biological functions, such as cell differentiation, proliferation and embryonic development in vertebrates.[1] The pleiotropic activities of retinoic acid (**1a**) are mediated by binding to and activating two classes of nuclear receptors, retinoic acid receptors (RAR α, β, γ) and retinoid X receptors (RXR α, β, γ), both of which belong to the steroid/thyroid hormone nuclear receptor superfamily, and are ligand-inducible transcription factors.[2] Studies using various synthetic analogs of retinoic acid (**1a**), so-called retinoids, show that the heterodimerization of RARs and RXRs, activated by RAR lignds, predominantly controls the retinoidal actions *in vivo*. RXRs, whose endogenous ligand is considered to be 9*Z*-retinoic acid (**1b**), do not require a ligand for RXR–RAR heterodimer activation, but RXR ligands are known to exhibit synergism with RAR ligands.[3,4] Furthermore, RXR ligands can regulate the activities of other hormones besides retinoic acid (**1a**), since RXRs form heterodimers with other nuclear receptors, such as thyroid hormone receptors, vitamin D_3 receptors, and peroxisome proliferative activated receptors (PPARs).[2,5]

Clinically, retinoids are used in the treatment of proliferative dermatological diseases and leukemia, and in the prevention of some tumors.[6] However, the applicability of retinoid therapy is restricted by the toxic effects of retinoic acid itself or synthetic retinoids. Therefore, the development of new-type retinoids with different pharmacological activities is important to extend the clinical utility of retinoids.

Recently, several thiazolidines were reported to bind to some nuclear receptors. CGP52608, possessing antiarthritic activity in rat adjuvant arthritis, showed affinity to the orphan nuclear receptor, retinoid Z receptor/retinoic acid receptor-related orphan receptor α (RZR/RORα).[7] More importantly, thiazolidinediones with antidiabetic activities, such as ciglitazone and pioglitazone, bind to and activate PPARγ,[8,9] and a relationship of PPARγ to non-insulin-dependent diabetes has been suggested. PPARγ can bind to 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2, a possible endogenous ligand,[9] and also to non-steroidal anti-inflammatory drugs (NSAIDs), including indomethacin and ibuprofen.[10] These results indicated that the thiazolidinedione skeleton may interact with the same site of the PPARγ ligand binding domain as the carboxyl group of prostaglandin or NSAIDs. This idea is supported by the fact that replacement of the thiazolidinedione group of ciglitazone analogs with an α-alkoxy or α-alkylthio carboxyl group did not destroy the glucose-lowering potency.[11] To examine whether the apparent biological isosteric relationship between the thiazolidinedione moiety and the carboxyl group applies to retinoid structures, we designed several thiazolidines bearing a polyene chain (**2**—**9**, Chart 1) as new-type retinoid candidates. Here, we describe the synthesis and retinoidal activities of these thiazolidine derivatives.

Chemistry

The synthetic routes to the thiazolidine derivatives **2**—**4** are shown in Chart 2. Horner–Emmons reaction of β-ionone (**10**) with triethyl (*E*)-phosphonocrotonate using NaH as a base afforded a mixture of two isomeric tetraenoic esters (**11a**, **11b**) in 16% yield. The reaction using other bases such as *n*-BuLi or lithium diisopropylamide (LDA), or Reformatsky reaction of β-ionone (**10**) with ethyl (*E*)-bromocrotonate resulted in a lower yield of **11**. After reduction of the ester group with diisobutylaluminium hydride (DIBAL), the two isomers were separated, and each was oxidized with active MnO_2 to the all-*E*-aldehyde **13a** and its 4*Z* isomer **13b**, whose stereochemistries were determined by ^{1}H-NMR spectra. The Knoevenagel reaction of the all-*E*-aldehyde **13a** and 2,4-thiazolidinedione or 2-thioxo-4-thiazolidinone in the pres-

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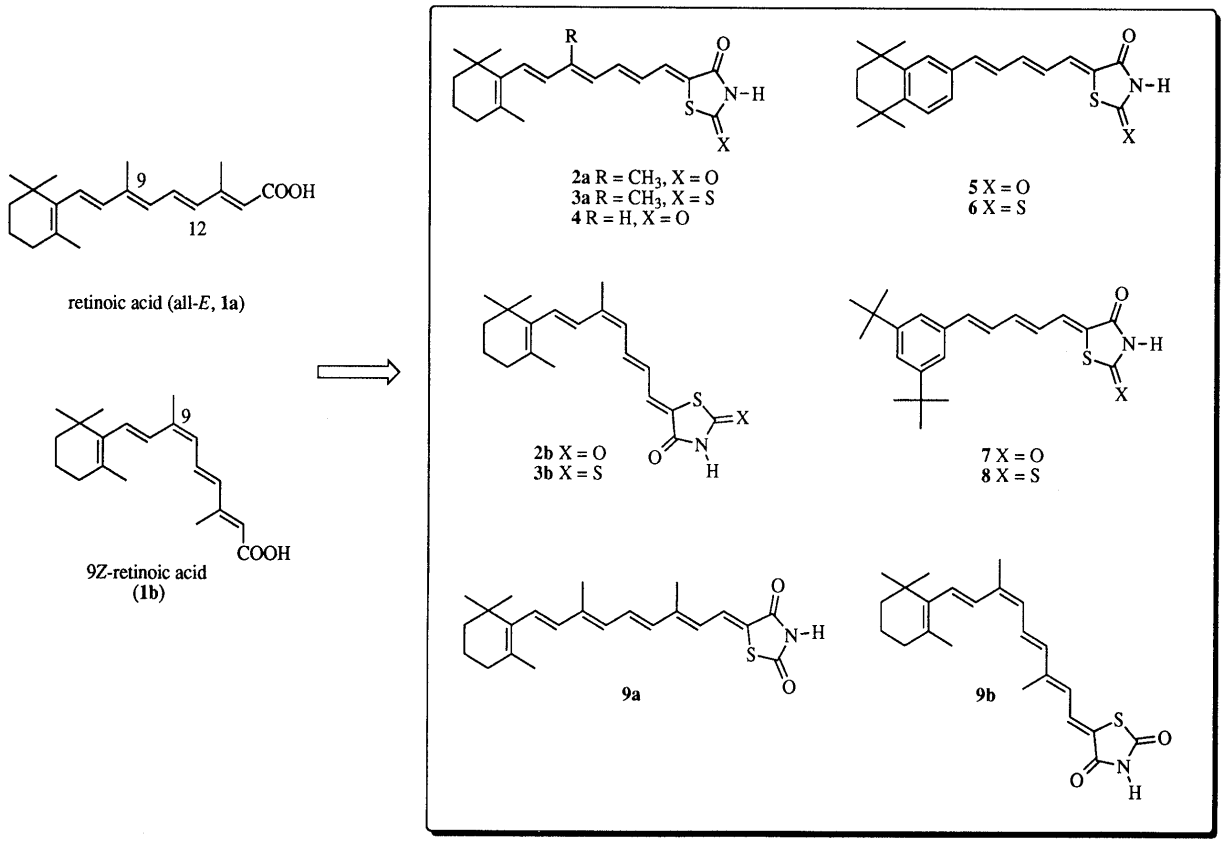
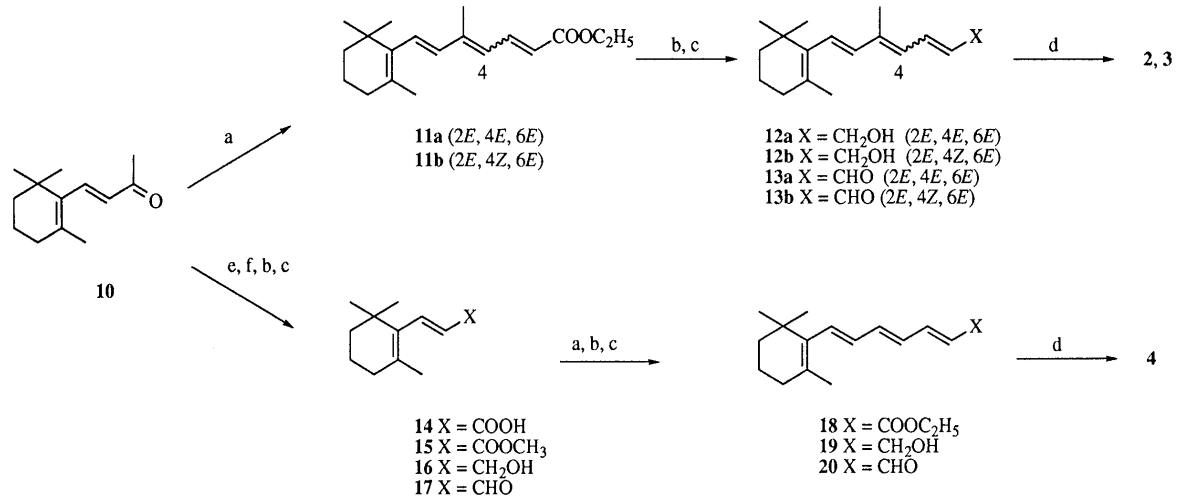


Chart 1



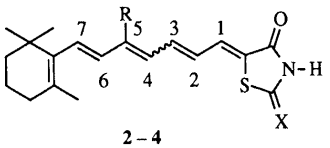
(a) NaH or *n*-BuLi / THF; (*E*)-$(EtO)_2P(O)CH_2CH{=}CHCOOC_2H_5$, (b) DIBAL / THF (c) active MnO_2 / CH_2Cl_2 (d) 2,4-thiazolidinedione or 2-thioxo-4-thiazolidinone / piperidine / AcOH / toluene (e) Br_2 / NaOH (f) $TMSCHN_2$ / CH_3OH

Chart 2

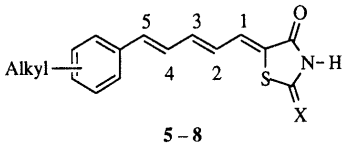
ence of piperidine and acetic acid in toluene gave a single condensed product, **2a** or **3a**, respectively. In the case of **13b**, the isomerization occurred even at rather lower temperature to afford a mixture of two isomers (**2a** : **2b** = 1 : 1 for 2,4-thiazolidinedione or **3a** : **3b** = 2 : 3 for 2-thioxo-4-thiazolidinone).

Compound **4**, the desmethyl analog of **2a**, was also prepared from β-ionone (**10**, Chart 2). The haloform reaction of β-ionone (**10**) using bromine under the basic conditions afforded (*E*)-3-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-propenoic acid (**14**),[12] which was converted to the corresponding aldehyde (**17**). Horner–Emmons reaction of **17** with triethyl (*E*)-phosphonocrotonate afforded a (2*E*,4*E*,6*E*)-tetraenoic ester **18** as a single product. The stereochemistry of **18** was determined from the ^{1}H-NMR chemical shifts and coupling constants (J = 14—16 Hz). After transformation of the ester group of **18** to the aldehyde, the Knoevenagel condensation of **20** with 2,4-

Table 1. ^{1}H-NMR Data for Thiazolidines (**2**—**8**)



2-4



5-8

Compound	Chemical shifts (ppm) and coupling constants (Hz, in parenthesis)						
	H_1	H_2	H_3	H_4	H_5	H_6	H_7
2a	7.47 (11.7)	6.15 (14.3, 12.1)	7.11 (14.3, 11.7)	6.22 (11.7)	—	6.18 (16.1)	6.43 (15.7)
2b	7.46 (12.1)	6.08 (14.7, 11.7)	7.20 (14.3, 12.1)	6.13 (11.7)	—	6.68 (15.8)	6.41 (15.8)
3a	7.32 (11.7)	6.16 (13.9, 12.5)	7.16 (13.9, 12.1)	6.24 (12.1)	—	6.20 (16.1)	6.47 (16.1)
3b	7.31 (11.7)	6.10 (14.3, 11.7)	7.24 (14.3, 11.7)	6.14 (13.2)	—	6.70 (15.8)	6.48 (15.8)
4	7.42 (11.4)	6.11 (14.3, 11.7)	6.78 (14.7, 11.4)	6.34 (14.7, 11.7)	6.60 (14.7, 11.0)	6.22 (15.4, 10.6)	6.43 (15.4)
5	7.46 (11.7)	6.25 (13.4, 11.9)	6.89 (13.7, 9.7)	6.84 (14.8, 10.4)	6.89 (14.3)	—	—
6	7.30 (11.7)	6.26 (13.4, 11.7)	6.91 (17.2, 9.2)	6.87 (16.1, 9.9)	6.93 (15.8)	—	—
7	7.47 (11.7)	6.28 (14.2, 11.7)	6.92 (14.3, 12.1)	6.91 (13.9, 11.7)	6.95 (15.4)	—	—
8	7.31 (11.0)	6.29 (14.3, 12.1)	6.95 (18.5, 12.3)	6.93 (15.8, 12.6)	6.98 (15.3)	—	—

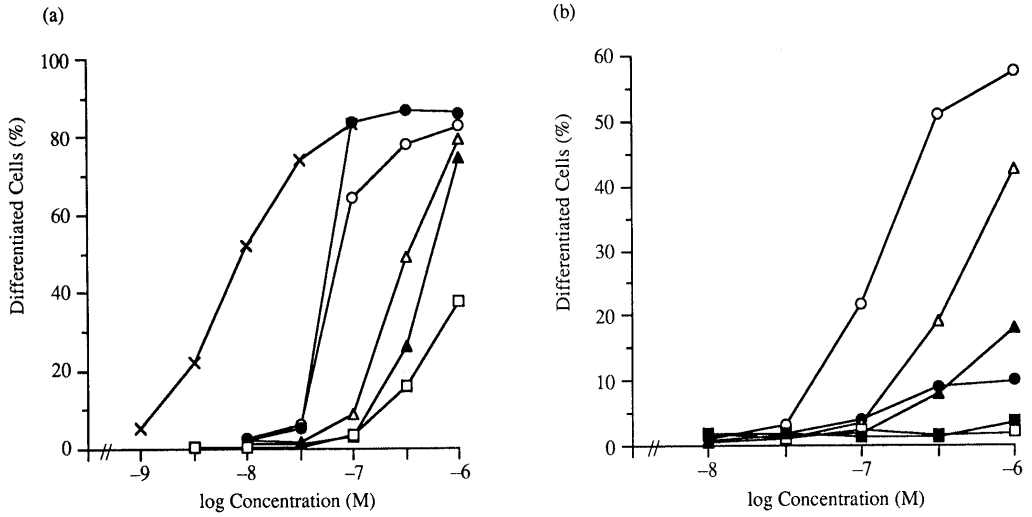


Fig. 1. Differentiation-Inducing Activity of Thiazolidine Derivatives in HL-60 Cells

The vertical scale is the percentage of differentiated cells evaluated from NBT reduction assay data, and the horizontal scale is the molar concentration of test compounds. Added compound: (a) retinoic acid (×), **2a** (○), **2b** (●), **3a** (△), **3b** (▲), or **4** (□), and (b) **5** (△), **6** (▲), **7** (○), **8** (●), **9a** (□), or **9b** (■).

thiazolidinedione afforded **4**.

Thiazolidines (**5**—**8**) with an aromatic ring as the hydrophobic region were synthesized similarly to compound **4**, using the corresponding alkylated benzaldehydes, which were prepared by Friedel–Craft reaction of toluene with 2,5-dichloro-2,5-dimethylhexane or *tert*-butyl chloride, followed by oxidation of the methyl group on the aromatic ring with ceric ammonium nitrate.[13] Two isomeric thiazolidinediones (**9**) having a longer polyene chain were obtained as a mixture by the reaction of 9*Z*-retinal with 2,4-thiazolidinedione, and were purified by column chromatography and recrystallization.

Stereochemistries of the thiazolidines synthesized were determined by analysis of the ^{1}H-NMR spectra (Table 1). The (4*E*)/(4*Z*) isomers of **2** and **3** can be distinguished in terms of the chemical shifts of H_3, H_4 and H_6. In particular, the signals of the H_6 protons of **2a** (6.18 ppm) and **3a** (6.20 ppm) are observed at 0.5 ppm higher field than those of **2b** (6.68 ppm) and **3b** (6.70 ppm), which correlates well with the difference between all-*E*- (**1a**) and 9*Z*-retinoic acid (**1b**). The stereochemistries of the side chains in **4**—**8** can be easily assigned as (2*E*,4*E*) from the couping constants (13—16 Hz). In each Knoevenagel reaction, only one isomer concerning the geometry of the double bond attached to the thiazolidine ring was obtained. From a consideration of the condensation mechanism, steric control would favor the formation of *Z*-isomers. The signals of the H_1 protons are observed at low field (7.42—7.47 ppm for thiazolidinediones, and 7.30—7.32 for 2-thioxo-4-thiazolidinones), and those of the H_2 protons at rather high field (6.08—6.30 ppm). The chemical shifts of H_{12} (corresponding H_2 in the thiazolidines, Chart 1) of all-*E*-retinoic acid (**1a**) and its 13*Z* isomer are 6.31 and 7.74 ppm, respectively. Therefore, the double bond attached to the thiazolizine ring of **2**—**9** could be assigned as (*Z*)-form with the carbonyl group directed toward the H_1 protons.

Biological Activity

Retinoidal activities of polyenylidene thiazolidines (**2**—

9) were examined based on differentiation-inducing ability towards human promyelocytic leukemia HL-60 cells (Fig. 1)[14] and their EC_{50} values were calculated from the nitro-blue tetrazolium (NBT) reduction assay data (Table 2).[15] The thiazolidines **2**—**8** induced HL-60 cell differentiation into mature granulocytes in the same manner as retinoic acid (**1a**). The thiazolidinediones (**2**) are more active than the corresponding 2-thioxo-4-thiazolidinones (**3**) by one order of magnitude. In both cases, there is no significant difference in activity between the (4*E*)- and (4*Z*)-isomers, as in the case of retinoic acid (**1a**) and 9*Z*-retinoic acid (**1b**), in HL-60 differentiation assay.[16] The potencies of **2a** (EC_{50}, 8.9×10^{-8} M) and **2b** (6.0×10^{-8} M) are about one-tenth of that of retinoic acid (**1a**, 8.7×10^{-9} M). Two isomeric compounds having a longer polyene chain were completely inactive below 1.0×10^{-6} M.

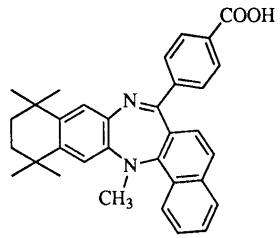
Compound **4**, a 9-desmethyl analog of **2a**, showed only weak differentiation-inducing activity towards HL-60 cells. Similarly, the thiazolidinediones with an aromatic moiety instead of the trimethylcyclohexenyl ring of **2** exhibited weak activity. These aromatic derivatives also lack the methyl group on the polyene chain.

In order to clarify the action mechanism of the thiazolidines, we examined the effects of retinoid-regulatory azepine derivatives (Chart 3) on the HL-60 differentiation induced by the thiazolidines. LE540 is an RAR pan-antagonist,[17] and HX600 and HX630 are RXR pan-agonists which exhibit no retinoidal activity alone, but enhance the activities of various RAR agonists in HL-60 assay.[3] As shown in Fig. 2, the differentiation-inducing activities of three thiazolidinediones (**2a**, **2b**, **5**) were completely inhibited by addition of 1.0×10^{-6} M LE540. Similarly, LE540 inhibited HL-60 differentiation induced by 2-thioxo-4-thiazolidinones **3** (data not shown). On the other hand, the retinoid synergists HX600 and HX630 increased the potency of all the thiazolidines in HL-60 cell

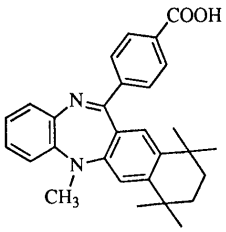
Table 2. Retinoidal Activities of Thiazolidine Derivatives

Compound	HL-60 assay; EC_{50}, M[a]			IL-6 assay IC_{50}, M
	Alone[b]	+HX600 (1.0×10^{-7} M)	+HX630 (1.0×10^{-7} M)	
Retinoic acid (**1a**)	8.7×10^{-9}	—	—	3.0×10^{-9}
2a	8.9×10^{-8}	3.6×10^{-8}	1.9×10^{-8}	1.4×10^{-8}
2b	6.0×10^{-8}	4.1×10^{-8}	2.3×10^{-8}	1.9×10^{-8}
3a	2.9×10^{-7}	7.9×10^{-8}	3.5×10^{-8}	—
3b	4.6×10^{-7}	1.2×10^{-7}	5.5×10^{-8}	—
4	$>1.0\times10^{-6}$	8.6×10^{-8}	2.7×10^{-8}	—
5	$>1.0\times10^{-6}$	6.3×10^{-8}	2.3×10^{-8}	—
6	$>1.0\times10^{-6}$	8.5×10^{-8}	4.4×10^{-8}	—
7	3.2×10^{-7}	5.2×10^{-8}	1.7×10^{-8}	1.6×10^{-8}
8	$>1.0\times10^{-6}$	1.3×10^{-7}	3.5×10^{-8}	2.7×10^{-7}
9a	Inactive	$>1.0\times10^{-6}$	8.0×10^{-7}	—
9b	Inactive	1.0×10^{-6}	3.2×10^{-7}	—

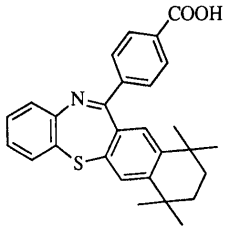
a) EC_{50} value was calculated from NBT reduction assay data. *b*) "Inactive" means there was no activity at 1.0×10^{-6} M test compound. "$>1.0\times10^{-6}$ M" means there was slight activity at 1.0×10^{-6} M test compound.



LE540
(RAR Antagonist)



HX600
(RXR Agonist)



HX630
(RXR Agonist)

Chart 3

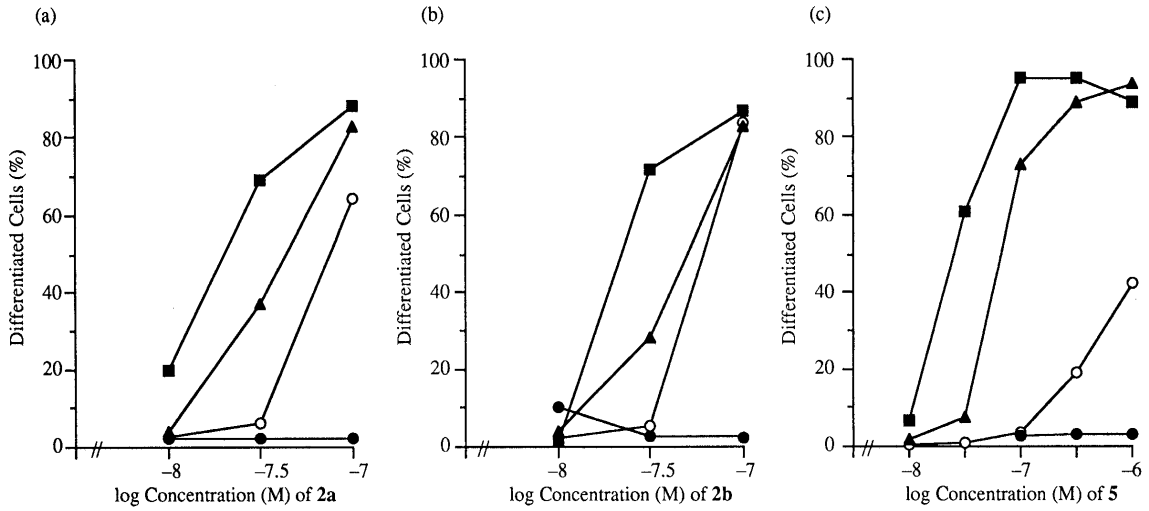


Fig. 2. Effects of a Retinoid Antagonist and Retinoid Synergists on Differentiation-Inducing Activity of Thiazolidine Derivatives **2a** (a), **2b** (b), and **5** (c) in HL-60 Cells

The vertical scale is the percentage of differentiated cells evaluated from NBT reduction assay data, and the horizontal scale is the molar concentration of thiazolidines. Added compound: none (thiazolidine only, ○), 1.0×10^{-6} M LE540 (●), 1.0×10^{-7} M HX600 (▲) or 1.0×10^{-7} M HX630 (■).

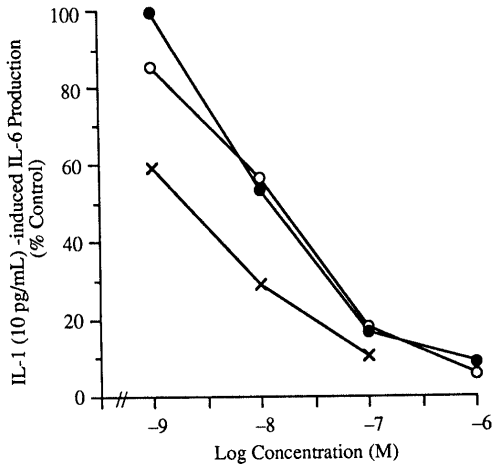


Fig. 3. Inhibition of IL-1-Induced IL-6 Production by Thiazolidine Derivatives (**2**) in MC3T3-E1 Cells

The vertical scale is IL-1α-induced IL-6 production (percentage of control, IL-1a only), and the horizontal scale is the molar concentration of retinoic acid or thiazolidines. Added compound: retinoic acid (**1a**, ×), **2a** (○) or **2b** (●).

differentiation assay (Table 2). The apparent EC_{50} value of **2a** (alone, 8.9×10^{-8} M) was reduced to half (3.6×10^{-8} M) or one-fourth (1.9×10^{-8} M) by the addition of 1.0×10^{-7} M HX600 or HX630, respectively. Drastic increases in the activity caused by the addition of a retinoid synergist were observed for weak differentiation-inducers such as **5** and **7**, which were as active as **2a** in the presence of 1.0×10^{-7} M HX630. Even the inactive compounds **9** showed significant activity under this condition. Thus, the differentiation-inducing activities of the thiazolidines were inhibited by the addition of a retinoid antagonist and enhanced by retinoid synergists.

The inhibition of the production of the multifunctional cytokine interleukin-6 (IL-6) by retinoids is significant, since abnormal expression of IL-6 is related to the pathogenesis of several diseases, such as psoriasis and rheumatoid arthritis,[18] and the suppression of IL-6 production by retinoids has raised the possibilty of clinical applicability of retinoids in various IL-6 associated diseases.[19] We therefore examined the effects of four selected thiazolidines on IL-6 production in mouse 3T3-E1 cells. Thiazolidines **2** alone did not affect IL-6 production, but inhibited IL-6 production induced by IL-1α (50 pg/ml) in the cells in a dose-dependent manner (Fig. 3). Thus, addition of 1.0×10^{-7} M **2a** or **2b** decreased IL-6 production to the basal level (EtOH only). The IC_{50} values are 1.4×10^{-8} M for **2a** and 1.9×10^{-8} M for **2b**, being about one-fifth of that of retinoic acid (**1a**, 3.0×10^{-9} M). Interestingly, compounds **7** and **8**, which alone are very weak inducers of HL-60 cell differentiation, inhibited IL-6 production, and **7** is as active as **2** with an IC_{50} value of 10 nM order.

Discussion

In this decade, studies on structure-activity relationships of retinoids, coupled with the finding of specific nuclear receptors and the elucidation of their physiological functions, have led to the development of various retinoids with characteristic properties, including receptor-selective or antagonistic compounds.[1] Retinoids consist structurally of three parts, that is, a hydrophobic region, a terminal polar group, and a linking group of appropriate conformation. In contrast to the marked variability that is permitted in the hydrophobic moiety and the linking group,[20] the replacement of the carboxyl group in retinoidal benzoic acids with a bioisosteric group, such as an aminosulfonyl, amidino or tetrazolyl group, causes the loss of the activity in HL-60 differentiation assay.[21] The results of this study show that the retinoidal carboxylic acid can be replaced with a thiazolidinone moiety, and compounds **2a** and **2b** are potent differentiation-inducers of HL-60 cells. Among the synthesized compounds, the thiazolidinedione derivatives were always more active than the corresponding 2-thioxo-4-thiazolidinones, suggesting that the thioxo group has an unfavorable electronic or steric interaction with the receptors.

The structure–activity relationship of the thiazolidines shows some similarity to that of retinoic acid derivatives, as follows: (1) The difference between the activities of the isomers, corresponding to the 9-position of retinoic acid (Chart 1), is small (**2a** *vs.* **2b**, or **3a** *vs.* **3b**) in the HL-60 assay. (2) The elongation of the polyene chain, yielding compounds **9**, diminished the activity. (3) The introduction of a methyl group on the polyene chain of **2a** increased the differentiation-inducing potency, compared to the weak activity of **4** or the aromatic derivatives (**5**, **7**). Concerning the last point, the role of the 19-methyl group of retinoic acid (**1a**) seems not to be significant in view of the structures of potent retinoidal benzoic acids.[1,20] In the case of polyenecarboxylic acids, the introduction of a polar group on the 19-methyl group of retinoic acid (**1a**) or the replacement of the 9,10-*trans*-olefinic bond with a secondary amide bond decreased the differentiation-inducing activity.[12,16] Some ealier studies on vitamin A suggested the significance of the methyl group on the side chain of retinol for mouse growth promotion[22] or in the case of retinal in the visual system.[23] The 9-methyl group may have some role related to the conformation, isomerization or pharmacological behavior of retinoic acid (**1a**).

Comparison of the structure–activity relationships suggests that the thiazolidines (**2**—**8**) act as retinoids, and this idea is biologically supported by the results of the experiments using retinoid regulatory compounds. Thus, the activities of the thiazolidines were suppressed by an RAR antagonist (LE540) and enhanced by RXR agonists (HX600 and HX630). HX 630 is a more potent synergist with thiazolidines than HX600, as is the case in their synergism with various retinoids. Therefore, we concluded that the thiazolidines bind to and activate the RAR site of RAR-RXR heterodimers to elicit differentiation-inducing activities. The inhibitory effects on IL-1-induced IL-6 production by retinoids can also be ascribed to the binding affinities to RARs, and correlate well to the potency in HL-60 assay.[19] The activity of the thiazolidines in IL-6 production assay also supported their identification as retinoids (RAR agonists). Recently, various thiazolidine derivatives have been synthesized as candidate anti-diabetes drugs, and some of them have been proven to bind to and activate PPARs.[8] Since the polyenylidene thiazolidines are structurally hybrids of retinoic acid and thiazolidinedione, the thiazolidines **2**—**9** may

activate other PPAR-related functions, in addition to their retinoidal activities. In order to elucidate further their biological behavior, we are investigating their binding and activating abilities towards various nuclear receptors including RARs, RXRs, and PPARs.

In conclusion, several polyene-substituted thiazolidines were synthesized and their retinoidal activities were examined. The finding that the retinoidal carboxylic acid moiety can be replaced with a thiazolidine ring with retention of activity should make possible further structural evolution of retinoids with novel pharmacological characteristics.

Experimental

General Melting points were determined by using a Yanagimoto hot-stage melting point apparatus and are uncorrected. Elemental analysis were carried out in the Microanalytical Laboratory, Faculty of Pharmaceutical Sciences, University of Tokyo, and were within ±0.4% of the theoretical values. NMR spectra were recorded on a JEOL JNM-GX400 (400 MHz) spectrometer. Chemical shifts are expressed in ppm relative to tetramethylsilane. Mass spectra were recorded on a JEOL JMS-DX303 spectrometer. IR spectra were taken with a Shimadzu IR-408 IR spectrometer and values are expressed in cm^{-1}.

Ethyl (2*E*,4*E*,6*E*)-5-Methyl-7-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6-heptatrienoate (11a) and the (2*E*,4*Z*,6*E*)-Isomer (11b) A solution of triethyl (*E*)-phosphonocrotonate (5.206 g, 20.81 mmol), prepared from ethyl (*E*)-3-bromocrotonate, in THF (5 ml) was added slowly to a suspension of NaH (60% in oil, 0.834 g, 20.88 mmol) in THF (15 ml) at room temperature in an Ar atmosphere. After 1 h, a solution of β-ionone (**10**, freshly distilled, 4.001 g, 20.81 mmol) in THF (5 ml) was added to the above dark red solution at 0 °C over 20 min, and the mixture was stirred at room temperature for 1 h, and then at 50 °C for 15 h. The reaction mixture was poured into ice water, and extracted with AcOEt. The organic layer was washed with brine, and dried over Na_2SO_4. After evaporation, the residue was purified by silica gel flash column chromatography (AcOEt : *n*-hexane = 1 : 10) to give a mixture of **11a** and **11b** (orange oil, 0.815 g, 16%, *E* : *Z* = 54 : 46). The isomers were used for the next reaction without separation. **11a**: ^{1}H-NMR ($CDCl_3$) δ 1.03 (6H, s), 1.31 (3H, t, *J* = 7.1 Hz), 1.48 (2H, m), 1.61 (2H, m), 1.71 (3H, s), 2.03 (2H, t, *J* = 5.9 Hz), 2.04 (3H, s), 4.22 (2H, q, *J* = 7.1 Hz), 5.87 (1H, d, *J* = 15.0 Hz), 6.15 (1H, d, *J* = 12.8 Hz), 6.15 (1 H, d, *J* = 15.8 Hz), 6.39 (1 H, d, *J* = 15.8 Hz), 7.72 (1H, dd, *J* = 15.0, 12.1 Hz); **11b**: ^{1}H-NMR ($CDCl_3$) δ 1.03 (6H, s), 1.30 (3H, t, *J* = 7.1 Hz), 1.48 (2H, m), 1.61 (2H, m), 1.73 (3H, s), 2.03 (2H, t, *J* = 5.9 Hz), 2.04 (3H, s), 4.21 (2H, q, *J* = 7.2 Hz), 5.81 (1H, d, *J* = 15.0 Hz), 6.06 (1H, d, *J* = 11.7 Hz), 6.35 (1H, d, *J* = 15.0 Hz), 6.72 (1H, d, *J* = 16.1 Hz), 7.79 (1H, dd, *J* = 15.0, 12.1 Hz).

(2*E*,4*E*,6*E*)-5-Methyl-7-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6-heptatrien-1-ol (12a) and the (2*E*,4*Z*,6*E*)-Isomer (12b) DIBAL (1.0 M in toluene, 1.2 ml) was added slowly to a solution of the mixture of **11a** and **11b** (118 mg, 0.42 mmol) in THF (4 ml) at −60 °C. After 5 min, the reaction mixture was poured into 1 N hydrochloric acid, and extracted with AcOEt. The organic layer was washed with brine, and dried over Na_2SO_4. After evaporation, the residue was purified by silica gel flash column chromatography (AcOEt : *n*-hexane = 1 : 5) to give **12a** (21 mg, 20%) and **12b** (20 mg, 20%). **12a**: Yellow oil; ^{1}H-NMR ($CDCl_3$) δ 1.02 (6H, s), 1.46 (2H, m), 1.61 (2H, m), 1.70 (3H, s), 1.93 (3H, s), 2.01 (2H, t, *J* = 6.2 Hz), 4.24 (2H, d, *J* = 5.9 Hz), 5.86 (1H, td, *J* = 15.0, 5.9 Hz), 6.04 (1H, d, *J* = 10.3 Hz), 6.08 (1H, d, *J* = 15.8 Hz), 6.17 (1H, d, *J* = 16.1 Hz), 6.64 (1H, dd, *J* = 15.0, 11.4 Hz); **12b**: Yellow oil; ^{1}H-NMR ($CDCl_3$) δ 1.02 (6H, s), 1.47 (2H, m), 1.63 (2H, m), 1.72 (3H, s), 1.93 (3H, s), 2.02 (2H, t, *J* = 6.0 Hz), 4.22 (2H, d, *J* = 5.9 Hz), 5.80 (1H, td, *J* = 15.0, 6.1 Hz), 5.96 (1H, d, *J* = 11.3 Hz), 6.18 (1H, d, *J* = 16.1 Hz), 6.59 (1H, d, *J* = 15.8 Hz), 6.73 (1H, dd, *J* = 15.0, 11.4 Hz).

(2*E*,4*E*,6*E*)-5-Methyl-7-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6-heptatrienal (13a) Active MnO_2 (Aldrich, 1.639 g, 16.02 mmol) was added to a solution of **12a** (0.248 g, 1.01 mmol) in dry CH_2Cl_2 (15 ml), and the mixture was stirred at room temperature for 6 h. After filtration, the filtrate was evaporated to afford **13a** (163 mg, 66%). **13a**: Yellow oil; ^{1}H-NMR ($CDCl_3$) δ 1.04 (6H, s), 1.48 (2H, m), 1.63 (2H, m), 1.73 (3H, s), 2.04 (2H, t, *J* = 6.0 Hz), 2.10 (3H, s), 6.17 (1H, dd, *J* = 15.0, 8.1 Hz), 6.21 (1H, d, *J* = 16.1 Hz), 6.29 (1H, d, *J* = 11.7 Hz), 6.51 (1H, d, *J* = 15.8 Hz), 7.54 (1H, dd, *J* = 15.0, 11.7 Hz), 9.61 (1H, d, *J* = 8.1 Hz).

12b was similarly oxidized with active MnO_2 to give (2*E*,4*Z*,6*E*)-5-methyl-7-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6-heptatrienal (**13b**, 88%). **13b**: Yellow oil; ^{1}H-NMR ($CDCl_3$) δ 1.05 (6H, s), 1.49 (2H, m), 1.63 (2H, m), 1.76 (3H, s), 2.06 (2H, t, *J* = 6.2 Hz), 2.08 (3H, s), 6.11 (1H, dd, *J* = 14.8, 7.9 Hz), 6.21 (1H, d, *J* = 11.7 Hz), 6.48 (1H, d, *J* = 15.8 Hz), 6.72 (1H, d, *J* = 15.8 Hz), 7.61 (1H, dd, *J* = 15.0, 12.1 Hz), 9.59 (1H, d, *J* = 8.1 Hz).

(*Z*)-5-[(2*E*,4*E*,6*E*)-5-Methyl-7-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6-heptatrienylidene]-2,4-thiazolidinedione (2a) A solution of piperidine (8 mg, 0.095 mmol) and acetic acid (6 mg, 0.093 mmol) in dry toluene (2 ml) was added to a solution of **13a** (26 mg, 0.104 mmol) and 2,4-thiazolidinedione (13 mg, 0.114 mmol) in dry toluene (0.5 ml), and the mixture was heated at 120 °C for 2 h. The reaction mixture was poured into ice water, and extracted with AcOEt. The organic layer was washed with brine, and dried over Na_2SO_4. After evaporation, the residue was purified by silica gel flash column chromatography (AcOEt : *n*-hexane = 1 : 4) to give **2a** (14 mg, 40%). **2a**: Red crystals (CH_2Cl_2–*n*-hexane); mp 202—203 °C; ^{1}H-NMR ($CDCl_3$) δ 1.04 (6H, s), 1.47 (2H, m), 1.62 (2H, m), 1.72 (3H, s), 2.03 (2H, t, *J* = 6.0 Hz), 2.05 (3H, s), 6.15 (1H, dd, *J* = 14.3, 12.1 Hz), 6.18 (1H, d, *J* = 16.1 Hz,), 6.22 (1H, d, *J* = 11.7 Hz), 6.43 (1H, d, *J* = 15.7 Hz) 7.11 (1H, dd, *J* = 14.3, 11.7 Hz), 7.47 (1H, d, *J* = 11.7 Hz), 8.06 (1H, br s); IR (KBr) 1668, 1732 cm^{-1} (C=O); HR-MS Calcd for $C_{20}H_{25}NO_2S$: 343.1607. Found: 343.1617; *Anal.* Calcd for $C_{20}H_{25}NO_2S \cdot 1/4H_2O$: C, 69.03; H, 7.39; N, 4.02. Found: C, 69.21; H, 7.48; N. 3.95.

13b was similarly condensed with 2,4-thiazolidinedione to give (*Z*)-5-[(2*E*,4*Z*,6*E*)-5-methyl-7-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6-heptatrienylidene]-2,4-thiazolidinedione (**2b**, 26%), together with the isomer **2a** (27%), which was separated by silica gel flash column chromatography (AcOEt : *n*-hexane = 1 : 4). **2b**: Orange crystals (CH_2Cl_2–*n*-hexane); mp 195—196 °C; ^{1}H-NMR ($CDCl_3$) δ 1.05 (6H, s), 1.49 (2H, m), 1.63 (2H, m), 1.76 (3H, s), 2.05 (2H, m), 2.05 (3H, s), 6.08 (1H, dd, *J* = 14.7, 11.7 Hz), 6.13 (1H, d, *J* = 11.7 Hz), 6.41 (1H, d, *J* = 15.8 Hz), 6.68 (1H, d, *J* = 15.8 Hz), 7.20 (1H, dd, *J* = 14.3, 12.1 Hz), 7.46 (1H, d, *J* = 12.1 Hz), 8.11 (1H, br s); IR (KBr) 1680, 1730 cm^{-1} (C=O); HR-MS Calcd for $C_{20}H_{25}NO_2S$: 343.1607. Found: 343.1623; *Anal.* Calcd for $C_{20}H_{25}NO_2S$: C, 69.94; H, 7.34: N, 4.08. Found: C, 70.20; H, 7.60; N, 3.79.

(*Z*)-5-[(2*E*,4*E*,6*E*)-5-Methyl-7-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6-heptatrienylidene]-2-thioxo-4-thiazolidinone (3a) A solution of piperidine (8 mg, 0.095 mmol) and acetic acid (6 mg, 0.093 mmol) in dry toluene (2.5 ml) was added to a solution of **13a** (30 mg, 0.139 mmol) and 2-thioxo-4-thiazolidinone (19 mg, 0.139 mmol) in dry toluene (0.5 ml), and the mixture was heated at 120 °C for 40 min. The reaction mixture was poured into ice water, and extracted with AcOEt. The organic layer was washed with brine, and dried over Na_2SO_4. After evaporation, the residue was purified by silica gel flash column chromatography (AcOEt : *n*-hexane = 1 : 5) to give **3a** (11 mg, 26%). **3a**: Red crystals (CH_2Cl_2–*n*-hexane); mp 206—207 °C; ^{1}H-NMR ($CDCl_3$) δ 1.04 (6H, s), 1.48 (2H, m), 1.62 (2H, m), 1.73 (3H, s), 2.04 (2H, t, *J* = 6.0 Hz), 2.04 (3H, s), 6.16 (1H, dd, *J* = 13.9, 12.5 Hz), 6.20 (1H, d, *J* = 16.1 Hz), 6.24 (1H, d, *J* = 12.1 Hz), 6.47 (1H, d, *J* = 16.1 Hz), 7.16 (1H, dd, *J* = 13.9, 12.1 Hz), 7.32 (1H, d, *J* = 11.7 Hz), 9.20 (1H, br s); IR (KBr) 1205 cm^{-1} (S=O), 1689 cm^{-1} (C=O); HR-MS Calcd for $C_{20}H_{25}NOS_2$: 359.1409. Found: 359.1404; *Anal.* Calcd for $C_{20}H_{25}NOS_2 \cdot 1/4H_2O$: C, 65.98; H, 7.06; N, 3.85. Found: C, 66.04; H, 7.10; N, 3.72.

13b was similarly condensed with 2-thioxo-4-thiazolidinone at 60 °C for 1 h to give (*Z*)-5-[(2*E*,4*Z*,6*E*)-5-methyl-7-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6-heptatrienylidene]-2-thioxo-4-thiazolidinone (**3b**, 33%), together with the isomer **3a** (21%), which was separated by silica gel flash column chromatography (AcOEt : *n*-hexane = 1 : 5). **3b**: Black crystals (CH_2Cl_2–*n*-hexane); mp 131—132 °C; ^{1}H-NMR ($CDCl_3$) δ 1.05 (6H, s), 1.48 (2H, m), 1.64 (2H, m), 1.76 (3H, s), 2.05 (3H, s), 2.06 (2H, t, *J* = 6.6 Hz), 6.10 (1H, dd, *J* = 14.3, 11.7 Hz), 6.14 (1H, d, *J* = 13.2 Hz), 6.48 (1H, d, *J* = 15.8 Hz), 6.70 (1H, d, *J* = 15.8 Hz), 7.24 (1H, dd, *J* = 14.3, 11.7 Hz), 7.31 (1H, d, *J* = 11.7 Hz), 9.24 (1H, br s); IR (KBr) 1210 cm^{-1} (S=O) 1680 cm^{-1} (C=O); HR-MS Calcd for $C_{20}H_{25}NOS_2$: 359.1409. Found: 359.1374.

(*E*)-3-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-propenal (17) Trimethylsilyldiazomethane (2.0 M in *n*-hexane, 50 ml) was added to a solution of (*E*)-3-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-propenoic acid[12] (**14**, 2.60 g, 13.4 mmol) in methanol (75 ml) at 0 °C, and the mixture was stirred for

1 h. After removal of the solvent, the residue was chromatographed on silica gel (AcOEt : *n*-hexane = 1 : 8) to give methyl (*E*)-3-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-propenoate (**15**, colorless oil, 2.79 g, quant). **15**: ^{1}H-NMR ($CDCl_3$) δ 1.06 (6H, s), 1.48 (2H, m), 1.60 (2H, m), 1.76 (3H, s), 2.06 (2H, m), 3.76 (3H, s), 5.82 (1H, d, J = 16.1 Hz), 7.43 (1H, d, J = 16.1 Hz).

DIBAL (1.0 M in toluene, 40.8 ml) was added slowly to a solution of **15** (2.83 g, 13.6 mmol) in THF (80 ml) at −78 °C under an Ar atmosphere with protection from light. After 1 h, the reaction mixture was poured into 1 N hydrochloric acid, and extracted with CH_2Cl_2. The organic layer was washed with brine, and dried over Na_2SO_4. After evaporation, the residue was chromatographed on silica gel (AcOEt : *n*-hexane = 1 : 6) to give **16** (colorless oil, 2.18 g, 89%). **16**: ^{1}H-NMR ($CDCl_3$) δ 1.00 (6H, s), 1.45 (2H, m), 1.60 (2H, m), 1.68 (3H, s), 1.99 (2H, m), 4.21 (2H, dd, J = 5.9, 1.5 Hz), 5.62 (1H, dd, J = 15.8, 5.9 Hz), 6.11 (1H, d, J = 15.8 Hz).

Active MnO_2 (Aldrich, 1.70 g, 19.4 mmol) was added to a solution of **16** (0.350 g, 1.94 mmol) in dry CH_2Cl_2 (15 ml), and the mixture was stirred at room temperature with protection from light for 2 d. After filtration and the evaporation, the residue was chromatographed on silica gel (AcOEt : *n*-hexane = 1 : 8) to give (*E*)-3-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-propenal (**17**, 301 mg, 87%). **17**: Colorless oil; ^{1}H-NMR ($CDCl_3$) δ 1.11 (6H, s), 1.51 (2H, m), 1.63 (2H, m), 1.82 (3H, s), 2.12 (2H, m), 6.20 (1H, m), 7.28 (1H, d, J = 16.1 Hz), 9.54 (1H, d, J = 7.7 Hz).

(*Z*)-5-[(2*E*,4*E*,6*E*)-7-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2,4,6-heptatrienylidene]-2,4-thiazolidinedione (4) *n*-BuLi (1.6 M in *n*-hexane, 12.5 ml) was added slowly to a solution of triethyl (*E*)-phosphonocrotonate (5.0 g, 20.0 mmol) in THF (20 ml) at −78 °C under an Ar atmosphere over 10 min. After 3 h, a solution of **17** (1.79 g, 10.0 mmol) in THF (10 ml) was added to the orange solution at −78 °C over 5 min, and the mixture was stirred while the temperature was raised slowly to room temperature. After 18 h, the reaction mixture was poured into ice water, and extracted with AcOEt. The organic layer was washed with brine, and dried over $MgSO_4$. After evaporation, the residue was chromatographed on silica gel (AcOEt : *n*-hexane = 1 : 8) to give **18** (1.16 g, 43%) as a single isomer. **18**: ^{1}H-NMR ($CDCl_3$) δ 1.04 (6H, s), 1.30 (3H, t, J = 7.3 Hz), 1.48 (2H, m), 1.62 (2H, m), 1.73 (3H, s), 2.04 (2H, m), 4.21 (2H, q, J = 7.3 Hz), 5.85 (1H, d, J = 15.4 Hz), 6.18 (1H, dd, J = 15.4, 10.6 Hz), 6.27 (1H, dd, J = 14.3, 10.6 Hz), 6.39 (1H, d, J = 15.0 Hz), 6.62 (1H, dd, J = 15.4, 10.6 Hz), 7.34 (1H, dd, J = 15.4, 11.4 Hz).

Compound **18** was converted to the thiazolidinedione **4** (three steps, 17%) by a similar method to that employed to obtain **2a** from **11a** (described above). **4**: Red crystals (CH_2Cl_2–*n*-hexane); mp 223—224 °C; ^{1}H-NMR ($CDCl_3$) δ 1.05 (6H, s), 1.47 (2H, m), 1.62 (2H, m), 1.75 (3H, s), 2.06 (2H, m), 6.11 (1H, dd, J = 14.3, 11.7 Hz), 6.22 (1H, dd, J = 15.4, 10.6 Hz), 6.34 (1H, dd, J = 14.7, 11.7 Hz), 6.43 (1H, d, J = 15.4 Hz), 6.60 (1H, dd, J = 14.7, 11.0 Hz), 6.78 (1H, dd, J = 14.7, 11.4 Hz), 7.42 (1H, d, J = 11.4 Hz), 8.10 (1H, br s); IR (KBr) 1680, 1730 cm^{-1} (C=O); HR-MS Calcd for $C_{19}H_{23}NO_2S$: 329.1450. Found: 329.1452. *Anal.* Calcd for $C_{19}H_{23}NO_2S \cdot 1/2H_2O$: C, 67.42; H, 7.15; N, 4.14. Found: C, 67.75; H, 7.00; N, 3.99.

(*Z*)-5-[(2*E*,4*E*)-5-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethylnaphthalen-2-yl)-2,4-pentadienylidene]-2,4-thiazolidinedione (5) $AlCl_3$ (1.181 g, 8.11 mmol) was added slowly to a solution of 2,5-dichloro-2,5-dimethylhexane (20.8 g, 0.115 mol) in dry toluene (100 ml) at 0 °C, and the mixture was stirred at 0 °C for 1 h. The reaction mixture was poured into ice water, and extracted with ether. The organic layer was washed successively with water, 1 N Na_2CO_3 and brine, and dried over $MgSO_4$. After evaporation, the crude product was distilled under vacuum and then recrystallized from methanol to give 1,2,3,4-tetrahydro-1,1,4,4,6-pentamethylnaphthalene (16.5 g, 71%, colorless needles; mp 33—34 °C; bp 95 °C (5 mmHg)).

A solution of ceric ammonium nitrite[13] (175.6 g, 0.32 mol) in hot water (250 ml) was added dropwise to a solution of 1,2,3,4-tetrahydro-1,1,4,4,6-pentamethylnaphthalene (16.2 g, 0.08 mol) in acetic acid (200 ml) at 100 °C over 50 min, and the mixture was stirred at 100 °C for 1 h. The reaction mixture was cooled, diluted with water (200 ml), and extracted with CH_2Cl_2. The organic layer was washed successively with 2 N NaOH, water and brine, and dried over $MgSO_4$. After evaporation, the residue was chromatographed on silica gel (CH_2Cl_2 : *n*-hexane = 1 : 1) and then recrystallized from *n*-hexane to give 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenecarbaldehyde (13.6 g, 78%). 5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenecarbaldehyde: Colorless plates (*n*-hexane); mp 52—53 °C; ^{1}H-NMR ($CDCl_3$) δ 1.31 (6H, s), 1.33 (6H, s), 1.72 (4H, s), 7.46 (1H, d, J = 8.1 Hz), 7.63 (1H, dd, J = 8.1, 1.8 Hz), 7.83 (1H, d, J = 1.8 Hz), 9.95 (1H, s); IR (KBr) 1675 cm^{-1} (C=O); *Anal.* Calcd for $C_{15}H_{20}O \cdot 1/10H_2O$: C, 82.60; H, 9.33. Found: C, 82.76; H, 9.12.

n-BuLi (1.5 M in hexane, 33.5 ml) was added slowly to a solution of triethyl (*E*)-phosphonocrotonate (12.59 g, 50.34 mmol) in THF (30 ml) at −78 °C in an Ar atmosphere over 20 min. After 3 h, a solution of 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenecarbaldehyde (5.55 g, 25.65 mmol) in THF (15 ml) was added to the orange solution at −78 °C over 15 min, and the mixture was stirred while the temperature was slowly raised to room temperature. After 17 h, the reaction mixture was poured into ice water, and extracted with AcOEt. The organic layer was washed with brine, and dried over Na_2SO_4. After evaporation, the residue was chromatographed on silica gel (CH_2Cl_2 : *n*-hexane = 1 : 1) and then recrystallized from *n*-hexane to give ethyl (2*E*,4*E*)-5-(5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphthalen-2-yl)-2,4-pentadienoate (5.38 g, 67%). Ethyl (2*E*,4*E*)-5-(5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphthalen-2-yl)-2,4-pentadienoate: Colorless prisms (*n*-hexane); mp 81—82 °C; ^{1}H-NMR ($CDCl_3$) δ 1.28 (6H, s) 1.30 (6H, s,) 1.31 (3H, t, J = 7.1 Hz), 1.69 (4H, s), 4.23 (2H, q, J = 7.1 Hz), 5.97 (1H, d, J = 15.0 Hz), 6.82 (1H, dd, J = 15.4, 9.9 Hz), 6.88 (1H, d, J = 15.8 Hz), 7.26 (1H, dd, J = 8.1, 1.8 Hz), 7.30 (1H, t, J = 8.4 Hz), 7.36 (1H, d, J = 1.5 Hz), 7.44 (1H, dd, J = 15.4, 9.9 Hz); IR (KBr) 1697 cm^{-1} (C=O); *Anal.* Calcd for $C_{21}H_{28}O_2$: C, 80.73; H, 9.03. Found: C, 80.92; H, 8.93.

Ethyl (2*E*,4*E*)-5-(5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphthalen-2-yl)-2,4-pentadienoate was converted to the thiazolidinedione **5** (three steps, 44%) by a similar method to that used to obtain **2a** from **11a** (described above). **5**: Orange cotton (AcOEt–*n*-hexane); mp 220—222 °C; ^{1}H-NMR ($CDCl_3$) δ 1.28 (6H, s), 1.30 (6H, s), 1.69 (4H, s), 6.25 (1H, dd, J = 13.4, 11.9 Hz), 6.84 (1H, dd, J = 14.8, 10.4 Hz), 6.89 (1H, dd, J = 13.7, 9.7 Hz), 6.89 (1H, d, J = 14.3 Hz), 7.26 (1H, dd, J = 8.2, 1.7 Hz), 7.31 (1H, d, J = 8.1 Hz), 7.37 (1H, d, J = 1.8 Hz), 7.46 (1H, d, J = 11.7 Hz), 8.29 (1H, br s); IR (KBr) 1678, 1739 cm^{-1} (C=O); HR-MS Calcd for $C_{22}H_{25}NO_2S$: 367.1607. Found: 367.1597; *Anal.* Calcd for $C_{22}H_{25}NO_2S$: C, 71.90; H, 6.86: N, 3.81. Found: C, 71.87; H, 6.98; N, 3.65.

(*Z*)-5-[(2*E*,4*E*)-5-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethylnaphthalen-2-yl)-2,4-pentadienylidene]-2-thioxo-4-thiazolidinone (6) Compound **6** was prepared similarly to **3a** (19%), as described above. **6**: Orange crystals (AcOEt–*n*-hexane); mp 227—229 °C; ^{1}H-NMR ($CDCl_3$) δ 1.28 (6H, s), 1.30 (6H, s), 1.69 (4H, s), 6.26 (1H, dd, J = 13.4, 11.9 Hz), 6.87 (1H, dd, J = 16.1, 9.9 Hz), 6.91 (1H, dd, J = 17.2, 9.2 Hz), 6.93 (1H, d, J = 15.8 Hz), 7.27 (1H, dd, J = 8.2, 1.8 Hz), 7.30 (1H, d, J = 11.7 Hz), 7.31 (1H, d, J = 8.4 Hz), 7.38 (1H, d, J = 1.8 Hz), 9.04 (1H, br s); IR (KBr) 1195 cm^{-1} (C=S), 1712 cm^{-1} (C=O); HR-MS Calcd for $C_{22}H_{25}NOS_2$: 383.1379. Found: 383.1393; *Anal.* Calcd for $C_{22}H_{25}NOS_2$: C, 68.89; H, 6.57: N, 3.65. Found: C, 68.84; H, 6.67; N, 3.54.

(*Z*)-5-[(2*E*,4*E*)-5-(3,5-Di-*tert*-butylphenyl)-2,4-pentadienylidene]-2,4-thiazolidinedione (7) $AlCl_3$ (2.7 g) was added slowly to a solution of *tert*-butyl chloride (100 g) in dry toluene (50 g) at 5 °C, and the mixture was stirred at room temperature for 20 h. The reaction mixture was poured into ice water, and extracted with ether. The organic layer was washed successively with water, 1 N $NaHCO_3$, and water, and dried over Na_2SO_4. After removal of the solvent, the residue was distilled under vacuum to give 3,5-di-*tert*-butyltoluene (60 g, 54%) as a colorless oil; bp 90—97 °C (6 mmHg).

A solution of ceric ammonium nitrite[13] (217.5 g, 0.40 mol) in hot water (225 ml) was added dropwise to a solution of 3,5-di-*tert*-butyltoluene (20.3 g, 0.10 mol) in acetic acid (200 ml) at 100 °C over 20 min, and the mixture was stirred at 100 °C for 1 h. The reaction mixture was cooled, diluted with water (200 ml), and extracted with CH_2Cl_2. The organic layer was washed successively with 2 N NaOH, water and brine, and dried over $MgSO_4$. After evaporation, the residue was chromatographed on silica gel (CH_2Cl_2 : *n*-hexane = 1 : 2) and then recrystallized from *n*-hexane to give 3,5-di-*tert*-butylbenzaldehyde (12.8 g, 59%). 3,5-Di-*tert*-butylbenzaldehyde: Colorless needles (*n*-hexane); mp 85—86 °C; ^{1}H-NMR ($CDCl_3$) δ 1.37 (18H, s), 7.71 (1H, t, J = 1.8 Hz), 7.73 (2H, d, J = 1.8 Hz), 10.01 (1H, s); IR (KBr) 1682 cm^{-1} (C=O).

n-BuLi (1.5 M in hexane, 33.5 ml) was added slowly to a solution of triethyl (*E*)-phosphonocrotonate (12.6 g, 50.19 mmol) in THF (30 ml) at −78 °C in an Ar atmosphere over 10 min. After 1 h, a solution of 3,5-di-*tert*-butylbenzaldehyde (5.5 g, 25.14 mmol) in THF (15 ml) was added to the yellow solution at −78 °C over 10 min, and the mixture was stirred while the temperature was slowly raised to 0 °C. After 5 h, the reaction mixture was poured into ice water, and extracted with

AcOEt. The organic layer was washed with brine, and dried over Na_2SO_4. After evaporation, the residue was chromatographed on silica gel (CH_2Cl_2 : *n*-hexane = 1 : 1) and then recrystallized from *n*-hexane to give ethyl (2*E*,4*E*)-5-(3,5-di-*tert*-butylphenyl)-2,4-pentadienoate (4.4 g, 56%). Ethyl (2*E*,4*E*)-5-(3,5-di-*tert*-butylphenyl)-2,4-pentadienoate: Colorless prisms (*n*-hexane); mp 71 °C; ^{1}H-NMR ($CDCl_3$) δ 1.32 (3H, t, J=7.1 Hz) 1.34 (18H, s), 4.23 (2H, q, J=7.0 Hz), 5.99 (1H, d, J=15.4 Hz), 6.87 (1H, dd, J=15.4, 10.4 Hz), 6.91 (1H, d, J=15.4 Hz), 7.31 (2H, d, J=1.8 Hz), 7.40 (1H, t, J=1.8 Hz), 7.46 (1H, dd, J=15.2, 10.1 Hz); IR (KBr) 1698 cm^{-1} (C=O); *Anal.* Calcd for $C_{21}H_{30}O_2$: C, 80.21; H, 9.62. Found: C, 79.93; H, 9.47.

Ethyl (2*E*,4*E*)-5-(3,5-di-*tert*-butylphenyl)-2,4-pentadienoate was converted to the thiazolidinedione **7** (three steps, 41%) by a similar method to that used to obtain **2a** from **11a** (described above). **7**: pale orange leaflets (AcOEt–*n*-hexane); mp 254—255 °C; ^{1}H-NMR ($CDCl_3$) δ 1.34 (18H, s), 6.28 (1H, dd, J=14.2, 11.7 Hz), 6.91 (1H, dd, J=13.9, 11.7 Hz), 6.92 (1H, dd, J=14.3, 12.1 Hz), 6.95 (1H, d, J=15.4 Hz), 7.31 (2H, d, J=1.5 Hz), 7.40 (1H, t, J=1.7 Hz), 7.47 (1H, d, J=11.7 Hz), 8.33 (1H, br s); IR (KBr) 1682, 1730 cm^{-1} (C=O); HR-MS Calcd for $C_{22}H_{27}NO_2S$: 369.1764. Found: 369.1779; *Anal.* Calcd for $C_{22}H_{27}NO_2S$: C, 71.51; H, 7.36: N, 3.79. Found: C, 71.47; H, 7.44; N, 4.06.

(*Z*)-5-[(2*E*,4*E*)-5-(3,5-Di-*tert*-butylphenyl)-2,4-pentadienylidene]-2-thioxo-4-thiazolidinone (8) Compound **8** was prepared similarly to **3a** (19%), as described above. **8**: Orange needles (CH_2Cl_2–*n*-hexane); mp 226—228 °C; ^{1}H-NMR ($CDCl_3$) δ 1.35 (18H, s), 6.29 (1H, dd, J=14.3, 12.1 Hz), 6.93 (1H, dd, J=15.8, 12.6 Hz), 6.95 (1H, dd, J=18.5, 12.3 Hz), 6.98 (1H, d, J=15.3 Hz), 7.31 (1H, d, J=11.0 Hz), 7.32 (2H, d, J=1.8 Hz), 7.42 (1H, t, J=1.8 Hz), 9.14 (1H, br s); IR (KBr) 1210 cm^{-1} (C=S), 1688 cm^{-1} (C=O); HR-MS Calcd for $C_{22}H_{27}NOS_2$: 385.1535. Found: 385.1537; *Anal.* Calcd for $C_{22}H_{27}NOS_2$: C, 68.53; H, 7.06: N, 3.63. Found: C, 68.49; H, 7.05; N, 3.48.

(*Z*)-5-[(2*E*,4*E*,6*E*,8*E*)-3,7-Dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenylidene]-2,4-thiazolidinedione (9a) and (*Z*)-5-[(2*E*,4*E*,6*Z*,8*E*)-3,7-Dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenylidene]-2,4-thiazolidinedione (9b) A solution of piperidine (36 mg, 0.404 mmol) and acetic acid (24 mg, 0.412 mmol) in dry toluene (2 ml) was added to a solution of 9*Z*-retinal (100 mg, 0.352 mmol) and 2,4-thiazolidinedione (47 mg, 0.44 mmol) in dry toluene (6 ml), and the mixture was heated at 60 °C for 1 h. The reaction mixture was poured into ice water, and extracted with AcOEt. The organic layer was washed with brine, and dried over Na_2SO_4. After evaporation, the residue was purified by silica gel flash column chromatography (AcOEt : *n*-hexane = 1 : 4) to give **9a** (34 mg, 25%) and **9b** (35 mg, 26%). **9a**: Black crystals (AcOEt–*n*-hexane); mp 222—223 °C; ^{1}H-NMR ($CDCl_3$) δ 1.04 (6H, s), 1.47 (2H, m), 1.62 (2H, m), 1.73 (3H, s), 2.02 (3H, s), 2.03 (2H, t, J=6.0 Hz), 2.13 (3H, s), 6.00 (1H, d, J=12.5 Hz), 6.16 (1H, d, J=16.1 Hz,), 6.17 (1H, d, J=9.9 Hz), 6.32 (1H, d, J=16.5 Hz), 6.42 (1H, d, J=15.0 Hz), 6.97 (1H, dd, J=14.8, 11.5 Hz), 7.74 (1H, d, J=12.5 Hz), 8.09 (1H, br s); IR (KBr) 1680, 1730 cm^{-1} (C=O); HR-MS Calcd for $C_{23}H_{29}NO_2S$: 383.1919. Found: 383.1910; *Anal.* Calcd for $C_{23}H_{29}NO_2S$: C, 72.03; H, 7.62: N, 3.65. Found: C, 71.73; H, 7.47; N, 3.45. **9b**: Red crystals (AcOEt–*n*-hexane); mp 217—219 °C; ^{1}H-NMR ($CDCl_3$) δ 1.05 (6H, s), 1.49 (2H, m), 1.64 (2H, m), 1.76 (3H, s), 2.02 (3H, s), 2.03 (2H, t, J=6.0 Hz), 2.12 (3H, s), 5.99 (1H, d, J=12.8 Hz), 6.08 (1H, d, J=11.4 Hz,), 6.28 (1H, d, J=15.4 Hz), 6.35 (1H, d, J=15.4 Hz), 6.67 (1H, d, J=15.8 Hz), 7.06 (1H, dd, J=14.7, 11.7 Hz), 7.73 (1H, d, J=12.5 Hz), 8.03 (1H, br s); IR (KBr) 1680, 1730 cm^{-1} (C=O); HR-MS Calcd for $C_{23}H_{29}NO_2S$: 383.1919. Found: 383.1902; *Anal.* Calcd for $C_{23}H_{29}NO_2S$: C, 72.03; H, 7.62: N, 3.65. Found: C, 71.83; H, 7.58; N, 3.42.

Cells and Culture The human promyelocytic leukemia cell line HL-60[14] was provided by Prof. F. Takaku (Faculty of Medicine, University of Tokyo) in 1980, and has been maintained since then, cultured in plastic flasks in RPMI1640 medium, supplemented with 5% fetal bovine serum (FBS) and antibiotics (penicillin G and streptomycin), in a humidified atmosphere of 5% CO_2 in air at 37 °C. The mouse osteogenic fibroblast cell line MC3T3-E1 was established by H. Kodama, Second Department of Anatomy, Tohoku Dental University, and the cells were cultured in plastic flasks in modified Eagle's minimum essential medium (MEM, Flow Laboratories), supplemented with 10% FBS and antibiotics, in a humidified atmosphere of 5% CO_2 in air at 37 °C.

HL-60 Cell Differentiation Assay Test compounds were dissolved in ethanol at 2 mM and added to HL-60 cells, which were seeded at about 8×10^4 cells/ml; the final ethanol concentration was kept below 0.5%. Control cells were given only the same volume of ethanol. Retinoic acid or Am80, as a positive control, was always assayed at the same time. The cells were incubated for 4 d and stained with Wright–Giemsa to visualize any morphological change. The percentages of the differentiated cells were determined by NBT reduction assay as described.[15] Cells were incubated for 20 min at 37 °C in RPMI1640 medium (5% FBS) and an equal volume of phosphate-buffered saline (PBS) containing NBT (0.2%) and 12-*O*-tetradecanoylphorbol-13-acetate (TPA; 200 ng/ml). The percentage of cells containing blue-black formazan was determined on a minimum of 200 cells. The evaluation of the differentiation from NBT reduction assay always coincided well with the morphological result. The assays of test compounds were performed at least twice. EC_{50} values of active compounds were calculated from the NBT reduction assay data by means of the van der Waerden method.

IL-6 Production Assay MC3T3-E1 cells (0.5 ml of 1.2×10^5 cells/ml suspension) were seeded in each well of 24-well plates, and were incubated for 24 h at 37 °C in a CO_2 incubator. The medium was exchanged for RPMI1640 (0.5 ml, without phenol red) with 5% FBS (dicyclohexylcarbodiimide-treated). After 24 h, mouse IL-1α (50 pg/ml) and/or a test compound (ethanol solution) were added to the cells. Control cells were given only the same volume (2.5 μl) of ethanol. The cells were incubated for 24 h and IL-6 production was examined using a commercial ELISA kit (Amersham).

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