Expedited Articles

Discovery of Novel Retinoic Acid Receptor Agonists Having Potent Antiproliferative Activity in Cervical Cancer Cells

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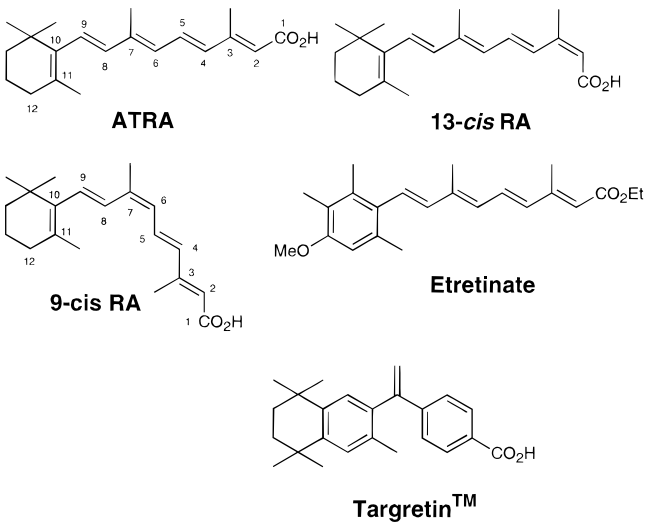
Retinoic acid receptor (RAR) active retinoids have proven therapeutically useful for treating certain cancers and dermatological diseases. Herein, we describe the discovery of two new RAR active trienoic acid retinoids, (2*E*,4*E*,6*E*)-7-(3,5-di-*tert*-butylphenyl)-3-methylocta-2,4,6-trienoic acid (**10a**, ALRT1550) and (2*E*,4*E*,6*Z*)-7-(3,5-di-*tert*-butylphenyl)-3-methylocta-2,4,6-trienoic acid (**10b**, LG100567). ALRT1550 is a RAR selective retinoid which exhibits exceptional potency in both competitive binding and cotransfection assays. Moreover, it is the most potent antiproliferative retinoid described to date and thus has implications for the treatment of certain cancers. LG100567 is a potent panagonist which activates both RARs and retinoid X receptors.

Retinoids have found utility for treatment of numerous diseases including acne, psoriasis, and cancer.[1−3] The therapeutic effects of retinoids result from their ability to control abnormal cellular processes by modulating cell differentiation, inhibiting cell proliferation, and regulating apoptosis. These processes are initiated by the formation of an active ligand−receptor complex with one or more of six intracellular (IR) retinoid receptors, RARα,*β*,*γ* and RXRα,*β*,*γ*.[4] This produces transcriptional activation which results in physiological responses. Interestingly, in addition to positively regulating gene expression, retinoids may also regulate the expression of certain genes by inhibiting the enhancer effects of transcription factors such as AP-1,[5−7] a transcription factor complex composed of the oncogenes Fos and Jun.

Currently, several retinoids are marketed or in clinical trials for treatment of cancer and skin disease.[1,2] These include ATRA (*all-trans*-retinoic acid), 13-*cis*-RA (13-*cis*-retinoic acid), etretinate, 9-*cis*-RA (9-*cis*-retinoic acid), Tazarotene,[8] and Targretin (LGD1069) (see Chart 1).[9,10] Apart from Targretin, an RXR selective retinoid, all of these compounds have significant RAR activity. Unfortunately, compounds such as ATRA, 13-*cis*-RA and etretinate have the potential liability of exhibiting hypervitaminosis-A-like side effects with chronic treatment.[11−13] In addition, chronic treatment with ATRA results in a rapid induction of metabolism[14] and, subsequently, reduced therapeutic efficacy.

The promising clinical results experienced by acute promyelocytic leukemia (APL) patients[15,16] treated with ATRA has inspired continued research to identify other RAR active agents for the treatment of cancer. Our goal was to identify novel analogs of ATRA and 9-*cis*-RA which exhibit (1) similar receptor profiles, (2) antiproliferative activity against a broad spectrum of cancer cells, and (3) increased chemical and metabolic stability. Additionally, it is desirable to obtain compounds which show an increase in therapeutic index. Our synthetic strategy was to substitute the *β*-ionene moiety **1** (see Figure 1) of ATRA and 9-*cis*-RA with a lipophilic bioisostere such as a dialkylphenyl group. The utility of such bioisosteres has been demonstrated by Shudo *et al.*[17,18] with the design of synthetic retinoids **3** and **4** which contain a di-*tert*-butylphenyl moiety **2.** These compounds were effective in differentiating human leukemia (HL-60) cells.[19] Substitution of the *β*-ionene moiety **1** of ATRA and 9-*cis*-RA with a di-*tert*-butylphenyl function would eliminate the oxidizable C-12 allylic position (see Chart 1 for numbering) and shorten the side chain by one conjugated double bond. Thus, we hypothesized that dialkylphenyl-substituted analogs of ATRA and 9-*cis*-RA may result in compounds with biological activity against cancer cells as well as increased chemical and metabolic stability.

Chart 1



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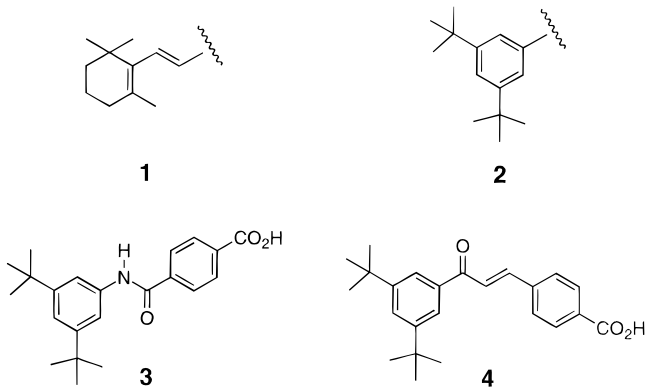


Figure 1.

Herein, we describe the synthesis and biological activity of two new retinoids, ALRT1550 (**10a**, Scheme 1) and LG100567 (**10b**). These compounds are analogs of ATRA and 9-*cis*-RA and differ from the parent compounds by having a 3,5-di-*tert*-butyl phenyl group in place of the β-ionene moiety. Our data indicate that ALRT1550 is one of the most potent and RAR selective retinoids discovered to date. It is 10−100 times more potent than ATRA in competitive binding and cotransfection assays and 300 times more potent than ATRA in its ability to inhibit cellular proliferation in cervical carcinoma cells.

Chemistry

ALRT1550 (**10a**) and LG100567 (**10b**) were synthesized from 3,5-di-*tert*-butylbenzoic acid (**5**) in five steps as shown in Scheme 1. Benzoic acid **5** was treated with 2 equiv of methyllithium at −78 °C to give acetophenone **6.** Condensation of the acetophenone **6** with the anion of diethyl (cyanomethyl)phosphonate resulted in a 10:1 mixture of two isomeric nitrile alkenes, **7a** (2*E*) and **7b** (2*Z*), which were separated by preparative thin-layer chromatography (PTLC). Nitrile **7a** was treated with 2 equiv of diisobutylaluminum hydride (DIBAL) to provide aldehyde **8a**. Horner−Emmons condensation of the aldehyde **8a** with diethyl[3-(ethoxycarbonyl)-2-methylprop-2-enyl]phosphonyl anion gave the ethyl trienoate **9a**. Saponification (KOH, MeOH) of **9a** gave the carboxylic acid **10a** (ALRT1550). Similarly, nitrile **7b** was converted to the triene carboxylic acid **10b** (LG100567). ALRT1550 and LG100567 were purified by crystallization from EtOH as fine, pale yellow needles. Analytical data, including high-resolution mass spectra, ^{1}H-NMR spectra, and elemental analysis, are consistent with the structures assigned for ALRT1550 and LG100567. The stereochemistry of aldehydes **8a** and **8b** was unambiguously assigned from nuclear Overhauser enhancement (NOE) experiments. A 6% NOE in the intensity of the olefinic proton was observed upon irradiation of the methyl group in **8b**. No such enhancement was observed for **8a**. This is consistent with the assignments for **8a** (*trans*) and **8b** (*cis*).

Biology

Binding Studies and Cotransfection Studies. Receptor binding assays for RARs and RXRs were performed in a similar manner as described in Boehm *et al.*[9] using [^{3}H]-9-*cis*-RA[20] as the radioligand for the RXRs and [^{3}H]ATRA for the RARs. K_i values (nM) for the analogues were determined by application of the Cheng−Prussof equation.[21] Cotransfection assays were performed as described,[8,22−24] and the values (EC_{50}) are reported in nM.

Cell Culture. ME-180 cells (ATCC HTB 33)[25] were obtained from the American Type Culture Collection (ATCC, Rockville, MD).

Incorporation of [5′-^{3}H]Thymidine. The method used for determining radiolabeled thymidine incorporation was adapted from the procedure described by Shrivastav *et al.*[26]

Biological Results. Compounds ALRT1550, LG-100567, ATRA, and 9-*cis*-RA were evaluated in the competitive binding assay which characterizes the ligand's ability to bind directly to each of the six retinoid receptor subtypes and the cotransfection assay which measures the ability of compounds to activate gene expression at each of the six retinoid receptors and reflects the compound's functional activity. K_i and EC_{50} values for ALRT1550 and LG100567, shown in comparison to ATRA and 9-*cis*-RA, are reported in Table 1 for the six known retinoid receptors: RARα, RARβ, RARγ, RXRα, RXRβ, and RXRγ. ALRT1550, like ATRA, is RAR selective; however, unlike ATRA, which is only 15−50 times more potent at the RARs than at the RXRs, ALRT1550 is 100−800 more potent in the binding assay and at least 2000 times more potent in the cotransfection assay to the RARs than to the RXRs. In addition it is at least 10-fold more potent than ATRA at the RARs in both assays. Although weak binding was observed at the RXRs for ALRT1550, no RXR activity was measured in the cotransfection assay at up to 1 μM. Further, ALRT1550 is not an antagonist of Targretin at the RXRs in the cotransfection assay (data not shown). In contrast, although LG100567 is active at the RARs, the binding and cotransfection values are at least 50 times less than ALRT1550. LG100567 is also active at the RXRs and exhibits a "pan-agonist" receptor profile (active at all six receptors) that is similar to that of 9-*cis*-RA in the cotransfection and binding assays. Interestingly, both LG100567 and 9-*cis*-RA exhibit somewhat higher binding affinities for the RXRs than for the RARs.

These retinoids were further examined for their antiproliferative activity in human cervical carcinoma cells (ME180) (shown in Figure 2). This assay measures the extent of incorporation of [^{3}H]thymidine into DNA with increasing concentrations of the four retinoids as compared to untreated cells. All four retinoids exhibit concentration-dependent inhibition of [^{3}H]thymidine incorporation into DNA. The IC_{50} values for LG100567, ALRT1550, ATRA, and 9-*cis*-RA are 20, 1, 300, and 500 nM, respectively, in this assay. On the basis of these values, ALRT1550 is 300 times more potent than ATRA and LG100567 is 25 times more potent than 9-*cis*-RA in their ability to inhibit cellular proliferation.

Discussion

In this study we identified two novel analogs of retinoic acid, namely, ALRT1550 and LG100567, which exhibit potent retinoid activity in binding and cotransfection assays and inhibit growth of ME180 cancer cells. The structural similarity of these compounds to ATRA and 9-*cis*-RA is evident in the configuration of the olefinic side chain. All four of these retinoids have

Table 1. Competition Binding (K_i)[a] and Cotransfection (EC_{50} and Percent Efficacy)[b] Data

		RAR			RXR		
compound	nM	α	*β*	*γ*	α	*β*	*γ*
ATRA	K_i	18.7 ± 1.7	17.4 ± 1.2	18.5 ± 1.9	350 ± 3.7	881 ± 9.0	288 ± 6.9
	EC_{50}	*563 ± 30*	*105 ± 8.0*	*33 ± 6.0*	*1084 ± 33*	*1394 ± 49*	*1255 ± 39*
	efficacy	100	100	100	100	100	100
9-*cis*-RA	K_i	22.3 ± 6.0	10.9 ± 1.4	20.0 ± 1.9	8.4 ± 0.7	7.4 ± 0.6	13.0 ± 1.7
	EC_{50}	*304 ± 20*	*52 ± 4.6*	*74 ± 9.0*	*316 ± 19*	200 ± 16	*219 ± 13*
	efficacy	81	81	93	143	128	132
ALRT1550	K_i	1.1 ± 0.1	0.7 ± 0.3	1.9 ± 0.2	223 ± 43	560 ± 130	320 ± 62
	EC_{50}	4.0 ± 0.6	*2.2 ± 0.7*	*0.34 ± 0.04*	NA	NA	NA
	efficacy	31	40	48			
LG100567	K_i	63.5 ± 8.5	90.4 ± 9.0	124 ± 18	4.6 ± 0.6	10.2 ± 3.7	9.3 ± 1.5
	EC_{50}	*65 ± 12*	*24 ± 3.0*	*16 ± 1.0*	*134 ± 16*	*87 ± 44*	*77 ± 26*
	efficacy	39	45	58	56	27	40

[a] All K_i values are mean ± SEM of an average of four or five experiments with triplicate determinations in baculovirus. [b] All EC_{50} values were determined from full dose−response curves ranging from 10^{-12} to 10^{-5} M in CV-1 cells. Values are represented as the mean ± SEM of at least three separate experiments with triplicate determinations. Percent efficacy is reported relative to ATRA (ATRA = 100%). NA = no measurable transactivation above the control at 1 μM.

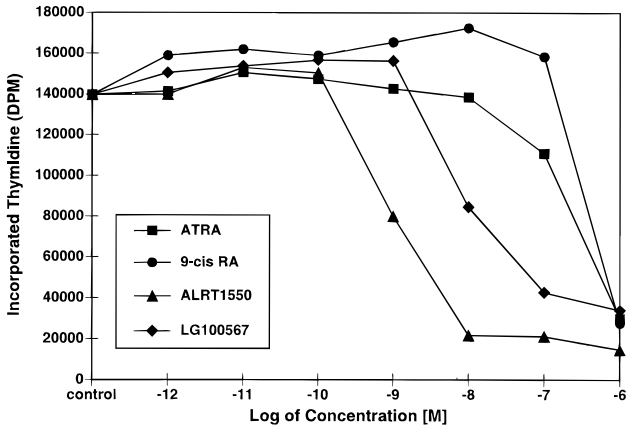
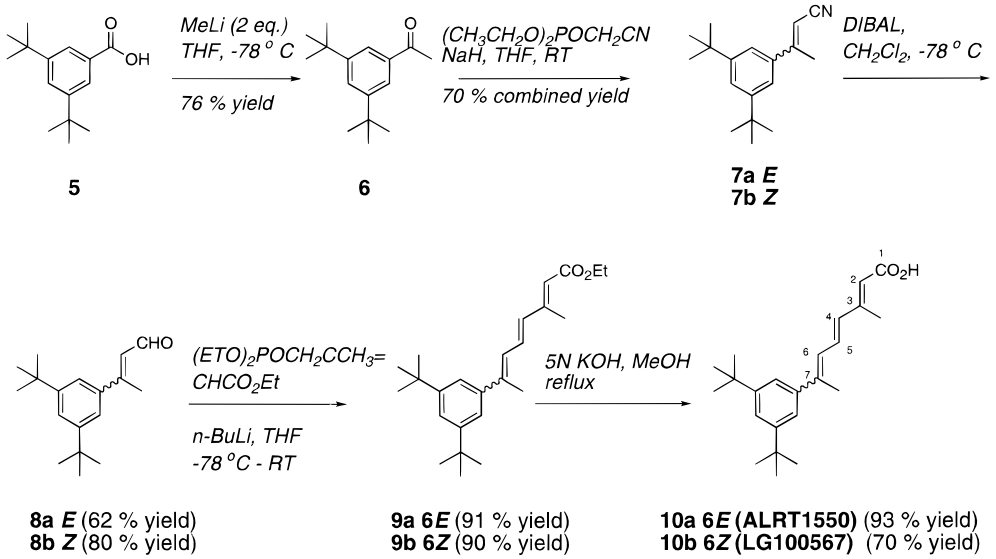


Figure 2. Antiproliferative effects of various retinoids on ME 180 cervical carcinoma cells. The extent of proliferation of ME-180 cells was determined by incorporation of [^{3}H]thymidine into DNA. The retinoids were added to appropriate wells at the concentrations indicated. The amount of incorporated radioactivity (DPM) was determined by liquid scintillation counting. The numbers represent the mean disintegrations per minute of incorporated thymidine from triplicate wells.

methyl groups at the C-3 and C-7 positions as well as olefins in the *E* configuration at the C-2 and C-4 positions (see Chart 1 and Scheme 1 for numbering). From the binding and cotransfection data it is apparent that the receptor selectivity of these compounds is largely determined by the configuration of the C-6 olefin bond. The 6*E* configuration of ATRA and ALRT1550 imparts RAR selectivity, whereas the 6*Z* configuration of 9-*cis*-RA and LG100567 imparts a pan-agonist profile. On the basis of our experience, we expected the respective isomer pairs (ATRA/ALRT1550, and 9-*cis*-RA/LG100567) to exhibit similar profiles in biological assays. Indeed, the results presented here demonstrate that LG100567 shows comparable activity to that of 9-*cis*-RA in both the binding and cotransfection assays. Surprisingly, however, comparison of the *all-trans*-retinoids reveals that ALRT1550 is significantly more potent and RAR selective than ATRA in the binding and cotransfection assays.

Both ALRT1550 and LG100567 are particularly effective in the antiproliferative assay against ME-180 cells where ALRT1550 is 300 times more potent than ATRA and LG100567 is 25 times more potent than 9-*cis*-RA. To the best of our knowledge, such a dramatic increase in activity among similar retinoid isomers is unprecedented. In the antiproliferative assay ALRT1550 is fully efficacious, showing inhibition of growth similar to that exhibited by ATRA, whereas in the cotransfection assay the efficacy of ALRT1550 is approximately one-third to one-half that of ATRA.

Scheme 1



RXR selective retinoids, such as Targretin, are minimally active or inactive in this assay (data not shown), suggesting that the mechanism of action may be mediated by transactivation of RARs or possibly via transrepression of AP-1-related signaling pathways.[7] The potent antiproliferative activity of ALRT1550 may be explained by its subnanomolar cotransfection activity at RARγ based on the observation that retinoid modulation of ME-180 cell growth is regulated by the epidermal growth factor receptor (EGFR) whose promoter is in turn regulated by RARγ.[27] However, other mechanisms may contribute to its potent antiproliferative activity as shown by the negative regulation of AP-1 genes with ATRA.[7]

One further benefit of this structural class of compounds may be their increased chemical and metabolic stability. Replacement of the β-ionene moiety of ATRA and 9-*cis*-RA with the di-*tert*-butylphenyl function results in elimination of the oxidizable C-12 allylic position as well as one less conjugated olefin in the side chain. As a result, it is likely that these compounds exhibit increased chemical and metabolic stability over that of ATRA and 9-*cis*-RA.

In conclusion, the potent activity of ALRT1550 and LG100567 in binding, cotransfection, and antiproliferative assays suggests that these retinoids may provide significant chemotherapeutic efficacy *in vivo*. Our preliminary data indicate that ALRT1550 is highly efficacious in inhibiting tumor growth of a human head and neck carcinoma in an *in vivo* xenograft mouse model.[28] Moreover, we believe that exceptionally potent RAR modulators such as ALRT1550 may provide substantial chemotherapeutic efficacy upon short-term administration and thus may reduce the incidence of hypervitaminosis-A, which is often observed with chronic administration of retinoids. This assumption remains to be evaluated in further *in vivo* studies. On the basis of the data presented here and the activity in numerous other *in vitro* and *in vivo* assays (to be presented in future reports), ALRT1550 has recently been selected as a clinical candidate for the acute treatment of various cancers.

Experimental Section

Unless otherwise stated, all reactions were carried out under a nitrogen atmosphere. The organic solvents were purchased from Fisher Scientific. TLC was performed with Merck Kieselgel 60 F-254 plates. ^{1}H-NMR spectra were determined on a Bruker 400 MHz instrument. Mass spectra were recorded on a Hewlett-Packard GCMS Model 5890 mass spectrometer. Melting points were obtained with Mettler FP62 and Mel-Temp II instruments. Elemental analyses were performed on a modified Coulometrics Carbon Analyzer Model 120 and a Carlo Erba Nitrogen Analyzer Model NA1500.

3,5-Di-*tert*-butylacetophenone (6). To 20.0 g (85.5 mmol) of 3,5-di-*tert*-butylbenzoic acid **2** in 100 mL of dry THF at −78 °C was added 94.0 mL (188.0 mmol) of a 2 N ether solution of MeLi. The reaction mixture was warmed slowly to room temperature, stirred for an additional 30 min, and then poured into saturated aqueous NH_4Cl (200 mL). The organic product was extracted with hexanes (2 × 100 mL), dried over $MgSO_4$, filtered, concentrated, and purified by chromatography (SiO_2, 2% EtOAc−hexanes) to give 15.0 g (64.7 mmol) of ketone **6** (76% yield): TLC (5% EtOAc−95% hexanes) R_f 0.8; ^{1}H-NMR ($CDCl_3$) δ 1.39 (s, 18H, 6(CH_3)), 2.61 (s, 3H, CH_3), 7.64 (t, J = 1 Hz, 1H, Ar-H), 7.80 (d, J = 1 Hz, 2H, Ar-H).

3-(3,5-Di-*tert*-butylphenyl)but-2-enenitriles (7a and 7b). To 2.43 g (13.7 mmol) of diethyl cyanomethylphosphonate in 10 mL of dry THF was added 440 mg (10.96 mmol) of NaH. The reaction mixture was stirred for 30 min, followed by addition of 1.59 g (6.85 mmol) of ketone **6** in 5 mL of dry THF. After 3 h of stirring, the reaction mixture was quenched with saturated aqueous NH_4Cl (50 mL), and the products were extracted with ether (2 × 50 mL). The combined ether extract was washed (water then brine), dried over $MgSO_4$, filtered, concentrated, and purified by preparative TLC (SiO_2, 2.5% EtOAc−hexanes) to give 1.1 g (4.4 mmol) of the *E* isomer **7a** and 104 mg (0.4 mmol) of the *cis* isomer **7b** (70% combined yield). *E* isomer **7a**: TLC (5% EtOAc−95% hexanes) R_f 0.9; ^{1}H-NMR ($CDCl_3$) δ 1.32 (s, 18H, 6(CH_3)), 2.49 (s, 3H, CH_3), 5.59 (s, 1H, =CH), 7.25 (d, J = 1 Hz, 2H, Ar-H), 7.50 (d, J = 1 Hz, 1H, Ar-H). *Z* isomer **7b**: TLC (5% EtOAc−95% hexanes) R_f 0.8; ^{1}H-NMR ($CDCl_3$) δ 1.42 (s, 18H, 6(CH_3)), 2.31 (s, 3H, CH_3), 5.34 (s, 1H, =CH), 7.39 (d, J = 1 Hz, 2H, Ar-H), 7.49 (t, J = 1 Hz, 1H, Ar-H).

3-(3,5-Di-*tert*-butylphenyl)but-2-enal (8a, *E* isomer). To 736 mg (2.89 mmol) of **7a** in 5 mL of CH_2Cl_2 at −78 °C was added 2.31 mL (3.47 mmol) of a 1.5 M solution of DIBAL in toluene. After 15 min of stirring at −78 °C, the reaction mixture was quenched with 10 mL of a saturated aqueous solution of Rochelle salt. The product was extracted with ether (2 × 20 mL), washed (water then brine), dried over $MgSO_4$, filtered, concentrated, and purified by chromatography (SiO_2, 3% EtOAc−hexanes) to give 462.3 mg (1.80 mmol) of **8a** (62% yield): TLC (10% EtOAc−90% hexanes) R_f 0.5; ^{1}H-NMR ($CDCl_3$) δ 1.34 (s, 18H, 6(CH_3)), 2.59 (s, 3H, CH_3), 6.50 (d, J = 8.0 Hz, 1H, =CH), 7.39 (d, J = 1 Hz, 2H, Ar-H), 7.51 (t, J = 1 Hz, 1H, Ar-H), 10.18 (d, J = 8.0 Hz, 1H, CHO).

3-(3,5-Di-*tert*-butylphenyl)but-2-enal (8b, *Z* isomer). The *Z* isomer **8b** was prepared from the corresponding *Z* isomer **7b** using the same method as described for **8a** (80% yield): TLC (10% EtOAc−90% hexanes) R_f 0.55; ^{1}H-NMR ($CDCl_3$) δ 1.34 (s, 18H, 6(CH_3)), 2.34 (s, 3H, CH_3), 6.12 (d, J = 8.0 Hz, 1H, =CH), 7.10 (d, J = 1 Hz, 2H, Ar-H), 7.46 (t, J = 1 Hz, 1H, Ar-H), 9.45 (d, J = 8.0 Hz, 1H, CHO).

Ethyl (2*E*,4*E*,6*E*)-7-(3,5-Di-*tert*-butylphenyl)-3-methylocta-2,4,6-trienoate (9a). To 790 mg (3.0 mmol) of triethyl 3-methyl-4-phosphonocrotonate in 8 mL of dry THF at −78 °C was added 1.2 mL of a 2.5 M nBuLi solution in hexanes. After stirring for 15 min, the solution containing the ylide of triethyl phosphonocrotonate was added to 258 mg (1.0 mmol) of the *E* isomer **8a** in 8 mL of dry THF at −78 °C. The reaction mixture was warmed to room temperature, and quenched with saturated aqueous NH_4Cl (20 mL), and the products were extracted with ether (2 × 50 mL). The combined ether extract was washed (water then brine), dried over $MgSO_4$, filtered, concentrated, and purified by column chromatography (SiO_2, 5% EtOAc−hexanes) to give 335 mg (0.91 mmol) of **9a** (91% yield): TLC (5% EtOAc−95% hexanes) R_f 0.78; ^{1}H-NMR ($CDCl_3$) δ 1.30 (t, J = 7.7 Hz, 3H, CH_2CH_3), 1.34 (s, 18H, 6(CH_3)), 2.28 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 4.17 (m, 2H, CH_2-CH_3), 5.82 (s, 1H, =CH), 6.40 (d, J = 15 Hz, 1H, =CH), 6.54 (d, J = 12 Hz, 1H, =CH), 7.04 (m, 1H, =CH), 7.21 (d, J = 1 Hz, 2H, Ar-H), 7.39 (t, J = 1 Hz, 1H, Ar-H).

Ethyl (2*E*,4*E*,6*Z*)-7-(3,5-Di-*tert*-butylphenyl)-3-methylocta-2,4,6-trienoate (9b). The 2*E*,4*E*,6*Z* isomer **9b** was prepared in the same manner as the 2*E*,4*E*,6*E* isomer **9a** except that **8b** was used instead of the **8a**. Compound **9b** was obtained in 90% yield: TLC (5% EtOAc−95% hexanes) R_f 0.82; ^{1}H-NMR ($CDCl_3$) δ 1.27 (t, J = 7.7 Hz, 3H, CH_2CH_3), 1.34 (s, 18H, 6(CH_3)), 2.17, (s, 3H, CH_3), 2.22 (s, 3H, CH_3), 4.15 (m, 2H, CH_2CH_3), 5.74 (s, 1H, =CH), 6.25 (d, J = 11 Hz, 1H, =CH), 6.27 (d, J = 15 Hz, 1H, =CH), 7.10 (d, J = 1 Hz, 2H, Ar-H), 7.37 (t, J = 1 Hz, 1H, Ar-H).

(2*E*,4*E*,6*E*)-7-(3,5-Di-*tert*-butylphenyl)-3-methylocta-2,4,6-trienoic Acid (10a). To 180 mg (0.49 mmol) of the (2*E*,4*E*,6*E*)-ethyl ester **9a** in 5 mL of MeOH was added 1 mL of 5 N aqueous NaOH solution. The mixture was heated at reflux for 10 min, cooled to room temperature, and acidified with 20% aqueous HCl solution, and the organics were extracted with ether (2 × 10 mL). The ether layer was washed (H_2O, brine), dried over $MgSO_4$, filtered, and concentrated. Purification by column chromatography (SiO_2, 20% EtOAc−hexanes) gave 157 mg (0.46 mmol) of the 2*E*,4*E*,6*E* isomer **10a** (93% yield): TLC (10% MeOH−90% $CHCl_3$) R_f 0.6; mp 196−

198 °C; ^{1}H-NMR ($CDCl_3$) δ 1.35 (s, 18H, 6(CH_3)), 2.29, (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 5.84 (s, 1H, =CH), 6.41 (d, J = 15 Hz, 1H, =CH), 6.54 (d, J = 11 Hz, 1H, =CH), 7.08 (m, 1H, =CH), 7.32 (d, J = 1 Hz, 2H, Ar-H), 7.39 (t, J = 1 Hz, 1H, Ar-H); HRMS found 340.2394, calcd 340.2402. Anal. ($C_{23}H_{32}O_2$) C, H.

(2*E*,4*E*,6*Z*)-7-(3,5-Di-*tert*-butylphenyl)-3-methylocta-2,4,6-trienoic Acid (10b). The 2*E*,4*E*,6*Z* isomer **10b** was prepared in the same manner as **10a** except that **9b** was used instead of **9a**. Compound **10b** was obtained in 70% yield: TLC (10% MeOH−90% $CHCl_3$) R_f 0.57; mp 221−222 °C; ^{1}H-NMR ($CDCl_3$) δ 1.34 (s, 18H, 6(CH_3)), 2.18, (s, 3H, CH_3), 2.23 (s, 3H, CH_3), 5.77 (s, 1H, =CH), 6.25 (d, J = 11 Hz, 1H, =CH), 6.29 (d, J = 15 Hz, 1H, =CH), 6.84 (m, 1H, =CH), 7.10 (d, J = 1 Hz, 2H, Ar-H), 7.37 (t, J = 1 Hz, 1H, Ar-H); HRMS found 340.2425, calcd 340.2402. Anal. ($C_{23}H_{32}O_2$) C, H.

Cell Culture. ME-180 cells (ATCC HTB 33) were obtained from the American Type Culture Collection (ATCC, Rockville, MD). They were grown and maintained in McCoy's 5a medium (Gibco) supplemented with 10% fetal bovine serum, glutamine, and antibiotics. The cells were maintained as monolayer cultures grown at 37 °C in a humidified atmosphere of 5% CO_2 in air.

Incorporation of [5′-^{3}H]Thymidine. The method used for determining radiolabeled thymidine incorporation was adapted from the procedure described by Shrivastav *et al.*[26] ME-180 cells, harvested by trypsinization, were plated in a 96-well flat bottom microtiter plate (Costar) at a density of 2000 cells/well. To appropriate wells were added retinoid test compounds (solubilized in 10% DMSO-90% EtOH then diluted in cell culture media) at final concentrations ranging from 10^{-12} to 10^{-6} in a final volume of 100 μL/well. After the appropriate incubation time (4 days), 1 μCi of [5′-^{3}H]thymidine (Amersham, U.K., 43 Ci/mmol) in 25 μL of culture medium was added to each well, and the cells were incubated for an additional 6 h. The supernatant was then removed, and the cells were washed with Versene (EDTA). ME-180 cells were briefly treated with 25 μL of 0.5% trypsin to dislodge the cells from the plate. The DNA was precipitated with 2% trichloroacetic acid onto glass fiber filter mats utilizing a SKATRON multiwell cell harvester (Skatron Instruments, Sterling, VA).

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