Design and Synthesis of Potent Retinoid X Receptor Selective Ligands That Induce Apoptosis in Leukemia Cells

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Structural modifications of the retinoid X receptor (RXR) selective compound 4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoic acid (LGD1069), which is currently in phase I/IIA clinical trials for cancer and dermatological indications, have resulted in the identification of increasingly potent retinoids with >1000-fold selectivity for the RXRs. This paper describes the design and preparation of a series of RXR selective retinoids as well as the biological data obtained from cotransfection and competitive binding assays which were used to evaluate their potency and selectivity. The most potent and selective of the analogs is 6-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopropyl]nicotinic acid (**12d**; LG100268). This compound has proven useful for investigating RXR dependent biological pathways including the induction of programmed cell death (PCD) and transglutaminase (TGase) activity. Our studies indicate that the induction of PCD and TGase in human leukemic myeloid cells is dependent upon activation of RXR-mediated pathways.

Introduction

Recently, we reported on the design and synthesis of a novel class of retinoid X receptor (RXR) selective compounds.[1] These compounds are potentially useful as therapeutic agents to treat cancer and dermatological diseases. One of these RXR selective retinoids, LGD1069, is currently in phase I/IIA clinical studies and represents the first RXR selective retinoid to be administered to humans. The biological profile of such compounds may provide significant advantages over currently administered retinoids which primarily activate the retinoic acid receptors (RARs). We have continued to explore the structure–activity relationships of the LGD1069 class of compounds and have now identified other potent RXR selective retinoids including a novel series of nicotinic acid derivatives.

Retinoids, such as *all-trans*-retinoic acid (ATRA), 13-*cis*-retinoic acid (13-*cis*-RA), and synthetic etretinate, have been administered for treatment of numerous skin diseases[2] including psoriasis[3] and acne.[4] ATRA and 13-*cis*-RA also have shown utility for treatment of carcinomas and for cancer chemoprevention.[5,6] The therapeutic actions of retinoids are due to their ability to regulate cellular processes *in vivo* such as cellular differentiation, proliferation, and modulation of apoptosis, also known as programmed cell death.[7,8] Unfortunately, associated with some of these processes are high incidences of undesirable side effects including skin irritation, lipid and bone toxicity, visual effects (night blindness, dry eye), and teratogenicity.[8] This provides impetus for identifying new retinoids which have unique biological profiles and potentially greater therapeutic indices.

The retinoid receptors are members of the superfamily of intracellular hormone receptors which function as regulators of gene transcription.[9,10] These receptors comprise two distinct families, the RARs and the RXRs, each of which embody three closely homologous receptors, RARα,β,γ and RXRα,β,γ. The classification of retinoid receptors is based upon differences in amino acid structure, responsiveness toward natural and synthetic ligands, and ability to modulate expression of various target genes. Ligands which interact with these receptors, the retinoids, make up a structurally diverse group of molecules (for reviews, see refs 11 and 12). Among these are (1) molecules containing a tetraene moiety including the endogenous retinoids ATRA, 13-*cis*-RA, 9-*cis*-RA, and 11-*cis*-retinal and synthetic derivatives such as eterinate and acitretin, (2) synthetic retinoids based upon the structure of stilbene, including TTNPB and 3-methyl-TTNPB, (3) analogs of TTNPB utilizing an amide functionality in place of the olefin which include AM80 and AM580,[13,14] (4) compounds containing an alkyne in place of the olefin such as Tazarotene which has shown utility for treatment of psoriasis,[15] and (5) recently identified compounds based upon benzophenone carboxylic acid, including 4-[(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic acid,[16] SR11237,[17] and LGD1069.[1]

These and other known retinoids are thought to exert their biological action by binding to one or more of the six known retinoid receptors resulting in the formation of a receptor–DNA complex and subsequent activation of gene expression. As a result of their diverse structues, the retinoids mentioned above exhibit a wide range of receptor selectivities. For example, *all-trans* tetraenoic acid derivatives, such as ATRA, primarily activate the RARs.[18] Similarly, TTNPB, AM80, and AM580 are activators of RARs.[13,14,18] In contrast, the isomer of ATRA, 9-*cis* RA, and the 3-methyl derivative of TTNPB, 3-methyl-TTNPB, are activators of all six retinoid receptors.[18,19] Finally, the recently identified

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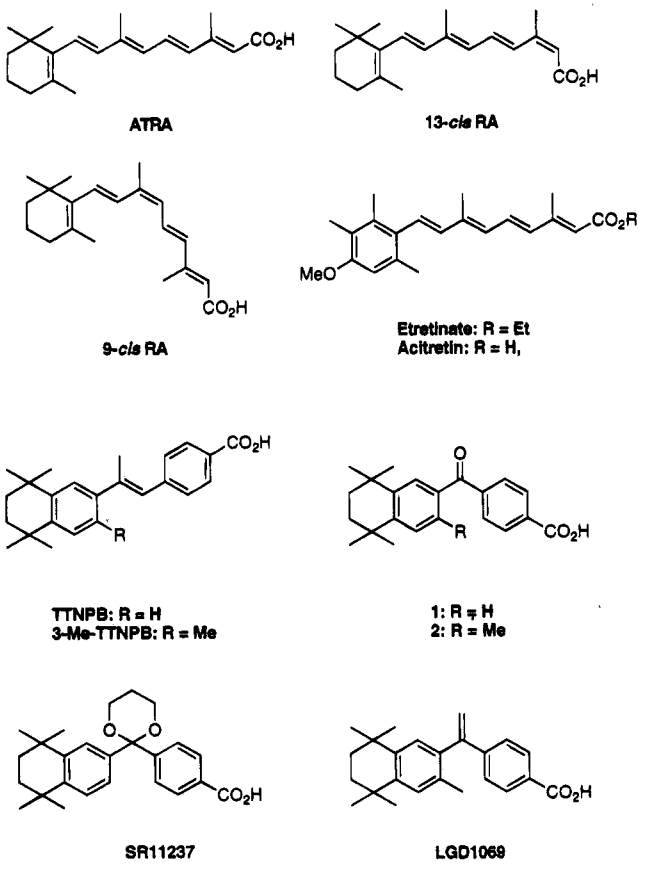
Chart 1



retinoids LGD1069 and SR11237 are selective activators of the RXRs with little or no activation of the RARs.[1,17]

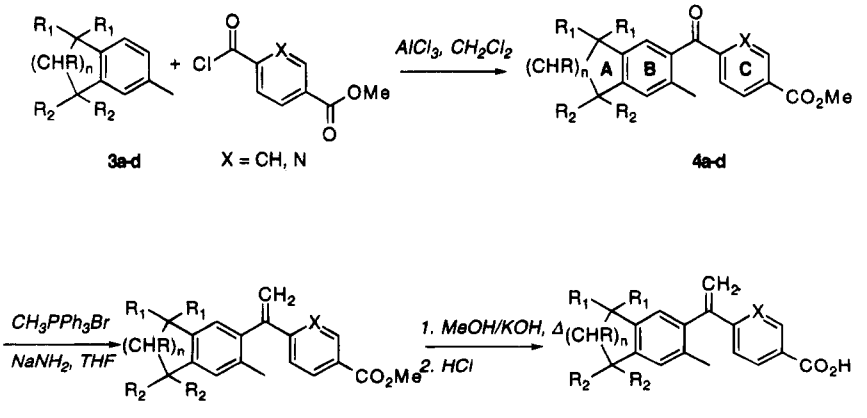
In our continuing examination of the chemistry and biology of RXR selective compounds, we have identified increasingly potent and selective analogs. These compounds serve as important probes to study the mode of action of retinoid X receptors. For example, it has been shown that retinoids may play a role in the regulation of apoptosis.[7] Recent studies have indicated that dying tumor cells may actively participate in their own demise.[20] This process, termed apoptosis, was first defined as a cascade of characteristic morphological features within the dying cell. In contrast to necrosis, cells triggered to undergo apoptosis are characterized by cell swelling, chromatin flocculation, rapid loss of membrane integrity, and rapid cell lysis.[21] The modes of induction of apoptosis are numerous and diverse, ranging from treatment with cytotoxic drugs[22] exposure to gluccocorticoids[23] or treatment with other biological modifiers.[24] Additionally, it has been shown that RXRs form dimers with other members of the superfamily of hormone receptors. For example, RXRs have been shown to form heterodimers with RARs upon activation with RXR selective ligands.[25,26] It has also been postulated that the RXRs form heterodimers with the thyroid hormone receptor (TR) and peroxisome proliferator-activated receptors (PPAR).[27,28] Thus, potent RXR selective ligands will increasingly provide tools for identifying new biological pathways which may have therapeutic utility for control of abnormal cellular processes.

In this report we describe structure–activity relationship studies of the RXR selective compound LGD1069 including the identification of a new, potent nicotinic acid derivative, **12d** (designated LG100268). These compounds were tested in two primary assays, a cotransfection assay and a competitive binding assay. In addition, experimental data using LG100268 demonstrate a link between ligand activation of RXR and the induction of apoptosis and tissue transglutaminase activity in human leukemia cells (HL-60 cells).

Chemistry

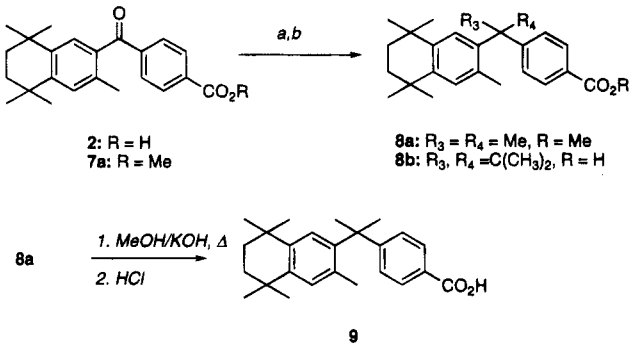
Analogs **4a–19b** were synthesized by the various routes described below. Compounds with modifications in the A-ring were prepared according to Scheme 1 (the A-, B-, and C-rings are defined as shown for structures **4a–d**). Using similar methodology as was described in Boehm *et al.*,[1] the appropriate tetrahydronaphthyl or indane derivative **3a–d** was acylated with chloromethylterephthalate or chloromethylnicotinate under Friedel–Crafts conditions to give ketones **4a–d**. Olefination of **4a–d** with the methyltriphenylphosphorylide gave esters **5a–d** which were saponified with methanolic KOH and acidified to give carboxylic acids **6a–d**.

Scheme 1



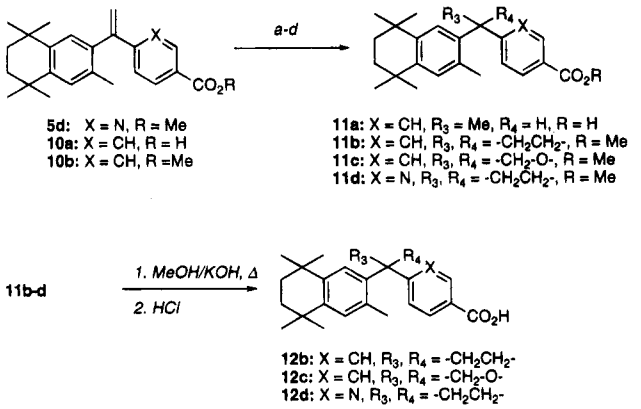
	R	R_1	R_2	n	X (4-6)
a	H	Me	H	2	CH
b	H	H	Me	2	CH
c	Me	Me	Me	1	CH
d	H	Me	Me	2	N

Scheme 2



[a] (a) $(CH_3)_2Zn$, $TiCl_4$, CH_2Cl_2 (**8a**); (b) $(CH_3)_2CHMgCl$, THF, HCl/MeOH (**8b**).

Scheme 3



[a] (a) H_2, 10% Pd/C (**11a**); (b) Zn, CuCl, CH_2I_2, Et_2O (**11b**); (c) *m*-CPBA, CH_2Cl_2 (**11c**); (d) Et_2Zn, $ClCH_2I$, CH_2Cl_2 (**11d**).

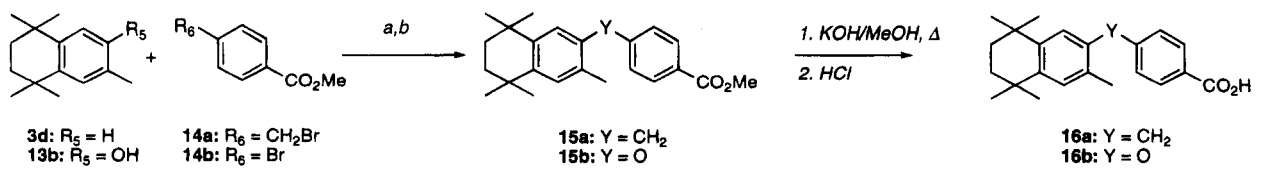
Compounds with modifications at the bridgehead (sp^2) position were prepared as shown in Schemes 2–5. In Scheme 2, treatment of ketone **7a** with dimethylzinc and titanium tetrachloride gave the dimethylated ester **8a**, which was saponified to give carboxylic acid **9**. Treatment of **2** with isopropylmagnesium chloride followed by dehydration gave carboxylic acid **8b**. In Scheme 3, treatment of **10a** with hydrogen gas over a heterogeneous catalyst such as Pd/C gave **11a**. Cyclopropanation of **10b** with zinc dust, cuprous chloride, and diiodomethane gave **11b**, while oxidation of **10b** with *m*-chloroperbenzoic acid gave epoxide **11c**. Treatment of nicotinate **5d** with diethylzinc and chloroiodomethane gave cyclopropyl compound **11d**. Saponification of esters **11b–d** gave carboxylic acids **12b–d**. In Scheme 4, 1,1,4,4,6-pentamethyltetrahydronaphthalene (**3d**) was alkylated with methyl (bromomethyl)benzoate **14a** under Friedel–Crafts conditions to give ester **15a**. The ether **15b** was prepared by treating phenol **13b** and methyl bromobenzoate **14b** with copper(I) oxide in refluxing collidine. Methyl esters **15a,b** were saponified and acidified to give carboxylic acids **16a,b**. The meta and ortho isomers of LGD1069 were prepared as shown in Scheme 5. Acylation of **3d** with the appropriate (chloromethyl)benzoate **17a,b** gave ketones **18a,b** which were saponified and acidified to afford compounds **19a,b**.

Biological Studies

Compounds **6a–d**, **8b**, **9**, **11a**, **12b–d**, **16a,b**, and **19a,b** were evaluated in a cotransfection assay,[29–31] which measures the ability of compounds to activate gene expression at each of the six retinoid receptors and reflects the compound's functional activity, and a competitive binding assay,[1] which characterizes the ligand's ability to bind directly to each of the six receptor subtypes. EC_{50} and K_d values are reported for RXRα, RXRβ, and RXRγ in Table 1. EC_{50} and K_d values for the RARs are >10 000 and >1000 nm, respectively, and, thus, are not shown.

The first series of analogs of LGD1069, **6a–c**, comprise modifications in the A-ring. Compounds **6a,b** were designed to establish the importance of the geminal dimethyl groups on the A-ring. Removal of the dimethyl groups at the 5-position (compound **6a**) showed a 100-fold decrease in potency in the cotransfection assay (Table 1). Similarly, removal of the geminal dimethyl function at the 8-position (compound **6b**) resulted in a 30–40-fold decrease in potency. These results were confirmed by competitive binding data which indicated >1000 nM binding for all six retinoid receptors for **6a** and only weak binding at the RXRs for **6b**. The indane derivative **6c** provided information about the biological effect of a smaller (five-membered) A-ring substitution. Compound **6c** was approximately 10-fold less effective than LGD1069 in inducing transcription or in the competitive binding assay. Thus, comparison of transcriptional activation of LGD1069 to the data from compounds **6a–c** shows that the most favorable A-ring

Scheme 4



[a] (a) $AlCl_3$, CH_2Cl_2 (**15a**); (b) Cu_2O, collidine, Δ (**15b**).

Scheme 5

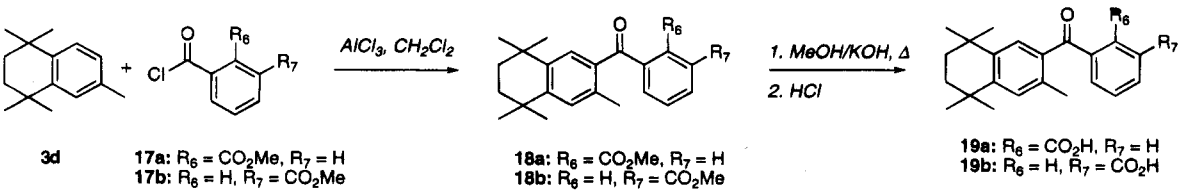
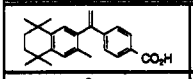
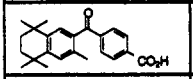
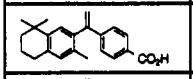
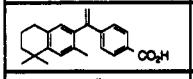
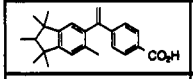
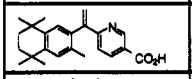
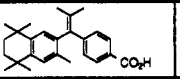
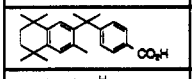
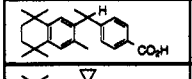
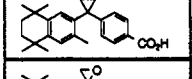
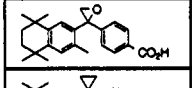
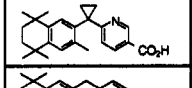
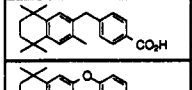
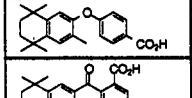
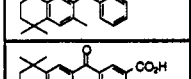
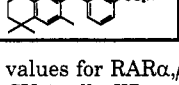


Table 1. Cotransfection and Competitive Binding Data For Synthetic Retinoids

Structure	Comp. #	RXR (EC$_{50}$, nM)* α	β	γ	RXR (K_d, nM)** α	β	γ
	LGD 1069	28±1	25±2	20±1	36±18	21±8	29±2
	2	279±43	213±81	246±29	138±8	191±45	299±75
	6a	2400	2400	2600	>1000	>1000	>1000
	6b	1200	770	600	1159±29	853±40	1736±490
	6c	300	280	500	176±9	291±195	283±72
	6d	6±1	9±2	5±1	22±8	61±40	39±8
	8b	300	130	240	50	160	150
	9	650	1900	650	>1000	>1000	>1000
	11a	470	310	340	110	150	200
	12b	26±1	22±5	17±5	46±23	70±8	25±3
	12c	51±7	67±18	47±6	670±100	190±16	547±284
	12d	4±0	3±1	4±0	3±1	3±1	3±1
	16a	460	1200	440	>1000	>1000	>1000
	16b	1000	1900	430	520	770	450
	19a	>10,000	>10,000	>10,000	>1000	>1000	>1000
	19b	>10,000	>10,000	>10,000	>1000	>1000	>1000

* EC_{50} values for RARα,β,γ are >10 000 nM. All EC_{50} values were determined from full dose–response curves ranging from 10^{-12} to 10^{-5} M in CV-1 cells. Where errors are indicated, values represent the standard error of the mean value of at least two separate experiments with triplicate determinations. Where no error range is indicated, values represent the EC_{50} determination of a single experiment with triplicate determinations. Standard errors for this assay system are, on average, *ca.* 15% of the mean values. ** K_d values for RARα,β,γ are >1000 nM. All K_d values are mean ± SEM of an average of three experiments in baculovirus. Where no error range is indicated, values represent the K_d determination of a single experiment with triplicate determinations. Standard errors for this assay system are, on average, *ca.* 15% of the mean values.

configuration is a six-membered ring containing two geminal dimethyl groups in both the 5- and 8-positions. Removal of either of the gem dimethyl groups resulted in inactive compounds (**6a,b**), while reduction of the size of the A-ring to give an indane reduced the potency and binding affinity by 10-fold (compound **6c**).

Modifications at the C-ring of this series include the ortho and meta isomers of the *p*-benzoic acid moiety of **2** (compounds **19a,b**) as well as introduction of a nicotinic acid isostere for the benzoic acid function in LGD1069 (compound **6d**). Unlike the para isomer **2**, the *o*- and *m*-benzoic acid isomers **19a,b** were completely inactive at all six receptors in both cotransfection and binding assays. Substitution of the benzoic acid group of LGD1069 with a nicotinic acid moiety (compound **6d**) showed a 4-fold increase in potency, with similar affinity in the competitive binding assay to LGD1069. Although the cotransfection assay shows higher potency for nicotinic acid derivative **6d** than for LGD1069, their corresponding binding affinities are comparable.

Examination of bridgehead ethenyl substitutions of LGD1069 (analogs **8b**, **9**, **11a**, **12b,c**, and **16a,b**) indicated that only the cyclopropyl group resulted in an analog with comparable or improved potency over the parent LGD1069. For example, alkyl groups such as methyl and dimethyl (compounds **9** and **11a**) diminished potency by at least 10-fold as did the dihydro compound **16a**. Similarly, replacement of the carbon bridge with an ether moiety (compound **16b**) resulted in loss of all activity. Larger functional groups such as the isopropenyl group in **8b**, as well as heterocyclic rings such as epoxide **12c**, decreased potency and binding affinity. However, introduction of a cyclopropyl group (compound **12b**) in place of the ethenyl moiety of LGD1069 resulted

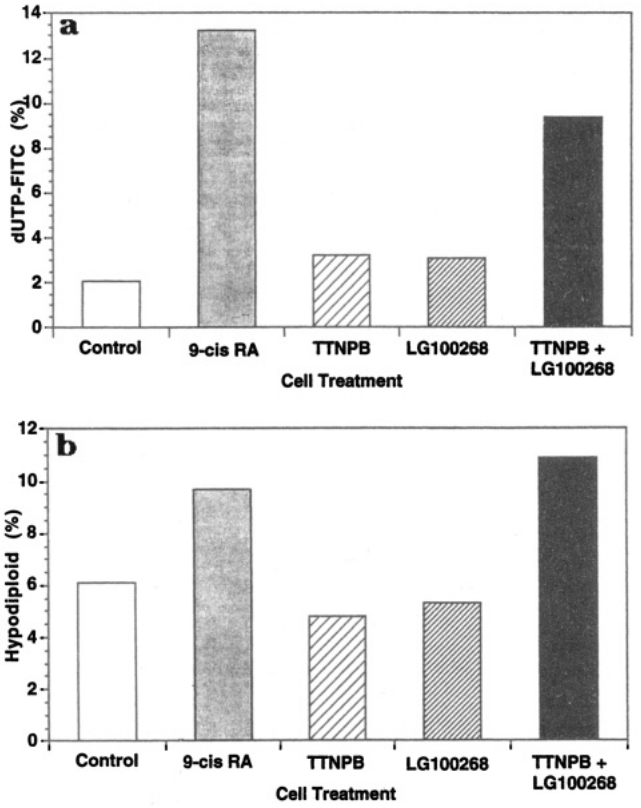


Figure 1. HL-60 cells were incubated with solvent control or with 1 μM 9-*cis*-RA, TTNPB, or LG100268 alone for 72 h followed by addition of fresh 10% FCS-RPMI medium for another 48 h. A second test group was incubated with 1 μM TTNPB alone for 72 h followed by addition of 1 μM LG100268 for another 48 h. After 5 days in culture, the cells were collected and analyzed for the induction of apoptosis as described in the Experimental Section. (a) The numbers represent the percentage of positive fluorescing cells as determined using a FACScan (Becton Dickinson). (b) The numbers represent the percentage of cells with less than 2 N DNA, hypodiploid, as determined by fluorescence of propidium iodide (PT) using a FACScan (Becton Dickinson).

in a slight increase of potency with similar binding affinity at RXRs.

Finally, the combination of the most favorable features in compounds **6d** and **12b** resulted in the highly potent and selective cyclopropylnicotinic acid derivative (LG100268) **12d**. LG100268 exhibited a substantial increase in RXR transcriptional activation as well as binding affinity (Table 1). As with many of the other analogs, LG100268 did not show any activity at the RARs (>1000-fold selective for the RXRs), and it is the most potent RXR selective compound reported to date.

RXR and RAR selective retinoids were further examined in the cell death assay (TUNEL assay) using the human promyelocytic leukemia HL-60 cell line. This assay is based on labeling of DNA strand breaks in individual cells with fluorescinated probes and subsequent analysis by flow cytometry.[32,33] Our experiments showed that cells treated for 5 days with 1 μM TTNPB, a RAR selective retinoid, or 1 μM LG100268, a RXR selective retinoid, did not undergo apoptosis as measured by the percentage of fluorescent positive cells as compared to the solvent control group (2%, 3%, and 3%, respectively; Figure 1a) or as a percentage of hypodiploid cells (6%, 5%, and 6% respectively; Figure 1b). In contrast, a 72 h treatment with TTNPB followed by a

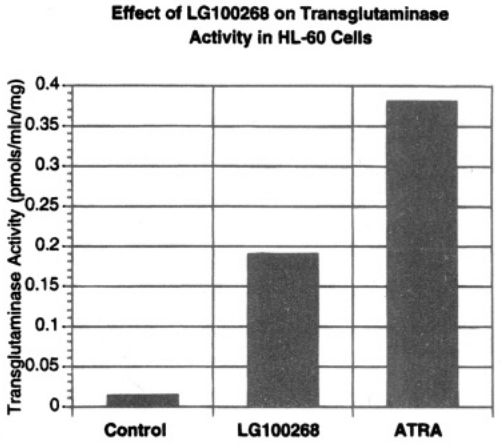


Figure 2. HL-60 cells were treated with LG100268 (10^{-8} M), *all-trans*-RA (10^{-6} M), or a solvent control under conditions described in detail in the Experimental Section. Values shown represent the mean of duplicate determinations of enzyme activity from four separate experiments. Error bars are ± standard deviation. Control = 0.014 ± 0.009; LG100268 = 0.19 ± 0.09; ATRA = 0.38 ± 0.07.

concurrent 48 h incubation with LG100268 caused a greater than additive increase in the percentage of cells labeling with the anti-digoxigenin FITC conjugated antibody (10%; Figure 1a) or in the percentage of hypodiploid cells (12%; Figure 1b). This suggests that the presence of both RAR- and RXR-activating components is required for induction of apoptosis. Similarly, a 5 day incubation of HL-60 cells with 1 μM 9-*cis*-RA induced 13.1% of the cells to undergo apoptosis (Figure 1a) and an increase in the percentage of hypodiploid cells relative to the solvent control (6% vs 9%, respectively; Figure 1b). Unlike TTNPB or LG100268 alone, 9-*cis*-RA induces apoptosis (Figure 1) which confirms observations of others[8] that retinoic acid isomers can induce HL-60 cells to undergo apoptosis. The activity of 9-*cis*-RA may be due to its ability to activate both RARs and RXRs.

Finally, LG100268 was examined for its ability to induce tissue transglutaminase (TGase) activity in HL-60 cells (Figure 2). Previous studies have shown that ATRA acts as an acute and specific inducer of tissue transglutaminase expression in both normal and leukemic myeloid cells.[34-36] Induction of TGase has also been associated with induction of apoptosis in these cells. Ligand activation of RXR with LG100268 (10^{-8} M) produced a significant increase in TGase activity. As a control, we used ATRA (10^{-6} M) which also showed an increase in TGase response. These data demonstrate a link between RXR activation and induction of TGase activity which is further supported by the observation that modulation of TGase by retinoids is regulated at the transcriptional level.[36]

Conclusions

Using the structure of LGD1069 as the basis for designing other RXR selective compounds, we identified additional functional modifications that yield increasingly potent RXR ligands. In our previous report (ref 1), we studied modifications of the B-ring of LGD1069 and identified functional groups at the 3-position including alkyls, halogens, and alkyl ethers which are essential for inducing RXR selectivity (the numbering scheme is defined for LGD1069 in Chart 1). In the

present series we compare a number of modifications of the A- and C-rings as well as modifications of the ethenyl group. In summary, we have shown in this series that the optimal configuration of a potent and selective RXR active retinoid consists of a tertramethyltetrahydronaphthyl group coupled to a homo- or heteroaromatic acid via an olefin or cyclopropyl moiety. The most potent compound in this series (analog LG100268, **12d**) was designed by combining features of two potent retinoids, **6d** and **12b**. Significantly, compounds such as LG100268 have utility for elucidating the action of the RXRs as exhibited in the apoptosis experiments, which indicate that RXR activation is necessary for induction of programmed cell death, and the transglutaminase assay, which indicates that its expression is an RXR-mediated process. These experiments imply that RXR active retinoids possess important biological functions and may provide novel drug therapies for cellular disorders.

Experimental Section

Unless otherwise stated, all reactions were carried out under a nitrogen atmosphere. The organic solvents were purchased from Fisher Scientific; monomethylterephthalic acid chloride was purchased from TCI America; 1,1,6- and 1,1,7-trimethyltetralin were purchased from K & K/ICN. 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).

Methyl 4-[(3,8,8-Trimethyl-5,6,7,8-tetrahydronaphthalen-2-yl)carbonyl]benzoate (4a). To 280 mg (1.41 mmol) of monomethylterephthalic acid chloride and 250 mg (1.44 mmol) of 1,1,6-trimethyltetralin (**3a**) in 5 mL of CH_2Cl_2 in a 25 mL, three-neck, round-bottom flask fitted with a magnetic stirring bar and reflux condenser was slowly added 575 mg (4.31 mmol) of aluminum chloride ($AlCl_3$). The brown mixture was stirred at room temperature for 10 min and then poured into 10 mL of ice water. After stirring for 10 min, the mixture was extracted with ether (3 × 20 mL), and the ether layer was washed with water (10 mL) and brine (10 mL), dried over $MgSO_4$, filtered, concentrated, and crystallized from EtOAc/MeOH to give 426 mg (1.27 mmol) of compound **4a** (88.4% yield): TLC (2% EtOAc–98% hexane) R_f 0.3; mp 94–96 °C; ^{1}H-NMR ($CDCl_3$) δ 1.23 (s, 6H, $2CH_3$), 1.70 (t, J = 10 Hz, 2H, CH_2), 1.85 (m, 2H, CH_2), 2.31 (s, 3H, CH_3), 2.78 (t, J = 10 Hz, 2H, CH_2), 3.98 (s, 3H, CO_2CH_3), 7.00 (s, 1H, Ar-CH), 7.27 (s, 1H, Ar-CH), 7.86 (d, J = 8.0 Hz, 2H, Ar-CH), 8.10 (d, J = 8 Hz, 2H, Ar-CH).

Methyl 4-[1-(3,8,8-Trimethyl-5,6,7,8-tetrahydronaphthalen-2-yl)ethenyl]benzoate (5a). To a 25 mL round-bottom flask containing 115 mg (0.321 mmol) of methyltriphenylphosphonium bromide in 8 mL of dry THF under dry N_2 was added 10 mg (0.268 mmol) of sodium amide. The reaction mixture was stirred at room temperature for 5 h to give a bright yellow solution, which was then slowly added to a 25 mL round-bottom flask containing 60 mg (0.179 mmol) of compound **4a** in 4 mL of dry THF. The formation of the olefin was monitored by TLC, and the reaction was complete in *ca.* 20 min. The reaction mixture was poured into 10 mL of cold water and extracted with ethyl acetate (2 × 20 mL). The organic layer was washed with water (10 mL) and brine (10 mL), dried over $MgSO_4$, filtered, and concentrated to give 59.0 mg (0.175 mmol) of compound **5a** (98% yield) which was used without further purification: TLC (20% EtOAc–80% hexane) R_f 0.6; ^{1}H-NMR ($CDCl_3$) δ 1.30 (s, 6H, $2CH_3$), 1.68 (t, J = 10 Hz, 2H, CH_2), 1.83 (m, 2H, CH_2),1.92 (s, 3H, CH_3), 2.75 (t, J = 10 Hz, 2H, CH_2), 3.92 (s, 3H, CH_3), 5.31 (s, 1H, CH=), 5.82 (s, 1H, CH=), 6.87 (s, 1H, Ar-CH), 7.15 (s, 1H, Ar-CH), 7.37 (d, J = 8.0 Hz, 2H, Ar-CH), 7.93 (d, J = 8.0 Hz, 2H, Ar-CH).

4-[1-(3,8,8-Trimethyl-5,6,7,8-tetrahydronaphthalen-2-yl)ethenyl]benzoic Acid (6a). To 40 mg (0.12 mmol) of the methyl ester **5a** suspended in 8 mL of MeOH in a 25 mL round-bottom flask equipped with a reflux condenser was added 1 mL of an aqueous 5 N KOH solution. The reaction was heated at reflux for 30 min or until hydrolysis was complete by TLC. After cooling to room temperature, the reaction mixture was poured into 20 mL of 20% aqueous HCl and extracted with EtOAc (2 × 20 mL). The EtOAc layer was washed with water (10 mL) and brine (10 mL), dried over $MgSO_4$, and crystallized from 1:4 EtOAc–hexane to give 30.4 mg (0.095 mmol) of **6a** (80% yield): TLC (10% MeOH–90% $CHCl_3$) R_f 0.5; mp: 180–182 °C; ^{1}H-NMR ($CDCl_3$) δ 1.28 (s, 6H, $2CH_3$), 1.68 (t, J = 10 Hz, 2H, CH_2), 1.83 (m, 2H, CH_2), 1.94 (s, 3H, CH_3), 2.72 (t, J = 10 Hz, 2H, CH_2), 5.32 (s, 1H, =CH), 5.84 (s, 1H, CH=), 6.88 (s, 1H, Ar-CH), 7.17 (s, 1H, Ar-CH), 7.38 (d, J = 8.0 Hz, 2H, Ar-CH), 8.04 (d, J = 8.0 Hz, 2H, Ar-CH). Anal. ($C_{22}H_{24}O_2 \cdot {}^1/_5H_2O$) C,H.

Methyl 4-[(3,5,5-Trimethyl-5,6,7,8-tetrahydronaphthalen-2-yl)carbonyl]benzoate (4b). Compound **4b** was synthesized from 1,1,7-trimethyltetralin (**3b**) following the representative procedure described for compound **4a**. Crystallization from 1:5 EtOAc–MeOH gave ester **4b** as white crystals (98% yield): TLC (5% EtOAc–95% hexane) R_f 0.4; mp 93–94 °C; ^{1}H-NMR ($CDCl_3$) δ 1.31 (s, 6H, $2CH_3$), 1.68 (t, J = 10 Hz, 2H, CH_2), 1.80 (m, 2H, CH_2), 2.30 (s, 3H, CH_3), 2.70 (t, J = 10 Hz, 2H, CH_2), 3.95 (s, 3H, CO_2CH_3), 7.00 (s, 1H, Ar-CH), 7.20 (s, 1H, Ar-CH), 7.81 (d, J = 8.0 Hz, 2H, Ar-CH), 8.09 (d, J = 8.0 Hz, 2H, Ar-CH).

Methyl 4-[1-(3,5,5-Trimethyl-5,6,7,8-tetrahydronaphthalen-2-yl)ethenyl]benzoate (5b). Ethenyl ester **5b** was synthesized in 80% yield from ketone **4b** following the representative procedure described for compound **5a**. Compound **5b** was used without further purification: TLC (5% EtOAc–95% hexane) R_f 0.5; ^{1}H-NMR ($CDCl_3$) δ 1.31 (s, 6H, $2CH_3$), 1.68 (t, J = 10 Hz, 2H, CH_2), 1.80 (m, 2H, CH_2), 1.94 (s, 3H, CH_3), 2.73 (t, J = 10 Hz, 2H, CH_2), 3.88 (s, 3H, CO_2CH_3), 5.28 (s, 1H, CH=), 5.78 (s, 1H, CH=), 6.87 (s, 1H, Ar-CH), 7.09 (s, 1H, Ar-CH), 7.32 (d, J = 8.0 Hz, 2H, Ar-CH), 7.94 (d, J = 16 Hz, 2H, Ar-CH).

4-[1-(3,5,5-Trimethyl-5,6,7,8-tetrahydronaphthalen-2-yl)ethenyl]benzoic Acid (6b). Acid **6b** was synthesized from ester **5b** following the representative procedure described for acid **6a**. Crystallization gave compound **6b** as a white solid in 80% yield: TLC (10% MeOH–90% $CHCl_3$) R_f 0.5; mp 240–242 °C; ^{1}H-NMR ($CDCl_3$) δ 1.31 (s, 6H, $2CH_3$), 1.69 (t, J = 10 Hz, 2H, CH_2), 1.82 (m, 2H, CH_2), 1.96 (s, 3H, CH_3), 2.74 (t, J = 10 Hz, 2H, CH_2), 5.32 (s, 1H, CH=), 5.82 (s, 1H, CH=), 6.88 (s, 1H, Ar-CH), 7.10 (s, 1H, Ar-CH), 7.38 (d, J = 8.0 Hz, 2H, Ar-CH), 8.02 (d, J = 16 Hz, 2H, Ar-CH). Anal. ($C_{22}H_{24}O_2{}^2/_5H_2O$) C,H.

Methyl 4-[(1,1,2,3,3,6-Hexamethylindan-5-yl)carbonyl]benzoate (4c). Ester **4c** was synthesized from the racemic compound **3c**[37] following the resentative procedure described for ketone **4a**. The racemic ester **4c** was obtained as a white solid in 59% yield: TLC (20% EtOAc–80% hexane) R_f 0.6; mp 128–130 °C; ^{1}H-NMR ($CDCl_3$) δ 1.02 (d, J = 8 Hz, 3H, CH_3), 1.04 (s, 3H, CH_3), 1.11 (s, 3H, CH_3), 1.21 (s, 3H, CH_3), 1.31 (s, 3H, CH_3), 1.90 (m, 1H, CH), 2.38 (s, 3H, CH_3), 3.97 (s, 3H, $COOCH_3$), 7.07 (s, 1H, Ar-CH), 7.08 (s, 1H, Ar-CH), 7.85 (d, J = 8.4 Hz, 2H, Ar-CH), 8.11 (d, J = 8.4 Hz, 2H, Ar-CH).

Methyl4-[1-(1,1,2,3,3,6-Hexamethylindan-5-yl)ethenyl]benzoate (5c). Ester **5c** was synthesized in 83% yield from ketone **4c** following the representative procedure described for compound **5a**. The racemic ester **5c** was used without further purification: TLC (20% EtOAc–80% hexane) R_f 0.8; ^{1}H-NMR ($CHCl_3$) δ 1.02 (d, J = 8.0 Hz, 3H,CH_3), 1.08 (s, 3H, CH_3), 1.11 (s, 3H, CH_3), 1.30 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), 1.90 (m, 1H, CH), 2.00 (s, 3H, CH_3), 3.90 (s, 3H, $COOCH_3$), 5.31 (s, 1H, CH=), 5.82 (s, 1H, CH=), 6.95 (s, 1H, Ar-CH), 6.98 (s, 1H, Ar-CH), 7.34 (d, J = 8.4 Hz, 2H, Ar-CH), 7.98 (d, J = 8.4 Hz, 2H, Ar-CH).

4-[1-(1,1,2,3,3,6-Hexamethylindan-5-yl)ethenyl]benzoic Acid (6c). Acid **6c** was synthesized from ester **5c** following

the representative procedure described for acid **6a**. The racemic compound **6c** was purified by column chromatography (SiO_2, 10% MeOH–90% $CHCl_3$) in 81% yield. The compound was further purified by semipreparative ODC-HPLC.: mp 212–214 °C; TLC (10% MeOH–90% $CHCl_3$) R_f 0.5; ^{1}H-NMR ($CDCl_3$) δ 1.02 (d, J = 8.0 Hz, 3H, CH_3), 1.09 (s, 3H, CH_3), 1.11 (s, 3H, CH_3), 1.28 (s, 3H, CH_3), 1.31 (s, 3H, CH_3), 1.89 (m, 1H, CH), 2.00 (s, 3H, CH_3), 5.37 (s, 1H, CH=), 5.84 (s, 1H, CH=), 6.96 (s, 1H, Ar-CH), 6.98 (s, 1H, Ar-CH), 7.40 (d, J = 8.4 Hz, 2H, Ar-CH), 8.00 (d, J = 8.4 Hz, 2H, Ar-CH); HRFAB-MS (M + H) calcd for $C_{24}H_{29}O_2$ 349.2089, found 349.2055.

Methyl 6-(Chlorocarbonyl)nicotinate.[38,39] To a 100 mL round-bottom flask containing 15.0 g (68.45 mmol) of pyridine-2,5-dicarboxylic acid 5-methyl ester was slowly added 50 mL of thionyl chloride. The reaction mixture was heated at reflux for 30 min and then cooled to room temperature. The excess thionyl chloride was removed by vacuum distillation, and the product was dried under vacuum for several hours to obtain *ca.* 13.0 g (65 mmol) of crude methyl 6-(chlorocarbonyl)-nicotinate which was directly used in the next step: ^{1}H-NMR ($CDCl_3$) δ 4.03 (s, 3H, $COOCH_3$), 8.18 (d, J = 10 Hz, 1H, pyridine-CH), 8.50 (d, J = 10 Hz, 1H, pyridine-CH), 9.37 (s, 1H, pyridine-CH).

Methyl 6-[(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)carbonyl]nicotinate (4d). Ketone **4d** was synthesized from methyl 6-(chlorocarbonyl)nicotinate and pentamethyltetrahydronaphthalene **3d** following the representative procedure described for compound **4a**. Ketone **4d** was obtained by column chromatography (SiO_2, 5% EtOAc–95% hexane) in 50% yield: TLC (20% EtOAc–80% hexane) R_f 0.7; mp 119–121 °C; ^{1}H-NMR ($CDCl_3$) δ 1.21 (s, 6H, $2CH_3$), 1.32 (s, 6H, $2CH_3$), 1.69 (s, 4H, $2CH_2$), 2.40 (s, 3H, CH_3), 3.99 (s, 3H, $COOCH_3$), 7.21 (s, 1H, Ar-CH), 7.42 (s, 1H, Ar-CH3), 8.08 (d, J = 10 Hz, 1H, pyridine-CH), 8.47 (d, J = 10 Hz, 1H, pyridine-CH), 9.28 (s, 1H, pyridine-CH).

Methyl 6-[1-(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)ethenyl]nicotinate (5d). Compound **5d** was synthesized from ketone **4d** following the representative procedure described for compound **5a** and purified by column chromatography (SiO_2, 5% EtOAc–95% hexane) in 64% yield: TLC (20% EtOAc–80% hexane) R_f 0.8; mp 163–165 °C; ^{1}H-NMR ($CDCl_3$) δ 1.30 (s, 6H, $2CH_3$), 1.33 (s, 6H, $2CH_3$), 1.72 (s, 4H, $2CH_2$), 2.00 (s, 3H, CH_3), 3.94 (s, 3H, $COOCH_3$), 5.51 (s, 1H, CH=), 6.52 (s, 1H, CH=), 7.01 (d, J = 10 Hz, 1H, pyridine-CH), 7.10 (s, 1H, Ar-CH), 7.12 (s, 1H, Ar-CH), 8.12 (d, J = 10 Hz, 1H, pyridine-CH), 9.21 (s, 1H, pyridine-CH).

6-[1-(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)ethenyl]nicotinoic Acid (6d). Acid **6d** was synthesized from ester **5d** following the representative procedure described for compound **6a.** Crystallization from 1:4 EtOAc–hexane gave **6d** as pale yellow crystals in 90% yield: TLC (10% MeOH–90% $CHCl_3$) R_f 0.6; mp 244–245 °C; ^{1}H-NMR ($CDCl_3$) δ 1.28 (s, 6H, $2CH_3$), 1.32 (s, 6H, $2CH_3$), 1.71 (s, 4H, $2CH_2$), 2.00 (s, 3H, CH_3), 5.55 (s, 1H, CH=), 6.58 (s, 1H, CH=), 7.05 (d, J = 10 Hz, 1H, pyridine-CH), 7.12 (s, 1H, Ar-CH), 7.16 (s, 1H, Ar-CH), 8.20 (d, J = 10 Hz, 1H, pyridine-CH), 9.30 (s, 1H, pyridine-CH). Anal. ($C_{23}H_{27}NO_2 \cdot {}^1/_5H_2O$) C,H,N.

Methyl 4-[1-Methyl-1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)ethyl]benzoate (8a). To a 100 mL round-bottom flask containing 782 mg (4.12 mmol) of titanium(IV) chloride in 10 mL of dry CH_2Cl_2 (under N_2) at −30 °C was added 393 mg (4.12 mmol) of dimethylzinc. The reaction mixture was stirred for 30 min at −30 °C followed by addition of 500 mg (1.37 mmol) of ketone **7a** in 2 mL of dry CH_2Cl_2. After stirring for 30 min, the reaction mixture was warmed to room temperature and the reaction quenched by pouring the contents into a beaker containing methanol and dry ice. When the evolution of gas ceased, the mixture was diluted with saturated ammonium carbonate, extracted with CH_2Cl_2, and dried over $MgSO_4$, to give 380 mg (1.00 mmol) of compound **8a** (73% yield). The compound was used without further purification: TLC (3% EtOAc–97% hexane) R_f 0.3; ^{1}H-NMR ($CDCl_3$) 1.24 (s, 6H, $2CH_3$), 1.31 (s, 6H, $2CH_3$), 1.65 (s, 10H, $2CH_3$ + $2CH_2$), 1.67 (s, 3H, CH_3), 3.88 (s, 3H, $COOCH_3$), 6.89 (s, 1H, Ar-CH), 7.22 (d, J = 8.4 Hz, 2H, Ar-CH), 7.44 (s, 1H, Ar-CH), 7.90 (d, J = 8.4 Hz, 2H, Ar-CH).

4-[1-Methyl-1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)ethyl]benzoic Acid (9). Acid **9** was synthesized from ester **8a** following the representative procedure described for compound **6a.** Crystallization from 1:4 EtOAc–hexane gave **9** as white crystals (73% yield): TLC (10% MeOH–90% $CHCl_3$) R_f 0.5; mp 278–280 °C; ^{1}H-NMR ($CDCl_3$) δ 1.25 (s, 6H, $2CH_3$), 1.33 (s, 6H, $2CH_3$), 1.67 (s, 10H, $2CH_3$ + $2CH_2$), 1.68 (s, 3H, CH_3), 6.91 (s, 1H, Ar-CH), 7.27 (d, J = 8.4 Hz, 2H, Ar-CH), 7.45 (s, 1H, Ar-CH), 7.97 (d, J = 8.4 Hz, 2H, Ar-CH). Anal. ($C_{25}H_{32}O_2$) C,H.

4-[2-Methyl-1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)propenyl]benzoic Acid (8b). To a 150 mL round-bottom flask containing 2.0 g (5.7 mmol) of compound **2** in 20 mL of dry Et_2O at 0 °C was slowly added 3.14 mL (6.28 mmol) of isopropylmagnesium chloride. The reaction mixture was warmed to room temperature and the reaction quenched with saturated aqueous NH_4Cl. The mixture was extracted with Et_2O, washed with water and brine, and then concentrated. To the crude material in 15 mL of MeOH was added 0.22 mL of concentrated HCl. The reaction mixture was heated to reflux for 10 min and then cooled to room temperature, the reaction was quenched with water, and the mixture was extracted with Et_2O, washed with water and brine, concentrated, dried over $MgSO_4$, and crystallized from 1:4 Et_2O-hexane to give 1.6 g (4.25 mmol) of acid **8b** (74.4% yield): TLC (10% MeOH–90% $CDCl_3$) R_f 0.6; mp 248–249 °C; ^{1}H-NMR ($CDCl_3$) δ 1.25 (s, 12H, $4CH_3$), 1.64 (s, 3H, CH_3), 1.66 (s, 3H, CH_3), 1.87 (s, 4H, $2CH_2$), 1.99 (s, 3H, CH_3), 7.00 (s, 1H, Ar-CH), 7.03 (s, 1H, Ar-CH), 7.25 (d, J = 8.0 Hz, 2H, Ar-CH), 7.98 (d, J = 8.0 Hz, 2H, Ar-CH). Anal. ($C_{26}H_{32}O_2$) C,H.

4-[1-(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)ethyl]benzoic Acid (11a). To 60 mg (0.172 mmol) of LGD1069 **(10a)** in 5 mL of EtOAc in a 15 mL round-bottom flask was added 10 mg of 10% Pd/C. The solution was degassed under vacuum, and H_2 gas was added. The reaction mixture was stirred under an H_2 atmosphere for 1 h and then filtered, concentrated, and crystallized from 1:4 EtOAc–hexane to give 56 mg (0.16 mmol) of acid **11a** (93% yield): TLC (10% MeOH–90% $CHCl_3$) R_f 0.5; mp 210–212 °C; ^{1}H-NMR ($CDCl_3$) δ 1.24 (s, 3H, CH_3), 1.25 (s, 3H, CH_3), 1.26 (s, 3H, CH_3), 1.28 (s, 3H, CH_3), 1.62 (d, J = 11.4 Hz, 3H, CH_3), 1.67 (s, 4H, $2CH_2$), 2.12 (s, 3H, CH_3), 4.30 (m, 1H, CH), 7.01 (s, 1H, Ar-CH), 7.20 (s, 1H, Ar-CH), 7.23 (d, J = 8.4 Hz, 2H, Ar-CH_2), 7.99 (d, J = 8.4 Hz, 2H, Ar-CH). Anal. ($C_{24}H_{30}O_2$) C,H.

Methyl 4-[1-(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopropyl]benzoate (11b). A 100 mL, three-neck, round-bottom flask was fitted with a condenser and a pressure-equalized dropping funnel and equipped for overhead mechanical stirring. To the flask were added 722 mg (11.65 mmol) of zinc dust, 109 mg (1.10 mmol) of cuprous chloride, 7.5 mL of anhydrous ether, and 1.48 g (5.53 mmol) of CH_2I_2. The addition funnel was charged with 1 g (2.76 mmol) of compound **10b** in 10 mL of anhydrous ether. The reaction mixture was heated to 45–50 °C, and the content of the addition funnel was added dropwise over 10 min. The reaction mixture was stirred under reflux for an additionl 3 h and then cooled in an ice bath, diluted with 50 mL of ether, and treated by dropwise addition with 20 mL of saturated aqueous NH_4Cl. The organic layer was separated, and the aqueous layer was extracted with Et_2O (1 × 20 mL). The combined organic layer was washed with 10% NaOH (3 × 20 mL) and brine (1 × 20 mL), dried over anhydrous $MgSO_4$, and concentrated to give 500 mg (1.33 mmol) of ester **11b** (49% yield). The compound was used without further purification: TLC (5% EtOAc–95% hexane) R_f 0.4; ^{1}H-NMR ($CDCl_3$) δ 1.29 (s, 12H, $4CH_3$), 1.40 (s, 4H, $2CH_2$), 1.68 (s, 4H, $2CH_2$), 2.12 (s, 3H, CH_3), 3.89 (s, 3H, $COOCH_3$), 6.97 (d, J = 8.4 Hz, 2H, Ar-CH), 7.07 (s, 1H, Ar-CH), 7.30 (s, 1H, Ar-CH), 7.86 (d, J = 8.4 Hz, 2H, Ar-CH).

4-[1-(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopropyl]benzoic Acid (12b). Acid **12b** was synthesized from ester **11b** following the representative procedure described for compound **6a.** Crystallization from 1:4 EtOAc–hexane gave **12b** as a white solid (70% yield): TLC (10% MeOH–90% $CHCl_3$) R_f 0.5; mp 252–254 °C; ^{1}H-NMR ($CDCl_3$) δ 1.31 (s, 12H, $4CH_3$), 1.42 (s, 4H, $2CH_2$), 1.73 (s, 4H,

$2CH_2$), 2.14 (s, 3H, CH_3), 6.99 (d, J = 8.4 Hz, 2H, Ar-CH), 7.06 (s, 1H, Ar-CH), 7.29 (s, 1H, Ar-CH), 7.90 (d, J = 8.4 Hz, 2H, Ar-CH); HRFAB-MS (M + H) calcd for $C_{25}H_{31}O_2$ 363.2324, found 363.2325.

Methyl 4-[2-(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)oxiranyl]benzoate (11c). To 500 mg (1.38 mmol) of benzoate **10b** in 25 mL of CH_2Cl_2 was added 953 mg (2.76 mmol) of *m*-CPBA (50% by weight) at room temperature. The reaction mixture was stirred for 2 h at room temperature, the reaction quenched with methyl sulfide, and the mixture extracted with ether, washed with water and then brine, dried over $MgSO_4$, filtered, concentrated, and purified by column chromatography (2% EtOAc–98% hexane) to give 482 mg (1.27 mmol) of epoxide **11c** (92% yield): TLC (5% EtOAc–95% hexane) R_f 0.5; mp 193–195 °C; ^{1}H-NMR ($CDCl_3$) δ 1.26 (s, 3H, CH_3), 1.27 (s, 3H, CH_3), 1.29 (s, 3H, CH_3), 1.30 (s, 3H, CH_3), 1.69 (s, 4H, $2CH_2$), 2.13 (s, 3H, CH_3), 3.14 (d, J = 5.8 Hz, 1H, CH), 3.40 (d, J = 5.8 Hz, 1H, CH), 3.89 (s, 3H, $COOCH_3$), 7.09 (s, 1H, Ar-CH), 7.24 (d, J = 8.4 Hz, 2H, Ar-CH), 7.31 (s, 1H, Ar-CH), 7.95 (d, J = 8.4 Hz, 2H, Ar-CH).

4-[2-(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)oxiranyl]benzoic Acid (12c). Acid **12c** was prepared from ester **11c** following the representative procedure described for compound **6a.** Crystallization from 1:4 EtOAc–hexane gave **12c** as white crystals in 88% yield: TLC (10% MeOH–90% $CDCl_3$) R_f 0.5; mp 186–188 °C; ^{1}H-NMR ($CDCl_3$) δ 1.26 (s, 3H, CH_3), 1.27 (s, 3H, CH_3), 1.29 (s, 3H, CH_3), 1.30 (s, 3H, CH_3), 1.69 (s, 4H, $2CH_2$), 2.14 (s, 3H, CH_3), 3.15 (d, J = 5.7 Hz, 1H, CH), 3.42 (d, J = 5.7 Hz, 1H, CH), 7.09 (s, 1H, Ar-CH), 7.26 (d, J = 8.3 Hz, 2H, Ar-CH), 7.31 (s, 1H, Ar-CH), 8.01 (d, J = 8.3 Hz, 2H, Ar-CH). Anal. ($C_{24}H_{28}O_3$) C,H.

Methyl 6-[1-(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopropyl]nicotinate (11d). In a 50 mL three-neck flask fitted with a reflux condenser and under nitrogen gas, a solution of 1.00 g (2.76 mmol) of nicotinate **5d** in 10 mL of dichloroethane was cooled to 0 °C and 1.70 g (13.77 mmol) of diethylzinc was added via syringe. To this solution was added 4.86 g (27.55 mmol) of CH_2ICl dropwise via syringe. The solution was stirred for 20 min at 0 °C and warmed to 50–55 °C for 1 h. The reaction mixture was then cooled in an ice bath, diluted with 50 mL of Et_2O, and, while stirring, treated by dropwise addition of 20 mL of saturated aqueous NH_4Cl. The organic layer was separated, and the aqueous layer was extracted with ether (1 × 20 mL). The combined organic layer was washed with water (2 × 20 mL) and brine (1 × 20 mL), dried over $MgSO_4$, filtered, concentrated, and crystallized from 1:4 EtOAc–MeOH to give 669 mg (1.77 mmol) of cyclopropylnicotinate **11d** (65% yield): TLC (20% EtOAc–20% hexane) R_f 0.85; mp 177–179 °C; ^{1}H-NMR ($CDCl_3$) δ 1.28 (s, 6H, $2CH_3$), 1.31 (s, 6H, $2CH_3$), 1.35 (d, J = 4 Hz, 2H, CH_2), 1.68 (s, 4H, $2CH_2$), 1.82 (d, J = 4 Hz, 2H, CH_2), 2.11 (s, 3H, CH_3), 3.90 (s, 3H, $COOCH_3$), 6.73 (d, J = 10 Hz, 1H, pyridine-CH), 7.10 (s, 1H, Ar-CH), 7.26 (s, 1H, Ar-CH), 7.95 (d, J = 10 Hz, 1H, pyridine-CH), 9.06 (s, 1H, pyridine-CH).

6-[1-(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopropyl]nicotinic Acid (12d). Nicotinic acid **12d** was synthesized from nicotinate **11d** following the representative procedure described for compound **6a.** Crystallization from 1:4 EtOAc–hexane gave **12d** as white crystals (80% yield): TLC (10% MeOH–90% $CHCl_3$) R_f 0.6; mp 277–279 °C; ^{1}H-NMR ($CDCl_3$) δ 1.28 (s, 6H, $2CH_3$), 1.32 (s, 6H, $2CH_3$), 1.38 (d, J = 4.0 Hz, 2H, CH_2), 1.72 (s, 4H, $2CH_2$), 1.87 (d, J = 4.0 Hz, 2H, CH_2), 2.12 (s, 3H, CH_3), 6.78 (d, J = 10 Hz, 1H, pyridine-CH), 7.11 (s, 1H, Ar-CH), 7.26 (s, 1H, Ar-CH), 8.00 (d, J = 10 Hz, 1H, pyridine-CH), 9.14 (s, 1H, pyridine-CH). Anal. ($C_{24}H_{29}NO_2$) C,H,N.

3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphthalen-2-ol (13b). Alcohol **13b** was synthesized from 2,5-dichloro-2,5-dimethylhexane and *o*-cresol following the representative procedure described for 1,2,3,4-tetrahydro-1,1,4,4,6-pentamethylnaphthalene (**3d**):[1] TLC (20% EtOAc–80% hexane) R_f 0.75; mp 118–120 °C; ^{1}H-NMR ($CDCl_3$) δ 1.24 (s, 12H, $4CH_3$),1.65 (s, 4H, $2CH_2$), 2.20 (s, 3H, CH_3), 4.46 (s, 1H, OH), 6.70 (s, 1H, Ar-CH), 7.03 (s, 1H, Ar-CH).

Methyl 4-[(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)methyl]benzoate (15a). To 6.0 g (29.7 mmol) of compound **3d** in 60 mL of CH_2Cl_2 was added 4.0 g (17.5 mmol) of methyl 4-(bromomethyl)benzoate (**14a**), followed by addition of 3.0 g (22.5 mmol) of $AlCl_3$. The reaction mixture was heated at reflux for 1 h and then cooled to room temperature, poured into 100 mL of ice water, stirred, and extracted with ether. The ether layer was washed (water and then brine), dried over $MgSO_4$, filtered, concentrated, and crystallized from hot MeOH to give methyl ester **15a**: TLC (5% EtOAc–95% hexane) R_f 0.4; mp 138–140 °C; ^{1}H-NMR ($CDCl_3$) δ 1.21 (s, 6H, $2CH_3$), 1.27 (s, 6H, $2CH_3$), 1.66 (s, 4H, $2CH_2$), 2.15 (s, 3H, CH_3), 3.90 (s, 3H, $COOCH_3$), 4.00 (s, 2H, CH_2), 7.00 (s, 1H, Ar-CH), 7.07 (1H, Ar-CH), 7.20 (d, J = 8.1 Hz, 2H, Ar-CH), 7.94 (d, J = 8.1 Hz, 2H, Ar-CH).

4-[(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)methyl]benzoic Acid (16a). Acid **16a** was synthesized from ester **15b** following the representative procedure described for compound **6a**: TLC (10% MeOH–90% $CDCl_3$); R_f 0.5; mp 239–242 °C; ^{1}H-NMR ($CDCl_3$) δ 1.22 (s, 6H, $2CH_3$), 1.27 (s, 6H, $2CH_3$), 1.67 (s, 4H, $2CH_2$), 2.16 (s, 3H, CH_3), 4.00 (s, 2H, CH_2), 7.00 (s, 1H, Ar-CH), 7.07 (s, 1H, Ar-CH), 7.23 (d, J = 8.1 Hz, 2H,Ar-CH), 8.01 (d, J = 8.1 Hz, 2H, Ar-CH). Anal. ($C_{23}H_{28}O_2$) C,H.

Methyl 4-[(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)oxy]benzoate (15b). To 2.0 g (9.17 mmol) of alcohol **13b** in 15 mL of collidine was added 1.5 g (6.98 mmol) of methyl 4-bromobenzoate and a catalytic amount (100 mg) of Cu_2O. The reaction mixture was heated at reflux for 48 h and cooled to room temperature, the reaction quenched with 5% aqueous HCl, and the mixture extracted with EtOAc, washed (water and then brine), dried over $MgSO_4$, and crystallized from MeOH to give 1.0 g (2.84 mmol) of ester **15b** (41% yield): TLC (20% EtOAc–80% hexane) R_f 0.7; mp 103–104 °C; ^{1}H-NMR ($CDCl_3$) δ 1.21 (s, 6H, $2CH_3$), 1.29 (s, 6H, $2CH_3$), 1.68 (s, 4H, $2CH_2$), 2.10 (s, 3H, CH_3), 3.88 (s, 3H, $COOCH_3$), 6.86 (d, J = 8.5 Hz, 2H, Ar-CH), 6.90 (s, 1H, Ar-CH), 7.16 (s, 1H, Ar-CH), 7.97 (d, J = 8.5 Hz, 2H, Ar-CH).

4-[(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)oxy]benzoic Acid (16b). Acid **16b** was synthesized from ester **15b** following the representative procedure described for compound **6a.** Crystallization from 1:4 EtOAc–hexane gave **16b** as white crystals (85% yield): TLC (10% MeOH–90% $CHCl_3$) R_f 0.5; mp 219–220 °C; ^{1}H-NMR ($CDCl_3$) δ 1.22 (s, 6H, $2CH_3$), 1.29 (s, 6H, $2CH_3$), 1.69 (s, 4H, $2CH_2$), 2.10 (s, 3H, CH_3), 6.89 (d, J = 8.9 Hz, 2H, Ar-CH), 6.92 (s, 1H, Ar-CH), 7.17 (s, 1H, Ar-CH), 8.04 (d, J = 8.9 Hz, 2H, Ar-CH). Anal. ($C_{22}H_{26}O_3$) C,H.

Methyl 2-[(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)carbonyl]benzoate (18a). Benzoate **18a** was synthesized from compound **3d** and monomethylphthalic acid chloride (**17a**) following the representative procedure described for ketone **4a.** Crystallization from MeOH gave compound **18a** as white crystals (91% yield): TLC (20% EtOAc–80% hexane) R_f 0.5; mp 105–107 °C; ^{1}H-NMR ($CDCl_3$): δ 1.07 (s, 6H, $2CH_3$), 1.28(s, 6H, $2CH_3$), 1.63 (dd, J = 12 Hz, 4.0 Hz, 4H, $2CH_2$), 2.57 (s, 3H, CH_3), 3.57(s, 3H, $COOCH_3$), 7.15 (s, 1H, Ar-CH), 7.18 (s, 1H, Ar-CH), 7.47 (d, J = 7.2 Hz, 1H, Ar-CH), 7.58 (m, 2H, Ar-CH), 7.90 (d, J = 7.2 Hz, 1H, Ar-CH).

2-[(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)carbonyl]benzoic Acid (19a). Acid **19a** was synthesized from ester **18a** following the representative procedure described for compound **6a.** Crystallization from 1:4 EtOAc–hexane gave **19a** as white crystals (90% yield): TLC (10% MeOH–90% $CHCl_3$) R_f 0.5; mp 187–188 °C; ^{1}H-NMR ($CDCl_3$) 1.05 (s, 6H, $2CH_3$), 1.26 (s, 6H, $2CH_3$), 1.62 (dd, J = 12, 4 Hz, 4H, $2CH_2$), 2.53 (s, 3H, CH_3), 7.14 (s, 1H, Ar-CH), 7.15 (s, 1H, Ar-CH), 7.42 (d, J = 7.2 Hz, 1H, Ar-CH), 7.55 (t, J = 7.6 Hz, 1H, Ar-CH), 7.61 (t, J = 7.6 Hz, 1H, Ar-CH), 8.01 (d, J = 7.2 Hz, 1H, Ar-CH); HRFAB-MS (M + H) calcd for $C_{23}H_{27}O_3$ 351.1992, found 351.1960.

Methyl 3-[(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)carbonyl]benzoate (18b). Benzoate **18b** was synthesized from compound **3d** and monomethylisophthalic acid chloride (**17b**) following the representative

procedure described for ketone **4a**. Crystallization from MeOH gave compound **18b** as white crystals (80% yield): TLC (20% EtOAc–80% hexane) R_f 0.5; mp 110–112 °C; ^{1}H-NMR ($CDCl_3$) δ 1.21 (s, 6H, $2CH_3$), 1.32 (s, 6H, $2CH_3$), 1.69 (s, 4H, $2CH_2$), 2.35 (s, 3H, CH_3), 3.92 (s, 3H, $COOCH_3$), 7.22 (s, 1H, Ar-CH), 7.28 (s, 1H, Ar-CH), 7.56 (t, J = 7.8 Hz, 1H, Ar-CH), 8.04 (d, J = 7.8 Hz, 1H, Ar-CH), 8.24 (d, J = 7.8 Hz, 1H, Ar-CH), 8.46 (s, 1H, Ar-CH).

3-[(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)carbonyl]benzoic Acid (19b). Acid **19b** was synthesized from ester **18b** following the representative procedure described for compound **6a**. Crystallization from 1:4 EtOAc–hexane gave **19b** as white crystals (80% yield): TLC (10% MeOH–90% $CHCl_3$) R_f 0.5; mp 192–194 °C; ^{1}H-NMR ($CDCl_3$) δ 1.21 (s, 6H, $2CH_3$), 1.32 (s, 6H, $2CH_3$), 1.70 (s, 4H, $2CH_2$), 2.18 (s, 3H, CH_3), 7.22 (s, 1H, Ar-CH), 7.28 (s, 1H, Ar-CH), 7.60 (t, J = 7.8 Hz, 1H, Ar-CH), 8.10 (d, J = 7.8 Hz, 1H, Ar-CH), 8.30 (d, J = 7.8 Hz, 1H, Ar-CH), 8.51 (s, 1H, Ar-CH). Anal. ($C_{23}H_{26}O_3$) C,H.

Biology. Cotransfection Assay. Cotransfections were carried out in 96-well plates in an automated workstation with CV-1 cells as previously described.[1, 29–31] All cotransfections were carried out in CV-1 cells as previously described, but modified for automation (Beckman Biomek Automated Workstation) and the use of 96-well plates.[1,30]

Binding Studies. Receptor binding assays for RARs and RXRs were performed in a similar manner as described in Boehm *et al.*[1] using [^{3}H]-9-*cis*-RA[18] as the radioligand for RXRs and [^{3}H]ATRA (purchased from NEN-DuPont) for the RARs. K_d values for the analogs were determined by application of the Cheng–Prussof equation.[40]

IdT-Mediated dUTP *in Situ* Nick End Labeling (TUNEL) Assay.[32,33] Individual cells were labeled with exogenous terminal deoxynucleotidyltransferase (TdT). TdT is a primer dependent DNA polymerase that catalyzes the repetitive addition of deoxyribonucleotide from deoxynucleotide triphosphates to the terminal 3′-hydroxyl of a DNA or RNA strand with the release of inorganic pyrophosphate. The incorporated dUTP which was conjugated to digoxigenin was in turn detected by fluorescinated anti-digoxigenin. The DNA was counterstained with propidium iodide (PI) to correlate the presence of DNA strand breaks in individual cells with their DNA ploidy and position in the cell cycle. The experimental method was as follows: To 5 mL of HL-60 cells at 5 × 10^4/mL in 10% FCS-RPMI in 6-well tissue culture plates was added 1 μM retinoid test compounds alone or in combination with other retinoids. These cells were incubated for 5 days at 37 °C under 5% CO_2 and then collected, washed twice with PBS, and fixed with 3 mL of 1% paraformaldehyde for 15 min at 4 °C. The cells were then washed an additional two times with PBS and stained following the protocol described in the ApopTag *in situ* apoptosis detection kit from ONCOR (Gaithersburg, MD). Following the staining procedure the cells were resuspended in 1 mL of PBS containing 5 mg/mL PI. Flow cytometric analysis of retinoid-treated cells was done on a FACSCAM flow cytometer (Beckton-Dickinson).

Transglutaminase Assay. HL-60 cdm-1 cells were cultured under conditions described in detail previously.[35] Cells in log-phase growth (2 × 10^{-5} cells/mL) in RPMI 1640 (Fisher Scientific) supplemented with insulin, transferrin, and sodium selenide (TIS; Sigma) were pretreated with 1.25% dimethyl sulfoxide (DMSO) for 18 h. Cells were then sedimented and resuspended in RPMI-TIS containing retinoids or an equivalent solvent control (0.1% ethanol). After culture for 24 h, cells were again sedimented, washed once and lysed, and the transglutaminase activity was assayed by measuring the covalent and Ca^{2+} dependent conjugation of [^{3}H]putrescine to *N,N*-dimethylcasein.[35]

References

(1) Boehm, M. F.; Zhang, L.; Badea, B. A.; White, S. K.; Mais, D. E.; Berger, E.; Suto, C. M.; Goldman, M. E.; Heyman, R. A. Synthesis and Structure-Activity Relationships of Novel Retinoid X Receptor Selective Retinoids. *J. Med. Chem.* **1994**, *37*, 2930–2941.

(2) Orfanos, C. E.; Ehlert, R.; Gollnick, H. The Retinoids. A Review of Their Clinical Pharmacology and Therapeutic Use. *Drugs* **1987**, *34*, 459–503.

(3) Biro, D. E.; Shalita, A. R. Clinical Aspects of Topical Retinoids. *Skin Pharmacol.* **1993**, *6* (Suppl. 1), 24–34.

(4) Shahidallah, M.; Tham, S. N.; Goh, C. L. Isotretinoin Therapy in Acne Vulgaris, a 10 Year Retrospective Study in Singapore. *Int. J. Dermatol.* **1994**, *33*, 60–63.

(5) Smith, M. A.; Parkinson, D. R.; Cheson, B. D.; Friedman, M. A. Retinoids in Cancer Therapy. *J. Clin. Oncol.* **1992**, *10*, 839–864.

(6) Vokes, E. E.; Weichselbaum, R. R.; Lippman, S. M.; Hong, W. K. Head and Neck Cancer. *N. Engl. J. Med.* **1993**, *328*, 184–194.

(7) Yang, Y.; Vacchio, M. S.; Ashwell, J. D. 9-*Cis*-Retinoic Acid Inhibits Activation-Driven T-Cell Apoptosis: Implications for Retinoid X Receptor Involvement in Thymocyte Development. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 6170–6174.

(8) Delia, D.; Aiello, A.; Lombardi, L.; Pelicci, P. G.; Grignani, F.; Formelli, F.; Menard, S.; Costa, A.; Veronesi, V.; Pierotti, M. A. N-(4-Hydroxyphenyl)Retinamide Induces Apoptosis of Malignant Hemopoietic Cell Lines Including Those Unresponsive to Retinoic Acid. *Cancer Res.* **1993**, *53*, 6036–6041.

(9) Mangelsdorf, D. J.; Umesono, K.; Evans, R. M. The Retinoid Receptors. *The Retinoids*; Academic Press: Orlando, FL, 1994: pp 319–349.

(10) Leid, M.; Kastner, P.; Chambon, P. Multiplicity Generates Diversity in the Retinoic Acid Signalling Pathways. *Trends Biochem. Sci.* **1992**, *17*, 427–433.

(11) *Chemistry and Biology of Synthetic Retinoids*; Dawson, M. I., Okamura, W. H., Eds.; CRC Press: Boca Raton, FL, 1990.

(12) *The Retinoids. Biology, Chemistry and Medicine*, 2nd ed; Sporn, M. B., Roberts, A. B., Goodman, D. S., Eds.; Raven Press: New York, 1994.

(13) Koizumi, H.; Sato, K. C.; Kazuko, C.; Ohkawara, A. Effects of Novel Synthesized Retinobenzoic Acid (AM80) on Adenylate Cyclase and Arachidonic Acid Release in Pig Epidermis. *J. Dermatol. Sci.* **1992**, *3*, 97–102.

(14) Fukasawa, H.; Iijima, T.; Kagechika, H.; Hashimoto, Y.; Shudo, K. Expression of the Ligand Binding Domain-Containing Region of Retinoic Acid Receptors alpha, beta, and gamma in Escherichia Coli and Evaluation of Ligand-Binding Selectivity. *Biol. Pharmacol. Bull.* **1993**, *16*, 343–348.

(15) Esgleyes-Ribot, T.; Chandraratna, R. A.; Lew-Kaya, D. A.; Sefton, J.; Duvic, M. Response of Psoriasis to a New Topical Retinoid, AGN190168. *J. Am. Acad. Dermatol.* **1994**, *30*, 581–590.

(16) Maignan, J.; Lang, G.; Malle, G.; Restle, S.; Shroot, B. Bicyclic Aromatic Derivatives, Method for Preparing Them, and Their Use in Human and Veterinary Medicine and in Cosmetics. U.S. Patent 4,833,240, 1989.

(17) Lehmann, J. M.; Jong, L.; Fanjul, A.; Cameron, J. F.; Lu, X. P.; Haefner, P.; Dawson, M. I.; Pfahl, M. Retinoids Selective for Retinoid X Receptor Response Pathways. *Science* **1992**, *258*, 1944–1946.

(18) Boehm, M. F.; McClurg, M. M.; Pathirana, C.; Mangelsdorf, D.; White, S. K.; Hebert, J.; Winn, D.; Goldman, M. E.; Heyman, R. A. Synthesis of High Specific Activity [^{3}H]-9-*cis* Retinoic Acid and its Application for Identifying Retinoids with Unusual Binding Properties. *J. Med. Chem.* **1994**, *37*, 408–414.

(19) Heyman, R. A.; Mangelsdorf, D. J.; Dyck, J. A.; Stein, R. B.; Eichele, G.; Evans, R. M.; Thaller, C. 9-Cis Retinoic Acid as a High Affinity Ligand for Retinoid X Receptors. *Cell* **1992**, *68*, 397–406.

(20) Moore, J. V. Death of cells and necrosis of tumors. In *Perspectives on mammalian cell death*; Potten, C. S., Ed.; Oxford University Press: Oxford, U.K., 1991; p 295.

(21) Kerr, J. F. R.; Wyllie, A. H.; Currie, A. R. Apoptosis: a basic biological phenonemon with wide-ranging implications in tissue kinetics. *Br. J. Cancer* **1972**, *26*, 239–257.

(22) Lennon, S. V.; Martin, S. J.; Cotter, T. G. Dose-dependent induction of apoptosis in human tumor cell lines by widely diverging stimuli. *Cell Prolif.* **1991**, *24*, 203–214.

(23) Wyllie, A. H. Gluccocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. *Nature* **1980**, *284*, 555–556.

(24) Askew, D. S.; Ashmun, R. A.; Simmons, B. C.; Cleveland, J. J. Constitutive c-myc expression in IL-3 dependent myeloid cell line suppresses cycle arrest and accelerates apoptosis. *Oncogene* **1991**, *6*, 1915–1922.

(25) Leid, M.; Kastner, P.; Lyons, R.; Nakshatn, H.; Saunders, M.; Zacharewski, T.; Chen, J.-Y.; Staub, A.; Garnier, J.-M.; Mader, S.; Chambon, P. Purification, Cloning, and RXR Identity of the HeLa Cell Factor with Which RAR pr TR Heterodimerizes to Bind Target Sequences Efficiently. *Cell* **1992**, *68*, 377–395.

(26) Kurokawa, R.; DiRenzo, J.; Boehm, M. F.; Sugerman, J.; Gloss, B.; Rosenfeld, M. G.; Heyman, R. A.; Glass, C. K. Regulation of Retinoid Signalling by Receptor Polarity and Allosteric Control of Ligand Binding. *Nature* **1994**, *371*, 528–531.

(27) Kliewer, S. A.; Umesono, K.; Noonan, D. J.; Heyman, R. A.; Evans, R. M. Convergence of 9-*cis* Retinoic Acid and Peroxisome Proliferator Signalling Pathways Through Heterodimer Formation of Their Receptors. *Nature* **1992**, *358*, 771–774.
(28) Berrodin, T. J.; Marks, M. S.; Ozato, K.; Linney, E.; Lazar, M. A. Heterodimerization Among Thyroid Hormone Receptor, Retinoic Acid Receptor, Retinoid X Receptor, Chicken Ovalbumin Upstream Promoter Transcription Factor, and an Endogenous Liver Protein. *Mol. Endocrinol.* **1992**, *6*, 1468–1478.
(29) Mangelsdorf, D. J.; Ong, E. S.; Dyck, J. A.; Evans, R. M. A Nuclear Receptor That Identifies a Novel Retinoic Acid Response Pathway. *Nature* **1990**, *345*, 224–229.
(30) Berger, T. S.; Parandoosh, Z.; Perry, B. W.; Stein, R. B. Interaction of Glucocorticoid Analogues With the Human Glucocorticoid Receptor. *J. Steroid Biochem. Mol. Biol.* **1992**, *41*, 733–738.
(31) Umesono, K.; Giguere, V.; Glass, C. K.; Rosenfeld, M. G.; Evans, R. M. Retinoic Acid and Thyroid Hormone Induce Gene Expression Through a Common Responsive Element. *Nature* **1988**, *336*, 262–265.
(32) Darzynkiewics, Z.; Bruno, S.; Del Bino, G.; Gorczyca, W.; Hotz, M. A.; Lassota, P.; Traganos, F. Features of apoptotic cells measured by flow cytometry. *Cytometry* **1992**, *13*, 795–808.
(33) Gorczyca, W.; Melamed, M. R.; Darrzykiewics, Z. Apoptosis of S phase HL-60 cell induced by DNA topoisomerase inhibitors: Detection of DNA strand breaks by flow cytometry using the in situ nick translation assay. *Int. J. Oncol.* **1992**, *1*, 639–648.
(34) Moore, W. T.; Murtaugh, M. P.; Davies, P. J. A. Retinoic Acid-induced Expression of Tissue Transglutaminase in Mouse Peritoneal Macrophages. *J. Biol. Chem.* **1984**, *259*, 12794–12802.
(35) Davies, P. J. A.; Murtaugh, M. P.; Moore, W. T.; Johnson, G. S.; Lucas, D. Retinoic Acid-induced Expression of Tissue Transglutaminase in Human Promyelocytic leukemia (HL-60) Cells. *J. Biol. Chem.* **1985**, *260*, 5166–5174.
(36) Chiocca, E. A.; Davies, P. J. A.; Stein, J. P. The Molecular Basis of Retinoid Action: Transcriptional Regulation of Tissue Transglutaminase Expression in Macrophages. *J. Biol. Chem.* **1988**, *263*, 11584–11589.
(37) Polak's Frutal Works, Inc. Procedure for the Preparation of Musk Perfumes. French Patent 1,392,804, 1964.
(38) Isigawa, K.; Kawai, M.; Fushizaki, Y. *Nippon Kagaku Zasshi* **1967**, *88*, 553–556.
(39) Baader, E.; Bickel, M.; Gunzler-Pukall, V. 4-or 5-Substituted Pyridine-2-carboxylic acids, Procedure for Their Preparation as Well as Their Use as Drugs. European Patent Publication, EPO479178A2, 1991.
(40) Cheng, Y.-C.; Prusoff, W. F. Relationship Between the Inhibition Constant (K_i) and the Concentration of Inhibitor Which Causes 50% Inhibition (I_{50}) of an Enzymatic Reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.

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