Conformationally Defined Retinoic Acid Analogues. 4. Potential New Agents for Acute Promyelocytic and Juvenile Myelomonocytic Leukemias

Donald D. Muccio,*,† Wayne J. Brouillette,*,† Theodore R. Breitman,‖ Mohammed Taimi,‖ Peter D. Emanuel,§ Xiao-kun Zhang,⊥ Guo-quan Chen,⊥ Brahma P. Sani,‡ Pratap Venepally,‡ Lakshmi Reddy,‡ Muzaffar Alam,† Linda Simpson-Herren,‡ and Donald L. Hill‡

Departments of Chemistry and Medicine, University of Alabama at Birmingham, Birmingham, Alabama 35294, Laboratory of Biological Chemistry, Division of Basic Sciences, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892, The Burnham Institute, La Jolla Cancer Research Center, 10901 North Torrey Pines Road, La Jolla, California 92037, and Departments of Biochemistry and Toxicology, Southern Research Institute, 2000 Ninth Avenue South, Birmingham, Alabama 35205

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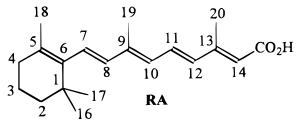
We recently synthesized several conformationally constrained retinoic acid (RA) analogues [8-(2′-cyclohexen-1′-ylidene)-3,7-dimethyl-2,4,6-octatrienoic acids with different alkyl substituents at 2′ (R_1) and 3′ (R_2) positions on the cyclohexene ring] (Muccio et al. *J. Med. Chem.* **1996**, *39*, 3625) as cancer chemopreventive agents. UAB8 (R_1 = Et; R_2 = ^{i}Pr), which contains sufficient steric bulk at the terminal end of the polyene chain to mimic the trimethylcyclohexenyl ring of RA, displayed biological properties similar to those of RA. To explore the efficacy of this retinoid in acute promyelocytic leukemia (APL) and juvenile myelomonocytic leukemia (JMML), we evaluated UAB8 isomers in in vitro assays which measure the capacity of retinoids to inhibit aberrant myeloid colony growth from blood or bone marrow cells obtained from human JMML patients and in assays measuring the potential of retinoids to differentiate NB4 cells (an APL cell line). Both (*all-E*)- and (13*Z*)-UAB8 were 2-fold more active than RA in the NB4 cell differentiation assay; however, only (*all-E*)-UAB8 had comparable activity to the natural retinoids in the JMML cell assays. These results were compared to the biological effectiveness of a new retinoid, UAB30 [8-(3′,4′-dihydro-1′(2′*H*)-naphthalen-1′-ylidene)-3,7-dimethyl-2,4,6-octatrienoic acid], which had different nuclear receptor binding and transactivational properties than UAB8. Relative to (*all-E*)-RA and (*all-E*)-UAB8, (*all-E*)-UAB30 bound well to RARα but did not activate transcription-mediated RARα homodimers, even though it was effective in RARβ- and RARγ-mediated transactivational assays. In APL assays, this retinoid had much reduced activity and was only moderately effective in JMML assays and in cancer chemoprevention assays.

Introduction

Retinoid receptors and other members of this superfamily of nuclear receptors (that include the steroid, thyroid, and vitamin D hormone receptors and other 'orphan' receptors without known ligands) are new targets for drug development.[1] It is thought that retinoic acid (RA) and synthetic retinoids act as ligand-dependent transcription factors with different members of nuclear retinoid receptors to control gene transcription responsible for cellular proliferation, differentiation, development, and cell death.[2] Two classes of nuclear retinoid receptors (RARs and RXRs) have been identified so far, and each has several different subtypes (α, β, γ). Both (*all-E*)- and (9*Z*)-RA bind to RARs and activate transcription mediated by RAR/RXR heterodimers, but (9*Z*)-RA is the only known natural ligand for the RXRs which mediate transcription by forming homodimers or heterodimers.

Recent advances in chemoprevention have heightened interest in the use of retinoids in several types of solid



RA

organ tumors, and major therapeutic successes have been demonstrated with retinoids in certain leukemias.[3] (*all-E*)-RA treatment of patients with acute promyelocytic leukemia (APL) leads to a 90% complete remission rate in these patients by inducing normal maturation and apoptosis of APL myeloblasts to neutrophils, but this differentiation therapy is transient and is commonly followed by relapse within 3−15 months, probably due to the development of resistance to RA.[4] (13*Z*)-RA effectively controls the excessive myeloproliferation in up to 50% of children with juvenile myelomonocytic leukemia (JMML).[5] However, this treatment is not curative and at best can lead to a period of prolonged stabilization of disease, but ultimately patients need to undergo allogenic bone marrow transplantation.[4,6]

To provide new, more effective therapeutic agents with the beneficial effects of RA but with reduced side effects, we designed[7] conformationally locked retinoids. We previously demonstrated[7,8] that (*all-E*)- and (9*Z*)-UAB8 retinoids (containing large R_1 and R_2 substituents in the ring region of the UAB retinoid general structure)

* To whom correspondence should be addressed.
† Department of Chemistry, University of Alabama at Birmingham.
§ Department of Medicine, University of Alabama at Birmingham.
‖ National Institutes of Health.
⊥ La Jolla Cancer Research Center.
‡ Southern Research Institute.

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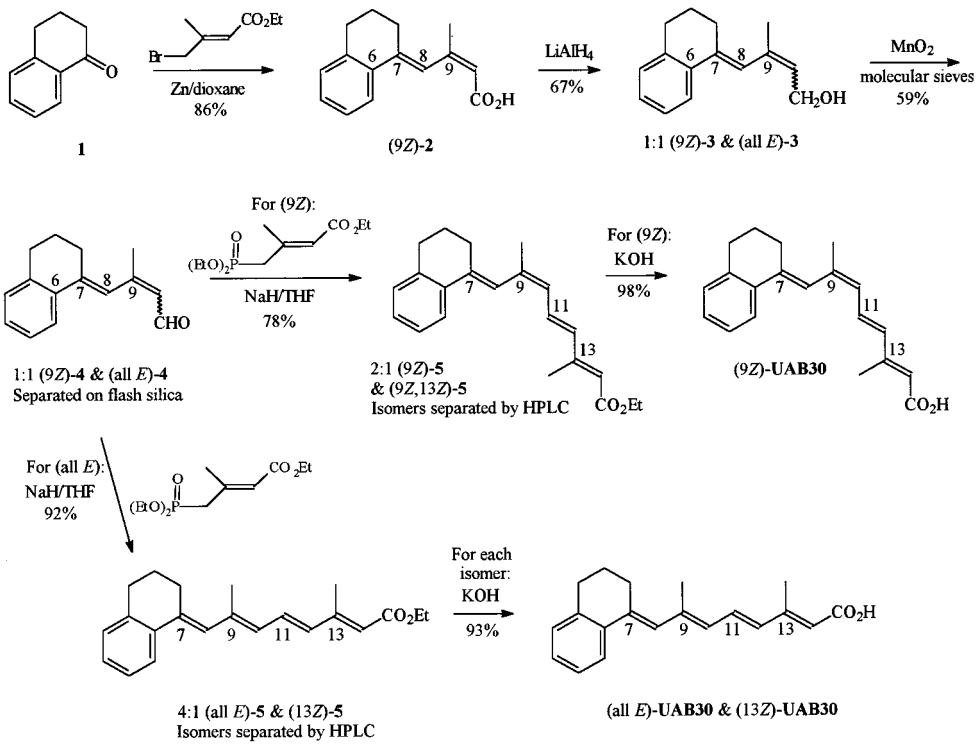
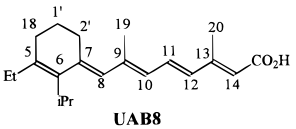
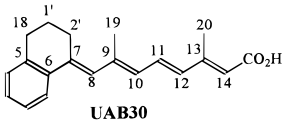
Scheme 1



were selective RAR and RXR agonists and further showed a correlation with enhanced biological activity in skin. In this study we examine three UAB8 isomers in in vitro differentiation and antiproliferation assays that predict for efficacy in APL and JMML therapy, respectively, and show equal or greater efficacy with the (*all-E*)- and (13*Z*)-UAB8 isomers as compared to the natural retinoids. Additionally we generate a new UAB retinoid, UAB30, whose *all-E*-isomer has different nuclear receptor selectivity (RARβ and RARγ subtype-selective retinoid and RARα antagonist) than (*all-E*)-UAB8. A comparison of the activities of UAB8 and UAB30 isomers in these in vitro assays suggests the importance of RARα binding/activation in ligand-induced differentiation of APL cells and RAR binding in antiproliferative effects in JMML.



UAB8



UAB30

Chemistry

Scheme 1 summarizes the methods employed to prepare UAB30. The approach was similar to that which we previously reported for the syntheses of the alkyl-substituted UAB retinoids (e.g., UAB8),[9,10] except that α-tetralone was used as the starting enone (**1** in Scheme 1). (Note that atom numbering in Scheme 1 is nonsystematic to allow ready comparison with RA.) As noted before,[9] a δ-lactone intermediate was not detected under these conditions but was assumed to be an intermediate in the production of (9*Z*)-**2**. Unlike earlier reports for related compounds,[8−10] the reduction of acid (9*Z*)-**2** to alcohol **3** was accompanied by isomerization to give a 1:1 mixture of *all-E*- and 9*Z*-isomers. This mixture was oxidized with MnO_2 to give a comparable mixture of aldehydes (**4**), which were preparatively separated using flash chromatography on silica gel. Each pure isomer of **4** was olefinated as shown to provide a mixture of either (9*Z*)- and (9*Z*,13*Z*)-**5** or (*all-E*)- and (13*Z*)-**5**. Each mixture was preparatively separated by HPLC on silica gel (0.5% Et_2O, 0.1% THF in hexane for the former and 1% Et_2O, 0.5% THF in hexane for the latter) using methods similar to those we previously described.[8−11] Individual isomers of ester **5** were then hydrolyzed to the corresponding acids in KOH, without *E*/*Z*-isomerization.[12] The isomeric purity for the final acids (>97%) was verified by NMR and reverse-phase HPLC.[8−11] Experimental yields and selected data for the intermediates and products in Scheme 1 are summarized in Table 1. The UV/vis spectral data of the purified isomers are given in Table 1, and the complete ^{1}H NMR assignments are contained in Table 2.

Biology

Using competitive binding assays as described elsewhere,[8−10] each isomer was evaluated for its ability to bind to cytoplasmic retinoic acid binding protein (CRABP) and nuclear retinoid receptors (RARα, RARβ, RARγ, and RXRα). The 50% inhibitory concentrations (IC_{50} values) were determined from a retinoid dose−response curve. Functional assays (transient transfection assays) were used to measure the capacity of retinoids to activate RARα, RARβ, and RARγ homodimers, RXRα homodimers, or an RXRα/RARα heterodimer using a chloramphenicol acetyltransferase (CAT) reporter gene containing TREpal with a thymidine kinase promoter [(TREpal)$_2$-tk-CAT] as previously described.[9,13] Dose−response curves of RA and UAB retinoids were used to determine the concentrations causing 50% of the maxi-

Table 1. Selected Data for New Compounds Produced in Scheme 1

compound	isolated percent yield	R_f[a]	UV/vis[b] λ_{max}	UV/vis[b] ϵ	IR[c] C=O	IR[c] C=C	MS *m/z*
(9*Z*)−2	86	0.35	310	14 000	1673	1618	ND
(9*Z*)- and (*all-E*)−3	67	0.21	264	12 000	(3334)	1630	214.1
(9*Z*)−4	59[d]	0.48	295	6 000	1662	1609	212.1
(*all-E*)−4	59[d]	0.42	298	8 200	1662	1605	212.1
(9*Z*)−5[e]	78	0.59	328	29 300	1701	1602	322.2
(*all-E*)−5	92	0.57	358	35 000	1708	1605	322.2
(13*Z*)−5	92	0.57	358	30 000	1709	1604	322.2
(9*Z*)-UAB30[f]	98	0.12	328	30 200	1672	1594	294.2
(*all-E*)-UAB30	93	0.09	358	32 500	1672	1598	294.2
(13*Z*)-UAB30	93	0.12	359	26 000	1673	1596	294.2

[a] Values are on silica gel using ether/hexane as eluent: 30% Et_2O, 3 and 4; 20% Et_2O, 2; 10% Et_2O, 5 and UAB30. [b] The wavelength maximum (nm) and extinction coefficients (M^{-1} cm^{-1}) were obtained in cyclohexane (5) or methanol (UAB30, 4) at room temperature. ND means not determined. [c] The IR stretching frequencies (cm^{-1}) were obtained as thin films on NaCl disks. Compound 3 OH stretching frequency is reported in parentheses. [d] Yield for the mixture of *E*/*Z*-isomers. [e] Ester 5 was preparatively separated on a Whatman Partisil 10 M20/50 normal phase column with 1.0% Et_2O and 0.5% THF in hexane with a 5-mL/min flow rate.[9] Retention times were 138.6 min (13*Z*), 140.2 min (9*Z*), and 142.6 min (*all-E*). Re-injected fractions showed one isomer when monitoring at either 300 or 340 nm. [f] UAB30 isomers were analyzed for isomeric purity by reverse-phase chromatography using a Spherisorb ODS C-18 column with 1% acetic acid in acetonitrile (3:7) with a 1.0 mL/min flow rate. Using 340-nm detection (where each isomer had very similar extinction coefficients), the isomeric ratios were determined from the integrated peak areas. The isomeric ratios were greater than 95% for each isomer.

mum effect (EC_{50} values). To compare UAB30 retinoids to other conformationally restricted UAB retinoids, namely UAB8, these isomers were evaluated in a skin antipapilloma assay which measured the capacity of retinoids to prevent chemically induced papillomas in mice.[14] This assay uses a two-stage model for carcinogenesis involving initiation by dimethylbenz[*a*]anthracene (DMBA) and promotion by 12-*O*-tetradecanoylphorbol 13-acetate (TPA). RA or retinoid application before promotion prevents papillomas in a dose-dependent manner, and the 50% effective doses (ED_{50} values) were measured as reported previously.[8−10] For evaluation of effectiveness in APL, UAB8 and UAB30 isomers were screened in an in vitro assay that measures the capacity of retinoids to induce differentiation of NB4 cells. This cell line was established from an APL patient and carries the (15;17) translocation which involves the RARα receptor. These assays were performed according to the recently published methods of Breitman and co-workers.[15] For the evaluation of retinoids in JMML, the UAB8 and UAB30 retinoids were screened for their ability to inhibit spontaneous granulocyte-macrophage colony-forming units (CFU-GM) using an in vitro assay with human cells derived from different JMML patients. These were performed by the methods developed by Emanuel et al.,[5,16] who first correlated the in vitro activity of (13*Z*)-RA to its therapeutic benefit in JMML patients.

Results and Discussion

Structural Studies. We recently reported[17] the X-ray crystal structure of (9*Z*)-**2**, which crystallized in an 8-*s*-*trans*-conformation. To compare the X-ray structure of (9*Z*)-**2** to that generated by molecular mechanics, Allinger's MM3(94) calculations were performed as we described previously for calculating the structures of UAB1−UAB8 retinoids.[8] The calculated structure of this intermediate was very similar to the solid-state structure. The $\psi_{5,6,7,8}$ torsional angles were nearly planar (−160°) in each structure, like other UAB retinoids with alkyl substituents.[8] The $\psi_{7,8,9,10}$ torsional angle was −133° for the molecular mechanics structure and −134.3° in the crystral structure. This angle is nonplanar due to steric interactions between the C2′ methylene and the C19 methyl group. The molecular mechanics structures of (9*Z*)- and (*all-E*)-UAB30 retinoids were also generated. When compared to those of UAB8 isomers,[8] the expected low-energy conformers are very similar; the aromatic ring of UAB30 retinoids occupies space that is very similar to that of the alkyl groups (R_1 = Et; R_2 = ^{i}Pr) of UAB8.

We previously noted that the 8-*s*-*cis*-conformer was as stable as the 8-*s*-*trans*-conformer for different UAB retinoids.[8] The low-energy structures of the 8-*s*-*cis*-conformer of UAB30 isomers were also calculated. The $\psi_{5,6,7,8}$ torsional angles were nearly identical to that of the 8-*s*-*trans*-conformer, and the $\psi_{7,8,9,10}$ angle was −70°. Further, the 8-*s*-*cis*-conformer was slightly more stable ($\Delta G \approx 1$ kcal/mol) than the corresponding 8-*s*-*trans*-conformer.

Nuclear Overhauser experiments (NOESY) (2D) were performed on (9*Z*)-**2** in $CDCl_3$. Significant NOEs were observed between H-19 and H-2′ (consistent with the 8-*s*-*trans*-conformer) and between H-19 and H-8 (consistent with the 8-*s*-*cis* conformer). Relative to the very intense cross-peak intensity found between the H-19 methyl protons and H-10 (indicating the 9*Z*-configuration), the intensities of the negative cross-peaks observed between H-19/H-2′ and H-19/H-8 were about one-half this integrated intensity. Similar results were obtained from NOESY experiments on *all-E*- and 9*Z*-isomers of UAB30. The NOE results are in support of the MM3 calculations which identified two low-energy conformers about the C8−C9 bond in the polyene chain of these retinoids.

CRABP Binding Affinity. The IC_{50} values were evaluated in a chick skin CRABP radioligand binding assay with [^{3}H]-(*all-E*)-RA as the radioligand.[18] At 100-fold excess of unlabeled UAB retinoid, (*all-E*)-UAB30 inhibited the binding of [^{3}H]RA by only 75%; (13*Z*)-UAB30 (28% inhibition) and (9*Z*)-UAB30 (5% inhibition) had even less affinity for CRABP. The lower affinity of UAB30 isomers (IC_{50} > 2000 nM) relative to the *all-E*-isomers of RA (IC_{50} = 600 nM) is surprising especially since UAB7 and UAB8 retinoids had binding affinities comparable to that of RA. Apparently, the aromatic ring residue of UAB30 does not interact as well as the bulky alkyl groups of UAB8 in the RA binding site of CRABP, even though these groups are positioned in a similar region of space in solution.

Nuclear Receptor Binding Affinity and Transcriptional Activity. The *all-E*-, 9*Z*-, and 13*Z*-isomers of UAB30 were evaluated for their ability to inhibit the binding of [^{3}H]-(*all-E*)-RA to nuclear retinoid receptors. To survey the inhibition process, nuclear receptors (RARα, RARβ, RARγ, and RXRα) were first exposed to 1000 nM UAB retinoids and 5 nM [^{3}H]-(*all-E*)-RA. This

Table 2. ^{1}H NMR Chemical Shift Assignments (ppm, TMS) for UAB30 Retinoids at 400 MHz

compound	H-1′	H-2′	H-4	H-3	H-2	H-1	H-8	H-10	H-11	H12	H-14	H-18	9Me	13Me
α-tetralone	2.11	2.63	7.22	7.44	7.27	8.01						2.94		
(9*Z*)−2	1.84	2.57	7.09	7.17	7.16	7.66	7.14	5.79	11.79			2.80	2.13	
(*all-E*)−3	1.82	2.70	7.09	7.16	7.15	7.58	6.39	5.62	4.29			2.80	1.86	
(9*Z*)−3	1.82	2.36	7.08	7.16	7.14	7.58	6.36	5.55	4.06			2.83	1.85	
(*all-E*)−4	1.86	2.76	7.14	7.22	7.20	7.61	6.49	6.04	10.10			2.83	2.35	
(9*Z*)−4	1.86	2.50	7.14	7.23	7.19	7.64	6.57	6.01	9.65			2.86	2.09	
(*all-E*)−5	1.85	2.78	7.09	7.15	7.15	7.58	6.51	6.23	6.96	6.28	5.79	2.81	2.08	2.37
(9*Z*)−5	1.83	2.41	7.12	7.19	7.19	7.64	6.47	6.11	6.64	6.23	5.75	2.85	1.98	2.23
(13*Z*)−5	1.84	2.78	7.09	7.15	7.15	7.58	6.50	6.34	6.95	7.79	5.65	2.80	2.07	2.08
(*all-E*)-UAB30	1.85	2.78	7.10	7.17	7.15	7.58	6.52	6.25	7.01	6.32	5.82	2.81	2.09	2.38
(9*Z*)-UAB30	1.84	2.41	7.12	7.20	7.20	7.59	6.48	6.12	6.69	6.25	5.77	2.85	1.99	2.23
(13*Z*)-UAB30	1.84	2.79	7.08	7.15	7.15	7.58	6.51	6.35	7.00	7.74	5.56	2.79	2.08	2.12

Table 3. Summary of IC_{50} and EC_{50} Values[a](nM) for UAB8 and UAB30 Retinoids in Nuclear Receptor Binding and Transcriptional Activation Assays

	IC_{50} (nM)				EC_{50} (nM)				
retinoid isomer	RARα	RAR*β*	RAR*γ*	RXRα	RARα	RAR*β*	RAR*γ*	RXRα	RARα/RXRα
(*all-E*)-RA	6	5	4	>2000	22	2	6	>2000	32
(9*Z*)-RA[b]	31	8	60	82	18	27	10	27	17
(*all-E*)-UAB8	14	6	10	>2000	33	20	2	>2000	68
(13*Z*)-UAB8[b]	900	371	708	>1000	51	150	42	>2000	450
(9*Z*)-UAB8	>1000	>1000	>1000	868	>1000	>2000	190	220	>2000
(*all-E*)-UAB30	35	49	55	>2000	>2000	110	370	>2000	>1000
(13*Z*)-UAB30	>1000	>1000	>1000	>2000	>2000	>2000	>2000	>2000	>2000
(9*Z*)-UAB30	>2000	>2000	>2000	284	>2000	>2000	>2000	118	>1000

[a] The IC_{50} and EC_{50} values were calculated by a Probit analysis of a dose−response curve using concentrations between 1 and 1000 nM. The standard error in the reported IC_{50} values was ±2.0 nM or less. The standard error in the reported EC_{50} values was ±2.5 nM or less, except for the EC_{50} value reported for the activation of RAR*γ* by (9*Z*)-RA, which was ±4 nM. [b] IC_{50} values were taken from Alam et al.[9]

was compared to a positive control using 1000 nM unlabeled (*all-E*)-RA and a control using no retinoid. For active retinoids, IC_{50} values were determined by titration with varying concentrations of UAB retinoid.

(*all-E*)-UAB30 was the only UAB30 isomer which efficiently bound to RARs. The IC_{50} values (30−50 nM) for RARs were only about 5-fold greater than that for (*all-E*)-RA or (*all-E*)-UAB8 (Table 3) and comparable to that for (9*Z*)-RA. The IC_{50} values for (9*Z*)-UAB30 were >1000 nM indicating that it did not bind to RAR receptors (Table 3), which is consistent with data on (9*Z*)-UAB8. The low affinity for RARs is not shared by (9*Z*)-RA, which binds nearly as well as (*all-E*)-RA to these receptors. Also, (13*Z*)-UAB30 was an ineffective binder to the RAR subtypes, which is different from the binding properties of (13*Z*)-UAB8 to these nuclear receptors.[9]

UAB30 isomers were evaluated in assays which measured their functional activity within cells to induce RARα-, RAR*β*-, and RAR*γ*-mediated transcriptional activity. CV-1 cells were transiently transfected with DNA plasmids from the appropriate RAR subtype and $(TREpal)_2$-tk-CAT.[9,19] Using a dose−response curve, the ED_{50} values for retinoid-induced transcription of UAB30 isomers were measured and then compared to those found for (*all-E*)- and (9*Z*)-RA and for (*all-E*)- and (9*Z*)-UAB8 (Table 3). (*All-E*)-UAB30 induced RAR*β*-mediated receptor-activated transcription; however, 5-fold higher concentrations were needed to achieve similar activation to those observed for (9*Z*)-RA or (*all-E*)-UAB8. (*all-E*)-UAB30 displayed moderate activity in inducing RAR*γ*-mediated transcription and had no activity for inducing RARα transcription. As such this UAB30 isomer displays RAR*β* and RAR*γ* subtype selectivity and RARα antagonist properties, which is in sharp contrast to (*all-E*)-UAB8—a very efficient *pan*-RAR agonist. (13*Z*)-UAB30 displayed much different nuclear receptor transcriptional properties than its UAB8 analogue; it had no activity in inducing RAR-mediated gene expression, whereas (13*Z*)-UAB8 is an efficient activator of gene transcription mediated by RARs. (9*Z*)-UAB30 was even a poorer activator of reporter genes mediated by RARs; it was much less active in these assays than (9*Z*)-UAB8.

The binding affinity of the UAB30 isomers to RXRα was examined next. Only (9*Z*)-UAB30 inhibited the binding of 20 nM [^{3}H]-(9*Z*)-RA to RXRα (Table 3). At 100-fold excess of unlabeled UAB retinoid, (9*Z*)-UAB30 inhibited 70% of the activity relative to (9*Z*)-RA. The IC_{50} value for this isomer was better than that for (9*Z*)-UAB8 and only 4-fold higher than that of (9*Z*)-RA. The other isomers were much less effective (IC_{50} > 2000 nM). When the effect on RXRα homodimer-mediated transcriptional activity was studied, (9*Z*)-UAB30 was the only efficient modulator of the reporter gene (Table 3). Relative to other 9*Z*-isomers, transcriptional activation by (9*Z*)-UAB30 was about 4-fold less efficient than that by (9*Z*)-RA but 2-fold better than that by (9*Z*)-UAB8. Of the (9*Z*)-UAB isomers, UAB30 is the most potent and selective ligand for activating RXRs.

Since (*all-E*)-UAB30 bound well to RARα (IC_{50} = 35 nm) but did not activate this receptor well (EC_{50} > 1000 nM), its potential to act as an RARα antagonist was next evaluated. Fixed (*all-E*)-RA concentrations ranging between 1 and 1000 nM were evaluated in the transcriptional assay mediated by RARα homodimers using two concentrations of (*all-E*)-UAB30 (10 or 100 nM). These were compared to a negative control of (*all-E*)-RA alone and a positive control using Ro-41-5253, a known RARα antagonist, at 10 and 100 nM. For 50 and 100 nM (*all-E*)-RA concentrations, the relative CAT activity of the cells treated with 100 nM (*all-E*)-UAB30

Table 4. ED_{50} Values[a] (nM) for 4-Day Induction of Differentiation of HL-60 and NB4 Cells and Normalized Percent Inhibition[b] of CFU-GM Colony Growth of JMML Cells from 3 Patients

	ED_{50} (nM)		patient J43 (%)		patient J83 (%)		patient J84 (%)		
retinoid isomer	HL-60	NB4	10^{-6} M	10^{-7} M	10^{-6} M	10^{-7} M	10^{-6}M	10^{-7} M	10^{-8} M
(*all-E*)-RA	120	610	100	74	100	88	100	52	33
(13*Z*)-RA	NT[c]	NT	109	100	94	86	87	52	5
(9*Z*)-RA	NT	NT	100	98	82	72	95	NT	NT
(*all-E*)-UAB8	>10 000	320	98	85	NT		103	51	NT
(13*Z*)-UAB8	>10 000	280	80	61	NT		49	18	0
(9*Z*)-UAB8	3 400	910	79	52	NT		59	38	13
(9*Z*,13*Z*)-UAB8	>10 000	>10 000	59	32	NT		44	23	20
(*all-E*)-UAB30	>10 000	>10 000	NT		72	68	57	48	28
(13*Z*)-UAB30	>10 000	>10 000	NT		88	82	80	72	25
(9*Z*)-UAB30	8 000	10 000	NT		79	76	39	44	3

[a] ED_{50} values were determined from a dose−response curve using four concentrations of retinoids ranging from 10^{-2} to 10 μM. [b] Normalized percent inhibition was calculated by (% inhibition of retinoid)/(% inhibition of (*all-E*)-RA at 10^{-6} M). The percent inhibition of (*all-E*)-RA at 10^{-6} M was 85%, 78%, and 61% for the J43, J83, and J84 patient cells, respectively. [c] NT means not tested.

decreased relative to those containing only (*all-E*)-RA. This decrease in CAT activity is consistent with RA antagonism by (*all-E*)-UAB30, but the magnitude of these effects was 2-fold less than that demonstrated by Ro-41-5253.

Chemoprevention of Mouse Skin Papilloma. The UAB30 retinoids were next evaluated for their activity in preventing the chemical induction of papillomas on mouse skin.[14] Both (*all-E*)-RA and (*all-E*)-UAB8 were very effective in this chemopreventive assay. At a 45.9-nmol dose, they completely prevented tumor formation (95% for RA; 99% for UAB8). The ED_{50} values for these retinoids were 3.0 (RA) and 2.5 (UAB8) nmol.[8] (*all-E*)-UAB30 was less effective than this RA or UAB8 isomer in the skin antipapilloma assay; it only reduced 59% of the papillomas at 45.9 nmol. Using dose−response curves, an ED_{50} could not be accurately determined for this retinoid, consistent with our efforts to quantify the activity of other marginally active UAB retinoids.[8] Previously, we[8] correlated (in structure−activity relationships) the high activity of (*all-E*)-UAB8 (containing large alkyl groups at R_1 and R_2) to its efficient binding and/or activation of RARs. Using these trends, the reduced activity of (*all-E*)-UAB30 may be due to its poor ability to activate RARγ and/or lack of ability to activate RARα (Table 3), especially in comparison to (*all-E*)-UAB8 which has excellent RARγ activity. The (9*Z*)-UAB30 isomer was also less effective than (9*Z*)-RA in this assay; it prevented only 54% of tumor formation at 45.9 nmol, a dose at which (9*Z*)-RA was highly (95%) active.[8] This low activity, however, is comparable to that of (9*Z*)-UAB8 in this assay.[8] Since both (9*Z*)-UAB8 and (9*Z*)-UAB30 are RXR-selective retinoids, they are not effective activators of RAR-mediated pathways, particularly those mediated by RARβ and RARγ nuclear receptors. Chandraratna et al.[20] showed that RARβ- and RARγ-selective retinoids are excellent inhibitors of TPA-induced ornithine decarboxylase (ODC) activity in skin, an assay which correlates well with the skin antipapilloma assay.[14] They suggested that these RARβ- and RARγ-selective retinoids are potent inhibitors of ODC activity in vivo by antagonizing AP-1-mediated gene expression through RARα binding. If this is the case, then (*all-E*)-UAB30 (a weak RARα antagonist) should not be an efficient AP-1 antagonist, but (*all-E*)-UAB8 should have activity to repress AP-1 gene transcription in addition to its demonstrated potent transactivation activity of RAR homodimers (Table 3).

Inhibition of JMML CFU-GM Colony Formation. In 1991, Emanuel et al.[21] demonstrated that the spontaneous CFU-GM colony formation observed in JMML is related to the selective hypersensitivity of JMML hematopoietic progenitor cells to granulocyte-macrophage colony-stimulating factor (GM-CSF). Since this initial report by the UAB group, others[22] have confirmed these observations of GM-CSF hypersensitivity as a pathogenetic mechanism in JMML. Additionally in 1991, Castleberry, Emanuel, and co-workers also presented preliminary evidence of the ability of (13*Z*)-RA to inhibit in vitro JMML spontaneous CFU-GM growth as well as evidence for its possible clinical effectiveness in JMML.[21] The final results of the pilot clinical trial were published in 1994 and suggested that the effects of (13*Z*)-RA in the in vitro screen agree with its efficacy in treating patients with JMML in the clinic.[5] In this study, we first evaluated (*all-E*)- and (9*Z*)-RA in this in vitro assay and showed that these RA isomers also have excellent potential to inhibit spontaneous CFU-GM colony growth in cells isolated from three patients with JMML (Table 4). We next studied the effects of UAB8 and UAB30 isomers on the inhibition of proliferation of human JMML cells. UAB8 isomers were evaluated in this assay using cells from two patients (Table 4). (*all-E*)-UAB8 was as effective as (*all-E*)-RA in controlling the spontaneous growth of these CFU-GM colonies. However, (9*Z*)- and (13*Z*)-UAB8 were less effective than the corresponding RA isomers in controlling growth. In particular for patient J84 cells, there is a 2-fold decrease in activity of these retinoids. For UAB30 isomers, the most potent isomer is (13*Z*)-UAB30 which has activity in two patients only slightly less than that of RA. In contrast to UAB8 isomers, the *all-E*-isomer of this retinoid is less active than RA and (*all-E*)-UAB8. Also, (9*Z*)-UAB30 exhibits lower activity in this assay, similar to that of (9*Z*)-UAB8.

There are no consistent chromosomal abnormalities in JMML, and none that appear to involve the retinoid receptors. However one very plausible mechanism[23] to explain the apparent modulating effects of RA on GM-CSF hypersensitivity in JMML is through the RA-induced antagonistic effects of RARα on the transcription factor, AP-1 (c-*jun*/c-*fos*). The GM-CSF signal transduction cascade through Ras appears to have as one of its major endpoints an upregulation or activation

of the AP-1 response element. This response element is activated by c-*jun*/c-*fos*, and when stimulated by extracellular signaling (e.g., GM-CSF), induction of AP-1 leads to cellular proliferation, among other processes. In fact in Northern blot experiments investigating early response gene expression in JMML, Emanuel and coworkers[22] have noted an apparent constitutive upregulation of both c-*jun* and c-*fos*, but not c-*myc*. As reviewed by Pfahl[24] and demonstrated in epithelial cells,[25] the mechanism by which RA represses gene transcription mediated by AP-1 is cell line-specific and may be mediated by either RARs or RXRs.

The precise mechanism for the high activity of (13*Z*)-RA in inhibiting the proliferation of these myeloid progenitor cells in JMML remains an area of investigation. Assuming a receptor-based mechanism, (13*Z*)-RA isomer may isomerize to either (*all-E*)- or (9*Z*)-RA and interact with RAR/RXR receptors. We show here for the first time that these latter two isomers are as active as (13*Z*)-RA in preventing proliferation in JMML cells, supporting this assumption. To probe RAR/RXR-mediated pathways, we investigated the ability of receptor-selective retinoids to prevent proliferation of JMML cells. The high activity of (*all-E*)-UAB8 in this in vitro JMML assay and reduced activity of either (9*Z*)-UAB8 or (9*Z*)-UAB30, two RXR-selective retinoids, are consistent with a RAR-mediated pathway of transrepression. (*all-E*)-UAB30 is less active than the other natural RA isomers and (*all-E*)-UAB8. Since it binds well to each RAR subtype, but does not activate transcription for RARα (an RARα antagonist), this lower activity in the in vitro JMML assay relative to (*all-E*)-UAB8 may be due to its RARα antagonism, or in other words, lack of AP-1 antagonism. Ligand binding is a necessary but not sufficient condition for AP-1 antagonism. This is not an unusual property of retinoids, since both Dawson and Chandraratna's group[26] have identified retinoids that are RARα antagonists but do not induce RAR-mediated transrepression of AP-1-mediated transcription.

Induction of Differentiation in HL-60 and NB4 Cells. To explore the potential of UAB retinoids for treatment of APL, we evaluated UAB8 and UAB30 isomers in in vitro assays which measure their ability to induce differentiation of two APL leukemia cell lines, HL-60 and NB4 cells. Even though HL-60 cells were isolated from APL patients, only the NB4 cell line contains the t(15;17) translocation involving the RARα to form the RARα/PML fusion protein. Because this characteristic mutation is stably carried in NB4 cells, it is a better model for judging retinoid efficacy for this disease. In NB4 cells, the ED_{50} values of two UAB retinoids were much lower than that of RA after treatment for 3 days: (*all-E*)-UAB8 (ED_{50} = 475 nM) was 10-fold more active and (13*Z*)-UAB8 was 3-fold more active (ED_{50} = 1400 nM) than (*all-E*)-RA (ED_{50} = 4700 nM) in this differentiation assay. The 9*Z*- and 9*Z*,-13*Z*-isomers of UAB8 and the three UAB30 isomers were essentially inactive (ED_{50} >10 000 nM) at this time point. After day 4, the (*all-E*)- and (13*Z*)-UAB8 isomers were 2-fold more active than RA (Table 4). In contrast to UAB8 isomers, none of the UAB30 isomers were active. Using receptor-selective retinoids, Kizaki et al.[27] and Chen et al.[28] recently demonstrated that retinoid activity to induce terminal differentiation in this cell line correlates well with their ability to activate RARα/RXRα-mediated transcription that presumably removes the negative block to normal myeloid stem cell differentiation. The data presented here support this idea. As we previously demonstrated,[8] both (*all-E*)-UAB8 and (13*Z*)-UAB8 efficiently activate this heterodimer, whereas the RXR-selective retinoids, (9*Z*)-UAB8 or (9*Z*)-UAB30, do not. As shown in Table 3, (*all-E*)-UAB30 binds well but does not activate RARα-mediated transcription, consistent with its inactivity in this assay.

In conclusion, we have shown that substituting a fused benzyl ring for alkyl groups has little effect on the conformation on the polyene chain between C-8 and C-15, but it markedly changes the biological properties of the conformationally constrained UAB retinoids. In particular, the nuclear receptor transactivational profiles of *all-E*-isomers are modified dramatically. For UAB30, this isomer displays selectivity for activation of RARβ- and RARγ-mediated transcription (an RARα antagonist), whereas (*all-E*)-UAB8 is a potent *pan*-RAR agonist. The transactivational profiles of the 9*Z*-isomers are very similar; both 9*Z*-isomers of UAB8 and UAB30 are RXRα-selective compounds, with the latter displaying the most potency and selectivity. These retinoids also exhibit very different efficacies in several biological assays, and the changes in their activities correlate well with their individual transcriptional properties. For example, the high activity of (*all-E*)-UAB8 over (*all-E*)-UAB30 and (9*Z*)-UAB8/(9*Z*)-UAB30 in the prevention of skin papillomas, in the induction of differentiation of NB4 cells, and in the inhibition of proliferation of JMML cells is consistent with its demonstrated potent activity to activate gene expression mediated by each RAR subtype and/or its putative activity to repress gene expression of AP-1 through RAR subtypes. The most exciting aspect of the work presented here is the higher efficacy of (*all-E*)- and (13*Z*)-UAB8 over (*all-E*)-RA in their ability to differentiate NB4 cells. The *all-E*-isomer of UAB8 is 2-fold less toxic than this isomer of RA,[29] and its pharmacokinetics allows higher concentrations in plasma for prolonged times. Taken together, these two UAB8 isomers may be improved agents for clinical use in the treatment of APL patients.

Experimental Section

Chemistry. ^{1}H NMR spectra were obtained at 400.1 MHz (Bruker DRX spectrometer) in $CDCl_3$. NOE experiments were performed on degassed samples using 2D phase-sensitive NOESY experiments with different mixing times (between 250 and 2000 ms). Typically, 1 s mixing times were used with 16 pulses for phase cycling and 2 dummy scans. The data were processed with line broadening of 0.3 Hz in each dimension and zero-filled to yield 512 × 4096 2D contour plots. The integrated intensities of the negative cross-peaks were determined using standard Bruker NMR software features.

UV/vis spectra were recorded on an AVIV 14DS spectrophotometer in cyclohexane or methanol solutions (Fisher, Spectrograde). IR spectra were recorded using a Nicolet FT IR spectrometer on thin films. HPLC separations were performed on a Gilson HPLC gradient system using 25-mL pump heads and an ISCO V^4 variable wavelength detector. The column employed was a Whatman Partisil 10 M20/50 (500- × 22-mm i.d.) with a flow rate of 5 mL/min and monitoring by UV/vis detection at 340 nm. TLC chromatography was performed on precoated 250-μm silica gel GF glass

plates (Analtech, Inc.; 5 × 10 cm). Solvents and liquid starting materials were distilled prior to use. Reactions and purifications were conducted with deoxygenated solvents, under inert gas (N_2) and subdued lighting. Selected data for the intermediates and products in Scheme 1 are contained in Table 1.

(2*Z*,4*E*)-4-(3′,4′-Dihydro-1′(2′*H*)-naphthalen-1′-ylidene)-3-methyl-2-butenoic Acid ((9*Z*)-2). Zinc dust (1.75 g, 26.8 mmol) was stirred with 5% HCl (5 mL) for 2 min at room temperature. The mixture was allowed to settle, and the liquid was carefully removed by pipet. In a similar fashion the Zn was washed, under nitrogen, with water (3 × 5 mL), acetone (3 × 5 mL), and ether (2 × 8 mL). After residual ether was removed under a stream of nitrogen, the flask containing the Zn dust was strongly heated with a Bunsen burner flame for 30 s. The cooled Zn dust was suspended in anhydrous dioxane (3 mL), and the stirred suspension was heated to reflux. A solution of freshly distilled α-tetralone (0.520 g, 3.56 mmol), ethyl bromosenecioate (1.47 g, 7.12 mmol), and anhydrous dioxane (2.5 mL) was prepared under nitrogen, and a portion of this solution (0.5 mL) was added to the heated Zn suspension. This produced an exothermic reaction, and the remainder of the solution containing α-tetralone was then added during 10 min at a rate sufficient to control reflux. The final reaction mixture was stirred at reflux for 8 h and then cooled to room temperature. Water (5 mL) was added, the mixture was stirred for 20 min, and ether (20 mL) was added. The suspension was filtered through a pad of Celite and the filter washed well with ether (40 mL). The filtrate was extracted with 15% HCl (40 mL), water (40 mL), 1 N NaOH (40 mL), and an additional amount of water (30 mL). The basic wash and final water wash were combined, adjusted to pH 1−2 with 15% HCl, and extracted with ether (2 × 100 mL). The organic layer was dried (Na_2SO_4) and concentrated under vacuum to provide (9*Z*)-**2** as yellow crystals (0.70 g, 86% yield): mp 153−154 °C (1:1 hexane−ethyl acetate).

(2*Z*,4*E*)- and (2*E*,4*E*)-4-(3′,4′-Dihydro-1′(2′*H*)-naphthalen-1′-ylidene)-3-methyl-2-buten-1-ol ((9*Z*)- and (*all-E*)-3). A solution of acid (9*Z*)-**2** (0.11 g, 0.48 mmol) in anhydrous THF (10 mL) was cooled to −78 °C, and 1 M $LiAlH_4$/ether (0.50 mL, 0.50 mmol) was added dropwise with stirring under nitrogen. The dry ice−acetone bath was removed, and the reaction mixture was brought to room temperature and stirred for 3 h. The reaction mixture was cooled to 0 °C, and methanol (0.2 mL) followed by 10% HCl (10 mL) was added dropwise. This was allowed to warm to room temperature, and the mixture was extracted with ether (2 × 20 mL). The ether layer was washed with brine (1 × 20 mL), dried (Na_2SO_4), and concentrated under vacuum to give an oily residue (0.113 g). This was placed on a flash silica gel column (1 × 30 cm) and eluted with 30% Et_2O−hexane to give a 1:1 mixture of (9*E*)- and (*all-E*)-**3** (0.068 g, 67% yield). The alcohol mixture was carried on in this form without further purification.

(2*Z*,4*E*)- and (2*E*,4*E*)-4-(3′,4′-Dihydro-1′(2′*H*)-naphthalen-1′-ylidene)-3-methyl-2-butenal ((9*Z*)- and (*all-E*)-4). To a stirred solution of alcohol **3** (0.065 g, 0.30 mmol) in dry CH_2Cl_2 (10 mL) at 0 °C, under nitrogen, was added 4 Å molecular sieves (1.5 g) followed by activated MnO_2 (0.53 g, 6.1 mmol), and the mixture was stirred at 0 °C for 3 h. The reaction mixture was filtered through a pad of flash silica gel, and the filter was washed with cold 50% CH_2Cl_2/ether (100 mL). The filtrate was concentrated to dryness under vacuum to give a residual oil (0.05). This was placed on a flash silica gel column (1 × 15 cm) and eluted with 10% Et_2O−hexane to give (9*Z*)-**4** (0.018 g), (*all-E*)-**4** (0.016 g), and starting alcohol **3** (0.007 g). The total yield of **4** was 0.034 g (59% yield based on unrecovered starting alcohol).

General Procedure for the Olefination of Individual Isomers of Aldehyde 4. NaH (1.2 equiv) was washed in dry THF three times to eliminate mineral oil. At 0 °C under N_2, freshly distilled triethyl phosphonosenecioate (1.1 equiv) in THF was added. After the mixture stirred for 30 min, HMPA (0.2 equiv) followed by either (9*Z*)- or (*all-E*)-**4** (1 equiv, final concentration 0.8 M) was added. After the mixture reacted for 120 min at room temperature, water was added and the reaction mixture was partitioned between ether and water. The ether layer was washed once with brine, dried (Na_2SO_4), and concentrated under vacuum to give ester as a mixture of mainly two configurational isomers. By this method were prepared the following.

(2*E*,4*E*,6*Z*,8*E*)- and (2*Z*,4*E*,6*Z*,8*E*)-Ethyl 8-(3′,4′-Dihydro-1′(2′*H*)-naphthalen-1′-ylidene)-3,7-dimethyl-2,4,6-octatrienoate ((9*Z*)- and (9*Z*,13*Z*)-5). This preparation employed a suspension of NaH (0.060 g, 2.5 mmol) in dry THF (1 mL), a solution of triethyl phosphonosenecioate (0.14 g, 0.53 mmol) in dry THF (1 mL), HMPA (0.041 g, 0.21 mmol), and a solution of (9*Z*)-**4** (0.089 g, 0.42 mmol) in dry THF (1 mL) to give a 2:1 mixture of esters (9*Z*)- and (9*Z*,13*Z*)-**5** (0.12 g, 78% yield). This mixture was separated by HPLC on silica gel using 0.5% Et_2O, 0.1% THF in hexane.

(2*E*,4*E*,6*E*,8*E*)- and (2*Z*,4*E*,6*E*,8*E*)-Ethyl 8-(3′,4′-Dihydro-1′(2′*H*)-naphthalen-1′-ylidene)-3,7-dimethyl-2,4,6-octatrienoate ((*all-E*)- and (13*Z*)-5). This preparation employed a suspension of NaH (0.050 g, 2.1 mmol) in dry THF (1 mL), a solution of triethyl phosphonosenecioate (0.11 g, 0.40 mmol) in dry THF (1 mL), HMPA (0.048 g, 0.22 mmol), and a solution of (*all-E*)-**4** (0.065 g, 0.31 mmol) in dry THF (1 mL) to give a 2:1 mixture of esters (*all-E*)- and (13*Z*)-**5** (0.12 g, 78% yield). This mixture was separated by HPLC on silica gel using 1% Et_2O, 0.5% THF in hexane.

General Procedure for the Hydrolysis of Individual Isomers of Ester 5. To a solution of the ester **5** (1 equiv) in methanol (final concentration 0.061 M) was added an aqueous solution of 2 M KOH (10 equiv). The solution was heated at reflux, and the reaction progress was monitored by TLC. After 90 min the hot solution was poured into a beaker of ice (40 g) and acidified with 10% HCl until pH 2. The mixture was then extracted with Et_2O, which was washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure to give the product. NMR revealed that the hydrolysis occurred without isomerization. The following acids were synthesized by this method.

(2*E*,4*E*,6*E*,8*E*)-8-(3′,4′-Dihydro-1′(2′*H*)-naphthalen-1′-ylidene)-3,7-dimethyl-2,4,6-octatrienoic Acid ((*all-E*)-UAB30). This preparation utilized a solution of KOH (0.258 g, 4.61 mmol) in water (2 mL) and a warm solution of ester (*all-E*)-**5** (0.122 g, 0.378 mmol) in methanol (10 mL) to provide acid (*all-E*)-UAB30 (0.104 g, 93% yield) as a yellow solid: mp 192−197 °C (cyclohexane).

(2*E*,4*E*,6*Z*,8*E*)-8-(3′,4′-Dihydro-1′(2′*H*)-naphthalen-1′-ylidene)-3,7-dimethyl-2,4,6-octatrienoic Acid ((9*Z*)-UAB30). This preparation utilized a solution of KOH (0.15 g, 2.8 mmol) in water (1 mL) and a warm solution of ester (9*Z*)-**5** (0.073 g, 0.23 mmol) in methanol (3 mL) to give acid (9*Z*)-UAB30 (0.065 g, 98% yield) as a yellow solid: mp 182−185 °C (cyclohexane).

(2*Z*,4*E*,6*E*,8*E*)-8-(3′,4′-Dihydro-1′(2′*H*)-naphthalen-1′-ylidene)-3,7-dimethyl-2,4,6-octatrienoic Acid ((13*Z*)-UAB30). This preparation employed a solution of KOH (0.085 g, 1.50 mmol) in water (1 mL) and a warm solution of ester (13*Z*)-**5** (0.040 g, 0.12 mmol) in methanol (4 mL) to give acid (13*Z*)-UAB30 (0.034 g, 93% yield) as a yellow solid: mp 180−185 °C (cyclohexane).

Biology. The chick skin CRABP binding assay measured IC_{50} values for retinoid binding to CRABP-II using a radiolabeled competition assay.[8−10] The IC_{50} values for retinoids with RARs and RXRs were measured with a radioligand competition assay.[8−10] The nuclear receptor transcriptional activity assays were performed using CV-1 cells. Transient transfection of these cells with a DNA plasmid was performed essentially as described in Alam et al.[9] The mouse skin antipapilloma assay measured the ED_{50} values for retinoid tumor inhibition on the dorsal skin of mice. The inhibition of mouse skin papilloma by retinoids was performed according to a modification of the procedure developed by Verma and Boutwell[14] as reported previously by Muccio et al.[8]

Inhibition of (*all-E*)-RA Induced-RARα Transcriptional Activity in CV-1 Cells. The antagonistic effects of UAB30 retinoids on the transcription of RARα were determined in CV-1 cells. The methods are nearly identical to those

used to perform transcriptional assays. These studies utilized the CAT reporter gene containing TREpal with a thymidine kinase promoter [$(TREpal)_2$-tk-CAT] as previously described by Vaezi et al.[11] Twenty-four hours after transfection, cells were treated with (*all-E*)-RA (1−1000 nM) alone or together with the indicated UAB retinoid (100 or 1000 nM). After 24 h, cells were harvested and the transcriptional effects were measured in relative CAT activity, after correction with β-gal activity for transfection efficiency. Similar studies were done with a known RARα antagonist, Ro-41-5253. All experiments were performed in triplicate, and the data were averaged. Antagonism was defined as a lowering of the relative CAT activity of transcription induced by (*all-E*)-RA in the presence of retinoid as compared to positive controls which did not contain retinoid. Both (*all-E*)-UAB30 and Ro-41-5253 exhibited antagonism at each concentration of (*all-E*)-RA used to induce transcription, but the effects were most noticeable when (*all-E*)-RA was 50 nM.

Induction of Differentiation of HL-60 and NB4 Cells. Human leukemia cell lines HL-60 and NB4 were used for measuring the ability of retinoids to induce terminal differentiation. Both cell lines are predominantly promyelocytes and are induced by RA to mature to cells with many characteristics of mature granulocytes. The induction of this differentiation is the basis for assaying one of the biological activities of RA analogues. For the differentiation assay, HL-60 or NB4 cells were grown in nutrient medium with fetal bovine serum as detailed previously by Breitman.[15] The UAB retinoids and RA were prepared in ethanol at concentrations from 0.01 to 10 mM and then diluted 100-fold into cell cultures. After 3- and 4-day incubation periods, the ability of the cells to reduce nitroblue tetrazolium was measured. The percentage of cells that gained this marker was measured microscopically. The percentage of positive cells was then plotted as a function of the retinoid concentration to determine ED_{50} values.

Inhibition of JMML Spontaneous CFU-GM Colony Formation. (*all-E*)- and (9*Z*)-RA, UAB8, and UAB30 retinoids were tested for their ability to inhibit spontaneous CFU-GM colony growth in three JMML patient samples as described in detail elsewhere by Emanuel et al.[5,16] Briefly, after obtaining parental consent and with the approval of the Institutional Review Board, JMML peripheral blood and bone marrow samples were collected from patients and shipped by overnight delivery to the University of Alabama at Birmingham. Mononuclear cells were separated by density gradient centrifugation, washed, and frozen in aliquots. Aliquots of JMML patient samples were later thawed, washed, and set up in 0.3% soft agar clonal assays in McCoys' 5A medium supplemented with nutrients as well as 15% fetal bovine serum. No growth factors or other exogenous stimuli were added to the cultures. UAB retinoids and RA isomers were dissolved in either 100% ethanol or DMSO. Appropriate dilutions were made, and controls using the respective ethanol or DMSO concentration were set up in parallel. Varying doses of retinoids were added only once, 24 h after initiation of the soft agar assays, by pipetting the retinoid solution on top of the soft agar and allowing the solution to enter the agar and interact with the cells by diffusion. The cultures were incubated for 14 days at 37 °C in a humidified atmosphere with 5% CO_2. CFU-GM colonies (1 colony has >40 cells) were scored, and the resultant amount of inhibition of spontaneous CFU-GM colony growth was compared to the (13*Z*)-RA.

Molecular Modeling. Retinoid structures were generated with Sybyl version 6.2 (Tripos Inc., St. Louis, MO) on a Silicon Graphics Indigo 2 workstation. The structure of (9*Z*)-**2** was built using the recently reported X-ray crystal structure.[17] This structure crystallized in the 8-*s*-*trans*-conformation. To generate 8-*s*-*cis*-conformations, this structure was rotated about the C8−C9 bond followed by energy minimization using Allinger's MM3(94) force fields. The structures of final UAB30 acids were generated from these 8-*s*-*cis*- and 8-*s*-*trans*-structures of **2**. The thermodynamic parameters were calculated according to previously described methods.[8]

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