Conformational Effects on Retinoid Receptor Selectivity. 1. Effect of 9-Double Bond Geometry on Retinoid X Receptor Activity

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A major challenge is the development of retinoids with selective biological activities. Recently, studies on retinoid response mechanisms indicate that retinoids activate two classes of nuclear receptor proteins, the retinoic acid receptors (RARs) and the retinoid X receptors (RXRs). Here, we analyze the activity of a series of (*E*)- and (*Z*)-stilbenecarboxylic acids for gene transcriptional activation of the RARs and RXR-α to determine the optimum pharmacophore for receptor activation. The data obtained indicate that RAR and RXR response pathways can be separated by using the appropriate ligand. The conformations of (*Z*)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)propen-1-yl]benzoic acid and (*Z*)-4-[1-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)propen-2-yl]benzoic acid were examined by experimental and theoretical methods to establish the appropriate conformation of the latter that specifically activated the retinoid RXR. A palladium(0)-catalyzed aryl bromide–arylboronic acid coupling under nonanhydrous conditions was used to construct a biaryl bond in the conformationally restricted retinoid 2′-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)biphenyl-4-carboxylic acid, which had RXR activity.

Introduction

Retinoids are important therapeutic agents in the treatment of cancer and proliferative diseases of the skin.[1-4] Retinoic acid (RA) and its synthetic analogs (retinoids) effect a wide array of biological processes by activating two distinct classes of nuclear receptor proteins, the retinoic acid receptors (RARs)[5-10] and the retinoid X receptors (RXRs).[11-15] These retinoid receptors belong to the steroid/thyroid hormone receptor superfamily,[16,17] and for each class three receptor subtypes, RAR-α, β, and γ, and RXR-α, β, and γ, have been identified. An increasing body of evidence has established a central role for RXR in regulating several distinct hormonal response pathways. One function of RXR is to act as an auxiliary receptor for several nuclear receptors including the RARs, thyroid hormone receptors, vitamin D receptor, and the peroxisome proliferator-activated receptor.[13,14,18-21] Heterodimers of RXR with these receptors form in solution[18] and bind selectively with high affinity to specific hormone response elements.[22] RXRs also function independently as homodimers,[23] which form in the presence of the 9-*cis* isomer of *all-trans*-RA [(9*Z*)-RA (**2**)] and have different response element specificities than the RAR:RXR heterodimers.[22,23] Therefore, the two retinoid receptor response pathways activate different sets of genes. RXRs do not bind *all-trans*-RA [(*E*)-RA, (**1**)] and only bind and are activated by (9*Z*)-RA. In contrast, RARs bind both ligands with high affinity.[24-26] Thus, compounds that activate only one of the pathways are useful in separating these pathways, and their more restricted activity may result in fewer side effects in therapeutic applications.

Several reports[27,28] indicate that retinoid binding data correlate directly with their transcriptional activation activity. Here, we employ a transcription activation assay to define retinoids that selectively activate one of the retinoid response pathways.

Conformationally restricted retinoids, in which the bonds corresponding to the double bonds of the RA side chain are constrained in aromatic systems, have been used to probe retinoid structure–activity relationships. (*E*)-4-[2-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)propen-1-yl]benzoic acid (**3**) is one of the most active and toxic retinoids known,[29] having activity comparable or above that of RA in bioassays for retinoid activity.[29-31] The double bonds of this retinoid corresponding to the 5,7-double bonds and 11,13-double bonds of (*E*)-RA are restricted to planar cisoid conformations by incorporation into tetrahydronaphthalene and phenyl ring systems, respectively. Metabolism at the 5-position of the tetrahydronaphthalene ring, which corresponds to the 4-position on the β-cyclogeranylidene ring of RA, is blocked to oxidative metabolic deactivation by the *gem*-dimethyl group. In the region of **3** corresponding to the tetraene chain of RA only those C–C bonds linking the aromatic rings with the central double bond, which corresponds to the 9-double bond of RA, are capable of rotational freedom. We earlier established that **3** was an effective activator of the RARs.[32] Because of the ability of (9*Z*)-RA to activate both RARs and RXRs, we assessed the effects of **3** and its *Z* isomer on receptor activation, along with a series of analogs.

Results

Synthetic Chemistry. Wittig and Horner–Wadsworth–Emmons olefination methodologies were used to introduce the double bonds of the 4-(arylpropenyl)benzoic acids **3** to **12**, 4-(butadienyl)benzoic acids **13** and **15**, and 4-(butenyl)benzoic acids **14** and **16**, the syntheses of which have been described elsewhere.[31,33,34] *E* and *Z* isomers were separated and characterized. Bond geometry was established by comparisons of the ^{1}H NMR and UV spectra of the isomeric pairs. The ^{1}H NMR spectra showed that the

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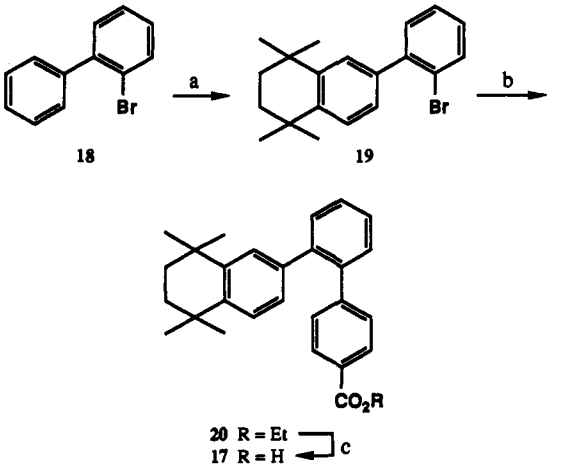
Scheme I[a]



[a] (a) $ClMe_2C(CH_2)_2CMe_2Cl$, $AlCl_3$, CH_2Cl_2. (b) $(PPh_3)_4Pd$, $MeOCH_2CH_2OMe$; 4-carbethoxyphenylboronic acid, EtOH, aqueous $NaHCO_3$. (c) Aqueous KOH, MeOH; 2 N HCl.

signals of the aromatic protons of the *Z* isomers **5**, **6**, **9**, **10**, and **12** were shifted upfield compared with those of the *E* isomers **3**, **4**, **7**, **8**, and **11**. More significant, the signals of the vinylic protons and the aromatic protons (meta to the carboxyl group) of the *Z* isomers appeared at 0.3–0.45 ppm higher field than those of the *E* isomers. Similar upfield shifts were found for the vinylic protons on the trisubstituted double bonds of the *Z* isomers **15** and **16** compared with those of the *E* isomers **13** and **14**, respectively. Invariably, the UV maxima of the *E* isomers were at longer wavelengths and had larger extinction coefficients than those of the *Z* isomers. These spectral differences between isomers have been described previously[33-35] and confirmed by X-ray crystallography.[35,36]

The 4-[2-(dihydrobenzothiopyranyl)prop-1-enyl]benzoic acids **4** and **6** are analogs of **3** and **5** and have a sulfur atom at the position corresponding to 5-position of the tetrahydronaphthalene ring. Retinoids **7** to **10** are analogs having a methyl group on the double bond corresponding to the 10-position of RA rather than the 9-position found in retinoids **3** to **6**. The benzonorbornenes **11** and **12** are less sterically bulky than their tetramethylated cousins **3** and **5**. The benzoic acids **13** to **16** are only conformationally constrained adjacent to the polar terminus and, therefore, are capable of greater mobility in the side-chain region adjacent to the hydrophobic terminus.

2′-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)biphenyl-4-carboxylic acid (**17**) has not been previously reported. This retinoid possesses the conformational restrictions of **5** and, in addition, has the propenyl bond system replaced by a 1,2-disubstituted benzene ring that holds the double bond corresponding to the 9*Z*-olefinic bond permanently *cis*. For the synthesis of **17**, the starting material 2-bromobiphenyl (**18**) was cyclialkylated with 2,5-dichloro-2,5-dimethylhexane in the presence of $AlCl_3$ to introduce the tetrahydrotetramethylnaphthalene ring of **19** (Scheme I), using conditions similar to those developed by Loeliger *et al.*[29] Cyclialkylation occurred predominantly on the more activated, nonbrominated ring, however bis-cyclialkylated material was also obtained. The benzoic acid group was introduced using a palladium(0)-catalyzed aryl bromide–arylboronic acid coupling,[37] which is novel to the synthesis of aromatic retinoids and, unlike the Negishi-type aryl bromide couplings,[38] can readily be conducted under nonanhydrous conditions. Treatment of aryl bromide **19** with tetrakis(triphenylphosphine)-palladium(0) in 1,2-dimethoxyethane generated an arylpalladium species, which was coupled to 4-carbethoxyphenylboronic acid in the presence of aqueous $NaHCO_3$ as base to afford ethyl ester **20** in 91% yield. Base hydrolysis and acidification afforded benzoic acid **17**.

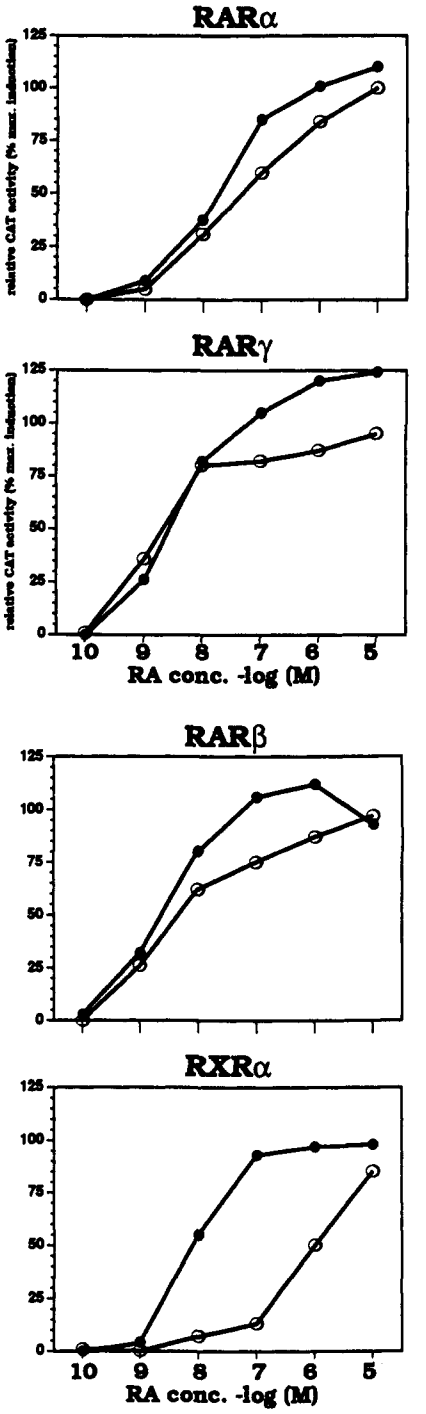


Figure 1. Dose–response curves of (*E*)-RA (○, **1**) and (9*Z*)-RA (●, **2**) for receptor activation. CV-1 cells were transiently transfected with the expression plasmid for RAR-α, RAR-β, RAR-γ, or RXR-α, the TREpal-*tk*-CAT reporter plasmid, and the β-galactosidase vector pCH110 as an internal standard. Cells were subsequently treated for 24 h with retinoids at the indicated concentrations. CAT activity was normalized to β-galactosidase activity and was expressed as the percent of transcriptional activation obtained for RAR-α at 10^{-5} M (*E*)-RA for each RAR and for RXR-α at 10^{-5} M (9*Z*)-RA after subtracting receptor basal activity. The average of three independent experiments is shown.

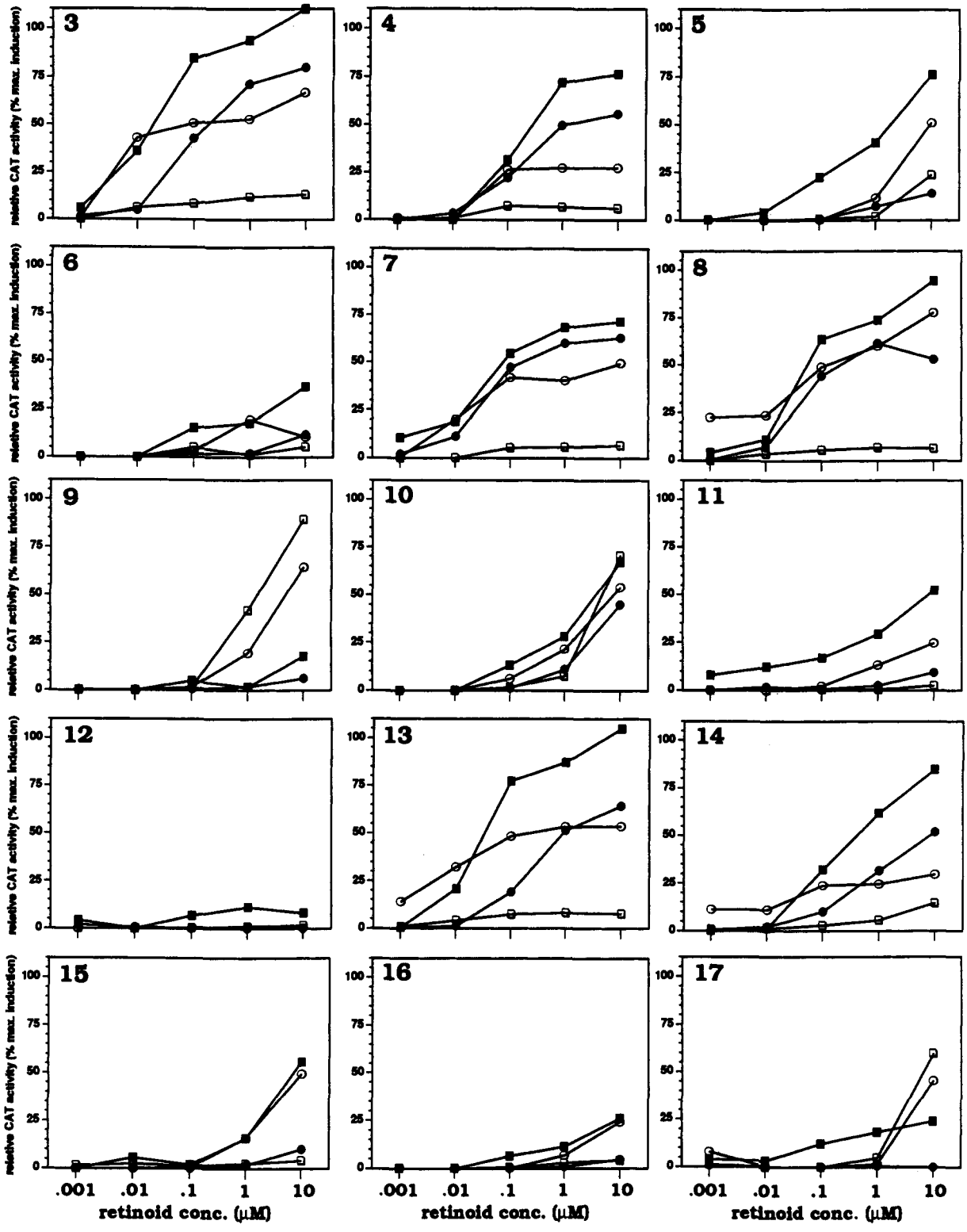


Figure 2. Dose–response curves of retinoids 3–17 for receptor activation. Cells were transiently transfected with the expression plasmid for RAR-α (●), RAR-β (○), RAR-γ (■), or RXR-α (□), as described in Figure 1.

Gene Transactivation and Structure–Activity Correlations. Retinoids 1 through 17 were assayed for their ability to induce gene activation in CV-1 cells transiently transfected with expression vectors for either RAR-α, β, or γ, or RXR-α, and the TREpal-*tk*-CAT reporter plasmid. TREpal is a retinoid response element that can be activated by RARs as well as by RXR homodimers[23,24] (Figures 1 and 2, and Table I). Retinoid activities were normalized after subtracting basal level activities by expressing the amount of transcriptional activation as the percent of the activation of RA at 10^{-5} M on the RARs and of (9*Z*)-RA at 10^{-5} M on RXR-α.[28] Consistent with previous publications,[23,24] we found that (9*Z*)-RA is a much more potent ligand for RXR than is (*E*)-RA, which showed no significant activation at 10^{-7} M (Figure 1). (9*Z*)-RA is also more potent than (*E*)-RA in activating the RARs.

The activity of these retinoids was assessed as a function of the lipophilicity of the β-cyclogeranylidene ring region of the molecule, the variation of the position of the methyl group on the olefinic bond corresponding to the 9-double

Table I. Activity of Retinoids 3–17 Compared to (*E*)-RA (1) and (9*Z*)-RA (2) in Activating RARs and RXR-α, Respectively, for Gene Transcriptional Activation in Transfected CV-1 Cells

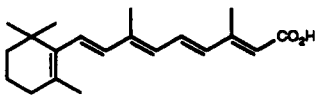
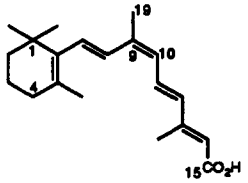
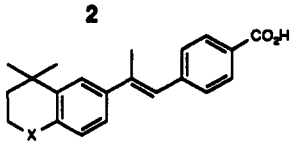
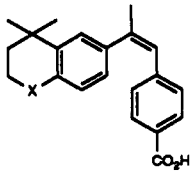
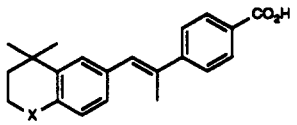
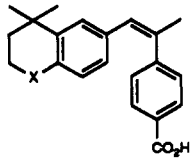
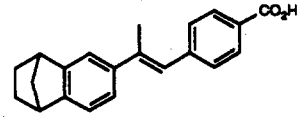
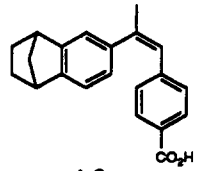
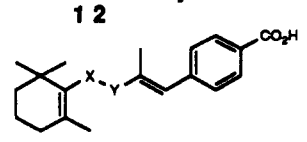
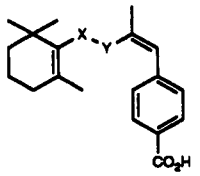
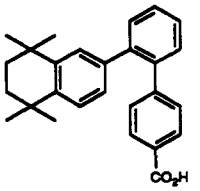
compd	retinoid activity (%)[a]				EC_{50} (nM)			
	RAR-α	RAR-β	RAR-γ	RXR-α	RAR-α[b]	RAR-β[b]	RAR-γ[b]	RXR-α[c]
								
1	100	97	98	86	27	12	8	430
								
2	110	86	127	100	45	22	12	13
								
3 X = CMe_2	80	69	112	13	85	6	24	nc[d]
4 X = S	56	29	76	6	27	15	30	nc
								
5 X = CMe_2	14	53	76	24	1000	2200	700	2700
6 X = S	12	11	38	5	2300	250	1100	nc
								
7 X = CMe_2	63	51	74	6	37	25	19	nc
8 X = S	53	79	96	6	45	40	47	nc
								
9 X = CMe_2	6	66	18	89	nc	1900	2700	1100
10 X = S	45	56	68	71	2100	1500	1500	2800
								
11	9	26	53	2	nc	1100	550	nc
								
12	0	0	8	2	nc	nc	70	nc
								
13 X, Y = (*E*)-HC=CH	64	55	107	8	240	5	35	nc
14 X, Y = $(CH_2)_2$	52	31	87	15	550	20	210	1500

Table I (Continued)

compd	retinoid activity (%)[a] RAR-α	RAR-β	RAR-γ	RXR-α	EC_{50} (nM) RAR-α[b]	RAR-β[b]	RAR-γ[b]	RXR-α[c]
								
15 X, Y = (*E*)-HC=CH	10	52	57	4	5000	1800	2000	nc
16 X, Y = $(CH_2)_2$	5	25	27	4	nc	2000	1300	nc
								
17	0	46	24	60	nc	3200	100	2800

[a] Relative retinoid activity for each RAR as percent of that of 10^{-5} M (*E*)-RA on RAR-α or for RXR-α as percent of that of 10^{-5} M (9*Z*)-RA. Retinoid molar concentration, determined graphically, at which activity was 50% that of maximum activity, providing that the activity was at least 10% of [b](*E*)-RA or [c](9*Z*)-RA. [d] EC_{50} values for retinoids having relative activity below 10% of that of (*E*)-RA or (9*Z*)-RA were not calculated.

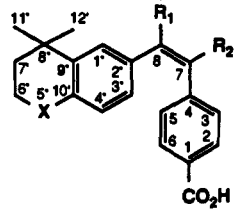
bond of RA, and the spatial relationship between the olefinic bond substituents. None of the retinoids was as active as (*E*)- or (9*Z*)-RA at activating the RARs. Biological activities reported for these retinoids that are higher than those of RA may be due to differences in pharmacokinetics or metabolism. Unlike the native ligands, in this aromatic series the *E* isomers were by far the most active. However, the *Z* isomers of the most potent (*E*)-retinoids did demonstrate some activity for RAR-β and RAR-γ at the higher concentrations, indicating that these *Z* isomers either had low affinity for the ligand-binding site of the RARs or that a small amount of bond isomerization to the *E* isomer did occur. Activation of endogenous CV-1 cell receptors that function comparably to the RARs also cannot be excluded.

In the *E* isomers, shifting the methyl group on the ethenyl bridge from the carbon adjacent to the tetrahydronaphthalene ring (**3**) to the carbon adjacent to the benzoic acid ring (**7**) decreased activity, however the converse occurred in the dihydrobenzothiopyranyl analogs (**4** and **8**). Decreasing lipophilic bulk in the region corresponding to the β-cyclogeranylidene ring of RA decreased transcriptional activation activity as demonstrated for both the dihydrobenzothiopyranyl and benzonorbornenyl analogs (**4** and **11**). The butadienylbenzoic acid **13** had activity comparable to that of **3** with RAR-β and RAR-γ but was less active with RAR-α. Saturation of the olefinic bond (**14**) corresponding to the 7-double bond of RA decreased activity by one-half at 10^{-7} M retinoid concentration. Retinoid **17** did not activate RAR-α, and only activated RAR-β at 10^{-5} M, where the response was 45% of that of (*E*)-RA. It was a weak activator of RAR-γ with a maximal response of 24% of that of (*E*)-RA at 10^{-5} M.

None of the *E* isomers was able to activate RXR-α. In fact, all could be considered essentially inactive, whereas (*E*)-RA had at least 20% of the activity of (9*Z*)-RA at 10^{-7} M and about 50% of the activity at 10^{-6} M. Isomerization of (*E*)-RA to (9*Z*)-RA also may have occurred under the assay conditions. *Z* isomers **5** and **6**, with a methyl group on the ethenyl bridge corresponding to the 19-methyl group of (9*Z*)-RA, were inactive. Only analogs **9** and **10** that have the methyl group shifted to the adjacent carbon of the ethenyl bridge, which corresponds to the 10-position of (9*Z*)-RA, displayed activity. Retinoid **17** had 60% of the activity of (9*Z*)-RA at 10^{-5} M but was inactive at lower concentrations. Considering the biological activity of **17**, it did not appear plausible that activity differences between **5** and **9** and between **6** and **10** were the result of changes in steric bulk caused by shifting of the methyl group to the adjacent olefinic carbon, but rather that the mutual orientation of the two aromatic rings contributed to the favorable interactions of **9** and **10** with RXR-α. Because these results were intriguing, we used a combination of experimental (NMR spectroscopy and X-ray crystallography) and theoretical methods to examine the conformations of retinoids **5** and **9** to establish why shifting a methyl group to the adjacent carbon would have such a profound effect on RXR transactivation activity. Determination of the preferred comformation is extremely important if structure–activity studies leading to the design of more effective analogs are to be guided by the hypothesis that the minimum-energy conformation of the ligand is initially recognized or bound by the receptor.

NMR Studies. Comparison of the ^1H NMR spectra of retinoids **5** and **6** with those of **9** and **10**, respectively, showed large differences in chemical shifts (Table II). Generally, the signals for the aromatic protons ($H_{1'}$, $H_{3'}$, and $H_{4'}$) on the tetrahydronaphthalene ring of **9**, as well as those on the dihydrobenzothiopyranyl ring of **10**, were shifted to higher fields than the corresponding protons of **5** and **6**. The aliphatic protons ($H_{11'}$ and $H_{12'}$ on the tetrahydronaphthalene ring methyl groups and $H_{7'}$ on the ring $C_{7'}$-methylene group) of **9** and **10** were more shielded relative to those of **5** and **6**. The signal for $H_{3'}$ of **10** appeared at 0.24 ppm higher field than that of **6**. This trend continued, but to a lesser degree (0.05 ppm), between **9** and **5**. In the benzoic acid region, the signals for H_2, H_3, H_5, and H_6 were shifted to lower fields in **9** and **10**, than in **5** and **6**. These results indicated the interactions

Table II. ^{1}H NMR Spectra of Retinoids 5, 6, 9, and 10



retinoid	substituents X	R_1	R_2	^{1}H NMR shift (ppm)[a] 5'-CMe_2	8'-CMe_2	H_8	$H_{7'}$	$H_{1'}$	$H_{3'}$	$H_{4'}$	H_2,H_6	H_3,H_5
5	CMe_2	Me	H	1.28	1.05		1.64	7.01	6.80	7.24	7.80	7.02
9	CMe_2	H	Me	1.20	0.96		1.58	6.79	6.75	7.07	8.05	7.32
6	S	Me	H		1.11	2.99	1.91	7.09	6.88	7.02	7.80	7.03
10	S	H	Me		1.01	2.95	1.84	6.86	6.64	6.83	7.98	7.27

[a] The chemical shifts of the 5'- and 8'-*gem*-dimethyl groups of retinoids 5 and 9 were confirmed by NOE spectroscopy.

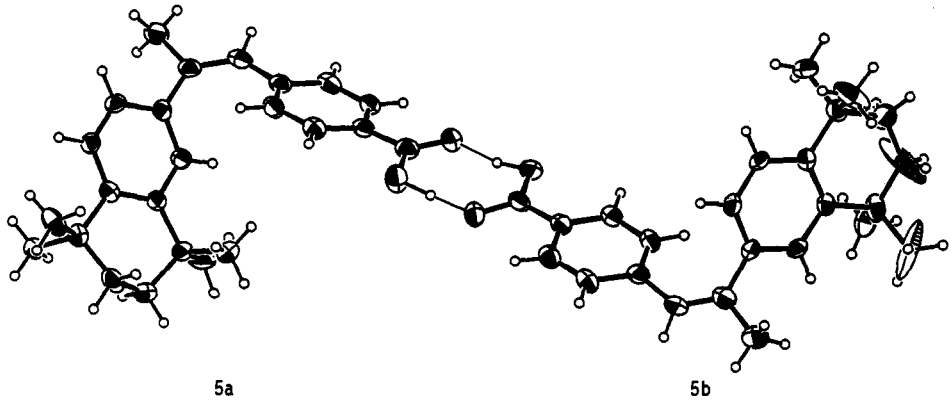


Figure 3. Solid-state conformations of the two crystallographically independent molecules (**5a** and **5b**) of **5** in the asymmetric crystal unit.

between the two aromatic rings of **5** and **6** were very different from those of **9** and **10**.

X-ray Crystallography. Examination of the solid-state conformation of **5** by single-crystal X-ray analysis revealed two rotational isomers (Figure 3), which were generated by rotation about the C2'-C8 bond, which is not a symmetry element of the molecule. The two rotamers **5a** and **5b** in the asymmetric unit constituted a hydrogen-bonded pair. In rotamer **5a**, the $H_{1'}$ proton on the tetrahydronaphthalene ring is directed toward the benzoic acid ring, whereas in rotamer **5b** the $H_{3'}$ proton is directed toward the benzoic acid ring. In contrast to retinoid **5**, only rotamer **9a**, in which the $H_{1'}$ proton is directed toward the benzoic acid ring, was observed in the crystal structure of retinoid **9** (Figure 4).

Computational Analysis. It was possible to refine the model of the RXR binding site using (9*Z*)-RA as the template.[39] (9*Z*)-RA having five rotatable bonds is conformationally flexible, however rotation is somewhat restricted by conjugation. Approximately 30 low-energy conformers of (9*Z*)-RA within 2 kcal/mol of the global energy minima were generated by the use of the RANDOMSEARCH command in SYBYL 5.5 (Tripos Associates, St. Louis, MO). Two groups of low-energy conformers were identified, and both are energetically acceptable. Conformer **2A** has a s-transoid 11,13-double bond system, and a distance of 9.6 Å from the C4 of the β-cyclogeranylidene ring to the C15 of the carboxylic acid terminus, whereas conformer **2B** has a s-cisoid 11,13-double bond system and a C4–C15 distance of 7.7 Å. Some flexibility of these precise conformations is possible (<10° for each rotatable bond). The selection of conformer **2A** as the functionally active conformer was based on comparison

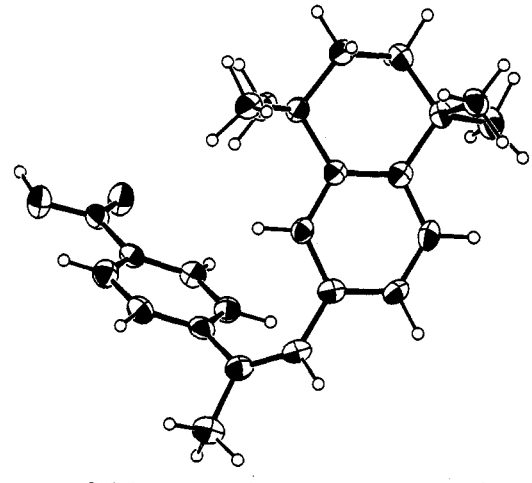


Figure 4. Solid-state conformation (**9a**) of **9** determined crystallographically.

with the conformers of the RXR-α-specific retinoids 2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-2-(4-carboxyphenyl)-1,3-dithiane (**21**), 4-[1-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-napthalenyl)-2-methyl-1-propenyl]benzoic acid (**22**), and 2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-2-(4-carboxyphenyl)-1,3-dioxolane (**23**) (Figure 5)][39].

Conformational analyses of **5** and **9**, using SYBYL 5.5 on the X-ray crystallographic structures, produced two distinct conformations for **5** and **9**, respectively, within 2 kcal/mol of the "global" minimum. For **5**, rotamer **5A** had torsion angles $\alpha_1 = -49°$ and $\alpha_2 = -45°$ (*i.e.*, α_1 is defined by the atoms C1'–C2'–C8–C7, and α_2 by C8–C7–C4–C3), whereas rotamer **5B** had the C2'–C8 bond twisted 180°

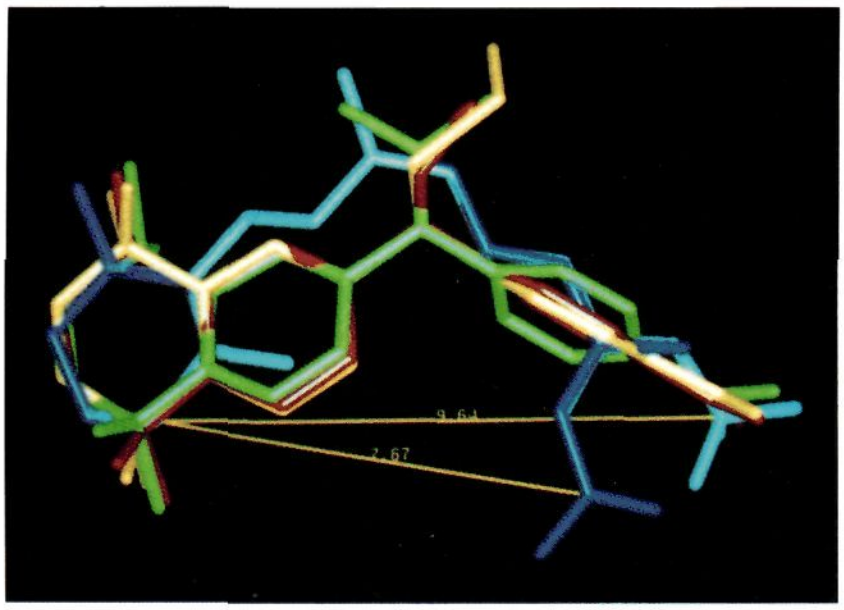


Figure 5. Low-energy minima of (9*Z*)-RA [**2a** (cyan) and **2b** (blue)] compared to three RXR-α-selective retinoids 2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-2-(4-carboxyphenyl)-1,3-dithiane (**21**, yellow), 4-[1-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-2-methyl-1-propenyl]benzoic acid (**22**, green), and 2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-2-(4-carboxyphenyl)-1,3-dioxolane (**23**, red) with the use of the FIT option in SYBYL 5.5. Hydrogen atoms were omitted for clarity.

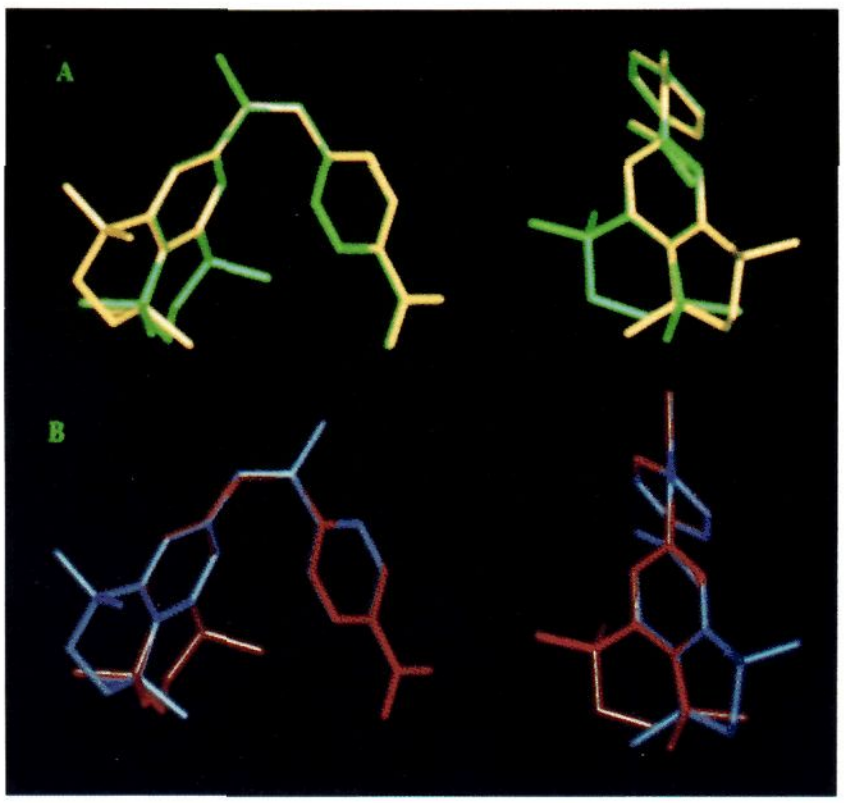


Figure 6. (A) Low-energy minima of (*Z*)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)propenyl]benzoic acid (**5**), rotamer **5A** (green), and rotamer **5B** (yellow); (B) low-energy minima of (*Z*)-4-[1-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)propen-2-yl]benzoic acid (**9**), rotamer **9A** (red) and rotamer **9B** (purple).

relative to that of the rotamer **5A**, with $\alpha_1 = 130°$ and α_2 $-44°$ (Figure 6A). Steric interaction of the methyl group on C8 of the double bond with the ring aromatic protons caused α_1 to be larger than α_2 in both rotamers of **5**. Rotamers **9A** and **9B** of **9** had torsion angles $\alpha_1 = -45°$ and $\alpha_2 = -50°$, and $\alpha_1 = 134°$ and $\alpha_2 = -48°$, respectively (Figure 6B). Computational analysis showed that rotamers **5A** and **9A** having the $H_{1'}$ proton oriented toward the benzoic acid ring were 2 kcal/mol lower in energy than rotamers **5B** and **9B**.

Discussion

To establish the relationship between retinoid structure and activity in the transfection assay, it was necessary to determine which of the two very different candidate conformations (**5A** or **5B** and **9A** or **9B**) of **5** and **9**, respectively, was the conformation that activated RXR-α. The rotamer **9A**, corresponding to conformer **9a** determined by X-ray crystallography, in which $H_{1'}$ is directed toward the benzoic acid ring, was selected as the "active conformation", because the ^{1}H NMR spectrum indicated that the $H_{1'}$ and aliphatic protons of **9** were shifted to higher field than those of **6** as a result of being strongly shielded by the benzoic acid ring. The ^{1}H NMR spectrum also indicated that the conformation found in the crystal was largely maintained in solution. Although two conformations (**5a** and **5b**) were observed on X-ray crystallography of **5** and although computational analysis of **5** indicated that of the corresponding conformers **5A** and **5B** the former was 2 kcal/mol lower in energy, rotamer **5B** having $H_{3'}$ directed toward the benzoic acid ring was selected as the "active" conformation and was considered to be the predominant conformer in solution based on comparisons of the ^{1}H NMR spectra of **5** and **9**.

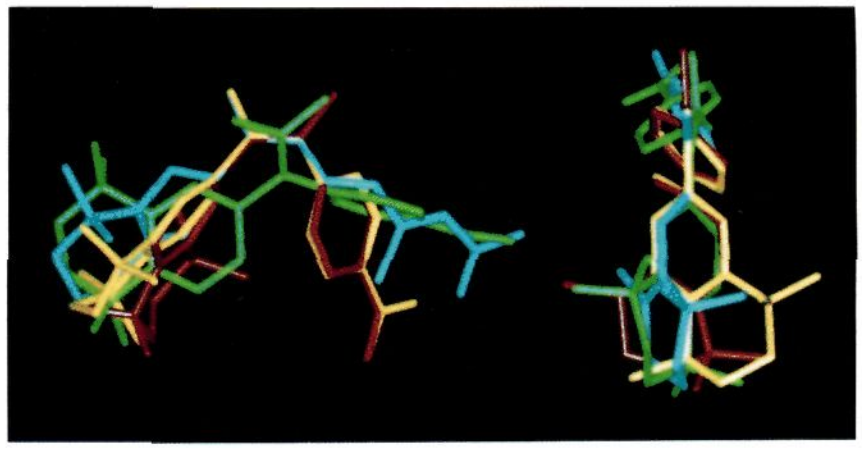


Figure 7. The "active" conformations of **5** and **9**, **5B** (yellow) and **9A** (red), respectively, compared to **2A** (cyan) and **22** (green) using the FIT option in SYBYL 5.5.

Two retinoids (9*Z*)-RA and 4-[1-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-2-methyl-1-propenyl]-benzoic acid (**22**) were selected as the templates for superimposition with retinoids **5** and **9**. (9*Z*)-RA is the natural ligand for RXR-α, but is not selective between RAR and RXR, whereas the conformationally restricted retinoid **22** is the most active and specific ligand for RXR-α reported[39] thus far. Retinoids **22** and **2A** were superimposed using C8′, C5′, C9, CO_2H of **22** and C1, C4, C19, C15 of (9*Z*)-RA as the fitting points to provide information about the RXR pharmacophoric requirements. Conformers **5B**, **9A**, and **2A** of retinoids **5** and **9** and (9*Z*)-RA, respectively, were superimposed using four atoms of the central double bond as the fitting points [C2′–C8–C7–C4 of **5** and **9** and C8–C9–C10–C11 of (9*Z*)-RA] with the FIT command in SYBYL 5.5 (Figure 7). As can be seen in Figure 7, shifting the methyl group from the C8 to C7 carbon changes the conformational relationship between the tetrahydronaphthalene ring and the aromatic ring, which dramatically affects biological activity.

Our model suggests that aliphatic interaction may play an important role in retinoid activation of RXR-α. Retinoids **9** and **10** having aliphatic rings that occupy spatial positions similar to those of (9*Z*)-RA and **22** activated RXR-α. The low RXR activity of **5** may therefore be attributed to the unfavorable steric interaction of its predominant conformer **5B** with RXR-α. Computational analysis of **17** provided a global energy-minimized structure, the orientation of whose aromatic rings and C5′-CO_2H distance resemble those of **9A**. The structural

similarity is consistent with the similar RXR-activation activities of 17 and 9. Optimal activation of RXR-α was found in those retinoids having a distance of 9.6–10 Å from C5′ of the tetrahydronaphthalene ring to the carboxylic acid carbon.[39] Therefore, analogs of retinoids 9, 10, and 17 having a greater C5′-CO_2H interatomic distance may have enhanced activity.

In conclusion, improving our knowledge of retinoid structure–activity relationships will allow the design of receptor subtype-selective retinoids that should have fewer side effects in therapeutic applications. The (9Z)-RA analogs described here (9 and 10) represent a promising step in this direction. These retinoids show higher activity with RXR than with the RARs, thus separating the activity of RXR homodimer from that of RAR:RXR heterodimer response pathways.

Experimental Section

General Methods. Solvents were used as purchased, unless otherwise stated. All glassware, syringes, magnetic stirrers, and needles were dried (100 °C) and allowed to cool in a dessicator before use. Merck silica gel-60 was used for preparative chromatography. Melting points were uncorrected. IR solution spectra were recorded on either a Perkin Elmer 710B IR or a 1600 FTIR spectrophotometer. UV spectra were taken using a Perkin Elmer UV/VIS Lambda 2 spectrophotometer. NMR spectra were recorded on Gemini 300 (300 MHz) and XL 400 Varian (400 MHz) spectrometers. Mass spectral analyses were conducted on a LKB Model 9000 combination gas chromatograph–mass spectrometer.

Retinoids. Retinoic acid (1) was obtained from Eastman and recrystallized (MeOH). (9Z)-Retinoic acid (2) was prepared by Corey oxidation (NaCN, MnO_2/HOAc/MeOH)[40] of (9Z)-retinal, followed by hydrolysis of the methyl ester.[41] (E)- and (Z)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)propen-1-yl]benzoic acids (3, Ro13-7410, and 5) were prepared by the modification[34] of the procedure of Loeliger *et al.*[29] (E)- and (Z)-6-[1-(4-carboxyphenyl)propen-2-yl]-3,4-dihydro-4,4-dimethyl-2H-1-benzothiopyrans (4 and 6) were prepared by a Wittig reaction between 1-(3,4-dihydro-4,4-dimethyl-2H-1-benzothiopyran-6-yl)ethyltriphenylphosphonium bromide and 4-carbethoxybenzaldehyde, followed by hydrolysis.[33] (E)- and (Z)-4-[1-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)propen-2-yl]benzoic acids (7 and 9) were prepared by a Wittig reaction between (5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-6-naphthalenyl)-methyltriphenylphosphonium bromide and 4-cyanoacetophenone, followed by hydrolysis.[31] (E)- and (Z)-6-[2-(4-carboxyphenyl)propen-1-yl]-3,4-dihydro-4,4-dimethyl-2H-1-benzothiopyrans (8 and 10) were prepared by a Wittig reaction between (3,4-dihydro-4,4-dimethyl-2H-1-benzothiopyran-6-yl)-methyltriphenylphosphonium bromide and 4-carbethoxyacetophenone, followed by hydrolysis.[31] (E)- and (Z)-4-[2-(1,4-methano-1,2,3,4-tetrahydronaphthalen-6-yl)propen-1-yl]-benzoic acids (11 and 12) were prepared by a Horner–Emmons reaction between 6-acetyl-1,4-methano-1,2,3,4-tetrahydronaphthalene and diethyl carbethoxybenzylphosphonate and hydrolysis.[33] (E)- and (1Z,3E)-4-[2-methyl-4-(2,6,6-trimethylcyclohexen-1-yl)buta-1,3-dien-1-yl]benzoic acids (13 and 15) were obtained by a Wittig reaction between β-ionyltriphenylphosphonium bromide and 4-carbethoxybenzaldehyde, followed by hydrolysis.[42] (E)- and (Z)-4-[2-methyl-4-(2,6,6-trimethylcyclohexenyl)but-1-en-1-yl]benzoic acids (14 and 16) were derived from Horner–Wadsworth–Emmons reaction of 7,8-dihydro-β-ionone and diethyl 4-carbethoxybenzylphosphonate, followed by hydrolysis.[33] Retinoid isomers were chromatographically purified as their esters before hydrolysis to the acids. All compounds were fully characterized and found to be greater than 99% isomerically pure by HPLC analysis.

2-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)bromobenzene (19). To a solution of 2-bromobiphenyl (18) (1.0 g, 4.2 mmol) in 7 mL of 1,2-dichloroethane under argon was added $AlCl_3$ (0.07 g, 0.51 mmol) with magnetic stirring. The suspension was cooled to −20 °C before 2,5-dichloro-2,5-dimethylhexane (0.80 g, 4.40 mmol) in 5 mL of 1,2-dichloroethane was introduced. The cooling bath was removed, and the dark-red mixture was allowed to slowly warm until the temperature reached −5 °C, when it was poured onto cracked ice and extracted with methylene chloride. The organic layer was washed twice with 40-mL portions of H_2O and dried ($MgSO_4$). After removal of the solvent, a colorless, viscous liquid resulted. The product was isolated by chromatography (50% hexanes/chloroform), followed by recrystallization from hot hexanes, to give 0.72 g (32%) of 19 as a white microcrystalline solid: mp 99–100 °C; IR (KBr) 2960, 2940, 1460, 1020, 830, 760 cm^{-1}; 300-MHz ^1H NMR ($CDCl_3$) δ 1.31 and 1.33 (2 s, 12, 5′,8′-CMe_2), 1.71 (s, 4, 6′,7′-CH_2), 7.20–7.22 (m, 2, Naph-H), 7.35–7.45 (m, 4, Naph-H, Ar-H), 7.72 (d, J = 8 Hz, 1, Ar-H ortho to Br); MS 344 (M^+ + 2, 40), 342 (M^+, 40), 329 (100), 327 (100), and small peaks at m/e 422 and m/e 420 indicating doublely brominated material. Anal. ($C_{20}H_{23}Br$) C, H, Br.

Ethyl 2′-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-4-biphenylcarboxylate (20). To a solution of 19 (0.17 g, 0.50 mmol) in 1,2-dimethoxyethane (10 mL) under argon was added tetrakis(triphenylphosphine)palladium (40 mg, 7 mol %), and the suspension was stirred for 15 min. Then, 4-carbethoxyphenylboronic acid (0.10 g, 0.55 mmol) dissolved in 1.0 mL of EtOH was added, followed by 3 mL of saturated aqueous $NaHCO_3$. The mixture was heated at reflux under argon for 2 h, then cooled to ambient temperature, and extracted with dichloromethane (20 mL). The extract was washed twice with brine and dried ($MgSO_4$). TLC (silica gel, 40% CH_2Cl_2/hexanes) showed a major spot at R_f 0.5. The major component was isolated by chromatography (hexanes), followed by triturations from cold hexanes, and crystallization from hot hexanes, to yield 0.19 g (91%) of 20 as white crystals: mp 104–105 °C; IR ($CHCl_3$) 2960, 1689, 1609, 1473, 1417, 1283, 1180, 754 cm^{-1}; 300-MHz ^1H NMR ($CDCl_3$) δ 0.91 (s, 6, 5′-CH_3), 1.25 (s, 6, 8′-CH_3), 1.37 (t, J = 7.1 Hz, 3, CH_2CH_3), 1.53–1.66 (m, 4, 6′,7′-CH_2), 4.37 (q, J = 7.1 Hz, 2, OCH_2), 6.85 (d, J = 1.9 Hz, 1, 1′-Naph-H), 7.04 (dd, J = 8.3 Hz, J = 1.9 Hz, 1, 3′-Naph-H), 7.19 (d, J = 8 Hz, 2, Ar-H meta to CO_2Et), 7.22 (d, J = 8.3 Hz, 1, 4′-Naph-H), 7.39–7.49 (m, 4, Ar-H), 7.89 (d, J = 8 Hz, 2, Ar-H ortho to CO_2Et); MS 412 (M^+, 78), 397 (100). Anal. ($C_{29}H_{32}O_2$) C, H.

2′-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-4-biphenylcarboxylic Acid (17). A suspension of 20 (0.20 g, 0.48 mmol) in MeOH (7 mL) was degassed under Ar and then heated to 65 °C, while a solution of KOH (8 mmol) in H_2O (1 mL) and MeOH (1.3 mL) was added dropwise over a period of 5 min. The mixture was stirred under argon for 3 h at 85 °C and then filtered while hot. The filtrate was cooled to 0 °C and diluted with 2 N HCl (15 mL). The white precipitate was washed with a small amount of CH_2Cl_2 and dried. The crude product was dissolved in hot dioxane and cooled to yield 0.15 g (82%) of 17 as a microcrystalline, white solid: mp 218–219 °C; IR (KBr) 2960, 2890, 1690, 1610, 1410, 1275, 755 cm^{-1}; 400-MHz ^1H NMR (DMSO-d_6) δ 0.82 (s, 6, 5′-CH_3), 1.21 (s, 6, 8′-CH_3), 1.55 (t, J = 1.3 Hz, 4, 6′,7′-CH_2), 6.74 (d, J = 2.0 Hz, 1, 1′-Naph-H), 7.12 (dd, J = 8.7 Hz, J = 1.9 Hz, 1, 3′-Naph-H), 7.21 (d, J = 8.2 Hz, 2, Ar-H meta to CO_2H), 7.30 (d, J = 8.2 Hz, 1, 4′-Naph-H), 7.40–7.50 (m, 4, Ar-H), 7.80 (d, J = 8.2 Hz, 2, Ar-H ortho to CO_2H), 12.8 (broad, 1, CO_2H); UV (EtOH) λ_{max} 264 nm (e 1.6 × 10^4), shoulder at 286 nm. Anal. ($C_{27}H_{28}O_2$) C, H.

Computational Analyses. Molecular modeling was performed using the SYBYL 5.5 software package (Tripos Associates, St. Louis, MO) on an IRIS 4D workstation. Retinoids 21, 22, 23, and (9Z)-RA[39] were built within SYBYL, and bond angles and lengths were optimized with MAXIMIN2 program. The atomic coordinates of 5 and 9, taken from X-ray data, were optimized using the MAXIMIN2. Atomic point charges were computed by using the Gasteiger–Hückel method. Conformational analyses were performed by employing the RANDOMSEARCH option within SYBYL. The C2′-C8 and C7-C4 bonds of 5 and 9, respectively, were defined as the rotatable bonds and the default parameters were used. The SYBYL RANDOMSEARCH function was used to locate the various energy minima available to each molecule by randomly perturbing torsions, minimizing, and eliminating duplicates. Low-energy conformers were overlapped

with the use of FIT command within SYBYL. Hydrogen atoms were included during the optimization process but omitted for display.

Transient Transfection of CV-1 cells. CV-1 cells (7×10^4) grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum were seeded in a volume of 1 mL/well in 24-well tissue-culture plates (Costar, Cambridge, MA). The fetal calf serum had been pretreated with charcoal (12 mg/mL of serum) to remove retinoids. The following day cells were transiently transfected using a calcium phosphate precipitation procedure as described previously.[43,44] Briefly, each well was incubated overnight with a calcium phosphate precipitate containing 100 ng of TREpal-*tk*-CAT reporter plasmid (a RA-inducible gene), 50 ng of pECE expression plasmid for either RAR-α, -β, or -γ or RXR-α, 100 ng of β-galactosidase expression vector (pCH110, Pharmacia, Piscataway, NJ), and 750 ng of carrier DNA (pBluescript, Strategene, La Jolla, CA) to reach a total of 1 μg of DNA. After removal of the precipitate, retinoids were applied for 24 h. Chloramphenicol acetyl transferase (CAT) activity and β-galactosidase activity were assayed as described.[43] CAT activity was normalized to β-galactosidase activity to correct for variations in transfection and harvesting efficiencies.

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Supplementary Material Available: Tables of X-ray data (fractional coordinates, anisotropic thermal parameters, and bond distances and angles) for compounds **5** and **9** (61 pages); tables of observed and calculated structure factors for **5** and **9** (15 pages). Ordering information is given on any current masthead page.

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