Synthesis and Structure–Activity Relationships of Novel Retinoid X Receptor-Selective Retinoids

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Two series of potent retinoid X receptor (RXR)-selective compounds were designed and synthesized based upon recent observation that (*E*)-4-[2-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthalenyl)-1-propenyl]benzoic acid (TTNPB) binds and transactivates only the retinoic acid receptor (RAR) subtypes whereas (*E*)-4-[2-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthalenyl)-1-propenyl]benzoic acid (3-methyl TTNPB) binds and transactivates both the RAR and RXR subfamilies. Addition of functional groups such as methyl, chloro, bromo, or ethyl to the 3-position of the tetrahydronaphthalene moiety of 4-[(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic acid (**5a**) and 4-[1-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoic acid (**6a**) results in compounds which elicit potent and selective activation of the RXR class. Such RXR-selective compounds offer pharmacological tools for elucidating the biological role of the individual retinoid receptors with which they interact. Activation profiles in cotransfection and competitive binding assays as well as molecular modeling calculations demonstrate critical structural determinants that confer selectivity for members of the RXR subfamily. The most potent compound of these series, 4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoic acid (**6b**), is the first RXR-selective retinoid (designated as LGD1069) to enter clinical trials for cancer indications.

Introduction

Retinoids play important roles in a variety of biological processes including mediation of cell growth and differentiation. The ability of retinoids to modulate proliferation and differentiation of both normal and malignant cells *in vivo* and *in vitro* has significant implications for the treatment of dermatological diseases, such as psoriasis,[1] and cancer including chemotherapeutic and chemopreventative applications.[2,3] Accordingly, the naturally occurring retinoids, *all-trans*-retinoic acid (ATRA) and 13-*cis*-retinoic acid (13-*cis*-RA), and synthetic etretinate (Chart 1) are currently marketed for the treatment of dermatological diseases, and several are being evaluated experimentally for oncological applications. However, widespread use of these agents has been limited due to the observation of numerous undesirable side effects upon short and long term administration. Such side effects of retinoids may arise from their ability to activate multiple retinoid receptors in a wide variety of target tissues. Thus, identification and development of novel, receptor-selective retinoids are critical to further elucidate the molecular mechanisms underlying retinoid transcriptional activity as well as to identify novel compounds having potential for an improved therapeutic index.

The retinoid receptors, members of the superfamily of intracellular receptors (IRs), have been classified into two subfamilies, the retinoic acid receptors (RARs) and the retinoid X receptors (RXRs), which function as ligand-dependent transcription factors.[4,5] The classification of the subfamilies is based primarily on differences in amino acid structure, responsiveness to different naturally occurring and synthetic retinoids, and the ability to modulate expression of different target genes. Each RAR and RXR subfamily is further made up of three distinct isoforms designated RARα,β,γ and RXRα,β,γ.

Although the biological and toxicological properties of RAR-selective compounds such as (*E*)-4-[2-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthalenyl)-1-propenyl]benzoic acid (TTNPB) and ATRA have been extensively studied,[6,7] little is known about the biological properties of RXR-active compounds. This is largely due to a paucity of RXR-selective ligands, which have only recently been described.[8,9]

Recently, we reported that addition of a methyl group to the 3-position of TTNPB (Chart 2) significantly altered the transcriptional activation profile of this compound.[10] Both TTNPB and 3-methyl-TTNPB activate the RARα,β,γ subtypes in a cotransfection assay with EC_{50} values ranging from 1 to 30 nM for TTNPB and 180 to 340 nM for 3-methyl-TTNPB. However, TTNPB does not activate the RXRs (EC_{50} values >10 000 nM). In contrast, 3-methyl-TTNPB activates the RXRs with EC_{50} values in the range of 1175–1500 nM. Similarly, the 3-ethyl and 3-isopropyl derivatives exhibit increased activity at the RXRs and decreased activity at the RARs. These data indicate that introduction of an alkyl substituent in the 3-position of TTNPB results in an enhancement in the affinity for RXRs and a decrease in the affinity for RARs.

We have extended the above observation to identify two series of 3-functionalized retinoids that exhibit increased potency and selectivity for the RXR subclass of receptors. The synthesis and structure–activity

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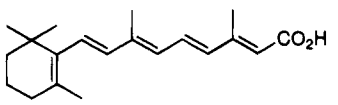
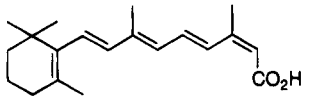
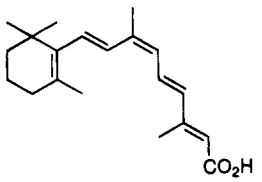
Chart 1



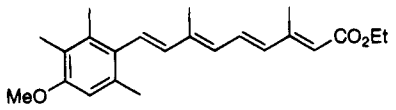
ATRA



13-*cis* RA



9-*cis* RA



Etretinate

Scheme 1

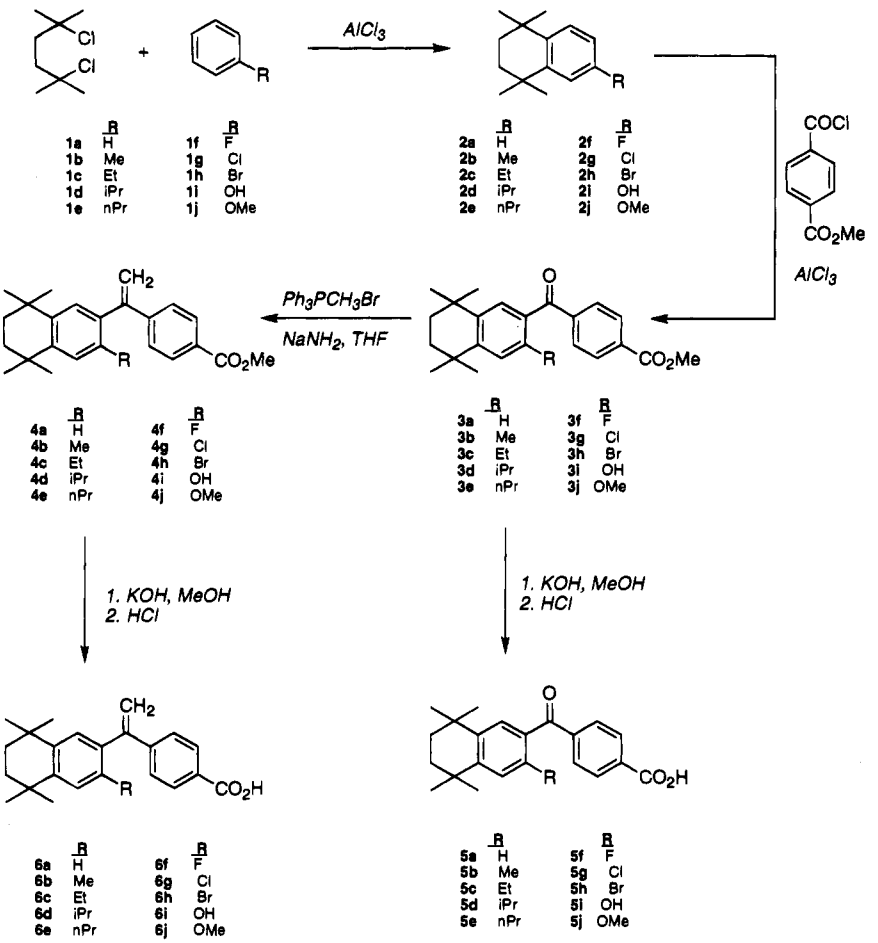
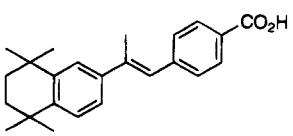
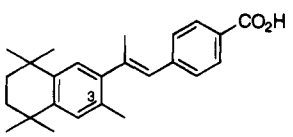


Chart 2



TTNPB



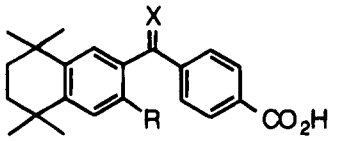
3-Me-TTNPB

relationships of these highly potent, receptor-selective, 3-substituted tetrahydronaphthyl derivatives are described herein. The two series of compounds, **5a–j** and **6a–j**, depicted in Scheme 1, were evaluated for their ability to regulate gene expression and bind to retinoid receptors using a receptor/reporter cotransfection assay system[11–13] in CV-1 cells and a competitive binding assay with [^{3}H]-9-*cis*-retinoic acid[10,14] and [^{3}H]ATRA as radioligands.

Chemistry

The 3-substituted tetrahydronaphthylbenzoic acids highlighted in Tables 1 and 2 were synthesized as outlined in Scheme 1. Compounds **5a–j** were prepared in three steps via Friedel–Crafts alkylation of the appropriate substituted benzene (**1a–j**) with 2,5-dichloro-2,5-dimethylhexane to give 1,2,3,4-tetrahydronaphthalenes **2a–j** followed by acylation with monomethyl-terephthalic acid chloride to provide methyl esters **3a–j**. Hydrolyses of **3a–j** in refluxing methanolic KOH followed by acidification with HCl gave the ketones **5a–j**. Condensation of the ketones **3a–j** with methyltriphenylphosphonium bromide–sodium amide in tetrahydrofuran afforded the corresponding olefins **4a–j**, which upon hydrolysis in methanolic KOH followed by acidification with HCl gave analogs **6a–j**. Synthesis of the bromo derivative **3h** and the methoxy derivative **3j**

Table 1. Cotransfection Data for Synthetic Retinoids in CV-1 Cells



compd no.	R	X	EC50 (nM)[a] RARα	RARβ	RARγ	RXRα	RXRβ	RXRγ
5a	H	O	>10 000	1388 ± 464	2043 ± 5	2971 ± 49	937	2836 ± 156
5b	Me	O	>10 000	>10 000	>10 000	379 ± 43	213 ± 81	246 ± 29
5c	Et	O	>10 000	>10 000	>10 000	514 ± 64	810 ± 422	384 ± 47
5d	iPr	O	>10 000	>10 000	>10 000	381 ± 16	313	357 ± 39
5e	nPr	O	>10 000	>10 000	>10 000	>10 000	>10 000	>10 000
5f	F	O	2856	395	1016	1820	1976	2246
5g	Cl	O	>10 000	>10 000	>10 000	294 ± 25	262	225 ± 17
5h	Br	O	>10 000	>10 000	>10 000	332	120	261
5i	OH	O	>10 000	>10 000	>10 000	>10 000	>10 000	>10 000
5j	OMe	O	>10 000	>10 000	>10 000	2019	2200	2191
6a	H	CH_2	>10 000	304 ± 70	266 ± 9	409 ± 3	486 ± 106	404 ± 36
6b	Me	CH_2	>10 000	>10 000	>10 000	33 ± 2	24 ± 4	25 ± 2
6c	Et	CH_2	>10 000	>10 000	>10 000	115	145	136
6d	iPr	CH_2	>10 000	>10 000	>10 000	207	289	227
6e	nPr	CH_2	>10 000	>10 000	>10 000	>10 000	>10 000	>10 000
6f	F	CH_2	>10 000	337	397	197	189	383
6g	Cl	CH_2	>10 000	436 ± 145	1446 ± 69	52 ± 10	33 ± 1	36 ± 13
6h	Br	CH_2	>10 000	>10 000	>10 000	51 ± 6	59 ± 11	43 ± 5
6i	OH	CH_2	>10 000	2238	>10 000	455	202	558
6j	OMe	CH_2	>10 000	>10 000	>10 000	83	198	230

[a] EC_{50} values were determined from full dose–response curves ranging from 10^{-12} to 10^{-5} M. Retinoid activity was normalized relative to that of ATRA and is expressed as potency (EC_{50}), which is the concentration of retinoid required to produce 50% of the maximal observed response. 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, approximately 15% of the mean values.

using CH_2Cl_2 as solvent for the Friedel–Crafts acylation resulted in loss of the bromine and cleavage of the methyl ether, respectively. However, when CH_2Cl_2 was replaced with hexane as the solvent, the reactions proceeded smoothly with no loss of bromine or hydrolysis of the methyl ether. Compound **5a** was synthesized as described previously.[15]

Biological Studies

The biological properties of the above retinoids were characterized using a cotransfection assay to examine their ability to interact individually with each of the six retinoid receptors and activate gene expression, as well as using a competitive binding assay to measure ligand-binding affinities at each receptor subtype.[11-13] The cotransfection assay can detect functional effects on gene expression of small molecules which interact with members of the IR superfamily. Two genes are introduced into cells; the IR under control of a promoter that synthesizes large quantities of the protein and a reporter molecule which is under the control of a hormone response element recognized by the IR being studied. In this case, the firefly luciferase gene is utilized as the reporter because it can readily be assayed and its catalytic activity directly reflects the amount of enzyme present. The ligand-binding assay is useful to characterize the direct interaction of the IRs with the compound being tested. A baculovirus expression system was employed which allows overexpression of the specific retinoid receptor of interest. This overexpression has been shown to result in full length functional receptors for all of the RAR and RXR subtypes.[14]

On the basis of the findings in our earlier studies with TTNPB and 3-Me-TTNPB,[10] which showed a shift of biological activity from RAR to RXR upon addition of the methyl group to the 3-position of TTNPB, we examined 3-functionalized retinoid analogs of 4-[(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic acid (**5a**) and 4-[1-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoic acid (**6a**). Cotransfection studies demonstrate that compound **5a** is weakly active (EC_{50} = 937–2971 nM) at RARβ,γ and RXRα,β,γ (Table 1). Substitution of a methylene group for the ketone (analog **6a**) demonstrated an increase in potency (EC_{50} values of 304 and 266 nM, respectively, for RARβ,γ, and 409, 486, and 404 nM, respectively, for RXRα,β,γ). Compounds **5a** and **6a** did not exhibit receptor subtype selectivity for either RARs or RXRs. In contrast, addition of a methyl group to the 3-positions of **5a** and **6a** resulted in a dramatic shift in biological activity from weakly RAR- and RXR-active compounds to potent, RXR-selective derivatives. For example, the EC_{50} values for the 3-methyl derivative **5b** were >10 000 nM for RARα,β,γ and 379, 213, and 246 nM, respectively, for RXRα,β,γ. Similarly, the EC_{50} values for the methylene derivative **6b** were >10 000 nM for RARα,β,γ and 33, 24, and 25 nM, respectively, for RXRα,β,γ.

These findings can be more clearly depicted in graphical form of the cotransfection data (Figures 1). The cotransfection data of the 3-methyl derivative **5b** (Figure 1, right) are shown in comparison to its parent **5a** (Figure 1, left). All six retinoid receptors are represented for each of the two analogs (as observed in the two figures, the background activation of RXRβ is consistently 20% higher than for the other five receptors). Figure 1, left, shows that **5a** is weakly active at micromolar concentrations for RARβ,γ and RXRα,β,γ, while **5b** is inactive at RARα,β,γ but is a potent

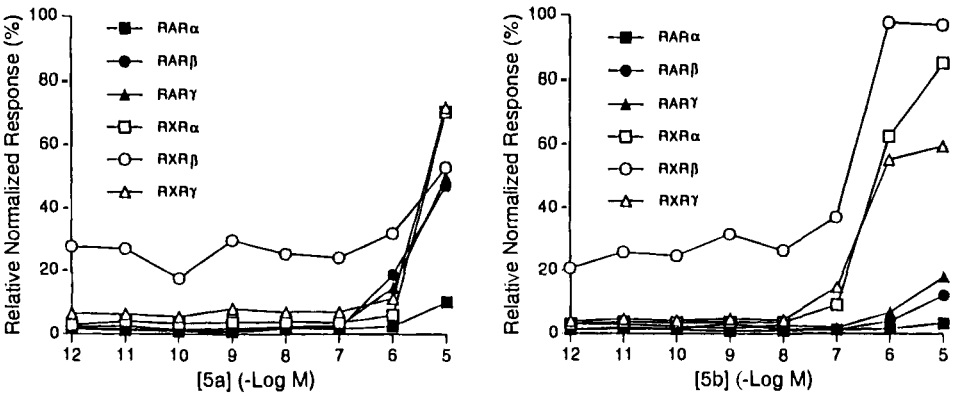
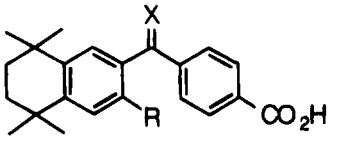


Figure 1. Transactivation dose–response curves for RARα,β,γ and RXRα,β,γ for **5a** (left) and **5b** (right). Retinoid activity was normalized relative to that of ATRA and is expressed as a percent of ATRA control.

Table 2. Competitive Binding Data for Synthetic Retinoids in Baculovirus-Expressed Retinoid Receptor Isoforms



			K_d (nM)[a]					
compd no.	R	X	RARα	RARβ	RARγ	RXRα	RXRβ	RXRγ
5a	H	O	>1000	>1000	>1000	>1000	>1000	>1000
5b	Me	O	>1000	>1000	>1000	138 ± 8	191 ± 45	299 ± 75
5c	Et	O	>1000	>1000	>1000	195 ± 11	232 ± 66	424 ± 67
5d	iPr	O	>1000	>1000	>1000	113 ± 13	155 ± 39	309 ± 60
5e	nPr	O	>1000	>1000	>1000	269 ± 52	371 ± 124	704 ± 151
5f	F	O	>1000	>1000	>1000	706 ± 148	902 ± 98	911 ± 89
5g	Cl	O	>1000	>1000	>1000	130 ± 37	110 ± 20	135 ± 31
5h	Br	O	>1000	>1000	>1000	105 ± 7	150 ± 18	150 ± 10
5i	OH	O	>1000	>1000	>1000	818 ± 182	>1000	>1000
5j	OMe	O	>1000	>1000	>1000	>1000	>1000	>1000
6a	H	CH_2	944 ± 56	909 ± 91	887 ± 114	150 ± 35	199 ± 48	290 ± 70
6b	Me	CH_2	>1000	>1000	>1000	14 ± 3	21 ± 4	29 ± 7
6c	Et	CH_2	>1000	>1000	>1000	31 ± 8	44 ± 2	59 ± 15
6d	iPr	CH_2	>1000	>1000	>1000	55 ± 12	75 ± 20	142 ± 70
6e	nPr	CH_2	>1000	>1000	>1000	137 ± 35	236 ± 76	332 ± 91
6f	F	CH_2	>1000	>1000	>1000	69 ± 21	108 ± 27	94 ± 36
6g	Cl	CH_2	>1000	>1000	>1000	27 ± 11	44 ± 19	44 ± 28
6h	Br	CH_2	>1000	>1000	>1000	28 ± 3	34 ± 11	35 ± 11
6i	OH	CH_2	>1000	>1000	>1000	487 ± 12	822 ± 178	812 ± 188
6j	OMe	CH_2	>1000	>1000	>1000	96 ± 46	191 ± 61	113 ± 10

[a] Values are mean ± SEM of an average of three experiments.

activator of RXRα,β,γ (Figure 1, right). The cotransfection profile for the methylene derivative **6b** (not shown) is analogous to that for ketone **5b**, albeit 10-fold more potent.

Investigation of other 3-functionalized analogs of **5a** and **6a**, including 3-ethyl, 3-isopropyl, 3-chloro, 3-bromo, and 3-methoxy (Table 1), showed that the activation of gene expression by the RXRs was significantly enhanced by addition of a 3-functional group. In contrast, the 3-fluoro, 3-hydroxy, and 3-*n*-propyl derivatives of **5a** and the 3-fluoro and 3-*n*-propyl derivatives of **6a** were either inactive or nonselective for the RXRs.

These compounds were further examined in a competitive binding assay using [^{3}H]ATRA and [^{3}H]-9-*cis*-RA[10,14] as radioligands for RARs and RXRs, respectively. The binding activity of these compounds correlated well with the data from the cotransfection assay (Table 2 and Figures 2 and 3). For example, comparison of compounds **5a** (Figure 2, left) and **6a** (Figure 3, left) with their respective methyl derivatives **5b** (Figure 2, right) and **6b** (Figure 3, right), showed that the desmethyl analog **5a** only marginally displaced [^{3}H]ATRA and [^{3}H]-9-*cis*-RA at all six receptors. Similarly, **6a** weakly displaced the radioligands at all six receptors, exhibiting slight selectivity for the RXR subclass. In contrast, **5b** and **6b** selectively competed with [^{3}H]-9-*cis*-RA at the RXRs with **6b** showing greater than 50-fold selectivity for the RXRs. For both of the latter compounds, competitive binding studies with [^{3}H]ATRA at the RARs showed little or no displacement (Figures 2, right, and 3, right). The other 3-functionalized derivatives also were examined in competitive binding assays. The results (shown in Table 2) compare favorably to those of cotransfection assays and demonstrate that 3-functionalization provides compounds that can directly and selectively bind to the RXRs. As in the parent compounds **5a** and **6a**, a direct comparison of the methylenic derivatives **6b–j** to their ketone counterparts **5b–j** demonstrates that, in general, the methylenic derivatives are more potent binders and activators than the corresponding ketones. Interestingly, although compounds **5e** and **6e** are inactive in the cotransfection assay, they exhibit favorable binding properties. We are currently exploring the possibility that these compounds possess antagonist activity at the RXRs.

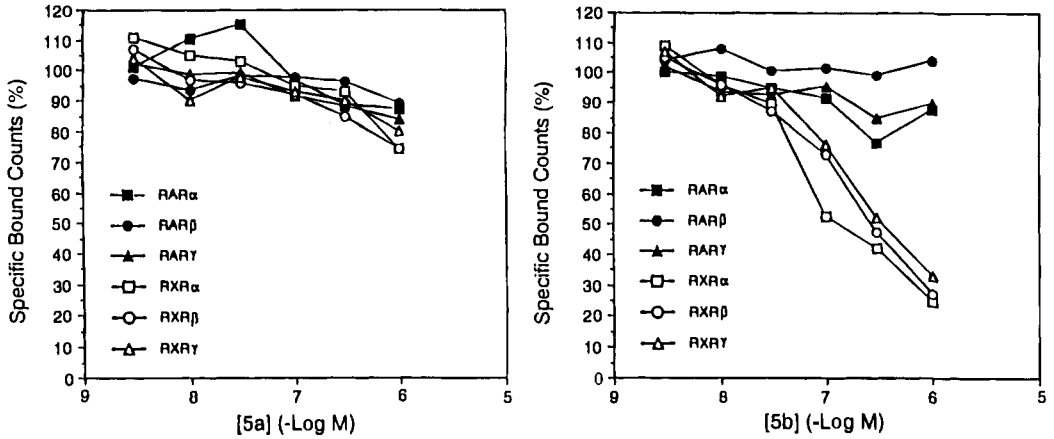


Figure 2. Competition binding of **5a** (left) and **5b** (right) to RARα,β,γ and RXRα,β,γ. The radioligand was [^{3}H]ATRA for the RARs and [^{3}H]-9-*cis*-RA for the RXRs. Percent bound radioligand is plotted against the negative log of the concentration of the analog. Each graph represents the mean of two to three separate determinations.

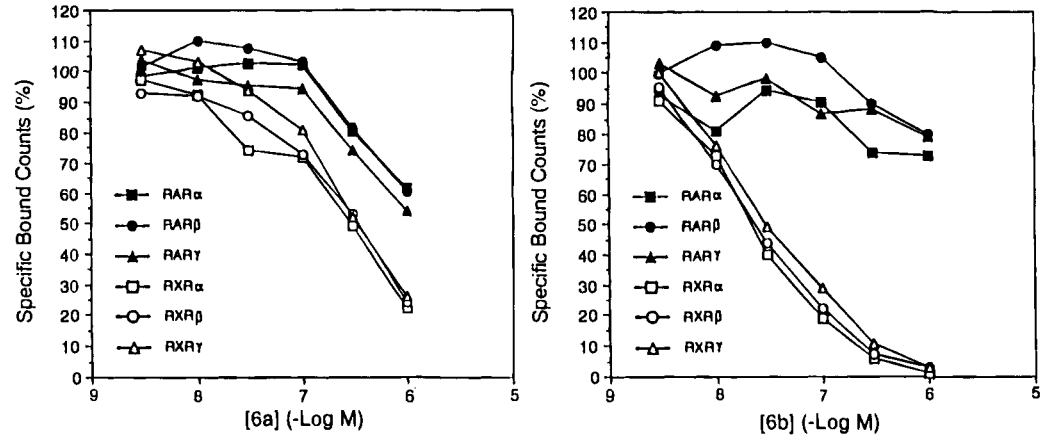


Figure 3. Competition binding of **6a** (left) and **6b** (right) to RARα,β,γ and RXRα,β,γ. The radioligand was [^{3}H]ATRA for the RARs and [^{3}H]-9-*cis*-RA for the RXRs. Percent bound radioligand is plotted against the negative log of the concentration of the analog. Each graph represents the mean of two to three separate determinations.

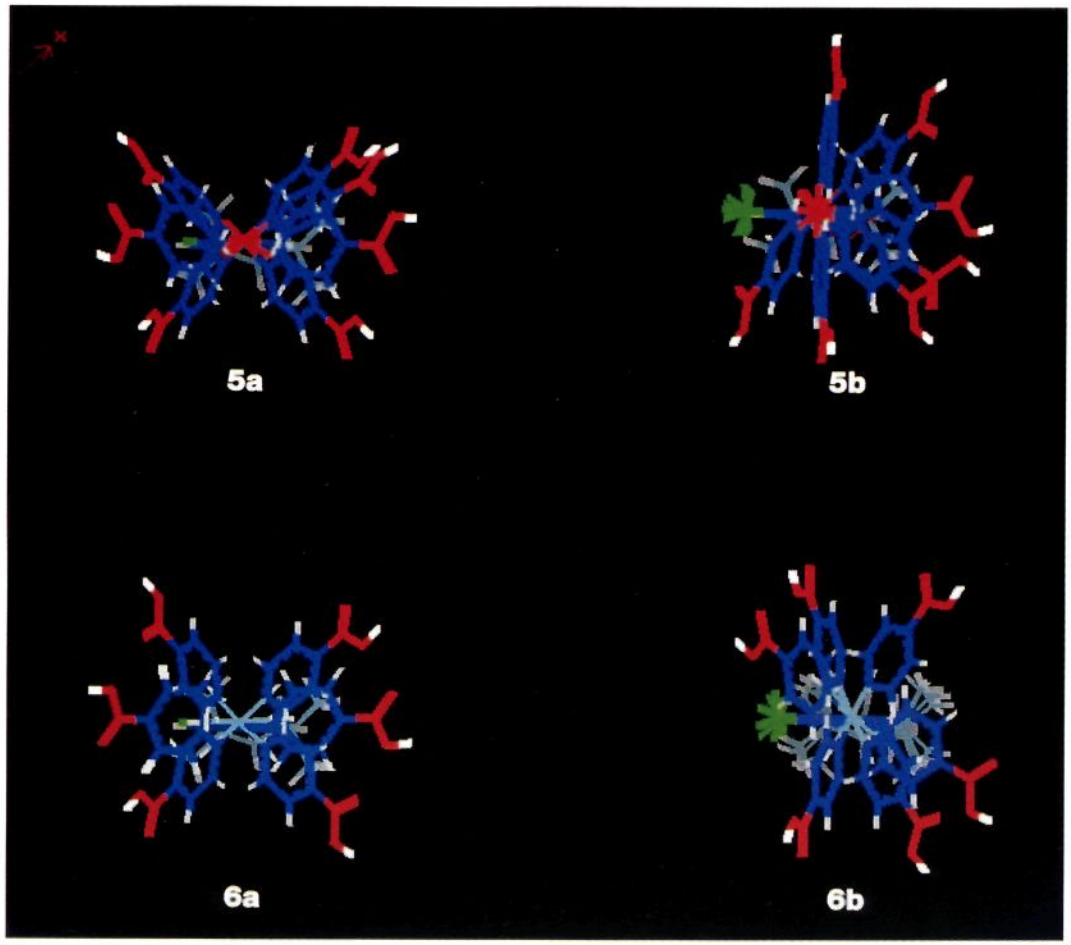
Molecular Modeling

To further explore the nature of the RXR selectivity of this series of molecules, molecular modeling studies were conducted on the parent compounds **5a** and **6a** and their corresponding methyl derivatives **5b** and **6b**. Conformational search routines were performed on these analogs to aid in identifying the effects of the 3-methylation on conformational mobility of **5b** and **6b**. The grid search routines were executed using a 20° torsion angle rotation window with a fixed torsion angle during subsequent energy minimization. Cluster analysis of all conformations within 3.5 kcal of the minimum energy conformation revealed six to eight representative structures, shown in Figure 4, top. Figure 4, bottom, shows a plot of the energy for each minimized structure versus the torsion angle (ϕ) of the B-ring/exo-sp^2 bond. Two important features are evident in these figures: (1) the parent compounds (**5a** and **6a**) show low energy conformations which occupy B-ring/C-ring coplanarity and (2) the 3-methyl analogs (**5b** and **6b**) show low energy conformations having the C-ring orthogonal to the B-ring. Quantitative structure–activity relationship parameters were calculated using the Molecular Simulations, Inc., program QSAR.[16] These calculations revealed a loss in conformational entropy of ca. 0.42–0.45 kcal/mol for the 3-methyl analogs **5b** and **6b** (Table 3) with respect to the parent compounds **5a** and **6a**.

Discussion and Conclusions

Evaluation of the biological profiles of the above 3-functionalized derivatives indicates that substituents in the 3-position can dramatically increase the affinity for the RXRs and decrease the affinity for the RARs. The binding activity profile of the analogs shown in Table 2 suggests that as the 3-substituent increases in effective volume (H < F < Me ≤ Cl ≤ Br < ethyl), the analogs become increasingly potent and selective for the RXR class with an optimal potency shown for the Me, Cl, and Br derivatives (**5b**,**g**,**h** and **6b**,**g**,**h**). Additionally, as RXR activity increases, binding activity for the RAR class decreases. However, when the substituent size increases beyond that of the ethyl group, as shown by the isopropyl and propyl derivatives **5d**, **6d**, **5e**, and **6e**, binding affinity decreases. Hence, the bulk tolerance for hydrophobic functional groups is limited to smaller moieties such as methyl, chloro, bromo, and ethyl. In general, although many of the ketone derivatives (**5b–j**) are quite potent and selective, their corresponding methylene derivatives are at least 5–10-fold more potent while still retaining a high degree of RXR selectivity.

In comparing the biological profiles of the 3-hydroxy (**5i** and **6i**) and 3-methoxy (**5j** and **6j**) derivatives to the corresponding alkylated derivatives **5b**,**c** and **6b**,**c**, it appears that electronic effects from substituents in the 3-position contribute only weakly to the biological



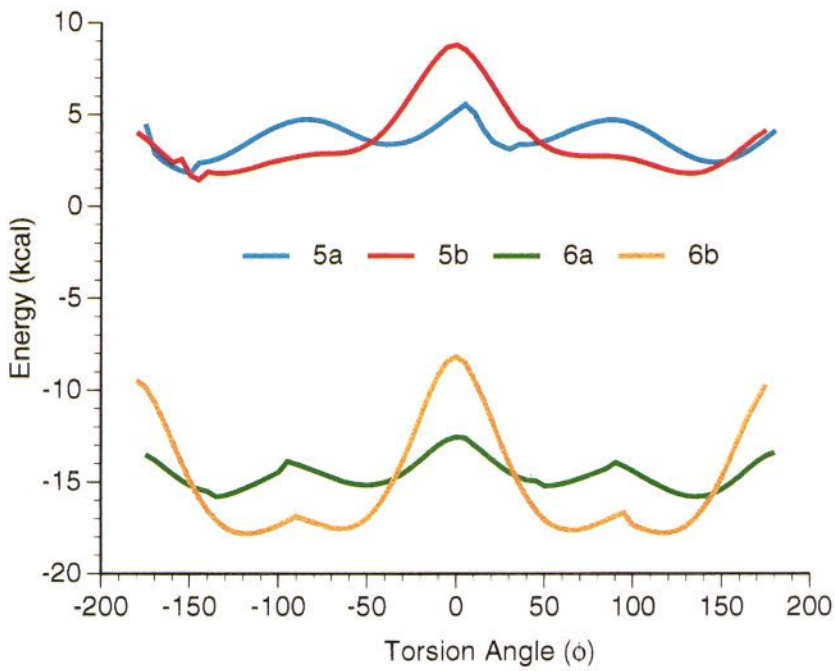


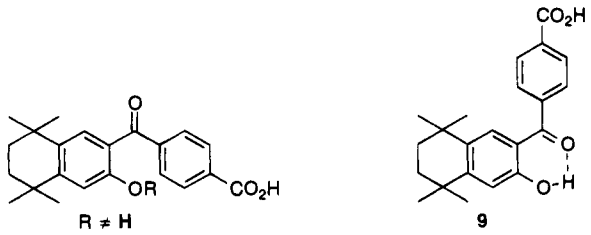
Figure 4. (top) Low energy minima conformations of unsubstituted parent compounds **5a** and **6a** compared with low energy minima conformations of 3-methyl analogs **5b** and **6b**. Note that the orthogonal orientations in the 3-methyl analogs are not evident in the parent compounds **5a** and **6a**. Images were created with superimposition of the aryl B-ring which is perpendicular to the plane of the figure. The 3-methyl groups for compounds **5b** and **6b** and the 3-H groups for compounds **5a** and **6a** are shown in green. (bottom) Energy as a function of the torsion angle defined by the aryl B-ring bond to the ketone or exo-methylene. The 3-methyl analogs **5b** and **6b** show large energy differences between orthogonal ($\phi = \pm 90°$) and coplanar orientations ($\phi = \pm 0°$, $\pm 180°$).

activity of this class of retinoid. While RXR selectivity is maintained by **5j** and **6i,j** (Table 1), small hydrophobic groups in the 3-position are preferred. It is interesting to note that **5i** is inactive at all six retinoid receptors

Table 3. Quantitative Comparison of Physicochemical Properties of Key Compounds

structure	energy cutoff (kcal)	no. of conformers	lowest energy	entropy
5a	3.5	296	29.754	2.98078
5b	3.5	161	31.162	2.53894
6a	3.5	195	19.462	2.85272
6b	3.5	105	20.555	2.43397

Chart 3



whereas **6i** is active at RARβ,γ and RXRα,β,γ. Comparison of the ^{1}H-NMR spectra of **5i** and **6i** shows that the hydroxyl group resonance of **5i** is shifted downfield by 1.4 ppm with respect to the hydroxyl resonance of **6i** (10.29 vs 8.89 ppm in DMSO-d_6), suggesting that the hydroxyl of **5i** is hydrogen bonded to the ketone. It is possible that the biological inactivity of **5i** is a result of a change in conformation of this molecule as compared to the methylene analog **6i** and other 3-substituted analogs that do not posses hydrogen-bonding capabilities (see Chart 3).

The excellent correlation of the binding data to the cotransfection data for these compounds implies that the analogs act directly at the receptor, resulting in gene expression, and thus it is unlikely that the biological activity is a result of metabolites produced from CV-1 cells in the cotransfection assay.

The shift in receptor selectivity which occurs upon addition of the 3-methyl group to compounds **5a** and **6a** and the increased potency of the methylene derivative **6b** over that of **5b** may be partially explained by the modeling studies. The free energy difference between an unbound ligand and the ligand–receptor complex is important for the characterization of binding. This free energy change (ΔG) is related to the equilibrium constant (K) for the ligand receptor interaction ($\Delta G = -2.30RT \log K$), as well as to the change in enthalpy and entropy ($\Delta G = \Delta H - T\Delta S$). The entropy of a ligand is reduced by the conformational restrictions imposed by the receptor binding site upon formation of the ligand–receptor complex. This change in entropy on binding is a measure of the flexibility within a given ligand. For example, freely rotatable bonds in ligands lose much of their rotational freedom when bound to the receptor. The energy cost associated with reduction of the ligand's entropy can reduce the ligand activity by as much as an order of magnitude.[17] Consequently, if one or more bonds in the ligand are fixed in a conformation that is preferred by the receptor, without introducing repulsive interactions into the ligand–receptor complex, then substantial decreases in the entropy loss are possible, which result in increased ligand activity.

From Figure 4, it is evident that the presence of the methyl group on compounds **5b** and **6b** results in eclipsed torsional interactions which restrict conformational freedom, thus reducing the entropy (Table 3). Similarly, the protons on the methylene derivative **6b** additionally restrict rotation by increasing the rotational barrier, thereby further decreasing the entropy (Table 3). These restrictions result in the majority of low energy conformations placing the C-ring orthogonal to the B-ring ($\phi = 90°$) and may be preferred RXR-binding conformations.

In summary, we have described the identification of two series of potent and highly selective retinoids which are active on the RXR family of retinoid receptors. The addition of a methyl group to compounds **5a** and **6a** not only increases potency by 10-fold but also increases selectivity at least 50-fold for RXR over RAR. These data are consistent with our previous observations using TTNPB and 3-methyl-TTNPB, although the effect of a 3-methyl substituent on TTNPB is not nearly as dramatic as the one observed for a 3-methyl substituent on compounds **5a** and **6a**. The structure–activity relationship data obtained from the cotransfection and competitive binding assays for these novel derivatives also indicate that other 3-substituents such as ethyl, propyl, and halogens of **5a** and **6a** significantly increase RXR activity and diminish RAR activity. From molecular modeling studies, the enhanced activity and selectivity of these RXR compounds can be explained, in part, by the loss of entropy which results from conformational restrictions imposed by functionalizing the 3-position of **5a** and **6a**. Of the two series of compounds, 4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)-ethenyl]benzoic acid (**6b**) is clearly the most biologically active analog, in both the cotransfection and binding assays. This compound, designated as LGD1069, is the first RXR-selective compound to enter clinical trials for treatment of various cancers.

Experimental Section

Unless otherwise stated, all reactions were carried out under a nitrogen atmosphere. The organic solvents were purchased from Fisher Scientific, 1,2,3,4-tetrahydro-1,1,4,4-tetramethylnaphthalene (**2a**) was purchased from Maybridge, and monomethylterephthalic acid chloride was purchased from TCI America. Thin layer chromatography (TLC) was performed with Merck Kieselgel 60 F-254 plates; ^{1}H-NMR spectra were determined on Bruker 300 and 400 MHz instruments. UV spectra were measured on a Kontron Uvikon Model 941 instrument, and 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.

2,5-Dichloro-2,5-dimethylhexane. The preparation of this material was modified from the one described by Bruson *et al.*[18a] Dry hydrogen chloride gas was bubbled through a vigorously stirring suspension of 500 g (3.40 mol) of 2,5-dimethyl-2,5-hexanediol in 1500 mL of EtOH in a 3000 mL round-bottomed flask. The temperature rose to 60 °C, and the solution turned a brown color. After the solution had cooled to 5 °C in an ice bath, white crystals formed which were filtered and washed 3× with water followed by cold EtOH. The white crystals were dried under vacuum to give 350 g (1.91 mol) of the desired dichloro compound (56% yield): TLC (5% ethyl acetate–95% hexanes) R_f 0.8; mp 63–65 °C (lit.[18a] mp 63–64 °C, lit.[18b] mp 64 °C); ^{1}H-NMR ($CDCl_3$) δ 1.60 (s, 12H, CH_3), 1.95 (s, 4H, CH_2). Anal. ($C_8H_{16}Cl_2$) C, H, Cl.

Methyl 4-[(5,5,8,8-Tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoate (3a). Compound **3a** was synthesized in a similar fashion to that described by Maignan *et al.*[15] To a 250 mL three-necked round-bottomed flask fitted with a magnetic stirring bar and reflux condenser containing 10.0 g (50.5 mmol) of monomethylterephthalic acid chloride and 50 mL of dichloromethane was added 10.0 g (53.2 mmol) of 1,1,4,4-tetramethyl-1,2,3,4-tetrahydronaphthalene (**2a**) fol-

lowed by slow addition (0.5 g aliquots) of 14.3 g (107.5 mmol) of aluminum chloride ($AlCl_3$). The brown mixture was heated at reflux for 15 min, and a 1 mL aliquot was quenched in 5 mL of 20% aqueous hydrochloric acid, extracted with ethyl acetate (EtOAc), and monitored by ^{1}H-NMR to determine if all of the chloromethyl terephthalate was consumed. Additional aluminum chloride (1–3 g) was often necessary to effect completion of the reaction. The cooled reaction mixture was poured into 200 mL of a vigorously stirred ice solution followed by acidification with 20% aqueous hydrochloric acid (50 mL) and addition of 100 mL of ethyl acetate. Stirring was continued until the organic layer was yellow (15 min). The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (2 × 100 mL). The combined ethyl acetate extract was washed with water (100 mL) and brine (50 mL), dried over magnesium sulfate, filtered, concentrated, and crystallized from 50 mL of hot ethyl acetate by addition of 100 mL of MeOH and cooling to room temperature to give 17.0 g (48.6 mmol) of ketone **3a** as white crystals (96% yield): TLC (20% ethyl acetate–80% hexanes) R_f 0.5; mp 134–136 °C (lit.[15] mp 136 °C); ^{1}H-NMR ($CDCl_3$) δ 1.28 (s, 6H, CH_3), 1.32 (s, 6H, CH_3), 1.71 (s, 4H, CH_2), 3.94 (s, 3H, CO_2CH_3), 7.41 (d, J = 8.0 Hz, 1H, Ar-CH), 7.50 (dd, J = 1.7, 8.0 Hz, 1H, Ar-CH), 7.77 (d, J = 1.7 Hz, 1H, Ar-CH), 7.81 (d, J = 8 Hz, 2H, Ar-CH), 8.12 (d, J = 8 Hz, 2H, Ar-CH). Anal. ($C_{23}H_{26}O_3$) C, H.

4-[(5,5,8,8-Tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic Acid (5a). Compound **5a** was synthesized in a similar fashion to that described by Maignan *et al.*[15] To 2.0 g (5.7 mmol) of methyl ester **3a** in 10 mL of MeOH was added 2 mL (10 mmol) of a 5 N aqueous KOH solution. The mixture was heated at reflux for 30 min, cooled to room temperature, and acidified with 10 mL of 20% aqueous HCl. The solution was extracted twice with 25 mL of EtOAc, and the combined organic extract was dried over $MgSO_4$, filtered, diluted with 50 mL of hexanes, and concentrated to a volume of 20 mL. At that concentration, crystals formed and the solution was allowed to stand for an additional 1 h followed by filtration and drying to give 1.2 g (3.6 mmol) of **5b** as a white powder (63% yield): TLC (10% MeOH–90% $CHCl_3$) R_f 0.33; mp 191–194 °C (lit.[15] mp 193 °C); ^{1}H-NMR ($CDCl_3$) δ 1.31 (s, 6H, CH_3), 1.33 (s, 6H, CH_3), 1.73 (s, 4H, CH_2), 7.41 (d, J = 8.0 Hz, 1H, Ar-CH), 7.55 (dd, J = 1.7, 8.0 Hz, 1H, Ar-CH), 7.81 (d, J = 1.7 Hz, 1H, Ar-CH), 7.87 (d, J = 8 Hz, 2H, Ar-CH), 8.23 (d, J = 8 Hz, 2H, Ar-CH). Anal. ($C_{22}H_{24}O_3$) C, H.

Methyl 4-[1-(5,5,8,8-Tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoate (4a). To a 100 mL round-bottomed flask containing 4.6 g (12.9 mmol) of methyltriphenylphosphonium bromide in 15 mL of dry tetrahydrofuran (THF) (under dry N_2) was added 670 mg (17.6 mmol) of sodium amide. The reaction mixture was stirred for 15 h to give a bright yellow solution, which was then added slowly to a 100 mL round-bottomed flask containing 3.0 g (8.6 mmol) of ketone **3a** in 20 mL of dry tetrahydrofuran. The rate of addition of methyltriphenylphosphonium bromide–sodium amide was adjusted so that the reaction temperature never exceeded 50 °C. The formation of the olefin was complete in 30 min and was directly monitored by TLC. After formation of the olefin, the reaction mixture was poured into 200 mL of cold water and extracted with 2 × 200 mL of ethyl acetate. The organic layer was washed with water and brine, dried over magnesium sulfate, filtered, concentrated, and crystallized from 1:2 ethyl acetate–methanol (first, the product was dissolved in a minimum amount of ethyl acetate (2 mL) followed by addition of 15 mL of methanol and removal of ethyl acetate by rotary evaporation) to give 2.6 g (7.5 mmol) of olefin **4a** (87% yield): TLC (20% ethyl acetate–80% hexanes) R_f 0.6; mp 115–117 °C; ^{1}H-NMR ($CDCl_3$) δ 1.21 (s, 6H, CH_3), 1.29 (s, 6H, CH_3), 1.68 (s, 4H, CH_2), 3.99 (s, 3H, CO_2CH_3), 5.45 and 5.53 (d, J = 1 Hz, 2H, =CH_2), 7.08 (dd, J = 1.7, 8.0 Hz, 1H, Ar-CH), 7.20 (d, J = 1.7 Hz, 1H, Ar-CH), 7.24 (d, J = 8.0 Hz, 1H, Ar-CH), 6.90 (d, J = 8.4 Hz, 2H, Ar-CH), 7.98 (d, J = 8.4 Hz, 2H, Ar-CH). Anal. ($C_{24}H_{28}O_2$) C, H.

4-[1-(5,5,8,8-Tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoic Acid (6a). To 1.5 g (4.3 mmol) of the methyl ester **4a** suspended in 20 mL of methanol in a 50 mL round-bottomed flask equipped with a reflux condenser was added 2 mL (10 mmol) of an aqueous 5 N potassium hydroxide solution. The reaction was refluxed for 30 min–1 h or until hydrolysis was complete by TLC. After cooling to room temperature, the reaction mixture was poured into 250 mL of 20% aqueous hydrochloric acid, and the organics were extracted with ethyl acetate (2 × 200 mL). The ethyl acetate layer was washed with water and brine, dried over magnesium sulfate, concentrated, and crystallized from EtOAc–hexane (the acid **4** was dissolved in 5 mL of hot EtOAc followed by addition of 5 mL of hexane). After cooling to room temperature, the solids were filtered to give 1.2 g (3.6 mmol) of acid **6a** as fine white crystals (83% yield): TLC (10% methanol–90% chloroform) R_f 0.5; mp 217–219 °C; ^{1}H-NMR ($CDCl_3$) δ 1.5 (s, 6H, CH_3), 1.30 (s, 6H, CH_3), 1.70 (s, 4H, CH_2), 5.50 and 5.56 (s, 2H, =CH_2), 7.08 (dd, J = 1.7, 8.0 Hz, 1H, Ar-CH), 7.24 (d, J = 1.7 Hz, 1H, Ar-CH), 7.28 (d, J = 8.0 Hz, 1H, Ar-CH), 7.48 (d, J = 8.4 Hz, 2H, Ar-CH), 8.09 (d, J = 8.4 Hz, 2H, Ar-CH). Anal. ($C_{23}H_{26}O_2$) C, H.

1,2,3,4-Tetrahydro-1,1,4,4,6-pentamethylnaphthalene (2b). (Compound **2b** was synthesized as described.[19] A representative example is provided here.) To a 250 mL round-bottomed flask fitted with a magnetic stirring bar and reflux condenser were added 10.0 g (54.5 mmol) of 2,5-dichloro-2,5-dimethylhexane, 10.0 g (110.0 mmol) of toluene, and 50 mL of dichloromethane. To this vigorously stirred solution was slowly added 100 mg (0.75 mmol) of aluminum chloride which resulted in rapid evolution of gaseous hydrochloric acid. The reaction mixture was stirred at room temperature for 30 min followed by reflux for an additional 15 min to give a red solution containing 1,1,4,4,6-pentamethyl-1,2,3,4-tetrahydronaphthalene (**2a**). After cooling, 10 mL of 20% aqueous hydrochloric acid was added to the stirred solution, and the reaction mixture turned clear/white. The organics were extracted with 2 × 100 mL of hexanes, washed with water and brine, dried over magnesium sulfate, filtered, concentrated, and distilled (100–105 °C, 1 mmHg) to give 10.0 g (49.5 mmol) of 1,2,3,4-tetrahydro-1,1,4,4,6-pentamethylnaphthalene (91% yield): mp 31–32 °C (lit.[19] mp 29 °C); ^{1}H-NMR ($CDCl_3$) δ 1.26 (s, 6H, CH_3), 1.27 (s, 6H, CH_3), 1.67 (s, 4H, CH_2), 2.30 (s, 3H, CH_3), 6.95 (d, J = 7.7 Hz, 1H, Ar-CH), 7.12 (s, 1H, Ar-CH), 7.20 (d, J = 7.7 Hz, 1H, Ar-CH). Anal. ($C_{15}H_{22}$) C, H.

Methyl 4-[(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoate (3b). Compound **3b** was synthesized from **2b** following the representative procedure described for **3a**. Crystallization gave **3b** as white crystals (72% yield): TLC (20% ethyl acetate–80% hexanes) R_f 0.5; mp 142–143 °C; ^{1}H-NMR ($CDCl_3$) δ 1.20 (s, 6H, CH_3), 1.32 (s, 6H, CH_3), 1.69 (s, 4H, CH_2), 2.35 (s, 3H, CH_3), 3.96 (s, 3H, CO_2CH_3), 7.21 (s, 1H, Ar-CH), 7.26 (s, 1H, Ar-CH), 7.86 (d, J = 8.4 Hz, 2H, Ar-CH), 8.12 (d, J = 8.4 Hz, 2H, Ar-CH), EI-MS m/z 364 (M^+), 349 ($M^+ - CH_3$), 305 ($M^+ - CO_2Me$). Anal. ($C_{24}H_{28}O_3$) C, H.

4-[(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic Acid (5b). Compound **5b** was synthesized from **3b** following the representative procedure described for **5a**. The desired acid was obtained as a white powder (67% yield): TLC (10% MeOH–90% $CHCl_3$) R_f 0.33; mp 198–199 °C; ^{1}H-NMR ($CDCl_3$) δ 1.20 (s, 6H, CH_3), 1.35 (s, 6H, CH_3), 1.75 (s, 4H, CH_2), 2.31 (s, 3H, CH_3), 7.21 (s, 1H, Ar-CH), 7.23 (s, 1H, Ar-CH), 7.86 (d, J = 8.4 Hz, 2H, Ar-CH), 8.18 (d, J = 8.4 Hz, 2H, Ar-CH); HRFAB-MS (M + H) calcd for $C_{23}H_{27}O_3$ 351.1960, found 351.1954. Anal. ($C_{23}H_{26}O_3$) C, H.

Methyl 4-[1-(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoate (4b). Compound **4b** was synthesized from **3b** following the representative procedure described for **4a**. Compound **4b** was obtained as a white powder (68% yield): TLC (20% ethyl acetate–80% hexanes) R_f 0.6; mp 160–161 °C; ^{1}H-NMR ($CDCl_3$) δ 1.27 (s, 6H, CH_3), 1.30 (s, 6H, CH_3), 1.70 (s, 4H, CH_2), 1.94 (s, 3H, CH_3), 3.91 (s, 3H, CO_2CH_3), 5.32 and 5.81 (d, J = 1 Hz, 2H, =CH_2), 7.07 (s, 1H, Ar-CH), 7.12 (s, 1H, Ar-CH), 7.34 (d, J = 8.4 Hz, 2H, Ar-CH), 7.95 (d, J = 8.4 Hz, 2H, Ar-CH); EI-MS m/z 362 (M^+), 347 ($M^+ - CH_3$), 303 ($M^+ - CO_2Me$). Anal. ($C_{24}H_{28}O_2$) C, H.

4-[1-(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoic Acid (6b). Compound **6b** was synthesized from **4b** following the representative procedure described for **6a**. Fine white crystals of **6b** were obtained in

93% yield: TLC (10% methanol–90% chloroform) R_f 0.5; mp 234 °C; UV λ_{MeOH} 264 nm (ϵ 16 400); ^{1}H-NMR ($CDCl_3$) δ 1.28 (s, 6H, CH_3), 1.31 (s, 6H, CH_3), 1.70 (s, 4H, CH_2), 1.95 (s, 3H, CH_3), 5.35 and 5.83 (s, 2H, =CH_2), 7.08 (s, 1H, Ar-CH), 7.13 (s, 1H, Ar-CH), 7.38 (d, J = 8.1 Hz, 2H, Ar-CH), 8.03 (d, J = 8.1 Hz, 2H, Ar-CH); HRFAB-MS (M + H) calcd for $C_{24}H_{29}O_2$ 349.2168, found 349.2178. Anal. ($C_{24}H_{28}O_2$) C, H, O.

Methyl 4-[(3-Ethyl-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoate (3c). Compound **3c** was synthesized from **2c**[19] following the representative procedure described for **3a**. Compound **3c** was obtained as white crystals (52% yield): TLC (20% ethyl acetate–80% hexanes) R_f 0.7; mp 89–90 °C; ^{1}H-NMR ($CDCl_3$) δ 1.16 (t, J = 8 Hz, 3H, $-CH_2CH_3$), 1.20 (s, 6H, CH_3), 1.32 (s, 6H, CH_3), 1.70 (s, 4H, CH_2), 2.70 (q, J = 8 Hz, 2H, $-CH_2CH_3$), 3.96 (s, 3H, CO_2CH_3), 7.20 (s, 1H, Ar-CH), 7.25 (s, 1H, Ar-CH), 7.87 (d, J = 8.5 Hz, 2H, Ar-CH), 8.10 (d, J = 8.5 Hz, 2H, Ar-CH). Anal. ($C_{25}H_{30}O_3$) C, H.

4-[(3-Ethyl-5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic Acid (5c). Compound **5c** was synthesized from **3c** following the representative procedure described for **5a**. Crystallization gave compound **5c** as a white powder (85% yield): TLC (10% MeOH–90% $CHCl_3$) R_f 0.3; mp 226 °C; ^{1}H-NMR ($CDCl_3$) δ 1.16 (t, J = 7.5 Hz, 3H, $-CH_2CH_3$), 1.19 (s, 6H, CH_3), 1.32 (s, 6H, CH_3), 1.69 (s, 4H, CH_2), 2.69 (q, J = 7.5 Hz, 2H, $-CH_2CH_3$), 7.20 (s, 1H, Ar-CH), 7.25 (s, 1H, Ar-CH), 7.87 (d, J = 8.4 Hz, 2H, Ar-CH), 8.20 (d, J = 8.4 Hz, 2H, Ar-CH). Anal. ($C_{24}H_{28}O_3$) C, H.

Methyl 4-[1-(3-Ethyl-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoate (4c). Compound **4c** was synthesized from **3c** following the representative procedure described for **4a**. Compound **4c** was obtained as a white powder (75% yield): TLC (20% ethyl acetate–80% hexanes) R_f 0.8; mp 129–130 °C; ^{1}H-NMR ($CDCl_3$) δ 0.98 (t, J = 7.6 Hz, 3H, $-CH_2CH_3$), 1.27 (s, 6H, CH_3), 1.31 (s, 6H, CH_3), 1.70 (s, 4H, CH_2), 2.29 (q, J = 7.6 Hz, 2H, $-CH_2CH_3$), 3.90 (s, 3H, CO_2CH_3), 5.34 (s, 1H, =CH), 5.80 (s, 1H, =CH), 7.08 (s, 1H, Ar-CH), 7.12 (s, 1H, Ar-CH), 7.32 (d, J = 8 Hz, 2H, Ar-CH), 8.96 (d, J = 8 Hz, 2H, Ar-CH); HRFAB-MS (M + H) calcd for $C_{26}H_{33}O_2$ 377.2481, found 377.2485. Anal. ($C_{26}H_{32}O_2$) C, H.

4-[1-(3-Ethyl-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoic Acid (6c). Compound **6c** was synthesized from **4c** following the representative procedure described for **6a**. Crystallization of acid **6c** gave fine white crystals (75% yield): TLC (10% MeOH–90% $CHCl_3$) R_f 0.5; mp 238–239 °C; ^{1}H-NMR ($CDCl_3$) δ 0.99 (t, J = 7.6 Hz, 3H, $-CH_2CH_3$), 1.27 (s, 6H, CH_3), 1.31 (s, 6H, CH_3), 1.70 (s, 4H, CH_2), 2.29 (q, J = 7.6 Hz, 2H, $-CH_2CH_3$), 5.34 (s, 1H, =CH), 5.83 (s, 1H, =CH), 7.08 (s, 1H, Ar-CH), 7.12 (s, 1H, Ar-CH), 7.38 (d, J = 8 Hz, 2H, Ar-CH), 8.00 (d, J = 8 Hz, 2H, Ar-CH); FAB-MS m/z 363 (M + H). Anal. ($C_{25}H_{30}O_2$) C, H.

Methyl 4-[(3-Isopropyl-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoate (3d). Compound **3d** was synthesized from **2d**[19] following the representative procedure described for **3a**. White crystals of **3d** were obtained after crystallization (94% yield): TLC (20% ethyl acetate–80% hexanes) R_f 0.7; mp 138–140 °C; ^{1}H-NMR ($CDCl_3$) δ 1.19 (d, J = 7 Hz, 6H, $-CH(CH_3)_2$), 1.21 (s, 6H, CH_3), 1.33 (s, 6H, CH_3), 1.70 (bs, 4H, CH_2), 3.12 (q, J = 7 Hz, 1H, $-CH(CH_3)_2$), 3.95 (s, 3H, CO_2CH_3), 7.12 (s, 1H, Ar-CH), 7.35 (s, 1H, Ar-CH), 7.89 (d, J = 8 Hz, 2H, Ar-CH), 8.11 (d, J = 8 Hz, 2H, Ar-CH). Anal. ($C_{26}H_{32}O_3$) C, H.

4-[(3-Isopropyl-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic Acid (5d). Compound **5d** was synthesized from **3d** following the representative procedure described for **5a**. Crystallization gave acid **5d** as a white powder (68% yield): TLC (10% MeOH–90% $CHCl_3$) R_f 0.3; mp 254 °C; ^{1}H-NMR ($CDCl_3$) δ 1.19 (d, J = 7 Hz, 6H, $-CH(CH_3)_2$), 1.21 (s, 6H, CH_3), 1.33 (s, 6H, CH_3), 1.70 (s, 4H, CH_2), 3.12 (q, J = 7 Hz, 1H, $-CH(CH_3)_2$), 7.14 (s, 1H, Ar-CH), 7.37 (s, 1H, Ar-CH), 7.92 (d, J = 8 Hz, 2H, Ar-CH), 8.18 (d, J = 8 Hz, 2H, Ar-CH). Anal. ($C_{25}H_{30}O_3$) C, H.

Methyl 4-[1-(3-Isopropyl-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoate (4d). Compound **4d** was synthesized from **3d** following the representative procedure described for **4a**. Compound **4d** was obtained as a white powder in 69% yield: TLC (20% ethyl acetate–80% hexanes) R_f 0.8; mp 145–147 °C; ^{1}H-NMR ($CDCl_3$) δ 1.19 (d, J = 7 Hz, 6H, $-CH(CH_3)_2$), 1.21 (s, 6H, CH_3), 1.33 (s, 6H, CH_3), 1.70 (s, 4H, CH_2), 3.12 (q, J = 7 Hz, 1H, $-CH(CH_3)_2$), 3.91 (s, 3H, CO_2CH_3), 5.33 (s, 1H, =CH), 5.85 (s, 1H, =CH), 7.07 (s, 1H, Ar-CH), 7.22 (s, 1H, Ar-CH), 7.35 (d, J = 8 Hz, 2H, Ar-CH), 8.12 (d, J = 8 Hz, 2H, Ar-CH). Anal. ($C_{27}H_{34}O_2$) C, H.

4-[1-(3-Isopropyl-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoic Acid (6d). Compound **6d** was synthesized from **4d** following the representative procedure described for **6a**. White crystals of **6d** were obtained in 91% yield: TLC (10% MeOH–90% $CHCl_3$) R_f 0.5; mp 252 °C; ^{1}H-NMR ($CDCl_3$) δ 1.05 (d, J = 7 Hz, 6H, $-CH(CH_3)_2$), 1.27 (s, 6H, CH_3), 1.32 (s, 6H, CH_3), 1.70 (s, 4H, CH_2), 2.73 (q, J = 7 Hz, 1H, $CH(CH_3)_2$), 5.32 (s, 1H, =CH), 5.87 (s, 1H, =CH), 7.06 (s, 1H, Ar-CH), 7.23 (s, 1H, Ar-CH), 7.40 (d, J = 8 Hz, 2H, Ar-CH), 8.04 (d, J = 8 Hz, 2H, Ar-CH); FAB-MS m/z 377 (M + H). Anal. ($C_{26}H_{32}O_2$) C, H.

1,2,3,4-Tetrahydro-1,1,4,4-tetramethyl-6-propylnaphthalene (2e). Compound **2e** was synthesized from **1e** following the representative procedure described for **2b**. Compound **2e** was distilled at 142–150 °C, 1 mmHg, to give a viscous oil (8% yield): ^{1}H-NMR ($CDCl_3$) δ 0.89 (t, J = 7.5 Hz, 3H, $-CH_2CH_2CH_3$), 1.55 (dt, J = 7.5, 8.0 Hz, 2H, $-CH_2CH_2CH_3$), 2.62 (t, J = 8 Hz, 2H, $-CH_2CH_2CH_3$), 6.85 (d, J = 7.7 Hz, 1H, Ar-CH), 7.10 (s, 1H, Ar-CH), 7.15 (d, J = 7.7 Hz, 1H, Ar-CH). Anal. ($C_{17}H_{26}$) C, H.

Methyl 4-[(3-Propyl-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoate (3e). Compound **3e** was synthesized from **2e** following the representative procedure described for **3a**. White crystals of **3e** were obtained in 95% yield: TLC (20% ethyl acetate–80% hexanes) R_f 0.7; mp 112–114 °C; ^{1}H-NMR ($CDCl_3$) δ 0.89 (t, J = 7.5 Hz, 3H, $-CH_2CH_2CH3$), 1.20 (s, 6H, CH_3), 1.31 (s, 6H, CH_3), 1.55 (dt, J = 7.5, 8.0 Hz, 2H, $-CH_2CH_2CH_3$), 1.69 (s, 4H, CH_2), 2.64 (t, J = 8 Hz, 2H, $-CH_2CH_2CH_3$), 3.95 (s, 3H, CO_2CH_3), 7.20 (s, 1H, Ar-CH), 7.22 (s, 1H, Ar-CH), 7.89 (d, J = 8 Hz, 2H, Ar-CH), 8.19 (d, J = 8 Hz, 2H, Ar-CH). Anal. ($C_{23}H_{32}O_3$) C, H.

4-[(3-Propyl-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic Acid (5e). Compound **5e** was synthesized from **3e** following the representative procedure described for **5a**. Crystallization gave **5e** as a white powder (93% yield): TLC (10% MeOH–90% $CHCl_3$) R_f 0.3; mp 252–254 °C; ^{1}H-NMR ($CDCl_3$) δ 0.89 (t, J = 7.5 Hz, 3H, $-CH_2CH_2CH_3$), 1.20 (s, 6H, CH_3), 1.31 (s, 6H, CH_3), 1.55 (dt, J = 7.5, 8.0 Hz, 2H, $-CH_2CH_2CH_3$), 1.69 (s, 4H, CH_2), 2.64 (t, J = 8 Hz, 2H, $-CH_2CH_2CH_3$), 7.20 (s, 1H, Ar-CH), 7.25 (s, 1H, Ar-CH), 7.90 (d, J = 8 Hz, 2H, Ar-CH), 8.20 (d, J = 8 Hz, 2H, Ar-CH). Anal. ($C_{25}H_{30}O_3$) C, H.

Methyl 4-[1-(3-Propyl-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoate (4e). Compound **4e** was synthesized from **3e** following the representative procedure described for **4a**. Benzoate **4e** was obtained as white crystals (78% yield): TLC (20% ethyl acetate–80% hexanes) R_f 0.8; mp 120–121 °C; ^{1}H-NMR ($CDCl_3$) δ 0.73 (t, J = 7.5 Hz, 3H, $-CH_2CH_2CH_3$), 1.26 (s, 6H, CH_3), 1.30 (s, 6H, CH_3), 1.39 (dt, J = 7.5, 8.0 Hz, 2H, $-CH_2CH_2CH_3$), 1.70 (s, 4H, CH_2), 2.24 (t, J = 8 Hz, 2H, $-CH_2CH_2CH_3$), 3.90 (s, 3H, CO_2CH_3), 5.30 (s, 1H, =CH), 5.80 (s, 1H, =CH), 7.08 (s, 1H, Ar-CH), 7.09 (s, 1H, Ar-CH), 7.33 (d, J = 8 Hz, 2H, Ar-CH), 7.94 (d, J = 8 Hz, 2H, Ar-CH). Anal. ($C_{27}H_{34}O_2$) C, H.

4-[1-(3-Propyl-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoic Acid (6e). Compound **6e** was synthesized from **4e** following the representative procedure described for **6a**. Crystallization gave acid **6e** as white crystals (83% yield): TLC (10% MeOH–90% $CHCl_3$) R_f 0.5; mp 263–265 °C; ^{1}H-NMR ($CDCl_3$) δ 0.73 (t, J = 7.5 Hz, 3H, $-CH_2CH_2CH_3$), 1.26 (s, 6H, CH_3), 1.30 (s, 6H, CH_3), 1.39 (dt, J = 7.5, 8.0 Hz, 2H, $-CH_2CH_2CH_3$), 1.70 (s, 4H, CH_2), 2.23 (t, J = 8 Hz, 2H, $-CH_2CH_2CH_3$), 5.31 (s, 1H, =CH), 5.81 (s, 1H, =CH), 7.08 (s, 1H, Ar-CH), 7.09 (s, 1H, Ar-CH), 7.36 (d, J = 8 Hz, 2H, Ar-CH), 8.00 (d, J = 8 Hz, 2H, Ar-CH); FAB-MS m/z 377 (M + H). Anal. ($C_{26}H_{32}O_2$) C, H.

1,2,3,4-Tetrahydro-1,1,4,4-tetramethyl-6-fluoronaphthalene (2f). Compound **2f** was synthesized from **1f** following the representative procedure described for **2b**. Compound **2f** was distilled at 185–195 °C, 4 mmHg, to afford a viscous oil (23% yield): ^{1}H-NMR ($CDCl_3$) δ 1.25 (s, 6H, CH_3), 1.26 (s, 6H,

CH_3), 1.67 (s, 4H, CH_2), 6.80 (dd, J = 2, 9 Hz, 1H, Ar-CH), 7.05 (dd, J = 2, 8 Hz, 1H, Ar-CH), 7.13 (dd, J = 8, 9 Hz, 1H, Ar-CH). Anal. ($C_{14}H_{19}F$) C, H.

Methyl 4-[(3-Fluoro-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoate (3f). Compound **3f** was synthesized from **2f** following the representative procedure described for **3a**. White crystals of compound **3f** were obtained in 87% yield: TLC (20% ethyl acetate–80% hexanes) R_f 0.6; mp 112–114 °C; ^{1}H-NMR ($CDCl_3$) δ 1.28 (s, 6H, CH_3), 1.31 (s, 6H, CH_3), 1.72 (s, 4H, CH_2), 3.96 (s, 3H, CO_2CH_3), 7.04 (d, J = 13 Hz, 1H, Ar-CH), 7.35 (d, J = 8 Hz, 1H, Ar-CH), 7.88 (d, J = 8 Hz, 2H, Ar-CH), 8.12 (d, J = 8 Hz, 2H, Ar-CH); EI-MS m/z 368 (M^+), 353 ($M^+ - CH_3$). Anal. ($C_{23}H_{25}O_3F$) C, H.

4-[(3-Fluoro-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic Acid (5f). Compound **5f** was synthesized from **3f** following the representative procedure described for **5a**. Crystallization gave compound **5f** as a white powder (94% yield): TLC (10% MeOH–90% $CHCl_3$) R_f 0.3; mp 199–201 °C; ^{1}H-NMR ($CDCl_3$) δ 1.29 (s, 6H, CH_3), 1.32 (s, 6H, CH_3), 1.72 (s, 4H, CH_2), 7.05 (d, J = 13 Hz, 1H, Ar-CH), 7.57 (d, J = 8 Hz, 1H, Ar-CH), 7.90 (d, J = 8 Hz, 2H, Ar-CH), 8.21 (d, J = 8 Hz, 2H, Ar-CH); HRFAB-MS (M + H) calcd for $C_{22}H_{24}O_2F$ 355.1709, found 355.1714. Anal. ($C_{22}H_{23}O_2F$) C, H.

Methyl 4-[1-(3-Fluoro-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoate (4f). Compound **4f** was synthesized from **3f** following the representative procedure described for **4a**. Benzoate **4f** was obtained as a white powder (60% yield): TLC (20% ethyl acetate–80% hexanes) R_f 0.8; mp 105–107 °C; ^{1}H-NMR ($CDCl_3$) δ 1.25 (s, 6H, CH_3), 1.29 (s, 6H, CH_3), 1.70 (s, 4H, CH_2), 3.95 (s, 3H, CO_2CH_3), 5.50 (s, 1H, =CH), 5.80 (s, 1H, =CH), 6.95 (d, J = 11 Hz, 1H, Ar-CH), 7.15 (d, J = 8 Hz, 1H, Ar-CH), 7.40 (d, J = 8 Hz, 2H, Ar-CH), 8.18 (d, J = 8 Hz, 2H, Ar-CH); HRFAB-MS (M + H) calcd for $C_{24}H_{28}O_2F$ 367.2073, found 367.2073. Anal. ($C_{24}H_{27}O_2F$) C, H.

4-[1-(3-Fluoro-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)1-ethenyl]benzoic Acid (6f). Compound **6f** was synthesized from **4f** following the representative procedure described for **6a**. Fine white crystals of **6f** were obtained in 87% yield: TLC (10% MeOH–90% $CHCl_3$) R_f 0.5; mp 215–217 °C; ^{1}H-NMR ($CDCl_3$) δ 1.25 (s, 6H, CH_3), 1.29 (s, 6H, CH_3), 1.70 (s, 4H, CH_2), 5.54 (s, 1H, =CH), 5.80 (s, 1H, =CH), 6.98 (d, J = 11 Hz, 1H, Ar-CH), 7.19 (d, 8 Hz, 1H, Ar-CH), 7.44 (d, J = 8 Hz, 2H, Ar-CH), 8.08 (d, J = 8 Hz, 2H, Ar-CH); HRFAB-MS (M + H) calcd for $C_{23}H_{26}O_2F$ 353.1917, found 353.1910. Anal. ($C_{23}H_{25}O_2F$) C, H.

1,2,3,4-Tetrahydro-1,1,4,4-tetramethyl-6-chloronaphthalene (2g). Compound **2g** was synthesized from **1g** following the representative procedure described for **2b**. Distillation of the product at 104 °C, 1 mmHg, gave, after cooling to room temperature, **2g** as a white solid (90% yield): mp 30–32 °C; ^{1}H-NMR ($CDCl_3$) 1.25 (s, 6H, CH_3), 1.26 (s, 6H, CH_3), 1.67 (s, 4H, CH_2), 7.07 (dd, J = 2, 8 Hz, 1H, Ar-CH), 7.21 (d, J = 8 Hz, 1H, Ar-CH), 7.24 (d, J = 2 Hz, 1H, Ar-CH). Anal. ($C_{14}H_{19}Cl$) C, H.

Methyl 4-[(3-Chloro-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoate (3g). Compound **3g** was synthesized from **2g** following the representative procedure described for **3a**. White crystals of **3g** were obtained in 66% yield: TLC (20% ethyl acetate–80% hexanes) R_f 0.6; mp 192–193 °C; ^{1}H-NMR ($CDCl_3$) δ 1.28 (s, 6H, CH_3), 1.36 (s, 6H, CH_3), 1.76 (s, 4H, CH_2), 3.92 (s, 3H, CO_2CH_3), 7.49 (s, 2H, Ar-CH), 7.85 (d, J = 8 Hz, 2H, Ar-CH), 8.18 (d, J = 8 Hz, 2H, Ar-CH). Anal. ($C_{23}H_{25}O_3Cl$) C, H.

4-[(3-Chloro-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic Acid (5g). Compound **5g** was synthesized from **3g** following the representative procedure described for **5a**. Compound **5g** was obtained as a white powder (80% yield): TLC (10% MeOH–90% $CHCl_3$) R_f 0.4; mp 254 °C; ^{1}H-NMR ($CDCl_3$) δ 1.26 (s, 6H, CH_3), 1.32 (s, 3H, CH_3), 1.72 (s, 4H, CH_2), 7.35 (s, 1H, Ar-CH), 7.36 (s, 1H, Ar-CH), 7.91 (d, J = 8 Hz, 2H, Ar-CH), 8.19 (d, J = 8 Hz, 2H, Ar-CH); HRFAB-MS (M + H) calcd for $C_{22}H_{24}O_3Cl$ 371.1414, found 371.1420. Anal. ($C_{22}H_{23}O_3Cl$) C, H.

Methyl 4-[1-(3-Chloro-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoate (4g). Compound **4g** was synthesized from **3g** following the representative procedure described for **4a**. Compound **4g** was obtained as a white powder (75% yield): TLC (20% ethyl acetate–80% hexanes) R_f 0.8; mp 149–150 °C; ^{1}H-NMR ($CDCl_3$) δ 1.28 (s, 6H, CH_3), 1.31 (s, 6H, CH_3), 1.71 (s, 4H, CH_2), 3.90 (s, 3H, CO_2CH_3), 5.40 (s, 1H, =CH), 5.89 (s, 1H, =CH), 7.22 (s, 1H, Ar-CH), 7.29 (s, 1H, Ar-CH), 7.39 (d, J = 8 Hz, 2H, Ar-CH), 8.00 (d, J = 8 Hz, 2H, Ar-CH); HRFAB-MS (M + H) calcd for $C_{24}H_{28}O_2Cl$ 383.1778, found 383.1775. Anal. ($C_{24}H_{27}O_2Cl$) C, H.

4-[1-(3-Chloro-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoic Acid (6g). Compound **6g** was synthesized from **4g** following the representative procedure described for **6a**. Fine white crystals of **6g** were obtained in 96% yield: TLC (10% MeOH–90% $CHCl_3$) R_f 0.5; mp 233 °C; ^{1}H-NMR ($CDCl_3$) δ 1.28 (s, 6H, CH_3), 1.31 (s, 6H, CH_3), 1.71 (s, 4H, CH_2), 5.42 (s, 1H, =CH), 5.89 (s, 1H, =CH), 7.23 (s, 1H, Ar-CH), 7.28 (s, 1H, Ar-CH), 7.37 (d, J = 8 Hz, 2H, Ar-CH), 8.03 (d, J = 8 Hz, 2H, Ar-CH); HRFAB-MS (M + H) calcd for $C_{23}H_{26}O_2Cl$ 349.1621, found 369.1610. Anal. ($C_{23}H_{25}O_2Cl$) C, H.

1,2,3,4-Tetrahydro-1,1,4,4-tetramethyl-6-bromonaphthalene (2h). Compound **2h** was synthesized from **1h** following the representative procedure described for **2b** except that hexanes were used as solvent instead of CH_2Cl_2. The compound was distilled at 112–114 °C, 1 mmHg, to give, after cooling to room temperature, **2h** as a white solid (92% yield): mp 40–42 °C; ^{1}H-NMR ($CDCl_3$) 1.25 (s, 6H, CH_3), 1.26 (s, 6H, CH_3), 1.66 (s, 4H, CH_2), 7.16 (d, J = 8 Hz, 1H, Ar-CH), 7.22 (dd, J = 2, 8 Hz, 1H, Ar-CH), 7.39 (d, J = 2 Hz, 1H, Ar-CH). Anal. ($C_{14}H_{19}Br$) C, H.

Methyl 4-[(3-Bromo-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoate (3h). Compound **3h** was synthesized from **2h** following the representative procedure described for **3a** except that hexanes were used instead of CH_2Cl_2. Ketone **3h** was obtained as white crystals (56% yield): TLC (20% ethyl acetate–80% hexanes) R_f 0.6; mp 182–183 °C; ^{1}H-NMR ($CDCl_3$) δ 1.25 (s, 6H, CH_3), 1.32 (s, 6H, CH_3), 1.71 (s, 4H, CH_2), 3.96 (s, 3H, CO_2CH_3), 7.29 (s, 1H, Ar-CH), 7.53 (s, 1H, Ar-CH), 7.89 (d, J = 8 Hz, 2H, Ar-CH), 8.12 (d, J = 8 Hz, 2H, Ar-CH); HRFAB-MS (M + H) calcd for $C_{23}H_{26}O_3Br$ 429.1065, found 429.1069. Anal. ($C_{23}H_{25}O_3Br$) C, H.

4-[(3-Bromo-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic Acid (5h). Compound **5h** was synthesized from **3h** following the representative procedure described for **5a**. Compound **5h** was obtained as a white powder (57% yield): TLC (10% MeOH–90% $CHCl_3$) R_f 0.3; mp 275 °C; ^{1}H-NMR ($CDCl_3$) δ 1.25 (s, 6H, CH_3), 1.32 (s, 6H, CH_3), 1.71 (s, 4H, CH_2), 7.30 (s, 1H, Ar-CH), 7.54 (s, 1H, Ar-CH), 7.90 (d, J = 8 Hz, 2H, Ar-CH), 8.18 (d, J = 8 Hz, 2H, Ar-CH); HRFAB-MS (M + H) calcd for $C_{22}H_{24}O_3Br$ 415.0909, found 415.0905. Anal. ($C_{22}H_{23}O_3Br$) C, H.

Methyl 4-[1-(3-Bromo-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoate (4h). Compound **4h** was synthesized from **3h** following the representative procedure described for **4a**. Crystallization gave **4h** as a white powder (67% yield): TLC (20% ethyl acetate–80% hexanes) R_f 0.8; mp 161–162 °C; ^{1}H-NMR ($CDCl_3$) δ 1.27 (s, 6H, CH_3), 1.30 (s, 6H, CH_3), 1.70 (s, 4H, CH_2), 3.90 (s, 3H, CO_2CH_3), 5.37 (s, 1H, =CH), 5.89 (s, 1H, =CH), 7.21 (s, 1H, Ar-CH), 7.33 (d, J = 8 Hz, 2H, Ar-CH), 7.46 (s, 1H, Ar-CH), 7.99 (d, J = 8 Hz, 2H, Ar-CH). Anal. ($C_{24}H_{27}O_2Br$) C, H.

4-[1-(3-Bromo-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoic Acid (6h). Compound **6h** was synthesized from **4h** following the representative procedure described for **6a**. Fine white crystals of **6h** were obtained in 96% yield: TLC (10% MeOH–90% $CHCl_3$) R_f 0.5; mp 235 °C; ^{1}H-NMR ($CDCl_3$) δ 1.27 (s, 6H, CH_3), 1.31 (s, 6H, CH_3), 1.71 (s, 4H, CH_2), 5.40 (s, 1H, =CH), 5.90 (s, 1H, =CH), 7.26 (s, 1H, Ar-CH), 7.36 (s, 1H, Ar-CH), 7.43 (d, J = 8 Hz, 2H, Ar-CH), 8.04 (d, J = 8 Hz, 2H, Ar-CH); HRFAB-MS (M + H) calcd for $C_{23}H_{26}O_2Br$ 415.1116, found 413.1110. Anal. ($C_{23}H_{25}O_2Br$) C, H.

1,2,3,4-Tetrahydro-1,1,4,4-tetramethyl-6-hydroxynaphthalene (2i). Compound **2i** was synthesized from **1i** following the representative procedure described for **2b** except that 1:1 CH_2Cl_2–hexane was used as solvent instead of CH_2Cl_2. The compound was distilled at 154–160 °C, 1 mmHg, to give, after

cooling to room temperature, **2i** as a white solid (54% yield): mp 116–119 °C; ^{1}H-NMR ($CDCl_3$) δ 1.18 (s, 6H, CH_3), 1.30 (s, 6H, CH_3), 1.70 (m, 4H, CH_2), 6.62 (dd, J = 3, 11 Hz, 1H, Ar-CH), 6.75 (d, J = 3 Hz, 1H, Ar-CH), 7.16 (d, J = 8 Hz, 1H, Ar-CH). Anal. ($C_{14}H_{20}O$) C, H.

Methyl 4-[(3-Hydroxy-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoate (3i). Compound **3i** was synthesized from **2i** following the representative procedure described for **3h**. Bright yellow crystals of **3i** were obtained from hot MeOH (54% yield): TLC (20% ethyl acetate–80% hexanes) R_f 0.6; mp 164–166 °C; ^{1}H-NMR ($CDCl_3$) δ 1.20 (s, 6H, CH_3), 1.35 (s, 6H, CH_3), 1.72 (m, 4H, CH_2), 3.95 (s, 3H, CO_2CH_3), 7.00 (s, 1H, Ar-CH), 7.55 (s, 1H, Ar-CH), 7.83 (d, J = 8 Hz, 2H, Ar-CH), 8.21 (d, J = 8 Hz, 2H, Ar-CH), 11.20 (s, 1H, –OH); HRFAB-MS (M + H) calcd for $C_{23}H_{27}O_4$ 367.1909, found 367.1904. Anal. ($C_{23}H_{26}O_4$) C, H.

4-[(3-Hydroxy-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic Acid (5i). Compound **5i** was synthesized from **3i** following the representative procedure described for **5a**. Acid **5i** was obtained as bright yellow crystals (68% yield): TLC (10% MeOH–90% $CHCl_3$) R_f 0.3; mp 269–271 °C; ^{1}H-NMR ($CDCl_3$) δ 1.20 (s, 6H, CH_3), 1.35 (s, 6H, CH_3), 1.72 (m, 4H, CH_2), 7.00 (s, 1H, Ar-CH), 7.55 (s, 1H, Ar-CH), 7.84 (d, J = 8 Hz, 2H, Ar-CH), 8.25 (d, J = 8 Hz, 2H, Ar-CH), 11.20 (s, 1H, –OH); ^{1}H-NMR (DMSO-d_6) δ 10.29 (s, 1H, –OH); EI-MS m/z 352 (M^+), 337 (M^+ – CH_3). Anal. ($C_{22}H_{24}O_4$) C, H.

Methyl 4-[1-(3-Hydroxy-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoate (4i). Compound **4i** was synthesized from **3i** following the representative procedure described for **4a**. Bright yellow crystals of **4i** were obtained in 74% yield: TLC (20% ethyl acetate–80% hexanes) R_f 0.6; mp 134–136 °C; ^{1}H-NMR ($CDCl_3$) δ 1.20 (s, 6H, CH_3), 1.30 (s, 6H, CH_3), 1.67 (s, 4H, CH_2), 3.92 (s, 3H, CO_2CH_3), 4.88 (s, 1H, –OH), 5.51 (d, J = 1 Hz, 1H, =CH), 5.92 (d, J = 0.8 Hz, 1H, =CH), 6.86 (s, 1H, Ar-CH), 6.99 (s, 1H, Ar-CH), 7.44 (d, J = 8 Hz, 2H, Ar-CH), 8.00 (d, J = 8 Hz, 2H, Ar-CH); HRFAB-MS (M + H) calcd for $C_{24}H_{29}O_3$ 365.2117, found 365.2120. Anal. ($C_{24}H_{28}O_3$) C, H.

4-[1-(3-Hydroxy-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl) ethenyl]benzoic Acid (6i). Compound **6i** was synthesized from **4i** following the representative procedure described for **6a**. Compound **6i** was obtained as fine yellow crystals (88% yield): TLC (10% MeOH–90% $CHCl_3$) R_f 0.3; mp 216 °C; ^{1}H-NMR ($CDCl_3$) δ 1.20 (s, 6H, CH_3), 1.30 (s, 6H, CH_3), 1.67 (s, 4H, CH_2), 5.55 (s, 1H, =CH), 5.96 (s, 1H, =CH), 6.86 (s, 1H, Ar-CH), 7.00 (s, 1H, Ar-CH), 7.49 (d, J = 8 Hz, 2H, Ar-CH), 8.09 (d, J = 8 Hz, 2H, Ar-CH); ^{1}H-NMR (DMSO-d_6) δ 8.89 (s, 1H, –OH); FAB-MS m/z 351 (M + H). Anal. ($C_{23}H_{26}O_3$) C, H.

1,2,3,4-Tetrahydro-1,1,4,4-tetramethyl-6-methoxynaphthalene (2j). Compound **2j** was synthesized from **1j** following the representative procedure described for **2b** except that anisole (**1j**) was used as solvent and reagent. The compound was distilled at 142 °C, 1 mmHg, to give a clear liquid which immediately formed hard white crystals of 1,2,3,4-tetrahydro-1,1,4,4-tetramethyl-6-methoxynaphthalene (**2j**) (86% yield): mp 40–42 °C; ^{1}H-NMR ($CDCl_3$) δ 1.28 (s, 6H, CH_3), 1.29 (s, 6H, CH_3), 2.42 (s, 4H, CH_2), 3.66 (s, 3H, $-OCH_3$), 7.50 (s, 1H, Ar-CH), 7.69 (d, J = 7.5 Hz, 1H, Ar-CH), 8.13 (d, J = 7.5 Hz, 1H, Ar-CH). Anal. ($C_{15}H_{22}O$) C, H.

Methyl 4-[(3-Methoxy-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoate (3j). Compound **3j** was synthesized from **2j** following the representative procedure described for **3a** except that hexanes were used as solvent instead of CH_2Cl_2. Crystallization from hot methanol gave ketone **3j** as white crystals (35% yield): TLC (20% ethyl acetate–80% hexanes) R_f 0.5; mp 145–147 °C; ^{1}H-NMR ($CDCl_3$) δ 1.25 (s, 6H, CH_3), 1.33 (s, 6H, CH_3), 1.71 (s, 4H, CH_2), 3.66 (s, 3H, $-OCH_3$), 3.95 (s, 3H, CO_2CH_3), 6.86 (s, 1H, Ar-CH), 7.38 (s, 1H, Ar-CH), 7.88 (d, J = 8 Hz, 2H, Ar-CH), 8.08 (d, J = 8 Hz, 2H, Ar-CH); HRFAB-MS (M + H) calcd for $C_{24}H_{29}O_4$ 381.2066, found 381.2075. Anal. ($C_{24}H_{28}O_4$) C, H.

4-[(3-Methoxy-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic Acid (5j). Compound **5j** was synthesized from **3j** following the representative procedure described for **5a**. Crystallization gave **5j** as a white powder (49% yield): TLC (10% MeOH–90% $CHCl_3$) R_f 0.3; mp 195–197 °C; ^{1}H-NMR ($CDCl_3$) δ 1.25 (s, 6H, CH_3), 1.34 (s, 6H, CH_3), 1.71 (s, 4H, CH_2), 3.66 (s, 3H, $-OCH_3$), 6.89 (s, 1H, Ar-CH), 7.40 (s, 1H, Ar-CH), 7.89 (d, J = 8 Hz, 2H, Ar-CH), 8.19 (d, J = 8 Hz, 2H, Ar-CH); EI-MS m/z 366 (M^+), 351 (M^+ – CH_3). Anal. ($C_{23}H_{26}O_4$) C, H.

Methyl 4-[1-(3-Methoxy-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoate (4j). Compound **4j** was synthesized from **3j** following the representative procedure described for **4a**. White crystals of **4j** were obtained in 60% yield: TLC (20% ethyl acetate–80% hexanes) R_f 0.7; mp 137–138 °C; ^{1}H-NMR ($CDCl_3$) δ 1.26 (s, 6H, CH_3), 1.33 (s, 6H, CH_3), 1.71 (s, 4H, CH_2), 3.60 (s, 3H, $-OCH_3$), 3.95 (s, 3H, CO_2CH_3), 5.42 (s, 1H, =CH), 5.84 (s, 1H, =CH), 7.20 (s, 1H, Ar-CH), 7.35 (s, 1H, Ar-CH), 7.45 (d, J = 8 Hz, 2H, Ar-CH), 7.95 (d, J = 8 Hz, 2H, Ar-CH). Anal. ($C_{25}H_{30}O_3$) C, H.

4-[1-(3-Methoxy-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoic Acid (6j). Compound **6j** was synthesized from **4j** following the representative procedure described for **6a**. Fine white crystals of **6j** were obtained in 96% yield: TLC (10% MeOH–90% $CHCl_3$) R_f 0.5; mp 199–200 °C; ^{1}H-NMR ($CDCl_3$) δ 1.27 (s, 6H, CH_3), 1.32 (s, 6H, CH_3), 1.71 (s, 4H, CH_2), 3.58 (s, 3H, $-OCH_3$), 5.45 (s, 1H, =CH), 5.79 (s, 1H, =CH), 6.80 (s, 1H, Ar-CH), 7.14 (s, 1H, Ar-CH), 7.40 (d, J = 8 Hz, 2H, Ar-CH), 8.00 (d, J = 8 Hz, 2H, Ar-CH); FAB-MS m/z 364 (M^+), 365 (M + H); HRFAB-MS (M + H) calcd for $C_{24}H_{29}O_3$ 365.2117, found 365.2120. Anal. ($C_{24}H_{28}O_3$) C, H.

Molecular Modeling Studies. Conformational searching was performed using the Quanta/Charmm molecular modeling software package from Molecular Simulations, Inc.[16] Each minimized structure was subjected to a grid scan conformational search using a 20° torsion angle window and 50 steps of conjugate gradient minimization on each fixed torsion angle conformer. The Quanta cluster analysis routine was used on all conformers within 3.5 kcal of the minima structure using a 3.4 rms cutoff over all non-hydrogen atoms. The resulting minima structure of each cluster was displayed to prepare Figure 4, top.

Biology. Cotransfection Assay. The receptor expression vectors used in the cotransfection assay have been described previously (pRShRARα,[20] pRShRARβ,[21] pRShRARγ,[21] pRShRXRα,[11] pRSmRXRβ,[14] pRSmRXRγ[22]). Briefly, the appropriate cDNAs were cloned into an expression vector in which expression is under the control of the RSV-LTR (rous sarcoma virus long terminal repeat). A basal reporter plasmid, MTV-LUC, containing two copies of the palindromic thyroid hormone response element (TREp2)[13] was used for all cotransfections with RARs. The RXR-responsive reporter plasmids were constructed by inserting an oligonucleotide containing either the CRBPII (cellular retinoic acid-binding protein II)-responsive element (for RXRα,γ)[23] or the CPRE3 (COUP response element 3)-responsive element (for RXRβ)[24] into the thymidine kinase luciferase plasmid. All cotransfections were carried out in CV-1 cells as previously described[11] but modified for automation (Beckman Biomek automated workstation) and the use of 96-well plates.[12] The plasmids were transiently transfected into CV-1 cells by the calcium phosphate coprecipitation method.[25]

Binding Assay. [^{3}H]-*all-trans*-Retinoic acid (40–60 Ci/mmol) was purchased from New England Nuclear (Boston, MA). ATRA was purchased from Sigma Chemical Co. (St. Louis, MO). [^{3}H]-9-*cis*-Retinoic acid (29 Ci/mmol) and unlabeled 9-*cis* retinoic acid were synthesized as previously described.[10] All other reagents were obtained from Sigma Chemical Co. The six retinoic acid receptor subtypes (RARα,β,γ and RXRα,β,γ) were derived from the cDNAs expressed in a baculovirus expression system.[14,26] The methods concerning growth, purification, and assays of recombinant viruses followed the protocol outlined by Summers and Smith.[27] The recombinant plasmids were cotransfected into SF21 cells with wild-type AcNPV DNA, and the recombinant viruses were plaque purified. For the mock (control) extracts, wild-type AcNPV-infected cells were used.

A receptor extract was prepared from the baculovirus system for each receptor, and aliquots were stored at –80 °C until used. Typical protein concentrations for these extracts were between 10 and 20 mg/mL. All the receptors were human

except for RXRβ and RXRγ which were derived from mouse cDNA. Stock solutions of ATRA, 9-*cis*-RA, or other competing compounds were prepared as either 5 mM ethanol or DMSO stock solutions, and serial dilutions were carried out in 1:1 DMSO–ethanol. The assay buffer consisted of the following for all six receptor assays: 8% glycerol, 120 mM KCl, 8 mM Tris, 5 mM CHAPS, 4 mM DTT, and 0.24 mM PMSF, pH = 7.4, at room temperature.

Receptor assays for all six receptors were performed in a similar manner with a final volume of 250 μL containing from 10 to 40 μg of extract protein, depending on the receptor being assayed, plus 5 nM [^{3}H]ATRA (RARα,β,γ) or 10 nM [^{3}H]-9-*cis*-RA (RXRα,β,γ) and varying concentrations of competing ligand ($0-10^{-5}$ M). Assays were set up, in part, using in-house computer programs developed for the Biomek and formatted for a 96-well minitube system. Incubations were carried out at 4 °C for 18 h. Equilibrium under these conditions of buffer and temperature was achieved by 4 h. Nonspecific binding was defined as that binding remaining in the presence of 1000 nM appropriate unlabeled retinoic acid isomer. At the end of the incubation period, 50 μL of 6.25% hydroxyapatite was added in wash buffer (see below). Specific ligand binding to receptor was determined by a hydroxyapatite-binding assay according to the protocol of Wecksler and Norman.[28] Hydroxyapatite absorbs the receptor–ligand complex, allowing for the separation of bound from free radiolabeled ligand. The wash buffer consisted of 100 mmol of KCl, 10 mmol of Tris, and either 5 mmol of CHAPS (RXRα,β,γ) or 0.5% Triton X-100 (RARα,β,γ). The mixture was vortexed and incubated for 10 min at 4 °C and centrifuged, and the supernatant was removed. The hydroxyapatite pellet was washed three more times with the appropriate wash buffer. The amount of receptor–ligand complex was determined by liquid scintillation counting of the hydroxyapatite pellet.

After correcting for nonspecific binding, IC_{50} values were determined. The IC_{50} value is defined as the concentration of competing ligand required to decrease specific binding by 50%; the IC_{50} values were determined graphically from a log–logit plot of the data. K_d values for the analogs were calculated by application of the Cheng–Prussof equation.[29] K_d values for ATRA or 9-*cis*-RA were determined by use of a modified Cheng–Prussof equation as described by Motulsky.[30]

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