Synthesis and Structure–Activity Relationships of Stilbene Retinoid Analogs Substituted with Heteroaromatic Carboxylic Acids

Richard L. Beard,[†] Roshantha A. S. Chandraratna,*[,†,‡] Diana F. Colon,[†] Samuel J. Gillett,[†] Elizabeth Henry,[†] Deborah K. Marler,[†] Tae Song,[†] Lydia Denys,[†] Michael E. Garst,[†] Taghreed Arefieg,[‡] Elliott Klein,[‡] Daniel W. Gil,[‡] Larry Wheeler,[‡] Devendra M. Kochhar,[§] and Peter J. A. Davies[⊥]

Retinoid Research, Departments of Chemistry and Biology, Allergan, Incorporated, Irvine, California 92715-1599, Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson University, Philadelphia, Pennsylvania 19107, and Departments of Pharmacology and Medicine, University of Texas Medical School, Houston, Texas 77225

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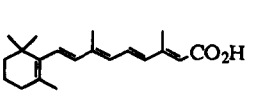
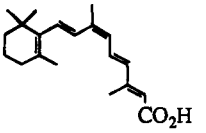
Retinoids elicit biological responses by activating a series of nuclear receptors. Six retinoid receptors belonging to two families are currently known: retinoic acid receptors ($RAR_{\alpha,\beta,and\gamma}$) and retinoid X receptors ($RXR_{\alpha,\beta,and\gamma}$). Stilbene retinoid analogs of retinoic acid (RA), such as (*E*)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)propen-1-yl]benzoic acid (TTNPB, **1**) and (*E*)-4-[2-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl)propen-1-yl]benzoic acid (3-methyl-TTNPB, **2**), display differential RAR and RXR activities, depending on the substituent at C3 of the naphthalene ring. We report here structural modifications of the benzoate moiety of **2** that result in analogs with greater RXR selectivity as well as those with pan-agonist (activate both RAR and RXR receptors) activities, analyze the structural features that impart receptor selectivity, and describe a stereoselective method for the synthesis of these analogs. The biological activities associated with the RAR and RXR receptors were examined by testing representative examples with different receptor activation profiles for their ability to induce tissue transglutaminase (Tgase) activity in a human promyelocytic leukemia cell line (HL-60 cdm-1) and to inhibit tumor-promoter-induced ornithine decarboxylase (ODC) activity in hairless mouse skin. These results suggest that RAR agonists and RXR agonists may have different therapeutic applications. Finally, we show that RXR agonists are significantly reduced in teratogenic potency relative to RAR agonists and may therefore have significant advantages in clinical practice.

Introduction

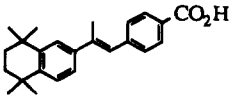
The physiological retinoids are hormones that regulate a wide variety of basic biological functions including cell differentiation and proliferation.[1] Because of their far ranging biological effects, retinoids have found clinical applications in dermatology[2] and oncology[3] and show promise in other diverse therapeutic areas including arthritis,[4] dyslipidemias,[5] and the prevention of HIV-induced lymphopenia.[6] Retinoids are believed to function primarily by regulating gene transcription through nuclear receptors.[7] There are six known retinoid receptors which belong to two families: the retinoic acid receptor family ($RAR_{\alpha,\beta,and\gamma}$)[8] and the retinoid X receptor family ($RXR_{\alpha,\beta,and\gamma}$).[9] *all-trans*-Retinoic acid (RA), the physiological hormone for the RAR family, specifically transactivates only the RARs. However, 9-*cis*-retinoic acid (9-*cis*-RA), the putative hormone for the RXRs, binds to and transactivates both RXRs and RARs.[10] The biology associated with the retinoid receptors is further complicated by the fact that they interact with the promoter regions of genes as dimers and that both homodimers and heterodimers are possible. Under physiological conditions the RARs are always believed to form heterodimers with RXRs.[11] RAR ligands can clearly initiate RAR hormonal pathways through the RAR–RXR heterodimers, and it is possible that RXR ligands may modulate these pathways by binding to the RXR component of the heterodimer. The RXRs can also form homodimers in the presence of RXR ligands and regulate gene transcription.[12] RXRs also form heterodimers with the thyroid, vitamin D, and other receptors,[11] and the question remains as to whether RXR ligands can modulate these hormonal pathways as well. There are numerous examples of RAR active ligands, and the biology associated with the RAR family as a whole is fairly well-defined. However, there have been only a few reports on RXR-selective ligands,[13] and as a consequence the biology associated with the RXR family is not well-understood.



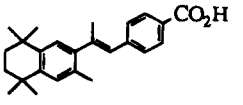
RA



9-*cis*-RA



TTNPB (1)



3-Methyl TTNPB (2)

Stilbene analogs of RA, such as (*E*)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)propen-1-yl]benzoic acid (TTNPB, **1**), are potent RAR agonists.

* Corresponding author: Roshantha A. S. Chandraratna, Director, Retinoid Research, Department of Chemistry, Allergan, Inc., 2525 Dupont Dr., Irvine, CA 92713–9534.
[†] Department of Chemistry, Allergan.
[‡] Department of Biology, Allergan.
[§] Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson University.
[⊥] Departments of Pharmacology and Medicine, University of Texas.
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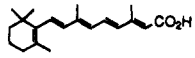
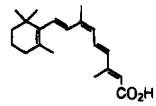
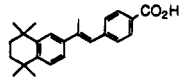
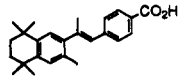
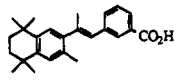
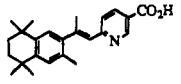
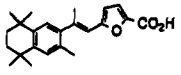
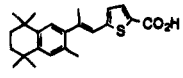
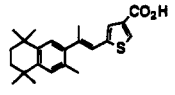
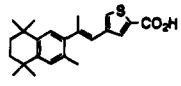
Recently, we[14] and others[15] reported that simple modifications (substitution at C3) of the stilbene skeleton of TTNPB can lead to retinoid analogs of significant potency at the RXR receptor. Thus, 3-methyl-TTNPB (**2**) is an effective activator of RXR_α while TTNPB is essentially inactive at this receptor. We ascribed this RXR selective activity primarily to a conformational effect resulting from the steric interaction between the C3 substituent and the C10 hydrogen. These findings prompted us to further investigate structural changes of the stilbene skeleton to explore the structure–activity relationships associated with RAR or RXR activity of 3-substituted stilbene analogs such as 3-methyl-TTNPB. We report here structural modifications of the benzoate moiety of 3-methyl-TTNPB that result in analogs with greater RXR selectivity as well as those with pan-agonist (activate both RAR and RXR receptors) activities, examine the structural features that impart receptor selectivity, and describe a stereoselective method for the synthesis of these analogs. We also demonstrate biological activities in nontransfected cells that are associated with the RXR activity of these compounds, suggesting that RXR agonists may have therapeutic applications that are not available to RAR agonists. Finally, we show that RXR agonists are significantly reduced in teratogenic potency relative to RAR agonists and may therefore have significant advantages in clinical practice.

Results and Discussion

Chemistry. The analogs used in this study are summarized in Table 1. 9-*cis*-RA, TTNPB (**1**), and 3-methyl-TTNPB (**2**) were synthesized according to methods described elsewhere.[16] Analogs **3** and **5** were prepared from ketone **12**[17] by Horner–Emmons–Wadsworth (HEW) methodology as depicted in Scheme 1. Thus, 3-carboxybenzyl bromide (**10**) was esterified and then heated with triethyl phosphite to give phosphonate **11** in 42% overall yield. The phosphonate was coupled to ketone **12** to give the *E*- and *Z*-olefins as a 1:2 mixture in 81% yield. We used a phosphazene base (P_4-*t*-Bu)[18] for the HEW coupling reaction since other anionic bases (NaH, $NaCH_2SOCH_3$, $NaN(TMS)_2$, $KN(TMS)_2$) were either unreactive or produced complex reaction mixtures. Although the *E*:*Z* ratio of stilbene retinoid analogs can sometimes be increased by subjecting them to various equilibrating conditions,[19] we found that the same conditions applied to 3-methyl analogs generally favor the formation of the *Z*-olefins. Thus, subjecting this mixture to various equilibrating conditions did not alter the *E*:*Z* olefin ratio. After HPLC separation, ester **13** was hydrolyzed to give (*E*)-3-[2-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl)propen-1-yl]benzoic acid (**3**). Phosphonate **14**[20] was treated with sodium bis(trimethylsilyl)amide and the resulting ylide coupled to **12** to give the olfein as 1:3 mixture of *E*- and *Z*-isomers in 51% yield. The pure *E*-isomer **15** was obtained by recrystallization. Hydrolysis of the ester produced (*E*)-5-[2-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl)propen-1-yl]-2-furoic acid (**5**) in 95% yield.

Thiophene analogs **6**–**8** were prepared by Wittig reactions involving phosphonium salt **17**[17] as shown in Scheme 2. The 2,5-substituted thiophene analog **6** was prepared by a Wittig reaction of the ylide of **17** and commercially available 5-bromo-2-thiophenecarboxaldehyde (**16**), followed by carboxylation (*n*-BuLi, CO_2, H^+) of the resulting aryl bromide. The 2,4-substituted thiophene analog **7** was prepared by the same method. Aldehyde **19**[21] was coupled with the ylide of **17** and the resulting ester (**20**) hydrolyzed with aqueous lithium hydroxide in THF to give the alternate 2,4-substituted analog **8**. These Wittig reactions with the thiophene aldehydes were highly stereoselective, producing the *E*-isomers exclusively.

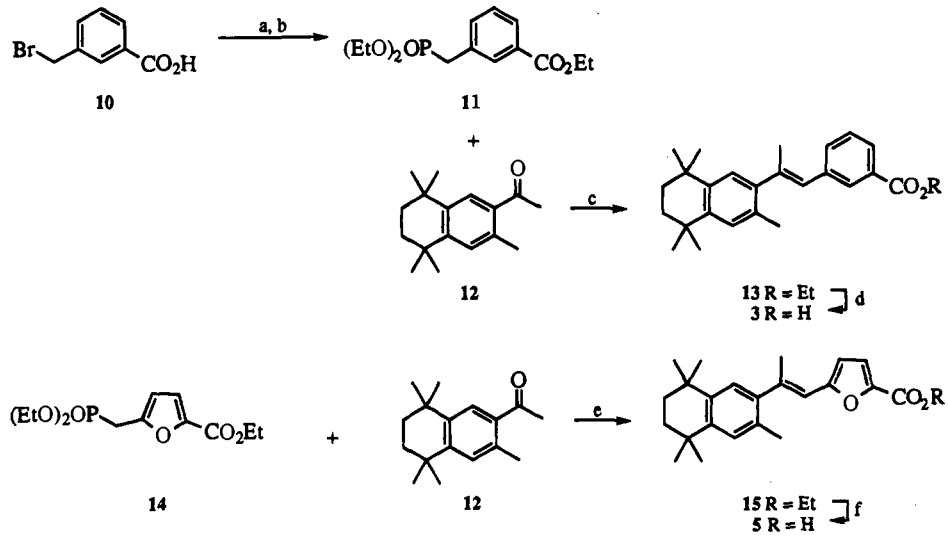
Table 1.[a] Transcriptional Activation Assay Data for Analogs of 3-Methyl-TTNPB[26]

entry	Structure	number/ name	EC_{50} (nM) RAR_α	RAR_β	RAR_γ	RXR_α
i		RA	5.0	1.5	0.5	NA
ii		9-*cis* RA	102	3.3	6.0	13
iii		**1**	21	4.0	2.4	NA
iv		**2**	4580	74	152	385
v		**3**	NA	NA	NA	NA
vi		**4**	2690	21	374	105
vii		**5**	NA	NA	NA	592
viii		**6**	NA	23	37	11
ix		**7**	NA	NA	NA	201
x		**8**	NA	1080	NA	112

[a] NA indicates not active (i.e., $EC_{50} > 10^4$ nmol).

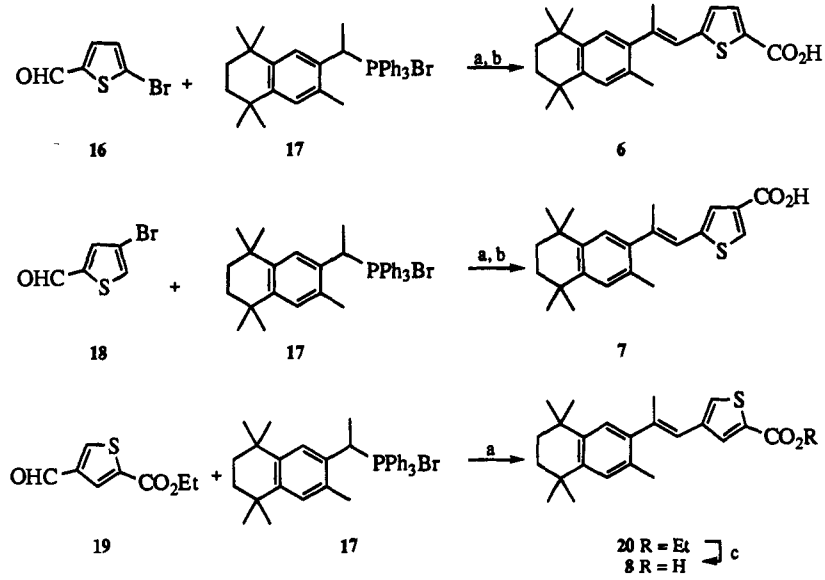
Each of the synthetic methods described above has distinct drawbacks: the HEW reaction is not very stereoselective and often produces the *Z* isomer as the major product, while the Wittig couplings are relatively low-yielding. Moreover, the starting materials for some systems are difficult to prepare, and the methodology is not always successful. In order to overcome these difficulties, a new approach to these compounds was investigated. Acetylenes are known to undergo regioselective carbometalation reactions with a number of organometallic reagents.[22] Negishi's carboalumination procedure[23] is particularly well-suited for our purposes since the carbometalated product can be coupled to aryl halides, the starting materials could be prepared in large quantities by literature procedures and the reactions proceeded in a regio- and stereospecific manner. The methodology we utilized is illustrated by the example shown in Scheme 3. Thus, in a one-pot reaction, acetylene **21** was carboaluminated with trimethylaluminum and coupled to ethyl 6-iodonicotinate

Scheme 1[a]



[a] (a) EtOH, H_2SO_4; (b) $P(OEt)_3$; (c) P_4-*t*-Bu, THF; (d) aqueous KOH, EtOH; 10% HCl; (e) $NaN(TMS)_2$, THF; (f) aqueous LiOH, THF; 10% HCl.

Scheme 2[a]



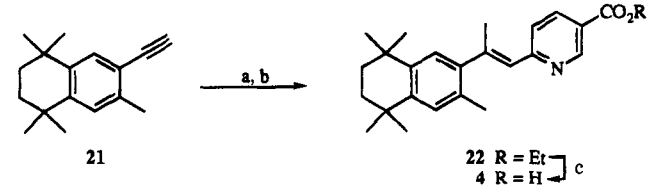
[a] (a) **17**, *n*-BuLi, THF; aldehyde; (b) *n*-BuLi, THF, −78 °C; CO_2; H^+; (c) aqueous LiOH, THF; 10% HCl.

to give isomerically pure stilbene **22** in 52% yield. Saponification of the ester gave carboxylic acid **4**.

The *E*/*Z*-geometry of these analogs was assigned on the basis of the ^{13}C NMR chemical shift of the C9 methyl group: chemical shifts for the *E*-isomers range from 17 to 22 ppm and for the *Z*-isomers are >27 ppm as reported by Strickland *et al.*[24] In all cases, the olefin geometry was confirmed by ^1H NMR NOE difference experiments.

Biology. We determined the transactivation properties of the retinoid analogs by measuring their ability to induce gene transcription in cells transiently cotransfected with a chimeric receptor gene construct and a reporter gene. Retinoid receptors are members of the steroid receptor superfamily of nuclear receptors that are characterized by homologous functional domains that can be interchanged among the receptors with retention of function. In our assays, we used chimeric receptors that contain the amino terminus and DNA-binding domain of the estrogen receptor (ER) and the

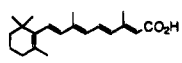
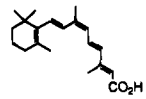
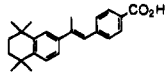
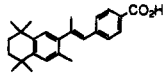
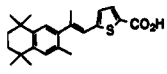
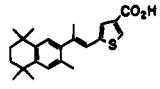
Scheme 3[a]



[a] (a) $AlMe_3$, $ZrCp_2Cl_2$; (b) ethyl 6-iodonicotinate, $Pd(PPh_3)_4$; (c) aqueous KOH, EtOH; 10% HCl.

hormone-binding domain of the retinoid receptors (RAR or RXR). These chimeric receptors (ER-RAR or ER-RXR) bind to and activate transcription from promoter sequences recognized by the estrogen receptor (estrogen response element, ERE) but do so in response to a retinoid.[25] In conjunction with the chimeric receptors, we used a chloramphenicol transferase (CAT) reporter gene which was under the control of an estrogen responsive promoter. This ERE-CAT reporter could be activated in the presence of an appropriate retinoid

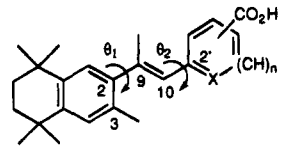
Table 2. Biological Assay Data for Representative Retinoid Analogs

Compound		Tgase Induction	ODC Inhibition	Chondrogenesis Inhibition
number	structure	EC_{50} (nM)	IC_{80} (nmol)	IC_{50} (nM)
RA		5	1.4	31
9-*cis*-RA		2	3.6	58
1		>1000	0.08	0.06
2		10	1.2	8.0
6		5	5.4	15
7		20	> 300	63

agonist by the transfected ER-RAR (or ER-RXR) receptors, but not by the complement of endogenous retinoid receptors. Thus, this stratagem provides a clean readout of activity at only the transfected receptor and avoids confounding effects due to endogenous retinoid receptors which are present in every mammalian cell. We determined the transactivational potencies of analogs at each of the RAR subtypes (α, β, and γ) and at RXR_α (Table 1).[26] In this assay, RA is a specific activator of only the RARs while 9-*cis*-RA activates RXR_α and the RARs. As reported previously,[14] TTNPB (**1**) is a potent and specific activator of the RARs. The introduction of an α-methyl group as in 3-methyl-TTNPB (**2**) results in a dramatic reversal of activity in that 3-methyl-TTNPB (**2**) is significantly more potent than TTNPB (**1**) at RXR_α while it is less potent at all the RARs. The *meta*-substituted analog, **3**, is completely inactive at RXR_α and the RARs. The nicotinic acid analog, **4**, has an essentially identical receptor profile to the benzoic acid congener. The thiophene analog, **6**, is very active at RXR_α, being equipotent with the putative physiological hormone 9-*cis*-RA. Interestingly, compound **6** is also quite active at RAR_β and RAR_γ and has a pan-agonist profile similar to 9-*cis*-RA except that **6** is inactive at RAR_α. The 3-carboxylthiophene analog, **7**, has no activity at the RARs and is a RXR-specific compound. The thiophene analog with reversed connectivity, compound **8**, is also RXR selective but it has measurable activity at RAR_β. The 2,5-disubstituted furan analog **5** is also a RXR-specific agonist although of reduced potency.

We also wanted to determine the biological activity of these analogs in nontransfected cells in order to determine whether RXR agonists can elicit activity through the endogenous complement of receptors. The ability to induce tissue transglutaminase (Tgase) activity in a human promyelocytic leukemia cell line (HL-60 cdm-1)[27] and to inhibit tumor-promoter-induced ornithine decarboxylase (ODC) activity in hairless mouse skin[28] were determined for representative examples of retinoid analogs (Table 2). The natural retinoids, RA and 9-*cis*-RA, both induce Tgase activity in HL-60 cells in a potent manner. However, the specific RAR agonist, TTNPB (**1**), was essentially inactive in this assay. In contrast, the structurally related analogs, **2**, **6**, and **7**, which all activated RXR_α, also induced Tgase activity. RA, 9-*cis*-RA, and all the analogs that had significant activity at the RARs (compounds **1**, **2**, and **6**) all inhibited ODC induction in an effective manner. In striking contrast, the RXR specific analog, **7**, was quite ineffective in inhibiting ODC activity. Finally, we determined the activity of these analogs in an assay of retinoid toxicity, namely that of inhibition of chondrogenesis in mouse embryo limb bud mesenchymal cells (Table 2).[29] This assay is predictive of the teratogenic potential of retinoids.[30] RA and 9-cis-RA are moderately potent in chondrogenesis inhibition while TTNPB (**1**) is about a 100 times more potent in keeping with its arotinoid structure.[31] However, the 3-methyl homolog, **2**, which also has an arotinoid structure is more than a 100-fold less potent than TTNPB. The RXR-specific compound, **7**, is the least active in this assay being about a 1000-fold less potent than TTNPB.

Table 3. Dihedral Angles for the Aromatic Analogs in Table 1[a,33]



no.	θ_1, deg	θ_2, deg	no.	θ_1, deg	θ_2, deg
1	−38.7	48.7	5	53.6	−179.1
2	71.7	52.6	6	58.8	31.6
3	72.8	44.0	7	59.2	30.1
4	72.2	−164.0	8	59.0	28.8

[a] Structures were minimized using the AM1 semiempirical model from the Cache MOPAC application developed by Tektronix, Irvine, CA.

Molecular Modeling. In order to explain the differences in the receptor activity of these compounds, it is helpful to examine their energy-minimized structures. In an earlier report,[14] we attributed differences in receptor selectivity between TTNPB (**1**) and 3-methyl-TTNPB (**2**) to the difference in the dihedral angle θ_1 about the C2−C9 single bond resulting from the steric interaction between the C3 substituent and the C10 hydrogen in 3-methyl-TTNPB (**2**). In examining modifications of the right half of the stilbene skeleton, it is also important to consider the dihedral angle θ_2 about the C10−C2′ bond. We used a computer-assisted molecular modeling program[32] to examine conformational differences in the energy minimized structures of each of the analogs described in Table 1. The results are summarized in Table 3.

Discussion

We reported[14] previously that the RXR selective activity of C3-substituted stilbenes can be ascribed to a conformational effect about the C2−C9 bond resulting from the steric interaction between the C3 substituent and the C10 hydrogen. In addition to causing a twist of the C2−C9 bond, the C3 substituent may contribute to the RXR activity of these compounds by occupying the same binding pocket in the RXRs that is occupied by the C9 methyl of 9-*cis*-RA. The data in Table 1 show that structural differences in the right-half aromatic ring profoundly influence the receptor activity profiles

exhibited by these stilbene-like analogs. The overall picture that emerges is one in which the receptor selectivities of the 3-methyl-substituted analogs are determined primarily by the orientation of the carboxyl group relative to the lipophilic left-hand ring. Since the dihedral angle θ_1 is relatively constant for all of the 3-substituted analogs, the differences in orientation result primarily from variations in the dihedral angle θ_2, which is dependent on a steric interaction between X on the right-hand aromatic ring and the C9 methyl substituent (Table 3).[33] The calculations summarized in Table 3 indicate that when the large S atom is present adjacent to the vinyl substituent (**6** and **7**) the right-hand aromatic ring is twisted out of the plane of the double bond and the S atom points away from the C9 methyl substituent. Likewise, if X is a large CH group, then the right-hand ring is similarly twisted as is the case in the phenyl analogs **2** and **3** and in the 2,4-substituted thiophene analog **8**. However, if X is a small atom such as N or O (**4** or **5**), then the right-hand aromatic ring is in the same plane as the double bond and X is pointed toward the C9 methyl group. Obviously, the relative orientation of the carboxyl group and the left-hand lipophilic ring would not be affected by differences in θ_2 in analogs which are symmetrically substituted about the right-hand ring. Thus, the symmetrically substituted analogs, 3-methyl-TTNPB (**2**) and the nicotinic acid analog **4**, have essentially identical receptor activity profiles in spite of the fact that the aromatic rings occupy quite different positions, being twisted in the former and coplanar in the latter relative to the double bond. The position occupied by the right-hand ring *per se* appears unimportant and the presence of a heteroatom as in **4** also appears to have no effect, suggesting that electronic effects are unimportant. Both compounds **2** and **4** have pan-agonist activity similar to 9-*cis*-RA in that they activate both RARs and RXR_α. In contrast, the unsymmetrically substituted *meta* derivative **3** is completely inactive at all the receptors.

For compounds possessing five-membered aromatic rings, the relative position of the carboxyl group is highly dependent on θ_2 and the receptor data on these compounds are quite informative. The thiophenecarboxylic acid analog **7** and the furoic acid analog **5** are selective RXR activators while the thiophenecarboxylic acid **6** is an effective activator of RXR_α and the RAR_β and RAR_γ subtypes. Interestingly, according to the calculations summarized in Table 3, the furan ring in **5** is twisted approximately 150° relative to the thiophene ring in **6** and **7**. This places the carboxyl group of **5** in about the same position as the carboxyl group of **7** but in a position different from the carboxyl of **6**. Thus, it would be expected that **5** and **7** have similar receptor activation properties and these would be different from those of **6**, and indeed this is what is observed.

Most of the extensive biology ascribed to retinoids that is described in the literature is that associated with RA and its synthetic analogs. Moreover, since 9-*cis*-RA, the putative hormone for the RXRs, also has RAR activating properties, it is virtually impossible to differentiate between the RAR- and RXR-mediated functions of 9-*cis*-RA. We tested representative examples of these analogs with different receptor activation profiles in some *in vitro* and *in vivo* assays of retinoid activity in order to determine whether they exhibit differential activity in nontransfected cells. This type of testing is of critical importance since transactivation assays are carried out under conditions in which large, nonphysiologic amounts of retinoid receptors are expressed in the target cell and the readout is made from multiple copies of a reporter gene which are not incorporated in the host genome. While these receptor-specific transactivation assays are powerful analytical tools, they may not necessarily reflect the physiological situation. The inhibition of tumor-promoter-induced ODC activity is a classical assay of the antiproliferative activity of retinoids. ODC catalyzes the synthesis of putrescine and is a rate-limiting enzyme in the biosynthesis of polyamines prior to the hyperproliferative response of cells.[34] Moreover, since ODC activity is elevated in psoriasis, its down-regulation by retinoids may have predictive value for retinoids effectiveness in the treatment of psoriasis and other hyperproliferative skin diseases.[35] TTNPB (**1**) and RA, which are RAR-specific compounds, are both effective inhibitors of TPA-induced ODC activity in hairless mouse skin with TTNPB being particularly potent in its inhibitory activity in keeping with its "arotinoid" nature (Table 2). The compounds that have pan-agonist receptor activity (9-*cis*-RA, **2** and **6**) also inhibit ODC activity with potencies more or less in keeping with their RAR-activating potencies. However, the RXR specific analog **7** is essentially inactive in this assay in spite of it possessing an arotinoid structure. These results suggest that only RAR-active compounds and not RXR-active compounds will have antiproliferative activities in skin by this mechanism.

Tgase activity is believed to be an important early marker of retinoid effects on gene expression.[27] The transglutaminases are a group of enzymes that covalently cross-link proteins,[36] and the induction of Tgase has been linked to the differentiation of several normal and transformed cell lines.[37] We examined the ability of these analogs to induce Tgase activity in a human promyelocylic leukemia cell line (HL-60), an activity that may be relevant to retinoid treatment of myeloid leukemias. TTNPB (**1**) which lacks any RXR activity was completely inactive in this assay while all analogs that had RAR and RXR activity (9-*cis*-RA, **2**, **6**) and the RXR specific analog **7** induced Tgase effectively (Table 3). Interestingly, RA also induced Tgase activity presumably because of conversion to 9-*cis*-RA under the assay conditions. These results suggest that RXR agonists have therapeutically pertinent biological effects in myeloid cells and may be useful in treating disorders of myeloid cells.

The clinical use of retinoids is often complicated by toxic side effects, most prominent of which is teratogenesis.[38] In order to determine whether there are any therapeutic advantages in terms of reduced toxicity for the use of RXR-specific analogs, we tested these analogs in an *in vitro* assay of retinoid teratogenicity, namely that of inhibition of chondrogenesis in mouse embryo limb bud cells.[29] Although all the compounds tested show some activity in this assay (Table 2), it is very informative to compare the activities of TTNPB (**1**) and its 3-methyl homolog **2**. While TTNPB (**1**) is very potent in this assay, 3-methyl-TTNPB (**2**) is more than 100-fold less potent, a differential which is reflective of their relative abilities to activate RARs. It is important to note that while 3-methyl-TTNPB (**2**) can also activate

RXR, TTNPB (**1**) cannot, indicating minimally that RXR activation is not required for chondrogenesis inhibition and therefore for teratogenicity. The RXR analog **7** is more than 1000-fold less active than TTNPB in this assay. The chondrogenic activity of **7** may be due to some residual RAR activity or due to weak intrinsic activity through RXRs. In any event, examination of the data in Tables 1 and 3 clearly indicated that *in vitro* teratogenicity parallels RAR activity and not RXR activity. These results indicate that the clinical use of RXR-specific agonists may be associated with a much lower risk of teratogenicity although more detailed studies are clearly warranted.

Conclusions

In summary, we have shown that heteroaromatic substitution of the benzoate moiety of 3-methyl-TTNPB results in analogs with greater RXR selectivity as well as those with pan-agonist activities. The RXR selectivity of these analogs is attributed to the relative position of the carboxylic acid moiety, which is influenced by changes in the dihedral angle about the C10−C2′ bond. These analogs were useful in elucidating differential biological activities of RXR- and RAR-selective compounds in nontransfected cells. Finally, we have demonstrated that the teratogenic potency of RXR selective agonists is decreased relative to RAR agonists. These studies also suggest that RXR agonists may have therapeutic applications that are not available to RAR agonists.

Experimental Section

General Procedures. RA (1) was obtained from Sigma Chemical Co. 9-*cis*-RA (**2**) was prepared by the procedure of Jong *et al.*[39] Analogs of TTNPB were prepared according to the procedures of Strickland *et al.*[16a] Solvents were used as purchased unless otherwise noted. When deemed necessary, reaction flasks containing magnetic stirbars were flame-dried, cooled under vacuum (<1 Torr), and flushed several times with dry argon before any reagents were added. Most reactions were monitored by analytical thin-layer chromatography (TLC) using Merck TLC glass plates precoated with silica gel 60 F_{254} (0.2 mm thick). Flash chromatography was performed using Merck silica gel 60. Melting points and boiling points are uncorrected. Unless the use of an internal thermometer is indicated, temperatures are reported as bath temperatures. IR spectra were obtained on a Mattson Galaxy Series 3000 FTIR spectrophotometer. NMR spectra were recorded on Gemini 300 (300 MHz) and XL 300 (300 MHz) Varian spectrometers. Mass spectral analyses were conducted on an EG 7070E organic mass spectrometer. Elemental analyses were conducted at Robertson Microlit Laboratories, Inc., Madison, NJ.

Triethyl 3-Phosphonobenzoate (11). Concentrated H_2SO_4 (6 mL) was added to a stirring suspension of 3-(bromomethyl)benzoic acid (20.0 g, 93 mmol) and 50 mL of ethanol. The suspension was heated to 70 °C for 16 h, cooled to room temperature, and basified with saturated aqueous $NaHCO_3$. The solution was concentrated under reduced pressure, and the products were extracted with ether (3×). The combined ether extracts were washed with water and brine and dried, (K_2CO_3) and the solvent was removed *in vacuo*. The crude ester was treated with triethyl phosphite (23.9 mL, 140 mmol) and heated to 150 °C for 12 h. The excess triethyl phosphite was removed *in vacuo* and the residue purified by Kugelrohr distillation (250 °C, 0.5 Torr) to give 11 (15.7 g, 56%) as a clear, colorless oil: IR 1720 cm^{-1} (C=O); ^1H NMR (300 MHz, $CDCl_3$) δ 1.26 (t, 6 H, J = 7.0 Hz), 1.40 (t, 3 H, J = 7.1 Hz) 3.20 (d, 2 H, J = 21.6 Hz), 4.03 (m, 4 H), 4.37 (q, 2 H, J = 7.1 Hz), 7.42 (m, 1 H), 7.52 (d, 1 H, J = 7.7 Hz), 7.94 (m, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.1, 16.1, 32.4, 34.2, 60.8, 61.9, 127.9, 128.4, 130.6, 131.9, 132.0, 134.0, 166.1; MS (EI, 70 eV) *m/z* 300 (M^+, 6), 299 (14), 254 (43), 266 (47), 164 (37), 118 (100), 109 (38), 90 (58); HRMS *m/z* (M^+) calcd 300.1126, obsd 300.1148. Anal. ($C_{14}H_{21}O_5P$) C, H.

Ethyl (*E*)-3-[2-(5,6,7,8-Tetrahydro-3,5,5,8,8-pentamethyl-2-naphthyl)propen-1-yl]benzoate (13) and Ethyl (*Z*)-3-[2-(5,6,7,8-Tetrahydro-3,5,5,8,8-pentamethyl-2-naphthyl)-propen-1-yl]benzoate. To a solution of phosphonate 11 (527 mg, 1.84 mmol) and 2.5 mL of THF at room temperature was added a 1.0 M solution of phosphazene base P_4-*t*-Bu[18] in hexane (1.84 mL, 1.84 mmol). The red solution was stirred at room temperature for 5 min, and ketone **12**[17] (300 mg, 1.23 mmol) was added in one portion. The solution was stirred until all of the ketone was consumed (about 1 h) and then diluted with water (0.5 mL) and ether (50 mL). The layers were separated, and the aqueous layer was extracted with ether (3×). The collected organic extracts were washed with 10% aqueous HCl and brine and dried ($MgSO_4$), and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (SiO_2, 5% ethyl acetate in hexanes) to give 390 mg of a yellow oil containing a 1:2 mixture of the *E*- and *Z*-isomers, respectively. Separation of the isomers was achieved by HPLC (Partisil-10 PAC column, 99:1 hexanes:ethyl acetate) to give ethyl (*Z*)-3-[2-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthyl)propen-1-yl]benzoate [IR 1716 cm^{-1} (C=O); ^1H NMR (300 MHz, $CDCl_3$) δ 1.18 (s, 3 H), 1.28 (2 s, 9 H), 1.33 (t, 3 H, J = 7.1 Hz), 1.56 (s, 4 H), 2.04 (s, 3 H), 2.17 (s, 3 H), 4.28 (q, 2 H, J = 7.1 Hz), 6.49 (s, 1H), 6.88 (d, 1 H, J = 7.7 Hz), 6.97 (s, 1 H), 7.07 (m, 2 H), 7.58 (s, 1 H), 7.69 (d, 1 H, J = 7.7 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.4, 18.9, 27.2, 31.8, 31.9, 33.8, 33.9, 35.15, 35.2, 60.7, 125.9, 126.0, 127.0, 127.7, 128.2, 129.5, 130.1, 131.1, 131.8, 138.0, 138.5, 140.7, 142.8, 143.6, 166.6; MS (EI, 70 eV) *m/z* 390 (M^+, 12), 389 (42), 376 (29), 374 (100), 328 (13); HRMS *m/z* (M^+) calcd 390.2559, obsd 390.2541. Anal. ($C_{27}H_{34}O_2$) C, H] and ethyl (*E*)-3-[2-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthyl)propen-1-yl]benzoate (**13**): IR 1722 cm^{-1} (C=O); ^1H NMR (300 MHz, $CDCl_3$) δ 1.30 (s, 12 H), 1.41 (t, 3 H, J = 7.1 Hz), 1.69 (s, 4 H), 2.19 (s, 3 H), 2.31 (s, 3 H), 4.40 (q, 2 H, J = 7.1 Hz), 6.41 (s, 1 H), 7.12 (s, 2 H), 7.43 (t, 1 H, J = 7.7 Hz), 7.55 (d, 1 H, J = 7.7 Hz), 7.92 (d, 1 H, J = 7.7 Hz), 8.06 (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.3, 19.7, 20.0, 31.8, 31.9, 33.9, 35.2, 61.0, 126.0, 127.3, 128.1, 128.1, 129.9, 130.4, 131.6, 133.2, 138.4, 140.6 142.2, 142.6, 143.4, 166.7; MS (EI, 70 eV) *m/z* 390 (M^+, 37), 376 (30), 375 (100), 329 (11); HRMS *m/z* (M^+) calcd 390.2559, obsd 390.2570. Anal. ($C_{27}H_{34}O_2$) C, H.

(*E*)-3-[2-(5,6,7,8-Tetrahydro-3,5,5,8,8-pentamethyl-2-naphthyl)propen-1-yl]benzoic Acid (3). To a solution of ester **13** (65.2 mg, 0.167 mmol) and 2.5 mL of EtOH was added 0.5 mL of 2 N aqueous KOH and the resulting solution stirred at 50 °C for 3 h. The solution was cooled to room temperature, diluted with 3 mL water, and washed with 1 mL of diethyl ether. The layers were separated, and the aqueous layer was treated with 5 mL of ether and acidified with 10% aqueous HCl. The layers were separated, and the aqueous layer was extracted with ether (3×). The collected organic extracts were washed with brine and dried ($MgSO_4$), and the solvent was removed under reduced pressure. The residue was recrystallized (1% ethyl acetate in hexanes) to give 49 mg (81%) of **3** as a colorless, crystalline solid: mp 216−218.5 °C; IR 3300−2500 (COOH), 1686 (C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.30 (s, 12 H), 1.69 (s, 4 H), 2.21 (s, 3 H), 2.32 (s, 3 H), 6.43 (s, 1 H), 7.12 (s, 2 H), 7.51 (t, 1 H, J = 7.7 Hz), 7.61 (d, 1 H, J = 7.7 Hz), 7.80 (d, 1 H, J = 7.7 Hz), 8.15 (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.7, 20.1, 31.9, 31.9, 33.9, 35.2, 126.0, 127.8, 128.0, 128.1, 128.4, 129.1, 130.5, 131.6, 134.3, 138.7, 141.0, 142.2, 142.6, 143.5, 171.6; MS (EI, 70 eV) *m/z* 362 (M^+, 29), 348 (27), 347 (100); HRMS *m/z* (M^+) calcd 362.2245, obsd 362.2243. Anal. ($C_{25}H_{30}O_2$) C, H.

Ethyl (*E*)-5-[2-(5,6,7,8-Tetrahydro-3,5,5,8,8-pentamethyl-2-naphthyl)propen-1-yl]-2-furancarboxylate (15). A solution of sodium bis(trimethylsilyl)amide (2.57 g, 12.6 mmol) and ethyl 5-[(diethylphosphono)methyl]-2-furancarboxylate (**14**)[20] (3.65 g, 12.6 mmol) in 15 mL of THF was stirred at −10 to 0 °C for 45 min. Methyl 3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl ketone (2.02 g, 8.3 mmol)[17] was added, and the

resulting mixture was allowed to warm to room temperature and stirred for 48 h. The mixture was concentrated and the residue taken up in water and extracted with methylene chloride and then ether. The organic layers were combined, washed with brine, dried ($MgSO_4$), and concentrated *in vacuo* to give an orange solid. The residue was purified by flash chromatography on silica gel (1% ethyl acetate in hexanes, then 5% ethyl acetate in hexanes) followed by recrystallization from hexane to yield 1.61 g (51%) of **15** as a white solid: mp 145–148 °C; IR (KBr) 1722 (C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.27 (s, 6 H), 1.29 (s, 6 H), 1.39 (t, 3 H, J = 7.1 Hz), 1.68 (s, 4 H), 2.27 (s, 3 H), 2.35 (s, 3 H), 4.37 (q, 2 H, J = 7.1 Hz), 6.31 (s, 1 H), 6.43 (d, 1 H, J = 3.6 Hz), 7.07 (s, 1 H), 7.10 (s, 1 H), 7.22 (d, 1 H, J = 3.6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.4, 19.9, 21.2, 31.8, 31.8, 33.9, 35.1, 35.1, 60.8, 110.3, 117.2, 119.4, 125.8, 128.3, 131.4, 142.1, 142.2, 142.7, 143.3, 143.9, 157.0, 158.9; MS (EI, 70 eV) m/z 380 (M^+, 100), 365 (60), 319 (15.5). Anal. ($C_{25}H_{32}O_3$) C, H.

(*E*)-5-[2-(5,6,7,8-Tetrahydro-3,5,5,8,8-pentamethyl-2-naphthyl)propen-1-yl]-2-furancarboxylic Acid (5). A solution of lithium hydroxide monohydrate (0.32 g, 7.5 mmol) and 0.5 mL of water, 4.0 mL of ethanol, and 25 mL of methanol was added to ethyl ester **15** (0.20 g, 0.53 mmol). To this was added 6.0 mL of methylene chloride and the resulting solution stirred at room temperature for 80 h. The solvent was removed *in vacuo* and the resulting solid taken up in water, acidified with 2 N HCl, and extracted with ether. The ether extracts were washed with water and brine and dried ($MgSO_4$). The solvent was removed *in vacuo* to yield 0.177 g (95 %) of **5** as a white solid: mp 231–232.5 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 1.28 (s, 6 H), 1.29 (s, 6 H), 1.68 (s, 4 H), 2.28 (s, 3 H), 2.37 (s, 3 H), 6.33 (s, 1 H), 6.48 (d, 1 H, J = 3.4 Hz), 7.08 (s, 1 H), 7.11 (s, 1 H), 7.38 (d, 1 H, J = 3.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.9, 21.3, 31.8, 31.8, 33.9, 35.1, 110.7, 117.1, 121.9, 125.8, 128.3, 131.4, 141.5, 141.9, 142.3, 144.0, 144.5, 158.2, 163.5; MS (EI, 70 eV) m/z 352 ($M^{\cdot+}$, 83), 337 (100); HRMS calcd for $C_{23}H_{28}O_3$ 352.2038, found 352.2066. Anal. ($C_{23}H_{28}O_3$) C, H.

(*E*)-5-Bromo-2-[2-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthyl)propen-1-yl]thiophene. To a suspension of the phosphonium salt **17**[17] (2.71 g 4.75 mmol) in 17 mL of DME at 0 °C under argon was added dropwise *n*-BuLi (6.7 mL, 10.7 mmol, 1.6 M in hexanes). The resulting red mixture was stirred at 0 °C to room temperature for 3 h. 5-Bromo-2-thiophenecarboxaldehyde (0.63 g, 0.39 mL, 3.28 mmoL) was added dropwise and the resulting mixture stirred for 72 h at room temperature. The reaction was cooled to 0 °C, brine was added, and the products were extracted two times with ether. The ether extracts were dried ($MgSO_4$), and the solvent was removed *in vacuo*. The resulting residue was purified by flash chromatography (SiO_2, 1% ethyl acetate in hexanes) to yield 1.10 g (58 %) of the title compound as a white solid: ^1H NMR (300 MHz, $CDCl_3$) δ 1.28 (s 12 H), 1.67 (s, 4 H), 2.23 (s, 3 H), 2.25 (s, 3 H), 6.45 (s, 1 H), 6.75 (d, 1 H, J = 3.9 Hz), 6.99 (d, 1 H, J = 3.9 Hz), 7.07 (s, 1 H), 7.09 (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.6, 20.7, 31.7, 31.7, 33.8, 35.0, 111.7, 122.3, 126.2, 127.1, 128.4, 129.8, 131.8, 138.6, 142.5, 142.8, 143.3, 143.9; m/z 402 ($M^{\cdot+}$, 81), 389 (100), 387 (96); HRMS calcd for $C_{22}H_{27}SBr$ 402.1017, found 402.1004.

(*E*)-5-[2-(5,6,7,8-Tetrahydro-3,5,5,8,8-pentamethyl-2-naphthyl)propen-1-yl]-2-thiophenecarboxylic Acid (6). To a solution of (*E*)-5-bromo-2-[2-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthyl)propen-1-yl]thiophene (2.16 g, 5.36 mmol) in 40 mL of THF was added *n*-BuLi (5.0 mL, 8.0 mmol, 1.6 M in hexanes) under argon at −78 °C. The resulting solution was stirred for 1 h, purged with carbon dioxide, and allowed to warm to room temperature slowly. After 24 h, the mixture was cooled to 0 °C, water was added, and the resulting mixture was partially concentrated *in vacuo*. The residue was acidified with concentrated HCl and extracted two times with a mixture of ether and methylene chloride (3:1). The organic layers were washed with brine and dried ($MgSO_4$). The solvent was removed *in vacuo* and the resulting solid recrystallized from methanol to give 1.22 g (62%) of **6** as a light green solid: mp 237–240 °C dec; IR (KBr) 3400 (COOH), 1664 (C=O) cm^{-1}; ^1H NMR (300 MHz, d_6-DMSO) δ 1.21 (s, 12 H), 1.60 (s, 4 H), 2.19 (s, 3 H), 2.24 (s, 3 H), 6.61 (s, 1 H), 7.06 (s, 1 H), 7.19 (d, 1 H, J = 4.0 Hz), 7.68 (d, 1 H, J = 4.0 Hz); ^{13}C NMR (75 MHz, d_6-DMSO) δ 19.4, 20.9, 31.5, 31.5, 33.5, 34.6, 34.6, 122.4, 125.6, 128.3, 128.9, 131.4, 133.4, 133.4, 140.9, 142.0, 142.4, 143.6, 147.2, 163.4; MS (EI, 70 eV) m/z 368 ($M^{\cdot+}$, 46), 353 (100), 309 (24). Anal. ($C_{23}H_{28}O_2S$) C, H, S.

(*E*)-4-Bromo-2-[2-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthyl)propen-1-yl]thiophene. To a suspension of phosphonium salt **17**[17] (0.56 g, 0.98 mmol) and THF (11 mL) at −78 °C under argon was added dropwise *n*-BuLi (0.61 mL, 0.98 mmol, 1.6 M in hexanes). The resulting suspension was allowed to warm to room temperature, and then a solution of 4-bromo-2-thiophenecarboxaldehyde (**18**) (0.28 g, 1.47 mmol) and 2 mL of THF was added. The resulting mixture was stirred for 20 h at room temperature. The solvent was removed *in vacuo* and the resulting solid taken up in water, acidified using 1 N HCl, and extracted three times with ether. The ether extracts were washed with water and brine and dried ($MgSO_4$). The solvent was removed *in vacuo* and the residue purified by flash chromatography (SiO_2, 0.5% ethyl acetate in hexanes) to yield 0.10 g (25%) of the title compound: ^1H NMR (300 MHz, $CDCl_3$) δ 1.27 (s, 6 H), 1.29 (s, 6 H), 1.68 (s, 4 H), 2.26 (s, 3 H), 2.27 (s, 3 H), 6.45 (s, 1 H), 6.75 (s, 1 H), 6.95 (s, 1 H), 7.07 (s, 1 H), 7.17 (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.6, 20.7, 31.7, 33.7, 35.0, 110.1, 121.3, 121.9, 126.1, 128.4, 129.0, 131.8, 140.1, 142.5, 142.6, 142.7, 144.0

(*E*)-2-[2-(5,6,7,8-Tetrahydro-3,5,5,8,8-pentamethyl-2-naphthyl)propen-1-yl]-4-thiophenecarboxylic Acid (7). To a solution of (*E*)-4-bromo-2-[2-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthyl)propen-1-yl]thiophene (500 mg, 1.24 mmol) and 15 mL of THF under argon at −100 °C was added *n*-BuLi (0.78 mL, 1.24 mmol, 1.6 M in hexanes). The reaction was stirred for 2 min and purged with carbon dioxide for 20 min. The reaction mixture was then allowed to warm to room temperature, acidified, and extracted with ether. The ether extracts were washed with water and brine and dried ($MgSO_4$). The solvent was removed *in vacuo* and the resulting residue taken-up in aqueous 2 N sodium hydroxide and washed with ether. The separated aqueous layer was acidified using 1 N HCl and extracted with ether. The combined ether layers were washed with water and brine and dried ($MgSO_4$). The solvent was removed *in vacuo* and the resulting material purified by flash chromatography on silica gel (10% ethyl acetate in hexanes) to yield 0.11 g (24 %) of **7** as a white solid: mp 223–225 °C; IR (KBr) 3400 (COOH), 1670 (C=O) cm^{-1}; ^1H NMR (300-MHz, d_6-DMSO) δ 1.23 (s, 12 H), 1.62 (s, 4 H), 2.21 (s, 3 H), 2.23 (s, 3 H), 6.56 (s, 1 H), 7.07 (s, 1 H), 7.13 (s, 1 H), 7.45 (s, 1 H), 8.24 (s, 1 H); ^{13}C NMR (75 MHz, d_6-DMSO) δ 19.5, 20.6, 31.6, 33.5, 34.6, 34.7, 121.9, 125.8, 127.9, 128.3, 131.4, 132.7, 134.2, 138.5, 141.6, 142.0, 143.4, 163.9; MS (EI, 70 eV, 400 °C) m/z 370 (M^+ + H, 4.6), 369 (15.6), 368 (M^+ − H, 58.4) 353 (100). Anal. ($C_{23}H_{28}O_2S$) C, H, S.

Ethyl (*E*)-4-[2-(5,6,7,8-Tetrahydro-3,5,5,8,8-pentamethyl-2-naphthyl)propen-1-yl]-2-thiophenecarboxylate (20). To a suspension of phosphonium salt **17** (1.24 g, 2.17 mmol) in THF (50 mL) was added *n*-BuLi (1.6 M in hexanes, 1.5 mL, 2.4 mmol). The resulting red solution was stirred for 30 min, and then aldehyde **19** (330 mg, 1.81 mmol) in 1.5 mL of THF was added. The solution was stirred at room temperature for 18 h to yield a peach-colored suspension. The solvent was evaporated and the residue partitioned between ether and saturated aqueous NH_4Cl solution. The organic layer was dried ($MgSO_4$), the solvent evaporated, and the resulting brown oil purified by flash chromatography (SiO_2, 5% ethyl acetate in hexanes) to give 323 mg (38%) of **20** as a clear, colorless oil: ^1H NMR (300 MHz, $CDCl_3$) δ 1.28 (s, 6 H), 1.29 (s, 6 H), 1.38 (t, 3 H, J = 7.1 Hz), 1.68 (s, 4 H), 2.23 (s, 3 H), 2.27 (s, 3 H), 4.36 (q, 2 H, J = 7.1 Hz), 6.30 (s, 1 H), 7.08 (s, 1 H), 7.11 (s, 1 H), 7.41 (d, 1 H, J = 1.2 Hz), 7.85 (d, 1 H, J = 1.2 Hz); ^{13}C (75 MHz, $CDCl_3$) δ 14.3, 19.8, 20.6, 31.8, 31.9, 33.9, 35.2, 61.2, 122.0, 126.0, 128.1, 129.0, 131.6, 133.3, 134.5, 139.9, 140.1, 142.2, 142.6, 143.5, 162.3; HRMS m/z (M^+) calcd 396.2123, found 396.2112.

(*E*)-4-[2-(5,6,7,8-Tetrahydro-3,5,5,8,8-pentamethyl-2-naphthyl)propen-1-yl]-2-thiophenecarboxylic Acid (8). To a suspension of ester **20** (48 mg, 0.12 mmol) and THF (5

mL) was added LiOH (2 mmol) as a 2 N solution in MeOH. The resulting solution was stirred for 24 h, and the solvents were removed *in vacuo*. The residue was dissolved in water (10 mL) and washed with ether. The aqueous layer was then treated with 10% aqueous HCl to pH = 1. The product was extracted with ether, the collected organic fractions were washed with brine and dried ($MgSO_4$), and the solvent was evaporated to give 40 mg (91%) of **8**: mp 197–199 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29 (s, 6 H), 1.30 (s, 6 H), 1.69 (s, 4 H), 2.24 (s, 3 H), 2.28 (s, 3 H), 6.31 (s, 1 H), 7.09 (s, 1 H), 7.11 (s, 1 H), 7.52 (d, 1 H, J = 1.2 Hz), 7.95 (d, 1 H, J = 1.2 Hz); ^{13}C (75 MHz, $CDCl_3$) δ 19.8, 20.6, 31.9, 31.9, 33.9, 35.2, 122.2, 125.0, 128.2, 130.8, 131.5, 132.1, 136.1, 140.3, 140.6, 142.5, 143.6, 167.8; MS (EI, 70 eV) 368 (M^+), 353 (100), 324, 309, 111; HRMS *m/z* (M^+) calcd 368.1810, obsd 368.1826. Anal. ($C_{23}H_{28}O_2S$) C, H.

(5,6,7,8-Tetrahydro-3,5,5,8,8-pentamethyl-2-naphthyl)-acetylene (21). A solution of *n*-BuLi and hexanes (12.2 mL, 19.6 mmol, 1.6 M) was added to a solution of freshly distilled diisopropylamine (3.01 mL, 21.5 mmol) and THF (41 mL) at 0 °C under argon. After 10 min, the solution was cooled to −78 °C, and a solution of ketone **12** and THF (10 mL) was added dropwise. The solution was stirred for 1 h at −78 °C, and then diethyl chlorophosphate (3.27 mL, 22.6 mmol) was added. The dry ice bath was removed, and the solution was allowed to warm to room temperature over 1 h and added dropwise to a −78°C solution of LDA (43.1 mmol) and THF (82 mL) prepared as described above. The resulting mixture was warmed to room temperature over 3 h and the reaction quenched with water. The products were extracted with hexanes, washed with 1 N HCl, water, and brine, dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by flash chromatography (SiO_2, hexanes) to give **21** as a colorless solid (4.3 g, 93%): mp 41–43 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23 (s, 6 H), 1.64 (s, 4 H), 2.38 (s, 3 H), 3.20 (s, 1 H), 7.10 (s, 1 H), 7.41 (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.2, 31.7, 31.8, 31.9, 33.8, 34.2, 35.0, 79.5, 83.0, 119.2, 127.5, 130.8, 137.3, 142.3, 145.9. Anal. ($C_{17}H_{22}$) C, H.

Ethyl (*E*)-6-[2-(5,6,7,8-Tetrahydro-3,5,5,8,8-pentamethyl-2-naphthyl)propen-1-yl]nicotinate (22). A solution of 2.88 mL of Me_3Al and hexanes (5.75 mmol, 2 M) was added to a solution of zirconocene dichloride (0.84 g, 2.87 mmol) and 10 mL of 1,2-dichloroethane at room temperature under argon. A solution of acetylene **21** (0.65 g, 2.87 mmol) and 2 mL of 1,2-dichloroethane was added to the resulting yellow-green solution and the mixture stirred for 26 h at room temperature. A solution of ethyl 6-iodonicotinate (0.61 g, 2.21 mmol) and THF (14 mL) was degassed with argon for 10 min and treated with tetrakis(triphenylphosphine)palladium. This solution was then added to the first solution and the reaction mixture stirred at room temperature for 12 h. Aqueous $NaHCO_3$ (5%) was added carefully and the organic material extracted with dichloromethane, washed with brine, dried ($MgSO_4$), and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (hexanes/ethyl acetate = 9:1) to give **22** as a light yellow solid (448 mg, 52%): mp 115.5–116.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.28 (s, 6 H), 1.30 (s, 6 H), 1.42 (t, 3 H, J = 7.1 Hz), 1.69 (s, 4 H), 2.31 (s, 3 H), 2.48 (s, 3 H), 4.42 (q, 2 H, J = 7.1 Hz), 6.47 (s, 1 H), 7.12 (s, 1 H), 7.13 (s, 1 H), 7.31 (d, 1 H, J = 8.2 Hz), 8.24 (dd, 1 H, J = 2.2, 8.2 Hz), 9.23 (d, 1 H, J = 2.2 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.3, 19.7, 20.9, 31.8, 31.9, 33.9, 35.2, 35.2, 61.2, 123.0, 123.7, 125.7, 127.5, 128.2, 131.2, 137.0, 142.2, 142.7, 143.8, 147.8, 150.4, 160.8, 165.4; HRMS *m/z* (M^+) calcd 391.2511, found 391.2516. Anal. ($C_{26}H_{33}NO_2$) C, H, N.

(*E*)-6-[2-(5,6,7,8-Tetrahydro-3,5,5,8,8-pentamethyl-2-naphthyl)propen-1-yl]nicotinic Acid (4). Nicotinate **24** (0.30 g, 0.77 mmol) was dissolved in 4 mL of ethanol and the resulting solution treated with 1 mL of 2 N aqueous KOH. The solution was stirred at room temperature for 30 min and acidified (pH = 4.0) with 1 M aqueous H_3PO_4. The organic material was extracted with methylene chloride, dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (hexanes/ethyl acetate, 1:1.5) to give the title compound as a light yellow solid (235 mg, 84%): mp 181–182 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.28 (s, 6 H), 1.30 (s, 6 H), 1.69 (s, 4 H), 2.33 (s, 3 H), 2.48 (s, 3 H), 6.54 (s, 1 H), 7.13 (s, 1 H), 7.15 (s, 1 H), 7.40 (d, 1 H, J = 8.3 Hz), 8.35 (dd, 1 H, J = 2.2 Hz, J = 8.3 Hz), 9.37 (d, 1 H, J = 2.2 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.7, 20.9, 31.9, 33.9, 35.2, 123.6, 125.3, 127.2, 128.2, 131.3, 137.7, 142.1, 142.5, 143.7, 147.2, 150.2, 159.4, 143.4, 169.8. Anal. ($C_{24}H_{29}NO_2$) C, H, N.

Receptor Transactivation Assays. Activation of retinoid receptors was determined by measuring the ability of retinoid analogs to induce transcription in cells transiently transfected with a hybrid receptor gene construct and a reporter gene. We used hybrid receptors that contain the amino terminus and DNA-binding domain of the estrogen receptor and the hormone-binding domain of specific retinoid receptor subtypes. These ER-RAR or ER-RXR receptors were used to activate transcription of a reporter gene from a promoter sequence containing an estrogen response element (ERE).[25]

The receptor hybrids were constructed in the lab of M. Pfahl (La Jolla Cancer Research Foundation) using polymerase chain reaction to generate an identical splice junction for each of the receptor subtypes.[40] The hybrid receptor DNA is inserted into the pECE eukaryotic expression vector, which contains SV40 promoter and 3′ untranslated sequences.[41] The reporter gene encodes the easily quantifiable enzyme, chloramphenicol acetyltransferase (CAT). The pBL-CAT-2 vector, containing a thymidine kinase promoter,[42] was placed under the control of the estrogen-responsive vitellogenin A2 promoter[43] so that it is inducible by the hybrid receptor.

We developed a procedure for rapid and efficient screening of retinoid analogs using the cotransfection technique. HeLa cells are plated in 12-well dishes at a density of 50 000 cells per well in DMEM (Gibco BRL) supplemented with 10% fetal bovine serum (FBS). The cells are incubated 20–24 h at 37 °C in 5% CO_2. For each well, plasmid DNA (0.5 μg of ERE-tk-CAT and 0.1 μg of ER/RAR) is combined and diluted in 25 μL of Opti-Mem (Gibco BRL). Lipofectin, a commercially available liposome cocktail (Gibco BRL), is separately diluted to a concentration of 2 μg/25 μL in Opti-Mem. The transfection mix is prepared by swirling the DNA and lipofectin together, wating 10 min for complexes to form, and further diluting with Opti-Mem to 500 μL/well. The cells are transfected for 5 h at 37 °C, 5% CO_2 prior to addition of 500 μL of DMEM supplemented with 20% charcoal-treated FBS. Various concentrations of retinoids are added 18 h after the start of transfection and incubation continues another 20–24 h at 37 °C, 5% CO_2.

The cells are washed and lysed directly in the wells for 60 min using 100 μL of an ice-cold hypotonic buffer containing 1 mM Tris-Cl, pH 7.8, 2, mM EDTA, 1% Triton X-100, and 0.4 mg/mL DNase 1. CAT enzyme activity is determined in 50 μL of the cell extract using a mixed phase scintillation counting method to measure the amount of ^{3}H-acetylated chloramphenicol product, which partitions into the nonpolar phase.[44] The substrate, [^{3}H]acetyl coenzyme A, remains in a urea-containing aqueous phase. The extract is assayed in a total volume of 100 μL containing 27 μL of buffer I (250 mM Tris-Cl, pH 7.8, 5 mM EDTA), 20 μL of 5 mM chloramphenicol in buffer I, 0.75 μL of 4 mM acetyl CoA, 0.8 μL of 2.5 mM [^{3}H]-acetyl CoA (0.4 μCi; New England Nuclear), and 1.5 μL of 1 mM HCl. For assay blanks, the chloramphenicol is replaced by buffer I. The extract is incubated for 2 h at 37 °C and then quenched with 100 μL of 7 M urea. The reaction is transferred to a scintillation vial, and 1 mL of 0.8% 2,5-diphenyloxazole (PPO) in toluene is added. Following vortexing and phase separation for 15 min, cpm in the PPO/toluene phase are counted.

Transcriptional induction of CAT activity is expressed as a percent of maximal induction by *all-trans*-retinoic acid. Typically, there is a 15-fold induction by ER/RAR$_\alpha$, 8-fold by ER/RAR$_\beta$, and 6-fold by ER/RAR$_\gamma$. EC_{50} values for the retinoid compounds are representative of determinations from two to four independent experiments.

ODC Inhibition. Inhibition of ODC by retinoids was determined in TPA-treated female hairless mice as described.[28] In brief, retinoids were applied dorsally in 100 μL of acetone 1 h prior to TPA treatment (40 nmol/mouse in 100 μL of acetone). Animals were sacrificed 4 h later, and the epidermis

was scraped from the dermis following treatment at 55 °C for 30 s. The supernatant of epidermal homogenate (30 min, 20000*g*) was stored at −70 °C and ODC assayed essentially as described except that benzethonium hydroxide was used as a trapping agent for released $^{14}CO_2$. Protein concentration in the epidermal extracts was determined by the Comassie binding method (Bio-Rad, Oakland). ODC activity was normalized to total epidermal protein content. The dose of retinoid (in nmol per mouse) causing 80% inhibition of TPA-induced ODC activity (IC80) was estimated graphically in multidose assays.

Tgase Induction. HL-60 cdm-1 cells were cultured under conditions described in detail previously.[27] Cells in log-phase growth (2×10^{-5} cells/mL) in RPMI 1640 (Fisher Scientific) supplemented with insulin, transferrin, and sodium selenide (TIS, Sigma) were pretreated with 1.25% dimethyl sulfoxide (DMSO) for 18 h. Cells were then sedimented and resuspended in RPMI-TIS containing retinoids or an equivalent solvent control (0.1% ethanol). After culture for 24 h, cells were again sedimented, washed once, and lysed, and the transglutaminase activity was assayed by measuring the covalent and Ca^{2+}-dependent conjugation of [^{3}H]putrescine to *N,N*-dimethylcasein.[27] The EC_{50} value was defined as the concentration required to induce 50% of the maximal induction of transglutaminase activity achieved with that compound.

Chondrogenesis Inhibition. The *in vitro* bioassay employed high-density micromass cultures of day 11 embryonic limb bud cells as described.[29] Briefly, forelimb buds were dissociated in a trypsin−EDTA solution, and the resultant single-cell suspension was plated as 20 μL spots (200 000 cells/spot) on plastic culture dishes. Retinoid concentrations ranging from 0.01 ng/mL to 10 μg/mL (0.03 nM to 30 μM) were added to the culture medium (Eagle's MEM + 10% fetal bovine serum, GIBCO) 24 h after initial plating. Control cultures received only the vehicle (ethanol, concentration <1% by volume). The cultures were terminated 96 h after plating, at which time the medium was removed and the cells were fixed for 1 h in 10% formalin containing 0.5% cetylpyridinium chloride. The cultures were rinsed with acetic acid, then dehydrated in ethanol, and scored for chondrogenesis under the microscope. An absence or reduction in the number of cartilage nodules as compared to control cultures was taken as a measure of suppression of chondrogenesis. The number of cartilage nodules stained in the whole spot were counted by automated image scan using the NIH Image-1.52 application. Mean number of nodules and standard deviations were calculated for four replicate cultures per concentration. The median concentration of each retinoid causing 50% inhibition of chondrogenesis compared with controls (IC_{50}) was calculated by logarithmic curve-fitting of the dose−response data.

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