JOURNAL OF
MEDICINAL
CHEMISTRY

Volume 39, Number 17 August 16, 1996

Communications to the Editor

Identification of the First Retinoid X Receptor Homodimer Antagonist

Stacie S. Canan Koch,*,† Laura J. Dardashti,† Jonathan J. Hebert,† Steven K. White,† Glenn E. Croston,‡ Karen S. Flatten,▽ Richard A. Heyman,§ and Alex M. Nadzan†

Departments of Retinoid Chemistry Research, New Leads Discovery, Endocrine Research, and Retinoid Research, Ligand Pharmaceuticals, Inc., 10255 Science Center Drive, San Diego, California 92121

Received April 26, 1996

Introduction. Retinoids regulate numerous biological functions, including cell growth, cellular differentiation, and modulation of apoptosis.[1,2] Retinoids are currently employed for the treatment of dermatological diseases such as acne and psoriasis[3] and are being studied in clinical trials for their potential in the treatment of oncological diseases.[4]

The retinoid receptors mediate the biological effects of retinoid ligands and are members of the superfamily of intracellular proteins which function as ligand-activated transcription factors. The retinoid receptors are classified into two subfamilies: the retinoic acid receptors (RARα, RARβ, RARγ) and the retinoid X receptors (RXRα, RXRβ, RXRγ).[5] These receptors modulate gene expression by binding as dimers to specific DNA sequences, the retinoic acid response elements.[5a,6] The RXRs can function as homodimers or can heterodimerize with RAR and other members of the intracellular receptor superfamily, including peroxisome proliferator-activated receptors (PPARs), vitamin D receptor (VDR), and thyroid hormone receptor (TR).[7] Hence, RXRs play a critical role in the response of a variety of structurally diverse hormones.

all-trans-Retinoic acid (ATRA)[8] functions as the natural ligand for the RARs, while 9-*cis*-retinoic acid (9-*cis*-RA; see Figure 1)[9] has been identified as an endogenous ligand for both RARs and RXRs. Although numerous RAR-selective synthetic retinoids (agonists and antagonists) have been described,[10,11] only recently have synthetic compounds been reported that interact selectively with the RXRs.[12] Our research group has previously disclosed three related series of compounds which selectively bind with high affinity to the RXRs and function as potent RXR agonists. These include Targretin (LGD1069, **1**, X = CH_2, Y = CH, R = CH_3),[12c] the oxime-linked (tetrahydronaphthyl)benzoic acid derivatives (**1**, X = NOH or $NOCH_3$, Y = CH, R = CH_3),[13] and LG100268 (**1**, X = CH_2CH_2, Y = N, R = CH_3).[12h] The RXR selectivity and potency of these retinoids were shown to depend on the nature of the C-3 substituent attached to the tetrahydronaphthyl moiety. For example, analogues containing a C-3 halogen or alkyl substituent exhibited significant RXR agonist activity.

As part of our program to further explore the importance of substituent effects on the retinoid activity of tetrahydronaphthalene derivatives, and to expand our program to the synthesis of 9-*cis*-RA analogues, we have designed a novel series of (tetrahydrotetramethylnaphthyl)octatrienoic acids containing 3-alkoxy substituents that exhibit agonist or antagonist activity depending on the size of the substituent (see Figure 1 for position numbering). Specifically, we have identified an interesting structure−activity profile for LG100754 (**2**) which suggests that this analogue is functioning as an RXR homodimer antagonist. Based on the expansive role that RXRs play as heterodimeric partners, both silent and active, in the control of intracellular receptor pathways,[7] such an RXR antagonist could function as a versatile biological tool for deciphering specific components of transcriptional responses. To our knowledge, this report represents the first disclosure of an RXR antagonist.

Chemistry. The synthetic RXR retinoids reported here are geometrically defined 7-(3-alkoxytetrahydrotetramethylnaphthyl)-6-*cis*-octatrienoic acids. Three analogues in the present series (methoxy **11**, ethoxy **12**, and *n*-propoxy **2**; see Table 1) have been prepared and analyzed for retinoid activity. The selective synthesis for the 6-*cis*-triene isomer is shown in Scheme 1. The key step, introduction of the 6-*cis*-double bond, is accomplished by the stereoselective 1,4-conjugate ad-

† Department of Retinoid Chemistry Research.
‡ Department of New Leads Discovery.
▽ Department of Endocrine Research.
§ Department of Retinoid Research.

S0022-2623(96)00311-1 CCC: $12.00

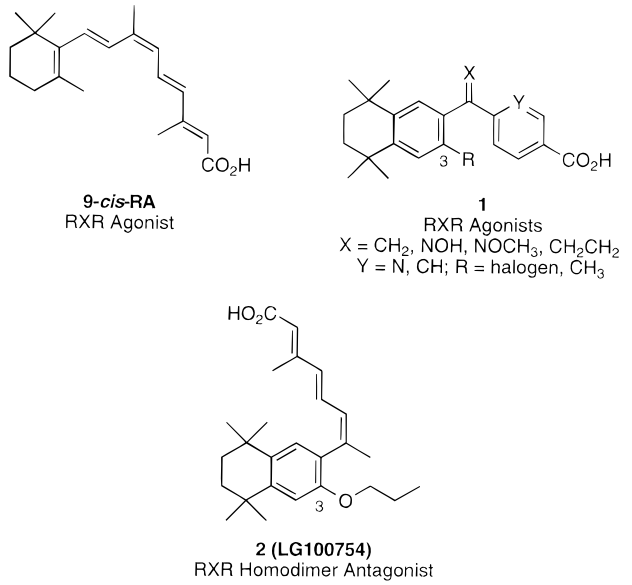


Figure 1. Selected RXR retinoids.

dition of dimethyl cuprate to an alkynenitrile. A similar methodology has been recently reported for the stereoselective synthesis of 9-*cis*-RA.[14]

Under Friedel−Crafts conditions ($AlCl_3$/dichloromethane), phenol is alkylated and cyclized with 2,5-dichloro-2,5-dimethylhexane to afford the tetrahydrotetramethylnaphthol intermediate **3** in 83% yield. The naphthol is converted into the acetate which undergoes a facile Lewis acid-catalyzed Fries rearrangement ($AlCl_3$/hexane/dichloromethane/reflux) to give the acylnaphthol **4** in 78% yield. *O*-Alkylation of the naphthol is accomplished in a routine manner by treatment with KOH and the corresponding alkyl bromide in DMSO to provide the keto ether **5** in 90−100% yield. The keto ether intermediate **5** is converted into the terminal acetylene **6** (39−57%) via a chloro enal by treatment first with phosphorus oxychloride and sodium bicarbonate in DMF followed by treatment with sodium hydroxide in dioxane/water at 80 °C.[15] The acetylene is deprotonated with ethylmagnesium bromide in THF at reflux temperature, and the anion is quenched with phenyl cyanate to afford the acetylenenitrile **7** in 80−85% yield.[16] Methyl cuprate addition to the cyanoalkyne selectively produces the *cis*-cyano olefin **8** in a 20:1 ratio over the *trans*-isomer in excellent yields (94−98%).[17] The crude cyano olefin product is reduced with DIBAL at −78 °C to provide the intermediate enal **9** in 78−92% yield with no loss in the geometric ratio. The olefin isomers may be separated at this or a later stage by silica gel chromatography. The *cis*-enal is treated with the lithium salt of diethyl 3-(ethoxycarbonyl)-2-methylprop-2-enylphosphonate in THF at reduced temperature in a Horner−Wadsworth−Emmons olefination to provide the trienoate ester **10** as a mixture of C-2 olefins in a 9:1 ratio of 2-*trans*:2-*cis*. The olefination is typically conducted in the presence of 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone (DMPU). The crude ester is readily saponified with KOH/methanol to give the trienoic acid (80−94% overall yield for two steps). The 6-*cis*-trienoic acid isomer (**11**, **12**, or **2**) is available as the major product and can be readily isolated by recrystallization or silica gel chromatography.

Biological Methods. To examine the ability of the synthetic ligands to bind to the retinoid receptors, we performed ligand binding assays employing recombinantly expressed RARα, RARβ, RARγ and RXRα, RXRβ, RXRγ in a baculovirus system as described previously.[9a,12c,18] The binding affinities (K_i values) are determined for the test retinoids by competition of 5 nM [^{3}H]-*all-trans*-RA (for RARs) or [^{3}H]Targretin ([^{3}H]-LGD1069; Ligand Pharmaceuticals, Inc.) (for RXRs); K_i values are determined by application of the Cheng−Prussof method.[18a] Binding data are shown in Table 1. To examine transcriptional activity (EC_{50} values), cotransfection assays are performed in CV-1 cells transfected with an expression vector for each of the RAR and RXR receptors and a luciferase reporter gene under the control of the appropriate RAR (RAREs) or RXR (RXREs) response elements.[9a,19] A ΔMTV-TREp-Luc reporter construct is used for the RARs, a CRBPII-tk-Luc reporter is used for the RXRα and RXRγ receptors, and a CPRE-tk-Luc reporter is used for the RXRβ receptor.[2c,6a,9a] Compounds are tested in three separate experiments in log dilutions from 1×10^{-5} to 1×10^{-12} M with triplicate determinations at each concentration. Typically, CV-1 cells are transiently cotransfected with the receptor expression vector, the reporter plasmid, and a β-galactosidase (RSV-β-Gal) internal control (used to calculate transfection efficiency). The cells are incubated in the presence of compound and assayed for luciferase and β-galactosidase activity as described previously.[9a] The luciferase data are presented as percent normalized response with the maximal response (100%) elicited by the control retinoids (9-*cis*-RA for RXRs and *all-trans*-RA for RARs). Standard errors for this assay system are, on average, *ca.* 15% of the mean value. Cotransfection data are shown in Table 1 and Figures 2 and 3. To detect RXR antagonist activity (IC_{50} values), the cotransfection assay is carried out in the presence of a fixed concentration of the known RXR agonist Targretin (LGD1069). The agonist is applied at a concentration (3.2×10^{-8} M) approximate to its EC_{50} value.[12c,20] The antagonist data for the compounds are graphically represented as percent luciferase activity relative to this control (100% luciferase activity at 3.2×10^{-8} M Targretin) and are shown in Figure 4.

Results and Discussion. RXR Activity. The 7-(3-alkoxytetrahydrotetramethylnaphthyl)-6-*cis*-octatrienoic acids (compounds **11**, **12**, and **2**) bind to all of the RXRs with high affinity, yet are distinguishable by their transactivation profiles (see Table 1 and Figures 2 and 4). The 7-(3-methoxytetrahydrotetramethylnaphthyl)-6-*cis*-octatrienoic acid (**11**) binds with high affinity to the RXRs (2−4 nM) and is a correspondingly potent activator of all three RXR subtypes (5−9 nM). In fact, compound **11** is one of the most potent RXR agonists reported to date.

The ethoxy ether analogue 7-(3-ethoxytetrahydrotetramethylnaphthyl)-6-*cis*-octatrienoic acid (**12**) has a ligand binding affinity for the RXRs which is comparable to that of the parent methoxy compound (4−17 nM). Although this compound is capable of activating the RXRs, it is considerably less efficacious (17−31%) than the methoxy analogue, and, in fact, analysis of the full dose−response curve suggests that the ethoxy compound **12** functions only as a partial agonist (see, for example, Figure 2).

The most interesting analogue of this study is LG100754 [7-(3-propoxytetrahydrotetramethylnaphthyl)-

Scheme 1. Synthesis of 7-(3-Alkoxytetrahydrotetramethylnaphthyl)-6-*cis*-trienoic Acids

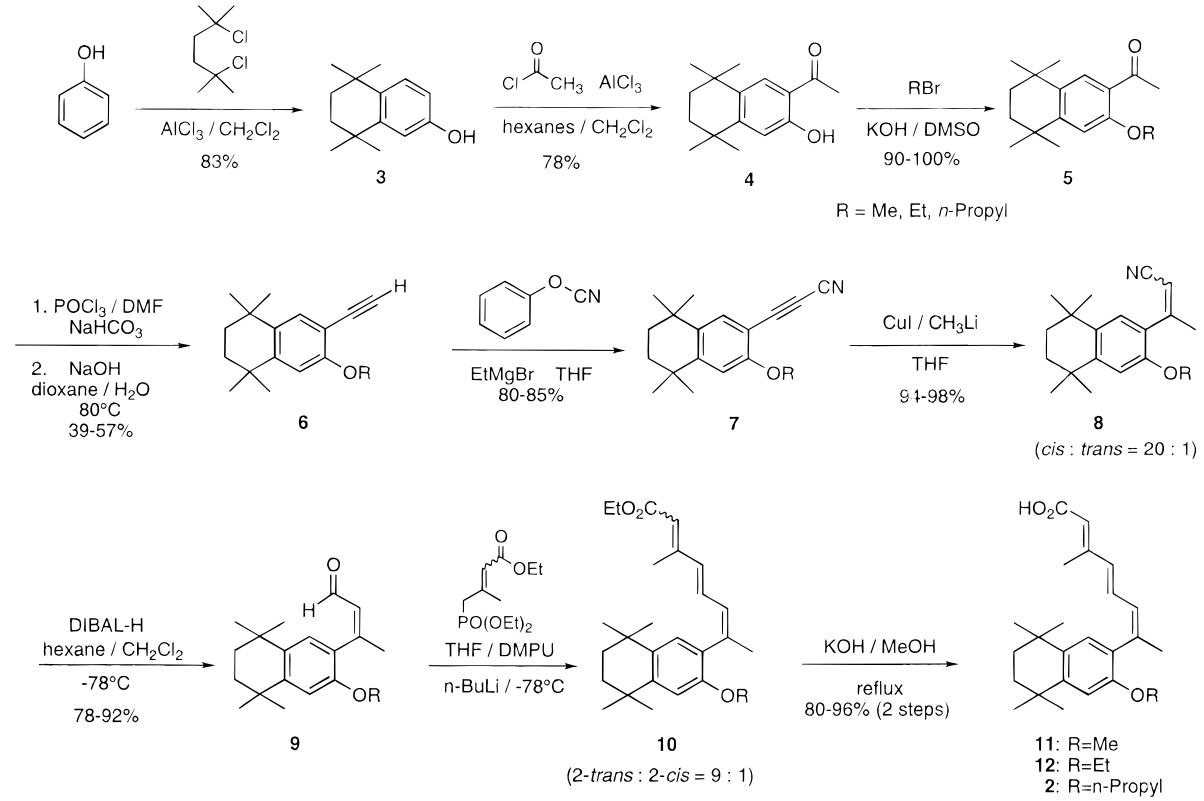
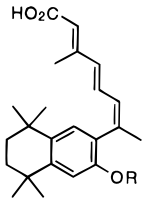


Table 1. Binding Affinity and Agonist Cotransfection Activity of 7-(3-Alkoxytetrahydrotetramethylnaphthyl)-6-*cis*-octatrienoic Acids on Retinoid Receptors



		binding affinity[a] K_i, nM			cotransfection activity EC_{50}, nM (efficacy)[b]		
no.	R	RXRα	RXRβ	RXRγ	RXRα	RXRβ	RXRγ
11	CH_3	2	2	4	5 (51%)	8 (102%)	9 (68%)
12	CH_2CH_3	4	12	17	8 (17%)	9 (31%)	7 (25%)
2	$CH_2CH_2CH_3$	8[c]	9[c]	14[c]	−[d] (2%)	− (13%)	− (4%)
		RARα	RARβ	RARγ	RARα	RARβ	RARγ
11	CH_3	306	400	437	40 (35%)	8 (54%)	17 (66%)
12	CH_2CH_3	2582	3115	4206	− (14%)	31 (27%)	121 (42%)
2	$CH_2CH_2CH_3$	1791	2587	6094	− (4%)	− (10%)	192 (27%)

[a] Binding affinities (K_i values) are determined for the test retinoids by competition of 5 nM [^{3}H]-*all-trans*-RA (for RARs) or [^{3}H]Targretin (LGD1069) (for RXRs). [b] Efficacy is calculated as a percent of maximal induction normalized to 9-*cis*-RA for the RXRs and *all-trans*-RA for the RARs. [c] Similar K_i values for LG100754 (**2**) are observed with [^{3}H]-9-*cis*-RA: K_i = 3, 10, and 12 nM for RXRα, RXRβ, and RXRγ, respectively. [d] EC_{50} is not calculated where the relative efficacy is less than 15%.

6-*cis*-octatrienoic acid, **2**], which exhibits a very unique RXR activity profile. LG100754 (**2**) retains high-affinity binding for the RXRs (8−14 nM) yet is completely inactive as an agonist on the RXRs (efficacy = 2−13%), even at receptor-saturating concentrations (see Figure 2). These data imply that this analogue may function as an RXR homodimer antagonist. Thus, we examined the ability of LG100754 (**2**) to block agonist-induced gene transcription in the CV-1 cell assay. Indeed, when the cotransfection assay is conducted in the presence of a fixed concentration of Targretin (LGD1069; 3.2×10^{-8} nM), coadministration of LG100754 (**2**) produces a concentration-dependent inhibition of transactivation activity of the RXR agonist. At equimolar concentrations of agonist and antagonist, LG100754 (**2**) inhibits RXR transactivation by approximately 50%. At a 100-fold excess, LG100754 (**2**) inhibits the transactivation activity on the RXRs by 90%. LG100754 (**2**) functions as an RXR antagonist for all three RXR subtypes with an IC_{50} = 16, 28, and 19 nM for RXRα, RXRβ, and RXRγ, respectively (see Figure 4). This unique profile effectively establishes LG100754 (**2**) as the first RXR homodimer antagonist.

We have observed identical antagonist activity for LG100754 (**2**) in other RXR-based assays, such as dose-dependent inhibition of receptor activation by other

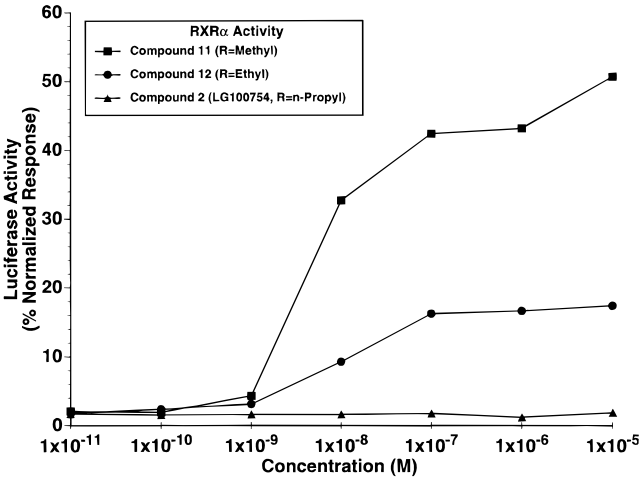


Figure 2. Ligand-induced transactivation of RXRα by 7-(3-alkoxytetrahydrotetramethylnaphthyl)-6-*cis*-octatrienoic acids. RXR subtypes are cotransfected into CV-1 cells along with a reporter plasmid as described in Biological Methods. Cells are incubated in the presence of ligand, and luciferase activity is determined. The activity is normalized relative to the standard 9-*cis*-RA.

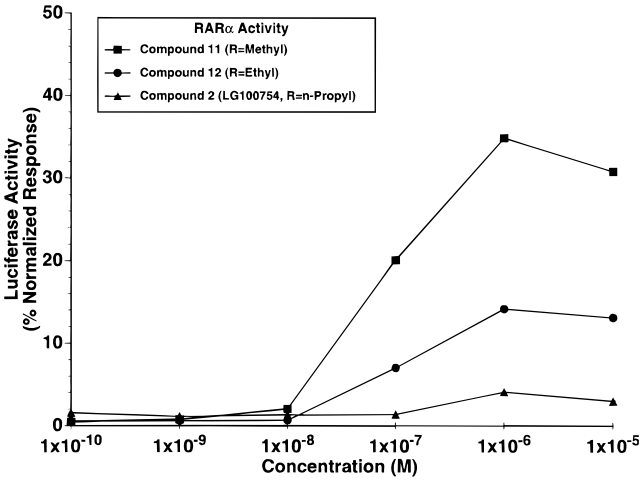


Figure 3. Ligand-induced transactivation of RARα by 7-(3-alkoxytetrahydrotetramethylnaphthyl)-6-*cis*-octatrienoic acids. RAR subtypes are cotransfected into CV-1 cells along with a reporter plasmid as described in Biological Methods. Cells are incubated in the presence of ligand, and luciferase activity is determined. The activity is normalized relative to the standard *all-trans*-RA.

agonists (i.e., 9-*cis*-RA or LG100268[12h]) and concentration-dependent inhibition of Targretin-induced transglutaminase expression in HL-60 cells (data not shown). Notably, compounds **11** and **12** are strictly RXR agonists and did not display any antagonist activity. Interestingly, the corresponding *all-trans* analogues of the above C-3-alkoxytetrahydrotetramethylnaphthyl trienoic acids do not exhibit RXR antagonist activity (data not shown).[21]

RAR Activity. Although the above 7-(3-alkoxytetrahydrotetramethylnaphthyl)-6-*cis*-octatrienoic acids display RXR activities, this class of retinoids has considerably weaker interactions with the three RAR subtypes in this reporter assay (see Figure 3). Compound **11** has approximately 100-fold less binding affinity for the RAR subtypes (306−437 nM) than the RXRs and induces only moderate transactivation of the RARs (8−40 nM, 35−66% efficacy). Compound **12** displays very weak binding affinity (2.6−4 μM) for the RARs, does not induce RARα, and is only a weak activator of RARβ and RARγ (31−

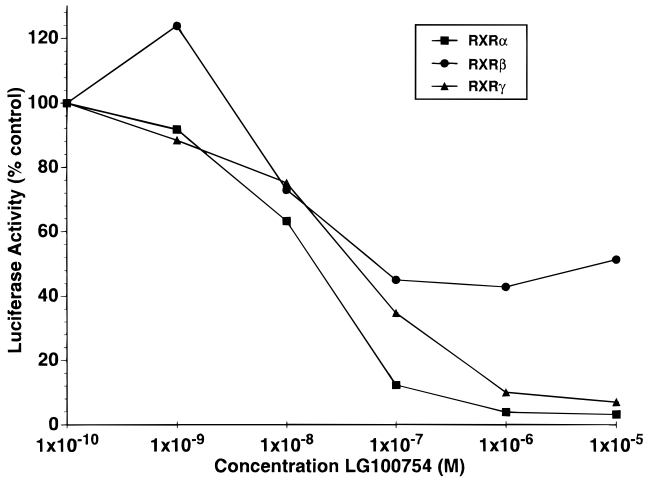


Figure 4. LG100754 (**2**) functions as an antagonist on RXRs. RXR subtypes are cotransfected into CV-1 cells along with a reporter plasmid as described in Biological Methods. Cells are treated with a fixed concentration of the RXR agonist Targretin (LGD1069; 3.2×10^{-8} M) in the presence of increasing concentrations of LG100754 (**2**). Luciferase activity is determined and is reported relative to cells treated with Targretin alone (100%).

121 nM, 27−42% efficacy). LG100754 (**2**) also has very low-affinity binding for the RARs (1.7−6 μM) and displays minimal activity on RARγ only (192 nM, 27% efficacy). Overall, the 7-(3-alkoxytetrahydrotetramethylnaphthyl)-6-*cis*-octatrienoic acids display weak or no activation of the RARs but exhibit very selective interactions with the RXRs.

Conclusions. We have designed a series of 7-(3-alkoxytetrahydrotetramethylnaphthyl)-6-*cis*-octatrienoic acids which displays high-binding affinities for the RXRs and activities ranging from RXR agonist to RXR homodimer antagonist. For this series, the nature of the 3-alkoxy group clearly defines the agonist or antagonist activity of these retinoid ligands. The data presented here demonstrate that LG100754 (**2**) functions as an RXR homodimer antagonist. LG100754 (**2**) displays high-binding affinity for the RXRs and is a potent inhibitor of the known RXR agonist Targretin (LGD1069) at all three RXR subtypes. The structural attributes of LG100754 (**2**) necessary for RXR homodimer antagonist activity include the size of the 3-alkoxy group on the tetrahydronaphthyl moiety and the nature of the olefin geometry at C-6. Thus, in the 6-*cis*-octatrienoic acid series reported here, the 3-methoxy substituent imparts agonist activity, the 3-ethoxy substituent imparts partial agonist activity, and the 3-propoxy group induces antagonist activity. Additionally, for the 3-propoxy analogue, the geometry of the trienoic acid moiety is an important structural component for imparting RXR antagonist activity. The 6-*cis*-geometry is essential for potent RXR antagonist activity, since the *all-trans*-triene analogue of LG100754 does not have significant antagonist activity.[22] Studies are being conducted to investigate the structure−activity relationship (SAR) of related retinoid antagonists, as well as to extend the SAR to other classes of retinoids.

Additionally, investigations are currently underway to decipher the biological and therapeutic significance of this unique retinoid modulator. Since RXRs serve as dimeric partners in the control of numerous intracellular receptor pathways, an RXR antagonist could

function as a versatile biological tool to dissect RXR-dependent transcriptional activities. The evidence that LG100754 (**2**) functions as an antagonist suggests that this unique ligand interacts with the RXR ligand binding domain[23] but may somehow inactivate or fail to induce the AF-2 activation domain, possibly because of the increased bulk of the 3-propoxy substituent. Thus, LG100754 (**2**) may prevent the optimal structural changes necessary for activating transcription. Other transcriptional responses have been investigated with LG100754 (**2**) and RXR heterodimers, and some selective interactions with certain cofactors and basal transcriptional proteins have been observed.[24] As is the case for anti-estrogen and anti-androgen hormones, RXR antagonists, such as LG100754 (**2**), could help define the specific transcriptional activities necessary for receptor activation and hormone signaling.

Acknowledgment. The authors wish to thank Y. L. Bennani for helpful synthetic discussions.

Supporting Information Available: Experimental details and full spectral characterization of compounds (8 pages). Ordering information is given on any current masthead page.

References

(1) For reviews, see: (a) Orfanos, C. E.; Ehlert, R.; Gollnick, H. The Retinoids. A Review of Their Clinical Pharmacology and Therapeutic Use. *Drugs* **1987**, *34*, 459−503. (b) Linney, E. Retinoic Acid Receptors: Transcription Factors Modulating Gene Regulation, Development, and Differentiation. *Current Topics in Developmental Biology*; Academic Press, Inc.: Orlando, FL, 1992; pp 309−350. (c) *The Retinoids: Biology, Chemistry, and Medicine*, 2nd ed.; Sporn, M. B., Roberts, A. B., Goodman, D. S., Eds.; Raven Press, Ltd.: New York, 1994.

(2) (a) Fanjul, A.; Dawson, M. I.; Hobbs, P. D.; Jong, L.; Cameron, J. F.; Harlev, E.; Graupner, G.; Lu, X.-P.; Phfahl, M. A New Class of Retinoids with Selective Inhibition of AP-1 Inhibits Proliferation. *Nature* **1994**, *372*, 107−111. (b) Lee, M.-O.; Hobbs, P. D.; Zhang, X.-K.; Dawson, M. I.; Pfahl, M. A Synthetic Retinoid Antagonist Inhibits the Human Immunodeficiency Virus Type 1 Promoter. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 5632−5636. (c) Bissonnettee, R. P.; Brunner, T.; Lazarchik, S. B.; Yoo, N. J.; Boehm, M. F.; Green, D. R.; Heyman, R. A. 9-*cis*-Retinoic Acid Inhibition of Activation-Induced Apoptosis is Mediated via Regulation of Fas Ligand and Requires Retinoic Acid Receptor and Retinoid X Receptor Activation. *Mol. Cell. Biol.* **1995**, *15*, 5576.

(3) (a) Pharmacology of Retinoids in the Skin. *Pharmacology and the Skin*; Reichert, U., Shroot, B., Eds.; Karger AG: Basel, 1989; Vol. 3. (b) Retinoids: Present and Future. Proceedings of a Symposium Held at the 18th World Congress of Dermatology; Shalita, A. R., Fritsch, P. O., Eds.; Supplement to *J. Am. Acad. Dermatol.* **1992**, *27*, part 2. (c) Esgleyes-Ribot, T.; Chandraratna, R. A.; Lew-Kaya, D. A.; Sefton, J.; Duvic, M. Response of Psoriasis to a New Topical Retinoid, AGN 190168. *J. Am. Acad. Dermatol.* **1994**, *30*, 581−590.

(4) (a) Smith, M. A.; Parkinson, D. R.; Cheson, B. D.; Friedman, M. A. Retinoids in Cancer Therapy. *J. Clini. Oncol.* **1992**, *10*, 839−864. (b) Tallman, M. S.; Wiernick, P. H. Retinoids in Cancer Treatment. *J. Clin. Pharmacol.* **1992**, *32*, 868−888. (c) Vokes, E. E.; Weichselbaum, R. R.; Lippman, S. M.; Hong, W. K. Head and Neck Cancer. *N. Engl. J. Med.* **1993**, *328*, 184−194. (d) Lotan, R.; Dawson, M. L.; Zou, C.-C.; Jong, L.; Lotan, D.; Zou, C. P. Enhanced Efficacy of Combination of Retinoic Acid- and Retinoic X Receptor-Selective Retinoids and α-Interferon in Inhibition of Cervical Carcinoma Cell Proliferation. *Cancer Res.* **1995**, *55*, 232.

(5) (a) See ref 1c: Mangelsdorf, D. J.; Umesono, K.; Evans, R. M. The Retinoid Receptors, pp 319−349. (b) Leid, M.; Kastner, P.; Chambon, P. Multiplicity Generates Diversity in the Retinoic Acid Receptor. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 3559−3563. (c) Mangelsdorf, D. J.; Borgmeyer, U.; Heyman, R. A.; Zhou, Y. U.; Ong, E. S.; Oro, A. E.; Kakizuka, A.; Evans, R. M. Characterization of Three RXR Genes that Mediate the Action of 9-*cis*-Retinoic Acid. *Genes Dev.* **1992**, 6, 329−344.

(6) (a) Umesono, K.; Giguère, V.; Glass, C. K.; Rosenfeld, M. G.; Evans, R. M. Retinoic Acid and Thyroid Hormone Induced Gene Expression Through a Common Responsive Element. *Nature* **1988**, *336*, 262−265. (b) Umesono, K.; Evans, R. M. Determinants of Target Gene Specificity for Steroid/Thyroid Hormone Receptors. *Cell* **1989**, *57*, 1139−1146. (c) Umesono, K.; Murakami, K. K.; Thompson, C. C.; Evans, R. M. Direct Repeats as Selective Response Elements for the Thyroid Hormone, Retinoic Acid, and Vitamin D_3 Receptors. *Cell* **1991**, *65*, 1255−1266. (d) Yang, N.; Schule, R.; Mangelsdorf, D. J.; Evans, R. M. Characterization of DNA Binding and Retinoic Acid Binding Properties of Retinoic Acid Receptor. *Proc. Natl. Acad. Sci U.S.A.* **1991**, *88*, 3559−3563. (e) Näär, A. M.; Boutin, J.-M.; Lipkin, S. M.; Yu, V. C.; Holloway, J. M.; Glass, C. K.; Rosenfeld, M. G. The Orientation and Spacing of Core DNA-Binding Motifs Dictate Selective Transcriptional Responses to Three Nuclear Receptors. *Cell*, **1991**, *65*, 1267−1279. (f) Rottman, J. N.; Widom, R. L.; Nadal-Ginard, B.; Mahdavi, V.; Karathanasis, S. K. A Retinoic Acid-Responsive Element in the Apoliopoprotein A1 Gene Distinguishes Between Two Different Retinoic Acid Response Pathways. *Mol. Cell. Biol.* **1991**, *11*, 3814−3820.

(7) (a) Yu, V. C.; Delsert, C.; Andersen, B.; Holloway, J. M.; Devary, O. V.; Näär, A. M.; Kim, S. Y.; Boutin, J.-M.; Glass, C. K.; Rosenfeld, M. G. RXRβ: A Coregulator That Enhances Binding of Retinoic, Thyroid Hormone, and Vitamin D Receptors to Their Cognate Response Elements. *Cell* **1991**, *67*, 1251−1266. (b) Kliewer, S. A.; Umesono, K.; Noonan, D. J.; Heyman, R. A.; Evans, R. M. 9-*cis*-Retinoic Acid and Peroxisome Proliferator Signaling Pathways Converge Through RXRα-PPAR Heterodimer Formation. *Nature* **1992**, *358*, 771. (c) Zhang, X.-K.; Lehmann, J.; Hoffmann, B.; Dawson, M. I.; Cameron, J.; Graupner, G.; Hermann, T.; Tran, P.; Pfahl, M. Homodimer Formation of Retinoid X Receptor by 9-*cis*-Retinoic Acid. *Nature* **1992**, *358*, 587−591. (d) Isseman, I.; Prince, R. A.; Tugwood, J. D.; Green, S. The Retinoid X Receptor Enhances the Function of the Peroxisome Proliferator Activated Receptor. *Biochemie* **1993**, *75*, 251. (e) Keller, H.; Dreyer, C.; Medin, J.; Mahfoudi, A.; Ozato, K.; and Wahli, W. Fatty Acids and Retinoids Control Lipid Metabolism Through Activation of Peroxisome Proliferator-Activated Receptor-Retinoid X Receptor-Heterodimers. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 2160. (f) Mukherjee, R.; Jow, L.; Noonan, D.; McDonnell, D. P. Human and Rat Peroxisome Proliferator Activated Receptors (PPARs) Demonstrate Similar Tissue Distribution but Different Responsiveness to PPAR Activators. *J. Steroid Biochem. Mol. Biol.* **1994**, *51*, 157. (g) Rastinejad, F.; Perlmann, T.; Evans, R. M.; Sigler, P. B. Structural Determinants of Nuclear Receptor Assembly on DNA Direct Repeats. *Nature* **1995**, *375*, 203−211. (h) Forman, B. M.; Umesono, K.; Chen, J.; Evans, R. M. Unique Response Pathways are Established by Allosteric Interactions. *Cell* **1995**, *81*, 541−550. (i) Willy, P. J.; Umesono, K.; Ong, E. S.; Evans, R. M.; Heyman, R. A.; Mangelsdorf, D. J. LXR: A Nuclear Receptor that Defines a Distinct Retinoid Response Pathway. *Genes Dev.* **1995**, *9*, 1033−1045. (j) Wagner, R. L.; Apriletti, J. W.; McGrath, M. E.; West, B. L.; Baxter, J. D.; Fletterick, R. J. A Structural Role for Hormone in the Thyroid Hormone Receptor. *Nature* **1995**, *378*, 690−697.

(8) (a) Giguère, V.; Ong, E. S.; Segui, P.; Evans, R. M. Identification of a Receptor for the Morphogen Retinoic Acid. *Nature* **1987**, *330*, 624−629. (b) Petrovich, M.; Brand, N. J.; Krust, A.; Chambon, P. A Human Retinoic Acid Receptor Which Belongs to the Family of Nuclear Receptors. *Nature* **1987**, *330*, 444−450. (c) Allenby, G.; Bocquel, M.-T.; Saunders, M.; Kazmer, S.; Speck, J.; Rosenberger, M.; Lovey, A.; Kastner, P.; Grippo, J. F.; Chambon, P.; Levin, A. A. Retinoic Acid Receptors and Retinoid X Receptors: Interactions with Endogenous Retinoic Acids. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 30−34.

(9) (a) Heyman, R. A.; Mangelsdorf, D. J.; Dyck, J. A.; Stein, R. B.; Eichele, G.; Evans, R. M.; Thaller, C. 9-*cis*-Retinoic Acid is a High Affinity Ligand for the Retinoid X Receptor. *Cell* **1992**, *68*, 397−406. (b) Levin, A. A.; Sturzenbecker, L. J.; Kazmer, S.; Bosakowski, T.; Huselton, C.; Allenby, G.; Speck, J.; Kratzeisen, C.; Rosenberger, M.; Lovey, A.; Grippo, J. F. 9-*cis*-Retinoic Acid Stereoisomer Binds and Activates the Nuclear Receptor RXRα. *Nature* **1992**, *355*, 359−361. (c) Boehm, M. F.; McClurg, M. R.; Pathirana, C.; Mangelsdorf, D. J.; White, S. W.; Hebert, J. J.; Winn, D.; Goldman, M. E.; Heyman, R. A. Synthesis of High Specific Activity [^{3}H]-9-*cis*-Retinoic Acid and Its Application for Identifying Retinoids with Unusual Binding Properties. *J. Med. Chem.* **1994**, *37*, 408−414.

(10) Hobbs, P. D.; Dawson, M. I. The Synthetic Chemistry of Retinoids In *The Retinoids: Biology, Chemistry, and Medicine*, 2nd ed.; Sporn, M. B., Roberts, A. B., Goodman, D. S., Eds.; Raven Press Ltd.: New York, 1994; pp 5−178.

(11) (a) Kaneko, S.; Kagechika, H.; Kawachi, E.; Hashimoto, Y.; Shudo, K. Retinoid Antagonists. *Med. Chem. Res.* **1991**, *1*, 220−225. (b) Apfel, C.; Bauer, F.; Crettaz, M.; Forni, L.; Kamber, M.; Kaufmann, F.; LeMotte, P.; Pirson, W.; Klaus, M. A Retinoic Acid Receptor α Antagonist Selectively Counteracts Retinoic Acid Effects. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 7129−7133. (c) Eyrolles, L.; Kagechika, H.; Kawachi, E.; Fukasawa, H.; Iijima, T.; Matsushima, Y.; Hashimoto, Y. ; Shudo, K. Retinobenzoic Acids. 6. Retinoid Antagonists with a Heterocyclic Ring. *J. Med. Chem.* **1994**, *37*, 1508−1517. (d) Johnson, A. T.; Klein, E. S.; Gillett, S. J.; Wang, L.; Song, T. K.; Pino, M. E.; Chan-

draratna, R. A. S. Synthesis and Characterization of a Highly Potent and Effective Antagonist of Retinoic Acid Receptors. *J. Med. Chem.* **1995**, *38*, 4764−4767. (e) Yoshimura, H.; Hagai, M.; Hibi, S.; Kikuchi, K.; Abe, S.; Hida, T.; Higashi, S.; Hishinuma, I.; Yamannada, T. A Novel Type of Retinoic Acid Receptor Antagonist: Synthesis and Structure-Activity Relationships of Heterocyclic Ring-Containing Benzoic Acid Derivatives. *J. Med. Chem.* **1995**, *38*, 3163−3173.

(12) (a) Lehmann, J. M.; Jong, L.; Fanjul, A.; Cameron, J. F.; Lu, X. P.; Haefner, P.; Dawson, M. I.; Pfahl, M. Retinoids Selective for Retinoid X Receptor Response Pathways. *Science* **1992**, *258*, 1944−1946. (b) Boehm, M. F.; Heyman, R. A.; Zhi, L. Compounds Having Selective Activity for Retinoid X Receptors and Means for Modulation of Processes Mediated by Retinoid X Receptors. Eur. Pat. Appl. WO93/21146, Oct. 28, 1993. (c) Boehm, M. F.; Zhang, L.; Badea, B. A.; White, S. K.; Mais, D. E.; Berger, E.; Suto, C. M.; Goldman, M. E.; Heyman, R. A. Synthesis and Structure-Activity Relationships of Novel Retiniod X Receptor-Selective Retinoids. *J. Med. Chem.* **1994**, *37*, 2930−2941. (d) Boehm, M. F.; Heyman, R. A.; Zhi, L.; Canan Koch, S. Compounds Having Selectivity for Retinoid X Receptors. Eur. Pat. Appl. WO 94/15902, July 21, 1994. (e) Gendimenico, G. J.; Stim, T. B.; Corbo, M.; Janssen, B.; Mezick, J. A. A Pleotropic Response Is Induced in F9 Embryonal Carcinoma Cells and Rhino Mouse Skin by *all-trans*-Retinoic Acid, a RAR Agonist but Not by SR11237, a RXR Selective Agonist. *J. Investi. Dermatol.* **1994**, *102*, 676−680. (f) Beard, R. L.; Chandraratna, R. A. S.; Colon, D. F.; Gillett, S. J.; Henry, E.; Marler, D. K.; Song, T.; Denys, L.; Garst, M. E.; Arefieg, T.; Klein, E.; Gil, D. W.; Wheeler, L.; Kochhar, D. M.; Davies, P. J. A. Synthesis and Structure-Activity Relationships of Stilbene Retinoid Analogs Substituted with Heteroaromatic Carboxylic Acids. *J. Med. Chem.* **1995**, *38*, 2820. (g) Dawson, M. I.; Jong, L.; Hobbs, P. D.; Cameron, J. F.; Chao, W.; Pfahl, M.; Lee, M.-O.; Shroot, B. Conformational Effects on Retinoid Receptor Selectivity. 2. Effects of Retinoid Bridging Group on Retinoid X Receptor Activity and Selectivity. *J. Med. Chem.* **1995**, *38*, 3368. (h) Boehm, M. F.; Zhang, L.; Zhi, L.; McClurg, M. R.; Berger, E.; Wagoner, M.; Mais, D. E.; Suto, C. M.; Davies, P. J. A.; Heyman, R. A.; Nadzan, A. M. Design and Synthesis of Potent Retinoid X Receptor Selective Ligands That Induce Apoptosis in Leukemia Cells. *J. Med. Chem.* **1995**, *38*, 3146. (i) Nadzan, A. M.; Boehm, M. F.; Zhang, L.; Badea, B. A.; Zhi, L.; White, S. K.; Mais, D. E.; Berger, E.; Suto, C. M.; McClurg, M. R.; Davies, P. J. A.; Heyman, R. A. Design of Novel RXR Selective Retinoids. *Eur. J. Med. Chem.* **1995**, *30*, 520s−533s. (j) Vuligonda, V.; Lin, Y.; Chandraratna, R. A. S. Synthesis of Highly Potent RXR-Specific Retinoids: The Use of a Cyclopropyl Group as a Double Bond Isostere. *Bio. Med. Chem. Lett.* **1996**, *6*, 213−218.

(13) (a) Canan Koch, S. S.; Dardashti, L. J.; Zhang, L.; Badea, B. A.; Boehm, M. F.; Suto, C. M.; Goldman, M. E.; Enyeart, D. L.; Berger, E. M.; Mais, D. E.; Nadzan, A. M. Synthesis of Potent Class of Oxime Retinoid X Receptor Selective Ligands. 209th American Chemical Society National Meeting, Anaheim, CA, Medicinal Chemistry Division, Apr. 6, 1995; Abstract No. 238. (b) Canan Koch, S. S.; Dardashti, L. J.; Zhang, L.; Badea, B. A.; Boehm, M. F.; Suto, C. M.; Goldman, M. E.; Enyeart, D. L.; Berger, E. M.; Mais, D. E.; Nadzan, A. M. Synthesis of Potent Retinoid X Receptor Selective Ligands. Retinoids '95 Conference, European Retinoid Research Group, Nice, France, Oct. 10, 1995, Abstract No. 98.

(14) Bennani, Y. L. An Efficient and Stereoselective Synthesis of 9-*cis*-Retinoic Acid. *J. Org. Chem.* **1996**, *61*, 3542−3544.

(15) (a) Bodendorf, K.; Kloss, P. Acetylene Derivatives from a Fragmentation Reaction. *Angew. Chem., Int. Ed. Engl.* **1963**, *2*, 98−99. (b) Bodendorf, K.; Mayer, R. Über die Darstellung und Fragmentierung von β-Chloracroleinen. *Chem. Ber.* **1965**, *98*, 3554−3559. (c) Gagan, J. M. F.; Lane, A. G.; Lloyd, D. The Stereochemistry of β-Chlorovinyl-Aldehydes. *J. Chem. Soc., C* **1970**, 2484−2488. (d) Royles, B. J. L.; Smith, D. M. The Inverse Electron-Demand Diels-Alder Reaction in Polymer Synthesis. Part 1. A Convenient Synthetic Route to Diethynyl Aromatic Compounds. *J. Chem. Soc., Perkin Trans. I* **1994**, 355−358.

(16) (a) Murray, R. E.; Zweifel, G. Preparation of Phenyl Cyanate and its Utilization for the Synthesis of α,β-Unsaturated Nitriles. *Synthesis* **1980**, 150. (b) Martin, D. and Bauer, M. Cyanic Acid Esters from Phenols: Phenyl Cyanate. *Organic Syntheses*; Wiley: New York, 1990; Collect. Vol. VII, p 435.

(17) Westmijze, H.; Kleijn, H.; Vermeer, P. A New, Stereospecific Synthesis of 2-Alkenenitriles from 2-Alkynenitriles and Organocuprates. *Synthesis* **1978**, *6*, 454−456.

(18) (a) Cheng, Y.-C.; Prussof, W. H. The Relationship Between the Inhibitory Constant K_i and the Concentration of Inhibitor Which Causes 50% Inhibition (IC_{50}) of an Enzymatic Reaction. *Biochem. Pharm.* **1973**, *22*, 3099−3108. (b) Christensen, K.; Estes, P. A.; Onate, S. A.; Beck, C. A.; DeMarzo, A. Characterization and Functional Properties of the A and B Forms of Human Progesterone Receptors Synthesized in a Baculovirus System. *Mol. Endocrinol.* **1991**, *5*, 1755−1770. (c) Allegretto, E. A.; McClurg, M. R.; Lazarchik, S. B.; Clemm, D. L.; Kerner, S. A.; Elgort, M. G.; Boehm, M. F.; White, S. K.; Pike, J. W.; Heyman, R. A. Characterization and Comparison of Hormone Binding and Transactivation Properties of Retinoic Acid and Retinoid X Receptors Expressed in Mammalian Cells and Yeast. *J. Biol. Chem.* **1993**, *268*, 26625−26633.

(19) (a) Umesono, K.; Giguère, V.; Glass, C. K.; Rosenfeld, M. G.; Evans, R. M. Retinoic Acid and Thyroid Hormone Induced Gene Expression Through a Common Responsive Element. *Nature* **1988**, *336*, 262−265. (b) Mangelsdorf, D. J.; Ong, E. S.; Dyck, J. A.; Evans, R. M. A Nuclear Receptor That Identifies a Novel Retinoic Acid Response Pathway. *Nature* **1990**, *345*, 224−229. (c) Berger, T. S.; Parandoosh, S.; Perry, B. W.; Stein, R. B. Interaction of Glucocorticoid Analogues with the Human Glucocorticoid Receptor. *J. Steroid Biochem. Mol. Biol.* **1992**, *41*, 733−738.

(20) Targretin (LGD1069) has EC_{50} = 28, 25, and 20 nM on RXRα, RXRβ, and RXRγ, respectively, in the described cotransfection assay in CV-1 cells.

(21) The *all-trans*-7-(3-methoxytetrahydrotetramethylnaphthyl)-octatrienoic acid analogue has been described; see: Loeliger, P. Polyene Compounds. U.S. Patent No. 4,193,931, Mar. 18, 1980.

(22) The *all-trans*-7-(3-propoxytetrahydrotetramethylnaphthyl)octatrienoic acid analogue has IC_{50} = 708, 109, and 203 nM on RXRα, RXRβ, and RXRγ, respectively, in the described antagonist cotransfection assay vs Targretin in CV-1 cells.

(23) Bourguet, W.; Ruff, M.; Chambon, P.; Gronemeyer, H.; Moras, D. Crystal Structure of the Ligand-Binding Domain of the Human Nuclear Receptor RXR-α. *Nature* **1995**, *375*, 377−382.

(24) Lala, D.; Mukherjee, R.; Schulman, I. G.; Canan Koch, S. S.; Dardashti, L. J.; Nadzan, A. M.; Croston, G. E.; Evans, R. M.; Heyman, R. A. A Novel RXR Homodimer Antagonist is Converted to an Agonist on Specific RXR Heterodimers. *Nature*, accepted for publication.

JM960311D