Published in final edited form as:

Bioorg Med Chem. 2014 January 1; 22(1): 178–185. doi:10.1016/j.bmc.2013.11.039.

Methyl-Substituted Conformationally Constrained Rexinoid Agonists for the Retinoid X Receptors Demonstrate Improved Efficacy for Cancer Therapy and Prevention

Anil Desphande^a, Gang Xia^a, LeeAnn J. Boerma^b, Kimberly K Vines^a, Venkatram R. Atigadda^a, Susan Lobo-Ruppert^c, Clinton J. Grubbs^d, Fariba L Moeinpour^d, Craig D. Smith^e, Konstantin Christov^f, Wayne J. Brouillette^{a,*}, and Donald D. Muccio^{a,*}

^aDepartment of Chemistry, University of Alabama at Birmingham, Birmingham Alabama 35294 USA.

^bDepartment of Biochemistry and Molecular Genetics, University of Alabama at Birmingham, Birmingham Alabama 35294 USA.

^cDepartment of Medicine, University of Alabama at Birmingham, Birmingham Alabama 35294 USA.

^dDepartment of Surgery, University of Alabama at Birmingham, Birmingham Alabama 35294 USA.

^eDepartments of Vision Sciences, University of Alabama at Birmingham, Birmingham Alabama 35294 USA.

^fDepartment of Surgical Oncology, University of Illinois, Chicago, Illinois 60612.

Abstract

(2*E*,4*E*,6*Z*,8*Z*)-8-(3',4'-Dihydro-1'-(2*H*)-naphthalen-1'-ylidene)-3,7-dimethyl-2,3,6-octatrienoic acid, 9cUAB30, is a selective rexinoid for the retinoid X nuclear receptors (RXR). 9cUAB30 displays substantial chemopreventive capacity with little toxicity and is being translated to the clinic as a novel cancer prevention agent. To improve on the potency of 9cUAB30, we synthesized 4-methyl analogs of 9cUAB30, which introduced chirality at the 4-position of the tetralone ring. The syntheses and biological evaluations of the racemic homolog and enantiomers are reported. We demonstrate that the *S*-enantiomer is the most potent and least toxic even though these enantiomers bind in a similar conformation in the ligand binding domain of RXR.

Keywords

Conformationally Constrained Retinoids; Rexinoids

© 2013 Elsevier Ltd. All rights reserved.

*Corresponding Authors: 1 205 9348288 (WJB). 1 205 9348285 (DDM). Fax: 1 205 9342543. wbrou@uab.edu or muccio@uab.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

Nuclear receptor (NR) proteins are ligand-inducible transcription factors that up-regulate target genes. NR signaling is tightly regulated in a ligand and coactivator/corepressor-dependent mechanism. Vitamin A acids (all-trans-RA; 9-cis-RA) bind to two classes of NR proteins, Retinoic Acid Receptors (RARs) and Retinoid X Receptors (RXRs), and activate transcription by recruitment of coactivator proteins.¹ Many classes of RXR agonists have been designed to selectively enhance signaling through RXRs independent of RAR-signaling.² Selective RXR agonists (rexinoids) are effective in cancer therapy and prevention, and they are less toxic than RAR agonists since they do not hyperactivate RAR signaling.

Targretin (bexarotene) was the first FDA-approved rexinoid, and it is clinically used to treat refractory cutaneous T-cell lymphoma.³ Phase I trials revealed that Targretin was better tolerated than all-trans-RA or 9cRA in humans.^{4, 5} Hyperlipidemia was the dose-limiting toxicity of oral Targretin treatment. To reduce toxicity, we pursued the design of potent rexinoids that were tissue-selective. Tissue-selective rexinoids, which do not exhibit dose-limiting lipid toxicities, could be administered chronically for cancer prevention. 9cUAB30 was designed by substituting a tetralone ring for the trimethylcyclohexenyl ring of 9cRA (Figure 1).^{6, 7} 9cUAB30 and Targretin each reduced proliferation, enhanced apoptosis in mammary tumors, and efficiently prevented mammary cancers in rodent models.⁸ However, unlike Targretin, 9cUAB30 did not induce hyperlipidemia, providing a clinical advantage.⁹ To improve upon the potency of 9cUAB30, we have designed and synthesized a series of methyl derivatives of 9cUAB30. In the X-ray crystal structure of hRXR α -LBD homodimers bound to 9cUAB30 with the coactivator peptide GRIP-1, we revealed that the binding pocket contained enough space to accommodate a small lipophilic group on the tetralone ring.¹⁰ Methyl substitution has proven to be very effective in improving activity through lipophilic interactions and/or from energetic gains realized by burial of the methyl group in the hydrophobic pockets of the protein.¹¹ Racemic **1** (($\pm$)-**1**) possesses a methyl group at the 4-position of the tetralone ring, and it is also known as 4-methyl-9c-UAB30 in the literature (Figure 1). ($\pm$)-**1** has been evaluated in cancer prevention studies. It has been compared directly to its parent rexinoid, 9cUAB30, and to other rexinoids including Targretin.⁸ ($\pm$)-**1** reduced cancer cell proliferation more than 9cUAB30 and prevented formation of mammary cancers *in vivo* better than 9cUAB30. However, this single methyl substitution substantially enhanced lipogenesis. The serum triglycerides from dosing of ($\pm$)-**1** were elevated to the levels displayed by Targretin dosing.⁸ Recently, gene expression in liver tissues from rats dosed with 9cUAB30, Targretin, and ($\pm$)-**1** were compared.⁹ This study demonstrated that Targretin and ($\pm$)-**1** stimulated lipogenesis using signaling pathways in the liver involving RXR heterodimers with the liver X receptors (LXRs), but 9cUAB30 had little effect on these lipogenic pathways.

In this manuscript, we prepare the R/S-enantiomers of **1**. We compare how each R/S-enantiomer activates the RXR receptor, prevents oncogenesis, and reduces proliferation of established mammary cancers relative to 9cUAB30. We also examine if lipid biosynthesis is induced by each enantiomer. Further, we report the X-ray crystal structures of each enantiomer bound to the ligand-binding domain (LBD) of human RXR α in the presence of

the coactivator peptide, GRIP-1. We compare these crystal structures to those we obtained for 9cRA, 9cUAB30 and Targretin in the hopes of revealing how this single methyl substitution improves potency and controls lipid biosynthesis.^{10, 12, 13}

2. Results and discussion

2.1 Chemistry

Scheme 1 summarizes the methods employed to prepare the new retinoids. Enantiomerically pure tetralones (**R**)-**2** and (**S**)-**2** were synthesized utilizing a previously reported procedure.^{14, 15} The syntheses of compounds ($\pm$)-**1**, (**R**)-**1** and (**S**)-**1** began with the condensation between ($\pm$)-4-methyl-1-tetralone (**2**) or enantiomerically pure (**R**)- or (**S**)-4-methyl-1-tetralone and ethyl 4-bromo-3-methyl-2-butenate in 1,4-dioxane under Reformatsky reaction conditions to give lactones **3**. The intermediate lactones were treated with NaOMe/methanol to generate the 9*Z*-acids ($\pm$)-**4**, (**R**)-**4**, and (**S**)-**4** in 60–86% yield. The carboxyl groups in compounds ($\pm$)-**4**, (**R**)-**4**, and (**S**)-**4** were subjected to reductive conditions in the presence of LiAlH₄ to produce the desired 9*Z*-alcohols ($\pm$)-**5**, (**R**)-**5** and (**S**)-**5** in quantitative yield. The alcohol functional groups in compounds ($\pm$)-**5**, (**R**)-**5** and (**S**)-**5** were then oxidized in the presence of IBX to provide the desired 9*Z*-aldehydes ($\pm$)-**6**, (**R**)-**6** and (**S**)-**6** containing minor amounts of all *E*-aldehyde (5–7%). Pure 9*Z* isomers ($\pm$)-**6**, (**R**)-**6** and (**S**)-**6** were isolated in 68–80% yields following column chromatography. Olefination of aldehydes ($\pm$)-**6**, (**R**)-**6** and (**S**)-**6** in the presence of triethyl phosphonoselenoate under *Horner-Emmons* conditions provided the esters ($\pm$)-**7**, (**R**)-**7** and (**S**)-**7** as an 85:15 mixture of (9*Z*,13*E*) and (9*Z*,13*Z*)-isomers. The isomers were separated by flash silica column chromatography to obtain the desired (9*Z*,13*E*) isomers in 70–85% yield. Subsequent hydrolysis of (9*Z*,13*E*)-esters ($\pm$)-**7**, (**R**)-**7** and (**S**)-**7** under basic conditions provided the final compounds ($\pm$)-**1**, (**R**)-**1** and (**S**)-**1** in 70–85% yields after recrystallization.

2.2 Binding and transactivation of the RXRs

9cRA is a potent pan-agonist for both RAR and RXR.¹⁶ Using fluorescence quenching, the 9cRA:hRXR α -LBD homodimer complex has a dissociation constant (K_d) of 14 nM (Table 1).¹⁰ This was consistent with a previously reported result.¹⁷ Using this method, the K_d for the 9cUAB30:hRXR α -LBD homodimer complex was determined to be 33 ± 5 nM, which was about 2-fold weaker than 9cRA binding. Targretin exhibited a K_d in between that of 9cRA and 9cUAB30 (Table 1). These fluorescence titrations were also performed for the compounds ($\pm$)-**1**, (**R**)-**1** and (**S**)-**1**. Methyl substitution of 9cUAB30 at the 4-position lowered K_d values to 13 ± 4 nM [(**S**)-**1**] and 23 ± 1 nM [(**R**)-**1**] (Table 1).

Previous studies have shown that 9cUAB30 is an RXR-selective agonist in two cell lines while 9cRA is a potent pan-agonist.^{7, 18} In RK3E cells, the EC₅₀ value for 9cUAB30 activating RXR α transcription is about 6-fold weaker than for 9cRA activation (Table 1). Using 9cRA as a positive control, 9cUAB30 did not activate RAR α -mediated transcription at a high concentration (<1% at 10⁻⁶ M). Using these receptor reporter assays in RK3E cells, we evaluated the methyl-substituted 9cUAB30 analogs. The EC₅₀ values for RXR α activation by (**R**)-**1** and (**S**)-**1** were much lower than the EC₅₀ for 9cUAB30. They were

similar to those found for 9cRA (Table 1). When RAR α activation was examined, (*R*)-**1** and (*S*)-**1** each activated transcription by only 17% at the high dose (10⁻⁶ M). Enantiomers (*R*)-**1** and (*S*)-**1** exhibited increased potency but reduced selectivity for the RXR receptors over the parent rexinoid 9cUAB30.

2.3 Inhibition of oncogenic transformation

Previously we have shown the pan-agonist 9cRA and two RXR-selective agonists, 9cUAB30 and Targretin, inhibited transformation of rat kidney epithelial (RK3E) cells infected with the oncogene KLF4.^{10, 18} 9cUAB30 was 3.6-fold less effective than 9cRA in this assay (Table 1). The inhibition of transformation correlated well with reduction of squamous cell carcinoma (SCC) in transgenic mice models.¹⁸ Here, the 4-methyl derivatives of 9cUAB30 were compared. Relative to DMSO controls, the EC₅₀ values for inhibition of KLF4-ER mediated transformation are 19 and 27 nM for (*R*)-**1** and (*S*)-**1**, respectively (Table 1). These data indicate (*R*)-**1** and (*S*)-**1** may be more effective in preventing SCC than 9cUAB30.

2.4 In vivo triglyceride levels and inhibition of proliferation in mammary cancers

The elevation of serum triglycerides (TG) is the dose-limiting toxicity of rexinoids like Targretin.⁵ 9cUAB30 exhibited no increase in serum TG in its preclinical evaluation in rats and dogs or in human trials.^{19, 20} We previously reported that serum TG increased by 566% over controls when rats were fed 200 mg/kg diet of ($\pm$)-**1** for 7 days.⁸ Each enantiomer of **1** was evaluated in a similar manner and compared to ($\pm$)-**1**. The study conducted here used older rats (216 days of age versus 50 days). As displayed in Table 1, both (*R*)-**1** and (*S*)-**1** increased serum TG; however TG levels due to (*R*)-**1** dosing were more than 2-fold higher than for (*S*)-**1**. The body weights of the rats increased by about 3% for all treated groups, but the increase was slightly larger for rats fed (*R*)-**1**. Liver weights were normalized to body weight. The livers from rats fed (*R*)-**1** were larger than those fed (*S*)-**1** (4.2 $\pm$ 0.1 g/100 g BW for (*R*)-**1**; 3.8 $\pm$ 0.1 g/100g BW for (*S*)-**1**; 3.3 g/100 g BW for controls). Each enantiomer of **1** induced hyperlipidemia and hepatomegaly to a different extent.

Previously we demonstrated that ($\pm$)-**1** was very potent as an antiproliferative agent, which correlated well with its capacity to prevent estrogen receptor-positive mammary cancers.⁸ We evaluated each enantiomer of **1** in the same antiproliferative assays. Using mammary cancers of about 200 mm² that have been chemically induced with *N*-methyl-nitrosourea in rats, the animals were treated orally for 7 days and cancers removed. As displayed in Table 1, the proliferation index decreased by 90% relative to controls when rats containing existing mammary cancers were fed a diet containing 200 mg/kg of either ($\pm$)-**1** or (*S*)-**1**. The decrease in proliferation index was only 16% in rat tumors treated with oral dosing of (*R*)-**1** (Table 1). We reported that the apoptotic index increased for mammary tumors treated with potent chemopreventive rexinoids.⁸ For each treated group, the apoptotic index increased, but the increase observed for ($\pm$)-**1** or for (*S*)-**1** was twice that of (*R*)-**1** (Table 1). Taken together these data clearly demonstrate that (*S*)-**1** is the major contributor toward the potency of ($\pm$)-**1** and (*S*)-**1** is also the enantiomer that is least likely to induce lipid toxicity.

2.5 Crystal structures of hRXR α -LBD homodimers bound with (*R*)-1 or (*S*)-1

We have determined the X-ray crystal structures of hRXR α -LBD homodimers bound to 9cRA (3OAP), 9cUAB30 (pdb ID: 4K4J) and Targretin (pdb ID: 4K6I) with the coactivator peptide GRIP-1 (⁶⁸⁶KHKILHRLQLDSS⁶⁹⁸).^{10,12} Here we report two crystal structures of hRXR α -LBD containing either (*R*)-1 or (*S*)-1 in the presence of GRIP-1 (4M8H and 4M8E). The summary of the X-ray crystallography and refinement statistics for the two structures are listed in Table 2.

The structures were highly similar to previously reported structure of hRXR α -LBD.^{10, 12} Each structure was overlaid with the structure of hRXR α -LBD bound to 9cUAB30 and GRIP-1. RMSD values were 0.137 and 0.126 for (*R*)-1 and (*S*)-1 (Figure 2). The active conformation was present in each structure, which had helix 12 folded back to the core of the hRXR α -LBD to form the coactivator binding site along with helix 3 and helix 4. In each structure, the ILxxLL motif of GRIP-1 bound to the hydrophobic pocket and the charge clamps identified previously were present.¹⁰

2.6 Ligand binding pocket of hRXR α -LBD

The ligand binding pocket is defined by helices 3, 5, 7, 11 and the beta sheet. Interactions between coactivator binding site and ligand binding pocket were present in each structure of hRXR α -LBD homodimers containing (*R*)-1 or (*S*)-1. 9cRA, 9cUAB30 and Targretin adopted an L-shaped conformation inside the ligand binding pocket (LBP) of hRXR α -LBD.¹⁰ For 9cUAB30, the C8–C9 torsional angle was 121.4°. The twisted conformation reduced steric effect between C2 and C3 methylene groups of the tetralone ring and the methyl group at C9 (C19). Both (*R*)-1 and (*S*)-1 also adopted an L-shaped conformation inside the LBP of hRXR α -LBD (Figure 3). However, when the polyene chain of (*R*)-1 or (*S*)-1 were overlaid with that of 9cUAB30, the tetralone ring was in a different orientation. The C8–C9 dihedral angle was –64.4° for (*R*)-1 and was –56.6° for (*S*)-1. In the 9cUAB30 structure, the benzyl ring of the tetralone was pointed to helix 7 (V342, I345, F346, V349) (Figure 3B). In the structures containing (*R*)-1 and (*S*)-1, this part of the tetralone rings were pointed toward helix 11 residues (H435, L436 and F439) (Figure 3C and 3D).

The tetralone ring of each rexinoid adopted an envelope conformation for 9cUAB30, (*R*)-1 and (*S*)-1. For each rexinoid, C3 of the tetralone was out of plane relative to the benzyl ring. C2 and C3 were very close to the phenyl ring of F313 on helix 5, but there were little interactions between the tetralone ring and F313 in the 9cUAB30 structure. The surface area contact between (*R*)-1 or (*S*)-1 and helix 5 residues increased relative to that of 9cUAB30. This increase was more apparent for (*R*)-1 (20% increase) than (*S*)-1 (7% increase). The difference in surface area contact to helix 5 residues for the enantiomers was due to the 0.8 Å translational shift of (*R*)-1 relative to (*S*)-1 in the ligand binding pocket (Figure 3A). The surface area contact between (*R*)-1 and (*S*)-1 and helix 7 residues also increased relative to those found for 9cUAB30. This increase in helix 5 contacts occurred mostly for (*S*)-1 (26% increase) relative to (*R*)-1 (14% increase). There were fewer interactions between helix 11 residues and (*R*)-1 (18% decrease) and (*S*)-1 (9% decrease) than observed for 9cUAB30.

In the structures of (**R**)-**1** and (**S**)-**1**, the 4-methyl groups were each in a *pseudo*-equatorial position of the puckered tetralone ring. The 4-methyl group carbons were about 3.5 Å from the phenyl ring of F346. The tetralone rings of (**R**)-**1** and (**S**)-**1** were essentially mirror images of each other with the 4-methyl group the same side of the plane as C3 (Figure 3E and 3F). The puckered tetralone was also observed in the structure of 9cUAB30 bound to this LBD and in crystal structures without the protein (unpublished data).¹⁰

3. Conclusion

In this study we demonstrate that methyl substitution of 9cUAB30 improved the potency of the selective RXR agonist, 9cUAB30, by nearly 8-fold. This is consistent with observations of how methyl substitution affects the potency for other drugs.¹¹ Methyl substitution also improved inhibition of KLF4-ER mediated transformation by more than 10-fold when compared with 9cUAB30. While potency was increased, ($\pm$)-**1** and its enantiomers were less selective than 9cUAB30. ($\pm$)-**1** was more potent than 9cUAB30 in receptor activation and cancer chemoprevention. However, ($\pm$)-**1** also demonstrated a profound and undesired increase in lipogenesis, which reduces its value as a chemopreventive agent for cancer. But since ($\pm$)-**1** and its *S* enantiomer are more potent than 9cUAB30 and as potent as Targretin, these rexinoids may be candidates for cancer therapy. When each enantiomer was evaluated for potency and toxicity, we demonstrate here that (**S**)-**1** significantly reduced mammary cancer cell proliferation *in vivo* suggesting that this enantiomer contributes most to the demonstrated chemopreventive capacity of ($\pm$)-**1**. Further, (**S**)-**1** displayed significantly lower TG biosynthesis and it should be less toxic than (**R**)-**1** or Targretin. The separation of potency using chiral rexinoids was also observed by the Chandraratna group for another class of RXR agonists.¹³ The X-ray crystal structures revealed that both enantiomers had similar, twisted structures in the LBP of hRXR α -LBD. However, the tetralone ring regions of (**S**)-**1** and (**R**)-**1** were oriented differently from the tetralone ring of 9cUAB30 in the chiral ligand binding pocket. The differences observed in the structures of (**R**)-**1** and (**S**)-**1** with the surrounding residues on the LBD, as well as differences in the pharmacokinetic parameters (transport, distribution, and metabolism), may contribute to the way these enantiomers produce differential potency and toxicity.

4. Experimental section

Detailed experimental procedures for the synthesis of (**S**)-**1** are described here and these same procedures were followed to synthesize (**R**)-**1** and ($\pm$)-**1**. ¹H and ¹³C NMR spectra were recorded on Bruker ARX 300 or DRX 400 spectrometers. IR spectra were recorded using a Bomem FTIR spectrometer. UV-vis spectra were recorded on a Varian (Cary 100) spectrophotometer in methanol (Aldrich, spectrograde). Mass spectra were measured on a Hewlett-Packard 1100 LC-MS instrument using electrospray ionization (ESI). All optical rotations were recorded at room temperature (25 °C) using Jasco P-1030 polarimeter. Melting points were recorded on an Electrothermal melting point apparatus and were uncorrected. Combustion analyses were performed by Atlantic Microlabs, Inc. (Norcross, GA). All reactions, unless otherwise mentioned, were monitored by thin layer chromatography (TLC) on 0.25 mm silica gel plates (60F-254, E. Merck or Silicycle). Flash chromatography was performed using Silicycle silica gel (40–63 μ m). Reactions and

purifications were conducted with nitrogen-saturated solvents and under subdued lighting. Ethyl 4-bromo-3-methylbut-2-enoate was prepared by the reaction of ethyl 3,3-dimethylacrylate with *N*-bromosuccinimide. Triethyl phosphonoseneoate was prepared *via* the Arbusov reaction.

4.1. General procedure for the preparation of compounds

4.1.1. (9*S*)-7,8-Benzo-4,9-dimethyl-1-oxaspiro[5,5]undec-3-ene-2-one [(*S*)-3]—A suspension of Zn dust (5.76 g, 88.0 mmol) and copper (II) acetate (0.580 g) in glacial acetic acid (25 mL) was stirred under nitrogen for 1 h in a 250 mL, round-bottomed flask. The mixture was diluted with anhydrous ether (50 mL), vacuum-filtered, and the Zn-Cu complex was washed successively with anhydrous ether (3 × 20 mL) and anhydrous benzene (3 × 20 mL). This complex was dried under vacuum for 1 h and transferred to a 250 mL, flame-dried, three-neck flask fitted with a condenser, addition funnel and nitrogen inlet. Anhydrous THF (40 mL) was transferred to the flask and this suspension was heated to 90 °C in an oil bath. This reaction mixture was then treated dropwise with a solution of (*S*)-4-methyl tetralone [(*S*)-2] (4.00 g, 25.0 mmol) and ethyl 4-bromo-3-methylbut-2-enoate (10.4 g, 50.0 mmol) in dry THF (40 mL). Vigorous bubbling was noticed during the addition process and the reaction mixture was stirred at reflux for 4 h and then cooled to room temperature. Water (40 mL) and 2N HCl (40 mL) were added. The mixture was diluted with ether (100 mL) and allowed to stir for 10 min. The mixture was filtered and the acidic layer was separated. The organic layer was washed successively with water (50 mL), 1N NaOH (2 × 40 mL), water (40 mL) and brine (40 mL). The organic layer was dried (Na₂SO₄) and concentrated under vacuum to result in thick oil. The unreacted tetralone and the other volatile materials were removed under high vacuum distillation to give δ -lactone (*S*)-3 (4.1 g, 68 % yield, mixture of isomers) as a cream colored solid, which was used in the next reaction without further purification.

4.1.2. (2*Z*,4*E*)-4-[(4'*S*)-methyl-3',4'-dihydro-1'(2'*H*)-naphthalen-1'-ylidene]-3-methyl-2-butenic acid [(*S*)-4]—A dry, 100 mL, two-neck round-bottomed flask fitted with a reflux condenser under nitrogen atmosphere was charged with anhydrous methanol (15 mL). To this Na (0.506 g, 23.0 mmol) pieces were added slowly at room temperature followed by the addition of δ -lactone (*S*)-3 (4.0 g, 16.5 mmol) in anhydrous methanol (10 mL). The reaction mixture was stirred at reflux for 1.5 h, cooled to room temperature, and the solvent was removed on a rotary evaporator under vacuum. The resulting crude oil was diluted with ice-cold water (100 mL), acidified with HCl (5N) to pH 1–2 and washed with ether (2 × 50 mL). The combined organic layers were washed with water (25 mL), brine (25 mL), dried (Na₂SO₄) and evaporated under vacuum to give the acid (*S*)-4 (4.0 g) in quantitative yield; mp 98–99 °C [ether/ hexanes (1:2)]; UV λ_{max} 309 nm (ϵ = 12242); IR (KBr): 2937 (OH), 1677 (C=O), 1606 (C=C) cm⁻¹; [α]_D -44.5° (*c* 0.42, MeOH); MS *m/z* 243 (MH⁺); ¹H NMR (300 MHz, CDCl₃) δ 7.63 (d, *J* = 7.5 Hz, 1H), 7.26-7.14 (m, 4H), 5.79 (t, *J* = 1.3 Hz, 1H), 2.97-2.89 (m, 1H), 2.71-2.64 (m, 1H), 2.57-2.49 (m, 1H), 2.15 (s, 3H), 1.99-1.91 (m, 1H), 1.65-1.53 (m, 1H), 1.29 (d, *J* = 7.0 Hz, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 171.14, 156.14, 143.24, 140.53, 135.63, 128.17, 127.84, 126.38, 125.24, 122.37, 118.08, 33.22, 30.97, 25.92, 25.91, 21.82; Anal calcd. for C₁₆H₁₈O₂: C, 79.31; H, 7.49. Found: C, 79.22; H, 7.47.

4.1.3.(2Z,4E)-4-[(4'*R*)-methyl-3',4'-dihydro-1'(2'*H*)-naphthalen-1'-ylidene]-3-methyl-2-butenic acid [(*R*)-4]—A solution of (*R*)-4-methyl tetralone [(*R*)-2] (3.50 g, 22.0 mmol) and bromoester (9.06 g, 44.0 mmol) was employed as described for (*S*)-4. $[\alpha]_{\text{D}} +44.7^{\circ}$ (*c* 0.64, MeOH). All other spectral data were identical to its enantiomer.

4.1.4. (2Z,4E)-4-[(4'*S*)-methyl-3',4'-dihydro-1'(2'*H*)-naphthalen-1'-ylidene]-3-methyl-2-butenol [(*S*)-5]—To a flame-dried two-neck round-bottomed flask fitted with a nitrogen inlet and addition funnel was added acid (*S*)-4 (4.00 g, 16.5 mmol) and anhydrous ether (50 mL). The flask was cooled to 0 °C in an ice bath and the reaction mixture was treated with 1 M LiAlH₄/ether (22 mL, 22.0 mmol) dropwise. The reaction mixture was stirred for 2 h at 0 °C, cooled to –80 °C in a dry ice/acetone bath, and slowly quenched with methanol (2 mL) followed by 1 N HCl (40 mL). The reaction mixture was allowed to come to room temperature and extracted with ether (2 × 75 mL). The combined ether layers were washed with water (40 mL), brine (40 mL), dried (Na₂SO₄) and concentrated under vacuum to give the alcohol (*S*)-5 (3.6 g, 96 %); mp 42–48 °C [ether/hexanes (1:2)]; UV λ_{max} 265 nm (ϵ = 19165); IR (KBr): 3347 (OH), 1605 (C=C) cm^{–1}; $[\alpha]_{\text{D}} -31.6^{\circ}$ (*c* 1.55, MeOH); MS *m/z* 229 (MH⁺); ¹H NMR (300 MHz, CDCl₃) δ 7.57 (d, *J* = 7.3 Hz, 1H), 7.23–7.04 (m, 3H), 6.34 (s, 1H), 5.56 (t, *J* = 6.7 Hz, 1H), 4.07 (d, *J* = 6.7 Hz, 2H), 2.96 (sex, *J* = 6.7 Hz, 1H), 2.43–2.37 (m, 1H), 2.33–2.28 (m, 1H), 1.95–1.82 (m, 1H), 1.87 (s, 3H), 1.80 (brs, 1H), 1.62–1.56 (m, 1H), 1.30 (d, *J* = 7.0 Hz, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 142.57, 138.00, 136.50, 135.40, 128.23, 127.76, 126.44, 126.17, 124.50, 122.31, 61.13, 33.23, 31.50, 25.20, 24.20, 22.31; Anal calcd. for C₁₆H₂₀O: C, 84.16; H, 8.83. Found: C, 84.06; H, 9.17.

4.1.5.(2Z,4E)-4-[(4'*R*)-methyl-3',4'-dihydro-1'(2'*H*)-naphthalen-1'-ylidene]-3-methyl-2-butenol [(*R*)-5]—A solution of acid (*R*)-4 (4.3 g, 7.7 mmol) was employed as described for the preparation of (*S*)-5. $[\alpha]_{\text{D}} +32.7^{\circ}$ (*c* 0.81, MeOH). All other spectral data were identical to its enantiomer.

4.1.6.(2Z,4E)-4-[(4'*S*)-methyl-3',4'-dihydro-1'(2'*H*)-naphthalen-1'-ylidene]-3-methyl-2-butenal [(*S*)-6]—A single-neck, round-bottomed flask fitted with a reflux condenser was charged with *o*-iodoxybenzoic acid (IBX) (21.0 g, 75.0 mmol) and acetone (75 mL) and warmed to 50–55 °C in an oil bath. A solution of alcohol (*S*)-5 (3.53 g, 15.0 mmol) in acetone (25 mL) was added all at once to the reaction mixture. The reaction was then allowed to stir at 50–55 °C for 1.5 h. The reaction mixture was cooled to 0 °C in an ice bath, diluted with ether (50 mL) and filtered through a sintered glass funnel. The filtrate was washed with ether (2 × 50 mL) and the combined organic layers were concentrated under vacuum to furnish the crude product (*S*)-6 (2.5 g, 74% yield). This was purified by flash column chromatography using 15% ether in hexane to give pure 9Z-aldehyde (*S*)-6 (2.1 g, 62% yield); UV λ_{max} 306 nm (ϵ = 8937); IR (neat): 1673 (C=O), 1611 (C=C) cm^{–1}; $[\alpha]_{\text{D}} -51.7^{\circ}$ (*c* 0.86, MeOH); MS *z/e* 227 (MH⁺); ¹H NMR (300 MHz, CDCl₃) δ 9.66 (d, *J* = 8.3 Hz, 1H), 7.61 (d, *J* = 7.8 Hz, 1H), 7.35–7.15 (m, 3H), 6.54 (s, 1H), 6.01 (dt, *J* = 1.1 Hz & 8.2 Hz, 1H), 3.03–2.93 (m, 1H), 2.62–2.53 (m, 1H), 2.48–2.44 (m, 1H), 2.09 (s, 3H), 2.06–1.92 (m, 1H), 1.64–1.59 (m, 1H), 1.31 (d, *J* = 7.0, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 193.40, 159.58, 143.17, 142.46, 134.47, 129.14, 128.78, 128.46, 126.33, 124.71, 120.43, 33.09,

31.48, 25.71, 25.43, 22.22. Anal calcd. for C₁₆H₁₈O: C, 84.92; H, 8.02. Found: C, 84.71; H, 8.27.

4.1.7.(2Z,4E)-4-[(4'*R*)-methyl-3',4'-dihydro-1'(2'*H*)-naphthalen-1'-ylidene]-3-methyl-2-butenal [(*R*)-6]—A solution of alcohol [(*R*)-5] (4.0 g, 17.5 mmol) was employed. [α]_D +52.1° (*c* 0.84, MeOH). All other spectral data were identical to its enantiomer.

4.1.8.(2E,4E,6Z,8E)-Ethyl-8-[(4'*S*)-methyl-3',4'-dihydro-1'(2'*H*)-naphthalen-1'-ylidene]-3,7-dimethyl-2,4,6-octatrienoate [(*S*)-7]—To a flame-dried, 250 mL, three-neck round-bottomed flask fitted with a nitrogen inlet, addition funnel and rubber septum was added NaH (60% suspension in mineral oil, 0.440 g, 11.0 mmol). Dry THF (25 mL, distilled over Na/benzophenone) was added to the flask followed by a solution of triethyl phosphonoseneoate (2.90 g, 11.0 mmol) in dry THF (25 mL). The resulting solution was stirred for 15 min and then freshly distilled HMPA (7.5 mL) was added under a nitrogen atmosphere. The flask was covered with aluminum foil and stirred for 15 min. A solution of aldehyde (*S*)-6 (2.03 g, 9.00 mmol) in dry THF (25 mL) was added dropwise through the addition funnel, and the mixture was then stirred for 2.5 h. The reaction mixture was quenched with water (25 mL) and extracted with ether (2 × 75 mL). The combined ether layers were washed with brine (60 mL), dried over Na₂SO₄ and concentrated under vacuum to provide the crude product as an oil. The product was purified by column chromatography (*n*-hexane/ether: 9/1) to give 2.35 g of (*S*)-7 as a 85:15 mixture of (9Z) : (9Z,13Z)-7S (78% combined yield). Separation of these isomers was achieved by column chromatography using *n*-hexane/benzene (1:1) to obtain the pure (9Z)-isomer of (*S*)-7 (1.8 g, 61% yield) as a yellow oil; UV λ_{max} 331 nm (ϵ = 30079); IR (Neat) 1708 (C=O), 1603 (C=C) cm⁻¹; [α]_D -61.2° (*c* 1.1, MeOH); MS *m/z* 337.65 (MH⁺); ¹H NMR (300 MHz, CDCl₃) δ 7.63 (d, *J* = 7.2 Hz, 1H), 7.26-7.17 (m, 3H), 6.65 (dd, *J* = 4.3 Hz & 11.1 Hz, 1H), 6.45 (s, 1H), 6.24 (d, *J* = 15.4 Hz, 1H), 6.13 (d, *J* = 11.1 Hz, 1H), 5.75 (s, 1H), 4.15 (q, *J* = 7.1 Hz, 2H), 3.01-2.91 (m, 1H), 2.55-2.45 (m, 1H), 2.40-2.30 (m, 1H), 2.23 (s, 3H), 1.98 (s, 3H), 2.00-1.90 (m, 1H), 1.65-1.50 (m, 1H), 1.31 (d, *J* = 7.0 Hz, 3H), 1.28 (t, *J* = 7.1 Hz, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 167.39, 153.11, 142.86, 140.69, 138.52, 135.57, 134.21, 133.06, 128.25, 127.93, 127.67, 126.24, 124.73, 122.78, 118.61, 59.83, 33.27, 31.53, 25.76, 24.87, 22.22, 14.56, 14.03; Anal calcd. for C₂₃H₂₈O₂: C, 82.10; H, 8.39. Found: C, 82.39; H, 8.63.

4.1.9.(2E,4E,6Z,8E)-Ethyl-8-[(4'*R*)-methyl-3',4'-dihydro-1'(2'*H*)-naphthalen-1'-ylidene]-3,7-dimethyl-2,4,6-octatrienoate [(*R*)-7]—A solution of aldehyde (*R*)-6 (2.00 g, 8.85 mmol) was employed. [α]_D +60.4° (*c* 1.1, MeOH). All other spectral data were identical to its enantiomer.

4.1.10.(2E,4E,6Z,8E)-8-[(4'*S*)-methyl-3',4'-dihydro-1'(2'*H*)-naphthalen-1'-ylidene]-3,7-dimethyl-2,4,6-octatrienoic Acid [(*S*)-1]—The (9Z)-ester (*S*)-7 (1.68 g, 5.00 mmol) was suspended in methanol (75 mL) and warmed to about 70 °C in an oil bath. An aqueous solution of 1.25 N KOH (40 mL, 50.0 mmol) (prepared with distilled and degassed water) was added to the above suspension and stirred under reflux for 1 h. Then the reaction mixture was cooled in an ice bath, diluted with ice-cold water (50 mL) and

acidified to pH 1–2 with ice-cold 2 N HCl. The resulting precipitate was extracted with ether (2×75 mL), washed with brine (40 mL), dried over Na_2SO_4 and concentrated under vacuum to furnish the final acid (*S*)-**1** (1.45 g, 95.0 % yield) as a yellow solid, which was crystallized from ether/*n*-hexanes (1:1) to furnish pure (9*Z*)-isomer of (*S*)-**1** (0.955, 62.0 % yield); mp 166–167 °C [ether/hexanes (1:1)]; UV λ_{max} 331 nm ($\epsilon = 28616$); IR (KBr) 2937 (–OH), 1667 (C=O), 1590 (C=C) cm^{-1} ; $[\alpha]_{\text{D}} -70.5^\circ$ (*c* 0.72, MeOH); MS m/z 309.41 (MH^+); ^1H NMR (300 MHz, CDCl_3) δ 10.9 (brs, 1H), 7.63 (d, $J = 7.2$ Hz, 1H), 7.26–7.17 (m, 3H), 6.70 (dd, $J = 4.3$ & 11.1 Hz, 1H), 6.46 (s, 1H), 6.26 (d, $J = 15.4$ Hz, 1H), 6.13 (d, $J = 11.1$ Hz, 1H), 5.77 (s, 1H), 2.97 (m, 1H), 2.54–2.43 (m, 1H), 2.40–2.31 (m, 1H), 2.23 (s, 3H), 1.99 (s, 3H), 2.00–1.90 (m, 1H), 1.64–1.54 (m, 1H), 1.30 (d, $J = 7.00$ Hz, 3H); ^{13}C NMR (75 MHz, CDCl_3) δ 172.50, 155.74, 142.90, 141.54, 38.71, 135.54, 134.01, 128.28, 127.99, 127.61, 126.26, 124.75, 122.75, 117.74, 33.27, 31.53, 25.78, 24.93, 22.23, 14.22; Anal calcd. for $\text{C}_{21}\text{H}_{24}\text{O}_2$: C, 81.78; H, 7.84. Found: C, 81.92; H, 7.74.

4.1.11. (2*E*,4*E*,6*Z*,8*E*)-8-[(4' *R*)methyl-3',4'-dihydro-1'(2' *H*)-naphthalen-1'-ylidene]-3,7-dimethyl-2,4,6-octatrienoic Acid [(*R*)-1**]**—A solution of ester (*R*)-**7** (1.4 g, 4.2 mmol) was employed. $[\alpha]_{\text{D}} +70.6^\circ$ (*c* 1.1, MeOH). All other spectral data were identical to its enantiomer.

4.2 Binding affinity and transient transfection and luciferase reporter assays

The binding affinities of compounds ($\pm$)-**1**, (*R*)-**1** and (*S*)-**1** to hRXR α -LBD homodimer were measured using a fluorescence quenching method.²¹ hRXR α -LBD homodimers (1 μM) were excited at 280 nm and the protein fluorescence was measured at 337 nm with Cary Eclipse fluorescence spectrophotometer (Varian, Palo Alto, CA). The binding dissociation constant K_d was calculated by using a nonlinear least square regression to fit a single-site binding model to the raw data.²¹

Transient transfection and luciferase reporter assays were performed using a previously reported protocol.¹⁸ At 24 h prior to transfection, human embryonic kidney (HEK) 293 cells were plated at 2×10^5 cells per well in six-well plates. Transfection mixtures included 0.2 μg of the Gal4 reporter plasmid pGL4.31[luc2P/Gal4UAS/Hygro] (Promega), 0.5 μg of pCMXGal4-hRAR α or pCMX-Gal4-hRXR α expression vector, and 0.01 μg of Renilla luciferase reporter plasmid, pRL-TK. TransIT-LT1 Transfection Reagent (Mirus) was used. At 24 h post-transfection, retinoid was added to the culture medium. At 48 h post-transfection, reporter activity was determined using the Dual-Luciferase Reporter Assay (Promega). The EC_{50} values from the luciferase assay were determined using a dose response model containing 2 duplicates at 4 different concentrations (1 nM, 10 nM, 100 nM, 1000 nM).

4.3 Inhibition of oncogenic transformation assay

BOSC23 ecotropic packaging cells at 80–90% confluence were transfected with 30 μg of KLF4-ER, ErB2 or pBpuro (control) plasmid DNA. Virus was collected and filtered at 24 h and 48 h post transfection and frozen in liquid nitrogen. Following viral titer, RK3E cells with 30% confluence were infected with virus in the presence of 10 $\mu\text{g}/\text{ml}$ Polybrene. After 15 h infection, virus was removed and RK3E were supplemented with DMEM. To cells

infected with KLF4-ER were added DMEM supplemented with DMSO (control), 9cRA, 9cUAB30, ($\pm$)-**1**, (**R**)-**1** and (**S**)-**1** every other day in duplicate at 3 different concentrations. Three weeks after post-infection, transformed foci were fixed, stained, counted and analyzed relative to DMSO control.

4.4. In vivo triglyceride levels assay and antiproliferative and apoptosis assays

The triglyceride, antiproliferative and apoptosis assays were conducted on female Sprague Dawley rats having small mammary tumors. The animals were fed a Teklad diet according to previous reports.⁸ The retinoids tested were mixed into the diet according to the previously published protocols and fed for 7 days.⁸ For the evaluation of the compounds on serum triglycerides, blood was collected from the inferior vena cava at the time of sacrifice of the animals. The blood was kept at 5 °C during centrifugation (3800 rpm for 15 minutes). Serum was immediately collected and frozen at -85 °C until it was analyzed for triglycerides.²² The infinity triglycerides assay kit was purchased from Thermo DMA. For evaluation of antiproliferative index, animals were injected with bromodeoxyuridine (BRDU) 2 h prior to euthanasia using CO₂. Cancers were removed and fixed with 10% formalin. Cell proliferation and cell apoptosis were performed as previously reported.⁸ The proliferation/apoptotic indices were calculated as the ratio of proliferation/apoptosis of treated animals to vehicle fed animals.

4.5. Protein purification, crystallization and X-ray crystallography

The hRXR α -LBD (T₂₂₃-T₄₆₂) was overexpressed in *Escherichia coli* and purified using AKTA purifier system.¹² The hRXR α -LBD homodimers were isolated from Nickelating column followed by size exclusion column. The protein was mixed with 4-fold excess of UAB retinoids and then 5-fold excess of GRIP-1 coactivator peptide (⁶⁸⁶KHKILHRLQLQDSS⁶⁹⁸). The ternary complexes were crystallized using vapor diffusion technique in hanging drops.¹² Crystals with suitable diffraction size appeared in 2 weeks with conditions of 2–10% PEG4000, 4–12% Glycerol and 0.1M Bis-Tris, pH 7.0. Crystals were flash frozen in liquid nitrogen using a reservoir cryoprotectant solution containing glycerol. The crystals were placed in stepwise increasing concentration of glycerol (10.0%, 12.5%, 15%, 17.5%, 20.0%, 22.5%, 25.0%) in order to avoid damage to the crystals due to the sharp increase in glycerol concentrations. Diffraction data of crystals were collected on a Rigaku IV+ diffractometer with a copper rotating anode and processed using the program D*Trek.²³ The structures were solved using the molecular replacement method with a high-resolution hRXR α -LBD structure with its ligand deleted as a search model (pdb ID: 3OAP). The ligand files and their associated topology and parameter files were created by PRODRG.²⁴ The structures were refined using CNS software.²⁵ A complete summary of data for these structures was listed in Table 2. Interactions between ligand/peptide and hRXR α -LBD were analyzed using a program Ligand-Protein Contacts (LPC)/Contacts of Structural Units (CSU).²⁶ All structures were prepared using PyMol (*The PyMOL Molecular Graphics System, Version 0.99. Schrödinger, LLC*).

Acknowledgements

We thank Dr. Ellen Li (Washington University in St. Louis) for providing the protein expression vector. We appreciate critical reading and helpful comments provided by Dr. Matthew B Renfrow. We also appreciate the help

from Dr. Bingdong Sha in the refinement of the X-ray crystal structures. This work was supported by grants from the National Institutes of Health (NIH) 2 P50 CA089019 (DDM) and the Komen Foundation (BCTR 20000690) (DDM)

Abbreviations

RXR	Retinoid X receptor
RAR	retinoic acid receptor
RA	retinoic acid
LBD	ligand binding domain
IBX	2-iodoxybenzoic acid
RKE	rat kidney epithelial
SCC	squamous cell carcinoma
TG	triglycerides
LBP	ligand binding pocket
THF	tetrahydrofuran
HMPA	hexamethylphosphoramide
TLC	thin layer chromatography
DMEM	Dulbecco's Modified Eagle Medium
ER	Estrogen Receptor
GRIP-1	Glucocorticoid Receptor Interacting Protein-1
KLF4	Kruppel-Like Factor 4
LXR_s	Liver X Receptors
NR	Nuclear Receptor
PDB	Protein Data Bank
RMSD	Root Mean Square Deviation
SCC	Squamous Cell Carcinoma

References and notes

1. Mangelsdorf DJ, Ong ES, Dyck JA, Evans RM. Nature. 1990; 345:224. [PubMed: 2159111]
2. Lehmann JM, Jong L, Fanjul A, Cameron JF, Lu XP, Haefner P, Dawson MI, Pfahl M. Science. 1992; 258:1944. [PubMed: 1335166]
3. Boehm MF, Zhang L, Badea BA, White SK, Mais DE, Berger E, Suto CM, Goldman ME, Heyman RA. J. Med. Chem. 1994; 37:2930. [PubMed: 8071941]
4. Duvic M, Martin AG, Kim Y, Olsen E, Wood GS, Crowley CA, Yocum RC. Arch. Dermatol. 2001; 137:581. [PubMed: 11346336]
5. Miller VA, Benedetti FM, Rigas JR, Verr AL, Pfister DG, Straus D, Kris MG, Crisp M, Heyman R, Loewen GR, Truglia JA, Warrell RP Jr. J. Clin. Oncol. 1997; 15:790. [PubMed: 9053506]
6. Atigadda VR, Vines KK, Grubbs CJ, Hill DL, Beenken SL, Bland KI, Brouillette WJ, Muccio DD. J. Med. Chem. 2003; 46:3766. [PubMed: 12904083]

7. Muccio DD, Brouillette WJ, Breitman TR, Taimi M, Emanuel PD, Zhang X, Chen G, Sani BP, Venepally P, Reddy L, Alam M, Simpson-Herren L, Hill DL. *J. Med. Chem.* 1998; 41:1679. [PubMed: 9572893]
8. Grubbs CJ, Lubet RA, Atigadda VR, Christov K, Deshpande AM, Tirmal V, Xia G, Bland KI, Eto I, Brouillette WJ, Muccio DD. *Carcinogenesis*. 2006; 27:1232. [PubMed: 16344269]
9. Vedell PT, Lu Y, Grubbs CJ, Yin Y, Jiang H, Bland KI, Muccio DD, Cvetkovic D, You M, Lubet R. *Mol. Pharmacol.* 2013; 83:698. [PubMed: 23292798]
10. Boerma LJ, Xia G, Qui C, Cox BD, Chalmers MJ, Smith CD, Lobo-Ruppert S, Griffin P, Muccio DD, Renfrow MB. *J. Biol. Chem.* 2013 in press.
11. Leung CS, Leung SS, Tirado-Rives J, Jorgensen WL. *J. Med. Chem.* 2012; 55:4489. [PubMed: 22500930]
12. Xia G, Boerma LJ, Cox BD, Qiu C, Kang S, Smith CD, Renfrow MB, Muccio DD. *Biochemistry*. 2011; 50:93. [PubMed: 21049972]
13. Vuligonda V, Thacher SM, Chandraratna RA. *J. Med. Chem.* 2001; 44:2298. [PubMed: 11428923]
14. Barry J, Kagan HB, Snatzke G. *Tetrahedron*. 1971; 27:4737.
15. Premasagar V, Palaniswamy VA, Eisenbraun EJ. *J. Org. Chem.* 1981; 46:2974.
16. Allegretto EA, McClurg MR, Lazarchik SB, Clemm DL, Kerner SA, Elgort MG, Boehm MF, White SK, Pike JW, Heyman RA. *J. Biol. Chem.* 1993; 268:26625. [PubMed: 8253793]
17. Bourguet W, Ruff M, Chambon P, Gronemeyer H, Moras D. *Nature*. 1995; 375:377. [PubMed: 7760929]
18. Jiang W, Deng W, Bailey SK, Nail CD, Frost AR, Brouillette WJ, Muccio DD, Grubbs CJ, Ruppert JM, Lobo-Ruppert SM. *Cancer Biol. Ther.* 2009; 8:289. [PubMed: 19197145]
19. Lindeblad M, Kapetanovic IM, Kabirov KK, Dinger N, Mankovskaya I, Morrissey R, Martin-Jimenez T, Lyubimov A. *Drug Chem. Toxicol.* 2011; 34:300. [PubMed: 21649485]
20. Kolesar JM, Hoel R, Pomplun M, Havighurst T, Stublaski J, Wollmer B, Krontiras H, Brouillette W, Muccio D, Kim K, Grubbs CJ, Bailey HE. *Cancer Prev. Res. (Phila)*. 2010; 3:1565. [PubMed: 21149332]
21. Cheng L, Norris AW, Tate BF, Rosenberger M, Grippo JF, Li E. *J. Biol. Chem.* 1994; 269:18662. [PubMed: 8034615]
22. Hartge P, Hoover R, Leshner LP, McGowan L. *Jama*. 1983; 250:1844. [PubMed: 6620481]
23. Pflugrath JW. *Acta. Crystallogr., Sect. D: Biol. Crystallogr.* 1999; 55:1718. [PubMed: 10531521]
24. Schuttelkopf AW, van Aalten DM. *Acta. Crystallogr., Sect. D: Biol. Crystallogr.* 2004; 60:1355. [PubMed: 15272157]
25. Brunger AT, Adams PD, Clore GM, DeLano WL, Gros P, Grosse-Kunstleve RW, Jiang JS, Kuszewski J, Nilges M, Pannu NS, Read RJ, Rice LM, Simonson T, Warren GL. *Acta. Crystallogr. D*. 1998; 54:905. [PubMed: 9757107]
26. Sobolev V, Sorokine A, Prilusky J, Abola EE, Edelman M. *Bioinformatics*. 1999; 15:327. [PubMed: 10320401]

Figure 1.
The structures of 9cRA, 9cUAB30, Targretin and 4-methyl-9cUAB30, and numbering schemes for the tetralone ring of 9cUAB30 and 4-methyl-9cUAB30.

Figure 2.
Overlay of X-ray crystal structures of hRXRα-LBD bound to 9cUAB30 (green), (*R*)-**1** (yellow) and (*S*)-**1** (magenta). GRIP-1 is highlighted in red.

Figure 3.

Conformation of rexinoids in the LBP of holo-hRXR α -LBD (A) Overlay of 9cUAB30 (green), (R)-1 (yellow) and (S)-1 (magenta) in the ligand binding pocket. Electron density maps (2F_o-F_c) for the ligand binding pocket of hRXR α -LBD homodimers containing (B) 9cUAB30; (C) (R)-1 and (D) (S)-1. (E) Overlay of 9cUAB30 (green), (R)-1 (yellow) and (S)-1 (magenta) in the ligand binding pocket from the point of view of the tetralone ring with C2 and C3 in front. (F) Puckered conformations of the 4-methyl tetralone ring in (R)-1 and (S)-1.

Scheme 1.

Table 1

Summary of Biological Data for (±)-**1** and Enantiomers

Retinoid Treatment	RXR α Binding ^a	RXR α Activation ^b	KLF4 Inhibition ^c	Serum Triglyceride ^d	Proliferation Index ^d	Apoptotic Index ^d
	K_d (nM)	EC_{50} (nM)	EC_{50} (nM)	Increase (%)	Decrease (%)	Increase (%)
9cRA	14 ± 3	120 ± 3	110 ± 13	326% ^d	56% ^d	67% ^d
Targretin	26 ± 3	40 ± 6	ND ^e	456% ^d	89% ^d	483% ^d
9cUAB30	33 ± 5	820	400 ± 90	65% ^d	65% ^d	33% ^d
(±)- 1	23 ± 5	±70	ND ^e	370%	89%	300%
(<i>R</i>)- 1	25 ± 5	110 ± 5	19 ± 4	420%	16%	150%
(<i>S</i>)- 1	13 ± 4	120 ± 5	27 ± 1	175%	89%	300%

^aThe dissociation constant K_d for retinoids and RXR α LBD was measured using fluorescence quenching method as reported by Xia *et al.*¹²^bThe EC_{50} values for RXR α transcriptional activation in RK3E cells was measured using a Luciferase assay as reported by Jiang *et al.*¹⁸^cThe EC_{50} value for retinoid inhibition of KLF4 transformation in RK3E cells was described by Jiang *et al.*¹⁸ and Boerma *et al.*¹⁰^dThe serum triglyceride levels, proliferation index, and apoptotic index were measured in female rats with MNU-initiated mammary tumors as reported by Grubbs *et al.*⁸ Data for 9cRA, Targretin, and 9cUAB30 were taken from Grubbs *et al.*⁸^eNot Determined.

Table 2Crystallographic data-collection and refinement statistics for hRXR α -LBD:4Me UAB30: GRIP-1 complexes

Data set PDB ID	(R)-1 4M8H	(S)-1 4M8E
Unit Cell parameters (Å, °)	a=65.92, b=65.92, c=111.65 $\alpha=90, \beta=90, \gamma=90$	a=66.28, b=66.28, c=111.01 $\alpha=90, \beta=90, \gamma=90$
Space group	P4 ₃ 2 ₁ 2	P4 ₃ 2 ₁ 2
Resolution (last shell) (Å)	29.30-2.20 (2.28-2.20)	26.15-2.40 (2.49-2.40)
No. of total/unique reflections	34390/12013	103513/10066
Rmerge (last shell) (%)	0.078 (0.218)	0.076 (0.314)
Redundancy (last shell)	2.86 (2.66)	10.28 (10.03)
Completeness (last shell) (%)	91.8 (94.5)	98.7 (99.3)
I/sigma (last shell)	8.7 (4.3)	17.0 (6.5)
Refinement		
no. of residues	224	224
no. of protein atoms	1785	1785
no. of water molecules	130	138
no. of ligand atoms	23	23
Rcryst (%)	22.81	20.85
Rfree (%)	26.52	24.68
R.M.S. deviation from ideality		
bond length (Å)	0.0081	0.0074
bond angles (°)	1.115	1.091
Ramachandran Plot		
most favored regions (%)	94.06	93.61
additional allowed regions (%)	3.65	4.57
generously allowed regions (%)	2.28	1.83
average B factor (Å ²)	29.22	40.06
non-hydrogen protein atoms (Å ²)	28.74	39.75
non-hydrogen ligand atoms (Å ²)	31.67	44.75
water molecules (Å ²)	36.00	37.92