BEXAROTENE METABOLISM IN RAT, DOG, AND HUMAN, SYNTHESIS OF OXIDATIVE METABOLITES, AND IN VITRO ACTIVITY AT RETINOID RECEPTORS

STANLEY R. HOWELL, MICHAEL A. SHIRLEY, TIMOTHY A. GRESE, DAVID A. NEEL, KIRK E. WELLS, AND EDGAR H. ULM


(Received October 17, 2000; accepted February 25, 2001)

This paper is available online at http://dmd.aspetjournals.org

ABSTRACT:

The metabolism of bexarotene, a rexinoid recently approved in the United States for treatment of cutaneous T-cell lymphoma, was studied using liver slices from untreated rats and dogs, liver microsomes from untreated and pretreated rats, and pooled human liver microsomes. Metabolite profiles were examined in bile and plasma from rats and dogs, and plasma from humans treated with bexarotene. Four metabolites, racemic 6-hydroxy-bexarotene, racemic 7-hydroxy-bexarotene, 6-oxo-bexarotene, and 7-oxo-bexarotene, were synthesized and their binding to, and transactivation of retinoid receptors were examined. Qualitatively similar metabolite profiles were observed in the microsomal and liver slice extracts; the predominant metabolites were 6-hydroxy-bexarotene and glucuronides of parent or hydroxylated metabolites. Pretreatment of rats with bexarotene induced hepatic microsomal bexarotene metabolism. The hydroxy and oxo metabolites were observed in plasma of rats, dogs, and humans treated with bexarotene and 6-hydroxy-bexarotene was a major circulating metabolite. The oxidative metabolites were more abundant relative to parent in plasma from humans than from rat or dog. The predominant biliary metabolites in rat and dog were bexarotene acyl glucuronide and a glucuronide of oxidized bexarotene, respectively. Since bexarotene elimination is primarily biliary in these species, these metabolites represent the main bexarotene metabolites in rats and dogs. The binding of synthetic metabolites to retinoid receptors was much reduced relative to parent compound. The metabolites exhibited minimal activity in transactivating retinoic acid receptors and had reduced activity at retinoid X receptors relative to bexarotene. Thus, while there is substantial systemic exposure to the oxidative metabolites of bexarotene, they are unlikely to elicit significant retinoid receptor activation following bexarotene administration.

Retinoids are compounds that bind to and activate one or more of the known nuclear retinoid receptor subtypes to modulate gene expression. There are two classes of retinoid receptors, RARs and RXRs, within each of which three subtypes (α, β, and γ) are recognized. RXRs can form homodimers or heterodimers with other ligand-bound nuclear receptors, such as RAR, peroxisome proliferator-activated receptor, vitamin D receptor, and thyroid hormone receptor (Chambon, 1996). This dimer formation can modulate the activity of these receptors, making RXR an important regulator of the activity of nonsteroid nuclear receptors (Mangelsdorf and Evans, 1995).

Numerous synthetic and endogenous retinoid receptor binding ligands have been identified and they display varying affinities for RARs and RXRs; the term “rexinoid” has been coined to refer to compounds that are selective for RXR (Mukherjee et al., 1997). Bexarotene (Fig. 1A) in an oral formulation (Targretin capsules) is a rexinoid that was recently approved in the United States for use in the treatment of cutaneous T-cell lymphoma. In the rat, bexarotene is metabolized to at least four phase I metabolites (6- and 7-hydroxy-bexarotene and 6- and 7-oxo-bexarotene) and a number of phase II metabolites, primarily glucuronides (Shirley et al., 1997). To examine metabolism of bexarotene across species, various biological matrices from rats, dogs, and humans treated with bexarotene and tissue or subcellular preparations incubated with bexarotene in vitro were analyzed by gradient HPLC with UV detection.

To evaluate whether circulating metabolites of bexarotene contribute to in vivo retinoid receptor activity following bexarotene administration, synthetic racemic 6-hydroxy-bexarotene, racemic 7-hydroxy-bexarotene, 6-oxo-bexarotene, and 7-oxo-bexarotene were analyzed for their binding to and transactivation of the six subtypes of RAR and RXR.

Materials and Methods

Chemicals. Except where otherwise indicated, reagents were purchased from Sigma Chemical Co., St. Louis, MO, or Aldrich Chemical Co., Milwaukee, WI. Bexarotene was supplied by the Medicinal Chemistry Department of Ligand Pharmaceuticals, Inc. or Raylo Chemical (Edmonton, Canada). Oxidative metabolites of bexarotene were synthesized at Eli Lilly & Co. as summarized in Scheme 1. HPLC solvents were purchased from Fisher Scientific (Fair Lawn, NJ). Super-refined sesame oil was purchased from Crodia Inc. (Parsippany, NJ).

Liver Slice. Standard techniques for liver slice studies were followed (Brendel et al., 1993). A male Sprague-Dawley rat (~300 g) was anesthetized with carbon dioxide and then killed by cervical dislocation before removal of its liver. A male beagle was anesthetized with intravenous pentobarbital and sacrificed by exsanguination before removal of its liver. For both rat and dog,
the liver was immediately placed in ice-cold SACKS buffer (0.75 g/l KH$_2$PO$_4$, 1.2 g/l NaHCO$_3$, 9.5 g/l K$_2$HPO$_4$, 37.5 g/l mannitol, 0.6 g/l KHCO$_3$, 0.75 g/l MgCl$_2$). Precision-cut liver slices (225 ± 25 μm) were prepared from liver cores using a Brendel-Vitron tissue slicer (Vitron Inc., Tucson, AZ) under ice-cold SACKS buffer and incubated in a dynamic organ slice incubator at 37°C in an atmosphere of 95% O$_2$ and 5% CO$_2$. After 5 h (rat) or 6 h (dog), the slices and media were homogenized together in 1 ml of ethanol, chilled at 5°C for 1 h to precipitate proteins, and then centrifuged. The supernatant was removed and evaporated in vacuo. The residue was then dissolved in 40% acetonitrile/glacial acetic acid (100:1) in 10 mM ammonium acetate/glacial acetic acid (100:1) for HPLC separation of metabolites.

Liver Microsomes. Hepatic microsomes were isolated from male Sprague-Dawley rats (300 g) that had received four daily doses of either bexarotene (100 mg/kg/day p.o., as an aqueous microparticulate suspension) or vehicle. Livers were homogenized in 50 mM Tris-HCl (pH 7.4) containing 150 mM KCl and 2 mM EDTA (Guengerich, 1982). Microsomes were prepared by ultracentrifugation (100,000 g for 60 min) of the postmitochondrial supernatant (10,000 g for 20 min). The first microsomal pellet was resuspended in 10 mM EDTA, pH 7.4, containing 150 mM KCl and resolubilized by ultracentrifugation. Pooled (11 donors, male and female) human liver microsomes were purchased from Human Biologics, Inc. (Phoenix, AZ).

Both rat and human microsomes were incubated at a protein concentration of 1 mg/ml and a starting bexarotene concentration of 100 μM. This concentration is consistent with the liver concentration estimated to occur in rats receiving tolerated oral doses of bexarotene.$^2$ Incubation mixtures contained an NADPH-generating system (Arlotto et al., 1987) and 0.5% (v/v) ethanol. After incubation at 37°C for 4 h, the reactions were quenched with 1.5 ml of ice-cold ethanol. Samples were then chilled at <5°C for 1 h to precipitate proteins and centrifuged. Supernatants were removed and evaporated in vacuo. The residues were then dissolved in 40% acetonitrile/glacial acetic acid (100:1) in 10 mM ammonium acetate/glacial acetic acid (100:1) for HPLC separation of metabolites.

Bile. To investigate biliary metabolites, two fed male rats (~300 g) that had received four daily doses of either bexarotene (100 mg/kg/day p.o., as an aqueous microparticulate suspension) or vehicle. Livers were homogenized in 50 mM Tris-HCl (pH 7.4) containing 150 mM KCl and 2 mM EDTA (Guengerich, 1982). Microsomes were prepared by ultracentrifugation (100,000 g for 60 min) of the postmitochondrial supernatant (10,000 g for 20 min). The first microsomal pellet was resuspended in 10 mM EDTA, pH 7.4, containing 150 mM KCl and resolubilized by ultracentrifugation. Pooled (11 donors, male and female) human liver microsomes were purchased from Human Biologics, Inc. (Phoenix, AZ).

Both rat and human microsomes were incubated at a protein concentration of 1 mg/ml and a starting bexarotene concentration of 100 μM. This concentration is consistent with the liver concentration estimated to occur in rats receiving tolerated oral doses of bexarotene.$^2$ Incubation mixtures contained an NADPH-generating system (Arlotto et al., 1987) and 0.5% (v/v) ethanol. After incubation at 37°C for 4 h, the reactions were quenched with 1.5 ml of ice-cold ethanol. Samples were then chilled at <5°C for 1 h to precipitate proteins and centrifuged. Supernatants were removed and evaporated in vacuo. The residues were then dissolved in 40% acetonitrile/glacial acetic acid (100:1) in 10 mM ammonium acetate/glacial acetic acid (100:1) for HPLC separation of metabolites.

Plasma. Plasma samples were obtained from rats that received either single or 15 daily oral doses of bexarotene, female dogs that received a single oral 25-mg/kg dose of bexarotene, and from patients with advanced cancer that...
received one or 15 to 16 daily 230- to 400-mg/m² (-6–10-mg/kg) doses of bexarotene. Plasma samples were extracted with 5 volumes of methanol; after chilling to -20°C and centrifuging at 4°C, the supernatants were dried by evaporation in vacuo and reconstituted in 40% acetonitrile in 10 mM ammonium acetate/glacial acetic acid (100:1).

Analytical Procedures. Metabolite molar extinction coefficients were determined by preparing 20.0 μM solutions of each metabolite and measuring UV absorbance at λ_{max} using a Beckman DU600 spectrophotometer (Beckman Instruments, Fullerton, CA). For analysis of plasma, bile, liver slice, and microsome extracts, gradient reverse phase HPLC (Hewlett-Packard model 1090 or 1050) was used to separate bexarotene and its metabolites. Peak detection was at 262 nm. The analytical HPLC column (Microsorb-MV 5 μm, 4.6 × 250 mm, C18) was obtained from Rainin Instrument Co., Inc. (Woburn, MA) and maintained at 40°C. The solvent program ramped linearly from 20 to 80% acetonitrile/glacial acetic acid (100:1) in 10 mM ammonium acetate/glacial acetic acid (100:1) over 20 min and was maintained for 15 min at 80% acetonitrile.

Identity of Metabolite Peaks. The identities of the various bexarotene metabolites detected by HPLC were assigned based on their retention times relative to metabolite peaks previously identified using mass spectrometry (6-hydroxy-bexarotene, 7-hydroxy-bexarotene, 6-oxo-bexarotene, 6/7-ether glucuronide of bexarotene, acyl glucuronide of 6/7-hydroxy-bexarotene, bexarotene taurine conjugate, and bexarotene acyl glucuronide; Shirley et al., 1997) and by coelution with synthetic standards (6-hydroxy-bexarotene, 7-hydroxy-bexarotene, 6-oxo-bexarotene, and 7-oxo-bexarotene). With the chromatographic method used, the enantiomers of the hydroxylated metabolites were not resolved and the C-6 and C-7 isomers of the hydroxy and oxo metabolites were incompletely resolved; therefore, some of the results for these metabolites are reported as the sum of the isomers, denoted by the prefix “6/7-.”

In Vitro Binding and Activity of Metabolites. Synthetic racemic 6-hydroxy-bexarotene, racemic 7-hydroxy-bexarotene, 6-oxo-bexarotene, and 7-oxo-bexarotene were analyzed for their binding to RAR and RXR subtypes (Boehm et al., 1994). Samples were also assessed in transactivation assays for RXR and RAR activity (Berger et al., 1992). Potency was calculated as the concentration of each compound that caused an activation of the receptor to 50% of its maximum activation by that compound (EC50). Efficacy was calculated as the maximum activation caused by each compound, expressed as a percentage of the maximum activation caused by a standard pan agonist (LG100351, Fig. 1B; Krebs, 1985). LG100351 was used as a comparator to provide the ability to assess the activity of bexarotene in parallel with the oxidative bexarotene metabolites, thereby enabling relative retinoid receptor selectivities to be demonstrated.

Results

Synthetic Metabolites. The synthetic route developed for compound 2 is regiochemically unambiguous and can only give rise to the 7-oxo regioisomer (Scheme 1). Although the synthesis of 1 could conceivably lead to either regioisomer, independent synthesis of 17-oxo regioisomer (Scheme 1). Therefore, HPLC-UV peak areas were used to estimate the relative abundance of bexarotene and its oxidative metabolites. Recoveries of bexarotene and its metabolites were assumed to be equivalent.

Metabolites Formed in the Rat. A number of metabolites were formed by rat liver slices incubated with bexarotene (Fig. 2). Based on their retention times relative to previously identified metabolite peaks (Shirley et al., 1997), the predominant metabolites were products of oxidation at C-6, C-7, and/or glucuronidation, the latter occurring either at the added oxygen (ether glucuronides) or the carboxyl moiety of the parent compound (acyl glucuronide) or hydroxy metabolites. Significant amounts of the nonhydroxylated acyl glucuronide and nonconjugated 6/7-hydroxy-bexarotene were formed. 6/7-Oxo-bexarotene was present at lower concentrations than 6/7-hydroxy-bexarotene. While the C-6 and C-7 isomers were not completely resolved, coelution assessments for both the hydroxy and oxo metabolites indicated that the predominant peaks were C-6 isomers and the later-eluting shoulders were C-7 isomers.

HPLC analysis of metabolites formed by cytochrome P450 in rat liver microsomes from vehicle-treated rats revealed that 6/7-hydroxy-bexarotene was the only significant metabolite formed after 4 h of incubation (Fig. 3A). Pretreatment of the rats with bexarotene induced oxidation at the C-6 and C-7 positions and several minor unidentified metabolites that eluted between 15 and 19 min (Fig. 3B).

By far the most abundant metabolite measured in rat bile was bexarotene acyl glucuronide (Fig. 4). Glucuronide conjugates of hydroxy-bexarotene, the taurine conjugate of bexarotene, and a trace amount of the parent were also present.

In comparing male to female rats, there were no discernible differences between their plasma metabolite profiles following a single dose, or before and after 15 daily doses of bexarotene (100 mg/kg/day). 6/7-Hydroxy-bexarotene predominated over 6/7-oxo-bexarotene (Fig. 5) and for both metabolites, the C-6 isomer predominated over the C-7 isomer. The 6/7-hydroxy and 6/7-oxo metabolites were less abundant than parent compound in rat plasma after a single dose and after multiple doses of bexarotene (Fig. 6). Both the metabolites and parent were less abundant on day 15 than on day 1, but the ratio of the metabolites to parent in plasma did not change appreciably during this time. Both 6/7-hydroxy-bexarotene and parent compound were detectable before dosing on day 15.

Metabolites Formed in the Dog. HPLC analysis of extracts from dog liver slices incubated with bexarotene for 6 h revealed a metabolite profile in which the acyl glucuronide of bexarotene predominated (Fig. 7). Smaller amounts of 6/7-hydroxy- and 6/7-oxo-bexarotene were observed. As in rat, the C-6 isomers of both the oxo and hydroxy metabolites were more abundant than the C-7 isomers. Two peaks eluted at retention times consistent with those of the ether glucuronides observed in the rat.

While bile collected from a bexarotene-treated (25-mg/kg) dog contained the acyl glucuronide of bexarotene, the predominant metabolite was the acyl glucuronide of hydroxylated bexarotene (Fig. 8).
Smaller amounts of the ether glucuronides were observed. Unconjugated oxidative metabolites and parent compound were not readily apparent.

As in rat, the 6/7-hydroxy and 6/7-oxo metabolites were less abundant than parent compound in plasma samples from dogs that received bexarotene (Figs. 9 and 10). Again, both hydroxy- and oxo-bexarotene were present primarily as the C-6 isomer.

**Metabolites Formed in Humans.** The predominant metabolites formed by human liver microsomes were 6/7-hydroxy- and 6/7-oxo-bexarotene (Fig. 11). While still less abundant than the hydroxylated metabolites, the 6/7-oxo metabolites were more abundant relative to the 6/7-hydroxy metabolites than in rat microsomes. As in the rat and dog, the C-6 isomer of the hydroxy and oxo metabolites predominated over the C-7 isomer. A number of minor peaks presumed to be metabolites were also observed.

Plasma samples from five patients enrolled in Phase 1–2 clinical studies were analyzed. The metabolite profiles observed were similar on Day 1 and Day 15–16 of dosing. The predominant plasma metabolites were 6/7-hydroxy- and 6/7-oxo-bexarotene (Fig. 12). As in rat and dog plasma, the C-6 isomer predominated over the C-7 isomer for both hydroxy-bexarotene and oxo-bexarotene. Overall, the abundance of the 6/7-hydroxy (primarily 6-hydroxy) metabolite was greater than that of parent in patients following both single and multiple doses (Fig. 13; Table 1). The abundance of the 6/7-oxo (primarily 6-oxo) metabolite was lower than or equivalent to that of parent after a single dose and after multiple doses.

**In Vitro Binding of Metabolites.** The binding of synthetic bexarotene metabolites to retinoid receptors was weak (Table 2). In only one of three experiments did they display any RAR binding (at RARα only) and then only with very high Kd values. The binding of metabolites to RXR was stronger than that to RAR, but it was much reduced relative to the parent compound; the only RXR binding affinity of less than 1000 nM was that of 7-oxo-bexarotene, the least.
abundant of the human plasma metabolites. The most abundant human plasma metabolite, 6-hydroxy-bexarotene, displayed the weakest binding at RXR ($K_D \approx 3000$ nM).

**In Vitro Transactivation by Metabolites.** HPLC-UV analysis confirmed that the compounds were stable for the duration of the transactivation assay. Consistent with historical data, bexarotene was about 100-fold more potent at RXR relative to RAR (Table 3). In six tests (duplicate assays on each of three occasions), the metabolites exerted little activity at RAR, exhibiting efficacies that were quite low ($\leq 20\%$) compared with those of LG100351, the standard pan agonist. All four metabolites displayed lower efficacy than bexarotene at RAR$\alpha$ and RAR$\beta$. While the efficacies of the metabolites at RAR$\alpha$ ($\leq 22\%$) were greater than bexarotene’s (7%), they are considered low in absolute terms. The potencies of the four metabolites at the RAR subtypes were weak ($>1000$ nM) and were equal to or lower than those of the parent. The metabolite efficacies at RXR were similar to that of the parent for all three subtypes, but their potencies were substantially reduced. Three of the four metabolites displayed EC$_{50}$ values at all three RXR subtypes that were at least 10 times less potent than bexarotene, and the 7-oxo metabolite was at least 3 times less potent. None of the metabolites displayed any activity at RAR or RXR when assayed in the antagonist mode (ability to counteract the effect of an EC$_{50}$ concentration of a standard agonist, LG100351).
In general, the qualitative metabolite profiles were similar in rat, dog, and human as demonstrated in the representative chromatograms shown in Figs. 2 to 5, 7 to 9, and 11 to 12, respectively. Consistent with findings for the rat (Shirley et al., 1997), the significant routes of metabolism of bexarotene in the dog and human were oxidation at C-6 and C-7 and glucuronidation, with oxidation at C-6 predominating over that at C-7 in all three species. Metabolite profiles for bexarotene in rat liver microsomes were consistent with those in rat liver slices. Although hydroxylated bexarotene was detected previously in the bile of bexarotene-treated rats, the mass spectral method used was not quantitative (Shirley et al., 1997), and the small amounts present were not detectable by UV absorbance. In the present study, other than the absence of phase I metabolites, the metabolite profiles in the bile of rats and a dog were qualitatively similar to the profiles in the liver slice model. The presence of the oxidized metabolites in plasma but not bile suggests selective transport of these compounds into the bloodstream, probably due to the action of transporter enzymes. A species difference in biliary metabolite profile existed in that bexarotene acyl glucuronide was the predominant metabolite in rat, while in dog the major metabolite was hydroxylated bexarotene acyl glucuronide. This is opposite the relationship observed in liver slices, in which the major glucuronides for rat were of oxidative metabolites while for the dog, the major glucuronide was that of the parent compound. Since the primary route of bexarotene excretion is hepatobiliary in rats and dogs\(^2\), glucuronidation is likely the primary route of bexarotene metabolism in these species.

6/7-Hydroxy-bexarotene and, to a lesser extent, 6/7-oxo-bexarotene appeared to be the major drug-related species in plasma from rats, dogs, and humans having received bexarotene. For both the hydroxy and acetoxy metabolites, the C-6 isomer was predominant. Therefore, 6-hydroxy-bexarotene was the major circulating metabolite in all three species. A difference in circulating metabolite levels was observed between humans and the other species; while in the rat and dog the parent was more abundant than either metabolite, in humans, 6/7-hydroxy-bexarotene was more abundant than parent and the abundance of 6/7-oxo-bexarotene was less than or equivalent to that of parent. This difference may be related to involvement of different cytochrome P450 isozymes in the different species. While CYP3A has been demonstrated to be involved in the oxidation of bexarotene in microsomes from both rat and human livers, CYP2B was also implicated in rat and CYP2C9 and CYP2C19 may be involved in human (Hein et al., 1996).

Synthetic bexarotene metabolites exhibited in vitro activity at retinoid receptors that was much reduced relative to parent, albeit each of the tested 6-hydroxy and 7-hydroxy metabolites was a mixture of two enantiomers, which could have different activities at RXR. The ratios of these enantiomers in the metabolite peaks appearing in patients’ plasma may not have been the same as those in the racemic mixtures tested for biological activity. Nevertheless, data derived with the racemates indicate that binding of the metabolites to retinoid receptors is weak and retinoid receptor transactivation potency of the metabolites is low. Thus, even at the highest concentrations achieved in patients, the contribution of metabolites to the clinical retinoid receptor activity of bexarotene is probably insignificant.

### Appendix

#### Synthesis of Metabolites

The 6- and 7-oxo-bexarotene and 6- and 7-hydroxy-bexarotene metabolites were prepared unambiguously as described in Scheme 1. Thus, Friedel-Crafts acylation of 1 (prepared by the method of Bruson et al., 1958; Zhang et al., 1995) followed by Wittig olefination of the benzophenone carbonyl provided the ester 5.

Hydrolysis of 5 then provided 6-oxo-bexarotene (7). Alternatively, selective reduction of the ketone moiety in 5 followed by hydrolysis provided racemic 6-hydroxy-bexarotene (9). The isomeric 7-hydroxy-bexarotene (8) and racemic 7-hydroxy-bexarotene (10) were prepared in similar manner from 2, available in four steps from 4-methylbenzyl cyanide (Barclay et al., 1970).

#### 3,4-Dihydro-1,1,4,4,7-pentamethyl-2(1H)-naphthalenone (1)

A solution of dihydro-2,2,5,5-tetramethyl-3(2H)-furanone (24.0 g, 190 mmol) in 100 ml of toluene at 5°C was treated slowly with aluminum chloride (45.50 g, 341 mmol, 1.8 eq). The solution was stirred for 30 min and then filtered. The filtrate was evaporated in vacuo. The dark brown residue was chromatographed on 1 kg of Silica 60 with 0 to 5% ethyl acetate in hexanes. The resulting material was recrystallized from hexanes to yield 9.5 g (23%) white needles.

#### 4-Methylbenzyl cyanide

4-Methylbenzyl cyanide (66.5 g, 0.5 mol), methyl iodide (78 ml, 1.25 mol, 2.5 eq), and 18-crown-6 (13.2 g, 0.05 mol, 0.1 eq) were combined in dry THF (2 liters) and cooled to -75°C under nitrogen. Potassium t-butoxide (140.3 g, 1.25 mol, 2.5 eq) was added in four portions over 5 min and the reaction mixture was heated at 75°C for 15 min. After cooling in an ice bath the reaction was poured into 300 ml of ice water and extracted with diethyl ether (2 × 250 ml). The organic portions were combined, dried (MgSO\(_4\)), filtered, and concentrated in vacuo. The dark residue was chromatographed on 1 kg of Silica 60 with 0 to 5% ethyl acetate in hexanes. The material was recrystallized from hexanes to yield 9.5 g (23%) white needles.

#### 3,4-Dihydro-1,1,4,4,7-pentamethyl-2(1H)-napthalenone (1)

3,4-Dihydro-1,1,4,4,7-pentamethyl-2(1H)-napthalenone (1) was prepared as described in Scheme 1. Thus, Friedel-Crafts acylation of 1 (prepared by the method of Bruson et al., 1958; Zhang et al., 1995) followed by Wittig olefination of the benzophenone carbonyl provided the ester 5.

Hydrolysis of 5 then provided 6-oxo-bexarotene (7). Alternatively, selective reduction of the ketone moiety in 5 followed by hydrolysis provided racemic 6-hydroxy-bexarotene (9). The isomeric 7-hydroxy-bexarotene (8) and racemic 7-hydroxy-bexarotene (10) were prepared in similar manner from 2, available in four steps from 4-methylbenzyl cyanide (Barclay et al., 1970).

#### 4-Methylbenzyl cyanide

4-Methylbenzyl cyanide (66.5 g, 0.5 mol), methyl iodide (78 ml, 1.25 mol, 2.5 eq), and 18-crown-6 (13.2 g, 0.05 mol, 0.1 eq) were combined in dry THF (2 liters) and cooled to -75°C under nitrogen. Potassium t-butoxide (140.3 g, 1.25 mol, 2.5 eq) was added in four portions over 5 min and the reaction mixture was heated at 75°C for 15 min. After cooling in an ice bath the reaction was poured into 300 ml of ice water and extracted with diethyl ether (2 × 250 ml). The organic portions were combined, dried (MgSO\(_4\)), filtered, and concentrated in vacuo. The dark residue was chromatographed on 1 kg of Silica 60 with 0 to 5% ethyl acetate in hexanes. The resulting material was recrystallized from hexanes to yield 9.5 g (23%) white needles.

#### 3,4-Dihydro-1,1,4,4,7-pentamethyl-2(1H)-napthalenone (1)

3,4-Dihydro-1,1,4,4,7-pentamethyl-2(1H)-napthalenone (1) was prepared as described in Scheme 1. Thus, Friedel-Crafts acylation of 1 (prepared by the method of Bruson et al., 1958; Zhang et al., 1995) followed by Wittig olefination of the benzophenone carbonyl provided the ester 5.

Hydrolysis of 5 then provided 6-oxo-bexarotene (7). Alternatively, selective reduction of the ketone moiety in 5 followed by hydrolysis provided racemic 6-hydroxy-bexarotene (9). The isomeric 7-hydroxy-bexarotene (8) and racemic 7-hydroxy-bexarotene (10) were prepared in similar manner from 2, available in four steps from 4-methylbenzyl cyanide (Barclay et al., 1970).
was added and the reaction heated to 100°C. Additional 2-methoxy ethanol (300 ml) was added to effect solution and the reaction was heated at 103°C for 64 h. The solvent (460 ml) was removed by distillation while the temperature remained at 115°C. Water (1 liter) was added and the reaction allowed to cool to room temperature. The aqueous portion was first washed with CH₂Cl₂ (3 × 500 ml) and then filtered to remove a small amount of insoluble material. The aqueous solution was then cooled to 5°C and treated with 5 N HCl (600 ml), filtered (using coarse filter paper), and dried in vacuo for 18 h at 40°C to obtain 73.7 g (87%) of an off-white solid. The material was recrystallized from hexanes/diethyl ether to obtain 62.6 g of white crystals. A second crop of 3.5 g was obtained for a combined yield of 78%. ¹H NMR (CDCl₃): δ 1.54 (6H, s), 2.29 (3H, s), 7.10 (2H, d, J = 8 Hz), 7.25 (2H, d, J = 8 Hz); MS (ES) m/z 178 (M + H)⁺. Anal. calcld. for C₁₁H₁₄O₂: C, 74.13; H, 7.92. Found: C, 74.43; H, 7.94.

**α,α,4-Trimethylbenzenacetal Chloride.** α,α,4-Trimethylbenzenecacetic acid (62.4 g, 350 mmol) was dissolved in thionyl chloride (300 ml) under nitrogen and heated at 65°C for 22 h. The thionyl chloride was removed in vacuo, dry CH₂Cl₂ was added, and the material reconstituted to provide 67.4 g (98%) of an orange oil. ¹H NMR (CDCl₃): δ 1.63 (6H, s); 2.31 (3H, s); 7.17 (4H, m).

**4-Dihydro-1,1,4,4,6-pentamethyl-2(1H)-naphthalenone (2).** The acid chloride (25.00 g, 127 mmol) was dissolved in dry CH₂Cl₂ (400 ml) under nitrogen and the solution was cooled in an ethylene glycol/dry ice bath. Tin (IV) chloride (17 ml, 17 mmol, 0.13 eq of a 1.0 M solution in CH₂Cl₂) was added at -11°C and the solution turned dark purple. In a graduated cylinder cooled to -78°C was condensed

### TABLE 1

Mean and median ratio of area under the chromatographic peak area versus time curves (AUC) of metabolites to those of bexarotene in plasma of patients (N = 5) treated with bexarotene for 1 or 15 to 16 days (240–400 mg/m²/day, by mouth).

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Ratio of AUC</th>
<th>Day 1</th>
<th>Day 15 or 16</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± S.D.</td>
<td>Median (Range)</td>
<td>Mean ± S.D.</td>
</tr>
<tr>
<td>6/7-Hydroxy-bexarotene</td>
<td>1.9 ± 0.7</td>
<td>1.9 (0.8–2.8)</td>
<td>3.0 ± 2.7</td>
</tr>
<tr>
<td>6/7-oxo-Bexarotene</td>
<td>0.5 ± 0.2</td>
<td>0.5 (0.2–0.6)</td>
<td>0.7 ± 0.4</td>
</tr>
</tbody>
</table>

### TABLE 2

Binding of synthetic bexarotene metabolites and parent compound to RAR and RXR subtypes in vitro.

<table>
<thead>
<tr>
<th>Compound</th>
<th>K_D (nM)</th>
<th>RARα</th>
<th>RARβ</th>
<th>RARγ</th>
<th>RXRα</th>
<th>RXRβ</th>
<th>RXRγ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bexarotene</td>
<td>8.17 ± 0.1</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>3,464 ± 474</td>
<td>4,205 ± 1,003</td>
<td>4,831 ± 1,354</td>
<td></td>
</tr>
<tr>
<td>LG100351</td>
<td>7.90 ± 0.1</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>1,324 ± 253</td>
<td>2,265 ± 634</td>
<td>1,702 ± 797</td>
<td></td>
</tr>
</tbody>
</table>

### TABLE 3

Efficacy and potency (EC₅₀) of synthetic bexarotene metabolites in trans-activating RAR and RXR subtypes in vitro.

Efficacy of compounds is calculated relative to LG100351, a reference pan agonist. Potency for any given assay set at 10,000 when efficacy is <10%. Values are presented as mean ± S.E.

<table>
<thead>
<tr>
<th>Compound</th>
<th>RARα</th>
<th>RARβ</th>
<th>RARγ</th>
<th>RXRα</th>
<th>RXRβ</th>
<th>RXRγ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bexarotene</td>
<td>7 ± 1</td>
<td>20 ± 5</td>
<td>12 ± 3</td>
<td>57 ± 17</td>
<td>292 ± 19</td>
<td>32 ± 2</td>
</tr>
<tr>
<td>LG100351</td>
<td>112 ± 15</td>
<td>97 ± 3</td>
<td>15 ± 3</td>
<td>7013 ± 2987</td>
<td>292 ± 19</td>
<td>32 ± 2</td>
</tr>
<tr>
<td>6-Hydroxy-bexarotene</td>
<td>16 ± 6</td>
<td>31 ± 6</td>
<td>14 ± 5</td>
<td>4414 ± 1800</td>
<td>1462 ± 328</td>
<td>602 ± 259</td>
</tr>
<tr>
<td>6-oxo-Bexarotene</td>
<td>22 ± 11</td>
<td>36 ± 6</td>
<td>14 ± 5</td>
<td>4199 ± 1852</td>
<td>2590 ± 419</td>
<td>602 ± 259</td>
</tr>
<tr>
<td>7-Hydroxy-bexarotene</td>
<td>14 ± 4</td>
<td>12 ± 3</td>
<td>15 ± 3</td>
<td>5133 ± 2176</td>
<td>2590 ± 419</td>
<td>602 ± 259</td>
</tr>
<tr>
<td>7-oxo-Bexarotene</td>
<td>13 ± 5</td>
<td>36 ± 6</td>
<td>15 ± 3</td>
<td>4179 ± 1851</td>
<td>602 ± 259</td>
<td>602 ± 259</td>
</tr>
</tbody>
</table>

*α,α,4-Trimethylbenzenacetal Chloride. α,α,4-Trimethylbenzenecacetic acid (62.4 g, 350 mmol) was dissolved in thionyl chloride (300 ml) under nitrogen and heated at 65°C for 22 h. The thionyl chloride was removed in vacuo, dry CH₂Cl₂ was added, and the material reconstituted to provide 67.4 g (98%) of an orange oil. ¹H NMR (CDCl₃): δ 1.63 (6H, s); 2.31 (3H, s); 7.17 (4H, m).

**4-Dihydro-1,1,4,4,6-pentamethyl-2(1H)-naphthalenone (2).** The acid chloride (25.00 g, 127 mmol) was dissolved in dry CH₂Cl₂ (400 ml) under nitrogen and the solution was cooled in an ethylene glycol/dry ice bath. Tin (IV) chloride (17 ml, 17 mmol, 0.13 eq of a 1.0 M solution in CH₂Cl₂) was added at -11°C and the solution turned dark purple. In a graduated cylinder cooled to -78°C was condensed
isobutylene (approximately 45 ml, 500 mmol, 4 eq). The liquid isobutylene was then added via cannula to the acid chloride at −28°C approximately 15 to 20 min after addition of the tin chloride. The temperature was maintained at −10−0°C over the next hour. TLC (9:1 hexanes/EtOAc) showed a new spot (Rf = 0.55), which was thought to be the intermediate unsaturated ketone and not the eventual product (Rf = 0.40). The cooling bath was removed and the reaction allowed to warm to room temperature while stirring for 18 h. TLC indicated the first spot was still present (5−10%) and a new spot corresponding to product had appeared. More tin chloride (1.2 ml, 0.01 eq) was added and after 1 h the first spot was entirely gone. The mixture was poured into water (150 ml), the organic layer was separated, and the aqueous was washed with more CH₂Cl₂ (200 ml). The organic portions were combined and washed with brine (200 ml), dried (MgSO₄), filtered, and concentrated in vacuo to give 33.3 g of a green oil, which solidified on standing. The material was purified by silica pad filtration using 98:2 hexanes/EtOAc and then 9:1 hexanes/EtOAc to provide 23.1 g of a yellow solid. The solid was recrystallized from hexane (130−150 ml) to provide 9.05 g of white crystals. mp 76–77°C. Purification of the mother liquor by preparative HPLC using hexane (93:7)→ethyl acetate (1:1) to give 4.59 g of a light pink solid. The material was purified by flash chromatography (9:1 hexanes/EtOAc) to provide 557 mg (63%), which was recrystallized from pentane to give 342 mg (39%) of white crystals. mp 148−150°C.

Methyl 4-[1-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-7-hydroxy-2-naphthalenyl)carbonyl]-benzoate (3). Prepared from 1 by the method described for 4 below. 1H NMR (CDCl₃): δ 1.26 (6H, s), 1.39 (6H, s), 2.31 (3H, s), 2.59 (2H, s), 7.04 (2H, d, J = 8 Hz); 7.13 (1H, s), 7.17 (2H, d, J = 8 Hz); MS (ES) m/z 217 (M + H)⁺. Anal. calc. for C₁₅H₂₀O₃: C, 76.17; H, 9.32. Found: C, 76.18; H, 9.31.

Methyl 4-[1-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-6-oxo-2-naphthalenyl)carbonyl]-benzoate (4). Prepared from 5 in 58% yield by the method described for 6 below. 1H NMR (CDCl₃): δ 1.12 (4H, d, J = 6 Hz), 1.49 (s, 6H), 2.35 (s, 3H), 2.65 (s, 2H), 3.97 (s, 3H), 7.23 (s, 1H), 7.32 (1H, J = 8 Hz); 8.14 (d, J = 8 Hz); MS (ES) m/z 379 (M + H)⁺. Anal. calc. for C₂₅H₃₀O₃: C, 79.49; H, 7.92.

Methyl 4-[1-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-7-oxo-2-naphthalenyl)ethyl]-benzoate (5). Prepared from 3 in 65% by the method described for 6 below. 1H NMR (CDCl₃): δ 1.31 (s, 6H), 1.48 (s, 6H), 1.99 (s, 3H), 2.66 (s, 2H), 3.91 (s, 3H), 5.34 (d, 1H, J = 8 Hz), 5.86 (d, 1H, J = 8 Hz), 7.10 (s, 1H), 7.19 (s, 1H), 7.34 (d, 1H, J = 8.4), 7.97 (d, 1H, J = 8.4 Hz); MS (ES) m/z 377 (M + H)⁺.

Methyl 4-[1-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-7-oxo-2-naphthalenyl)ethyl]-benzoate (6). A stirred suspension of methyltriphenylphosphonium bromide (2.09 g, 5.85 mmol) in toluene (50 ml) at 0°C under nitrogen was treated with a 0.5 M (12.3 ml, 6.14 mmol) solution of potassium bis(trimethylsilylamide) in toluene. The diketone (886 mg, 2.34 mmol) in toluene (20 ml) was added dropwise and the reaction mixture was stirred 3 h at 0−5°C. Water (10 ml) was added dropwise at 0°C and the mixture partitioned between diethyl ether (40 ml) and water (40 ml). The layers were separated and the aqueous washed again with diethyl ether (40 ml). The ether portions were combined and washed with brine, dried (Na₂SO₄), and concentrated in vacuo to give 2.27 g of a light pink solid. The material was purified by flash chromatography (9:1 hexanes/EtOAc) and then 4:1 hexanes/EtOAc) to provide 557 mg (63%), which was recrystallized from pentane to give 342 mg (39%) of white crystals. mp 148−150°C.

Methyl 4-[1-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-7-oxo-2-naphthalenyl)ethyl]-benzoate (7). Prepared from 5 in 58% yield by the method described for 6 below. 1H NMR (CDCl₃): δ 1.26 (6H, s), 1.39 (s, 6H), 1.95 (s, 3H), 2.65 (s, 2H), 3.53 (br m, 1H), 5.97 (br m, 1H), 7.18 (1H, J = 8 Hz), 7.29 (s, 1H), 7.36 (d, 1H, J = 8.5 Hz), 7.92 (d, 1H, J = 8.5 Hz), 12.97 (br s, 1H); MS (ES) m/z 361 (M−H)⁻. Anal. calc. for C₂₄H₂₆O₃ · 0.25 H₂O: C, 78.53; H, 7.29. Found: C, 78.70; H, 7.22.

Methyl 4-[1-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-7-oxo-2-naphthalenyl)ethyl]-benzoate (8). The methyl ester (1.80 g, 4.78 mmol) was suspended in MeOH (50 ml). KOH (5 N, 2.6 ml) was added and the reaction refluxed gently for 2 h. The reaction was cooled to 0°C and 5 N HCl (10 ml) was slowly added. The mixture was washed with EtOAc (300 ml, 2× 100 ml). The combined organic layers were washed with brine (100 ml), dried (MgSO₄), filtered, and concentrated in vacuo to provide 1.9 g of a solid. The solid was recrystallized from 1:1 hexanes/EtOAc to give 1.11 g (64%) of white crystals. mp 254−257°C. 1H NMR (DMSO-δ₆): δ 1.26 (6H, s), 1.37 (s, 6H), 1.96 (s, 3H), 2.65 (s, 2H), 5.31 (s, 1H), 5.97 (s, 1H), 7.19 (1H, J = 8 Hz), 7.27 (s, 1H), 7.36 (d, 2H, J = 9 Hz), 7.92 (d, 2H, J = 9 Hz), 12.93 (bs, 1H); MS (ES) m/z 363 (M + H)⁺. Anal. calc. for C₂₅H₂₈O₃: C, 79.73; H, 7.23. Found: C, 79.81; H, 7.25.

Methyl 4-[1-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-7-hydroxy-2-naphthalenyl)ethyl]-benzoate (9). The ketone 5 (1.25 g, 3.32 mmol) was partially dissolved in methanol (50 ml) followed by dropwise addition of dichloromethane until a solution was achieved. Sodium borohydride (750 mg, 19.8 mmol) was added in 250-mg portions and the reaction stirred for 6 h at room temperature. A saturated solution of ammonium chloride was added dropwise to destroy the excess sodium borohydride and the product extracted with dichloromethane. The organic portion was washed with saturated ammonium chloride, dried (MgSO₄), filtered, and concentrated in vacuo to provide 1.21 g (97%) of a white solid. 1H NMR (CDCl₃): δ 1.07 (3H, s), 1.24 (3H, s), 1.26 (3H, s), 1.33 (3H, s), 1.61 (dd, 1H), 1.76 (t, 1H), 1.90 (s, 3H), 3.67 (dt, 1H), 3.84 (3H, s), 4.65 (d, 1H), 5.28 (br s, 1H), 5.92 (br s, 1H), 7.05 (s, 1H), 7.16 (s, 1H), 7.36 (d, 1H, J = 8.3 Hz), 7.92 (d, 1H, J = 8.3 Hz); MS (ES) m/z 379 (M + H)⁺. Anal. calc. for C₂₃H₂₈O₃: C, 79.33; H, 7.99. Found: C, 79.13; H, 8.18.

Hydrolysis of this material by the method described for 6 above provided the title compound in 90% yield. 1H NMR (CDCl₃): δ 1.07
(s, 3H), 1.24 (s, 3H), 1.26 (s, 3H), 1.33 (s, 3H), 1.61 (dd, 1H), 1.77 (t, 1H), 1.90 (s, 3H), 3.68 (dt, 1H), 4.68 (d, 1H), 5.27 (br s, 1H), 5.91 (br s, 1H), 7.05 (s, 1H), 7.16 (s, 1H), 7.33 (d, 1H, J = 5.83 Hz), 7.90 (d, 1H, J = 5.83 Hz), 12.97 (br s, 1H). MS (ES) m/z 363 (M-H)⁺. Anal. calcd. for C₂₄H₂₈O₃: C, 79.08, H, 7.74. Found: C, 79.03, H, 7.62.

4-[1-(5,6,7,8-Tetrahydro-3,5,5,8,8-pentamethyl-7-hydroxy-2-naphthalenyl)ethenyl]benzoic acid (10). Reduction of the ketone 6 by the method described above provided 3.12 g of a white solid, which was recrystallized from hexanes/diethyl ether to give 2.57 g (83%) of the intermediate ester as a white powder. mp 163–165°C. ¹H NMR (CDCl₃) δ 1.18 (s, 3H), 1.32 (s, 3H), 1.37 (s, 3H), 1.40 (s, 3H), 1.75 (dd, 1H, J = 6 Hz, J = 13 Hz), 1.91 (t, 1H, J = 6 Hz), 1.93 (s, 3H), 3.90 (s, 3H), 3.90 (m, 1H), 5.33 (s, 1H), 5.82, s, 1H), 7.05 (s, 1H), 7.16 (s, 1H), 7.34 (d, 2H, J = 9 Hz), 7.95 (d, 2H, J = 9 Hz); MS (ES) m/z 379 (M⁺). Anal. calcd. for C₂₅H₃₀O₃: C, 79.33; H, 7.99. Found: C, 78.89, H, 8.17.

Hydrolysis of this material by the method described for 8 above provided the title compound in 43% yield as white crystals. mp 254 –257°C. ¹H NMR (DMSO -d₆) δ 1.06 (s, 3H), 1.27 (s, 3H), 1.30 (s, 3H), 1.31 (s, 3H), 1.62 (dd, 1H, J = 6 Hz, J = 13 Hz), 1.78 (t, 1H, J = 6 Hz), 3.67 (m, 1H), 4.65 (d, 1H, J = 6 Hz), 5.28 (s, 1H), 5.92 (s, 1H), 7.08 (s, 1H), 7.13 (s, 1H), 7.35 (d, 2H, J = 9 Hz), 7.90 (d, 2H, J = 9 Hz), 12.91 (bs, 1H); MS (ES) m/z 365 (M + H)⁺. Anal. calcd. for C₂₄H₂₈O₃: C, 79.08; H, 7.74. Found: C, 79.21, H, 7.73.