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Modeling, Synthesis, and Biological Evaluation of Potential Retinoid X Receptor (RXR) Selective Agonists: Novel Analogues of 4-[1-(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)ethynyl]benzoic Acid (Bexarotene) and (*E*)-3-(3-(1,2,3,4-tetrahydro-1,1,4,4,6-pentamethylnaphthalen-7-yl)-4-hydroxyphenyl)acrylic Acid (CD3254)

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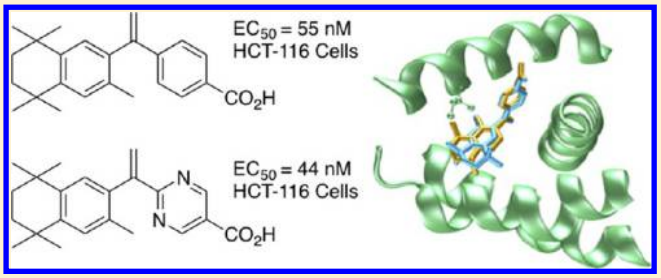
Supporting Information

ABSTRACT: Three unreported analogues of 4-[1-(3,5,5,8,8-pentamethyl-5-6-7-8-tetrahydro-2-naphthyl)ethynyl]benzoic acid (**1**), otherwise known as bexarotene, as well as four novel analogues of (*E*)-3-(3-(1,2,3,4-tetrahydro-1,1,4,4,6-pentamethylnaphthalen-7-yl)-4-hydroxyphenyl)acrylic acid (CD3254), are described and evaluated for their retinoid X receptor (RXR) selective agonism. Compound **1** has FDA approval as a treatment for cutaneous T-cell lymphoma (CTCL), although treatment with **1** can elicit side-effects by disrupting other RXR-heterodimer receptor pathways. Of the seven modeled novel compounds, all analogues stimulate RXR-regulated transcription in mammalian 2 hybrid and RXRE-mediated assays, possess comparable or elevated biological activity based on EC_{50} profiles, and retain similar or improved apoptotic activity in CTCL assays compared to **1**. All novel compounds demonstrate selectivity for RXR and minimal crossover onto the retinoic acid receptor (RAR) compared to all-*trans*-retinoic acid, with select analogues also reducing inhibition of other RXR-dependent pathways (e.g., VDR-RXR). Our results demonstrate that further improvements in biological potency and selectivity of bexarotene can be achieved through rational drug design.



INTRODUCTION

The human retinoid X receptors (hRXRs) consist of three identified isoforms (α, β, γ)[1] that function as transcription factors, often in partnership with other members of a larger nuclear receptor (NR) superfamily of transcriptional regulators including the thyroid hormone receptor (TR), the vitamin D receptor (VDR), the liver X receptor (LXR), the peroxisome proliferator-activated receptor (PPAR), and the retinoic acid receptor (RAR), to name a few. All of these nuclear receptors generally promote or modulate gene transcription in the presence of the appropriate receptor ligand. This ligand, usually comprising an endogenous molecule, binds to the receptor ligand-binding domain (LBD), effecting a conformational shift that enables the receptor to associate with high affinity to its specific hormone responsive element (HRE) on DNA. While an increasing number of HREs have been located at a considerable distance either upstream or downstream from their regulated genes, many HREs are often found within or close to the promoter region of the regulated gene. Typically, HREs consist of minimal core hexad sequences comprising two half-sites punctuated by a nucleotide spacer of variable length between direct, inverted, or everted repeats.[2] Nuclear receptor proteins, as homodimers or heterodimers, regulate transcription when binding to their corresponding half-sites within the HREs.

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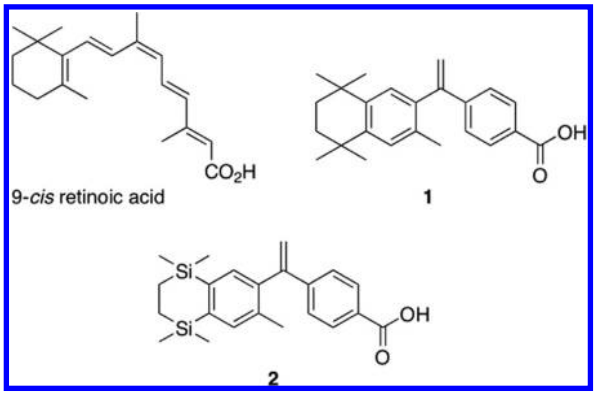
While RAR, VDR, and TR were initially thought to bind to their HREs as homodimers,[3] they actually bind as heterodimers with RXR.[4] When RXR binds to its endogenous 9-*cis*-retinoic acid (9-*cis*-RA) agonist ligand, an RXR homodimer forms that subsequently binds to the RXR responsive element (RXRE). However, when RXR operates as a heterodimeric partner with other nuclear receptors, it can do so either in the presence or absence of a ligand in the RXR LBD. For the RXR-VDR heterodimer, evidence suggests that RXR is unoccupied.[5] Alternatively, data suggest that RXR may possess a ligand when it operates as a heterodimer with LXR.[6] Notably, RXR has been described as the central NR regulator because it often plays a critical role in heterodimer complex formation with the other NRs to allow DNA binding to their respective response elements.[7]

These and other observations employing a number of studies with RXR, rexinoids, and a wide range of nuclear receptors, have led to the classification of at least two types of RXR-heterodimer complexes. These have been termed non-permissive or permissive heterodimers. Purely nonpermissive heterodimers can only be activated by the ligand of the RXR partner receptor, while permissive heterodimers that can be activated either by an RXR ligand or by a partner-specific ligand.[8] The nonpermissive heterodimers include RXR−TR, RXR−VDR, and RXR−RAR. Under most (but not all) conditions, RXR is completely "silent" in TR and VDR heterodimers, while RXR−RAR heterodimers can be further activated by select RXR ligands in the presence of an RAR ligand. Furthermore, in some cases, unique rexinoids have also been identified that stimulate RXR−RAR, even in the absence of a RAR ligand.[9] Thus, RXR−RAR can also be considered conditionally nonpermissive. In contrast, the fully permissive heterodimers include the RXR−LXRs, RXR−PPARs, and RXR−FXR.

The development of RXR-specific ligands for therapeutic use is complicated by the observation that not only are some RXR heterodimers permissive but also that under certain cellular conditions, or in particular cell types, the pool of RXR may be somewhat limiting. This has led to the observation that RXR ligands, like 9-*cis* RA for example, can inhibit nonpermissive nuclear receptor transactivation by such receptors as VDR[10] and TR.[11] Moreover, nuclear receptor ligands like thyroid hormone or 1,25-dihydroxyvitamin D_3 (1,25D) that stimulate RXR heterodimerization with their cognate receptor are thought to participate in crosstalk inhibition of signaling by other receptors that also utilize the limited intracellular RXR pool, and this has been reported for T_3−TR−RXR-mediated inhibition of VDR transactivation[12] and 1,25D−VDR−RXR-directed inhibition of TR activity[12b,13] although the mechanism involved in cross-receptor squelching likely extends beyond just sequestration of RXR. Nonetheless, a central issue in the development of novel therapeutic rexinoids is the creation of compounds that are likely to achieve a greater degree of heterodimer selectivity. Thus, changing the structure of the central RXR heteropartner ligand may result in specific NR modulators (SNuRMs) that possess unique characteristics that may exert a novel combination of NR control.[14]

RXR selective ligand (rexinoid) SNuRMs present attractive medicinal targets because selectively activating RXR versus RAR confers chemotherapeutic effects for several human cancers without incurring negative crossover RAR effects.[15] Following lengthy synthesis and testing[16] of molecules modeled after 9-*cis* RA, 4-[1-(3,5,5,8,8-pentamethyltetralin-2-yl)ethynyl]-benzoic acid (**1**)[17] was shown to be a highly specific RXR agonist that Ligand Pharmaceuticals Inc. further developed to become an FDA approved drug, commonly known as bexarotene, for treatment of cutaneous T-cell lymphoma. Several analogues of bexarotene, some quite similar such as the disilabexarotene (**2**),[18] have since been reported and shown to possess similarly specific RXR agonism.



While **1** is primarily used to treat CTCL, it has also been explored as a potential treatment for colon cancer[19] and breast cancer,[20] and it has even been applied as an off-label treatment for lung cancer[21] because promoting the transcription of RXR-regulated genes seems to suppress uncontrolled cell proliferation and predispose cancer cells to undergo apoptosis during chemotherapy.[20] Several RXR selective agonists, including compound **1**, have been explored in mouse models of noninsulin-dependent diabetes mellitus (NIDDM) because of the ability of RXR to partner with PPAR.[22] Despite the specificity that compound **1** displays for RXR in comparison to RAR, the known side effects of **1** include hypothyroidism,[23] cutaneous toxicity, and hyperlipidemia. These side effects of **1** are likely incurred either by nonpermissive receptor antagonism, as has been noted for TR[24] to explain RXR agonist induced hypothyroidism, or permissive receptor agonism, such as LXR to effect hyperlipidemia[25] or RAR to effect cutaneous toxicity[26] at typical dose concentrations. Thus, there is compelling motivation to develop novel RXR selective agonists whose effects on other receptor mediated pathways are attenuated or altogether absent. In addition to this motivation, several RXR agonists are being studied to treat other diseases through RXR-impacted pathways, now including two separate clinical trials of **1** in Alzheimer's disease (AD) based on up-regulation of apoE in mouse models of AD.[27]

There are several RXR specific agonists modeled on 9-*cis* RA and **1** reported in literature. For example, the cyclopropyl dienoic acid (**3**)[28] is a potent RXR agonist. Novel aza-retinoids, exemplified by compound **4**,[29] as well as amide retinoids,[30] have been described. RXR agonists based on aryl-trienoic acid compounds either unbranched[31] or locked with one[32] or multiple-fused[33] ring systems were developed by Boehm and co-workers, of which **5**[33] demonstrates the latter. Our group has reported that the addition of a single fluorine atom *ortho* to the carboxylic acid group of **1**, giving compound **6**,[34] and the addition of two fluorine atoms, giving compound **7**,[35] increases RXR agonism in human colon cancer cell lines, Caco-2 and HCT-116, respectively. Incorporating a pyridine ring, compound **8**[36] has been reported to be equally potent as **1**, and substituting the alkene bridging group in **8** for a cyclopropyl bridging group yields LGD100268 (**9**),[36] with a reported potency 1 order of magnitude greater than **1** in a CV-1 cell

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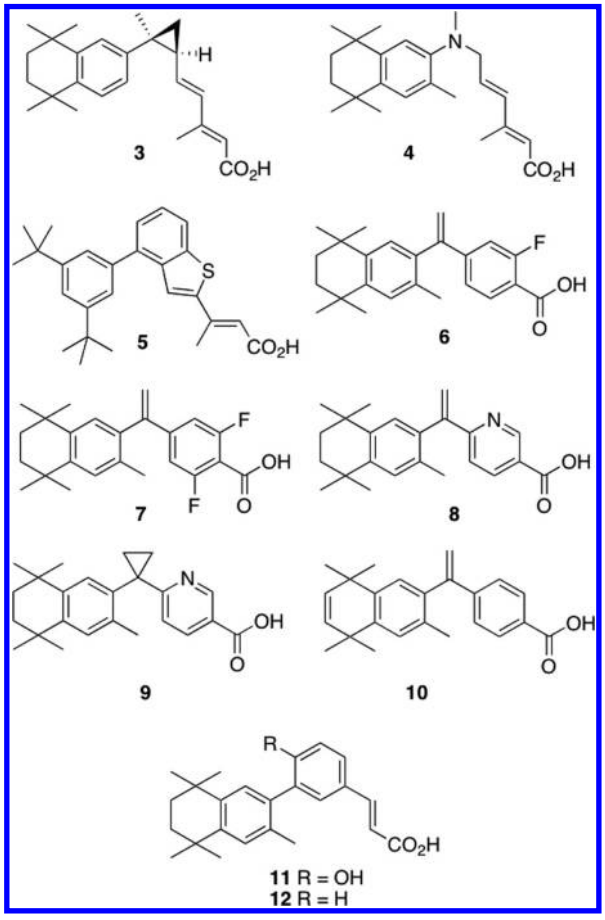
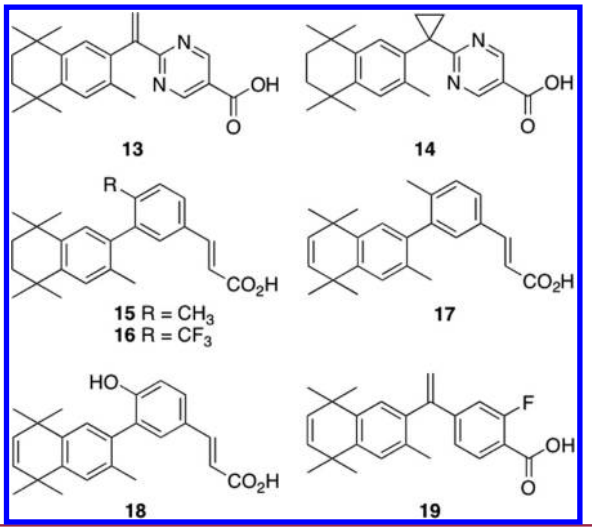
line.[36] An unsaturated analogue of **1**, compound **10**,[37] has a reported synthetic route that can provide preclinical trial quantities,[38] although to our knowledge, the ability of **10** to bind and activate RXR has not been reported. Finally, acrylic acid **11**[39] demonstrates potent, selective RXR agonism, and acrylic acid CD2915 (**12**)[40] is also a known RXR agonist.



Because of the potent and selective RXR agonism reported for compounds **8**, **9**, and **11** as well as a lack of reported biological evaluation of **10**, recent work in our laboratories has focused on synthesizing seven novel analogues, **13**−**19**, modeled on compounds **8**−**12** and subsequently assessing these novel compounds for RXR-selective activation alongside **1** and their parent compounds, **8**−**12**.

RESULTS AND DISCUSSION

Molecular Modeling. Docking results for compounds **8**−**19** relative to **1** are given in Figure 1. Compounds were scored by the Autodock binding free energy estimate, with negative values indicating better binding than **1**. Docks were performed with both the Autodock and OpenBabel charges; the latter tend to show less overpolarization.[35] All compounds were predicted to bind nearly as well or better than **1**, with predicted binding affinities generally within 0.5 kcal/mol of **1**. Overlays of docking poses in the L-shaped binding pocket showed that the phenyl ring was held in nearly identical position for most ligands, while small adjustments were observed in the position of the double ring system. This is illustrated in Figure 2, which shows an overlay of the top docking binding poses of **1** and **13** in the binding pocket. The observation that chemical substitutions are accommodated by small shifts of the fused ring system indicates the presence of available space around the fused ring, which suggests that the fused ring might be a good target for future chemical modifications.



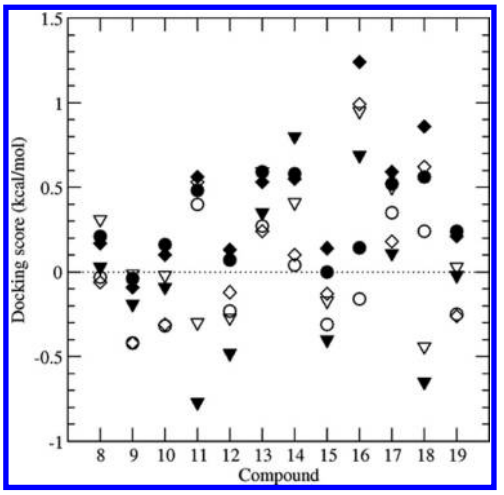


Figure 1. Docking results for compounds **8**−**19** relative to **1**. Calculated Autodock binding free energies were used to score the ligands, with negative values indicating better binding than **1**. Circles, docking to 1MVC; diamonds, docking to 1H9U; triangles, docking to 3FUG; open symbols, using Babel charges; filled symbols, using Autodock charges.

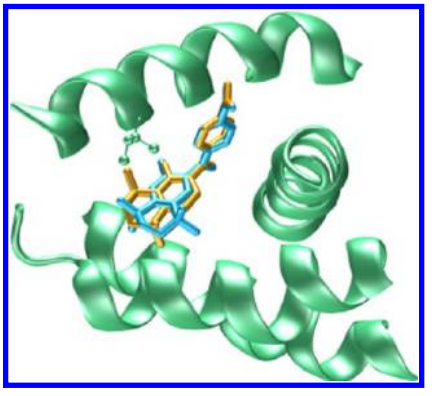


Figure 2. Overlay of best poses for **1** (orange) and compound **13** (blue) in 1MVC using OpenBabel charges. Ile268, which makes important hydrophobic contacts, and was treated flexible in the docking studies, is shown as ball-and-stick.

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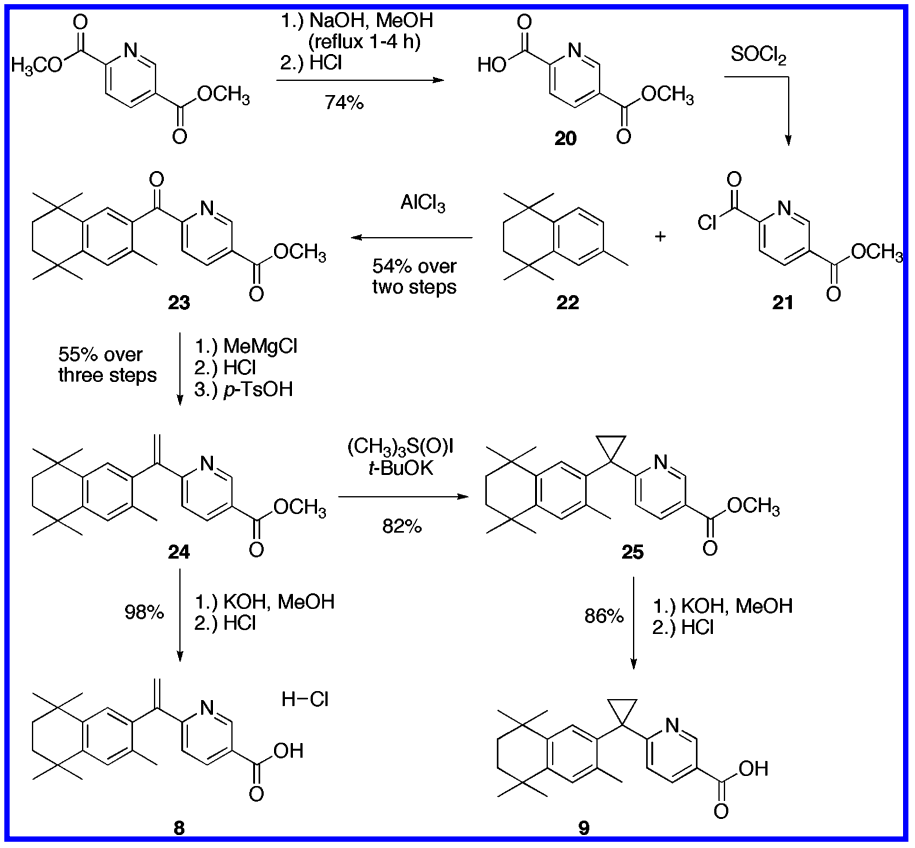
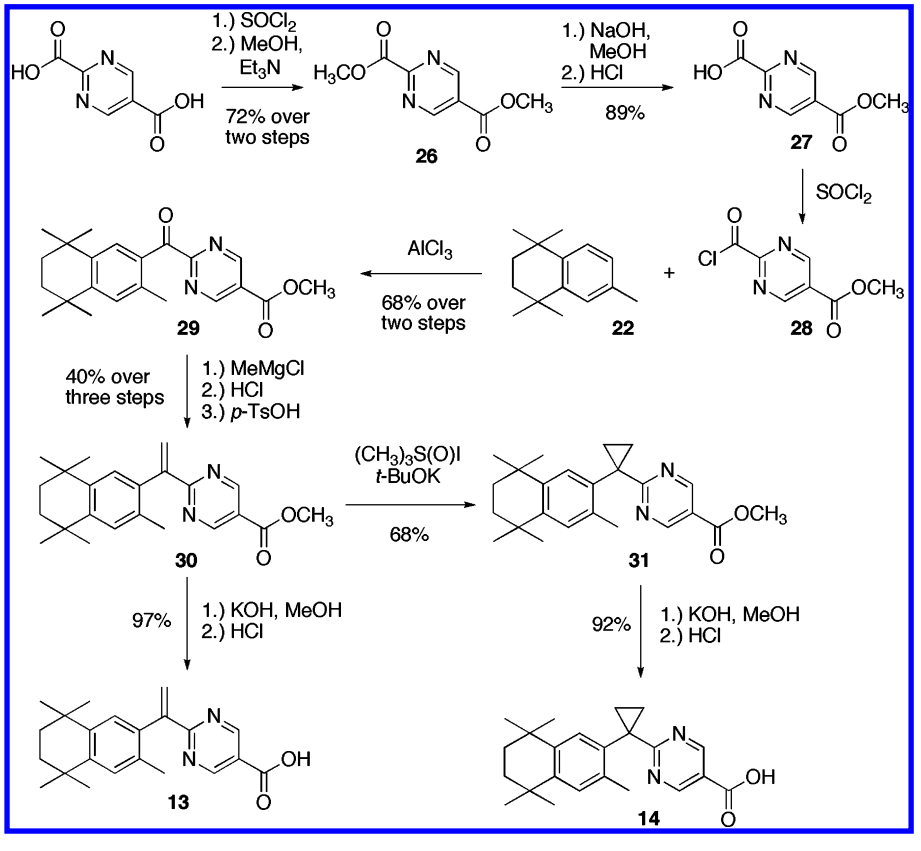
Scheme 1



Scheme 2



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Chemistry. The synthesis of pyrimidine-bexarotene analogue **13** and the pyrimidine-**9** analogue **14** follows a scaled down methodology[38] that was recently reported for the synthesis of pyridine-bexarotene analogues **8** and **9** (Scheme 1). We also undertook the synthesis of bexarotene analogue **8** and **9** to have standards against which to test **13** and **14**. Commercially available dimethyl pyridine-2,5-dicarboxylate was selectively saponified by the addition of solid sodium hydroxide pellets to a suspension in methanol that was then refluxed for 4 h followed by acidification to give crude 5-(methoxycarbonyl)-pyridine-2-carboxylic acid (**20**)[38] in 74% after filtration and recrystallization from boiling water. Carboxylic acid **20** was converted to acid chloride **21**[38] by treatment with thionyl chloride, and acid chloride **21** was used in the aluminum chloride catalyzed Friedel−Crafts acylation reaction with compound **22**[34] to give ketone **23**[38] in 54% yield over two steps. Ketone **23** was treated with a 3.0 M solution of methyl magnesium chloride in THF at 0 °C, followed by quenching with a 1 N solution of hydrochloric acid and extraction, and the concentrated residue was refluxed with *para*-toluene sulfonic acid monohydrate to give alkene **24**[38] in 55% yield over three steps. Alkene **24** was converted to the cyclopropyl methyl ester **25**[38] in 82% yield by adding a solution of **24** in THF to a DMSO solution of trimethylsulfoxonium iodide treated with 22 wt % potassium *tert*-butoxide in THF. Both alkene **24** and cyclopropyl methyl ester **25** were saponified by treatment with aqueous potassium hydroxide and refluxing in methanol to give, after precipitation with 20% HCl, the corresponding carboxylic acids **8** and **9**, respectively, in near quantitative crude yields. The carboxylic acids were purified by recrystallization from hot ethyl acetate and hexanes.

Commercially available pyrimidine-2,5-dicarboxylic acid was refluxed in thionyl chloride with a few drops of catalytic DMF for 4 h, and excess thionyl chloride was removed in vacuo to give the solid diacid chloride that was dissolved in toluene and added to a solution of triethyl amine in methanol to yield the dimethyl pyrimidine-2,5-dicarboxylate **26** in 72% yield over two steps. A suspension of dimethyl ester **26** in methanol was then treated with sodium hydroxide pellets and refluxed for 4 h, followed by acidification with 2N HCl, to give 5-(methoxycarbonyl) pyrimidine-2,5-dicarboxylate (**27**) in 89% yield after filtration. Acid **27** was converted to acid chloride **28** by treatment with thionyl chloride, and acid chloride **28** was used in the aluminum chloride catalyzed Friedel−Crafts acylation reaction with compound **22** to give ketone **29** in 68% yield over two steps. Ketone **29** was treated with a 3.0 M solution of methyl magnesium chloride in THF at 0 °C followed by quenching with a 1 N solution of hydrochloric acid and then extracted, and the concentrated residue was refluxed with *para*-toluene sulfonic acid monohydrate to give alkene **30** in 40% yield over three steps. Alkene **30** was converted to the cyclopropyl methyl ester **31** in 68% yield by adding a solution of **30** in THF to a DMSO solution of trimethylsulfoxonium iodide treated with 22 wt % potassium *tert*-butoxide in THF. Both alkene **30** and cyclopropyl methyl ester **31** were saponified by treatment with aqueous potassium hydroxide and refluxing in methanol to give, after precipitation with 20% HCl, the corresponding carboxylic acids **13** and **14**, respectively, in near quantitative crude yields. The carboxylic acids were purified by recrystallization from hot ethyl acetate and hexanes (Scheme 2).

Fortunately, compound **14** yielded single, transparent crystals from ethyl acetate, suitable for X-ray diffraction, and an X-ray diffraction study was performed (Figure 3).

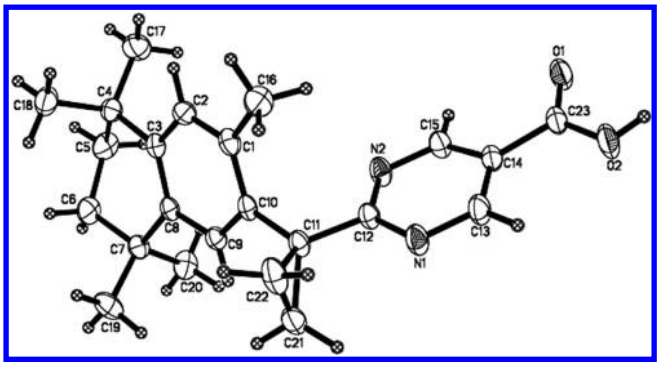


Figure 3. X-ray crystal structure of **14**. The thermal ellipsoid plot is shown at the 50% probability level.

To synthesize a sample of compound **10**, a modified literature procedure[37] was used. Toluene was reacted with dihydro-2,2,5,5-tetramethylfuran-3(2*H*)-one (**32**) with aluminum chloride as a catalyst to provide 3,4-dihydro-1,1,4,4,7-pentamethylnaphthalen-2(1*H*)-one (**33**)[37] in 44% yield. Ketone **33** was reacted with bromine and an aluminum chloride catalyst to afford 6-bromo-3,4-dihydro-1,1,4,4,7-pentamethylnaphthalen-2(1*H*)-one (**34**). Ketone **34** was condensed with *para*-toluenesulfonyl hydrazide to give compound **35** in 50% yield. Compound **35** was treated with methyl lithium to provide 6-bromo-1,4-dihydro-1,1,4,4,7-pentamethylnaphthalene (**36**)[38] in a Shapiro reaction[41] in 90% yield (Scheme 3).

With alkene **36** in hand, we proceeded to make the appropriate coupling partner for it en route to compound **10** by reacting 4-cyanobenzoyl chloride (**37**) with *N*,*O*-hydroxylamine hydrochloride to give the Weinreb amide (**38**)[38] in 88% yield (Scheme 4).

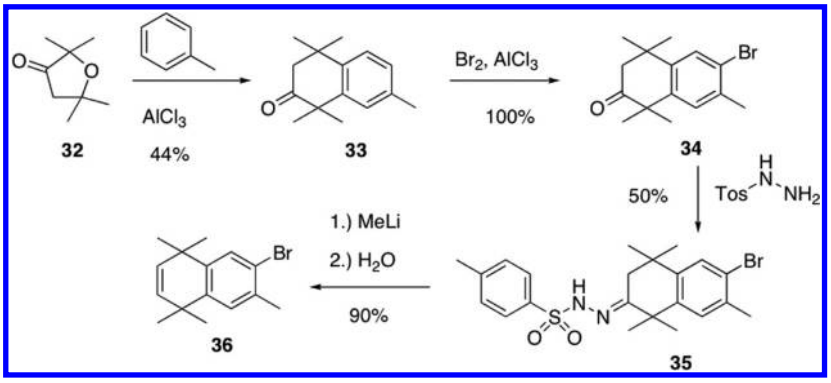
To complete the synthesis of compound **10**, we treated alkene **36** with butyl lithium followed by amide **38** to give ketone **39**[38] after aqueous acidic workup in 82% overall yield. The nitrile group of ketone **39** was hydrolyzed with potassium hydroxide to give carboxylic acid **40**[38] after aqueous acidic workup in 96% yield (Scheme 5).

Acid **40** was treated with methyl magnesium chloride, followed by aqueous acidic workup to give alcohol **41**[38] that was subsequently refluxed with *para*-toluenesulfonic acid monohydrate in toluene to give alkene **10** in 91% overall yield (Scheme 5).

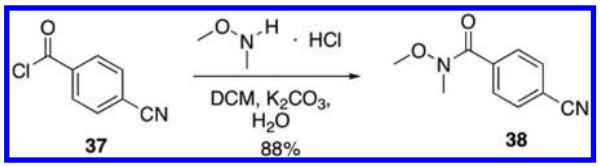
To synthesize compound **15**, a strategy using the Suzuki−Miyura coupling reaction[42] as the key step was followed. Compound **22** was treated with bromine in DCM to give compound **42**[16b] in 60% yield. Bromide **42** was treated with butyl lithium followed by triisopropylborate and aqueous acidic workup to give boronic acid **43** in 45% yield over three steps (Scheme 6).

To prepare the Suzuki coupling partner for boronic acid **43**[38] en route to **15**, 3-bromo-4-methylbenzoic acid (**44**) was treated with borane·THF to give a benzyl alcohol that was subsequently oxidized to aldehyde **45**[43] with manganese(IV)-oxide in 74% overall yield. Aldehyde **45** was converted to the alkene ester **46** by a Horner−Wadsworth−Emmons reaction.[44] With the alkene ester **46** in hand, we proceeded to couple it

Scheme 3



Scheme 4

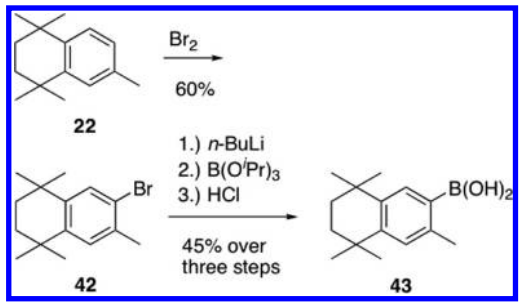


with boronic acid **43** via a Suzuki–Miyura reaction to give biphenyl **47** in 80% yield. The ester group of biphenyl **47** was saponified with KOH to give the carboxylic acid **15** in 75% yield after aqueous acidic workup (Scheme 7).

Next, we targeted the synthesis of a trifluoro-methyl analogue of **11**, compound **16**, because we wished to compare the substitution of the hydroxyl group of **11** with a nonpolar methyl group (**15**) versus a much more polar trifluoro-methyl group (**16**). Hence, we performed the Horner–Wadsworth–Emmons reaction on commercially available aldehyde **48** to give ester **49** in 98% yield. With aromatic bromine **49** in hand, we proceeded to couple it with boronic acid **43** via a Suzuki–Miyura reaction to give biphenyl **50** in 71% yield. The ester group of biphenyl **50** was saponified with KOH to give the carboxylic acid **16** in 75% yield after aqueous acidic workup (Scheme 8).

Next, we set out to synthesize an analogue of **15** with an alkene in the aliphatic ring: compound **17**. Thus, we converted alkene **36** to boronic acid **51**[38] by treating **36** with butyl lithium followed by triisopropyl borate and an aqueous acidic workup. With boronic acid **51** in hand, we proceeded to couple it with ester **46** via a Suzuki–Miyura reaction to give biphenyl **52** in 72% yield. The ester group of biphenyl **52** was saponified with KOH to give the carboxylic acid **17** in 75% yield after aqueous acidic workup (Scheme 9).

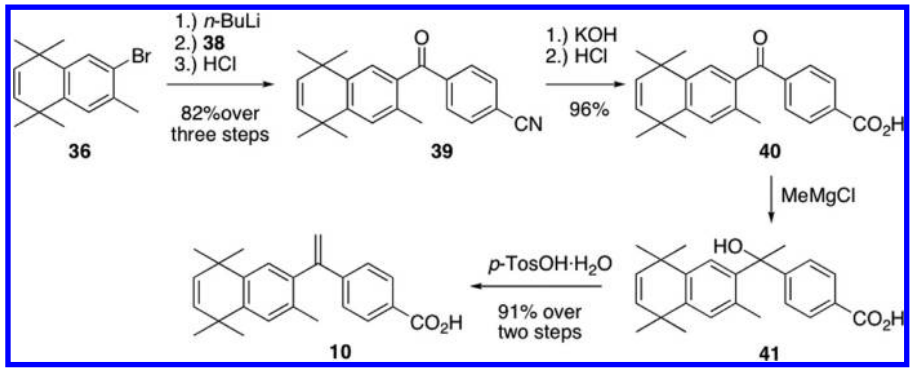
Scheme 6



To compare the effect of placing the alkene in the aliphatic ring of **11**, we prepared compound **18**. First, we made the appropriate aromatic halide coupling partner for boronic acid **51** by acetylating 3-bromo-4-hydroxybenzaldehyde (**53**) to give aldehyde **54**[39] in 90% yield, and then we carried out the Horner–Wadsworth–Emmons reaction on **54** to give ester **55**[39] in 84% yield. With aromatic bromide **55** in hand, we proceeded to couple it with boronic acid **51** via a Suzuki–Miyura reaction to give biphenyl **56** in 54% yield. The ester group of biphenyl **56** was saponified with KOH to give the carboxylic acid **18** in 89% yield after aqueous acidic workup (Scheme 10).

Because of our recent finding[34] that the addition of a fluorine atom *ortho* to the carboxylic acid group of **1** increases its binding and activation of RXR, we prepared **19** to investigate both the addition of an alkene in the aliphatic ring of **1** and fluorination *ortho* to the carboxylic acid. The synthesis of **19** begins with the sodium carbonate mediated conversion of methyl-4-acetyl-2-fluorobenzoate **57** to the enol triflate **58** in 27% yield. The enol triflate **58** was then coupled to **51** to give

Scheme 5



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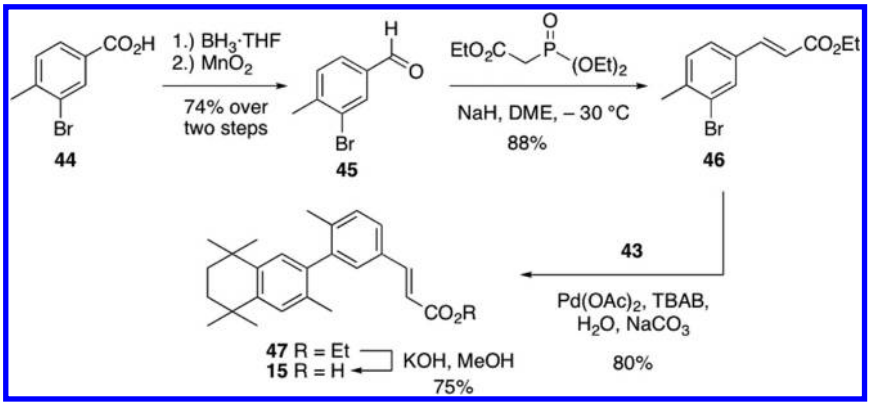
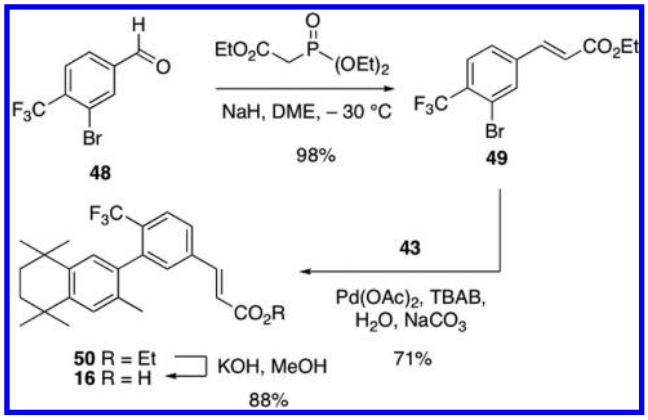
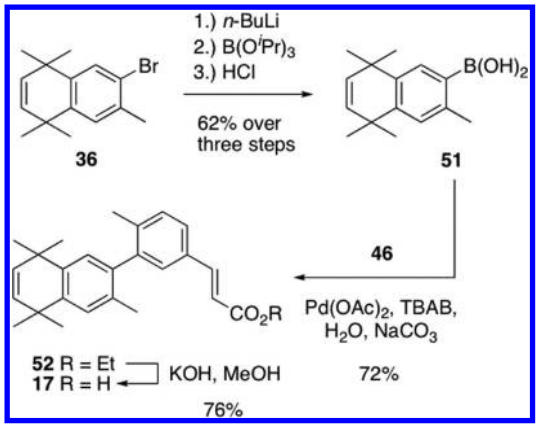
Scheme 7



Scheme 8



Scheme 9



methyl ester **59** in 55% yield, and ester **59** was saponified with KOH in methanol to give **19** in 70% yield (Scheme 11).

Finally, we prepared a sample of acrylic acid **12** to compare against **11** and the other novel acrylic acid analogues (**15**–**18**) in this study. Thus, 3-bromobenzaldehyde **60** was converted to ethyl acrylate **61** in 98% yield. The ethyl acrylate **61** was coupled to boronic acid **43** to give ethyl ester **62** in 70%, and **62** was subsequently saponified with KOH in methanol to yield **12** in 84% yield (Scheme 12).

Biological Assays and Rationale. *A Mammalian Two-Hybrid Assay to Assess RXR Transcriptional Response of Analogues Compared to* ***1****.* Biological assessment of a subset of analogues described above (compounds **8**–**19**) was first carried out employing the mammalian two-hybrid assay in human colon cancer (HCT-116) cells (Figure 4). This assay tests for ligand binding to a recombinant, full length RXR containing an activation domain. If the ligand–RXR complex then homodimerizes with an RXR–Gal4 fusion protein, the luciferase reporter gene will be transcribed, as the luciferase gene is downstream of Gal4p DNA binding elements.

We tested the indicated compounds at two different concentrations (25 and 100 nM) in this system. The 100 nM assay (Figure 4B) revealed that five compounds (**8**, **9**, **11**, **13**, and **15**) exhibited high activity compared with compound **1** (bexarotene). Moreover, the 25 nM assay of compounds shows an even wider range of receptor binding and RXR homodimerization ability because the 100 nM assay likely approaches RXR binding saturation while the 25 nM assay represents a subsaturating concentration of ligand (see also EC_{50} values in Table 1). Compounds **8**, **9**, **11**, **13**, and **15** possess significantly (using a one-way ANOVA, $p < 0.01$) greater activity than compound **1** in the 25 nM assay (Figure 4A), with compound **18** also approaching statistical significance. Additionally, compounds **12**, **17**, and **19** display a smaller degree of RXR transcriptional response (using a one-way ANOVA, $p < 0.01$) relative to compound **1** in the 25 nM assay, and compounds **12** and **17** also possess significantly lower binding than compound **1** in the 100 nM assay, but **12**, **17**, and **19** still have high activity compared to the ethanol vehicle control (Figure 4A,B). These results imply that compounds modeled after **1** can be synthesized successfully and can mediate RXR transcriptional activation, and we are interested in elucidating the factors responsible for eliciting the different response-ranges observed. We have also utilized the mammalian two-hybrid assay in human HCT-116 cells to assess dose–response relationships (see Supporting Information Figures 1–3), and EC_{50} values as described below.

Novel Analogues of ***1*** *Bind to RXR and Mediate Transactivation in the Context of an RXRE.* It is important to note that the use of the mammalian two-hybrid assay as an initial screen for agonist binding to and activation of RXR is useful because of the speed, convenience, and sensitivity of the assay. However, one potential drawback of the mammalian two-hybrid assay is that the RXR–agonist complex may be dependent on the correct biologically relevant DNA platform, or retinoid X receptor responsive element (RXRE), that specifically associates with the RXR homodimer in vivo. The RXRE DNA sequence is usually found in the promoter region and also up- or downstream of the genes controlled by the RXR homodimer in response to the endogenous 9-*cis* RA ligand or when RXR is bound to a synthetic rexinoid. It is possible that

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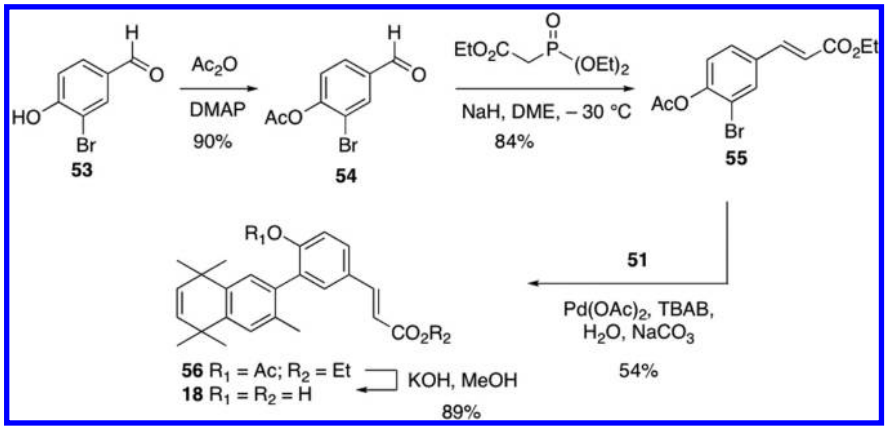
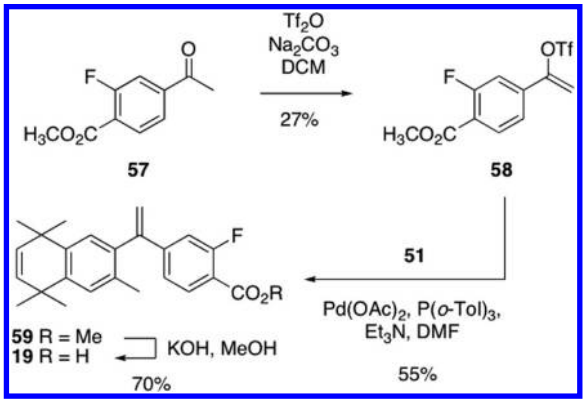
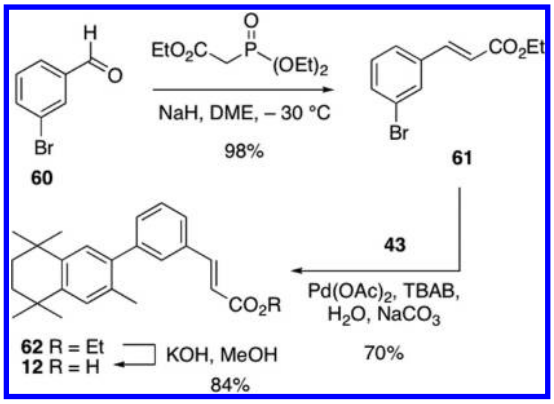
Scheme 10



Scheme 11



Scheme 12



the RXRE may allosterically influence the affinity and/or selectivity of the RXR protein toward potential ligands. Thus, a second screening protocol for our collection of possible RXR agonists included transfection of HCT-116 cells with an expression vector for wild-type human RXRα along with a reporter construct that contains an authentic RXRE driving the expression of the luciferase reporter gene. The most frequent side effect of bexarotene administration is an increase in plasma triglycerides as a result of liver RXR/LXR-directed activation of lipid metabolism.[45] Thus, we chose to first assess the ability of our analogues to bind/activate RXRα because the liver expresses predominantly the α isoform,[46] and this isoform is likely responsible for the elevated triglyceride synthesis in the liver. We selected HCT-116 as a cell line that has a relatively low expression of RXRα[47] and other receptors of interest to this study so we could quantitate most of the measurable effects as resulting from the expression of the transfected, rather than endogenous, receptor. The expression levels (as assessed by Western blotting) and ligand responsiveness of the HCT-116 cell line transfected with and without each of the receptors evaluated in this study are shown in Supporting Information Figure 4.

We tested the indicated compounds at two different concentrations (25 and 100 nM) in the RXRE assay. The results in Figure 5 reveal that compounds **8**, **9**, **10**, **11**, **13**, **15**, and **18** displayed transcriptional activity consistently above the compound **1** level in both assays (Figure 5A,B). In particular, compounds **8**, **11**, **15**, and **18** possess a significantly greater activity than compound **1** at both concentrations tested (using a one-way ANOVA, $p < 0.05$ for compound **8**, **15**, and **18**, and $p < 0.01$ for compound **11**). Importantly, the profile of analogue activity in the RXRE-based assays (Figure 5) is almost identical to the profile of analogues identified in the mammalian two-hybrid assay (Figure 4), thus validating the mammalian two-hybrid assay, and reinforcing the identification and reproducibility of those analogues with the greatest potency in terms of RXR activation.

Evaluation of EC_{50} Values and Quantitation of RAR Agonist Activity. We utilized our mammalian two-hybrid assay in human colon cancer cells (Figure 4) to evaluate a much larger array of **1** and analogue concentrations. In addition to employing a single ligand concentration as in Figure 4, dose–response assays were carried out with ligand concentrations ranging from 10^{-9} M up to 0.3×10^{-5} M of each compound as well as **1** (Supporting Information Figures 1–3). Utilizing this collection of dose–response experiments, we were able to calculate EC_{50} values, which are listed in Table 1. Our EC_{50} value for **1**, which is an estimate of ligand affinity for RXR, is similar to values obtained previously by another group.[16b,17] In fact, Boehm and co-workers show that the different isoforms of RXR have similar EC_{50} values, within the same order of magnitude, not only for **1**, but several analogues of **1**.[17] Moreover, the data in Figures 4 and 5 suggest that compounds **12**, **16**, **17**, and **19** possess consistently lower RXR-mediated transcriptional activity while compounds **8**, **9**, **11**, **13**, **15**, and **18** are consistently more active, observations that are entirely consistent with the EC_{50} values in Table 1. We also performed an analysis of the "residual" retinoic acid receptor (RAR) agonist activity of **1**, as well as for each analogue versus the known RAR ligand (all-*trans* retinoic acid). The results of this

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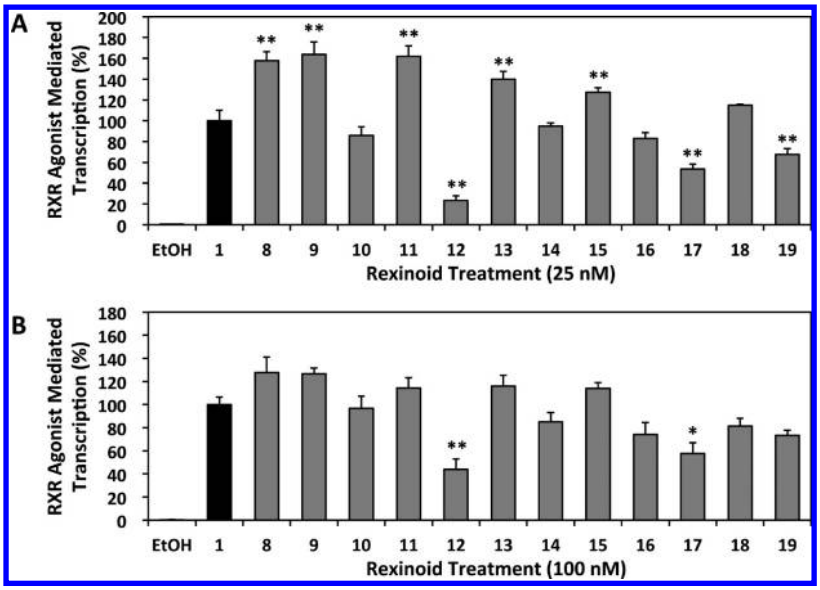

Figure 4. Identification of RXR agonists via a mammalian two-hybrid screening assay in human colon cancer cells. HCT-116 human colon cancer cells were cotransfected using a pCMV-hRXR binding domain vector (BD) as well as an hRXR-activation domain (AD) plasmid along with a pFR-Luc reporter gene containing BD-binding sites and renilla control plasmid. Cells were transfected for 24 h utilizing a liposome-mediated transfection protocol and then treated with ethanol vehicle and either 25 or 100 nM of the indicated compound. After a 24 h incubation, cells were lysed and a dual luciferase assay was completed. Analogue-mediated RXR binding and homodimerization, as measured by luciferase output, was compared to the RXR agonist parent compound **1** (value set to 1.0). Values are means ± SD $*p < 0.05$, $**p < 0.01$ vs compound **1**.

assay, which employed expression of human RARα (the most widely distributed isoform of RAR in human tissues) and a retinoic acid responsive element (RARE)-luciferase reporter system, revealed that compounds **8**, **10**, **13**, **14**, **15**, **17**, and **19** possess greater RAR agonist activity, compounds **9** and **18** are approximately equal to **1** in its activation of RAR, and compounds **11**, **12**, and **16** possess slightly lower RAR-directed activity. Taken together, these results suggest that modification of **1** with more polar atoms in the aromatic ring that bears the carboxylic acid may not only increase the analogues' ability to activate RAR (compounds **8**, **13**, **14**, **19**), but also increase its ability to activate RXR (analogues **8** and **13**).

Analysis of Apoptotic Activity by Bexarotene and Novel Analogues in a CTCL System. One potential mechanism to explain the efficacy of **1** in treating CTCL effectively is that this rexinoid induces apoptosis and/or cytotoxicity in the T-lymphocyte.[48] Thus, we next tested CTCL (Hut-78) cells treated with **1** and the indicated analogues for their ability to induce classic apoptosis, using an assay for caspases 3 and 7, two caspases that mediate apoptosis.[49] We tested each compound (**8**–**19**) by treatment for either 24 or 48 h (Figure 6). The results of the apoptosis assay suggest that most of the analogues possess some pro-apoptotic activity. Two compounds (analogues **8** and **13**) that exhibit higher RXR binding affinity (lower EC_{50}, Table 1) and greater potency in the RXR transcription assays (Figures 4 and 5) are also more active (Figure 6) than compound **1** in the apoptotic assay (using a one-way ANOVA, $p < 0.05$ for compound **8**, $p < 0.01$ for compound **13** both at 48 h, and $p < 0.05$ for compound **13** at 24 h). Several analogues reveal activity that is greater than the ethanol vehicle control (Figure 6) in eliciting apoptosis at 24 h (using a one-way ANOVA, $p < 0.05$ for compounds **1**, **9**, **10**, **12**, **14**, and **18**, $p < 0.01$ for compounds **8**, **13**, **16**, **17**, and **19**) or 48 h (using a one-way ANOVA, $p < 0.05$ for compounds **1**, **9**, **14**, **17**, **18**, and **19**, $p < 0.01$ for compounds **8**, **13**). A potent known apoptotic inducer (sodium butyrate, NaBu) serves as a positive control in this system.

*Evaluation of **1** and Analogues As Inhibitors of VDR–RXR Activity Due to Analogue-Mediated Formation of RXR–RXR Homodimers.* **1** binds to RXR and induces RXR–RXR homodimerization, thus there may be less RXR available in the intracellular pool for the creation of heterodimers with other nuclear receptors such as the formation of VDR–RXR heterodimers. This disruption of RXR heterodimer pathways is one of several potential molecular mechanisms that may be responsible for side effects of **1** in humans. Therefore, we seek to develop RXR analogues that are not only more robust in their RXR biopotency via RXRE-mediated transcription but also that are less disruptive to RXR heterodimerization pathways. As a result, we measured vitamin D responsive element (VDRE)-mediated transcriptional activity in the presence of the active 1,25-dihydroxyvitamin D_3 metabolite (1,25D; 10^{-9} M) to assess the level of RXR–RXR "diversion" and thus likely inhibition of VDR–RXR-directed transcription at two different concentrations of RXR analogues (Figure 7A, rexinoid 10^{-7} M; Figure 7B, rexinoid 10^{-6} M). Compound **1** inhibited VDR activity by approximately 50% in the 10^{-6} M assay (Figure 7B) and by 30% in the 10^{-7} M assay (Figure 7A). Compounds **8**, **10**, **13**, **14**, and **17** inhibited VDR activity less compared with compound **1** in *both* assays (Figure 7A,B). Statistically, compounds **1**, **9**, **11**, and **15** are significantly lower (using a one-way ANOVA, $p < 0.05$ for compounds **1**, $p < 0.01$ for compounds **9**, **11**, and **15**) than 1,25D alone in the 100 nM assay (Figure 7A). Most compounds (except for **10**) are significantly lower (using a one-way ANOVA, $p < 0.05$ for compounds **17**, $p < 0.01$ for compounds **1**, **8**, **9**, **11**, **12**, **13**, **14**, **15**, **16**, **18**, and **19**) than 1,25D alone in the 1000 nM assay (Figure 7B). These results suggest that the expected side effects of compound **8**, **10**, **13**, **14**, and **17** due to RXR–RXR diversion may be smaller than compound **1**. It is important to note that some previous reports[50] have demonstrated a stimulatory effect

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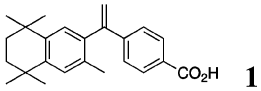
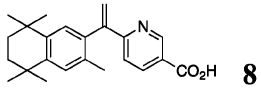
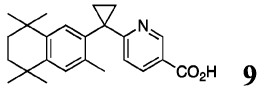
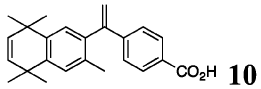
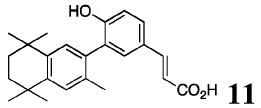
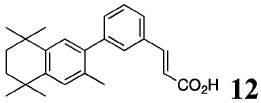
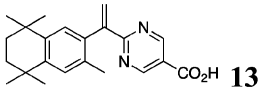
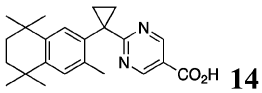
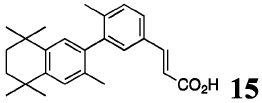
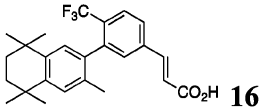
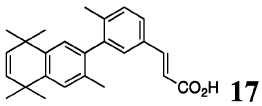
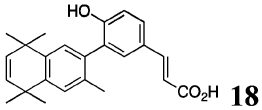
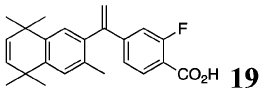
Table 1. Determination of EC_{50} Values in RXR M2H Assay and Quantitation of RAR Agonist Activity[a]

Compound	EC_{50} value[1] nM (± S.D.)	% RAR Agonist Activity[2] at 100 nM (± S.D.)	% RAR Agonist Activity[2] at 1 μM (± S.D.)
 **1**	55 (6)	22 (5)	25 (4)
 **8**	21 (2)	32 (4)	39 (6)
 **9**	15 (3)	25 (2)	29 (1)
 **10**	74 (7)	28 (6)	39 (2)
 **11**	13 (3)	18 (4)	20 (5)
 **12**	109 (8)	14 (3)	21 (5)
 **13**	44 (12)	27 (6)	37 (9)
 **14**	50 (10)	28 (5)	30 (7)
 **15**	42 (3)	31 (6)	39 (8)
 **16**	69 (7)	15 (2)	20 (3)
 **17**	72 (11)	31 (6)	35 (8)
 **18**	15 (2)	24 (4)	28 (5)
 **19**	71 (10)	48 (10)	56 (16)

[a](1) EC_{50} values were determined from full dose−response experiments with each compound in the range of 10^{-9} to 10^{-5} M in transfected HCT-116 cells using an RXR mammalian two-hybrid system. (2) RAR agonist activity was derived from an RAR/RARE reporter system in transfected HCT-116 cells treated with all-*trans* RA or indicated analogue at 100 nM or 1 μM. The activity with analogue (or compound **1**) divided by the activity with all-*trans* RA expressed as a percentage represents the RAR agonist activity.

of 9-*cis* RA/rexinoids on 1,25D−VDR−VDRE-mediated transcription. However, several other studies have revealed the opposite effect, i.e., suppression of VDR−RXR activity (see Introduction). Therefore, this remains a controversial theme in the vitamin D field. To address this important question, we conducted additional transcriptional assays with VDR−RXR employing different classes of vitamin D responsive elements (VDREs) beyond the ER6 VDRE used in Figure 7A,B. These experiments (Figure 7C−F) reveal that the effect of rexinoids (**1**/analogues) on 1,25D−VDR−RXR transcriptional response

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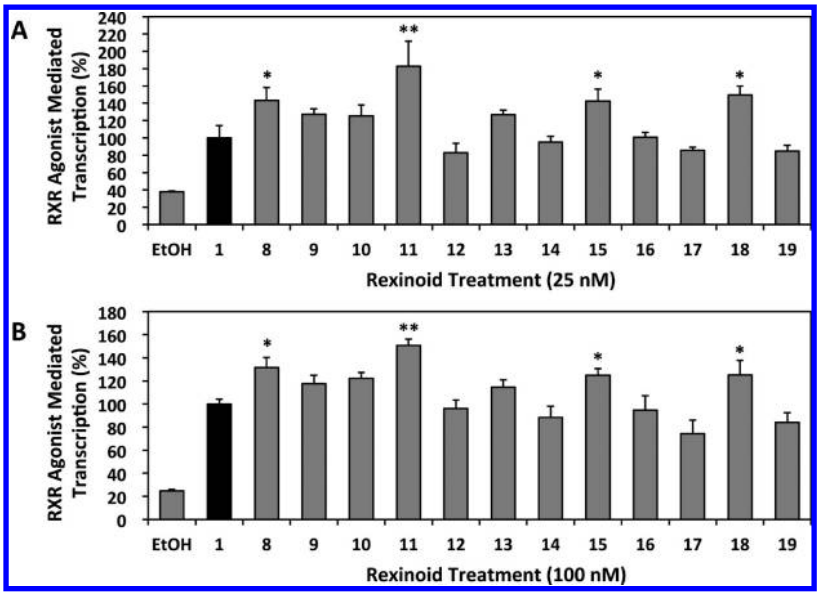

Figure 5. Evaluation of RXR agonists via an RXRE-luciferase reporter-based assay in human colon cancer cells. HCT-116 cells were transfected with human RXRα, RXRE-luciferase reporter gene, and renilla control plasmid. Cells were transfected for 24 h utilizing a liposome-mediated transfection protocol as in Figure 4 and then treated with ethanol vehicle and 25 or 100 nM of the indicated compound. After a 24 h incubation, cells were lysed and a luciferase assay was completed. Analogue-stimulated, RXR-mediated transcription, as measured by luciferase output, was compared to the RXR agonist parent compound **1** (value set to 1.0). Values are means ± SD $^{*}p < 0.05$, $^{**}p < 0.01$ vs compound **1**.

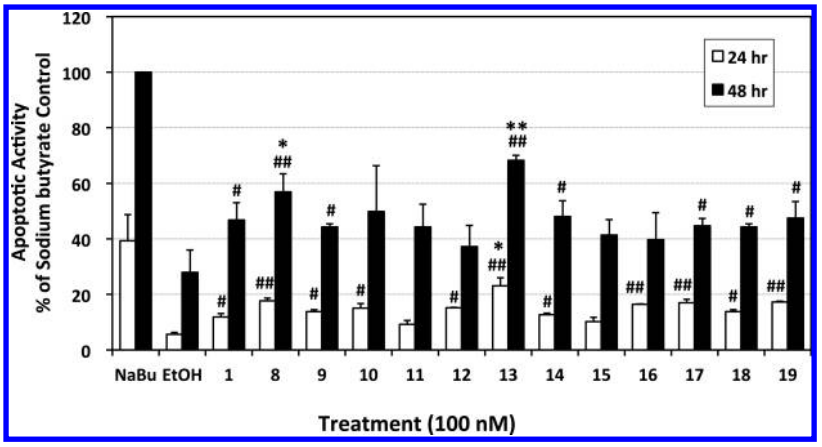

Figure 6. Assessment of bexarotene and analogues for apoptotic activity in CTCL cells. Human cutaneous T lymphocytes (Hut-78) were plated and subsequently dosed with the indicated treatments. Cells were allowed to incubate for 24 or 48 h, followed by measurement of apoptosis with a commercial kit (see Experimental Section). Sodium butyrate (NaBu), a known inducer of apoptosis in this system, was used as a positive control. Values are means ± SD $^{*}p < 0.05$, $^{**}p < 0.01$ vs compound **1**. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs ethanol.

(e.g., repression, no effect, stimulation) is dependent on the nature of the VDRE. For example, as shown in Figure 7A,B, there is a reproducible inhibition with **1** and most analogues on VDR activity (as described above) when the VDR DNA platform is an everted repeat-6 (ER6) from the CYP3A4 gene. In contrast, there is a stimulation in VDR-mediated activity when using the more classical direct repeat-3 (DR3) VDRE (Figure 7C,D) in the presence of 1,25D and some of the analogues compared to treatment with 1,25D alone. Compounds **1** and **8** are significantly higher (using a one-way ANOVA, $p < 0.05$) than 1,25D alone, and compound **12** and **17** are significantly lower (using a one-way ANOVA, $p < 0.05$) than 1,25D alone in the 100 nM assay (Figure 7C). Additionally, compounds **1**, **8**, and **15** are significantly higher (using a one-way ANOVA, $p < 0.05$ for compounds **8** and **15**, $p < 0.01$ for compounds **1**) than 1,25D alone in the 1000 nM assay (Figure 7D). The CYP24 natural promoter, which contains 2 VDREs, has a net zero effect of most rexinoids on 1,25D−VDR transactivation (Figure 7E,F) with only a few analogues showing significant inhibiton (using a one-way ANOVA, $p < 0.05$ for compounds **18** and **19**, $p < 0.01$ for compounds **12** in the 100 nM assay, $p < 0.05$ for compounds **12** and **18** in the 1000 nM assay). Related findings have been reported for a TR−RXRβ−TRE system where RXRβ can either enhance, not alter, or inhibit TR-dependent transcription depending on the nature of TRE.[51] These novel results with VDR not only address the need to evaluate the effect of **1**/rexinoids on 1,25D−VDR−RXR transcriptional activity in the context of multiple VDREs, but they may also provide a compelling resolution to the conflicting data that have been reported in the literature with respect to the actions of rexinoids on vitamin D signaling.

While much of the comparative discussion has focused on how a given novel analogue (**13**−**19**) performs in these assays

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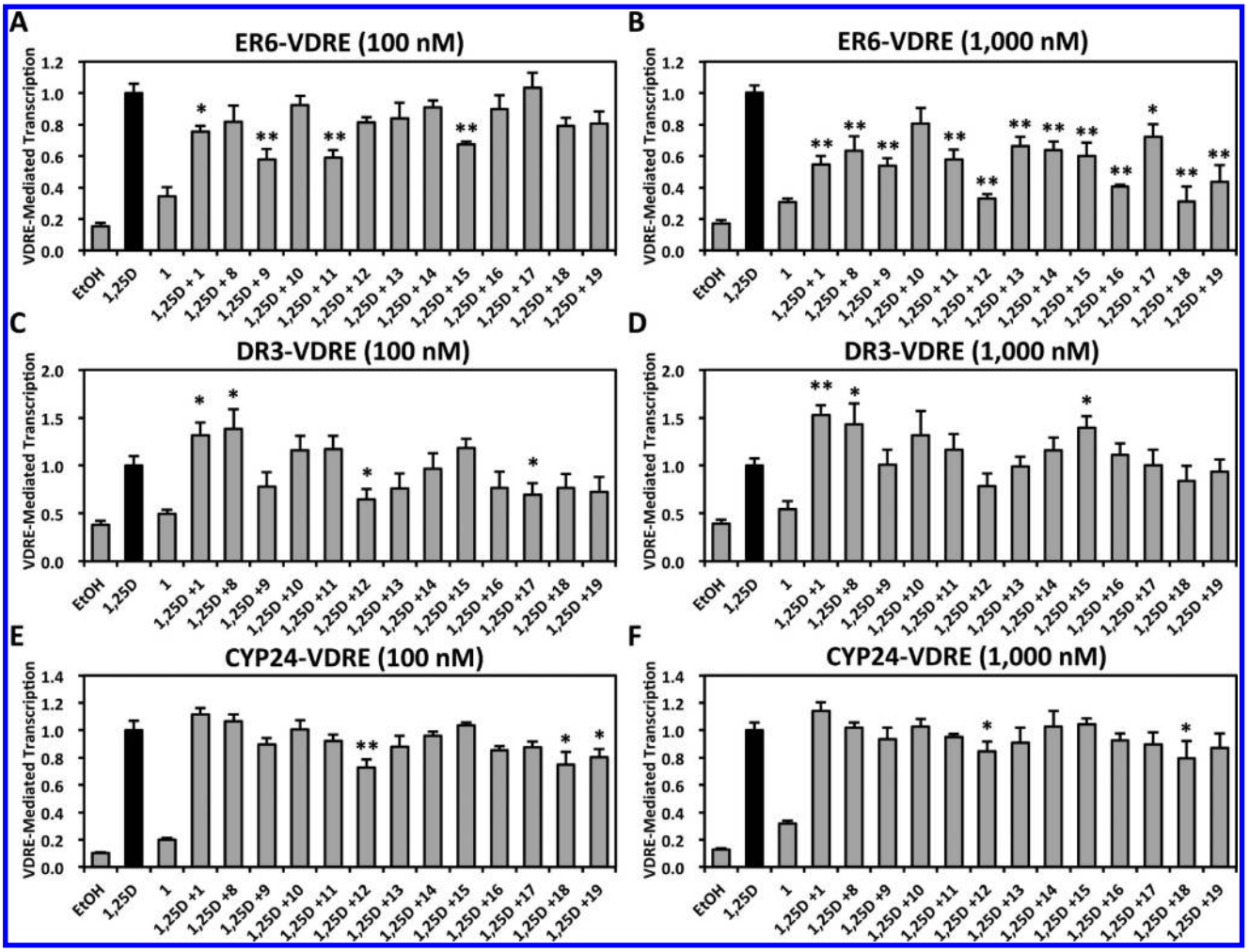

Figure 7. Evaluation of bexarotene and selected analogues for modulation of VDR−VDRE-mediated transcription via formation and/or diversion of RXR homodimers. HCT-116 cells were transfected with hVDR, VDRE-luciferase reporter plasmids (proximal ER6 or distal DR3 from the human CYP3A4 gene or the natural human CYP24A1 promoter-5.5kb), and a renilla control plasmid. Cells were transfected for 24 h utilizing a liposome-mediated transfection protocol and then treated with ethanol vehicle, 1 nM 1,25D, and 100 or 1000 nM of the indicated compound in the presence of 1 nM 1,25D. After a 24 h incubation, cells were lysed and a luciferase assay was completed. 1,25D-stimulated, VDRE-mediated transcription in the presence of rexinoids (as measured by luciferase output) was compared to the 1,25D-only group (value set to 1.0). (A) ER6-mediated activity in the presence of 1 nM 1,25D and 100 nM analogues. (B) ER6-mediated activity in the presence of 1 nM 1,25D and 1000 nM analogues. (C) DR3-mediated activity in the presence of 1 nM 1,25D and 100 nM analogues. (D) DR3-mediated activity in the presence of 1 nM 1,25D and 1000 nM analogues. (E) hCYP24A1 promoter activity in the presence of 1 nM 1,25D and 100 nM analogues. (F) hCYP24A1 promoter activity in the presence of 1 nM 1,25D and 1000 nM analogues. Values are means ± SD $*p < 0.05$, $**p < 0.01$ vs 1 nM 1,25D only (black bar).

versus **1**, it is also useful to examine how each novel analogue performs relative to a known compound (**8**−**12**) possessing a correspondingly more similar structure. Substituting a pyrmidine ring in **13** and **14** versus a pyridine ring in **8** and **9** resulted in higher RXRα EC_{50} values, whereas the RAR agonist activity was similar, as was the ability to modulate VDR activity on ER6 and CYP24, and the ability of **13** and **14** to stimulate apoptosis. On the other hand, the fluorine substitution in novel analogue **19** versus the hydrogen atom in **10** resulted in similar RXRα EC_{50} values, whereas the RAR agonist activity for **19** was much greater than **10**, but the apoptosis and ER6/CYP24 VDRE transcription levels were similar for both **10** and **19**. Substituting a methyl group, **15**, or trifluoromethyl group, **16**, for the hydroxyl group of **11** led to higher RXRα EC_{50} values, whereas the RAR agonist activity was higher in **15** but nearly identical in **16**. The apoptosis profile and activity on all three VDREs were similar for **11**, **15**, and **16**. Interestingly, introducing an unsaturation in the aliphatic ring of **11** to give analogue **18** conferred similar RXRα EC_{50} values and RAR agonist activities between **18** and **11** as well as similar apoptosis and VDRE transcription levels. However, substituting a methyl group for the hydroxyl group in **11** while simultaneously introducing an unsaturation in the aliphatic ring to give analogue **17** resulted in a higher RXRα EC_{50} value as well as higher RAR agonist activities than observed in **11**.

Bexarotene and the Novel Analogues Are Not Mutagenic. Utilizing the EPA-approved *Saccharomyces cerevisiae* mutagenesis assay,[34] all compounds were tested for their ability to damage DNA. DNA damage in this assay leads to either a point mutation (reversion of a phenotype of isoleucine auxotroph to isoleucine prototroph) or substantial damage leading to subsequent repair. In this way, DNA mutagenesis in this strain can be quantified by colony formation on selective media, indicative of a phenotype change induced by DNA damage. All compounds were tested at concentrations up to 0.15% w/v and

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none increased colony formation above vehicle control (data not shown).

CONCLUSIONS

1 is an FDA approved drug to treat cutaneous T-cell lymphoma and is being explored to treat a myriad of additional types of cancer. Moreover, recent data suggest that **1** is also a promising compound in the continued fight against AD.[27,52] Our work was motivated by the untoward side effects of **1** because the drug appears to be safe and efficacious in treatment of some CTCL patients, but the side effects include hyperlipidemia and hypothyroidism due to the ability of **1** to serve as an RXR ligand with little heterodimer-specific selectivity. The potential benefit of exploring additional RXR ligands for therapeutic treatments is further motivated by the opportunities of rational drug design, and as we have shown,[34,35] modeling can help drive synthesis of additional potent RXR ligands. Our continued work has produced seven novel compounds, four of which possess EC_{50} values lower than **1** and two of which possess superior pro-apoptotic profiles in CTCL cells. We are thus hopeful that an RXR ligand with a lower EC_{50} will translate into a pharmaceutical that would allow for a lower effective dose regime. Furthermore, side effects of hyperlipidemia and hypothyroidism are an ongoing consideration in treatment with **1**. We address concerns about cross-talk by RXR ligands with other RXR nuclear receptor pathways, as well as the potential for new rexinoids to titrate away RXR from nonpermissive heteropartners by assessing our novel compounds for their ability to modulate VDR−RXR-directed transcription utilizing multiple VDREs. We demonstrate that many of our novel analogues are superior at mitigating RXR heterodimerization disruption compared to **1**. Taken together, our novel RXR ligands appear more potent, demonstrate higher efficacy, and are less likely to lead to side effects than the parent compound. Work is ongoing to analyze further biological parameters of these compounds and develop additional novel analogues of **1** with improved side effect profiles.

EXPERIMENTAL SECTION

Mammalian Two-Hybrid Assay. HCT-116 colorectal carcinoma cells were plated overnight at 80000 cells/well in a 24-well plate and maintained in DMEM/high glucose (Hyclone) enhanced with 10% FBS (Invitrogen), 1 mM sodium pyruvate (Invitrogen), 100 μg/mL streptomycin, and 100 unit/mL penicillin. The cells were cotransfected using a human RXR binding domain (BD) vector, a human RXR activation domain (AD) vector, a luciferase reporter gene containing BD-binding sites, and renilla control plasmid. A liposome-mediated transfection was completed according to the manufacturer's protocol using 2 μL/well of Express-IN transfection reagent (Thermo Fisher Scientific, Lafayette, CO) and allowed to incubate for 24 h. The cells were then treated with media containing ethanol vehicle (0.1%), bexarotene or analogues at a final concentration of 25 nM or 100 nM and incubated for 24 h. The amount of rexinoid activity was measured by luciferase output utilizing a dual-luciferase reporter assay system according to the manufacturer's protocol (Promega, Madison, WI) in a Sirus luminometer (Berthold Detection System, Pforzheim, Germany). Three independent assays were conducted with triplicate samples for each treatment group.

RXRE-Mediated Transcription Assay. The RXRE assays were completed using HCT-116 cells plated at 80000 cells/well in a 24-well plate and maintained as described above. The cells were cotransfected using 250 ng of RXRE-luciferase reporter gene (RXRE from the naturally occurring responsive element in the rat cellular retinol binding protein II gene:[53] AAAATGAACTGTGACCTGTGACCTGTGACCTGTGAC), 50 ng of pSG5-human RXRα, and 20 ng of the renilla control plasmid and 2 μL/well of Express-IN was again used for the liposome-mediated delivery. The cells were incubated for 24 h post-transfection and then treated with media containing ethanol vehicle (0.1%), or analogues (final concentration of 100 nM or 1 μm). After a 24 h incubation period, the amount of retinoid activity was measured using the same luciferase assay described above. Three independent assays were conducted with triplicate samples for each treatment group.

EC_{50} Determination. EC_{50} values were determined from full dose−response curves ranging from 1×10^{-9} to 0.3×10^{-5} M in transfected HCT-116 cells using an RXR mammalian two-hybrid system. HCT-116 cells were plated overnight at 80000 cells/well in a 24-well plate and maintained as described above. The cells were cotransfected using a human RXR binding domain (BD) vector, a human RXR activation domain (AD) vector, a luciferase reporter gene containing BD-binding sites, and renilla control plasmid. Transfection was achieved via 2 μL/well of Express-IN transfection reagent which was allowed to incubate for 24 h with the cells. Then, the cells were treated with ethanol vehicle (0.1%) or analogues (1.0, 2.5, 5.0, 7.5, 10, 25, 50, 75, 100, 250, 500 nM, 1, 2, 3 μM) and incubated for 24 h. The amount of rexinoid activity at each concentration was measured using the same luciferase assay described above, and EC_{50} values were derived from dose−response curves of ligand concentration versus normalized luciferase activity

RARE-Mediated Transcription Assay. The RARE assay was completed using HCT-116 cells plated at 80000 cells/well in a 24-well plate and maintained as described above. The cells were cotransfected using 250 ng of pTK-DR5(x2)-luciferase reporter gene. This RARE is an optimized element that has been described previously[54] and is responsive to the RAR ligand, all-*trans* retinoic acid. Also included in the transfection were 50 ng of pCMX-human RARα and 20 ng of the renilla control plasmid, along with 2 μL/well of Express-IN for liposome-mediated delivery of the DNA into the cells. The cells were incubated for 24 h post-transfection and then treated with ethanol (0.1%) or analogues (final concentration of 100 nM or 1 μM). After a 24 h incubation period, the amount of retinoid activity was measured using the same luciferase assay described above. The activity with analogue (or compound **1**) divided by the activity with all-*trans* RA (expressed as a percentage) represents the RAR agonist activity. Three independent assays were conducted with triplicate samples for each treatment group.

Apoptosis Assay. Apoptotic activity was assessed by the Caspase-Glo 3/7 assay (Promega, Madison, WI) according to the manufacturer's instructions. The Caspase-Glo 3/7 assay is based on the cleavage of the DEVD sequence of a luminogenic substrate by caspases 3 and 7, which results in a luminescent signal. HuT-78 (human T-cell lymphoma) cells were distributed (1×10^4 cells/well) in white-walled 96-well microplates (Corning, NY) in 100 μL of medium and incubated with 500 μM sodium butyrate (NaBu), 100 nM **1**, or rexinoid analogues for 24 and 48 h. The Caspase-Glo 3/7 reagent was then added to each well and incubated for an additional 1 h at room temperature. The luminescence was measured in a luminometer (Safire2, Tecan, US). NaBu, a known inducer of apoptosis in HuT-78, was used as a positive control. Each treatment group was dosed in triplicate, and at least two independent experiments were performed. Numbers were standardized to sodium butyrate (set at 100%).

VDR−VDRE Assay. The VDR−VDRE assay was completed using HCT-116 cells plated at 80000 cells/well in a 24-well plate and maintained as described above. The cells were cotransfected using 250 ng of the indicated VDRE-luciferase reporter gene (proximal ER6 or distal DR3 VDRE from the human CYP3A4 gene, or the natural human CYP24A1 promoter-5.5kb), 50 ng of pSG5-hVDR, and 20 ng of the renilla control plasmid with 2 μL/well of Express-IN used for liposome-mediated DNA delivery. The cells were incubated for 24 h post-transfection and then further treated with ethanol vehicle (0.1%), 10^{-9} M 1,25-dihydroxyvitamin D_3 (1,25D) alone, or 10^{-9} M 1,25D in combination with analogues (final concentration of 10^{-6} M or 10^{-7} M). After a 24 h incubation period, the amount of luciferase activity was measured using the luminescence assay described above. Three

independent assays were conducted with triplicate samples for each treatment group. Modulation of vitamin D activity was determined by evaluation of 1,25D-treated cells compared to cells treated with 1,25D plus bexarotene/analogue.

Mutagenicity Assays. Mutagenicity assays utilized the D7 strain of *Saccharomyces cerevisiae*.[55] D7 cells were incubated at 30 °C with shaking for 3 h with the compound solubilized in DMSO in increasing concentrations up to 0.15% w/v, and then aliquots were plated on selective media as described in Marshall[56] and allowed to incubate for 4 days. Colony number was then scored and compared to DMSO alone (negative) and ethidium bromide (positive) controls.

Docking Studies. Docking studies on the human RXRα (Protein Data Bank codes 1MVC[57] and 3FUG,[39] respectively) and RXRβ (Protein Data Bank code 1H9U[58]) were performed using the Autodock 4.2 software.[59] As in our previous studies,[34] protein residues Arg316 and Ile268 (residue numbering as in 1MVC) were treated flexibly and both Autodock and OpenBabel 2.3.0 charges[60] were used. Docking used the Lamarckian genetic algorithm with 25 000 000 energy evaluations per dock and a total of 250 docks per compound. Calculated binding free energies were used to score the compounds.

Instrumentation. A 400 MHz Bruker spectrometer was used to acquire ^{1}H NMR and ^{13}C NMR spectra. Chemical shifts (δ) are listed in ppm against residual nondeuterated solvent peaks in a given deuterated solvent (e.g., $CHCl_3$ in $CDCl_3$) as an internal reference. Coupling constants (J) are reported in Hz, and the abbreviations for splitting include: s, single; d, doublet; t, triplet; q, quartet; p, pentet; m, multiplet; br, broad. All ^{13}C NMR spectra were acquired on a Bruker instrument at 100.6 MHz. Chemical shifts (δ) are listed in ppm against deuterated solvent carbon peaks as an internal reference. High resolution mass spectra were recorded using either a JEOL GCmate (2004), a JEOL LCmate (2002) high resolution mass spectrometer, or an ABI Mariner (1999) ESI-TOF mass spectrometer.

General Procedures. Removal of volatile solvents transpired under reduced pressure using a Büchi rotary evaporator and is referred to as removing solvents in vacuo. Thin layer chromatography was conducted on precoated (0.25 mm thickness) silica gel plates with 60F-254 indicator (Merck). Column chromatography was conducted using 230−400 mesh silica gel (E. Merck reagent silica gel 60). All tested compounds were analyzed for purity by combustion analysis through Columbia Analytical Services (formerly Desert Analytics in Tucson, AZ) and were found to be >95% pure.

5-(Methoxycarbonyl)pyridine-2-carboxylic Acid (20). The protocol of Faul et al. was followed.[38] To a suspension of dimethyl pydrine-2,5-dicarboxylate (10.09 g, 51.7 mmol) in methanol (130 mL) was added sodium hydroxide pellets (2.20 g, 55.0 mmol), and the heterogeneous reaction was stirred and refluxed for 4 h. After cooling to 65 °C, 2.0 N HCl (38 mL, 76 mmol) was slowly added, and over the course of addition, the solid dissolved and a precipitate formed. The reaction solution was allowed to cool to room temperature, and then it was cooled in an ice-bath and filtered. The filter-cake was washed with water to give an off-white product (7.71 g, 82%). A sample of this crude, filtered product (1.09 g) was dissolved in boiling water (70 mL), and the solution was slowly cooled to room temperature and then cooled in an ice-bath before it was filtered to give a white, crystalline powder (0.98 g) at a 74% overall yield, mp 189−190 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.15 (dd, J = 2.0, 0.8, 1H), 8.44 (dd, J = 8.0, 2.0, 1H), 8.15 (dd, J = 8.0, 0.8, 1H), 3.91 (s, 3H), 3.34 (br s, 1H). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 165.5, 164.6, 151.7, 149.8, 138.3, 127.9, 124.6. IR (neat) ν 3440, 2969, 1717, 1694 cm^{-1}. LC-FAB-MS (M + H)$^+$ calcd for $C_8H_8NO_4$ 182.0453, found 182.0458.

Methyl 6-[(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)carbonyl]nicotinate (23). The procedure of Faul and co-workers was followed.[38] Methyl 6-(chlorocarbonyl)-pyridine-3-carboxylate (**21**) was synthesized by refluxing 5-(methoxycarbonyl)-pyridine-2-carboxylic acid (**20**) (1.20 g, 6.62 mmol) in thionyl chloride (12.0 mL, 165 mmol) in a 100 mL one-neck round-bottom flask fitted with a water-cooled reflux condenser. Excess thionyl chloride was removed in vacuo to give crude **21** as an off-white solid, and this solid was dissolved in dry benzene (ca. 20 mL) and evaporated to dryness to remove residual thionyl chloride. The acid chloride **21** was dried on high vacuum to remove residual benzene. To a two-neck, 50 mL round-bottom flask equipped with a reflux condenser and magnetic stir-bar was added **22** (1.40 g, 6.91 mmol) followed by a solution of crude acid chloride **21** (6.62 mmol) in DCM (15 mL). Aluminum chloride (2.20 g, 16.5 mmol) was added to the reaction solution at room temperature slowly, with stirring, and the reaction solution turned from colorless to red accompanied by the evolution of gas and heat. The reaction was stirred for 5 min then heated to reflux for 15 min. The reaction was judged to be complete by TLC, and the solution was poured into an ice solution (25 mL) acidified with a 20% HCl solution (8 mL), and ethyl acetate was added (13 mL). The aqueous and organic layers were separated, and the aqueous layer was extracted with ethyl acetate (15 mL, twice). The combined organics were washed with water and brine, dried over sodium sulfate, filtered, and concentrated to give crude **23**. Crude **23** was purified by column chromatography (250 mL SiO_2, hexanes:ethyl acetate 95:5 to 85:15) to give **23** (1.30 g, 54%) as an off-white, crystalline solid, mp 122−125 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.26 (dd, J = 2.0, 0.8, 1H), 8.49 (dd, J = 8.0, 2.0, 1H), 8.10 (dd, J = 8.0, 0.8, 1H), 7.42 (s, 1H), 7.21 (s, 1H), 3.99 (s, 3H), 2.39 (s, 3H), 1.68 (s, 4H), 1.29 (s, 6H), 1.20 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 195.8, 165.1, 158.6, 149.9, 149.2, 141.6, 138.1, 135.7, 133.2, 130.3, 129.7, 127.5, 123.8, 52.7, 34.8, 34.8, 34.4, 33.8, 31.6, 31.5, 20.5. IR (neat) 2919, 1720, 1688 cm^{-1}. LC-MS (M + H)$^+$ calcd for $C_{23}H_{28}NO_3$ 366.2069, found 366.2070.

Methyl 6-[(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)ethenyl]nicotinate (24). The procedure of Faul and co-workers was followed.[38] To a 100 mL round-bottom flask charged with **23** (1.0077 g, 2.757 mmol) was added toluene (10 mL), and the solution was cooled in a salt-water ice bath to −15 °C with stirring, under nitrogen. To this solution was added a 22 wt % solution of MeMgCl in THF (1.20 mL, 3.60 mmol), and the reaction was stirred for 15 min at −15 °C and then warmed to room temperature and stirred for 35 min before quenching with 1N HCl (7 mL, 7 mmol). The reaction mixture was then separated, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were filtered and a small yellow filter-cake was dissolved in chloroform and added to the organic filtrate, and the combined organic filtrate was concentrated in vacuo to give a crude alcohol intermediate that was used without further purification. To this crude intermediate in a 100 mL round-bottom flask was added *p*-toluenesulfonic acid monohydrate (0.5247 g, 2.76 mmol) and toluene (40 mL), and the reaction was refluxed for 3 h into a Dean−Stark apparatus half-filled with toluene (6 mL). After the reaction had cooled to room temperature, it was added to a solution of sodium carbonate (0.78 g) in water (15 mL), shaken vigorously, and the layers were separated. The aqueous layer was extracted with ethyl acetate, and the combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated in vacuo to give a crude brown product that was purified by column chromatography (150 mL SiO_2, hexanes:ethyl acetate 97.5:2.5 to 95:5) to give **24** (0.5595 g, 55%) as a white, fiber-like solid, mp 166−167 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.22 (dd, J = 2.0, 0.8, 1H), 8.15 (dd, J = 8.0, 2.0, 1H), 7.14 (s, 1H), 7.11 (s, 1H), 7.02 (dd, J = 8.4, 0.8, 1H), 6.54 (d, J = 2.0, 1H), 5.51 (d, J = 2.0, 1H), 3.94 (s, 3H), 1.98 (s, 3H), 1.69 (s, 4H), 1.31 (s, 6H), 1.26 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 165.8, 161.1, 150.6, 148.0, 144.5, 142.5, 137.6, 136.7, 132.7, 128.0, 124.1, 121.1, 121.0, 52.2, 35.1, 35.0, 33.9, 33.8, 31.9, 31.8, 19.8. IR (neat) 2959, 1722, 1591 cm^{-1}. LC-MS (M + H)$^+$ calcd for $C_{24}H_{30}NO_2$ 364.2277, found 364.2272.

Methyl 6-[(3,5,8,8-Pentamethyl-5.6,7,8-tetrahydronaphthalen-2-yl)cyclopropyl]nicotinate (25). The procedure of Faul and co-workers was followed.[38] To a suspension of trimethylsulfoxonium iodide (0.365 g, 1.66 mmol) in DMSO (1.2 mL) was added a 20 wt % solution of potassium *tert*-butoxide in THF (0.94 mL, 1.67 mmol). A solution of **24** (0.40 g, 1.10 mmol) in THF (4.8 mL) was added dropwise over 20 min at 30−34 °C with stirring. The reaction was stirred for 60 min at 35 °C, then cooled to room temperature and quenched with 1N HCl (5 mL). The layers were separated, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and

concentrated to give an off-white crude solid that was purified by column chromatography (150 mL SiO_2, hexanes:ethyl acetate 97.5:2.5 to 90:10) to give **25** (0.3411 g, 82%) as a white crystalline solid, mp 168−170 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.08 (dd, J = 2.4, 0.8, 1H), 7.98 (dd, J = 8.4, 2.4, 1H), 7.27 (s, 1H), 7.11 (s, 1H), 6.74 (dd, J = 8.4, 0.8, 1H), 3.90 (s, 3H), 2.11 (s, 3H), 1.82−1.83 (m, 2H), 1.69 (s, 4H), 1.35−1.36 (m, 2H), 1.30 (s, 6H), 1.27 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 169.2, 166.1, 150.4, 143.8, 142.6, 137.1, 136.5, 135.7, 129.2, 128.3, 122.1, 120.7, 52.0, 35.1, 34.0, 33.9, 31.9, 31.8, 30.3, 20.2, 19.2. IR (neat) 2958, 1714, 1595 cm^{-1}. LC-MS (M + H)$^+$ calcd for $C_{25}H_{32}NO_2$ 378.2433, found 378.2422.

Methyl 6-(1-(1,2,3,4-Tetrahydro-1,1,4,4,6-pentamethylnaphthalen-7-yl)vinyl)pyridine-3-carboxylate (8).[36] To a suspension of **24** (0.2898 g, 0.797 mmol) in methanol (5.0 mL) was added a solution of KOH (0.116 g) in water (0.18 mL), and the reaction was refluxed at 85 °C for 1 h. The reaction solution was cooled to room temperature and quenched with 20% HCl (26 mL). The crude precipitate was filtered and dried to give a crude white product (0.2753 g, 98%) that was recrystallized from hexanes:ethyl acetate 4:1 to give the pure **8** as a white crystalline solid, mp 243−245 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.51 (br s, 1H), 9.47 (s, 1H), 8.82 (dd, J = 8.4, 2.0, 1H), 7.41 (d, J = 8.4, 1H), 7.23 (s, 1H), 7.15 (s, 1H), 7.10 (s, 1H), 6.08 (s, 1H), 2.01 (s, 3H), 1.68 (s, 4H), 1.29 (s, 6H), 1.24 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 161.8, 154.3, 146.4, 146.3, 143.6, 139.9, 133.0, 132.5, 130.0, 128.8, 128.6, 128.1, 125.6, 34.8, 34.1, 33.9, 31.8, 31.7, 19.7. IR 2948, 1706, 1590 cm^{-1}. LC-MS (M + H)$^+$ calcd for $C_{23}H_{28}NO_2$ 350.2120, found 350.2111. Anal. Calcd for $C_{24}H_{29}NO_2$·HCl: C, 71.58; H, 7.31; N, 3.63. Found: C, 72.64; H, 7.35; N, 3.62.

6-[1-(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopropyl]nicotinic Acid (9).[36] To a suspension of **25** (0.3043 g, 0.806 mmol) in methanol (8.0 mL) was added a solution of KOH (0.1287 g) in water (0.18 mL), and the reaction was refluxed at 85 °C for 1 h. The reaction solution was cooled to room temperature and quenched with 20% HCl (27 mL). The crude precipitate was filtered and dried to give a crude white product (0.2543 g, 86%) that was recrystallized from hexanes:ethyl acetate 4:1 to give pure **9** as a white crystalline solid (0.147 g, 50%), mp 273−274 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.69 (br s, 1H), 9.18 (dd, J = 2.0, 0.8, 1H), 8.05 (dd, J = 8.4, 2.0, 1H), 7.27 (s, 1H), 7.12 (s, 1H), 6.79 (dd, J = 8.4, 0.8, 1H), 2.13 (s, 3H), 1.86−1.87 (m, 2H), 1.69 (s, 4H), 1.39−1.40 (m, 2H), 1.31 (s, 6H), 1.27 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 170.8, 170.2, 151.0, 143.9, 142.7, 137.2, 136.9, 135.7, 129.2, 128.3, 121.4, 120.9, 35.1, 34.0, 33.9, 31.9, 31.8, 30.5, 20.5, 19.2. IR (neat) 2952, 1686, 1593 cm^{-1}. LC-MS (M + H)$^+$ calcd for $C_{24}H_{30}NO_2$ 364.2277, found 364.2265. Anal. Calcd for $C_{24}H_{29}NO_2$: C, 79.30; H, 8.04; N, 3.85. Found: C, 78.76; H, 7.93; N, 3.76.

Dimethyl Pyrimidine-2,5-dicarboxylate (26). To a 100 mL round-bottom flask charged with pyrimidine-2,5-dicarboxylic acid (5.067 g, 30.1 mmol) was slowly added thionyl chloride (21 mL, 290 mmol) and 3 drops of DMF, and the reaction was refluxed in an oil bath at 85 °C for 3 h. Excess thionyl chloride was removed in vacuo and benzene (20 mL) was added to the crude solid and the benzene was removed in vacuo to give crude pyrimidine-2,5-dicarboyl dichloride that was used without further purification. To the crude pyrimidine-2,5-dicarboyl dichloride was added toluene (13.6 mL), and this solution was added dropwise to a solution of triethylamine (16.5 mL, 118 mmol) in methanol (86 mL). After stirring for 1 h at room temperature, the reaction was quenched with 1 N HCl (120 mL). The reaction was poured into ethyl acetate (80 mL), the layers were separated, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with saturated $NaHCO_3$ (75 mL) and brine, dried over sodium sulfate, and removed in vacuo to give crude **26**. Crude **26** was purified by column chromatography (250 mL SiO_2, hexanes:ethyl acetate 2:3) to give **26** (4.26 g, 72%) as a colorless, crystalline solid (mp 140−142 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.40 (s, 2H), 4.07 (s, 3H), 3.99 (s, 3H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 163.0, 162.9, 158.8, 158.3, 125.4, 53.8, 53.0. IR (neat) 2969, 1709, 1579. 1551 cm^{-1}. GC-MS (M)$^+$ calcd for $C_8H_8N_2O_4$ 196.0484, found 196.0485.

5-(Methoxycarbonyl)pyrimidine-2-carboxylic Acid (27). To a suspension of of dimethyl pyrimidine-2,5-dicarboxylate (**26**) (3.34 g, 17.0 mmol) in methanol (30 mL) was added sodium hydroxide pellets (0.748 g, 18.7 mmol), and the heterogeneous reaction was stirred and refluxed for 4 h. After cooling to 65 °C, 2.0 N HCl (14 mL, 28 mmol) was slowly added. The reaction solution was allowed to cool to room temperature, and then it was concentrated in vacuo to give a solid. The solid was filtered with cold water, and the filter-cake was washed with a small amount of cold water to give **27** as a white crystalline solid (mp 152−154 °C) (2.765 g, 89%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.89 (br s, 1H), 9.39 (s, 2H), 3.97 (s, 3H). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 164.3, 163.4, 159.6, 158.4, 124.8, 52.9. IR (neat) 3493, 2970, 1716, 1584, 1558 cm^{-1}. GC-MS (M)$^+$ calcd for $C_7H_6N_2O_4$ 182.0328, found 182.0335.

Methyl 2-[(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)carbonyl]pyrimidine-5-carboxylate (29). Methyl 2-(chlorocarbonyl)-pyrimidine-5-carboxylate (**28**) was synthesized by refluxing 5-(methoxycarbonyl)pyrimidine-2-carboxylic acid (**27**) (1.22 g, 6.70 mmol) in thionyl chloride (12.0 mL, 165 mmol) in a 100 mL one-neck round-bottom flask fitted with a water-cooled reflux condenser. Excess thionyl chloride was removed in vacuo to give crude **28** as an off-white solid, and this solid was dissolved in dry benzene (ca. 20 mL) and evaporated to dryness to remove residual thionyl chloride. The acid chloride **28** was dried on high vacuum to remove residual benzene. To a two-neck, 50 mL round-bottom flask equipped with a reflux condenser and magnetic stir-bar was added **22** (1.45 g, 7.16 mmol) followed by a solution of crude acid chloride **28** (6.70 mmol) in DCM (15 mL). Aluminum chloride (2.20 g, 16.5 mmol) was added to the reaction solution at room temperature slowly, with stirring, and the reaction solution turned from colorless to red accompanied by the evolution of gas and heat. The reaction was stirred for 5 min then heated to reflux for 15 min. The reaction was judged to be complete by TLC, and the solution was poured into an ice solution (25 mL), acidified with a 20% HCl solution (8 mL), and ethyl acetate added (13 mL). The aqueous and organic layers were separated, and the aqueous layer was extracted with ethyl acetate (15 mL, twice). The combined organics were washed with water and brine, dried over sodium sulfate, filtered, and concentrated to give crude **29**. Crude **29** was purified by column chromatography (250 mL SiO_2, hexanes:ethyl acetate 95:5 to 85:15) to give **29** (1.677 g, 68%) as an off-white, crystalline solid, mp 94−96 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.42 (s, 2H), 7.40 (s, 1H), 7.21 (s, 1H), 4.03 (s, 3H), 2.43 (s, 3H), 1.66 (s, 4H), 1.28 (s, 6H), 1.17 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 192.8, 165.9, 163.5, 158.4, 150.3, 142.0, 136.7, 131.9, 130.8, 130.0, 124.0, 52.9, 34.7, 34.6, 34.4, 33.8, 31.6, 31.4, 20.9. IR (neat) 2951, 1715, 1683, 1577, 1549 cm^{-1}. LC-MS (M + H)$^+$ calcd for $C_{22}H_{27}N_2O_3$ 367.2022, found 367.2017.

Methyl 2-[(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)ethenyl]pyrimidine-5-carboxylate (30). To a 100 mL round-bottom flask charged with **29** (1.0373 g, 2.83 mmol) was added toluene (10 mL), and the solution was cooled in a salt-water ice bath to −15 °C with stirring, under nitrogen. To this solution was added a 22 wt % solution of MeMgCl in THF (1.20 mL, 3.60 mmol), and the reaction was stirred for 15 min at −15 °C and then warmed to room temperature and stirred for 35 min before quenching with 1N HCl (7 mL, 7 mmol). The reaction mixture was then separated, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were filtered and a small yellow filter-cake was dissolved in chloroform and added to the organic filtrate and the combined organic filtrate and combined organic filtrate was concentrated in vacuo to give a crude alcohol intermediate that was used without further purification. To this crude intermediate in a 100 mL round-bottom flask was added *p*-toluenesulfonic acid monohydrate (0.5247 g, 2.76 mmol) and toluene (40 mL), and the reaction was refluxed for 3 h into a Dean−Stark apparatus half-filled with toluene (6 mL). After the reaction had cooled to room temperature, it was added to a solution of sodium carbonate (0.78 g) in water (15 mL), shaken vigorously, and the layers separated. The aqueous layer was extracted with ethyl acetate, and the combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated in vacuo to

give a crude brown product that was purified by column chromatography (150 mL SiO_2, hexanes:ethyl acetate 97.5:2.5 to 95:5) to give **30** (0.4127 g, 40%) as a white, fiber-like solid, mp 189−191 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.25 (s, 2H), 7.17 (s, 1H), 7.11 (s, 1H), 6.83 (d, J = 2.0, 1H), 5.80 (d, J = 2.0, 1H), 3.97 (s, 3H), 1.98 (s, 3H), 1.69 (s, 4H), 1.31 (s, 6H), 1.28 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 168.6, 164.4, 158.2, 148.3, 144.4, 142.1, 136.3, 132.7, 128.0, 127.9, 126.7, 121.1, 52.5, 35.1, 34.0, 33.8, 31.9, 31.8, 20.0. IR (neat) 2959, 1717, 1582 cm^{-1}. LC-MS (M + H)$^+$ calcd for $C_{23}H_{29}N_2O_2$ 365.2229, found 365.2232.

Methyl 2-[(3,5,8,8-Pentamethyl-5.6,7,8-tetrahydronaphthalen-2-yl)cyclopropyl]pyrimidine-5-carboxylate (31). To a suspension of trimethylsulfoxonium iodide (0.365 g, 1.66 mmol) in DMSO (1.2 mL) was added a 20 wt % solution of potassium *tert*-butoxide in THF (0.94 mL, 1.67 mmol). A solution of **30** (0.4062 g, 1.11 mmol) in THF (4.8 mL) was added dropwise over 20 min at 30−34 °C with stirring. The reaction was stirred for 60 min at 35 °C, then cooled to room temperature and quenched with 1N HCl (5 mL). The layers were separated, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and concentrated to give an off-white crude solid that was purified by column chromatography (150 mL SiO_2, hexanes:ethyl acetate 97.5:2.5 to 90:10) to give **31** (0.2884 g, 68%) as a white crystalline solid, mp 202−203 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.07 (s, 2H), 7.23 (s, 1H), 7.09 (s, 1H), 3.92 (s, 3H), 2.12 (s, 3H), 1.87−1.88 (m, 2H), 1.67 (s, 4H), 1.46−1.47 (m, 2H), 1.29 (s, 6H), 1.27 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 176.8, 164.7, 157.7, 143.2, 141.8, 136.9, 135.9, 128.6, 127.8, 119.9, 52.3, 35.2, 34.0, 33.9, 31.9, 31.8, 31.7, 21.6, 19.4. IR (neat) 2951, 1719, 1588 cm^{-1}. LC-MS (M + H)$^+$ calcd for $C_{24}H_{31}N_2O_2$ 379.2386, found 379.2385.

Methyl 2-(1-(1,2,3,4-Tetrahydro-1,1,4,4,6-pentamethylnaphthalen-7-yl)vinyl)pyridine-5-carboxylate (13). To a suspension of **30** (0.828 g, 2.27 mmol) in methanol (15.0 mL) was added a solution of KOH (0.3627 g) in water (0.54 mL), and the reaction was refluxed at 85 °C for 1 h. The reaction solution was cooled to room temperature and quenched with 20% HCl (60 mL). The crude precipitate was filtered and dried to give a crude white product (0.7741 g, 97%) that was recrystallized from hexanes:ethyl acetate 4:1 to give the pure **13** as a white crystalline solid (0.3869 g, 48%), mp 254−255 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.11 (br s, 1H), 9.31 (s, 2H), 7.17 (s, 1H), 7.12 (s, 1H), 6.86 (d, J = 1.6, 1H), 5.86 (d, J = 1.6, 1H), 1.99 (s, 3H), 1.68 (s, 4H), 1.28 (s, 6H), 1.27 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 169.0, 168.2, 158.8, 148.0, 144.5, 142.2, 136.0, 132.7, 128.0, 127.9, 127.3, 120.7, 35.1, 34.0, 33.8, 31.9, 31.8, 20.0. IR (neat) 2963, 1682, 1580 cm^{-1}. LC-MS (M + H)$^+$ calcd for $C_{22}H_{27}N_2O_2$ 351.2073, found 351.2082. Anal. Calcd for $C_{22}H_{26}N_2O_2$: C, 75.40; H, 7.48; N, 7.99. Found: C, 75.35; H, 7.54; N, 7.91.

2-[1-(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopropyl]pyrimidine-2-carboxylic Acid (14). To a suspension of **31** (0.287 g, 0.758 mmol) in methanol (5.0 mL) was added a solution of KOH (0.126 g) in water (0.18 mL), and the reaction was refluxed at 85 °C for 1 h. The reaction solution was cooled to room temperature and quenched with 20% HCl (32 mL). The crude precipitate was filtered and dried to give a crude white product (0.2568 g, 92%) that was recrystallized from hexanes:ethyl acetate 4:1 to give the pure **14** as a white crystalline solid (0.2433 g, 88%), mp 266−267 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.18 (s, 2H), 7.23 (s, 1H), 7.09 (s, 1H), 2.13 (s, 3H), 1.90−1.91 (m, 2H), 1.66 (s, 4H), 1.50−1.51 (m, 2H), 1.27 (s, 6H), 1.26 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 177.6, 168.6, 158.3, 143.3, 141.9, 136.6, 135.9, 128.6, 127.9, 119.2, 35.2, 34.0, 33.9, 32.0, 31.9, 31.8, 22.1, 19.4. IR (neat) 2954, 1679, 1586 cm^{-1}. LC-MS (M + H)$^+$ calcd for $C_{23}H_{29}N_2O_2$ 365.2229, found 365.2225. Anal. Calcd for $C_{23}H_{28}N_2O_2$: C, 75.79; H, 7.74; N, 7.69. Found: C, 75.67; H, 7.95; N, 7.32.

3,4-Dihydro-1,1,4,4,7-pentamethylnaphthalen-2(1*H*)-one (33).[37] A modified procedure of Boehm and co-workers was used.[37] To a 100 mL round-bottom flask charged with dihydro-2,2,5,5-tetramethylfuran-3(2*H*)-one (**32**) (2.4 g, 17 mmol) and toluene (10.0 mL, 94 mmol) at 0 °C was added aluminum chloride (4.55 g, 34 mmol) in one portion with stirring. The reaction was stirred at 0 °C for 30 min, then warmed to room temperature and stirred for 2 h, and then heated to 50−55 °C with stirring for 15 min. The reaction was cooled to room temperature and then poured onto ice water (50 mL) and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated in vacuo. The resulting crude solid was purified by column chromatography (150 mL SiO_2, hexanes:ethyl acetate 98:2) to give **33** (1.6348 g, 44%) as a white crystalline solid, 49−51 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (d, J = 8.0, 1H), 7.13 (s, 1H), 7.05 (d, J = 8.0, 1H), 2.63 (s, 2H), 2.35 (s, 3H), 1.44 (s, 6H), 1.31 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 214.5, 142.9, 140.6, 136.3, 127.7, 127.4, 124.3, 51.5, 47.9, 37.6, 30.6, 28.4, 21.0. IR (neat) 2968, 1705 cm^{-1}. GC-MS (M)$^+$ calcd for $C_{15}H_{20}O$ 216.1514, found 216.1523.

6-Bromo-3,4-dihydro-1,1,4,4,7-pentamethylnaphthalen-2(1*H*)-one (34). A DCM (22.0 mL) solution of **33** (3.193 g, 14.76 mmol) at 0 °C was stirred during the addition of aluminum chloride (3.993 g, 29.95 mmol), and the mixture was stirred at 0 °C for 5 min. To this solution was added a solution of bromine (0.90 mL, 17.47 mmol) in DCM (11.0 mL) dropwise, and the reaction solution was stirred for 30 min at 0 °C. The reaction solution was poured onto ice (50 mL) and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and concentrated in vacuo to give a crude product that was purified by column chromatography (150 mL SiO_2, hexanes:ethyl acetate 98:2) to give **34** (4.36 g, 100%) as a white crystalline solid, mp 114−117 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (s, 1H), 7.15 (s, 1H), 2.60 (s, 2H), 2.38 (s, 3H), 1.42 (s, 6H), 1.28 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 213.6, 143.2, 142.4, 136.3, 129.5, 128.3, 123.1, 51.2, 47.6, 37.5, 30.4, 28.3, 22.6. IR (neat) 2951, 1706 cm^{-1}. GC-MS (M)$^+$ calcd for $C_{15}H_{19}BrO$ 294.0619, found 294.0617.

(*E*)-1-(6-Bromo-3,4-dihydro-1,1,4,4,7-pentamethylnaphthalen-2(1*H*)-ylidene)-2-tosylhydrazine (35). To a suspension of **34** (3.0664 g, 10.4 mmol) and *p*-toluenesulfonylhydrazide (2.2158 g, 11.9 mmol) in methanol (61 mL) was added *p*-toluenesulfonic acid monohydrate (0.4977 g, 2.616 mmol), and the reaction solution was stirred and refluxed under nitrogen for 24 h. The reaction was then cooled to room temperature and then it was stirred in a salt-water ice-bath at −15 °C for 1 h and the resulting precipitate was filtered and rinsed with cold methanol to afford **35** as a white crystalline solid (2.41 g, 50%), mp 181−184 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (br s, 1H), 7.84 (d, J = 8.0, 2H), 7.34 (s, 1H), 7.28 (d, J = 8.4, 2H), 7.12 (s, 1H), 2.41 (s, 2H), 2.38 (s, 3H), 2.33 (s, 3H), 1.37 (s, 6H), 1.08 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 163.2, 144.0, 143.1, 142.7, 136.0, 134.9, 129.3, 128.3, 128.0, 122.7, 42.8, 36.8, 36.2, 29.9, 29.89, 22.5, 21.5. IR (neat) 3251, 2971 cm^{-1}. LC-MS (M + H)$^+$ calcd for $C_{22}H_{28}N_2O_2SBr$ 463.1055, found 463.1048.

6-Bromo-1,1,4,4,7-pentamethyl-1,4-dihydronaphthalene (36).[38] To a suspension of **35** (1.0127 g, 2.19 mmol) in MTBE (20 mL) was added a methyl lithium LiBr complex solution (1.5 M) in ether (4.40 mL, 6.60 mmol) at room temperature with stirring under nitrogen. The solution turned yellow with the evolution of gas (presumably nitrogen), and a fine off-white precipitate formed. The heterogeneous solution was stirred for 1 h, cooled to 0 °C, and then quenched with water (25.0 mL). The reaction was extracted with ethyl acetate, and the organic layers were combined, washed with brine, dried over sodium sulfate, filtered, and concentrated in vacuo to give a crude off-white solid that was purified by column chromatography (150 mL SiO_2, hexanes) to give **36** (0.5463 g, 90%) as a white solid (mp 109−111 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (s, 1H), 7.21 (s, 1H), 5.49 (s, 2H), 2.38 (s, 3H), 1.32 (s, 12H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 142.3, 141.8, 135.0, 132.7, 132.6, 129.8, 128.5, 122.4, 35.0, 34.9, 32.5, 32.4, 22.6. IR (neat) 3013, 2958, 1483, 1455, 1079, 889 cm^{-1}. GC-MS (M)$^+$ calcd for $C_{15}H_{19}Br$ 278.0670, found 278.0655.

(4-Cyanophenyl)-*N*-methoxy-*N*-methylformamide (38).[38] The method of Faul and co-workers was used.[38] To a suspension of *N*,*O*-dimethylhydroxyamine hydrochloride (7.07 g, 72.5 mmol) and K_2CO_3 (10.0 g, 72.5 mmol) in ACN (100 mL) and water (50 mL) was added 4-cyanobenzoyl chloride (**37**) (8.00 g, 48.3 mmol), and the reaction was stirred for 2 h at room temperature. The reaction solution

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was poured into water (50 mL) and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and concentrated in vacuo to give a crude solid that was purified by column chromatography (250 mL SiO_2, hexanes:ethyl acetate 45:55 to 1:1) to give **38** (8.12 g, 88%) as a white crystalline solid, mp 78−79 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, J = 8.4, 2H), 7.69 (d, J = 8.4, 2H), 3.50 (s, 3H), 3.36 (s, 3H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 167.8, 138.2, 131.8, 128.7, 118.1, 114.0, 61.2, 33.0. IR (neat) 2947, 1627 cm^{-1}. GC-MS $(M)^+$ calcd for $C_{10}H_{10}N_2O_2$ 190.0742, found 190.0739.

4-[(3,5,5,8,8-Pentamethyl-2-5,8-dihydronaphthyl)carbonyl]-benzenecarbonitrile (39).[38] The method of Faul and co-workers was followed.[38] To a solution of **35** (2.00 g, 7.16 mmol) in THF (25 mL) at −78 °C under nitrogen was added a 1.6 M solution of *n*-BuLi in hexanes (5.40 mL, 8.60 mmol) over 10 min, and the solution was stirred for 20 min at −78 °C. This reaction solution was transferred via airtight syringe to a solution of **38** (1.23 g, 6.47 mmol) in THF (10 mL) at −78 °C, and the combined mixture was stirred for 15 min at −78 °C and then warmed to room temperature before 1.0 N HCl (75 mL) was added to quench the reaction. The solution was poured into ethyl acetate, the layers were separated, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and concentrated in vacuo to give a crude product that was purified by column chromatography (150 mL SiO_2, hexanes:ethyl acetate 95:5) to give **39** (1.7865 g, 82%) as a white crystalline solid, mp 117−119 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, J = 8.4, 2H), 7.75 (d, J = 8.4, 2H), 5.52 (s, 2H), 2.37 (s, 3H), 1.37 (s, 6H), 1.26 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 196.6, 146.2, 141.6, 139.8, 135.0, 134.4, 132.6, 132.6, 129.2, 128.0, 118.0, 115.9, 35.3, 34.8, 32.4, 32.3, 20.0. IR (neat) 2960, 1669 cm^{-1}. GC-MS $(M)^+$ calcd for $C_{23}H_{23}NO$ 329.1780, found 329.1788.

4-[(3,5,5,8,8-Pentamethyl-2-5,8-dihydronaphthyl)carbonyl]-benzoic Acid (40).[38] The method of Faul and co-workers was followed.[38] To a heterogeneous solution of **39** (1.62 g, 4.92 mmol) in 2-methoxyethanol (20 mL) was added a solution of KOH (1.64 g, 24.5 mmol) in water (10 mL). The reaction was heated in an oil bath at reflux temperature and stirred under nitrogen for 16 h. The reaction was allowed to cool to room temperature before it was quenched with 1 N HCl (50 mL). The solution was poured into ethyl acetate, the layers were separated, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and concentrated in vacuo with additional toluene to azeotrope off 2-methoxyethanol to give **40** (1.65 g, 96%) as a white powder, mp 199−201 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.36 (br s, 1H), 8.08 (d, J = 8.4, 2H), 7.80 (d, J = 8.4, 2H), 7.42 (s, 1H), 7.34 (s, 1H), 5.54 (s, 2H), 2.25 (s, 3H), 1.33 (s, 6H), 1.22 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 197.0, 166.6, 145.1, 140.7, 139.3, 135.2, 134.5, 133.9, 132.6, 132.5, 129.7, 129.6, 128.8, 127.1, 34.9, 34.5, 32.0, 19.4. IR (neat) 2970, 1745 cm^{-1}. LC-MS $(M + H)^+$ calcd for $C_{23}H_{25}O_3$ 349.1804, found 349.1805.

4-[1-(3,5,5,8,8-Pentamethyl-2-5,8-dihydronaphthyl)vinyl]-benzoic Acid (10).[38] The procedure of Faul and co-workers was followed.[38] To a 100 mL round-bottom flask charged with a 3.0 M solution of MeMgCl (1.53 mL, 4.60 mmol) was added THF (3 mL), and the solution was cooled to −10 °C in a salt−water ice bath with stirring under nitrogen. To this solution was added a solution of **40** (0.40 g, 1.15 mmol) in THF (4 mL), dropwise, and the reaction was stirred at 0 °C for 4 h. The reaction was quenched with 1.0 N HCl (15 mL), the solution was extracted with ethyl acetate, and the combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated in vacuo to give an intermediate alcohol that was used without further purification. The intermediate alcohol was dissolved in toluene (30 mL), and to this solution was added *p*-toluenesulfonic acid monohydrate (0.02 g, 0.116 mmol) and the solution was refluxed into a Dean−Stark apparatus prefilled with toluene. After the solution was refluxed for 2 h, it was cooled to room temperature and poured into ethyl acetate and water. The aqueous layer was extracted with ethyl acetate, and the combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated in vacuo to give a crude product that was purified by column chromatography (25 mL SiO_2, ethyl acetate) to give **10** (0.3628 g, 91%) as a white crystalline solid (mp 210−213 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.98 (br s, 1H), 8.05 (d, J = 8.8, 2H), 7.40 (d, J = 8.4, 2H), 7.20 (s, 1H), 7.15 (s, 1H), 5.86 (d, J = 1.2, 1H), 5.54 (s, 2H), 5.38 (d, J = 1.2, 1H), 1.99 (s, 3H), 1.37 (s, 6H), 1.34 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 172.0, 149.0, 146.3, 142.0, 140.1, 138.2, 133.1, 133.1, 133.0, 130.3, 128.0, 127.7, 127.7, 126.6, 117.3, 35.0, 34.9, 32.7, 32.6, 20.0. IR (neat) 2953, 1688, 1606 cm^{-1}. LC-MS $(M + H)^+$ calcd for $C_{24}H_{27}O_2$ 347.2011, found 347.1997. Anal. Calcd for $C_{24}H_{26}O_2$: C, 83.20; H, 7.56. Found: C, 82.73; H, 7.54.

6-Bromo-1,2,3,4-tetrahydro-1,1,4,4,7-pentamethylnaphthalene (42).[16b] The method of Dawson and co-workers was used to synthesize **42**.[16b] To a solution of **22** (1.32 g, 6.52 mmol) in chloroform (6.0 mL) was added bromine (0.5 mL, 9.71 mmol) at room temperature, and the reaction was stirred for 30 min and then diluted with ethyl acetate. The solution was poured into an aqueous saturated solution of Na_2SO_3, and the biphasic mixture was shaken, the layers were separated, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and concentrated in vacuo to give an oil that was purified by column chromatography (150 mL SiO_2, ethyl acetate) to give an inseparable 2:1 mixture of **42**:**22** (1.679 g, 60% yield for **42**) as a white crystalline solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (s, 1H), 7.14 (s, 1H), 2.34 (s, 3H), 1.66 (s, 4H), 1.27 (s, 12H). GC-MS $(M)^+$ calcd for $C_{15}H_{21}Br$ 280.0827, found 280.0812.

5,6,7,8-Tetrahydro-3,5,5,8,8-pentamethylnaphthalen-2-yl-2-boronic Acid (43).[38] The method of Faul and co-workers was used.[38] To a 100 mL round-bottom flask containing THF (30 mL) was added a 1.6 M solution of *n*-BuLi in hexanes (8.0 mL, 12.8 mmol), and the resulting solution was cooled in a dry ice acetone bath to −78 °C with stirring, under nitrogen. To this solution was added a solution of the 2:1 mixture of **42**:**22** (3.3587 g, 7.88 mmol) in THF (8 mL) over 20 min and the reaction was stirred at −78 °C for 10 min and a mixture of triisopropylborate (4.9 mL, 21.3 mmol) in THF (10 mL) was added dropwise over 20 min. The reaction was stirred at −78 °C for 1 h and then warmed to room temperature and stirred for 2 h. The reaction was then quenched with 3 N HCl (35 mL), and after stirring for 2 h, it was poured into ethyl acetate, the layers were separated, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and concentrated in vacuo to give a crude product that was purified by column chromatography (150 mL SiO_2, ethyl acetate:hexanes 1:3) to give **43** (0.8838 g, 45%) as a white crystalline solid, mp 220−225 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (s, 1H), 7.21 (s, 1H), 2.82 (s, 3H), 1.72 (s, 4H), 1.34 (s, 6H), 1.33 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 149.4, 142.8, 141.4, 136.3, 128.5, 35.1, 35.0, 34.3, 33.8, 31.8, 31.5, 22.6. IR (neat) 2958, 1602, 1335 cm^{-1}.

3-Bromo-4-methylbenzaldehyde (45).[43] The method of Adams and co-workers was followed.[43] To a solution of 3-bromo-4-methylbenzoic acid (**44**) (5.08 g, 23 mmol) in THF (50 mL) stirring under nitrogen at 0 °C was added a 1 M borane·THF solution (34.6 mL, 34.6 mmol) dropwise. The reaction was warmed to room temperature, stirred for 18 h, then cooled to 0 °C and quenched by the slow addition of water (10 mL). The reaction was warmed to room temperature, and the solvents were removed in vacuo. The crude product was dissolved in ethyl acetate, washed with a 1 M aqueous sodium carbonate solution then brine, and the organic layer was dried over sodium sulfate and concentrated in vacuo to give a benzyl alcohol product (4.75 g, 100%) that was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (d, J = 0.8, 1H), 7.20 (d, J = 8.0, 1H), 7.17 (dd, J = 7.6, 1.6, 1H), 4.61 (s, 2H), 2.38 (s, 3H), 2.05 (br s, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 140.1, 137.0, 130.8, 130.7, 125.8, 124.9, 64.2, 22.5. To a solution of the benzyl alcohol intermediate (4.5 g, 22.4 mmol) in chloroform (100 mL) was added manganese dioxide (15 g, 172 mmol). The reaction was refluxed with stirring in an oil bath at 70 °C for 18 h. Then it was filtered through Celite, and solvents were removed in vacuo to give a crude product that was purified by column chromatography (150 mL SiO_2, ethyl acetate:hexanes 1:9) to give **45** (3.2974 g, 74%) as a white crystalline solid, mp 45−51 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.91 (s, 1H), 8.02 (d, J =

1.6, 1H), 7.70 (dd, J = 7.6, 1.6, 1H), 7.39 (d, J = 8.0, 1H), 2.47 (s, 3H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 190.4, 145.1, 135.8, 133.4, 131.3, 128.3, 125.6, 23.4. IR (neat) 2980, 1682, 1598 cm^{-1}. GC-MS $(M)^+$ calcd for C_8H_7OBr 197.9680, found 197.9665.

(*E*)-Ethyl 3-(3-Bromo-4-methylphenyl)acrylate (46). To a solution of a 60% dispersion of NaH in mineral oil (0.29 g, 7.25 mmol) in DME (2 mL) at −30 °C was added a solution of ethyl 2-phosphonoacetate (1.46 mL, 7.29 mmol) in DME (13 mL), and the mixture was stirred at this temperature for 30 min. To this solution was added a solution of **45** (1.32 g, 6.63 mmol) in DME (3 mL), and the reaction was stirred at −30 °C for 1.5 h and then poured into water (50 mL) and extracted with ethyl acetate. The combined organic layers were washed with an aqueous saturated NH_4Cl solution and then brine, dried over sodium sulfate, filtered, and concentrated in vacuo to give a crude product that was purified by column chromatography (150 mL SiO_2, ethyl acetate:hexanes 1:9) to give **46** (1.576 g, 88%) as a colorless crystalline solid, mp 46−47 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (d, J = 1.6, 1H), 7.57 (d, J = 16.0, 1H), 7.35 (dd, J = 8.0, 1.6, 1H), 7.23 (d, J = 7.6, 1H), 6.38, (d, J = 16.0, 1H), 4.25 (q, J = 7.2, 2H), 2.41 (s, 3H), 1.33 (t, J = 7.2, 3H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 166.7, 142.8, 140.1, 133.9, 131.6, 131.1, 126.8, 125.3, 118.6, 60.5, 22.9, 14.2. IR (neat) 2984, 1718, 1698, 1634 cm^{-1}. LC-MS $(M + H)^+$ calcd for $C_{12}H_{14}O_2Br$ 269.0177, found 269.0171.

(*E*)-Ethyl 3-(3-(1,2,3,4-Tetrahydro-1,1,4,4,6-pentamethylnaphthalen-7-yl)-4-methylphenyl)acrylate (47). To a 50 mL Schlenk flask charged with bromide **46** (0.4317 g, 1.60 mmol), boronic acid **43** (0.4010 g, 1.63 mmol), TBAB (0.52 g), Na_2CO_3 (0.51 g, 4.81 mmol), and water (3.7 mL), was added $Pd(OAc)_2$ (0.0203 g, 0.09 mmol), and the flask was evacuated and backfilled with nitrogen three times. The reaction was stirred at room temperature for 15 min and then placed in an oil bath preheated to 150 °C and stirred for 5 min. The reaction was allowed to cool to room temperature, and the black residue was taken up in ethyl acetate and water. The layers were separated, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated in vacuo to give a crude product that was purified by column chromatography (150 mL SiO_2, ethyl acetate:hexanes 1:9) to give **47** (0.5032 g, 80%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (d, J = 16.0, 1H), 7.41 (dd, J = 8.0, 2.0, 1H), 7.33 (d, J = 1.6, 1H), 7.27 (d, J = 8.0, 1H), 7.16 (s, 1H), 7.00 (s, 1H), 6.39, (d, J = 16.0, 1H), 4.25 (q, J = 7.2, 2H), 2.09 (s, 3H), 2.01 (s, 3H), 1.70 (s, 4H), 1.33 (t, J = 7.2, 3H), 1.32 (s, 6H), 1.26 (s, 3H), 1.24 (s, 3H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 171.1, 167.2, 144.6, 143.8, 143.1, 142.5, 142.1, 141.6, 139.0, 138.8, 137.7, 132.8, 132.3, 131.7, 130.3, 129.3, 127.9, 127.6, 127.4, 127.2, 126.6, 117.2, 60.4, 60.3, 35.2, 35.1, 34.0, 33.9, 32.0, 31.9, 31.8, 31.8, 21.0, 20.0, 19.8, 19.5, 14.3, 14.1. IR (neat) 2957, 1711, 1635 cm^{-1}. LC-MS $(M + H)^+$ calcd for $C_{27}H_{35}O_2$ 391.2637, found 391.2655.

(*E*)-3-(3-(1,2,3,4-Tetrahydro-1,1,4,4,6-pentamethylnaphthalen-7-yl)-4-methylphenyl)acrylic Acid (15). To a 100 mL round-bottom flask containing **47** (0.3288 g, 0.84 mmol) suspended in methanol (5.0 mL) was added a solution of KOH (0.1412 g, 2.5 mmol) in water (0.18 mL), and the solution was refluxed in an oil bath preheated to 85 °C for 1 h. The reaction was allowed to cool to room temperature and acidified with an aqueous 20% HCl solution (28 mL). The resulting precipitate was filtered and washed with copious amounts of water, and the crude white powder was purified by column chromatography (25 mL SiO_2, ethyl acetate:hexanes 15:85) to give **15** (0.2295 g, 75%) as a white crystalline solid, mp 189−196 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, J = 16.0, 1H), 7.44 (dd, J = 8.0, 1.6, 1H), 7.37 (d, J = 1.6, 1H), 7.29 (d, J = 7.6, 1H), 7.17 (s, 1H), 7.01 (s, 1H), 6.42, (d, J = 16.0, 1H), 2.11 (s, 3H), 2.02 (s, 3H), 1.71 (s, 4H), 1.33 (s, 3H), 1.32 (s, 3H), 1.27 (s, 3H), 1.24 (s, 3H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 172.6, 147.1, 143.8, 142.7, 142.1, 139.7, 137.5, 132.3, 131.4, 130.4, 129.6, 127.7, 126.9, 116.3, 35.2, 35.1, 34.0, 33.9, 32.0, 31.9, 31.8, 20.1, 19.5. IR (neat) 2957, 1680, 1627 cm^{-1}. LC-MS $(M + H)^+$ calcd for $C_{25}H_{31}O_2$ 363.2324, found 363.2311. Anal. Calcd for $C_{25}H_{30}O_2$: C, 82.83; H, 8.34. Found: C, 81.84; H, 8.28.

(*E*)-Ethyl 3-(3-Bromo-4-(trifluoromethyl)phenyl)acrylate (49). To a solution of a 60% dispersion of NaH in mineral oil (0.31 g, 7.75 mmol) in DME (2 mL) at −30 °C was added a solution of ethyl 2-phosphonoacetate (1.46 mL, 7.29 mmol) in DME (13 mL), and the mixture was stirred at this temperature for 30 min. To this solution was added a solution of 3-bromo-4-(trifluoromethyl)-benzaldehyde (**48**) (1.68 g, 6.63 mmol) in DME (3 mL), and the reaction was stirred at −30 °C for 1.5 h and then poured into water (50 mL) and extracted with ethyl acetate. The combined organic layers were washed with an aqueous saturated NH_4Cl solution and then brine, dried over sodium sulfate, filtered, and concentrated in vacuo to give a crude product that was purified by column chromatography (150 mL SiO_2, ethyl acetate:hexanes 5:95) to give **49** (2.1188 g, 98%) as a colorless crystalline solid, mp 75−76 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (s, 1H), 7.69 (d, J = 8.0, 1H), 7.59 (d, J = 16.0, 1H), 7.52 (d, J = 8.0, 1H), 6.50, (d, J = 16.0, 1H), 4.27 (q, J = 7.2, 2H), 1.34 (t, J = 7.2, 3H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 165.9, 141.0, 139.2, 133.8, 131.4, 130.7, 130.4, 128.3, 128.2, 128.1, 128.1, 126.4, 123.9, 122.2, 121.2, 120.6, 120.5, 60.9, 14.2. IR (neat) 2923, 1711, 1638, 1603 cm^{-1}. GC-MS $(M)^+$ calcd for $C_{12}H_{10}F_3O_2Br$ 321.9816, found 321.9805.

(*E*)-Ethyl 3-(4-(Trifluoromethyl)-3-(1,2,3,4-tetrahydro-1,1,4,4,6-pentamethylnaphthalen-7-yl)phenyl)acrylate (50). To a 50 mL Schlenk flask charged with bromide **49** (1.03 g, 3.20 mmol), boronic acid **43** (0.8040 g, 3.27 mmol), TBAB (1.04 g), Na_2CO_3 (1.02 g, 9.62 mmol), and water (7.4 mL) was added $Pd(OAc)_2$ (0.0406 g, 0.18 mmol), and the flask was evacuated and backfilled with nitrogen three times. The reaction was stirred at room temperature for 15 min and then placed in an oil bath preheated to 150 °C and stirred for 5 min. The reaction was allowed to cool to room temperature, and the black residue was taken up in ethyl acetate and water. The layers were separated, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated in vacuo to give a crude product that was purified by column chromatography (150 mL SiO_2, ethyl acetate:hexanes 1:9) to give **50** (1.0138 g, 71%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, J = 8.4, 1H), 7.68 (d, J = 16.0, 1H), 7.57 (d, J = 8.0, 1H), 7.45 (s, 1H), 7.13 (s, 1H), 7.02 (s, 1H), 6.51 (d, J = 16.0, 1H), 4.26 (q, J = 7.2, 2H), 1.98 (s, 3H), 1.69 (s, 4H), 1.32 (t, J = 7.2, 3H), 1.31 (s, 6H), 1.24 (s, 3H), 1.22 (s, 3H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 166.4, 144.5, 142.6, 141.9, 141.3, 137.1, 135.1, 132.3, 131.1, 130.2, 129.9, 127.6, 127.3, 126.7, 126.6, 126.3, 125.0, 122.3, 120.8, 60.7, 35.1, 35.0, 33.9, 33.8, 31.9, 31.8, 31.7, 19.7, 14.2. IR (neat) 2960, 1716, 1641 cm^{-1}. GC-MS $(M)^+$ calcd for $C_{27}H_{31}F_3O_2$ 444.2276, found 444.2253.

(*E*)-3-(4-(Trifluoromethyl)-3-(1,2,3,4-tetrahydro-1,1,4,4,6-pentamethylnaphthalen-7-yl)phenyl)acrylic Acid (16). To a 100 mL round-bottom flask containing **50** (0.4196 g, 1.00 mmol) suspended in methanol (5.0 mL) was added a solution of KOH (0.1706 g, 3.04 mmol) in water (0.22 mL), and the solution was refluxed in an oil bath preheated to 85 °C for 1 h. The reaction was allowed to cool to room temperature and acidified with an aqueous 20% HCl solution (33 mL). The resulting precipitate was filtered and washed with copious amounts of water, and the crude white powder was purified by column chromatography (25 mL SiO_2, ethyl acetate:hexanes 15:85) to give **16** (0.3445 g, 88%) as a white crystalline solid, mp 217−221 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, J = 15.6, 1H), 7.78 (d, J = 8.4, 1H), 7.61 (d, J = 8.4, 1H), 7.48 (s, 1H), 7.15 (s, 1H), 7.03 (s, 1H), 6.52, (d, J = 16.0, 1H), 2.00 (s, 3H), 1.70 (s, 4H), 1.36 (s, 6H), 1.25 (s, 3H), 1.23 (s, 3H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 171.7, 145.2, 144.6, 142.1, 141.3, 136.6, 134.9, 132.3, 131.4, 130.7, 130.4, 127.6, 127.3, 126.7, 126.6, 124.9, 122.2, 119.8, 35.1, 35.0, 34.0, 33.8, 31.9, 31.8, 31.7, 19.7. IR (neat) 2961, 1688, 1635 cm^{-1}. LC-MS $(M + H)^+$ calcd for $C_{25}H_{28}F_3O_2$ 417.2041, found 417.2068. Anal. Calcd for $C_{25}H_{27}F_3O_2$: C, 72.10; H, 6.53; F, 13.69. Found: C, 71.42; H, 6.45; F, 14.3.

5,8-Dihydro-3,5,5,8,8-pentamethylnaphthalen-2-yl-2-boronic Acid (51).[38] The method of Faul and co-workers was used.[38] To a 100 mL round-bottom flask containing THF (20 mL) was added a 1.6 M solution of *n*-BuLi in hexanes (4.94 mL, 7.90 mmol), and the

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resulting solution was cooled in a dry ice acetone bath to −78 °C with stirring, under nitrogen. To this solution was added a solution of **36** (2.1244 g, 7.61 mmol) in THF (5 mL) over 20 min and the reaction was stirred at −78 °C for 10 min and a mixture of triisopropylborate (3.5 mL, 15.2 mmol) in THF (5 mL) was added dropwise over 20 min. The reaction was stirred at −78 °C for 2 h and then warmed to room temperature and stirred for 1 h. The reaction was then quenched with 3 N HCl (30 mL), and after stirring for 30 min, it was poured into ethyl acetate, the layers were separated, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and concentrated in vacuo to give a crude product that was purified by column chromatography (150 mL SiO_2, ethyl acetate:hexanes 1:3) to give **51** (1.1639 g, 62%) as a white crystalline solid, mp 162−166 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (s, 1H), 7.28 (s, 1H), 5.56 (s, 2H), 2.87 (s, 3H), 1.41 (s, 6H), 1.39 (s, 6H). IR (neat) 3213 (br), 2958, 1605 cm^{-1}.

(2*E*)-Ethyl 3-(3-(1,4-Dihydro-1,1,4,4,6-pentamethylnaphthalen-7-yl)-4-methylphenyl)acrylate (52). To a 50 mL Schlenk flask charged with bromide **46** (0.6506 g, 2.41 mmol), boronic acid **51** (0.2032 g, 0.83 mmol), TBAB (0.26 g), Na_2CO_3 (0.256 g, 2.42 mmol), and water (1.85 mL) was added $Pd(OAc)_2$ (0.0136 g, 0.061 mmol), and the flask was evacuated and backfilled with nitrogen three times. The reaction was stirred at room temperature for 15 min and then placed in an oil bath preheated to 150 °C and stirred for 5 min. The reaction was allowed to cool to room temperature, and the black residue was taken up in ethyl acetate and water. The layers were separated, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated in vacuo to give a crude product that was purified by column chromatography (150 mL SiO_2, ethyl acetate:hexanes 2.5:97.5) to give **52** (0.2256 g, 72%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (d, J = 16.0, 1H), 7.42 (dd, J = 8.0, 2.0, 1H), 7.36 (d, J = 2.0, 1H), 7.29 (d, J = 8.0, 1H), 7.22 (s, 1H), 7.06 (s, 1H), 6.41, (d, J = 16.0, 1H), 5.53 (s, 2H), 4.25 (q, J = 7.2, 2H), 2.10 (s, 3H), 2.04 (s, 3H), 1.38 (s, 6H), 1.33 (t, J = 7.2, 3H), 1.32 (s, 3H), 1.30 (s, 3H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 167.8, 144.5, 142.4, 141.5, 139.8, 139.0, 138.1, 133.1, 133.0, 132.7, 131.8, 130.4, 129.3, 127.3, 126.8, 126.6, 117.3, 60.3, 35.0, 34.9, 32.7, 32.6, 32.6, 20.0, 19.6, 14.3, 14.1. IR (neat) 2948, 1698, 1634 cm^{-1}. GC-MS (M)$^+$ calcd for $C_{27}H_{32}O_2$ 388.2402, found 388.2414.

(2*E*)-3-(3-(1,4-Dihydro-1,1,4,4,6-pentamethylnaphthalen-7-yl)-4-methylphenyl)acrylic Acid (17). To a 100 mL round-bottom flask containing **52** (0.5555 g, 1.43 mmol) suspended in methanol (5.0 mL) was added a solution of KOH (0.2454 g, 4.37 mmol) in water (0.30 mL), and the solution was refluxed in an oil bath preheated to 85 °C for 1 h. The reaction was allowed to cool to room temperature and acidified with an aqueous 20% HCl solution (50 mL). The resulting precipitate was filtered and washed with copious amounts of water, and the crude white powder was purified by column chromatography (25 mL SiO_2, ethyl acetate:hexanes 15:85 to 2:3) to give **17** (0.3917 g, 76%) as a white crystalline solid, mp 183−185 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, J = 15.6, 1H), 7.46 (dd, J = 8.0, 2.0, 1H), 7.39 (d, J = 1.6, 1H), 7.31 (d, J = 8.0, 1H), 7.24 (s, 1H), 7.07 (s, 1H), 6.43, (d, J = 16.0, 1H), 5.54 (s, 2H), 2.12 (s, 3H), 2.05 (s, 3H), 1.39 (s, 6H), 1.33 (s, 3H), 1.31 (s, 3H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 172.6, 147.8, 142.5, 141.6, 139.9, 137.9, 133.1, 133.0, 132.7, 131.4, 130.5, 129.6, 127.4, 127.0, 126.8, 116.3, 35.0, 34.9, 32.7, 32.6, 32.6, 20.1, 19.6. IR (neat) 2956, 1689, 1624 cm^{-1}. GC-MS (M)$^+$ calcd for $C_{25}H_{28}O_2$ 360.2089, found 360.2089. Anal. Calcd for $C_{25}H_{27}O_2$: C, 83.29; H, 7.83. Found: C, 82.75; H, 7.83.

2-Bromo-4-formylphenyl Acetate (54).[39] The method of Gronemeyer and co-workers was followed.[39] To a 100 mL round-bottom flask charged with 3-bromo-4-hydroxybenzaldehyde (2.018 g, 10.0 mmol) was added DMAP (0.066 g, 0.54 mmol) and acetic anhydride (11.0 mL, 116 mmol), a reflux condenser was appended, the apparatus was evacuated and backfilled with nitrogen, and the solution was heated to 135 °C in a preheated oil bath for 8 min. The reaction was cooled to room temperature and then poured into water and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated in vacuo to give a crude product that was purified by column chromatography (150 mL SiO_2, ethyl acetate:hexanes 1:9) to give **54** (2.44 g, 90%) as a white crystalline solid, mp 58−60 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.94 (s, 1H), 8.13 (d, J = 2.0, 1H), 7.85 (dd, J = 8.4, 2.0, 1H), 7.31 (d, J = 8.4, 1H), 2.38 (s, 3H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 189.5, 167.8, 152.8, 135.2, 134.5, 129.8, 124.5, 117.4, 20.7. IR (neat) 2944, 1743, 1697, 1686 cm^{-1}. GC-MS (M)$^+$ calcd for $C_9H_7O_3Br$ 241.9579, found 241.9574.

(*E*)-Ethyl 3-(4-Acetoxy-3-bromophenyl)acrylate (55).[39] The method of Gronemeyer and co-workers was followed.[39] To a solution of a 60% dispersion of NaH in mineral oil (0.31 g, 7.75 mmol) in DME (2 mL) at −30 °C was added a solution of ethyl 2-phosphonoacetate (1.46 mL, 7.29 mmol) in DME (13 mL), and the mixture was stirred at this temperature for 30 min. To this solution was added a solution of **54** (1.61 g, 6.62 mmol) in DME (3 mL), and the reaction was stirred at −30 °C for 1.5 h and then poured into water (50 mL) and extracted with ethyl acetate. The combined organic layers were washed with an aqueous saturated NH_4Cl solution and then brine, dried over sodium sulfate, filtered, and concentrated in vacuo to give a crude product that was purified by column chromatography (150 mL SiO_2, ethyl acetate:hexanes 1:9) to give **55** (1.7598 g, 84%) as a colorless crystalline solid, mp 71−72 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, J = 2.0, 1H), 7.58 (d, J = 16.0, 1H), 7.46 (dd, J = 8.0, 2.0, 1H), 7.14 (d, J = 8.4, 1H), 6.38, (d, J = 16.0, 1H), 4.25 (q, J = 7.2, 2H), 2.36 (s, 3H), 1.33 (t, J = 7.2, 3H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 168.2, 166.4, 149.3, 141.9, 133.9, 132.5, 127.9, 124.0, 119.8, 116.8, 60.6, 20.7, 14.2. IR (neat) 2981, 1758, 1715 cm^{-1}. GC-MS (M)$^+$ calcd for $C_{13}H_{13}O_2Br$ 311.9997, found 311.9988.

(2*E*)-Ethyl-3-(4-acetoxy-3-(1,4-dihydro-1,1,4,4,6-pentamethylnaphthalen-7-yl)phenyl)acrylate (56). To a 50 mL Schlenk flask charged with bromide **55** (0.5038 g, 1.60 mmol), boronic acid **51** (0.4040 g, 1.65 mmol), TBAB (0.52 g), Na_2CO_3 (0.51 g, 4.81 mmol), and water (3.70 mL) was added $Pd(OAc)_2$ (0.0277 g, 0.123 mmol), and the flask was evacuated and backfilled with nitrogen three times. The reaction was stirred at room temperature for 15 min and then placed in an oil bath preheated to 150 °C and stirred for 5 min. The reaction was allowed to cool to room temperature, and the black residue was taken up in ethyl acetate and water. The layers were separated, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated in vacuo to give a crude product that was purified by column chromatography (150 mL SiO_2, ethyl acetate:hexanes 5:95 to 3:7) to give **56** (0.3673 g, 54%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (d, J = 16.0, 1H), 7.54 (dd, J = 8.0, 2.0, 1H), 7.50 (d, J = 2.0, 1H), 7.23 (s, 1H), 7.17 (d, J = 8.4, 1H), 7.10 (s, 1H), 6.41, (d, J = 16.0, 1H), 5.52 (s, 2H), 4.25 (q, J = 7.2, 2H), 2.13 (s, 3H), 1.91 (s, 3H), 1.36 (s, 6H), 1.33 (t, J = 7.2, 3H), 1.30 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 169.1, 166.8, 149.9, 143.5, 142.1, 139.6, 135.6, 133.5, 133.3, 133.0, 132.9, 132.2, 131.0, 127.8, 127.6, 127.5, 123.1, 118.5, 60.5, 35.0, 34.9, 32.6, 20.4, 19.6, 14.2. IR (neat) 3317, 2970, 1680, 1632 cm^{-1}. LC-MS (M + H)$^+$ calcd for $C_{28}H_{33}O_4$ 433.2379, found 433.2371.

(2*E*)-3-(3-(1,4-Dihydro-1,1,4,4,6-pentamethylnaphthalen-7-yl)-4-hydroxyphenyl)acrylic Acid (18). To a 100 mL round-bottom flask containing **56** (0.3622 g, 0.87 mmol) suspended in methanol (5.0 mL) was added a solution of KOH (0.3326 g, 5.93 mmol) in water (0.48 mL), and the solution was refluxed in an oil bath preheated to 85 °C for 1 h. The reaction was allowed to cool to room temperature and acidified with an aqueous 20% HCl solution (50 mL). The resulting precipitate was filtered and washed with copious amounts of water, and the crude white powder was purified by column chromatography (25 mL SiO_2, ethyl acetate:hexanes 10:90 to 2:5) to give **18** (0.2820 g, 89%) as a white crystalline solid, mp 163−168 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, J = 15.6, 1H), 7.51 (dd, J = 8.4, 2.0, 1H), 7.39 (d, J = 2.4, 1H), 7.39 (s, 1H), 7.21 (s, 1H), 7.03 (d, J = 8.4, 1H), 6.34, (d, J = 16.0, 1H), 5.54 (s, 2H), 2.15 (s, 3H), 1.39 (s, 6H), 1.33 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 172.6, 155.2, 146.7, 143.4, 141.2, 134.2, 132.9, 132.8, 132.0, 130.8, 129.5, 128.6, 128.5, 128.0, 126.6, 115.9, 114.7, 60.4, 35.1, 35.0, 32.6, 32.5, 21.0, 19.4, 14.1. IR (neat) 2959, 1682, 1629 cm^{-1}. LC-MS (M + H)$^+$ calcd for

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$C_{24}H_{27}O_3$ 363.1960, found 363.1967. Anal. Calcd for $C_{24}H_{26}O_3$: C, 79.53; H, 7.23. Found: C, 78.33; H, 7.14.

1-(4-(Methoxycarbonyl)-3-fluorophenyl)vinyl Trifluoromethanesulfonate (58). Following a procedure similar to that used by Faul and co-workers,[38] to a solution of methyl 4-acetyl-2-fluorobenzoate (**57**) (1.972 g, 10.05 mmol) in dichloromethane (15.0 mL) was added finely ground anhydrous Na_2CO_3 (1.71 g, 16.1 mmol) followed by trifluoromethanesulfonic anhydride (3.4 mL, 20 mmol). The reaction was stirred under nitrogen for 24 h, at which point an additional amount of finely ground anhydrous Na_2CO_3 (0.42 g, 4.0 mmol) followed by trifluoromethanesulfonic anhydride (1.7 mL, 10 mmol) was added, and the reaction was stirred for an additional 48 h. The mixture was filtered, concentrated in vacuo to an oil, and loaded directly onto a silica gel column (2.5% ethyl acetate in hexanes). The product-containing fractions were combined to give **58** as a crystalline solid (0.9011 g, 27%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (t, J = 8.0, 1H), 7.38 (dd, J = 8.0, 1.6, 1H), 7.31 (dd, J = 11.2, 1.6, 1H), 5.76 (d, J = 4.0, 1H), 5.55 (d, J = 4.4, 1H), 3.94 (s, 3H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 163.9, 163.9, 163.1, 160.5, 150.9, 150.9, 137.9, 137.8, 132.9, 120.6, 120.5, 120.0, 119.9, 116.8, 114.0, 113.8, 107.4, 52.6. IR (neat) 2970, 1691, 1414 cm^{-1}. LC-MS $(M)^+$ calcd for $C_{11}H_9F_4O_5S$ 329.0107, found 329.0106.

Methyl 2-Fluoro-4-(1-(1,4-dihydro-1,1,4,4,6-pentamethylnaphthalen-7-yl)vinyl)benzoate (59). A solution of boronic acid (**51**) (0.294 g, 1.20 mmol), triflate **58** (0.429 g, 1.31 mmol), $Pd(OAc)_2$ (0.014 g, 0.062 mmol), P(*o*-Tol)$_3$ (0.030 g, 0.099 mmol), and Et_3N (0.34 mL, 2.4 mmol) in DMF (4.0 mL) was heated to 50 °C and stirred for 2 h. After cooling to room temperature, the reaction was poured into water and extracted with ethyl acetate. The combined organic layers were washed with water and brine and then dried over sodium sulfate, filtered, and concentrated in vacuo to give a crude oil that was purified by column chromatography (silica gel, 2.5% ethyl acetate in hexanes) to give **59** as a white, crystalline solid: (0.252 g, 55%), mp 124−126 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (t, J = 8.0, 1H). 7.17−7.14 (m, 3H), 7.03 (dd, J = 12.4, 1.6, 1H), 5.85 (d, J = 1.2, 1H), 5.53 (s, 2H), 5.39 (d, J = 1.2, 1H), 3.92 (s, 3H), 1.98 (s, 3H), 1.37 (s, 6H), 1.34 (s, 3H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 164.7, 164.7, 163.3, 160.7, 148.0, 147.9, 147.7, 147.6, 142.3, 140.2, 137.6, 133.0, 133.0, 1132.0, 127.8, 127.6, 122.0, 122.0, 117.8, 117.1, 117.0, 114.9, 114.7, 52.2, 35.0, 34.9, 32.6, 32.6, 19.9. IR (neat) 2952, 1712, 1618 cm^{-1}. LC-MS $(M + H)^+$ calcd for $C_{25}H_{28}FO_2$ 379.2073, found 379.2069.

2-Fluoro-4-(1-(1,4-dihydro-1,1,4,4,6-pentamethylnaphthalen-7-yl)vinyl)benzoic Acid (19). To a suspension of **59** (0.2475 g, 0.65 mmol) in methanol (3.4 mL) was added a solution of KOH (0.1009 g) in water (0.18 mL), and the reaction was refluxed at 85 °C for 1 h. The reaction solution was cooled to room temperature and quenched with 1N HCl (50 mL). The crude precipitate was filtered and dried to give a crude white product (0.2135 g, 89%) that was purified by column chromatography (silica gel, 10−30% ethyl acetate in hexanes) to give **19** as a crystalline solid (0.1664 g, 70%), mp 187−188 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.29 (br s, 1H), 7.97 (t, J = 8.0, 1H), 7.20−7.16 (m, 3H), 7.07 (dd, J = 12.4, 1.2, 1H), 5.88 (d, J = 0.8, 1H), 5.54 (s, 2H), 5.42 (d, J = 0.8, 1H), 2.00 (s, 3H), 1.37 (s, 6H), 1.34 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 169.5, 169.4, 164.0, 161.4, 148.8, 148.7, 147.9, 142.4, 140.2, 137.5, 133.0, 133.0, 132.7, 127.8, 127.6, 122.1, 122.1, 118.3, 116.0, 115.9, 115.1, 114.8, 35.0, 34.9, 32.6, 32.6, 19.9. IR (neat) 2958, 1701, 1617 cm^{-1}. LC-MS $(M + H)^+$ calcd for $C_{24}H_{26}O_2F$ 365.1917, found 365.1923. Anal. Calcd for $C_{24}H_{25}O_2F$: C, 79.09; H, 6.91; F, 5.21. Found: C, 78.74; H, 6.76; F, 5.10.

***(E)*-Ethyl 3-(3-Bromophenyl)acrylate (61).** To a solution of a 60% dispersion of NaH in mineral oil (0.31 g, 7.75 mmol) in DME (2 mL) at −30 °C was added a solution of ethyl 2-phosphonoacetate (1.46 mL, 7.29 mmol) in DME (13 mL), and the mixture was stirred at this temperature for 30 min. To this solution was added a solution of 3-bromobenzaldehyde (**60**) (1.23 g, 6.65 mmol) in DME (3 mL), and the reaction was stirred at −30 °C for 1.5 h and then poured into water (50 mL) and extracted with ethyl acetate. The combined organic layers were washed with an aqueous saturated NH_4Cl solution and then brine, dried over sodium sulfate, filtered, and concentrated in vacuo to give a crude product that was purified by column chromatography (150 mL SiO_2, ethyl acetate:hexanes 5:95) to give **61** (1.6796 g, 98%) as a colorless crystalline solid, mp 34−36 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (t, J = 1.6 Hz, 1H), 7.59 (d, J = 16.0, 1H), 7.49 (dq, J = 7.6, 0.8 Hz, 1H), 7.42 (d, J = 7.6, 0.8 Hz, 1H), 7.25 (d, J = 8.0 Hz, 1H), 6.42 (d, J = 16.0, 1H), 4.27 (q, J = 7.2, 2H), 1.33 (t, J = 7.2, 3H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 166.5, 142.8, 136.5, 132.9, 130.6, 130.3, 126.6, 122.9, 119.7, 60.6, 14.2. IR (neat) 2978, 1708, 1640 cm^{-1}. GC-MS $(M)^+$ calcd for $C_{11}H_{11}BrO_2$ 253.9942, found 253.9946.

***(E)*-Ethyl 3-(3-(1,2,3,4-Tetrahydro-1,1,4,4,6-pentamethylnaphthalen-7-yl)phenyl)acrylate (62).** To a 50 mL Schlenk flask charged with bromide **61** (0.4125 g, 1.61 mmol), boronic acid **43** (0.4020 g, 1.64 mmol), TBAB (0.52 g), Na_2CO_3 (0.52 g, 4.81 mmol), and water (3.7 mL), was added $Pd(OAc)_2$ (0.0203 g, 0.09 mmol), and the flask was evacuated and backfilled with nitrogen three times. The reaction was stirred at room temperature for 15 min and then placed in an oil bath preheated to 150 °C and stirred for 5 min. The reaction was allowed to cool to room temperature, and the black residue was taken up in ethyl acetate and water. The layers were separated, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated in vacuo to give a crude product that was purified by column chromatography (150 mL SiO_2, ethyl acetate:hexanes 1:9) to give **62** (0.4295 g, 70%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (d, J = 16.0, 1H), 7.51−7.49 (m, 2H), 7.45−7.36 (m, 2H), 7.21 (s, 1H), 7.16 (s, 1H), 6.46 (d, J = 16.0, 1H), 4.27 (q, J = 7.2, 2H), 2.24 (s, 3H), 1.71 (s, 4H), 1.36−1.26 (m, 15H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 167.0, 144.6, 144.2, 142.8, 142.5, 138.3, 134.2, 132.1, 131.2, 129.0, 128.5, 128.3, 127.8, 126.1, 118.3, 60.5, 35.1, 33.9, 33.9, 31.9, 31.8, 21.0, 20.1, 14.3, 14.1. IR (neat) 2957, 1711, 1637 cm^{-1}. GC-MS $(M)^+$ calcd for $C_{26}H_{32}O_2$ 376.2402, found 376.2390.

***(E)*-3-(3-(1,2,3,4-Tetrahydro-1,1,4,4,6-pentamethylnaphthalen-7-yl)phenyl)acrylic Acid (12).** To a 100 mL round-bottom flask containing **62** (0.400 g, 1.06 mmol) suspended in methanol (5.0 mL) was added a solution of KOH (0.2002 g, 3.57 mmol) in water (0.26 mL), and the solution was refluxed in an oil bath preheated to 85 °C for 1 h. The reaction was allowed to cool to room temperature and acidified with an aqueous 20% HCl solution (30 mL). The resulting precipitate was filtered and washed with copious amounts of water, and the crude white powder was purified by column chromatography (25 mL SiO_2, ethyl acetate:hexanes 15:85) to give **12** (0.3119 g, 84%) as a white crystalline solid, 205−207 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (d, J = 16.0 Hz, 1H), 7.54−7.53 (m, 2H), 7.46 (t, J = 7.6, 1H), 7.41 (dt, J = 8.4, 1.6 Hz, 1H), 7.22 (s, 1H), 7.17 (s, 1H), 6.49, (d, J = 16.0, 1H), 2.25 (s, 3H), 1.73 (s, 4H), 1.33 (s, 6H), 1.31 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 172.2, 147.2, 144.3, 143.0, 142.6, 138.1, 133.8, 132.1, 131.8, 129.3, 128.6, 128.4, 127.8, 126.5, 117.3, 35.1, 34.0, 33.9, 31.9, 31.8, 20.1. IR (neat) 2955, 1706, 1630 cm^{-1}. LC-MS $(M + H)^+$ calcd for $C_{24}H_{29}O_2$ 349.2168, found 349.2168. Anal. Calcd for $C_{24}H_{28}O_2$: C, 82.72; H, 8.10. Found: C, 82.05; H, 7.94.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H NMR and ^{13}C NMR spectra of all compounds reported in the experimentals and dose−response curves for all novel and reported rexinoids. The X-ray data for compound **14** (CCDC 942622) can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare the following competing financial interest(s): Patent applications covering the technologies described in this work have been applied for on behalf of the Arizona Board of Regents.

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■ ABBREVIATIONS USED

RXR, retinoid X receptor; RAR, retinoic acid receptor; CTCL, cutaneous T-cell lymphoma; RXRE, retinoid X receptor element; HRE, hormone responsive element; TR, thyroid hormone receptor; VDR, vitamin D receptor; SNuRMs, specific nuclear receptor modulators; NR, nuclear receptor; LXR, liver X receptor; PPAR, peroxisome proliferator activating receptor; LBD, ligand binding domain; NaBu, sodium butyrate

■ REFERENCES

(1) (a) Mangelsdorf, D. J.; Umesono, K.; Evans, R. M. The Retinoid Receptors. In *The Retinoids*; Academic Press: Orlando, FL, 1994; pp 319−349. (b) Leid, M.; Kastner, P.; Chambon, P. Multiplicity Generates Diversity in the Retinoic Acid Signaling Pathways. *Trends Biochem. Sci.* **1992**, *17*, 427−433.

(2) Olefsky, J. M. Nuclear Receptor Minireview Series. *J. Biol. Chem.* **2001**, *276*, 36863−36864.

(3) Forman, B. M.; Yang, C.-R.; Au, M.; Casanova, J.; Ghysdael, J.; Samuels, H. H. A domain containing leucine-zipper-like motifs mediates novel in vitro interactions between the thyroid hormone and retinoic acid receptors. *Mol. Endocrinol.* **1989**, *3*, 1610−1626.

(4) Mangelsdorf, D. J.; Evans, R. M. The RXR heterodimers and orphan receptors. *Cell* **1995**, *83*, 841−850.

(5) Thompson, P. D.; Remus, L. S.; Hsieh, J.-C.; Jurutka, P. W.; Whitfield, G. K.; Galligan, M. A.; Encinas Dominguez, C.; Haussler, C. A.; Haussler, M. R. Distinct retinoid X receptor activation function-2 residues mediate transactivation in homodimeric and vitamin D receptor heterodimeric contexts. *J. Mol. Endocrinol.* **2001**, *27*, 211−227.

(6) Svensson, S.; Ostberg, T.; Jacobsson, M.; Norstrom, C.; Stefansson, K.; Hallen, D.; Johansson, I. C.; Zachrisson, K.; Ogg, D.; Jendeberg, L. Crystal structure of the heterodimeric complex of LXRα and RXRβ ligand-binding domains in a fully agonistic conformation. *EMBO J.* **2003**, *22*, 4625−4633.

(7) Nahoum, V.; Perez, E.; Germain, P.; Rodriquez-Barrios, F.; Manzo, F.; Kammerer, S.; Lemaire, G.; Hirsch, O.; Royer, C. A.; Gronemeyer, H.; de Lera, A. R.; Bourguet, W. Modulators of the structural dynamics of the retinoid X receptor to reveal receptor function. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 17323−17328.

(8) Forman, B. M.; Umesono, K.; Chen, J.; Evans, R. M. Unique response pathways are established by allosteric interactions among nuclear hormone receptors. *Cell* **1995**, *81*, 541−550.

(9) Lala, D. S.; Mukherjee, R.; Schulman, I. G.; Canan Koch, S. S.; Dardashti, L. J.; Nadzan, A. M.; Croston, G. E.; Evans, R. M.; Heyman, R. A. Activation of specific RXR heterodimers by an antagonist of RXR homodimers. *Nature* **1996**, *383*, 450−453.

(10) (a) Lemon, B. D.; Freedman, L. P. Selective effects of ligands on vitamin D3 receptor- and retinoid X receptor-mediated gene activation in vivo. *Mol. Cell. Biol.* **1996**, *16*, 1006−1016. (b) MacDonald, P. N.; Dowd, D. R.; Nakajima, S.; Galligan, M. A.; Reeder, M. C.; Haussler, C. A.; Ozato, K.; Haussler, M. R. Retinoid X receptors stimulate and 9-*cis* retinoic acid inhibits 1,25-dihydroxyvitamin D3-activated expression of the rat osteocalcin gene. *Mol. Cell. Biol.* **1993**, *13*, 5907−5917. (c) Thompson, P. D.; Jurutka, P. W.; Haussler, C. A.; Whitfield, G. K.; Haussler, M. R. Heterodimeric DNA binding by the vitamin D receptor and retinoid X receptors is enhanced by 1,25-dihydroxyvitamin D3 and inhibited by 9-*cis* retinoic acid: evidence for allosteric receptor interactions. *J. Biol. Chem.* **1998**, *273*, 8483−8491.

(11) Lehmann, J. M.; Zhang, X. K.; Graupner, G.; Lee, M. O.; Hermann, T.; Hoffmann, B.; Pfahl, M. Formation of Retinoid X Receptor Homodimers Leads to Repression of T3 Response: Hormonal Cross Talk by Ligand-Induced Squelching. *Mol. Cell. Biol.* **1993**, *13*, 7698−7707.

(12) (a) Raval-Pandya, M.; Freedman, L. P.; Li, H.; Christakos, S. Thyroid Hormone Receptor Does Not Heterodimerize with the Vitamin D Receptor but Represses Vitamin D Receptor-Mediated Transactivation. *Mol. Endocrinol.* **1998**, *12*, 1367−1379. (b) Thompson, P. D.; Hsieh, J.-C.; Whitfield, G. K.; Haussler, C. A.; Jurutka, P. W.; Galligan, M. A.; Tillman, J. B.; Spindler, S. R.; Haussler, M. R. Vitamin D receptor displays DNA binding and transactivation as a heterodimer with the retinoid X receptor, but not with the thyroid hormone receptor. *J. Cell. Biochem.* **1999**, *75*, 462−480.

(13) Yen, P. M.; Liu, Y.; Sugawara, A.; Chin, W. W. Vitamin D Receptors Repress Basal Transcription and Exert Dominant Negative Activity on Triiodothyronine-Mediated Transcriptional Activity. *J. Biol. Chem.* **1996**, *271*, 10910−10916.

(14) Altucci, L.; Leibowitz, M. D.; Ogilvie, K. M.; de Lera, A. R.; Gronemeyer, H. RAR and RXR modulation in cancer and metabolic disease. *Nature Rev. Drug Discovery* **2007**, *6*, 793−810.

(15) 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 pathways. *Science* **1992**, *258*, 1944−1946.

(16) (a) Jong, L.; Lehmann, J. M.; Hobbs, P. D.; Harlev, E.; Huffman, J. C.; Pfahl, M.; Dawson, M. I. Conformational effects on retinoid receptor selectivity. 1. Effect of 9-double bond geometry on retinoid X receptor activity. *J. Med. Chem.* **1993**, *36*, 2605−2613. (b) Dawson, M. I.; Jong, L.; Hobbs, P. D.; Cameron, J. F.; Chao, W.-R.; Pfahl, M.; Lee, M.-O.; Shroot, B.; Pfahl, M. Conformational Effects on Retinoid Receptor Selectivity. 2. Effects of Retinoid Bridging Group on Retinoid X Receptor Activity and Selectivity. *J. Med. Chem.* **1995**, *38*, 3368−3383.

(17) 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 Retinoid X Receptor-Selective Retinoids. *J. Med. Chem.* **1994**, *37*, 2930−2941.

(18) Daiss, J. O.; Burschka, C.; Mills, J. S.; Montana, J. G.; Showell, G. A.; Fleming, I.; Gaudon, C.; Ivanova, D.; Gronemeyer, H.; Tacke, R. Synthesis, Crystal Structure Analysis, and Pharmacological Characterization of Disila-bexarotene, a Disila-Analogue of the RXR-Selective Retinoid Agonist Bexarotene. *Organometallics* **2005**, *24*, 3192−3199.

(19) Cesario, R. M.; Stone, J.; Yen, W.-C.; Bissonnette, R. P.; Lamph, W. W. Differentiation and growth inhibition mediated via the RXR:PPAR$_\gamma$ heterodimer in colon cancer. *Cancer Lett.* **2006**, *240*, 225−233.

(20) Yen, W. -C.; Prudente, R. Y.; Lamph, W. W. Synergistic effect of a retinoid X receptor-selective ligand bexarotene (LGD1069, Targretin) and paclitaxel (Taxol) in mammary carcinoma. *Breast Cancer Res. Treat.* **2004**, *88*, 141−148.

(21) (a) Yen, W.-C.; Corpuz, M. R.; Prudente, R. Y.; Cooke, T. A.; Bissonnette, R. P.; Negro-Vilar, A.; Lamph, W. W. A Selective Retinoid X Receptor Agonist Bexarotene (Targretin) Prevents and Overcomes Acquired Paclitaxel (Taxol) Resistance in Human Non-Small Cell Lung Cancer. *Clin. Cancer Res.* **2004**, *10*, 8656−8664. (b) Dragnev, K. H.; Petty, W. J.; Shah, S. J.; Lewis, L. D.; Black, C. C.; Memoli, V.;

Nugent, W. C.; Hermann, T.; Negro-Vilar, A.; Rigas, J. R.; Dmitrovsky, E. A Proof-of-Principle Clinical Trial of Bexarotene in Patients with Non-Small Cell Lung Cancer. *Clin. Cancer Res.* **2007**, *13*, 1794−1800.
(22) Mukherjee, R.; Davies, P. J. A.; Crombie, D. L.; Bischoff, E. D.; Cesario, R. M.; Jow, L.; Hamanns, L. G.; Boehm, M. F.; Mondon, C. E.; Nadzan, A. M.; Paterniti, J. R.; Heyman, R. A. Sensitization of diabetic and obese mice to insulin by retinoid X receptor agonists. *Nature* **1997**, *386*, 407−410.
(23) Sherman, S. I.; Gopal, J.; Haugen, B. R.; Chiu, A. C.; Whaley, K.; Nowlakha, P.; Duvic, M. Central hypothyroidism associated with retinoid X receptor-selective ligands. *N. Engl. J. Med.* **1999**, *340*, 1075−1079.
(24) Li, D.; Li, T.; Wang, F.; Tian, H.; Samuels, H. H. Functional Evidence for Retinoid X Receptor (RXR) as a Nonsilent Partner in the Thyroid Hormone Receptor/RXR Heterodimer. *Mol. Cell. Biol.* **2002**, *22*, 5782−5792.
(25) (a) Murthy, S.; Born, E.; Mathur, S. N.; Field, F. J. LXR/RXR activation enhances basolateral efflux of cholesterol in CaCo-2 cells. *J. Lipid Res.* **2002**, *43*, 1054−1064. (b) Field, F. J.; Born, E.; Mathur, S. N. LXR/RXR ligand activation enhances basolateral efflux of beta-sitosterol in CaCo-2 cells. *J. Lipid Res.* **2004**, *45*, 905−913.
(26) Thacher, S. M.; Standeven, A. M.; Athanikar, J.; Kopper, S.; Castilleja, O.; Escobar, M.; Beard, R. L.; Chandraratna, R. A. S. Receptor Specificity of Retinoid-Induced Epidermal Hyperplasia: Effect of RXR-Selective Agonists and Correlation with Topical Irritation. *JPET* **1997**, *282*, 528−534.
(27) (a) Cramer, P. E.; Cirrito, J. R.; Wesson, D. W.; Lee, C. Y. D.; Karlo, J. C.; Zinn, A. E.; Casali, B. T.; Restivo, J. L.; Goebel, D. A.; James, M. J.; Brunden, K. R.; Wilson, D. A.; Landreth, G. E. ApoE-Directed Therapeutics Rapidly Clear β-Amyloid and Reverse Deficits in AD Mouse Models. *Science* **2012**, *335*, 1503−1506. (b) Landreth, G. E.; Cramer, P. E.; Lakner, M. M.; Cirrito, J. R.; Wesson, D. W.; Brunden, K. R.; Wilson, D. A. Response to Comments on 'ApoE-Directed Therapeutics Rapidly Clear β-Amyloid and Reverse Deficits in AD Mouse Models.'. *Science* **2013**, *340*, 924.
(28) Vuligonda, V.; Thacher, S. M.; Chandraratna, R. A. S. Enantioselective Synthesis of Potent Retinoid X Receptor Ligands: Differential Biological Activities of Individual Antipodes. *J. Med. Chem.* **2001**, *44*, 2298−2303.
(29) Farmer, L. J.; Marron, K. S.; Canan Koch, S. S.; Hwang, C. K.; Kallel, E. A.; Zhi, L.; Nadzan, A. M.; Robertson, D. W.; Bennani, Y. L. Aza-retinoids as novel retinoid X receptor-specific agonists. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2352−2356.
(30) Kagechika, H.; Kawachi, E.; Hashimoto, Y.; Himi, T.; Shudo, K. Retinobenzoic Acids. 1. Structure−Activity Relationships of Aromatic Amides with Retinoidal Activity. *J. Med. Chem.* **1988**, *31*, 2182−2192.
(31) Michellys, P. Y.; Ardecky, R. J.; Chen, J. H.; Crombie, D. L.; Etgen, G. J.; Faul, M. M.; Faulkner, A. L.; Grese, T. A.; Heyman, R. A.; Karanewsky, D. S.; Klausing, K.; Leibowitz, M. D.; Liu, S.; Mais, D. A.; Mapes, C. M.; Marschke, K. B.; Reifel-Miller, A.; Ogilvie, K. M.; Rungta, D.; Thompson, A. W.; Tyhonas, J. S.; Boehm, M. F. Novel (2*E*,4*E*,6*Z*)-7-(2-Alkoxy-3,5-dialkylbenzene)-3-methylocta-2,4,6-trienoic Acid Retinoid X Receptor Modulators are Active in Models of Type 2 Diabetes. *J. Med. Chem.* **2003**, *46*, 2683−2696.
(32) Michellys, P.-Y.; Ardecky, R. J.; Chen, J.-H.; D'Arrigo, J.; Grese, T. A.; Karanewsky, D. S.; Leibowitz, M. D.; Liu, S.; Mais, D. A.; Mapes, C. M.; Montrose-Rafizadeh, C.; Ogilvie, K. M.; Reifel-Miller, A.; Rungta, D.; Thompson, A. W.; Tyhonas, J. S.; Boehm, M. F. Design, Synthesis, and Structure−Activity Relationship Studies of Novel 6,7-Locked-[7-(2-alkoxy-3,5-dialkylbenzene)-3-methylocta]-2,4,6-trienoic Acids. *J. Med. Chem.* **2003**, *46*, 4087−4103.
(33) Michellys, P.-Y.; Darrigo, J.; Grese, T. A.; Karanewsky, D. S.; Leibowitz, M. D.; Mais, D. A.; Mapes, C. M.; Reifel-Miller, A.; Rungta, D.; Boehm, M. F. Design, synthesis and structure−activity relationship of novel RXR-selective modulators. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1593−1598.
(34) Wagner, C. E.; Jurutka, P. W.; Marshall, P. A.; Groy, T. L.; van der Vaart, A.; Ziller, J. W.; Furmick, J. K.; Graeber, M. E.; Matro, E.; Miguel, B. V.; Tran, I. T.; Kwon, J.; Tedeschi, J. N.; Moosavi, S.; Danishyar, A.; Philp, J. S.; Khamees, R. O.; Jackson, J. N.; Grupe, D. K.; Badshah, S. L.; Hart, J. W. Modeling, Synthesis and Biological Evaluation of Potential Retinoid X Receptor (RXR) Selective Agonists: Novel Analogues of 4-[1-(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)ethynyl]benzoic Acid (Bexarotene). *J. Med. Chem.* **2009**, *52*, 5950−5966.
(35) Furmick, J. K.; Kaneko, I.; Walsh, A. N.; Yang, J.; Bhogal, J. S.; Gray, G. M.; Baso, J. C.; Browder, D. O.; Prentic, J. L. S.; Montano, L. A.; Huynh, C. C.; Marcus, L. M.; Tsosie, D. G.; Kwon, J. S.; Quezada, A.; Reyes, N. M.; Lemming, B.; Saini, P.; van der Vaart, A.; Groy, T. L.; Marshall, P. A.; Jurutka, P. W.; Wagner, C. E. Modeling, Synthesis and Biological Evaluation of Potential Retinoid X Receptor-Selective Agonists: Novel Halogenated Analogues of 4-[1-(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)ethynyl]benzoic Acid (Bexarotene). *ChemMedChem* **2012**, *7*, 1551−1566.
(36) 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*, 3146−3155.
(37) Zhang, L.; Badea, B. A.; Enyeart, D.; Berger, E. M.; Mais, D. E.; Boehm, M. F. Synthesis of Isotopically Labeled 4-[1-(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoic Acid (LGD1069). *J. Labelled Compd. Radiopharm.* **1995**, *36*, 701−712.
(38) Faul, M. M.; Ratz, A. M.; Sullivan, K. A.; Trankle, W. G.; Winneroski, L. L. Synthesis of Novel Retinoid X Receptor-Selective Retinoids. *J. Org. Chem.* **2001**, *66*, 5772−5782.
(39) Santin, E. P.; Germain, P.; Quillard, F.; Khanwalkar, H.; Rodriguez-Barrios, F.; Gronemeyer, H.; de Lera, A. R.; Bourguet, W. Modulating Retinoid X Receptor with a Series of (*E*)-3-[4-Hydroxy-3-(3-alkoxy-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-phenyl]acrylic Acids and Their 4-Alkoxy Isomers. *J. Med. Chem.* **2009**, *52*, 3150−3158.
(40) Gianni, M.; Ponzanelli, I.; Mologni, L.; Reichert, U.; Rambaldi, A.; Terao, M.; Garattini, E. Retinoid-dependent growth inhibition, differentiation and apoptosis in acute promyelocytic leukemia cells. Expression and activation of caspases. *Cell Death Differ.* **2000**, *7*, 447−460.
(41) (a) Shapiro, R. H.; Heath, M. J.; Tosylhydrazones, V. Reaction of Tosylhydrazones with Alkyllithium Reagents. A New Olefin Synthesis. *J. Am. Chem. Soc.* **1967**, *89*, 5734−5735. (b) Shapiro, R. H.; Lipton, M. F.; Kolonko, K. J.; Buswell, R. L.; Capuano, L. A. Tosylhydrazones and alkyllithium reagents: more on the regiospecificity of the reaction and the trapping of three intermediates. *Tetrahedron Lett.* **1975**, *16*, 1811−1814.
(42) Miyaura, N.; Yamada, K.; Suzuki, A. A new stereospecific cross-coupling by the palladium-catalyzed reaction of 1-alkenylboranes with 1-alkenyl or 1-alkynyl halides. *Tetrahedron Lett.* **1979**, *20*, 3437−3440.
(43) Adams, N. D.; Adams, J. L.; Burgess, J. L.; Chaudhari, A. M.; Copeland, R. A.; Donatelli, C. A.; Drewry, D. H.; Fisher, K. E.; Hamajima, T.; Hardwicke, M. A.; Huffman, W. F.; Koretke-Brown, K. K.; Lai, Z. V.; McDonald, O. B.; Nakamura, H.; Newlander, K. A.; Oleykowski, C. A.; Parrish, C. A.; Patrick, D. R.; Plant, R.; Sarpong, M. A.; Sasaki, K.; Schmidt, S. J.; Silva, D. J.; Sutton, D.; Tang, J.; Thompson, C. S.; Tummino, P. J.; Wang, J. C.; Xiang, H.; Yang, J.; Dhanak, D. Discovery of GSK1070916, a Potent and Selective Inhibitor of Aurora B/C Kinase. *J. Med. Chem.* **2010**, *53*, 3973−4001.
(44) (a) Horner, L.; Hoffmann, H. M. R.; Wippel, H. G. Phosphororganische Verbindungen, XII. Phosphinoxyde als Olefinierungsreagenzien. *Chem. Ber.* **1958**, *91*, 61−63. (b) Wadsworth, W. S., Jr.; Emmons, W. D. The Utility of Phosphonate Carbanions in Olefin Synthesis. *J. Am. Chem. Soc.* **1961**, *83*, 1733.
(45) Lalloyer, F.; Pedersen, T. A.; Gross, B.; Lestavel, S.; Yous, S.; Vallez, E.; Gustafsson, J.-A.; Mandrup, S.; Fievet, C.; Staels, B.; Tailleux, A. Rexinoid Bexarotene Modulates Triglyceride but not Cholesterol Metabolism via Gene-Specific Permissivity of the RXR/LXR Heterodimer in the Liver. *Arterioscler. Thromb. Vasc. Biol.* **2009**, *29*, 1488−1495.

(46) Wan, Y.-J. Y.; An, D.; Cai, Y.; Repa, J. J.; Chen, T. H.-P.; Flores, M.; Postic, C.; Magnuson, M. A.; Chen, J.; Chien, K. R.; French, S.; Mangelsdorf, D. J.; Sucov, H. M. Hepatocyte-Specific Mutation Establishes Retinoid X Receptor α as a Heterodimeric Integrator of Multiple Physiological Processes in the Liver. *Mol. Cell. Biol.* **2000**, *20*, 4436−4444.
(47) van der Leede, B.-j. M.; van den Brink, C. E.; van der Saag, P. T. Retinoic Acid Receptor and Retinoid X Receptor Expression in Retinoic Acid-Resistant Human Tumor Cell Lines. *Mol. Carcinog.* **1993**, *8*, 112−122.
(48) Zhang, C.; Hazarika, P.; Ni, X.; Weidner, D. A.; Duvic, M. Induction of Apoptosis by Bexarotene in Cutaneous T-Cell Lymphoma Cells. *Clin. Cancer Res.* **2002**, *8*, 1234−1240.
(49) Budihardjo, I.; Holt, O.; Lutter, M.; Luo, X.; Wang, X. Biochemical Pathways of Caspase Activation During Apoptosis. *Annu. Rev. Cell Dev. Biol.* **1999**, *15*, 269−290.
(50) (a) Kotani, H.; Tanabe, H.; Mizukami, H.; Amagaya, S.; Inoue, M. A Naturally Occurring Rexinoid, Honokiol, Can Serve as a Regulator of Various Retinoid X Receptor Heterodimers. *Biol. Pharm. Bull.* **2012**, *35*, 1−9. (b) Zou, A.; Elgort, M. G.; Allegretto, E. A. Retinoid X receptor (RXR) ligands activate the human 25-hydroxyvitamin D3−24-hydroxylase promoter via RXR heterodimer binding to two vitamin D-responsive elements and elicit additive effects with 1,25-dihydroxyvitamin D3. *J. Biol. Chem.* **1997**, *272*, 19027−19034.
(51) Hallenbeck, P. L.; Phyillaier, M.; Nikodem, V. M. Divergent effects of 9-*cis*-retinoic acid receptor on positive and negative thyroid hormone receptor-dependent gene expression. *J. Biol. Chem.* **1993**, *268*, 3825−3828.
(52) (a) Hong-Qi, Y.; Zhi-Kun, S.; Sheng-Di, C. Current advances in the treatment of Alzheimer's disease: focus on considerations targeting Aβ and tau. *Transl. Neurodegener.* **2012**, *1*, 21. (b) Gross, M. Understanding amyloid and Alzheimer's disease. *Curr. Biol.* **2012**, R381−R384. (c) Appleby, B. S.; Nacopoulos, D.; Milano, N.; Zhong, K.; Cummings, J. L. A Review: Treatment of Alzheimer's Disease Discovered in Repurposed Agents. *Dementia Geriatr. Cognit. Disord.* **2013**, *35*, 1−22.
(53) Mangelsdorf, D. J.; Umesono, K.; Kliewer, S. A.; Borgmeyer, U.; Ong, E. S.; Evans, R. M. A direct repeat in the cellular retinol-binding protein type II gene confers differential regulation by RXR and RAR. *Cell* **1991**, *66*, 555−561.
(54) Forman, B. M.; Umesono, K.; Chen, J.; Evans, R. M. Unique response pathways are established by allosteric interactions among nuclear hormone receptors. *Cell* **1995**, *81*, 541−550.
(55) (a) Zimmerman, F. K. Procedures used in the induction of mitotic recombination and mutation in the yeast *Saccharomyces cerevisiae*. *Mutat. Res.* **1975**, *31*, 71−86. (b) Zimmermann, F. K.; Kern, R.; Rasenberger, H. A yeast strain for simultaneous detection of mitotic crossing over, mitotic gene conversion, and reverse mutation. *Mutat. Res.* **1975**, *28*, 381−388.
(56) Marshall, P. A. Using *Saccharomyces cerevisiae* to Test the Mutagenicity of Household Compounds: An Open Ended Hypothesis-Driven Teaching Lab. *CBE−Life Sci. Educ.* **2007**, *6*, 307−315.
(57) Egea, P. F.; Mitschler, A.; Moras, D. Molecular Recognition of Agonist Ligands by RXRs. *Mol. Endocrinol.* **2002**, *16*, 987−997.
(58) Love, J. D.; Gooch, J. T.; Benko, S.; Li, C.; Nagy, L.; Chatterjee, V. K. K.; Evans, R. M.; Schwabe, J. W. R. The Structural Basis for the Specificity of Retinoid-X Receptor-selective Agonists: New Insights Into the Role of Helix H12. *J. Biol. Chem.* **2002**, *277*, 11385−11391.
(59) Morris, G. M.; Huey, R.; Lindstrom, W.; Sanner, M. F.; Belew, R. K.; Goodsell, D. S.; Olson, A. J. AutoDock4 and AutoDock Tools4: automated docking with selective receptor flexibility. *J. Comput. Chem.* **2009**, *30*, 2785−2791.
(60) O'Boyle, N. M.; Banck, M.; James, C. A.; Morley, C.; Vandermeersch, T.; Hutchison, G. R. Open Babel: an open chemical toolbox. *J. Cheminf.* **2011**, *3*, 33.