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Modeling, Synthesis and Biological Evaluation of Potential Retinoid-X-Receptor (RXR) Selective Agonists: Novel Analogs 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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Abstract

Three unreported analogs 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 analogs 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); though, treatment with **1** can elicit side-effects by disrupting other RXR-heterodimer receptor pathways. Of the 7 modeled novel compounds, all analogs stimulate RXR-regulated transcription in mammalian-2-hybrid and RXRE-mediated assays, possess comparable or elevated biological activity based on EC₅₀ 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 analogs 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.

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Supporting Information: ¹H-NMR and ¹³C-NMR spectra of all compounds reported in the experimentals and dose-response curves for all novel and reported retinoids in the manuscript. This material is available free of charge via the internet at <http://pubs.acs.org>.

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.

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.

While RAR, VDR and TR were initially thought to bind to their HREs as homodimers³, 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 nonpermissive 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 VDR¹⁰ and TR.¹¹ Moreover, nuclear receptor ligands like thyroid hormone or 1,25-dihydroxyvitamin D₃ (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 T₃-TR-RXR-mediated inhibition of VDR transactivation¹² and 1,25D-VDR-RXR-directed inhibition of TR activity^{13, 12(b)}

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 testing¹⁶ 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 analogs 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 cancer¹⁹, breast cancer²⁰ and it has even been applied as an off-label treatment for lung cancer²¹, 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 non-insulin-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 hypothyroidism²³, cutaneous toxicity, and hyperlipidemia. These side effects of **1** are likely incurred either by non-permissive receptor antagonism, as has been noted for TR²⁴ to explain RXR agonist induced hypothyroidism, or permissive receptor agonism, such as LXR to effect hyperlipidemia²⁵ or RAR to effect cutaneous toxicity²⁶, 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 dienolic acid (**3**)²⁸ is a potent RXR agonist. Novel aza-retinoids, exemplified by compound **4**²⁹, as well as amide retinoids³⁰ have been described. RXR agonists based on aryl-trienoic acid compounds either unbranched³¹, or locked with one³² or multiple-fused³³ 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 one order of magnitude greater than **1** in a CV-1 cell line.³⁶ An unsaturated analog of **1**, compound **10**³⁷, has a reported synthetic route that can provide preclinical trial quantities³⁸, though 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 analogs, **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 Fig. 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 Fig. 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.

The Chemistry

The synthesis of pyrimidine-bexarotene analog **13** and the pyrimidine-LDG100268 analog **14** follows a scaled down methodology³⁸ that was recently reported for the synthesis of pyridine-bexarotene analog **8** and LGD100268 (**9**) (Scheme 1). We also undertook the synthesis of bexarotene analog **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 hours 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 the extracted, 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 di-acid chloride that was dissolved in toluene and added to a solution of tri-ethyl 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 hours, 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 the extracted, 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** could be 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).

To synthesize a sample of compound **10**, a modified literature procedure³⁷ 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 Reaction⁴¹ 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 reaction⁴² as the key step was followed. Compound **22** was treated with bromine in DCM to give compound **42**^{16(b)} 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 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 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 analog of **11**, compound **16**, since we wished to compare the substitution of the hydroxyl group of **11** with a non-polar 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 analog 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).

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 finding³⁴ 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** is then coupled to **51** to give methyl ester **59** in 55% yield, and ester **59** is 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 analogs (**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 analogs compared to bexarotene—Biological assessment of a subset of analogs 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 2 different concentrations (25 nM and 100 nM) in this system. The 100 nM assay (Figure 4B) revealed that 5 compounds (**8, 9, 11, 13** and **15**) exhibited high activity compared with compound **1** (bexarotene). More importantly, 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 saturation while the 25 nM assay represents a sub-saturating concentration of ligand (see also EC₅₀ 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 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 and B). These results imply that compounds modeled after the bexarotene parent molecule 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 Supplemental Figures 1-3) and EC₅₀ values as described below.

Novel analogs of bexarotene 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 down-stream 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 retinoid. It is possible that the RXRE may allosterically influence the affinity and/or selectivity of the RXR protein towards 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 RXR α 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 analogs to bind/activate RXR α since the liver expresses predominantly the α isoform⁴⁶, 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 RXR α ⁴⁷ and other receptors of interest to this study so we could quantitate most of the measureable 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 Supplemental Figure 4.

We tested the indicated compounds at two different concentrations (25 nM and 100 nM). 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 and B). In particular, compounds **8**, **11**, **15** and **18** are significantly greater than the activity of 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 analog activity in the RXRE-based assays (Figure 5) is almost identical to the profile of analogs identified in the mammalian two-hybrid assay (Figure 4), thus validating the mammalian two-hybrid assay, and reinforcing the identification and reproducibility of those analogs with the greatest potency in terms of RXR activity.

Evaluation of EC₅₀ 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 bexarotene and analog 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 10⁻⁵ M of each compound as well as bexarotene (Supplemental Figures 1-3). Utilizing this collection of dose-response experiments, we were able to calculate EC₅₀ values, which are listed in Table 1. Our EC₅₀ value for bexarotene, which is an estimate of ligand affinity for RXR, is similar to values obtained previously by another group.^{16(b), 17} In fact, Boehm and co-workers show that the different isoforms of RXR have similar EC₅₀ values—within the same order of magnitude—not only for bexarotene, but several analogs of bexarotene.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 EC₅₀ values in Table 1. We also performed an analysis of the “residual” retinoic acid receptor (RAR) agonist activity of the parent bexarotene compound, as well as for each analog versus the known RAR ligand (*all-trans* retinoic acid). The results of this assay, which employed expression of human RAR α (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 bexarotene 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 increase the activation of RAR (compounds **8**, **13**, **14**, **19**), but also increase its ability to activate RXR (analogs **8** and **13**).

Analysis of apoptotic activity by bexarotene and novel analogs in a CTCL system—One potential mechanism to explain the efficacy of bexarotene 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 bexarotene and the indicated analogs 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 hours (Figure 6). The results of the apoptosis assay suggest that most of the analogs possess some pro-apoptotic activity. Two compounds (analogs **8** and **13**) that exhibit higher RXR binding affinity (lower EC₅₀, 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 hr; and $p < 0.05$ for compound **13** at 24 hr). Several analogs reveal activity that is greater than the ethanol vehicle control (Figure 6) in eliciting apoptosis at 24 hours (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 hours (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 bexarotene and analogs as inhibitors of VDR-RXR activity due to analog-mediated formation of RXR-RXR homodimers—Bexarotene 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 bexarotene side effects in humans. Therefore, we seek to develop RXR analogs that are not only more robust in their RXR bio-potency 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 D₃ 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 analogs (Figure 7A: rexinoid 10⁻⁶ M, Figure 7B: rexinoid 10⁻⁷ 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 and 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 (Fig. 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 1,000 nM assay (Fig. 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 reports⁵⁰ have demonstrated a stimulatory effect 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. In order 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 Fig. 7AB. These experiments (Fig. 7C-F) reveal that the effect of rexinoids (**1**/analogs) on 1,25D-VDR-RXR transcriptional response (e.g., repression, no effect, stimulation) is dependent on the nature of the VDRE. For example, as shown in Figure 7AB, there is a reproducible inhibition with bexarotene and most analogs 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 (Fig. 7CD) in the presence of 1,25D and some of the analogs 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 (Fig. 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 1,000 nM assay (Fig. 7D). The CYP24 natural promoter, which contains 2 VDREs, has a net zero effect of most rexinoids on 1,25D-VDR transactivation (Fig. 7EF) with only a few analogs showing significant inhibition (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 1,000 nM assay). Related findings have been reported for a TR-RXR β -TRE system where RXR β 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 analog (**13-19**) performs in these assays versus **1**, it is also useful to examine how each novel analog performs relative to a known compound (**8-12**) possessing a correspondingly more similar structure. Substituting a pyrimidine ring in **13** and **14** versus a pyridine ring in **8** and **9** resulted in higher RXR α EC₅₀ values, whereas the RAR agonist activity was similar, as were the VDR agonist activity and the ability of **13** and **14** to stimulate apoptosis. On the other hand, the fluorine substitution in novel analog **19** versus the hydrogen atom in **10** resulted in similar RXR α EC₅₀ values, whereas the RAR agonist activity for **19** was much

greater than **10**, but the apoptosis and 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 RXR α EC₅₀ values, whereas the RAR agonist activity was higher in **15** but nearly identical in **16**. Again, the apoptosis and VDRE transcription levels were similar for **11**, **15** and **16**. Interestingly, introducing an unsaturation in the aliphatic ring of **11** to give analog **18** conferred similar RXR α EC₅₀ 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 analog **17** resulted in a higher RXR α EC₅₀ value as well as higher RAR agonist activities than observed in **11**.

Bexarotene and the novel analogs are not mutagenic—Utilizing the EPA-approved *Saccharomyces cerevisiae* mutagenesis assay³⁴, 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 isoleucone 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 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**, since 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 7 novel compounds, 4 of which possess EC₅₀s lower than **1** and 2 of which possess superior pro-apoptotic profiles in CTCL cells. We are thus hopeful that an RXR ligand with a lower EC₅₀ 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 bexarotene treatment. 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 non-permissive 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 analogs 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 analogs of **1** with improved side effect profiles.

Experimental Section

Mammalian Two-Hybrid Assay

HCT-116 colorectal carcinoma cells were plated overnight at 80,000 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 μ g/mL streptomycin and 100 unit/mL penicillin. The cells were co-transfected 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 μ L/well of Express-IN transfection reagent (Thermo Fisher Scientific, Lafayette, CO) and allowed to incubate for 24 hours. The cells were then treated with media containing ethanol vehicle (0.1%), bexarotene or analogs at a final concentration of 25 nM or 100 nM and incubated for 24 hours. 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 Sirius 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 80,000 cells/well in a 24 well plate and maintained as described above. The cells were co-transfected using 250 ng of RXRE-luciferase reporter gene (RXRE from the naturally occurring responsive element in the rat cellular retinoid binding protein II gene⁵³: AAAATGAACTGTGACCTGTGACCTGTGACCTGTGAC), 50 ng of pSG5-human RXR α , and 20 ng of the renilla control plasmid and 2 μ L/well of Express-IN was again used for the liposome-mediated delivery. The cells were incubated for 24 hours post-transfection and then treated with media containing ethanol vehicle (0.1%), or analogs (final concentration of 25 or 100 nM). After a 24-hour 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.

EC₅₀ Determination

EC₅₀ 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 80,000 cells/well in a 24 well plate and maintained as described above. The cells were co-transfected 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 μ L/well of Express-IN transfection reagent which was allowed to incubate for 24 hours with the cells. Then, the cells were treated with ethanol vehicle (0.1%) or analogs (1.0, 2.5, 5.0, 7.5, 10, 25, 50, 75, 100, 250, 500 nM, 1, 2, 3 μ M) and incubated for 24 hours. The amount of rexinoid activity at each concentration was measured using the same luciferase assay described above, and EC₅₀ 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 80,000 cells/well in a 24 well plate and maintained as described above. The cells were co-transfected using 250 ng of pTK-DR5(x2)-luciferase reporter gene. This RARE is an optimized element that has been described previously⁵⁴ and is responsive to the RAR ligand, all-trans retinoic acid. Also included in the transfection were 50 ng of pCMX-human RAR α and 20 ng of the renilla control plasmid, along with 2 μ L/well of Express-IN for liposome-mediated delivery of the DNA into the cells. The cells were incubated for 24 hours post-transfection and then treated with ethanol (0.1 %) or analogs (final concentration of 25 nM or 100 nM). After a 24-hour incubation period the amount of retinoid activity was measured using the same luciferase assay described above. The activity with analog (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 μ l of medium and incubated with 500 μ M sodium butyrate (NaBu), 100 nM bexarotene (Bex) or rexinoid analogs for 24 and 48 hours. The Caspase-Glo[®] 3/7 reagent was then added to each well and incubated for an additional 1 hour 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 80,000 cells/well in a 24 well plate and maintained as described above. The cells were co-transfected 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 μ L/well of Express-IN used for liposome-mediated DNA delivery. The cells were incubated for 24 hours post-transfection and then further treated with ethanol vehicle (0.1%), 10^{-9} M 1,25-dihydroxyvitamin D₃ (1,25D) alone, or 10^{-9} M 1,25D in combination with analogs (final concentration of 10^{-6} M or 10^{-7} M). After a 24-hour 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/analog.

Mutagenicity Assays

Mutagenicity assays utilized the D7 strain of *Saccharomyces cerevisiae*.⁵⁵ D7 cells were incubated at 30°C with shaking for three hours 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 Marshall⁵⁶ 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 RXR α (protein data bank code 1MVC⁵⁷ and 3FUG³⁹, respectively) and RXR β (protein data bank code 1H9U⁵⁸) were performed using the Autodock 4.2 software.⁵⁹ As in our previous studies³⁴, protein residues Arg316 and Ile268 (residue numbering as in 1MVC) were treated flexible and both Autodock and OpenBabel 2.3.0 charges⁶⁰ 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 ¹H NMR and ¹³C NMR spectra. Chemical shifts (δ) are listed in ppm against residual non-deuterated solvent peaks in a given deuterated solvent (e.g. CHCl₃ in CDCl₃) 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 ¹³C 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 pyridine-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, m.p. 189-190 °C: ¹H NMR (400 MHz, d₆-DMSO) 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); ¹³C NMR (100.6 MHz, d₆-DMSO) 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 C₈H₈NO₄ 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 2-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 SiO₂, hexanes:ethyl acetate 95:5 to 85:15) to give **23** (1.30 g, 54%) as an off-white, crystalline solid, m.p. 122-125 °C: ¹H NMR (400 MHz, CDCl₃) 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 ($\text{M} + \text{H}$) $^+$ calcd for $\text{C}_{23}\text{H}_{28}\text{NO}_3$ 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 dried over sodium sulfate, filtered and concentrated 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, m.p. $166\text{--}167^\circ\text{C}$: ^1H 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, $J = 2.0, 1\text{H}$), 5.51 (d, $J = 2.0, 1\text{H}$), 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 ($\text{M} + \text{H}$) $^+$ calcd for $\text{C}_{24}\text{H}_{30}\text{NO}_2$ 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 $30\text{--}34^\circ\text{C}$ 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, m.p. $168\text{--}170^\circ\text{C}$: ^1H 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 ($\text{M} + \text{H}$) $^+$ calcd for $\text{C}_{25}\text{H}_{32}\text{NO}_2$ 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, m.p. 243-245 °C: ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (100.6 MHz, CDCl₃) 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 C₂₃H₂₈NO₂ 350.2120, found 350.2111. Anal. Calcd for C₂₄H₂₉NO₂·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%), m.p. 273-274 °C: ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (100.6 MHz, CDCl₃) 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 C₂₄H₃₀NO₂ 364.2277, found 364.2265. Anal. Calcd for C₂₄H₂₉NO₂: 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 3h. 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-dicarboxyl dichloride that was used without further purification. To the crude pyrimidine-2,5-dicarboxyl 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 NaHCO₃ (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 SiO₂, hexanes:ethyl acetate 2:3) to give **26** (4.26 g, 72%) as a colorless, crystalline solid (m.p. 140-142 °C): ¹H NMR (400 MHz, CDCl₃) δ 9.40 (s, 2H), 4.07 (s, 3H), 3.99 (s, 3H); ¹³C NMR (100.6 MHz, CDCl₃) 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 C₈H₈N₂O₄ 196.0484, found 196.0485.

5-(Methoxycarbonyl)pyrimidine-2-carboxylic acid (27)

To a suspension 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 (m.p. 152-154 °C) (2.765 g, 89%): ¹H NMR (400 MHz, d₆-DMSO) δ 13.89 (br s, 1H), 9.39 (s, 2H), 3.97 (s, 3H); ¹³C NMR (100.6 MHz, d₆-DMSO) 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 C₇H₆N₂O₄ 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 2-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 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 **29**. Crude **29** was purified by column chromatography (250 mL SiO₂, hexanes:ethyl acetate 95:5 to 85:15) to give **29** (1.677 g, 68%) as an off-white, crystalline solid, m.p. 94-96 °C: ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (100.6 MHz, CDCl₃) 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 C₂₂H₂₇N₂O₃ 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 filtered, and a small yellow filter-cake was dissolved in chloroform and added to the 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 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 SiO₂, hexanes:ethyl acetate 97.5:2.5 to 95:5) to give **30** (0.4127 g, 40%) as a white, fiber-like solid, m.p. 189-191 °C: ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (100.6 MHz, CDCl₃) 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 C₂₃H₂₉N₂O₂ 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 SiO₂, hexanes:ethyl acetate 97.5:2.5 to 90:10) to give **31** (0.2884 g, 68%) as a white crystalline solid, m.p. 202-203 °C: ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (100.6 MHz, CDCl₃) 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 C₂₄H₃₁N₂O₂ 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%), m.p. 254-255 °C: ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (100.6 MHz, CDCl₃) 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 C₂₂H₂₇N₂O₂ 351.2073, found 351.2082. Anal. Calcd for C₂₂H₂₆N₂O₂: 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%), m.p. 266-267 °C: ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (100.6 MHz, CDCl₃) 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 C₂₃H₂₉N₂O₂ 365.2229, found 365.2225. Anal. Calcd for C₂₃H₂₈N₂O₂: 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(1H)-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(2H)-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 2h, 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 SiO₂, hexanes:ethyl acetate 98:2) to give **33** (1.6348 g, 44%) as a white crystalline solid, 49-51 °C: ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (100.6 MHz, CDCl₃) 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 C₁₅H₂₀O 216.1514, found 216.1523.

6-Bromo-3,4-dihydro-1,1,4,4,7-pentamethylnaphthalen-2(1H)-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 SiO₂, hexanes:ethyl acetate 98:2) to give **34** (4.36 g, 100%) as a white crystalline solid, m.p. 114-117 °C: ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (100.6 MHz, CDCl₃) 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 C₁₅H₁₉BrO 294.0619, found 294.0617.

(E)-1-(6-bromo-3,4-dihydro-1,1,4,4,7-pentamethylnaphthalen-2(1H)-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 hour, and the resulting precipitate was filtered and rinsed with cold methanol to afford **35** as a white crystalline solid (2.41 g, 50%), m.p. 181-184 °C: ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (100.6 MHz, CDCl₃) 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 $\text{C}_{22}\text{H}_{28}\text{N}_2\text{O}_2\text{SBr}$ 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 (m.p. 109-111 °C): ^1H 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 $\text{C}_{15}\text{H}_{19}\text{Br}$ 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 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, m.p. 78-79 °C: ^1H 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 $\text{C}_{10}\text{H}_{10}\text{N}_2\text{O}_2$ 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 air-tight 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, m.p. 117-119 °C: ^1H 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 $\text{C}_{23}\text{H}_{23}\text{NO}$ 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, m.p. 199-201 °C: ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (100.6 MHz, CDCl₃) 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 C₂₃H₂₅O₃ 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 pre-filled 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 column chromatography (25 mL SiO₂, ethyl acetate) to give **10** (0.3628 g, 91%) as a white crystalline solid (m.p. 210-213 °C): ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (100.6 MHz, CDCl₃) 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 C₂₄H₂₇O₂ 347.2011, found 347.1997. Anal. Calcd for C₂₄H₂₆O₂: 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**)^{16(b)}

The method of Dawson and co-workers was used to synthesize **42**.^{16(b)} 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 Na₂SO₃ 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 SiO₂, ethyl acetate) to give an inseparable 2:1 mixture of **42:22** (1.679 g, 60% yield for **42**) as a white crystalline solid: ¹H NMR (400 MHz, CDCl₃) 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 C₁₅H₂₁Br 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 SiO₂, ethyl acetate:hexanes 1:3) to give **43** (0.8838 g, 45%) as a white crystalline solid, m.p. 220-225 $^{\circ}\text{C}$: ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (100.6 MHz, CDCl₃) 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: ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (100.6 MHz, CDCl₃) 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 SiO₂, ethyl acetate:hexanes 1:9) to give **45** (3.2974 g, 74%) as a white crystalline solid, m.p. 45-51 $^{\circ}\text{C}$: ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (100.6 MHz, CDCl₃) 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 C₈H₇OBr 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 NH₄Cl 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 SiO₂, ethyl acetate:hexanes 1:9) to give **46** (1.576 g, 88%) as a colorless crystalline solid, m.p. 46-47 $^{\circ}\text{C}$: ¹H NMR (400 MHz, CDCl₃) 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 $\text{C}_{12}\text{H}_{14}\text{O}_2\text{Br}$ 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 $\text{Pd}(\text{OAc})_2$ (0.0203 g, 0.09 mmol), and the flask was evacuated and back-filled with nitrogen three times. The reaction was stirred at room temperature for 15 min and then placed in an oil bath pre-heated 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: ^1H 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 $\text{C}_{27}\text{H}_{35}\text{O}_2$ 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 pre-heated 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, m.p. 189-196 °C: ^1H 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 $\text{C}_{25}\text{H}_{31}\text{O}_2$ 363.2324, found 363.2311. Anal. Calcd for $\text{C}_{25}\text{H}_{30}\text{O}_2$: 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 SiO₂, ethyl acetate:hexanes 5:95) to give **49** (2.1188 g, 98%) as a colorless crystalline solid, m.p. 75-76 °C: ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (100.6 MHz, CDCl₃) 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 C₁₂H₁₀F₃O₂Br 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), Na₂CO₃ (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 back-filled with nitrogen three times. The reaction was stirred at room temperature for 15 min and then placed in an oil bath pre-heated 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 SiO₂, ethyl acetate:hexanes 1:9) to give **50** (1.0138 g, 71%) as a colorless oil: ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (100.6 MHz, CDCl₃) 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 C₂₇H₃₁F₃O₂ 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 pre-heated 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 SiO₂, ethyl acetate:hexanes 15:85) to give **16** (0.3445 g, 88%) as a white crystalline solid, m.p. 217-221 °C: ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (100.6 MHz, CDCl₃) 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 C₂₅H₂₈F₃O₂ 417.2041, found 417.2068. Anal. Calcd for C₂₅H₂₇F₃O₂: 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-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 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, m.p. $162\text{--}166^{\circ}\text{C}$: ^1H 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} .

(2E)-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 $\text{Pd}(\text{OAc})_2$ (0.0136 g, 0.061 mmol), and the flask was evacuated and back-filled with nitrogen three times. The reaction was stirred at room temperature for 15 min and then placed in an oil bath pre-heated 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: ^1H 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 $\text{C}_{27}\text{H}_{32}\text{O}_2$ 388.2402, found 388.2414.

(2E)-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 pre-heated 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, m.p. $183\text{--}185^{\circ}\text{C}$: ^1H 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 $\text{C}_{25}\text{H}_{28}\text{O}_2$ 360.2089, found 360.2089. Anal. Calcd for $\text{C}_{25}\text{H}_{27}\text{O}_2$: 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 back-filled with nitrogen, and the solution was heated to 135 °C in a pre-heated 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 SiO₂, ethyl acetate:hexanes 1:9) to give **54** (2.44 g, 90%) as a white crystalline solid, m.p. 58-60 °C: ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (100.6 MHz, CDCl₃) 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 C₉H₇O₃Br 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 NH₄Cl 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 SiO₂, ethyl acetate:hexanes 1:9) to give **55** (1.7598 g, 84%) as a colorless crystalline solid, m.p. 71-72 °C: ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (100.6 MHz, CDCl₃) 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 C₁₃H₁₃O₂Br 311.9997, found 311.9988.

(2E)-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), Na₂CO₃ (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 back-filled with nitrogen three times. The reaction was stirred at room temperature for 15 min and then placed in an oil bath pre-heated 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 SiO₂, ethyl acetate:hexanes 5:95 to 3:7) to give **56** (0.3673 g, 54%) as a colorless oil: ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (100.6 MHz, CDCl₃) 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 C₂₈H₃₃O₄ 433.2379, found 433.2371.

(2E)-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 pre-heated 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 SiO₂, ethyl acetate:hexanes 10:90 to 2:5) to give **18** (0.2820 g, 89%) as a white crystalline solid, m.p. 163-168 °C: ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (100.6 MHz, CDCl₃) 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 C₂₄H₂₇O₃ 363.1960, found 363.1967; Anal. Calcd for C₂₄H₂₆O₃: 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-workers³⁸, 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 Na₂CO₃ (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 Na₂CO₃ (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%): ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (100.6 MHz, CDCl₃) 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 C₁₁H₉F₄O₅S 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 mmols), triflate **58** (0.429 g, 1.31 mmols), Pd(OAc)₂ (0.014 g, 0.062 mmols), P(*o*-Tol)₃ (0.030 g, 0.099 mmols), and Et₃N (0.34 mL, 2.4 mmols) in DMF (4.0 mL) was heated to 50 °C and stirred for 2h. After cooling to room temperature, the reaction was poured into water and extracted with ethyl acetate. The combined organic layers were washed with water, 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%), m.p. 124-126 °C: ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (100.6 MHz, CDCl₃) 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 C₂₅H₂₈FO₂ 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 purified by column chromatography (silica gel, 10% to 30% ethyl acetate in hexanes) to give **19** as a crystalline solid (0.1664 g, 70%), m.p. 187-188 °C: ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (100.6 MHz, CDCl₃) 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 C₂₄H₂₆O₂F 365.1917, found 365.1923. Anal. Calcd for C₂₄H₂₅O₂F: 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 NH₄Cl 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 SiO₂, ethyl acetate:hexanes 5:95) to give **61** (1.6796 g, 98%) as a colorless crystalline solid, m.p. 34- 36 °C: ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (100.6 MHz, CDCl₃) 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 C₁₁H₁₁BrO₂ 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), Na₂CO₃ (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 back-filled with nitrogen three times. The reaction was stirred at room temperature for 15 min and then placed in an oil bath pre-heated 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 SiO₂, ethyl acetate:hexanes 1:9) to give **62** (0.4295 g, 70%) as a colorless oil: ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (100.6 MHz, CDCl₃) 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 C₂₆H₃₂O₂ 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 pre-heated 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 SiO₂, ethyl acetate:hexanes 15:85) to give **12** (0.3119 g, 84%) as a white crystalline solid, 205-207 °C: ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (100.6 MHz, CDCl₃) 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 C₂₄H₂₉O₂ 349.2168, found 349.2168. Anal. Calcd for C₂₄H₂₈O₂: C, 82.72; H, 8.10. Found: C, 82.05; H, 7.94.

Supplementary Material

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Acknowledgments

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References

1. (a) Mangelsdorf, DJ.; Umesono, K.; Evans, RM. The Retinoids. Academic Press; Orlando, FL: 1994. The Retinoid Receptors; p. 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. [PubMed: 1333659]
2. Olefsky JM. Nuclear Receptor Minireview Series. J Biol Chem. 2001; 276(40):36863-36864. [PubMed: 11459855]
3. Forman BM, Yang CR, Au M, Casanova J, Ghysdael J, Samuels HH. 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. [PubMed: 2558297]
4. Mangelsdorf DJ, Evans RM. The RXR heterodimers and orphan receptors. Cell. 1995; 83:841-850. [PubMed: 8521508]
5. Thompson PD, Remus LS, Hsieh JC, Jurutka PW, Whitfield GK, Galligan MA, Encinas Dominguez C, Haussler CA, Haussler MR. Distinct retinoid X receptor activation function-2 residues mediate transactivation in homodimeric and vitamin D receptor heterodimeric contexts. J Mol Endocrinol. 2001; 27(2):211-227. [PubMed: 11564604]
6. Svensson S, Ostberg T, Jacobsson M, Norstrom C, Stefansson K, Hallen D, Johansson IC, Zachrisson K, Ogg D, Jendeborg 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. [PubMed: 12970175]
7. Nahoum V, Perez E, Germain P, Rodriguez-Barrios F, Manzo F, Kammerer S, Lemaire G, Hirsch O, Royer CA, Gronemeyer H, de Lera AR, Bourguet W. Modulators of the structural dynamics of the retinoid X receptor to reveal receptor function. PNAS. 2007; 104:17323-17328. [PubMed: 17947383]

8. Forman BM, Umesono K, Chen J, Evans RM. Unique response pathways are established by allosteric interactions among nuclear hormone receptors. *Cell*. 1995; 81:541–550. [PubMed: 7758108]
9. Lala DS, Mukherjee R, Schulman IG, Canan Koch SS, Dardashti LJ, Nadzan AM, Croston GE, Evans RM, Heyman RA. Activation of specific RXR heterodimers by an antagonist of RXR homodimers. *Nature*. 1996; 383:450–453. [PubMed: 8837780]
10. (a) Lemon BD, Freedman LP. Selective effects of ligands on vitamin D3 receptor- and retinoid X receptor-mediated gene activation in vivo. *Mol Cell Biol*. 1996; 16:1006–1016. [PubMed: 8622645] (b) MacDonald PN, Dowd DR, Nakajima S, Galligan MA, Reeder MC, Haussler CA, Ozato K, Haussler MR. 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. [PubMed: 8395017] (c) Thompson PD, Jurutka PW, Haussler CA, Whitfield GK, Haussler MR. 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. 1998. [PubMed: 9525962]
11. Lehmann JM, Zhang XK, Graupner G, Lee MO, 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. [PubMed: 8246986]
12. (a) Raval-Pandya M, Freedman LP, 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. [PubMed: 9731705] (b) Thompson PD, Hsieh JC, Whitfield GK, Haussler CA, Jurutka PW, Galligan MA, Tillman JB, Spindler SR, Haussler MR. 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. [PubMed: 10536369]
13. Yen PM, Liu Y, Sugawara A, Chin WW. Vitamin D Receptors Repress Basal Transcription and Exert Dominant Negative Activity on Triiodothyronine-mediated Transcriptional Activity. *J Biol Chem*. 1996; 271:10910–10916. [PubMed: 8631908]
14. Altucci L, Leibowitz MD, Ogilvie KM, de Lera AR, Gronemeyer H. RAR and RXR modulation in cancer and metabolic disease. *Nature Rev Drug Discov*. 2007; 6:793–810. [PubMed: 17906642]
15. Lehmann JM, Jong L, Fanjul A, Cameron JF, Lu XP, Haefner P, Dawson MI, Pfahl M. Retinoids selective for retinoid X receptor pathways. *Science*. 1992; 258:1944–1946. [PubMed: 1335166]
16. (a) Jong L, Lehmann JM, Hobbs PD, Harlev E, Huffman JC, Pfahl M, Dawson MI. 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. [PubMed: 8410972] (b) Dawson MI, Jong L, Hobbs PD, Cameron JF, Chao WR, Pfahl M, Lee MO, 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. [PubMed: 7650690]
17. Boehm MF, Zhang L, Badea BA, White SK, Mais DE, Berger E, Suto CM, Goldman ME, Heyman RA. Synthesis and Structure-Activity Relationships of Novel Retinoid X Receptor-Selective Retinoids. *J Med Chem*. 1994; 37:2930–2941. [PubMed: 8071941]
18. Daiss JO, Burschka C, Mills JS, Montana JG, Showell GA, 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 RM, Stone J, Yen WC, Bissonnette RP, Lamph WW. Differentiation and growth inhibition mediated via the RXR:PPAR γ heterodimer in colon cancer. *Cancer Letters*. 2006; 240:225–233. [PubMed: 16271436]
20. Yen WC, Prudente RY, Lamph WW. 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. [PubMed: 15564797]
21. (a) Yen WC, Corpuz MR, Prudente RY, Cooke TA, Bissonnette RP, Negro-Vilar A, Lamph WW. 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. [PubMed: 15623650] (b) Dragnev KH, Petty WJ, Shah SJ, Lewis LD, Black CC, Memoli V, Nugent WC, Hermann T, Negro-Vilar A, Rigas JR, 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. [PubMed: 17363535]
22. Mukherjee R, Davies PJA, Crombie DL, Bischoff ED, Cesario RM, Jow L, Hamanns LG, Boehm MF, Mondon CE, Nadzan AM, Paterniti JR, Heyman RA. Sensitization of diabetic and obese mice to insulin by retinoid X receptor agonists. *Nature.* 1997; 386:407–410. [PubMed: 9121558]
23. Sherman SI, Gopal J, Haugen BR, Chiu AC, Whaley K, Nowlakha P, Duvic M. Central hypothyroidism associated with retinoid X receptor-selective ligands. *N Engl J Med.* 1999; 340:1075–1079. [PubMed: 10194237]
24. Li D, Li T, Wang F, Tian H, Samuels HH. 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. [PubMed: 12138189]
25. (a) Murthy S, Born E, Mathur SN, Field FJ. LXR/RXR activation enhances basolateral efflux of cholesterol in CaCo-2 cells. *J Lipid Res.* 2002; 43:1054–1064. [PubMed: 12091489] (b) Field FJ, Born E, Mathur SN. LXR/RXR ligand activation enhances basolateral efflux of beta-sitosterol in CaCo-2 cells. *J Lipid Res.* 2004; 45:905–913. [PubMed: 14993242]
26. Thacher SM, Standeven AM, Athanikar J, Kopper S, Castilleja O, Escobar M, Beard RL, Chandraratna RAS. 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 PE, Cirrito JR, Wesson DW, Lee CYD, Karlo JC, Zinn AE, Casali BT, Restivo JL, Goebel DA, James MJ, Brunden KR, Wilson DA, Landreth GE. ApoE-Directed Therapeutics Rapidly Clear β -Amyloid and Reverse Deficits in AD Mouse Models. *Science.* 2012; 335:1503–1506. [PubMed: 22323736] (b) Landreth GE, Cramer PE, Lakner MM, Cirrito JR, Wesson DW, Brunden KR, Wilson DA. Response to Comments on ‘ApoE-Directed Therapeutics Rapidly Clear β -Amyloid and Reverse Deficits in AD Mouse Models.’. *Science.* 2013; 340:924. [PubMed: 23704556]
28. Vuligonda V, Thacher SM, Chandraratna RAS. Enantioselective Synthesis of Potent Retinoid X Receptor Ligands: Differential Biological Activities of Individual Antipodes. *J Med Chem.* 2001; 44:2298–2303. [PubMed: 11428923]
29. Farmer LJ, Marron KS, Canan Koch SS, Hwang CK, Kallel EA, Zhi L, Nadzan AM, Robertson DW, Bennani YL. Aza-retinoids as novel retinoid X receptor-specific agonists. *Bioorg Med Chem Lett.* 2006; 16:2352–2356. [PubMed: 16364638]
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. [PubMed: 3184125]
31. Michellys PY, Ardecky RJ, Chen JH, Crombie DL, Etgen GJ, Faul MM, Faulkner AL, Grese TA, Heyman RA, Karanewsky DS, Klausing K, Leibowitz MD, Liu S, Mais DA, Mapes CM, Marschke KB, Reifel-Miller A, Ogilvie KM, Rungta D, Thompson AW, Tyhonas JS, Boehm MF. Novel (2E,4E,6Z)-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. [PubMed: 12801232]
32. Michellys PY, Ardecky RJ, Chen JH, D'Arrigo J, Grese TA, Karanewsky DS, Leibowitz MD, Liu S, Mais DA, Mapes CM, Montrose-Rafizadeh C, Ogilvie KM, Reifel-Miller A, Rungta D, Thompson AW, Tyhonas JS, Boehm MF. 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. [PubMed: 12954061]
33. Michellys PY, Darrigo J, Grese TA, Karanewsky DS, Leibowitz MD, Mais DA, Mapes CM, Reifel-Miller A, Rungta D, Boehm MF. Design, synthesis and structure-activity relationship of novel RXR-selective modulators. *Bioorg Med Chem Lett.* 2004; 14:1593–1598. [PubMed: 15006411]
34. Wagner CE, Jurutka PW, Marshall PA, Groy TL, van der Vaart A, Ziller JW, Furmick JK, Graeber ME, Matro E, Miguel BV, Tran IT, Kwon J, Tedeschi JN, Moosavi S, Danishyar A, Philp JS, Khamees RO, Jackson JN, Grupe DK, Badshah SL, Hart JW. 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. [PubMed: 19791803]
35. Furmick JK, Kaneko I, Walsh AN, Yang J, Bhogal JS, Gray GM, Baso JC, Browder DO, Prentic JLS, Montano LA, Huynh CC, Marcus LM, Tsosie DG, Kwon JS, Quezada A, Reyes NM, Lemming B, Saini P, van der Vaart A, Groy TL, Marshall PA, Jurutka PW, Wagner CE. 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. [PubMed: 22927238]
36. Boehm MF, Zhang L, Zhi L, McClurg MR, Berger E, Wagoner M, Mais DE, Suto CM, Davies PJA, Heyman RA, Nadzan AM. Design and Synthesis of Potent Retinoid X Receptor Selective Ligands That Induce Apoptosis in Leukemia Cells. *J Med Chem.* 1995; 38:3146–3155. [PubMed: 7636877]
37. Zhang L, Badea BA, Enyeart D, Berger EM, Mais DE, Boehm MF. Synthesis of Isotopically Labeled 4-[1-(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)ethynyl]benzoic Acid (LGD1069). *Journal of Labelled Compounds and Radiopharmaceuticals.* 1995; 36:701–712.
38. Faul MM, Ratz AM, Sullivan KA, Trankle WG, Winneroski LL. Synthesis of Novel Retinoid X Receptor-Selective Retinoids. *J Org Chem.* 2001; 66:5772–5782. [PubMed: 11511251]
39. Santin EP, Germain P, Quillard F, Khanwalkar H, Rodriguez-Barrios F, Gronemeyer H, de Lera AR, 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. [PubMed: 19408900]
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. [PubMed: 10800078]
41. (a) Shapiro RH, Heath MJ. Tosylhydrazones. V. Reaction of Tosylhydrazones with Alkylolithium Reagents. A New Olefin Synthesis. *J Am Chem Soc.* 1967; 89:5734–5735. (b) Shapiro RH, Lipton MF, Kolonko KJ, Buswell RL, Capuano LA. Tosylhydrazones and alkylolithium reagents: More on the regioselectivity 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 ND, Adams JL, Burgess JL, Chaudhari AM, Copeland RA, Donatelli CA, Drewry DH, Fisher KE, Hamajima T, Hardwicke MA, Huffman WF, Koretke-Brown KK, Lai ZV, McDonald OB, Nakamura H, Newlander KA, Oleykowski CA, Parrish CA, Patrick DR, Plant R, Sarpong MA, Sasaki K, Schmidt SJ, Silva DJ, Sutton D, Tang J, Thompson CS, Tummino PJ, Wang JC, 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. [PubMed: 20420387]
44. (a) Horner L, Hoffmann HMR, Wippel HG. Phosphororganische Verbindungen, XII. Phosphinoxyde als Olefinierungsreagenzien. *Chem Ber.* 1958; 91:61–63. (b) Wadsworth WS Jr, Emmons WD. The Utility of Phosphonate Carbanions in Olefin Synthesis. *J Am Chem Soc.* 1961; 83:1733.
45. Lalloyer F, Pedersen TA, Gross B, Lestavel S, Yous S, Vallez E, Gustafsson JA, Mandrup S, Fievet C, Staels B, Tailleux A. Retinoid 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. [PubMed: 19592467]
46. Wan YJY, An D, Cai Y, Repa JJ, Chen THP, Flores M, Postic C, Magnuson MA, Chen J, Chien KR, French S, Mangelsdorf DJ, Sucov HM. 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. [PubMed: 10825207]
47. van der Leede, BJM; van den Brink, CE.; van der Saag, PT. Retinoic Acid Receptor and Retinoid X Receptor Expression in Retinoic Acid-Resistant Human Tumor Cell Lines. *Mol Carcinog.* 1993; 8:112–122. [PubMed: 7691069]

48. Zhang C, Hazarika P, Ni X, Weidner DA, Duvic M. Induction of Apoptosis by Bexarotene in Cutaneous T-Cell Lymphoma Cells. *Clinical Cancer Research*. 2002; 8:1234–1240. [PubMed: 12006543]
49. Budihardjo I, Holt O, Lutter M, Luo X, Wang X. Biochemical Pathways of Caspase Activation During Apoptosis. *Annual Review of Cell and Developmental Biology*. 1999; 15:269–290.
50. (a) Kotani H, Tanabe H, Mizukami H, Amagaya S, Inoue M. A Naturally Occurring Retinoid, Honokiol, Can Serve as a Regulator of Various Retinoid X Receptor Heterodimers. *Biol Pharm Bull*. 2012; 35:1–9. [PubMed: 22223330] (b) Zou A, Elgort MG, Allegretto EA. 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. [PubMed: 9228086]
51. Hallenbeck PL, Phyllaier M, Nikodem VM. 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. [PubMed: 7680034]
52. (a) Hong-Qi Y, Zhi-Kun S, Sheng-Di C. Current advances in the treatment of Alzheimer's disease: focus on considerations targeting A β and tau. *Translational Neurodegeneration*. 2012; 1:21.(b) Gross M. Understanding amyloid and Alzheimer's disease. *Current Biology*. 2012;R381–R384. [PubMed: 22799004] (c) Appleby BS, Nacopoulos D, Milano N, Zhong K, Cummings JL. A Review: Treatment of Alzheimer's Disease Discovered in Repurposed Agents. *Dement Geriatr Cogn Disord*. 2013; 35:1–22. [PubMed: 23307039]
53. Mangelsdorf DJ, Umesono K, Kliewer SA, Borgmeyer U, Ong ES, Evans RM. A direct repeat in the cellular retinol-binding protein type II gene confers differential regulation by RXR and RAR. *Cell*. 1991; 66:555–561. [PubMed: 1651173]
54. Forman BM, Umesono K, Chen J, Evans RM. Unique response pathways are established by allosteric interactions among nuclear hormone receptors. *Cell*. 1995; 81:541–550. [PubMed: 7758108]
55. (a) Zimmerman FK. Procedures used in the induction of mitotic recombination and mutation in the yeast *Saccharomyces cerevisiae*. *Mutat Res*. 1975; 31:71–86. [PubMed: 235086] (b) Zimmermann FK, 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 PA. Using *Saccharomyces cerevisiae* to Test the Mutagenicity of Household Compounds: An Open Ended Hypothesis-Driven Teaching Lab. *CBE-LSE*. 2007; 6:307–315. [PubMed: 18056302]
57. Egea PF, Mitschler A, Moras D. Molecular Recognition of Agonist Ligands by RXRs. *Mol Endocrinol*. 2002; 16:987–997. [PubMed: 11981034]
58. Love JD, Gooch JT, Benko S, Li C, Nagy L, Chatterjee VKK, Evans RM, Schwabe JWR. 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. [PubMed: 11782480]
59. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ. AutoDock4 and AutoDock Tools4: Automated docking with selective receptor flexibility. *J Comput Chem*. 2009; 30:2785–2791. [PubMed: 19399780]
60. O'Boyle NM, Banck M, James CA, Morley C, Vandermeersch T, Hutchison GR. Open Babel: An open chemical toolbox. *J Cheminf*. 2011; 3:33.

Abbreviations

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

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.

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

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 hours utilizing a liposome-mediated transfection protocol, and then treated with either ethanol vehicle, 25 nM or 100 nM of the indicated compound. After a 24-hour incubation, cells were lysed and a dual luciferase assay was completed. Analog-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 $\pm$ SD. * $p < 0.05$, ** $p < 0.01$ vs compound **1**.

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 RXR α , RXRE-luciferase reporter gene, and renilla control plasmid. Cells were transfected for 24 hours utilizing a liposome-mediated transfection protocol as in Figure 4, and then treated with ethanol vehicle, 25 nM or 100 nM of the indicated compound. After a 24-hour incubation, cells were lysed and a luciferase assay was completed. Analog-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 $\pm$ SD. * p <0.05, ** p <0.01 vs compound **1**.

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

Figure 7. Evaluation of bexarotene and selected analogs 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 hours utilizing a liposome-mediated transfection protocol, and then treated with ethanol vehicle, 1 nM 1,25D, 100 nM or 1,000 nM of the indicated compound in the presence of 1 nM 1,25D. After a 24-hour 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 analogs. **B)** ER6-mediated activity in the presence of 1 nM 1,25D and 1,000 nM analogs. **C)** DR3-mediated activity in the presence of 1 nM 1,25D and 100 nM analogs. **D)** DR3-mediated activity in the presence of 1 nM 1,25D and 1,000 nM analogs. **E)** hCYP24A1 promoter activity in the presence of 1 nM 1,25D and 100 nM analogs. **F)** hCYP24A1 promoter activity in the presence of 1 nM 1,25D and 1,000 nM analogs. Values are means $\pm$ SD. * $p < 0.05$, ** $p < 0.01$ vs. 1 nM 1,25D only (black bar).

Scheme 1.

Scheme 2.

Scheme 3.

Scheme 4.

Scheme 5.

Scheme 6.

Scheme 7.

Scheme 8.

Scheme 9.

Scheme 10.

Scheme 11.

Scheme 12.

9-*cis* retinoic acid **1** **2****Structures 1.**

Structures 2.

Structures 3.

Table 1
Determination of EC₅₀ Values in RXR M2H Assay and Quantitation of RAR Agonist Activity

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)

¹EC₅₀ 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.

²RAR agonist activity was derived from an RAR/RARE reporter system in transfected HCT-116 cells treated with all-trans RA or indicated analog at 100 nM or 1 μM. The activity with analog (or compound **1**) divided by the activity with all-trans RA expressed as a percentage represents the RAR agonist activity.