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Modeling, Synthesis and Biological Evaluation of Potential Retinoid-X-Receptor (RXR) Selective Agonists: Novel Halogenated Analogs of 4-[1-(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)ethynyl]benzoic Acid (Bexarotene)

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Abstract

The synthesis of halogenated analogs of 4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)ethynyl]benzoic acid (**1**), known commonly as bexarotene, and their evaluation for retinoid-X-receptor (RXR)-specific agonist performance is described. Compound **1** is FDA approved to treat cutaneous T-cell lymphoma (CTCL); however, bexarotene treatment can induce hypothyroidism and elevated triglyceride levels, presumably by disrupting RXR heterodimer pathways for other nuclear receptors. The novel halogenated analogs in this study were modeled and assessed for their ability to bind to RXR and stimulate RXR homodimerization in an RXRE-mediated transcriptional assay as well as an RXR mammalian-2-hybrid assay. In an array of 8 novel compounds, 4 analogs were discovered to promote RXR-mediated transcription with comparable EC_{50} values as **1** and are selective RXR agonists. Our approach also uncovered a periodic trend of increased binding and homodimerization of RXR when substituting a halogen atom for a proton *ortho* to the carboxylic acid on **1**.

Keywords

Bexarotene; Cutaneous T Cell Lymphoma; Retinoic Acid Receptor; Retinoid X Receptor; Rexinoid

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Supporting Information Available: 1H-NMR and 13C-NMR spectra of all compounds reported in the experimentals as well as X-ray data for compounds **12, 14, 16, 18** and **52**. The Xray data can be found at the Cambridge Crystallographic Data Centre under the identification numbers: 859464-859468.

Introduction

Retinoids are small molecules that interact with at least two specific nuclear receptors and regulate cellular processes such as gene transcription, proliferation and differentiation. The two major nuclear receptors that retinoids target are retinoid X receptors (RXRs) and retinoic acid receptors (RARs), and both receptors have three known subtypes: α, β and γ. [1] Retinoid receptors, in addition to other lipophilic hormone molecule receptors, are part of a larger receptor superfamily, including the vitamin D receptor (VDR) and thyroid hormone receptor (TR), all of which essentially function to promote transcription in the presence of an appropriate molecular signal. This molecular signal, usually comprised of an endogenous ligand, binds to the protein's ligand binding pocket (LBP), inducing a conformational change that ultimately enables the protein to bind to a hormone responsive element (HRE) specific for the receptor on DNA. Many HREs are located in or proximal to the promoter regions for the genes they regulate, although a growing number of these elements can be found at large distances upstream or downstream of the gene they control. Nonetheless, HREs are typically constructed of minimal core hexad sequences consisting of half-sites interrupted by nucleotide spacers of variable length between inverted, everted or direct repeats. [2] Nuclear receptors activate transcription by binding to the HREs as heterodimers or homodimers, where each partner binds to a half-site in the element.

Although initially proposed to operate as homodimers [3], HRE high affinity binding by RAR, VDR and TR actually proceeds via an RXR heterodimer. [4] When RXR binds to its natural 9-*cis* retinoic acid (9-*cis* RA) ligand, it forms a homodimer that associates with the RXR responsive element or RXRE, but when functioning as a heterodimeric partner with other receptors, RXR can be either liganded or unliganded. For example, in the case of the RXR-VDR heterodimer, RXR is believed to be unliganded. [5] There are a few nuclear receptors, such as the liver X receptor (LXR), that form heterodimers with liganded RXR. [6] Thus, RXR often plays the role of the "master" partner, controlling the operation of several nuclear receptors through liganded and unliganded heterodimers that effect specific physiological equilibria via control of gene expression. [7]

Notably, ligand-promoted RXR homodimer transcriptional activity is suppressed for most cases where RXR is coordinated in a heterodimer with an endogenous ligand-bound receptor such as TR or VDR, and the TR and VDR partners in these RXR heterodimers are termed "nonpermissive" partners for RXR. [5] In contrast to TR and VDR, RAR forms a heterodimer with RXR when RAR binds to all-*trans*-retinoic acid, but the RXR partner is still capable of binding 9-*cis*-RA, and both retinoids act in synergy to promote RAR/RARE-mediated gene transcription. When synthetic high-affinity RXR binding ligands (rexinoids) or 9-*cis*-RA are available, even in the presence of the TR and VDR agonists for the TR-RXR or VDR-RXR heterodimers, the rexinoids divert RXR proteins from heterodimer formation, instead promoting RXR homodimer formation and reducing thyroid hormone and $1,25(OH)_2D_3$ responsiveness. Altering the binding ligand structure for a given nuclear receptor (NR), especially the RXR master heteropartner ligand, produces specific NR modulators (SNuRMs) that have unique properties that exert novel influences on the NR activity. [8]

RXR selective molecule (rexinoid) SNuRMs have been recent targets for medicinal chemistry, since selective RXR activation versus RAR appears to confer chemotherapeutic effects [8] in a number of cancers without inciting concomitant negative side-effects from RAR interaction. [9] After extensive synthesis [10] of molecules modeled in part on the endogenous 9-*cis* RA (shown below), Ligand Pharmaceuticals, Inc., demonstrated a highly selective RXR agonist, 4-[1-(3,5,5,8,8-pentamethyltetralin-2-yl)ethenyl]benzoic acid (**1**) [11], commonly named bexarotene. A disilabexarotene (**2**) [12]Compound, modeled on **1**

but substituting two silicon atoms for two carbon atoms in the aliphatic ring system, demonstrated similar RXR specific agonism.

Compound **1** has been FDA approved to treat cutaneous T-cell lymphoma (CTCL), has recently been employed off-label to treat lung cancer [13], and has been analyzed as a treatment for breast cancer [14], colon cancer [15] and other uncontrolled cell proliferation diseases, because promoted expression of RXR regulated genes appears to slow or arrest cell proliferation as well as predisposing cancerous cells to apoptosis in the presence of a chemotherapeutic agent. [14] Additionally, compound **1** and several of its analogs have been considered in non-insulin-dependent diabetes mellitus (NIDDM) mouse models. [16] Despite the ability of compound **1** to specifically activate RXR, versus RAR, the primary drawbacks to treatment with **1** include hyperlipidemia, hypothyroidism [17], and cutaneous toxicity. Many of these side-effects occur either because of antagonism of a non-permissive receptor, as in the case of TR for hypothyroidism [18], or agonism of a permissive receptor, as in the cases of LXR for hyperlipidemia [19] and RAR for cutaneous toxicity [20], at the typical dose concentration. Hence, there is ample motivation to explore novel RXR agonists that may attenuate or avoid these side effects.

There are a plethora of demonstrated RXR agonists modeled on **1**. For example, cyclopropyl dienoic acid (**3**) [21], and several novel aza-retinoids, of which compound **4** [22] is representative, in addition to amide retinoids [23]Have all been disclosed in literature. The thiocarbamate bexarotene analog (**5**) [24] induces apoptosis when administered to leukemia HL-60 cells. Substituting pyridine for one of the aromatic rings has led to several analogs of **1**, such as compound **6** [25], and analogs of **1** possessing unsaturation in the aliphatic ring, as in compound **7** [26], have also been reported. Studies describing the development of selective RXR agonists containing aryl-trienoic acid moieties, either alone [27], or locked by one [28] or two [29] ring systems, were disclosed by Boehm et al., for which compound **8** is representative of the latter. Acetal **9** [30] and compound **10** [7] are both selective, potent RXR agonists, with the latter serving as a model to design a potent RXR antagonist. Also, our group recently synthesized an analog of **1** bearing a fluorine atom *ortho* to the carboxylic acid moiety, 2-fluoro-4-(1-(1,2,3,4-tetrahydro-1,1,4,4,6-pentamethylnaphthalen-7-yl)vinyl)benzoic acid (**11**) [31], that demonstrated slightly higher RXR activation in Caco-2 colon cancer cells and possessed a slightly lower K_d than **1**.

Thus, our recent studies have systematically examined by modeling, synthesis, and *in vitro* biological evaluation eight novel analogs of **11** bearing different or additional halogen atoms, exemplified by compounds **12–19**.

Herein, we report the synthesis of compounds **12–19** and evaluate their potency and activity in biologically relevant systems including mammalian 2-hybrid (M2H) assays, RAR- and RXR-response element (RARE and RXRE) transcriptional activation assays in cultured human cell lines, as well as in mutagenicity and apoptosis assays. We have assessed these novel analogs in comparison to compound **1** as well as its ketone analog (**20**).

Results and Discussion

Molecular Modeling

Computational docking studies were performed to help guide the selection of ligands for synthesis. Analysis of the top binders in a large library of bexarotene-like analogs showed a high preference for electron withdrawing groups *ortho* to the carboxylic acid of the phenyl group; nearly all the top binders in the docking studies contain a halogen or a nitro group in this position. Structural comparisons of the docked poses showed that the torsional angle between the phenyl ring and the bridge head changed by ~15 to 30° in the *ortho*-halogenated

compounds compared to the non-halogenated compounds. The effect of this rotation was a strengthening of the interactions between Ile268 and the hydrophobic portion of the ligand, as measured by decreased distances in the poses. Halogenation did not change hydrogen bonding with Arg316, however. The degree of rotation was inversely related to the size of the halogen atom, with fluorine incurring the largest rotation and stabilization of the hydrophobic group, and iodine having a smaller rotation and stabilization. Addition of a second halogen amplified the effect. Replacement of the phenyl group by a heteroatomic ring generally decreased binding by disrupting interactions between the ring and Leu309, Ala271, and Leu326. In the docking studies ligands with methylene and cyclopropane bridge heads were predicted to bind more favorably than ligands with ketone bridge heads. Structural analyses of the docked poses showed that methylene and cyclopropane bridge heads were bound in a small hydrophobic pocket, while the ketone bridge head tended to vacate the hydrophobic pocket, disrupting interactions with the hydrophobic ring system of the ligand. This disruption led to a rotation of the ring system away from Ile268, weakening the hydrophobic interactions with this residue. The hydrophobic ring system was accommodated in a large hydrophobic pocket, with interactions between the ligand and Ile345, Ile268, and Leu436.

Given the highly favorable docking energies of the class of *ortho*-halogenated compounds, and the relative synthetic accessibility of this class, we decided to synthesize and test compounds **12–18**, as well as compounds **19–20**. *Ortho*-halogenated compounds **12–17** were found to be within the best 20 binders for the library, and the binding of compound **18** was also predicted to be more favorable than bexarotene. The calculated relative binding free energy for compounds **12–20** as predicted by the docking studies are shown in Fig. 1. These calculated binding free energies were merely used to score the ligands; reported values are relative to the calculated binding free energy of docked bexarotene, with negative numbers indicating better binding. An example of a low energy docking pose for compound **18** is shown in Fig. 2.

The Chemistry

The synthesis of compounds **12–19** started with the radical di-bromination of commercially available 3-chloro-4-methylbenzoic acid (**21**) and 3-bromo-4-methylbenzoic acid (**22**) to give dibromides **23** and **24**, respectively, which were smoothly converted to aldehydes **25** and **26** upon treatment with silver nitrate in ethanol and water according to the method Kishida et al. reported. [32] We initially envisioned the synthesis of the 3,5-difluoro-formylbenzoic acid (**27**) [33] to follow the same route as used for aldehydes **25** and **26**, beginning with the known 3,5-difluoro-4-methylbenzoic acid. [34]However, we chose to implement a three-step, one-pot Bouveault aldehyde synthesis [35] in which 3,5-difluorobenzoic acid (**28**) was treated with *tert*-butyl lithium, followed by addition of dimethylformamide and hydrolysis with hydrochloric acid to give **27** in 36% yield according to the method of Anderson and co-workers, instead. [33]Continuing with the general method of Kishida et al. [32], the carboxylic acid aldehydes **25–27** were benzyl-protected by treatment with sodium hydride and benzyl bromide to give benzyl ester aldehydes **29–31**, respectively. The ester aldehydes **29–31** were oxidized with sodium chlorite to give the benzyl ester acids **32–34**, respectively, whose carboxylic acid moieties were converted to methyl esters **35–37** by treatment with thionyl chloride followed by methanol. [32] The benzyl esters of **35–37** were converted to carboxylic acids **38–40** by treatment with 10% palladium on carbon and hydrogen, however, while **35** and **36** were converted smoothly to **38** and **39** at room temperature and atmospheric pressure hydrogen, benzyl ester **37** required a higher temperature of 70 °C and higher pressure of hydrogen (10–15 bar) that was safely achieved [36] to give quantitative yield of **40**. The carboxylic acids **38–40** were then quantitatively converted to the acid chlorides **41–43** with thionyl chloride (Scheme 1). [32]

A slightly different route was taken to prepare the iodinated analogs **16** and **17**. 4-(Methoxycarbonyl)-3-aminobenzoic acid (**44**) was treated with sodium nitrite and hydrochloric acid followed by potassium iodide to give 4-(methoxycarbonyl)-3-iodobenzoic acid (**45**) [37] in 77% yield, and acid **45** was converted to acid chloride **46** with thionyl chloride (Scheme 2).

Finally, 2,5-dimethyl-2,5-hexanediol was converted to 2,5-dichloro-2,5-dimethylhexane (**47**) [38],[11] in 73% yield by treatment with concentrated hydrochloric acid, and the dichloride **47** was reacted with toluene and catalytic aluminum chloride to give 1,2,3,4-tetrahydro-1,1,4,4,6-pentamethylnaphthalene (**48**) [11], [31] in 94% yield.

With compound **48** in hand, acid chlorides **41–43** and **46** were converted to ketones **49–52** according to the general method of Boehm and co-workers. [11] Iodo-ketone methyl-ester **51** was observed to possess a small percentage (~7%) of the chloro-ketone methyl-ester **49** by ^{1}H-NMR analysis, and the iodine atom may be labile under reflux conditions with aluminum trichloride. Compounds **49–52** were either saponified with potassium hydroxide in methanol, followed by acidic work-up, to give analogs **13, 15, 17**, and **19** respectively, or they were treated with triphenylphosphine methylide to give alkenes **53–56**, respectively. The bromo-ketone **15** also possessed minor amounts of unidentifiable impurities that could not be removed by chromatography or crystallization. Alkenes **53–56** were then saponified with potassium hydroxide in methanol, followed by acidic work-up, to give analogs **12, 14, 16**, and **18**, respectively (Scheme 3). While the iodo-ketone acid **17** contained ~9% of the chloro-ketone acid **13** and could not be purified by column chromatography or attempted crystallization, the minor amount of chloro-alkene acid **12** could be removed from iodo-alkene **16** by crystallization from ethyl acetate.

The X-ray crystal structures of compounds **12, 14**, and **16** are shown in Figure 3.

The X-ray crystal structures of compounds **18** and **52** are shown in Figure 4.

Biological Assays and Rationale

A Mammalian Two-Hybrid Assay Demonstrates that Several Novel Analogs bind RXR in an Agonist Fashion

Biological evaluation of the synthetic analogs described above (compounds **12–19**) was first carried out in a mammalian two-hybrid assay in human colon cancer (Caco-2) cells then repeated in a different colorectal carcinoma line (HCT-116) (Figure 5 a) and b), respectively). This assay tests the ability of the analog to bind to the recombinant human RXR receptor and induce homodimerization as measured by luciferase output.

Four compounds were identified in this initial evaluation whose agonist activity ranged from 20 to 400% of the binding of compound **1** in the two separate cell lines. More specifically **12, 14**, and **16** are able to bind and mediate homodimerization with about 80, 60, and 20% respectively of the parent compound's efficiency independent of the cell line. They were also found to be significantly better than the ethanol control vehicle (P values of < 0.05 for all, using one-tailed heteroscadastic t test). Compound **18**, however, was found to induce receptor binding and homodimerization better than that of the parent compound **1** to a degree considered statistically significant. Moreover, in comparison to compound **1, 18** was about 1.5 times more potent in its ability to induce RXR homodimerization than **1** in the Caco-2 cells and about 3.5 times better in HCT-116 cells (P values of < 0.05 for all, using one-tailed heteroscedastic t test). These results further support that compounds modeled after **1** can be successfully synthesized to possess RXR binding ability [31] and agonistic properties.

Novel Analogs of 1 are able to direct RXR/RXRE Homodimer Mediated Transcription

The mammalian two-hybrid assay is useful as an initial screen for RXR agonist induced homodimerization because of its accessibility, speed and sensitivity. However, because this assay employs a system that utilizes a synthetic binding domain (BD) and synthetic activating domain (AD), it is possible that within this artificial context the RXR-agonist complex may have an altered ligand or transcriptional coactivator affinity. Thus, it is important to test our collection of potential RXR agonists for the ability to mediate transcription in a more natural setting. Hence, a second screening protocol included the transfection of both Caco-2 and HCT-116 cells with human RXRα and an authentic RXRE from the naturally occurring responsive element in the rat cellular retinol binding protein II gene [39] linked to a luciferase reporter gene. The results in Figure 6 demonstrate that compounds **12, 14, 18**, and **19** are able to activate RXR homodimer-mediated transcription significantly better than that of the ethanol vehicle (for compounds **12, 14**, and **18** in Caco-2 cells, using a one-tailed heteroscedastic *t* test *P* values < 0.05; for these compounds in HCT-116 cells using a one-tailed heteroscedastic *t* test *P* values < 0.01; and for compound **19** in both cell lines, using a one-tailed heteroscadastic *t* test *P* value < 0.01).

In the Caco-2 cells, **16** was a weak agonist, while **17** was not able to activate transcription when compared to ethanol, but both analogs did display significant activity in the HCT-116 colorectal carcinoma (using a one-tailed heteroscedastic *t* test *P* value for **16** < 0.01 and <0.001 for **17**).

Importantly, compounds **12, 14, 16** and **18** displayed activity in both the mammalian two-hybrid assay (Fig. 5) and the RXRE assay (Fig. 6), thus revealing that both assays are not only valid, but generate consistent and complementary data when evaluating RXR agonists.

Determination of Ligand-Receptor Binding Affinity of the Most Active Compounds

To determine the relative binding affinity and effectiveness of our most active analogs, a mammalian two-hybrid assay was performed with ligand concentrations ranging from 10×10^{-10} M up to 0.5×10^{-5} M and EC50 values were calculated (Table 1). Notably, the difluorobexarotene analog (**18**) has an EC_{50} value of 34 +/− 6 nM in HCT116 cells versus an EC_{50} value of 55 +/− 6 nM for bexarotene (**1**). All of the other analogs possess EC_{50} values that mirror the results of the mammalian two-hybrid analysis in Figure 5, and the RXRE-based assays in Figure 6.

Analysis of RAR Agonist Activity by the Most Promising Compounds

Since compound **1** is known to have some “residual” retinoic acid receptor (RAR) agonist activity [31] we evaluated this group of analogs for activation of this closely related nuclear receptor. This assay utilizes the expression of human RAR and a retinoic acid responsive element (RARE)-luciferase reporter gene, and it demonstrates (Table 2) that compounds **12, 14**, and **18** are comparable to **1** (*P* value > 0.05 for all, using a one-tailed heteroscedastic *t* test). Table 2 also shows that **16** and **19** possess significantly lower RAR binding affinity (*P* value < 0.05 for both, using a one-tailed heteroscedastic *t* test). All-*trans* retinoic acid was used as the positive control in this experiment because it is the endogenous ligand for RAR. Taken together, these results not only further support our previous findings [31] that variation of **1** with a halogen atom on the aromatic that bears the carboxylic acid may reduce the activation of RAR (analogs **16** and **19**) or increase its capacity to bind and activate RXR (compound **18**), but they also strongly suggest a specific periodic trend for the substitution of a proton that may relate to the analog’s degree of agonist activity.

Apoptosis in a CTCL System

As previously reported [31], bexarotene and several of its analog are capable of inducing apoptosis. We were interested in determining the pro-apoptotic ability of these new analogs in comparison to compound **1**. Since compound **1** has been effectively employed in the treatment of CTCL because it induces apoptosis in the T-lymphocyte, we assayed HuT-78 lymphocytes treated with compound **1** or analogs for caspase 3 and 7 activity, a hallmark of apoptosis, and compared caspase activity to cells treated with ethanol vehicle and sodium butyrate (apoptotic positive control) (Figure 7). Similar to previously published results [31], bexarotene (**1**) and all of the analogs were better than vehicle control at inducing apoptosis (P values of < 0.01 using one-tailed heteroscadastic t test), after a 48 hour treatment period in our CTCL cells.

Conclusions

Here we report the modeling, synthesis, and biological evaluation of several analogs of compound **1**, in an extension of our earlier work. [31] We have shown that the addition of two fluorine atoms *ortho* to the carboxylic acid of **1** increases the ability of analog **18** to activate RXR. We have identified several new halogenated analogs of **1** that have apparent binding and biological activity similar to the parent compound, and even follow a periodic trend, with one (**18**) that capitalizes on the finding that adding one fluorine *ortho* to the carboxylic acid of **1** increases binding and activation of RXR. [31] With the fact that several RXR selective agonists are being explored to treat many conditions through biological pathways impacted by RXR, now including the demonstrated up-regulation of the apoE gene and the facilitated clearance of plaques by **1** in mouse models of Alzheimer's Disease [40], there is motivation to develop new RXR agonists. Our results indicate that novel analogs of **1** that substitute halogen atoms on the aromatic ring bearing the carboxylic acid can likely serve as effective, and perhaps more potent, ligands for RXR, which may also have reduced RAR agonist activity (Table 2); thus, these compounds may also possess less detrimental side-effects in cutaneous T-cell lymphoma patients.

Experimental Section

Docking Studies

Docking studies using Autodock 4.2 [41] were performed using the X-ray structures of human RXRα in complex with BMS 649 [42] and RXRβ in complex with LG100268 [43], protein data bank access codes 1MVC and 1H9U, respectively. In both cases, the Arg316 and Ile268 protein residues (numbering as in 1MVC) were treated flexible. Arg316 was selected to enable hydrogen bonding with carboxylate groups on the phenyl moiety, an important interaction identified in earlier studies. [11], [31] Ile268 was selected since structural overlays of 33 different ligand-bound RXR structures in the protein data bank showed large motions of Ile268, and in all cases Ile268 made significant interactions with hydrophobic portions of the ligands. Atomic charges generated by AutoDockTools [41] were frequently overpolarized for nitro groups (leading to a net negative charge on the nitro), while charges generated by OpenBabel 2.3.0 [44] did not have this artifact. Therefore, all docks were performed with both charge models, and results for nitro-compounds were omitted for the AutoDockTools-generated charges. Docking was performed with the Lamarckian genetic algorithm using a maximum of 25,000,000 energy evaluations per docking. The number of docks was set to 250 for three torsions; this number was increased by fifty for each additional torsion, up to a maximum of 400 for six or more torsions. The docking was performed with a large library of bexarotene-like analogs. These analogs differed in substituents on the phenyl ring (including halogenated, carboxylated, hydroxylated and nitrated rings) and fused phenyl ring (including methylated, aminated,

nitrated and halogenated rings), as well as changes in the identity of the bridge head between the ring systems (including methylene, ketone, and cyclopropane bridge heads, as well as the absence of a bridge head), and the identity of the aliphatic part of the fused ring system. Calculated AutoDock binding free energies were used to score the ligands.

Mammalian Two-Hybrid Assay

Caco-2 colorectal carcinoma cells were plated overnight at 70,000 cells/well in a 24 well plate and kept in minimum essential media (MEM) (Invitrogen, Carlsbad CA) enhanced with 20% fetal bovine serum (FBS) (Invitrogen), 1 mM sodium pyruvate (Invitrogen), 100 μg/mL streptomycin, and 100 unit/mL penicillin. HCT-116 colorectal carcinoma cells were plated overnight at 70,000 cells/well in a 24 well plate and kept in DMEM enhanced with 10% FBS (Invitrogen), 1 mM sodium pyruvate (Invitrogen), 100 μg/mL streptomycin, and 100 unit/mL penicillin. Both cell lines were co-transfected using a human RXR binding domain vector, a hRXR activation domain (AD), a luciferase reporter gene containing BD-binding sites, and a renilla control plasmid. A liposome-mediated transfection was completed according to the manufacturer's protocol using 2 μL/well of Express-In transfection reagent (Thermo Fisher Scientific, Lafayette, CO) and allowed to incubate for 7 hours. The cells were then treated with ethanol vehicle, **1**, or analogs at a final concentration of 10^{-7} M and incubated for 24 hours. The amount of rexinoid activity was measure by luciferase output utilizing a dual-luciferase reporter assay system according to the manufacturer's protocol (Promega, Madison, WI) in a Sirus FB12 luminometer (Berthold Detection Systems, Zylux Corporation, Huntsville, AL). Several independent assays were conducted with triplicate samples for each treatment group.

RXRE-Mediated Transcription Assay

The RXRE assays were completed using both Caco-2 and HCT-116 cells plated at 70,000 cells/well in a 24 well plate and maintained as described above. The cells were cotransfected using 250 ng of RXRE-luciferase reporter gene (RXRE from the naturally occurring responsive element in the rat cellular retinol binding protein II gene), 20 ng of the renilla control plasmid, 50 ng of human pSG5-RXRα, and 100 ng pTZ18U carrier DNA plasmid and 2 μL/well of Express-In was again used for the liposome mediated delivery. The cells were incubated for 7 hours post-transfection and then treated with ethanol, or 10^{-7} M of either the parent compound or the indicated analog. After a 24-hour incubation period the amount of retinoid activity was measured using the same luciferase assay described above.

RAR/RARE-Agonist Activity Assay

An embryonic kidney cell line, HEK-293, was used for the RARE transcription assay. The cells were plated at a concentration of 70,000 cells/well and maintain as described above. The cells were allowed to incubate over night to ensure attachment to the plate surface. The transfection protocol called for 20 ng of renilla null control plasmid to monitor transfection efficiency, 30 ng of pTZ18U carrier DNA plasmid, 50 ng of the pCMX-human RARα expression vector, and 250 ng of pTK-DR5(X2)-Luc plasmid. Specifics pertaining to this RARE-containing reporter vector have been described previously as well as the sequence of the double RARE. [45] The cells were incubated for 7 hours and then treated with ethanol, all-trans retinoic acid, or analogs at final concentration ranging from 10^{-6} M to 10^{-7} M for 24 hours. After the incubation period cell were lysed and the same luciferase assay was completed that was previously described.

Apoptosis Assay

Apoptotic activity was assessed by the Caspase-Glo® 3/7 Assay (Promega, Madison, WI) according to the manufacturer's instructions. The Caspase-Glo ® 3/7 Assay is based on the

cleavage of the DEVD sequence of a luminogenic substrate by caspases 3 and 7 which results in a luminescent signal. HuT-78 (human T-cell lymphoma) cells were distributed (1×10^4 Cells/well) in white-walled 96-well microplates (Corning, NY) in 100 μl of medium and incubated with 500 μM sodium butyrate (NaBu), 10 μM bexarotene (Bex) or rexinoid analogs for 24 and 48h. The Caspase-Glo® 3/7 Reagent was then added to each well and incubated for an additional 1 h at room temperature. The luminescence was measured in a luminometer (Safire2, Tecan, US). NaBu, a known inducer of apoptosis in HuT-78, was used as a positive control. Each treatment group was dosed in triplicate, and at least two independent experiments were performed. Numbers were standardized to sodium butyrate (set at 100%).

Mutagenicity

We tested the compounds for mutagenicity as in Wagner *et al.* [31] None of the compounds are mutagenic. This assay utilized a yeast strain, D7, that is genetically engineered to change phenotype upon a genotype change. [46] We used this strain to test the mutagenicity of the compounds by solubilizing the compounds in DMSO and performing a dose/response curve with the highest concentration being 0.15% w/v comparing to DMSO control for mutagenicity, scored as a phenotype change on agar plates. [47]

Instrumentation

A 400 MHz Bruker spectrometer was used to acquire ^{1}H NMR and ^{13}C NMR spectra. Chemical shifts (δ) are listed in ppm against residual non-deuterated solvent peaks in a given deuterated solvent (e.g. $CHCl_3$ in $CDCl_3$) as an internal reference. Coupling constants (*J*) are reported in Hz, and the abbreviations for splitting include: s, single; d, doublet; t, triplet; q, quartet; p, pentet; m, multiplet; br, broad. All ^{13}C NMR spectra were acquired on a Bruker instrument at 100.6 MHz. Chemical shifts (δ) are listed in ppm against deuterated solvent carbon peaks as an internal reference. High resolution mass spectra were recorded using either a JEOL GCmate(2004), a JEOL LCmate(2002) high resolution mass spectrometer or an ABI Mariner (1999) ESI-TOF mass spectrometer. HPLC traces were obtained on an Agilent 1100 LC with a Phenomenex Kinetex C18 10 cm by 2.1 mm column with 2.6u solid core particles.

General Procedures

Tetrahydrofuran, methylene chloride, diethyl ether, and benzene were dried by filtration through alumina according to the procedure described by Grubbs. [48] 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.

Benzyl-3-Chloro-4-formylbenzoate (29)

Compound **29** was synthesized according to the methods of Kishida and co-workers. [32] To a 500 mL round bottom flask charged with 3-chloro-4-methylbenzoic acid (**21**) (3.24 g, 19.0 mmol) was added NBS (8.00 g, 44.9 mmol), benzoylperoxide (0.23 g, 0.95 mmol), and carbon tetrachloride (37 mL). The reaction solution was heated to reflux under magnetic stirring for 36 h, cooled to room temperature, and solids were filtered and washed with carbon tetrachloride (~20 mL). The filtrate solvent was removed *in vacuo* and the crude 4-(dibromomethyl)-3-chlorobenzoic acid (**23**) was dried on high vacuum and used without

further purification. To a 500 mL round bottom flask charged with crude **23** (6.23 g, 19.0 mmol) was added ethanol (48 mL), and a solution of silver nitrate (6.64 g, 39.1 mmol) in warm water (9 mL) was added dropwise while the reaction solution was stirred in an oil bath preheated to 50–55 °C. Upon addition of the silver nitrate solution, a green precipitate formed. After stirring at 50 °C for 45 min, the reaction solution was cooled to room temperature and filtered to remove the green precipitate. The filtrate solvent was concentrated in vacuo, extracted with ethyl acetate, and the combined organic extracts were washed with water and brine, dried over sodium sulfate and removed in vacuo to give crude 3-chloro-4-formylbenzoic acid (**25**) (3.51 g, 100%) that was used without further purification. To a 500 mL round bottom flask charged with **25** (3.51 g, 19.0 mmol) was added dry dimethylformamide (37 mL) and a 60 wt % suspension of NaH in mineral oil (0.93 g, 23 mmol) in small aliquots over 20 min. The reaction solution was stirred an additional 20 min, and benzylbromide (2.8 mL, 23 mmol) was added to the red heterogeneous solution. After stirring 5 h, the reaction solution had become homogeneous, and it was poured into 1N HCl (100 mL), extracted with ethyl acetate (100 mL, twice), and the combined organic extracts were washed with saturated $NaHCO_3$ (75 mL) and brine, dried over sodium sulfate, and removed in vacuo to give crude **29**. Crude **29** was purified by column chromatography (150 mL SiO_2, hexanes:ethyl acetate 4:1) to give **29** (5.2 g, 99%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.52 (s, 1H), 8.15 (dd, J = 8.0, 1.6, 1H), 7.97–8.04 (m, 2H), 7.37–7.48 (m, 5H), 5.39 (s, 2H); IR (neat): ν = 2968, 2855, 2656, 2560, 1724, 1687, 1605, 1556, 1485 cm^{-1} ; GC-MS [M^+] calcd for $C_{15}H_{11}O_3Cl$: 274.0397, found: 274.0390.

4-((Benzyloxy)carbonyl)-2-chlorobenzoic Acid (32)

Compound **32** was prepared according to the method of Kishida and co-workers. [32] To a 100 mL round bottom flask charged with **29** (5.22 g, 19.0 mmol), sulfamic acid (1.86 g, 19.2 mmol), water (20 mL), and ACN (15 mL) was added a solution of 80% $NaClO_2$ (1.79 g, 19.8 mmol) in water (10 mL). After stirring for 1 h, the reaction solution was poured into saturated Na_2SO_3 (25 mL) and 1N HCl (50 mL), and the resulting solution was extracted with ethyl acetate (50 mL, thrice). The combined organic extracts were washed with brine, dried over sodium sulfate, and removed in vacuo to give crude **32** (4.97 g, 90%) that was used without further purification. A small sample was purified by column chromatography (25 mL SiO_2, hexanes:ethyl acetate 1:1) to give pure **32** as a white powder, m.p. 120–122 °C: ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.71 (br s, 1H), 8.17 (d, J = 1.6, 1H), 8.05 (d, J = 8.0, 1H), 8.02 (dd, J = 8.0, 1.6, 1H), 7.36–7.47 (m, 5H), 5.40 (s, 2H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 170.1, 164.4, 135.2, 134.8, 134.7, 132.4, 132.3, 132.2, 128.7, 128.6, 127.6, 67.6; IR (neat): ν = 2964, 1724, 1685, 1603, 1557, 1484, 1455 cm^{-1} ; LC-FAB-MS [M^+ +H] calcd for $C_{15}H_{12}O_4Cl$: 291.0424, found: 291.0434.

4-Benzyl 1-methyl 2-chlorobenzene-1,4-dioate (35)

Compound **35** was synthesized according to the method of Kishida and co-workers. [32] To a 100 mL round bottom flask charged with compound **32** (5.5 g, 18.9 mmol) was added $SOCl_2$ (16.0 mL, 220 mmol) and the reaction solution was refluxed for 1h in an oil bath at 84 °C. The reaction solution was cooled to room temperature and the excess thionyl chloride was removed *in vacuo* to give crude 4-chlorocarbonyl-2-chlorobenzoic acid benzyl ester. The crude 4-chlorocarbonyl-2-chlorobenzoic acid in dry toluene (8 mL) was added dropwise to a solution of triethylamine (5.2 mL, 37 mmol) in methanol (53 mL, 1.3 mol) over 10 min. The reaction solution was stirred 1h and poured into 1N HCl (150 mL) and extracted with ethyl acetate (80 mL, thrice). The combined organic extracts were washed with brine, dried over sodium sulfate, and removed in vacuo to give crude **35**. Crude **35** was purified by column chromatography (150 mL SiO_2, hexanes:ethyl acetate 95:5) to give pure **35** as a white solid (5.05 g, 87%), m.p. 46–48 °C: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (d, J = 1.6,

1H), 7.98 (dd, *J* = 8.0, 1.6, 1H), 7.85 (d, *J* = 8.0, 1H), 7.36–7.46 (m, 5H), 5.38 (s, 2H), 3.95 (s, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 165.5, 164.4, 135.3, 134.0, 133.8, 133.7, 132.0, 131.2, 128.7, 128.6, 128.4, 127.6, 67.4, 52.7; IR (neat): ν = 3033, 2958, 1735, 1716, 1560, 1498, 1482 cm^{-1}; GC-MS [M^+] calcd for $C_{16}H_{13}O_4Cl$: 304.0502, found: 304.0498.

4-(Methoxycarbonyl)-3-chlorobenzoic Acid (38)

Compound **38** was synthesized according to the methods of Kishida and co-workers. [32] A 3-neck 250 mL round bottom flask charged with **35** (4.94 g, 16.2 mmol), 10% Pd/C (0.499 g), ethanol (28.0 mL), and ethyl acetate (28.0 mL) was evacuated and back-filled with hydrogen gas from a balloon three times, and the reaction solution was allowed to stir under hydrogen at room temperature overnight. The reaction solution was filtered through celite, and the solvents were removed in vacuo to give crude **38** (2.97 g, 85%) as a white crystalline solid that was used without further purification. A small sample of crude **38** was purified by recrystallization from hot ethyl acetate to give pure **38**, m.p. 156–157 °C: ^{1}H NMR (400 MHz, DMSO-d6) δ 13.64 (br s, 1H), 7.99 (d, *J* = 1.6, 1H), 7.95 (dd, *J* = 8.0, 1.6, 1H), 7.89 (d, *J* = 8.0, 1H), 3.89 (s, 3H); ^{13}C NMR (100.6 MHz, DMSO-d6) δ 165.4, 165.1, 134.9, 133.7, 131.8, 131.2, 130.9, 128.0, 52.8; IR (neat): ν = 2961, 2824, 2545, 1717, 1687, 1557, 1488 cm^{-1}; LC-APCI-MS [M^+] calcd for $C_9H_7O_4Cl$: 214.0033, found: 214.0027.

Methyl 2-chloro-4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)carbonyl)benzoate (49)

Compound **49** was synthesized according to the method of Boehm and co-workers. [11] Methyl 4-(chlorocarbonyl)-2-chlorobenzoate (**41**) was synthesized by refluxing 4-(methoxycarbonyl)-3-chlorobenzoic acid (**38**) (1.35 g, 6.29 mmol) in thionyl chloride (10.0 mL, 137 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 **41** as an off-white solid, and this solid was dissolved in dry benzene (ca. 20 mL) and evaporated to dryness three times to remove residual thionyl chloride. The acid chloride **41** 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 **48** (1.38 g, 6.82 mmol) followed by a solution of crude acid chloride **41** (6.29 mmol) in DCM (15 mL). Aluminum chloride (2.0 g, 15 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 **49**. Crude **49** was purified by column chromatography (250 mL SiO_2, hexanes:ethyl acetate 95:5 to 92.5:7.5) to give **49** (2.22 g, 88%) as a white, crystalline solid, m.p. 97–98 °C: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (d, *J* = 8.0, 1H), 7.88 (d, *J* = 1.6, 1H), 7.70 (dd, *J* = 8.0, 1.6, 1H), 7.25 (s, 1H), 7.21 (s, 1H), 3.96 (s, 3H), 2.35 (s, 3H), 1.69 (s, 4H), 1.31 (s, 6H), 1.20 (s, 6H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ196.0, 165.7, 148.8, 142.0, 141.8, 134.8, 133.9, 133.7, 133.3, 132.3, 131.1, 129.6, 128.6, 127.8, 52.7, 34.8, 34.7, 34.3, 33.8, 31.6, 31.5, 20.0; IR (neat): ν = 2961, 2864, 1738, 1665, 1547, 1484 cm^{-1}; GC-MS [M^+] calcd for $C_{24}H_{27}O_3Cl$: 398.1649, found: 398.1657.

Methyl 2-chloro-4-(1-(1,2,3,4-tetrahydro-1,1,4,4,6-pentamethylnaphthalen-7-yl)vinyl)benzoate (53)

Compound **53** was synthesized according to the method of Boehm and co-workers. [11] To a 20-dram vial containing **49** (0.830 g, 2.08 mmol) and dry THF (3 mL) at room temperature with a Teflon magnetic stir-bar was slowly added a triphenylphosphonium methylide solution prepared as follows: to a 100 mL round bottom flask equipped with a Teflon magnetic stir-bar and containing dry THF (2.0 mL) was added $^{i}Pr_2NH$ (0.66 mL, 4.67 mmol) and a 2.5M solution of n-butyl lithium in hexanes (1.7 mL, 4.25 mmol), and the solution was stirred for 30 min at room temperature at which point, methyl triphenylphosphonium bromide (1.13 g, 3.19 mmol) was added and the solution was stirred an additional 20 min to provide a homogeneous dark yellow ylide solution. The reaction was monitored by TLC, and when the reaction was judged to be complete, the reaction solution was poured into water (50 mL) and the aqueous solution was extracted with ethyl acetate (50 mL, twice). The combined organic extracts were washed with water and brine, dried over sodium sulfate, and concentrated in vacuo to give crude **53** which was purified by column chromatography (25 mL SiO_2, hexanes:ethyl acetate 97.5:2.5) to give **53** (0.283 g, 34%) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, *J* = 8.0, 1H), 7.39 (d, *J* = 1.6, 1H), 7.18 (dd, *J* = 8.0, 1.6, 1H), 7.10 (s, 1H), 7.09 (s, 1H), 5.80 (d, *J* = 1.2, 1H), 5.35 (d, *J* = 1.2, 1H), 3.92 (s, 3H), 1.95 (s, 3H), 1.70 (s, 4H), 1.31 (s, 6H), 1.27 (s, 6H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 165.9, 147.9, 145.7, 144.6, 142.4, 137.2, 133.9, 132.6, 131.5, 128.9, 128.2, 128.1, 128.0, 124.8, 117.7, 52.3, 35.1, 35.0, 33.9, 33.8, 31.9, 31.8, 19.9; IR (neat): ν = 2955, 2924, 2860, 1734, 1714, 1598, 1542, 1497, 1456 cm^{-1}; GC-MS [M^+] calcd for $C_{25}H_{29}O_2Cl$: 396.1856, found: 396.1850.

2-Chloro-4-(1-(1,2,3,4-tetrahydro-1,1,4,4,6-pentamethylnaphthalen-7-yl)vinyl)benzoic acid (12)

Compound **12** was synthesized following the methods of Boehm and co-workers. [11] To a 100 mL round bottom flask charged with **53** (0.353 g, 0.89 mmol) and methanol (5 mL) was added a 5M aqueous solution of potassium hydroxide (0.4 mL, 2.0 mmol). A reflux condenser was fitted to the round bottom flask and the reaction solution was refluxed and monitored by TLC. After 1 h at reflux, the reaction solution was cooled to room temperature and quenched with 20% HCl (42 mL). The aqueous solution was extracted with ethyl acetate (50 mL, twice) and the organic extracts were combined, washed with water and brine, dried over sodium sulfate, and concentrated in vacuo to give crude **12**. Crude **12** was purified by column chromatography (25 mL SiO_2, hexanes:ethyl acetate 9:1) to give **12** (0.31 g, 91%) as a white crystalline solid, m.p. 200–201 °C: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, *J* = 8.0, 1H), 7.43 (d, *J* = 1.6, 1H), 7.21 (dd, *J* = 8.0, 1.6, 1H), 7.11 (s, 1H), 7.09 (s, 1H), 5.83 (d, *J* = 0.8, 1H), 5.38 (d, *J* = 0.8, 1H), 1.96 (s, 3H), 1.71 (s, 4H), 1.31 (s, 6H), 1.28 (s, 6H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 170.5, 147.8, 146.8, 144.7, 142.5, 137.1, 135.0, 132.6, 132.5, 129.3, 128.2, 128.0, 126.5, 124.9, 118.2, 35.1, 34.0, 33.9, 31.9, 31.8, 19.9; IR (neat): ν = 2958, 1694, 1596, 1488 cm^{-1}; LC-APCI-MS [M^++H] calcd for $C_{24}H_{28}O_2Cl$: 383.1778, found: 383.1778. Anal. Calcd for $C_{24}H_{27}O_2Cl$: C 75.28; H 7.11; Cl 9.26. Found: C 75.20; H 6.93; Cl 9.60.

2-Chloro-4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)carbonyl)benzoic acid (13)

Compound **13** was synthesized following the method of Boehm and co-workers. [11] To a 100 mL round bottom flask charged with **49** (0.353 g, 0.88 mmol) and methanol (4 mL) was added a 5M aqueous solution of potassium hydroxide (0.4 mL, 2.0 mmol). A reflux condenser was fitted to the round bottom flask and the reaction solution was refluxed and monitored by TLC. After 1 h at reflux, the reaction solution was cooled to room temperature

and quenched with 20% HCl (20 mL). The precipitate was filtered and washed with water to give crude **13** (0.335 g, 98%). Crude **13** was purified by column chromatography (25 mL SiO_2, hexanes:ethyl acetate 9:1) to give **13** (0.13 g, 40%) as a white crystalline solid, m.p. 174–175 °C: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, *J* = 8.0, 1H), 7.92 (d, *J* = 1.6, 1H), 7.74 (dd, *J* = 8.0, 1.6, 1H), 7.27 (s, 1H), 7.23 (s, 1H), 2.37 (s, 3H), 1.70 (s, 4H), 1.32 (s, 6H), 1.22 (s, 6H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 196.0, 169.9, 149.0, 142.6, 142.1, 134.9, 134.7, 133.7, 132.7, 132.1, 131.6, 129.7, 128.7, 127.9, 34.8, 34.7, 34.4, 33.9, 31.6, 31.5, 20.1; IR (neat): ν = 2955, 2926, 1704, 1667, 1543, 1458 cm^{-1}; LC-APCI-MS $[M^+ + H]$ calcd for $C_{23}H_{26}O_3Cl$: 385.1571, found: 385.1564. Anal. Calcd for $C_{23}H_{25}O_3Cl$: C 71.77; H 6.55; Cl 9.21. Found: C 71.45; H 6.30; Cl 9.2.

Benzyl-3-bromo-4-formylbenzoate (30)

Compound **30** was synthesized according to the methods of Kishida and co-workers. [32] To a 500 mL round bottom flask charged with 3-chloro-4-methylbenzoic acid (**22**) (8.17 g, 38.0 mmol) was added NBS (16.10 g, 90.45 mmol), benzoylperoxide (0.45 g, 1.8 mmol), and carbon tetrachloride (74 mL). The reaction solution was heated to reflux under magnetic stirring for 36 h, cooled to room temperature, and solids were filtered and washed with carbon tetrachloride (~20 mL). The filtrate solvent was removed *in vacuo* and the crude 4-(dibromomethyl)-3-bromobenzoic acid (**24**) was dried on high vacuum and used without further purification. To a 500 mL round bottom flask charged with crude **24** (14.16 g, 38.0 mmol) was added ethanol (96 mL), and a solution of silver nitrate (13.28 g, 78.18 mmol) in warm water (18 mL) was added dropwise while the reaction solution was stirred in an oil bath preheated to 50–55 °C. Upon addition of the silver nitrate solution, a green precipitate formed. After stirring at 50 °C for 45 min, the reaction solution was cooled to room temperature and filtered to remove the green precipitate. The filtrate solvent was concentrated in vacuo, extracted with ethyl acetate, and the combined organic extracts were washed with water and brine, dried over sodium sulfate and removed in vacuo to give crude 3-bromo-4-formylbenzoic acid (**26**) (8.7 g, 100%) that was used without further purification. To a 500 mL round bottom flask charged with **26** (8.7 g, 38.0 mmol) was added dry dimethylformamide (74 mL) and a 60 wt % suspension of NaH in mineral oil (1.86 g, 46.5 mmol) in small aliquots over 20 min. The reaction solution was stirred an additional 20 min, and benzylbromide (5.60 mL, 46.8 mmol) was added to the red heterogeneous solution. After stirring 5 h, the reaction solution had become homogeneous, and it was poured into 1N HCl (200 mL), extracted with ethyl acetate (100 mL, twice), and the combined organic extracts were washed with saturated $NaHCO_3$ (75 mL) and brine, dried over sodium sulfate, and removed in vacuo to give crude **30**. Crude **30** was purified by column chromatography (150 mL SiO_2, hexanes:ethyl acetate 4:1) to give **30** (12.1 g, 99%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.40 (d, *J* = 0.8, 1H), 8.33 (d, *J* = 1.6, 1H), 8.10–8.08 (ddd, *J* = 0.8, 1.6, 8.0, 1H), 7.95 (d, *J* = 8.0 5H), 7.37–7.47 (m, 5H), 5.39 (s, 2H); IR (neat): ν = 2962, 1723, 1683, 1601, 1556, 1479 cm^{-1}; GC-MS $[M^+]$ calcd for $C_{15}H_{11}O_3Br$: 317.9892, found: 317.9887.

4-((Benzyloxy)carbonyl)-2-bromobenzoic Acid (33)

Compound **33** was prepared according to the method of Kishida and co-workers. [32] To a 100 mL round bottom flask charged with **30** (12.1 g, 37.9 mmol), sulfamic acid (4.06 g, 41.8 mmol), water (45 mL), and ACN (32 mL) was added a solution of 80% $NaClO_2$ (3.84 g, 42.5 mmol) in water (20 mL). After stirring for 1 h, the reaction solution was poured into saturated Na_2SO_3 (50 mL) and 1N HCl (100 mL), and the resulting solution was extracted with ethyl acetate (100 mL, thrice). The combined organic extracts were washed with brine, dried over sodium sulfate, and removed in vacuo to give crude **33** (10.5 g, 82%) that was used without further purification. A small sample was purified by column chromatography (25 mL SiO_2, hexanes:ethyl acetate 1:1) to give pure **33** as a white powder: ^{1}H NMR (400

MHz, $CDCl_3$) δ 8.37 (d, *J* = 1.6, 1H), 8.08 (dd, *J* = 8.0, 1.6, 1H), 8.01 (d, *J* = 8.0, 1H), 7.34–7.47 (m, 5H), 5.39 (s, 2H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 170.3, 164.2, 135.6, 135.2, 134.4, 134.2, 132.1, 128.7, 128.6, 128.4, 128.2, 122.3, 67.6; IR (neat): ν = 2963, 2538, 1681, 1602, 1551, 1481 cm^{-1}; LC-APCI-MS $[M^+ + H]$ calcd for $C_{15}H_{12}O_4Br$: 334.9919, found: 334.9934.

4-Benzyl 1-methyl 2-bromobenzene-1,4-dioate (36)

Compound **36** was synthesized according to the method of Kishida and co-workers. [32] To a 100 mL round bottom flask charged with compound **33** (10.5 g, 31.3 mmol) was added $SOCl_2$ (24.0 mL, 330 mmol) and the reaction solution was refluxed for 1h in an oil bath at 84 °C. The reaction solution was cooled to room temperature and the excess thionyl chloride was removed *in vacuo* to give crude 4-chlorocarbonyl-2-bromobenzoic acid benzyl ester. The crude 4-chlorocarbonyl-2-bromobenzoic acid in dry toluene (16 mL) was added dropwise to a solution of triethylamine (10.4 mL, 74 mmol) in methanol (106 mL, 2.6 mol) over 10 min with vigorous stirring. The reaction solution was stirred 1h and poured into 1N HCl (300 mL) and extracted with ethyl acetate (80 mL, thrice). The combined organic extracts were washed with brine, dried over sodium sulfate, and removed in vacuo to give crude **36**. Crude **36** was purified by column chromatography (150 mL SiO_2, hexanes:ethyl acetate 95:5) to give pure **36** as an oil (8.55 g, 78%): ^1H NMR (400 MHz, $CDCl_3$) δ 8.32 (d, *J* = 1.6, 1H), 8.03 (dd, *J* = 8.0, 1.6, 1H), 7.80 (d, *J* = 8.0, 1H), 7.36–7.46 (m, 5H), 5.38 (s, 2H), 3.95 (s, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 166.1, 164.3, 136.2, 135.3, 135.2, 133.7, 130.9, 128.7, 128.5, 128.4, 128.2, 121.4, 67.4, 52.7; IR (neat): ν = 2964, 2558, 1723, 1688, 1603, 1557, 1484 cm^{-1}; GC-MS $[M^+]$ calcd for $C_{16}H_{13}O_4Br$: 347.9997, found: 348.0013.

4-(Methoxycarbonyl)-3-bromobenzoic Acid (39)

Compound **39** was synthesized according to the methods of Kishida and co-workers. [32] A 3-neck 250 mL round bottom flask charged with **36** (8.55 g, 24.4 mmol), 10% Pd/C (1.8 g), ethanol (50.0 mL), and ethyl acetate (50.0 mL) was evacuated and back-filled with hydrogen gas from a balloon three times, and the reaction solution was allowed to stir under hydrogen at room temperature for 48 h. The reaction solution was filtered through celite, and the solvents were removed in vacuo to give crude **39** (6.15 g, 96%) as a white crystalline solid that was used without further purification. A small sample of crude **39** was purified by recrystallization from hot ethyl acetate to give pure **39**, m.p. 138–140 °C: ^1H NMR (400 MHz, DMSO-d6) δ 13.59 (br s, 1H), 8.15 (d, *J* = 1.6, 1H), 8.00 (dd, *J* = 8.0, 1.6, 1H), 7.85 (d, *J* = 8.0, 1H), 3.89 (s, 3H); ^{13}C NMR (100.6 MHz, DMSO-d6) δ 165.8, 165.3, 136.1, 134.7, 134.0, 130.9, 128.4, 119.9, 52.8; IR (neat): ν = 2959, 2844, 2545, 1716, 1686, 1603, 1551, 1485 cm^{-1}; LC-GC-MS $[M^+]$ calcd for $C_9H_7O_4Br$: 257.9528, found: 257.9502.

Methyl 2-bromo-4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)carbonyl)benzoate (50)

Compound **50** was synthesized according to the method of Boehm and co-workers.[11] Methyl 4-(chlorocarbonyl)-2-bromobenzoate (**42**) was synthesized by refluxing 4-(methoxycarbonyl)-3-bromobenzoic acid (**39**) (1.64 g, 6.31 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 **42** as an off-white solid, and this solid was dissolved in dry benzene (ca. 20 mL) and evaporated to dryness three times to remove residual thionyl chloride. The acid chloride **42** 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 **48** (1.38 g, 6.82 mmol) followed by a solution of crude acid chloride **42** (6.31 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 **50**. Crude **50** was purified by column chromatography (250 mL SiO_2, hexanes:ethyl acetate 95:5 to 92.5:7.5) to give **50** (2.37 g, 84%) as a white, crystalline solid, m.p. 109–111 °C: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, *J* = 1.6, 1H), 7.83 (d, *J* = 8.0, 1H), 7.75 (dd, *J* = 8.0, 1.6, 1H), 7.25 (s, 1H), 7.22 (s, 1H), 3.97 (s, 3H), 2.35 (s, 3H), 1.69 (s, 4H), 1.31 (s, 6H), 1.20 (s, 6H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 195.8, 166.2, 148.8, 142.0, 141.6, 135.6, 135.4, 134.8, 133.8, 130.9, 129.6, 128.7, 128.5, 121.5, 52.7, 34.8, 34.7, 34.3, 33.8, 31.6, 31.5, 20.0; IR (neat): ν = 3015, 2953, 2931, 2863, 1736, 1666, 1605, 1544, 1496, 1459 cm^{-1}; GC-MS [M^+] calcd for $C_{24}H_{27}O_3Br$: 442.1144, found: 442.1135.

Methyl 2-bromo-4-(1-(1,2,3,4-tetrahydro-1,1,4,4,6-pentamethylnaphthalen-7-yl)vinyl)benzoate (54)

Compound **54** was synthesized according to the method of Boehm and co-workers. [11] To a 20-dram vial containing **50** (0.923 g, 2.08 mmol) and dry THF (3 mL) at room temperature with a Teflon magnetic stir-bar was slowly added a triphenylphosphonium methylide solution prepared as follows: to a 100 mL round bottom flask equipped with a Teflon magnetic stir-bar and containing dry THF (2.0 mL) was added ^{i}Pr$_2$NH (0.66 mL, 4.67 mmol) and a 2.5M solution of n-butyl lithium in hexanes (1.7 mL, 4.25 mmol), and the solution was stirred for 30 min at room temperature at which point, methyl triphenylphosphonium bromide (1.13 g, 3.19 mmol) was added and the solution was stirred an additional 20 min to provide a homogeneous dark yellow ylide solution. The reaction was monitored by TLC, and when the reaction was judged to be complete, the reaction solution was poured into water (50 mL) and the aqueous solution was extracted with ethyl acetate (50 mL, twice). The combined organic extracts were washed with water and brine, dried over sodium sulfate, and concentrated in vacuo to give crude **54** which was purified by column chromatography (25 mL SiO_2, hexanes:ethyl acetate 97.5:2.5) to give **54** (0.712 g, 77%) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (d, *J* = 8.0, 1H), 7.62 (d, *J* = 1.6, 1H), 7.20 (dd, *J* = 8.0, 1.6, 1H), 7.10 (s, 1H), 7.08 (s, 1H), 5.80 (d, *J* = 1.2, 1H), 5.34 (d, *J* = 0.8, 1H), 3.92 (s, 3H), 1.95 (s, 3H), 1.70 (s, 4H), 1.31 (s, 6H), 1.27 (s, 6H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ166.3, 147.8, 145.7, 144.6, 142.4, 137.1, 132.6, 132.1, 131.3, 130.2, 128.1, 128.0, 125.4, 121.9, 117.8, 52.4, 35.1, 35.0, 33.9, 33.8, 31.9, 31.8, 20.0; IR (neat): ν = 2956, 2921, 1731, 1596, 1541, 1497, 1485 cm^{-1}; GC-MS [M^+] calcd for $C_{25}H_{29}O_2Br$: 440.1351, found: 440.1379.

2-Bromo-4-(1-(1,2,3,4-tetrahydro-1,1,4,4,6-pentamethylnaphthalen-7-yl)vinyl)benzoic acid (14)

Compound **14** was synthesized following the methods of Boehm and co-workers. [11] To a 100 mL round bottom flask charged with **54** (0.4056 g, 0.9189 mmol) and methanol (4 mL) was added a 5M aqueous solution of potassium hydroxide (0.4 mL, 2.0 mmol). A reflux condenser was fitted to the round bottom flask and the reaction solution was refluxed and monitored by TLC. After 1 h at reflux, the reaction solution was cooled to room temperature and quenched with 20% HCl (42 mL). The aqueous solution was extracted with ethyl acetate (50 mL, twice) and the organic extracts were combined, washed with water and brine, dried over sodium sulfate, and concentrated in vacuo to give crude **14**. Crude **14** was purified by column chromatography (25 mL SiO_2, hexanes:ethyl acetate 9:1) to give **14** (0.273 g, 70%)

as a white crystalline solid, m.p. 199–200 °C: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (d, *J* = 8.0, 1H), 7.67 (d, *J* = 1.6, 1H), 7.24 (dd, *J* = 8.0, 1.6, 1H), 7.10 (s, 1H), 7.09 (s, 1H), 5.82 (d, *J* = 0.8, 1H), 5.37 (d, *J* = 0.8, 1H), 1.96 (s, 3H), 1.70 (s, 4H), 1.30 (s, 6H), 1.28 (s, 6H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 170.4, 147.7, 146.6, 144.7, 142.4, 137.0, 132.6, 132.5, 128.4, 128.2, 128.0, 125.6, 122.8, 118.3, 35.1, 35.0, 34.0, 33.9, 31.9, 31.8, 20.0; IR (neat): ν = 2959, 1697, 1594, 1484, 1452 cm^{-1}; LC-APCI-MS [M^++H] calcd for $C_{24}H_{28}O_2Br$: 427.1273, found: 427.1287. Anal. Calcd for $C_{24}H_{27}O_2Br$: C 67.45; H 6.37; Br 18.7. Found: C 68.16; H 6.07; Br 18.1.

2-Bromo-4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)carbonyl)benzoic acid (15)

Compound **15** was synthesized following the method of Boehm and co-workers. [11] To a 100 mL round bottom flask charged with **50** (0.395 g, 0.89 mmol) and methanol (4 mL) was added a 5M aqueous solution of potassium hydroxide (0.4 mL, 2.0 mmol). A reflux condenser was fitted to the round bottom flask and the reaction solution was refluxed and monitored by TLC. After 1 h at reflux, the reaction solution was cooled to room temperature and quenched with 20% HCl (20 mL). The precipitate was filtered and washed with water to give crude **15** (0.3722 g, 97%). Crude **15** was purified by column chromatography (25 mL SiO_2, hexanes:ethyl acetate 9:1) to give pure **15** (0.25 g, 65%) as a white crystalline solid, m.p. 166–167 °C: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (d, *J* = 1.6, 1H), 8.05 (d, *J* = 8.0, 1H), 7.80 (dd, *J* = 8.0, 1.6, 1H), 7.27 (s, 1H), 7.23 (s, 1H), 2.38 (s, 3H), 1.70 (s, 4H), 1.32 (s, 6H), 1.21 (s, 6H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 195.7, 170.6, 149.0, 143.1, 142.2, 142.0, 136.4, 135.1, 133.6, 131.6, 129.7, 129.4, 129.0, 94.1, 34.8, 34.7, 34.4, 33.9, 31.6, 31.5, 20.1; IR (neat): ν = 2955, 2924, 1703, 1667, 1607, 1542, 1456 cm^{-1}; LC-APCI-MS [M^++H] calcd for $C_{23}H_{26}O_3Br$: 429.1065, found: 429.1074. Anal. Calcd for $C_{23}H_{25}O_3Br$: C 64.34; H 5.87; Br 18.61. Found: C 65.08; H 5.59; Br 17.1.

4-(Methoxycarbonyl)-3-iodobenzoic acid (45)

The method of Bertozzi and co-workers was followed to synthesize **45**. [37] To a 100 mL round bottom flask charged with 1-mehtyl-2-aminoterephthalate (0.503 g, 2.58 mmol) was added concentrated HCl (5 mL) followed by the dropwise addition of a solution of sodium nitrite (0.185 g, 2.68 mmol) in water (1 mL), during which addition, orange gas evolved. The reaction was stirred (30 min) at room temperature, filtered through glass wool, and then a solution of potassium iodide (4.31 g, 2.6 mmol) in water (7 mL) was added, dropwise. The resulting red solution was stirred (1h) and then diluted with DCM (100 mL). The layers were separated, and the aqueous layer was washed with DCM (10 mL, twice), followed by saturated Na_2SO_3, water, and then brine. The aqueous layers were then back extracted with DCM (20 mL), and the combined organic layers were dried over sodium sulfate and concentrated to give crude **45**. Crude **45** was dissolved in hot methanol (15 mL), and water was added (15 mL), and the resulting solution was cooled in an ice bath and the precipitate was filtered to give pure **45** (0.318 g, 40%) as a yellow powder, m.p. 160–163 °C: ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.00 (br s, 1H), 8.68 (d, *J* = 1.6, 1H), 8.11 (dd, *J* = 8.0, 1.6, 1H), 7.83 (d, *J* = 8.0, 1H), 3.97 (s, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 169.7, 166.5, 142.6, 140.1, 132.4, 130.5, 129.4, 93.3, 52.8. IR (neat): ν = 3289, 2952, 2652, 1736, 1693, 1551, 1480 cm^{-1}.

Methyl 2-iodo-4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)carbonyl)benzoate (51)

Compound **51** was synthesized according to the method of Boehm and co-workers. [11] Methyl 4-(chlorocarbonyl)-2-iodobenzoate (**46**) was synthesized by refluxing 4-(methoxycarbonyl)-3-iodobenzoic acid (**45**) (1.93 g, 6.31 mmol) in thionyl chloride (10.0

mL, 137 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 **46** as an off-white solid, and this solid was dissolved in dry benzene (ca. 20 mL) and evaporated to dryness three times to remove residual thionyl chloride. The acid chloride **46** 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 **48** (1.38 g, 6.82 mmol) followed by a solution of crude acid chloride **46** (6.31 mmol) in DCM (15 mL). Aluminum chloride (2.30 g, 17.2 mmol) was added to the reaction solution at room temperature slowly, with stirring, and the reaction solution turned from colorless to dark 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 **51**. Attempted purification of crude **51** by column chromatography (250 mL SiO_2, hexanes:ethyl acetate 95:5 to 92.5:7.5) gave **51** (2.91 g, 89%) that was ~ 7 mol % **49** as a white, crystalline solid, m.p. 115–116 °C: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (d, *J* = 1.6, 1H), 7.83 (d, *J* = 8.0, 1H), 7.79 (dd, *J* = 8.0, 1.6, 1H), 7.25 (s, 1H), 7.22 (s, 1H), 3.97 (s, 3H), 2.37 (s, 3H), 1.69 (s, 4H), 1.31 (s, 6H), 1.21 (s, 6H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 195.7, 166.6, 148.8, 142.5, 141.9, 141.4, 138.5, 135.0, 133.7, 130.4, 129.6, 129.3, 128.8, 93.5, 52.7, 34.8, 34.7, 34.3, 33.8, 31.6, 31.5, 20.1; IR (neat): ν = 2953, 1729, 1667, 1540, 1459 cm^{-1}; GC-MS [M^+] calcd for $C_{24}H_{27}O_3I$: 490.1005, found: 490.0995.

methyl 2-iodo-4-(1-(1,2,3,4-tetrahydro-1,1,4,4,6-pentamethylnaphthalen-7-yl)vinyl)benzoate (55)

Compound **55** was synthesized according to the method of Boehm and co-workers. [11] To a 20-dram vial containing **51** (1.0123 g, 2.06 mmol) and dry THF (3 mL) at room temperature with a Teflon magnetic stir-bar was slowly added a triphenylphosphonium methylide solution prepared as follows: to a 100 mL round bottom flask equipped with a Teflon magnetic stir-bar and containing dry THF (2.0 mL) was added ^{i}Pr$_2$NH (0.66 mL, 4.67 mmol) and a 2.5M solution of n-butyl lithium in hexanes (1.7 mL, 4.25 mmol), and the solution was stirred for 30 min at room temperature at which point, methyl triphenylphosphonium bromide (1.13 g, 3.19 mmol) was added and the solution was stirred an additional 20 min to provide a homogeneous dark yellow ylide solution. The reaction was monitored by TLC, and when the reaction was judged to be complete, the reaction solution was poured into water (50 mL) and the aqueous solution was extracted with ethyl acetate (50 mL, twice). The combined organic extracts were washed with water and brine, dried over sodium sulfate, and concentrated *in vacuo* to give crude **55**. Attempted purification of **55** by column chromatography (25 mL SiO_2, hexanes:ethyl acetate 97.5:2.5) gave **55** (0.3502 g, 32%) that possessed ~ 9 mol% **53** as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (d, *J* = 1.6, 1H), 7.72 (d, *J* = 8.0, 1H), 7.21 (dd, *J* = 8.0, 1.6, 1H), 7.09 (s, 1H), 7.08 (s, 1H), 5.78 (d, *J* = 1.2, 1H), 5.33 (d, *J* = 0.8, 1H), 3.92 (s, 3H), 1.95 (s, 3H), 1.70 (s, 4H), 1.31 (s, 6H), 1.27 (s, 6H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 166.6, 147..6, 145.6, 144.5, 142.4, 139.1, 137.2, 133.1, 132.6, 130.8, 128.1, 128.0, 126.3, 117.8, 94.4, 52.4, 35.1, 33.9, 33.8, 31.9, 31.8, 20.0; IR (neat): ν = 2957, 2926, 2862, 1726, 1664, 1590, 1541, 1497, 1456 cm^{-1}; GC-MS [M^+] calcd for $C_{25}H_{29}O_2I$: 488.1213, found: 488.1222.

2-Iodo-4-(1-(1,2,3,4-tetrahydro-1,1,4,4,6-pentamethylnaphthalen-7-yl)vinyl)benzoic acid (16)

Compound **16** was synthesized following the methods of Boehm and co-workers. [11] To a 100 mL round bottom flask charged with **55** (0.3156 g, 0.646 mmol) and methanol (4 mL) was added a 5M aqueous solution of potassium hydroxide (0.4 mL, 2.0 mmol). A reflux

condenser was fitted to the round bottom flask and the reaction solution was refluxed and monitored by TLC. After 1 h at reflux, the reaction solution was cooled to room temperature and quenched with 20% HCl (42 mL). The aqueous solution was extracted with ethyl acetate (50 mL, twice) and the organic extracts were combined, washed with water and brine, dried over sodium sulfate, and concentrated *in vacuo* to give crude **16**. Crude **16** was purified by column chromatography (25 mL SiO_2, hexanes:ethyl acetate 9:1) to give **16** (0.105 g, 34%), and this material was crystallized from ethyl acetate to provide pure **16** (0.063, 20%) as a white crystalline solid, m.p. 199–200 °C: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, *J* = 2.0, 1H), 7.94 (d, *J* = 8.0, 1H), 7.25 (dd, *J* = 8.0, 2.0, 1H), 7.10 (s, 1H), 7.09 (s, 1H), 5.81 (d, *J* = 0.8, 1H), 5.37 (d, *J* = 1.2, 1H), 1.97 (s, 3H), 1.70 (s, 4H), 1.31 (s, 6H), 1.28 (s, 6H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 170.9, 147.5, 146.5, 144.6, 142.4, 139.7, 137.0, 132.5, 132.0, 131.2, 128.2, 128.0, 126.4, 118.2, 95.0, 35.0, 34.0, 33.8, 31.9, 31.8, 20.0; IR (neat): ν = 2954, 2909, 1695, 1591, 1539, 1481 cm^{-1}; LC-APCI-MS $[M^+ + H]$ calcd for $C_{24}H_{28}O_2I$: 475.1134, found: 475.1126. Anal. Calcd for $C_{24}H_{27}O_2I$: C 60.77; H 5.74; I 26.75. Found: C 61.04; H 5.64; I 26.4.

2-Iodo-4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)carbonyl)benzoic acid (17)

Compound **17** was synthesized following the method of Boehm and co-workers. [11] To a 100 mL round bottom flask charged with **51** (0.437 g, 0.89 mmol) and methanol (4 mL) was added a 5M aqueous solution of potassium hydroxide (0.4 mL, 2.0 mmol). A reflux condenser was fitted to the round bottom flask and the reaction solution was refluxed and monitored by TLC. After 1 h at reflux, the reaction solution was cooled to room temperature and quenched with 20% HCl (20 mL). The precipitate was filtered and washed with water to give crude **17** (0.4043 g, 95%). Attempted purification of crude **17** by column chromatography (25 mL SiO_2, hexanes:ethyl acetate 9:1) gave **17** (0.34 g, 74%) that contained ~9 mol % **13** as a white crystalline solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 (d, *J* = 1.6, 1H), 8.05 (d, *J* = 8.0, 1H), 7.84 (dd, *J* = 8.0, 1.6, 1H), 7.27 (s, 1H), 7.23 (s, 1H), 2.38 (s, 3H), 1.70 (s, 4H), 1.32 (s, 6H), 1.22 (s, 6H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 195.7, 170.6, 149.0, 143.1, 142.2, 142.0, 136.4, 135.1, 133.6, 131.6, 129.7, 129.4, 129.0, 94.1, 34.8, 34.7, 34.4, 33.9, 31.6, 31.5, 20.1; IR (neat): ν = 2958, 1703, 1661, 1542, 1458 cm^{-1}; LC-APCI-MS $[M^+ + H]$ calcd for $C_{23}H_{26}O_3I$: 477.0927, found: 477.0922. Anal. Calcd for $C_{23}H_{25}O_3I$: C 57.99; H 5.29; I 26.64. Found: C 59.15; H 5.14; I 24.5.

3,5-Difluoro-4-formylbenzoic acid (27)

The method of Anderson and co-workers was followed to synthesize **27**. [33] To a 1 L round bottom flask charged with 3,5-difluorobenzoic acid (10.00 g, 63.3 mmol) and THF (290 mL) cooled to –78 °C was added a 1.7 M solution of *tert*-butyl lithium in pentane (93.0 mL, 158 mmol), dropwise. The reaction was stirred at –78 °C for 30 min, and then dimethylformamide (12.4 mL, 158 mmol) was added. The reaction was allowed to stir at –78 °C for 1 h, and then stirred at 0 °C for 1 h, and then allowed to warm to room temperature and stirred for 16 h. The reaction was carefully quenched with concentrated HCl (by slow addition) until pH 1 (about 30 mL), and then concentrated in vacuo. The residue was extracted with DCM (100 mL, twice), and the organic layers were combined and concentrated in vacuo. The residue was diluted with DCM (50 mL) and then washed with saturated $NaHCO_3$ (75 mL, twice). The aqueous extracts were acidified with concentrated HCl (18 mL), and the resulting precipitate was filtered and dried to give pure **27** (4.28 g, 36%) as a white powder, m.p. 194 – 216 °C: ^{1}H NMR (400 MHz, DMSO-d6) δ 13.98 (br s, 1H), 10.23 (s, 1H), 7.64 (d, *J* = 9.2, 1H); ^{13}C NMR (100.6 MHz, DMSO-d6) δ 184.9, 184.9, 184.8, 164.6, 164.5, 164.5, 163.3, 163.2, 160.7, 160.6, 138.4, 138.3, 138.2, 116.5, 116.4, 116.3, 113.4, 113.3, 113.2, 113.1; IR (neat): ν = 3078, 2935, 2615, 1717,

1679, 1633, 1573, 1475 cm^{-1}; GC-MS [M^+] calcd for $C_8H_4O_3F_2$: 186.0129, found: 186.0130.

Benzyl-3,5-difluoro-4-formylbenzoate (31)

Compound **31** was synthesized according to the methods of Kishida and co-workers. [32] To a 500 mL round bottom flask charged with **27** (8.8 g, 47.2 mmol) was added dry dimethylformamide (100 mL) and a 60 wt % suspension of NaH in mineral oil (2.93 g, 73.3 mmol) in small aliquots over 20 min. The reaction solution was stirred an additional 20 min, and benzylbromide (8.10 mL, 67.7 mmol) was added to the red heterogeneous solution. After stirring 5 h, the reaction solution had become homogeneous, and it was poured into 1N HCl (250 mL), extracted with ethyl acetate (100 mL, twice), and the combined organic extracts were washed with saturated $NaHCO_3$ (75 mL) and brine, dried over sodium sulfate, and removed in vacuo to give crude **31**. Crude **31** was purified by column chromatography (150 mL SiO_2, hexanes:ethyl acetate 4:1) to give **31** (12.1 g, 93%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.32 (s, 1H), 7.66 (d, J = 11.2, 1H), 7.36–7.46 (m, 5H), 5.38 (s, 2H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 186.2, 186.2, 184.0, 184.0, 183.9, 164.0, 164.0, 163.1, 163.1, 163.1, 161.4, 161.3, 137.4, 137.2, 137.1, 134.8, 134.6, 128.7, 128.7, 128.7, 128.5, 128.4, 116.8, 116.7, 116.6, 113.8, 113.7, 113.7, 113.6, 113.5, 113.5, 113.0, 107.9, 107.6, 68.4, 68.0; IR (neat): ν = 3072, 2668, 2548, 1722, 1697, 1632, 1573, 1484 cm^{-1}; GC-MS [M^+] calcd for $C_{15}H_{10}O_3F_2$: 292.0547, found: 292.0548.

4-((Benzyloxy)carbonyl)-2,6-difluorobenzoic Acid (34)

Compound **34** was prepared according to the method of Kishida and co-workers. [32] To a 100 mL round bottom flask charged with **31** (12.1 g, 43.8 mmol), sulfamic acid (4.60 g, 47.4 mmol), water (75 mL), and ACN (38 mL) was added a solution of 80% $NaClO_2$ (5.43 g, 60.0 mmol) in water (25 mL). After stirring for 1 h, the reaction solution was poured into saturated Na_2SO_3 (80 mL) and 1N HCl (150 mL), and the resulting solution was extracted with ethyl acetate (100 mL, thrice). The combined organic extracts were washed with brine, dried over sodium sulfate, and removed in vacuo to give crude **34** (11.4 g, 89%) that was used without further purification. A small sample was purified by column chromatography (25 mL SiO_2, hexanes:ethyl acetate 1:1) to give pure **34** as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.83 (br s, 1H), 7.67 (d, J = 8.0, 1H), 7.36–7.46 (m, 5H), 7.34–7.47 (m, 5H), 5.39 (s, 2H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 165.6, 163.4, 163.4, 162.1, 162.0, 159.5, 159.5, 135.6, 135.5, 135.4, 134.8, 128.7, 128.4, 113.6, 113.5, 113.4, 113.3, 113.3, 113.2, 67.9; IR (neat): ν = 3071, 2896, 2668, 2548, 1723, 1694, 1632, 1572, 1484 cm^{-1}; LC-APCI-MS [M^++H] calcd for $C_{15}H_{10}O_3F_2$: 276.0598, found: 276.0604.

4-Benzyl 1-methyl-2,6-difluorobenzene-1,4-dioate (37)

Compound **37** was synthesized according to the method of Kishida and co-workers. [32] To a 100 mL round bottom flask charged with compound **33** (11.4 g, 39.0 mmol) was added $SOCl_2$ (25.0 mL, 340 mmol) and the reaction solution was refluxed for 1h in an oil bath at 84 °C. The reaction solution was cooled to room temperature and the excess thionyl chloride was removed in vacuo to give crude 4-chlorocarbonyl-2,6-difluorobenzoic acid benzyl ester. The crude 4-chlorocarbonyl-2,6-difluorobenzoic acid in dry toluene (25 mL) was added dropwise to a solution of triethylamine (13.2 mL, 95 mmol) in methanol (70 mL, 1.7 mol) over 10 min with vigorous stirring. The reaction solution was stirred 1h and poured into 1N HCl (300 mL) and extracted with ethyl acetate (80 mL, thrice). The combined organic extracts were washed with brine, dried over sodium sulfate, and removed in vacuo to give crude **37**. Crude **37** was purified by column chromatography (150 mL SiO_2, hexanes:ethyl acetate 95:5) to give pure **37** as an oil (9.67 g, 81%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, J = 7.6, 2H), 7.36–7.48 (m, 5H), 5.38 (s, 2H), 3.97 (s, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$)

δ 163.5, 163.4, 163.4, 161.5, 161.4, 161.2, 158.9, 158.9, 134.9, 134.6, 134.5,134.4, 128.7, 128.6, 128.4, 115.0,114.8, 114.6, 113.3, 113.3, 113.3, 113.1, 113.1, 113.0, 67.7, 53.1; IR (neat): ν = 2956, 1728, 1634, 1574, 1488 cm^{-1}; LC-MS $[M^+ + H]$ calcd for $C_{16}H_{13}O_4F_2$: 307.0782, found: 307.0784.

4-(Methoxycarbonyl)-3,5-difluorobenzoic Acid (40)

Compound **40** was synthesized as follows. A 0.05 M solution of **37** (9.67 g, 31.6 mmol) in ethanol (630 mL) was passed through a 10% Pd/C cartridge in the ThalesNano H-cube® at 70 °C and 14 bar. The resulting solution was concentrated in vacuo to give crude **40** (6.54 g, 96%) as a white crystalline solid that was used without further purification. A small sample of crude **40** was purified by recrystallization from hot ethyl acetate to give pure **40**, m.p. 148–150 °C: ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.65 (br s, 1H), 7.67 (d, *J* = 7.6, 2H), 3.99 (s, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 169.2, 161.5, 161.4, 161.1, 158.9, 158.9, 133.5, 133.4, 133.3, 115.9, 115.7, 115.5, 113.9, 113.8, 113.6, 113.6, 53.2; IR (neat): ν = 3080, 2840, 2653, 2577, 1739, 1697, 1632, 1575, 1489 cm^{-1}; LC-MS $[M^+ + H]$ calcd for $C_9H_7O_4F_2$: 217.0312, found: 217.0300.

Methyl 2,6-difluoro-4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)carbonyl)benzoate (52)

Compound **52** was synthesized according to the method of Boehm and co-workers. [11] Methyl 4-(chlorocarbonyl)-3,5-difluorobenzoate (**43**) was synthesized by refluxing 4-(methoxycarbonyl)-3,5-difluorobenzoic acid (**40**) (1.47 g, 6.80 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 **43** as an off-white solid, and this solid was dissolved in dry benzene (ca. 20 mL) and evaporated to dryness three times to remove residual thionyl chloride. The acid chloride **43** 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 **48** (1.47 g, 7.26 mmol) followed by a solution of crude acid chloride **43** (6.80 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 **52**. Crude **52** was purified by column chromatography (250 mL SiO_2, hexanes:ethyl acetate 95:5 to 92.5:7.5) to give **52** (3.19 g, 58%) as a white, crystalline solid, m.p. 107–111 °C: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (d, *J* = 8.0, 2H), 7.23 (s, 1H), 7.21 (s, 1H), 3.98 (s, 3H), 2.34 (s, 3H), 1.69 (s, 4H), 1.31 (s, 6H), 1.21 (s, 6H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 194.7, 161.5, 161.4, 158.9, 158.9, 149.0, 142.5, 142.5, 142.1, 136.1, 134.7, 133.3, 133.1, 129.9, 129.7, 128.3, 114.1, 113.5, 113.2, 53.1, 52.6, 34.7, 34.7, 34.4, 33.9, 33.8, 31.6, 31.5, 20.6, 20.0; IR (neat): ν = 3072, 2957, 2922, 2858, 1747, 1670, 1630, 1567, 1455 cm^{-1}; LC-MS $[M^+ + H]$ calcd for $C_{24}H_{27}O_3F_2$: 401.1928, found: 401.1937.

Methyl 2,6-difluoro-4-(1-(1,2,3,4-tetrahydro-1,1,4,4,6-pentamethylnaphthalen-7-yl)vinyl)benzoate (56)

Compound **56** was synthesized according to the method of Boehm and co-workers. [11] To a 20-dram vial containing **52** (0.814 g, 2.03 mmol) and dry THF (3 mL) at room temperature with a Teflon magnetic stir-bar was slowly added a triphenylphosphonium methylide

solution prepared as follows: to a 100 mL round bottom flask equipped with a Teflon magnetic stir-bar and containing dry THF (2.0 mL) was added $^{i}Pr_2NH$ (0.66 mL, 4.67 mmol) and a 2.5M solution of n-butyl lithium in hexanes (1.7 mL, 4.25 mmol), and the solution was stirred for 30 min at room temperature at which point, methyl triphenylphosphonium bromide (1.13 g, 3.19 mmol) was added and the solution was stirred an additional 20 min to provide a homogeneous dark yellow ylide solution. The reaction was monitored by TLC, and when the reaction was judged to be complete, the reaction solution was poured into water (50 mL) and the aqueous solution was extracted with ethyl acetate (50 mL, twice). The combined organic extracts were washed with water and brine, dried over sodium sulfate, and concentrated in vacuo to give crude **56** which was purified by column chromatography (25 mL SiO_2, hexanes:ethyl acetate 97.5:2.5) to give **56** (0.36 g, 44%) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.09 (s, 1H), 7.08 (s, 1H), 6.86 (dd, *J* = 9.2, 4, 2H), 5.80 (d, *J* = 3.2, 1H), 5.36 (d, *J* = 3.2, 1H), 3.94 (s, 3H), 1.95 (s, 3H), 1.70 (s, 4H), 1.31 (s, 6H), 1.27 (s, 6H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 162.0, 159.5, 159.4, 147.3, 146.6, 146.4, 144.8, 144.8, 142.5, 136.6, 136.6, 132.5, 132.1, 132.0, 131.9, 128.5, 128.4, 128.2, 127.9, 118.2, 110.1, 109.8, 109.0, 61.8, 60.3, 52.69, 35.0, 35.0, 34.0, 33.8, 31.8, 31.8, 21.0, 19.8, 14.1, 14.1; IR (neat): ν = 2958, 2924, 2862, 1728, 1629, 1557, 1497, 1456 cm^{-1}; LC-MS $[M^+ + H]$ calcd for $C_{25}H_{29}O_2F_2$: 399.2136, found: 399.2138.

2,6-difluoro-4-(1-(1,2,3,4-tetrahydro-1,1,4,4,6-pentamethylnaphthalen-7-yl)vinyl)benzoic acid (18)

Compound **18** was synthesized following the method of Boehm and co-workers. [11] To a 100 mL round bottom flask charged with **56** (0.3586 g, 0.8999 mmol) and methanol (4 mL) was added a 5M aqueous solution of potassium hydroxide (0.4 mL, 2.0 mmol). A reflux condenser was fitted to the round bottom flask and the reaction solution was refluxed and monitored by TLC. After 1 h at reflux, the reaction solution was cooled to room temperature and quenched with 20% HCl (20 mL). The precipitate was filtered and washed with water to give crude **18** (0.3459 g, 100%). Crude **18** was purified by column chromatography (25 mL SiO_2, hexanes:ethyl acetate 9:1) to give pure **18** (0.25 g, 72%) as a white crystalline solid, m.p. 191–192 °C: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.23 (br s, 1H), 7.10 (s, 1H), 7.08 (s, 1H), 6.89 (d, *J* = 10, 2H), 5.84 (s, 1H), 5.41(s, 1H), 1.97 (s, 3H), 1.70 (s, 4H), 1.31 (s, 6H), 1.28 (s, 6H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 166.6, 162.8, 162.8, 160.2, 160.2, 147.9, 147.8, 147.7, 147.2, 144.9, 142.6, 136.5, 132.5, 128.2, 128.0, 118.7, 110.4, 110.3, 110.1, 107.8, 107.6, 107.5, 35.0, 35.0, 34.0, 33.8, 31.9, 31.8, 19.8; IR (neat): ν = 3748, 2956, 2349, 1695, 1625, 1556, 1487 cm^{-1}; LC-APCI-MS $[M^+ + H]$ calcd for $C_{24}H_{27}O_2F_2$: 385.1979, found: 385.1975. Anal. Calcd for $C_{24}H_{26}O_2F_2$: C, 74.98; H, 6.82; F, 9.88. Found: C, 74.74; H, 6.84; F, 9.10.

2,6-Difluoro-4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)carbonyl)benzoic acid (19)

Compound **19** was synthesized following the method of Boehm and co-workers. [11] To a 100 mL round bottom flask charged with **52** (0.692 g, 1.73 mmol) and methanol (9 mL) was added a 5M aqueous solution of potassium hydroxide (0.8 mL, 4.0 mmol). A reflux condenser was fitted to the round bottom flask and the reaction solution was refluxed and monitored by TLC. After 1 h at reflux, the reaction solution was cooled to room temperature and quenched with 20% HCl (42 mL). The precipitate was filtered and washed with water to give crude **19** (0.634 g, 95%). Crude **19** was purified by column chromatography (25 mL SiO_2, hexanes:ethyl acetate 9:1) to give **19** (0.54 g, 81%) as a white crystalline solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (br s, 1H), 7.39 (d, *J* = 8.4, 1H), 7.25 (s, 1H), 7.22 (s, 1H), 2.34 (s, 3H), 1.69 (s, 4H), 1.31 (s, 6H), 1.22 (s, 6H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 194.7, 165.6, 162.0, 162.0, 159.4, 159.4, 149.2, 143.2, 142.2, 134.8, 133.2, 129.7, 128.3, 113.7, 113.4, 34.7, 34.7, 34.4, 33.9, 31.6, 31.5, 20.0; IR (neat): ν = 3498, 2956, 2923, 2522,

1674, 1632, 1571, 1487 cm^{-1}; LC-APCI-MS $[M^+ + H]$ calcd for $C_{23}H_{25}O_3F_2$: 387.1772, found: 387.1757. Anal. Calcd for $C_{23}H_{24}O_3F_2$: C, 71.49; H, 6.26; F, 9.83. Found: C, 70.53; H, 6.05; F, 7.90.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

(h)RXR	(human) retinoid-X-receptor
RAR	retinoic-acid-receptor
CTCL	cutaneous T-cell lymphoma
RXRE	retinoid-X-receptor element
HRE	hormone response element
LBP	ligand binding pocket
LXR	liver X receptor
TR	thyroid hormone receptor
VDR	1,25-dihydroxyvitamin D receptor
SNuRMs	specific nuclear receptor modulators
TLC	thin layer chromatography
FDA	Food and Drug Administration
MEM	minimum essential media
FBS	fetal bovine serum
BD	binding domain
AD	activation domain
apoE	apolipoprotein E

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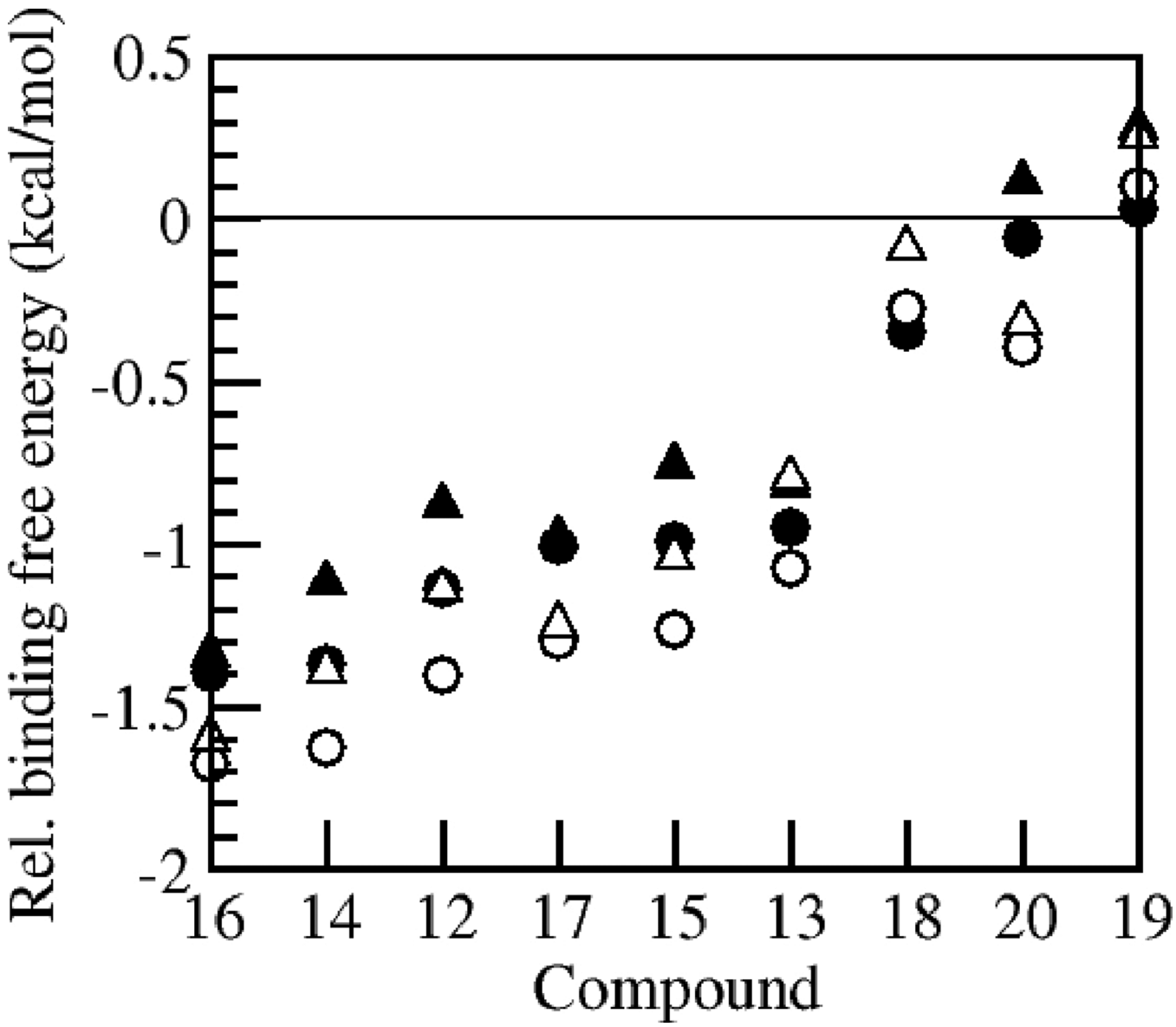


Figure 1.

Docking results for compounds **12–20**; calculated binding free energies are relative to the calculated binding free energy of docked bexarotene with negative numbers indicating better binding. Triangles represent docks to 1MVC, circles to 1H9U; open symbols represent AutoDockTool charges, and closed symbols OpenBabel charges. Compounds are listed from left to right by increasing docking free energies averaged over the various protocols.

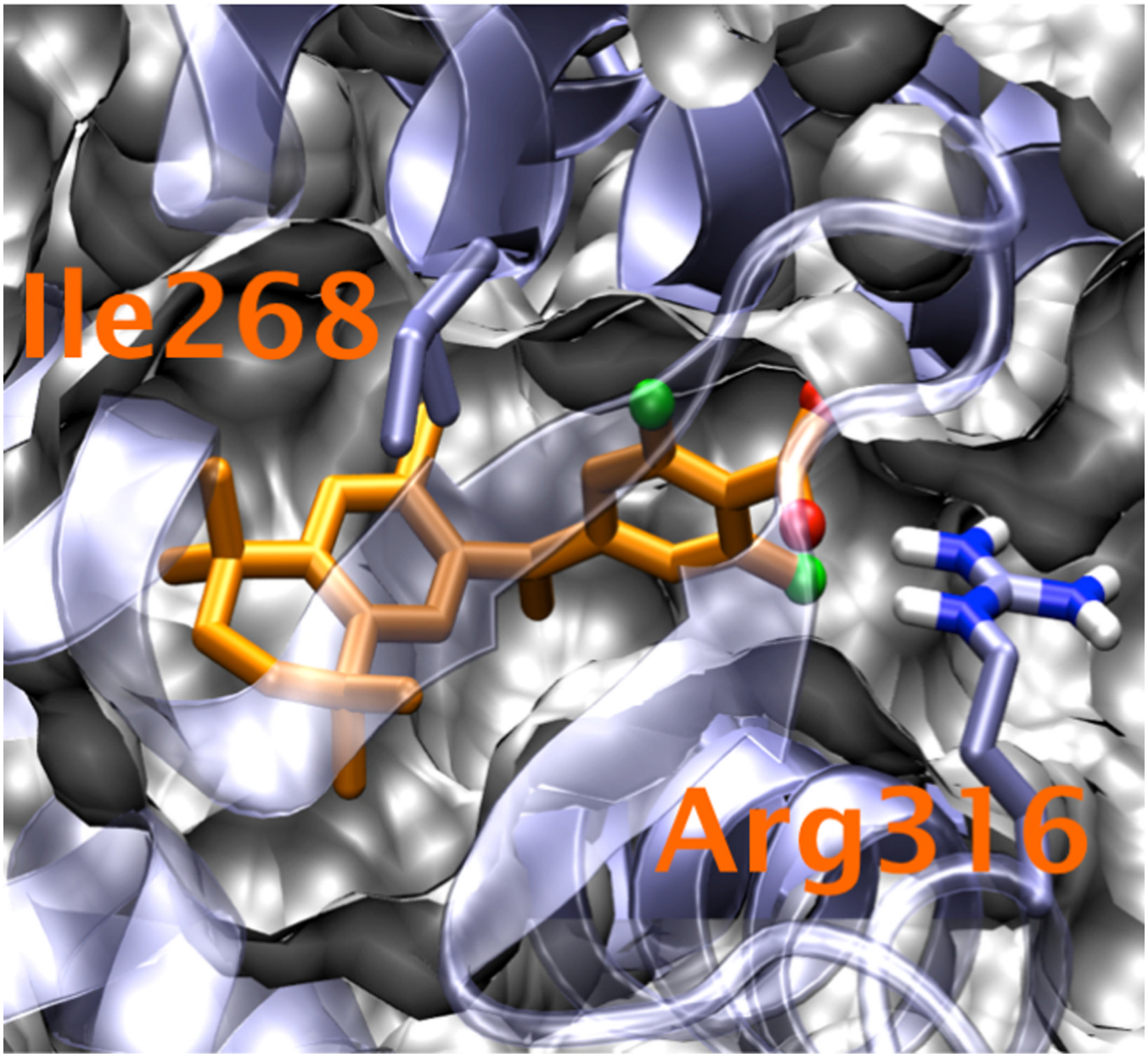


Figure 2.
Low energy docking pose for compound **18**.

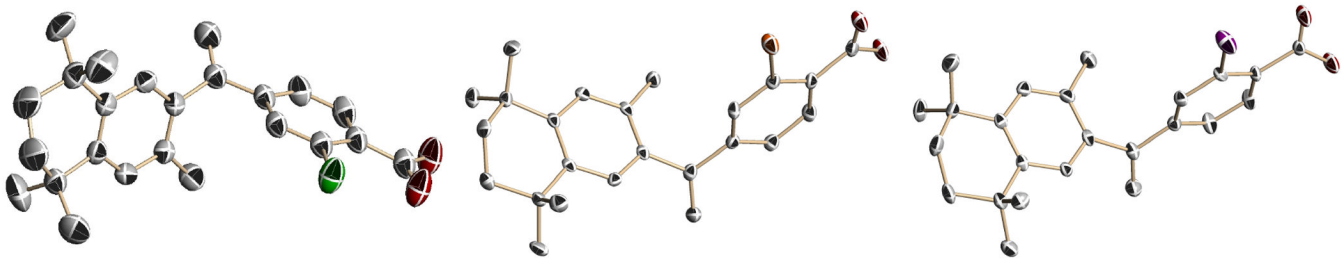


Figure 3.
The X-ray crystal structures of bexarotene analogs **12, 14**, and **16** shown without hydrogen atoms, for clarity, with thermal ellipsoids at the 50 % probability level.

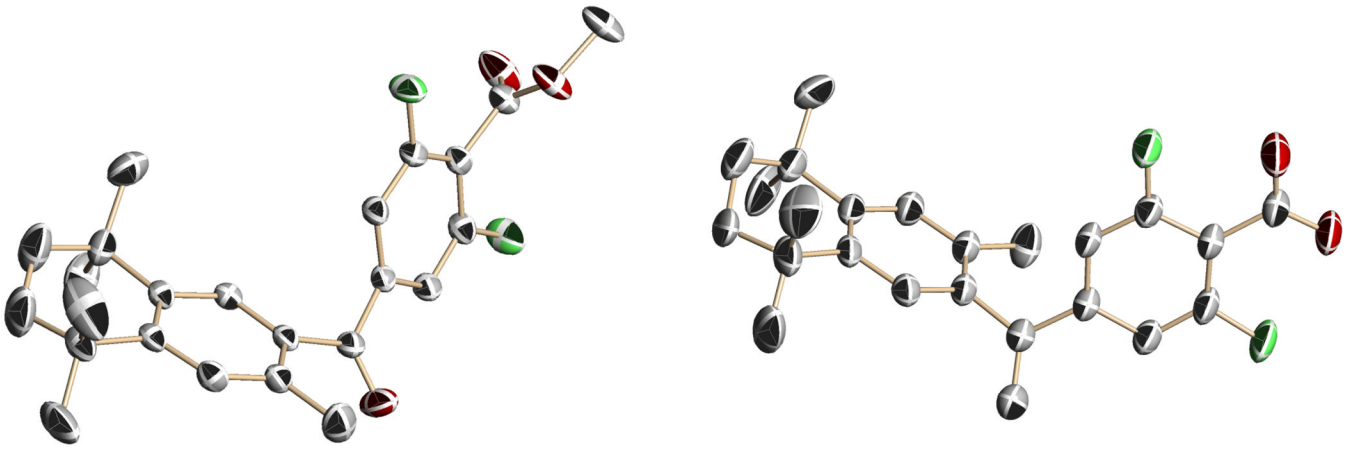


Figure 4.
The X-ray crystal structures of bexarotene analogs **52** and **18** shown without hydrogen atoms, for clarity, with thermal ellipsoids at the 30 % probability level. Compound **18** displayed twist-isomerism in the aliphatic ring. Hence, one twist isomer has been displayed.

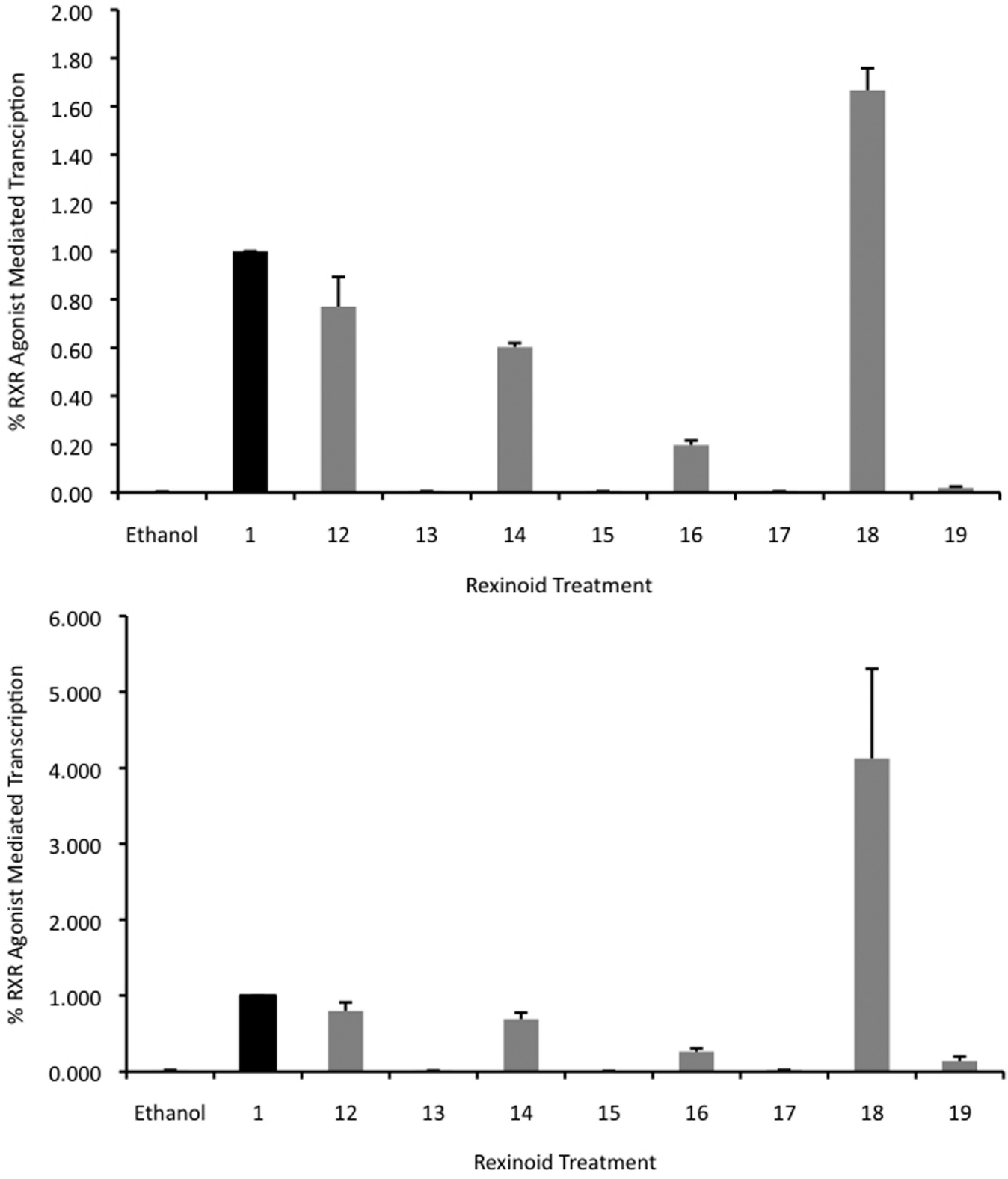


Figure 5.

a) and b). Evaluation of potential RXR selective agonists via a mammalian two-hybrid assay in two different types of human colon cancer cells, Caco-2 (a) and HCT-116 (b), respectively. Both cell lines were transfected with pCMV-BD-hRXR binding domain vector (BD), pCMV-AD-hRXR activation domain (AD), pFR-Luc reporter gene containing BD-binding sites, and a renilla control plasmid. Cells were transfected for 7 hours utilizing a liposome-mediated transfection protocol then exposed to either the ethanol vehicle or 10^{-7} M compound **1** or the indicated analog. After 24 hours the cells were lysed and a luciferase assay was completed. Analog-dependent RXR binding and homodimerization, as measured by luciferase output, was compared to the parent compound **1** (value set to 1.0).

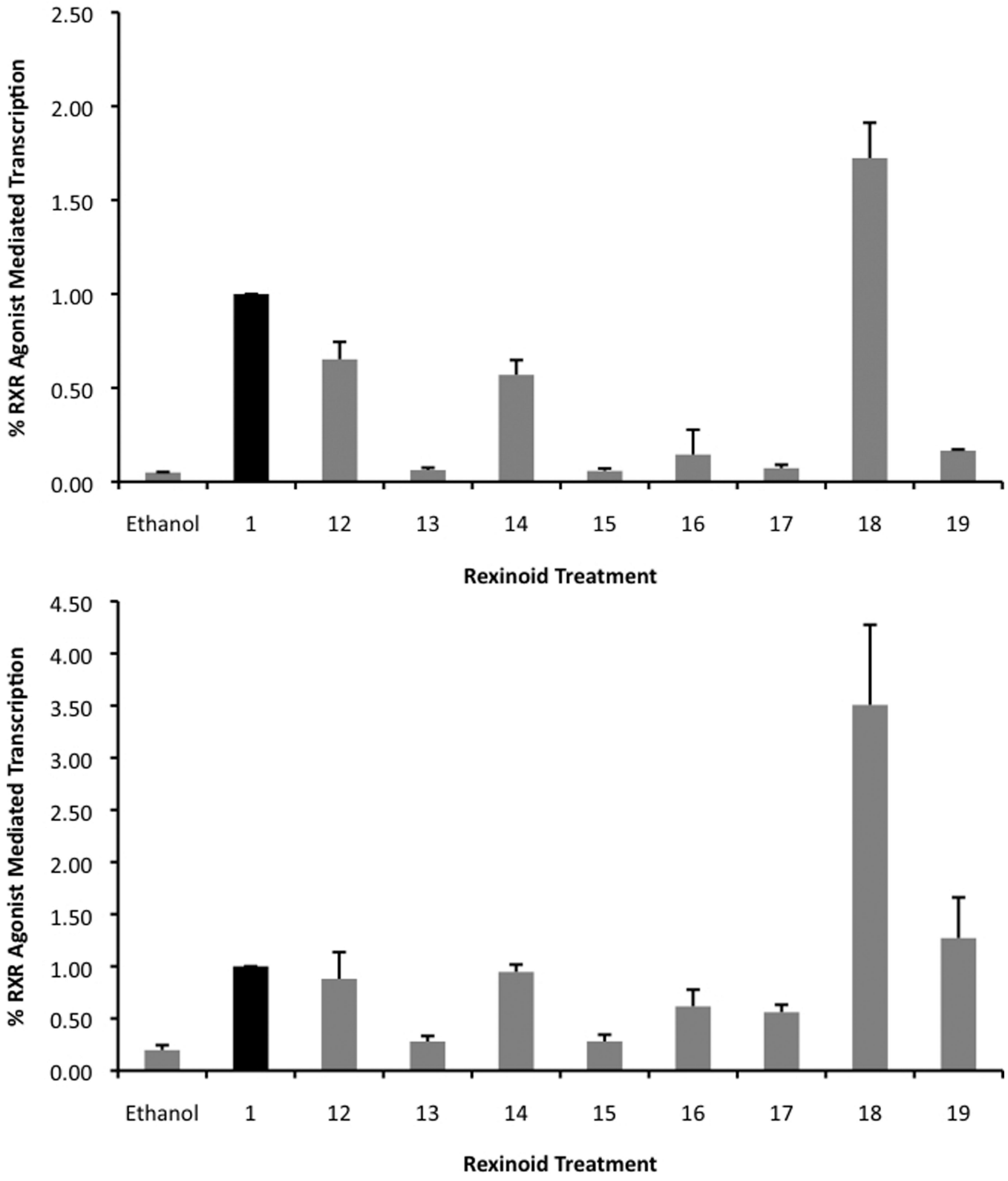


Figure 6.

a) and b). Detection of potential RXR agonists via an RXRE-luciferase based system utilizing human colon cancer cells, Caco-2 (a) and HCT-116 (b), respectively. Both cell lines were transfected with hRXRα, an RXRE luciferase reporter gene, renilla control plasmid, and carrier DNA (pTZ18U). Cells were transfected for 7 hours utilizing a liposome-mediated transfection protocol then exposed to either the ethanol vehicle or 10^{-7} M compound **1** or the indicated analog. After 24 hours the cells were lysed and a luciferase assay was completed. Analog-dependent, RXR-mediated transcription, as measured by luciferase output, was compared to the parent compound **1** (value set to 1.0).

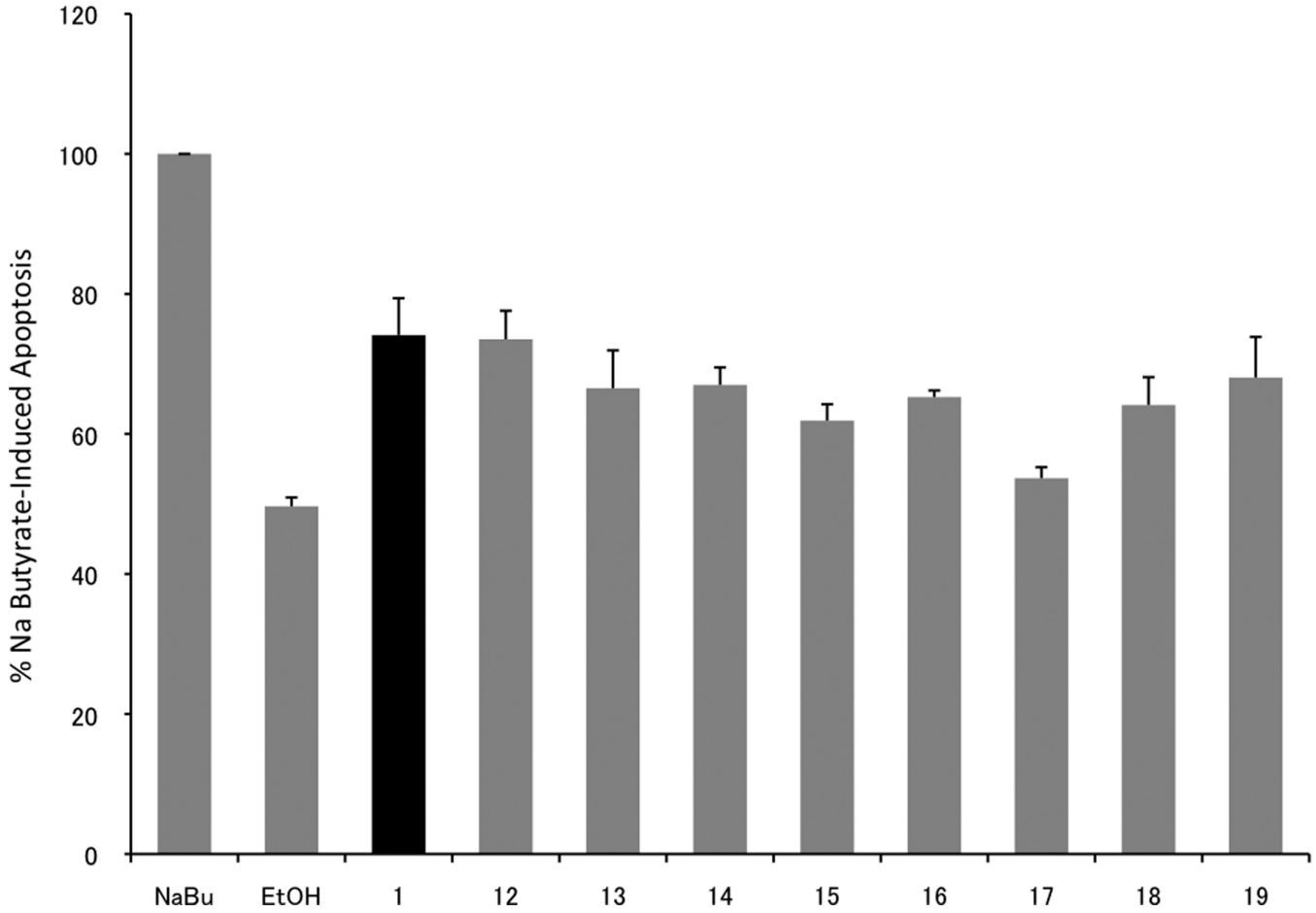
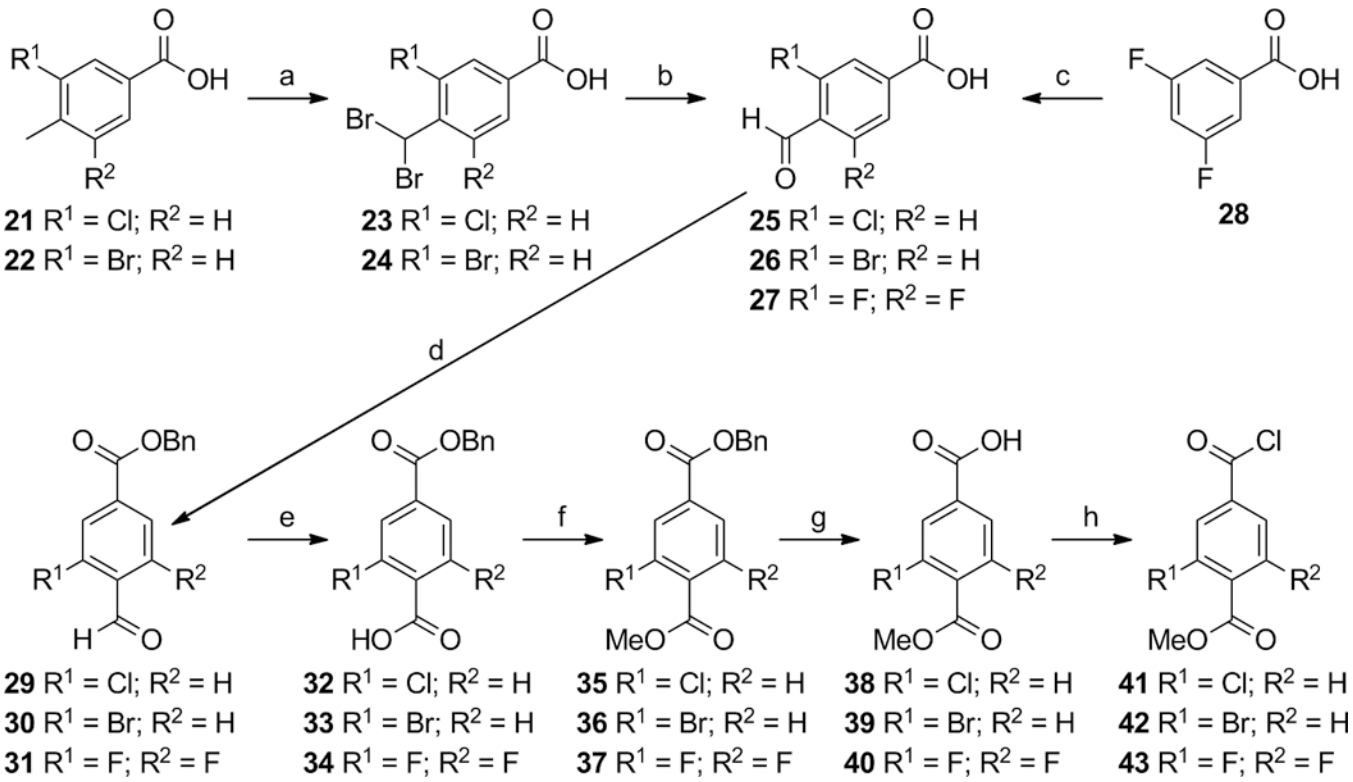
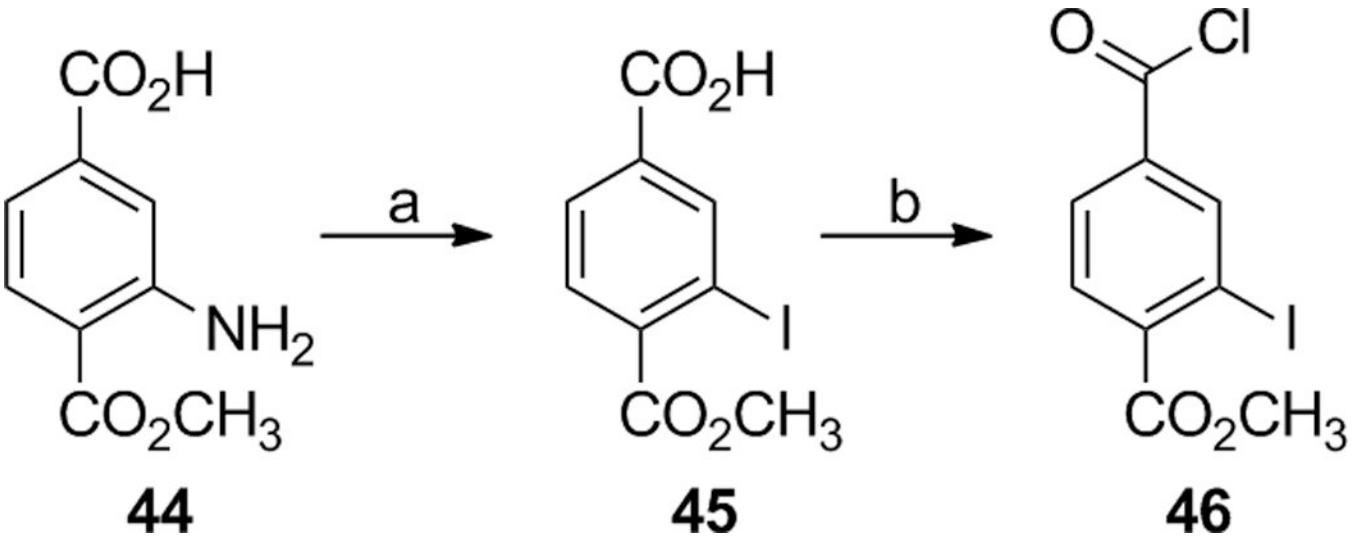


Figure 7.

Apoptosis analysis in a CTCL cell line. HuT-78 cells were treated for 48 hours with analog, ethanol vehicle, or sodium butyrate as a positive control. Cells were analyzed for apoptosis via a caspase assay (Promega Caspase-Glo 3/7 assay) according to manufacturer's instructions. Data is an average of six independent experiments and plotted as a percentage of sodium butyrate activity (set at 100%).

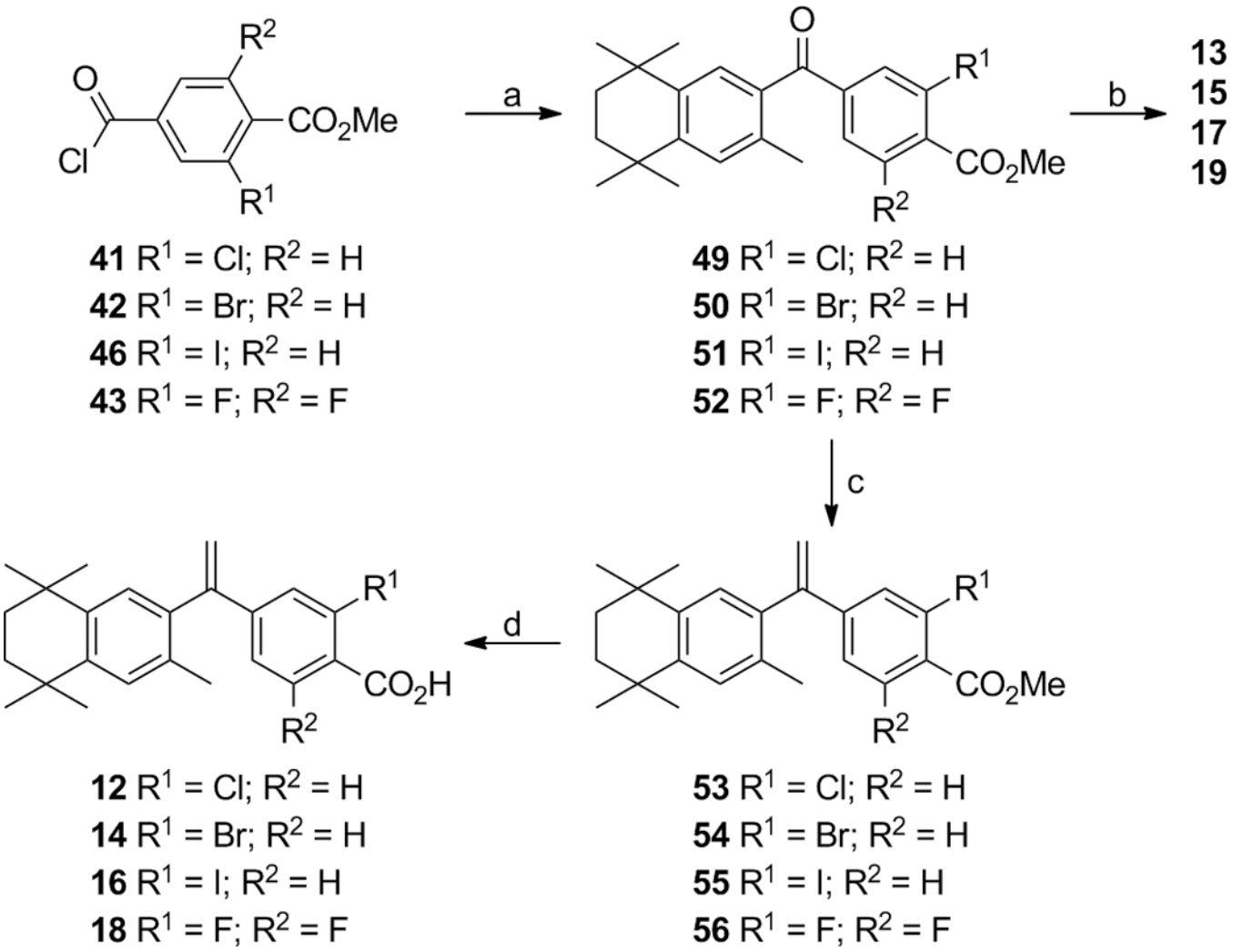


Scheme 1.

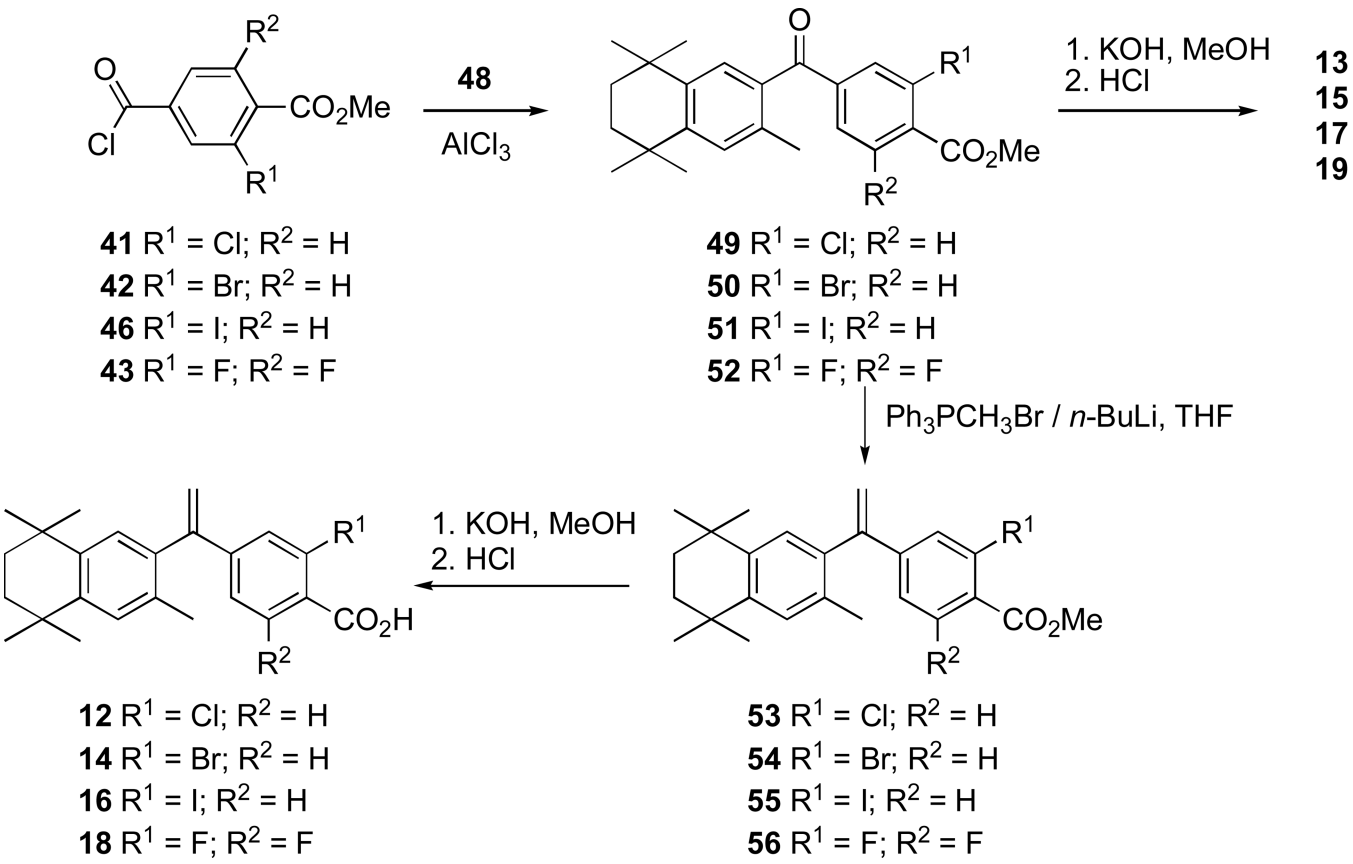


44 **45** **46**

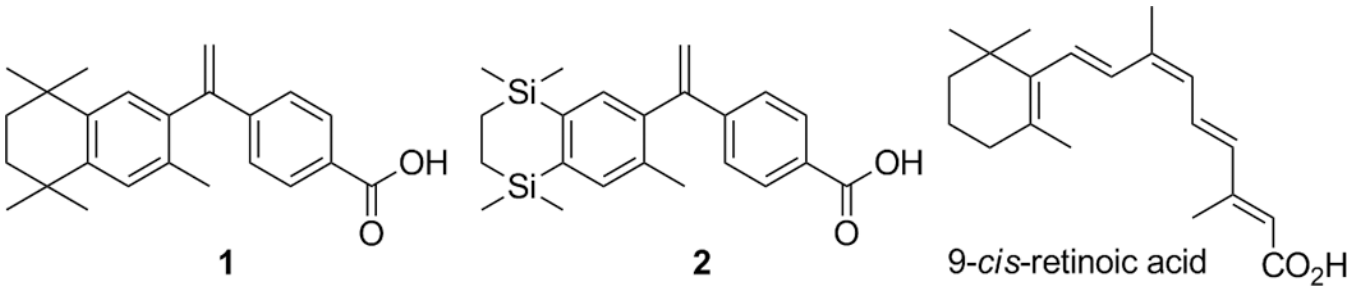
Scheme 2.



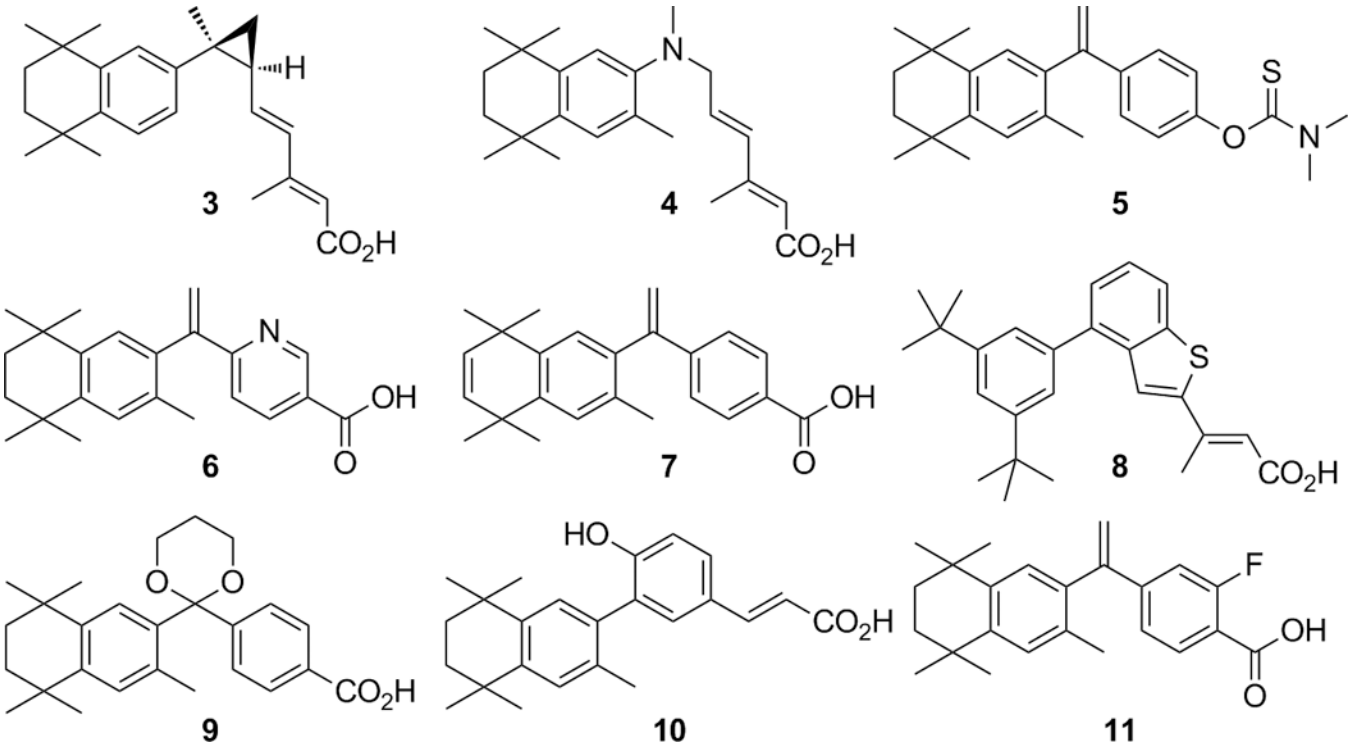
Scheme 3.



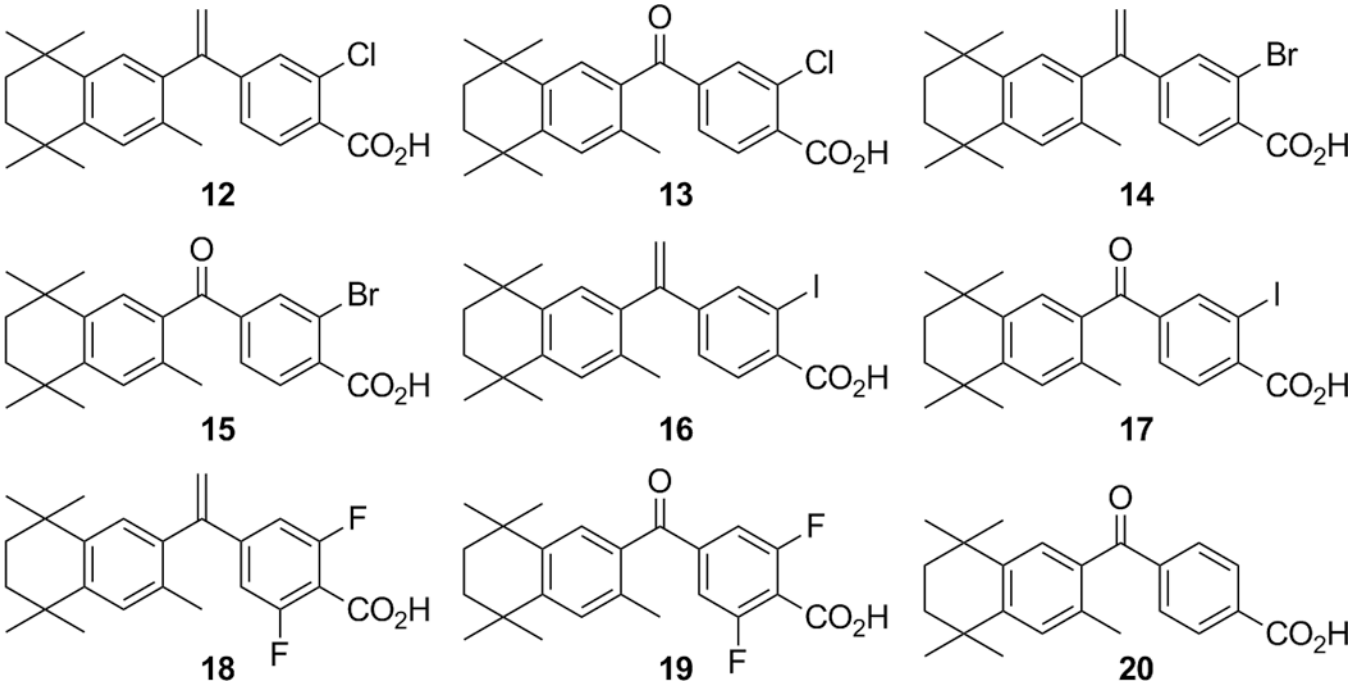
Scheme 4.



Formula 1.



Formula 2.



Formula 3.

Table 1

Determination of EC_{50} Values

Compound	EC 50 value, nM (± S.D.)
Bexarotene **1**	55 (± 6)
Analog **12**	90 (± 14)
Analog **14**	150 (± 18)
Analog **16**	280 (± 34)
Analog **18**	34 (± 6)
Analog **19**	550 (± 67)

EC_{50} values were determined from full dose-response curves ranging from 10^{-10} to 10^{-5} M in transfected HCT-116 cells using an RXR mammalian two-hybrid system as described in *Experimental Section*.

Table 2

Quantitation of RAR Agonist Activity

Compound	% RAR Agonist Activity at 100 nM (± S.D.)	% RAR Agonist Activity at 1 μM (± S.D.)
Bexarotene **1**	21 (4)	19 (3)
Analog **12**	13 (2)	14 (2)
Analog **14**	5 (1)	5 (1)
Analog **16**	5 (1)	5 (1)
Analog **18**	8 (1)	9 (2)
Analog **19**	4 (1)	8 (2)

RAR agonist activity was derived from an RAR/RARE reporter system in transfected HEK-293 cells treated with analog or all-*trans* retinoic acid (RA) at either 100 nM or 1 μM. The activity with analog (or Bex) divided by the activity with all-*trans* RA expressed as a percentage represents the RAR agonist activity.