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Synthesis and biological evaluation of halogenated curcumin analogs as potential nuclear receptor selective agonists

Shane Batie, Jamie H. Lee, Rabia A. Jama, Drew O. Browder, Luis A. Montano, Chanh C. Huynh, Lisa M. Marcus, Dorian G. Tsosie, Zeynab Mohammed, Vu Trang, Pamela A. Marshall, Peter W. Jurutka *, Carl E. Wagner *

School of Mathematical and Natural Sciences, New College of Interdisciplinary Arts and Sciences, Arizona State University, 4701 W. Thunderbird Road, Glendale, AZ 85306, United States

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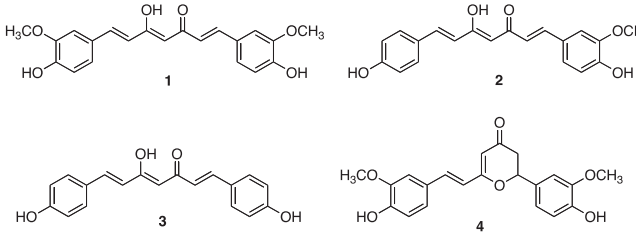
ABSTRACT

This report describes the synthesis of analogs of curcumin, and their analysis in acting as nuclear receptor specific agonists. Curcumin (CM), a turmeric-derived bioactive polyphenol found in curry, has recently been identified as a ligand for the vitamin D receptor (VDR), and it is possible that CM exerts some of its bioeffects via direct binding to VDR and/or other proteins in the nuclear receptor superfamily. Using mammalian-two-hybrid (M2H) and vitamin D responsive element (VDRE) biological assay systems, we tested CM and 11 CM synthetic analogs for their ability to activate VDR signaling. The M2H assay revealed that RXR and VDR association was induced by CM and several of its analogs. VDRE-based assays demonstrated that pure curcumin and eight CM analogs activated transcription of a luciferase plasmid at levels approaching that of the endocrine 1,25 dihydroxyvitamin D_3 (1,25D) ligand in human colon cancer cells (HCT-116). Additional experiments were performed in HCT-116 utilizing various nuclear receptors and hormone responsive elements to determine the receptor specificity of curcumin binding. CM did not appear to activate transcription in a glucocorticoid responsive system. However, CM along with several analogs elicited transcriptional activation in retinoic acid and retinoid X receptor (RXR) responsive systems. M2H assays using RXR–RXR, VDR–SRC1 and VDR–DRIP revealed that CM and select analogs stimulate RXR homodimerization and VDR–coactivator interactions. These studies may lead to the discovery of novel curcumin analogs that activate nuclear receptors, including RXR, RAR and VDR, resulting in similar health benefits as those for vitamins A and D, such as lowering the risk of epithelial and colon cancers.

1. Introduction

The yellow spice powder turmeric, extracted from the *Curcuma longa* L. rhizome, derives its power to stain rice a bright canary yellow in Chinese and Indian curries from a variety of closely related substances termed curcuminoids. The primary constituent of the curcuminoids, termed curcumin (**1**) (CM), accounts for 5% of turmeric powder by weight, with the other minor curcuminoids largely consisting of demethoxycurcumin (**2**) and bisdemethoxycurcumin (**3**), and a very small amount of cyclocurcumin (**4**).[1]



In addition to being an essential spice in Chinese and Indian cuisine, turmeric has a long history of use in traditional Chinese and Indian medicines. Recent studies have shown the principally active chemical component, curcumin, to possess a wide range of pharmacological and biological activities including antioxidant[2],

Abbreviations: CM, curcumin; RAR, retinoic acid receptor; HRE, hormone responsive element; M2H, mammalian two hybrid; RXR, retinoid X receptor; TR, thyroid hormone receptor; T_3, triiodothyronine; VDR, vitamin D receptor; VDRE, vitamin D responsive element; GRE, glucocorticoid responsive element; GR, glucocorticoid receptor; BEX, bexarotene; DEX, dexamethasone; SRC, steroid receptor coactivator; DRIP, vitamin D receptor interacting protein; SNuRMs, selective nuclear receptor modulators; AD, Alzheimer's disease; 1,25D, 1,25 dihydroxyvitamin D_3.

* Corresponding authors. Tel.: +1 602 543 6937; fax: +1 602 543 6073.
E-mail addresses: Peter.Jurutka@asu.edu (P.W. Jurutka), Carl.Wagner@asu.edu (C.E. Wagner).

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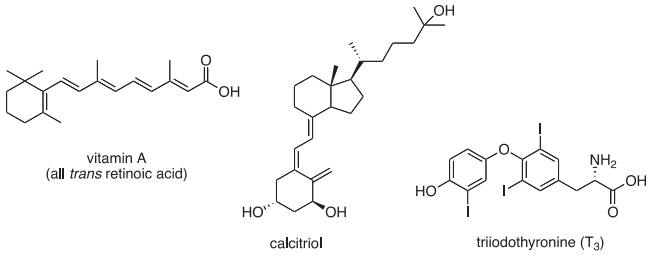
anti-angiogenic[3], anti-inflammatory[4], antiviral[5], antimicrobial, chemo-preventive[6] and anticancer[7] characteristics through biochemical pathways and bio-molecular receptor interactions including various transcriptional factors (NF-κB)[8], cell survival regulators (e.g., cFLIP, Bcl-xL, and Bcl-2)[9], cell proliferation regulators (e.g, c-myc, and cyclin D1)[10], caspase activation signals (e.g., caspase-3, caspase-8, and caspase-9)[11], death receptor regulators (e.g., DR5 and DR4)[12], tumor suppressor factors (e.g. p21 and p53)[13], protein kinase cascades (e.g., Akt, JNK, and AMPK)[14], and mitochondrial pathways. It is precisely this wide-range of pharmacological activities that may well endow curcumin with extraordinary potential in cancer chemoprevention.[15]

Recently, it has been shown that curcumin is a molecule that can bind to and activate the vitamin D receptor (VDR), a nuclear receptor that has been shown not only to regulate bone growth and mineral homeostasis but also cell proliferation through tumor suppressor factors such as p21.[16] Many, but not all, of the nuclear receptor proteins have an endogenous ligand(s) that binds to a specific pocket within the protein, altering the conformation of the receptor and allowing the protein to bind to a specific molecular scaffold on DNA. The association of the nuclear receptor protein with the DNA results in modulation of gene expression ultimately leading to a physiological effect. Examples of nuclear receptor endogenous ligands include vitamin A (the all trans retinoic acid form) as a biologically active ligand for the retinoic acid receptor (RAR)[17], the hormonally active form of vitamin D (calcitriol) as the endogenous ligand for VDR[18], and triiodothyronine (T3) as the ligand for the thyroid hormone receptor (TR).[19]

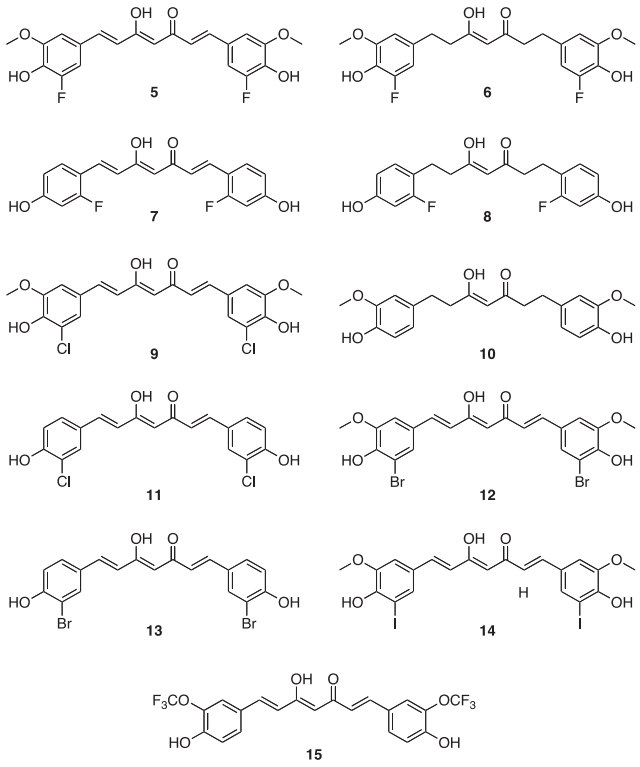


Appropriate nuclear receptor activity is vital to the healthy functioning of physiological processes. For example, the lack of biologically active vitamin D, either through malnutrition or a genetic disorder that prevents the body from being able to make calcitriol, results in a condition known as Rickets where there is softening of the bones.[20] Also, the potential for VDR to up-regulate the tumor suppressing gene p21, as well as the role of vitamin D in chemoprevention of colon and prostate cancers[21] suggests that if curcumin activates VDR as well as vitamin D, then curcumin and its analogs have tremendous pharmacological potential.

Despite the fact that curcumin has been shown to be safe, even at high doses[22] in human and animal models, it has not thus far been FDA approved or validated as a *bona fide* therapeutic agent. Three factors that may explain why, despite its multi-targeting of important biological pathways implicated in cancer, curcumin has not been FDA approved to treat cancer are its poor water solubility, its intense staining ability, and its seemingly low 'bioavailability' (ability to be absorbed, and access various affected systems, in the body).[1] While various approaches have been taken to develop curcumin formulations that increase bioavailability and limit its intense staining properties, there is still ample motivation to prepare novel molecular analogs of curcumin that vary in the substitution of various atoms or structural motifs in order to evaluate their efficacy vis-à-vis curcumin in VDR and other nuclear receptor binding/activation.

1.1. Chemistry

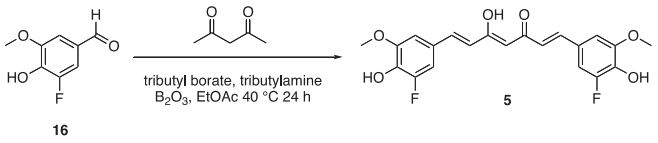
Towards the above two goals, we have synthesized a wide range of novel, halogenated curcumin analogs (**5**–**9**), as well as tetrahydrocurcumin (**10**)[1] and halogenated curcumin analogs that have been reported in literature (**11**–**15**).



Tetrahydrocurcumin (**10**)[1] is reported to have potent biological activities, without the deeply staining color of curcumin. The chlorinated curcumin analog **11** has been patented as a potential therapeutic target in the treatment of Alzheimer's disease.[23] The dibromo curcumin analog **12**[24] has been reported to have comparable antioxidant and cytotoxicity properties as curcumin. The dibromo curcumin analog **13**[25] has been reported to have a lower IC_{50} (33 μM)[25a] than curcumin (>400 μM) for the p300 histone acetyltransferase enzyme, an enzyme implicated in several human cancers and disease pathways. While **14**[26] was synthesized for examination as a potential curcumin analog to inhibit the human platelet 12-lipoxygenase (P-12-LOX) enzyme, curcumin possessed a much lower IC_{50} (66 μM) than **14** (>100 μM). Finally, analog **15**[27] has been shown to be nearly as potent as curcumin in its ability to bind to Alzheimer's amyloid-beta proteins. The fact that several of the reported halogenated curcumin analogs have moderate bioactivities, and the observation that halogen substitutions can occasionally increase binding and activation of nuclear receptors relative to a given agonist,[28] encouraged us to synthesize this array of halogenated curcumin analogs for testing as potential VDR agonists.

The synthesis of analogs **5**–**15** followed the reported synthetic routes[29] for curcumin (**1**) and tetrahydrocurcumin (**10**). For example, the difluoro analog (**5**) was synthesized from commercially available fluoro-vanillin (**16**), in a one-pot reaction that combined **16** with pentane-2,4-dione, tributylborate, tributylamine, and boric anhydride in ethyl acetate for 24 h at 40 °C according to Scheme 1.

The reduction of analog **5** to the difluoro tetrahydrocurcumin **6** was accomplished by hydrogenation with 10% Palladium on carbon



16 5

Scheme 1.

in a continuous hydrogenation flow reactor[30] as shown below (Scheme 2).

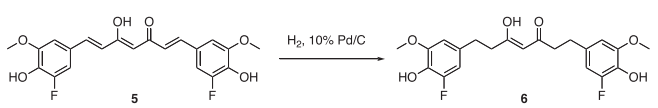
All analogs **5**–**15** were synthesized in methods similar to those in Schemes 1 and 2.

1.2. Biological assays and rationale

Biological evaluation of synthetic halogenated curcumin analogs (**5**–**9**), tetrahydrocurcumin (**10**), and those halogenated curcumin analogs reported in literature (**11**–**15**) was first carried out in two different VDRE-luciferase reporter systems and then repeated in a mammalian two-hybrid assay in colorectal carcinoma cells (HCT-116) (Fig. 1A–C, respectively). These assays test the ability of the analog to bind to or activate the vitamin D receptor (VDR) and induce transcription as measured by luciferase output.

Prior to testing the analogs in these assays, initial dose-response experiments were carried out with CM in the mammalian two-hybrid assay in colorectal carcinoma cells to determine the approximate EC50 for CM activity. The estimated EC50 from these initial experiments (data not shown) was approximately 20–40 μM, and this concentration of ligand was thus utilized in subsequent assays with CM and CM analogs. In the VDRE-based assay (Fig. 1A), in which the activation of VDR by a ligand is quantitated when the active VDR-analog complex is bound to a potent authentic VDRE from the CYP3A4 gene, the results indicate that analogs **6**–**8** do not activate VDR when compared to the DMSO vehicle control, while analogs **5** and **10** display a very mild (but statistically significant) stimulation of luciferase activity, and analogs **9**, **11**–**15** reveal significant activation in this system. Employing a different, less potent VDRE in the context of the natural promoter from the CYP24A1 gene (Fig. 1B), the profile of activation is similar to the CYP3A4 VDRE-based assay, although there is some expected attenuation of activity as a percentage of the DMSO control (compared to Fig. 1A) in this system, especially with the parent compound (curcumin) and with analogs **9** and **11**–**15** which display VDRE-mediated stimulation of luciferase similar to analogs **5** and **10**, while analogs **6**–**8** remain inactive in both systems. Finally, utilizing a mammalian two-hybrid (M2H) assay (Fig. 1C), which detects the ability of each analog to induce VDR-RXR heterodimerization, the range of VDR-RXR activation by our panel of analogs is similar to that of the two VDRE-based assays. The parent compound, along with analogs **5**, **9**, and **11**–**15** exhibit activation while **6**–**8** are inactive. Taken together, the results in Figure 1 demonstrate that curcumin, and select CM analogs (especially halogenated compounds **12**, **14**, **15**) can not only induce the RXR-VDR heterodimer that is required for biological activity (Fig. 1C), but the analogs can bind to VDR and drive transcription mediated by two distinct VDREs (Fig. 1A and B).

The M2H assay was also employed to further detect the ability of select halogenated analogs (**12**, **14**, and **15**) to bind to VDR and recruit the known VDR coactivators SRC-1 and $DRIP_{205}$, again via



5 6

Scheme 2.

quantitation of luciferase production in colorectal carcinoma cells (HCT-116) (Fig. 2A and B, respectively). In the VDR/SRC-1 assay (Fig. 2A), VDR-mediated recruitment of SRC-1 was exhibited with the endocrine VDR ligand (1,25D), as well as with the curcumin parent compound, and all three analogs tested (**12**, **14**, and **15**). The level of ligand-induced SRC binding to VDR was almost as potent (or more potent) with the curcumin compounds as it was with the authentic 1,25D hormone (Fig. 2A, black bar). Similar patterns of luciferase activation are seen in the VDR/$DRIP_{205}$ assay (Fig. 2B), with the parent compound displaying significantly more activation than in the VDR/SRC-1 assay when compared to the 1,25D control, and the CM analogs possessing almost equal activity as 1,25D. The results in Figure 2 demonstrate the ability of curcumin and select halogenated analogs to bind VDR and recruit additional co-factor proteins that are obligatory for VDR-directed transcriptional activation and control of vitamin D target genes.

We next probed whether receptor activation by curcumin and analogs (**5**–**15**) is a VDR-specific phenomenon by employing the glucocorticoid responsive element (GRE), retinoid X receptor responsive element (RXRE), and a retinoic acid receptor responsive element (RARE) (Fig. 3A–C, respectively) transfected in colorectal carcinoma cells (HCT-116). These assay systems are similar to the one employed in Figure 1, except different responsive element-luciferase constructs are employed, and the cells are treated with the appropriate receptor ligands. In the glucocorticoid receptor (GR) and responsive element system (Fig. 3A), the results indicate that while dexamethasone (DEX), a bona fide GR ligand, stimulates dramatic luciferase production, the parent CM compound and analogs **5**–**15** do not activate GR compared to the DMSO control vehicle as measured by activation of luciferase activity. Utilizing an RXRE (Fig. 3B) system with the known RXR ligand bexarotene (BEX) as a positive control, the parent compound along with analogs **9** and **11**–**15** induced transcription of the luciferase gene. When cells were transfected with the retinoic acid receptor and responsive element construct (Fig. 3C), again the parent compound along with analogs **9** and **11**–**15** induced transcription of the luciferase gene in excess of the negative control (DMSO). Interestingly, the pattern of activation between the RXR and RAR systems (Fig. 2), and the VDR–VDRE system (Fig. 1) is similar, perhaps with the exception of analog **5**. Thus, these experiments demonstrate that although CM does not activate the GR system, CM and analog activation of VDR is apparently not solely receptor specific because other proteins in the nuclear receptor superfamily like RXR and RAR are activated by CM and select analogs, at least under these conditions. Moreover, this pattern of receptor activation may be related to the observation that GR is exclusively in the homodimerizing class of nuclear receptors, while RXR, RAR and VDR are all in the heterodimer sub-family. If CM and CM analogs not only bind to VDR, as our results and those of Bartik et al.[16] demonstrate, but also bind to RXR, then this may explain why the pattern of activation is so similar between the RAR, RXR and VDR systems, since all three receptors contain RXR in their transcriptional activation complex.

Therefore, we next sought to further evaluate the potential for CM and CM analogs to directly stimulate RXR–RXR homodimerization using the M2H system (Fig. 4) in colorectal carcinoma cells (HCT-116). Similar to the RXRE experiment (Fig. 3B), analogs **9** and **11**–**15** were shown to induce RXR homodimerization and thus activate the luciferase gene. Taken together, the observations in Figures 3 and 4 further support the hypothesis that CM and select analogs may function as selective nuclear receptors modulators (SNuRMs), either by direct receptor binding in the ligand pocket, as is the case with VDR[31,16] or by modulating the transcriptional activation potential of the receptor, which may perhaps be the mechanism with RAR and RXR.

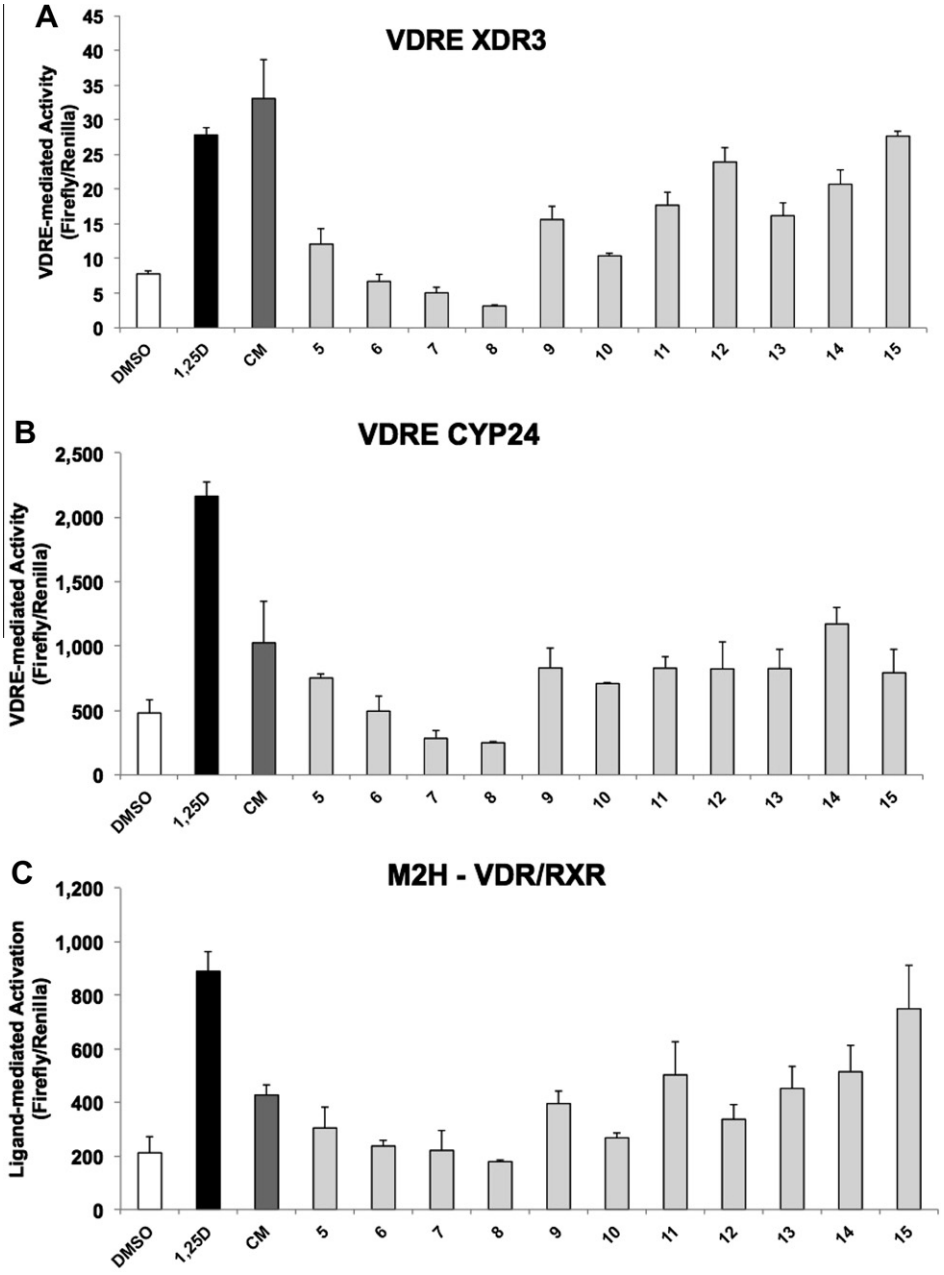


Figure 1. Evaluation of curcumin analogs in activating VDR using a vitamin D responsive element (VDRE)-luciferase reporter system and the mammalian two hybrid (M2H) system transfected into human colon cancer cells (HCT-116): Experiments were performed using 10^{-10} M 1,25-dihydroxyvitamin D, 3.75×10^{-5} M curcumin (CM) and analogs, or DMSO vehicle in complete media with 24 h of treatment. Error bars indicate standard deviation; the data shown are representative of three independent experiments with triplicate samples in each experiment. (A) Transfection with a firefly luciferase plasmid containing two copies of the vitamin D responsive element, XDR3 from the CYP3A4 gene. Utilizing a Students' unpaired, homoscedastic *t*-test, compounds **5** and **10** are significantly more active than DMSO (p <0.05) and 1,25D, CM, **9**, and **11–15** are significantly more active than DMSO (p <0.01). (B) Luciferase-based transcriptional assay using the natural promoter from the human CYP24A1 gene, which contains 2 VDREs. As analyzed by a Students' unpaired, homoscedastic *t*-test, 1,25D, CM, **5** and **9–15** are significantly more active than DMSO (p <0.03). (C) Cells transfected with the expression vectors VDR-AD (prey) and RXRα-BD (bait) along with a firefly luciferase reporter vector (pFR-luc) and Renilla luciferase control plasmid to measure the amount of ligand-stimulated VDR-RXR interaction and to quantitate transfection efficiency, respectively. 1,25D, CM, **9,** and **11–15** are able to drive VDR–RXR dimerization significantly more than DMSO (p <0.05), as determined by a Students' unpaired, homoscedastic *t*-test.

Finally, it has been reported that certain compounds can potentiate VDR activity.[32] Thus, we evaluated the ability of resveratrol, a phytoalexin found in the skin of red grapes, to elicit a synergistic effect when added to cells dosed with curcumin or 1,25D using the XDR3-luciferase reporter system (similar to Fig. 1A) in transfected colorectal carcinoma cells (Fig. 5). CM along with analogs **12**, **14** and **15** all activated transcription of the luciferase gene at approximately 3-fold over the negative control (DMSO). However, when cells were treated with resveratrol and curcumin in combination, CM along with analogs **12**, **14** and **15** again activated transcription of the luciferase gene but this time at levels of approximately 8-fold over DMSO. Similarly, 1,25D elicits an 8-fold activation of luciferase while 1,25D plus resveratrol achieves a 22-fold induction. These results suggest that resveratrol may be potentiating the activity of not only 1,25D-VDR, but also CM-VDR. This latter observation may be of particular significance in that nutritionally derived lipids with known or postulated health benefits (vitamin D, curcumin and

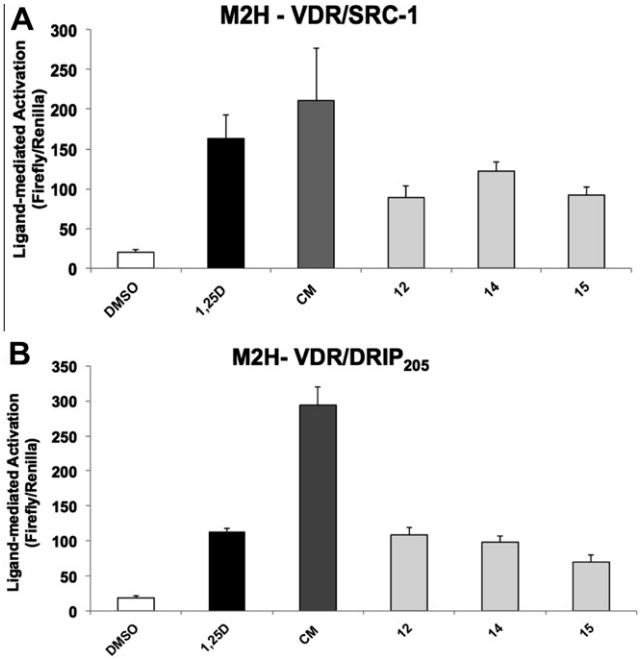


Figure 2. Analysis of CM and CM analogs to stimulate interaction of VDR with VDR coactivators, SRC-1 and DRIP in a mammalian two hybrid system (M2H): Experiments were performed using 10^{-10} M 1,25-dihydroxyvitamin D, 3.75×10^{-5} M curcumin (CM) and analogs, or DMSO vehicle in complete media with 24 h of treatment. Error bars indicate standard deviation; the data shown are representative of experiments in duplicate with triplicate samples in each experiment. (A) HCT-116 cells transfected with the expression vectors VDR-AD (prey) and SRC-1-BD (bait) along with a firefly luciferase reporter vector (pFR-luc) and Renilla luciferase control plasmid to measure the amount of ligand-stimulated VDR-SRC-1 interaction and to measure transfection efficiency, respectively. (B) Cells transfected with the expression vectors VDR-AD (prey) and $DRIP_{205}$-BD (bait) to test for association of VDR and DRIP after treatment with curcumin or analogs. Utilizing a Students' unpaired, homoscedastic *t*-test, all compounds are statistically significantly able to stimulate VDR-coactivator interaction, as compared to DMSO control, (p <0.001).

resveratrol) culminate in modulation of nuclear receptor signaling to exert healthful bioeffects.

The concentrations of CM and the CM analogs used in order to stimulate biological activity are within the range published in previous studies,[16] but may be much higher than needed in in vivo systems due to limitations of the cell culture model. Specifically, in vivo curcumin has been shown to be poorly bioavailable,[22c] but nonetheless metabolized to potentially active compounds[33] suggesting that other components found in vivo are missing in an in vitro system.[34] Additionally, in the gut, even very small levels of CM seem to be quite efficacious[34] suggesting that additional physiological mechanisms are at work in vivo that are attenuated in cell culture. Therefore, the cell culture system may require compensatory higher concentrations of CM and CM analogs to elicit a biological effect due to the loss of the in vivo milieu.

In order to assess the potential for these analogs to be utilized as pharmaceuticals, we employed a *Saccharomyces cerevisiae*-based mutagenicity assay.[28a] This assay uses a strain of yeast that will demonstrate a phenotypic change in response to a genome lesion. In this assay, we tested CM and the analogs for their ability to mutate DNA. DNA mutation is scored either as a color change from white to red on complete (YPD media) or as the ability to grow on synthetic media lacking either tryptophan or isoleucine. CM and all the analogs did not increase the number of red colonies on YPD nor did they increase the number of colonies on synthetic media over DMSO only control (data not shown). Thus, in our initial screen, the compounds were not mutagenic and have exciting potential as additional nutritional additives.

2. Conclusions

We have shown in this manuscript that CM[16] and CM analogs are potent ligands of the VDR. Moreover, our novel data also reveal that CM and CM analogs are able to activate transcription on RXR-homodimer responsive elements as well as RAR-responsive elements. The RXR activation is intriguing given the very recent reports surrounding the effects of RXR agonists on beta-amyloid plaque clearance in mouse models of Alzheimer's disease (AD).[35] Additional work has also shown that curcumin in vitro and in vivo has many therapeutic activities towards AD phenotypes.[36] We speculate that CM may modulate RXR homo- or heterodimerization activity to help alleviate beta-amyloid accumulation. This beta-amyloid clearing activity is of vast clinical significance as bexarotene, the therapeutic RXR analog that is utilized to activate RXR-mediated pathways and potentially treat AD[32] has untoward side effects of hyperlipidemia—likely due to RXR/LXR activation[37]—and hypothyroidism[38] while curcumin ingestion does not cause these issues. Furthermore, our enticing results that curcumin and its analogs demonstrate synergy with resveratrol in binding to or activating VDR could lead the way to data-driven medically sound advice about healthful diet choices, based upon therapeutic needs of the patient. In summary, curcumin, a nutritionally-derived bioactive lipid, and several curcumin analogs, bind to or activate multiple nuclear receptors/pathways, including VDR, RXR, and RAR, and further demonstrate synergy with resveratrol in VDR signaling.

3. Experimental section

3.1. Transfection of cultured mammalian cells and transcriptional activation assays

Cells (HCT-116 colorectal carcinoma) were grown at 37 °C under a humidified atmosphere of 5% carbon dioxide. All cell lines in this study originated from ATCC (Manassas, VA) and were transfected in Falcon 24-well plates from Beckton Dickinson using Express-In Transfection Reagent supplied by Thermo Scientific (Waltham, MA). HCT-116 cells were plated at a density of 90,000 cells/well approximately 24 h prior to transfection in Dulbecco's modified eagle medium (DMEM), supplemented with 10% fetal bovine serum (FBS), 0.1 mM nonessential amino acids, 100 U/ml penicillin and 100 μg/ml streptomycin. The transfection procedure was adapted from the manufacturer's protocol. Briefly, each well received 2 μl/well Express-In and the appropriate DNA plasmids as indicated in the figure legends, along with 20 ng of pRL-null (constitutively expressing low levels of Renilla reniformis luciferase) to monitor transfection efficiency. The luciferase-containing vectors were included in the amount of 250 ng of pLuc-MCS plasmid (Stratagene, La Jolla, CA, USA) containing an oligonucleotide (cloned between the HindIII and BglII sites) with two copies of a nuclear receptor responsive element upstream of the firefly (*Photinus pyralis*) luciferase gene. The VDRE, designated XDR3, was the distal element from the human cytochrome P450 (CYP) 3A4 gene.[39] The entire sequence inserted into pLuc-MCS reporter vector was CAGAGGGTCAGCAAGTTCATTCACAGAGGGTCAGCAAGTTCATTCA, with the half elements underlined. The VDRE, designated CYP24 contained 5.5 kb of the promoter region[40] of the human CYP24 gene (kindly provided by Drs. S. Christakos and J. W. Pike, New Jersey Medical School and University of Wisconsin, respectively) cloned into a firefly luciferase plasmid. The human CYP24 gene possesses two antisense DR3 VDREs (AGGTGAN$_3$AGGGCG

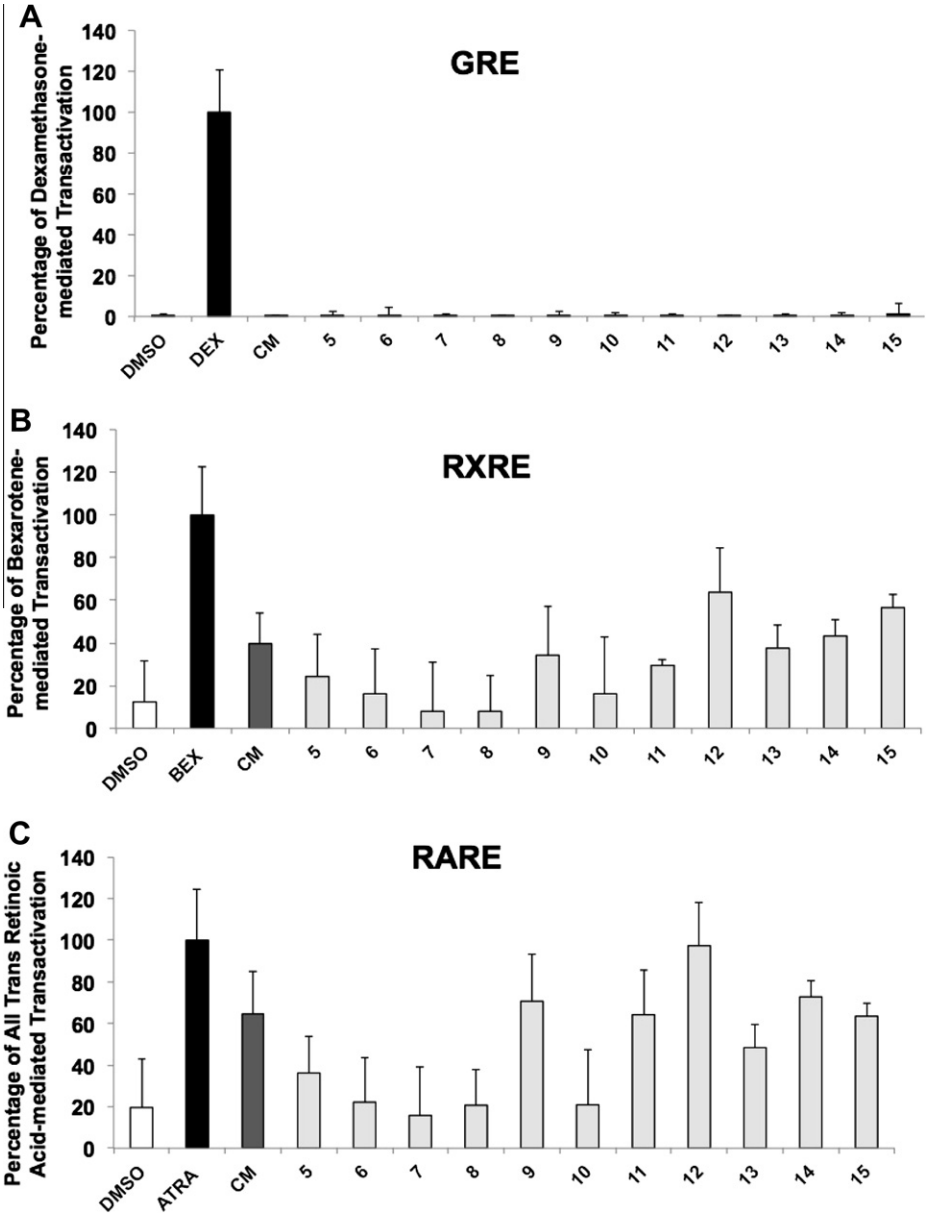


Figure 3. Examination of receptor specificity by curcumin and analogs in transfected human colon cancer cells (HCT-116): Experiments were performed using 2.4×10^{-5} M curcumin (CM) and analogs in complete media with 24 h of treatment, as well as DMSO vehicle. The data were normalized for transfection efficiency and expressed as a percent of ligand-mediated transactivation. Error bars indicate standard deviation. (A) Dexamethasone (10^{-7} M) along with CM and analogs were tested using a GRE-based luciferase system. Utilizing a Students' unpaired, homoscedastic *t*-test, DEX only is significantly different than DMSO (p <0.001). (B) Bexarotene (10^{-8} M) along with CM and analogs tested in an RXRE assay. As analyzed by a Students' unpaired, homoscedastic *t*-test, Bex, CM, **11**–**15** are significantly more able to stimulate transcription than DMSO (p <0.05). (C) All-trans retinoic acid (10^{-6} M) along with CM and analogs tested in the RARE system. ATRA, CM, **9**, and **11**–**15** are significantly more able to activate transcription than DMSO (p <0.05), as determined by a Students' unpaired, homoscedastic *t*-test.

and AGTTCAN$_3$GGTGTG) at −170 and −291 bp, respectively, relative to the transcription start site. A glucocorticoid responsive element (GRE) derived from the rat tyrosine aminotransferase gene was employed, and the sequence cloned into pLuc-MCS was AGCTAGAACATCCTGTACAGCAGAGCTAGAACATCCTGTACAGCAG.[41] The reporter construct containing a retinoid X receptor responsive element (RXRE) was based on a naturally occurring double-repeat responsive element from the rat cellular retinol binding protein II gene.[42] The sequence used was AAAATGAACTGTGACCTGTGACCTGTGACCTGTGAC. A retinoic acid responsive element (RARE) is an optimized element that has been described previously[43] and is responsive to the RAR ligand, all-*trans* retinoic acid. The sequence of the double RARE is (5′-AAAGGTCACCGAAAGGTCACCATCCCGGGAAAAGGTCACCGAAAGGTCACC-3′), with the half elements underlined. Following transfection, the cells were treated with dimethyl sulfoxide (DMSO) vehicle, 1,25-dihydroxyvitamin D$_3$, resveratrol, CM, or CM analogs as described in the figure legends, 18 h after completion of transfection; treatment times ranged from 22 to 26 h. After incubation with ligands, cells were collected and the amount of reporter gene product (luciferase) produced in the cells was measured using the Dual-Luciferase Reporter Assay System according to the manufacturer's protocol (Promega, Madison, WI, USA). To control for transfection efficiency, general cell death and ligand-induced cellular toxicity, the amount of luminescence produced by inducible firefly luciferase was divided by luminescence created by constitutively expressed Renilla luciferase; this ratio was then multiplied by 10,000 to simplify data presentation. The mean ratio of firefly luciferase to

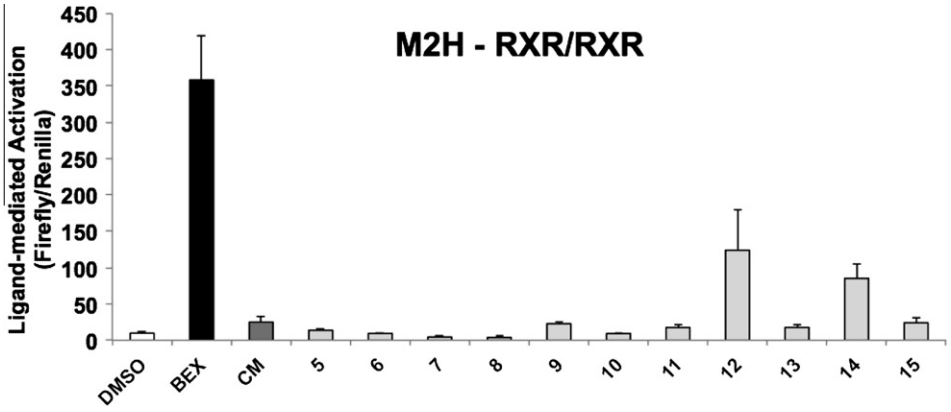


Figure 4. Ability of CM and analogs to stimulate RXR–RXR homodimerization using the M2H system in human colon cancer cells (HCT-116): Experiments performed using 10^{-8} M Bexarotene and 3.75×10^{-5} M curcumin (CM) and analogs in complete media with 24 h of treatment, as well as DMSO vehicle. Cells were transfected with the expression vectors RXRα-AD (prey) and RXRα-BD (bait) along with a firefly luciferase reporter vector (pFR-luc) and Renilla luciferase control plasmid to measure the amount of ligand-stimulated RXR–RXR interaction and to assess transfection efficiency, respectively. Error bars indicate standard deviation; the data shown are representative of three independent experiments with triplicate samples in each experiment. Bex, CM, **9**, and **11**–**15** are significantly more able to stimulate RXR homodimerization than DMSO (p <0.05), as analyzed by a Students' unpaired, homoscedastic t-test.

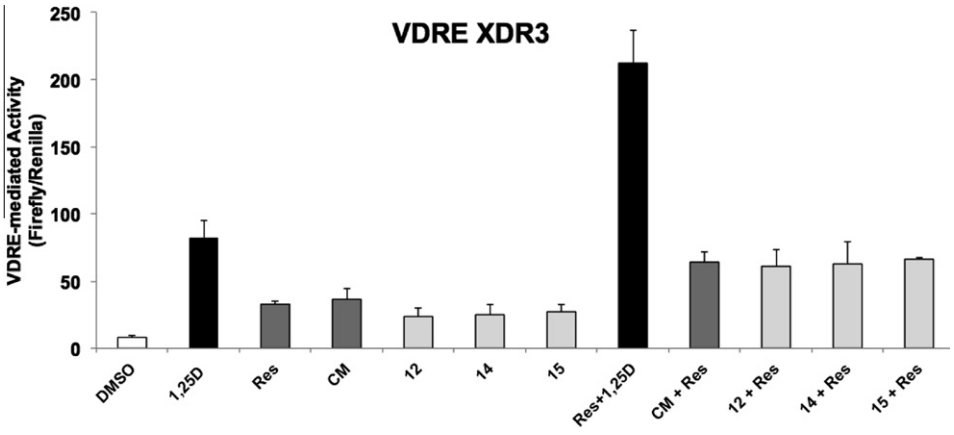


Figure 5. Evaluation of transcriptional synergism between curcumin, CM analogs and resveratrol employing a VDRE-luciferase reporter system in human colon cancer cells (HCT-116): Experiments were performed using either DMSO vehicle, 10^{-10} M 1,25-dihydroxyvitamin D, 3.75×10^{-5} M curcumin (CM) and analogs, or 2.8×10^{-5} M resveratrol (Res) in complete media with 24 h of treatment. Cells were transfected with a firefly luciferase plasmid containing two copies of the vitamin D responsive element, XDR3. Error bars indicate standard deviation; the data shown are representative of three independent experiments with triplicate samples in each experiment. Utilizing a Students' unpaired, homoscedastic t-test, all treatments are significantly more able to stimulate VDRE-mediated transcription (p <0.01) than DMSO alone.

Renilla luciferase signal was determined for each experimental group, and the standard deviation was calculated using MS Excel (expressed as error bars). All data are reported as either the average of two or more experiments, or are representative of two or more trials. Each experimental treatment group was replicated in at least three, and often as many as six, wells.

3.2. Mammalian two-hybrid (M2H) transfections

HCT-116 cells were transfected with components of the mammalian two-hybrid system from Agilent Technologies (Santa Clara, CA), employing similar procedures to those outlined above. RXRα was cloned into pCMV-BD (bait) and VDR into pCMV-AD (prey). SRC-1 was cloned into the pM vector from Clontech (Mountain View, CA, USA), which is similar to the pCMV-BD construct and is compatible with pCMV-AD. $DRIP_{205}$ was cloned into pCMV-BD. RXRα was likewise cloned into pCMV-AD in order to test RXR-RXR interactions. Each well received 50 ng of bait, 50 ng of prey vectors and 20 ng of pRL-null; 250 ng of pFR-luc, a firefly luciferase reporter construct, was also introduced into the cells. The cells were assayed for luciferase activity according to the manufacturer's protocol and results were analyzed as described above.

3.3. Saccharomyces mutagenicity assay

Yeast D7 strain was grown as described in Marshall[44] and one nonrevertant strain was selected. This strain was used for the remainder of the assays. Cells were incubated for two hours with compounds solubilized in DMSO or DMSO alone (control), at increasing concentrations from 0.008 mg/ml to 0.1 mg/ml and then plated on YPD or selective media lacking tryptophan or isoleucine. Growth was scored after 5 days at 30 °C, and compared to DMSO only control. Mutagenicity would be scored as the formation of colonies in numerical excess as compared to DMSO only control. Ethidium bromide was used as a positive control.

3.4. Statistical analysis

A Students' unpaired T-test, with two tails, homoscedastic variance was used to compare all data to controls and are as indicated in the figure legends.

3.5. 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. Melting points were assayed on a Thomas Hoover capillary melting point apparatus.

3.6. General procedures

Tetrahydrofuran, methylene chloride, diethyl ether, and benzene were dried by filtration through alumina according to the procedure described by Grubbs.[45] 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). 3-Fluoro-4-hydroxy-5-methoxybenzaldehyde was purchased from Apollo Scientific. 2-Fluoro-4-hydroxybenzaldehyde was purchased from Oakwood Products, Inc. 5-Iodovanillin, 2,4-pentanedione, *n*-butylamine, hydroxybenzaldehyde, and tributylborate were purchased from ACROS. 5-Bromovanillin was purchased from Indofine Chemicals. Boron oxide, 3-chloro-4-hydroxy-5-methoxybenzaldehyde, 3-chloro-4-hydroxybenzaldehyde, and 3-bromo-4-methoxybenzaldehyde were purchased from Aldrich. Trifluoromethylvanillin was purchased from JRD Fluorochem. All tested compounds were analyzed for purity by combustion analysis through Columbia Analytical Services (formerly Desert Analytics in Tucson, AZ).

3.7. General procedure to synthesize 5, 7, 9, and 11–15

3.7.1. (1*E*,4*Z*,6*E*)-1,7-Bis(3-fluoro-4-hydroxy-5-methoxyphenyl)-5-hydroxyhepta-1,4,6-trien-3-one (5)

The method of Muzumder and co-workers was followed.[29] To a solution of 3-fluoro-4-hydroxy-5-methoxybenzaldehyde (3.40 g, 20.0 mmol), 1,4-pentanedione (1.03 mL, 10.0 mmol), boron oxide (0.50 g, 7.2 mmol), and tributylborate (5.4 mL, 20.0 mmol) in ethyl acetate (10.0 mL) was added dropwise a solution of butyl amine (1.10 g, 15.0 mmol) in ethyl acetate (10.0 mL). The reaction was stirred at 40 °C for 24 h and then quenched by the addition of 10% HCl (10.0 mL) and stirring at 60 °C (1 h). The solution was poured into water (20 mL), and the organic layer was separated. The aqueous layer was extracted with ethyl acetate and the combined organic layers were washed with water, separated, and dried over sodium sulfate. The solvents were removed in vacuo to give a crude, dark powder that was purified by column chromatography (silica gel, hexanes/ethyl acetate 1:1) to give a deep red crystalline product (0.42 g, 10%): mp 221–222 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.89 (br s, 2H), 7.53 (d, J = 15.6 Hz, 2H), 7.25 (d, J = 11.2 Hz, 2H), 7.21 (s, 2H), 6.86 (d, J = 15.6 Hz, 2H), 6.03 (s, 1H), 3.87 (s, 6H), 3.16 (s, 1H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 183.1, 152.5, 150.1, 149.7, 149.6, 139.9, 136.9, 136.7, 125.4, 124.3, 122.7, 109.2, 109.0, 108.0, 101.5, 56.3, 48.6; LC-APCI-MS [M$^+$+H] calcd for $C_{21}H_{19}O_6F_2$ 405.1150, found 405.1143. Anal. Calcd for $C_{21}H_{18}O_6F_2$: C, 62.38; H, 4.49; F, 9.40. Found: C, 62.05; H, 4.87; F, 9.3.

3.7.2. (1*E*,4*Z*,6*E*)-1,7-Bis(2-fluoro-4-hydroxyphenyl)-5-hydroxyhepta-1,4,6-trien-3-one (7)

Bright yellow crystalline product (0.44 g, 12.6%): mp 213–216 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.58 (br s, 2H), 7.68 (t, J = 8.8 Hz, 2H), 7.60 (d, J = 16 Hz, 2H), 6.77 (d, J = 16 Hz, 2H), 6.70 (dd, J = 8.4, 2.4 Hz, 2H), 6.65 (dd, J = 12.8, 2.4 Hz, 2H), 6.09 (s, 1H), 3.16 (s, 2H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 183.0, 163.2, 161.4, 161.3, 160.7, 132.4, 132.4, 130.3, 130.2, 122.9, 122.8, 113.4, 113.3, 112.7, 103.1, 102.8, 101.6, 48.6; LC-APCI-MS [M$^+$+H] calcd for $C_{19}H_{15}O_4F_2$ 345.0938, found 345.0925. Anal. Calcd for $C_{19}H_{14}O_4F_2$: C, 66.28; H, 4.10; F, 11.04. Found: C, 65.35; H, 4.33; F, 10.6.

3.7.3. (1*E*,4*Z*,6*E*)-1,7-Bis(3-chloro-4-hydroxy-5-methoxyphenyl)-5-hydroxyhepta-1,4,6-trien-3-one (9)

Bright yellow powder (0.43 g, 9.8%): mp 227–230 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 16.29 (br s, 1H), 10.06 (br s, 2H), 7.53 (d, J = 16 Hz, 2H), 7.39 (d, J = 1.6 Hz, 2H), 7.34 (d, J = 1.6 Hz, 2H), 6.88 (d, J = 15.6 Hz, 2H), 6.05 (s, 1H), 3.89 (s, 6H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 183.1, 148.8, 144.9, 139.6, 126.5, 122.7, 120.2, 109.9, 101.5, 56.3; LC-APCI-MS [M$^+$+H] calcd for $C_{21}H_{19}O_6Cl_2$ 437.0559, found 437.0563. Anal. Calcd for $C_{21}H_{18}O_6Cl_2$: C, 57.68; H, 4.15; Cl, 16.22. Found: C, 57.48; H, 4.24; Cl, 16.30.

3.7.4. (1*E*,4*Z*,6*E*)-1,7-Bis(3-chloro-4-hydroxyphenyl)-5-hydroxyhepta-1,4,6-trien-3-one (11)

Bright yellow powder (0.70 g, 18.8%): mp 199–205 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 16.29 (br s, 1H), 10.82 (br s, 2H), 7.79 (s, 2H), 7.53 (d, J = 9.2 Hz, 2H), 7.51 (d, J = 15.6 Hz, 2H), 7.00 (d, J = 8.4 Hz, 2H), 6.81 (d, J = 16.0 Hz, 2H), 6.02 (s, 1H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 183.1, 155.1, 139.1, 129.9, 128.8, 127.1, 122.3, 120.5, 116.9, 101.6; LC-APCI-MS [M$^+$+H] calcd for $C_{19}H_{15}O_4Cl_2$ 377.0347, found 377.0349. Anal. Calcd for $C_{19}H_{14}O_4Cl_2$: C, 60.50; H, 3.74; Cl, 18.80. Found: C, 60.21; H, 3.99; Cl, 19.3.

3.7.5. (1*E*,4*Z*,6*E*)-1,7-Bis(3-bromo-4-hydroxy-5-methoxyphenyl)-5-hydroxyhepta-1,4,6-trien-3-one (12)[24]

Bright orange crystalline solid (0.65 g, 12.3%): mp 224–232 °C (lit. 236–238 °C);[24] ^{1}H NMR (400 MHz, DMSO-d_6) δ 16.30 (br s, 1H), 10.10 (s, 1H), 7.53 (d, J = 16.0 Hz, 2H), 7.52 (d, J = 2.0 Hz, 2H), 7.38 (d, J = 2.0 Hz, 2H), 6.88 (d, J = 16.0 Hz, 2H), 6.05 (s, 1H), 3.89 (s, 6H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 183.1, 148.5, 146.0, 139.4, 127.2, 125.6, 122.6, 110.4, 109.6, 101.4, 56.3; LC-APCI-MS [M$^+$+H] calcd for $C_{21}H_{19}O_6Br_2$ 526.9528, found 526.9539. Anal. Calcd for $C_{21}H_{18}O_6Br_2$: C, 47.94; H, 3.45; Br, 30.37. Found: C, 47.85; H, 3.40; Br, 29.4.

3.7.6. (1*E*,4*Z*,6*E*)-1,7-Bis(3-bromo-4-hydroxyphenyl)-5-hydroxyhepta-1,4,6-trien-3-one (13)[25a]

Bright orange crystalline solid (0.87 g, 18.5%): mp 218–222 °C (lit. 175–176 °C);[25a] ^{1}H NMR (400 MHz, DMSO-d_6) δ 16.29 (s, 1H), 10.89 (s, 2H), 7.93 (d, J = 2.0 Hz, 2H), 7.56 (dd, J = 8.4, 2.0, 2H), 7.51 (d, J = 16.0 Hz, 2H), 6.99 (d, J = 8.4 Hz, 2H), 6.80 (d, J = 16.0 Hz, 2H), 6.02 (s, 1H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 183.1, 156.1, 139.0, 132.9, 129.3, 127.6, 122.2, 116.5, 110.0, 101.6; LC-APCI-MS [M$^+$+H] calcd for $C_{19}H_{15}O_4Br_2$ 464.9337, found 464.9345. Anal. Calcd for $C_{19}H_{14}O_4Br_2$: C, 48.96; H, 3.03; Br, 34.28. Found: C, 48.93; H, 2.85; Br, 35.5.

3.7.7. (1*E*,4*Z*,6*E*)-5-Hydroxy-1,7-bis(4-hydroxy-3-iodo-5-methoxyphenyl)hepta-1,4,6-trien-3-one (14)[26]

Golden brown powder (0.96 g, 7.7%): mp 230–233 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 16.32 (br s, 1H), 10.19 (s, 2H), 7.70 (s, 2H), 7.55 (d, J = 15.6 Hz, 2H), 7.42 (s, 2H), 6.89 (d, J = 16 Hz, 2H), 6.10 (s, 1H), 3.92 (s, 6H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 183.1, 148.5, 147.1, 139.3, 131.4, 128.2, 122.4, 111.0, 101.3, 84.8, 56.2; LC-APCI-MS [M$^+$+H] calcd for $C_{21}H_{19}O_6I_2$ 620.9272, found 620.9300. Anal. Calcd for $C_{21}H_{18}O_6I_2$: C, 40.60; H, 3.08. Found: C, 40.93; H, 2.84.

3.7.8. (1*E*,4*Z*,6*E*)-5-Hydroxy-1,7-bis(4-hydroxy-3-(trifluoromethoxy)phenyl)hepta-1,4,6-trien-3-one (15)[27]

Golden brown powder (0.25 g, 11%): mp 144–153 °C (lit. 173–174 °C);[27] ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.88 (2.14), 7.70–7.60 (m, 4H), 7.57 (d, J = 16 Hz, 2H), 7.06 (d, J = 8.0 Hz, 2H), 6.82 (d, J = 16 Hz, 2H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 183.1, 152.0, 139.1, 136.3, 129.0, 126.6, 123.2, 122.6, 121.6, 119.1, 118.0, 101.5; LC-APCI-MS [M$^+$+H] calcd for $C_{21}H_{15}O_6F_6$ 477.0773, found 477.0776. Anal. Calcd for $C_{21}H_{14}O_6F_6$: C, 52.95; H, 2.96; F, 23.93. Found: C, 51.22; H, 3.25; F, 22.90.

3.8. General procedure to synthesize 6, 8, and 10

3.8.1. (*Z*)-1,7-Bis(3-fluoro-4-hydroxy-5-methoxyphenyl)-5-hydroxyhept-4-en-3-one (6)

A 0.25 M solution of **5** (0.1403 g, 0.34 mmol) in a 1:1 ethyl acetate/ethanol solution (1.4 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 **6** that was purified by column chromatography (silica gel, hexanes/ethyl acetate 1:1) to give a colorless crystalline product (0.068 g, 49%): mp 136–139 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.40 (br s, 1H), 6.55 (dd, J = 10.8, 2.0 Hz, 2H), 6.48 (d, J = 1.6 Hz, 2H), 5.41 (s, 1H), 5.31 (br s, 2H), 3.87 (s, 6H), 2.80 (t, J = 8.0 Hz, 4H), 2.53 (t, J = 8.0 Hz, 4H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 202.9, 192.7, 151.5, 149.1, 148.0, 147.9, 132.0, 131.9, 131.8, 108.7, 108.5, 106.7, 106.7, 106.6, 106.6, 99.8, 57.5, 56.3, 45.1, 39.9, 31.1, 31.0, 28.9; LC-APCI-MS [M$^+$] calcd for $C_{21}H_{22}O_6F_2$ 408.1385, found 408.1405. Anal. Calcd for $C_{21}H_{22}O_6F_2$: C, 61.76; H, 5.43. Found: C, 61.84; H, 5.41.

3.8.2. (*Z*)-1,7-Bis(2-fluoro-4-hydroxyphenyl)-5-hydroxyhept-4-en-3-one (8)

Off white, crystalline solid (0.1295 g, 76%): mp 89–91 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.29 (br s, 1H), 6.94–6.98 (m, 2H), 6.50–6.54 (m, 2H), 6.51 (s, 2H), 5.42 (br s, 2H), 5.41 (s, 1H), 2.85 (t, J = 8.0 Hz, 4H), 2.53 (t, J = 8.0 Hz, 4H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 193.3, 162.6, 160.1, 155.3, 155.2, 131.0, 130.9, 119.2, 119.1, 111.1, 111.0, 103.3, 103.0, 99.8, 38.6, 24.5; LC-APCI-MS [M$^+$] calcd for $C_{19}H_{19}O_4F_2$ 349.1251, found 349.1247. Anal. Calcd for $C_{19}H_{19}O_4F_2$: C, 65.51; H, 5.21; F, 10.91. Found: C, 65.20; H, 5.25; F, 10.9.

3.8.3. (*Z*)-5-Hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)hept-4-en-3-one (10)

White, crystalline solid (0.0929 g, 61%): mp 95–97 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.83–6.80 (m, 2H), 6.68 (s, 2H), 6.67–6.65 (m, 2H), 5.53 (br s, 2H), 5.42 (s, 1H), 3.85 (s, 6H), 2.86–2.76 (m, 4H), 2.57–2.53 (t, J = 7.2, 4H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 203.4, 193.2, 146.3, 143.9, 132.5, 120.7, 114.2, 110.9, 110.8, 99.8, 57.6, 55.8, 45.4, 40.3, 31.2, 29.0. GC-MS [M$^+$] calcd for $C_{21}H_{24}O_6$ 372.1573, found 372.1574. Anal. Calcd for $C_{21}H_{24}O_6$: C, 67.73; H, 6.50. Found: C, 68.32; H, 6.41.

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Supplementary data

Supplementary data (^{1}H NMR and ^{13}C NMR spectra of all compounds reported in the experimentals) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.11.033.

References and notes

1. Anand, R.; Thomas, S. G.; Kunnumakkara, A. B.; Sundaram, C.; Harikumar, K. B.; Sung, B.; Tharakan, S. T.; Misra, K.; Priyadarsini, I. K.; Rajasekharan, K. N.; Aggarwal, B. B. *Biochem. Pharm.* **2008**, *76*, 1590.
2. Ruby, A. J.; Kuttan, G.; Dinesh Babu, K. V.; Rajasekharan, K. N.; Kuttan, R. *Cancer Lett.* **1995**, *94*, 79.
3. Adams, B. K.; Ferstl, E. M.; Davis, M. C.; Herold, M.; Kurtkaya, S.; Camalier, R. F.; Hollingshead, M. G.; Kaur, G.; Sausville, E. A.; Rickles, F. R.; Snyder, J. P.; Liotta, D. C.; Shoji, M. *Bioorg. Med. Chem.* **2004**, *12*, 3871.
4. Zambre, A. P.; Kulkarni, V. M.; Padhye, S.; Sandur, S. K.; Aggarwal, B. B. *Bioorg. Med. Chem.* **2006**, *14*, 7196.
5. Vajragupta, O.; Boonchoong, P.; Morris, G. M.; Olson, A. J. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3364.
6. Zhang, Q.; Zhong, Y.; Yan, L.-N.; Sun, X.; Gong, T.; Zhang, Z.-R. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 1010.
7. Surh, Y. J. *Rev. Cancer* **2003**, *3*, 768.
8. Singh, S.; Aggarwal, B. B. *Biol. Chem.* **1995**, *270*, 24995.
9. Aggarwal, S.; Ichikawa, H.; Takada, Y.; Sandur, S. K.; Shishodia, S.; Aggarwal, B. B. *Pharmacology* **2006**, *69*, 195.
10. (a) Dorai, T.; Aggarwal, B. B. *Cancer Lett.* **2004**, *215*, 129; (b) Mukhopadhyay, A.; Banerjee, S.; Stafford, L. J.; Xia, C.; Liu, M.; Aggarwal, B. B. *Oncogene* **2002**, *21*, 8852.
11. Shikora, E.; Bielak-Zmijewska, A.; Magalska, A.; Piwocka, K.; Mosieniak, G.; Kalinowska, M.; Widlak, P.; Cymerman, I. A.; Bujnicki, J. M. *Cancer Ther.* **2006**, *5*, 927.
12. Jung, E. M.; Lim, J. H.; Lee, T. J.; Park, J.-W.; Choi, K. S.; Kwon, T. K. *Carcinogenesis* **1905**, *2005*, 26.
13. Choudhuri, T.; Pal, S.; Agwarwal, M. L.; Das, T.; Sa, G. *FEBS Lett.* **2002**, *512*, 334.
14. (a) Woo, J.-H.; Kim, Y.-H.; Lee, K.-S.; Bae, J. H.; Min, D. S.; Chang, J.-S.; Jeong, Y.-J.; Lee, Y. H.; Park, J.-W.; Kwon, T. K. *Carcinogenesis* **2003**, *24*, 1199; (b) Siwak, D. R.; Shishodia, S.; Aggarwal, B. B.; Kurzrock, R. *Cancer* **2005**, *104*, 879.
15. Ravindran, J.; Prasad, S.; Aggarwal, B. B. *AAPS J.* **2009**, *11*, 495.
16. Bartik, L.; Whitfield, G. K.; Kaczmarska, M.; Lowmiller, C. L.; Moffet, E. W.; Furmick, J. K.; Hernandez, Z.; Haussler, C. A.; Haussler, M. R.; Jurutka, P. W. *J. Nutr. Biochem.* **2010**, *21*, 1153.
17. Dawson, M. I.; Jong, L.; Hobbs, P. D.; Cameron, J. F.; Chao, W.; Pfahl, M.; Lee, M.-O.; Shroot, B.; Pfahl, M. *J. Med. Chem.* **1995**, *38*, 3368.
18. Wu-Wong, J. R.; Nakane, M.; Ma, J.; Dixon, D.; Gagne, G. *Leuk. Lymphoma* **2006**, *47*, 727.
19. Baxter, J. D.; Goede, P.; Apriletti, J. W.; West, B. L.; Feng, W.; Mellstrom, K.; Fletterick, R. J.; Wagner, R. L.; Kushner, P. J.; Ribeiro, R. C. J.; Webb, P.; Scanlan, T. S.; Nilsson, S. *Endocrinology* **2002**, *143*, 517.
20. Haussler, M. R.; Whitfield, G. K.; Haussler, C. A.; Hsieh, J.-C.; Jurutka, P. W. Nuclear Vitamin D Receptor: Natural Ligands, Molecular Structure–Function, and Transcriptional Control of Vital Genes. In *Vitamin D*; Feldman, D., Pike, J. W., Adams, J. S., Eds., 3rd ed.; Academic Press: Amsterdam, 2011; pp 137–170. Vol. 1.
21. Haussler, M. R.; Haussler, C. A.; Whitfield, G. K.; Hsieh, J.-C.; Thompson, P. D.; Barthel, T. K.; Bartik, L.; Egan, J. B.; Wu, Y.; Kubicek, J. L.; Lowmiller, C. L.; Moffet, E. W.; Forster, R. E.; Jurutka, P. W. *J. Steroid Biochem. Mol. Biol.* **2010**, *121*, 88.
22. (a) Sharma, R. A.; Euden, S. A.; Platton, S. L.; Cooke, D. N.; Shafayat, A.; Hewitt, H. R.; Marczylo, T. H.; Morgan, B.; Hemingway, D.; Plummer, S. M.; Pirohamed, M.; Gescher, A. J.; Steward, W. P. *Clin. Cancer Res.* **2004**, *10*, 6847; (b) Lao, C. D.; Ruffin, M. T., IV; Normolle, D.; Heath, D. D.; Murray, S. I.; Bailey, J. M.; Boggs, M. E.; Crowell, J.; Rock, C. L.; Brenner, D. E. *BMC Complement. Altern. Med.* **2006**, *6*, 6; (c) Dhillon, N.; Aggarwal, B. B.; Newman, R. A.; Wolff, R. A.; Kunnumakkara, A. B.; Abbruzzese, J. L.; Ng, C. S.; Badmaev, V.; Kurzrock, R. *Clin. Cancer Res.* **2008**, *14*, 4491.
23. (a) Cashman, J.R.; Fiala, M. PCT Int. Appl. WO 2008048410 A2 20080424, 2008.; (b) Takahasi, T.; Hijikuro, I.; Sugimoto, H.; Kihara, T.; Shimmyo, Y.; Niidome, T.

PCT Int. Appl. WO 2008066151 A1 20080605, 2008.; (c) Cashman, J.R.; Abel, K.J. PCT Int. Appl. WO2010068935 A2 20100617, 2010.
24. Venkateswarlu, S.; Ramachandra, M. S.; Subbaraju, G. V. *Bioorg. Med. Chem.* **2005**, *13*, 6374.
25. (a) Costi, R.; Di Santo, R.; Artico, M.; Miele, G.; Valentini, P.; Novellino, E.; Cereseto, A. *J. Med. Chem.* **2007**, *50*, 1973; (b) Mai, A.; Cheng, D.; Bedford, M. T.; Valente, S.; Nebbioso, A.; Perrone, A.; Brosch, G.; Sbardella, G.; De Bellis, F.; Miceli, M.; Altucci, L. *J. Med. Chem.* **2008**, *51*, 2279.
26. Jankun, J.; Aleem, A. M.; Malgorzewicz, S.; Szkudlarek, M.; Zavodszky, M. I.; DeWitt, D. L.; Feig, M.; Selman, S. H.; Skrzypczak-Jankun, E. *Mol. Cancer Ther.* **2006**, *5*, 1371.
27. Yanagisawa, D.; Shirai, N.; Amatsubo, T.; Taguchi, H.; Hirao, K.; Urushitani, M.; Morikawa, S.; Inubushi, T.; Kato, M.; Kato, F.; Morino, K.; Kimura, H.; Nakano, I.; Yoshida, C.; Okada, T.; Sano, M.; Wada, K.-N.; Yamamoto, A.; Tooyama, I. *Biomaterials* **2010**, *31*, 4179.
28. (a) Wagner, C. E.; Jurutka, P. W.; Marshall, P. A.; Groy, T. L.; van der Vaart, A.; Ziller, J. W.; Furmick, J. K.; Graeber, M. E.; Matro, E.; Miguel, B. V.; Tran, I. T.; Kwon, J.; Tedeschi, J. N.; Moosavi, S.; Danishyar, A.; Philp, J. S.; Khamees, R. O.; Jackson, J. N.; Grupe, D. K.; Badshah, S. L.; Hart, J. W. *J. Med. Chem.* **2009**, *52*, 5950; (b) Furmick, J. K.; Kaneko, I.; Walsh, A. N.; Yang, J.; Bhogal, J. S.; Gray, G. M.; Baso, J. C.; Browder, D. O.; Prentice, J. L. S.; Montano, L. A.; Huynh, C. C.; Marcus, L. M.; Tsosie, D. G.; Kwon, J. S.; Quezada, A.; Reyes, N. M.; Lemming, B.; Saini, P.; van der Vaart, A.; Groy, T. L.; Marshall, P. A.; Jurutka, P. W.; Wagner, C. E. *ChemMedChem* **2012**, *7*, 1551.
29. Muzumder, A.; Neamati, N.; Sunder, S.; Schulz, J.; Pertz, H.; Eich, E.; Pommier, Y. *J. Med. Chem.* **1997**, *40*, 3057.
30. The ThalesNano H-Cube® was used for this hydrogenation reaction.
31. Menegaz, D.; Mizwicki, M. T.; Barrientos-Duran, A.; Chen, N.; Henry, H. L.; Norman, A. W. *Mol. Endocrinol.* **2011**, *25*, 1289.
32. Dampf-Stone, A. et al *Proc. Ariz.-Nev. Acad. Sci.* **2012**, *47*(1), 17.
33. Ireson, C. R.; Jones, D. J.; Orr, S.; Coughtrie, M. W.; Boocock, D. J.; Williams, M. L.; Farmer, P. B.; Steward, W. P.; Gescher, A. J. *Cancer Epidemiol. Biomarkers Prev.* **2002**, *11*, 105.
34. Irving, G. R. B.; Karmokar, A.; Berry, D. P.; Brown, K.; Steward, W. P. *Best Pract. Res. Clin. Gastroenterol.* **2001**, *25*, 519.
35. Cramer, P. E.; Cirrito, J. R.; Wesson, D. W.; Lee, C. Y. D.; Karlo, J. C.; Zinn, A. E.; Casali, B. T.; Restivo, J. L.; Goebel, D. A.; James, M. J.; Brunden, K. R.; Wilson, D. A.; Landreth, G. E. *Science* **2012**, *335*, 1503.
36. (a) Hamaguchi, T.; Ono, K.; Yamada, M. *CNS Neurosci. Ther.* **2010**, *16*, 285; (b) Yang, F.; Lim, G. P.; Begum, A. N.; Ubeda, O. J.; Simmons, M. R.; Ambegaokar, S. S.; Chen, P. P.; Kayed, R.; Glabe, C. G.; Frautschy, S. A.; Cole, G. M. *J. Biol. Chem.* **2005**, *280*, 5892.
37. (a) Murthy, S.; Born, E.; Mathur, S. N.; Field, F. J. *J. Lipid Res.* **2002**, *43*, 1054; (b) Field, F. J.; Born, E.; Mathur, S. N. *J. Lipid Res.* **2004**, *45*, 905.
38. Sherman, S. I.; Gopal, J.; Haugen, B. R.; Chiu, A. C.; Whaley, K.; Nowlakha, P.; Duvic, M. *N. Engl. J. Med.* **1999**, *340*, 1075.
39. Thompson, P. D.; Jurutka, P. W.; Whitfield, G. K.; Myskowski, S. M.; Eichhorst, K. R.; Dominguez, C. E.; Haussler, C. A.; Haussler, M. R. *Biochem. Biophys. Res. Commun.* **2002**, *299*, 730.
40. Jin, C. H.; Kerner, S. A.; Hong, M. H.; Pike, J. W. *Mol. Endocrinol.* **1996**, *10*, 945.
41. Jantzen, H. M.; Strahle, U.; Gloss, B.; Stewart, F.; Schmid, W.; Boshart, M.; Miksicek, R.; Schutz, G. *Cell* **1987**, *49*, 29.
42. Mangelsdorf, D. J.; Umesono, K.; Kliewer, S. A.; Borgmeyer, U.; Ong, E. S.; Evans, R. M. *Cell* **1991**, *66*, 555.
43. Forman, B. M.; Umesono, K.; Chen, J.; Evens, R. M. *Cell* **1995**, *81*, 541.
44. Marshall, P. A. *CBE-LSE* **2007**, *6*, 307.
45. Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J. *Organometallics* **1996**, *15*, 1518.