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Derivation of a Retinoid X Receptor Scaffold from Peroxisome Proliferator-Activated Receptor γ Ligand 1-Di(1*H*-indol-3-yl)methyl-4-trifluoromethylbenzene

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Abstract

1-Di(1*H*-indol-3-yl)methyl-4-trifluoromethylbenzene (DIM-Ph-4-CF₃) is reported to inhibit cancer cell growth and to act as a transcriptional agonist of peroxisome proliferator-activated receptor γ (PPAR γ) and nuclear receptor 4A subfamily member 1 (NR4A1). In addition, DIM-Ph-4-CF₃ exerts anticancer effects independent of these receptors because PPAR γ antagonists do not block its inhibition of cell growth, and the small pocket in the NR4A1 crystal structure suggests no ligand can bind. Because PPAR γ and NR4A1 heterodimerize with retinoid X receptor (RXR), and several PPAR γ ligands transcriptionally activate RXR, DIM-Ph-4-CF₃ was investigated as an RXR ligand. DIM-Ph-4-CF₃ displaces 9-*cis*-retinoic acid from RXR α but does not transactivate RXR α . Structure-based design using DIM-Ph-4-CF₃ as a template led to the RXR α transcriptional agonist (*E*)-3-[5-di(1-methyl-1*H*-indol-3-yl)methyl-2-thienyl]acrylic acid. Its docked pose in the RXR α ligand binding domain suggests that binding is stabilized by interactions of its carboxylate group with arginine 316, its indoles with cysteines 269 and 432, and its 1-methyl groups with hydrophobic residues lining the binding pocket. As is expected of a selective activator of RXR α , but not of RARs and PPAR γ , this RXR α agonist, unlike DIM-Ph-4-CF₃, does not appreciably decrease cancer cell growth or induce apoptosis at pharmacologically relevant concentrations.

Keywords

antitumor agents; dimethylarenes; receptors; retinoids; RXR; TR3

Introduction

Only one retinoid X receptor (RXR)-selective retinoid, bexarotene,[1–4] is approved for cancer treatment.[5] Despite evaluation in clinical trials alone or in combination with other anticancer drugs, dose-limiting toxicities[6,7] or lack of objective responses[8–10] limit its use to oral and topical treatment of cutaneous T-cell lymphoma.[11] In addition to transcriptionally activating RXR nuclear receptors, bexarotene can transactivate retinoic acid receptors (RARs) on *trans*-retinoic acid (*trans*-RA)-sensitive response elements (RAREs)[1] and permissive nuclear receptors such as peroxisome proliferator-activated receptors (PPARs) heterodimerized with RXR on PPAR response elements (PPREs).[12] Thus, to improve therapeutic indices, the search to identify a retinoid selective for RXR in the context of its heterodimer with a specific nuclear receptor has continued.

The orphan nuclear receptor (NR) family member 4A subtype 1 (NR4A1; human TR3, mouse Nur77, rat NGFI-B α) exhibits genomic activity as a transcription factor in the cell nucleus[13,14] and nongenomic activity as a cytosolic mediator of apoptosis. In the cytosol, it converts Bcl-2 function from mitochondrial protection to apoptosis induction.[15–17] Recently, Safe and co-workers reported that several 4-substituted di(1*H*-indol-3-yl)methylbenzenes (DIM-arenes) induce colon and prostate cancer cell apoptosis and decrease cancer xenograft growth in animal models.[18] Using reporter assays in these cell lines and NR co-activator recruitment assays, they correlated NR4A1/TR3 transcriptional activation by DIM-arenes with their abilities to inhibit cell proliferation. The most potent transactivators were DIM-Ph-4-CF₃ and DIM-Ph-4-OMe (**1** and **2** in Figure 1, respectively). In contrast, DIM-Ph-4-OH (**3**) was inactive at concentrations up to 20 μ M on the GAL4–Nur77(domain E/F) chimeric receptor bound to the pGAL4–LUC reporter construct, but antagonized transactivation induced by **1** or **2**.

Reports on other NR4A subfamily members did not support small-molecule binding by the NR4A ligand binding domain (LBD or E domain), but suggested that NR4A interaction with small molecules was atypical: 1) Mutational studies showed that, rather than the LBD, the N-terminus or AB domain of NR4A subgroup members NR4A2/Nurr1 and NR4A3/NOR1, which contains the ligand-independent activation function-1 (AF-1), is responsible for transcriptional activation induced by a 6-thiopurine metabolite.[19–21] 2) Although prostaglandin A₂ transactivates NR4A3/NOR1 and interacts with the NR4A3/NOR1 LBD, its low affinity and AF-1 domain requirement suggested indirect effects.[22] 3) NMR studies suggested a unique binding surface on the NR4A2/Nurr1 LBD by showing that peptides derived from transcriptional co-repressors SMRT and NCoR and the NR4A2/Nurr1 N-terminal peptide (residues 1–13) interact with a hydrophobic surface located between helices H11 and H12.[23] 4) Other NMR studies suggested that several benzimidazole and isoxazolopyridinone NR4A2/Nurr1 activators, which were identified in reporter assays,[24] interact with a surface formed from regions of its LBD helices H4, H11, and H12.[25]

Moreover, crystallography of the NR4A2/Nurr1 LBD revealed that the region occupied by the ligand binding pocket (LBP) is filled with hydrophobic amino acid side chains.[26] The sequence identity of ~60% among the NR4A LBDs[21] suggests that the NR4A1/TR3 and NR4A3/NOR1 LBDs would have similar tertiary structures. Subsequently, crystal structures of the NR4A1/NGFI-B LBD[27] and NR4A1/TR3 LBD[28] confirmed the absence of accessible LBPs. For example, the latter structure (PDB code: 2QW4) revealed that the LBP

region is predominantly occupied by hydrophobic side chains of H3 Phe75, H5 Phe112, β 1– β 2 loop Phe133, and H7 Phe148 and Tyr151.[28] These results suggest the absence of a typical NR LBP, and reports that, in addition to acting as a monomer, homodimer, and NR4A1/TR3–NR4A2/Nurr1 heterodimer, NR4A1/TR3 and NR4A2/Nurr1 heterodimerize with RXR[29–32] suggested that DIM-Ph-4-CF₃ (**1**) could function as an RXR ligand in the context of the RXR–NR4A1/TR3 heterodimer or another heterodimer on an RXR response element. Our premise was partly supported by reports that: 1) Although **1** functions as a PPAR γ agonist in PPAR γ –GAL4/pGAL4–LUC reporter assays,[33] it decreases the survival of several cancer cell lines in the presence of a PPAR γ transcriptional antagonist.[34,35] 2) Natural products that are PPAR γ or PPAR α transcriptional agonists— β -apo-14'-carotenal, [36] arachidonic acid,[37] docosahexaenoic acid,[38] and phytanic acid[39]—are also RXR agonists.[36,40–42] On this basis, if **1** were an RXR ligand, it could provide a new template for RXR ligand design. Herein we report the design, synthesis, RXR α LBD binding affinities, and biological activities of several **1**-derived analogues (compounds **4–14** in Figure 1) in comparison with **1** and **3**. Interestingly, **1** binds to the RXR α LBD but is unable to transcriptionally activate the RXR α homodimer on the TREpal or the RXR α –NR4A1/TR3 heterodimer on the β RARE in reporter assays.

Results and Discussion

Design and synthesis

Analogue design was based on docking of DIM-Ph-4-CF₃ (**1**) to the RXR α LBD in the crystal structure (PDB code: 1MVC) of its complex with transcriptional agonist 2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)-2-(4-carboxyphenyl)-1,3-dioxolane (BMS649, **15**),[43] first reported as SR11237.[44] The docked pose for **1** (Figure 2A) reveals that dihedral angles formed by the indole ring C2 and C3 atoms, the methine carbon, and the 1-aryl carbon permitted us to superpose **1** and **15** in the LBP. The indole ring of **1**, with a dihedral angle of 47°, resides in same region of the LBP as the 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl ring of **15**, whereas the indole ring, with a dihedral angle of –100°, is adjacent to the 1,3-dioxolane ring of **15**. These orientations cause the phenyl rings of **1** and **15** to overlap. In this docked pose, the electron-donating CF₃ group of **1** is near the CO₂H group of **15** (C–C: $d=2.0$ Å). Although **1** lacks the ability to form robust ionic and hydrogen bonding interactions with the guanidinium NH groups of Arg316 in helix H5 and the β sheet backbone NH group of Ala327, as observed for the carboxyl groups of **15** and 9-*cis*-retinoic acid (9-*cis*-RA) in the RXR α LBD,[43,45] the distances (4–6 Å) between the guanidinium nitrogen atoms and the fluorine atoms of CF₃ suggest that binding could be stabilized by N–H–F bonding if the Arg316 side chain shifts during formation of the complex between RXR α LBD and compound **1**.

The docked pose for **1** suggests that DIM-arenes might assist in suppressing cancer cell growth by functioning as RXR ligands in RXR heterodimers with PPAR γ , NR4A1/TR3, or another NR. To validate this hypothesis, we determined whether **1** could bind to RXR α . Next, because **1** was shown to have anticancer effects at 10–20 μ M,[18] we employed structure-based design to identify analogues that could have higher affinities for RXR α LBD. The overlap of **1** with **15** (Figure 2A) suggested that binding affinity might be improved if the 4-CF₃ group of **1** were replaced by CO₂H, as found in analogue **4**, whereas deletion of the phenyl ring from **4**, as in **6**, was expected to decrease activity. The overlap also suggested that alkylation at the 1-position of the indoles, as in **5**, could enhance hydrophobic contacts with residues lining the LBP. To determine whether interactions between the oxygen atoms of CO₂H and the guanidinium NH groups of Arg316 would be enhanced by decreasing the inter-group distance, the Ph-4-CO₂H of **4** was replaced by Ph-3-(*E*-CH=CHCO₂H), as in **7** and **8**, and by 2-thienyl-5-(*E*-CH=CHCO₂H), as in **9–14**. The effects of increasing LBP contact were assessed by **10–14**, having a hydrophobic methyl

group at the 1-, 5-, or 7-indole ring position. All compounds except **6** assumed similar poses on docking to the RXR α LBD in that their polar termini were directed toward the Arg316 guanidinium group and their indoles faced the hydrophobic portion of the LBP. In contrast, the pose for **6** had a reverse orientation.

Compounds were synthesized as outlined in Scheme 1. The DIM moiety was introduced by electrophilic substitution reactions of indoles **16–21** with 4-substituted benzaldehydes **22–24**, ethyl glyoxalate (**27**), 3-substituted benzaldehyde **30**, or 5-substituted thiophene-2-carboxaldehyde **34** using a procedure described by Safe and co-workers,[18] namely, the cerium chloride and sodium iodide on silica gel catalyst developed by Bartoli et al.[46] Wittig mono-olefinations of 1,3-phthalaldehyde (**29**) and thiophene-2,5-dicarboxaldehyde (**33**) introduced the 3- and 5-(*E*-CH=CHCO₂Et) groups of **30** and **34**, respectively. While **1** was produced directly, base hydrolysis was used to remove the ester protecting groups of **25**, **26**, **28**, **31**, **32**, and **35–40** to provide **4–14**, respectively. Phenol **3** was prepared by condensation of indole (**16**) and 4-hydroxybenzaldehyde (**23**) using iodine in acetonitrile as the catalyst.[47]

Competitive binding to the RXR α LBD

In competitive binding assays with RXR transcriptional agonist [11,12-³H₂]9-*cis*-RA, the DIM-arenes **1**, **4**, and **10** most effectively displaced bound label from recombinant human RXR α LBD (Table 1). On the basis of IC₅₀ values, **10** had the highest affinity for RXR α , whereas **1** and **4** had 53 and 17% of the affinity, respectively. Analogues **5**, **7**, **8**, and **12** had IC₅₀ values in the mid-micromolar range.

Docking was conducted to understand how **4** and **10** could interact with RXR α LBP residues (Figure 2 B). The indole rings in the docked poses for **1**, **4**, and **10** reside in the same regions of the LBP, whereas superposing with RXR ligand **15** suggests that the CO₂H carbon atoms of **4** and **10** are closer to that of **15** (0.7 and 0.5 Å, respectively). The N–O interatomic distances between the guanidinium group of Arg316 and the CO₂H group of the ligand measured for **4** (3.4–4.6 Å) and **10** (2.7–3.4 Å) suggest why both DIM-arenes bind to RXR α and why **10**, for which these distances are shorter, has higher affinity.

Most interesting in the docked pose for **1** is the proximity of one indole ring nitrogen to the sulfur atom of Cys269 on H3, and the other indole nitrogen atom to the sulfur of Cys432 on H11 (3.5 and 4.4 Å, respectively), which suggests possible N–H–S bonding interactions. The proximity between the cysteine S atoms and indole N atoms was recapitulated in the docked poses for **10** and **4** (S–N interatomic distances: **10**, 3.5–4.2 Å; **4**, 3.3–9.8 Å). The docked pose for **10** also suggests potential hydrophobic interactions between the 1-methyl group of one indole and a methyl group of H3 Val265, and the other 1-methyl group with alkyl side chains of H7 Ile345 and H11 Ile428. Such hydrophobic stabilizing interactions are absent in docked poses for **1** and **4**.

Although the structural similarity of **9** to **10** suggests that **9** would bind RXR α with similar potency, compound **9**, at concentrations as high as 100 μ M, could not displace radiolabeled 9-*cis*-RA from the RXR α LBD. While the docked pose for **9** shows its CO₂H group almost coincident with that of **10** and one indole N atom 3.5 Å from the Cys432 S atom, the second indole N is 5.5 Å from Cys432 S and 5.9 Å from C269 S. In addition, the second indole and the thiophene of **9** are almost orthogonal to those rings in the docked pose for **10**. Thus, the decreased N–H–S interactions and dissimilar docking poses suggest why **9** does not displace 9-*cis*-RA from RXR α . Similarly, the docked pose for **3** suggests that the O–N interatomic distances of 4.9–5.1 Å between its phenolic oxygen atom and the NH groups of Arg316 would not permit robust N–H–O interactions required for high-affinity binding.

Activation of RXR α and NR4A1/TR3 in reporter assays

Reporter assays were conducted to determine whether the DIM-arenes transactivate RXR α or NR4A1/TR3 (Figure 3). CV-1 kidney cells were transfected with the *nr4a1/tr3* gene plasmid and the everted repeat NurRE–thymidine kinase–chloramphenicol acetyl transferase (NurRE–*tk*–CAT) reporter, which is activated by the NR4A1/TR3 homodimer;[32] the *rxra* gene construct and the palindromic TREpal–*tk*–CAT reporter, which is activated by an RXR homodimer–agonist complex; or with genes for both receptors and the DR-5 β RARE–CAT reporter, which we found to be activated by the RXR–NR4A1/TR3 heterodimer–RXR agonist complex.[30] Cells were then treated with a DIM-arene: **1** and **3–14** at 20 μ M, which was the highest concentration of **1** and **2** used by Safe and co-workers to demonstrate NurRE–LUC reporter activation.[18] In this group of compounds, only **10** at 1.0 μ M effectively activated RXR α on the TREpal (63% of that produced by 9-*cis*-RA at 0.1 μ M, or 7-fold above the vehicle-treated control; Figure 3A and Table 1). The other analogues were essentially inactive. Activation by **10** at 20 μ M rose to 1.9-fold that of 0.1 μ M 9-*cis*-RA, whereas the other DIM-arenes only weakly activated (16–36%) or were inactive (Table 1). Although capable of binding to RXR α in the micromolar range, compound **1** did not activate the RXR α homodimer on the TREpal. Because PPAR target genes containing PPRES have been reported to be activated *in vivo* by RXR homodimers in the presence of 9-*cis*-RA,[48] the converse may occur so that **1** could function as an RXR transcriptional antagonist in certain contexts to prevent PPRES activation by RXR homodimers.

At 20 μ M, neither **1** nor its analogues significantly activated the NR4A1/TR3 homodimer on the NurRE above the basal level (termed 100%) in *nr4a1/tr3* plus NurRE–*tk*–CAT-transfected CV-1 kidney cells (Figure 3 B). The weak decrease (30%) in basal activation of NR4A1/TR3 by **3** and **10** suggested that in the context of this homodimer both function as weak antagonists either directly by binding to NR4A1/TR3 or indirectly by binding to another NR that preferentially recruits NR4A1/TR3 co-activators. The antagonism demonstrated by **3** partially supports the results reported by Safe and colleagues, that compound **3** at 20 μ M, while unable to activate endogenous NR4A1/Nur77 on the NurRE or the chimeric GAL4–Nur77 receptor on the pGAL4–LUC in transfected pancreatic cancer cells, completely blocked activation of the latter reporter induced by 20 μ M **1** or **2**. [18] The differences between the present results and those previously reported[18] may be due to differences among cell lines or the possibility that endogenous PPAR γ or another NR was also activated by the DIM-arenes.

Earlier, the research group of Zhang established that the β RARE response element, which is typically activated by binding of an RXR–RAR α heterodimer–RAR α agonist complex, was also activated by an RXR α –NR4A1/TR3 heterodimer–RXR agonist complex.[30] Thus, in CV-1 cells transfected with plasmids for both *rxra* and *nr4a1/tr3* genes and the β RARE–*tk*–CAT reporter construct, compound **10** at 20 μ M was the most potent transactivator (83% that of 0.1 μ M 9-*cis*-RA) followed by **3** and **7–9** (47–56%) (Table 1). Least active were compounds **1**, **11**, and **12**.

DIM-arene **10** was next examined for its abilities to transactivate the RARs on the TREpal in CV-1 cells. Although it does not significantly transactivate any RAR subtype alone (Figure 4A–C) and does not significantly enhance the activation of RAR α (Figure 4A) or RAR β (Figure 4B) induced by 1.0 μ M *trans*-RA, compound **10** at 1.0 and 10 μ M does enhance *trans*-RA-induced RAR γ activation (1.28- and 1.54-fold, respectively; Figure 4 C). At 10 μ M, **10** suppresses RAR β basal activation (29% decrease) and very weakly inhibits (5% decrease) that induced by *trans*-RA.

DIM-arenes **1**, **3**, and **10** were then examined for PPAR γ transactivation on the PPRES (Figure 4D). The RXR–PPAR heterodimer on a PPRES can be activated by an agonist

binding to RXR or PPAR or by both agonists binding, in which case activation can be enhanced.[49–52] Analogues **1** and **3** behaved as transcriptional agonists, with **1** being the more potent, but at 20 μM , compound **1** is cytotoxic. When compared on the basis of fold-activation above that of the PPARE-transfected vehicle-alone control, **1**, at 10 μM , was as potent (18.3-fold) as the PPAR γ agonist troglitazone at 20 μM (15.6-fold) and **3** at 40 μM (18.5-fold) in the PPARE and PPAR γ co-transfected cells. In contrast, **10** behaved as a weak partial agonist of the RXR α –PPAR γ heterodimer on the PPARE (4.5-fold activation above the PPARE control at 2.0 μM and 3.5-fold at 20 μM). At 40 μM , **10** was cytotoxic.

Growth inhibition and apoptosis induction

We next investigated the abilities of the DIM-arenes shown in Figure 1 to inhibit the proliferation of androgen-dependent retinoid-sensitive LNCaP and androgen-independent retinoid-resistant DU-145 prostate cancer cells, and drug-resistant KG-1 acute myelocytic leukemia (AML) cells. According to Western blot analysis, LNCaP cells express NR4A1/TR3 protein.[18] Treatment of LNCaP cells with compound **1** at 10 or 20 μM for 48 h was reported to inhibit cell growth[35] and to induce apoptosis.[18] Values for 50% growth inhibition by **1** and **3–14** were determined under similar conditions except that cells were grown in 10% delipidized serum (Table 2). On the basis of IC₅₀ values after treatment for 48 h, compound **12** (IC₅₀=2.4 μM) was the most potent inhibitor of LNCaP cell growth followed by **1** (IC₅₀=13 μM), whereas **11** was less active (IC₅₀ value 3.6-fold higher than that of **12**). Analogues **5**, **7**, and **9** had IC₅₀ values in the 20–40 μM range, whereas 20 μM **6** and **10** had only low inhibitory activity, and **3** was inactive up to 100 μM .

DIM-arenes **1**, **3**, **10**, **11**, and **14** inhibited DU-145 cell growth with IC₅₀ values in the 40–90 μM range. Analogues **5**, **6**, and **12** were least active (~20% growth inhibition at 100 μM). Only compound **1** inhibited KG-1 AML cell growth at 48 h (IC₅₀= 15 μM and 81% inhibition at 20 μM). The similar EC₅₀ value (23 μM) for KG-1 apoptosis induction by **1** at 48 h suggests that apoptosis was a major contributor to growth inhibition.

Because of its relative potency in inhibiting cancer cell growth, the efficacy of **1** was assessed against other human cancer cell lines: radiation- and cisplatin-resistant HeLa-S cervical adenocarcinoma; androgen- and anchorage-independent, FAS- and cyclophosphamide-resistant metastatic PPC-1 prostate adenocarcinoma with defective p53; and TRAIL-, cisplatin-, and radiation-resistant U2OS osteosarcoma. Potency in these tumor cell lines was compared with that in the Ki-ras mutated, 5-fluoro-2'-deoxyuridine-resistant HCT-116 colon cancer cell line. Safe and co-workers reported that **1** decreased HCT-116 cell numbers with an IC₅₀ value of 10 μM when cells were grown in medium containing 2.5% charcoal-stripped fetal bovine serum (FBS).[18] We elected to evaluate the cell lines in the presence of 10% FBS, which would provide more robust growth. As shown in Figure 5, HeLa-S, PPC-1, U2OS, and HCT-116 cell vitalities were decreased by 50% after 22 h with compound **1** at 10.0, 17.6, 12.6, and 9.3 μM , respectively, in comparing ATP levels with those of vehicle-alone-treated controls. These results indicate that even under more optimal growth conditions, **1** effectively inhibits the proliferation of these cancer cell lines by inducing cell death.

Conclusions

The results presented herein demonstrate that, in addition to several natural products, a synthetic ligand that activates PPAR γ , DIM-Ph-4-CF₃ (**1**), can interact with RXR α . Although unable to potently transactivate RXR α on the TRE β and β RARE, **1** was structurally modified by using docking to enhance pocket contacts to afford the higher-affinity RXR α ligand (1.9-fold binding enhancement) and transcriptional agonist 1,1'-Me₂-DIM-2-thienyl-5-(*E*-CH=CHCO₂H) (**10**), which at 1.0 μM , increased activation of the RXR α .

homodimer on the TREpal eightfold above the basal level. Similarly, structural modification of RAR-selective agonist 4-[3-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)phenyl]benzoic acid (AC_{50} values: RAR α , 0.2 nM; RAR β , 4.3 nM; RAR γ , 2.3 nM; RXR α 2.3 μ M)[53] by replacing its benzoic acid terminus by (2*E*,4*E*)-2-methyl-2,4-pentadienoic acid decreased RAR activation at 10 μ M (RAR α , 5%; RAR β , 30%; RAR γ , 68% and AC_{50} 4.5 μ M) but enhanced that of RXR α (93% and AC_{50} 1.5 μ M). These combined results suggest that increasing ligand flexibility next to its CO₂H group to allow a decrease in the O–N distance between the CO₂H group and Arg316 enhances both RXR selectivity and transactivational activity.

Although RXR ligands terminated by phenyl-3-(*E*-CH=CHCO₂H) and 2-furyl-5-(*E*-CH=CHCO₂H) groups and equipped with an alkyl-substituted sp³ carbon bridge to the hydrophobic tetrahydrotetramethylnaphthalene ring have been reported,[54–56] compound **10** is the first RXR agonist with an indole as the hydrophobic ring and another indole as the bridge substituent. A 9-*cis*-RA analogue with a 1-(methylethyl)-1,2,3,4-tetrahydroquinolin-6-yl group in place of the β -cyclogeranylidene ring and the 7,8*E* double bond was reported to selectively activate RXRs.[57] The docked pose of its low-energy conformer suggested to us that the quinoline N atom could stabilize binding by hydrogen bonding with the Cys432 SH group (N–S interatomic distance: 4.6 Å), as was observed in the docked pose for **10**. Recently, virtual ligand screening employing molecular interaction fingerprinting identified several new RXR scaffolds.[58] Interestingly, these compounds possessed indole, benzothiophene, and indane rings.

After the present studies were completed, cytosporone B [ethyl 2-(3,5-dihydroxy-2-octanoylphenyl)acetate] was reported to be an NR4A1/Nur77 agonist, which, at 1.0 μ M, activated the GAL4–Nur77 LBD on a GAL4 reporter 13.5-fold and Nur77 on a NurRE reporter 10-fold, and bound GST–Nur77 LBD with a K_d value of 1.9 μ M.[59] Cytosporone B also docked to the crystal structure of the NR4A1/TR3 LBD (PDB code: 2QW4) in the region typically occupied by NR LBPs, suggesting that other NR4A1/TR3 ligands could be identified. Interestingly, we observed that compound **1** could dock to the same region.

The retinoid bexarotene, which is capable of activating both RARs and RXRs,[1] is used clinically to inhibit CTCL cell growth. DIM-arene **10** activated RXR α but not the RARs or PPAR γ . Thus, as expected, a correlation between the abilities of **10** to transactivate RXR α and inhibit cancer cell growth or induce KG-1 cell apoptosis was not found. Previously, we observed that the induction of either activity by a selective RXR agonist required the presence of both the RXR heterodimeric partner RAR α or PPAR γ and its agonist. In those cases, the RXR agonist enhanced the activity induced by the agonist of its partner. For example, in head-and-neck squamous-cell carcinoma cell lines lacking RAR β , RXR-selective retinoids, which were inactive or poorly active alone, enhanced the ability of RAR-selective retinoids to inhibit cell growth.[60] Similar combinatorial effects were observed in leukemia cell lines.[61,62] PPAR γ ligand citaglitazone and RXR-selective agonist **15** cooperatively inhibited CaLu-6 lung and ZR-75-1 and T-47D breast cancer cell line viability.[12] However, RXR-selective retinoids alone can have growth inhibitory effects in certain contexts. In NR4A1/TR3-expressing, RAR α -null MDA-MB-231 breast cancer cells, **15** induced NR4A1/TR3 activation of the β RARE and inhibited cell viability;[30] however, in LNCaP cells, which express RAR α , RXR α , and NR4A1/TR3, **15** did not induce NR4A1/TR3 activation, nuclear export, or apoptosis, leading to the conclusion that RAR α outcompetes NR4A1/TR3 for RXR α . [15]

Of the DIM-arenes **4–14**, only **12** inhibits LNCaP growth with an IC₅₀ value <3 μ M. The data in Table 1 suggest that its selective activity against LNCaP cells is not due to affinity for RXR α or activation of RXR α or NR4A1/TR3. The similarity of PPAR γ expression

levels in the LNCaP and DU-145 cell lines and their comparable growth inhibition by PPAR γ agonist ciglitazone[63] further suggests that **12** does not function by activating PPAR γ . As LNCaP cells are reported to be somewhat more sensitive to all-*trans*-RA-induced growth inhibition than DU-145 cells,[64] and **12** is a poor inhibitor of DU-145 growth (Table 2), **12** may perhaps function through RAR activation. The other DIM-arene analogues only inhibit cell growth at high, non-pharmacologic concentrations and were not further explored.

Of the DIM-arenes evaluated, only compound **1** inhibited growth of both prostate cancer cell lines and KG-1 leukemia cells, and induced KG-1 apoptosis. Whether **1** exerts its effects by activating PPAR γ or inducing NR4A1/TR3 nuclear export remains to be explored. However, it appears unlikely that **1** functions by antagonizing RXR α -NR4A1/TR3 basal activation on the β RARE, because **11** and **12**, while similarly decreasing activation, have disparate effects on LNCaP growth inhibition. Using transfected CV-1 cells, we were unable to confirm that compound **1** at 20 μ M activated the NR4A1/TR3 homodimer on an NurRE reporter construct as was reported.[18] The requirement for a particular co-activator protein may have caused the differences between the results observed for this cell line and those reported earlier.[18] More recently, using RNA interference and receptor antagonists, the Safe research group also determined that DIM-arenes exert cancer growth inhibitory and apoptotic effects through PPAR γ - and NR4A1/TR3-independent pathways,[14] which include inducing: 1) CCAAT/enhancer binding protein homologous protein (CHOP)/GADD153 expression,[65] 2) pro-apoptotic nonsteroidal anti-inflammatory drug-activated gene-1 expression,[66] 3) *c*-Jun N-terminal kinase activation,[65] 4) endoplasmic reticulum-mediated stress,[67] and 5) loss of mitochondrial membrane potential and cytochrome *c* release.[66,68,69] Thus, in keeping with other anticancer drugs, the DIM-arenes appear to function through a variety of different signaling pathways; however, initial target(s) remain to be identified.

Experimental Section

Chemistry

General—Chemicals and solvents from commercial sources were used without purification unless specified. Reactions were conducted under Ar and monitored by thin-layer chromatography on silica gel (mesh size 60, F₂₅₄) with visualization under UV light. Organic extracts were dried using Na₂SO₄ unless otherwise specified and then concentrated at reduced pressure. Standard and flash column chromatography employed silica gel (Merck 60, 230–400 mesh). Experimental procedures were not optimized. Melting points of compounds were determined in capillaries using a Mel-Temp II apparatus and are uncorrected. ¹H NMR spectra were recorded on a 300 MHz Varian Unity Inova spectrometer, and shift values are expressed in ppm (δ) relative to CHCl₃ as an internal standard. Unless stated otherwise, NMR spectra were taken in CDCl₃ solution. IR spectra were obtained on powdered samples using an FTIR Mason Satellite spectrophotometer. HRMS data were recorded on an Agilent ESI-TOF mass spectrometer at The Scripps Research Institute (La Jolla, CA). ESMS was performed on an ABI EPI-3000 instrument.

1-Isopropyl-1*H*-indoles **18 and **20****—A reported method[70] was adapted. *N,N*-Dimethylformamide (DMF; 8 mL) was added to the indole (10.0 mmol) and 60% NaH dispersion (600 mg, 15 mmol) in mineral oil at 0 °C with stirring. 2-Bromopropane (1.23 g, 10 mmol) was then added dropwise with stirring. The reaction mixture was stirred with slow warming to room temperature until evolution of H₂ ceased. After the reaction was complete as indicated by TLC (~1 h), the solvent was removed, and the residue was extracted (CH₂Cl₂). The extract was washed (H₂O and brine) and dried. The residue obtained after concentration was subjected to chromatography (hexane) to give the 1-isopropylindole.

1-Isopropyl-1*H*-indole (18):[70]—Compound **16**, after reaction and chromatography, afforded **18** as a colorless oil (1.05 g, 66 %): TLC (20% EtOAc/hexane): R_f = 0.75; ^1H NMR: δ = 1.51 (d, J = 6.6 Hz, 6H, $(\text{CH}_3)_2\text{CH}$), 4.67 (sept, J = 6.6 Hz, 1H, $(\text{CH}_3)_2\text{CH}$), 6.51 (d, J = 3.3 Hz, 1H, 2-InH), 7.09 (dd, J = 6.9, 7.8 Hz, 1H, 5-ArH), 7.20 (dd, J = 6.9, 7.8 Hz, 1H, 6-ArH), 7.21 (d, J = 3.3 Hz, 1H, 3-InH), 7.37 (d, J = 7.8 Hz, 1H, 7-ArH), 7.63 ppm (d, J = 7.8 Hz, 1H, 4-ArH); IR: ν = 2974, 1509, 1459, 1221 cm^{-1} ; in agreement with reported data.[70]

1-Isopropyl-5-methyl-1*H*-indole (20)—5-Methyl-1*H*-indole (**19**), after reaction and chromatography, afforded **20** as a colorless oil (1.11 g, 64 %): TLC (20% EtOAc/hexane): R_f = 0.77; ^1H NMR: δ = 1.49 (d, J = 6.9 Hz, 6H, $(\text{CH}_3)_2\text{CH}$), 2.44 (s, 3H, CH_3 -Ar), 4.61 (sept, J = 6.9 Hz, 1H, $(\text{CH}_3)_2\text{CH}$), 6.41 (d, J = 3 Hz, 1H, 2-InH), 7.02 (d, J = 8.1 Hz, 1H, 6-ArH), 7.16 (d, J = 3.0 Hz, 1H, 3-InH), 7.25 (d, J = 8.1 Hz, 1H, 7-ArH), 7.41 ppm (s, 1H, 4-ArH); IR: ν = 2973, 1480, 1318, 1221 cm^{-1} ; HRMS: m/z [$M+\text{H}$] $^+$ calcd for $\text{C}_{12}\text{H}_{15}\text{N}$: 174.1277, found: 174.1275.

3-Arylacrylates 30 and 34 from arylaldehydes 29 and 33, respectively—A reported method[71] was adapted. Thus, ethyl(triphenyl-phosphoranylidene) acetate (3.48 g, 10.0 mmol) was added to a stirred mixture of the aldehyde (10.0 mmol) in PhMe (10 mL). This mixture was stirred at 80 °C for 1 h, quenched with H_2O (10 mL), and extracted with EtOAc (3 $\times$ 10 mL). The extract was washed (brine) and dried. The residue obtained after concentration was subjected to chromatography (33% EtOAc/hexane) to give the 3-arylacrylate.

Ethyl (*E*)-3-(3-formylphenyl)acrylate (30):[72]—Isophthalaldehyde (**29**) (1.34 g, 10.0 mmol), after reaction and chromatography, afforded **30** as a white solid (1.65 g, 81 %): TLC (20% EtOAc/hexane): R_f = 0.43; mp: 37–39 °C; ^1H NMR: δ = 1.35 (t, J = 6.9 Hz, 3H, CH_2CH_3), 4.29 (q, J = 6.9 Hz, 2H, CH_2CH_3), 6.54 (d, J = 15.9 Hz, 1H, $\text{CH}=\text{CHCO}$), 7.58 (t, J = 7.5 Hz, 1H, 5-ArH), 7.73 (d, J = 15.9 Hz, 1H, $\text{CH}=\text{CHCO}$), 7.70 (d, J = 8.7 Hz, 1H, 4-ArH), 7.89 (d, J = 7.5 Hz, 1H, 5-ArH), 8.03 (s, 1H, 2-ArH), 10.05 ppm (s, 1H, CHO); IR: ν = 2981, 1695, 1639, 1172 cm^{-1} ; in agreement with reported data.[72]

Ethyl (*E*)-3-(5-formyl-2-thienyl)acrylate (34)—Thiophene-2,5-dicarb-aldehyde (**33**), after reaction and chromatography, afforded **34** as a yellow solid (1.74 g, 83%): TLC (20% EtOAc/hexane): R_f = 0.31; mp: 45–47 °C; ^1H NMR: δ = 1.34 (t, J = 7.2 Hz, 3H, CH_2CH_3), 4.27 (q, J = 7.2 Hz, 2H, CH_2CH_3), 6.43 (d, J = 15.9 Hz, 1H, $\text{CH}=\text{CHCO}$), 7.32 (d, J = 3.9 Hz, 1H, 3-TpH), 7.70 (d, J = 3.9 Hz, 1H, 4-TpH), 7.75 (d, J = 15.9 Hz, 1H, $\text{CH}=\text{CHCO}$), 9.91 ppm (s, 1H, CHO); IR: ν = 1706, 1665, 1627, 1166 cm^{-1} ; HRMS: m/z [$M+\text{H}$] $^+$ calcd for $\text{C}_{10}\text{H}_{10}\text{O}_3\text{S}$: 211.0423, found: 211.0422.

Condensation of indoles 16–21 with 4-substituted benzaldehydes 22 and 24, ethyl glyoxalate (27), or arylaldehydes 30 and 33 to give di(indolyl)methylbenzenes 1, 25, and 26, di(indolyl)acetate 28, 3-di(indolyl)methylcinnamates 31 and 32, or 3-[5-di(indolyl)methyl-2-thienyl]acrylates 35–40, respectively—A reported procedure[46] was used. Briefly, silica gel (0.500 g) was added to a stirred mixture of $\text{CeCl}_3 \cdot 7\text{H}_2\text{O}$ (0.113 g, 0.300 mmol) and NaI (0.045 g, 0.300 mmol) in MeCN (8 mL). The mixture was stirred overnight and then concentrated to give a yellow solid, to which was then added the indole (2.00 mmol) and aldehyde (1.00 mmol) dissolved in MeCN (15 mL). The mixture was stirred for 4 h, diluted with Et_2O (20 mL), and filtered through a short pad of Celite (ether rinse). The crude product obtained on concentration was purified by chromatography.

3,3'-[(4-Trifluoromethylphenyl)methylene]bis(1*H*-indole) (1):[46]—Indole (16) (0.234 g) and 4-(trifluoromethyl)benzaldehyde (22) (0.174 g), after reaction and chromatography (33% EtOAc/hexane), gave **1** as a white solid (0.63 g, 86%): TLC (33% EtOAc/hexane): R_f = 0.43; mp: 85–87 °C; ^1H NMR: δ = 5.95 (s, 1H, PhCH), 6.67 (d, J = 1.5 Hz, 2H, 2-ArH), 7.00–7.04 (m, 2H, ArH), 7.16–7.22 (m, 2H, ArH), 7.35–7.39 (m, 4H, ArH), 7.46 (d, J = 8.1 Hz, 2H, 2, 6-ArH), 7.53 (d, J = 8.1 Hz, 2H, 3,5-ArH), 7.98 ppm (s, 2H, NH); IR (CHCl₃): ν = 3425, 3415, 3048, 2666, 2367, 1324 cm⁻¹; in agreement with reported data. [46]

Methyl 4-[di(1*H*-indol-3-yl)methyl]benzoate (25)—16 (0.234 g) and methyl 4-formylbenzoate (24) (0.164 g) gave, after reaction and chromatography (33% EtOAc/hexane), **25** as a white solid (0.677 g, 89%): TLC (33% EtOAc/hexane): R_f = 0.41; mp: 98–100 °C; ^1H NMR: δ = 3.89 (s, 3H, CH₃), 5.94 (s, 1H, CH), 6.60 (d, J = 1.5 Hz, 2H, C=CH-NH), 6.98–7.03 (m, 2H, ArH), 7.15–7.20 (m, 2H, ArH), 7.34–7.37 (m, 2H, ArH), 7.37 (d, J = 8.4 Hz, 2H, 2, 6-ArH), 7.42 (d, J = 8.4 Hz, 2H, 3,5-ArH), 7.94–7.97 (m, 2H, ArH), 7.97 ppm (s, 2H, NH); IR (CHCl₃): ν = 3425, 3403, 1702, 1282 cm⁻¹; HRMS: m/z [M -H]⁻ calcd for C₂₅H₂₀N₂O₂ : 379.1452, found: 379.1452.

Methyl 4-[di(1-methyl-1*H*-indol-3-yl)methyl]benzoate (26)—1-Methyl-1*H*-indole (17) (0.262 g) and **24** (0.164 g) gave, after reaction and chromatography (20% EtOAc/hexane), **26** as a white solid (0.342 g, 84%): TLC (20% EtOAc/hexane): R_f = 0.34; mp: 185–187 °C; ^1H NMR: δ = 3.68 (s, 6H, NCH₃), 3.89 (s, 3H, OCH₃), 5.92 (s, 1H, ArCH), 6.51 (s, 2H, 2-ArH), 7.00 (t, J = 7.2 Hz, 2H, ArH), 7.18–7.23 (m, 2H, ArH), 7.25–7.28 (m, 2H, ArH), 7.31–7.36 (m, 2H, ArH), 7.41 (d, J = 8.1 Hz, 2H, 3,5-PhH), 7.95 ppm (d, J = 8.1 Hz, 2H, 2,6-PhH); IR: ν = 1716, 1608, 1274 cm⁻¹; HRMS: m/z [M +Na]⁺ calcd for C₂₇H₂₄N₂O₂ : 431.1730, found: 431.1728.

Ethyl 2,2-di(1*H*-indol-3-yl)acetate (28):[46]—16 (0.234 g) and a 50% solution of ethyl glyoxalate (27) (1.0 mmol) in PhMe (0.198 mL) gave, after reaction and chromatography (33% EtOAc/hexane), **28** as a white solid (0.522 g, 82%): TLC (50% EtOAc/hexane): R_f = 0.62; mp: 69–71 °C; ^1H NMR: δ = 1.21 (t, J = 7.2 Hz, 3H, CH₂CH₃), 3.48 (q, J = 7.2 Hz, 2H, CH₂CH₃), 5.54 (s, 1H, Ar-CH), 7.07–7.10 (m, 2H, 5-ArH), 7.14 (s, 2H, 2-ArH), 7.17–7.21 (m, 2H, 6-ArH), 7.35 (d, J = 7.5 Hz, 2H, 7-ArH), 7.63 (d, J = 8.4 Hz, 2H, 4-ArH), 8.01 ppm (br s, 2H, NH); IR: ν = 3406, 1715, 1174 cm⁻¹; in agreement with reported data.[46]

Ethyl (*E*)-3-[3-di(1*H*-indol-3-yl)methylphenyl]acrylate (31)—16 (0.234 g) and ethyl (*E*)-3-(3-formylphenyl)acrylate (30) (0.204 g) gave, after reaction and chromatography (20% EtOAc/hexane), **31** as a white solid (0.365 g, 87%): TLC (50% EtOAc/hexane): R_f = 0.69; mp: 90–92 °C; ^1H NMR: δ = 1.27 (t, J = 7.2 Hz, 3H, CH₂CH₃), 4.23 (q, J = 7.2 Hz, 2H, CH₂CH₃), 5.90 (s, 1H, ArCH), 6.35 (d, J = 16.2 Hz, 1H, CH=CHCO), 6.66 (s, 2H, 2-ArH), 7.01 (t, J = 7.2 Hz, 2H, ArH), 7.18 (t, J = 7.5 Hz, 2H, ArH) 7.31 (d, J = 7.5 Hz, 1H, ArH), 7.36–7.38 (m, 6H, ArH), 7.51 (s, 1H, 2-PhH), 7.62 (d, J = 16.2 Hz, 1H, CH=CHCO), 7.95 ppm (br s, 2H, NH); IR: ν = 3399, 1693, 1635, 1272, 1182 cm⁻¹; HRMS: m/z [M +Na]⁺ calcd for C₂₈H₂₄N₂O₂ : 443.1730, found: 443.1715.

Ethyl (*E*)-3-[3-di(1-methyl-1*H*-indol-3-yl)methylphenyl]acrylate (32)—1-Methyl-1*H*-indole (17) (0.262 g) and **30** (0.204 g) gave, after reaction and chromatography (20% EtOAc/hexane), **32** as a white solid (0.352 g, 84%); TLC (20% EtOAc/hexane): R_f = 0.34; mp: 93–95 °C; ^1H NMR: δ = 1.29 (t, J = 7.2 Hz, 3H, CH₂CH₃), 3.69 (s, 6H, NCH₃), 4.23 (q, J = 7.2 Hz, 2H, CH₂CH₃), 5.89 (s, 1H, ArCH), 6.35 (d, J = 15.9 Hz, 1H, CH=CHCO), 6.52 (s, 2H, 2-ArH), 7.01 (t, J = 7.2 Hz, 2H, ArH), 7.20 (t, J = 7.2 Hz, 2H, ArH) 7.30 (d, J = 8.4 Hz, 2H, ArH), 7.35–7.38 (m, 4H, ArH), 7.50 (s, 1H, 2-PhH), 7.63 ppm

(d, $J = 15.9$ Hz, 1H, $\text{CH}=\text{CHCO}$); IR: $\tilde{\nu} = 1705, 1633, 1614, 1468, 1176\text{ cm}^{-1}$; HRMS: m/z $[M+\text{Na}]^+$ calcd for $\text{C}_{30}\text{H}_{28}\text{N}_2\text{O}_2$: 471.2043, found: 471.2055.

Ethyl (E)-3-[5-(di(1H-indol-3-yl)methyl-2-thienyl)acrylate (35)]—16 (0.234 g) and ethyl (E)-3-(5-formyl-2-thienyl)acrylate (**34**) (0.210 g) gave, after reaction and chromatography (20% EtOAc/hexane), **35** as a white solid (0.360 g, 87%): TLC (50% EtOAc/hexane): $R_f = 0.67$; mp: 95–97 °C; ^1H NMR: $\delta = 1.28$ (t, $J = 7.2$ Hz, 3H, CH_2CH_3), 4.20 (q, $J = 7.2$ Hz, 2H, CH_2CH_3), 6.05 (d, $J = 15.9$ Hz, 1H, $\text{CH}=\text{CHCO}$), 6.12 (s, 1H, ArCH), 6.60 (d, $J = 3.6$ Hz, 1H, TpH), 6.88 (s, 2H, 2-ArH), 7.02–7.06 (m, 2H, ArH), 7.07 (d, $J = 3.6$ Hz, 1H, TpH), 7.17–7.22 (m, 2H, ArH), 7.38 (d, $J = 7.8$ Hz, 2H, ArH), 7.46 (d, $J = 7.8$ Hz, 2H, ArH), 7.68 (d, $J = 15.9$ Hz, 1H, $\text{CH}=\text{CHCO}$), 8.00 ppm (br s, 2H, NH); IR: $\nu = 3400, 1690, 1619, 1456, 1175\text{ cm}^{-1}$; HRMS: m/z $[M+\text{Na}]^+$ calcd for $\text{C}_{26}\text{H}_{22}\text{N}_2\text{O}_2\text{S}$: 427.1475, found: 427.1455.

Ethyl (E)-3-[5-di(1-methyl-1H-indol-3-yl)methyl-2-thienyl]acrylate (36)]—1- Methyl-1H-indole (**17**) (0.262 g) and **34** (0.210 g) gave, after reaction and chromatography (20% EtOAc/hexane), **36** as a white solid (0.366 g, 83%): TLC (20% EtOAc/hexane): $R_f = 0.33$; mp: 158–160 °C; ^1H NMR: $\delta = 1.28$ (t, $J = 7.2$ Hz, 3H, CH_2CH_3), 3.71 (s, 6H, NCH_3), 4.20 (q, $J = 7.2$ Hz, 2H, CH_2CH_3), 6.04 (d, $J = 15.6$ Hz, 1H, $\text{CH}=\text{CHCO}$), 6.10 (s, 1H, ArCH), 6.73 (s, 2H, 2-ArH), 6.84 (d, $J = 2.7$ Hz, 1H, TpH), 7.01–7.06 (m, 2H, ArH), 7.07 (d, $J = 2.7$ Hz, 1H, TpH), 7.20–7.25 (m, 2H, ArH), 7.31 (d, $J = 8.1$ Hz, 2H, ArH), 7.45 (d, $J = 8.1$ Hz, 2H, ArH), 7.69 ppm (d, $J = 15.9$ Hz, 1H, $\text{CH}=\text{CHCO}$); IR: $\nu = 1702, 1618, 1465, 1159\text{ cm}^{-1}$; HRMS: m/z $[M+\text{Na}]^+$ calcd for $\text{C}_{28}\text{H}_{26}\text{N}_2\text{O}_2\text{S}$: 455.1788, found: 455.1779.

Ethyl (E)-3-[5-[di(1-isopropyl-1H-indol-3-yl)methyl-2-thienyl]acrylate (37)]—1- Isopropyl-1H-indole (**18**) (0.318 g) and **34** (0.210 g) gave, after reaction and chromatography (50% EtOAc/hexane), **37** as a white solid (0.914 g, 92%): TLC (50% EtOAc/hexane): $R_f = 0.86$; mp: 83–85 °C; ^1H NMR: $\delta = 1.28$ (t, $J = 7.2$ Hz, 3H, CH_2CH_3), 1.46 (d, $J = 6.6$ Hz, 12 H, $\text{CH}(\text{CH}_3)_2$), 4.20 (q, $J = 7.2$ Hz, 2H, CH_2CH_3), 4.63 (sept, $J = 6.6$ Hz, 2H, $\text{CH}(\text{CH}_3)_2$), 6.04 (d, $J = 15.6$ Hz, 1H, $\text{CH}=\text{CHCO}$), 6.10 (s, 1H, ArCH), 6.82 (d, $J = 3.3$ Hz, 1H, 4-TpH), 6.91 (s, 2H, 2-ArH), 7.01 (dd, $J = 7.8, 7.2$ Hz, 2H, 6-ArH), 7.06 (d, $J = 3.3$ Hz, 1H, 3-TpH), 7.18 (dd, $J = 7.8, 7.2$ Hz, 2H, 5-ArH), 7.37 (d, $J = 7.8$ Hz, 2H, 7-ArH), 7.43 (d, $J = 7.8$ Hz, 2H, 4-ArH), 7.69 ppm (d, $J = 15.6$ Hz, 1H, $\text{CH}=\text{CHCO}$); IR: $\nu = 2974, 1704, 1620, 1461, 1194\text{ cm}^{-1}$; HRMS: m/z $[M+\text{Na}]^+$ calcd for $\text{C}_{32}\text{H}_{34}\text{N}_2\text{O}_2\text{S}$: 533.2233, found: 533.2232.

Ethyl (E)-3-[5-(di(1-isopropyl-5-methyl-1H-indol-3-yl)methyl-2-thienyl]acrylate (38)]—1- Isopropyl-5-methyl-1H-indole (**20**) (0.346 g) and **34** (0.210 g) gave, after reaction and chromatography (20% EtOAc/hexane), **38** as a white solid (0.969 g, 93%): TLC (50% EtOAc/hexane): $R_f = 0.86$; mp: 86–88 °C; ^1H NMR: $\delta = 1.29$ (t, $J = 7.2$ Hz, 3H, CH_2CH_3), 1.44 (d, $J = 7.2$ Hz, 12H, $\text{CH}(\text{CH}_3)_2$), 2.36 (s, 6H, $\text{CH}_3\text{-Ar}$), 4.21 (q, $J = 7.2$ Hz, 2H, CH_2CH_3), 4.58 (sept, $J = 7.2$ Hz, 2H, $\text{CH}(\text{CH}_3)_2$), 6.04 (s, 1H, ArCH), 6.05 (d, $J = 15.6$ Hz, 1H, $\text{CH}=\text{CHCO}$), 6.82 (d, $J = 3.6$ Hz, 1H, 4-TpH), 6.84 (s, 2H, 2-ArH), 7.04 (d, $J = 8.1$ Hz, 2H, 6-ArH), 7.07 (d, $J = 3.3$ Hz, 1H, 3-TpH), 7.22 (s, 2H, 4-ArH), 7.26 (d, $J = 8.1$ Hz, 2H, 7-ArH), 7.70 ppm (d, $J = 15.6$ Hz, 1H, $\text{CH}=\text{CHCO}$); IR: $\nu = 2976, 1708, 1662\text{ cm}^{-1}$; HRMS: m/z $[M+\text{Na}]^+$ calcd for $\text{C}_{34}\text{H}_{38}\text{N}_2\text{O}_2\text{S}$: 561.2546, found: 561.2542.

Ethyl (E)-3-[5-(di(5-methyl-1H-indol-3-yl)methyl-2-thienyl]acrylate (39)]—5- Methyl-1H-indole (**19**) (0.262 g) and **34** (0.210 g) gave, after reaction and chromatography (20% EtOAc/hexane), **39** as a white solid (0.479 g, 89%): TLC (50% EtOAc/hexane): $R_f = 0.66$; mp: 96–98 °C; ^1H NMR: $\delta = 1.28$ (t, $J = 7.2$ Hz, 3H, CH_2CH_3), 2.38 (s, 6H, $\text{CH}_3\text{-Ar}$), 4.20 (q, $J = 7.2$ Hz, 2H, CH_2CH_3), 6.05 (s, 1H, ArCH), 6.05 (d, $J = 15.6$ Hz, 1H,

CH=CHCO), 6.81 (s, 2H, 2-ArH), 6.84 (d, $J = 3.6$ Hz, 1H, 4-TpH), 7.02 (d, $J = 7.8$ Hz, 2H, 6-ArH), 7.08 (d, $J = 3.6$ Hz, 1H, 3-TpH), 7.25 (s, 2H, 4-ArH), 7.26 (d, $J = 7.8$ Hz, 2H, 7-ArH), 7.69 (d, $J = 15.6$ Hz, 1H, CH=CHCO), 7.90 ppm (s, 2H, NH); IR: $\nu = 3418, 1696, 1619$ cm⁻¹; HRMS: m/z [$M+H$]⁺ calcd for C₂₈H₂₆N₂O₂S: 455.1788, found: 455.1776.

Ethyl (E)-3-[5-(di(7-methyl-1H-indol-3-yl)methyl-2-thienyl)acrylate (40)]—7-

Methyl-1H-indole (**21**) (0.262 g) and **34** (0.210 g) gave, after reaction and chromatography (20% EtOAc/hexane), **40** as a white solid (0.468 g, 87%): TLC (50% EtOAc/hexane): $R_f = 0.61$; mp: 94–96 °C; ¹H NMR: $\delta = 1.28$ (t, $J = 6.3$ Hz, 3H, CH₂CH₃), 2.49 (s, 6H, CH₃-Ar), 4.20 (q, $J = 6.3$ Hz, 2H, CH₂CH₃), 6.03 (d, $J = 15.3$ Hz, 1H, CH=CHCO), 6.10 (s, 1H, ArCH), 6.84 (d, $J = 3.6$ Hz, 1H, 4-TpH), 6.86 (s, 2H, 2-ArH), 6.98 (dd, $J = 6.3, 6.3$ Hz, 2H, 5-ArH), 6.99 (d, $J = 6.3$ Hz, 2H, 6-ArH), 7.06 (d, $J = 3.6$ Hz, 1H, 3-TpH), 7.31 (d, $J = 6.3$ Hz, 2H, 4-ArH), 7.68 (d, $J = 15.6$ Hz, 1H, CH=CHCO), 7.90 ppm (s, 2H, NH); IR (CHCl₃): $\nu = 2970, 2945, 2360, 2342, 1734, 1053$ cm⁻¹; HRMS: m/z [$M+H$]⁺ calcd for C₂₈H₂₆N₂O₂S: 455.1788, found: 455.1788.

4-Di(1H-indol-3-yl)methylphenol (3):[47]—A reported procedure[47] was used. 4-Hydroxybenzaldehyde (**23**) (0.233 g, 1.00 mmol), indole (**16**) (0.234 g, 2.00 mmol), and I₂ (0.051 mg, 0.2 mmol) in MeCN (3 mL) was stirred for 1 min, at which time TLC [(33% EtOAc/hexane): $R_f = 0.25$] indicated complete reaction. The mixture was treated with 5% Na₂S₂O₃(aq) (5 mL) and extracted with EtOAc (3 × 5 mL). The extract was washed (H₂O and brine) and dried. Concentration and chromatography (33% EtOAc/hexane) afforded **3** as a red solid (0.331 g, 98%): TLC (33% EtOAc/hexane): $R_f = 0.25$; mp: 110–114 °C; ¹H NMR: $\delta = 4.66$ (s, 1H, ArOH), 5.83 (s, 1H, ArCH), 6.65 (s, 2H, 2-ArH), 6.73 (d, $J = 8.4$ Hz, 2H, 2,6-PhH), 6.98–7.03 (m, 2H, 5-ArH), 7.15 (d, $J = 7.2$ Hz, 2H, 4-ArH), 7.20 (d, $J = 8.4$ Hz, 2H, 3,5-PhH), 7.34–7.40 (m, 2H, 6-ArH), 7.38 (d, $J = 8.4$ Hz, 2H, 7-ArH), 7.91 ppm (s, 2H, NH); IR (CHCl₃): $\nu = 2966, 2215$ cm⁻¹; in agreement with reported data.[47]

Hydrolysis of esters 25, 26, 28, 31, 32 and 35–40 to carboxylic acids 4–14, respectively—A reported procedure[73] was adapted. Briefly, LiOH·H₂O (0.033 g, 0.789 mmol) was added to a solution of ester (0.158 mmol) in MeOH (1.5 mL). This mixture was heated at reflux under Ar for 3 h, cooled to room temperature, acidified with 1 N HCl (pH ~ 1), and extracted with EtOAc (10 mL). The extract was washed (H₂O and brine).

Concentration and drying or chromatography (50% EtOAc/hexane) gave the pure carboxylic acid. Acids were found to be unstable to analytical RP-HPLC conditions. Therefore, the corresponding esters were analyzed. Chromatograms indicated that esters were > 98% pure (see Supporting Information).

4-Di(1H-indol-3-yl)methylbenzoic acid (4)—After hydrolysis, concentration, and drying, **25** (0.060 g, 0.158 mmol) yielded **4** as a white solid (0.049 g, 85%): TLC (50% EtOAc/hexane): $R_f = 0.28$; mp: 248–250 °C (dec); ¹H NMR: $\delta = 5.92$ (s, 1H, CH), 6.66 (s, 2H, C=CHNH), 6.86–6.90 (m, 2H, ArH), 7.03–7.08 (m, 2H, ArH), 7.26 (d, $J = 7.8$ Hz, 2H, 2,6-ArH), 7.33 (d, $J = 7.8$ Hz, 2H, 3,5-ArH), 7.41–7.43 (m, 2H, ArH), 7.91–7.94 (m, 2H, ArH), 10.17 ppm (s, 1H, CO₂H); IR (CHCl₃): $\nu = 2970, 2945, 2360, 2342, 1734, 1053$ cm⁻¹; HRMS: m/z [$M+Na$]⁺ calcd for C₂₄H₁₈N₂O₂: 389.1260, found: 389.1273.

4-Di(1-methyl-1H-indol-3-yl)methylbenzoic acid (5)—After hydrolysis and chromatography, **26** (0.122 g, 0.30 mmol) yielded **5** as a pale-yellow solid (0.105 g, 89%): TLC (50% EtOAc/hexane): $R_f = 0.49$; mp: 257–259 °C (dec); ¹H NMR (CD₃OD): $\delta = 3.70$ (s, 6H, NCH₃), 5.92 (s, 1H, ArCH), 6.60 (s, 2H, 2-ArH), 7.00 (t, $J = 7.2$ Hz, 2H, ArH), 6.89–6.94 (m, 2H, ArH), 7.11–7.16 (m, 2H, ArH), 7.26–7.28 (m, 2H, ArH), 7.43 (d, $J = 8.4$

Hz, 2H, 3,5-PhH), 7.93 ppm (d, $J = 8.4$ Hz, 2H, 2,6-PhH); IR: $\tilde{\nu} = 3384, 1680, 1607, 1291$ cm^{-1} ; HRMS: m/z $[M-H]^-$ calcd for $\text{C}_{26}\text{H}_{22}\text{N}_2\text{O}_2$: 393.1608, found: 393.1610.

2,2-Di(1*H*-indol-3-yl)acetic acid (6):[74]—After hydrolysis and chromatography, **28** (0.095 g, 0.30 mmol) yielded **6** as a pale-yellow solid (0.076 g, 87%): TLC (50% EtOAc/hexane): $R_f = 0.29$; mp: 187–189 °C; ^1H NMR ($[\text{D}_6]\text{DMSO}$): $\delta = 5.34$ (s, 1H, ArCH), 6.95 (dd, $J = 7.5, 6.6$ Hz, 2H, 5-ArH), 7.06 (dd, $J = 7.2, 6.6$ Hz, 2H, 6-ArH), 7.21 (s, 2H, 2-ArH), 7.36 (d, $J = 7.2$ Hz, 2H, 7-ArH), 7.55 (d, $J = 7.5$ Hz, 2H, 4-ArH), 10.92 (s, 2H, NH), 12.36 ppm (br s, 1H, CO_2H); IR: $\tilde{\nu} = 3400, 1702, 1338, 1198$ cm^{-1} ; in agreement with reported data.[74]

(*E*)-3-[3-Di(1*H*-indol-3-yl)methylphenyl]acrylic acid (7)—After hydrolysis and chromatography, **31** (0.105 g, 0.25 mmol) yielded **7** as a pale-yellow solid (0.088 g, 90 %): TLC (50% EtOAc/hexane): $R_f = 0.25$; mp: 137–139 °C; ^1H NMR (CD_3OD): $\delta = 5.89$ (s, 1H, ArCH), 6.35 (d, $J = 16.2$ Hz, 1H, $\text{CH}=\text{CHCO}$), 6.66 (s, 2H, 2-ArH), 6.85–6.90 (m, 2H, ArH), 7.03–7.08 (m, 2H, ArH) 7.26–7.29 (m, 2H, ArH), 7.32 (s, 2H, NH), 7.32–7.34 (m, 4H, ArH), 7.42–7.45 (m, 2H, ArH), 7.59 ppm (d, $J = 16.2$ Hz, 1H, $\text{CH}=\text{CHCO}$); IR: $\tilde{\nu} = 3427, 3400, 1683, 1630, 1274$ cm^{-1} ; HRMS: m/z $[M-H]^-$ calcd for $\text{C}_{26}\text{H}_{20}\text{N}_2\text{O}_2$: 391.1452, found: 391.1448.

(*E*)-3-[3-Di(1-methyl-1*H*-indol-3-yl)methylphenyl]acrylic acid (8)—After hydrolysis and chromatography, **32** (0.09 g, 0.20 mmol) yielded **8** as a pale-yellow solid (0.076 g, 90%): TLC (50% EtOAc/hexane): $R_f = 0.36$; mp: 140–142 °C; ^1H NMR: $\delta = 3.69$ (s, 6H, NCH_3), 5.89 (s, 1H, ArCH), 6.35 (d, $J = 15.9$ Hz, 1H, $\text{CH}=\text{CHCO}$), 6.52 (s, 2H, 2-ArH), 7.00 (t, $J = 7.2$ Hz, 2H, ArH), 7.21 (t, $J = 7.2$ Hz, 2H, ArH) 7.29–7.31 (m, 2H, ArH), 7.35–7.38 (m, 4H, ArH), 7.51 (s, 1H, 2-PhH), 7.71 (d, $J = 15.9$ Hz, 1H, $\text{CH}=\text{CHCO}$), 10.28 ppm (br s, 1H, CO_2H); IR: $\tilde{\nu} = 3382, 1691, 1469, 1014$ cm^{-1} ; HRMS: m/z $[M+\text{Na}]^+$ calcd for $\text{C}_{28}\text{H}_{24}\text{N}_2\text{O}_2$: 443.1730, found: 443.1732.

(*E*)-3-[5-Di(1*H*-indol-3-yl)methyl-2-thienyl]acrylic acid (9)—After hydrolysis and chromatography, **35** (0.082 g, 0.20 mmol) yielded **9** as a pale-yellow solid (0.07 g, 91 %): TLC (50% EtOAc/hexane): $R_f = 0.26$; mp: 196–198 °C; ^1H NMR (CD_3OD): $\delta = 6.05$ (d, $J = 15$ Hz, 1H, $\text{CH}=\text{CHCO}$), 6.09 (s, 1H, Ar-CH), 6.60 (d, $J = 3.6$ Hz, 1H, TpH), 6.88 (s, 2H, 2-ArH), 7.02–7.06 (m, 2H, ArH), 7.07 (d, $J = 3.6$ Hz, 1H, TpH), 7.17–7.22 (m, 2H, ArH), 7.38 (d, $J = 7.8$ Hz, 2H, ArH), 7.46 (d, $J = 7.8$ Hz, 2H, ArH), 7.68 (d, $J = 15.9$ Hz, 1H, $\text{CH}=\text{CHCO}$), 8.00 ppm (br s, 2H, NH); IR: $\tilde{\nu} = 3366, 3335, 1678, 1454, 1015$ cm^{-1} ; HRMS: m/z $[M+\text{H}]^+$ calcd for $\text{C}_{24}\text{H}_{18}\text{N}_2\text{O}_2\text{S}$: 399.1162, found: 399.1147.

(*E*)-3-[5-Di(1-methyl-1*H*-indol-3-yl)methyl-2-thienyl]acrylic acid (10)—After hydrolysis and chromatography, **36** (0.088 g, 0.20 mmol) yielded **10** as a pale-yellow solid (0.077 g, 93%): TLC (50% EtOAc/hexane): $R_f = 0.39$; mp: 120–122 °C; ^1H NMR (CD_3OD): $\delta = 3.72$ (s, 6H, $\text{N}(\text{CH}_3)_2$), 6.01 (d, $J = 15.9$ Hz, 1H, $\text{CH}=\text{CHCO}$), 6.11 (s, 1H, ArCH), 6.81 (s, 2H, 2-InH), 6.86–6.88 (m, 1H, TpH), 6.93–6.98 (m, 2H, ArH), 7.12–7.15 (m, 1H, TpH), 7.15–7.17 (m, 2H, ArH), 7.34 (d, $J = 8.1$ Hz, 2H, ArH), 7.37 (d, $J = 8.1$ Hz, 2H, ArH), 7.69 ppm (d, $J = 15.9$ Hz, 1H, $\text{CH}=\text{CHCO}$); IR: $\tilde{\nu} = 3362, 1677, 1613, 1262$ cm^{-1} ; HRMS: m/z $[M+\text{Na}]^+$ calcd for $\text{C}_{26}\text{H}_{22}\text{N}_2\text{O}_2\text{S}$: 449.1294, found: 449.1287.

(*E*)-3-[5-(Di(1-isopropyl-1*H*-indol-3-yl)methyl-2-thienyl]acrylic acid (11)—After hydrolysis and chromatography, **37** (0.497 g, 1.00 mmol) yielded **11** as a pale-yellow solid (0.413 g, 88 %): TLC (50% EtOAc/hexane): $R_f = 0.41$; mp: 248–250 °C (dec); ^1H NMR: $\delta = 1.40$ (d, $J = 6.3$ Hz, 12H, $\text{CH}(\text{CH}_3)_2$), 4.71 (sept, $J = 6.3$ Hz, 2H, $\text{CH}(\text{CH}_3)_2$), 5.96 (d, $J = 15.6$ Hz, 1H, $\text{CH}=\text{CHCO}$), 6.16 (s, 1H, ArCH), 6.92 (d, $J = 7.8$ Hz, 2H, 7-ArH), 6.94 (d, $J =$

3.6 Hz, 1H, 4-TpH), 7.09 (dd, $J = 6.6, 7.8$ Hz, 2H, 6-ArH), 7.28 (d, $J = 9$ Hz, 2H, 4-ArH), 7.30 (d, $J = 3.6$ Hz, 1H, 3-TpH), 7.45 (s, 2H, 2-ArH), 7.46 (dd, $J = 9.0, 6.6$ Hz, 2H, 5-ArH), 7.63 (d, $J = 15.6$ Hz, 1H, $\text{CH}=\text{CHCO}$), 12.22 ppm (s, 1H, CO_2H); IR: $\nu = 3571, 2976, 1677, 1610, 1460, 1194$ cm^{-1} ; HRMS: m/z $[M+\text{Na}]^+$ calcd for $\text{C}_{30}\text{H}_{30}\text{N}_2\text{O}_2\text{S}$: 505.1920, found: 505.1923.

(E)-3-[5-(Di(1-isopropyl-5-methyl-1H-indol-3-yl)methyl-2-thienyl)]acrylic acid (12)—After hydrolysis and chromatography, **38** (0.088 g, 0.20 mmol) yielded **12** as a pale-yellow solid (0.077 g, 93%): TLC (50% EtOAc/hexane): $R_f = 0.40$; mp: 251–253 °C(dec); ^1H NMR: $\delta = 1.39$ (d, $J = 6.3$ Hz, 12H, $\text{CH}(\text{CH}_3)_2$), 2.28 (s, 6H, $\text{CH}_3\text{-Ar}$), 4.67 (sept, $J = 6.3$ Hz, 2H, $\text{CH}(\text{CH}_3)_2$), 5.96 (d, $J = 15.6$ Hz, 1H, $\text{CH}=\text{CHCO}$), 6.07 (s, 1H, ArCH), 6.89 (d, $J = 3.6$ Hz, 1H, 4-TpH), 6.91 (d, $J = 7.8$ Hz, 2H, 6-ArH), 7.19 (s, 2H, 2-ArH), 7.20 (s, 2H, 4-ArH), 7.30 (d, $J = 3.6$ Hz, 1H, 3-TpH), 7.35 (d, $J = 7.8$ Hz, 2H, 7-ArH), 7.62 (d, $J = 15.6$ Hz, 1H, $\text{CH}=\text{CHCO}$), 12.20 ppm (s, 1H, CO_2H); IR: $\nu = 3617, 2971, 1680, 1613, 1197$ cm^{-1} ; HRMS: m/z $[M+\text{Na}]^+$ calcd for $\text{C}_{32}\text{H}_{34}\text{N}_2\text{O}_2\text{S}$: 533.2233, found: 533.2237.

(E)-3-[5-(Di(5-methyl-1H-indol-3-yl)methyl-2-thienyl)]acrylic acid (13)—After hydrolysis and chromatography, **39** (0.091 g, 0.20 mmol) yielded **13** as a pale-yellow solid (0.078 g, 91%): TLC (50% EtOAc/hexane): $R_f = 0.18$; mp: 172–174 °C; ^1H NMR (CD_3OD): $\delta = 2.32$ (s, 6H, $\text{CH}_3\text{-Ar}$), 6.00 (d, $J = 15.6$ Hz, 1H, $\text{CH}=\text{CHCO}$), 6.04 (s, 1H, ArCH), 6.80 (s, 2H, 2-ArH), 6.84 (d, $J = 3.6$ Hz, 1H, 4-TpH), 6.91 (d, $J = 7.2$ Hz, 2H, 6-ArH), 7.15 (s, 2H, 4-ArH), 7.16 (d, $J = 3.6$ Hz, 1H, 3-TpH), 7.23 (d, $J = 7.8$ Hz, 2H, 7-ArH), 7.70 ppm (d, $J = 15.6$ Hz, 1H, $\text{CH}=\text{CHCO}$); IR: $\nu = 3589, 3407, 1677, 1612, 1265$ cm^{-1} ; HRMS: m/z calcd $[M+\text{H}]^+$ for $\text{C}_{26}\text{H}_{22}\text{N}_2\text{O}_2\text{S}$: 427.1475, found: 422.1468.

(E)-3-[5-(Di(7-methyl-1H-indol-3-yl)methyl-2-thienyl)]acrylic acid (14)—After hydrolysis and chromatography, **40** (0.091 g, 0.20 mmol) yielded **14** as a pale-yellow solid (0.077 g, 90%): TLC (50% EtOAc/hexane): $R_f = 0.18$; mp: 175–177 °C; ^1H NMR (CD_3OD): $\delta = 2.48$ (s, 6H, $\text{CH}_3\text{-Ar}$), 6.00 (d, $J = 15.6$ Hz, 1H, $\text{CH}=\text{CHCO}$), 6.08 (s, 1H, ArCH), 6.84 (d, $J = 3.6$ Hz, 1H, 4-TpH), 6.87 (dd, $J = 7.8, 7.8$ Hz, 2H, 5-ArH), 6.86 (s, 2H, 2-ArH), 7.20 (d, $J = 7.8$ Hz, 2H, 6-ArH), 7.15 (d, $J = 3.6$ Hz, 1H, 3-TpH), 7.20 (d, $J = 7.8$ Hz, 2H, 4-ArH), 7.69 ppm (d, $J = 15.6$ Hz, 1H, $\text{CH}=\text{CHCO}$); IR: $\nu = 3629, 3409, 1679, 1612, 1263$ cm^{-1} ; HRMS: m/z $[M+\text{H}]^+$ calcd for $\text{C}_{26}\text{H}_{22}\text{N}_2\text{O}_2\text{S}$: 427.1475, found: 422.1466.

Computational methods

Three-dimensional structures of potential DIM-arenes were constructed using the SKETCH module in Sybyl 7.1 (Tripos, St. Louis, MO, USA). Energy was minimized with the Tripos force-field using the conjugated gradient method until a convergence value of 0.005 $\text{kcal}\text{\AA}^{-1}\text{mol}^{-1}$ was achieved. Docking was performed using the automated docking program AutoDock 4.0.[75] Grid maps for docking simulations were generated with a grid of 50 points in the x , y , and z directions using the Arg316 residue of the RXR α LBD (PDB code: 1MVC) as the central residue and 0.375 Å as the grid spacing. A distance-dependent function of the dielectric constant was used for the energetic map calculations. The AutoDockTools suite[75] was used to identify the torsion angles of the ligands and assign Gasteiger and Kollman partial atomic charges to DIM-arenes and the RXR α LBD, respectively. One hundred independent docking runs were performed for each DIM-arene using the Lamarckian genetic algorithm and a maximum number of 2 500 000 energy evaluations. Other parameters were assigned default values, implemented by the program. Cluster analysis performed on the results from 100 runs gave a root-mean-square tolerance of 2 Å.

Biological assays

Competitive binding to RXR α —Purified polyhistidine-tagged human RXR α LBD(223–462) (50 ng) was incubated with [11,12-³H]9-*cis*-RA (Amersham Biosciences, Piscataway, NJ, USA) in the presence of various concentrations of unlabeled 9-*cis*-RA or DIM-arene at 4 °C for 12 h. RXR α LBD was then captured by incubation with Ni-coated beads. The amount of specifically bound radiolabel was determined by scintillation counting of the beads followed by subtraction of nonspecifically bound radiolabel as described.[76]

Transient-transfection assays for transcriptional activation of RXR α , RARs, and NR4A1/TR3—CV-1 cells (5×10^4 per well) were plated in 24-well plates in Dulbecco's modified Eagle's medium (DMEM) containing 10% charcoal-stripped FBS. After 24 h, cells were transfected with the TREpal-*tk*-CAT reporter construct (100 ng) alone or with an *rxra*, *rara*, *rar* β , or *rarg* gene plasmid (25 ng); NurRE-*tk*-CAT alone or with the *nr4a1/tr3* plasmid; or β RARE-*tk*-CAT alone or with *rxra* and/or *nr4a1/tr3* as described[12,15,17,76] and, at the same time, the β -galactosidase gene (*gal*) (100 ng) was included as a control for transfection efficiency. Cells were then treated for 48 h with DMSO vehicle alone (control) or compound dissolved in DMSO as specified in Figures 3 and 4A–C, then collected for measurement of CAT enzyme activity, which was normalized for transfection efficiency on the basis of β GAL enzyme activity as a measure of co-transfected *gal* gene activity. Results reported are averages of duplicates.

PPRE-*tk*-LUC reporter assays—PPAR γ transcriptional activation was monitored by transfection of an (AOx)₃-*tk*-LUC plasmid, containing three copies of the acyl-CoA oxidase PPRE upstream of the basal thymidine kinase (*tk*) promoter in the *tk*-LUC plasmid.[76,77] HeLa cells were maintained in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin. Cells (7×10^4) in 1.0 mL of medium were transfected in 24-well plates using 1 μ g polyethylenimine (PEI, Polyscience Inc., Warrington, PA, USA), 0.2 μ g (AOx)₃-*tk*-LUC plasmid,[60] and either 0.3 μ g of human *ppary*[78] or pMSCVhph vector in 50 μ L serum-free DMEM per well. This PEI mixture had been incubated for 20 min at room temperature before addition to the cells, which previously had their medium replaced by fresh medium. Transfections also included Renilla luciferase (pRL-*tk*) plasmid as a control for transfection efficacy. After overnight incubation at 37 °C under 10% CO₂, medium was replaced with DMEM containing 10% FBS alone, or combined with 20 μ M troglitazone as the positive control, 0.2, 2.0, 10, 20, or 40 μ M (final concentration) **1**, **3**, or **10**, or DMSO vehicle alone up to a final maximal concentration of 0.5%, which did not affect the assay results. After 48 h incubation, cells were lysed in lysis buffer and assayed for luciferase activity using the Dual Luciferase kit (Promega Corp., Madison, WI, USA) according to the manufacturer's protocol. Luminescence was measured using a Veritas Microplate Luminometer (Turner Biosystems, Sunnyvale, CA, USA). Data were normalized to pRL-*tk* and expressed as fold-activation above (AOx)₃-*tk*-LUC activation induced by vehicle alone. Results expressed in Figure 4D are the averages of triplicates $\pm$ SD.

Growth inhibition—LNCaP and DU-145 prostate cancer cells were obtained from ATCC (Manassas, VA, USA) and grown at 37°C as described.[15,76] Compound-induced growth inhibition was assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay in 96-well microtiter plates. Cells were allowed to attach and then treated for 48 h with DMSO alone or by DIM-arene in DMSO at concentrations specified in Table 1. At 4 h before the end of treatment, MTT was added to each well to a final concentration of 1.0 mg mL⁻¹, and incubation was continued at 37 °C. The MTT reaction was terminated by removing the supernatant, and the dye was dissolved by adding DMSO (200 μ L) followed by thorough mixing. Plates were read at 550 nm using a microtiter plate reader. Means and standard deviations were derived from eight replicates.

KG-1 cells in medium containing 5% FBS were allowed to attach overnight, treated for the indicated times with analogues at the specified concentrations in DMSO or DMSO alone (0.1% final concentration), and then harvested as described.[62] Cell numbers were counted in triplicate wells using a hemocytometer.

Cell viability—HeLa-S cervical adenocarcinoma, PPC-1 prostate adenocarcinoma, U2OS bone osteosarcoma, and HCT-116 colon carcinoma cell lines (ATCC) were cultured in DMEM supplemented with 10% heat-inactivated FBS and 1% penicillin/streptomycin mixture in 10 cm dishes at 37°C, 10% CO₂, and 90% relative humidity. Cell suspensions (1×10^4 cells in 50 μ L per well) in white-bottomed Greiner 384-well plates were pre-incubated for 1 h under the same culture conditions and then treated with various concentrations of **1** that had been dissolved in DMSO (0.125% maximum concentration, which had no effect on cell growth) and diluted into DMEM containing 10% FBS. After 24 h incubation, plates were transferred to room temperature for 15 min. The luciferase assay was performed by adding ATPLite™ Assay System reagent (20 μ L per well; PerkinElmer, Boston, MA, USA) and 10 min later measuring light emission using a FlexStation 3 Microplate Reader (Molecular Devices, Sunnyvale, CA, USA). Experiments were performed in triplicate. Viability was calculated on the basis of maximum luminescence intensity observed for each cell line in the absence of compound **1** (100% value).

Cell apoptosis—KG-1 acute myelocytic leukemia cells were seeded and incubated as described in the growth inhibition experiment. Apoptotic cells were identified using acridine orange staining on at least three replicates as described.[77]

Supplementary Material

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Glossary

AML	acute myelocytic leukemia
DIM	di(1 <i>H</i> -indol-3-yl)methyl
GAL	β -galactosidase
H	helix
LBD	ligand binding domain
LBP	ligand binding pocket
LUC	luciferase
NBRE	Nur77-binding response element
NGFI-B	nerve growth factor-induced clone B
NOR1	neuron-derived orphan receptor 1
NR	nuclear receptor
NurRE	Nur response element
Nurr1	Nur-related factor 1
PPAR	peroxisome proliferator-activated receptor
PPRE	PPAR response element
RAR	retinoic acid receptor

RARE	retinoic acid response element
RXR	retinoid X receptor

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References

1. Dawson, MI.; Hobbs, PD.; Jong, L.; Cameron, JF.; Lehmann, JM.; Fanjul, A.; Lu, XP.; Haefner, P.; Pfahl, M. US Patent. 5 466 861. 1995.
2. Dawson MI, Jong L, Hobbs PD, Cameron JF, Chao WR, Pfahl M, Lee MO, Shroot B, Pfahl M. J. Med. Chem 1995;38:3368–3383. [PubMed: 7650690]
3. Boehm MF, Zhang L, Badea BA, White SK, Mais DE, Berger E, Suto CM, Goldman ME, Heyman RA. J. Med. Chem 1994;37:2930–2941. [PubMed: 8071941]
4. Boehm, MF.; Heyman, RA.; Zhi, L.; Koch, SC. US Patent . 6 320 074. 2001.
5. Hurst RE. Curr. Drug Metab. Curr. Opin. Investig. Drugs 2000;1:514–523.
6. Smit JW, Stokkel MP, Pereira AM, Romijn JA, Visser TJ. J. Clin. Endocrinol. Metab 2007;92:2496–2499. [PubMed: 17440015]
7. Heald P. Clin. Lymphoma 2000;1 Suppl. 1:S45–S49. [PubMed: 11707864]
8. Blumenschein GR Jr, Khuri FR, von Pawel J, Gatzemeier U, Miller WH Jr, Jotte RM, Le Treut J, Sun SL, Zhang JK, Dziewanowska ZE, Negro-Vilar A. J. Clin. Oncol 2008;26:1879–1885. [PubMed: 18398153]
9. Wildi JD, Baggstrom MQ, Suresh R, Read W, Fracasso PM, Govindan R. Chemotherapy 2008;54:125–130. [PubMed: 18332628]
10. Esteva FJ, Glaspy J, Baidas S, Laufman L, Hutchins L, Dickler M, Tripathy D, Cohen R, DeMichele A, Yocum RC, Osborne CK, Hayes DF, Hortobagyi GN, Winer E, Demetri GD. J. Clin. Oncol 2003;21:999–1006. [PubMed: 12637463]
11. Duvic M, Martin AG, Kim Y, Olsen E, Wood GS, Crowley CA, Yocum RC. Arch. Dermatol 2001;137:581–593. [PubMed: 11346336]
12. James SY, Lin F, Kolluri SK, Dawson MI, Zhang X-K. Cancer Res 2003;63:3531–3538. [PubMed: 12839938]
13. Kuang AA, Cado D, Winoto A. Eur. J. Immunol 1999;30:3722–3728. [PubMed: 10556828]
14. Cho SD, Yoon K, Chintharlapalli S, Abdelrahim M, Lei P, Hamilton S, Khan S, Ramaiah SK, Safe S. Cancer Res 2007;67:674–683. [PubMed: 17234778]
15. Li H, Kolluri SK, Gu J, Dawson MI, Cao X, Hobbs PD, Lin B, Chen G, Lu J, Lin F, Xie Z, Fontana JA, Reed JC, Zhang X. Science 2000;289:1159–1164. [PubMed: 10947977]
16. Lin B, Kolluri SK, Lin F, Liu W, Han YH, Cao X, Dawson MI, Reed JC, Zhang X-K. Cell 2004;116:527–540. [PubMed: 14980220]
17. Cao X, Liu W, Lin F, Li H, Kolluri SK, Lin B, Han YH, Dawson MI, Zhang X-K. Mol. Cell. Biol 2004;24:9705–9725. [PubMed: 15509776]
18. Chintharlapalli S, Burghardt R, Papineni S, Ramaiah S, Yoon K, Safe S. J. Biol. Chem 2005;280:24903–24914. [PubMed: 15871945]
19. Wansa KD, Harris JM, Muscat GE. J. Biol. Chem 2002;277:33001–33011. [PubMed: 12082103]
20. Ordentlich P, Yan Y, Zhou S, Heyman RA. J. Biol. Chem 2003;278:24791–24799. [PubMed: 12709433]
21. Wansa KD, Harris JM, Yan G, Ordentlich P, Muscat GE. J. Biol. Chem 2003;278:24776–24790. [PubMed: 12709428]

22. Kagaya S, Ohkura N, Tsukada T, Miyagawa M, Sugita Y, Tsujimoto G, Matsumoto K, Saito H, Hashida R. *Biol. Pharm. Bull* 2005;28:1603–1607. [PubMed: 16141523]
23. Codina A, Benoit G, Gooch JT, Neuhaus D, Perlmann T, Schwabe JW. *J. Biol. Chem* 2004;279:53338–53345. [PubMed: 15456745]
24. Hintermann S, Chiesi M, von Krosigk U, Mathe D, Felber R, Hengerer B. *Bioorg. Med. Chem. Lett* 2007;17:193–196. [PubMed: 17035009]
25. Poppe L, Harvey TS, Mohr C, Zondlo J, Tegley CM, Nuanmanee O, Cheetham J. *J. Biomol. Screening* 2007;12:301–311.
26. Wang Z, Benoit G, Liu J, Prasad S, Aarnisalo P, Liu X, Xu H, Walker NP, Perlmann T. *Nature* 2003;423:555–560. [PubMed: 12774125]
27. Flaig R, Greschik H, Peluso-Iltis C, Moras D. *J. Biol. Chem* 2005;280:19250–19258. [PubMed: 15716272]
28. Min JR, Schuetz A, Loppnau P, Weigelt J, Sundström M, Arrow-smith CH, Edwards AM, Bochkarev A, Plotnikov AN. RCSB Protein Data Bank. 2007 accession code 2QW4, DOI: 10.2210/pdb2qw4/pdb.
29. Okabe T, Nawata H. *Nippon Rinsho* 1996;54:1768–1772. [PubMed: 8741664]
30. Wu Q, Dawson MI, Zheng Y, Hobbs PD, Agadir A, Jong L, Li Y, Liu R, Lin B, Zhang X-K. *Mol. Cell. Biol* 1997;17:6598–6608. [PubMed: 9343423]
31. Maira M, Martens C, Philips A. J. Drouin, *Mol. Cell. Biol* 1999;19:7549–7557.
32. Maxwell MA, Muscat GE. *Nucl. Recept. Signal* 2006;4:e002. [PubMed: 16604165]
33. Chintharlapalli S, Smith R III, Samudio I, Zhang W, Safe S. *Cancer Res* 2004;64:5994–6001. [PubMed: 15342379]
34. Chintharlapalli S, Papineni S, Baek SJ, Liu S, Safe S. *Mol. Pharmacol* 2005;68:1782–1792. [PubMed: 16155208]
35. Chintharlapalli S, Papineni S, Safe S. *Mol. Pharmacol* 2007;71:558–569. [PubMed: 17093136]
36. Ziouzenkova O, Orasanu G, Sukhova G, Lau E, Berger JP, Tang G, Krinsky NI, Dolnikowski GG, Plutsky J. *Mol. Endocrinol* 2007;21:77–88. [PubMed: 17008383]
37. Lin Q, Ruuska SE, Shaw NS, Dong D, Noy N. *Biochemistry* 1999;38:185–190. [PubMed: 9890897]
38. Yamazaki Y, Kawano Y, Uebayasi M. *Life Sci* 2008;82:290–300. [PubMed: 18166202]
39. Heim M, Johnson J, Boess F, Bendik I, Weber P, Hunziker W, Fluhmann B. *FASEB J* 2002;16:718–720. [PubMed: 11923221]
40. Lengqvist J, Mata de Urquiza A, Bergman AC, Willson TM, Sjövall J, Perlmann T, Griffiths WJ. *Mol. Cell. Proteomics* 2004;3:692–703. [PubMed: 15073272]
41. Mata de Urquiza A, Liu S, Sjöberg M, Zetterström RH, Griffiths W, Sjövall J, Perlmann T. *Science* 2000;290:2140–2144. [PubMed: 11118147]
42. Lemotte PK, Keidel S, Apfel CM. *Eur. J. Biochem* 1996;236:328–333. [PubMed: 8617282]
43. Egea PF, Mitschler A, Moras D. *Mol. Endocrinol* 2002;16:987–997. [PubMed: 11981034]
44. Lehmann JM, Jong L, Fanjul A, Cameron JF, Lu XP, Haefner P, Dawson MI, Pfahl M. *Science* 1992;258:1944–1946. [PubMed: 1335166]
45. Egea PF, Mitschler A, Rochel N, Ruff M, Chambon P, Moras D. *EMBO J* 2000;19:2592–2601. [PubMed: 10835357]
46. Bartoli G, Bosco M, Foglia G, Giuliani A, Marcantoni E, Sambri L. *Synthesis* 2004;6:895–900.
47. Bandgar BP, Shaikh KA. *Tetrahedron Lett* 2003;44:1959–1961.
48. IJpenberg A, Tan NS, Gelman L, Kersten S, Seydoux J, Xu J, Metzger D, Canaple L, Chambon P, Wahli W, Desvergne B. *EMBO J* 2004;23:2083–2091. [PubMed: 15103326]
49. Diab A, Hussain RZ, Lovett-Racke AE, Chavis JA, Drew PD, Racke MK. *J. Neuroimmunol* 2004;148:116–126. [PubMed: 14975592]
50. Yamazaki K, Shimizu M, Okuno M, Matsushima-Nishiwaki R, Kanemura N, Araki H, Tsurumi H, Kojima S, Weinstein IB, Moriwaki H. *Gut* 2007;56:1557–1563. [PubMed: 17604322]
51. Zapata-Gonzalez F, Rueda F, Petriz J, Domingo P, Villarroja F, de Madariaga A, Domingo JC. *J. Immunol* 2007;178:6130–6139. [PubMed: 17475839]

52. Lu J, Chen M, Stanley SE, Li E. *Biochem. Biophys. Res. Commun* 2008;365:42–46. [PubMed: 17980149]
53. Dawson MI, Jong L, Hobbs PD, Xiao D, Feng KC, Chao WR, Pan C, Fontana JA, Zhang X-K. *Bioorg. Med. Chem. Lett* 2000;10:1311–1313. [PubMed: 10890153]
54. Bernardon, J-M.; Diaz, P. US Patent. 6 258 775. 2001.
55. Bernardon, J-M.; Diaz, P. US Patent. 6 825 360. 2004.
56. Haffner CD, Lenhard JM, Miller AB, McDougald DL, Dwornik K, Ittoop OR, Gampe RT Jr, Xu HE, Blanchard S, Montana VG, Consler TG, Bledsoe RK, Ayscue A, Croom D. *J. Med. Chem* 2004;47:2010–2029. [PubMed: 15056000]
57. Hibi S, Kikuchi K, Yoshimura H, Nagai M, Tai K, Hida T. *J. Med. Chem* 1998;41:3245–3252. [PubMed: 9703470]
58. Venhorst J, Nunez S, Terpstra JW, Kruse CG. *J. Med. Chem* 2008;51:3222–3229. [PubMed: 18447336]
59. Zhan Y, Du X, Chen H, Liu J, Zhao B, Huang D, Li G, Xu Q, Zhang M, Weimer BC, Chen D, Cheng Z, Zhang L, Li Q, Li S, Zheng Z, Song S, Huang Y, Ye Z, Su W, Lin S-C, Shen Y, Wu Q. *Nat. Chem. Biol* 2008;4:548–556. [PubMed: 18690216]
60. Sun S-Y, Yue P, Dawson MI, Shroot B, Michel S, Lamph WW, Heyman RA, Teng M, Chandraratna RAS, Shudo K, Hong WK, Lotan R. *Cancer Res* 2007;67:4931–4939.
61. Kizaki M, Dawson MI, Heyman R, Elstner E, Morosetti R, Pakkala S, Chen DL, Ueno H, Chao W, Morikawa M, Ikeda Y, Heber D, Pfahl M, Koeffler HP. *Blood* 1996;87:1977–1984. [PubMed: 8634447]
62. Shiohara M, Dawson MI, Hobbs PD, Sawai N, Higuchi T, Koike K, Komiyama A, Koeffler HP. *Blood* 1999;93:2057–2066. [PubMed: 10068679]
63. Laidler P, Dulińska J, Mrozicki S. *Arch. Biochem. Biophys* 2007;462:1–12. [PubMed: 17466258]
64. Hsu JY, Pfahl M. *Cancer Res* 1998;58:4817–4822. [PubMed: 9809984]
65. Lei P, Abdelrahim M, Cho SD, Liu S, Chintharlapalli S, Safe S. *Carcinogenesis* 2008;29:1139–1147. [PubMed: 18460448]
66. Cho SD, Lei P, Abdelrahim M, Yoon K, Liu S, Guo J, Papineni S, Chintharlapalli S, Safe S. *Mol. Carcinog* 2008;47:252–263. [PubMed: 17957723]
67. York M, Abdelrahim M, Chintharlapalli S, Lucero SD, Safe S. *Clin. Cancer Res* 2007;13:6743–6752. [PubMed: 18006776]
68. Hong J, Samudio I, Chintharlapalli S, Safe S. *Mol. Carcinog* 2008;47:492–507. [PubMed: 18085536]
69. Safe S, Papineni S, Chintharlapalli S. *Cancer Lett* 2008;269:326–338. [PubMed: 18501502]
70. Lane BS, Brown MA, Sames D. *J. Am. Chem. Soc* 2005;127:8050–8057. [PubMed: 15926829]
71. Kumar SP, Nagaiah K. *Tetrahedron Lett* 2007;48:1391–1394.
72. Greenman KL, Van Vranken DL. *Tetrahedron Lett* 2005;61:6438–6441.
73. Corey EJ, Székely I, Shiner CS. *Tetrahedron Lett* 1977;18:3529–3532.
74. Sato S, Sato T. *Carbohydr. Res* 2005;340:2251–2255. [PubMed: 16098491]
75. Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, Olson AJ. *J. Comput. Chem* 1998;19:1639–1662.
76. Kolluri SK, Corr M, James SY, Bernasconi M, Lu D, Liu W, Cottam HB, Leoni LM, Carson DA, Zhang XK. *Proc. Natl. Acad. Sci. USA* 2005;102:2525–2530. [PubMed: 15699354]
77. Dawson MI, Xia Z, Liu G, Fontana JA, Farhana L, Patel BB, Arumugarajah S, Bhuiyan M, Zhang X-K, Han YH, Stallcup WB, Fukushi J, Mustelin T, Tautz L, Su Y, Harris DL, Waleh N, Hobbs PD, Jong L, Chao WR, Schiff LJ, Sani BP. *J. Med. Chem* 2007;50:2622–2639. [PubMed: 17489579]
78. Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK. *Nature* 1998;391:79–82. [PubMed: 9422508]

Figure 1. Structures of DIM-Ph-4-CF₃ (**1**), DIM-Ph-4-OMe (**2**), DIM-Ph-4-OH (**3**), analogues **4–14**, and RXR α agonist SR11237/BMS649 (**15**).

Figure 2.

Docking of DIM-arenes into the RXR α ligand binding pocket (LBP) as found in the crystal structure of recombinant human RXR α LBD complexed with **15** (PDB code: 1MVC). A) Docked low-energy pose of DIM-Ph-4-CF₃ (**1**, yellow C atoms) overlaps with the structure of RXR transcriptional agonist **15** (green C atoms). Ionic and hydrogen bonds of the CO₂H group O atoms of **15** with Arg316 guanidinium and Ala327 backbone NH groups are designated by dashed yellow lines. B) Low-energy conformers of DIM-Ph-4-CO₂H (**4**, magenta C atoms) and 1,1'-Me₂-DIM-2-thienyl-5-(*E*-CH=CHCO₂H) (**10**, cyan C atoms) dock into the RXR α LBP and overlap with **1** (yellow C atoms). Potential ionic and hydrogen bonds of Arg316 and Ala327 NH groups with CO₂H O atoms of **10** and Cys432 and Cys439

SH groups with indole N atoms of **10** are designated by dashed yellow lines. F, pale-blue; N, blue; O, red; S, gold. Docking was conducted as described in the Experimental Section.

Figure 3.

Effects of 1,1'-Me₂-DIM-2-thienyl-5-(*E*-CH=CHCO₂H) (**10**) and other DIM-arenes on RXR α and NR4A1/TR3 transcriptional activation of reporter constructs. A) Fold-activation of RXR α on the TREpal-*tk*-CAT reporter construct induced by **1** and **3–14** at 1.0 μ M relative to those of the vehicle-alone control (con) and 0.1 μ M 9-*cis*-retinoic acid (9c). B) Fold-activation of NR4A1/TR3 on the NurRE-*tk*-CAT reporter construct induced by **1** and **3–14** at 20 μ M relative to that of the vehicle-alone control (con). C) Fold-activation of RXR α plus NR4A1/TR3 on the β RARE-*tk*-CAT reporter construct induced by 20 μ M **1** and **3–14** alone (gray bars) or in the presence 0.1 μ M 9-*cis*-retinoic acid (9-*cis*-RA, 9c) (black bars) relative to that induced by vehicle alone control (con) (gray bar) or 0.1 μ M 9-*cis*-RA alone (black

bar). CV-1 cells were transfected with *rxra* and/or *NR4A1/tr3* genes plus the reporter constructs and treated as described in the Experimental Section. DMSO was used as the vehicle for solubilizing compounds. Results are averages of duplicates.

Figure 4.

Effects of 1,1'-Me₂-DIM-2-thienyl-5-(E-CH=CHCO₂H) (**10**) on RAR and PPAR γ transcriptional activation of their reporter constructs. Fold-activation of A) RAR α , B) RAR β , and C) RAR γ on the TREpal-*tk*-CAT reporter construct induced by 0.001, 0.01, 0.1, 1.0, and 10 μ M **10** alone (gray bars) or in the presence of 1.0 μ M *trans*-retinoic acid (*trans*-RA) (black bars) relative to those of the vehicle-alone control (con) (gray bar) and 1.0 μ M *trans*-RA (black bar). D) Fold-activation of co-transfected PPAR γ on the PPRE-*tk*-LUC reporter construct induced by the vehicle-alone control (con); 20 μ M troglitazone (Tg); or 0.2, 2.0, and 10 μ M DIM-Ph-4-CF₃ (**1**); 0.2, 2.0, 20, and 40 μ M DIM-Ph-4-OH (**3**); or 2 and 20 μ M **10** (dark-gray bars) relative to reporter activation without transfected PPAR γ (light-

gray bars). At 20 and 40 μM **1** was cytotoxic, as was **10** at 40 μM . CV-1 cells were co-transfected with the reporter construct and a receptor gene plasmid (A–C) or transfected with the reporter construct alone or combined with the receptor gene plasmid (D) and then treated as described in the Experimental Section. DMSO was used as the vehicle for solubilizing compounds.

Figure 5. DIM-Ph-4-CF₃ (**1**) decreases cancer cell viability as measured by ATP levels. Cells were treated with **1** for 22 h before their ATP levels were assessed as described in the Experimental Section. Viability is expressed as the average of triplicate experiments $\pm$ SD relative to that of the untreated controls (100%). Compound concentrations (μ M) are indicated at right.

Scheme 1.

Synthesis of DIM-arenes **1** and **3–14**. Reagents and conditions: a) **22**, **24**, **27**, **30**, **34**: $CeCl_3 \cdot 7H_2O$ – NaI – SiO_2 , MeCN; b) **23**: I_2 , MeCN; c) $LiOH \cdot H_2O$, MeOH, reflux; $HCl(aq)$; d) $Ph_3P=CHCO_2Et$, PhMe, $80^\circ C$.

Table 1

Effects of substituted DIM-arene analogues on competitive binding to RXR α and transcriptional activation of RXR α and/or NR4A1/TR3 on the TREpal, β RARE, and NurRE response elements.

RXR α Competitive Binding ^[a]					Transcriptional Activation of Reporter Construct [%] ^[b]					
Compd	R	R ¹	R ²	R ³	IC ₅₀ [μ M]	% at 10 or 100 μ M	RXR α on TREpal [%] ^[c]	NR4A1/TR3 on NurRE ^[d]	RXR α -NR4A1/TR3 on β RARE ^[e]	
1	H	H	H	Ph-4-CF ₃	3.2	98[g]	23 $\pm$ 1	(2 $\pm$ 1)	114 $\pm$ 9	15 $\pm$ 2
3	H	H	H	Ph-4-OH	>100	49[h]	26 $\pm$ 1	(9 $\pm$ 4)	67 $\pm$ 18	56 $\pm$ 5
4	H	H	H	Ph-4-CO ₂ H	10	50[g]	26 $\pm$ 8	(-1 $\pm$ 1)	105 $\pm$ 13	35 $\pm$ 34
5	Me	H	H	Ph-4-CO ₂ H	49	74[h]	16 $\pm$ 8	(6 $\pm$ 3)	81 $\pm$ 13	30 $\pm$ 4
6	H	H	H	CO ₂ H	>100	0[h]	36 $\pm$ 5	(-5 $\pm$ 3)	98 $\pm$ 4	22 $\pm$ 26
7	H	H	H	Ph-3-(<i>E</i> -CH=CCO ₂ H)	53	71[h]	28 $\pm$ 1	(4 $\pm$ 0)	79 $\pm$ 12	55 $\pm$ 10
8	Me	H	H	Ph-3-(<i>E</i> -CH=CCO ₂ H)	30	67[h]	23 $\pm$ 2	(6 $\pm$ 3)	85 $\pm$ 10	51 $\pm$ 3
9	H	H	H	2-thienyl-5(<i>E</i> -CH=CCO ₂ H)	>100	0[h]	17 $\pm$ 5	(8 $\pm$ 2)	81 $\pm$ 2	47 $\pm$ 2
10	Me	H	H	2-thienyl-5(<i>E</i> -CH=CCO ₂ H)	1.7	89[g]	188 $\pm$ 16	(63 $\pm$ 15)	68 $\pm$ 9	83 $\pm$ 5
11	<i>i</i> Pr	H	H	2-thienyl-5(<i>E</i> -CH=CCO ₂ H)	>100	45[h]	-4 $\pm$ 2	(0 $\pm$ 1)	90 $\pm$ 13	18 $\pm$ 2
12	<i>i</i> Pr	H	Me	2-thienyl-5(<i>E</i> -CH=CCO ₂ H)	69	62[h]	-2 $\pm$ 1	(4 $\pm$ 1)	108 $\pm$ 15	22 $\pm$ 0
13	H	H	Me	2-thienyl-5(<i>E</i> -CH=CCO ₂ H)	ND ^[f]	ND	ND	(3 $\pm$ 0)	ND	ND
14	H	Me	H	2-thienyl-5(<i>E</i> -CH=CCO ₂ H)	ND	ND	ND	(4 $\pm$ 2)	ND	ND

^[a] In competition with 7.5 nM [³H]9-*cis*-RA for binding to recombinant human RXR α LBD with incubation at 4 °C for 16 h; 9-*cis*-RA IC₅₀ = 0.067 μ M.

^[b] At a compound concentration of 20 μ M.

[c] Expressed relative to RXR α activation of TREpal by 0.1 μ M 9-*cis*-RA as 100% after subtraction of the vehicle-alone control; number in parentheses is the percent activation by 1.0 μ M compound relative to that of 0.1 μ M 9-*cis*-RA.

[d] Expressed relative to activation by NR4A1/TR3 of the NurRE in vehicle-alone-treated cells as 100% after subtraction of the NurRE-alone control.

[e] Expressed relative to RXR α + NR4A1/TR3 activation of the β RARE by 0.1 μ M 9-*cis*-RA as 100% after subtraction of the vehicle-alone control.

[f] Not determined. Number in parentheses reflects percent of label displaced at:

[g] 10 μ M or

[h] 100 μ M.

Effects of substituted DIM-arene analogues on inhibition of prostate cancer and leukemia cell growth and induction of leukemia cell apoptosis.

Table 2

Compd	R	R ¹	R ²	R ³	IC ₅₀ for Growth Inhibition [μM] ^[a]			IC ₅₀ for Apoptosis [μM] ^[b]	
					LNCaP PC ^[c]	DU-145 PC ^[d]	KG-1 AML ^[e]	KG-1 AML ^[f]	KG-1 AML ^[f]
1	H	H	H	Ph-4-CF ₃	13 (97)	47 (95)	15 (81)/g/	23/i/	
3	H	H	H	Ph-4-OH	>100 (0)	48 (99)	>20 (4)/g/	ND	
4	H	H	H	Ph-4-CO ₂ H	21 (100)	>100 (34)	>20 (10)/g/	>20 (7)/g/	
5	Me	H	H	Ph-4-CO ₂ H	36 (95)	>100 (20)	>20 (25)/g/	ND	
6	H	H	H	CO ₂ H	>100 (34)	>100 (15)	>20 (23)/g/	>20 (17)/g/	
7	H	H	H	Ph-3-(E-CH=CCO ₂ H)	43 (95)	>100 (28)	>20 (12)/g/	ND	
8	Me	H	H	Ph-3-(E-CH=CCO ₂ H)	>20 (36)	>100 (37)	>20 (10)/g/	ND	
9	H	H	H	2-thienyl-5(E-CH=CCO ₂ H)	55 (96)	90 (55)	>20 (15)/g/	ND	
10	Me	H	H	2-thienyl-5(E-CH=CCO ₂ H)	>20 (25)	46 (99)	>20 (7)/g/	ND	
11	iPr	H	H	2-thienyl-5(E-CH=CCO ₂ H)	26 (98)	52 (94)	>5 (12)/h/	>5 (0)/h/	
12	iPr	H	Me	2-thienyl-5(E-CH=CCO ₂ H)	2.4 (52)	>100 (21)	>5 (14)/h/	>5 (0)/h/	
13	H	H	Me	2-thienyl-5(E-CH=CCO ₂ H)	ND/f/	>100 (44)	>5 (2)/h/	>5 (0)/h/	
14	H	Me	H	2-thienyl-5(E-CH=CCO ₂ H)	ND	64 (78)	>5 (1)/h/	>5 (0)/h/	

^[a] Percent inhibition at highest concentration evaluated, 48 h.

^[b] Percent apoptosis at highest concentration evaluated, 48 h.

^[c] MTT assay results using 100 μM as the highest concentration of analogue evaluated with the exception of **1**, **8**, and **10**, for which the highest concentration was 20 μM; results are averages of triplicates in comparison with vehicle-alone-treated control (0 %).

[d] MTT assay results with 100 μM the highest concentration of analogue evaluated; also evaluated at 0.01, 0.1, 1.0, and 10 μM ; results are averages of triplicates in comparison with vehicle-alone-treated controls (0 %).

[e] Evaluated at 1.0, and 5.0 μM , or at 1.0, 5.0, and 20 μM ; results are averages of triplicates.

[f] Not determined. Number in parentheses reflects growth inhibition or apoptosis induction at:

[g] 20 μM or

[h] 5 μM .

[i] Extrapolated value.