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A parallel chemistry approach to identify novel nuclear receptor ligands based on the GW0742 scaffold

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Abstract

We describe the parallel synthesis of novel analogs of GW0742, a peroxisome proliferator-activated receptor δ (PPAR δ) agonist. For that purpose, modified reaction conditions were applied, such as a solid-phase palladium-catalyzed Suzuki coupling. In addition, tetrazole-based compounds were generated as a bioisostere for carboxylic acid-containing ligand GW0742. The new compounds were investigated for their ability to activate PPAR δ mediated transcription and their cross-reactivity with the vitamin D receptor (VDR), another member of the nuclear receptor superfamily. We identified many potent PPAR δ agonists that were less toxic than GW0742, where ~65 of the compounds synthesized exhibited partial PPAR δ -activity (23-98%) with EC₅₀ values ranging from 0.007 – 18.2 μ M. Some ligands, such as compound **32**, were more potent inhibitors of VDR-mediated transcription with significantly reduced PPAR δ activity than GW0742, however, none of the ligands were completely selective for VDR inhibition over PPAR δ activation of transcription.

Keywords

Nuclear Receptor (NR); Vitamin D Receptor (VDR); Peroxisome Proliferation-Activated Receptors α , δ , γ (PPAR α , δ , γ); GW0742; steroid receptor coactivator 2

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Supporting Information.

The supporting information is available free of charge on the ACS publication website at DOI: It includes detailed descriptions of the chemical synthesis and compound characterization as well as VDR activities for all compounds.

Author Contributions

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INTRODUCTION

Nuclear receptors (NRs) are ligand-activated transcription factors representing one of the largest classes of proteins targeted for drug intervention.¹ Thus far, 48 identified proteins belong to this superfamily, which is typically subdivided into three classes that share similar protein structure and ligand binding. These classes are steroid receptors, retinoid X receptor (RXR) heterodimers, and orphan receptors.² Regardless of their class, NRs are modular proteins that contain a DNA-binding domain (DBD) and a ligand-binding domain (LBD). The LBD is a highly structured region that contains the ligand-dependent activation function or AF-2, which acts as the major interface for the recruitment of coactivators or corepressors. In general, the NR-LBD consists of a three-layered α -helical sandwich containing up to 12 helices designated as H1–H12. In the absence of endogenous ligand, corepressor proteins of the SMRT/NCOR family might associate with NR-LBD and block transcription.³ In the presence of ligand, NRs undergo a conformational change that both stabilizes the binding of the ligand and accommodates coactivator proteins such as SRC-1 that bind the hydrophobic cleft formed by rearranged H12.⁴

The vitamin D receptor (VDR) and peroxisome proliferator-activated receptor δ (PPAR δ) form RXR heterodimers. The biological role of PPAR δ has only recently been characterized, in part, due to its broad tissue expression and the lack of selective chemical probes to distinguish the pharmacology between the PPAR isoforms (δ , α , γ). To help reveal the biological function of PPAR δ , GlaxoSmithKline (GSK) used combinatorial chemistry and structure-based drug design to develop GW0742 and GW501516, both highly selective and potent PPAR δ agonists (EC_{50} = ~ 0.001 μ M).⁵ Since their discovery, it has been revealed that PPAR δ plays a role in lipid, lipoprotein, and glucose homeostasis thus making it a suitable target for hypertension,⁶ diabetes⁷, obesity, inflammation⁸ and cancer⁹ therapies. Although GW501516 has shown beneficial effects in primate models of metabolic disorders,^{5b, 10} only PPAR α and PPAR γ ligands have been approved for human use. Fibrates (PPAR α ligands) and thiazolidinediones (PPAR γ ligands) are prescribed for the treatment of hypercholesterolemia and diabetes mellitus type 2, respectively, but have shown adverse side effects after long-term use possibly due to poor isoform selectivity and off-target effects.¹¹ Due to its complementary role in lipid homeostasis and energy metabolism, a strong interest in developing selective PPAR δ ligands without adverse side effects has emerged. VDR, on the other hand, mediates the biological action of the active form of vitamin D, 1 α ,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) and regulates calcium homeostasis, immunity, and cellular growth and differentiation.¹² Although a lot of research has been directed towards developing new VDR modulators, there still remains a need for potent and VDR selective drugs to treat diseases like osteoporosis, cancer, inflammation and cardiovascular disease that do not cause detrimental side effects such as hypercalcemia.¹³

High throughput screening (HTS) has quickly evolved over the last decades to become one of the main methods for the identification of lead compounds in drug discovery.¹⁴ The advantages of using HTS to identify hit compounds includes the quick retrieval of quality data from large libraries of compounds in a cost-effective manner.¹⁵ Previously we reported our collaborative work conducted with the NIH Chemical and Genomics Center (NCGC) to identify VDR ligands that did not possess the secosteroid scaffold of 1,25(OH)₂D₃ in order

to overcome hypercalcemia induced by these endogenous ligands.¹⁶ By using a fluorescence polarization (FP) assay employing VDR-LBD and a fluorescently labeled peptide, SRC2-3, we derived GW0742 as a non-secosteroid inhibitor of VDR ($IC_{50} = 27 \pm 2.7 \mu M$) out of a library of ~390,000 compounds (PubChem Assay Identifier (AID): 504847). We found GW0742 possessed moderate inhibitory effects for VDR and the androgen receptor (AR) in addition to greatly activating PPAR δ . Evaluation of VDR-target gene regulation in LNCaP cells in the presence of GW0742 resulted in negative regulation of *CYP24A1*, *IGFBP-3* and *TRPV6* when stimulated with 1, 25(OH) $_2$ D $_3$. Additionally, GW0742 was capable of inhibiting ($IC_{50} = 37.6 \mu M$) cell differentiation induced by 1,25(OH) $_2$ D $_3$ in HL-60 cells, a process governed by VDR gene expression.

Herein, we discuss the medicinal chemistry approach used to optimize GW0742 as a potent VDR antagonist with decreased PPAR δ activity. Four major regions of GW0742 were modified as described in Figure 1. The SAR included the replacement of the phenyl ring (pink region) with substituted-aryl or heteroaryl groups, exchanging of the methyl (cyan region) with a hydrogen, substitution of the linker atoms (green region) with oxygen, nitrogen or sulfur, and bioisosteric substitution of the carboxylic acid (blue region).

RESULTS AND DISCUSSION

Synthesis

Over 100 compounds were synthesized based on the GW0742 core scaffold using a parallel chemistry approach that efficiently produced the desired compounds at sufficient yields. Mono-, poly-, and aromatic-substituted GW0742 analogues were synthesized according to reaction Scheme 1. Sodium borohydride was used to reduce ethyl 2-bromo-4-methylthiazole-5-carboxylate to the corresponding primary alcohol **1a**. Subsequent reaction with thionyl chloride afforded **1b**, which was coupled with 4-hydroxy-3-methylthiophenol in the presence of cesium carbonate to give **1c**. Suzuki coupling methodology was applied to enable diversity in this position via different boronic acids and a unique solid supported diphenylphosphine palladium (II) heterogeneous catalyst that could be recovered and used again. The resulting esters were then cleaved with trifluoroacetic acid in CH $_2$ Cl $_2$ to afford the final carboxylic acid products (**1-78**).

Bioisosteric substitution is a common approach in medicinal chemistry to improve potency, selectivity and/or metabolic stability while maintaining similar physical (e.g. size, shape, polarity) and chemical properties (e.g. pKa). In our case, a tetrazole was introduced in place of the carboxylate as depicted in Scheme 2. 2-methyl-4-thiocyanatophenol and 2-bromoacetonitrile were coupled under basic conditions to produce **2a**. Reaction with Na $_2$ S yielded the free thiol that was sequentially coupled to **1b** to yield **2c**. The cyano-substituted analogues **2d-2g** were formed by Suzuki coupling and subsequently treated with sodium azide to form tetrazoles analogues (**79-82**) by [2+3] cycloaddition.

The synthesis of linker-modified GW0742 analogues (**87**, **92** and **93**) started with reduction of ethyl 2-bromo-4-methylthiazole-5-carboxylate to obtain **1a**.¹⁷ This compound was coupled with methyl 3-(4-hydroxyphenyl)propionate under Mitsunobu reaction conditions yielding thiazole ester **3a**.¹⁸ A microwave-assisted Suzuki reaction allowed for the

introduction of the third aromatic ring. Conveniently, the carbon-carbon bond formation and hydrolysis occurred under microwave conditions to form the final carboxylic acids **87**, **92** and **93**.

Biological Evaluation of GW0742 Analogues

GW0742 analogues were characterized using several biochemical and cell-based assays to determine their activity towards VDR and PPAR δ . Cytotoxicity of each compounds was also investigated in HEK293T cells. This included a high-throughput FP assay investigating the interaction between VDR-LBD and coactivator SRC2-3, VDR- and PPAR δ -mediated transcription assays and a cytotoxicity assay to differentiate between inhibition of transcription and cytotoxicity.^{16, 19} We report the results in the following tables: mono-substituted analogues (Table 1), poly-substituted analogues (Table 2), aromatic-substituted analogues (Table 3), tetrazole-substituted analogues (Table 4) and linker-substituted analogues (Table 5).

The mono-substituted GW0742 analogues exhibited a wide range of potencies (EC_{50} = 0.009 – <100 μ M) with respect to PPAR δ activation in cells (Table 1).

Three mono-substituted compounds modulated transcription with high potency. They were **13** (*p*-CF₃), **17** (*p*-OCF₃), and **22** (*p*-CO₂Et) with EC_{50} values lower than 15 nM. Compound **13**, also known as GW501516, was confirmed as a potent PPAR δ agonist.^{5a} The other two compounds have not been reported but possess similar electron donating properties and significant hydrophobicity. Notably, the positioning of substituents around the phenyl ring affected the ligand's ability to activate PPAR δ mediated transcription. A *para* positioned substituent, in most cases, resulted in a more potent PPAR δ agonist than compounds that bear the same group in the *ortho* or *meta* position. This relationship was observed with methyl (**2-4**), trifluoromethyl (**11-13**), trifluoromethoxy (**16-17**) and cyano (**18-19**) substituents. However, ligands with halide substituents, like Cl (**5-7**) and F (**8-10**), showed no significant activity difference between *ortho*, *meta*, or *para* positioning possibly due to their atomic size or change in orientation. Compounds with *para*-positioned fluorine and chlorine substituents had similar but relatively high EC_{50} values of ~0.14 μ M (compounds **10** and **7**). The least active PPAR δ agonist was polar sulfonamide **24**. As evidenced by the crystal structure of PPAR δ complexed with GW0742 (PDB: 3TKM), the binding pocket accommodates the pink region of Figure 1 of the ligand which is relatively large and hydrophobic due to residues V245, V305, V312, L317, and I328 (Figure 2).^{5a, 20} Therefore, depending on its position within the LBP, it is expected that extra hydrogen bonding capability is not favorable in this region of the ligand. Compound **29** bearing a piperazine was another example where polarity reduced activity towards PPAR δ with an EC_{50} = 16.8 $\pm$ 5.7 μ M.

Interestingly, while many ligands exhibited partial agonistic activity towards PPAR δ , we did not observe a trend between structure and efficacy. This is significant because some nuclear receptor partial agonists possess superior pharmacodynamics due to reduced side effects and moderate activation.²¹ Finally, most compounds exhibited CC₅₀ values, determined in the presence of HEK293 kidney cells, of 80 μ M and higher. An exception was compound **6** (*m*-Cl) with an CC₅₀ of 28.8 μ M.

Poly-substituted ligands found in Table 2 were also capable of activating PPAR δ mediated transcription (EC₅₀ = 0.0035 – 6.6 μ M).

The least active compound in our series was compound **51** (*o,o'*-Cl) with an EC₅₀ value of greater than 5 μ M, indicating that two substituents occupying both *ortho* positions of the phenyl ring is not favorable for PPAR δ activation. Interestingly, by moving just a chloride to the R₃ position (compound **52**) activity of this ligand is greatly increased by 230-fold when compared to **51**. The positioning of groups like CF₃, Cl, F and OCF₃ on phenyl ring positions gave some insight about the PPAR δ ligand binding pocket. For example, by switching the *p*-CF₃ and *m*-F substituents on GW0742 to make compound **41** (*m*-CF₃, *p*-F) a 140-fold decrease in ligand potency was observed. When *m*-F of GW0742 is exchanged for a *m*-Cl (compound **35**), no significant change in ligand potency was observed. When comparing compound **35** (*p*-CF₃, *m*-Cl) to compound **34** (*p*-CF₃, *o*-Cl), only a small decrease in PPAR δ activation was observed (4-fold change). When *p*-CF₃ of GW0742 was replaced with *p*-OCF₃ regardless of a Cl or F in the *meta* position (compounds **36** and **37**, respectively), PPAR δ activation was observed at low nanomolar concentrations. With respect to all fluorine substituents, it appeared that two fluorine substituents were better than one regardless of their positioning. The same trend was observed for chlorine substituents. The toxicity of poly-substituted GW0742 analogues was, in general, more pronounced than their mono-substituted counterparts, however none of them exhibited toxicity below 50 μ M.

Aromatic substituents were also coupled to the C-2 position of the thiazole ring and their biological activity is summarized in Table 3.

Five compounds activated PPAR δ with EC₅₀ values less than 75 nM (**56**, **58**, **68**, **72** and **78**). Of these, all but one had a bicyclic aromatic ring structure. This result confirmed earlier

observations that the LBD of PPAR δ is spacious enough to accommodate such ligands, possibly through a unique orientation unlike GW0742. It is worth noting that when compared to VDR, PPAR δ has a larger Y-shaped ligand binding pocket which can make contact with the ligand in three different regions, thus possibly explaining the accommodation for large ring systems.²⁰ From Table 1 we concluded that non-substituted phenyl ring structures such as compound **1** made poor PPAR δ agonists. Similarly, substitution of the phenyl ring with a pyridine, as in compound **64**, resulted in a poor binding. However, when the pyridine ring contained a trifluoromethyl group like compound **72**, a 620-fold increase in transcriptional activation was observed when compared to **64**. The positioning of heterocyclic rings may be important considering the effect compounds **77** and **78** have on PPAR δ activation. Both have an indole ring attached to C-2 of the thiazole but the 1*H*-indol-4-yl group (**77**) was nearly 3,000 times less potent than the 1*H*-indol-2-yl substituent (**78**).

Bioisosteric substitution of the carboxylate group with a tetrazole ring was used for several analogues to determine its effect on PPAR δ -mediated transcription (Table 4).

In general, increased toxicity and decreased potency was observed for tetrazole analogues when compared to their carboxylate counterparts. The tetrazole substitution of GW0742, resulting in compound **79**, had an 11-fold decrease in potency and a two-fold increase of toxicity. Although similar in acidity, the spacial ability of this particular tetrazole to form hydrogen bond interactions is different from a carboxylic acid. It is known that both oxygens on the carboxylate group make hydrogen bonds with key residues like His413 (helix 10/11), Try437 (helix 12), His287 (helix 7) of PPAR δ LBP and are implicated with maintaining the locked conformation of helix 12 (Figure 2).²⁰ Thus, the inability of the tetrazole to form all necessary hydrogen bonds might be responsible for low efficacy of these compounds ranging between 26-51%.

The replacement of the sulfur and oxygen linkers of GW0742 allowed structure-activity relationship investigations into the central part of PPAR δ agonists (Table 5).

In addition, the role of the *ortho* methyl group (R) was investigated in terms of favorable PPAR δ binding. Compound **85** is structurally similar to GW0742 except for the lack of methyl group in the R position: it was 7 times less potent than GW0742 and surprisingly,

behaved as a partial agonist (84% in comparison with GW0742). A large decrease in potency (~200-fold) was observed for compound **93** when Y was replaced with a smaller oxygen atom and X with a CH₂ in the absence of a fluoride and methyl group in positions R₁ and R, respectively. When compared to compound **87**, bearing a fluorine in the R₁ position, the activity difference was less, suggesting an additional hydrophobic interactions between **87** and PPAR δ LBP. This behavior was not observed for Y = nitrogen when comparing **89** and **90**. The presence or absence of an R positioned methyl had little effect on activation of transcription thus producing agonists that were ~50 times less potent than GW0742. However, CH₂ in place of oxygen (**91**) improved PPAR δ binding when compared to **89** and **90**. The least potent compound was **92**, bearing a *m*-CH₂OH substituent on the phenyl ring with linkers similar to that of **93**. This compound was approximately 5,000 times less potent than GW0742 confirming that hydrogen bond donor and acceptor moieties in the pink region of Figure 1 of ligands are not favorable for PPAR δ binding.

In addition to GW0742 and GW501516, compounds **7**, **10**, **32**, **46**, and **83** were patented by GSK.²² It was reported that most of the GW0742-like compounds showed ~50% activation of hPPAR δ at concentrations of 10 nM or less in a β -galactosidase transactivation assay using CV-1 cells. Other than compounds **32** and **46**, this correlates well with what we have observed in our PPAR δ -mediated transcription assay. Also, these compounds were reported being at least 10-fold selective for hPPAR δ over hPPAR α and PPAR γ .

The SAR study of GW0742 analogues has given us some insight into the structural requirements for these highly potent PPAR δ ligands. In addition, structural elements have been identified that significantly reduce the ability of ligands to activate PPAR δ -mediated transcription. The *in vitro* data was further supported by molecular modeling using a crystal structure of GW0742 bound to PPAR δ LBD (PDB: 3TKM). Using the Schrödinger software to perform standard precision grid-based ligand docking with energetics (Glide) studies, we were able to show that **32** aligns favorably with GW0742. However, occupation of the highly hydrophobic region on the left side of the pocket (Figure 2) differs significantly. It is evident that the addition of two bulky trifluoromethyl groups in both *meta* positions orients the pink region of Figure 1 of the molecule downwards therefore causing a 283-fold decrease in PPAR δ activity (GW0742, EC₅₀ = 0.0034 μ M and **32**, EC₅₀ = 0.99 μ M).

Having identified several PPAR δ ligands with EC₅₀ values greater than 1 μ M, we next set out to test their ability to bind to VDR. All “poor” PPAR δ ligands were investigated with respect to their ability to inhibit the interaction between VDR and coactivator peptide SRC2-3 and their ability to inhibit VDR-mediated transcription in cells. The results are summarized in Table 6.

Among the ligands investigated only **32**, bearing a CF₃ group in the *meta* position instead of the *para* position like GW0742, exhibited an IC₅₀ value below 10 μ M in the VDR FP-based assay. Many of the other ligands were not active. The activity of **32** was confirmed in cells with an IC₅₀ of 15 μ M for VDR-mediated transcription. Notably, most ligands including GW0742 in light of the FP-based assay and the VDR-mediated transcription assay, showed full antagonistic activity when compared to the DMSO control (Figures S2 and S3). Compounds whose IC₅₀ values were greater than 100 μ M appeared to be trending towards

full antagonistic activity although higher concentrations would need to be tested to confirm this. Unfortunately, none of the synthesized ligands exhibited high affinity for VDR. Interestingly, other identified VDR ligands that bear a carboxylic acid function such as lithocholic acid²³ or calcitric acid²⁴ exhibited binding constants toward VDR in the same range (1-10 μ M); thus the carboxylic acid function of these molecules might be inferior to the two hydroxyl groups of 1,25(OH)₂D₃ to bind VDR.²⁵ On the other hand, 1,25(OH)₂D₃ interacts with histidine residues via its tertiary alcohol. The only ligand bearing a hydroxyl function among the ligands generated is compound **92** that interacted with neither PPAR δ nor VDR. Thus, the current investigation illustrates that the change of substituents introduced here to design new VDR ligands based on the GW0742 scaffold is not sufficient. In the future, we will change the length of this scaffold by introducing smaller ring structures and linkers in addition to other carboxylic acid bioisosteres. However, it is encouraging that the simple removal of a *para* substituent and the addition of *meta* substituents on the phenyl ring (pink region, Figure 1), as in **32**, does greatly reduce PPAR δ activity. Thus, an introduction of a 3,4-bis(hydroxymethyl)phenyl group as shown for VDR ligands CD4420²⁶ might be advantageous to improve VDR binding and reduce PPAR δ activity. On the other hand, we did identify many new potent PPAR δ agonists and partial agonists during this study, however, further studies are needed to determine the PPAR subtype selectivity of these ligands. This will be essential to develop new drug candidates for lowering density lipoprotein therapeutics based on PPAR δ binding.

EXPERIMENTAL PROCEDURES

Chemistry

General Information for Compounds 1-86 and 88-91—All reagents and solvents used in this study were obtained from commercial suppliers and used without further purification. Preparative purification was performed on a Waters semi-preparative HPLC system using a Phenomenex Luna C18 (5 micron, 30 $\times$ 75 mm) at a flow rate of 45 mL/min. A gradient of 10% to 50% acetonitrile in water over 8 minutes (each containing 0.1% trifluoroacetic acid) was used as a mobile phase during the purification. Fraction collection was triggered by UV detection (220 nm). Analytical analysis was performed on an Agilent LC/MS (Agilent Technologies, Santa Clara, CA). Method t1: A 7 minute gradient of 4% to 100% Acetonitrile (containing 0.025% trifluoroacetic acid) in water (containing 0.05% trifluoroacetic acid) was used with an 8 minute run time at a flow rate of 1 mL/min. A Phenomenex Luna C18 column (3 micron, 3 $\times$ 75 mm) was used at a temperature of 50°C. Method t2: A 3 minute gradient of 4% to 100% Acetonitrile (containing 0.025% trifluoroacetic acid) in water (containing 0.05% trifluoroacetic acid) was used with a 4.5 minute run time at a flow rate of 1 mL/min. A Phenomenex Gemini Phenyl column (3 micron, 3 $\times$ 100 mm) was used at a temperature of 50° C. Method t3: Analysis was performed on an Agilent 1290 Infinity Series HPLC. UHPLC Long Gradient Equivalent 4% to 100% acetonitrile (0.05% trifluoroacetic acid) in water over 3.5 minutes run time of 4 minutes with a flow rate of 0.8 mL/min. > 95 % Purity was determined using an Agilent Diode Array Detector for both Method t1, Method t2 and Method t3. Mass determination was performed using an Agilent 6130 mass spectrometer with electrospray ionization in the positive mode. ¹H NMR spectra were recorded on Varian 400 MHz spectrometer. Chemical

shifts are reported in ppm with DMSO at 2.49 ppm as internal standard. High resolution mass spectrometry was recorded on Agilent 6210 Time-of-Flight LC/MS system. Confirmation of molecular formula was accomplished using electrospray ionization in the positive mode with the Agilent Masshunter software (version B.02). The synthesis of these compounds is described in more detail in the Supporting Information.

General Information for Compounds 87, 92-93—All starting reagents were purchased from Sigma-Aldrich. Anhydrous solvents were purchased in sure-seal bottles and handled under dry conditions using syringe technique. All glassware was dried overnight at 100°C before use. Thin layer chromatography was performed on pre-coated silica gel 60 F254 plates (Fisher Scientific). Microwave reactions were performed using a CEM Discover SP instrument. Synthesized compounds were purified by normal phase flash chromatography (SPI Biotage, silica gel 230-400 mesh) and concentrated under vacuum. All pure compounds were stored as solids at -20°C. Molecular masses and >95 % purities were determined with a Shimadzu 2020 LC-MS (single quadrupole) instrument. NMR spectra were recorded on a Bruker 500 MHz or 300 MHz instrument with samples diluted in either CDCl₃ or DMSO-D₆. Chemical shifts are reported in δ (parts per million: ppm) by reference to the hydrogenated residues of the deuterated solvent as an internal standard (CDCl₃: δ = 7.24 ppm (¹HNMR) and δ = 77 ppm (¹³CNMR) and DMSO-D₆: δ = 2.50 ppm (¹HNMR) and δ = 39.5 ppm (¹³CNMR). The synthesis of these compounds is described in more detail in the Supporting Information.

Biology

Reagents and Instrumentation—LG190178 (a VDR agonist) was synthesized following a published procedure²⁷ and used as a positive control. 3-dibutylamino-1-(4-hexyl-phenyl)-propan-1-one (a VDR-coactivator inhibitor) was used as a second positive control and was synthesized using a previously published method.²⁸ 1,25(OH)₂D₃ was purchased from Endotherm. The VDR-LBDmt DNA was provided by D. Moras²⁹ and cloned into pMAL-z2X vector (New England Biolabs). For a details on the expression and purification of VDR-LBD, see ref³⁰. AlexaFluor 647 -Labeled SRC2-3 was synthesized by coupling SRC2-3 (CKKKENALLRYLLDKDDTKD) with cysteine-reactive AlexaFluor 647 probe followed by reverse phase HPLC purification using a C18 column. VDR-CMV and CYP24A1-luciferase reporter plasmids were provide by D.D. Bikle.

VDR Binding Determined by a Fluorescence Polarization (FP) Assay—Was conducted in 384-well black polystyrene microplates (Corning, #3573). The assay solution contained buffer (25mM PIPES, 50mM NaCl (Fisher), and 0.01% NP-40, at pH 6.75), 0.1 μ M VDR-LBD and 7.5 nM Alexa Fluor 647-labeled SRC2-3. For competitive inhibition, 0.75 μ M LG190178 was also added to the solution. 10 mM stock solutions of synthesized compounds made in DMSO were serially diluted (1:3), and four 14 μ L aliquots of each compound concentration was transferred to opaque 384-well polypropylene plates for storage. Small volume transfers were performed by a Tecan Freedom EVO liquid handling system using a 50H hydrophobic coated pin tool that carried 100 nL (V&P Scientific). To obtain a maximum concentration of 300 μ M, 600 nl of each compound concentration was transferred into the 20 μ L assay solution. Agonistic binding was determined in the absence

of LG190178 while competitive inhibition binding was determined in the presence of LG190187. Fluorescence polarization was detected after 30 minutes at an emission/excitation wavelength of 635/685 nm (Alexa Fluor 647). LG190178 ($K_i = 150$ nM) and DMSO were used as positive and negative controls in the agonistic binding assay, respectively. In the competitive inhibition binding assay 3-dibutylamino-1-(4-hexyl-phenyl)-propan-1-one was the positive control while DMSO was used as a negative control. Controls were measured within each plate to determine the z' factor, which assessed the quality of the assay, and enable data normalization. Three independent experiments were carried out in quadruplicate, and data was analyzed using nonlinear regression with a variable slope (GraphPrism). Data reported in the Supporting Information.

VDR- and PPAR δ -Mediated Transcription Assays—Human embryonic kidney (HEK) 293T cells were purchased (ATCC) and cultured in 75 cm² flasks (CellStar) coated in matrigel (BD Bioscience, #354234). Cells were grown in DMEM/High Glucose (Hyclone, #SH3024301) media to which non-essential amino acids (Hyclone, #SH30238.01), 10 mM HEPES (Hyclone, #SH302237.01), 5×10^6 units of penicillin and streptomycin (Hyclone, #SV30010), and 10% of heat inactivated fetal bovine serum (Gibco, #10082147) were added. For the assay, cells at 70-80% confluency were transfected by lipid-based methods where 2 mL of untreated DMEM/High Glucose media (without additives) containing: 1) transcription assay: 0.7 μ g of VDR-CMV plasmid, 16 μ g of a CYP24A1-luciferase reporter gene, LipofectamineTM LTX (75 μ L, Life Technologies, #15338020), and PLUSTM reagent (25 μ L) or 2) Hybrid Assay: 1.5 μ g of CMV-PPAR δ -LBD-GAL4-DBD plasmid, 16 μ g of a 6xGal4-luciferase reporter gene, LipofectamineTM LTX (75 μ L) and PLUSTM reagent (25 μ L) was added to the flask. After 16 hours of incubation at 37°C with 5% CO₂, the cells were harvested with 3 mL of 0.05% Trypsin (Hyclone, #SH3023601), added to 10 mL of the assay buffer, DMEM/High Modified buffer without phenol red (Hyclone, #SH30284.01) containing all the above mentioned additives plus 10 mM sodium pyruvate and 2% percent charcoal treated FBS (Invitrogen, #12676-011) instead of HI FBS, and spun down for 2 minutes at 1000 rpm. The media was removed and cells were re-suspended in the assay buffer, DMEM/High Modified buffer without phenol red. Prior to adding cells to sterile white, optical bottom 384-well plates (NUNC, #142762), plates were treated with 20 μ L per well of a 0.25% Matrigel solution. To each well, 20 μ L of cells were added to yield a final concentration of 15,000 cells per well. After 4 hours, plated cells were treated with 200 nL of small molecules (1:3 serial dilution, with maximum final concentration at 100 μ M) and controls (VDR: DMSO and 10 nM 1,25(OH)₂D₃ [$EC_{50} = 1$ nM] and PPAR δ : DMSO and 30 nM GW0742 [PPAR δ $EC_{50} = 3.5$ nM]) which were added using a Tecan Freedom EVO liquid handling system with a 50H hydrophobic coated pin tool. In the competitive inhibition assay, 1,25(OH)₂D₃ (10 nM) was also added to the assay wells containing small molecule. After 16 hours of incubation at 37°C with 5% CO₂, 20 μ L of Bright-GloTM Luciferase Assay Kit (Promega, Madison, WI) was added to each well and luminescence was read. Controls were measured within each plate to determine the z' factor and to enable data normalization. At least two independent experiments were performed in quadruplicate and data was analyzed using nonlinear regression with variable slope (GraphPrism).

Cytotoxicity Assay—Hek293T cells were plated according to the transcription assay protocol and 200 nL of serially diluted (1:3 in DMSO) small molecules (final maximum concentration at 100 μ M) were transferred using a Tecan Freedom EVO liquid handling system with a 50H hydrophobic coated pin tool. The controls for the cytotoxicity assay used were 3-dibutylamino-1-(4-hexyl-phenyl)-propan-1-one (100 μ M in DMSO, positive) and DMSO (negative). After 18 hours, cell viability was evaluated by adding 20 μ L of Cell Titer-Glo™ Luminescence Assay Kit (Promega, Madison, WI) to each well and reading luminescence on a Tecan Infinite M1000 plate reader. Controls were measured within each plate to determine the z' factor and to enable data normalization. At least two independent experiments were performed in quadruplicate and data was analyzed using nonlinear regression with variable slope (GraphPrism).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

NR	Nuclear Receptor
VDR	Vitamin D Receptor
PPARδ, γ, α	Peroxisome Proliferator-Activated Receptor δ , γ , α
SAR	structure-activity relationship
DBD	DNA-Binding Domain
LBD	Ligand-Binding Domain
125(OH)$_2$D$_3$	1 α , 25-dihydroxyvitamin D $_3$
HTS	High Throughput Screening
FP	Fluorescence Polarization
AR	Androgen Receptor

Figure 1.
Design of GW0742 derivatives.

Figure 2.
Computer docking of **32** (blue) in the ligand binding pocket of PPARδ with GW0742 (green) (PDB: 3TKM). Major nonpolar and hydrogen bonding interactions have been labeled.

Scheme 1.

General synthetic route for mono, poly, and aromatic-substituted ligands.

i) NaBH₄, EtOH, R.T., 66%, ii) SOCl₂, CH₂Cl₂, R.T., 86%; iii) a) 4-hydroxy-3-methylthiophenol, Cs₂CO₃, MeCN, R.T.; b) *tert.* butyl bromoacetate, Cs₂CO₃, MeCN, R.T., overall yield = 89%; iv) Boronic acid Na₂CO₃, SiliaCat® DPP-Pd, DME, 150 °C, MW, 0.5 h, 33-60%; v) TFA, CH₂Cl₂, R.T., 1h, ~100%.

Scheme 2.

General synthetic route for bioisostere-substituted analogues.

i) Cs₂CO₃, MeCN, R.T., 12 h, 42%; ii) Na₂S, EtOH•H₂O, R.T., 3 h, 91%; iii) **1b**, Cs₂CO₃, MeCN, R.T., 1 h, 75%; iv) Boronic acid, Na₂CO₃, SiliaCat® DPP-Pd, DME, 150 °C, MW, 0.5 h; 36%-45%; v) NaN₃, NH₄Cl, DMF, 100°C, 12 h, 35-69%.

Scheme 3.

General synthetic route for linker-substituted GW0742 analogues.

i) PPh_3 , DIAD, CH_2Cl_2 , methyl 3-(4-hydroxyphenyl) propionate, R.T., 2 h, 30%; ii)

$\text{PdCl}_2(\text{PPh}_3)_2$, $\text{Na}_2\text{CO}_3 \cdot \text{H}_2\text{O}$, DME/ H_2O /EtOH, 160 °C, 10minutes, MW, 36%-45%.

Table 1

Evaluation of mono-substituted GW0742 analogues

Compound	R	PPAR δ EC ₅₀ (μ M) ^{a,b}	Toxicity CC ₅₀ (μ M) ^c
1	H	0.26 $\pm$ 0.35 (90 $\pm$ 7.5)	>100
2	<i>o</i> -CH ₃	0.60 $\pm$ 0.17 (66 $\pm$ 5.2)	>100
3	<i>m</i> -CH ₃	1.62 $\pm$ 0.41 (74 $\pm$ 4.3)	>80
4	<i>p</i> -CH ₃	0.05 $\pm$ 0.02 (104 $\pm$ 12)	>80
5	<i>o</i> -Cl	0.58 $\pm$ 0.20 (71 $\pm$ 4.8)	>80
6	<i>m</i> -Cl	0.50 $\pm$ 0.16 (74 $\pm$ 4.5)	28.8 $\pm$ 5.0
7	<i>p</i> -Cl	0.13 $\pm$ 0.04 (66 $\pm$ 5.8)	>100
8	<i>o</i> -F	0.26 $\pm$ 0.08 (94 $\pm$ 5.9)	>80
9	<i>m</i> -F	0.43 $\pm$ 0.23 (96 $\pm$ 12)	>100
10	<i>p</i> -F	0.16 $\pm$ 0.04 (93 $\pm$ 3.3)	Non-toxic
11	<i>o</i> -CF ₃	2.4 $\pm$ 0.95 (39 $\pm$ 2.8)	>100
12	<i>m</i> -CF ₃	1.1 $\pm$ 0.6 (79 $\pm$ 14)	>50
13 (GW501516)	<i>p</i> -CF ₃	0.013 $\pm$ 0.004 (95 $\pm$ 3.3)	>33
14	<i>o</i> -OCH ₃	2.3 $\pm$ 0.70 (27 $\pm$ 4.2)	>100
15	<i>m</i> -OCH ₃	2.36 $\pm$ 0.67 (96 $\pm$ 7.2)	>80
16	<i>m</i> -OCF ₃	0.45 $\pm$ 0.15 (40 $\pm$ 2.4)	>50
17	<i>p</i> -OCF ₃	0.014 $\pm$ 0.007 (49 $\pm$ 2.2)	>50
18	<i>m</i> -CN	3.2 $\pm$ 1.0 (74 $\pm$ 5.4)	>80
19	<i>p</i> -CN	0.12 $\pm$ 0.42 (126 $\pm$ 11)	>80
20	<i>p</i> -N(CH ₃) ₂	0.16 $\pm$ 0.02 (140 $\pm$ 14)	>50
21	<i>p</i> -NHCH ₃	0.57 $\pm$ 0.24 (83 $\pm$ 7.4)	Non-toxic
22	<i>p</i> -CO ₂ Et	0.009 $\pm$ 0.002 (123 $\pm$ 5.8)	>80
23	<i>m</i> -methylsulfinyl	6.7 $\pm$ 3.2 (59 $\pm$ 4.9)	Non-toxic
24	<i>p</i> -methylsulfonamido	>100	Non-toxic
25	<i>p</i> -NHCOCH ₃	3.9 $\pm$ 2.3 (97 $\pm$ 4.7)	>100
26	<i>m</i> -CONH(CH ₂) ₃ N(CH ₃) ₂	2.4 $\pm$ 1.3 (70 $\pm$ 7.5)	Non-toxic
27	<i>p</i> -CONH(CH ₂) ₂ N(CH ₃) ₂	2.1 $\pm$ 1.4 (65 $\pm$ 11)	Non-toxic
28	<i>p</i> -(4-methylpiperazinyl) methanone	6.9 $\pm$ 5.53 (70 $\pm$ 13)	Non-toxic
29	<i>p</i> -piperazinyl	16.8 $\pm$ 5.7 (37 $\pm$ 3.4)	>80
30	Morpholino-methanone	3.8 $\pm$ 1.5 (37 $\pm$ 2.6)	Non-toxic

^aTwo-hybrid assay using a CMV-PPAR δ -LBD-GAL4-DBD plasmid and a 6xGal4-luc reporter vector. The maximum concentration used for this assay was 100 μ M of each compound;

^bEfficacy in PPAR δ assay in respect to full activation with GW0742 are in parentheses;

^cCell-TiterGlo (Promega). The maximum concentration used for transcription and toxicity assay was 100 μ M of each compound. Data were analyzed using a nonlinear regression with a variable slope (GraphPad Prism).

Table 2

Evaluation of poly-substituted GW0742 analogues.

Compound	R	R ₁	R ₂	R ₃	R ₄	PPARδ EC ₅₀ (μM) ^{a, b}	Toxicity CC ₅₀ (μM) ^d
31 (GW0742)	H	H	CF ₃	F	H	0.0035 ± 0.0003 (97 ± 4.4)	>50
32	H	CF ₃	H	CF ₃	H	0.99 ± 0.42 (46 ± 5.4)	>50
33	CF ₃	H	CF ₃	H	H	0.20 ± 0.09 (50 ± 2.8)	>50
34	H	H	CF ₃	H	Cl	0.028 ± 0.013 (88 ± 2.8)	>50
35	H	H	CF ₃	Cl	H	0.007 ± 0.003 (75 ± 5.4)	>33
36	H	H	OCF ₃	Cl	H	0.009 ± 0.004 (69 ± 3.1)	>50
37	H	H	OCF ₃	F	H	0.020 ± 0.010 (100 ± 6.3)	>50
38	H	OCH ₃	OCH ₃	H	H	1.06 ± 0.47 (44 ± 4.6)	>100
39	H	F	OCH ₃	H	H	0.074 ± 0.031 (112 ± 5.2)	>100
40	H	H	OCH ₂ CF ₃	F	H	0.050 ± 0.028 (91 ± 7.9)	>50
41	H	CF ₃	F	H	H	0.49 ± 0.31 (116 ± 14)	>50
42	H	H	F	F	F	0.042 ± 0.009 (122 ± 7.3)	>80
43	H	F	F	F	H	0.046 ± 0.019 (106 ± 19)	>100
44	H	H	F	CN	H	1.07 ± 0.59 (150 ± 24)	>100
45	H	H	Cl	H	CF ₃	0.18 ± 0.12 (63 ± 5.3)	>50
46	H	H	Cl	Cl	H	0.692 ± 0.137 (79 ± 4.5)	>50
47	H	H	CN	H	Cl	0.066 ± 0.04 (108 ± 11)	>100
48	H	F	H	Cl	H	0.17 ± 0.05 (62 ± 3.4)	>50
49	H	Cl	H	CF ₃	H	0.60 ± 0.28 (23 ± 2.3)	>33
50	H	Cl	H	Cl	H	0.71 ± 0.27 (78 ± 6.0)	>50
51	Cl	H	H	H	Cl	6.6 ± 4.8 (84 ± 11)	>100
52	H	H	H	Cl	Cl	0.028 ± 0.017 (78 ± 7.5)	>100
53	H	Cl	H	H	Cl	1.40 ± 0.74 (75 ± 2.1)	>80
54	H	F	Benzyl-morpholine	H	H	4.3 ± 1.9 (50 ± 4.4)	>100

^aTwo-hybrid assay using a CMV-PPAR6-LBD-GAL4-DBD plasmid and a 6xGal4-luc reporter vector. The maximum concentration used for this assay was 100 μM of each compound;

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_b Efficacy in PPAR δ assay in respect to full activation with GW0742 are in parentheses;

_c Cell-TiterGlo (Promega). The maximum concentration used for transcription and toxicity assay was 100 μ M of each compound. Data were analyzed using a nonlinear regression with a variable slope (GraphPad Prism).

Table 3

Evaluation of substituted GW0742 analogues

Compound	R	PPAR δ EC ₅₀ (μ M) ^{a,b}	Toxicity CC ₅₀ (μ M) ^c
55	3,5-dimethylisoxazol-4-yl	0.29 $\pm$ 0.12 (103 $\pm$ 17)	>100
56	1 <i>H</i> -indazol-4-yl	0.052 $\pm$ 0.035 (101 $\pm$ 14)	>100
57	4-pyridine	9.1 $\pm$ 3.6 (81 $\pm$ 6.4)	>80
58	2-(benzofuran-2-yl)	0.061 $\pm$ 0.028 (97 $\pm$ 9.1)	>80
59	2,3-dihydrobenz[1,4]dioxin-6-yl	0.72 $\pm$ 0.23 (127 $\pm$ 6.2)	>80
60	Naphthalene-1-yl	1.69 $\pm$ 0.34 (36 $\pm$ 1.7)	>50
61	Benzo[1,3]dioxol-5-yl	0.26 $\pm$ 0.12 (52 $\pm$ 3.8)	>50
62	Benzo[1,2,5]oxadiazol-5-yl	0.16 $\pm$ 0.04 (35 $\pm$ 2.2)	>100
63	Furan-2-yl	6.0 $\pm$ 2.7 (96 $\pm$ 11)	>100
64	Pyridin-3-yl	8.1 $\pm$ 5.6 (87 $\pm$ 12)	>100
65	Piperazin-1-yl	0.57 $\pm$ 0.39 (46 $\pm$ 9.6)	>100
66	Pyrimidin-yl	1.57 $\pm$ 0.62 (51 $\pm$ 3.8)	Non-toxic
67	1 <i>H</i> -indazol-6-yl	3.18 $\pm$ 1.80 (122 $\pm$ 36)	>100
68	Benzothiazol-6-yl	0.073 $\pm$ 0.053 (99 $\pm$ 23)	100
69	Isoquinolin-4-yl	0.35 $\pm$ 0.18 (51 $\pm$ 6.7)	Non-toxic
70	6-fluoropyridin-3-yl	0.192 $\pm$ 0.082 (110 $\pm$ 9.5)	Non-toxic
71	1-methyl-1 <i>H</i> -indazol-6-yl	0.58 $\pm$ 0.34 (99 $\pm$ 6.7)	>100
72	6-(trifluoromethyl)pyridin-3-yl	0.013 $\pm$ 0.006 (91 $\pm$ 4.9)	>100
73	1-methyl-1 <i>H</i> -indol-6-yl	0.52 $\pm$ 0.29 (103 $\pm$ 5.0)	>100
74	1 <i>H</i> -pyrazol-4-yl	3.9 $\pm$ 1.7 (74 $\pm$ 5.7)	Non-toxic
75	1 <i>H</i> -indazol-5-yl	1.44 $\pm$ 0.66 (64 $\pm$ 5.5)	Non-toxic
76	Benzothiophen-5-yl	0.14 $\pm$ 0.08 (95 $\pm$ 12)	>50
77	1 <i>H</i> -indol-4-yl	>100	Non-toxic
78	1 <i>H</i> -indol-2-yl	0.035 $\pm$ 0.015 (108 $\pm$ 5.5)	>50

^a Two-hybrid assay using a CMV-PPAR δ -LBD-GAL4-DBD plasmid and a 6xGal4-luc reporter vector. The maximum concentration used for this assay was 100 μ M of each compound;

^b Efficacy in PPAR δ assay in respect to full activation with GW0742 are in parentheses;

^c Cell-TiterGlo (Promega). The maximum concentration used for transcription and toxicity assay was 100 μ M of each compound. Data were analyzed using a nonlinear regression with a variable slope (GraphPad Prism).

Table 4

Evaluation of tetrazole-substituted GW0742 analogues

Compound	R ₁	R ₂	R ₃	R ₄	PPARδ EC ₅₀ (μM) <i>a, b</i>	Toxicity CC ₅₀ (μM) <i>c</i>	Entry for Corresponding Carboxylic Acid
79	H	CF ₃	F	H	0.04 ± 0.018 (29 ± 1.5)	>33	31 (GW0742)
80	Cl	H	Cl	H	0.594 ± 0.435 (29 ± 5.2)	>50	53
81	H	Cl	H	CF ₃	0.51 ± 0.18 (51 ± 4.8)	>33	46
82	H	Cl	Cl	H	0.24 ± 0.11 (26 ± 1.9)	>33	49

*a*Two-hybrid assay using a CMV-PPARδ-LBD-GAL4-DBD plasmid and a 6xGal4-luc reporter vector. The maximum concentration used for this assay was 100 μM of each compound;

*b*Efficacy in PPARδ assay in respect to full activation with GW0742 are in parentheses;

*c*Cell-TiterGlo (Promega). The maximum concentration used for transcription and toxicity assay was 100 μM of each compound. Data were analyzed using a nonlinear regression with a variable slope (GraphPad Prism).

Table 5

Evaluation of linker-substituted GW0742 analogues.

Compound	R ₁	R ₂	Y	R	X	PPAR δ EC ₅₀ (μ M) <i>a,b</i>	Toxicity CC ₅₀ (μ M) <i>c</i>
83	H	CF ₃	S	H	CH ₂	0.039 $\pm$ 0.022 (87 $\pm$ 4.3)	>100
84	F	CF ₃	S	H	--	0.018 $\pm$ 0.010 (70 $\pm$ 3.7)	>100
85	F	CF ₃	S	H	O	0.026 $\pm$ 0.008 (84 $\pm$ 5.1)	>100
86	F	CF ₃	O	CH ₃	O	0.02 $\pm$ 0.02 (73 $\pm$ 11)	>100
87	F	CF ₃	O	H	CH ₂	0.032 $\pm$ 0.021 (77 $\pm$ 5.0)	>100
88	F	CF ₃	O	H	S	0.27 $\pm$ 0.023 (75 $\pm$ 5.0)	>100
89	F	CF ₃	N	CH ₃	O	0.18 $\pm$ 0.12 (61 $\pm$ 5.0)	>100
90	F	CF ₃	N	H	O	0.16 $\pm$ 0.045 (78 $\pm$ 6.0)	>80
91	F	CF ₃	N	H	CH ₂	0.058 $\pm$ 0.021 (95 $\pm$ 7.0)	>80
92	CH ₂ OH	H	O	H	CH ₂	18.2 $\pm$ 2.8 (47 $\pm$ 1.8)	Non-toxic
93	H	CF ₃	O	H	CH ₂	0.70 $\pm$ 0.11 (111 $\pm$ 6.5)	>100

^aTwo-hybrid assay using a CMV-PPAR δ -LBD-GAL4-DBD plasmid and a 6xGal4-luc reporter vector. The maximum concentration used for this assay was 100 μ M of each compound;

^bEfficacy in PPAR δ assay in respect to full activation with GW0742 are in parentheses;

^cCell-TiterGlo (Promega). The maximum concentration used for transcription and toxicity assay was 100 μ M of each compound. Data were analyzed using a nonlinear regression with a variable slope (GraphPad Prism).

Table 6Evaluation of “poor” PPAR δ ligands ($EC_{50} < 1\mu M$) in respect to VDR binding.

Entry	VDR-SRC2-3 interaction IC_{50} (μM) ^a	VDR transcription IC_{50} (μM) ^b	PPAR δ EC_{50} (μM) ^{a,b}
25	40.11 $\pm$ 9.26	inactive	3.9 $\pm$ 2.3 (97 $\pm$ 4.7)
11	32.27 $\pm$ 5.28	31.5 $\pm$ 9.1	2.4 $\pm$ 0.95 (39 $\pm$ 2.8)
14	45.18 $\pm$ 5.65	31.4 $\pm$ 8.1	2.3 $\pm$ 0.70 (27 $\pm$ 4.2)
18	>100	25.1 $\pm$ 10.0	3.2 $\pm$ 1.0 (74 $\pm$ 5.4)
23	>100	>100	6.7 $\pm$ 3.2 (59 $\pm$ 4.9)
26	30.28 $\pm$ 4.5	inactive	2.4 $\pm$ 1.3 (70 $\pm$ 7.5)
27	52.03 $\pm$ 12.79	>100	2.1 $\pm$ 1.4 (65 $\pm$ 11)
32	9.088 $\pm$ 0.982	15.0 $\pm$ 4.7	0.99 $\pm$ 0.42 (46 $\pm$ 5.4)
38	>100	>50	1.06 $\pm$ 0.47 (44 $\pm$ 4.6)
44	53.53 $\pm$ 15.95	23.7 $\pm$ 4.7	1.07 $\pm$ 0.59 (150 $\pm$ 24)
51	40.28 $\pm$ 8.07	33.0 $\pm$ 7.2	6.6 $\pm$ 4.8 (84 $\pm$ 11)
53	14.47 $\pm$ 2.21	>50	1.40 $\pm$ 0.74 (75 $\pm$ 2.1)
57	43.88 $\pm$ 8.54	inactive	9.1 $\pm$ 3.6 (81 $\pm$ 6.4)
63	20.19 $\pm$ 1.89	26.3 $\pm$ 0 8.3	1.69 $\pm$ 0.34 (36 $\pm$ 1.7)
30	>100	>100	3.8 $\pm$ 1.5 (37 $\pm$ 2.6)
54	68.79 $\pm$ 13.04	>50	4.3 $\pm$ 1.9 (50 $\pm$ 4.4)
63	>100	>50	6.0 $\pm$ 2.7 (96 $\pm$ 11)
64	>100	>100	8.1 $\pm$ 5.6 (87 $\pm$ 12)
66	>100	inactive	1.57 $\pm$ 0.62 (51 $\pm$ 3.8)
67	57.78 $\pm$ 8.69	inactive	3.18 $\pm$ 1.80 (122 $\pm$ 36)
74	>100	inactive	3.9 $\pm$ 1.7 (74 $\pm$ 5.7)
75	51.14 $\pm$ 5.55	inactive	1.44 $\pm$ 0.66 (64 $\pm$ 5.5)
77	28.93 $\pm$ 4.69	inactive	>100
3	47.45 $\pm$ 6.61	38.2 $\pm$ 8.6	1.62 $\pm$ 0.41 (74 $\pm$ 4.3)
12	24.5 $\pm$ 2.27	19.0 $\pm$ 6.01	1.1 $\pm$ 0.6 (79 $\pm$ 14)
15	48.82 $\pm$ 6.56	26.3 $\pm$ 6.9	2.36 $\pm$ 0.67 (96 $\pm$ 7.2)
28	>100	24.5 $\pm$ 6.1	6.9 $\pm$ 5.53 (70 $\pm$ 13)
29	>100	inactive	16.8 $\pm$ 5.7 (37 $\pm$ 3.4)
92	inactive	inactive	18.2 $\pm$ 2.8 (47 $\pm$ 1.8)

^aVDR-LBD concentration used was 0.1 μM . Inhibition of VDR-SRC2-3 interaction in the presence of LG190178 (0.75 μM). The maximum concentration used for this assay was 300 μM of each compound;

^bTranscription assay using a CMV-VDR plasmid and a luciferase reporter plasmid under control of a 24-hydroxylase promoter with GW0742 analogues. The maximum concentration used for this assay was 100 μM of each compound. Data were analyzed using a nonlinear regression with a variable slope (GraphPad Prism).