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Novel VDR antagonists based on the GW0742 scaffold

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Abstract

The vitamin D receptor is a nuclear hormone receptor that regulates cell proliferation, cell differentiation and calcium homeostasis. The receptor is endogenously activated by 1,25-dihydroxyvitamin D₃, which induces transcription of VDR targets genes regulated by coactivator binding. VDR antagonists and partial agonists have been developed based on the secosteroid scaffold of vitamin D. Only a few non-secosteroid VDR antagonists are known. Herein, we report the rational design of non-secosteroid VDR antagonists using GW0742 as a scaffold. GW0742 is a PPAR δ agonist previously identified by our group as a VDR antagonist. Several modifications including the replacement of the thiazole ring with an oxazole ring led to compound **7b**, which inhibited VDR-mediated transcription (IC₅₀ = 660 nM) without activating PPAR δ -mediated transcription. However, inhibition of transcription mediated by other nuclear receptors was observed.

Graphical abstract

Keywords

Nuclear Receptor; Vitamin D Receptor (VDR); Peroxisome Proliferation-Activated Receptor δ (PPAR δ); Antagonist; steroid receptor coactivator 2

The vitamin D receptor (VDR) is a transcription factor that belongs to the superfamily of nuclear receptors and mediates the transcription of genes responsible for cell differentiation, cell proliferation and calcium homeostasis.¹ The most potent endogenous agonist for VDR is 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) (Figure 1), which binds VDR with high

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Supplementary Material

Supplementary material can be found in the online version at <http://.....>

affinity.² In the cell nucleus, VDR binds DNA and forms a heterodimer with the retinoid X receptor (RXR).³ RXR is also a nuclear receptor and binds, among other ligands, 9-*cis* retinoic acid.⁴ In the absence of ligand, VDR can associate with corepressors such as the nuclear receptor corepressor (NCoR) and the silencing mediator of retinoic acid and thyroid hormone receptor (SMRT) and repress transcriptional activity.⁵ In the presence of 1,25(OH)₂D₃, a structural part of VDR, the ligand binding domain (LBD), undergoes a conformational change, which prevents corepressor binding and permits interactions with coactivator proteins such as steroid receptor coactivator 2, resulting in the formation of a multi-protein complex that activates VDR-mediated transcription.^{6–8}

Due to its role in gene expression, VDR is a promising pharmaceutical drug target for various diseases including skin disorders, autoimmune diseases and cancer. A mechanism to modulate VDR-mediated transcription are small molecules that inhibit the interactions between VDR and coregulators (corepressor and coactivators). Recently, VDR–coactivator inhibitors have been introduced by other groups and us.^{9–11} The inhibition of VDR–coregulator interactions has been shown to selectively modulate the expression of VDR target genes.

During the last decades, thousands of VDR agonists have been synthesized to identify new treatments for skin diseases, psoriasis, benign prostate hyperplasia, cancer, autoimmune diseases, microbial infections, and osteoporosis. The majority of these agonists are based on the secosteroid scaffold of 1,25(OH)₂D₃. In recent years, several non-secosteroidal VDR agonist¹² and their analogs were introduced such as diphenylmethane analog LG190178,¹³ bis-aromatic compound CD4528,¹⁴ and carboranes.¹⁵ In addition, a smaller number of VDR antagonists has been developed, which include the irreversible antagonist TEI-9647¹⁶ and those bearing bulky side chains such as 25-carboxylic esters (ZK168218 and ZK159222),¹⁷ 26-adamantly substituted antagonists (ADTT and analogs),¹⁸ and 22-butyl-branched compounds¹⁹ that ultimately destabilized the active conformation of VDR (Figure 1). Many of these antagonists are highly active but none of them have been further developed as therapeutics. In contrast to VDR agonists, VDR antagonists are almost exclusively based on the secosteroid scaffold. Recently, our group identified GW0742, a potent peroxisome proliferator activated receptor δ (PPAR δ) agonist,²⁰ that acted as a weak non-steroidal VDR antagonist.²¹ In addition, other nuclear receptor ligands were identified as novel VDR antagonists using virtual screening.²²

In collaboration with the NIH National Center for Advancing Translational Sciences (NCATS), compounds based on the GW0742 scaffold were synthesized and analyzed in respect to their ability to inhibit VDR-mediated transcription and activate PPAR δ -mediated transcription.²³ Among those compounds, NCGC00319047 and NCGC00319052 exhibited weak PPAR δ agonistic activity (EC_{50} = 2.25 $\pm$ 0.69 μ M and 2.36 $\pm$ 0.67 μ M, respectively) and moderate inhibition of VDR-mediated transcription (IC_{50} = 31.4 $\pm$ 8.11 μ M and 26.3 $\pm$ 6.93 μ M, respectively). In comparison, GW0742 activated PPAR δ at 3.5 $\pm$ 0.31 nM (EC_{50}) and inhibited VDR at 20.7 $\pm$ 4.5 μ M (IC_{50}). Furthermore, Sznajdman *et al.*²⁰ synthesized several oxazole analogues such as compound **7f** that showed weak activation of PPAR α , PPAR δ , and PPAR γ mediated transcription (Figure 2). It was hypothesized that **7f** was too

short to fit into the PPAR δ ligand binding site, in addition to an increased polar surface area of the oxazole-based ligand (73.3 Å²) in comparison to the thiazole-based ligand (49.2 Å²).

Herein, we report the synthesis and evaluation of a new series of oxazole compounds with the goal to develop a selective VDR antagonist that exhibits weak PPAR δ binding. In accordance with compound **7f**, the series includes changes of the carboxylic acid functionality. Based on previous studies by our group,²³ *o*- or *m*-methoxy aryl substituents were utilized. All ligands were characterized with respect to modulation of VDR and PPAR δ -mediated transcription in addition to preclinical characterization that include solubility and permeability measurements. Finally, we determined the selectivity of a number of analogs towards different nuclear receptors.

The synthesis of the oxazole ring was accomplished by heating 2- or 3-methoxybenzamide (**1a** and **1b**) and ethyl 2-chloroacetoacetate to form **2a** and **2b**, respectively (Scheme 1). Reduction with lithium aluminum hydride gave the corresponding primary alcohols **3a** and **3b**. The sequential addition of thionyl chloride to form the corresponding chloride followed by a reaction with different phenols produced esters **4a–8a** and **4b–8b**, which were hydrolyzed in the presence of sodium hydroxide to yield the final carboxylic acids **9a–13a** and **9b–13b**.

First, compounds were investigated whether they inhibit or promote the interaction between VDR and coactivator peptide SRC2–3 using a fluorescence polarization assay. Surprisingly none of the synthesized compounds exhibited any activity (Table S1), probably due to high specificity of this assay between VDR and this particular steroid receptor coactivator (SRC). However, activity in cells was observed for the majority of compounds determined with transcription assays mediating VDR or PPAR δ (Table 1). Esters with an *o*-OCH₃ substituent were able to inhibit VDR-mediated transcription while lacking the ability to activate PPAR δ -mediated transcription. On the contrary, PPAR δ -mediated transcription was activated by most of the *o*-OCH₃ derivatives without inhibiting VDR-mediated transcription. The most potent *o*-OCH₃ substituted VDR antagonist in this series was **5a** with an IC₅₀ of 2.5 μM and the inability to activate PPAR δ -mediated transcription at a concentration of 100 μM. In respect to GW0742, a 10-fold increased potency was achieved with **5a**. Overall, the series of *o*-OCH₃ and *m*-OCH₃ esters (**4a–8a** and **4b–8b**, respectively) were more potent VDR antagonists compared to their acid counterparts. Additionally, *o*-OCH₃ and *m*-OCH₃ esters selectively targeted VDR when compared to PPAR δ activation with the exception of **8b**, which partially (7.7 % in comparison to GW0742) activated PPAR δ with an EC₅₀ of 1.1 μM. However, *o*-OCH₃ and *m*-OCH₃ esters were more toxic at higher concentration in comparison to their corresponding acids. The most cytotoxic ester was **8a**. The most potent VDR antagonist was ester **7b** with an IC₅₀ of 660 nM. Compounds that activated PPAR δ -mediated transcription showed partial agonism between 2.2% and 48% with respect to GW0742. In general, partial agonism was weaker for the *m*-OCH₃ analogs than for *o*-OCH₃ analogs.

Specificity towards the VDR–SRC1 (steroid receptor coactivator 1) interaction in cells was determined by two-hybrid assay (Table 1). Unlike the VDR–SRC2-3 interaction, which was not inhibited by these compounds *in vitro* (Figure S1), IC₅₀ values as low as 6.7 μM were

observed for these compounds in cells for the VDR–SRC1 interaction. In general, higher IC₅₀ values were observed with the 2-hybrid assay in comparison to the transcription assay, although the differences were not always significant. The most active ester identified with this assay was compound **8b** ($6.7 \pm 3.4 \mu\text{M}$), whereas **12b** was the most active acid ($26.7 \pm 15.8 \mu\text{M}$). The esters and acids synthesized were evaluated for two physicochemical characteristics: aqueous solubility and permeability (Table 1). As expected, all esters were less water soluble than their corresponding acids. When compared to low, medium, and highly soluble control compounds, esters possessed low solubility while acids exhibited medium water solubility. In comparison to low, medium, and highly permeable control compounds, esters have medium permeability while acids were more comparable to Ranitidine with low permeability across a hydrophobic barrier at physiological pH.

Four different compound (**5a**, **7a**, **6b**, and **7b**) were selected for further investigation with other nuclear receptors such as PPAR α , PPAR γ , RXR α , thyroid receptors TR α and TR β , and the estrogen receptors ER α and ER β . The results are summarized in Table 2. Interestingly, all four compounds inhibited the transcription mediated by all nuclear receptors investigated. Minor selectivity was observed for each compound. For instance **5a** was more effective towards ER α than ER β , however its selectivity between TR and PPAR isoforms was marginal. Still, VDR-mediated transcription was inhibited at low concentration by **5a** with an IC₅₀ of $2.5 \mu\text{M}$. In addition, **7b** exhibited not only selectivity between ER isoforms but was also selective for PPAR α in comparison to PPAR γ and PPAR δ . Nevertheless, the IC₅₀ for VDR ($0.6 \mu\text{M}$) was still lower than for all other nuclear receptors. In order to ensure that the observed activity was not due to a possible inhibition of the luciferase enzyme employed for the transcription assay, a separate receptor independent assay was conducted. Therefore, adenosine triphosphate was added to compound and Cell Titer Glo™ (Promega). None of the four compounds inhibited the formation of light mediated by luciferase provided by the Cell Titer Glo (Figure S1).

A good structure activity relationship in respect to VDR could be established with *ortho*- and *meta*-substituted oxazole containing compounds bearing an ester or acid function. In cells, *o*-OCH₃ and *m*-OCH₃ substituted esters are potent VDR antagonists with reduced activation of PPAR δ -mediated transcription. *m*-OCH₃ substituted acids were able to inhibit VDR-mediated transcription and possessed partial PPAR δ agonism. *o*-OCH₃ substituted acids showed partial agonism for PPAR δ and lack of inhibition for VDR-mediated transcription. Most compounds exhibited a strong preference for the VDR–SRC1 interaction, while the VDR–SRC2-3 interaction was not inhibited *in vitro*. Based on profiling of four analogs, it can be concluded that compounds described here might fit the ligand binding pocket of several nuclear receptors. The affinity of the investigated compounds was moderate thus future investigations applying other heterocycles and substituents might enable the discovery of more selective and more potent nuclear receptor ligands. Current efforts to co-crystallize these ligands with VDR to obtain crystallographic data will guide future SAR approaches.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Jurutka PW, Whitfield GK, Hsieh JC, Thompson PD, Haussler CA, Haussler MR. Molecular nature of the vitamin D receptor and its role in regulation of gene expression. *Rev Endocr Metab Disord*. 2001; 2(2):203–216. [PubMed: 11705326]
2. Holick MF, Schnoes HK, DeLuca HF, Suda T, Cousins RJ. Isolation and identification of 1,25-dihydroxycholecalciferol. A metabolite of vitamin D active in intestine. *Biochemistry*. 1971; 10(14):2799–2804. [PubMed: 4326883]
3. Toell A, Polly P, Carlberg C. All natural DR3-type vitamin D response elements show a similar functionality in vitro. *Biochem J*. 2000;301–309. [PubMed: 11085922]
4. Mangelsdorf DJ, Evans RM. The R α r Heterodimers and Orphan Receptors. *Cell*. 1995; 83(6):841–850. [PubMed: 8521508]
5. Kim JY, Son YL, Lee YC. Involvement of SMRT corepressor in transcriptional repression by the vitamin D receptor. *Mol Endocrinol*. 2009; 23(2):251–264. [PubMed: 19098224]
6. Masuyama H, Brownfield CM, St-Arnaud R, MacDonald PN. Evidence for ligand-dependent intramolecular folding of the AF-2 domain in vitamin D receptor-activated transcription and coactivator interaction. *Mol Endocrinol*. 1997; 11(10):1507–1517. [PubMed: 9280066]
7. Hong H, Kohli K, Garabedian MJ, Stallcup MR. GRIP1, a transcriptional coactivator for the AF-2 transactivation domain of steroid, thyroid, retinoid, and vitamin D receptors. *Mol Cell Biol*. 1997; 17(5):2735–2744. [PubMed: 9111344]
8. Li H, Gomes PJ, Chen JD. RAC3, a steroid/nuclear receptor-associated coactivator that is related to SRC-1 and TIF2. *Proc Natl Acad Sci U S A*. 1997; 94(16):8479–8484. [PubMed: 9238002]
9. Mita Y, Dodo K, Noguchi-Yachide T, et al. LXXLL peptide mimetics as inhibitors of the interaction of vitamin D receptor with coactivators. *Bioorg Med Chem Lett*. 2010; 20(5):1712–1717. [PubMed: 20144545]
10. Sidhu PS, Nassif N, McCallum MM, et al. Development of Novel Vitamin D Receptor-Coactivator Inhibitors. *ACS Med Chem Lett*. 2014; 5(2):199–204. [PubMed: 24799995]
11. Nandhikonda P, Lynt WZ, McCallum MM, et al. Discovery of the First Irreversible Small Molecule Inhibitors of the Interaction between the Vitamin D Receptor and Coactivators. *J Med Chem*. 2012; 55(10):4640–4651. [PubMed: 22563729]
12. Yamada S, Makishima M. Structure-activity relationship of nonsecosteroidal vitamin D receptor modulators. *Trends Pharmacol Sci*. 2014
13. Boehm MF, Fitzgerald P, Zou A, et al. Novel nonsecosteroidal vitamin D mimics exert VDR-modulating activities with less calcium mobilization than 1,25-dihydroxyvitamin D₃. *Chem Biol*. 1999; 6(5):265–275. [PubMed: 10322128]
14. Perakyla M, Malinen M, Herzig KH, Carlberg C. Gene regulatory potential of nonsteroidal vitamin D receptor ligands. *Mol Endocrinol*. 2005; 19(8):2060–2073. [PubMed: 15860548]
15. Fujii S, Masuno H, Taoda Y, et al. Boron cluster-based development of potent nonsecosteroidal vitamin D receptor ligands: direct observation of hydrophobic interaction between protein surface and carborane. *J Am Chem Soc*. 2011; 133(51):20933–20941. [PubMed: 22066785]
16. Miura D, Manabe K, Ozono K, et al. Antagonistic action of novel 1 α ,25-dihydroxyvitamin D₃-26, 23-lactone analogs on differentiation of human leukemia cells (HL-60) induced by 1 α ,25-dihydroxyvitamin D₃. *J Biol Chem*. 1999; 274(23):16392–16399. [PubMed: 10347199]
17. Bury Y, Steinmeyer A, Carlberg C. Structure activity relationship of carboxylic ester antagonists of the vitamin D(3) receptor. *Mol Pharmacol*. 2000; 58(5):1067–1074. [PubMed: 11040055]

18. Igarashi M, Yoshimoto N, Yamamoto K, et al. Identification of a highly potent vitamin D receptor antagonist: (25S)-26-adamantyl-25-hydroxy-2-methylene-22,23-didehydro-19,27-dinor-20-epi-vitamin D3 (ADMI3). *Arch Biochem Biophys*. 2007; 460(2):240–253. [PubMed: 17214957]
19. Inaba Y, Yoshimoto N, Sakamaki Y, et al. A new class of vitamin D analogues that induce structural rearrangement of the ligand-binding pocket of the receptor. *J Med Chem*. 2009; 52(5): 1438–1449. [PubMed: 19193059]
20. Sznajdman ML, Haffner CD, Maloney P, et al. Novel Selective Small Molecule Agonists for Peroxisome Proliferator-Activated Receptor δ (PPAR δ)—Synthesis and Biological Activity. *Bioorg Med Chem Lett*. 2003; 13:1517–1521. [PubMed: 12699745]
21. Nandhikonda P, Yasgar A, Baranowski AM, et al. GW0742 interacts weakly with multiple nuclear receptors, including the vitamin D receptor. *Biochemistry*. 2013:4193–4203. [PubMed: 23713684]
22. Teske K, Nandhikonda P, Bogart JW, et al. Identification of VDR antagonists among nuclear receptor ligands using virtual screening. *Nuclear Receptor Research*. 2014; 1(1):1–8.
23. Teske KA, Rai G, Nandhikonda P, et al. Parallel chemistry approach to identify novel nuclear receptor ligands based on the GW0742 scaffold. *ACS Comb Sci*. 2017; 19(10):646–656. [PubMed: 28825467]

Figure 1.
Chemical structures of VDR agonist 1,25(OH)₂D₃ and VDR antagonists AD47, TEI-9647, and ZK159222.

Figure 2.
GW0742 and analogues.

Scheme 1.

Synthesis of novel oxazole-based VDR antagonists. i) Ethyl-2-chloroacetoacetate, neat, 120–125°C, 3 days, 50–66%; ii) LiAlH₄, THF, 0°C to rt, 3 h, 40–89%; iii) a) SOCl₂, DCM, 1–2 h, b) Cs₂CO₃, DMF, phenol, rt, overnight, 20–66%; iv) a) THF, 2 M NaOH aq., 40–70°C, overnight, b) HCl aq., 79–100%.

Table 1

Activity and pre-clinical properties for oxazole-based VDR antagonists.

Compd	VDR transcription IC ₅₀ ^a (μM)	PPARδ transcription EC ₅₀ (μM)	2-Hybrid VDR transcription ^c IC ₅₀ (μM)	% Toxicity ^d at 100 μM	Solubility ^e (μM)	Permeability ^f LogP (cm/s)
4a	3.25 ± 1.4	Inactive	20.2 ± 13.5	54.1 ± 5.3	185.4	-3.33
5a	2.50 ± 1.2	Inactive	7.33 ± 3.84	39.5 ± 6.7	44.1	-3.42
6a	4.85 ± 2.2	Inactive	17.9 ± 7.92	81.5 ± 1.6	62.6	-3.35
7a	3.82 ± 1.3	Inactive	11.13 ± 4.9	31.2 ± 5.1	61.6	-3.56
8a	10.1 ± 1.7	Inactive	13.6 ± 5.2	79.9 ± 12.3	62.6	-3.34
4b	6.75 ± 2.1	Inactive	15.4 ± 11.6	94.6 ± 2.1	52.8	-3.29
5b	5.56 ± 1.9	Inactive	17.1 ± 5.2	59.3 ± 6.8	24.1	-3.37
6b	5.67 ± 1.4	Inactive	8.89 ± 2.5	35.6 ± 5.2	18.5	-3.43
7b	0.66 ± 0.30	Inactive	13.9 ± 11.5	63.4 ± 1.6	45.3	-3.32
8b	2.88 ± 0.97	1.09 ± 0.58 (7.7%)	6.7 ± 3.4	67.6 ± 2.3	38.2	-3.34
9a	Inactive	Inactive	>80	4.1 ± 5.0	401.6	-3.63
10a	Inactive	0.88 ± 0.5 (22%)	>100	n.o.	423.1	-3.58
11a	Inactive	8.7 ± 6.4 (16%)	>80	n.o.	460.0	-3.67
12a	Inactive	11.0 ± 10.2 (48%)	>80	n.o.	436.0	-3.72
13a	>80	4.6 ± 3.1 (30%)	>100	2.1 ± 4.6	399.7	-3.59
9b	40.4 ± 13.4	Inactive	>33	37.3 ± 5	264.1	-3.63
10b	33.2 ± 15.2	Inactive	33.14 ± 13.1	23.9 ± 3.4	498.1	-3.88
11b	>100	2.6 ± 1.8 (2.1%)	>80	27.2 ± 2.1	457.3	-3.77
12b	3.60 ± 1.4	1.8 ± 0.8 (8.5%)	27.7 ± 17.3	34.5 ± 0.93	410.3	-3.88
13b	3.35 ± 1.5	1.4 ± 0.4 (13.7%)	26.7 ± 15.8	41.2 ± 5.0	234.4	-3.92

^aTranscription assay using a CMV-VDR plasmid and a luciferase reporter plasmid under control of a 24-hydroxylase promoter, maximum concentration used was 100 μM.^b2-hybrid assay using CMV-PPARδ-LBD-GAL4-DBD plasmid and a 6xGal4-luc reporter vector, maximum concentration used was 100 μM. Parenthesis indicate percent partial agonism with respect to GW0742.^cTwo-hybrid assay using VP16-VDR-LBD, SRC1-GAL4, and luciferase reporter plasmid vector, maximum concentration used was 100 μM.^dCell-TiterGlo (Promega), n.o.= not observed, maximum concentration used was 100 μM.

f_p Filter-based assay, maximum concentration was 500 μ M.

f_p PAMPA assay (Pion)

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Table 2

Activity of VDR antagonists against a panel of nuclear receptors.

Compd	PPAR α IC ₅₀ (μ M) ^a	PPAR γ IC ₅₀ (μ M) ^b	PPAR δ IC ₅₀ (μ M) ^c	RXR α IC ₅₀ (μ M) ^d	TR α IC ₅₀ (μ M) ^e	TR β IC ₅₀ (μ M) ^e	ER α IC ₅₀ (μ M) ^f	ER β IC ₅₀ (μ M) ^f
5a	6.6 $\pm$ 2.9	7.6 $\pm$ 3.4	10.9 $\pm$ 3.9	11.1 $\pm$ 4.6	10.1 $\pm$ 3.3	9.8 $\pm$ 2.9	5.2 $\pm$ 1.5	23.6 $\pm$ 17.2
7a	8.0 $\pm$ 6.6	6.3 $\pm$ 3.1	6.6 $\pm$ 2.9	4.4 $\pm$ 3.1	8.4 $\pm$ 2.7	7.6 $\pm$ 4.4	4.1 $\pm$ 1.7	10.0 $\pm$ 6.4
6b	3.5 $\pm$ 2.1	7.6 $\pm$ 3.4	10.9 $\pm$ 4.1	4.6 $\pm$ 2.6	5.4 $\pm$ 2.5	8.8 $\pm$ 3.0	4.7 $\pm$ 1.1	5.9 $\pm$ 2.8
7b	1.9 $\pm$ 1.3	6.7 $\pm$ 3.0	11.3 $\pm$ 6.8	2.0 $\pm$ 1.7	4.2 $\pm$ 2.2	0.97 $\pm$ 0.82	1.7 $\pm$ 0.96	7.4 $\pm$ 6.3

^aGW7647 (30 nM).^bRosiglitazone (300 nM).^cGW0742 (50 nM).^dBexarotene (200 nM).^eT3 (10 nM) and^fEstradiol (10 nM).The maximum concentration used was 100 μ M.