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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 D_3, 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 PPARδ 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 (IC_{50} = 660 nM) without activating PPARδ-mediated transcription. However, inhibition of transcription mediated by other nuclear receptors was observed.

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 D_3 ($1,25(OH)_2D_3$) (Fig. 1), which binds VDR with high 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 $1,25(OH)_2D_3$, a structural part of VDR, the ligand binding domain (LBD), undergoes a conformational change. This rearrangement prevents corepressor binding and permits interactions with coactivator proteins such as steroid receptor coactivator 2 and results 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 $1,25(OH)_2D_3$. In recent years, several non-secosteroidal VDR agonists[12] 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-9647[16] and those bearing bulky side chains such as 25-carboxylic esters (ZK168218 and ZK159222),[17] 26-adamantly substituted antagonists (AD47 and analogs),[18] and 22-butyl-branched compounds[19] that ultimately destabilized the active conformation of VDR (Fig. 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 (PPARδ) 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

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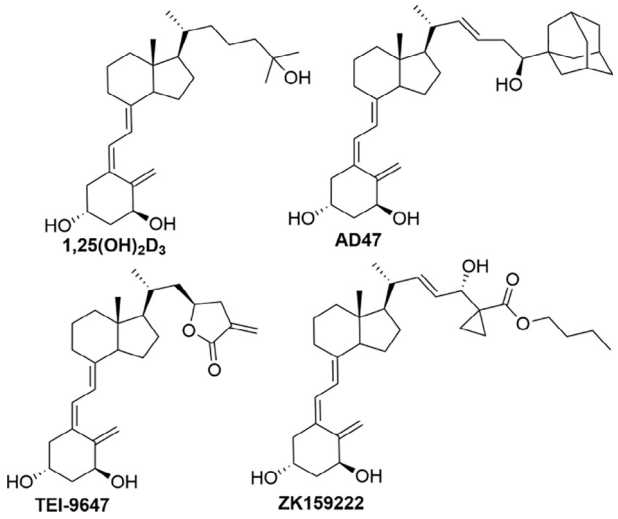

Fig. 1. Chemical structures of VDR agonist $1,25(OH)_2D_3$ and VDR antagonists AD47, TEI-9647, and ZK159222.

PPARδ-mediated transcription.[23] Among those compounds, NCGC00319047 and NCGC00319052 exhibited weak PPARδ agonistic activity (EC_{50} = 2.25 ± 0.69 μM and 2.36 ± 0.67 μM, respectively) and moderate inhibition of VDR-mediated transcription (IC_{50} = 31.4 ± 8.11 μM and 26.3 ± 6.93 μM, respectively). In comparison, GW0742 activated PPARδ at 3.5 ± 0.31 nM (EC_{50}) and inhibited VDR at 20.7 ± 4.5 μM (IC_{50}). Furthermore, Sznaidman et al.[20] synthesized several oxazole analogues such as compound **7f** that showed weak activation of PPARα, PPARδ, and PPARγ mediated transcription (Fig. 2). It was hypothesized that **7f** was too short to fit into the PPARδ ligand binding site, in addition to an increased polar surface area of the oxazole-based ligand (73.3 $Å^2$) in comparison to the thiazole-based ligand (49.2 $Å^2$).

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 PPARδ 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 PPARδ-mediated transcription in addition to preclinical characterization that include solubility and permeability measurements. Finally, we determined the selectivity of a number of analogues 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 addition of thionyl chloride formed the corresponding chlorides, which were reacted with different phenols to produce esters **4a–8a** and **4b–8b**. The esters were hydrolyzed in the presence of sodium hydroxide to yield the final carboxylic acids **9a–13a** and **9b–13b**.

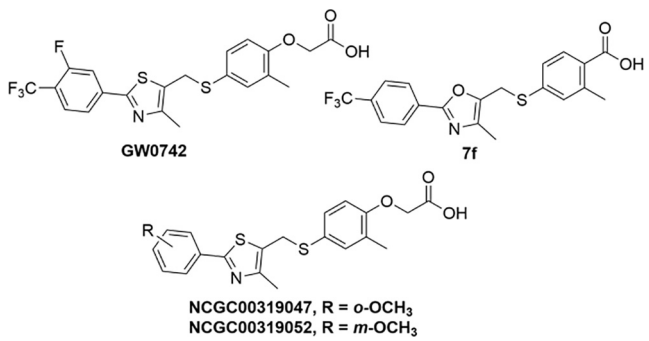

Fig. 2. GW0742 and analogues.

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 as determined by transcription assays mediating VDR or PPARδ (Table 1). Esters with an *o*-OCH_3 substituent were able to inhibit VDR-mediated transcription while lacking the ability to activate PPARδ-mediated transcription. On the contrary, PPARδ-mediated transcription was activated by most of the *o*-OCH_3 derivatives without inhibiting VDR-mediated transcription. The most potent *o*-OCH_3 substituted VDR antagonist in this series was **5a** with an IC_{50} of 2.5 μM and the inability to activate PPARδ-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*-OCH_3 and *m*-OCH_3 esters (**4a–8a** and **4b–8b**, respectively) were more potent VDR antagonists compared to their acid counterparts. Additionally, *o*-OCH_3 and *m*-OCH_3 esters selectively targeted VDR when compared to PPARδ activation with the exception of **8b**, which partially (7.7% in comparison to GW0742) activated PPARδ with an EC_{50} of 1.1 μM. However, *o*-OCH_3 and *m*-OCH_3 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 IC_{50} of 660 nM. Compounds that activated PPARδ-mediated transcription showed partial agonism between 2.2% and 48% with respect to GW0742. In general, partial agonism was weaker for the *m*-OCH_3 analogs than for *o*-OCH_3 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* (Fig. S1), IC_{50} values as low as 6.7 μM were observed for these compounds in cells for the VDR–SRC1 interaction. In general, higher IC_{50} values were observed with the two-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 ± 3.4 μM), whereas **12b** was the most active acid (26.7 ± 15.8 μM). 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 compounds (**5a**, **7a**, **6b**, and **7b**) were selected for further investigation with other nuclear receptors such as PPARα, PPARγ, RXRα, thyroid receptors TRα and TRβ, and the estrogen receptors ERα and ERβ. 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 ERα than ERβ, however its selectivity between TR and PPAR isoforms was marginal. Still, VDR-mediated transcription was inhibited at low concentration by **5a** with an IC_{50} of 2.5 μM. In addition, **7b** exhibited not only selectivity between ER isoforms but was also selective for PPARα in comparison to PPARγ and