Design, Synthesis, Evaluation and Structure of Allenic α,25-Dihydroxyvitamin D₃ Analogs with Locked Mobility at C-17

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In memory of Anthony Norman and Gary Posner.

Abstract: Vitamin D receptor ligands have potential for the treatment of hyperproliferative diseases and disorders related to the immune system. However, hypercalcemic effects limit their therapeutical uses and call for the development of tissue-selective new analogs. We have designed and synthesized the first examples of α,25-dihydroxyvitamin D₃ analogs bearing an allenic unit attached to the D ring to restrict the side-chain conformational mobility. The triene system was constructed by a Pd⁰-mediated cyclization/Suzuki-Miyaura cross-coupling process in the presence of an allenic side chain. The allenic moiety was built through an orthoester-Claisen rearrangement of a propargylic alcohol. The biological activity and structure of (22S)-1α,25-dihydroxy-17,20-dien-24-homo-21-nor-vitamin D₃ bound to binding domain of the vitamin D receptor, provide information concerning side-chain conformational requirements for biological activity.

Introduction

The active form of the secosteroid hormone vitamin D₃, 1α,25-dihydroxyvitamin D₃ [1, calcitriol, 1,25-(OH)₂D₃, 1,25D, Figure 1], exerts its biological functions by binding to the vitamin D receptor (VDR), a transcription factor of the nuclear receptor superfamily (NRs).[1] 1,25D was known for many years as a primary regulator of calcium homeostasis, but it also controls a wide range of other biological functions including cell differentiation, cell proliferation, and immune responses.[2,3] 1,25D induces the expression of more than 200 genes associated with several diseases such as arthritis, diabetes and cancer, suggesting that this secosteroid hormone might induce a wide range of biological functions.[4] Unfortunately, 1,25D induces hypercalcemic effects and disorders related to the immune system. However, hypercalcemic effects limit their therapeutical uses and call for the development of tissue-selective new analogs. We have designed and synthesized the first examples of α,25-dihydroxyvitamin D₃ analogs bearing an allenic unit attached to the D ring to restrict the side-chain conformational mobility. The triene system was constructed by a Pd⁰-mediated cyclization/Suzuki-Miyaura cross-coupling process in the presence of an allenic side chain. The allenic moiety was built through an orthoester-Claisen rearrangement of a propargylic alcohol. The biological activity and structure of (22S)-1α,25-dihydroxy-17,20-dien-24-homo-21-nor-vitamin D₃ bound to binding domain of the vitamin D receptor, provide information concerning side-chain conformational requirements for biological activity.

Figure 1. Structures of the natural hormone 1,25D (1), 1,25D analogs 2a–2e, calcipotriol and maxacalcitol.

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mia at the doses required for the treatment of hyperproliferative diseases such as cancer. This limitation has led to intense synthetic efforts towards VDR ligands with selective activities as potential therapeutic agents for the treatment of cancer, immunodeficiency syndromes, autoimmune diseases and skin disorders. Calcipotriol and maxacalcitol, examples of 1,25D analogs with a modified side-chain, exhibit similar or higher antiproliferative potency with reduced calcemic effects in comparison with natural hormone 1,25D (Figure 1). Other potent 1,25D analogs structurally modified at the CD-rings or triene system that induce low potency for calcium metabolism have also been synthesized. Representative examples of these compounds include inecalcitol (19-nor-1-4-epi-23-yne-1,25D, TX522) and PG-136, which exhibit anticancer properties, and paricalcitol (19-nor-1,25D), which induces immunomodulatory effects and was recently proposed as a therapeutic option for patients with severe COVID-19. Unfortunately, the mechanism underlying the dissociation of the antiproliferative effects from the calcemic effects, as well as the conformational requirements for selective biological functions, have not yet been established.

The crystallographic structures of 1,25D and various of its analogs in complex with the VDR ligand binding domain indicate that the corresponding side-chain-C25OH groups adopt similar positions. However, the side-chain flexibility makes difficult to predict the conformation associated with a particular biological function. Okamura and coworkers, in efforts to understand structure-activity relationships, pioneered the synthesis of 1,25D analogs with rigid structural units at the side chain to restrict its conformational mobility.

Results and Discussion

Design

Based on the crystal structure of the active hVDR ligand-binding domain (LBD) bound to 1,25D, we studied the docking of the analogs 2a–2e bearing an allenic unit at the side chain attached to C17 of the D-ring (Figure 1). We showed that analog 2d binds well (97%) to the VDR LBD in comparison with the native hormone 1,25D (100%) (Figure 2). This analog adopts the canonical active conformation as the natural hormone in the binding pocket, where the A, C and D rings, and the triene system occupy similar positions. The A-ring and side-chain hydroxyl groups form effective hydrogen bonds with the same amino acid residues (His-305, His-397, Ser-278, Arg-274, Tyr-143, Ser-237) as the natural hormone. The other allenic analogs 2a (87%), 2b (92%), 2c (86%), and 2e (84%) bind to the hVDR LBD less efficiently than 2d. On the basis of the in silico binding results, we chose compounds 2a, 2b and 2d as the synthetic targets.

Retrosthetic analysis of target compounds

The synthetic plan for the synthesis of target compounds 2a, 2b, and 2d is outlined in Scheme 1. The formation of the vitamin D triene system involves a stereoselective Pd0-catalyzed ring closure of enoltriflate 4 and subsequent Suzuki-Miyaura coupling with unprotected alkenyl-boronic ester 3 in protic medium following procedures developed in this laboratory. At this point, the stability of the allenic unit under the Pd-catalyzed reaction conditions was uncertain. The boronates 3 are envisaged to arise from the allene 5 by conventional
chemistry. The key allene 5 would arise from propargylic alcohol 6 through an orthoester-Claisen rearrangement.\[18\] Compound 6 would be prepared in several steps by degradation of vitamin D\(_2\).\[19\]

Synthesis of the upper boronate fragments

The synthesis of boronates 3a, 3b, and 3d is outlined in Scheme 2. Reaction of ketone 7 with lithium trimethylsilylacetylide in THF followed by desilylation with Na\(_2\)CO\(_3\) in methanol provided propargylic alcohol 6\[6\] in 86% yield. Exposure of 6 to orthoester Claisen rearrangement with triethyl orthoacetate and acetic acid delivered the (5)-allene 5 (84%) as a single isomer [\(^1\)H NMR δ 5.27 (C=C=CH)]. Treatment of 5 with methyl magnesium bromide in THF provided the tertiary alcohol 8a (98%), which was deprotected to alcohol 9a (98%) with tetrabutylammonium fluoride in THF. Pyridinium dichromate oxidation of 9a in CH\(_2\)Cl\(_2\) followed by Wittig-olefination of the resulting ketone 10a with ylide Ph\(_3\)P=CHBr\[21\] prepared from (Ph\(_3\)PCH\(_2\)Br)Br and KOtBu in toluene, led to (E)-alkenyl bromide 11a in 69% yield (two steps), which was converted to the desired upper boronate 3a (58% yield) without protection of the side-chain-hydroxyl group by Miyaura’s modified method\[17,22\] using bis(pinacolato)diboron and KOAc in the presence of catalytic [1,1’bis(diphenylphosphino)ferrocene]dichloropalladium(II) in DMSO.

The synthesis of boronate 3d began with ester 5, which was converted to iodide 13 by a three steps sequence (81% yield) involving reduction with diisobutylaluminum hydride in THF, treatment of the resulting alcohol 12 with p-toluenesulfonyl chloride in pyridine, and subsequent reaction of the resulting

Scheme 2. Synthesis of the upper boronates 3a, 3b, and 3d.
tosylate with sodium iodide in acetone. Chain extension by reaction of iodide 13 with the lithium anion derived from tert-butyl acetate in THF provided ester 14 in 95% yield, which was converted to diol 16 (90%) by methylation (MeLi, THF) and subsequent deprotection (nBu4NF, THF). Pyridinium dichromate oxidation of 16 in CH2Cl2 and subsequent Wittig-olefination of the resulting ketone 17 with ylide PhP=CHBr afforded the (E)-alkenyl bromide 18 in 82% yield (two steps), which was transformed into the desired upper boronate 3d (57% yield) by reaction with bis(pinacolato)diboron and KOAc in the presence of catalytic [1,1’bis(diphenylphosphino)ferrocene]dichloropalladium(II) in DMSO as above (23.3% overall yield from ketone 7, 12 steps). The allene moiety survived the Pd-catalyzed process during the formation of the vitamin D triene system though in moderate yield.  

Synthesis of target compounds

With the upper fragments in hand, we proceeded to the final convergent formation of the triene system of the target compounds 2a, 2b, and 2d (Scheme 3). Pd-catalyzed carbocyclization of enoltriflate 4 (1.1 equiv) and subsequent Suzuki-Miyaura coupling with boronate 3a (1 equiv) in the presence of catalytic bis(triphenylphosphine)palladium(II) in aqueous K2PO4 (2 M)/THF provided, after deprotection, the desired 1,25D-analog 2a in 51% yield (two steps) (13.7% overall yield from ketone 7, 14 steps). Same procedure was used for the preparation of allene analog 2b (16.4% overall yield from ketone 7, 14 steps). In a similar way, Pd-catalyzed carbocyclization/Suzuki-Miyaura cross coupling tandem process on 3c provided the protected 1,25D-analog 19, which upon desilylation, gave the desired allene 1,25D-analog 2d (54%, two steps) (14.5% overall yield from ketone 7, 14 steps).

Functional activity

As the in silico data indicate that the allene 2d binds to VDR with the highest efficiency, we determined its biological properties. Transactivation assay in HeLa cells transfected with human full length VDR showed that 1,25D (1) and 2d induce the expression of a luciferase reporter gene under the control of the promoter of human CYP24A1, the main vitamin D target gene (Figure 3, A). Note that at lower doses, 2d was less potent than 1. In addition, we monitored the expression of CYP24A1 transcripts in cells derived from prostate cancer metastasis (DU-145) treated for 24 h. Ligands 2d and 1 induced transcript levels at 100 nM and 10 nM, but the levels were lower in cells treated with 2 (Figure 3, B).

To determine the effects of 2d in vivo, wild type mice were daily treated with various doses of 2d and 1 for 5 days. In agreement with previous results, 1 1 μg/kg of 1 induced hypercalcemia and the transcript levels of the vitamin D target genes Cyp24a1 and S100g in the kidney (Figure 3, D–E). However, the renal transcript levels were similar in mice treated with 2d and with vehicle (Figure 3, D–E). The calcemic activity of 2d was evaluated by monitoring the serum calcium levels in the treated mice (Figure 3, C). Serum calcium levels were unaffected at 1 and 3 μg/kg. Thus, these results indicate that 2d is less potent than 1,25D, but behaves as a low-calcemic 1,25D analog.

Crystal structure

To determine the binding pose of the analog 2d, we co-crystallized the zebrafish wild-type VDR LBD[11c] in complex with

![Scheme 3. Synthesis of the target vitamin D analogs 2a, 2b, and 2d.](image_url)
the ligand and a coactivator peptide and solved the structure with a resolution up to 2.1 Å (Supporting Table 1). The overall structure is highly homologous to the VDR-1,25D structure with a root mean square deviation of 0.3 Å over 235 residues when comparing the Cα atoms of the two complexes. Superposition of the VDR ligand binding pocket in the presence of 2d and 1,25D shows similar positioning of the ligands (Figure 4, A), in agreement with the in silico docking data. The C1-OH, C3-OH and C25-OH groups of 2d form similar H-bonds as 1,25D (Figure 4A), however the C25-OH forms weaker H-bonds with His-333 and His-423 (3.2 Å and 3.0 Å for analog 2d compared to 2.8 Å and 2.8 Å for 1,25D). The secosteroidal part of the ligand forms similar interactions with the zVDR ligand binding pocket compared to 1,25D. The side chain of 2 d forms extensive interactions with Leu-255, Leu-258, Val-262, Ala-331, His-333, His-423, Tyr-427, Leu-430 at a 4.0 Å distance cutoff (Figure 4, B).

The difference in side chain position as a consequence of the rigid allenic unit attached to C17, results in a loss of interaction to residue Leu-337 in complex with 2d. Overall, the contacts formed by 2d in the zVDR complex stabilize the agonist conformation of VDR in agreement with the agonist potency of this novel compound. The weaker hydrogen bonds with His-333 and His-423 and loss of interaction with Leu-337 explain the observed difference in activity between the 1,25D and compound 2 d.

**Conclusion**

In summary, we have designed and synthesized three analogs of the hormone 1α,25-dihydroxyvitamin D3 bearing an allenic unit attached to C17 as the structural element to impart conformational rigidity near the D-ring. Highlights of the synthetic route (about 14% overall yield from bicyclic ketone 7, 14 steps), include the access to the allenic moiety by an orthoester-Claisen rearrangement and the formation of the triene system by a Pd2-catalyzed cyclization/cross coupling process involving an enoltriflate, precursor of the A-ring, and vinyl boronate containing an allenic side-chain unit corresponding to the CD-Side chain framework. Biological evaluation of 2 d with the highest affinity for VDR, reveals that the geometry imposed by the C17-allene moiety reduces the transcriptional potency and calcemic activity. The crystallographic structure of 2d in complex with the VDR LBD provides information concerning side-chain conformational requirements for the design of new noncalcemic vitamin D analogs of potential therapeutic interest.

**Experimental Section**

General: For details on the general materials and methods, see Supporting Information.

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**Conflict of Interest**

The authors declare no conflict of interest.

**Keywords:** allenes · orthoester-Claisen rearrangement · Pd-catalyzed reactions · synthesis · vitamin D analogs

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[1] R. M. Evans, Science 1988, 240, 889–895.
[2] M. R. Haussler, G. K. Whitfield, I. Kaneko, C. A. Haussler, D. Hsieh, J. C. Hsieh, P. W. Jurutka, Calcif. Tissue Int. 2013, 92, 77–98.
[3] a) R. Bouillon, W. H. Okamura, A. W. Norman, Endocr. Rev. 1995, 16, 200–257; b) Vitamin D (Eds.: D. Feldman, J. W. Pike), Academic Press, New York, 1997; c) Vitamin D (Eds.: D. Feldman, J. W. Pike, J. S. Adams), Two-Volume Set, Elsevier, Academic Press, New York, 2011.
[4] S. V. Ramagopalan, A. Heger, A. J. Berlanga, N. J. Maugeri, M. R. Lincoln, A. Burrell, L. Handunnetthi, A. E. Handel, G. Disanto, S.-M. Orton, C. T. Watson, J. M. Morahan, G. Giovannoni, C. P. Ponting, G. C. Ebers, Genome Res. 2010, 20, 1352–1360.
[5] a) L. L. Isa, M. G. Leong, R. L. Sutherland, J. A. Esman, J. Bone Miner. Res. 2002, 17, 879–890; b) A. Lori, L. A. Plum, H. F. DeLuca, Nat. Drug Discov. 2010, 8, 941–955; c) C. Leyssens, L. Verlinden, M. Verstuyf, Front. Plant Physiol. 2014, 5, 1–18.
[6] a) J. L. O’Neill, S. R. Feldman, Drugs Today 2010, 46, 351–60; b) M. A. Maestro, F. Molnár, C. Carlborg, J. Med. Chem. 2019, 62, 6854–6875.
[7] L. Verlinden, A. Verstuyl, M. Van Camp, S. Marcelis, K. Sabbe, X.-Y. Zhao, P. De Clercq, M. Vandewalle, R. Bouillon, Cancer Res. 2000, 60, 2673–2679.

[8] P. Gogoi, S. Seoane, R. Siguéiro, T. Guiberteau, M. A. Maestro, R. Pérez-Fernández, N. Rochel, A. Mourriño, J. Med. Chem. 2018, 61, 4928–4937.

[9] R. M. Evans, S. M. Lippman, Cell Metab. 2020, 32, 704–709.

[10] a) N. Rochel, J. M. Wurtz, A. Mitschler, B. Klaholz, D. Moras, Mol. Cell 2000, 5, 173–179; b) G. Tocchini-Valentini, N. Rochel, J. M. Wurtz, A. Mitschler, D. Moras, Proc. Nat. Acad. Sci. 2001, 98, 5491–5496; c) F. Ciesielski, N. Rochel, D. Moras, J. Steroid Biochem. Mol. Biol. 2007, 103, 235–242; d) C. Carlberg, F. Molnár, A. Mourriño, Expert Opin. Ther. Pat. 2012, 22, 417–435.

[11] a) B. Figadère, A. W. Norman, H. L. Henry, H. P. Koeffler, J-Y. Zhou, W. H. Okamura, J. Med. Chem. 1991, 34, 2452–2463; b) A. S. Craig, A. W. Norman, W. H. Okamura, J. Org. Chem. 1992, 57, 4374–4380; c) E. D. Collins, J. E. Bishop, C. M. Bula, A. Acevedo, W. H. Okamura, A. W. Norman, J. Steroid Biochem. Mol. Biol. 2005, 94, 279–288.

[12] a) J. A. Martínez-Pérez, L. Sarandeses, J. Granja, J. A. Palenzuela, A. Mourriño, Tetrahedron Lett. 1998, 39, 4725–4728.

[13] R. Riveiros, A. Rumbo, A. Sarandeses, A. Mourriño, J. Org. Chem. 2007, 72, 5477–5485.

[14] A. Fernández-Gacio, C. Vitale, A. Mourriño, J. Org. Chem. 2000, 65, 6978–6983.

[15] For a review on the chemistry of allenes, see: J. Ye, S. Ma, Acc. Chem. Res. 2014, 47, 989–1000.

[16] a) X. Pérez-García, A. Rumbo, M. J. Larriba, P. Ordóñez, A. Muñoz, A. Mourriño, Org. Lett. 2003, 5, 4033–4036; b) N. Rochel, S. Hourai, X. Pérez-García, A. Rumbo, A. Mourriño, D. Moras, Arch. Biochem. Biophys. 2007, 460, 172–176; c) R. Siguéiro, M. A. Maestro, A. Mourriño, Org. Lett. 2018, 20, 2641–2644.

[17] a) P. Gogoi, R. Siguéiro, S. Eduardo, A. Mourriño, Chem. Eur. J. 2010, 16, 1432–1435; b) D. Carballa, R. Siguéiro, Z. Rodríguez-Docampo, F. Zaccioni, M. A. Maestro, A. Mourriño, Chem. Eur. J. 2018, 24, 3314–3320.

[18] R. R. Fernandes, A. K. Chowdhury, P. Kattanguru, Eur. J. Org. Chem. 2014, 2014, 2833–2871.

[19] E. Moman, D. Nicoletti, A. Mourriño, J. Org. Chem. 2004, 69, 4615–4625.

[20] C. Fernández, O. Díouf, E. Momán, G. Gómez, Y. Fall, Synthesis 2005, 10, 1701–1705.

[21] B. M. Trost, J. Dumas, M. Villa, J. Am. Chem. Soc. 1992, 114, 9836–9845.

[22] T. Ishiyama, T. Murata, M. Miyaura, J. Org. Chem. 1995, 60, 7508–7510.

[23] For a review on the chemistry of allenes, see: J. Ye, S. Ma, Acc. Chem. Res. 2014, 47, 989–1000.

[24] D. Rovito, A. Y. Belorusova, S. Chalhoub, A. I. Rerra, E. Guiot, A. Molin, A. Linglart, N. Rochel, G. Laverny, D. Metzger, Nat. Commun. 2020, 11, 6249–6260.