



HAL
open science

Structural analysis and biological activities of C25-amino and C25-nitro vitamin D analogs

Uxía Gómez-Bouzó, Anna Y Belorusova, Marcos L Rivadulla, Hugo Santalla, Lieve Verlinden, Annemieke Verstuyf, Maria J Ferronato, Alejandro C Curino, Maria M Facchinetti, Yagamare Fall, et al.

► **To cite this version:**

Uxía Gómez-Bouzó, Anna Y Belorusova, Marcos L Rivadulla, Hugo Santalla, Lieve Verlinden, et al.. Structural analysis and biological activities of C25-amino and C25-nitro vitamin D analogs. *Bioorganic Chemistry*, 2023, 136, pp.106528. 10.1016/j.bioorg.2023.106528 . hal-04274216

HAL Id: hal-04274216

<https://hal.science/hal-04274216>

Submitted on 7 Nov 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Structural analysis and biological activities of C25-amino and C25-nitro vitamin D analogs

Uxía Gómez-Bouzó,¹ Anna Y Belorusova,² Marcos L. Rivadulla,¹ Hugo Santalla,¹ Lieve Verlinden,³ Annemieke Verstuyf,³ Maria J Ferronato,⁴ Alejandro C Curino,⁴ Maria M Facchinetti,⁴ Yagamare Fall,¹ Generosa Gómez^{1,*} and Natacha Rochel^{2,*}

¹ Departamento de Química Orgánica and Instituto de Investigación Sanitaria Galicia Sur (IISGS), Campus Lagoas Marcosende, Universidad de Vigo, 36310 Vigo, Spain

² Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC); Institut National de La Santé et de La Recherche Médicale (INSERM), U1258; Centre National de Recherche Scientifique (CNRS), UMR7104; Université de Strasbourg, Strasbourg, Illkirch 67400, France

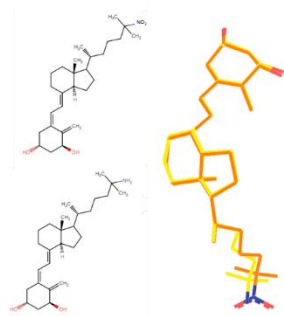
³ Clinical and Experimental Endocrinology, Department of Chronic Diseases and Metabolism, KU Leuven, 3000 Leuven, Belgium

⁴ Laboratorio de Biología del Cáncer, Instituto de Investigaciones Bioquímicas Bahía Blanca (INIBIBB-CONICET), Centro Científico Tecnológico Bahía Blanca, Bahía Blanca, Argentina

ABSTRACT

Intense synthetic efforts have been directed towards the development of noncalcemic analogs of 1,25-dihydroxyvitamin D₃. We describe here the structural analysis and biological evaluation of two derivatives of 1,25-dihydroxyvitamin D₃ with modifications limited to the replacement of the 25-hydroxyl group by a 25-amino or 25-nitro groups. Both compounds are agonists of the vitamin D receptor. They mediated biological effects similar to 1,25-dihydroxyvitamin D₃, the 25-amino derivative being the most potent one while being less calcemic than 1,25-dihydroxyvitamin D₃. The in vivo properties of the compounds make them of potential therapeutic value.

GRAPHIC abstract



1. Introduction

The bioactive form of vitamin D, 1α -25-dihydroxyvitaminD₃ ($1,25(\text{OH})_2\text{D}_3$) plays a key role in calcium homeostasis, and displays anti-proliferative and anti-inflammatory activities [1-3]. $1,25(\text{OH})_2\text{D}_3$ decreases the severity of the symptoms in preclinical models of autoimmune diseases and various types of cancer, but supra-physiological doses of $1,25(\text{OH})_2\text{D}_3$ required for such therapeutic effects induce hypercalcemia and mineralization of various tissues [1-3]. $1,25(\text{OH})_2\text{D}_3$ act via activation of the transcription factor VDR, an endocrine receptor and member of the superfamily of nuclear receptors [4]. To control gene expression, VDR binds as a heterodimer with retinoid X receptors (RXRs) to DNA sites and recruit co-regulatory proteins, such as Med1 and p160 family members, and the specific composition of this complex will result in promoter- and/or tissue-selective activities [5-7].

$1,25(\text{OH})_2\text{D}_3$ binds to VDR hydrophobic residues lining the ligand binding pocket (LBP) and to polar residues that anchor the ligand and form hydrogen bonds with the three hydroxyl groups of the ligand. The 1α -OH group interacts with Y143 (helix H1) and S278 (helix H5), the 3β -OH group contacts S237 (helix H3) and R274 (helix H5), and the 25-OH group interacts with H305 (loop between helices H6 and H7) and H397 (helix H11) [8]. In order to potentiate beneficial properties of $1,25(\text{OH})_2\text{D}_3$ and reduce its calcemic effects, thousands of analogs were synthesized based on medicinal chemistry approach [9], however few of them are of clinical interest [10,11]. Majority of these synthetic VDR ligands are direct derivatives of $1,25(\text{OH})_2\text{D}_3$ and have a similar binding mode to VDR as $1,25(\text{OH})_2\text{D}_3$ with the three OH

groups of each analog similarly interacting to the anchoring residues. Structural modifications induced by selective moieties (e.g. 20-epi, fluoro, oxolane or cyclopropyl groups, geminal methyl groups in the side chain, additional side chain) stabilize the agonist conformation of the complex through an increase of the constraint of the ligand and of the strength of the contacts with the VDR LBP [12-14].

In the present study, we investigated 1,25(OH)₂D₃ analogs with limited modifications corresponding to the replacement of the C25-OH group by a C25-NH₂ or C25-NO₂ groups [15] (Fig. 1). Drugs containing amino or nitro groups may change the hydrogen binding properties and have been widely used in medicinal chemistry [16,17]. We characterized the biological activity and the structures of the two analogs in complex with VDR ligand binding domain (LBD).

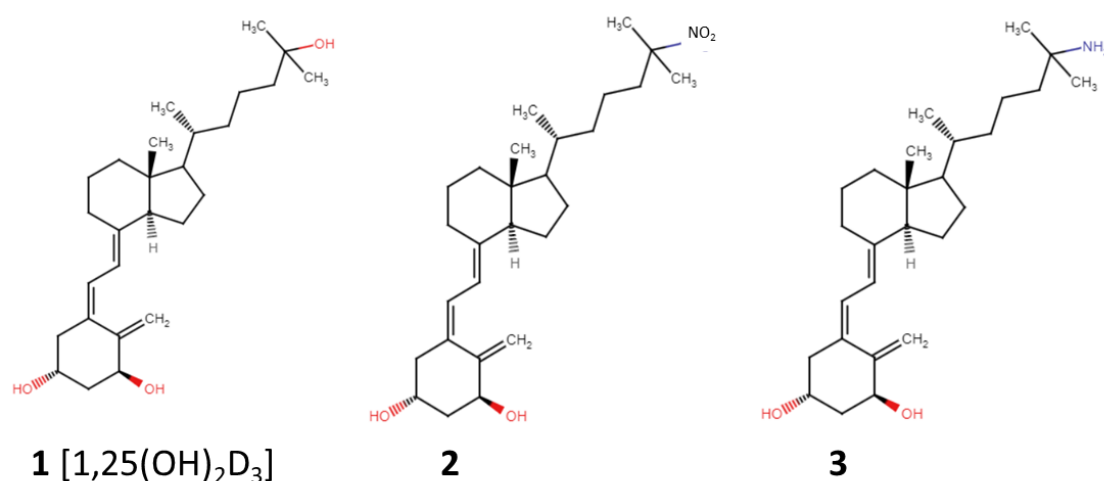


Figure 1. Structures of 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃, **1**), 25-nitro derivative (**2**) and 25-amino derivative (**3**).

2. Results and Discussion

2.1. Crystal structures of VDR- complexes with 1,25(OH)₂D₃ derivatives with C25-nitro or C25-amino groups.

To compare the binding mode of the C25-amino and C25-nitro compounds into the VDR LBD to that of the natural compound with a 25-hydroxyl group, we determined the crystal

structures of their complexes with zebrafish wild-type VDR LBD [18] in complex with the ligand and a coactivator peptide by X-ray crystallography. We have previously demonstrated by x-ray crystallography and hydrogen-deuterium exchange coupled to mass spectrometry that zVDR LBD and human VDR LBD bound to the natural hormone adopt the canonical agonist conformation, and that the binding mode of the ligand is similar in the zVDR LBD and in the hVDR LBD as well as in the full length hVDR [18-20], validating zVDR as an appropriate model for VDR structural studies. The structures were refined with a resolution up to 2.1 Å. The data collection and refinement statistics of the structure are summarized in Supplementary Table 1. The overall structures are highly homologous to the VDR-1,25(OH)₂D₃ structure with a root mean square deviations of 0.4 Å over 235 residues when comparing the C α atoms for the two complexes. Superposition of the VDR LBP in the presence of the analogs and 1,25(OH)₂D₃ shows similar positioning of the ligands (Fig. 2A). The C1-OH and C3-OH of **2** and **3** form similar H-bonds as 1,25(OH)₂D₃ (Fig. 2B-C).

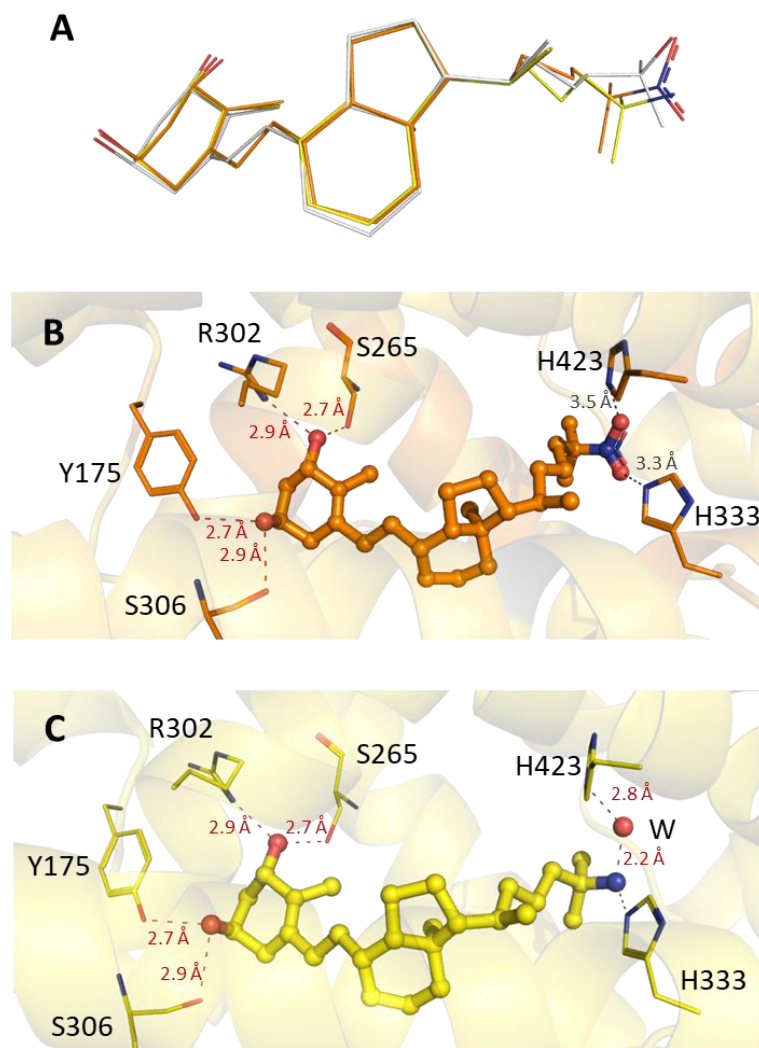


Figure 2. Crystal structures of zVDR LBD complexes. (A) Ligands **2** (orange) and **3** (yellow) superimposed with 1,25(OH)₂D₃ (PDB 2HC4) (grey). (B-C) Hydrogen bonds formed by compounds **2** (B) and **3** (C) are shown by red dotted lines. Red sphere corresponds to a water molecule.

Differences in interactions are only observed around the C-25 modified moieties. The larger NO₂ group compared to the OH group induces a slightly different position of geminal methyl groups (Fig. 3A) and form weaker interactions with the two anchoring histidines, His333 and His423 (3.3 Å and 3.5 Å for analog **2** compared to 2.8 Å and 2.8 Å for 1,25(OH)₂D₃). The C25-amino group of compound **3** is positioned differently compared to the hydroxyl group of 1,25(OH)₂D₃. The amino group is shifted by 1.5 Å compared to the hydroxyl group to allow a

water molecule to mediate interaction between NH_2 and His423 (Fig. 3B). The other histidine, His333, forms a direct hydrogen bond with the NH_2 group of analog **3**. The geminal methyl groups of **3** form stronger interactions with Leu258 and Leu440 and weaker interactions with Leu255, Tyr427 and Leu430 compared to $1,25(\text{OH})_2\text{D}_3$. Overall, the structural data indicate that the two compounds stabilize the agonist conformation of VDR and the C-25 amino and nitro groups efficiently replace the C-25 OH group and form hydrogen bonds with His333 and His423 that act as hydrogen bonds acceptor and donor, respectively. However, both analogs form weaker interactions with anchoring histidines compared to the natural ligand, in agreement with the expected strength of hydrogen bonding ($\text{OH} > \text{NH}_2 > \text{NO}_2$).

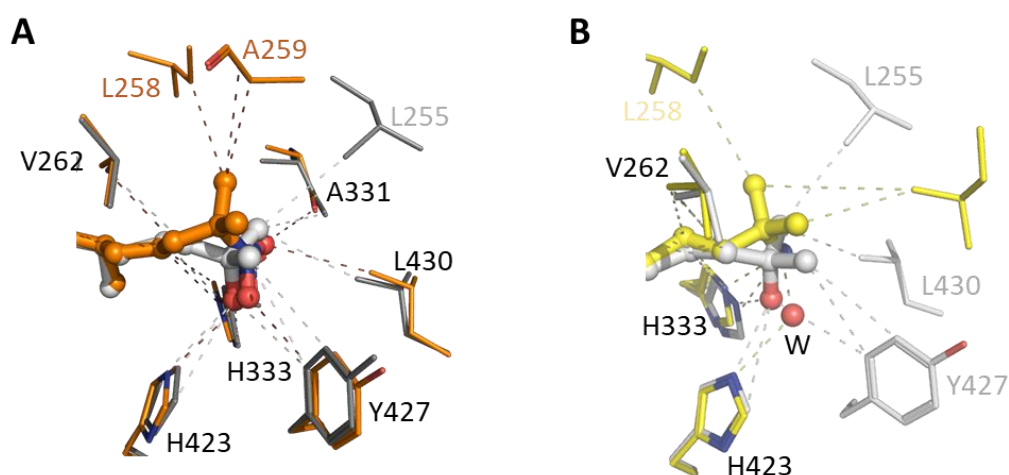


Figure 3. Details of the interactions mediated by the terminal groups of the side chains of $1,25(\text{OH})_2\text{D}_3$ (grey) and of the compounds **2 (A) and **3** (B) with residues of the zVDR LBD at a 4 Å distance cutoff.**

2.2. Biological activities.

To estimate agonistic properties of the C25-amino and C25-nitro analogs, we performed a transactivation assay in HEK293 cells using hCYP24-Tk-Luc reporter plasmid. The two compounds activate the reporter gene transcription at concentrations 10 times higher than $1,25(\text{OH})_2\text{D}_3$ but with similar efficacies at high doses, in comparison to $1,25(\text{OH})_2\text{D}_3$ (Fig. 4A).

Ligand **3** appears to be the most potent compound among the two compounds, displaying transcriptional activity at 10 nM.

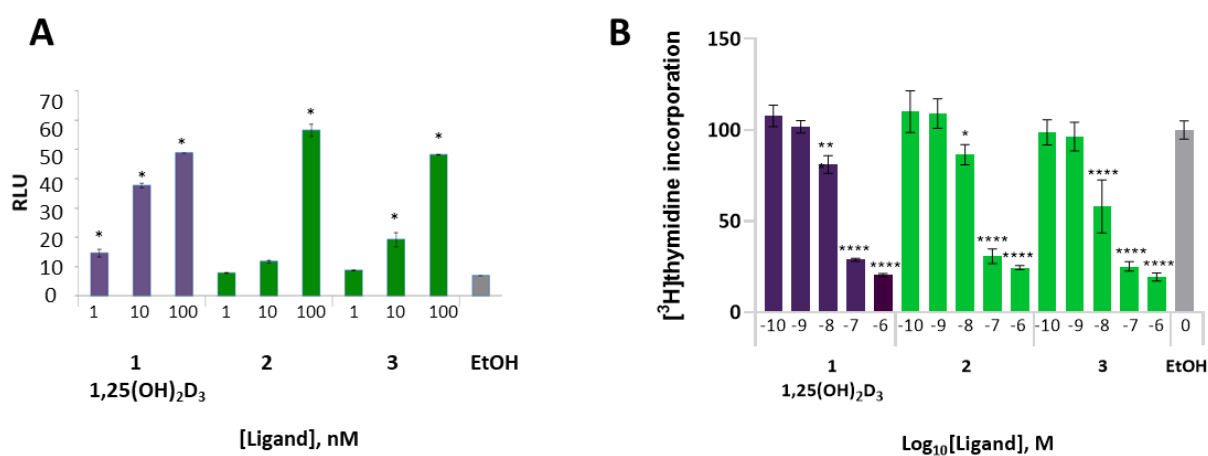


Figure 4. Transactivating and anti-proliferative effects of the C25-amino and C25-nitro analogs. (A) Relative activity of a reporter gene under the control of the promoter region of CYP24A1 in KEK293 cells transiently transfected with VDR. Cells were treated for 24 h with the indicated ligand. Normalized amounts of the expressed luciferase are presented in relative light units (RLU) intensity. n = 3 biological replicates/condition. * p < 0.05 vs. vehicle. (B) Cell proliferation in human breast adenocarcinoma MCF-7 cells after treatment with **1**, **2** and **3** for 72 h.

Subsequently, the antiproliferative activity of the C25-amino and C25-nitro analogs was investigated in estrogen receptor positive MCF-7 breast cancer cells. At high concentrations, compounds **2** and **3** were as potent as 1,25(OH)₂D₃ in inhibiting the proliferation of MCF7 cells, as demonstrated by the reduced incorporation of [³H]thymidine (Fig. 4B). At a concentration of 10 nM compound **3** was slightly more potent in reducing cell proliferation, in comparison to 1,25(OH)₂D₃.

Finally, we evaluated the calcemic activity of the most active analog, the C25-amino compound **3**, by the analysis of plasma calcium concentrations. Either 1,25(OH)₂D₃, compound **3** or vehicle were administered at continuous doses of 5 µg/Kg of body weight by intraperitoneal injections in CF1 mice. As shown in Figure 5, compound **3** maintained plasma

calcium within normal levels during 96 h and provoked hypercalcemia after 11 days of administration. Instead, 1,25(OH)₂D₃ induced an increase in plasma calcium levels at 24 h of administration which was maintained for a period of 72 h finally causing the death of the animals, as previously described [21,22].

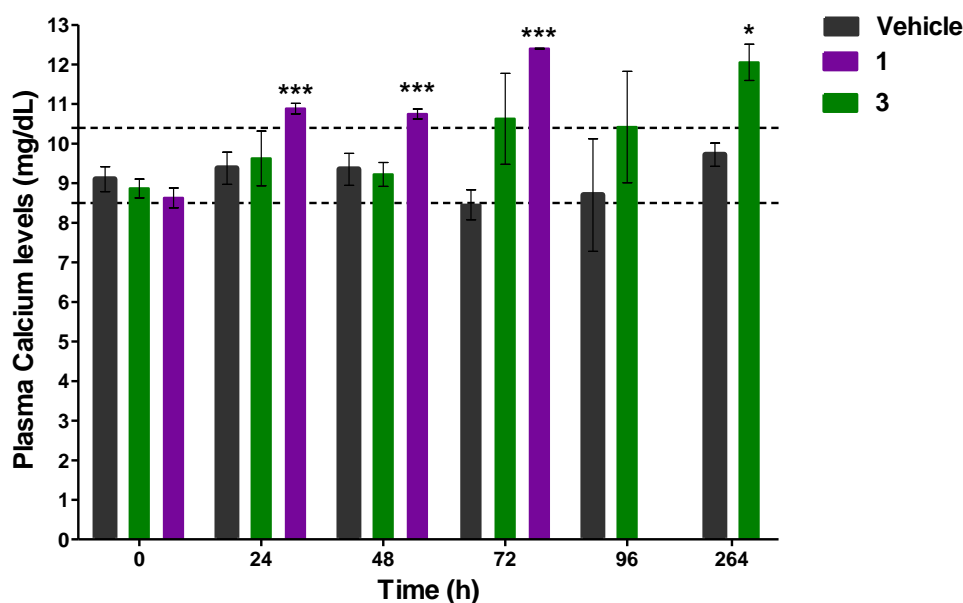


Figure 5. Effect of 25-amino compound 3 on plasma calcemic levels of CF1 mice. The animals were injected intraperitoneally with continuous doses of 5 µg/Kg of 1, 3 or vehicle over 11 days. The graphs show the mean ± SD. Values for 1 beyond 72 h were not available because the animals died following 3 days of treatment. Two-way ANOVA and Bonferroni posttest were applied. Dotted lines indicate normal range of total calcium values. *p < 0.05 and ***p < 0.001.

Overall our results show that the 25-amino or 25-nitro derivatives mediated biological effects similar to 1,25(OH)₂D₃, the 25-amino derivative being the most potent one. In contrast, previously described analogs with 1-amino or 3-amino groups were shown to bind poorly to VDR [23]. While the 1-amino derivative exhibited 30% biologic activities compared to 1,25(OH)₂D₃, the 3-amino analog was not active [23]. Replacement of the 25-OH group, although less critical than the replacement of the 2 other hydroxyl groups [24], allows to maintain significant biological activities while being normocalcemic. It remains to be clarified the exact mechanism underlying the dissociated profile of the 25-amino derivative.

3. Conclusion

In summary, we have characterized the binding mode to VDR and biological properties of two analogs of the hormone $1\alpha,25$ -dihydroxyvitamin D_3 with modifications limited to the replacement of the C-25 hydroxyl group by an amino or a nitro groups. The crystallographic structures of both compounds in complex with the VDR LBD efficiently stabilize the active VDR conformation. Biological evaluation indicates that both compounds are VDR agonists and mediated biological effects similar to $1,25(OH)_2D_3$. The 25-amino derivative appears to be the most potent one. Interestingly, replacement of the hydroxyl group by an amino reduces the calcemic activity of the compound.

4. Materials and methods

Ligands. The synthesis of ligands **2** and **3** was previously reported [15]. Ligands were dissolved in ethanol at 10^{-2} M and stored at -20 °C.

4.1. Crystallization and structure determination

Expression of zebrafish VDR LBD (residues 156–453) and the following purification were done by the procedure reported previously [18]. The protein was concentrated using Amicon ultra-30 (Millipore) to 3-7 mg/ml and incubated with a two-fold excess of ligand and a three-fold excess of the coactivator SRC-1 NR2 (686-RHKILHRLQLQEGSPS-700) peptide (IGBMC peptide synthesis common facility). Crystals were obtained in 50 mM Bis-Tris pH 6.5, 1.6 M lithium sulfate and 50 mM magnesium sulfate. Protein crystals were mounted in a fiber loop and flash-cooled under a nitrogen flux after cryo-protection with 20% glycerol. Data collection from a single frozen crystal was performed at 100 K on the PX2A beamline at SOLEIL (France). The raw data were processed with XDS [25] and scaled with AIMLESS [26] programs. The crystals belong to the space group $P6_522$, with one LBD complex per asymmetric unit. The structures were solved and refined using Phenix [27] and iterative

model building using COOT [28]. Crystallographic refinement statistics are presented in Supplementary data.

4.2. Biological evaluation

Tansactivation assay. HEK 293 EBNA cells were used for the luciferase gene reporter assay. Cells were plated into 24-well plates at 10^5 cells per well and grown overnight in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% charcoal- treated fetal bovine serum (FCS) and 40 $\mu\text{g}/\text{mL}$ gentamycin. At 80% confluence of the cells, 1 μg of pDNA was transfected per well using jetPEI (Polyplus transfection). Transfection was performed according to the manufacturer's instructions. For the luciferase gene reporter assay, HEK293 EBNA cells were transfected with 150 ng of the expression plasmid pSG5-hVDR encoding the full-length receptor, 150 ng of the CYP24a1 luciferase reporter plasmid, 3ng of the pRL plasmid (Promega) containing the Renilla luciferase gene (transfection and cell viability control), and 697 ng of the carrier plasmid pBlueScript (Stratagene). Eight hours post transfection, the analogs or vehicle were added. Cells were harvested after eighteen hours of incubation with ligands. The amounts of reporter gene product (firefly luciferase) and constitutively expressed Renilla luciferase produced in the cells were measured using Dual-Luciferase® Reporter Assay System (Promega) on a luminometer plate reader LB96P (Berthold Technologies). Luminescence of firefly luciferase values was normalized to the Renilla luciferase activity. Luciferase activities were expressed as relative light unit (RLU) intensities. Assays were performed in triplicate. For every triplicate, the mean and the standard deviation of the mean were calculated.

Proliferation. The human breast carcinoma (MCF-7) cell line was obtained from the American Tissue Culture Company (Rockville, MD). The antiproliferative activity of $1,25(\text{OH})_2\text{D}_3$ or the C25-amino and C25-nitro analogs on MCF-7 cells was assessed by evaluating [^3H]thymidine incorporation. Cells were seeded in 96-well plates (10,000 cells/well) and 1 μCi [methyl- ^3H]thymidine (ICN Biomedicals, Costa Mesa, CA) was added 72 h after the initiation of treatment. Cells were semi-automatically harvested after an additional 4 h of incubation on filterplates only retaining incorporated thymidine (GF/C Filter and

Filtermate Universal Harvester, Packard Instrument, Meriden, CT). Counting was performed using a microplate scintillation counter (Topcount, Packard).

Calcemic activity. Inbred normal CF1 mice aged 8 –10 weeks and weighing 40 g were obtained from the animal facility of the Biology, Biochemistry and Pharmacy Department of the Universidad Nacional del Sur (Bahía Blanca, Argentina) and treated in accordance with the institutional animal care and use committee guidelines. Mice were intraperitoneally injected with compound 1 (n=5), compound 3 (n=5) or vehicle (n=6) with nine total doses (five consecutive doses in one week and four consecutive doses in the following week), Blood samples were collected in heparinized capillary tubes from mice anesthetized with Acedan® (Holliday Scott, Argentina) 0.22 mg/Kg body weight (basal levels as well as at 24, 48, 72, 96 h and 264 h time points). Samples were held on ice, protected from light and processed at 4° C. Plasma was separated by centrifugation at 10 x g and stored at –20°C. Calcium concentration was determined using Ca-Color Arsenazo III AA kit (Wiener Lab, Argentina), measuring the absorbance at 650 nM using a spectrophotometer. The calcium concentration was calculated from calcium standards provided by the manufacturer. To adjust for differences in hemolysis among samples, blanks were prepared and the absorbance reading was subtracted from the test reading. The data was analyzed with Prism 5.0 (GraphPad Prism Software) and statistical significance was evaluated using the Student's t-test. A p value of less than 0.05 was considered significant.

Analysis of the results. One-way multiple comparison ANOVA test in concert with Uncorrected Fisher's LSD posttest was used to compare ligand-treated groups with the vehicle control using Prism V 7.0 (GraphPad Inc). The significance threshold was set at $p < 0.05$.

Associated content

Supporting Information (SI). Crystallographic refinement statistics of VDR complexes. These data can be found online at <https://doi.org/>

Declaration of Competing Interest

The authors declare no competing financial interest.

Acknowledgement

This research was funded by Agence Nationale de la Recherche (ANR-13-BSV8-0024-01 and ANR-21-CE17-0009-01) and by Fondation pour la Recherche Médicale (FRM-FDT20140930978), and supported by institutional funds from Instruct-ERIC for support and the use of resources of the French Infrastructure for Integrated Structural Biology (ANR-10-LABX-0030-INRT and ANR-10-IDEX-0002-02). We thank the IGBMC cell culture facility, P. Eberling for peptide synthesis, and A. McEwen for help in X-ray data collections. We thank the staff of SOLEIL for assistance in using the beamlines.

References

- [1] D. Bikle, S. Christakos, S. New aspects of vitamin D metabolism and action — addressing the skin as source and target. *Nat. Rev. Endocrinol.* 16 (2020) 234–252.
- [2] J.C. Fleet. The role of vitamin D in the endocrinology controlling calcium homeostasis. *Mol Cell Endocrinol.* 453 (2017) 36-45.
- [3] D. Goltzman D. Functions of vitamin D in bone. *Histochem Cell Biol.* 149(4) (2018) 305-312.
- [4] C. Carlberg. A Pleiotropic Nuclear Hormone Labelled Hundred Years Ago Vitamin D. *Nutrients.* 15(1) (2022) 171.
- [5] C. Carlberg. Vitamin D and Its Target Genes. *Nutrients.* 14(7) (2022) 1354.
- [6] J.W. Pike, M.B. Meyer. New Approaches to Assess Mechanisms of Action of Selective Vitamin D Analogues. *Int J Mol Sci.* 22(22) (2021) 12352.
- [7] N. Rochel. Vitamin D and Its Receptor from a Structural Perspective. *Nutrients.* 14(14) (2022) 2847.
- [8] N. Rochel, J.M. Wurtz, A. Mitschler, B. Klaholz, D. Moras. The crystal structure of the nuclear receptor for vitamin D bound to its natural ligand. *Mol Cell.* 5(1) (2000) 173-179.
- [9] M.A. Maestro, S. Seoane. The Centennial Collection of VDR Ligands: Metabolites, Analogs, Hybrids and Non-Secosteroidal Ligands. *Nutrients.* 14(22) (2022) 4927.
- [10] C. Leyssens, L. Verlinden, A. Verstuyf. The future of vitamin D analogs. *Front Physiol.* 5 (2014) 122.
- [11] G. Jones, M. Kaufmann. Update on pharmacologically-relevant vitamin D analogues. *Br J Clin Pharmacol.* 85(6) (2019) 1095-1102.
- [12] A.Y. Belorusova, N. Rochel. Structural Studies of Vitamin D Nuclear Receptor Ligand-Binding Properties. *Vitam Horm.* 100 (2016) 83-116.
- [13] M.A. Maestro, F. Molnár, A. Mouriño, C. Carlberg. Vitamin D receptor 2016: novel ligands and structural insights. *Expert Opin Ther Pat.* 26(11) (2016) 1291-1306.
- [14] A.Y. Belorusova, D. Rovito, Y. Chebaro, S. Doms, L. Verlinden, A. Verstuyf, D. Metzger, N. Rochel, G. Laverny. Vitamin D Analogs Bearing C-20 Modifications Stabilize the Agonistic Conformation of Non-Responsive Vitamin D Receptor Variants. *Int J Mol Sci.* 23(15) (2022) 8445.

- [15] a) M.L. Rivadulla, X. Pérez-García, M. Pérez, G. Gómez, Y. Fall. Pd-allylic substitution mediated synthesis of 25-amino vitamin D3 derivatives. *Tetrahedron Letters* 54 (2013) 3164-3166. b) M.J. Ferronato, D.J. Obiol, E.N. Alonso, J.A. Guevara, S.M. Grioli, M. Mascaró, M.L. Rivadulla, A. Martínez, G. Gómez, Y. Fall, M.A. Quevedo, A.C. Curino, M.M. Facchinetti. Synthesis of a novel analog of calcitriol and its biological evaluation as antitumor agent. *J Steroid Biochem Mol Biol.* 185 (2019) 118-136.
- [16] A. Palmieri. Special Issue "Recent Synthetic Aspects on the Chemistry of Nitro, Nitroso and Amino Compounds". *Molecules.* 22(1) (2016) 9.
- [17] K. Nepali, H.Y. Lee, J.P. Liou. Nitro-Group-Containing Drugs. *J Med Chem.* 62(6) (2019) 2851-2893.
- [18] F. Ciesielski, N. Rochel, D. Moras. Adaptability of the Vitamin D nuclear receptor to the synthetic ligand Gemini: remodelling the LBP with one side chain rotation. *J Steroid Biochem Mol Biol.* 103(3-5) (2007) 235-242.
- [19] Belorusova, A.Y.; Chalhoub, S.; Rovito, D.; Rochel, N. Structural Analysis of VDR Complex with ZK168281 Antagonist. *J Med Chem.* 63 (2020) 9457-9463.
- [20] Rovito, D.; Belorusova, A. Y.; Chalhoub, S.; Rerra, A.I.; Guiot, E.; Molin, A.; Linglart, A.; Rochel, N.; Laverny, G.; Metzger, D. Cytosolic sequestration of the vitamin D receptor as a therapeutic option for vitamin D-induced hypercalcemia. *Nat. Commun.* 11 (2020) 6249.
- [21] M.J. Ferronato, D.G. Salomón, M.E. Fermento, N.A. Gandini, A. López Romero, M.L. Rivadulla, X. Pérez-García, G. Gómez, M. Pérez, Y. Fall, M.M. Facchinetti, A.C. Curino. Vitamin D analogue: potent antiproliferative effects on cancer cell lines and lack of hypercalcemic activity. *Arch Pharm (Weinheim).* 348(5) (2015) 315-329.
- [22] D.G. Salomón, S.M. Grioli, M. Buschiazzo, E. Mascaró, C. Vitale, G. Radivoy, M. Perez, Y. Fall, E.A. Mesri, A.C. Curino, M.M. Facchinetti. Novel alkynylphosphonate analogue of calcitriol with potent antiproliferative effects in cancer cells and lack of calcemic activity. *ACS Med Chem Lett.* 2(7) (2011) 503-508.
- [23] Oves D, Fernández S, Ferrero M, Bouillon R, Verstuyf A, Gotor V. Versatile synthesis and biological evaluation of 1,3-diamino-substituted 1 α ,25-dihydroxyvitamin D3 analogues. *Bioorg Med Chem.* 14(4) (2006) 928-937.
- [24] Ostrem VK, Lau WF, Lee SH, Perlman K, Prah J, Schnoes HK, DeLuca HF. Induction of monocytic differentiation of HL-60 cells by 1,25-dihydroxyvitamin D analogs. *J Biol Chem.* 262(29) (1987) 14164-14171.
- [25] W. Kabsch. XDS. *Acta Crystallogr D Biol Crystallogr.* 66(Pt 2) (2010) 125-132.
- [26] P. Evans. Scaling and assessment of data quality. *Acta Crystallogr D Biol Crystallogr* 62(Pt 1) (2006) 72-82.
- [27] P.V. Afonine, R.W. Grosse-Kunstleve, P.D. Adams. A robust bulk-solvent correction and anisotropic scaling procedure. *Acta Crystallogr D Biol Crystallogr.* 61(Pt 7) (2005) 850-855.
- [28] P. Emsley, K. Cowtan. Coot: model-building tools for molecular graphics. *Acta Crystallogr D Biol Crystallogr.* 60(Pt 12 Pt 1) (2004) 2126-2132.