Journal of Steroid Biochemistry & Molecular Biology 121 (2010) 417–419

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

CD-ring modified vitamin D_3 analogs and their superagonistic action☆

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ARTICLE INFO

Article history:
Received 3 November 2009
Accepted 22 January 2010

Keywords:
Non-steroidal
CD-ring analog
Colon cancer
β-Catenin
Coactivator

ABSTRACT

Non-steroidal analogs of 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] represent a most particular class of analogs because they are either not directly derived from the core 1,25$(OH)_2D_3$-structure or they have modifications in the core structure that are so drastic that the steroidal structure is lost. Non-steroidal CD-ring analogs of 1,25$(OH)_2D_3$ have been developed to study the role of the central rigid CD-ring system in the biological activity of 1,25$(OH)_2D_3$. Here we review the different classes of CD-ring analogs and highlight some representative analogs such as the fluorinated D-ring analogs CD578, WU515 and WY1113 which show markedly increased differentiating activity on human SW480-ADH colon cancer cells, characterized by a stronger induction of the invasion suppressor E-cadherin and a stronger repression of the β-catenin/TCF target oncogene c-Myc. Correspondingly, CD578, WU515 and WY1113 are more potent inhibitors of β-catenin/TCF signaling than 1,25$(OH)_2D_3$ and induce stronger VDR–coactivator interactions. Underlying the increased biological potency of analog CD578 are additional contacts between the side chain fluorine atoms of the analog with specific residues of helix 12 (H12) of the Vitamin D Receptor (VDR) and subsequent stronger VDR–coactivator interactions.

1. Introduction

1,25-Dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] is a key regulator of calcium/phosphate levels and bone maintenance and acts through the Vitamin D Receptor (VDR), a member of the nuclear receptor superfamily, which can heterodimerize with the Retinoid X Receptor (RXR) and binds to Vitamin D Response Elements (VDREs) in the promoter region of target genes [1]. Beside the actions on calcium and bone, 1,25$(OH)_2D_3$ has a potent growth-inhibitory or antiproliferative and prodifferentiating action on different cell types including malignant cancer cells, mostly characterized by a G1/S arrest in the cell cycle [2]. It has become clear that, depending on the cell type, 1,25$(OH)_2D_3$ can use different pathways or combinations of pathways to establish its antiproliferative effect. Cyclins, cyclin dependent kinases (CDKs), CDK inhibitors, E2F transcription factors and their binding partners from the Retinoblastoma family are mostly the point of convergence between the different pathways [3].

In theory, its potent antiproliferative action combined with the presence of the VDR in a large array of cell types, would make 1,25$(OH)_2D_3$ a powerful drug to treat hyperproliferative disorders. However, the calcemic actions obstruct the clinical applicability of 1,25$(OH)_2D_3$. Administration of the required doses to fully benefit from the antiproliferative action, can result in severe hypercalcemia, hypercalciuria and bone resorption. For this reason, structural analogs of 1,25$(OH)_2D_3$ with an improved antiproliferative/prodifferentiating action and lower calcemic effects have been designed. The analogs can have modifications in the side chain of 1,25$(OH)_2D_3$, in the central CD-ring system, in the seco-B,A-ring system or can have combinations of modifications in two or even all three of these target regions [3]. Here we review a particular class of non-steroidal analogs which were developed in order to study the importance of the less accessible CD-ring system in the biological actions of 1,25$(OH)_2D_3$.

2. Non-steroidal CD-ring analogs of 1,25$(OH)_2D_3$

Different series of CD-ring modified analogs of 1,25$(OH)_2D_3$ have been designed among which analogs in which the steroidal structure was lost due to the drastic changes in this ring structure (Fig. 1). The most basic non-steroidal compound is the acyclic analog KS018 in which 1,25$(OH)_2D_3$ is completely stripped

☆ Special issue selected article from the 14th Vitamin D Workshop held at Brugge, Belgium on October 4–8, 2009.

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doi:10.1016/j.jsbmb.2010.01.010

to its five carbon backbone. KS018 does not bind to the VDR and lacks all biological activity. Introduction of an unnatural, five-membered 'E-ring' in KS018 (analog KS176) resulted in 10% VDR binding (in comparison with $1,25(OH)_2D_3$), minor antiproliferative action and negligible calcemic effects [4]. Remarkably, re-introduction of the six-membered C-ring (15-*nor* C-ring analogs, e.g. ZG1368) or the five-membered D-ring (9,11-*bisnor* D-ring analogs, e.g. SL117) generally results in an increased dissociation ratio between antiproliferative potency and calcemic effects in comparison with $1,25(OH)_2D_3$ [5]. As such the D-ring analog WU515, which has an additional 23-yne-26,27-F_6 modification, displays an 800-fold increased antiproliferative/calcemic ratio. The best selectivity profile within the C- and D-ring analogs is obtained with the 19-*nor*-16-ene-26,27-bishomo C-ring analog CY616 for which the antiproliferative/calcemic ratio is 1200-fold higher than for $1,25(OH)_2D_3$ [5]. The CE-ring and CF-ring analogs represent two additional classes of non-steroidal analogs in which the D-ring has been deleted and a connection was made between C12 and C21 or between C18 and C21, respectively. The CE-ring analogs (e.g. analogs XU248 and SD65) have very low or do not display antiproliferative actions on MCF-7 human breast cancer cells. The CF-ring analogs (e.g. SW123) have low VDR affinity (<10%) as well as low calcemic actions (<1%) in comparison with $1,25(OH)_2D_3$ [6,7]. SW123 is five times more potent in inhibiting MCF-7 cell proliferation and is more than 200 times less calcemic than $1,25(OH)_2D_3$. The introduction of a 22-oxa-23-yne-26,27-F_6 side chain (analog FDB198E) further enhanced the antiproliferative action and reduced the calcemic effects of SW123 [8].

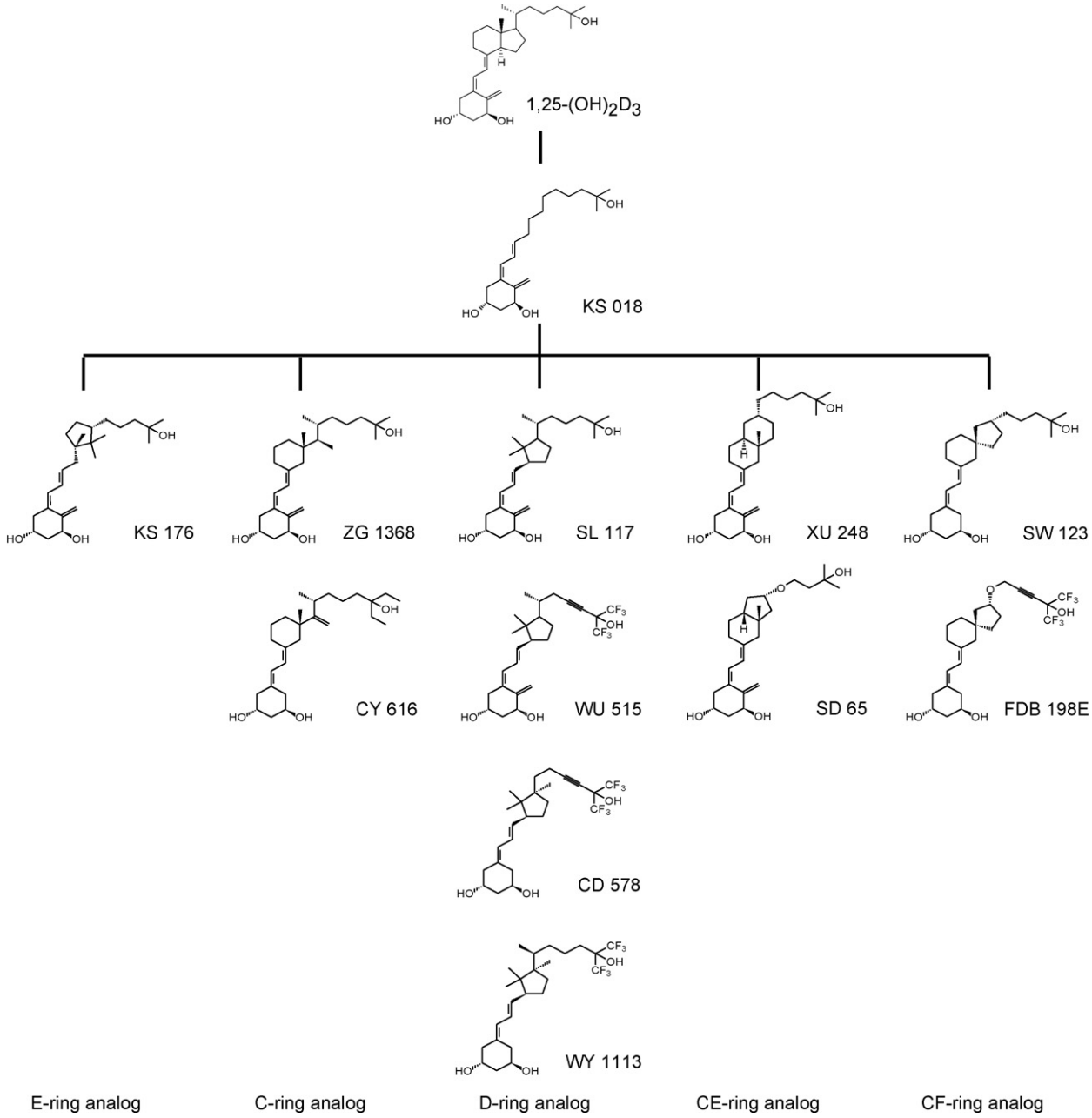

Fig. 1. Non-steroidal analogs of $1,25(OH)_2D_3$ with major modifications in the CD-ring system. In addition to the most basic non-steroidal, acyclic analog KS018 five different classes of non-steroidal analogs are shown with drastic modifications in the CD-ring system. For each class first an analog with the natural A-ring and natural side chain is shown (except for analog SW123 which has a 19-*nor* A-ring structure). Further down are analogs with additional A-ring or side chain modifications.

Table 1
Effects of non-steroidal D-ring analogs CD578, WU515 and WY1113 on β-catenin/TCF transcriptional activity and on VDR–coactivator interactions.

Compound	β-Catenin/TCF transcriptional activity ± SEM	VDR–coactivator (DRIP205) interaction ± SEM
$1,25(OH)_2D_3$	100 ± 17	100 ± 4
CD578	58 ± 3	1504 ± 137
WU515	54 ± 11	1787 ± 19
WY1113	51 ± 7	1645 ± 114

The data for β-catenin/TCF transcriptional activity and VDR–coactivator interaction have been previously described as well as the methods used to obtain the data [9]. All data are expressed relative to the values obtained for $1,25(OH)_2D_3$, which were set to 100%. For all compounds a dose of 10^{-8} M was used. SEM: standard error of the mean.

3. Superagonistic action of D-ring analogs CD578, WU515 and WY1113

Recently, we determined the actions of the three potent non-steroidal D-ring analogs CD578 (17-methyl-19-*nor*-21-*nor*-23-yne-26,27-F_6-$1,25(OH)_2D_3$-D-ring analog), WU515 (23-yne-26,27-F_6-$1,25(OH)_2D_3$-D-ring analog) and WY1113 (17-methyl-19-*nor*-20-*epi*-26,27-F_6-$1,25(OH)_2D_3$-D-ring analog) (Fig. 1) on colon cancer cells [9]. These D-ring analogs with fluorinated side chains have up to 200-fold higher antiproliferative action on MCF-7 breast cancer cells with calcemic actions ranging from equal to those of $1,25(OH)_2D_3$ to 100-fold lower. When treated with $1,25(OH)_2D_3$, SW480-ADH colon cancer cells undergo differentiation characterized by an increase in adhesiveness and the formation of cell islands [10]. CD578, WU515 and WY1113 have the same prodifferentiating action on SW480-ADH cells, albeit at a significantly lower dose than $1,25(OH)_2D_3$. The increased prodifferentiating action of the three analogs is characterized by a stronger induction of the invasion suppressor E-cadherin. Furthermore, treatment of SW480-ADH colon cancer cells with CD578, WU515 or WY1113 leads to a significantly more potent inhibition of β-catenin/TCF transcriptional activity in comparison with $1,25(OH)_2D_3$ (Table 1) and a subsequent lower expression of the β-catenin/TCF target oncogene c-Myc [9]. CD578, WU515 and WY1113 show an at least 2-fold stronger VDR-based transactivation potency in comparison with $1,25(OH)_2D_3$ and all three analogs are stronger inducers of VDR–coactivator interactions than $1,25(OH)_2D_3$ (Table 1). CD578, WU515 and WY1113 are fluorinated on C26 and C27 of the side chain, a chemical modification that is often used to make analogs of $1,25(OH)_2D_3$ more resistant to degradation by CYP24; a C–F bond better withstands cleavage and radical oxidation than a C–H bond. The basic fluorinated analog 26,27-F_6-$1,25(OH)_2D_3$ (falecalcitriol) was shown to be resistant to CYP24-mediated catabolism and had a higher $T_{1/2}$ than the parent compound [11]. Both antiproliferative and calcemic actions are tenfold more potent for falecalcitriol. The abovementioned stronger VDR–coactivator interactions for CD578, WU515 and WY1113 could not be explained by differences in resistance to catabolism between the three analogs and $1,25(OH)_2D_3$ as was demonstrated by the use of VID400, a selective inhibitor of the $1,25(OH)_2D_3$-metabolizing enzyme 24-hydroxylase (CYP24) [12]. Analog CD578 was cocrystallized in complex with the zebrafish (z)VDR and an LXXLL-motif containing SRC-1 peptide. The zVDR Ligand Binding Domain (LBD) was previously shown to be similar to the structure of the human VDR LBD in complex with $1,25(OH)_2D_3$ [13]. In comparison with $1,25(OH)_2D_3$, CD578 looses contact with residue Leu258 of the zVDR due to the missing C-ring. Furthermore, fewer interactions with Trp314 were observed. The fluorine atoms on CD578 interact with Val444 and Phe448 of the activation helix 12 (H12) of the zVDR and with Leu440 in the H11-H12 loop within a distance cutoff of 4.0 Å. $1,25(OH)_2D_3$ makes no contacts with H12 within the same distance cutoff. Stabilization of H12 in the zVDR-CD578 complex through the abovementioned additional contacts, causes the SRC-1 coactivator peptide to make additional contacts with the VDR in the zVDR-CD578 complex as compared to the zVDR-$1,25(OH)_2D_3$ complex [9].

4. Conclusions

The non-steroidal CD-ring analogs of $1,25(OH)_2D_3$ are an ultimate tool to study the impact of drastic changes at the CD-ring system, and of the combination of changes at the CD-ring with other modifications, on the biological activity of $1,25(OH)_2D_3$. The D-ring analogs CD578, WU515 and WY1113, for example, show a significantly stronger differentiating action on human colon cancer cells caused by a stronger inhibition of β-catenin/TCF signaling and more potent induction of VDR–coactivator interactions. In addition, the side chain fluorine atoms on these three analogs not only protect them from CYP24-mediated metabolic degradation but also contribute to increased stability of the activation H12 of the VDR.

Acknowledgements

The authors are supported by grants G.0587.09 and G.0553.06 from the 'Fonds voor Wetenschappelijk Onderzoek' (FWO) and a grant from the 'Stichting tegen Kanker'. G.E. is a postdoctoral researcher for the FWO.

References

[1] A.S. Dusso, A.J. Brown, E. Slatopolsky, Vitamin D, Am. J. Physiol. Renal. Physiol. 289 (1) (2005) F8–28.
[2] K.K. Deeb, D.L. Trump, C.S. Johnson, Vitamin D signalling pathways in cancer: potential for anticancer therapeutics, Nat. Rev. Cancer 7 (9) (2007) 684–700.
[3] G. Eelen, C. Gysemans, L. Verlinden, E. Vanoirbeek, P. De Clercq, D. Van Haver, C. Mathieu, R. Bouillon, A. Verstuyf, Mechanism and potential of the growth-inhibitory actions of vitamin D and analogs, Curr. Med. Chem. 14 (17) (2007) 1893–1910.
[4] A. Verstuyf, L. Verlinden, H. Van Baelen, K. Sabbe, C. D'Hallewyn, P. De Clercq, M. Vandewalle, R. Bouillon, The biological activity of nonsteroidal vitamin D hormone analogs lacking both the C- and D-rings, J. Bone Miner. Res. 13 (4) (1998) 549–558.
[5] A. Verstuyf, L. Verlinden, E. van Etten, L. Shi, Y. Wu, C. D'Halleweyn, D. Van Haver, G.D. Zhu, Y.J. Chen, X. Zhou, M.R. Haussler, P. De Clercq, M. Vandewalle, H. Van Baelen, C. Mathieu, R. Bouillon, Biological activity of CD-ring modified 1alpha,25-dihydroxyvitamin D analogues: C-ring and five-membered D-ring analogues, J. Bone Miner. Res. 15 (2) (2000) 237–252.
[6] S. Demin, D. Van Haver, M. Vandewalle, P.J. De Clercq, R. Bouillon, A. Verstuyf, Synthesis and biological activity of 22-oxa CD-ring modified analogues of 1alpha,25-dihydroxyvitamin D_3: cis-perhydrindane CE-ring analogues, Bioorg. Med. Chem. Lett. 14 (15) (2004) 3885–3888.
[7] W. Schepens, D.V. Van Haver, M. Vandewalle, P.J. De Clercq, R. Bouillon, A. Verstuyf, Synthesis and biological activity of 22-oxa CD-ring modified analogues of 1alpha,25-dihydroxyvitamin D_3: spiro[5,5]undecane CF-ring analogues, Bioorg. Med. Chem. Lett. 14 (15) (2004) 3889–3892.
[8] F. De Buysser, L. Verlinden, A. Verstuyf, P.J. De Clercq, Synthesis of 22-oxaspiro[4.5]decane CD-ring modified analogs of 1α,25-dihydroxyvitamin D_3, Tetrahedron Lett. 50 (2009) 4174–4177.
[9] G. Eelen, N. Valle, Y. Sato, N. Rochel, L. Verlinden, P. De Clercq, D. Moras, R. Bouillon, A. Munoz, A. Verstuyf, Superagonistic fluorinated vitamin D_3 analogs stabilize helix 12 of the vitamin D receptor, Chem. Biol. 15 (10) (2008) 1029–1034.
[10] H.G. Palmer, J.M. Gonzalez-Sancho, J. Espada, M.T. Berciano, I. Puig, J. Baulida, M. Quintanilla, A. Cano, A.G. de Herreros, M. Lafarga, A. Munoz, Vitamin D_3 promotes the differentiation of colon carcinoma cells by the induction of E-cadherin and the inhibition of beta-catenin signaling, J. Cell Biol. 154 (2) (2001) 369–387.
[11] D. Lohnes, G. Jones, Further metabolism of 1 alpha,25-dihydroxyvitamin D_3 in target cells, J. Nutr. Sci. Vitaminol (Tokyo) Spec No (1992) 75–78.
[12] I. Schuster, H. Egger, D. Bikle, G. Herzig, G.S. Reddy, A. Stuetz, P. Stuetz, G. Vorisek, Selective inhibition of vitamin D hydroxylases in human keratinocytes, Steroids 66 (3–5) (2001) 409–422.
[13] N. Rochel, D. Moras, Ligand binding domain of vitamin D receptors, Curr. Top. Med. Chem. 6 (12) (2006) 1229–1241.