

Supporting information

Vitamin D analogs bearing C-20 modifications stabilize the agonistic conformation of non-responsive vitamin

D receptor variants

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Content of SI:

X-ray crystal structure of BXL-62 bound to zVDR LBD. (Figure S1)

Schematic representation of the interactions of BXL-62 with zVDR or with zVDRgem. (Figure S2)

Close-up view around Leu(His)337. (Figure S3)

Distances between C20 atom of the ligand and Cy of H333 and H423. (Figure S4)

Distances between C21 and C21a atoms of BXL-62 and Cy of His333 and His423. (Figure S5)

Data collection and refinement statistics. (Table S1)

Protein - ligand aliphatic side chain distances (in Å) observed in the 1,25D, BXL-62 and Gemini-72 zVDR WT LBD complexes. (Table S2)

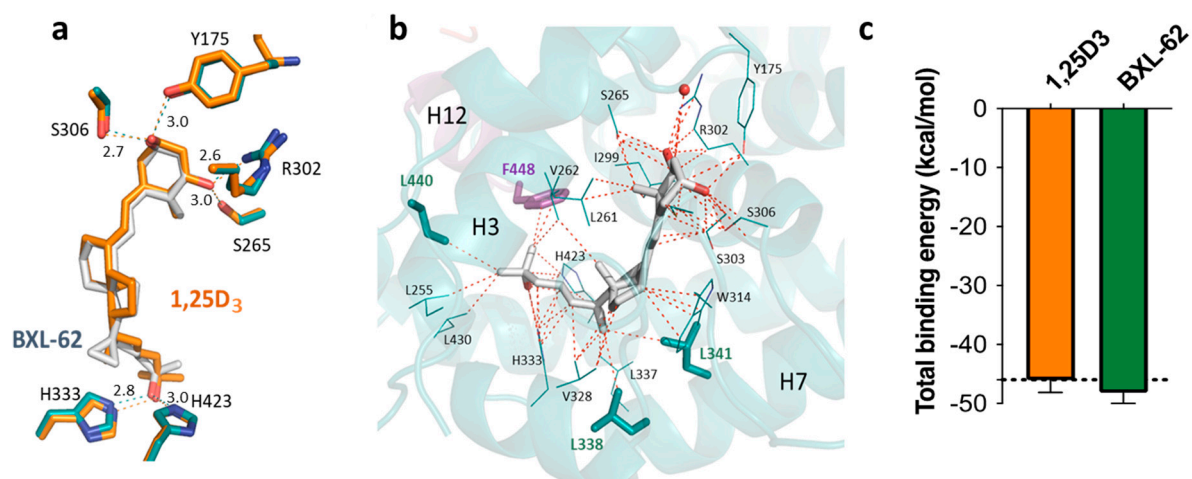
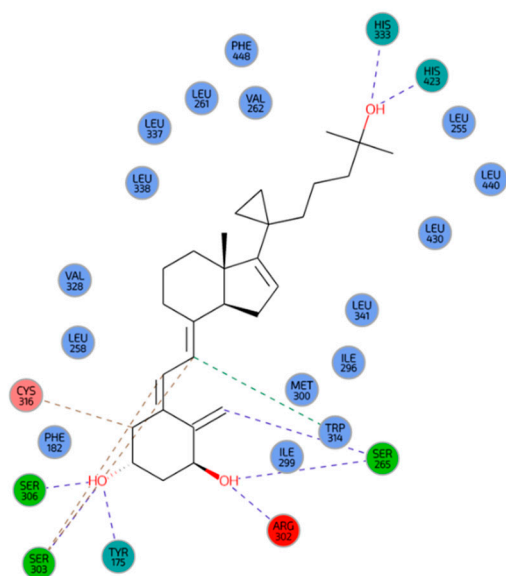


Figure S1: X-ray crystal structure of BXL-62 bound to zVDR LBD. a) Superposition of BXL-62 (gray) and 1,25D₃ (orange). Hydrogen bonds are shown by dotted lines. Distances in Å for hydrogen bonds formed by BXL-62. **b)** Interactions of BXL-62 with VDR residues within 4 Å distance are shown by red dotted lines. Residues that form stronger interactions with BXL-62 than with 1,25D₃ are shown in thicker. **c)** Total binding affinities for BXL-62 and 1,25D₃.

zVDR – BXL-62



zVDRgem – BXL-62

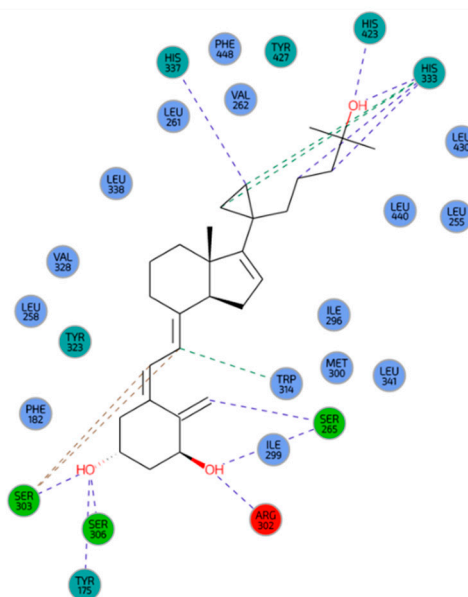


Figure S2. Schematic representation of the interactions of BXL-62 with zVDR, or with zVDRgem. Interactions of BXL-62 with zVDR or zVDRgem residues within 4 Å distance are shown by dotted lines. Color code: blue, hydrophobic residue; cyan, aromatic residue; pink, cysteine; red, positively charged residue; green, polar residues

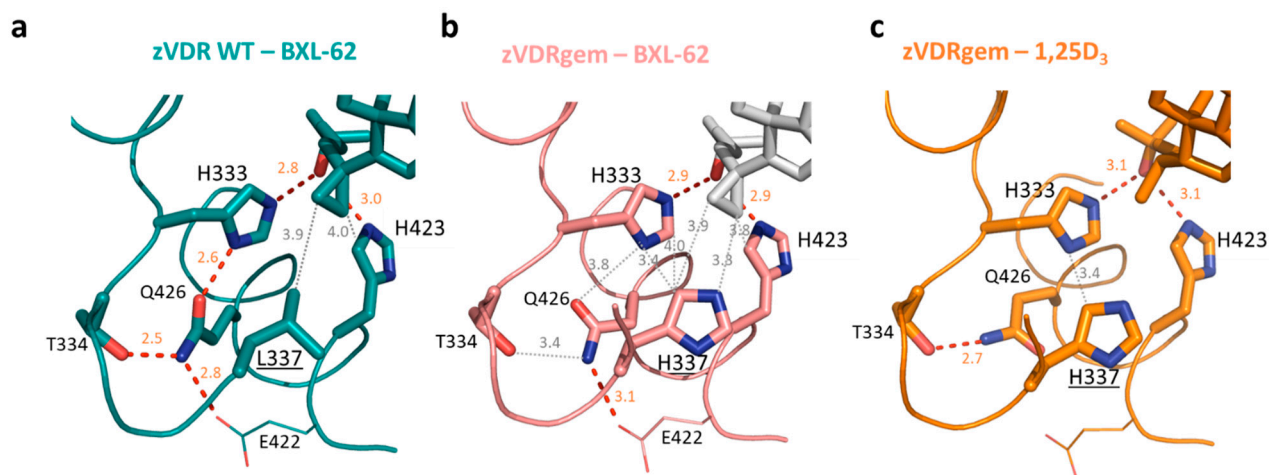


Figure S3: Close-up view around Leu(His)337 in the zVDR WT - BXL-62 (**a**), zVDRgem - BXL-62 (**b**) and zVDRgem – 1,25D₃ (**c**). Grey dashed lines correspond to ligand interactions with VDR residues within 4 Å distance and red dashed lines to hydrogen bonds. Distances are in Å.

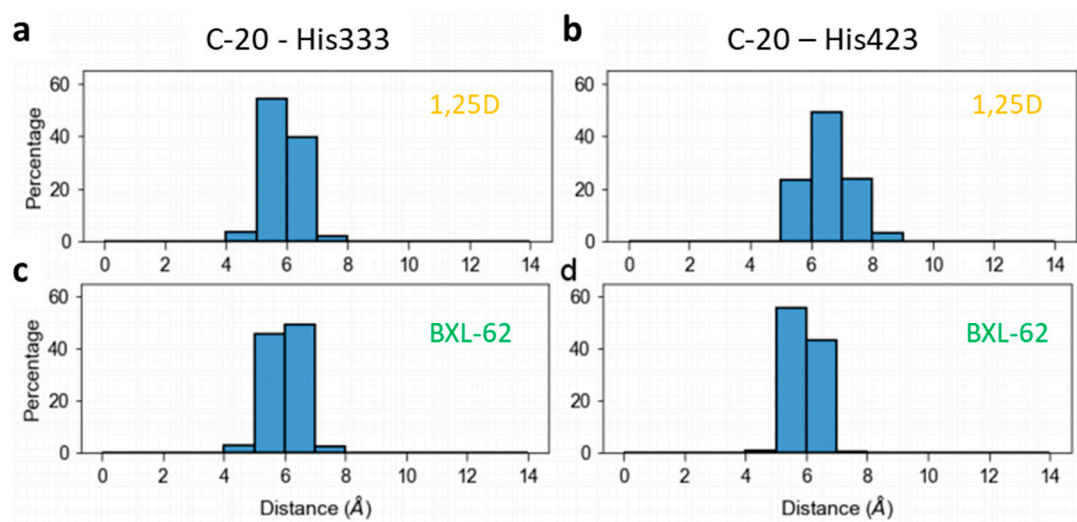


Figure S4: Distances between the C γ of H333 and H423 of VDRgem and the C20 atom of 1,25D₃ (**a,b**) and of BXL-62 (**c,d**).

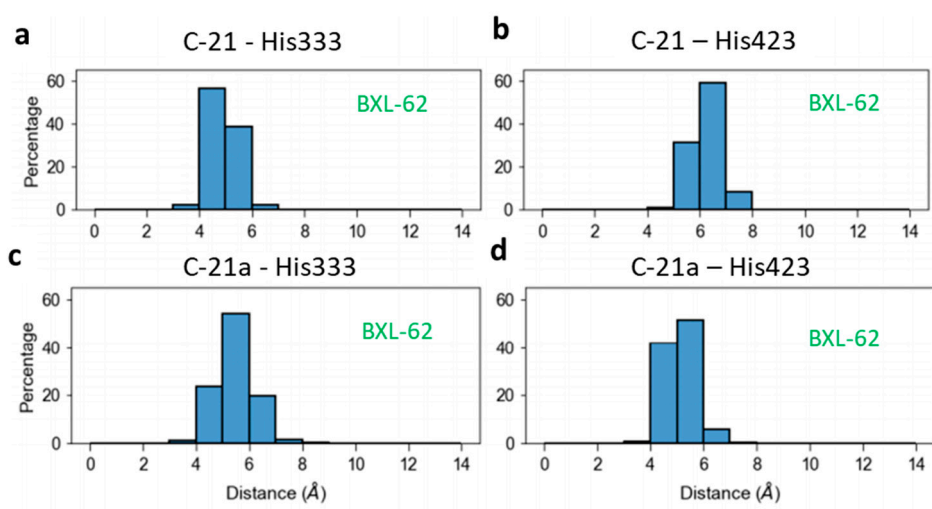


Figure S5: Distances between the C γ of H333 and H423 of VDRgem and the C21 and C21a atoms of 1,25D₃ (a,b) and of BXL-62 (c,d).

Table S1: Data collection and refinement statistics.

	zVDR WT - BXL-62	zVDRgem - BXL-62
PDB ID	7BNS	7BNU
Data Processing		
X-ray source	ID29	ID23-2
λ	0.98 Å	1.00 Å
Temperature	100 K	100k
Resolution (Å)	24.8-2.7	29.4-2.4
Crystal space group	P6 ₅ 22	P6 ₅ 22
Cell parameters (Å)	a=b=65.87; c=263.68	a=b=65.66 ; c=264.14
Unique reflections	9994	14068
Mean redundancy	1.3	2.0
Completeness (%)	98.38	99.17
Mean I/ σ	9.4	12.25
CC _{1/2}	1	1
Refinement		
Resolution (Å)	24.8-2.7	29.4-2.4
Number of non-hydrogen atoms		
Protein	2002	1988
Ligand	31	31
Water molecules	38	72
R _{cryst} (%)	17.94	19.0
R _{free} (%)	26.14	26.1
RMSD bond length (Å)	0.007	0.006
RMSD bond angles (°)	0.84	0.76
Ramachandran plot (%)		
Core	97.52	97.92
Allow	2.48	2.08

Table S2. Protein - ligand aliphatic side chain distances (in Å) observed in the 1,25D₃, BXL-62 and Gemini-72 zVDR WT LBD complexes excluding the interactions with the hydroxyl groups. A cutoff of 4.0 Å has been used. For comparison purposes, contacts with 1,25D₃ greater than 4.0 Å are indicated in italics.

Residue	Atom	zVDR WT			zVDRgem
		1,25D ₃	Gemini-72	BXL-62	BXL-62
		PDB: 2HC4	PDB: 3O1D	PDB: 7BNS	PDB: 7BNU
zLeu255(hLeu227) H3	CD1	C26(3.8)	F4(3.6),F5(3.6),C32(3.9)	C26(3.9)	C26(4.0)
zVal262(hVal234) H3	CG1	<i>C24(4.8)</i>	F2(3.7),F1(3.8)	C27(3.9)	C27(4.0)
	CG2	C24(3.7)	F2(3.3)	C27(3.7)	C27(4.0)
zIle296(hIle268) H5	CD1	> 5	C22(3.8)		
zMet300(hMet272) H5	CE	<i>C21(4.8)</i>	C24(3.5),C26(3.6)		
zVal328(hVal300) H6	CG1	C21(4.0)		C21(3.7),C22(3.9)	C21(3.8),C22(4.1)
zAla331(hAla303) H6	O	<i>C26(4.3)</i>	F6(3.3)		
zHis333(hHis305) L6-7	NE2	C23(3.7),C26(3.7),	F6(3.2),C28(3.7)	C22(3.8),C23(3.4)	C22(3.8),C23(3.5),
	CD2	<i>C24(4.1)</i>	C29(3.5)	C24(3.4)	C24(3.4)
	CE1	C23(3.9),C21(4.0)	C23(4.0),C24(3.8)	C22(3.6),C24(3.9)	C22(3.8),C24(4.1)
zLeu337(hLeu309) H7	CD2	C21(3.7)	C27(4.0)	C21a(3.8)	C21a(3.8)
zHis337 H7	CD2				C21a (3.8)
	NE2				C21a(3.3)
zLeu338(hIle310) H7	CD2	<i>C21(4.6)</i>	C27(3.8)	C21(3.8)	C21(3.9)
zLeu341(hLeu313) H7	CD2	<i>C21(4.4)</i>	C27(3.5)	C21(3.9)	C21(3.5)
zLeu419(hLeu393) H10	O	>5	C26(3.5)		
zHis423(hHis397) H11	NE2	C24(3.9)	F1(3.1),C30(4.0)	C27(3.8)	C27(3.7)
	CD2	<i>C21(4.3)</i>	C22(3.9),C24(3.7)	C21a(3.8)	C21a(3.8)
zLeu430(hLeu404) H11	CD2	C26(3.8)	F5(3.3),F6(3.3), C32(3.9)	C26(3.7)	C26(4.0)
zLeu440(hLeu414)L11-12	CD2	<i>C27(4.3)</i>	F5(3.6)	C26(4.0)	C26(4.1)
zVal444(hVal418) H12	CG1	C27(4.0)	F3(3.7)		
zPhe448(hPhe422) H12	CE2	<i>C26(4.4)</i>	F1(3.4)	C27(3.8)	C27(3.8)