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Novel vitamin D receptor ligands bearing a spherical hydrophobic core structure—Comparison of bicyclic hydrocarbon derivatives with boron cluster derivatives

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ABSTRACT

Vitamin D receptor (VDR) is a nuclear receptor for 1α,25-dihydroxyvitamin D_3 (1α,25$(OH)_2D_3$), and is an attractive target for multiple clinical applications. We recently developed novel non-secosteroidal VDR ligands bearing a hydrophobic *p*-carborane cage, thereby establishing the utility of this spherical hydrophobic core structure for development of VDR ligands. Here, we synthesized two series of novel non-secosteroidal VDR ligands with different spherical hydrophobic cores, that is, bicyclo[2.2.2]octane derivatives and *p*-carborane derivatives, and compared their biological activities in order to examine the difference between the interactions of the C–H hydrocarbon surface and the B–H carborane surface with the receptor. Carborane derivatives exhibited more potent differentiation-inducing activity toward HL-60 cells than did the corresponding bicyclo[2.2.2]octane derivatives. These results suggest that the hydrophobic carborane cage may interact more efficiently than the hydrocarbons with the hydrophobic surface of VDR. This finding further supports the view that carborane structure is a promising option for drug development.

Vitamin D receptor (VDR) is the ligand-inducible nuclear receptor for vitamin D,[1] and is involved in many physiological processes, including calcium and phosphate homeostasis, bone metabolism, immune regulation, cell proliferation and differentiation.[2] VDR is activated by binding of its endogenous agonist, 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$: **1**] (Fig. 1), and regulates expression of specific target genes. VDR and its ligands have significant roles in the pathogenesis and therapy of diseases such as osteoporosis, arthritis, psoriasis and cancers. Thus, VDR is an attractive target for drug discovery, and thousands of VDR ligands have been developed, leading to the clinical application of eldecalcitol (**2**) and maxacalcitol (**3**).[3] Most of the reported synthetic derivatives with high potency as VDR ligands possess the secosteroidal skeleton, like **1**, consisting of the A-ring bearing two hydroxyl groups, a conjugated olefin linker, the CD-ring and a side chain. Although modification of the secosteroid structure has provided highly active analogs, their structural complexity, synthetic inconvenience and chemical instability are disadvantageous for potential clinical application. Therefore, development of novel non-secosteroidal VDR ligands is important, and various non-steroidal analogs of steroid hormones, as well as non-secosteroidal VDR ligands with unique biological activities, such as bisphenol derivative **4**,[4] have been developed in the last decade.

Previously, we obtained novel non-secosteroidal VDR ligands by using dicarba-*closo*-dodecaboranes (carboranes) as the hydrophobic pharmacophore.[5] Carboranes are carbon-containing boron clusters, and have noteworthy chemicophysical characteristics, including spherical geometry and a hydrophobic B–H surface.[6,7] Carboranes can be used as the hydrophobic core of biologically active molecules, especially nuclear receptor ligands, such as androgen receptor ligands,[8] estrogen receptor ligands[9] and retinoids.[10] We developed a potent non-secosteroidal VDR ligand **5** using *p*-carborane (1,12-dicarba-*closo*-dodecaborane), instead of the CD ring of the secosteroid structure of **1**. X-ray crystallographic analysis of the VDR ligand-binding domain (LBD) complexed with **5** revealed that the carborane cage of **5** is surrounded by several hydrophobic amino acid residues in the ligand-binding pocket, and thus, the spherical B–H surface of carborane interacts effectively with hydrophobic sites at the protein surface.[5] Therefore, in the present work, we planned to synthesize and compare two series of novel VDR ligands: one in which the spherical hydrophobic core is a hydrocarbon derivative and the other in which it is a carborane derivative, in order to investigate the difference

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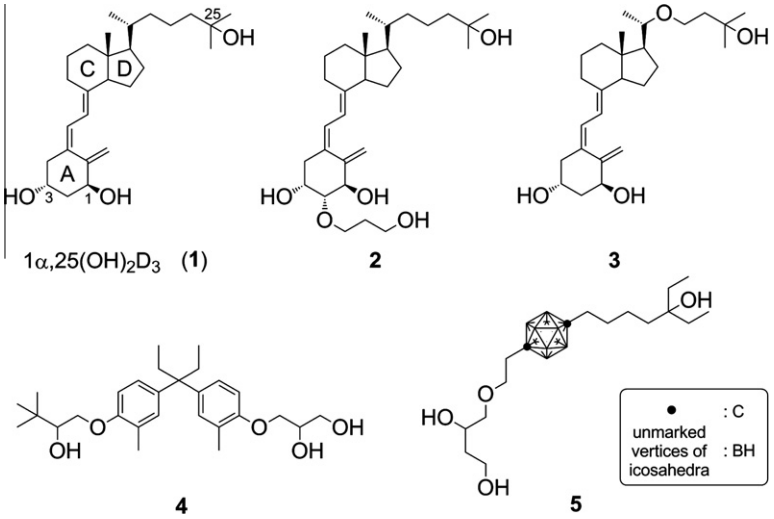


Figure 1. Structures of VDR ligands.

between the interactions of the C–H surface of hydrocarbons and the B–H surface of carborane with the receptor.

Four structural elements are required for effective binding to VDR; one is a hydrophobic core with appropriate bulkiness, and the others are three appropriately positioned hydroxyl groups, as in **1**. As a spherical hydrocarbon core structure of novel VDR ligand candidates, we chose the bicyclo[2.2.2]octane skeleton, based on its spatial volume and its symmetric structure, resembling that of *p*-carborane. As a side chain structure, we chose diethylcarbinol, as in **5**, in place of dimethylcarbinol, as in **1,** based on reports that the diethylcarbinol moiety enhances vitamin D potency.[11] Our previous study indicated that the 1,3-diol moiety of **5** corresponds to the two hydroxyl groups of the A-ring of **1**, and the 1,2-diol structure can also serve this purpose. Therefore, we designed bicyclo[2.2.2]octane derivatives with a 1,2- or 1,3-dihydroxylalkoxyl side chain **6**–**8**, as well as the corresponding *p*-carborane derivatives **9**–**11** (Fig. 2). The designed derivatives have the ether oxygen atom at a different position from the spherical core structure, compared to compound **5**. X-ray analysis of the complex of the VDR–LBD with **5** suggested that the ether oxygen atom of **5** does not form a hydrogen bond to amino acid residues of the receptor, and so the position of the oxygen atom may not affect the vitamin D potency. We anticipated that examination of the structure–activity relationships of compounds **6**–**11** would clarify this point.

Scheme 1 summarizes the synthetic route to the designed bicyclo[2.2.2]octane derivatives using 1,4-bisethoxycarbonylbicyclo[2.2.2]octane **12**[12] as the starting material. Reduction of **12** gave diol **13**, and ether formation using tosylates **14** or **15** corresponding to the 1,3-diol moiety afforded **16** and **17**, respectively. Oxidation of alcohol gave aldehydes **18** and **19**, and then Horner–Wadsworth–Emmons type reaction using phosphonate **20** afforded

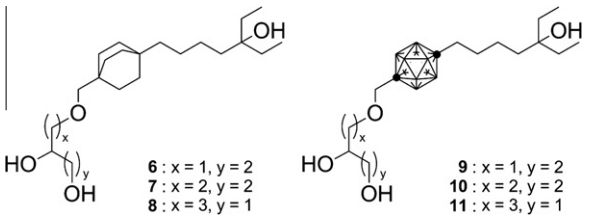
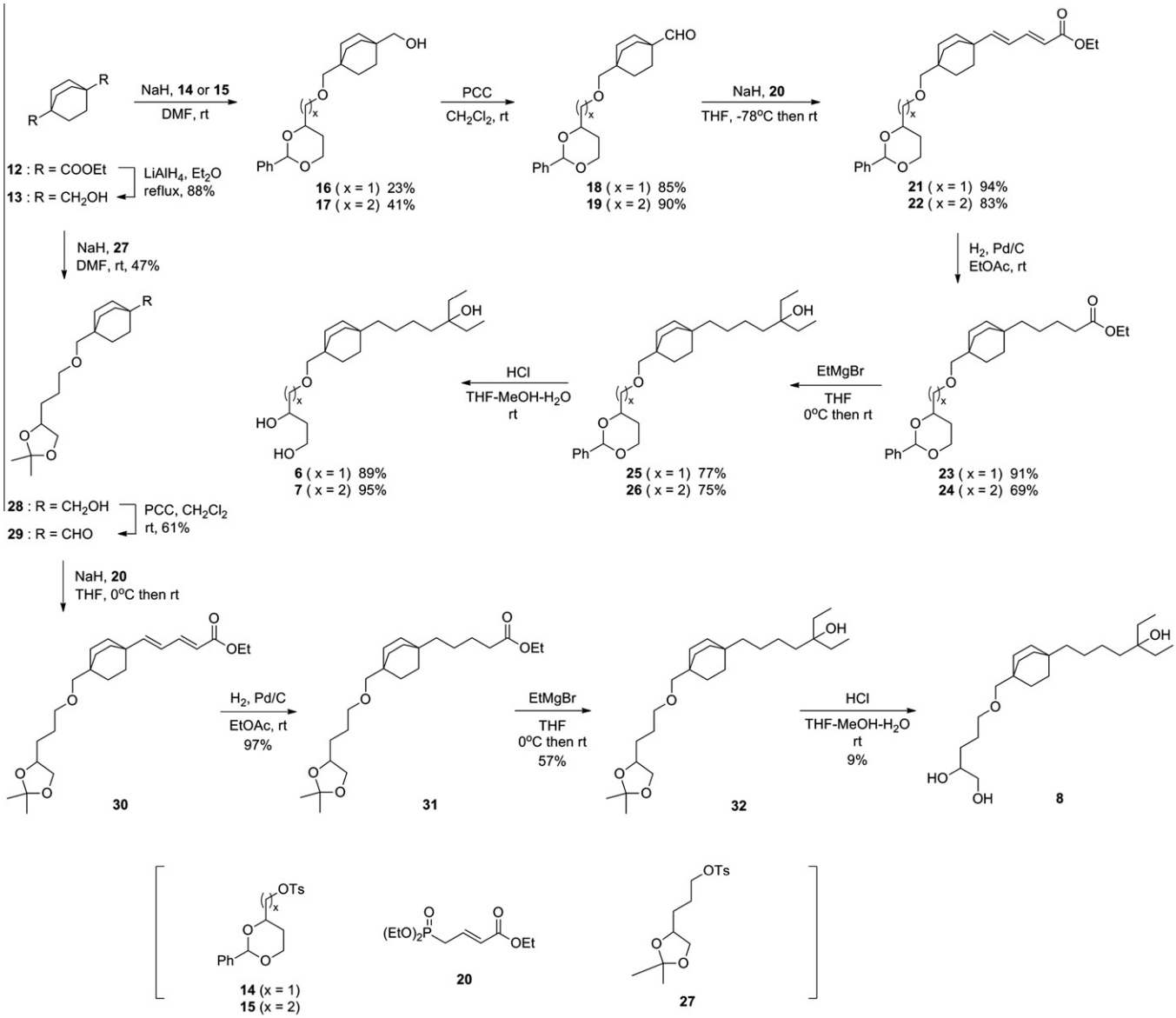


Figure 2. Structures of novel VDR ligand candidates bearing a spherical hydrophobic core.

dienes **21** and **22**, respectively. Hydrogenation, followed by Grignard reaction, gave diethylcarbinol **25** and **26**, and removal of the benzylidene group under acidic conditions gave the target compounds **6** and **7**, respectively. The 1,2-diol derivative **8** was synthesized by a similar method to that used for synthesis of **6** and **7**. Ether formation of **13** using tosylate **27** corresponding to the 1,2-diol moiety afforded **28**, and oxidation of alcohol gave aldehyde **29**. Then, the Horner–Wadsworth–Emmons type reaction afforded diene **30**, followed by hydrogenation, Grignard reaction and removal of the acetonide group to give the target compound **8**, although the yield was low due to the formation of by-product caused by ether cleavage reaction (Scheme 1).

The synthesis of the carborane derivatives is summarized in Scheme 2. Reaction of the C-lithiated form of carborane[13] and paraformaldehyde gave carboranylmethanol **34**, and ether formation using tosylates **14** or **15** afforded **35** and **36**, respectively. The side-chain moiety was introduced at the other carbon atom of carborane using bromide **37** to afford triol precursors **38** and **39**, respectively. Finally, removal of protective groups under acidic conditions gave the target compounds **9** and **10**. The 1,2-diol derivative **11** was similarly synthesized. Ether formation of **34** using tosylate **27** afforded **40**, and introduction of the side-chain part gave **41**. Then, removal of protective groups under acidic conditions gave the target compound **11** (Scheme 2).

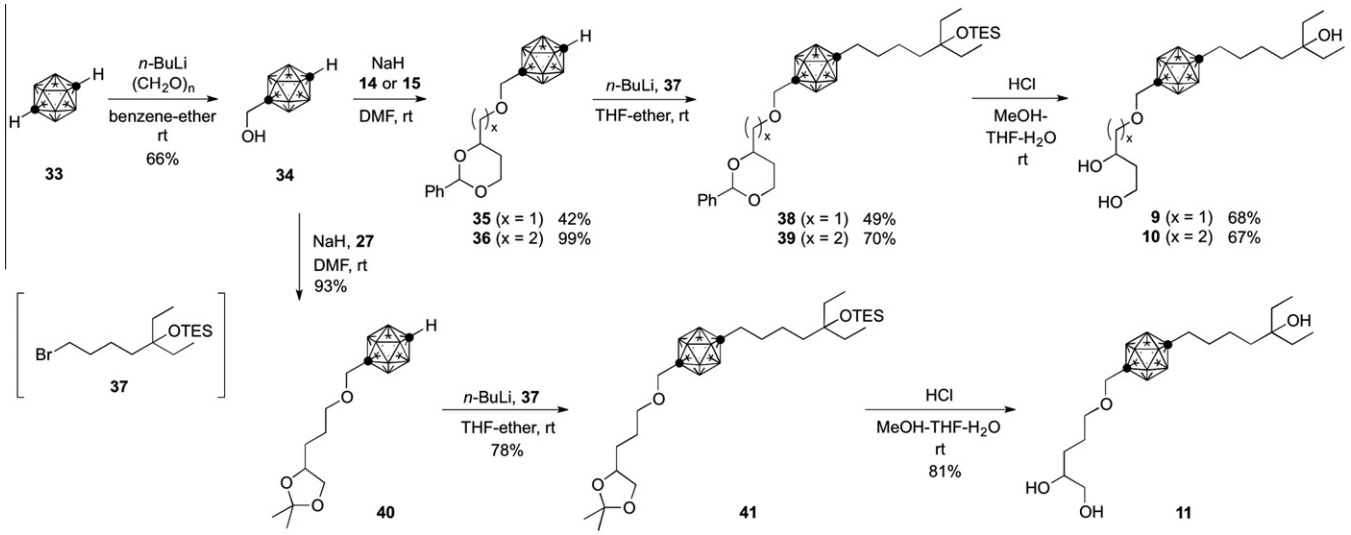
Vitamin D activity of the synthesized molecules was evaluated in terms of cell differentiation-inducing activity toward human acute promyelocytic leukemia cell line HL-60.[14] The bicyclo[2.2.2]octane derivatives exhibited HL-60 cell differentiation-inducing activity at the concentration of 10^{-5} M (Fig. 3A), and the most potent compound **7**, bearing 1,3-diol structure, exhibited moderate activity at the concentration of 10^{-6} M. The activity of compound **7** was weaker than that of **1** by approximately two orders of magnitude. Compound **6** bearing a shorter 1,3-dihydroxylalkoxyl structure than that of **7** and compound **8** with 1,2-diol structure exhibited lower potency than did **7**. On the other hand, the corresponding carborane derivatives exhibited more potent activity in HL-60 cell assay (Fig. 3B). Compound **10** with 1,3-diol structure exhibited the highest HL-60 cell differentiation-inducing potency among the three compounds. The potency of **10** was approximately one-tenth of that of **1**, and **10** is one of the most potent non-secosteroidal VDR ligands so far discovered. Compound **11** with 1,2-diol structure also exhibited potent activity, while compound **9**, bearing a shorter 1,3-dihydroxylalkoxyl structure than that of **10**, showed lower potency.



Scheme 1. Synthesis of bicyclo[2.2.2]octane derivatives.

The synthesized vitamin D analogs containing bicyclo[2.2.2]octane or *p*-carborane as the spherical hydrophobic core structure were found to exhibit differentiation-inducing activity toward HL-60 cells. Thus, the bicyclo[2.2.2]octane skeleton can function as the hydrophobic core structure of VDR ligands, as can *p*-carborane. However, the potencies of the compounds in the two series were quite different. The carborane derivatives exhibited higher activity than the corresponding bicyclo[2.2.2]octane derivatives by about one order of magnitude. It is noteworthy that compounds bearing similar spherical hydrophobic core structures and similarly directed substituents exhibit such different potency. This implies that the hydrophobic B–H surface of the *p*-carborane cage functions as a better anchor to the surface of VDR, compared with the hydrophobic C–H surface of the bicyclo[2.2.2]octane skeleton. The slight difference of spatial volume between these two spherical cores might also influence the ligand activity. In both series, the 3,5-dihydroxypentoxymethyl derivatives, namely **7** and **10**, exhibited the highest activity. The 1,3-diol structure and seven-atom separation between the terminal hydroxyl group and the core structure seem to be the most favorable for VDR agonistic activity, and this is in accord with our previous report on the structure–activity relationships of **5**.[5] Interestingly, compound **10** exhibited potent activity, similar to or greater than that of **5**, though still only about one-tenth or one-twentieth of that of **1**. These results suggest that the position of the ether oxygen atom is not important for the ligand potency. These findings are expected to be useful for further structural development of non-secosteroidal VDR ligands bearing novel hydrophobic core structures.

In conclusion, we have synthesized two series of novel non-secosteroidal VDR ligands bearing different kinds of spherical hydrophobic core structure, that is, bicyclo[2.2.2]octane and *p*-carborane, and demonstrated that both structures can function as the hydrophobic anchor of VDR ligands. Although corresponding compounds in the two series have similar spatial volume and similarly directed substituents, their VDR agonistic potency was quite different. Our results suggest that *p*-carborane is superior to bicyclo[2.2.2]octane as a hydrophobic core of VDR ligands. The precise nature of the difference between these two hydrophobic structures is under investigation. The results reported here are expected to contribute to the further development of non-secosteroidal VDR ligands.



Scheme 2. Synthesis of carborane derivatives.

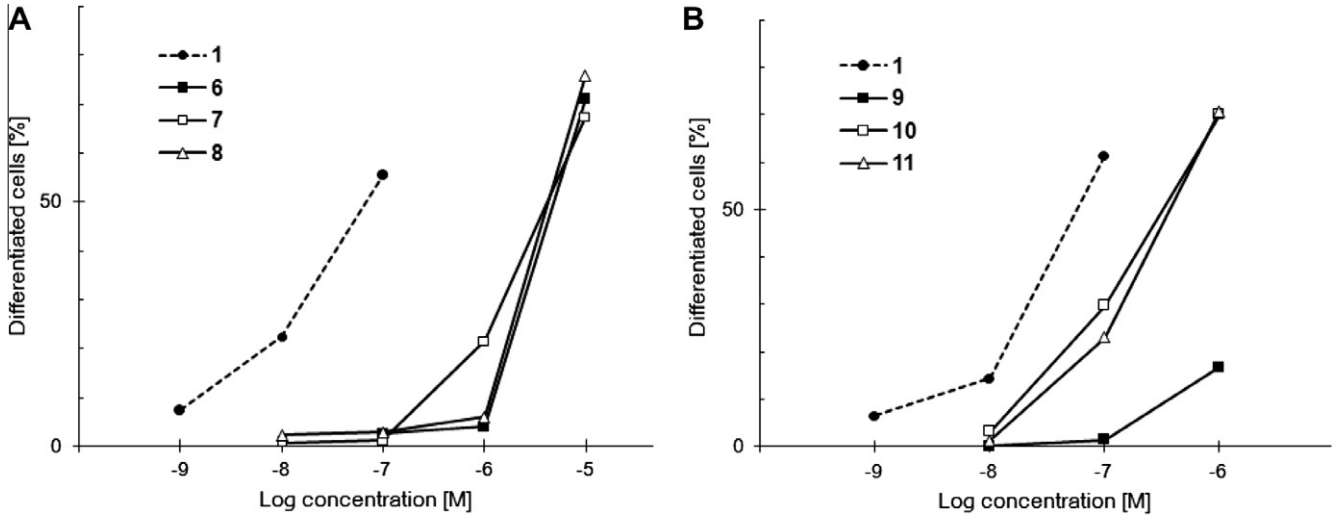


Figure 3. HL-60 cell differentiation-inducing potency of the synthesized bicyclo[2.2.2]octane derivatives (A) and carborane derivatives (B) in the concentration range of 10^{-9} to 10^{-5} M. Cell differentiation was determined as the ratio of NBT-positive cells.

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Supplementary data

Supplementary data (experimental procedures and characterization of compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.12.137.

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