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Structure–Activity Study of Retinoid Agonists Bearing Substituted Dicarba-*closo*-dodecaborane. Relation between Retinoidal Activity and Conformation of Two Aromatic Nuclei

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Abstract—We have investigated the structure–activity relationships of the potent retinoid agonist, 4-[4-(2-propyl-1,2-dicarba-*closo*-dodecaboran-1-yl)phenylamino]benzoic acid (BR403), which we have previously reported. Substitution of a methyl group on the aromatic nucleus or a methyl group on the nitrogen atom, or replacement of the amino group with ether, methylene, carboxyl or 1,1-ethylene greatly decreased the activity. The relatively planar conformation at the phenyl-*N*-phenyl moiety seems to play a critical role in the appearance of the biological activity.

Applications of the unique structural and chemical properties[1] offered by icosahedral carboranes (dicarba-*closo*-dodecaboranes) in the field of biomedical sciences, especially in boron neutron capture therapy (BNCT), have received increasing attention over the past 30 years.[2] We have focused on the possibility of using carboranes as a hydrophobic component in biologically active molecules which interact hydrophobically with receptors. Recently, we have reported examples of the design, synthesis and biological evaluation of nuclear receptor ligands (estrogens)[3] and other biologically active molecules[4] containing a carborane cage as a hydrophobic pharmacophore. We have also reported potent retinoid agonists[5] and antagonists[6] bearing dicarba-*closo*-dodecaborane as a hydrophobic pharmacophore. Among the synthesized compounds, 4-[4-(2-propyl-1,2-dicarba-*closo*-dodecaboran-1-yl)phenylamino]benzoic acid (BR403, **2a**) exhibited potent differentiation-inducing ability toward human promyelocytic leukemia HL-60 cells at the concentration of 10^{-9} M; its potency is almost equal to that of the native ligand, all-*trans*-retinoic acid (**1**) (Fig. 1). The biological activities of retinoids are mediated by binding to and activation of the retinoic acid receptors (RARs),[7] followed by modulation of target gene transcription by the complex. The availability of 3-D structural information[8] has revealed the structural requirements for the appearance of retinoidal activity. High binding affinity for RAR requires a carboxylic acid moiety and an appropriate hydrophobic group, such as in retinobenzoic acid, Am80 (**3**).[9] In addition, the role of the linking group between the hydrophobic and hydrophilic pharmacophores at the ends of the molecule is critical for the appearance of biological activity, especially for subtype selectivity of RARs, and selectivity between RARs and retinoid X receptors (RXRs). Compounds containing the bulky carboranyl group, which may fit the hydrophobic cavity of the RAR ligand binding domain (LBD), should be suitable for investigation of the effect of replacement of the linking group. In this paper, we describe the synthesis of compounds related to **2a**, and the importance of

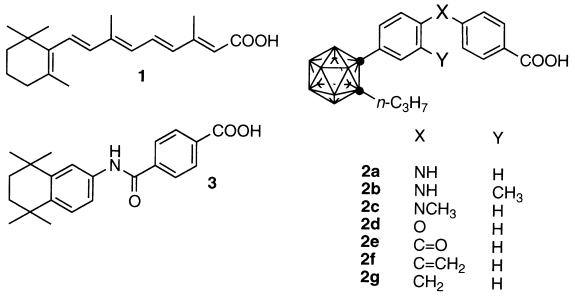

Figure 1. Structures of typical retinoid agonists and designed molecules bearing a carborane moiety. In icosahedral cage structures throughout this paper, closed circles (●) represent carbon atoms and other vertices represent BH units.

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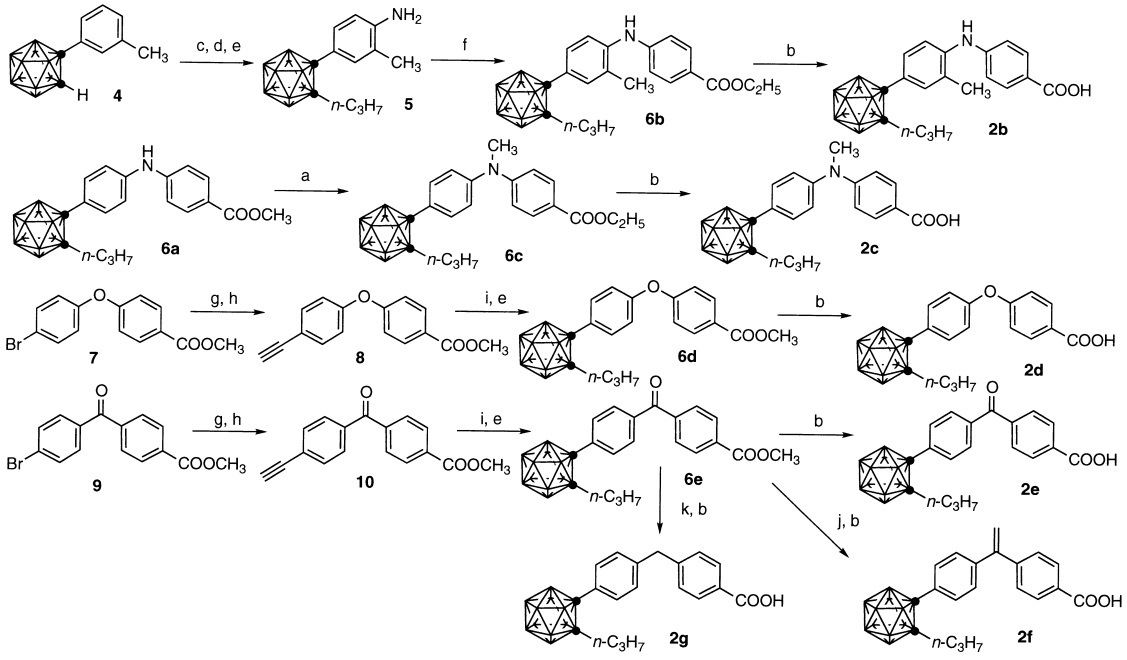
planar conformation at the phenyl-*N*-phenyl moiety for the appearance of the biological activity. We also report the results of transient transactivation assay to show that the retinoidal agonistic activity of the carborane-containing molecules is mediated through RARs.

The syntheses of the designed molecules are summarized in Scheme 1. BR408 (**2b**) was prepared from 1-(3-tolyl)-1,2-dicarba-*closo*-dodecaborane (**4**). Nitration of **4** with HNO_3–H_2SO_4 in CH_2Cl_2 afforded the 4-nitro derivative in 83% yield. After catalytic hydrogenation, an *n*-propyl group was introduced under basic conditions to give **5** (78%). Coupling of the amines with ethyl 4-iodobenzoate catalyzed by tris(dibenzylideneacetone) dipalladium(0) in the presence of (*R*)-BINAP[10] gave the diphenylamine derivative (**6b**) (57%). Hydrolysis of **6b** under acidic conditions afforded 4-[2-methyl-4-(2-*n*-propyl-1,2-dicarba-*closo*-dodecaboran-1-yl)phenylamino]benzoic acid (BR408, **2b**, 68%). BR413 (**2c**) was prepared from methyl 4-[4-(2-propyl-1,2-dicarba-*closo*-dodecaboran-1-yl)phenylamino]benzoate (**6a**),[5] a synthetic intermediate of **2a**, by using sodium hydride and methyl iodide, followed by hydrolysis (48%). The diphenylether congener, BR703 (**2d**) was prepared from methyl 4-(4-bromophenoxy)benzoate (**7**).[11] The bromide **7** was converted to **8** by means of palladium-catalyzed alkynylation with ethynyltrimethylsilane followed by desilylation (56%). Construction of the *o*-carborane cage from the alkyne **8** with *nido*-decaborane(14) followed by introduction of an *n*-propyl group on the carbon atom of carborane afforded **6d** (30%). Hydrolysis of **6d** under acidic conditions afforded 4-[4-(2-*n*-propyl-1,2-dicarba-*closo*-dodecaboran-1-yl)phenoxy]benzoic acid (BR703, **2d**, 95%). Methyl 4-(4-bromobenzoyl)benzoate (**9**)[12] was converted to methyl 4-[4-(2-*n*-propyl-1,2-dicarba-*closo*-dodecaboran-1-yl)benzoyl]benzoate (**6e**) in a manner similar to that described for **6d** (total 36%). Hydrolysis of **6e** under acidic conditions afforded 4-[4-(2-*n*-propyl-1,2-dicarba-*closo*-dodecaboran-1-yl)benzoyl]-benzoic acid (BR713, **2e**, 99%). The compound **6e** was converted to the 1,1-ethylene derivative, BR723 (**2f**) by Wittig olefination (26%), followed by hydrolysis (64%). The compound **6e** was also converted to the methylene derivative BR733 (**2g**) by reduction with triethylsilane under acidic conditions (90%), followed by hydrolysis (quantitative).

The biological activities of the compounds **2a**–**2g** were evaluated in terms of the activity to induce differentiation of HL-60 cells into mature granulocytes. The differentiated cells were identified by nitro blue tetrazolium (NBT) reduction assay.[13] BR403 (**2a**) exhibited a potent differentiation-inducing activity toward HL-60 cells, with an EC_{50} value of 3×10^{-9} M. The activity of **2a** is one order weaker than that of retinobenzoic acid Am80 (**3**), and comparable to that of all-*trans*-retinoic acid (**1**) as shown in Figure 2. Replacement of the *n*-propyl group on the carborane cage with a methyl group (BR401, **11**)[5] decreased the activity. Introduction of a methyl group on the aromatic nucleus (BR408, **2b**) decreased the activity by one order of magnitude. Methylation on the nitrogen atom (BR413, **2c**) also decreased the activity by two orders of magnitude, compared with **2a**. Replacement of the diphenylamine moiety in BR403 (**2a**) with a diphenylether moiety greatly decreased the activity. BR703 (**2d**) exhibited weak differentiation-inducing activity at the concentration of 10^{-6} M, while the cellular response to **2d** at 10^{-6} M (12%) was enhanced to 93% in the presence of 1×10^{-7} M HX630,[14] which is a potent retinoidal syner-



Scheme 1. Synthesis of the designed compounds bearing a carborane moiety. key: (a) (1) NaH/DMF; (2) CH_3I; (b) H_2SO_4/aq dioxane; (c) HNO_3–H_2SO_4/CH_2Cl_2; (d) H_2, Pd–C/C_2H_5OH; (e) (1) NaH/DMF; (2) *n*-C_3H_7I; (f) ethyl 4-iodobenzoate, Cs_2CO_3, $Pd_2(dba)_3$, BINAP/toluene; (g) ethynyltrimethylsilane, $(PPh)_3PdCl_2$, CuI, (*i*-$C_3H_7)_2NH$/THF; (h) K_2CO_3/CH_3OH; (i) decaborane(14), CH_3CN/benzene; (j) $NaNH_2$, $Ph_3P^+CH_3Br^-$/THF; (k) $(C_2H_5)_3SiH$/CF_3COOH–CH_2Cl_2.

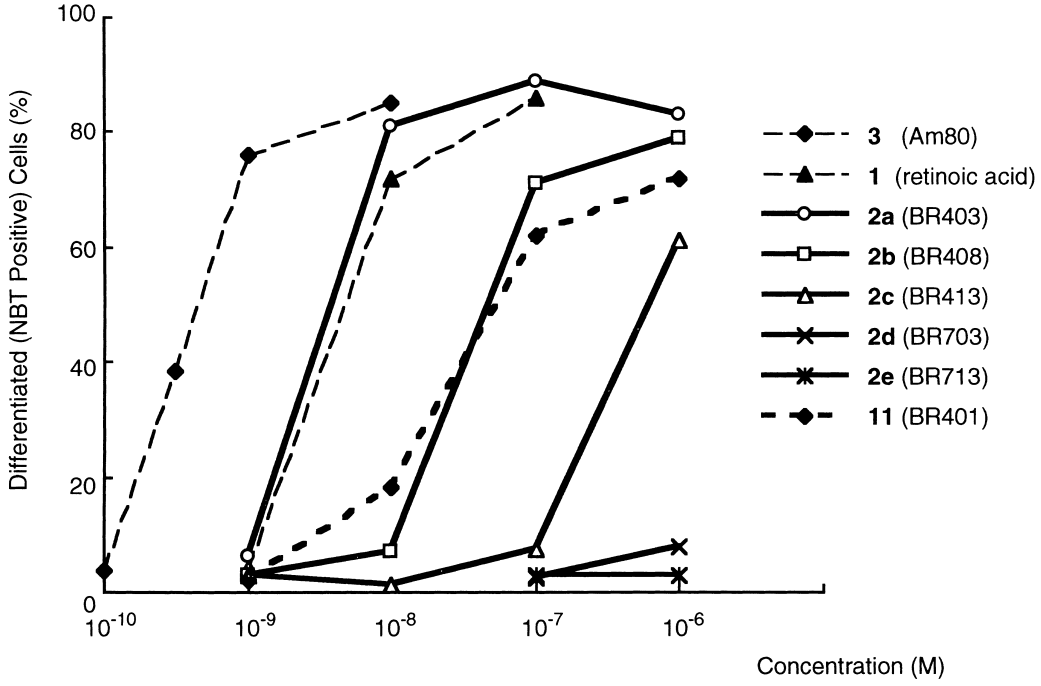


Figure 2. HL-60 differentiation-inducing activity of carborane-containing compounds. **2f** (BR723) and **2g** (BR733) were inactive below 10^{-6} M, as was **2e**.

gist. The compounds having a benzophenone moiety (BR713, **2e**), 1,1-diphenylethylene moiety (BR723, **2f**) and diphenylmethane moiety (BR733, **2g**) no longer exhibited any activity below 10^{-6} M, even in the presence of the retinoidal synergist.

In order to elucidate whether the potency of the active carborane-containing molecules in HL-60 cell assay can be ascribed to their ability to activate retinoid receptors, a transient transactivation assay using RARs was conducted for selected compounds.[15] We selected the most potent carborane-containing retinoid agonist, BR403 (**2a**), a weaker retinoid agonist, BR401 (**11**) (Fig. 3), and a typical retinobenzoic acid, Am80 (**3**). Figure 4 shows transcriptional activation by these compounds. Am80 (**3**) is known to be an RARα, β-selective agonist, and does not activate RARγ.[16] BR401 (**11**) and BR403 (**2a**) also showed RARα, β-selectivity. The results for these three compounds (**3**, **11**, and **2a**), suggest that the order of RARα-transactivation activity is consistent with that of the activity in HL-60 cell differentiation assay.

The substituent effects of a methyl group on the aromatic nucleus or a methyl group on the nitrogen atom may arise from changes in the twisting conformation at the phenyl-*N*-phenyl moiety. The present results suggest that a planar conformation at the phenyl-*N*-phenyl moiety is preferred for an RAR ligand, and that the bulky carboranyl moiety on the benzene nucleus is permitted as the hydrophobic region of the molecule. Docking simulations to RAR would be useful to clarify the relation between the three-dimensional structures and the activity of carborane-containing retinoids. Docking simulations in this study were performed using an automatic docking program (ADAM).[17] The structure of the human RARαLBD model was simulated from the crystal structure of the hRARγLBD complex (2LBD).[8] All-*trans*-retinoic acid (**1**) and Am80 (**3**) were well-fitted to the cavity of the LBD through two types of contacts, hydrogen bonding at the carboxylic acid and hydrophobic van der Waals contacts along the bulky hydrophobic substituents at the end of the molecule, as shown in Figure 5A and B. The most potent carborane-containing retinoid BR403 (**2a**) also fitted well to the cavity of the LBD, as shown in Figure 5C. The bulky alkylated carboranyl moiety fitted well to the hydrophobic region of the LBD. It is noteworthy that the phenyl-*N*-phenyl moiety maintains an almost planar conformation in the cavity, as found for Am80 (**3**). Structural modifications affected the planarity of the two benzene nuclei, resulting in diminution of the activity. An attempt at docking simulation of the *N*-methylated compound BR413 (**2c**) to the LBD failed in the general docking conditions.

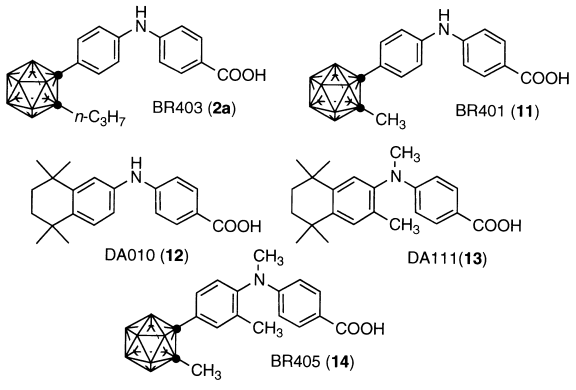


Figure 3. Compounds structurally related to carborane-containing retinoid agonists (**2a**).

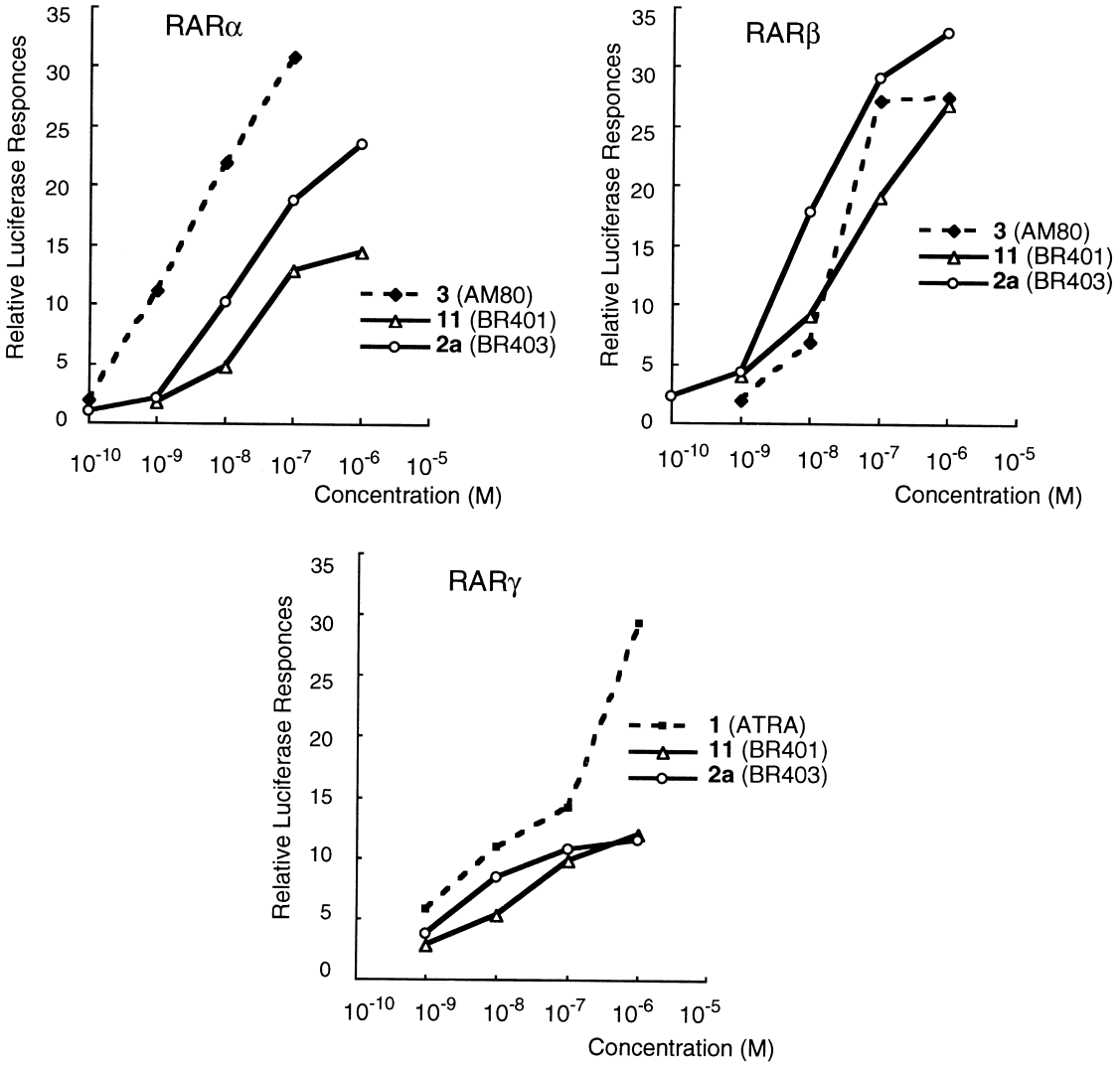


Figure 4. Transcriptional activation by BR401 (**11**) and BR403 (**2a**). COS-1 cells were transfected with TREpal×3-TKLUC and pSG-hRAR and incubated with the compounds at concentration of 10^{-10}–10^{-6} M. The results are shown as averages of triplicate transfections.

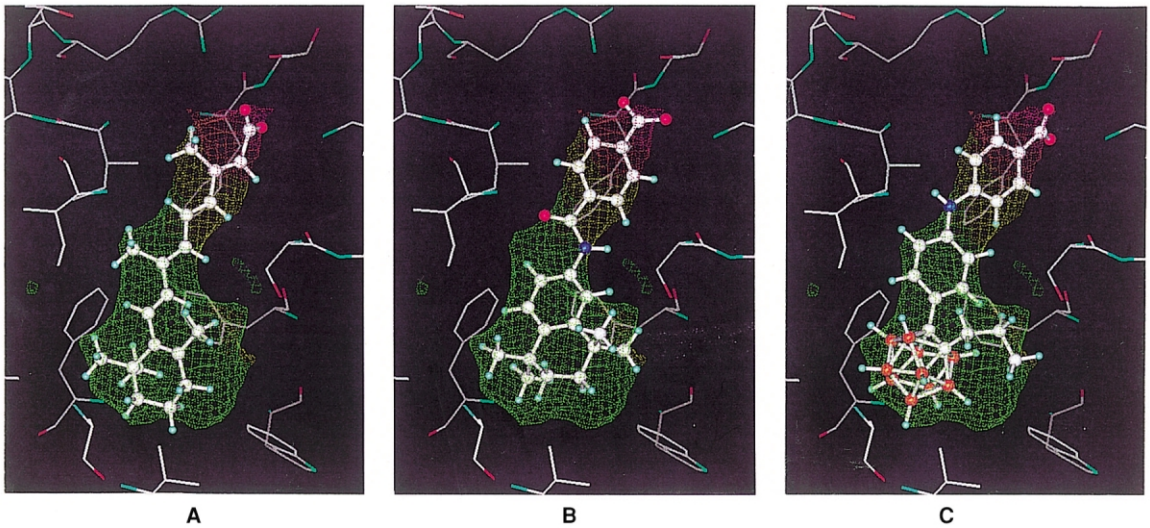


Figure 5. Stable docking models of retinoid agonists. (A) All-*trans*-retinoic acid (**1**); (B) Am80 (**3**); (C) BR403 (**2a**) in the RARα cavity simulated from the crystal structure of RARγ-all-*trans*-retinoic acid (2LBD).[8]

The relationship between the planarity of the two phenyl groups and retinoidal activity is also of interest in connection with RXRs. The RXR ligands (e.g., 9-*cis*-retinoic acid) bind to RXRs and activate RAR-RXR heterodimers, when an RAR agonist binds to RAR site. Thus, RXR ligands alone cannot exhibit retinoid activity, but they can increase the potency of RAR ligands. One of these retinoid synergists with a diphenylamine moiety, DA010 (**12**) (Fig. 3), exhibited weak retinoid activity and significant synergistic (RXR agonistic) activity.[18] Introduction of methyl groups on the aromatic nucleus and/or the nitrogen atom, resulted in disappearance of the retinoid activity and increase of the synergistic activity: DA111 (**13**) is one of the most potent selective RXR activators known.[18] The twisting conformation at the phenyl-*N*-phenyl moiety due to the *N*-alkyl and *ortho*-alkyl moieties is preferred for RXR ligands. However, the carborane-containing compounds **2a**, **11**, and **14**, which is a twisting conformation analogue of **11**, showed no synergistic activity. In contrast, the retinoid agonistic activity of BR403 (**2a**) is two orders stronger than that of DA010 (**12**). The results indicate that the bulky carboranyl moiety on the benzene nucleus fits well to the RAR cavity, but does not fit the RXR cavity. This suggests that the optimum distance between the hydrophobic group and carboxylic acid moiety of an RXR ligand is appreciably shorter than that of an RAR ligand.

In conclusion, we have investigated the structure–activity relationship of novel carborane-containing molecules with potent retinoid agonistic activity. The structural requirements for the activity elucidated in this study should be helpful in the design of RAR subtype-selective compounds and analysis of the distinction between RAR ligands and RXR ligands. Carborane-containing retinoid agonists, with their unique physicochemical properties compared with conventional active molecules, may be candidates for use in retinoid therapy.

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