Dicarba-*closo*-dodecaboranes as a Pharmacophore. Novel Potent Retinoidal Agonists

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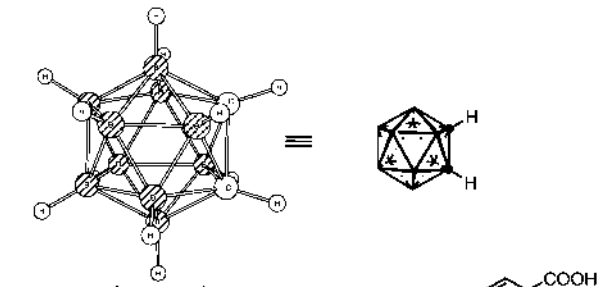
The synthesis and biological evaluation of the dicarba-*closo*-dodecaborane (carborane) derivatives of retinoids are described. Retinoidal activity was examined in terms of the differentiation-inducing ability toward human promyelocytic leukemia HL-60 cells. High retinoidal activity (agonist or antagonist for the retinoid receptor RAR) requires a carboxylic acid moiety and an appropriate hydrophobic group located at a suitable position on the molecule. 4-[4-(1,2-Dicarba-*closo*-dodecaboran-1-yl)phenylamino]benzoic acids and 4-[3-(1,2-dicarba-*closo*-dodecaboran-1-yl)phenylamino]benzoic acids showed potent agonistic activity at concentrations of 10^{-8}—10^{-9} M. The results indicate that carboranes are applicable as the hydrophobic moiety of biologically active molecules.

Key words carborane; dicarba-*closo*-dodecaborane; retinoid; differentiation; hydrophobic moiety

The carboranes (dicarba-*closo*-dodecaboranes)[1] are chemical building blocks of remarkable thermal stability and high boron content, resistant to attack by most types of reagent, and generally inactive toward biological systems. One of their most striking features is the ability of the 2 carbon atoms and 10 boron atoms to adopt icosahedral geometry in which the carbon and boron atoms are hexacoordinated. This feature of the structure gives rise to the unusual properties of such molecules and their carbon and boron derivatives. Their properties make them uniquely suitable for several specialized applications, including synthesis of polymers for high-temperature use and neutron shielding purposes.[2] In the field of medical and pharmaceutical sciences, incorporation of large numbers of boron atoms into tumor cells for boron neutron capture therapy (BNCT)[3] has become a topic of interest in recent years. Most carborane-containing compounds that have so far been synthesized are composed of cellular building blocks (nucleic acid,[4] amino acid,[5] *etc*.) to which carborane units are added. In contrast to the interest in carboranes for BNCT, little attention has been paid to them as building blocks of biologically active compounds. The exceptional hydrophobic character and spherical geometry of carboranes may allow their use as a hydrophobic pharmacophore in biologically active molecules that bind to receptors. Recently, we have reported the first example of the design, synthesis, and biological evaluation of retinoids containing a carborane cage as a hydrophobic pharmacophore.[6] In this article, we describe the synthesis and biological evaluation of potent carborane-containing retinoidal agonists.

Retinoic acid (all-*trans*, **1**) has a broad spectrum of biological activity related to cellular differentiation and proliferation[7] and is essential for normal embryonic development in vertebrates.[8] These biological responses are mediated by binding to and activation of the specific retinoic acid receptors (RARs)[9] followed by modulation of target gene transcription by the complex. The retinoidal actions are also modulated by retinoid X receptors (RXRs), which bind 9-*cis*-retinoic acid.[7] Recent work on the design of synthetic retinoids[10] and the availability of 3D structural information[11] have revealed the structural requirements for the appearance of retinoidal activity, including subtype selectivity. For example, Am80 (**2**), which activates RAR-α and -β, exhibits potent retinoidal activities and HX630 (**3**), which binds to the RXR site of RXR·RAR heterodimers, exhibits potent synergistic activity. Recently, we have reported potent retinoidal synergists such as DA013 (**4b**), which has a diphenylamine skeleton and no retinoid agonistic activity.[12] High binding affinity for RAR requires a carboxylic acid moiety and an appropriate hydrophobic group, such as in **2**. On the other hand, high binding affinity for RXR also requires a carboxylic acid moiety and an appropriate hydrophobic group, such as in **4**. Differences of the direction with respect to the carboxylic acid and of the optimum bulk of the hydrophobic group lead to drastic changes of binding selectivity for RAR and RXR. These investigations led us to synthesize and investigate compounds having carboranes as a hydrophobic moiety (**5** and **6**), as shown in Fig 1. In icosahedral cage structures throughout this paper, closed circles



	R_1	R_2	R_3
5a	H	H	H
5b	CH_3	H	H
5c	C_2H_5	H	H
5d	n-C_3H_7	H	H
5e	i-C_3H_7	H	H
5f	n-C_4H_9	H	H
5g	CH_3	H	CH_3
5h	CH_3	CH_3	CH_3

	R_1	R_2	R_3
6a	H	H	H
6b	CH_3	H	H
6c	C_2H_5	H	H
6d	n-C_3H_7	H	H
6e	i-C_3H_7	H	H
6f	n-C_4H_9	H	H
6g	CH_3	H	CH_3
6h	CH_3	CH_3	CH_3

Fig. 1

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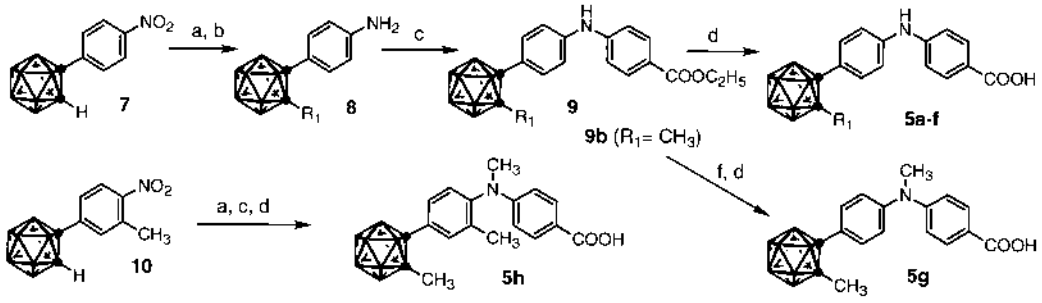

a) H_2, Pd-C/ EtOH; b) NaH, R_1I / DMF; c) ethyl p-iodobenzoate, Cs_2CO_3, $Pd_2(dba)_3$, BINAP/ toluene; d) KOH/ H_2O-THF; f) NaH, CH_3I/ DMF

Chart 1

Table 1. Retinoidal Activity of Carborane-containing Derivatives in HL-60 Cell Assay

Compound	R_1	R_2	R_3	EC_{50} (M)	Compound	R_1	R_2	R_3	EC_{50} (M)
5a	H	H	H	3.7×10^{-8}	**6a**	H	H	H	6.8×10^{-8}
5b	CH_3	H	H	5.4×10^{-8}	**6b**	CH_3	H	H	5.4×10^{-8}
5c	C_2H_5	H	H	4.3×10^{-9}	**6c**	C_2H_5	H	H	4.5×10^{-9}
5d	*n*-C_3H_7	H	H	1.5×10^{-9}	**6d**	*n*-C_3H_7	H	H	3.4×10^{-9}
5e	*i*-C_3H_7	H	H	2.9×10^{-9}	**6e**	*i*-C_3H_7	H	H	4.1×10^{-9}
5f	*n*-C_4H_9	H	H	3.1×10^{-9}	**6f**	*n*-C_4H_9	H	H	5.0×10^{-9}
5g	CH_3	H	CH_3	Inactive[a]	**6g**	CH_3	H	CH_3	Inactive
5h	CH_3	CH_3	CH_3	Inactive	**6h**	CH_3	CH_3	CH_3	Inactive

a) "Inactive" means there was no activity at test compound concentrations below 1.0×10^{-6} M.

(•) represent carbon atoms and other vertices represent BH units.

The syntheses of the designed molecules are summarized in Chart 1. Compounds **5a**—**f** were prepared from 4-(1,2-dicarba-*closo*-dodecaboran-1-yl)nitrobenzene (**7**), which is prepared by nitration of 1-phenyl-1,2-dicarba-*closo*-dodecaborane.[6] After catalytic hydrogenation of **7**, a suitable alkyl group (R_1) was introduced by lithiation of the carborane derivatives, followed by reaction with alkyl halide to give **8a**—**f**. Coupling of the amines **8a**—**f** with ethyl 4-iodobenzoate catalyzed by tris(dipenzylidenacetone)dipalladium (0) in the presence of (*R*)-BINAP[13] gave ethyl 4-(2-alkyl-1,2-dicarba-*closo*-dodecaboran-1-yl)phenylamino]benzoates (**9a**—**f**). Hydrolysis of **9a**—**f** under basic conditions afforded 4-(2-alkyl-1,2-dicarba-*closo*-dodecaboran-1-yl)phenylamino]benzoic acids (**5a**—**f**). The dimethylated compound **5g** was prepared from **9b** by N-methylation using sodium hydride and methyl iodide, followed by hydrolysis. The trimethylated compound **5h** was prepared from 4-(1,2-dicarba-*closo*-dodecaboran-1-yl)-3-methylnitrobenzene (**10**), which is easily prepared from 1-(3-tolyl)-1,2-dicarba-*closo*-dodecaborane. Compound **10** was converted to the diphenylamine derivative in a manner similar to that described for **9**. Methylation of carbon on the carborane cage and nitrogen, followed by hydrolysis, afforded **5h**. Compounds bearing 1,2-carborane at the 3-position of the benzene nucleus **6a**—**h** were prepared from 3-(1,2-dicarba-*closo*-dodecaboran-1-yl)nitrobenzene in the same manner as described for the *para*-carboranyl isomers.

The biological activities of compounds **5** and **6** were evaluated in terms of the activity to induce differentiation of HL-60 cells into mature granulocytes.[14] The differentiated cells were identified by the nitro blue tetrazolium (NBT) reduction assay, and the results are summarized in Table 1. The compound bearing 1,2-carborane at the 4-position of the benzene nucleus **5a** exhibited a potent differentiation-inducing activity toward HL-60 cells, with an EC_{50} value of 3.7×10^{-8} M, and showed no synergistic effect with the synthetic retinoid Am80 (**2**). The compounds bearing an alkyl group at the 2-position of the 1,2-carborane cage (**5b**—**f**) also exhibited potent retinoid agonistic activity. The agonistic activity was increased by introduction of an *n*-propyl or an iso-propyl group on the carborane cage; the EC_{50} values of **5d** and **5e** are 1.5×10^{-9} M and 2.9×10^{-9} M, respectively. The activities of **5d** and **5e** are comparable to that of all-*trans*-retinoic acid. Introduction of a longer alkyl group such as an *n*-butyl group diminished the differentiation-inducing activity. Compounds bearing 1,2-carborane at the 3-position of the benzene nucleus **6** also exhibited potent retinoid agonistic activity. The effect of the introduction of an alkyl group on the carborane cage of **6** was similar to that in the case of the *para*-isomers. The EC_{50} value of the most potent *meta*-isomer **6d** is 3.4×10^{-9} M. The differentiation-inducing activity of compounds bearing a methyl group on the aromatic nucleus (**5g**) or a methyl group on the nitrogen atom (**5h**) disappeared. These substituent effects may arise from changes in the twisting conformation at the phenyl-N-phenyl moiety caused by the alkyl group on the nitrogen atom and/or on the *ortho* position of the aromatic nucleus. The structure–activity relationships of the retinoidal agonists may be explained in terms of affinity for the nuclear receptor RARs. The present results suggest that a relatively planar conformation at the phenyl-N-phenyl moiety is preferred for an RAR ligand, and that the bulky carboranyl moiety on either the *meta*- or *para*-position of the benzene nucleus is permitted for the hydrophobic region of the molecule. On the other hand, the compounds with a diphenylamine skeleton **4a** exhibit weak agonistic activity

and significant synergistic activity.[12] The twisting conformation at the phenyl-N-phenyl moiety due to the *N*-alkyl and *ortho*-alkyl moiety is preferred for the appearance of the synergistic activity, which may be related to affinity for RXRs. Compounds **5h** and **6h** have no synergistic activity. In contrast, the retinoid agonistic activities of **5a** and **6a** are more potent than that of **4a**. 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 developed novel carborane-containing molecules with potent retinoidal activity. The unique character of biologically active molecules containing a carborane skeleton may give rise to unusual membrane transport characteristics and metabolism compared with conventional active molecules. The results of this study demonstrate that carboranes can be employed as the hydrophobic moiety of biologically active molecules.

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