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 $10^{-8}$—$10^{-11}$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) 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 carborane-containing retinoidal agonists.

Retinoic acid (all-trans, 1) has a broad spectrum of biological activity related to cellular differentiation and proliferation7) 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 recep-
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. 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$^{-8}$ 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.

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>R$_1$</th>
<th>R$_2$</th>
<th>R$_3$</th>
<th>EC$_{50}$ (m)</th>
<th>Compound</th>
<th>R$_1$</th>
<th>R$_2$</th>
<th>R$_3$</th>
<th>EC$_{50}$ (m)</th>
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<tbody>
<tr>
<td>5a</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>3.7×10$^{-8}$</td>
<td>6a</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>6.8×10$^{-8}$</td>
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<tr>
<td>5b</td>
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<td>H</td>
<td>H</td>
<td>5.4×10$^{-8}$</td>
<td>6b</td>
<td>CH$_3$</td>
<td>H</td>
<td>H</td>
<td>5.4×10$^{-8}$</td>
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<tr>
<td>5c</td>
<td>C$_2$H$_5$</td>
<td>H</td>
<td>H</td>
<td>4.3×10$^{-9}$</td>
<td>6c</td>
<td>C$_2$H$_5$</td>
<td>H</td>
<td>H</td>
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<tr>
<td>5d</td>
<td>n-C$_3$H$_7$</td>
<td>H</td>
<td>H</td>
<td>1.5×10$^{-9}$</td>
<td>6d</td>
<td>n-C$_3$H$_7$</td>
<td>H</td>
<td>H</td>
<td>3.4×10$^{-9}$</td>
</tr>
<tr>
<td>5e</td>
<td>i-C$_3$H$_7$</td>
<td>H</td>
<td>H</td>
<td>2.9×10$^{-9}$</td>
<td>6e</td>
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<td>H</td>
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<tr>
<td>5f</td>
<td>n-C$_4$H$_9$</td>
<td>H</td>
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<td>CH$_3$</td>
<td>CH$_3$</td>
<td>CH$_3$</td>
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<td>6g</td>
<td>CH$_3$</td>
<td>CH$_3$</td>
<td>CH$_3$</td>
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</tr>
<tr>
<td>5h</td>
<td>CH$_3$</td>
<td>CH$_3$</td>
<td>CH$_3$</td>
<td>Inactive</td>
<td>6h</td>
<td>CH$_3$</td>
<td>CH$_3$</td>
<td>CH$_3$</td>
<td>Inactive</td>
</tr>
</tbody>
</table>

(•) “Inactive” means there was no activity at test compound concentrations below 1.0×10$^{-6}$ M.

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. 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(diphenylidenacetone)dipalladium (0) in the presence of (R)-BINAP gave ethyl 4-(2-alkyl-1,2-dicarba-closo-dodecaboran-1-yl)phenylamino benzozoic acids (9a—f). Hydrolysis of 9a—f under basic conditions afforded 4-(2-alkyl-1,2-dicarba-closo-dodecaboran-1-yl)phenylaminobenzoic 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-toly)-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.
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 5\(h\) and 6\(h\) have no synergistic activity. In contrast, the retinoid agonistic activities of 5\(a\) and 6\(a\) are more potent than that of 4\(a\). 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.

References and Notes