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Utility of Boron Clusters for Drug Design. Relation Between Estrogen Receptor Binding Affinity and Hydrophobicity of Phenols Bearing Various Types of Carboranyl Groups

Yasuyuki Endo,[a,*] Keisuke Yamamoto[b] and Hiroyuki Kagechika[b]

[a]*Faculty of Pharmaceutical Sciences, Tohoku Pharmaceutical University, 4-4-1, Komatsushima, Aoba-ku, Sendai 981-8558, Japan*
[b]*Graduate School of Pharmaceutical Sciences, University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan*

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Abstract—High binding affinity for estrogen receptor and the appearance of estrogenic activity require a phenolic ring and an appropriate hydrophobic group adjacent to the phenolic ring. A quantitative structure–activity relationship analysis based on the values of log*P* and the pK_a of the phenolic group showed that the hydrophobicity of these compounds is highly correlated to the estrogen receptor α (ERα)-binding affinity. These results should be useful for application of these spherical boron clusters (dicarba-*closo*-dodecaboranes; carboranes) as hydrophobic pharmacophores in drug design, as well as for microscopic analysis of ER–ligand interactions.

The icosahedral carboranes (dicarba-*closo*-dodecaboranes)[1] have characteristic properties, such as high boron content, remarkable thermal and chemical stability, spherical geometry and exceptional hydrophobic character. 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[2,3] and retinoids[4]) and other biologically active molecules[5] containing a carborane cage as a hydrophobic pharmacophore. In view of the potential application of carboranes in the design of a wide range of drugs, we have also experimentally measured the partition coefficients, log*P*, for carboranylphenols by means of an HPLC method, and determined the values of the Hansch–Fujita hydrophobic parameter π of various carboranyl groups. The values (π = 2.71–4.47) depend on the position of substitution on the carborane cage and the isomeric form (*o*-, *m*-, *p*-carboranes).[6] In the work, we selected eight carboranylphenols (**1**–**8**) for the determination of the hydrophobic parameter π of the carboranyl group, because measuring partition coefficients of phenol derivatives is a fundamental method for determination of 'aromatic' substituent constants,[7] and because these carboranylphenols themselves exhibit potent estrogenic activity.[2] The potency of compound **1** was almost the same as that of 17β-estradiol, and was significantly higher than that of known estrogenic 4-alkylphenols[8] and 4-cycloalkylphenols[9] that are either present in the environment, or used as chemicals. Further, alteration of the position of substitution on the carborane cage affects the biological activities. The carboranylphenols (**1**–**8**) have the same molecular geometry and do not have conformational factors which would have to be considered in a structure–activity study. On the other hand, the acidity of the phenolic group may affect the binding affinity for ER through hydrogen-bonding, in addition to the hydrophobicity of the carboranes. Therefore, we considered that two parameters, log*P* and pK_a, would be sufficient for quantitative structure–activity relationship analysis in this system. We describe here measurements of pK_a and binding affinity to ERα for eight carboranylphenols (**1**–**8**), and we present quantitative structure–activity relationships based on log*P* and pK_a (Fig. 1).

The log*P* values of the carboranylphenols and the aromatic hydrophobic parameters π of the carboranyl groups, which have been reported in our previous paper,[6] are shown in Table 1. It is apparent that *C*-substituted

*Corresponding author. Tel.: +81-22-234-4183; fax: +81-22-275-2013; e-mail: yendo@tohoku-pharm.ac.jp

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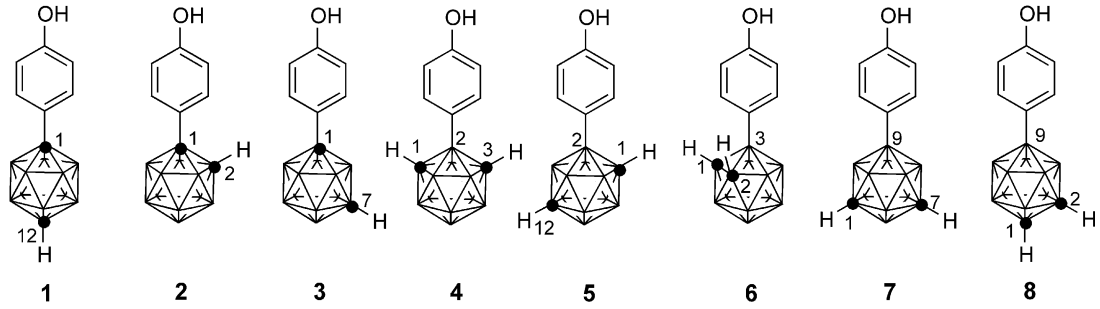

Figure 1. Designed carboranylphenols (**1**–**8**).

Table 1. Hydrophobic and electronic parameters, and ERα binding affinity of carboranylphenols (**1**–**8**)

Compd	Log*P*	π[a]	pK_a	σ^{-}[b]	IC_{50}[c]	Log (1/IC_{50})
4-(*p*-Carboran-1-yl)phenol (**1**)	5.89	+4.47	9.56	0.67	0.59	0.229
4-(*o*-Carboran-1-yl)phenol (**2**)	5.75	+4.33	9.16	1.07	2.85	−0.455
4-(*m*-Carboran-1-yl)phenol (**3**)	5.71	+4.29	9.46	0.77	3.60	−0.556
4-(*m*-Carboran-2-yl)phenol (**4**)	5.53	+4.11	9.67	0.55	9.57	−0.981
4-(*p*-Carboran-2-yl)phenol (**5**)	5.46	+4.04	9.91	0.32	6.88	−0.838
4-(*o*-Carboran-3-yl)phenol (**6**)	5.16	+3.74	9.61	0.62	71.6	−1.855
4-(*m*-Carboran-9-yl)phenol (**7**)	4.62	+3.20	10.19	0.04	23.4	−1.368
4-(*o*-Carboran-9-yl)phenol (**8**)	4.13	+2.71	10.25	−0.02	3.92	−0.593

[a]Hydrophobic parameter π for each substituent calculated on a basis of standard log*P* value of unsubstituted phenol (log*P* = 1.46).
[b]Substituent constant σ^{-} for each substituent calculated on a basis of standard pK_a value of unsubstituted phenol (pK_a = 10.23).
[c]Relative IC_{50} values based on competitive inhibition to 4 nM of ^{3}H-estradiol binding to ERα.

carboranyl groups [*p*-carboran-1-yl (**1**), *o*-carboran-1-yl (**2**), *m*-carboran-1-yl (**3**)] are more hydrophobic than *m*-carboran-2-yl (**4**) and *p*-carboran-2-yl (**5**) groups. The later two compounds are comparable to the adamantyl group. The hydrophobicity of other phenols decreases in the following order: *o*-carboran-3-yl (**6**) > -*m*-carboran-9-yl- (**7**) > *o*-carboran-9-ylphenol (**8**). The *o*-carboran-9-yl group is less hydrophobic than a cyclohexyl group. The π values of the test compounds were in the range of 2.71–4.47, which corresponds to those of various types of hydrocarbon groups (*n*-alkyl, cycloalkyl, etc.), even though all the carboranyl groups have the same molecular shape.

The electronic effect of carboranes on a substituent outside the cage indicates that the *C*-substituted carboranes behave as electron-withdrawing groups in the sequence *ortho* (σ_p = +0.38) > > *meta* (σ_p = +0.19) > *para* (σ_p = +0.12) >, while some *B*-substituted carboranes behave as weak electron-donating groups (9-substituted *o*-carborane, σ_p = −0.19).[10] The difference of the electronic effect of the various substitution types of carboranes should affect the pK_a values. The pK_a values were determined by measurement of the change of absorbance at λ_{max} in the pH-dependent UV spectra of ionic species in 20:80 (w/w) methanol–water*. The values of pH obtained with the pH meter were corrected by using the equation: pH* = pH (recorded) − δ (δ = 0.01),[11] because the measurements were performed in 20:80 (w/w) methanol-water as a solvent. The values of pK_a and the Hammett substituent constant σ_p^- of the carboranyl groups obtained in the present experiments (the pK_a of unsubstituted phenol used as a standard is 10.211), are shown in Table 1. The pK_a values of the test compounds were in the range of one pH unit (pK_a = 9.16–10.25). The most acidic phenol was *o*-carboran-1-ylphenol (**2**), which corresponds to 3-chlorophenol[12] (pK_a = 9.25). The pK_a values of the two *C*-substituted carboranyl compounds, *m*-carboran-1-ylphenol (**3**) and *p*-carboran-1-ylphenol (**1**), increased with decrease of electron-withdrawing effect. The pK_a values of *B*-substituted phenols increases in the following order: *o*-carboran-3-yl- (**6**) < *m*-carboran-2-yl- (**4**) < *p*-carboran-2-yl- (**5**) < *m*-carboran-9-yl- (**7**) < *o*-carboran-9-ylphenol (**8**). The pK_a values of carboranylphenols **7** and **8** were almost the same as that of unsubstituted phenol.

ERα binding assays were performed by measurement of inhibition of [6,7-^{3}H]17β-estradiol binding (K_d = 0.4 nM) to human recombinant ERα (PanVera) at 4 °C, using the nitrocellulose filter binding assay method. 4-(*p*-Carboran-1-yl)phenol (**1**) showed strong affinity for ERα; its potency was somewhat stronger than that of 17β-estradiol. The other *C*-substituted phenols, *o*-carboran-1-yl- (**2**) and *m*-carboran-1-ylphenol (**3**), exhibited potent affinity for ERα. The ERα binding affinity of *B*-substituted phenols decreased in the following order: > *o*-carboran-9-yl (**8**) > *p*-carboran-2-yl (**5**) > *m*-carboran-2-yl (**4**) > *m*-carboran-9-yl (**7**) > *o*-carboran-3-yl (**6**) derivatives. The relative IC_{50} values based on competitive inhibition of ^{3}H-estradiol binding to ERα are shown in Table 1.

Extensive quantitative structure–activity relationship (QSAR) analysis of estrogens has been reported.[13] In general, selection of parameters is extremely important in QSAR studies. However, steric and conformational factors could be excluded, and two well-defined factors, hydrophobicity and electronic effect, should explain the variations of biological activity in the present system.

We anticipated that the principal factor for ERα binding affinity would be hydrophobicity, but the two carboranylphenols with low hydrophobicity, *o*-carboran-9-yl- (**8**) and *m*-carboran-9-ylphenol (**7**), exhibited unexpectedly high binding affinity to ERα. First-order relationship between log*P* and the binding affinity was not observed. Therefore, we fitted the data to the following parabolic equation by regression analysis. This equation ($R^2 = 0.923$) shows that the principal factor determining the ERα binding affinity of carboranylphenols is hydrophobicity.

$$\log(1/IC_{50}) = 1.93(\pm 0.276)(\log P)^2 - 18.9(\pm 2.76)(\log P) + 44.4(\pm 6.80)$$
$$n = 8,\ R^2 = 0.923,\ s = 0.206$$

The following equation with regression coefficient $R^2 = 0.980$ was obtained by the addition of the electronic factor, pK_a (Table 2)

$$\log(1/IC_{50}) = 2.00(\pm 0.158)(\log P)^2 - 19.1(\pm 1.57)(\log P) + 0.779(\pm 2.30)(pK_a) + 36.3(\pm 4.54)$$
$$n = 8,\ R^2 = 0.980,\ s = 0.117$$

Table 2. QSAR analysis of carboranylphenols (**1**–**8**)

Compd	Log ($1/IC_{50}$)		
	Observed	Calcd from eq 2	Δ
4-(*p*-Carboran-1-yl)phenol (**1**)	0.229	0.286	−0.057
4-(*o*-Carboran-1-yl)phenol (**2**)	−0.455	−0.585	0.130
4-(*m*-Carboran-1-yl)phenol (**3**)	−0.556	−0.498	−0.058
4-(*m*-Carboran-2-yl)phenol (**4**)	−0.981	−0.911	−0.070
4-(*p*-Carboran-2-yl)phenol (**5**)	−0.838	−0.914	0.076
4-(*o*-Carboran-3-yl)phenol (**6**)	−1.855	−1.738	−0.117
4-(*m*-Carboran-9-yl)phenol (**7**)	−1.369	−1.443	0.074
4-(*o*-Carboran-9-yl)phenol (**8**)	−0.593	−0.558	−0.035

In general, the biological activity increases as log*P* increases until the maximum value is obtained. The value of log*P* at the maximum represents the optimum for biological activity. Beyond that point, further increase in log*P* results in a decrease of biological activity. It is apparent that the parabolic curve expressed by the above eq 2 is the reverse of the usual parabolic curve. However, in the present system, membrane transport and distribution do not affect the activity. Our results may effectively represent the microscopic QSAR at the ligand-binding cavity of the receptor. On this basis, the experimental results can be explained as follows. Recent studies on the three-dimensional structure of the complex formed by 17β-estradiol and the human ERα ligand binding domain (hERαLBD)[14] have revealed the structural requirements for the appearance of estrogenic activity. 17β-Estradiol is oriented in the ligand-binding pocket by two types of contacts: hydrogen bonding from the phenolic hydroxyl group to Glu-353 and Arg394, and from the 17-β-hydroxyl group to the δ-nitrogen of His-524, and hydrophobic interaction along the body of the skeleton. The hydrophobic interaction along the spherical carborane cage to hydrophobic residues (such as Leu384, Phe404, Leu428, Leu526) of the receptor cavity seems to produce a stronger interaction than that in the case of 17β-estradiol. This may be the reason for the high activity of compound **1**, which lacks a hydroxyl group for hydrogen-bonding to His-524. Therefore, it is reasonable that the ERα binding affinity increases as log*P* increases, even in the high log*P* range. The reason for increase of the activity in the low logP range can be explained by the difference in the natures of CH and BH on the carborane cage. The two carbon vertices of carboranes bear relatively acidic protons (p$K_a = 21$) and readily allow substitution with metals or organic groups, and this characteristic is exhibited in the sequence *o*- > *m*- > *p*-carborane. Recent studies on the interaction of CH on the cage include a crystallographic analysis of the hydrogen-bonding interactions between cage CH and diaza-18-crown-6.[15] Therefore, one CH on the cage of *o*-carboran-9-ylphenol (**8**) and *m*-carboran-9-ylphenol (**7**), which have two CH groups towards the outside, may have an electrostatic interaction with His-524.

In summary, we have measured pK_a and binding affinity to ERα for eight carboranylphenols and conducted a simple QSAR analysis of carboranylphenols for ERα binding affinity, employing log*P* and pK_a as coefficients. These results should be useful for application of carboranes in the design of a wide range of drugs, as well as for microscopic analysis of ER-ligand interactions.

References and Notes

1. For recent reviews on medicinal applications, see: Hawthorne, M. F. *Angew. Chem., Int. Ed. Engl* **1993**, *32*, 950. Soloway, A. H.; Tjarks, W.; Barnum, B. A.; Rong, F.-G.; Barth, R. F.; Codogni, I. M.; Wilson, J. G. *Chem. Rev.* **1998**, *98*, 1515.
2. Endo, Y.; Iijima, T.; Yamakoshi, Y.; Yamaguchi, M.; Fukasawa, H.; Shudo, K. *J. Med. Chem.* **2001**, *42*, 1501. Endo, Y.; Iijima, T.; Yamakoshi, Y.; Fukasawa, H.; Miyaura, C.; Kubo, A.; Itai, A. *Chem. Biol.* **1999**, *8*, 341.
3. Endo, Y.; Iijima, T.; Yamakoshi, Y.; Kubo, A.; Itai, A. *Bioorg. Med. Chem. Lett.* **2000**, *9*, 3313. Endo, Y.; Yoshimi, T.; Iijima, T.; Yamakoshi, Y. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3387. Endo, Y.; Yoshimi, T.; Yamakoshi, Y. *Chem. Pharm. Bull.* **1999**, *48*, 314.
4. Iijima, T.; Endo, Y.; Tsuji, M.; Kawachi, E.; Kagechika, H.; Shudo, K. *Chem. Pharm. Bull.* **1999**, *47*, 398. Endo, Y.; Endo, Y.; Iijima, T.; Ohta, K.; Kagechika, H.; Kawachi, E.; Shudo, K. *Chem. Pharm. Bull.* **1999**, *47*, 585. Endo, Y.; Yaguchi, K.; Kawachi, E.; Kagechika, H. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1733.
5. Endo, Y.; Yoshimi, T.; Kimura, K.; Itai, A. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2561. Tsuji, M.; Koiso, Y.; Takahashi, M.; Hashimoto, Y.; Endo, Y. *Biol. Pharm. Bull.* **2000**, *23*, 513.
6. Yamamoto, K.; Endo, Y. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2389.
7. Fujita, T.; Iwasa, J.; Hansch, C. *J. Am. Chem. Soc.* **1964**, *86*, 5175.

8. Routledge, E. J.; Sumpter, J. P. *J. Biol. Chem.* **1997**, *272*, 3280.
9. Yamakoshi, Y.; Otani, Y.; Fujii, S.; Endo, Y. *Biol. Pharm. Bull.* **2000**, *53*, 151.
10. Hawthorne, M. F.; Berry, T. E.; Wegner, P. A. *J. Am. Chem. Soc.* **1965**, *87*, 4746. Zakharkin, L. I.; Kalinin, V. N.; Rys, E. G. *Izv. Akad. Nauk SSSR, Ser. Khim.* **1974**, 2632.
11. Bates, R. G. *Determination of pH, Theory and Practice*; Wiley: New York, 1973.
12. Rochester, C. H.; Wilson, D. N. *J. Chem. Soc., Faraday Trans.* **1976**, *72*, 2930.
13. For review see: Gao, H.; Katzenellenbogen, J. A.; Garg, R.; Hansch, C. *Chem. Rev.* **1999**, *99*, 723.
14. Brzozowski, A. M.; Pike, A. C. W.; Dauter, Z.; Hubbard, R. E.; Bonn, T.; Engstrom, O.; Ohman, L.; Greene, G. L.; Gustafsson, J.; Carlquist, M. *Nature* **1997**, *389*, 753.
15. Godfrey, P. D.; Grigsby, W. J.; Nicjols, P. J.; Raston, C. L. *J. Am. Chem. Soc.* **1997**, *119*, 9283.