Stereo Structure-Controlled and Electronic Structure-Controlled Estrogen-Like Chemicals to Design and Develop Non-estrogenic Bisphenol A Analogs Based on Chemical Hardness Concept

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The aim of this study was to elucidate the structure–activity relationship of bisphenol A (BPA) analogs using absolute hardness (η) and absolute electronegativity (χ) (chemical hardness) and to design a non-estrogen active BPA. To determine the structure–activity relationships of BPA analogs, we investigated MCF-7 cell proliferation stimulated by BPA analogs and an η–χ diagram based on the electronic structure of the BPA analogs. The results show that the actions of the environmental hormones BPA analogs have two chemical properties; (i) 'stereo structure-controlled' and (ii) 'electronic structure-controlled' estrogen-like chemical activities. Therefore, we designed and synthesized BPA analogs which do not possess these 2 characteristics, ((i) and (ii)), and demonstrate the non-estrogen activity of the analog.

Key words environmental hormone; bisphenol A; chemical hardness; MCF-7 cell; η–χ diagram; cell proliferation

Recently, it has been reported that the chemicals 2,2-bis(*p*-hydroxyl phenyl)propane (bisphenol A, BPA),[1] nonylphenol (NP), and *trans*-diethyl stilbestrol (DES), as well as others, can mimic natural hormones, and may disrupt the endocrine system in animals and humans.[2] The chemicals BPA and NP are widely used in the manufacturing of polycarbonate (PC) and alkylphenolpolyethoxylate (APE), respectively. Trace levels of BPA and NP leach out from PC plastics[3] and APE, respectively, causing extensive damage to kidneys, liver, and lungs.[4] This has become a problem of international environmental pollution. The chemical structures and properties of BPA and NP are very similar to 17β-estradiol (estrogen, 17β-E_2) (Fig. 1), and they are hydrophobic rich and have a phenol-like aromatic system in the side chain of the molecules. Estrogens and similar substances express their biological effects by binding to estrogen receptors (ER), which are members of the superfamily of nuclear transcription factors.[5] It has been suggested that phenol-like aromatic and hydrophobic properties are requirements of a chemical structure showing estrogen-like properties.

Although a lot of biological data on BPA has been accumulating, the estrogen-like characteristics that are essential in the chemical structure of BPA are, as of yet poorly understood. What are the characteristics of the chemical structure or property that are essential to estrogen activity in an estrogen-like substance? Previously, we have first shown that the electronic structures of an estrogen-like substance can be classified into at least four groups in terms of the coordinate $\mathbf{r}(\chi, \eta)$ of the electronic structure on the η–χ diagram formed by plotting the values of absolute hardness (η) and absolute electronegativity (χ) calculated at the semi-empirical AM1 Hamiltonian level.[6—8] In the η–χ diagram, the electronic structures of estrogen-like chemicals also play an important role in the expression of estrogen activity. The $\mathbf{r}(\chi, \eta)$ belongs to that of 17β-E_2. If the values of η and χ for a target chemical are plotted in the diagram, we can determine the group to which a target belongs.

In order to understand the structure–activity relationships (SARs) of BPA analogs, in this study, we investigated the estrogen-like activity of BPA analogs obtained by growth assay of human breast cancer (MCF-7) cells. In addition, we show the coordinate $\mathbf{r}(\chi, \eta)$ of the electronic structures of BPA analogs using the *ab initio* restricted Hartree-Fock (RHF) method with a 6-31G(d) basis set. By comparing coordinates $\mathbf{r}(\chi, \eta)$ between 17β-E_2 and BPA analogs, we can estimate to which group the chemicals belong. Here, we demonstrate that two types, (a) *stereo structure-controlled* BPA analogs and (b) *electronic structure-controlled* BPA analogs, using the η–χ diagram are related to the binding between ER and BPA analogs.

Finally, the η–χ diagram is a new method for predicting the structure–activity relationship of estrogen-like chemicals and for the molecular design of non-estrogenic BPA analogs and new eco-materials.

Experimental

Chemicals The bisphenols analogs **1b**, **c**, **e**—**h** and **4** were purchased from Honshu-Kagaku Co. (Tokyo), and **2a**, **b** and **3** were obtained from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.), and **1d**[9] was purchased from SANBO CHEMICAL IND., LTD. (Osaka, Japan). Dimethylsulfoxide (DMSO) used as a solvent for the other BPA analogs and 17β-Estradiol was biochemical grade and obtained from Wako Junyaku Co. (Osaka, Japan). Penicillin G potassium and streptomycin sulfonate were purchased from Meiji Seika, Ltd. (Tokyo).

Cell Lines and Culture Breast cancer MCF-7 cells were obtained from Human Science of Japan (Osaka) and grown in phenol red-free RPMI1640 medium (GIBCO, Life Technologies, Basel, Switzerland) containing 23.8 mM $NaHCO_3$ and supplemented with 5% stripped fetal calf serum (FCS),[10] penicillin G (10000 units/l), and streptomycin sulfonate (10 mg/l) (the medium). MCF-7 cells were maintained in 75 cm^2 culture flasks and incubated in a humidified mixture of 5% CO_2 under atmospheric pressure at 37 °C.

Cell Growth Assays and Doubling Times For the cell proliferation assay,[11,12] MCF-7 cells were trypsinized, mechanically dissociated, and seeded at a density of 1×10^4 cells/ml in 12-well cell culture plates (Costar; Corning Incorporated, U.S.A.) in the medium. Cells were allowed to attach in the seeding medium for 24 h. The seeding medium was changed every 3 d. The cells were detached with trypsin, washed once with the medium, resuspended in 1 ml of the medium, and counted on days 0, 1, 7, 11, 18, and 23. The cells were incubated with a test compound (final concentrations, 1×10^{-7} M), 17β-E_2 as positive control (1×10^{-8} M), and chemical-free medium (control). The cell numbers (N (cells/ml)) are expressed as the mean for five determinations. The number of cells (cells/ml) was counted and the

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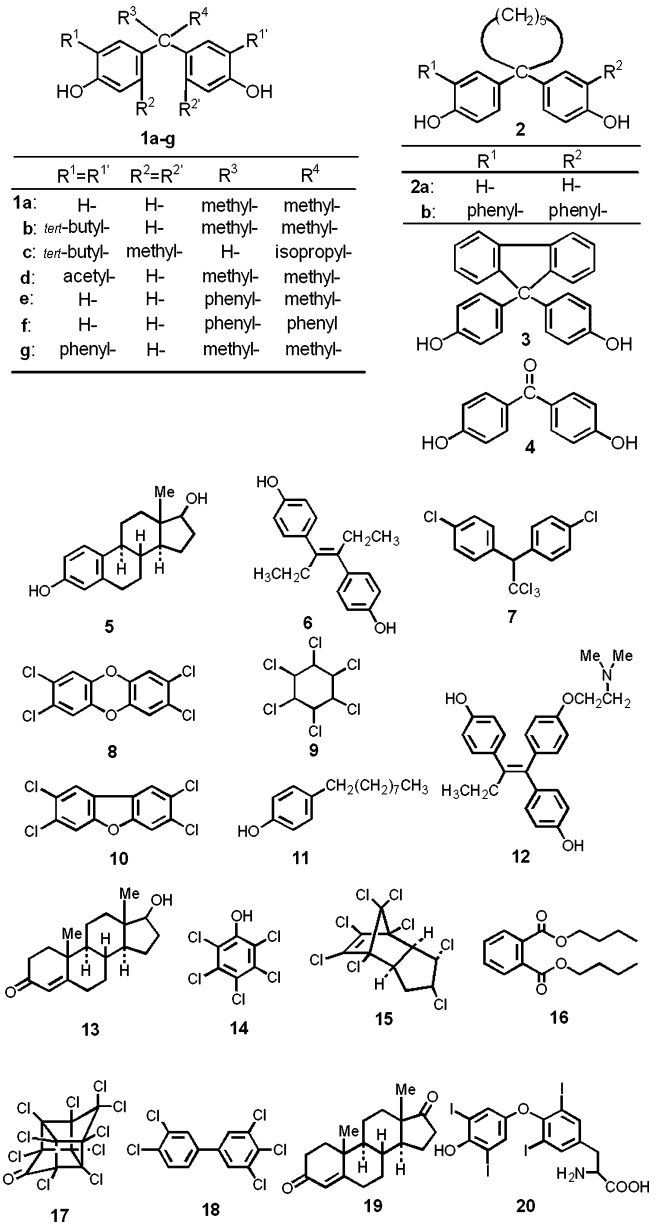

	R^1=$R^{1'}$	R^2=$R^{2'}$	R^3	R^4
1a:	H-	H-	methyl-	methyl-
b:	*tert*-butyl-	H-	methyl-	methyl-
c:	*tert*-butyl-	methyl-	H-	isopropyl-
d:	acetyl-	H-	methyl-	methyl-
e:	H-	H-	phenyl-	methyl-
f:	H-	H-	phenyl-	phenyl
g:	phenyl-	H-	methyl-	methyl-

	R^1	R^2
2a:	H-	H-
b:	phenyl-	phenyl-

Fig. 1. Structure of BPA Analogs and Other Related Chemicals

cells dyed with trypan blue[13] using a hemocytometer (Improved Neubauer deep 1/10 mm; Erma, Tokyo); N (cells/ml)=(counting cell number)×10000×dilution rate.

The estrogen-like activity of the chemicals was estimated using the doubling time (DT). DT was calculated by $DT=(t_2-t_1)/3.32\log(N_2/N_1)$, where N_1 and N_2 are the cell number counted at times t_1 and t_2 in the cell growth phase, respectively.

Computational Chemistry The lowest energy conformation determined with the CONFORMATIONAL SEARCH computation at the MMFF level was optimized using both the restricted Hartree-Fock (RHF) and RB3LYP levels with a 6-31G(d) basis set and we obtained the electronic structures and geometry of the optimized target chemicals. For T4 containing an iodine atom, we used a Los Alamos developed LACVP(d) basis set that includes an effective core potential DZ (double zeta). The values of absolute hardness (η) and absolute electronegativity (χ) were calculated by Eqs.1 and 2, as defined by Parr and Pearson.[14,15]

$$\chi=-\mu=-(\partial E/\partial N)_{v(r)}=(Ip+Ea)/2 \quad (1)$$

$$\eta=1/2\,(\partial\mu/\partial N)_{v(r)}=1/2\,(\partial^2E/\partial N^2)=(Ip-Ea)/2 \quad (2)$$

Table 1. Stereochemical Effect of BPA Analogs on Doubling Time of hMCF-7 Cell Proliferation

Compound	Doubling time (d)[c]
Without BPA analog	7.4[a]
5	3.2[a]
1a	7.0[a]
1b	10.5[a]
1c	10.4[a]
1g	6.5[b]
1f	7.8[a]
1e	8.5[a]
2a	4.2[b]
2b	6.3[b]
3	11.8[a]
4	7.9[a]

a) Cell density of 8.2×10^3/200 μl/well (**1a**, **1b**, **1c**, **1e**, **1f**, **4**, **5**) at 0 d. *b*) Cell density of 2.58×10^4/1000 μl/well (**1g**, **2a**, **2b**, **3**) at 0 d. *c*) The data are expressed as means for two determinations.

where E is the electronic energy of a molecule and N is the number of electrons, and v(r) is the external electrostatic potential. Ip and Ea are the ionization energy and the electron affinity (eV), respectively, and are used to calculate approximately the η and χ values of environmental hormones in this study, using Eqs. 3 and 4[15]

$$\chi=-1/2\,(\varepsilon_{HOMO}+\varepsilon_{LUMO}) \quad (3)$$

$$\eta=1/2\,(\varepsilon_{LUMO}-\varepsilon_{HOMO}) \quad (4)$$

Where ε_{HOMO} and ε_{LUMO} are the energy levels for the frontier orbitals. The Log P values as a hydrophobic parameter in BPA analogs were computed using the Villar-PM3 method.[16]

All calculations utilized the Spartan V.5.1.3 (Unix) and Spartan04 (Windows)[17] programs and were undertaken on a Compaq alphastation DS10 (alpha 21264A) or a Dell dimension 8250 (pentium 4) of personal computer, respectively.

Results and Discussion

MCF-7 Cell Growth Assay of BPA Analogs To determine the effects of BPA analogs on MCF-7 cell proliferation, we measured the cell growth number (cells/ml) and doubling time. Generally, the time required for the MCF-7 cell growth assay is 3—6 d. In this study, the medium exchanges and BPA analogs uptake in MCF-7 cells at a concentration of 1×10^{-7} M were about every 3—4 d for 20—25 d, according to the method described in the experimental section. The number of MCF-7 cells increased by treatment with 17β-E_2 at a concentration of 10^{-8} M. Table 1 is listed the doubling times (d) for MCF-7 cells proliferation stimulated with **1a**, **1g**, **2a**, and **2b** at a concentration of 10^{-7} M, and the results are shown in graph form in Figs. 3, 4. For example, the DT of MCF-7 cells stimulated by **1a** is 7.08 d; DT=25/3.32 log(94700/8200)=7.08.

The potency of estrogen activity, in decreasing order, of the BPA analogs was: **5**>**2a**>**1a**>**1f**>**1e**>**3** (Fig. 2). In contrast, at a concentration of 10^{-7} M, compound **3** (DT=11.77) did not affect MCF-7 cell growth any more than that of **2a**, **1f**, or **1a**. However, compound **2a** (DT=4.24), which contains a cyclohexyl side chain, accelerated MCF-7 cell growth activity at the same low concentration. Figure 3 shows the results for **1b**, **4**, and **1c** on MCF-7 growth. The relative order

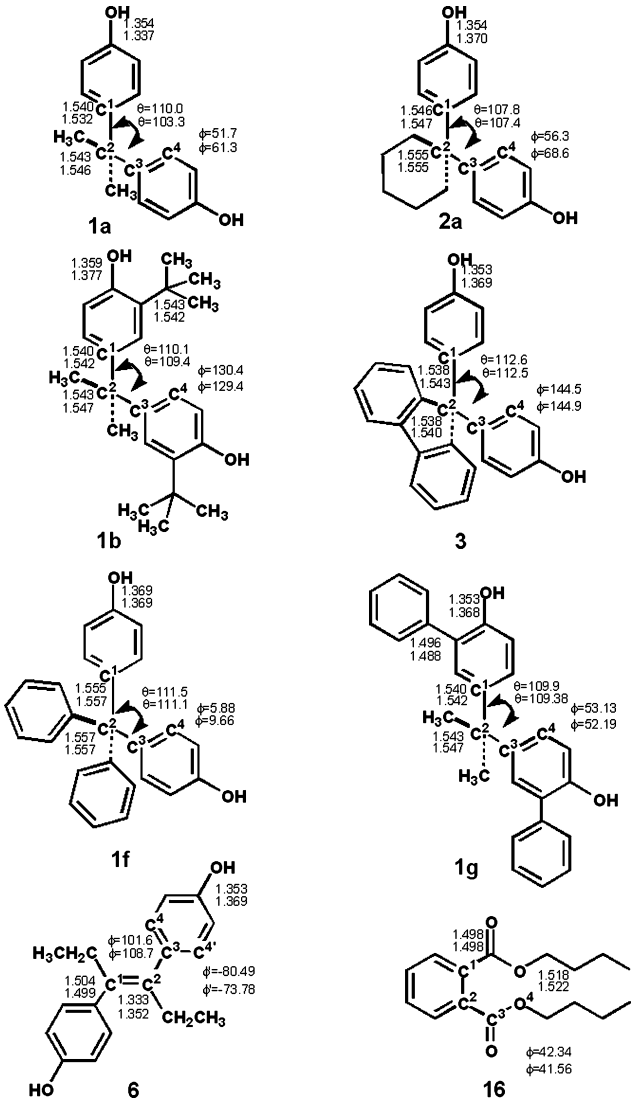

Fig. 2. Geometry of Optimized BPA Analogs by *ab initio* Molecular Orbital Calculation

a) Bond lengths, bond angles (θ), and dihedral angles (ϕ) for **1a**, **1b**, **1f**, **1g**, **2a**, **3**, **6**, and **16** at RHF/6-31G(d) (upper) and B3LYP/6-31G(d) (bottom) level; distances in Å, and angles in degrees.

of the growth activity was: **5**>**1a**>**4**>**1b**>**1c**. The BPA analogs containing an alkyl side chain in the phenol skeleton, such as compounds **1a**, **1c** and **2a** showed higher cell growth activity than those containing an aromatic side chain such as **3** and **1f**. Although the structure–activity relationships for estrogen activity of the BPA analogs are not well known at present, we are the first to show that the estrogen activity increased in the following order: **1b**, **1c**—**3**<**1e**<**1f**<**1a**<**4**<**2a**<**5**.

Interestingly, estrogen activity may be controlled by two requirements; first, *tert*-butyl *ortho*-containing (**1b**, **c**) and homo phase extended to the side chain-containing (**3**, **1f**) BPA analogs have lower estrogen activity than **1a**; second, BPA analogs possessed a similar electronic structure (**r**(χ, η); see next section) with 17β-E_2 having high estrogen activity. Next, we describe new relationships between the electronic structure and estrogen activity.

Electronic Structure-Controlled and Stereo Structure-Controlled Estrogen-Like Compounds In SAR studies,

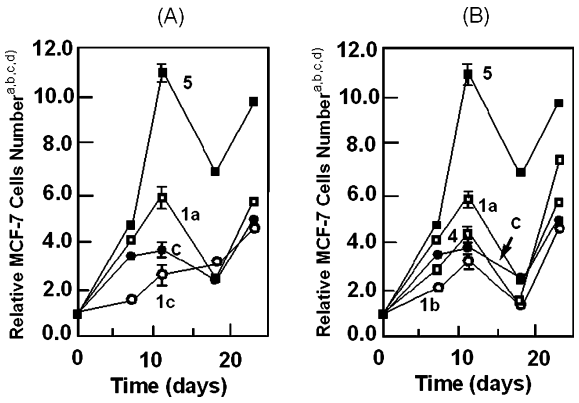

Fig. 3. Time Course of MCF-7 Cell Proliferation by Stimulation with BPA Analogs (10^{-7} mol/l) and 17β-Estradiol (10^{-8} mol/l)[a,b,c]

a) MCF-7 cells were stimulated with 1×10^{-7} mol/l of **1a**, **1c**, and without (**c**) (A) and **1a**, **1b**, **4**, and without (**c**) (B) in phenol red free RPMI 1640 supplemented with 5% stripped FCS serum. *b*) The cells were stimulated with 1×10^{-8} mol/l of **5** (17β-estradiol) in phenol red free RPMI 1640 supplemented with 5% stripped FCS serum. *c*) The cells were counted using a hemocytometer and the cell numbers were started 8.20×10^3 cells/200 ml/well the cells number was assumed to be one on the graph in relatively. The bars represent the standard deviation from triplicate wells. *d*) Relative MCF-7 cell number has plotted 8.20×10^3 cells as 1.0.

the octanol-water partition coefficient log P is a parameter widely used as a measure of the hydrophobicity of chemicals. Hydrophobicity is believed to play an important role in the molecular mechanism of hydrophobic chemical (drug)-receptor interactions, membrane penetration, and the toxicity of chemicals. In an attempt to elucidate the relationship between the estrogen activity and hydrophobicity of BPA analogs, log P values were computed using the Villar-PM3 method[16] and the results are listed in Table 2. If log P is small, the estrogen activity with the chemicals is higher since the log P of ligand 17β-E_2 is 2.75. However, chemicals with high estrogen activity do not necessarily have a small log P. Although the order for the estrogen activity of **1a**, **1g**, **2a**, **2b**, and **5** is **1a**<**2b**<**1g**<**2a**<**5**, the log P of compounds **1g** and **2b** is 6.72 and 7.27, respectively. In addition, the computed log P values of NP and DES, which have high estrogen activity, are 4.89 and 4.88, and the compounds are more hydrophilic than BPA. As a result, the log P of the BPA analogs has almost no correlation with their estrogen activity.

In order to further clarify the reason for the order for the strength of the estrogen activity, we studied the MO calculated chemical structures and electronic structures of optimized BPA analogs. The results of the RHF/6-31G(d) and B3LYP/6-31G(d) calculations of the BPA analogs used in this study are illustrated in Fig. 2. Although the dihedral angles, $\angle C^1$–C^2–C^3–C^4, (ϕ) between two phenol rings, **1a** and **2a**, are 51.7° and 56.3°, respectively, the ϕ of **3** and **1b** is 144.5° and 130.4°, respectively. The twisted geometry of **1a** (ϕ=51.7°) is a minimum 1.54 kcal/mol lower in total energy than the planar form (ϕ=0°) at the B3LYP/6-31G* calculation level. Due to this small energy difference, BPA is essentially a conformational flexible compound in comparison with **3** and **1b**. The steric repulsion of the fluorene ring and *tert*-butyl group in **3** and **1b** makes it difficult to produce a planar form. These results suggest that the estrogen activity of BPA analogs is related to the ring flexibility and steric repulsion in the side chain. For BPA analogs, obviously, higher estrogen activity is seen for the nonrigidly small dihedral angle, such as in compounds **1a** and **2a** (Table 1), and lower

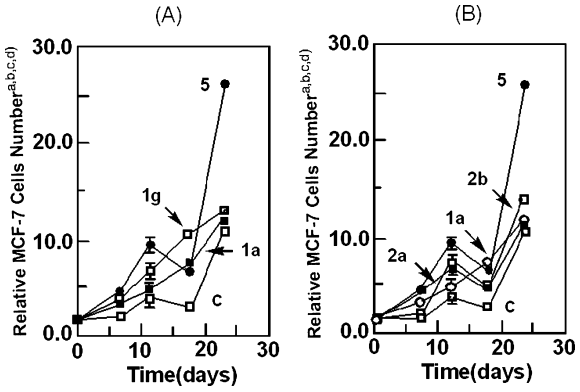

Fig. 4. Time Course of MCF-7 Cell Proliferation by Stimulation with BPA Analogs (10^{-7} mol/l) and 17β-Estradiol (10^{-8} mol/l)[a,b,c]

a) MCF-7 cells were stimulated with 1×10^{-7} mol/l of **1a**, **1g**, and without (**c**) (A) and **1a**, **2a**, **2b**, and without (**c**) (B) in phenol red free RPMI 1640 supplemented with 5% stripped FCS serum. *b*) The cells were stimulated with 1×10^{-8} mol/l of **5** (17β-estradiol) in phenol red free RPMI 1640 supplemented with 5% stripped FCS serum. *c*) The cells were counted using a hemocytometer and the cell numbers were started 2.58×10^4 cells/1000 ml/well the cells number was assumed to be one on the graph in relatively. The bars represent the standard deviation from triplicate wells. *d*) Relative MCF-7 cell number has plotted 2.58×10^4 cells as 1.0.

activity for a rigidly large dihedral angle, such as in **3** and **1b**. The electronic structure is closely related to the strength of the estrogen activity since the differences in the geometry are reflected in the electronic structures of BPA analogs.

We are very interested in the relationship between the electronic structure and estrogen activity of BPA analogs. Global absolute hardness (η) and electronegativity (χ) that are driving force of the interaction between BPA analogs and estrogen receptor (ER) in a living cell was computed (Table 2). In the hardness theory, the first derivative ($-\partial E/\partial N$) is equal to electronegativity (χ), while the second derivative ($\partial^2E/\partial N^2$) is equal to hardness (η).[18] Moreover, the χ is approximately equal to $|(\partial^2E/\partial N^2)+\varepsilon_{HOMO}|$. As one of the methods to predict estrogen-like properties of chemicals, we have been studying the coordinate $\mathbf{r}(\chi, \eta)=\mathbf{r}(-\partial E/\partial N, \partial^2E/\partial N^2)$ of the electronic structure to the chemicals.[6,8] Although the chemical structure of **1f** is similar to **3**, the η value of **1f** (**r**(2.395, 5.665)) computed using the HF/6-31G(d) is larger than **3** (**r**(2.420, 5.230)). The small η value of **3** indicates that **3** is chemically a more soft acid than **1f**. The η values of **1f** and **2a** (**r**(2.015, 5.805)) are nearly equal to estrogenic **1a** (**r**(2.100, 5.800)). In **1a** and **2a**, the differences $\Delta=|\eta_{2a}-\eta_{1a}|$ and $|\chi_{2a}-\chi_{1a}|$ are 0.005 and 0.085, respectively. The results show that the electronic structures of **2a** and **1f** are strikingly similar to that of **1a**. Interestingly, BPA is similar to the electronic structures of 17β-E_2 (**r**(2.050, 5.970)) as a ligand of ER. In such a case, we define it as an '**electronic structure-controlled**' estrogen-like chemical.

On the other hand, *tert*-butyl *ortho*-containing **1b** has an electronic structure similar to that of BPA and 17β-E_2, and compound **1b** has less estrogenic activity than **1a** and **2a**. *tert*-Butyl *ortho*-containing **1c** also has an action similar to that of **1a** and **2a**. We call this type of BPA analog in a '**stereo structure-controlled**' estrogen-like chemical. The electronic structures of dibutylphthalate (DBP) (**16**) and 2-diethylbutylphthalate (DEBP), which are used as plasticizers, differ from that of **1a**, **2a**, or **3**. Compound **16** acts as an electron acceptor since the difference $|\chi_{16}-\chi_{1a}|$ in absolute electronegativity of **1a** with **16** by calculation is 1.375.

Table 2. Calculated Absolute Hardness (η) and Absolute Electronegativity (χ) of BPA Analogs and Related Compounds Under Optimized Structures

Compound (glossary)	Absolute hardness (η)[a], eV	Absolute electronegativity (χ)[b], eV	Log P[c]
1a (BPA)	5.805	2.105	3.21
1b (BPAtB)	5.775	2.075	6.02
1c (BPIMtB)	5.850	1.890	6.92
1d (BPAA)	5.280	2.840	2.89
1e (BPAFM)	5.735	2.345	4.48
1f (BPADF)	5.665	2.395	5.53
1g (BPAOP)	5.490	2.325	6.72
2a (BPZ)	5.805	2.015	3.66
2b (BPZOP)	5.455	2.265	7.27
3 (BPF)	5.230	2.420	5.97
4 (DHBP)	5.610	3.070	1.94
5 (17β-E_2)	5.970	2.050	2.75
6 (DES)	5.885	2.085	4.89
7 (DDT)	5.800	3.220	6.23
8 (2378-TCDD)	5.525	3.015	4.85
9 (α-HCH)	7.310	4.780	2.68
10 (2378-TCDF)	5.145	3.565	5.80
11 (NP)	6.025	2.095	4.88
12 (*p*-OHtamoxifene)	5.360	2.010	5.29
13 (testosterone)	6.430	3.230	2.82
14 (PCP)	5.775	3.645	2.86
15 (Chlordene)	6.585	3.745	2.57
16 (DBP)	6.045	3.475	2.60
17 (Chlordecone)	6.668	4.899	3.14
18 (343′4′5′PCB)	5.480	3.610	6.55
19 (Androstenedione)	6.430	3.370	3.03
20 (Thyroxine)	5.305[d]	3.435[d]	—

a, *b*) At RHF level using the 6-31G(d) basis set. *c*) At Villar-PM3 level. *d*) At RHF level using the LACVP(d) basis set.

An η–χ Activity Diagram of Bisphenol A Analogs Concerning the structure–activity relationships of the BPA analogs suspected of being environmental hormones, we proposed an η–χ diagram as a coordinate for the electronic structures of environmental hormones calculated using the RHF method with a 6-31G(d) basis set. The results are shown in Fig. 5. The diagram shows a plot of η *vs.* χ using χ as the abscissa and η as the ordinate. We are able to classify environmental hormones into four groups; group I, **1a** and **6**, *etc.* are soft bases, and their endocrine disrupter effects are clearly confirmed; group II, androgens such as testosterone (**13**) and androstenedione (**18**), and their analogs are classified as hard acids; group III, aromatic polychlorinated hydrocarbons such as **7**, **8**, **10** whose analogs are potently toxic, and these chemicals are classified as soft acids; and group IV, aliphatic polychlorinated hydrocarbons such as **9** and **15**, that are less toxic than dioxins (group III), and these chemicals are classified as hard acids.[6]

From the plots, it is clear that the bisphenols are located in group I in the diagram (Fig. 5). The χ of compounds **1a** and **2a** are lower value than those of **7**, **8**, **14**, and **15**. The target receptors of 17β-E_2 and **6** are ERα or ERβ, which belong to the superfamily of nuclear receptors,[19] and the BPA analogs such as compounds **1a**, **2a**, and **1f** may target their receptors. The electronic structures are largely similar to that of the ligand, 17β-E_2 , which binds to the ERα or ERβ. The bisphenols are listed in ascending order of the values of absolute electronegativity: compounds **12**, **5**, **2a**, **1a**, **1f**, and **3**.

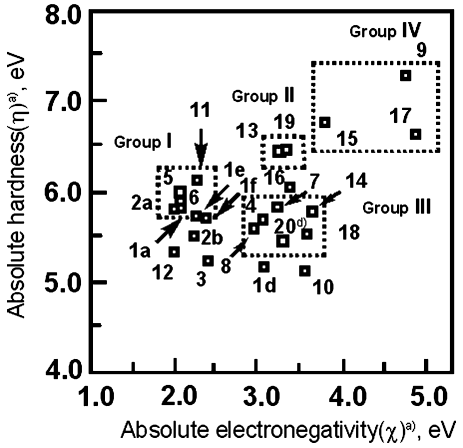

Fig. 5. Plot of the Absolute Hardness (η)–Absolute Electronegativity (χ) Diagram against BPA Analogs[a,b,c]

a) At RHF/6-31G(d) level. *b*) Compound numbers are listed in Fig. 1. *c*) (□) ligands; 17β-E_2 **5**, testosterone **13**, and thyroxine **20**. *d*) At RHF/LACVP(d) level.

That is, the greater the estrogen activity of the BPA analog, the lower the χ value. In addition, one of the other factors, η, must also satisfy the condition as one of the requirements of $|\eta_{17\beta\text{-E2}} - \eta_{\text{BPA}}| \sim 0$. Where, the symbol | | is the absolute value. Although the results suggest that a phenol-like aromatic system and hydrophobic properties are requirements of the chemical structure showing estrogen-like properties of BPA analogs, the electronic structure coordinates $\mathbf{r}(\chi, \eta)$ may also play an important role in expressing the estrogen activity. Side chains such as phenyl or fluorine groups reduced estrogen activity, while cyclohexyl groups increased estrogen activity. The estrogen activity of BPA analogs is related to the species of the substituted groups. A highly aromatic side chain in a BPA analog decreases the absolute hardness and increases the absolute electronegativity. However, high aliphaticity of the side chain inversely increases the absolute hardness and decreases the absolute electronegativity. From the results, the $\mathbf{r}(\chi, \eta)$ of the bisphenols such as **1a** and **2a** is similar to the 17β-E_2. The similarity of the $\mathbf{r}(\chi, \eta)$ of the electronic structure affects MCF-7 cell growth. Therefore, the estrogen activity of **1a** and **2a** is stronger than **3**, **1e**, and **1f**. For chemicals which have high estrogenic activity, it is essential that the $\mathbf{r}(\chi, \eta)$ must be similar to the value of 17β-E_2.

In other words, we can draw rules (Eqs. (i), (ii)) as requirements in order to be environmental hormone (eh).

$$|\eta_{\text{eh}} - \eta_{17\beta\text{E2}}| \sim 0 \quad \text{(i)}$$

$$|\chi_{\text{eh}} - \chi_{17\beta\text{E2}}| \sim 0 \quad \text{(ii)}$$

The $\mathbf{r}(\chi, \eta)$ of **3** has a large gap compared with the conditions of (i) and (ii), and differs from that of BPA, which suggests that compound **3** has no or lower estrogen activity. This is supported by the data from MCF-7 cell growth activity.

We were interested in the electronic structure of **3**, because no estrogen-like monomer of polycarbonate plastics can be designed by chemical modification of **3**. Compound **3** has a larger χ value. Because of one chemicals are estrogen-like compound, the following essential conditions must be satisfy;

Table 3. Classification of BPA Analogs Based on Stereo Structure- and Electronic Structure-Controlled Estrogen Activity

Type	Compounds	Relative MCF-7 growth activity[a]	Group[b]
Stereo structure-controlled	**1b**, **1c**	(−)	I
Electronic structure-controlled	**1a**, **1g**, **2a**, **2b**,	(+)	I
	5, **6**, **11**	(+)	I
Electronic structure-controlled	**1d**, **1f**, **3**,	(−)	ND
	4	(−)	III

a) Compared with the doubling time of MCF-7 cells treated with **1a**: (−), none active and (+), active. *b*) In Fig. 5.

Table 4. Electronic Structure Effect of BPA Analogs on Doubling Time of Human MCF-7 Cells

Compound	Doubling time (d)
Comtrol	7.4
1a	7.0[a]
1d	250[b]
5	3.2[a]

a) Cell density of 8.2×10^3/200 μl/well (**1a**, **5**) at 0 d. *b*) Cell density of 1.8×10^5/1000 μl/well (**1d**) at 0 d. The data are expressed as means for two determinations.

$$|\eta_{\text{eh}} - \eta_{17\beta\text{E2}}| > 0 \quad \text{(iii)}$$

$$|\chi_{\text{eh}} - \chi_{17\beta\text{E2}}| > 0 \quad \text{(iv)}$$

the chemicals have a non-estrogen-like effect. Therefore, Eqs. (iii) and (iv) can be used to judge whether they have the requirements of estrogen-like activity. The results indicate that dioxins, DDT, and PCB, which are grouped into group III in Fig. 5, interact easily with thyroid hormone receptors (TRs) more than ERα or ERβ.[20]

Molecular Design of Non-estrogenic BPA Analogs BPA analogs belonging to the estrogenic-like group (group I) had an $\mathbf{r}(\chi, \eta)$ similar to that of 17β-E_2. To develop non-estrogenic BPA analogs, therefore, it is necessary to design analogs which differ with respect to the coordinate $\mathbf{r}(\chi, \eta)$ for the estrogenic-like group (group I), as shown in Figs. 5 and 6. The Eqs. (iii) and (iv) show that the chemical characteristics as non-estrogenic BPA analogs are having electron withdrawing groups, –Cl, $-COCH_3$ **1d**,[9] $-NHCOCH_3$, and $-NO_2$, *etc.* in BPA analog. The electron withdrawing groups enlarge an absolute electronegativity and reduces an absolute hardness of target chemicals. Although the $\chi_{\mathbf{b}}$ value of compound **b** ($\mathbf{r}_{\mathbf{b}}(\chi_{\mathbf{b}}, \eta_{\mathbf{b}})$, is approximetly equal to the $\chi_{\mathbf{a}}$ value of compound **a** ($\mathbf{r}_{\mathbf{a}}(\chi_{\mathbf{a}}, \eta_{\mathbf{a}})$), the difference ($|\eta_{\mathbf{b}} - \eta_{\mathbf{a}}|$) of $\eta_{\mathbf{b}}$ and $\eta_{\mathbf{a}}$ is a large (in Fig. 6). Compound **b** is an antagonist for **a** (for example, compound **12**). Compound **c**, $\mathbf{r}_{\mathbf{c}}(\chi_{\mathbf{c}}, \eta_{\mathbf{c}})$, satisfies the requirement for being a non-estrogenic compound; $|\chi_{\mathbf{c}} - \chi_{17\beta\text{E2}}| > 0$ and $|\eta_{\mathbf{c}} - \eta_{17\beta\text{E2}}| > 0$.

We found that the electronic state of **1d**, $\mathbf{r}(3.085, 5.165)$ differs from that of 17β-E_2, **1a**, and **2a**. We designed and synthesized compound **1d** and tested it for MCF-7 cell growth activity. The results are presented as the doubling time (DT) of MCF-7 cell proliferation in Table 4. The DT of MCF-7 cells stimulated by **1d** is 250 d. The results suggest that the molecular design of the non-estrogen chemical shown in Fig. 6 is a useful method.

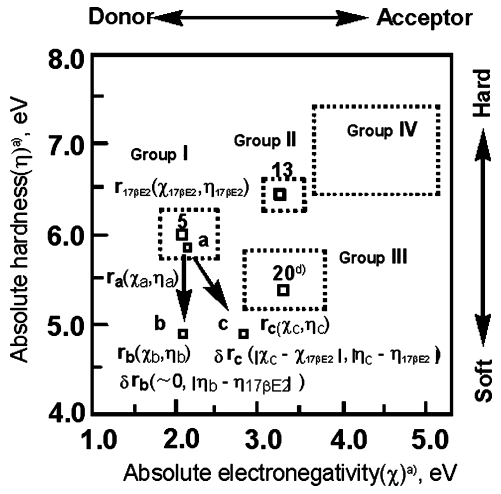

Fig. 6. Molecular Design of Non-estrogenic BPA Analogs Based on η–χ Diagram

a) At RHF/6-31G(d) level. *b*) Compound numbers are listed in Fig. 1. *c*) (□) ligands; 17β-E_2 **5**, testosterone **13**, and thyroxine **20**. *d*) At RHF/LACVP(d) level. Compounds **b** and **c** are an antagonist (for **5**) and a non-estrogenic BPA analogs, respectively. The δ represents the difference of the η and χ between ligand 17β-E_2 and compound **b** (or **c**).

Conclusion

In this study, we have presented a new useful method with which to predict and judge the estrogen activity of BPA analogs. The results show that the estrogen activity of BPA and other derivatives is dependent on not only the stereochemical but also the electronic structure, $\mathbf{r}(\chi, \eta)$. However, $\log P$ as a parameter of hydrophobicity does not play an important role in the structure–activity correlation of BPA analogs. We predicted, designed, and synthesized one non-estrogenic compound, **1d**, using the electronic structure coordinate $\mathbf{r}(\chi, \eta)$, defined using the two parameters absolute electronegativity (χ) and hardness (η).

In order to determine the structure–activity relationships (SARs) of BPA analogs, in this study, we first proved the SARs of BPA analogs by using the doubling time of human breast cancer (MCF-7) cells stimulated by target BPA analogs. Two types of properties which resulted from analysis of the coordinate $\mathbf{r}(\chi, \eta)$ are (a) *stereochemical-controlled* BPA analogs and (b) *electronic structure-controlled* BPA analogs. The two chemical properties are related to the binding affinity and the conformational changes following the complex local charge changing between ER and BPA analogs.

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