

Estrogenic/Antiestrogenic Activities of Polycyclic Aromatic Hydrocarbons and Their Monohydroxylated Derivatives by Yeast Two-Hybrid Assay

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Estrogenic/antiestrogenic activities of 14 polycyclic aromatic hydrocarbons (PAHs) and 63 monohydroxylated PAHs (OHPAHs) having 2 to 6 rings were evaluated by yeast two-hybrid assay expressing human estrogen receptor α . Relative effective potencies of estrogenic and antiestrogenic activities were calculated as the inverse values of the relative concentration of the test compound that gave the same activities of E_2 and 4-hydroxytamoxifen, respectively. PAHs did not show any estrogenic/antiestrogenic activity, but several OHPAHs having 3 to 5 rings showed activities. Especially, OHPAHs having 4 rings such as 3-, 4- and 10-hydroxybenz[*a*]anthracenes (3-, 4- and 10-OHBaAs) and 2-hydroxychrysene (2-OHCh) showed strongly estrogenic activity. Several other OHPAHs having 4 rings such as 2- and 3-hydroxybenzo[*c*]phenanthrenes (2-, 3- OHBcPhs), 2-OHBaA and 3-OHCh showed strongly antiestrogenic activity. The length-to-breadth (L/B) ratios of the rectangular van der Waals planes surrounding the ring molecules of estrogenic OHPAHs were in the narrow range from 1.599 to 1.734. The distances between the oxygen atom of the phenol group and farthest hydrogen atom (O-H distance) of the estrogenic OHPAHs ranged from 10.825 Å to 11.738 Å. The L/B ratios and O-H distances of antiestrogenic OHPAHs were in the wider ranges from 1.277 to 1.734 and from 8.47 Å to 11.681 Å, respectively. The partial charges (atomic unit) of the phenol group of both estrogenic and antiestrogenic OHPAHs were in the range from –0.250 atomic unit (au) to –0.253 au. The similarity of these values to those of E_2 and diethylstilbestrol suggested that the compositions of estrogenic OHPAHs were similar to them and that the compositional conditions of estrogenic OHPAHs were much smaller than those of antiestrogenic OHPAHs. These results raise the possibility of predicting the estrogenic/antiestrogenic activities of OHPAHs from their structural characteristics, although using only the above three parameters might not be enough for accurate estimations.

Key words—hydroxypolycyclic aromatic hydrocarbon, estrogen receptor, estrogenic activity, antiestrogenic activity, structure-activity relationship

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants. They are formed through incomplete combustion of fossil fuel, wood and other organic materials including automobile exhaust, domestic heating and industrial processes. Humans and animals are exposed to PAHs from environmental (air, water), dietary

and occupational sources, and also from cigarette smoke.¹⁾ Many PAHs are carcinogenic in human and laboratory animals²⁾ and the principal concern regarding exposure to PAHs is that they increase the risk of cancer.

In recent years there has been increasing interest in endocrine disruptors which may cause dysfunction of human and wildlife endocrine systems leading to cancers, reproductive system abnormalities and immune system deficiencies.^{3,4)} Several screening test methods have been developed to evaluate the endocrine-disrupting activities of chemicals. As for PAHs, antiestrogenic activity was observed in a yeast assay system⁵⁾ and estrogenic ac-

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tivity was found in MCF-7 cells.⁶⁾ Yeast two-hybrid assay system based on the ligand-dependent interaction of estrogen receptor (ER) and its co-activator has been developed as a simple and effective screening biological system. The estrogenic activities of 517 chemicals including several monohydroxylated derivatives of PAHs (OHPAHs) were evaluated by using this method. A common structure of the above estrogenic compounds was a phenol with a hydrophobic moiety at the *para*-position without a bulky group at the *ortho*-position.⁷⁾ This result suggested that the structural similarity of several OHPAHs to 17 β -estradiol, which binds to human estrogen receptor (hER), might account for their estrogenic or antiestrogenic activities.

OHPAHs are formed from the corresponding PAHs in the presence of cytochrome P450 enzymes (CYPs) in human and animals⁸⁾ as well as chemically in the atmosphere,⁹⁾ suggesting that the concentrations of OHPAHs may increase easily in the body. It is important to clarify whether these compounds have any biological effects. Several studies have been conducted to evaluate the estrogenic and antiestrogenic activities of OHPAHs such as hydroxybenzo[*a*]pyrene (OHBaP). Both 1- and 2-OHBaPs bound strongly to ER in rat cytosol but 4-, 5-, 6- and 12-OHBaPs bound poorly.¹⁰⁾ Both 3- and 10-OHBaPs showed antagonistic activity to a mixture of ER α and β .⁶⁾ Recently, we have tested twelve OHBaP isomers (1- through 12-OHBaPs) using the competition binding as-

say to ER α and β , and the gene expression assay of the yeast two-hybrid assay system, and found that 1-, 2-, 3- and 9-OHBaPs were estrogenic and 8-OHBaP was antiestrogenic.¹¹⁾ We also identified 2-hydroxyfluorene (2-OHFl), 2- and 3-hydroxyphenanthrenes, 1-hydroxypyrene (1-OHPy) and *n*-propyl-*p*-hydroxybenzoate in cigarette smoke condensate as estrogenic compounds.¹²⁾ These results suggested that the activities of OHPAHs might depend strongly on their structures. However, little is known about the activities of the hydroxyl derivatives of other PAHs.

In this study, we examined both estrogenic and antiestrogenic activities of 14 PAHs and 63 OHPAHs having two to six rings in the yeast two-hybrid assay system and discuss how their structural characteristics might affect the expression of both estrogenic and antiestrogenic activities.

MATERIALS AND METHODS

Chemicals— Chemical structures of PAHs and OHPAHs tested in this report are shown in Fig. 1. Naphthalene (Np), fluorene (Fl), phenanthrene (Ph) anthracene (Ant) and benzo[*k*]fluoranthene (BkFR) were purchased from Kanto Chemical (Tokyo, Japan). Fluoranthene (FR), benz[*a*]anthracene (BaA) and benzo[*ghi*]perylene (BgPe) were purchased from Nakalai (Kyoto, Japan). Benzo[*e*]fluoranthene (BeFR), benzo[*j*]fluoranthene

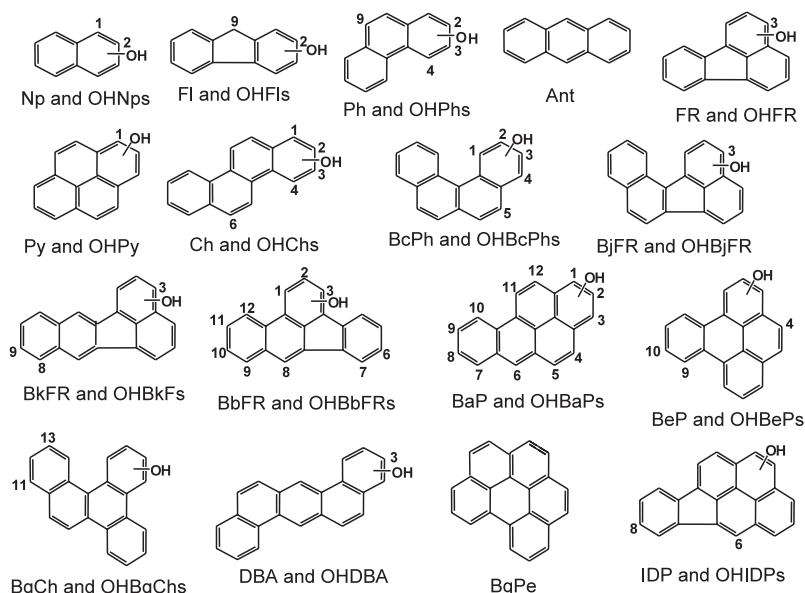


Fig. 1. Chemical Structures of PAHs and OHPAHs Tested

For abbreviations, see text. Numbers indicate the positions of hydroxyl groups.

(BjFR), 1- and 2-hydroxynaphthalenes (1- and 2-OHNps) and 17 β -estradiol (E₂) were purchased from Wako Pure Chemicals (Osaka, Japan). [2,4,6,7-³H(N)]-E₂ (72 Ci/mmol) was purchased from Dai-Ichi Pure Chemicals (Tokyo, Japan). Pyrene (Py), chrysene (Ch), benzo[*a*]pyrene (BaP), benzo[*c*]phenanthrene (BcPh), 2- and 9-OHFs and 1-OHPy were purchased from Aldrich (Milwaukee, WI, U.S.A.). 4-Hydroxytamoxifen and benzo[*e*]pyrene (BeP) were from Sigma (St. Louis, MO, U.S.A.). Benzo[*g*]chrysene (BgCh), dibenz[*a,h*]anthracene (DBA), 2-, 3-, 4- and 9-hydroxyphenanthrenes (2-, 3-, 4- and 9-OHPhs) were from Chiron AS (Trondheim, Norway). Indeno[1,2,3-*cd*]pyrene (IDP), 3-hydroxyfluoranthene (3-OHFR), 1-, 2-, 3-, 4-, 5-, 9-, 10- and 11-hydroxybenz[*a*]anthracenes (1-, 2-, 3-, 4-, 5-, 9-, 10- and 11-OHBaAs), 1-, 2-, 3-, 4- and 6-hydroxychrysenes (1-, 2-, 3-, 4- and 6-OHChs), 11- and 13-hydroxybenzo[*g*]chrysenes (11- and 13-OHBgChs), 1-, 2-, 3-, 6-, 7-, 8-, 9-, 10-, 11- and 12-hydroxybenzo[*b*]fluoranthenes (1-, 2-, 3-, 6-, 7-, 8-, 9-, 10-, 11- and 12-OHBbFRs), 3-hydroxybenzo[*j*]fluoranthene (3-OHBjFR), 3-, 8- and 9-hydroxybenzo[*k*]fluoranthenes (3-, 8- and 9-OHBkFRs), 1-, 2-, 3-, 4- and 5-hydroxybenzo[*c*]phenanthrenes (1-, 2-, 3-, 4- and 5-OHBcPhs), 1-, 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, 10-, 11- and 12-OHBaPs, 4-, 9- and 10-hydroxybenzo[*e*]pyrenes (4-, 9- and 10-OHBePs), 3-hydroxydibenz[*a,h*]anthracene (3-OHDBA) and 6- and 8-hydroxyindeno[1,2,3-*cd*]pyrenes (6- and 8-OHIDPs) were purchased from NCI Chemical Carcinogen Repository (Kansas City, MO, U.S.A.). Test compounds were dissolved in ethanol and stored at -20°C until use. All other chemicals were of the highest quality available from commercial sources.

Assay of Estrogenic and Antiestrogenic Activities — Yeast cells expressing hER α or β were prepared according to the previous report.¹²⁾ Estrogenic and antiestrogenic activities of PAHs and OHPAHs were evaluated by the yeast two-hybrid assay method using the above yeast cells as described.¹¹⁾ To examine the agonistic activity of PAHs and OHPAHs, the yeast cells were grown overnight at 27°C with shaking in synthetic defined medium free from tryptophan and leucine, and treated with each test compound in the concentration range from 1 \times 10⁻⁹ M to 1 \times 10⁻⁶ M at 27°C for 4 hr. After the incubation, the treated cells were collected and enzymatically digested with 1 mg/ml Zymolyase 20T

at 37°C for 20 min. 2-Nitrophenyl- β -D-galactoside (ONPG) was added to the lysate to a final concentration of 4 mg/ml. After incubation at 27°C for 20 min, the reaction was stopped by the addition of 1 M Na₂CO₃. The yeast debris was removed by centrifugation and β -galactosidase activity was assayed by measuring the absorbance of supernatant at 415 nm. Three independent experiments were run, and the mean values of the three were shown in this report. Relative effective potency of estrogenic activity (REP_E) was calculated as the inverse value of the relative concentration of the test compound that gave the same activity of E₂. Relative effective potency of antiestrogenic activity (REP_{AE}) was calculated as the inverse value of the relative concentration of the test compound that gave the same activity of 4-hydroxytamoxifen in the presence of 1 nM E₂.

Competition Binding Assay — Competition between the test compounds and E₂ was measured as described previously.¹³⁾ A crude extract prepared from Sf21 insect cells infected with hER α or β recombinant virus, kindly supplied by Professor T. Nishihara, Osaka University, was used for competition binding assay. Various concentrations of a test compound and 1.25 pmol of [2,4,6,7-³H(N)]-E₂ were incubated in 250 μ l of reaction mixture of TKE (20 mM Tris-HCl at pH 7.4, 20 mM KCl and 1 mM EDTA) containing 5 μ l hER α or β at 0°C for 16 hr. Free and bound ligands were separated by addition of an equal volume of dextran-coated charcoal. Samples were periodically mixed on ice for 5 min and centrifuged at 15000 rpm for 1 min. Aliquots (300 μ l) of the supernatant were used for scintillation counting.

Calculation of Physical Parameters — In order to examine the relationship between structure and activity, several physical parameters of PAHs and OHPAHs were used. The length-to-breadth (L/B) ratios¹⁴⁾ of the rectangular van der Waals plane surrounding each PAH molecule were obtained from the Polycyclic Aromatic Hydrocarbon Structure Index.¹⁵⁾ The O-H distance, the distance between the oxygen atom of the phenol group and the hydrogen atom located farthest from the phenol group, and the partial charge (atomic charge, au) of the phenol group were calculated with a molecular modeling program (CACHeworksystem ver.4.1.1 for Apple Macintosh, Fujitsu Co. Ltd., Chiba, Japan).

Determination of OHPAHs in Yeast Cells — Yeast cells incubated with test compound were enzymatically digested according to the procedures

described above (*Assay of estrogenic and antiestrogenic activities*). The digested yeast cells was mixed with acetonitrile and centrifuged. The upper (acetonitrile) phase was collected, filtered with a membrane and an aliquot of the filtrate was injected into the HPLC with a fluorescence detector. OHPAHs were quantified by using deuterated OHPAHs as internal standards according to our previous report,¹⁶⁾ with some modifications of mobile phases, detection wavelengths and time program.

RESULTS

Estrogenic Activity

None of the 14 PAHs (Np, Fl, Ph, Ant, FR,

Py, BaA, Ch, BbFR, BkFR, BaP, DBA, BgPe and IDP) showed estrogenic activity at concentrations between 1×10^{-6} M and 1×10^{-9} M. A significant increase of β -galactosidase activity ($REP_E > 1 \times 10^{-3}$) was observed for 3-, 4- and 10-OHBaAs and 2-OHCh. These were all 4-ring OHPAHs. Among them, 4-OHBaA exhibited the strongest estrogenic activity ($REP_E = 7.5 \times 10^{-3}$). 2-OHFl, 2-OHPh (3-ring), 3-OHFR, 1-OHPy, 1-OHCh, 12-OHBbFR, 3-OHBkFR (4-ring), and 4-OHBeP (5-ring) were also active ($1 \times 10^{-3} > REP_E \geq 1 \times 10^{-4}$). The estrogenic activities of the other OHPAHs were much weaker or undetectable (Table 1).

Antiestrogenic Activity

None of the 14 PAHs as tested for estrogenic

Table 1. Estrogenic and Antiestrogenic Activities of OHPAHs by Yeast Two-Hybrid Assay

No. of rings	Abbreviation	$REP_E^{a)}$ $\times 10^{-4}$	$REP_{AE}^{b)}$ $\times 10^{-2}$	No. of rings	Abbreviation	$REP_E^{a)}$ $\times 10^{-4}$	$REP_{AE}^{b)}$ $\times 10^{-2}$
2	1-OHNp	< 1	< 1	5	6-OHBbFR	< 1	3.6
	2-OHNp	< 1	< 1		7-OHBbFR	< 1	< 1
3	2-OHFl	5.6	< 1		8-OHBbFR	< 1	< 1
	9-OHFl	< 1	< 1		9-OHBbFR	< 1	< 1
	2-OHPh	7.5	< 1		10-OHBbFR	< 1	7.6
	3-OHPh	< 1	< 1		11-OHBbFR	< 1	< 1
	4-OHPh	< 1	< 1		12-OHBbFR	1	< 1
4	9-OHPh	< 1	< 1		3-OHBjFR	< 1	< 1
	3-OHFR	4.2	< 1		3-OHBkFR	1.3	< 1
	1-OHPy	2.4	< 1		8-OHBkFR	< 1	< 1
	1-OHBaA	< 1	< 1		9-OHBkFR	< 1	21
	2-OHBaA	< 1	42		1-OHBaP	< 1	< 1
	3-OHBaA	42	< 1	2-OHBaP	< 1	< 1	
	4-OHBaA	75	< 1	3-OHBaP	< 1	< 1	
	5-OHBaA	< 1	< 1	4-OHBaP	< 1	< 1	
	9-OHBaA	< 1	34	5-OHBaP	< 1	< 1	
	10-OHBaA	32	< 1	6-OHBaP	< 1	< 1	
	11-OHBaA	< 1	< 1	7-OHBaP	< 1	< 1	
	1-OHBcPh	< 1	< 1	8-OHBaP	< 1	43	
	2-OHBcPh	< 1	69	9-OHBaP	< 1	< 1	
	3-OHBcPh	< 1	190	10-OHBaP	< 1	< 1	
	4-OHBcPh	< 1	13	11-OHBaP	< 1	< 1	
	5-OHBcPh	< 1	1.2	12-OHBaP	< 1	< 1	
	1-OHCh	4.2	< 1	4-OHBeP	4.2	< 1	
2-OHCh	42	< 1	9-OHBeP	< 1	< 1		
3-OHCh	< 1	40	10-OHBeP	< 1	< 1		
4-OHCh	< 1	< 1	11-OHBgCh	< 1	29		
6-OHCh	< 1	30	13-OHBgCh	< 1	22		
5	1-OHBbFR	< 1	< 1	3-OHDBA	< 1	< 1	
	2-OHBbFR	< 1	< 1	6	6-OHIDP	< 1	< 1
	3-OHBbFR	< 1	< 1		8-OHIDP	< 1	< 1

a) Estrogenic activity of each test compound was assayed in the concentration range from 1×10^{-6} M to 1×10^{-9} M. REP_E was calculated from the value of E_2 as a positive control. b) Antiestrogenic activity of each test compound was assayed in the concentration range from 1×10^{-6} M to 1×10^{-9} M. REP_{AE} was calculated from the value of 4-hydroxytamoxifen as a positive control.

assay showed antiestrogenic activity at concentrations between 1×10^{-6} M and 1×10^{-9} M. On the other hand, several OHPAHs showed antiestrogenic activity. A significant decrease of β -galactosidase activity ($REP_{AE} > 1 \times 10^{-1}$) was observed for 2- and 9-OHBaAs, 2-, 3- and 4-OHBcPhs, 3- and 6-OHChs (4-ring), 9-OHBkFR, 8-OHBaP, 11- and 13-OHBgChs (5-ring). Among them, 3-OHBcPh exhibited the strongest antiestrogenic activity ($REP_{AE} = 1.9$). 5-OHBcPh, 6- and 10-OHBbFRs showed weak activity ($1 \times 10^{-1} \geq$

$REP_{AE} \geq 1 \times 10^{-2}$). The relative antiestrogenic activities of the other OHPAHs were much weaker or undetectable (Table 1).

Competition Binding Assay

No component from uninfected Sf2 cells (*i.e.* cells that did not express hER α and β) bound to hER non-specifically. Thus, any decrease of bound [2,4,6,7- 3 H(N)]-E $_2$ in the presence of one of the test compounds was attributed to competitive binding to hER. Four- and 5-ring PAHs did not bind to either hER α or β (relative binding affinity $\leq 5 \times 10^{-3}$), while each of the corresponding OHPAHs except for 1-OHBcPh bound to hER (relative binding affinity $> 1 \times 10^{-2}$). 2-OHPh, 3-OHFR, 3-, 4-, 9- and 10-OHBaAs, 3- and 4-OHBcPhs, 2-OHCh, 10-BkFR, 3- and 8-OHBaPs showed strong binding affinity to hER α (relative binding affinity $> 2 \times 10^{-1}$). Among them, 9-OHBaA and 2-OHCh showed the strongest binding affinities (relative binding affinity $\approx 4.1 \times 10^{-1}$) (Table 2). In general, these OHPAHs bound more strongly to hER β than to hER α , but the difference was small. So, the relative binding affinities to only hER α are listed in Table 2.

DISCUSSION

One of the way in which a compound can have estrogenic/antiestrogenic activity is to have an affinity for hER. Several 3-ring PAHs, such as Ant and Ph, have antiestrogenic activity.¹⁷⁾ However, in our present study, their activities were much weaker than the activity of 4-OHT. Moreover, 4-ring PAHs such as FR, Py, BaA, BcPh and Ch did not bind to hER (Table 2) and did not show any activities. Thirty-one OHPAHs having 3 to 5 rings bound to hER except for 1-OHBcPh, whose binding affinity was as low as the affinity of BcPh (Table 2). Among the OHPAHs tested, only a few compounds exhibited estrogenic or antiestrogenic activity in the yeast-two hybrid assay system (Table 1). In order to check the adsorption of OHPAHs into yeast cells, yeast cells were incubated with 1-, 2- and 4-OHBaAs as inactive, estrogenic and antiestrogenic compounds according to their REP_E and REP_{AE} values, respectively. When the cells were digested and the solution was extracted with acetonitrile, 38–42% of the three OHBaAs added were recovered from the yeast cells. These results mean that OHPAHs passed through the cell membrane and bound to hER in the nucleus. BaA was recovered from

Table 2. Binding Affinity of PAHs and OHPAHs to hER

No. of rings	Abbreviation	Relative binding affinity ^{a)}	
3	2-OHFl	0.128	
	2-OHPh	0.232	
4	Frt	0.005	
	3-OHFR	0.207	
	Py	0.004	
	1-OHPy	0.110	
	BaA	0.005	
	1-OHBaA	0.047	
	2-OHBaA	0.137	
	3-OHBaA	0.228	
	4-OHBaA	0.294	
	5-OHBaA	0.058	
	9-OHBaA	0.410	
	10-OHBaA	0.295	
	11-OHBaA	0.027	
	BcPh	0.002	
	1-OHBcPh	0.001	
	2-OHBcPh	0.185	
	3-OHBcPh	0.345	
	4-OHBcPh	0.210	
5-OHBcPh	0.044		
4	Ch	0.003	
	1-OHCh	0.126	
	2-OHCh	0.406	
	3-OHCh	0.138	
	4-OHCh	0.012	
	6-OHCh	0.090	
	5	10-OHBbFR	0.115
		3-OHBkFR	0.150
9-OHBkFR		0.205	
1-OHBaP		0.187	
3-OHBaP		0.394	
8-OHBaP		0.376	
4-OHBeP		0.117	
11-OHBgCh		0.197	
13-OHBgCh	0.135		

^{a)} hER α was used in this assay. ^{b)} Relative binding affinity of each test compound (1×10^{-5} M) was calculated from binding affinity of E $_2$ (1×10^{-7} M) as a competitor.

the yeast cells incubated with BaA, suggesting that PAH, that have a larger $P_{o/w}$ value than their corresponding OHPAH, also passed through the cell membrane. However, PAHs could not bind to the active site of hER because of the absence of a phenol group. This might be the reason for the large difference between PAHs and OHPAHs in estrogenic activities. The data in Table 1 suggest that the phenol group is necessary for OHPAHs to exhibit both estrogenic and antiestrogenic activities. However, we have recently found that several quinoid derivatives of PAHs showed strong antiestrogenic activities, suggesting hydroxyl group is not necessary for exhibiting antiestrogenic activity.

The binding affinities of the OHPAHs to hER α were correlated with their estrogenic activities (Fig. 2A; $R^2 = 0.6146$) and weakly correlated with their antiestrogenic activities (Fig. 2B; $R^2 = 0.4115$). However, 2-OHCh and 9-OHBaP, which had the strongest binding affinities to hER α , were not the strongest estrogenic and antiestrogenic OHPAHs, respectively. Thus, OHPAHs, which had the strongest affinity, did not always exhibit the strongest estrogenic or antiestrogenic activity, suggesting that the binding affinity to hER was not the only factor to control the estrogenic or antiestrogenic activity.

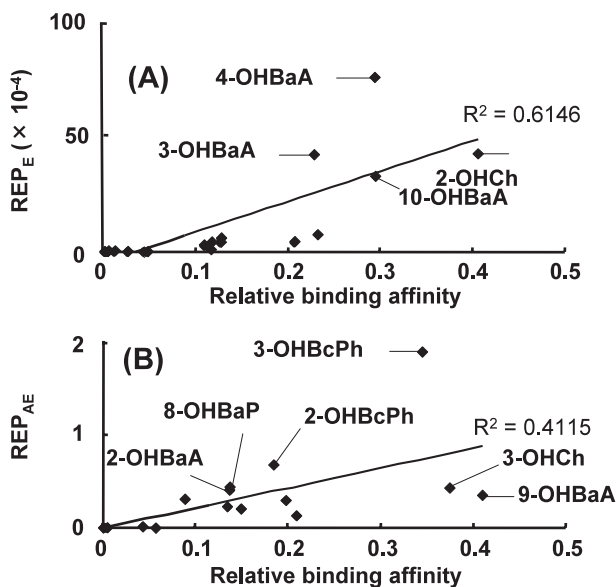


Fig. 2. Relationships between Binding Affinity to hER α and (A) REP_E and (B) REP_{AE} of OHPAHs by Yeast Two-Hybrid Assay

Relative binding affinity was calculated from binding affinity of E_2 as a competitor. REP_E and REP_{AE} were calculated from the values of E_2 and 4-OHT.

Strong estrogenic activity was mainly observed in 4-ring OHPAHs (Fig. 3A). Several 3-ring OHPAHs such as 2-OHFl, 2- and 3-OHFls were identified in cigarette smoke condensate as estrogenic compounds.¹²⁾ Among these 3-ring PAHs, 2-OHPh gave the largest REP_E ($= 7.5 \times 10^{-4}$) followed by 2-OHFl, but the value was 10 times smaller than that of 4-OHBaA which showed the strongest estrogenic activity. Strongly antiestrogenic activity was also observed in several OHPAHs having 4 and 5 rings (Fig. 3B). However, many other 4- and 5-ring OHPAHs were inactive (Table 1). Thus, the numbers of rings of OHPAH was one of factors controlling estrogenic or antiestrogenic activity.

It has been reported that the phenol group (OH-3) of E_2 makes hydrogen bonds with Glu353 and Arg394 of hER and H_2O and that the alcohol group (OH-17) of E_2 has an affinity for the nitrogen atom of His524 of hER. Among these hydrogen bonds, the distance between Glu353 and the phenol group was the shortest (2.37 Å), which suggests that this bond is the strongest. On the other hand, van der Waals interaction takes place between the benzene ring of E_2 and the benzene ring of Phe404 of the binding site of hER.^{18,19)} These reports suggest whether 4-ring OHPAHs interact with the binding site of hER depends on the conformation of the benzene ring and the position of the phenol group.

Estrogenic compounds usually consist of phenols with hydrophobic moiety at the *para*-position

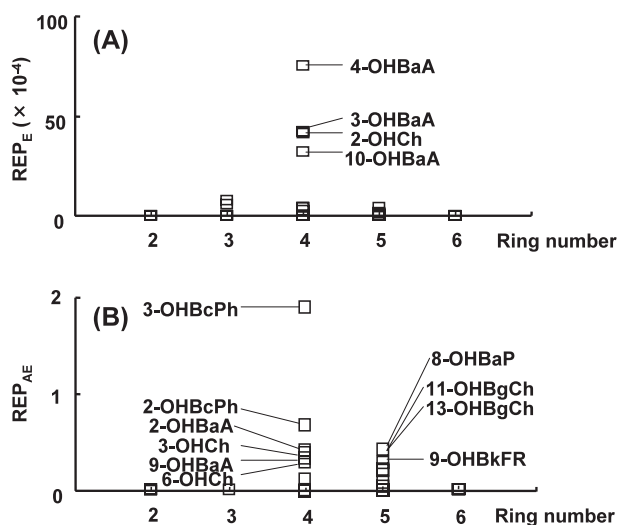


Fig. 3. Relationship between Number of Rings of OHPAHs and (A) REP_E and (B) Calculated from The Values of E_2 and 4-OHT

REP_E and REP_{AE} were calculated from the values of E_2 and 4-OHT.

without a bulky group at the *ortho*-position.⁷⁾ However, 4-OHBaA, which has a bulky group (Ant) at the *ortho*-position of phenol, was strongly estrogenic ($REP_E = 7.5 \times 10^{-3}$; Table 1). Our results suggest that bulkiness only at the *ortho*-position might not be enough to suppress estrogenic activity. As shown in Fig. 4, the L/B ratio and the O-H distance might also have an effect on the activity. The strongly estrogenic 4-ring OHPAHs [3-, 4- and 10-OHBaAs and 2-OHCh ($REP_E \geq 3.2 \times 10^{-3}$)], had L/B ratios in the range from 1.599 to 1.734 and O-H distances in the range from 10.825 Å to 11.738 Å. The other 4-ring OHPAHs that were outside this area did not have such strong estrogenic activity.

Compounds having a strong affinity to hER, such as E₂ and diethylstilbestrol (DES), have two hydroxyl groups with the appropriate O-O distance.¹⁸⁾ The L/B ratios of E₂ and DES were 1.545 and 1.515, respectively. The O-O distances of E₂ and DES were 10.97 Å and 12.03 Å, respectively. These L/B ratios and O-O distances were close to the values of L/B ratios and O-H distances of the above four strongly estrogenic OHPAHs (Fig. 5).

On the other hand, the strongly antiestrogenic 4- and 5-ring OHPAHs [2- and 9-OHBaAs, 2- and 3-OHBcPhs, 3- and 6-OHChs, 9-OHBkFR, 8-OHBaP and 11- and 13-OHBgChs ($REP_{AE} \geq 0.21$)], had L/B ratios of 1.277 to 1.734 and O-H distances of 8.47 Å to 11.681 Å. The area of the L/B ratio and OH distance of strongly antiestrogenic OHPAHs was much larger than that of strongly estrogenic OHPAHs described above. All of these antiestrogenic compounds except 9-OHBaA were outside the area of strong estrogenic compounds as

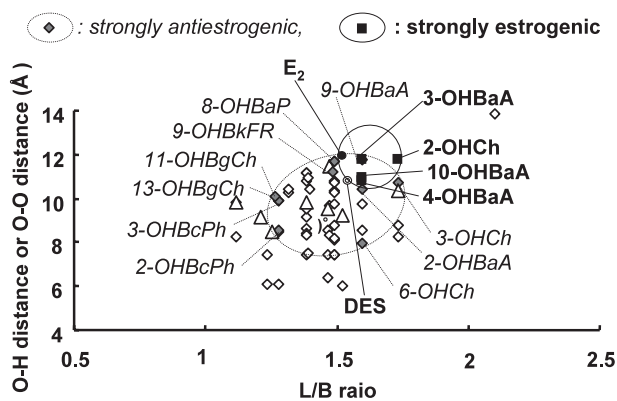


Fig. 4. Relationships between L/B Ratio and O-H Distance Values of Estrogenic and Antiestrogenic OHPAHs

hER α was used in the assay. Symbols: ■; $REP_E \geq 0.001$, ◇; $REP_{AE} > 0.1$, ○; E₂, ●; DES. In the cases of E₂ and DES, O-O distance was used instead of O-H distance.

described above. Although it is unclear why 9-OHBaA was an exception, this result suggests that the antagonistic OHPAHs can exhibit activity even when they bind to sites other than the active site of hER.

Finally, we examined the partial charge of the phenol group of OHPAHs that is peculiar to compounds elected by Austin Model 1 (AM1), since AM1 has often been used to quantify the structure-activity relationship as according to the semi-empirical molecular orbital theory.^{18,20,21)} The partial charge was related to estrogenic activity (Fig. 5A) as well as antiestrogenic activity (Fig. 5B). The partial charges of strongly estrogenic OHPAHs ($REP_E \geq 7.5 \times 10^{-4}$) ranged from -0.249 atomic unit (au) to -0.253 au for both agonistic and antagonistic effects. This value was very close to the values of phenol groups of E₂ (partial charge = -0.253 au) and DES (partial charge = -0.252 au), but not so close to that of the alcohol group of E₂ (partial charge = -0.318 au). The author also calculated other physical parameters such as ionization potential, dipole moment, dielectric energy and log P for all OHPAHs tested. However, there was not any relationship between these parameters and estrogenic/antiestrogenic activities.

It is interesting that 1- and 11-OHBaAs, whose phenol groups were on the Bay-region, did not

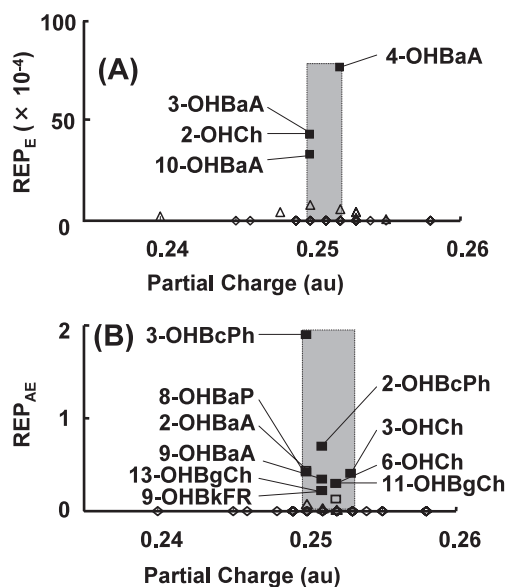


Fig. 5. Relationship between Partial Charge and (A) REP_E and (B) REP_{AE}

hER α was used in the assay. Symbols and relative activities are the same as in Fig. 3. REP_E and REP_{AE} were calculated from the values of E₂ and 4-OHT. Unit of partial charge, atomic unit.

6

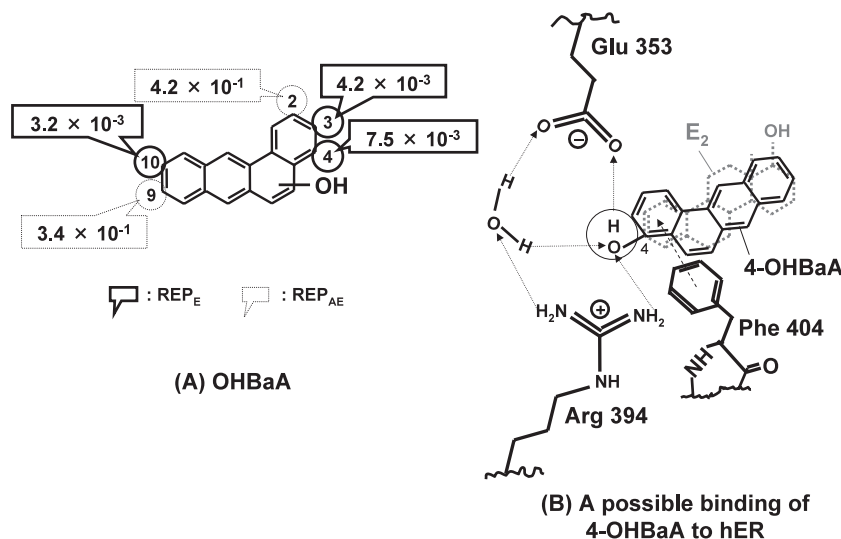


Fig. 6. Structures of (A) OHBaAs and (B) A Possible Binding of 4-OHBaP to hER Dotted line in Fig. 5B means E₂

show any activity and that 5-OHBaA, whose phenol group was on the K-region, did not. However, 3-, 4- and 10-OHBaAs showed strong estrogenic activity, while 2- and 9-OHBaAs showed strong antiestrogenic activity (Fig. 6A). All these active phenol groups were on the outside of the end rings of OHBaAs and the phenol groups of strongly antiestrogenic OHBaAs were next to the sites of the phenol groups of the above strongly estrogenic. As described above, the L/B ratios, O-H distances and partial charges of these 3-, 4- and 10-OHBaAs were similar to those of E₂ and DES (Fig. 4), suggesting that the phenol group is required to form hydrogen bonding at Glu353 of hER and to go into the active site of hER. Figure 6B shows a possible binding of 4-OHBaA to hER as an example of strongly estrogenic OHPAHs. The hydrogen bonds of 2- and 9-OHBaAs to Arg394 or His524 of hER may interfere with the insertion of E₂ into the active site.

As conclusions, the following facts were obtained from the assays of estrogenic and antiestrogenic activities of fourteen PAHs and sixty-three OHPAHs in the yeast two-hybrid system. Several OHPAHs having four rings showed strongly estrogenic/antiestrogenic activities. The most strongly estrogenic and antiestrogenic OHPAHs tested were 4-OHBaA and 3-OHBcP, respectively. A rectangular plane of four rings and a phenol group were necessary for OHPAHs to bind to the site of hER and exhibit the activities. OHPAHs with stronger affinities for hER tend to have stronger activity. Activities of OHPAHs were found to depend on the three physical parameters: L/B ratio, O-H distance

and partial charge of the phenol group. These findings suggested that estrogenic OHPAHs were similar to those of E₂ and DES and that the compositional flexibility of estrogenic OHPAHs was much smaller than that of antiestrogenic OHPAHs.

These facts suggest that the activities of OHPAHs can be roughly predicted from their physical parameters, although differentiation of agonistic and antagonistic effects is not easy. As mentioned above, several quinoid PAHs showed strongly antiestrogenic activities. Further studies of these compounds should clarify the relationship between structures and activities of PAH derivatives.

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