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Phosphine boranes as less hydrophobic building blocks than alkanes and silanes: Structure-property relationship and estrogen-receptor-modulating potency of 4-phosphinophenol derivatives

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ABSTRACT

Increasing structural options in medicinal chemistry is important for the development of novel and distinctive drug candidates. In this study, we focused on phosphorus-containing functionalities. We designed and synthesized a series of phosphinophenol derivatives and determined their physicochemical properties, including hydrophobicity parameter Log*P*, and their biological activity toward estrogen receptor (ER). Notably, the phosphine borane derivatives (**9** and **14**) exhibited potent ER-antagonistic activity, exceeding the potency of the corresponding alkane (**15**) and silane (**16**) derivatives, despite having a less hydrophobic nature. The determined physicochemical parameters will be helpful for the rational design of phosphorus-containing biologically active compounds. Our results indicate that phosphine boranes are a promising new chemical entry in the range of structural options for drug discovery.

1. Introduction

Phosphorus is ubiquitously present in biological systems, for example in the form of phosphates of nucleic acids or phosphorylated proteins.[1] Indeed, several series of phosphonic acid and phosphinic acid derivatives have been developed as therapeutic agents and drug candidates. For example, bisphosphonate agents such as etidronic acid (**1**) and zolendronic acid (**2**), bearing two phosphonic acid groups, are used clinically for the treatment of osteoporosis and related diseases.[2] Phosphonic acids are also used as mimics of phosphoric acids. Tenofovir (**3**), a nucleotide analog bearing a phosphonic acid moiety as a phosphoric acid isostere, is used in the treatment of human immunodeficiency virus (HIV) and hepatitis B virus (HBV) infections.[3] In addition to these nucleotide analogs, several applications of phosphonic acids as phosphoric acid mimics have been reported.[4,5] Furthermore, phosphinic peptides such as RXP407 (**4**; an ACE inhibitor)[6] are Zn binder-type or transition state analog (TSA)-type inhibitors of peptidases (Fig. 1).[7–9]

However, in contrast to these oxoacids of phosphorus, applications of other phosphorus-containing compounds, such as phosphines and related derivatives, in medicinal chemistry have been little investigated. Nevertheless, phosphorus is a versatile element which forms stable bonds with multiple elements, including sulfur, selenium and boron, as well as carbon and oxygen, and therefore phosphorus-containing functionalities including phosphines and related chemical species might provide a wide variety of chemical options for the structural development of biologically active compounds. Thus, elucidation of the physicochemical properties of various phosphorus-containing moieties as options for structural development of biologically active compounds is of interest. Here, we describe a systematic investigation of the structure-property relationship and structure-activity relationship of phosphine derivatives in order to throw light on their potential utility in medicinal chemistry.

2. Results and discussion

2.1. Compound design

Because systematic determination of the physicochemical parameters of phosphines and related substituents could be helpful in the rational design of novel biologically active compounds, we set out to synthesize a series of simple 4-phosphinophenol derivatives **5–14** bearing phosphine, phosphine oxide, phosphine sulfide, phosphine selenide, and phosphine borane functionalities and to examine their

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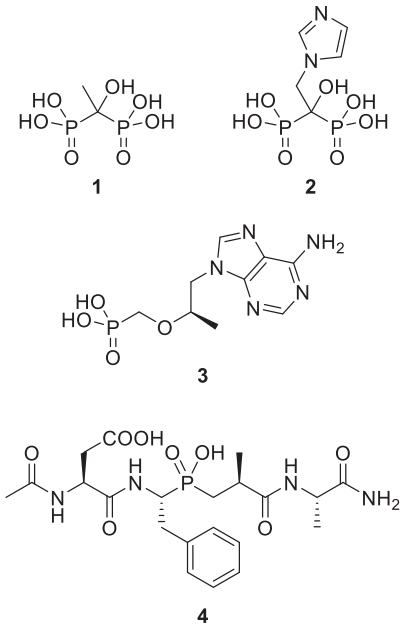


Fig. 1. Representative phosphorus-containing therapeutic agents **1–3** and phosphinic peptide **4**.

hydrophobicity, substituent constants, and biological activity. We also planned to compare their properties and activities with those of the corresponding alkane and silane analogs (**15** and **16**) (Fig. 2).

2.2. Synthesis

Synthesis of the designed phosphinophenol derivatives is summarized in Scheme 1. Using methylphenylphosphinic chloride **17** or diphenylphosphinic chloride **18** as the starting material, arylation reaction with 4-methoxyphenylmagnesium gave diphenyl phosphine oxide derivative **19** or **20**, respectively. Demethylation with borane tribromide gave the designed phosphine oxides **6** and **11**, respectively. Reduction of **6** and **11** with trichlorosilane gave the designed trivalent phosphines **5** and **10**, respectively.[10] Reduction of phosphine oxides **19** and **20** gave phosphines **21** and **22**, respectively, and then sulfidation of **21** and **22** with elemental sulfur gave phosphine sulfides **23** and **24**, respectively. The desired phosphine sulfides **7** and **12** were obtained by demethylation of **23** and **24**, respectively. Phosphine selenides **8** and **13** and phosphine boranes **9** and **14** were synthesized from phosphines **1** and **2** by reaction with elemental selenium or borane, respectively (Scheme 1).[11,12] The preparation of silicon analogue **16** was previously reported.[13]

2.3. Hydrophobicity

The hydrophobicity of the synthesized phosphinophenol compounds was determined as the octanol–water partition coefficient (*P*) using an HPLC method.[14] Table 1 summarizes the Log*P* values of the compounds and the Hansch–Fujita hydrophobicity parameter π of the substituents, deduced by subtraction of the log*P* value of phenol (1.46) from the observed log*P* value. Among derivatives bearing the same core structure, i.e., methyldiphenylphosphines **5–9** and triphenylphosphines **10–14**, larger hydrophobicity parameters were observed in the order of phosphine, phosphine borane, phosphine selenide, phosphine sulfide, and phosphine oxide. With regard to phosphine chalcogenides, compounds bearing chalcogen with a larger atomic number on phosphorus exhibited higher hydrophobicity. Phosphine boranes showed hydrophobicity parameters larger than those of phosphine chalcogenides and smaller than those of phosphines. These results suggest that phosphine-based substructures would represent a versatile structural option to produce a series of compounds with a wide range of hydrophobicity. Phosphine borane **9** is isoelectronic to alkane **15** and silane **16**, but showed a significantly smaller Log*P* value (Table 1).

2.4. Acidity

The acid dissociation constant pK_a of the phenolic hydroxyl group of each compound was determined from the pH-dependent change of absorbance spectra. Table 2 summarizes the pK_a values of the compounds and the differences in pK_a values between phenol and each compound. Large pK_a values were observed in the order of phosphine, phosphine borane, and phosphine chalcogenides. Both phosphine chalcogenides show similar pK_a values. Differences between the pK_a values of methyldiphenylphosphine derivatives and the corresponding triphenylphosphine derivatives are small. All phosphorus substituents investigated in this study function as strong electron-withdrawing groups in comparison with related alkyl and silyl groups, and phosphine boranes exhibit significant electron-withdrawing character, comparable to that of corresponding phosphine oxides and other phosphine chalcogenides (Table 2).

2.5. Activity toward estrogen receptor (ER)

Estrogen receptor (ER) is a member of the nuclear receptor superfamily.[15] ER has two subtypes (α and β), and their ligand is the endogenous estrogen estradiol (E_2). Estrogens are involved in regulation

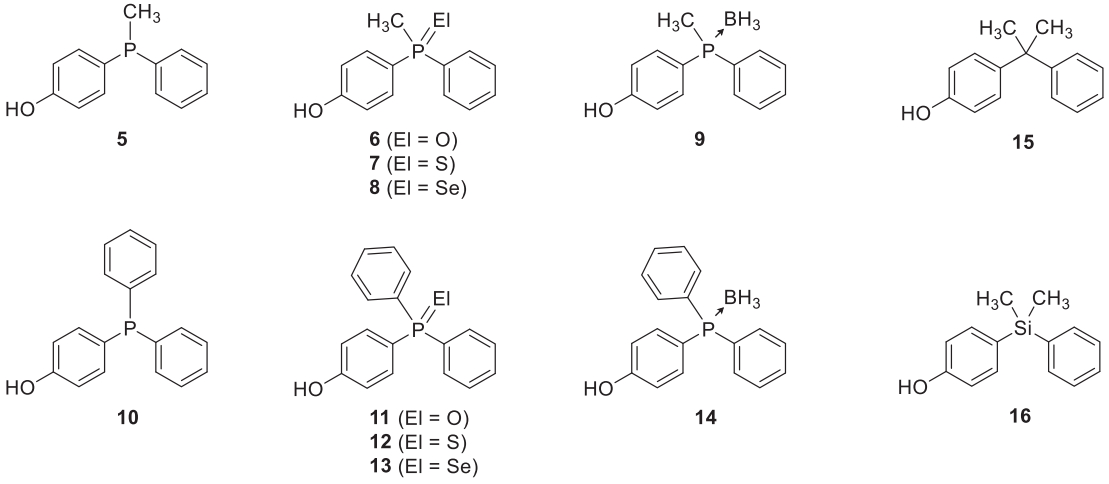
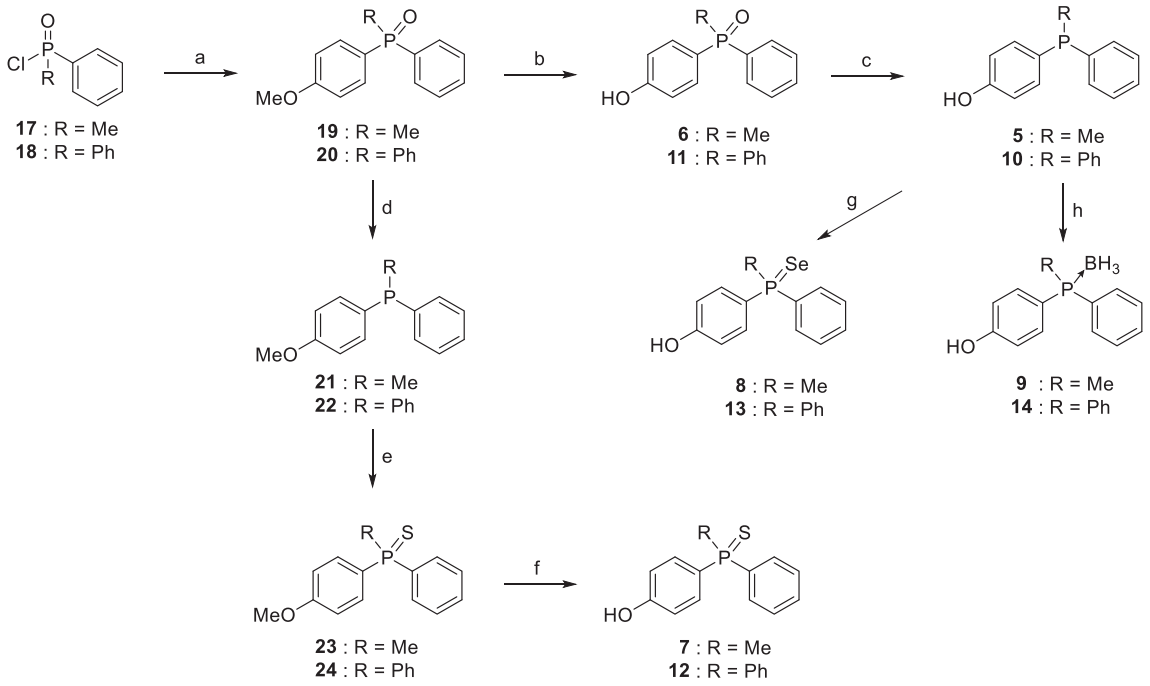
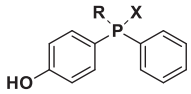


Fig. 2. Structures of compounds investigated in this study.



Scheme 1. Synthesis of the designed phosphinophenol derivatives. Reagents and conditions: (a) 4-methoxyphenylmagnesium bromide, THF, 0 °C to rt, 97% (**19**), quant. (**20**); (b) BBr_3, CH_2Cl_2, rt, quant. (**6**), 86% (**11**); (c) trichlorosilane, THF/toluene, rt to reflux, 81% (**5**), 93% (**10**); (d) trichlorosilane, THF/toluene, rt to reflux, 95% (**21**), 93% (**22**); (e) sulfur, THF, 120 °C microwave, 90% (**23**), 92% (**24**); (f) BBr_3, CH_2Cl_2, rt, 90% (**7**), 91% (**12**); (g) selenium, CH_2Cl_2, 100 °C, microwave, 97% (**8**), 89% (**13**); (h) BH_3-THF, THF, rt, quant. (**9**), 95% (**14**).

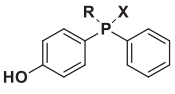
Table 1
Hydrophobicity parameter of phosphinophenol derivatives.



Compound	R	X	LogP	π[a]
Phenol	–		1.46	–
5	Me	–	3.67	+2.21
6		=O	1.88	+0.42
7		=S	2.69	+1.23
8		=Se	3.27	+1.81
9		$-BH_3$	3.38	+1.92
10	Ph	–	5.06	+3.60
11		=O	3.22	+1.76
12		=S	4.08	+2.62
13		=Se	4.73	+3.27
14		$-BH_3$	4.96	+3.50
15	–		4.20	+2.74
16	–		4.80	+3.34

[a] Hansch–Fujita hydrophobicity parameter of each substituent.

Table 2
pK_a Values of Phosphinophenol Derivatives.

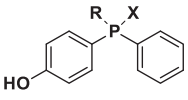


Compound	R	X	pK_a	ΔpK_a (phenol)
Phenol	–		10.44	–
5	Me	–	9.49	−0.95
6		=O	8.63	−1.81
7		=S	8.65	−1.79
8		=Se	8.64	−1.80
9		$-BH_3$	8.83	−1.61
10	Ph	–	9.48	−0.96
11		=O	8.51	−1.93
12		=S	8.60	−1.84
13		=Se	8.60	−1.84
14		$-BH_3$	8.81	−1.63
15	–		10.59	+0.15
16	–		10.25	−0.19

of the female and male reproductive systems, bone metabolism, and the cardiovascular system, as well as the central nervous system, and therefore ER ligands are attractive targets of drug discovery.[16,17] We have previously reported that simple 4-substituted phenols act as ER ligands,[13,18] and here we evaluated the estrogenic activities of our 4-phosphinophenol derivatives by means of luciferase reporter gene assay using HEK293 cells. The compounds showed no significant cytotoxicity toward HEK293 cells in the assay condition (data not shown). The concentration-dependent transcriptional activity of each compound was quantified; the agonistic activity was calculated as EC_{50} and the antagonistic activity as IC_{50} (Table 3).

Among the methyldiphenylphosphine derivatives **5**–**9**, phosphine **5** did not exhibit any activity toward ERα or ERβ. Phosphine chalcogenides **6**–**8** exhibited only weak partial agonistic activity toward both or either of ERα and ERβ. Interestingly, phosphine borane **9** exhibited antagonistic activity toward ERs, and its activity was similar to or greater than that of silane **16**, which is isoelectronic to phosphine borane **9**. None of the triphenylphosphine derivatives **10**–**14** exhibited agonistic activity toward ERs. Triphenylphosphinyl structure might be too bulky to induce ER-agonistic activity. Phosphine sulfide **12** and phosphine selenide **13** exhibited antagonistic activity toward ERα and ERβ, while phosphine borane **14** was an even more potent antagonist. The potency of ER ligands is influenced by the hydrophobicity of the compounds, and therefore it is reasonable that the relatively highly hydrophobic phosphine boranes are more potent than the corresponding phosphine chalcogenides. Though the trivalent phosphines **5** and **10** are more hydrophobic, the nucleophilic phosphines may be lost via reaction with electrophilic substances in the biological environment. We also examined membrane permeability of the compounds using immobilized artificial membrane column (IAM column), and the

Table 3
ER-modulating potency of the synthesized 4-phosphinophenol derivatives.



Compound	R	X	ERα		ERβ	
			EC_{50} (μM)[a]	IC_{50} (μM)[b]	EC_{50} (μM)[a]	IC_{50} (μM)[b]
5	Me	–	n.a.	n.a.	n.a.	n.a.
6		=O	19	n.a.	(36%)[c]	n.a.
7		=S	(24%)[c]	n.a.	(24%)[c]	n.a.
8		=Se	n.a.	n.a.	(24%)[c]	(30%) [d]
9		$-BH_3$	n.a.	9.8	(16%)[c]	2.5
10	Ph	–	n.a.	n.a.	n.a.	n.a.
11		=O	n.a.	n.a.	n.a.	n.a.
12		=S	n.a.	20	n.a.	13
13		=Se	n.a.	20	n.a.	10
14		$-BH_3$	n.a.	12	n.a.	2.0
15	–		4.4	24	(38%)[c]	13
16	–		> 30	13	(20%)[c]	5.0

[a] Concentration showing 50% of the maximal activity of E_2.
[b] concentration inhibiting the activity of 0.3 nM E2 by 50%.
[c] percentage of the maximal activation of E_2 at 30 μM.
[d] % inhibition at 30 μM, n.a: no activity.

results indicated the synthesized phosphine boranes can permeate through the lipid membrane (Table S1).

Among the synthesized phosphine derivatives, phosphine boranes exhibited the most potent activity. Therefore, we conducted a docking simulation of **9** with the X-ray crystal structure of the antagonist-bound form of hERβ ligand-binding domain (LBD) (PDB ID: 1L2J),[19] using AutoDock 4.2.[20] Compound **9** has two stereoisomers depending on the chirality of the phosphorus atom, and both isomers were docked. Fig. 3A shows the docking model of the phosphine borane **9** in the hERβ LBD as a superimposition of the two isomers. In the calculated structure, the two isomers occupy the same region of the ligand-binding pocket and take almost the same conformation. Namely, the phenolic hydroxyl group interacts with Glu305 and Arg346, with which estradiol forms key hydrogen bonds, and the methylphenylphosphine borane moiety occupies the hydrophobic cavity of the ligand-binding pocket. Next, we docked silane **16**, which is isoelectronic with phosphine borane **9**, to the hERβ LBD, and compared the binding mode with that of phosphine borane. Fig. 3B and 3C shows the docked structure of **16** superimposed on that of each isomer of **9**. In the docked structure, silane **16** adopts the same conformation as phosphine borane (Fig. 3). We also conducted a docking simulation of the compounds with hERαLBD (PDB ID: 3UUA),[21] and the results similar to those of hERβ were obtained from the calculation, namely, silane **16** adopts the same conformation as phosphine borane (Fig. 4). Biological evaluation revealed that phosphine borane **9** showed similar ERβ-inhibitory potency to that of silane **16** (IC_{50} = 2.5 μM and 5.0 μM, respectively). Though the hydrophobicity of phosphine borane **9** is smaller than that of silane **16** (there is a difference of 1.42 in the LogP values), the results suggest that phosphine boranes can function as isosteric building blocks of silanes, albeit with a less hydrophobic nature than silanes. Methods of decreasing the hydrophobicity of compounds are useful tools for medicinal chemists, and therefore phosphine boranes could be useful as less hydrophobic building blocks than alkanes and silanes.

3. Conclusion

In this study, using the phosphinophenol structure as a common platform, we investigated the structure-property relationship and ER-modulating potency of phosphine derivatives. The phosphine derivatives examined showed a wide range of hydrophobicity parameters, and these results suggest that phosphine-based substructures provide a versatile option to generate a series of compounds with a wide range of hydrophobicity. Phosphine boranes **9** and **14** exhibited significant ER-antagonistic activity, suggesting that phosphine boranes can function as isosteric building blocks of alkanes and silanes with a less hydrophobic nature. The results indicate the potential of phosphine boranes as new structural options for drug discovery. The parameters and structure–-activity relationship presented here should be helpful in the application of such phosphorus-containing compounds for drug development.

4. Experimental

4.1. Chemistry

4.1.1. General remarks. All reagents were purchased from Sigma-Aldrich Chemical Co., Tokyo Kasei Kogyo Co., FUJIFILM Wako Pure Chemical Industries, or Kanto Kagaku Co., Inc. Silica gel for column chromatography was purchased from Kanto Kagaku Co., Inc. ^{1}H NMR, ^{13}C NMR and ^{31}P NMR spectra were recorded on a JEOL JNM-GX500 (500 MHz, 125 MHz, and 202 MHz respectively) spectrometer. Chemical shifts for ^{1}H NMR and ^{13}C NMR are reported as parts per million (ppm) relative to chloroform (7.26 ppm for ^{1}H NMR and 77.00 ppm for ^{13}C NMR) or DMSO (2.50 ppm for ^{1}H NMR and 39.52 ppm for ^{13}C NMR) or methanol (3.31 ppm for ^{1}H NMR and 49.00 ppm for ^{13}C NMR). The following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = double doublet, td = triple doublet, br = broad. Electrospray ionization mass spectra (ESI-MS) were recorded on a Bruker micrOTOF II spectrometer with Low Concentration Tuning mix (G1969-85000).

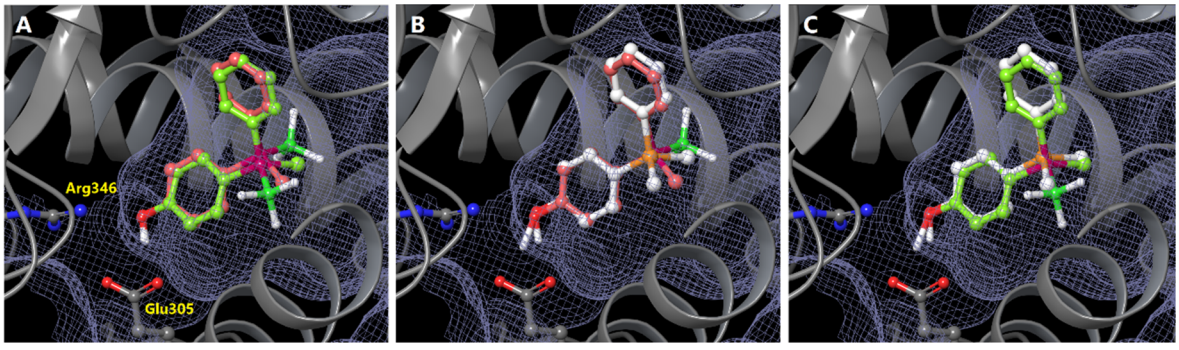


Fig. 3. Docking models of phosphine borane **9** with hERβ LBD (PDB ID: 1L2J) obtained with AutoDock 4.2. The protein surface is indicated as a blue mesh. A) Superimposition of docking models of (*R*)-**9** (pink) and (*S*)-**9** (green) in the hERβ LBD. B). Superimposition of docking models of (*R*)-**9** (pink) and silane **16** (white). C) Superimposition of docking models of (*S*)-**9** (green) and silane **16** (white). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

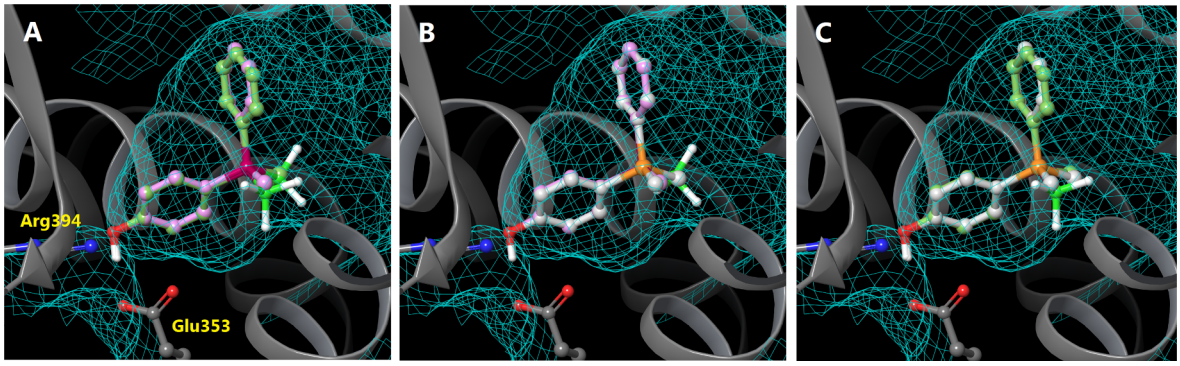


Fig. 4. Docking models of phosphine borane **9** with hERα LBD (PDB ID: 3UUA) obtained with AutoDock 4.2. The protein surface is indicated as a cyan mesh. A) Superimposition of docking models of (*R*)-**9** (pink) and (*S*)-**9** (green) in the hERα LBD. B). Superimposition of docking models of (*R*)-**9** (pink) and silane **16** (white). C) Superimposition of docking models of (*S*)-**9** (green) and silane **16** (white). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

*4.1.1. General procedure a for preparation of phosphine oxides **19** and **20**.*

To a solution of dialkylphosphinic chloride (**17** or **18**) in dry THF, 4-methoxyphenylmagnesium bromide solution (0.5 M in THF, 1.1 or 2.0 eq) was added dropwise at 0 ˚C under an argon atmosphere. The mixture was stirred at room temperature for 2 h, and then 1.0 M aqueous HCl was added. Stirring was continued for 10 min, and the mixture was extracted with $CHCl_3$. The organic layer was washed with saturated brine, dried over with sodium sulfate and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography on silica gel (ethyl acetate/methanol = 10/1) to isolate the product.

*4.1.2. General procedure B for cleavage of methoxy groups to afford compounds **6, 7, 11,** and **12**.*

To a solution of **13**, **14**, **17**, or **18** in dry CH_2Cl_2, boron tribromide (1.0 M in CH_2Cl_2, 5 eq) was added dropwise at 0 ˚C under an argon atmosphere. The reaction mixture was stirred at room temperature for 15 h, then quenched with water, and the mixture was extracted with $CHCl_3$. The organic layer was washed with saturated brine, dried over with sodium sulfate and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography on silica gel (ethyl acetate/methanol = 10/1) to isolate the product.

*4.1.3. General procedure C for reduction of phosphine oxide to afford phosphines **5, 10, 21,** and **22**.*

To a solution of **6**, **11**, **19**, or **20** in a mixture of dry THF and toluene (1:1), trichlorosilane (12–20 eq) was added at room temperature under an argon atmosphere. The reaction mixture was stirred for 2 h at 110 ˚C, then quenched with 10% aqueous NaOH solution, and extracted with $CHCl_3$. The organic layer was washed with saturated brine, dried over sodium sulfate, and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography on silica gel (chloroform/methanol = 10/1) to isolate the desired compounds.

*4.1.4. General procedure d for preparation of phosphine sulfides **23** and **24**.*

Compound **21** or **22** and elemental sulfur (15 eq) were dissolved in dry THF and the mixture was stirred for 30 min at 120 ˚C under microwave irradiation. The reaction mixture was washed with water and extracted with $CHCl_3$. The organic layer was washed with saturated brine, dried over sodium sulfate and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography on silica gel (hexane/ethyl acetate = 10/1) to isolate the product.

*4.1.5. General procedure e for preparation of phosphine selenides **8** and **13**.*

Compound **5** or **10** and selenium powder (10 eq) were dissolved in dry THF and the mixture was stirred for 30 min at 100 ˚C under microwave irradiation. The reaction mixture was filtered and the filtrate was concentrated under reduced pressure. The resulting residue was purified by flash column chromatography on silica gel (hexane/ethyl acetate = 2/1) to isolate the product.

*4.1.6. General procedure F for preparation of phosphine boranes **9** and **14**.*

To the solution of **5** or **10** in dry THF, BH_3 in THF (1.0 M, 3 eq) was added at room temperature under an argon atmosphere. The reaction mixture was stirred for 3 h at room temperature, then quenched with water and extracted with $CHCl_3$. The organic layer was washed with saturated brine, dried over with sodium sulfate and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography on silica gel (chloroform:ethyl acetate = 10:1) to isolate the product.

*4.1.7. (4-Methoxyphenyl)(methyl)(phenyl)phosphine oxide (**19**)*

Prepared by general procedure A (97%) as a white powder. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.71 (m, 2H), 7.66 (m, 2H), 7.53 (td, J = 7.5, 1.8 Hz, 1H), 7.48 (td, J = 7.5, 2.9 Hz, 2H), 6.99 (dd, J = 8.6, 2.9 Hz, 2H), 3.85 (s, 3H), 2.04 (d, J = 13.2 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.5 (d, J_{CP} = 2.4 Hz), 134.3 (d, J_{CP} = 101.3 Hz), 132.5 (d, J_{CP} = 10.5 Hz), 131.7 (d, J_{CP} = 2.4 Hz), 130.5 (d, J_{CP} = 10.7 Hz), 128.6 (d, J_{CP} = 11.9 Hz) , 124.9 (d, J_{CP} = 107.3 Hz), 114.3 (d, J_{CP} = 11.9 Hz), 55.4 (s), 16.8 (d, J_{CP} = 73.9 Hz). ^{31}P NMR (202 MHz, DMSO-d_6) δ 28.9 (s).

*4.1.8. (4-Methoxyphenyl)diphenylphosphine oxide (**20**)*

Prepared by general procedure A (quant.) as a white powder. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.66 (m, 4H), 7.58 (m, 2H), 7.53 (t, J = 7.5 Hz, 2H), 7.46 (m, 4H), 6.97 (dd, J = 8.6, 2.9 Hz, 2H), 3.85 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.6 (s), 134.1 (d, J_{CP} = 10.7 Hz), 132.9 (d, J_{CP} = 103.7 Hz), 132.1 (d, J_{CP} = 9.5 Hz), 131.9 (d, J_{CP} = 2.4 Hz), 128.6 (d, J_{CP} = 11.9 Hz) , 123.5 (d, J_{CP} = 109.3 Hz), 114.2 (d, J_{CP} = 13.1 Hz), 55.4 (s). ^{31}P NMR (202 MHz, DMSO-d_6) δ 26.1(s). ESIMS (positive) m/z 331 $[M+Na]^+$.

*4.1.9. (4-Hydroxyphenyl)(methyl)(phenyl)phosphine oxide (**6**)*

Prepared by general procedure B (quant.) as a white powder. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.66 (m, 2H), 7.51 (m, 1H), 7.46–7.41 (m, 4H), 6.98 (dd, J = 8.6, 2.3 Hz, 2H), 1.97 (d, J = 13.2 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 161.9 (s), 133.1 (d, J_{CP} = 102.5 Hz), 132.4 (d, J_{CP} = 10.7 Hz), 132.1 (s), 130.6 (d, J_{CP} = 9.5 Hz), 128.9 (d, J_{CP} = 11.9 Hz), 120.1 (d, J_{CP} = 114.6 Hz), 116.5 (d, J_{CP} = 13.1 Hz), 16.5 (d, J_{CP} = 73.9 Hz). ^{31}P NMR (202 MHz, DMSO-d_6) δ 29.2 (s). ESIMS (positive) m/z 255 $[M+Na]^+$.

*4.1.10. (4-Hydroxyphenyl)diphenylphosphine oxide (**11**)*

Prepared by general procedure B (86%) as a white powder. ^{1}H NMR (500 MHz, CD_3OD) δ 7.62 (m, 6H), 7.54–7.51 (m, 4H), 7.46–7.42 (m, 2H), 6.92 (dd, J = 8.6, 2.3 Hz, 2H). ^{13}C NMR (125 MHz, CD_3OD) δ 161.7 (s), 133.9 (d, J_{CP} = 11.9 Hz), 132.2 (d, J_{CP} = 2.4 Hz) 131.9 (d, J_{CP} = 104.9 Hz), 131.7 (d, J_{CP} = 10.7 Hz), 128.6 (d, J_{CP} = 11.9 Hz), 120.2 (d, J_{CP} = 113.3 Hz), 115.6 (d, J_{CP} = 13.1 Hz). ^{31}P NMR (202 MHz, DMSO-d_6) δ 26.3 (s). ESIMS (negative) m/z 293 $[M-H]^-$.

*4.1.11. (4-hydroxyphenyl)(methyl)(phenyl)phosphine (**5**)*

Prepared by general procedure C (81%) as a white powder. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40–7.26 (m, 7H), 6.86 (d, J = 6.9 Hz, 2H), 1.61 (br, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 161.0 (s), 134.4 (d, J_{CP} = 19.1 Hz), 132.5 (d, J_{CP} = 11.9 Hz), 131.7 (d, J_{CP} = 16.7 Hz), 130.6 (d, J_{CP} = 9.5 Hz), 128.9 (d, J_{CP} = 11.9 Hz), 121.8 (d, J_{CP} = 109.7 Hz), 116.4 (d, J_{CP} = 13.1 Hz), 16.5 (d, J_{CP} = 73.9 Hz). ^{31}P NMR (202 MHz, DMSO-d_6) δ −30.0 (s). ESIMS (positive) m/z 217 $[M + H]^+$.

*4.1.12. (4-hydroxyphenyl)diphenylphosphine (**10**)*

Prepared by general procedure C (93%) as a clear oil. ^{1}H NMR (500 MHz, CD_3OD) δ 7.29 (m, 6H), 7.21 (m, 4H), 7.13 (t, J = 8.0 Hz, 2H), 6.78 (d, J = 8.6 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.5 (s), 138.2 (d, J_{CP} = 10.7 Hz), 135.5 (d, J_{CP} = 21.5 Hz), 133.1 (d, J_{CP} = 19.1 Hz), 128.2 (d, J_{CP} = 4.8 Hz), 128.1 (s), 125.8 (d, J_{CP} = 6.0 Hz), 115.4 (d, J_{CP} = 8.4 Hz). ^{31}P NMR (202 MHz, DMSO-d_6) δ −8.10 (s). ESIMS (negative) m/z 277 $[M-H]^-$.

*4.1.13. (4-Methoxyphenyl)(methyl)(phenyl)phosphine (**21**)*

Prepared by general procedure C (74%) as an amorphous yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43–7.38 (m, 4H), 7.35–7.30 (m, 3H), 6.91 (d, J = 8.6 Hz, 2H), 3.82 (s, 3H), 1.63 (d, J = 2.9 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 160.4 (s), 141.5 (d, J_{CP} = 23.6 Hz), 134.3 (d, J_{CP} = 21.5 Hz), 131.7 (d, J_{CP} = 17.9 Hz), 130.3 (d, J_{CP} = 9.5 Hz), 128.9 (d, J_{CP} = 6.0 Hz), 128.5 (s), 114.8 (d, J_{CP} = 8.3 Hz), 55.6 (s), 12.4 (d, J_{CP} = 13.1 Hz).

*4.1.14. (4-Methoxyphenyl)diphenylphosphine (**22**)*

Prepared by general procedure C (93%) as a white powder. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35–7.31 (m, 12H), 6.91 (m, 2H), 3.81 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 160.7 (s), 137.0 (d, J_{CP} = 6.0 Hz), 135.7 (d, J_{CP} = 21.5 Hz), 133.5 (d, J_{CP} = 17.9 Hz), 128.9 (s), 128.6 (d, J_{CP} = 7.2 Hz) , 126.7 (d, J_{CP} = 2.4 Hz), 126.7 (s), 114.4 (d, J_{CP} = 8.4 Hz), 55.4 (d, J_{CP} = 19.1 Hz).

*4.1.15. (4-Methoxyphenyl)(methyl)(phenyl)phosphine sulfide (**23**)*

Prepared by general procedure D (90%) as an amorphous oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.80–7.72 (m, 4H), 7.48–7.44 (m, 3H), 6.96 (dd, J = 8.6, 2.3 Hz, 2H), 3.84 (s, 3H), 2.24 (d, J = 13.2 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.3 (s), 134.7 (d, J_{CP} = 82.3 Hz), 132.8 (d, J_{CP} = 11.9 Hz), 131.4 (d, J_{CP} = 2.4 Hz), 130.7 (d, J_{CP} = 10.7 Hz), 128.7 (d, J_{CP} = 13.1 Hz), 124.7 (d, J_{CP} = 88.2 Hz), 114.3 (d, J_{CP} = 13.1 Hz), 55.5 (s), 22.1 (d, J_{CP} = 59.6 Hz).

*4.1.16. (4-Methoxyphenyl)diphenylphosphine sulfide (**24**)*

Prepared by general procedure D (92%) as a white powder. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.71–7.61 (m, 6H), 7.47 (m, 2H), 7.41 (m, 4H), 6.93 (dd, J = 8.6, 2.3 Hz, 2H), 3.81 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.4 (d, J_{CP} = 2.4 Hz), 134.2 (d, J_{CP} = 11.9 Hz), 133.5 (d, J_{CP} = 85.9 Hz), 132.3 (d, J_{CP} = 10.7 Hz), 131.5 (d, J_{CP} = 2.4 Hz), 128.6 (d, J_{CP} = 11.9 Hz), 123.7 (d, J_{CP} = 90.6 Hz), 114.2 (d, J_{CP} = 13.1 Hz), 55.5 (s).

*4.1.17. (4-Hydroxyphenyl)(methyl)(phenyl)phosphine sulfide (**7**)*

Prepared by general procedure B (95%) as an amorphous oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (m, 2H), 7.58 (m, 2H), 7.47–7.42 (m, 3H), 6.85 (dd, J = 8.6, 2.3 Hz, 2H), 2.23 (d, J = 13.2 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.0 (d, J_{CP} = 2.4 Hz), 134.0 (d, J_{CP} = 83.5 Hz), 132.8 (d, J_{CP} = 13.1 Hz), 131.6 (s), 130.8 (d, J_{CP} = 10.7 Hz), 128.8 (d, J_{CP} = 13.1 Hz), 124.1 (d, J_{CP} = 89.4 Hz), 116.0 (d, J_{CP} = 14.3 Hz), 21.9 (d, J_{CP} = 59.6 Hz). ^{31}P NMR (202 MHz, DMSO-d_6) δ 35.6 (s). ESIMS (negative) m/z 247 $[M-H]^-$.

*4.1.18. (4-Hydroxyphenyl)diphenylphosphine sulfide (**12**)*

Prepared by general procedure B (91%) as a white powder. ^{1}H NMR (500 MHz, CD_3OD) δ 7.67–7.63 (m, 4H), 7.52–7.49 (m, 4H), 7.50–7.43 (m, 4H), 6.85 (dd, J = 8.6, 2.3 Hz, 2H). ^{13}C NMR (125 MHz, CD_3OD) δ 160.9 (d, J_{CP} = 2.4 Hz), 134.1 (d, J_{CP} = 84.7 Hz), 133.6 (d, J_{CP} = 84.7 Hz), 131.9 (d, J_{CP} = 10.7 Hz), 131.4 (d, J_{CP} = 2.4 Hz), 128.3 (d, J_{CP} = 11.9 Hz), 121.4 (d, J_{CP} = 93.0 Hz), 115.2 (d, J_{CP} = 13.1 Hz). ^{31}P NMR (202 MHz, DMSO-d_6) δ 42.6 (s). ESIMS (negative) m/z 309 $[M-H]^-$.

*4.1.19. (4-Hydroxyphenyl)(methyl)(phenyl)phosphine selenide (**8**)*

Prepared by general procedure E (97%) as an amorphous oil. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.83–7.79 (m, 2H), 7.70 (dd, J = 13.2, 2.3 Hz, 2H), 7.52–7.47 (m, 3H), 6.87 (dd, J = 11.5, 2.3 Hz, 2H), 2.45 (d, J = 13.7 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 161.1 (s), 134.6 (d, J_{CP} = 68.2 Hz), 133.6 (d, J_{CP} = 11.9 Hz), 131.7 (s), 131.2 (d, J_{CP} = 10.7 Hz), 129.0 (d, J_{CP} = 13.1 Hz), 121.6 (d, J_{CP} = 79.9 Hz), 116.0 (d, J_{CP} = 14.3 Hz), 21.7 (d, J_{CP} = 53.7 Hz). ^{31}P NMR (202 MHz, DMSO-d_6) δ 23.6 (s). ESIMS (negative) m/z 295 $[M-H]^-$.

*4.1.20. (4-Hydroxyphenyl)diphenylphosphine selenide (**13**)*

Prepared by general procedure E (89%) as a white powder. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.65–7.46 (m, 12H), 6.91 (dd, J = 13.8, 2.3 Hz, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 161.4 (s), 131.9 (d, J_{CP} = 13.1 Hz), 132.7 (d, J_{CP} = 76.3 Hz), 132.5 (d, J_{CP} = 10.7 Hz), 132.2 (s) , 129.2 (d, J_{CP} = 11.9 Hz), 119.7 (d, J_{CP} = 82.3 Hz), 116.3 (d, J_{CP} = 13.1 Hz). ^{31}P NMR (202 MHz, DMSO-d_6) δ 34.4 (s). ESIMS (negative) m/z 357 $[M-H]^-$.

*4.1.21. (4-hydroxyphenyl)(methyl)(phenyl)phosphine borane (**9**)*

Prepared by general procedure F (quant.) as an amorphous oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.61 (m, 2H), 7.54 (m, 2H), 7.47–7.41 (m, 3H), 6.90 (dd, J = 8.6, 1.7 Hz, 2H), 1.82 (d, J = 10.4 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.6 (d, J_{CP} = 2.4 Hz), 133.9 (d, J_{CP} = 10.7 Hz), 131.6 (d, J_{CP} = 9.6 Hz), 131.2 (d, J_{CP} = 56.0 Hz) 131.1 (s), 128.9 (d, J_{CP} = 10.7 Hz), 120.8 (d, J_{CP} = 62.0 Hz) , 116.2 (d, J_{CP} = 10.7 Hz), 12.3 (d, J_{CP} = 40.5 Hz). ^{31}P NMR (202 MHz, DMSO-d_6) δ 7.68 (q). ESIMS (negative) m/z 229 $[M-H]^-$.

*4.1.22. (4-Hydroxyphenyl)diphenylphosphine borane (**14**)*

Prepared by general procedure F (95%) as an amorphous oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.57–7.53 (m, 4H), 7.49–7.47 (m, 4H), 7.45–7.41 (m, 4H), 6.89 (dd, J = 8.6, 1.8 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.5 (s), 135.4 (d, J_{CP} = 10.7 Hz), 133.1 (d, J_{CP} = 9.5 Hz), 131.2 (d, J_{CP} = 2.4 Hz), 129.7 (d, J_{CP} = 57.2 Hz) 128.8 (d, J_{CP} = 10.7 Hz), 119.7 (d, J_{CP} = 62.0 Hz), 116.1 (d, J_{CP} = 11.9 Hz). ^{31}P NMR (202 MHz, DMSO-d_6) δ 18.8 (q). ESIMS (negative) m/z 291 $[M-H]^-$.

4.2. Determination of logP values

The 1-octanol/water partition coefficient, log P, was determined by means of an HPLC method based on the OECD Guideline for Testing Chemicals.[14] An Inertsil ODS-4 (5 μm, 4.6 × 150 mm, GL Sciences Inc., Japan) column was fitted on an HPLC instrument (Photodiode Array detector, MD-2018 Plus, JASCO) equipped with a pump (PU-980, JASCO) and oven (SSC-2120, Senshu Scientific Co., Ltd.). The injection volume was 10 μL, and the flow rate was 1.0 mL/min in all cases. The compounds were detected by measuring UV absorption at 240 and 230 nm. The temperature of the column was kept at 40.0 (± 0.1) °C

during the measurement. The mobile phase was a methanol–water system, and the methanol concentration was changed from 75% (v/v) to 60% (v/v) in 5% (v/v) steps.[22,23] The dead time t_0 was measured with thiourea as the unretained compound. Each measurement was performed in triplicate, and the mean value was used for further calculations. The capacity factor of each compound in 100% aqueous eluent, log k_w, was calculated by extrapolation of the line fitted to the measured log k values in the methanol–aqueous mobile phase. For the reference compounds (4-methoxyphenol, phenol, p-cresol, 4-chlorophenol, 4-phenylphenol, diphenylether), the calculated log k_w values were plotted against log P values to prepare a calibration graph (log P = 1.1155 log k_w + 0.0433, R^2 = 0.9968). The log P values of tested phenols were obtained by interpolation of the calculated capacity factors log k_w on the calibration graph.

4.3. Determination of pK_a.

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–phosphate buffer (in the pH range of 6.0–12.5).[24] The values of pH obtained with the pH meter were corrected by using the equation: pH* = pH (recorded) – d (d = 0.01). Each measurement was performed in triplicate.

4.4. ER reporter gene assay

The ER-agonistic and antagonistic activities of compounds were evaluated by means of reporter gene assay using a Gal4-human ERα or ERβ reporter system. A fragment of human ERα/β was inserted into the pCMX-GAL4 vector to obtain pCMX-GAL4-hER (pCMX-flag vector to make pCMX-ERα/β). GAL4-responsive MH100 (USA)x4-tk-LUK reporter was used. Human embryonic kidney (HEK 293) cells were cultivated in Dulbecco's modified Eagle's medium (DMEM without Phenol Red) containing 5% fetal bovine serum (FBS) and antibiotic-antimycotic mixture (Nacalai) at 37 °C in a humidified atmosphere of 5% CO_2 in air. Transfections were performed by the calcium phosphate coprecipitation method. Test compounds were added at 24 h after transfection. Cells were harvested 24 h after the treatment, and luciferase and β-galactosidase activities were assayed using a luminometer and a microplate reader. DNA cotransfection experiments were done with 50 ng of reporter plasmid, 15–20 ng pCMX-s-galactosidase, 10–15 ng of each receptor expression plasmid and pGEM carrier DNA to make a total of 150 ng DNA per well in a 96-well plate. Luciferase data were normalized to an internal β-galactosidase control, and reported values are means of triplicate assays. Antagonist activity was measured in the presence of 0.3 nM 17β-estradiol.

4.5. Docking simulation

The structure of the LBD of hERβ was prepared from the Protein Data Bank accession 1L2J, chain A. Polar hydrogens and partial atomic charges were assigned using AutoDockTools (ADT). Molecular docking was performed using AutoDock 4.2 with the Genetic Algorithm. AutoDock parameters for boron atoms were Rii = 4.08 and eii = 0.180, and those for silicon atoms were Rii = 4.30 and eii = 0.402.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

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