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6-Arylcoumarins as Novel Nonsteroidal Type Progesterone Antagonists: An Example with Receptor-Binding-Dependent Fluorescence

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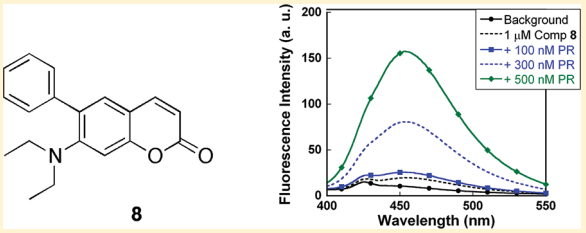
Supporting Information

ABSTRACT: Various 6-arylcoumarin derivatives were designed and synthesized as candidate nonsteroidal type progesterone antagonists. 6-Bromocoumarin derivatives were prepared from the corresponding 4-substituted 2-acetoxy-5-bromobenzaldehyde by employing the Still−Gennari modification of the Horner−Wadsworth−Emmons olefination reaction and were converted to 6-arylcoumarins by means of Suzuki−Miyaura cross-coupling reactions. The biological activities of these coumarin derivatives were evaluated by means of alkaline phosphatase assay in the T47D human breast carcinoma cell line. Among the synthesized compounds, **36** (IC_{50} = 0.12 μM) and **38** (IC_{50} = 0.065 μM), bearing a five-membered heterocycle, showed potent PR antagonist activity. Competitive binding assay showed that compounds **8** and **34** have potent PR binding affinity. The fluorescence of compound **8** was dependent on the solvent properties and was increased in the presence of PR ligand binding domain. This property might be applicable to the development of fluorescence probes for studies on PR.



INTRODUCTION

Progesterone (**1**, Figure 1) plays significant roles in female reproduction, including regulation of growth and differentiation of the uterus and mammary gland, ovulation, and establishment and maintenance of pregnancy.[1−4] Most progesterone actions are mediated by binding to and activation of the progesterone receptor (PR), which is a ligand-dependent transcriptional factor belonging to the nuclear receptor superfamily.[5] PR agonists are used in contraceptives, for hormone therapy in combination with estrogens, and in the treatment of gynecologic disorders such as endometriosis and uterine fibroids. PR antagonists also have potential clinical applications, though they are in only limited use at present. The development of novel PR-selective antagonists should be helpful in studies to clarify their potential therapeutic utility in various gynecological diseases, including hormone-dependent breast and prostate cancers,[6−8] nonmalignant chronic conditions such as fibroids,[9,10] and endometriosis.[11,12]

Mifepristone (**2**, Figure 1) was the first clinically available PR antagonist.[13] However, mifepristone and related derivatives bearing a steroid skeleton (some of which are classified as PR modulators) exhibit side effects partially related to cross-activity with steroid hormone receptors, such as estrogen receptor (ER), androgen receptor (AR), and glucocorticoid receptor (GR). In order to overcome these disadvantages, nonsteroidal PR antagonists have been developed,[14−24] though the available structural variation is still limited. Among these compounds, nitrogen-containing heterocycles fused with an aryl-substituted benzene ring, such as compounds **3**−**6**, have been widely studied (Figure 2).[25−29] These cyclic anilide-type compounds exhibit very potent PR ligand activity. However, subtle structural modifications can switch the activity between agonistic and antagonistic. For example, compounds **3a** and **4a** show PR antagonistic activity, while the thio analogues **3b** and **4b**, respectively, act as PR agonists. The pyrrole derivatives **4c** and **4d** are both agonists. The detailed structural requirements of these compounds for PR agonist or antagonist activity are still

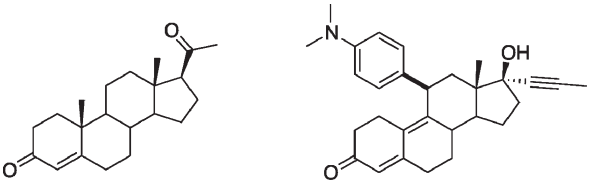


Figure 1. Structures of progesterone and mifepristone.

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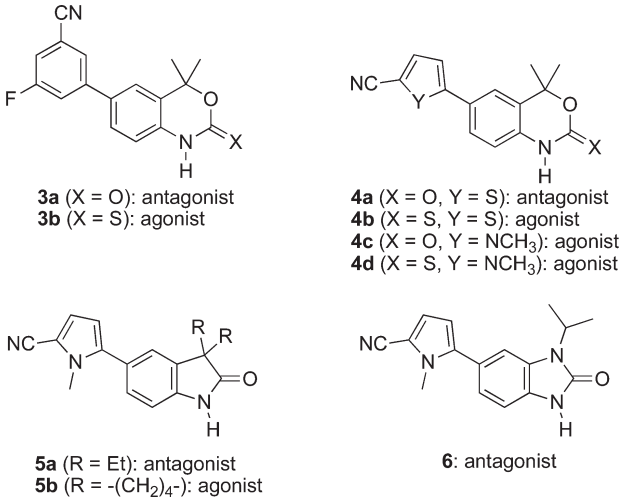


Figure 2. Structures of typical nonsteroidal PR ligands.

undefined, although there is clearly potential to develop various types of modulators of PR action.

We previously reported several ethyl 6-arylcoumarin-3-carboxylates as fluorescence sensor candidates, and some of them exhibited unique fluorescence properties, depending on the substituents on the 6-aryl ring or the environmental conditions.[30] This bicyclic coumarin structure is thought to be an alternative scaffold to the bicyclic anilide scaffold of previously reported PR ligands (i.e., benzoxadine, indole, and benzimidazole derivatives bearing a nitrogen atom in the bicyclic heterocyclic skeleton), and these coumarin-based compounds are considered to be good candidates for PR antagonists with novel biological and pharmacological properties. Indeed, some coumarin derivatives have already been developed as fluorescent sensors for hydroxysteroid dehydrogenase activity.[31,32] In addition, the coumarin derivatives with PR affinity may have potential as fluorescence probes for elucidation of the mechanisms of PR action and also for screening of novel PR ligands. Several types of fluorescence probes for steroid hormone receptors including green fluorescence protein-fused receptors[33] and the conjugated ligands with the fluorophore by the linking group were reported.[34] If we could find a PR ligand that itself changes the fluorescence properties upon binding to PR, it might be much useful as a chemical tool, like some reported for fluorescent ER ligands.[35] Therefore, we examined the PR activity of the compounds in our coumarin library and found that compound **7** (Figure 3) exhibited PR antagonistic activity, although its potency (IC_{50} = 4.07 μM) in alkaline phosphatase assay was very weak compared to that of mifepristone (IC_{50} = 0.216 nM). Here, we describe the development of various 6-arylcoumarin derivatives as candidate PR antagonists by using compound **7** as a lead compound. We also report the discovery of a compound showing an increase of fluorescence upon binding to the PR ligand binding domain.

CHEMISTRY

On the basis of a comparison of the structure of **7** with those of other nonsteroidal PR antagonists, we considered that the ester group at the 3-position of the coumarin ring of **7** would be unnecessary for the activity, and this was confirmed by examination of the activity of **8** in this study. Therefore, we designed the 6-arylcoumarin derivatives **8**−**38** (Figure 3, structures shown in

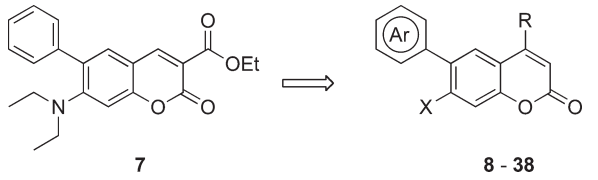


Figure 3. Concept underlying transformation of **7** to **8**−**38**.

tables). The substituent X at the 7-position is expected to influence both the biological activity and the fluorescence properties because of its intrinsic chemical properties and its effect on the dihedral angle between the aryl and coumarin rings.

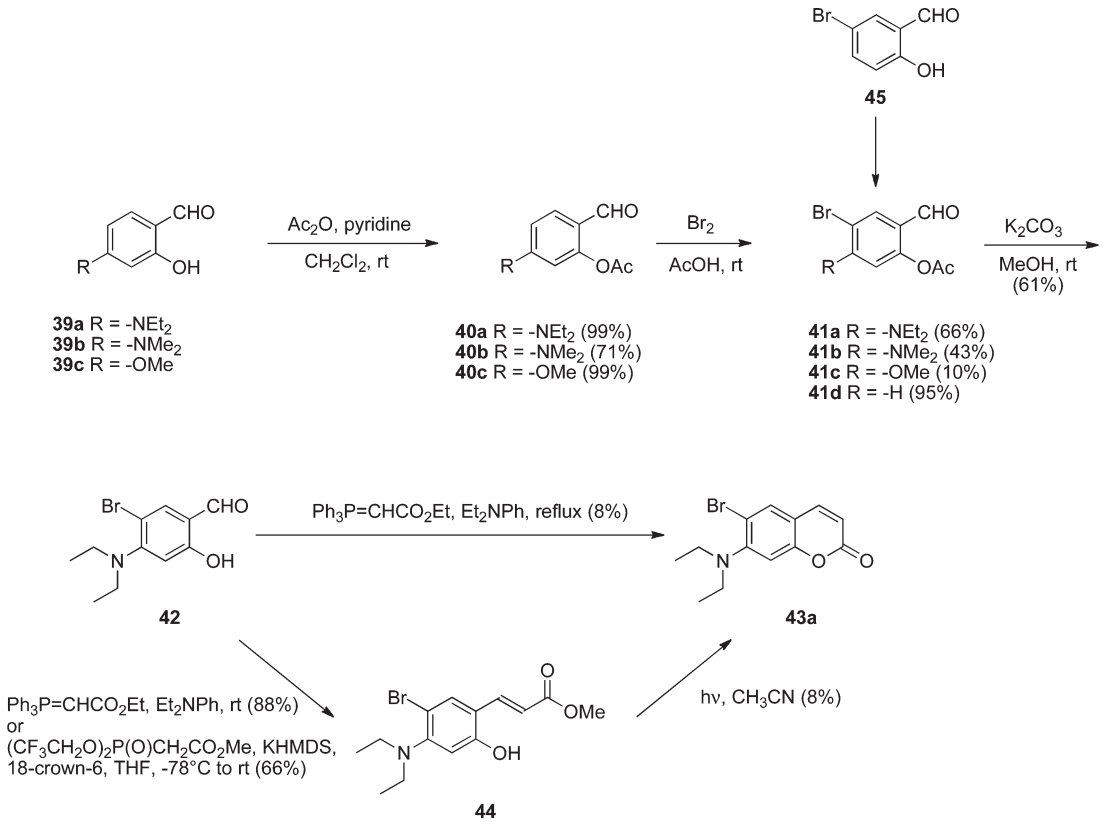
Our previous studies showed that 6-arylcoumarin derivatives could be obtained by Suzuki−Miyaura cross-coupling of the corresponding 6-bromocoumarins.[30] 5-Bromosalicylaldehydes, the precursors for coumarin synthesis, were synthesized as shown in Scheme 1. After acetylation (99%) of 4-diethylaminosalicylaldehyde (**39a**), **40a** was brominated (66%) and then hydrolyzed (61%) to afford compound **42**. First, we tried Wittig reaction of **42** with ethyl (triphenylphosphoranylidene)acetate in the presence of *N*,*N*′-diethylaniline at high temperature (220 °C), but the desired coumarin **43a** was obtained in only 8% yield. After Wittig reaction at lower temperature, the obtained styrene ester **44** was cyclized photochemically to afford **43a**, also in 8% yield.[36] The Still−Gennari modification of the Horner−Wadsworth−Emmons olefination is effective method for *Z*-olefin, but it afforded the trans olefin **44** in 66% yield.

Then we examined coumarin synthesis from acetylsalicylaldehyde **41a** by using 2,8,9-triisopropyl-2,5,8,9-tetraaza-1-phosphabicyclo-[3,3,3]undecane and 4 Å molecular sieves (Scheme 2).[37] This method is useful for the synthesis of coumarins without an electron-withdrawing group at the 3-position, but **41a** afforded **43a** in only 28% yield. Interestingly, the Still−Gennari modification of the Horner−Wadsworth−Emmons olefination of **41a** gave the coumarin **43a** in 60% yield via the cis isomer of **44**. The difference of the stereochemistry in the reactions between **42** and **41a** is unclear, while the hydroxyl group ortho to the aldehyde group of **42** would cause the formation of *E*-isomer. The other coumarin derivatives **43b**−**d** were synthesized similarly. Suzuki−Miyaura cross-coupling reaction of **43** with arylboronic acid or its pinacol ester afforded the 6-arylcoumarins. 7-Hydroxycoumarins **23** and **25** were prepared by demethylation of **22** and **24**, respectively, with borane tribromide.

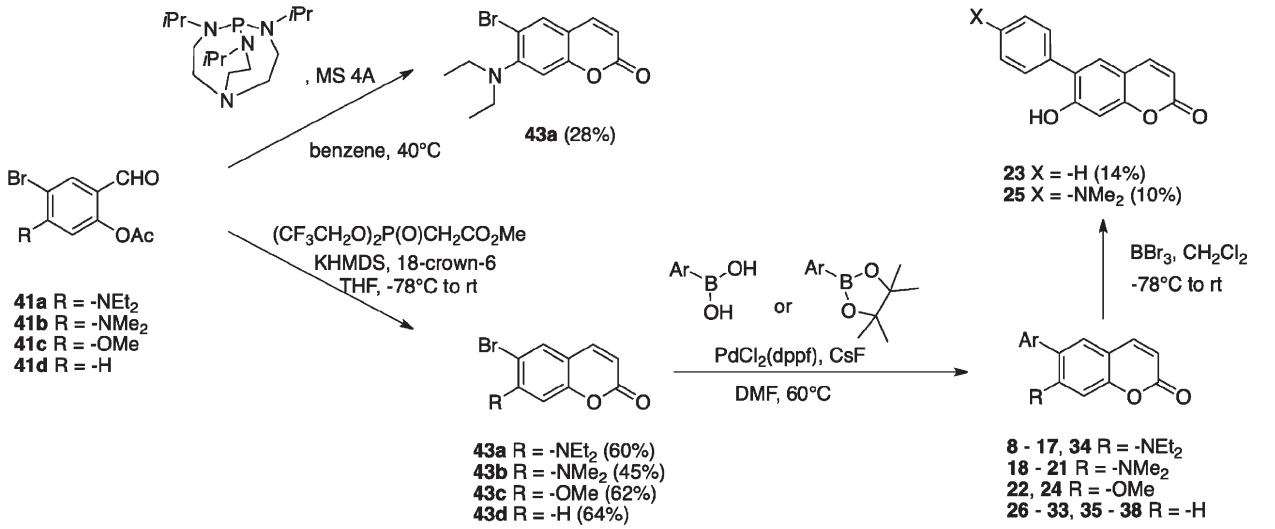
BIOLOGICAL ACTIVITY

The biological activity of the 6-arylcoumarins was tested by means of alkaline phosphatase (AP) assay in the T47D human breast carcinoma cell line.[38,39] None of the coumarin derivatives examined acted as PR agonists in AP assay. Next, PR antagonistic activity was examined in the presence of 1 nM progesterone, and the IC_{50} values were calculated. The coumarin derivative **7** in our library of fluorescent compounds exhibited PR antagonistic activity with an IC_{50} of 4.07 μM (Table 1). Compound **8** without the 3-substituent showed more potent PR antagonistic activity than **7** by 1 order of magnitude. This result is consistent with the structure−activity relationships of typical nonsteroidal anilide-type PR ligands (Figure 2) that have no electron-withdrawing substituent on the bicyclic heterocyclic skeleton. Therefore, we examined the activity of coumarin derivatives without any 3-substituent.

Scheme 1



Scheme 2



The substituent effects on the coumarin ring at the 6 and 7 positions of **8** were examined (Table 1). In the case of anilide-type PR antagonists such as **3**, a cyano or nitro group on the phenyl ring increased the potency. However, the 4-cyanophenyl (**9**) or 4-nitrophenyl derivative (**10**) showed similar or weaker activity, compared with **8** bearing an unsubstituted phenyl group. Introduction of an amino group or halogen also did not significantly change the PR antagonistic potency. Compound **17** bearing a cyano group at the meta position showed similar potency compared with the compounds with para substituents.

Compounds **18**—**21** bearing a 7-dimethylamino group showed a slight increase in antagonistic activity, compared with the corresponding compounds with a 7-diethylamino group, while replacement of the amino moiety with a 7-methoxyl or 7-hydroxyl group decreased the activity. Interestingly, the compound without any 7-substituent showed similar antagonistic activity, and

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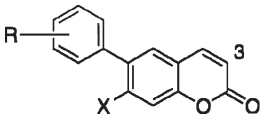
Table 1. PR Antagonistic Activity of 6-Arylcoumarins in Alkaline Phosphatase Assay



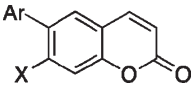
compd	X	R	AP assay, IC_{50} (μM)
7 (3-COOEt)	NEt_2	(none)	4.07
8	NEt_2	(none)	0.50
9	NEt_2	4-CN	0.50
10	NEt_2	4-NO_2	0.86
11	NEt_2	3-NO_2, 4-NH_2	0.51
12	NEt_2	4-NHAc	0.47
13	NEt_2	4-NMe_2	0.39
14	NEt_2	4-Ac	0.40
15	NEt_2	4-Cl	0.77
16	NEt_2	4-F	0.63
17	NEt_2	3-CN	0.38
18	NMe_2	(none)	0.36
19	NMe_2	4-CN	0.25
20	NMe_2	4-F	0.47
21	NMe_2	3-CN	0.26
22	OMe	(none)	0.88
23	OH	(none)	1.28
24	OMe	4-NMe_2	3.34
25	OH	4-NMe_2	2.59
26	H	(none)	0.51
27	H	4-CN	0.29
28	H	4-NMe_2	0.31
29	H	4-F	0.21
30	H	3-CN	1.11
31	H	3,5-F_2	0.56
32	H	3-CF_3	0.19
33	H	3-pyridyl	27.9

some compounds, such as **27** with a 4-cyanophenyl group or **29** with a 4-fluorophenyl group, had better IC_{50} values.

We next introduced a heterocyclic ring on the coumarin ring. The pyridyl derivative **33** was a very weak PR antagonist (Table 1), while the introduction of five-membered heterocycles was effective. Compounds **34** and **35** with a 6-thiophen-2-yl group showed potent PR antagonistic activity with IC_{50} values of 0.22 and 0.54 μM, respectively (Table 2). Introduction of a chloro group on the thiophene ring increased the activity, and compound **36** was 4.5 times more active than compound **35**. Introduction of a furan ring decreased the activity, compared to the 6-phenyl derivative, while introduction of the cyano-substituted pyrrole ring increased the potency. Thus, compound **38** was the most potent PR antagonist (IC_{50} = 65 nM) among the coumarin derivatives examined in this study.

The PR binding affinity of selected compounds was examined by competitive binding assay using recombinant hPR-LBD. The binding affinities toward other steroid hormone receptors (hAR, hER, hGR, and rMR) were also examined (Table 3). Compounds **8** and **34** bearing a 7-diethylamino group showed potent PR binding affinity, while compounds **35**, **36**, and **38** showed weaker binding affinity. The reason for the difference between the

Table 2. PR Antagonistic Activity of Coumarins Bearing a 6-Heterocyclic Substituent in Alkaline Phosphatase Assay



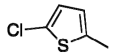
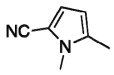
Compd	X	Ar	AP assay, IC_{50} (μM)
34	NEt_2		0.22
35	H		0.54
36	H		0.12
37	H		1.28
38	H		0.065

Table 3. Binding Affinity of Coumarin Derivatives toward Steroid Hormone Receptors[a]

	IC_{50} (μM)				
compd	hPR	hER	hAR	hGR	rMR
8	1.42	ND	10.0	3.54	ND
34	1.24	ND	9.7	4.96	ND
35	10.0	ND	ND	ND	ND
36	50.5	ND	ND	ND	ND
38	15.2	ND	ND	ND	ND

[a] ND: no detectable binding.

binding experiments and AP assay results of these compounds is unclear. Some nuclear receptor ligands with low receptor binding affinities were reported to exhibit potent biological activities in cell assay or vice versa.[40] The action of nuclear receptor ligands depends not only on the binding affinity but also on the binding feature and interaction between *holo*-receptor and cofactor proteins. In this case, the lipophilic (compounds **8** and **34**) or hydrophobic (compounds **35**, **36**, and **38**) character of the 7-substituent may have some effect on the activity. None of the coumarin derivatives examined bound to ER and MR, and only compounds **8** and **34** had weak AR and moderate GR binding affinities. Thus, the 7-diethylamino group may cause the binding ability of the coumarin derivatives to some steroid hormone receptors. The coumarin derivatives without 7-substituent have lower IC_{50} values but were PR-selective in the assay conditions, and therefore, these would be lead structures for PR-selective coumarin derivatives.

Recently, the crystal structure of PR ligand binding domain bound to compound **4d** was reported.[27] Nonsteroidal molecule **4d** occupied the same binding site as progesterone, and the cyano group on the pyrrole ring made hydrogen bonds with Gln725 and Arg766, similar to the 3-keto group of progesterone. Further, the cyclic thioamide group of compound **4d** made a hydrogen bond with Asn719, which is not observed for progesterone. The coumarin derivatives have similar molecular shape and sizes with the cyclic anlide-type PR ligands and would be able to occupy the

same binding site as compound **4d** or progesterone. One of the significant differences between compounds **4d** and the coumarin derivatives is the atom at the 1 position. The coumarin derivatives lack the thioamide group of compound **4d** that made hydrogen bond with Asn719. This would cause the decrease in the binding potency and also no agonistic activity of coumarin derivatives, compared to cyclic anilide-type PR ligands that showed agonistic and antagonistic activity, depending on the substituents.

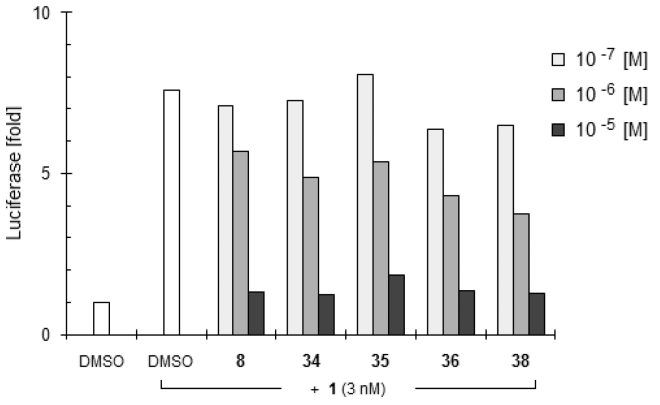


Figure 4. PR antagonistic activity of coumarins in transactivation assay. PR antagonistic activity of coumarins in transactivation assay. Inhibition of transcriptional activation of progesterone (**1**) by the test compounds. CHO cells were transfected with hPR expression vector and MMTV-Luc and incubated with the test compounds (10^{-7}–10^{-5} M) plus **1** (3×10^{-9} M). Values are normalized by the response in the absence of **1**.

In order to clarify the ability of the coumarin derivatives to modulate PR functions, the transactivation assay using PR (Figure 4) was conducted. All coumarin derivatives examined inhibited the progesterone-induced activation of PR in CHO cells. The potency of phenylcoumarin derivative **8** was similar to the potency of compounds **34**–**36** and **38** bearing a heterocyclic ring. Thus, the coumarin derivatives have the ability to inhibit PR functions, although the in vitro binding affinity was rather lower.

Compounds whose fluorescence properties change upon specific binding with a protein can be utilized as fluorescent sensors,[33–35,41] for example, to estimate the amount or activity of

Table 4. Fluorescence Properties of Coumarin Derivatives

		solvent[a]				
compd		CH_2Cl_2	$(CH_3)_2CO$	CH_3CN	CH_3OH	buffer
8	abs max	371	363	367	370	374
	em max	453	458	469	456	468
	Φ_{fl} [b]	0.744	0.141	0.047	0.007	0.007
34	abs max	369	366	366	370	374
	em max	460	467	472	460	468
	Φ_{fl} [b]	0.104	0.080	0.038	0.005	0.004

[a] All data were measured in the presence of 0.3% DMSO as a cosolvent. Buffer was 10 mM sodium phosphate buffer (pH 7.4). [b] Fluorescence quantum yields (Φ_{fl}) were determined using quinine sulfate (0.577) in 0.1 M H_2SO_4 as a standard.

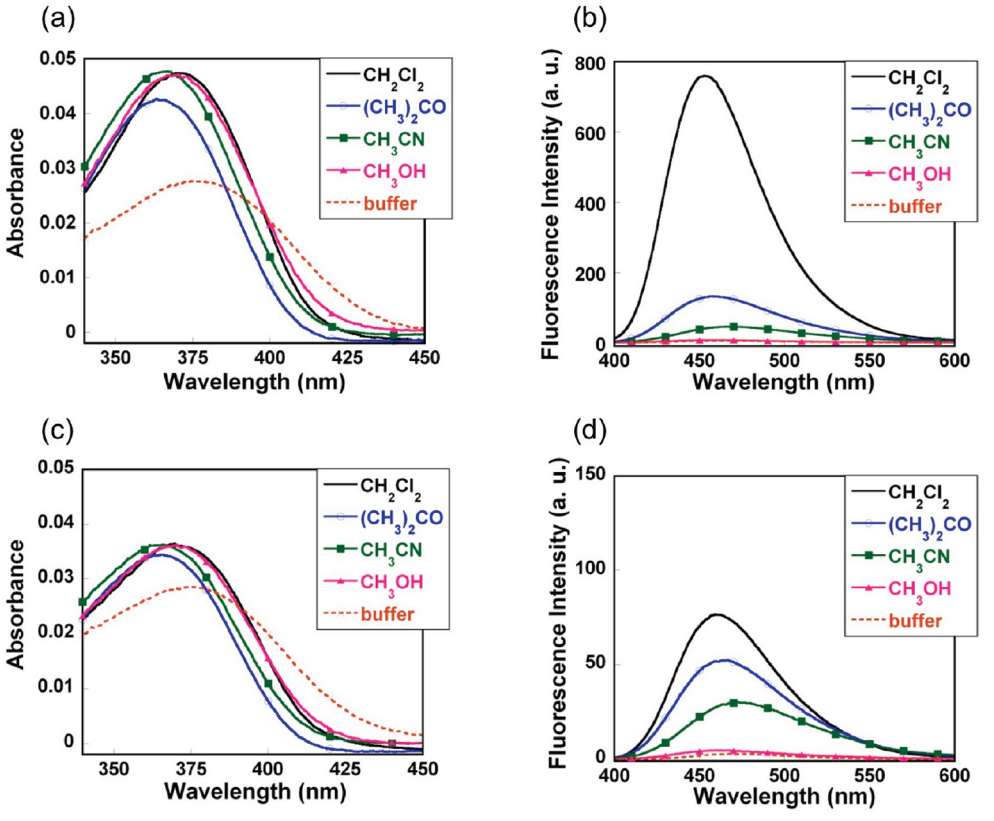


Figure 5. (a) Absorption spectra and (b) fluorescence spectra (excitation at 370 nm) of **8** (3 μM) in various solvents. Buffer is 10 mM sodium phosphate buffer (pH 7.4). Each solvent contained 0.3% DMSO as a cosolvent. (c) Absorption spectra and (d) fluorescence spectra (excitation at 370 nm) of compound **34** (3 μM) are also shown.

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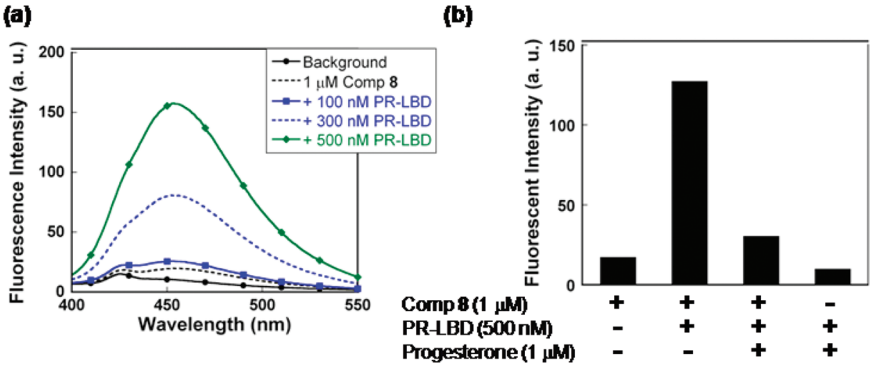

Figure 6. (a) Fluorescence spectra (excitation at 370 nm) of **8** (1 μM) in the presence of various concentrations of PR LBD. (b) Fluorescence properties of **8** (1 μM) in the presence of PR LBD and/or progesterone. The vertical scale is the fluorescence intensity at 453 nm (excitation at 370 nm).

a protein, or in ligand screening experiments. Since the environment inside proteins is less polar, a compound that has different fluorescence properties under polar and nonpolar conditions is a good candidate for a fluorescent sensor. Therefore, the fluorescence properties of selected compounds were examined (Figure 5 and Table 4). Compounds **35**, **36**, and **38**, which showed potent activity in AP assay, had only weak absorption at around 350 nm and exhibited almost no fluorescence in various solvents (data not shown). Compounds **8** and **34**, which had potent PR binding affinity, showed absorption in the region of 300−400 nm and exhibited fluorescence in the region of 400−550 nm. The fluorescence intensity of compounds **8** and **34** strongly depended on the solvent. Thus, compound **8** showed strong fluorescence in methylene chloride (quantum yield, 0.744) and moderate fluorescence in acetone (quantum yield, 0.141) or acetonitrile (quantum yield, 0.047). Further, weak fluorescence was observed in methanol and buffer, in which the quantum yields were estimated to be less than 0.01. Compound **34** showed similar fluorescence characteristics. Then we examined the fluorescence properties of **8** in the presence of PR (Figure 6). Compound **8** exhibited weak fluorescence in the absence of PR ligand binding domain (LBD) in buffer, while the addition of PR LBD substantially increased the fluorescence of **8**; that is, 500 nM PR-LBD induced about an 8-fold fluorescent enhancement. The ncreased fluorescence of **8** in the presence of PR LBD was blocked by the addition of progesterone. Similar experiments with ER did not result in any increase of the fluorescence of **8** in buffer (data not shown). These results indicate that compound **8** shows a specific fluorescence enhancement upon binding with the PR-LBD. Compound **8** is a candidate fluorescent sensor for PR, although improvement in the binding affinity and fluorescence quantum yield would be desirable.

CONCLUSION

6-Arylcoumarin derivatives were designed and synthesized as novel PR antagonist candidates. Compounds **36** and **38**, bearing a five-membered heterocycle at the 6-position of the coumarin ring, showed potent PR antagonist activity in AP assay. Compounds **8** and **34** exhibited potent PR binding affinity, and **8** showed a change in fluorescence properties upon binding to PR. The coumarin derivatives, such as **8**, have different structures and chemical properties, compared with the steroid-type and anilide-type PR antagonists so far known, and showed unique fluorescence enhancement upon binding to PR, which may be useful for developing fluorescent probes for the study of PR function and for searching for novel PR ligands.

EXPERIMENTAL SECTION

^{1}H and ^{13}C NMR spectra were recorded on a JNN-AL 400 or Bruker Avance 600 spectrometer. The ^{1}H NMR chemical shifts are reported in parts per million (ppm) relative to the centerline of a singlet signal of the solvent molecule (7.26 ppm for chloroform); coupling constants are given in hertz (Hz). The ^{13}C NMR chemical shifts are reported in ppm relative to the centerline of a triplet at 77.16 ppm for $CDCl_3$. Mass spectra were recorded on a Bruker Daltonics microTOF-2focus spectrometer in the positive ion detection mode. The purity of each test compound was determined by NMR and elemental analysis or HPLC (95%> purity; see Supporting Information).

Synthesis of 40a. Acetic anhydride (7 mL) and pyridine (10 mL) were added to a solution of **39a** (2.56 g, 13.3 mmol) in methylene chloride (30 mL), and the mixture was stirred for 1 h. After evaporation, the residue was purified by silica gel column chromatography (AcOEt/*n*-hexane = 1/3) to give compound **40a** (3.11 g, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.74 (s, 1 H), 7.64 (d, J = 9.3 Hz, 1 H), 6.55 (d, J = 6.8 Hz, 1 H), 6.27 (s, 1 H), 3.41 (q, J = 7.3 Hz, 4 H), 2.37 (s, 3 H), 1.21 (t, J = 7.1 Hz, 6 H).

Compounds **40b** and **40c**, were prepared similarly.

Compound 40b. Yield 71%; purple oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.78 (s, 1 H), 7.68 (d, J = 8.8 Hz, 1 H), 6.58 (d, J = 9.0 Hz, 1 H), 6.31 (s, 1 H), 3.07 (s, 6 H), 2.37 (s, 3 H).

Compound 40c. Yield 99%; yellow powder; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.95 (s, 1 H), 7.81 (d, J = 8.8 Hz, 1 H), 6.88 (dd, J = 8.3, 2.4 Hz, 1 H), 6.67 (d, J = 2.3 Hz 1 H), 3.88 (s, 3 H), 2.39 (s, 3 H).

Synthesis of 41a. A solution of bromine (0.908 g, 3.86 mmol) in acetic acid (10 mL) was added to a solution of **40a** (0.908 g, 3.86 mmol) in acetic acid (10 mL), and the mixture was stirred for 1 h. The mixture was poured into ice−water and extracted with ethyl acetate. The organic layer was washed successively with water, saturated $NaHCO_3$, and brine and dried over magnesium sulfate. After filtration and evaporation, the residue was purified by silica gel column chromatography (AcOEt/*n*-hexane = 1/8) to give compound **41a** (0.796 g, 66%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.85 (s, 1 H), 7.99 (s, 1 H), 6.69 (s, 1 H), 3.29 (br s, 4 H), 2.36 (s, 3 H), 1.12 (t, J = 7.1 Hz, 6 H).

Compounds **41b**, **41c**, and **41d** were prepared similarly.

Compound 41b. Yield 43%; brown oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.84 (s, 1 H), 7.98 (s, 1 H), 6.68 (s, 1 H), 2.97 (s, 6 H), 2.38 (s, 3 H).

Compound 41c. Yield 10%; yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.92 (s, 1 H), 8.05 (s, 1 H), 6.70 (s, 1 H), 3.97 (s, 3 H), 2.40 (s, 3 H).

Compound 41d. Yield 95%; colorless powder; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.05 (s, 1 H), 8.00 (s, 1 H), 7.73 (d, J = 8.8 Hz, 1 H), 7.10 (d, J = 8.8 Hz, 1 H), 2.39 (s, 3 H).

Synthesis of 43a. A solution of bis(2,2,2-trifluoroethyl)(methoxycarbonylmethyl)phosphonate (114.7 mg, 0.3587 mmol) and 18-crown-6 (221.7 mg, 0.8388 mmol) in dry THF (6 mL) was cooled to 0 °C under argon and treated with potassium bis(trimethylsilyl)amide (0.5 M in toluene, 1.4 mL, 0.7 mmol). The reaction mixture was cooled to −78 °C, and **41a** (104.7 mg, 0.3332 mmol) was added to it. The mixture was stirred for 4 h at −78 °C, then neutralized with saturated ammonium chloride and extracted with ethyl acetate. The organic layer was dried over magnesium sulfate and concentrated. The residue was purified by silica gel column chromatography (AcOEt/*n*-hexane = 1/10) to give **43a** (58.8 mg, 0.199 mmol, 60%) as an orange oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.66 (s, 1 H), 7.56 (d, J = 9.3 Hz, 1 H), 6.94 (s, 1 H), 6.28 (d, J = 9.3 Hz, 1 H), 3.22 (q, J = 6.8 Hz, 4 H), 1.10 (t, J = 6.8 Hz, 6 H).

Compounds **43b**−**d** were prepared similarly.

Compound 43b. Yield 45%; orange oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (s, 1 H), 7.55 (d, J = 9.8 Hz, 1 H), 6.93 (s, 1 H), 6.27 (d, J = 9.3 Hz, 1 H), 2.91 (s, 6 H).

Compound 43c. Yield 62%; colorless powder; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.66 (s, 1 H), 7.58 (d, J = 9.8 Hz, 1 H), 6.84 (s, 1 H), 6.30 (d, J = 9.8 Hz, 1 H), 3.97 (s, 3 H).

Compound 43d. Yield 64%; colorless powder; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (s, 1 H), 7.62 (m, 2 H), 7.23 (d, J = 9.8 Hz, 1 H), 6.47 (d, J = 9.8 Hz, 1 H).

Synthesis of 8. A solution of boronic acid ester (106 mg, 0.519 mmol), CsF (145 mg, 0.954 mmol), and $PdCl_2$(dppf)−CH_2Cl_2 (31 mg, 0.0385 mmol) in dry DMF (2.5 mL) was added to **43a** (51 mg, 0.174 mmol) at room temperature under argon. The reaction mixture was heated at 60 °C for 3 h and then cooled to room temperature. The mixture was extracted with ethyl acetate, washed successively with saturated ammonium chloride, water, and brine, dried over magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography (CH_2Cl_2/*n*-hexane = 2/1) to give **8** (31.1 mg, 0.106 mmol, 61%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.61 (d, J = 9.8 Hz, 1 H), 7.48 (d, J = 7.3 Hz, 2 H), 7.41 (t, J = 7.3 Hz, 2 H), 7.33 (t, J = 7.3 Hz, 1 H), 7.24 (s, 1 H), 6.94 (s, 1 H), 6.23 (d, J = 9.8 Hz, 1 H), 2.98 (q, J = 6.8 Hz, 4 H), 0.94 (t, J = 6.8 Hz, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.9, 154.9, 153.1, 143.7, 140.9, 132.4, 131.0, 128.9, 128.8, 127.4, 113.1, 112.9, 107.59, 46.1, 12.2. HRMS (ESI) Calcd for $C_{19}H_{20}NO_2$ $(M + H)^+$ 294.1488. Found 294.1491.

Compounds **9**−**17** and **34** were prepared similarly from compound 43a.

Compound 9. Yield 8%; colorless solid (ethyl acetate/*n*-hexane); mp 114 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ7.70 (d, J = 8.3 Hz, 2 H), 7.64 (d, J = 8.8 Hz, 2 H), 7.61 (d, J = 9.8 Hz, 1 H), 7.23 (s, 1 H), 6.95 (s, 1 H), 6.26 (d, J = 9.8 Hz, 1 H), 2.95 (q, J = 6.8 Hz, 4 H), 0.96 (t, J = 6.8 Hz, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.4, 155.5, 152.7, 145.7, 143.3, 132.8, 130.9, 130.6, 129.6, 119.2, 114.0, 113.5, 111.3, 108.5, 46.4, 12.12. HRMS (ESI) Calcd for $C_{20}H_{19}N_2O_2$ $(M + H)^+$ 319.1441. Found 319.1449. Anal. Calcd for $C_{20}H_{18}N_2O_2 \cdot {}^1/_4H_2O$: C, 74.40; H, 5.78; N, 8.68. Found: C, 74.61; H, 5.66; N, 8.44.

Compound 10. Yield 16%; orange oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (d, J = 8.8 Hz, 2 H), 7.71 (d, J = 9.3 Hz, 2 H), 7.62 (d, J = 9.3 Hz, 1 H), 7.27 (s, 1 H), 6.97 (s, 1 H), 6.22 (d, J = 9.8 Hz, 1 H), 2.97 (q, J = 6.8 Hz, 4 H), 0.97 (t, J = 6.8 Hz, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.4, 155.6, 147.7, 147.2, 143.3, 130.9, 130.9, 130.2, 129.6, 124.3, 114.0, 113.5, 108.6, 46.4, 12.1. HRMS (ESI) Calcd for $C_{19}H_{19}N_2O_4$ $(M + H)^+$ 339.1339. Found 339.1345.

Compound 11. Yield 61%; orange solid (ethyl acetate/*n*-hexane); mp 78 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (s, 1 H), 7.64 (d, J = 8.8 Hz, 1 H), 7.62 (d, J = 9.3 Hz, 1 H), 7.27 (s, 1 H), 6.94 (s, 1 H), 6.84 (d, J = 8.3 Hz, 1 H), 6.26 (d, J = 9.3 Hz, 1 H), 6.15 (s, 2 H), 3.00 (q, J = 6.8 Hz, 4 H), 0.98 (t, J = 6.8 Hz, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.6, 154.9, 144.2, 143.5, 136.6, 132.5, 130.6, 130.5, 129.5, 125.6, 119.2, 113.9, 113.7, 108.7, 46.0, 12.1. HRMS (ESI) Calcd for $C_{19}H_{20}N_3O_4$ $(M + H)^+$ 354.1448. Found 354.1439. Anal. Calcd for $C_{19}H_{19}N_3O_4$: C, 64.58; H, 5.42; N, 11.89. Found: C, 64.23; H, 5.57; N, 11.74.

Compound 12. Yield 63%; yellow solid (ethyl acetate/*n*-hexane); mp 195 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, J = 9.3 Hz, 1 H), 7.55 (d, J = 8.8 Hz, 2 H), 7.46 (d, J = 8.8 Hz, 2 H), 7.21 (br s, 2 H), 6.22 (d, J = 9.3 Hz, 1 H), 2.97 (q, J = 6.8 Hz, 4 H), 2.21 (s, 3 H), 0.94 (t, J = 6.8 Hz, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.8, 162.0, 154.8, 143.8, 137.4, 136.7, 131.9, 130.9, 130.8, 129.3, 120.2, 113.1, 107.8, 46.0, 25.0, 12.2. HRMS (ESI) Calcd for $C_{21}H_{23}N_2O_3$ $(M + H)^+$ 351.1703. Found 351.1709. Anal. Calcd for $C_{21}H_{22}N_2O_3$: C, 71.98; H, 6.33; N, 7.99. Found: C, 71.81; H, 6.37; N, 7.97.

Compound 13. Yield 59%; yellow solid (ethyl acetate/*n*-hexane); mp 112 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, J = 9.3 Hz, 1 H), 7.38 (d, J = 8.8 Hz, 2 H), 7.20 (s, 1 H), 6.88 (s, 1 H), 6.76 (d, J = 8.8 Hz, 2 H), 6.20 (d, J = 9.3 Hz, 1 H), 3.01 (q, J = 6.8 Hz, 4 H), 2.99 (s, 6 H), 0.96 (t, J = 6.8 Hz, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.1, 154.4, 153.2, 143.9, 143.8, 132.7, 130.6, 130.5, 129.4, 129.3, 112.9, 112.8, 107.6, 45.7, 40.9, 12.2. HRMS (ESI) Calcd for $C_{21}H_{25}N_2O_2$ $(M + H)^+$ 337.1910. Found 337.1913. Anal. Calcd for $C_{21}H_{24}N_2O_2$: C, 74.97; H,7.19; N, 8.33. Found: C, 75.20; H, 7.38; N,8.42.

Compound 14. Yield 34%; yellow oil; ^{1}H NMR (400 MHz, CD_3OD) δ 8.06 (d, J = 8.3 Hz, 2 H), 7.87 (d, J = 9.8 Hz, 1 H), 7.67 (d, J = 7.8 Hz, 2 H), 7.43 (s, 1 H), 7.01 (s, 1 H), 6.24 (d, J = 9.8 Hz, 1 H), 3.02 (q, J = 6.8 Hz, 4 H), 2.64 (s, 3 H), 0.96 (t, J = 7.3 Hz, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.9, 161.6, 155.3, 145.9, 143.5, 136.2, 131.2, 130.9, 129.1, 129.0, 113.6, 133.3, 108.2, 46.3, 26.9, 12.2. HRMS (ESI) Calcd for $C_{21}H_{22}NO_3$ $(M + H)^+$ 336.1594. Found 336.1590.

Compound 15. Yield 46%; yellow solid (ethyl acetate/*n*-hexane); mp 97 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ7.61 (d, J = 9.3 Hz, 1 H), 7.44 (d, J = 8.3 Hz, 2 H), 7.37 (d, J = 8.3 Hz, 2 H), 7.21 (s, 1 H), 6.92 (s, 1 H), 6.23 (d, J = 9.8 Hz, 1 H), 2.97 (q, J = 6.8 Hz, 4 H), 0.95 (t, J = 6.8 Hz, 6 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.5, 154,9, 143.3, 139.1, 133.2, 131.1, 130.6, 130.4, 129.9, 129.0, 113.3, 133.0, 108.0, 46.0, 12.0. HRMS (ESI) Calcd for $C_{19}H_{18}ClNNaO_2$ $(M + Na)^+$ 350.0918. Found 350.0913. Anal. Calcd for $C_{19}H_{18}ClNO_2$: C, 69.62; H, 5.53; N, 4.27. Found: C, 69.66; H, 5.61; N, 4.42.

Compound 16. Yield 31%; yellow solid (ethyl acetate/*n*-hexane); mp 107 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (d, J = 9.8 Hz, 1 H), 7.45 (dd, J = 8.8, 5.4 Hz, 2 H), 7.22 (s, 1 H), 7.11 (dd, J = 8.8, 8.3 Hz, 2 H), 7.03 (s, 1 H), 6.26 (d, J = 9.3 Hz, 1 H), 2.98 (q, J = 6.8 Hz, 4 H), 0.97 (t, J = 6.8 Hz, 6 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 162.9, 161.6, 161.3, 154.8, 153.0, 143.4, 136.6, 136.6, 131.4, 130.6, 130.3, 130.2, 115.7, 115.6, 113.2, 112.9, 107.8, 45.8, 12.0. HRMS (ESI) Calcd for $C_{19}H_{18}FNNaO_2$ $(M + Na)^+$ 334.1213. Found 334.1207. Anal. Calcd for $C_{19}H_{18}FNO_2$: C, 73.29; H, 5.83; N, 4.50. Found: C, 73.14; H, 5.89; N, 4.44.

Compound 17. Yield 65%; yellow crystals (ethyl acetate); mp 133 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (t, J = 1.6 Hz, 1 H), 7.76 (dt, J = 8.0, 1.2 Hz, 1 H), 7.62 (m, 2 H), 7.52 (t, J = 7.6 Hz, 1 H), 7.23 (s, 1 H), 6.96 (s, 1 H), 6.27 (d, J = 9.2 Hz, 1 H), 2.95 (q, J = 7.2 Hz, 4 H), 0.95 (t, J = 7.2 Hz, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.1, 155.0, 152.6, 143.0, 141.7, 133.0, 132.1, 130.7, 130.5, 130.0, 129.5, 118.7, 113.6, 113.1, 112.8, 108.2, 45.9, 11.8. HRMS (ESI) Calcd for $C_{20}H_{19}N_2O_2$ $(M + H)^+$ 319.1441. Found 319.1434. Anal. Calcd for $C_{20}H_{18}N_2O_2$: C, 75.45; H, 5.70; N, 8.80. Found: C, 75.39; H, 5.85; N, 8.82.

Compound 34. Yield 56%; yellow solid (ethyl acetate/*n*-hexane); mp 98 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (d, J = 9.3 Hz, 1 H), 7.49 (s, 1 H), 7.33 (d, J = 4.6 Hz, 1 H), 7.32 (d, J = 3.4 Hz, 1 H), 7.05 (dd, J = 4.9, 3.4 Hz, 1 H), 6.28 (d, J = 9.3 Hz, 1 H), 3.07 (q, J = 6.8 Hz, 4 H), 1.02 (t, J = 6.8 Hz, 6 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.3, 154.4, 143.3, 140.8, 129.5, 127.0, 126.6, 126.2, 125.4, 114.0, 109.7, 46.2, 11.6. HRMS (ESI) Calcd for $C_{17}H_{17}NNaO_2S$ $(M + Na)^+$ 322.0872. Found 322.0879. Anal. Calcd for $C_{17}H_{17}NO_2S$: C, 68.20; H, 5.72; N, 4.68. Found: C, 68.25; H, 5.81; N, 4.65.

Compounds **18**−**21** were prepared from **43b** according to the method described for **8**.

Compound 18. Yield 66%; orange crystals (ethyl acetate); mp 71 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, J = 9.3 Hz, 1 H), 7.49 (d, J = 7.3 Hz, 2 H), 7.42 (t, J = 7.3 Hz, 2 H), 7.33 (t, J = 7.3 Hz, 1 H), 7.24 (s, 1 H), 6.87 (s, 1 H), 6.21 (d, J = 9.3 Hz, 1 H), 2.64 (s, 6 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.8, 155.0, 154.8, 143.6, 140.9, 131.0, 129.9, 128.9, 128.4, 127.2, 112.5, 112.1, 104.4, 43.0; MS m/z 288 (M + Na^+). HRMS Calcd for $C_{17}H_{15}NNaO_2$ (M + Na^+) 288.0995. Found 288.0990. Anal. Calcd for $C_{17}H_{15}NO_2 \cdot {}^1/_4H_2O$: C, 75.68; H, 5.79; N, 5.19. Found: C, 75.74; H, 5.63; N, 5.06.

Compound 19. Yield 35%; yellow crystals (ethyl acetate); mp 185 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, J = 8.3 Hz, 2H), 7.64 (d, J = 8.8 Hz, 2 H), 7.61 (d, J = 9.3 Hz, 1 H), 7.24 (s, 1 H), 6.89 (s, 1 H), 6.24 (d, J = 9.3 Hz, 1 H), 2.64 (s, 6 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 161.2, 155.4, 154.4, 145.5, 143.0, 132.6, 130.8, 128.9, 127.7, 118.8, 113.0, 112.3, 110.8, 104.8, 43.1; MS m/z 284 (MH^+). HRMS Calcd for $C_{18}H_{15}N_2O_2$ (MH^+) 291.1128. Found 291.1131. Anal. Calcd for $C_{18}H_{14}N_2O_2$: C, 74.47; H, 4.86; N, 9.65. Found: C, 74.28; H, 4.91; N, 9.49.

Compound 20. Yield 85%; orange crystals (ethyl acetate); mp 125 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, J = 9.3 Hz, 1 H), 7.47 (dd, J = 8.8, 5.4 Hz, 2 H), 7.21 (s, 1 H), 7.11 (dd, J = 8.8, 6.3 Hz, 2 H), 6.87 (s, 1 H), 6.22 (d, J = 9.3 Hz, 1 H), 2.63 (s, 6 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 162.9, 161.7, 161.3, 155.0, 154.8, 143.4, 136.7, 130.8, 130.1, 130.0, 129.0, 115.9, 115.7, 112.8, 112.3, 104.7, 43.0; MS m/z 284 (M + $H)^+$. HRMS Calcd for $C_{17}H_{15}FNO_2$ (M + $H)^+$ 284.1081. Found 284.1079. Anal. Calcd for $C_{17}H_{14}FNO_2$: C, 72.07; H, 4.98; N, 4.94. Found: C, 72.00; H, 4.98; N, 4.87.

Compound 21. Yield 29%; yellow crystals (methanol); mp 147 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (t, J = 1.4 Hz, 1 H), 7.76 (dt, J = 7.8, 1.4 Hz, 1 H), 7.62 (dt, J = 7.8, 1.4 Hz, 1 H), 7.61 (d, J = 9.2 Hz, 1 H), 7.54 (t, J = 7.8 Hz, 1 H), 7.23 (s, 1 H), 6.90 (s, 1 H), 6.25 (d, J = 9.2 Hz, 1 H), 2.63 (s, 6 H). HRMS Calcd for $C_{18}H_{15}N_2O_2$ (M + $H)^+$ 291.1128. Found 291.1120. Anal. Calcd for $C_{18}H_{14}N_2O_2$: C, 74.47; H, 4.86; N, 9.65. Found: C, 74.19; H, 4.96; N, 9.48.

Compounds **22** and **24** were prepared from **43c** according to the synthetic method for **8**.

Compound 22. Yield 70%; colorless crystals (ethyl acetate/*n*-hexane); mp 133 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, J = 9.3 Hz, 1 H), 7.48 (d, J = 6.8 Hz, 2 H), 7.43 (dd, J = 7.3, 6.8 Hz, 2 H), 7.37 (d, J = 7.3 Hz, 1 H), 6.91 (s, 1 H), 6.57 (d, J = 9.3 Hz, 1 H), 3.89 (s, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.3, 160.0, 155.4, 143.6, 136.9, 129.6, 129.5, 128.5, 128.4, 127.7, 113.6, 112.5, 99.5, 56.3; MS m/z 275 (M + Na^+). HRMS Calcd for $C_{16}H_{12}O_3Na$ (M + Na^+) 275.0678. Found 275.0683. Anal. Calcd for $C_{16}H_{12}O_3$: C, 76.18; H, 4.79. Found: C, 75.90; H, 4.98.

Compound 24. Yield 25%; yellow crystals (ethyl acetate/*n*-hexane); mp 152 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, J = 9.3 Hz, 1 H), 7.40 (d, J = 8.3 Hz, 2 H), 7.36 (s, 1 H), 6.88 (s, 1 H), 6.79 (d, J = 7.8 Hz, 2 H), 6.26 (d, J = 9.3 Hz, 1 H), 3.88 (s, 3 H), 3.00 (s, 6 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.5, 160.1, 154.8, 150.1, 143.8, 130.3, 128.9, 128.8, 128.6, 113.3, 112.5, 112.3, 99.3, 56.2, 40.7; MS m/z 296 (M + $H)^+$. HRMS Calcd for $C_{18}H_{18}NO_3$ (M + $H)^+$ 296.1281. Found 296.1276. Anal. Calcd for $C_{18}H_{17}NO_3$: C, 73.2; H, 5.80; N, 4.74. Found: C, 72.96; H, 5.93; N, 4.85.

Synthesis of 23. Boron tribromide (1 M in dichloromethane, 0.60 mL, 0.60 mmol) was added to a solution of **22** (27 mg, 0.108 mmol) in dichloromethane (2 mL) at −78 °C under argon. The mixture was stirred at room temperature for 1 day, then poured into water and extracted with ethyl acetate. The organic layer was washed with water and brine, dried over magnesium sulfate, and filtered. The solvent was removed in vacuo, and the residue was purified by preparative thin-layer chromatography (silica gel, ethyl acetate/*n*-hexane = 1/4) to give **23** (2.0 mg, 0.0084 mmol, 8%) as a white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (d, J = 9.3 Hz, 1 H), 7.53 (dd, J = 7.3 Hz, 2 H), 7.45 (dd, J = 7.8, 6.8 Hz, 3 H), 7.36 (s, 1 H), 7.00 (s, 1 H), 6.29 (d, J = 9.3 Hz, 1 H), 6.08 (s, 1 H), 3.89 (s, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.4, 156.4, 155.3, 143.6, 135.4, 129.7, 129.3, 129.3, 128.7, 126.3, 113.7, 113.0, 103.8; MS m/z 239 (M + $H)^+$. HRMS Calcd for $C_{15}H_{11}O_3$ (M + $H)^+$ 239.0702. Found 239.0703.

Compound **25** was prepared similarly. Yield 33%; yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (d, J = 9.3 Hz, 1 H), 7.30 (s, 1 H), 7.29 (d, J = 8.3 Hz, 2 H), 6.94 (s, 1 H), 6.84 (d, J = 8.8 Hz, 2 H), 6.26 (d, J = 9.8 Hz, 1 H), 5.87 (s, 1 H), 3.02 (s, 6 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.5, 156.6, 154.9, 150.6, 143.7, 130.0, 128.9, 126.5, 122.2, 113.4, 113.3, 112.9, 103.4, 40.6; MS m/z 282 (M + $H)^+$. HRMS Calcd for $C_{17}H_{16}NO_3$ (M + $H)^+$ 282.1124. Found 282.1117.

Compounds **26**−**33** and **35**−**38** were prepared from **43d** according to the method described for **8**.

Compound 26. Yield 41%; colorless crystals (ethyl acetate/*n*-hexane); mp 112 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, J = 9.3 Hz, 1 H), 7.56 (dd, J = 8.5, 2.0 Hz, 1 H), 7.67 (d, J = 2.0 Hz, 1 H), 7.58 (d, J = 7.3 Hz, 2 H), 7.47 (t, J = 7.3 Hz, 2 H), 7.41 (d, J = 8.8 Hz, 1 H), 7.40 (d, J = 7.3 Hz, 1 H), 6.47 (d, J = 9.3 Hz, 1 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.9, 153.6, 143.7, 139.6, 138.0, 130.9, 129.2, 128.0, 127.2, 126.2, 119.2, 117.5, 117.2; MS m/z 223 (M + $H)^+$. HRMS Calcd for $C_{15}H_{11}O_2$ (M + $H)^+$ 223.0753. Found 223.0754.

Compound 27. Yield 67%; colorless solid (ethyl acetate/*n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (m, 4 H), 7.70 (m, 3 H), 7.45 (d, J = 8.3 Hz, 1 H), 6.51 (d, J = 9.8 Hz, 1 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.4, 154.4, 144.0, 143.2, 135.9, 133.0, 130.8, 127.8, 126.6, 119.5, 118.8, 117.9, 117.8, 111.7; MS m/z 248 (M + $H)^+$. HRMS Calcd for $C_{16}H_{10}NO_2$ (M + $H)^+$ 248.0706. Found 248.0705. Anal. Calcd for $C_{16}H_9NO_2$: C, 77.72; H, 3.67; N, 5.67. Found: C, 77.42; H, 3.91; N, 5.68.

Compound 28. Yield 62%; yellow solid (ethyl acetate/*n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, J = 9.8 Hz, 1 H), 7.71 (dd, J = 8.3, 2.0 Hz, 1 H), 7.61 (d, J = 2.0 Hz, 1 H), 7.49 (d, J = 8.8 Hz, 2 H), 7.36 (d, J = 8.3 Hz, 1 H), 6.82 (d, J = 7.8 Hz, 2 H), 6.44 (d, J = 9.3 Hz, 1 H), 3.02 (s, 6 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.4, 152.8, 150.3, 143.9, 138.1, 130.2, 127.8, 124.9, 119.2, 117.2, 116.9, 112.9, 40.7; MS m/z 266 (M + Na^+). HRMS Calcd for $C_{17}H_{16}NO_2$ (M + $H)^+$ 266.1175. Found 266.1181. Anal. Calcd for $C_{17}H_{15}NO_2 \cdot {}^1/_6H_2O$: C, 76.10; H, 5.76, N, 5.22. Found: C, 76.00; H, 5.60; N, 5.22.

Compound 29. Yield 21%; colorless solid (ethyl acetate/*n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, J = 9.3 Hz, 1 H), 7.70 (dd, J = 8.8, 2.4 Hz, 1 H), 7.62 (d, J = 2.4 Hz, 1 H), 7.54 (dd, J = 8.8, 4.9 Hz, 2 H), 7.41 (d, J = 8.3 Hz, 1 H), 7.16 (t, J = 8.5 Hz, 2 H), 6.48 (d, J = 9.3 Hz, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.7, 161.7, 160.6, 153.4, 143.3, 136.9, 135.6, 130.6, 128.7, 125.9, 119.1, 117.3, 116.0; MS m/z 241 (M + $H)^+$. HRMS Calcd for $C_{15}H_{10}FO_2$ (M + $H)^+$ 241.0659. Found 241.0652.

Compound 30. Yield 63%; colorless crystals (ethyl acetate); mp 170 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (t, J = 1.5 Hz, 1 H), 7.81 (dt, J = 7.8, 1.5 Hz, 1 H), 7.78 (d, J = 9.3 Hz, 1 H), 7.73 (dd, J = 8.8, 2.4 Hz, 1 H), 7.68 (dt, J = 7.8, 1.5 Hz, 1 H), 7.59 (dd, J = 7.8, 7.3 Hz, 1 H), 7.45 (d, J = 8.8 Hz, 1 H), 6.51 (d, J = 9.8 Hz, 1 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.6, 154.0, 143.4, 140.7, 135.5, 131.5, 131.3, 130.7, 130.6, 130.0, 126.4, 119.4, 118.7, 117.9, 117.6, 113.2; MS m/z 248 (M + $H)^+$, 270 (M + Na^+). HRMS Calcd for $C_{16}H_9NO_2Na$ (M + Na^+) 270.0525. Found 270.0525. Anal. Calcd for $C_{16}H_9NO_2$: C, 77.72; H, 3.67; N, 5.67. Found: C, 77.54; H, 3.96; N, 5.83.

Compound 31. Yield 37%; colorless powder (ethyl acetate/*n*-hexane); mp 162 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, J = 9.8 Hz, 1 H), 7.71 (dd, J = 8.8, 2.0 Hz, 1 H), 7.65(d, J = 2.0 Hz, 1 H), 7.43 (d, J = 8.8 Hz, 1 H), 7.10 (dd, J = 7.2, 2.0 Hz, 2 H), 6.84 (tt, J = 8.8, 2.2 Hz, 2 H), 6.50 (d, J = 9.3 Hz, 1 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 164.4, 164.3, 162.7, 162.6, 160.4, 154.1, 143.3, 142.8, 142.7, 142.7, 135.5, 135.5, 130.5, 126.3, 119.3, 117.7, 117.6, 110.2, 110.1, 110.0, 110.0, 103.3, 103.2, 103.0; MS m/z 259 (M + $H)^+$. HRMS Calcd for $C_{15}H_9F_2O_2$ (M + $H)^+$

259.0565. Found 259.0559. Anal. Calcd for $C_{15}H_8F_2O_2$: C, 69.77; H, 3.12. Found: C, 69.48; H, 3.47.

Compound 32. Yield 89%; colorless crystals (ethyl acetate/*n*-hexane); mp 117 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (s, 1 H), 7.78 (d, J = 9.3 Hz, 1 H), 7.76 (dd, J = 8.8, 2.4 Hz, 1 H), 7.76 (d, J = 7.8 Hz, 1 H), 7.69 (d, J = 2.0 Hz, 1 H), 7.66 (d, J = 7.8 Hz, 1 H), 7.60 (d, J = 7.8 Hz, 1 H), 6.50 (d, J = 9.3 Hz, 1 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.6, 154.0, 143.3, 140.4, 136.5, 131.7, 131.5, 130.8, 130.5, 129.7, 126.4, 125.1, 124.6, 124.6, 124.6, 124.0, 124.0, 123.9, 123.3, 119.4, 117.7, 117.6; MS m/z 291 $(M + H)^+$. HRMS Calcd for $C_{16}H_{10}F_3O_2$ $(M + H)^+$ 291.0627. Found 291.0629. Anal. Calcd for $C_{16}H_9F_3O_2$: C, 66.21; H, 3.13; N, 0. Found: C, 66.01; H, 3.48; N, 0.

Compound 33. Yield 8%; orange crystals (ethyl acetate/*n*-hexane); mp 161 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.87 (br s, 1 H), 8.66 (br s, 1 H), 7.92 (d, J = 7.9 Hz, 1 H), 7.79 (d, J = 9.5 Hz, 1 H), 7.75 (dd, J = 8.6, 1.7 Hz, 1 H), 7.69 (s, 1 H), 7.47 (d, J = 8.6 Hz, 1 H), 7.44 (d, J = 7.9 Hz, 1 H), 6.51 (d, J = 9.5 Hz, 1 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.5, 154.2, 148.3, 147.5, 143.2, 135.6, 135.2, 134.1, 130.8, 126.4, 124.2, 119.5, 118.0, 117.8; MS m/z 224 $(M + H)^+$. HRMS Calcd for $C_{14}H_{10}NO_2$ $(M + H)^+$ 224.0706. Found 224.0700.

Compound 35. Yield 57%; colorless solid (ethyl acetate/*n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (dd, J = 6.3, 2.0 Hz, 1 H), 7.74 (d, J = 9.8 Hz, 1 H), 7.68 (d, J = 2.0 Hz, 1 H), 7.35 (d, J = 7.8 Hz, 1 H), 7.33 (dd, J = 4.9, 1.5 Hz, 1 H), 7.32 (dd, J = 3.4, 0.98 Hz, 1 H), 7.11 (dd, J = 5.0, 4.2 Hz, 1 H), 6.47 (d, J = 9.8 Hz, 1 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.7, 153.4, 143.3, 142.5, 131.3, 129.7, 128.4, 125.6, 124.8, 123.9, 119.3, 117.6, 117.5; MS m/z 251 $(M + H)^+$ 229. HRMS Calcd for $C_{13}H_9O_2S$ $(M + H)^+$ 229,0317. Found 229.0317. Anal. Calcd for $C_{13}H_8O_2S$: C, 68.40; H, 3.53; N, 0. Found: C, 68.02; H, 3.80; N, 0.

Compound 36. Yield 18%; yellow crystals (ethyl acetate); mp 165 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, J = 9.8 Hz, 1 H), 7.66 (dd, J = 8.8, 2.4 Hz, 1 H), 7.57 (d, J = 2.0 Hz, 1 H), 7.35 (d, J = 8.3 Hz, 1 H), 7.07 (d, J = 3.8 Hz, 1 H), 6.92 (d, J = 3.8 Hz, 1 H), 6.47 (d, J = 9.8 Hz, 1 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.5, 153.6, 143.2, 140.9, 130.6, 130.1, 129.2, 127.5, 124.5, 123.1, 119.4, 117.8, 117.7; MS m/z 263 $(M + H)^+$. HRMS Calcd for $C_{13}H_8ClO_2S$ $(M + H)^+$ 262.9928. Found 262.9928. Anal. Calcd for $C_{13}H_7ClO_2S$: C, 59.43; H, 2.69. Found: C, 59.18; H, 2.95.

Compound 37. Yield 76%; yellow crystals (ethyl acetate/*n*-hexane); mp 105 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (dd, J = 8.5, 2.2 Hz, 1 H), 7.77 (d, J = 2.0 Hz, 1 H), 7.74 (d, J = 9.3 Hz, 1 H), 7.50 (d, J = 2.0 Hz, 1 H), 7.35 (d, J = 8.8 Hz, 1 H), 6.68 (d, J = 2.9 Hz, 1 H), 6.50 (dd, J = 3.4, 2.7 Hz, 1 H), 6.46 (d, J = 9.3 Hz, 1 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.6, 153.2, 152.4, 143.4, 142.7, 127.8, 127.5, 122.7, 119.2, 117.4, 117.3, 112.0, 105.7; MS m/z 213 $(M + H)^+$. HRMS Calcd for $C_{13}H_9O_3(M + H)^+$ 213.0546. Found 213.0549. Anal. Calcd for $C_{13}H_8O_3$: C, 73.58; H, 3.80; N, 0. Found: C, 73.43; H, 3.72; N, 0.

Compound 38. Yield 58%; colorless powder (ethyl acetate); mp 211 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, J = 9.3 Hz, 1 H), 7.55 (dd, J = 8.7, 2.0 Hz, 1 H), 7.50 (d, J = 2.0 Hz, 1 H), 7.43 (d, J = 8.8 Hz, 1 H), 6.87 (d, J = 3.9 Hz, 1 H), 6.51 (d, J = 9.8 Hz, 1 H), 6.26 (d, J = 4.4 Hz, 1 H), 3.75 (s, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.3, 154.0, 143.0, 138.2, 132.4, 128.3, 127.9, 119.8, 119.2, 117.9, 117.6, 114.1, 110.4, 106.3, 33.9; MS m/z 251 $(M + H)^+$. HRMS Calcd for $C_{15}H_{11}N_2O_2$ $(M + H)^+$ 251.0815. Found 251.0811. Anal. Calcd for $C_{15}H_{10}N_2O_2$: C, 71.99; H, 4.03; N, 11.19. Found: C, 71.64; H, 4.09; N, 11.23.

Alkaline Phosphatase Assay. T47D breast-carcinoma cells were cultured in RPMI 1640 medium with 10% (v/v) fetal bovine serum and penicillin−streptomycin mixed solution. Cells were plated in 96-well plates at 1×10^4 cells/well and incubated overnight (37 °C, 5% CO_2 in air). The next day, cells were treated with fresh medium containing test compound in the presence or absence of progesterone (1 nM) and further incubated for 24 h. The medium was aspirated, and the cells were fixed with 100 μL of 1.8% formalin (in PBS). The fixed cells were washed with PBS, and 75 μL of assay buffer (1 mg/mL *p*-nitrophenol phosphate in diethanolamine water solution, pH 9.0, 2 mM $MgCl_2$) was added. The mixture was incubated at room temperature with shielding from light for 2 h. Then the reaction was terminated by the addition of 100 μL of NaOH. The absorbance at 405 nm was measured.

hPR Binding Assay. hPR-binding assay was performed using recombinant hPR-LBD purchased from Invitrogen. hPR-LBD was diluted with buffer (20 mM Tris-HCl, 300 mM NaCl, 1 mM EDTA, 5 mM DTT, pH 8.0) to 5 nM, and 300 μL aliquots were incubated in the dark at 4 °C with 4 nM [1,2,6,7-^{3}H]PG (Perkin-Elmer) and reference or test compounds (dissolved in DMSO; final concentration of DMSO was 3%). Nonspecific binding was assessed by addition of a 200-fold excess of nonradioactive PG. After 24 h, 30 μL of dextran T-70/γ-globulin-coated charcoal suspension was added to the ligand/protein mixtures (1% activated charcoal, 0.05% γ-globulin, 0.05% dextran 70, final concentrations) and incubated at 4 °C for 5 min. The charcoal was removed by centrifugation for 5 min at 1300*g*, and the radioactivity of the supernatant was measured in ACS II scintillation cocktail (GE) by using a liquid scintillation counter. All experiments were performed in duplicate.

hAR Binding Assay. A hAR-LBD expression plasmid vector that encodes GST-hARLBD (627−919 aa, EF domain) fusion protein under the *lac* promoter (provided by Prof. S. Kato, University of Tokyo) was transfected into *E. coli* strain *HB-101*. An overnight culture (10 mL) of the bacteria was added to 1 L of LB medium and incubated at 27 °C until the optical density at 600 nm reached 0.6−0.7. Following the addition of IPTG to a concentration of 1 mM, incubation was continued for an additional 4.5 h. Cells were harvested by centrifugation at 4000*g* at 4 °C for 15 min and stored at −80 °C until use. All subsequent operations were performed at 4 °C. The bacterial pellet obtained from 40 mL of culture was resuspended in 1 mL of ice-cold TEGDM buffer (10 mM Tris-HCl, pH 7.4, 1 mM EDTA, 10% glycerol, 10 mM DTT, 10 mM sodium molybdate). The suspension was subjected to sonication using 10 × 10 s bursts on ice, and crude GST-hARLBD fraction was prepared by centrifugation of the suspension at 12000*g* for 30 min at 4 °C. The crude receptor fraction was diluted with buffer (20 mM Tris-HCl, pH 8.0, 0.3 M KCl, 1 mM EDTA) to a protein concentration of 0.3−0.5 mg/mL and used in binding assays as hAR-LBD fraction. Aliquots of the hAR-LBD fraction were incubated in the dark at 4 °C with [^{3}H]DHT (PerkinElmer, 4 nM final concentration) and reference or test compounds (dissolved in DMSO, final concentration of DMSO was 2%). Nonspecific binding was assessed by addition of a 200-fold excess of nonradioactive DHT. After 18 h, a dextran 70/γ-globulin-coated charcoal suspension was added to the ligand/protein mixture (1% activated charcoal, 0.05% γ-globulin, 0.05% dextran, 70 final concentrations) and the whole was incubated at 4 °C for 10 min. The charcoal was removed by centrifugation for 5 min at 1300*g*, and the radioactivity of the supernatant was measured in ACS II scintillation cocktail (GE) by using a liquid scintillation counter. All experiments were performed in duplicate.

hER Binding Assay. The recombinant human ER was purchased from Wako Pure Chemical Industries, Ltd. Bio-Gel HT hydroxylapatite (Bio-Rad, Hercules, CA) was washed five times with the buffer (50 mM Tris-HCl, 1 mM KH_2PO_4, pH 7.2). The hydroxylapatite slurry was adjusted to 50% (by volume) hydroxylapatite in the suspension. The ER was diluted with binding buffer (50 mM Tris, pH 7.5, 10% glycerol, 0.1 mM butylated hydroxyanisole, 10 mM mercaptoethanol, 0.5% yeast extract) to a protein concentration of 2−4 nM. Then 2 μL of 10^{-6} M [^{3}H]estradiol solution in DMSO was added to each tube, followed by 2 μL aliquots of the competitor solutions in DMSO. After addition of 200 μL of ER solution to each tube, the tubes were placed in the refrigerator. The final incubation conditions were the following: 10^{-8} M [^{3}H]-estradiol, 10^{-4}−10^{-8} M competitor. After 14−18 h, the bound ligand was assayed by adsorption on hydroxylapatite for 15 min at 0 °C, followed by three washes with 1 mL of 0.05 M Tris, pH 7.3. After the last wash, the hydroxylapatite pellet was resuspended in 0.3 mL of EtOH and

radioactivity was counted in 7 mL of scintillation cocktail (ACS II). All experiments were performed in duplicate.

hGR and rMR Binding Assay. hGR binding assays were performed at Caliper Life Science (U.S.) using the methods described below. The hGR was diluted with binding buffer (50 mM KH_2PO_4, pH 7.4, with 10 mM sodium molybdate and 1 mM dithiothreitol) to yield a final protein concentration of 1.25 nM in the assay tubes. The final incubation conditions were the following: [^{3}H]dexamethasone, 10^{-9} M; triamcinolone acetonide, 10^{-5} M (for nonspecific binding determination only); test compounds, 10^{-7}–10^{-5} M, 0.4% DMSO. After 18 h, the bound ligand was assayed by vacuum filtration onto glass fiber filters and radioactivity was counted in 50 μL of scintillation cocktail (Microscint-20). All experiments were performed in duplicate.

rMR binding assays were also performed at Caliper Life Science (U.S.) using the methods described below. The rMR was diluted with binding buffer (20 mM HEPES, pH 7.4, 15 mM sodium molybdate, 1 mM ditiothreitol, 10% glycerol, and 1 mM EDTA). The concentration for the MR receptor tissue preparation is optimized for each tissue preparation and is expressed in original tissue wet weight/volume. For the assays in question it was 16.7 mg wet weight/mL in the final reaction. The final incubation conditions were the following: [^{3}H]aldosterone, 2×10^{-9} M; spironolactone, 10^{-6} M (for nonspecific binding determination only); test compounds, 10^{-7}–10^{-5} M; 0.4% DMSO. After 18–20 h, the bound ligand was assayed by adsorption of unbound ligand onto dextran-coated charcoal, centrifugation of charcoal and unbound ligand, and removal of 200 μL of the supernatant. Unabsorbed radioactivity was counted in 6 mL of scintillation cocktail (Luma Safe). All experiments were performed in duplicate.

PR Transactivation Assay. Reporter gene assays using hPR were carried out with CHO (Chinese hamster ovary) cells. CHO cells were cultured in MEMα medium with 5% (v/v) fetal bovine serum. Transient transfections of CHO cells were performed using FuGENE 6 (Roche), according to the manufacturer's protocol. Transfections were done in 96-well plates at 2×10^4 cells/well with 5 ng/well pCMV-hPR and 95 ng/well pGL4-MMTV-luc (Promega). Twenty-four hours after addition of the sample (final concentration, 10^{-5}–10^{-7} M) and 3×10^{-9} progesterone, the medium was removed and 50 μL of Pica-Gene luminescence kit (Toyo Ink Manufacturing Company, Ltd.) was added. Incubation was continued at room temperature with shielding from light for 15 min, and then the luminescence was measured. All data points were measured in duplicate.

Fluorescence Study. All UV spectra were recorded with a JASCO V-550, and fluorescence spectra were recorded with a JASCO FP-6600. Fluorescence quantum yields (Φ_{fl}) were determined using quinine sulfate (0.577) in 0.1 M H_2SO_4 as a standard. Fluorescent spectra in the presence of PR-LBD were measured in the same buffer as that of hPR binding assay.

■ ASSOCIATED CONTENT

Supporting Information. HPLC charts for coumarin derivatives **8**, **10**, **14**, **23**, **25**, **26**, **29**, and **33**. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

PR, progesterone receptor; ER, estrogen receptor; AR, androgen receptor; GR, glucocorticoid receptor; AP, alkaline phosphatase

■ REFERENCES

(1) Psychoyos, A. Hormonal control of ovo-implantation. *Vitam. Horm.* **1973**, *31*, 201–256.

(2) Lydon, J. P.; Demayo, F. J.; Funk, C. R.; Mani, S. K.; Hughes, A. R.; Montgomery, C. A.; Shyamala, G.; Conneely, O. M.; O'Malley, B. W. Mice lacking progesterone receptor exhibit pleiotropic reproductive abnormalities. *Gene Dev.* **1995**, *9*, 2266–2278.

(3) Zhang, Z.; Funk, C.; Glasser, S. R.; Mulholland, J. Progesterone regulation of heparin-binding epidermal growth factor-like growth factor gene expression during sentitization and decidualization in the rat uterus: effects of the antiprogestin, ZK-98299. *Endocrinology* **1994**, *135*, 1256–1263.

(4) Mani, S. K.; Allen, J. M. C.; Clark, J. H.; Blaustein, J. D.; O'Malley, B. W. Convergent pathway for steroid hormone- and neurotransmitter-induced rat sexual behavior. *Science* **1994**, *265* (5176), 1246–1249.

(5) Mangelsdorf, D. J.; Thummel, C.; Beato, M.; Herrlich, P.; Schuetz, G.; Umesono, K.; Blumberg, B.; Kastner, P.; Mark, M. The nuclear receptor superfamily: the second decade. *Cell* **1995**, *83*, 835–839.

(6) Horwitz, K. B.; Tung, L.; Takimoto, G. S. Progestins, progesterone receptors, and breast cancer. *Horm. Cancer* **1996**, 283–306.

(7) Klijn, J. G. M.; Setyono-Han, B.; Foekens, J. A. Progesterone antagonists and progesterone receptor modulators in the treatment of breast cancer. *Steroids* **2000**, *65*, 825–830.

(8) Michna, H.; Parczyk, K.; Schneider, M. R.; Nishino, Y. Differentiation therapy with progesterone antagonists. *Ann. N.Y. Acad. Sci.* **1995**, *761*, 224–247.

(9) Murphy, A. A.; Kettel, L. M.; Morales, A. J.; Roberts, V. J.; Yen, S. S. Regression of uterine leiomyomata in response to the antiprogesterone RU486. *J. Clin. Endocrinol. Metab.* **1993**, *76*, 513–517.

(10) Murphy, A. A.; Morales, A. J.; Kettel, L. M.; Yen, S. S. Regression of uterine leiomyomata to the antiprogesterine RU486: dose-response effect. *Fertil. Steril.* **1995**, *64*, 187–190.

(11) Kettel, L. M.; Murphy, A. A.; Morales, A. J.; Yen, S. S. Preliminary report on the treatment of endometriosis with low-dose mifepristone (RU486). *Am. J. Obstet. Gynecol.* **1998**, *178*, 1151–1156.

(12) Kettel, L. M.; Murphy, A. A.; Morales, A. J.; Yen, S. S. Clinical Efficacy of the Antiprogesterone RU486 in the Treatment of Endometriosis and Uterine Fibroids. In *Human Reproduction*; Edwards, R. G., Ed.; Oxford University Press: Oxford, England, 1994; Vol. 9, Suppl. 1, pp 116–120.

(13) Teutsch, G.; Philibert, D. History and Perspectives of Anti-Progestins from the Chemist's Point of View. In *Human Reproduction*; Edwards, R. G., Ed.; Oxford University Press: Oxford, England, 1994; Vol. 9, Suppl. 1, pp 12–31.

(14) Winneker, R. C.; Fensome, A.; Zhang, P.; Yudt, M. R.; McComas, C. C.; Unwalla, R. J. A new generation of progesterone receptor modulators. *Steroids* **2008**, *73*, 689–701.

(15) Buijsman, R. C.; Hermkens, P. H. H.; van Rijn, R. D.; Stock, H. T.; Teerhuis, N. M. Non-steroidal steroid receptor modulators. *Curr. Med. Chem.* **2005**, *12*, 1017–1075.

(16) Palmer, S.; Campen, C. A.; Allan, G. F.; Rybczynski, P.; Haynes-Johnson, D.; Hutchins, A.; Kraft, P.; Kiddoe, M.; Lai, M.-T.; Lombardi, E.; Pedersen, P.; Hodgen, G.; Combs, D. W. Nonsteroidal progesterone

receptor ligands with unprecedented receptor selectivity. *J. Steroid Biochem. Mol. Biol.* **2001**, *75*, 33–42.

(17) Hamann, L. G.; Winn, D. T.; Pooley, C. L. F.; Tegley, C. M.; West, S. J.; Farmer, L. J.; Zhi, L.; Edwards, J. P.; Marschke, K. B.; Mais, D. E.; Goldman, M. E.; Jones, T. K. Nonsteroidal progesterone receptor antagonists based on a conformationally restricted subseries of 6-aryl-1,2-dihydro-2, 2, 4-trimethylquinolines. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2731–2736.

(18) Zhi, L.; Tegley, C. M.; Pio, B.; West, S. J.; Marschke, K. B.; Mais, D. E.; Jones, T. K. Nonsteroidal progesterone receptor antagonists based on 6-thiophenylhydroquinolines. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 415–418.

(19) Hamann, L. G.; Farmer, L. J.; Johnson, M. G.; Bender, S. L.; Mais, D. E.; Wang, M.-W.; Crombie, D.; Goldman, M. E.; Jones, T. K. Synthesis and biological activity of novel nonsteroidal progesterone receptor antagonists based on cyclocymopol monomethyl ether. *J. Med. Chem.* **1996**, *39*, 1778–1789.

(20) Pooley, C. L. F.; Edwards, J. P.; Goldman, M. E.; Wang, M. −W.; Marschke, K. B.; Crombie, D. L.; Jones, T. K. Discovery and preliminary SAR studies of a novel, nonsteroidal progesterone receptor antagonist pharmacophore. *J. Med. Chem.* **1998**, *41*, 3461–3466.

(21) Zhang, P.; Terefenko, E. A.; Wrobel, J.; Zhang, Z.; Zhu, Y.; Cohen, J.; Marschke, K. B.; Mais, D. Synthesis and progesterone receptor antagonist activities of 6-aryl benzimidazolones and benzothiazolones. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2747–2750.

(22) Comb, D. W.; Reese, K.; Philip, A. Nonsteroidal progesterone receptor ligands. 1. 3-Aryl-1-benzoyl-1,4,5,6-tetrahydropyridazines. *J. Med. Chem.* **1995**, *38*, 4878–4879.

(23) Kurihara, K.; Tanabe, K.; Yamamoto, Y.; Shinei, R.; Ajito, K.; Okonogi, T. Synthesis and structure−activity relationships of new nonsteroidal progesterone receptor ligands. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1837–1842.

(24) Kern, J. C.; Terefenko, E.; Trybulski, E.; Berrodin, T. J.; Cohen, J.; Winneker, R. C.; Yudt, M. R.; Zhang, Z.; Zhu, Y.; Zhang, P. 1-Methyl-1*H*-pyrrole-2-carbonitrile containing tetrahedronaphthalene derivatives as non-steroidal progesterone receptor antagonists. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4816–4818.

(25) Zhang, P.; Terefenko, E. A.; Fensome, A.; Wrobel, J.; Winneker, R.; Lundeen, S.; Marschke, K. B.; Zhang, Z. 6-Aryl-1,4-dihydro-benzo-[*d*][1,3]oxazin-2-ones: a novel class of potent, selective, and orally active nonsteroidal progesterone receptor antagonists. *J. Med. Chem.* **2002**, *45*, 4379–4382.

(26) Collins, M. A.; Hudak, V.; Bender, R.; Fensome, A.; Zhang, P.; Miller, L.; Winneker, R. C.; Zhang, Z.; Zhu, Y.; Cohen, J.; Unwalla, R. J.; Wrobel, J. Novel pyrrole-containing progesterone receptor modulators. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2185–2189.

(27) Fensome, A.; Bender, R.; Chopra, R.; Cohen, J.; Collins, M. A.; Hudak, V.; Malakian, K.; Lockhead, S.; Olland, A.; Svenson, K.; Terefenko, E. A.; Unwalla, R. J.; Wilhelm, J. M.; Wolfrom, S.; Zhu, Y.; Zhang, Z.; Zhang, P.; Winneker, R. C.; Wrobel, J. Synthesis and structure−activity relationship of novel 6-aryl-1,4-dihydrobenzo[*d*][1,3]oxazine-2-thiones as progesterone receptor modulators leading to the potent and selective nonsteroidal progesterone receptor agonist tanaproget. *J. Med. Chem.* **2005** *48*, 5092–5095.

(28) Terefenko, E. A.; Kern, J.; Fensome, A.; Wrobel, J.; Zhu, Y.; Cohen, J.; Winneker, R.; Zhang, Z.; Zhang, P. SAR studies of 6-aryl-1,3-dihydrobenzimidazol-2-ones as progesterone receptor antagonists. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3600–3603.

(29) Fensome, A.; Adams, W. R.; Adams, A. L.; Berrodin, T. J.; Cohen, J.; Huselton, C.; Illenberger, A.; Kern, J. C.; Hudak, V. A.; Marella, M. A.; Melenski, E. G.; McComas, C. C.; Mugford, C. A.; Slayden, O. D.; Yudt, M.; Zhang, Z.; Zhang, P.; Zhu, Y.; Winneker, R. C.; Wrobel, J. E. Design, synthesis, and SAR of new pyrrole-oxindole progesterone receptor modulators leading to 5-(7-fluoro-3,3-dimethyl-2-oxo-2,3-dihydro-1*H*-indol-5-yl)-1-methyl-1*H*-pyrrole-2-carbonitrile (WAY-255348). *J. Med. Chem.* **2008**, *51*, 1861–1873.

(30) Hirano, T.; Hiromoto, K.; Kagechika, H. Development of a library of 6-arylcoumarins as candidate fluorescent sensors. *Org. Lett.* **2007**, *9*, 1315–1318.

(31) Yee, D. J.; Balsanek, V.; Sames, D. New tools for molecular imaging of redox metabolism: development of a fluorogenic probe for 3α-hydroxysteroid dehydrogenases. *J. Am. Chem. Soc.* **2004**, *126*, 2282–2283.

(32) Yee, D. J.; Balsanek, V.; Bauman, D. R.; Penning, T. M.; Sames, D. Fluorogenic metabolic probes for direct activity readout of redox enzymes: selective measurement of human AKR1C2 in living cells. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 13304–13309.

(33) Htun, H.; Holth, L. T.; Walker, D.; Davie, J. R.; Hager, G. L. Direct visualization of the human estrogen receptor alpha reveals a role for ligand in the nuclear distribution of the receptor. *Mol. Biol. Cell* **1999**, *10*, 471–486.

(34) (a) Adamczyk, M.; Reddy, R. E.; Yu, Z. Synthesis of a novel fluorescent probe for estrogen receptor. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1283–5. (b) Rickert, E. L.; Oriana, S.; Hartman-Frey, C.; Long, X.; Webb, T. T.; Nephew, K. P.; Weatherman, R. V. Synthesis and characterization of fluorescent 4-hydroxytamoxifen conjugates with unique antiestrogenic properties. *Bioconjugate Chem.* **2010**, *21*, 903–10.

(35) Hwang, K.-J.; Carlson, K. E.; Anstead, G. M.; Katzenellenbogen, J. A. Donor−acceptor tetrahydrochrysenes, inherently fluorescent, high-affinity ligands for the estrogen receptor: binding and fluorescence characteristics and fluorometric assay of receptor. *Biochemistry* **1992**, *31*, 11536–11545.

(36) Horaguchi, T.; Hosokawa, N.; Tanemura, K.; Suzuki, T. Photocyclization reactions. Part 8. Synthesis of 2-quinolone, quinoline and coumarin derivatives using trans−cis isomerization by photoreaction. *J. Heterocycl. Chem.* **2002**, *39*, 61–67.

(37) Kisanga, P.; Fei, X.; Verkade, J. P$(RNCH_2CH_2)_3$N: an efficient promoter for the synthesis of 3-substituted coumarins. *Synth. Commun.* **2002**, *32*, 1135–1144.

(38) Zhang, Z.; Lundeen, S. G.; Zhu, Y.; Carver, J. M.; Winneker, R. C. In vitro characterization of trimegestone: a new potent and selective progestin. *Steroids* **2000**, *65*, 637–643.

(39) Beck, C. A.; Weigel, N. L.; Moyer, M. L.; Nordeen, S. K.; Edwards, D. The progesterone antagonist RU486 acquires agonist activity upon stimulation of cAMP signaling pathways. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 4441–4445.

(40) Umemiya, H.; Fukasawa, H.; Ebisawa, M.; Eyrolles, L.; Kawachi, E.; Eisenmann, G.; Gronemeyer, H.; Hashimoto, Y.; Shudo, K.; Kagechika, H. Regulation of retinoidal actions by diazepinylbenzoic acids. Retinoid synergists which activate the RXR-RAR heterodimers. *J. Med. Chem.* **1997**, *40*, 4222–4234.

(41) Vendrell, M.; Lee, J.-S.; Chang, Y.-T. Diversity-oriented fluorescence library approaches for probe discovery and development. *Cur. Opin. Chem. Biol.* **2010**, *14*, 383–389 and references therein.