5-Benzylidene 1,2-Dihydrochromeno[3,4-*f*]quinolines, A Novel Class of Nonsteroidal Human Progesterone Receptor Agonists

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A novel series of nonsteroidal progestins, 5-benzylidene-1,2-dihydrochromeno[3,4-*f*]quinolines (**2**), was discovered, and a preliminary structure−activity relationship study around the 5-benzylidene ring generated several potent human progesterone receptor agonists (compounds **8**, **16**). These new progestins showed biological activities (EC_{50} = 5.7 and 7.6 nM) similar to progesterone (EC_{50} = 2.9 nM) in the cotransfection assay with high efficacy (132% and 166%) and binding affinity (K_i = 0.66 and 0.83 nM) similar to medroxyprogesterone acetate (MPA) (K_i = 0.34 nM). A representative analogue, **8**, demonstrated similar oral potency to MPA in the uterine wet weight/mammary gland morphology assay in ovariectomized rats.

Introduction

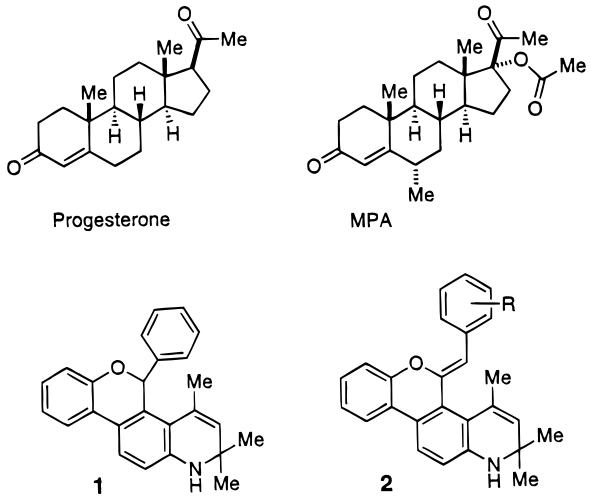
Progestins have been widely used in many therapeutic areas which include oral contraception,[1] hormone replacement therapy[2] (in combination with estrogens), and treatment of dysmenorrhea[3] and breast cancer.[4] Despite the structural, pharmacokinetic, and hormonal potency differences among the commercial progestins, they all share the common steroidal skeleton, which may exhibit various degrees of side effects due to cross-reactivities with other steroidal hormone receptors (androgen, glucocorticoid, mineralocorticoid, and estrogen) and other classes of receptors (e.g., GABA and glycine).[5] Development of cell-based high-throughput cotransfection assays affords opportunities to discover and optimize novel nonsteroidal selective progestins or progestins with a desired cross-reactivity profile.[6]

Recently, we reported the discovery of 5-aryl-1,2-dihydrochromeno[3,4-*f*]quinoline **1** as a novel pharmocophore and the development of a series of orally available potent human progesterone receptor (hPR) agonists.[7,8] On the basis of the result that the 5-aryl group was responsible for progestational activity of the dihydroquinoline **1**,[7] we continued to optimize the 5-substituent and discovered a novel class of potent progestins of general structure **2** with a 5-benzylidene functionality (Chart 1). These 5-benzylidene analogues exhibit high binding affinity to hPR-A (human A-subtype) and potent progestational activity in the cotransfection assay with high efficacy.

Chemistry

The first 5-benzylidene compound, **6** (LG120716), was isolated as a byproduct in a reaction to prepare 5-benzyl

Chart 1



compound **5** according to a previously reported procedure[7] in which a Grignard reagent was added to lactone **3** to give the cyclic hemiacetal **4** followed by reduction with triethylsilane in the presence of trifluoroacetic acid (Scheme 1). A modified procedure consisting of treatment of intermediate **4** directly with a catalytic amount of *p*-toluenesulfonic acid in dichloromethane at room temperature afforded **6** exclusively. The analogues **2** were prepared in excellent yield as a single *Z*-isomer[9] by a procedure similar to that of **6** (Scheme 2). The substituted benzyl Grignard reagent was prepared from a corresponding benzyl bromide or chloride, and it was found that diethyl ether is superior to THF as a solvent for benzyl Grignard formation. Unequivocal proof of the novel 5-benzylidene structure **2** was obtained by an X-ray single-crystal structure analysis of the HCl salt of **6** (Figure 1 and Supporting Information).

Results and Discussion

In the process of optimizing the original hPR modulator lead (**1**), we explored different substituents at C(5)

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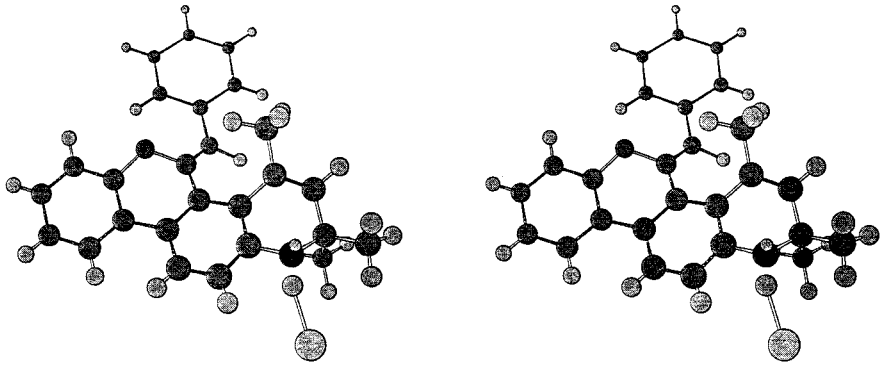
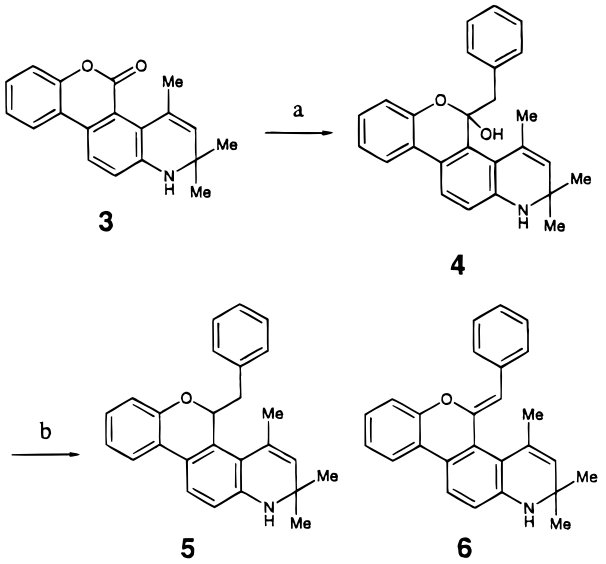


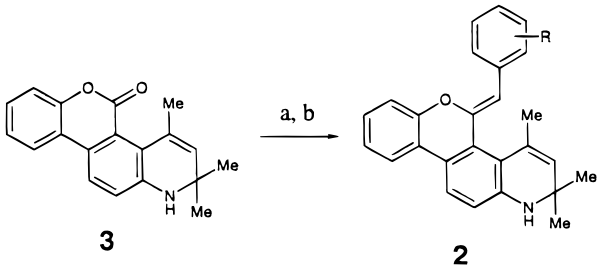
Figure 1. Structure of compound **6**·HCl as determined by X-ray diffraction.

Scheme 1[a]



[a] Reagents: (a) BnMgX, Et_2O or THF, rt; (b) Et_3SiH, TFA, CH_2Cl_2, rt.

Scheme 2[a]



[a] Reagents: (a) BnMgX, Et_2O or THF, rt; (b) pTsOH, CH_2Cl_2, rt.

of 1,2-dihydrochromeno[3,4-*f*]quinoline. The 5-benzyl analogue **5** significantly improved potency as a partial agonist compared to **1** in the hPR cotransfection assay. The 5-benzylidene compound **6**, isolated as an unexpected byproduct, showed great improvement over **1** in both potency and efficacy as a pure hPR agonist with binding affinity similar to that of progesterone. The potent biological activity and lack of chirality of compound **6** encouraged us to initiate a structure−activity relationship (SAR) study around the benzylidene ring system.

Table 1 summarizes the preliminary SAR results of compounds **2** in both cotransfection and competitive binding assays. A fluoro group on the benzylidene ring was well tolerated in all positions, and meta substitution generated the most active analogue, **8** (LG120744). In contrast to monofluoro analogues, *m*-bromo compound **11** was the least active among the three bromo analogues. Substitution of a chloro group at the meta or para position also gave less active compounds. Introduction of a methyl group at the ortho position (**16**, LG120838) generated one of the most potent compounds in this series; however, a methyl group at the meta or para positions decreased or eliminated agonist activity. The 3-methoxy analogue (**19**) showed no agonist activity but moderate antagonist activity with weak binding affinity. Ortho substitution was optimal for hPR agonist activity.

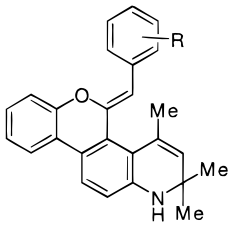
To explore disubstitution patterns, all the combinations of difluoro 5-benzylidene compounds were tested, and 2,5- and 3,4-disubstitution (**22** and **24**) provided the most active analogues in the difluoro series.

A group of representative hPR agonists (**6**, **8**, **16**, **22**, and **24**) were tested in other steroidal hormone receptor assays to profile their cross-reactivity. They demonstrated better hPR selectivities over human androgen receptor (hAR) and human glucocorticoid receptor (hGR) than did progesterone and mehydroxyprogesterone acetate (MPA) in the competitive binding assay (Table 2) and showed no agonist activity but weak antagonist activity in some of the hAR, hGR, and hMR cotransfection assays (Table 3).

Inhibitory effects of compound **8** and MPA on estrogen-induced uterine wet weight and stimulation of mammary alveolar bud formation were evaluated to access in vivo activity of the new progestins according to a previously described method.[7] MPA significantly increased ($p < 0.05$) rat mammary gland lobular bud formation over vehicle or estrone (E) alone after 3 days of oral administration at 1.0 or 3.0 mg/rat (Figure 2). Compound **8** was able to increase lobular bud formation greater than 2-fold over E-treated animals and demonstrated better potency and efficacy than MPA in this experiment. Compound **8** was also effective in decreasing E-induced uterine wet weight in the same test animals with similar potency and efficacy to MPA (Figure 3).

In summary, the new nonsteroidal hPR agonists exhibited potent progestational activity in both binding and cotransfection assays, and a representative analogue **8** demonstrated similar oral potency to MPA in the uterine wet weight/mammary gland morphology assay in ovariectomized rats. The nonchiral 5-benzylidenechromenoquinoline skeleton (**2**) provides a chemi-

Table 1. Cotransfection and Competitive Binding Data for the Quinoline Analogues[a]



		hPR agonist[b]		hPR antagonist[b]		hPR-A binding
compd	R	eff (%)	EC_{50} (nM)[c]	eff (%)	IC_{50} (nM)[c]	K_i (nM)
progesterone		100	2.9 ± 0.9	–[d]	–	3.5 ± 0.2
MPA		80 ± 7	0.15 ± 0.05	–	–	0.34 ± 0.04
1	(5-aryl)	42 ± 10	156 ± 71	75 ± 4	290 ± 121	3.6 ± 0.7
5	(benzyl)	49.6 ± 7.0	16.8 ± 10.4	32 ± 11*	270 ± 240*	2.1 ± 0.3
6	H	126 ± 24	24.4 ± 12.7	–	–	4.9 ± 1.9
7	2-F	98 ± 21	29.0 ± 11.3	–	–	5.6 ± 2.6
8	3-F	132 ± 17	7.6 ± 3.7	–	–	0.83 ± 0.04
9	4-F	150 ± 21*	59.5 ± 33.2*	–	–	3.5 ± 2.2
10	2-Br	127 ± 25	57.8 ± 22.5	–	–	5.5 ± 2.3
11	3-Br	59 ± 5	37 ± 10	–	–	22.1 ± 4.8
12	4-Br	114 ± 14	31.5 ± 15.7	–	–	8.7 ± 2.5
13	2-Cl	127 ± 25	57.8 ± 22.5	–	–	1.3 ± 0.6
14	3-Cl	83 ± 12	117 ± 89	–	–	18.7 ± 4.1
15	4-Cl	22 ± 3	139 ± 88	59	880	60.0 ± 7.6
16	2-Me	166 ± 35	5.7 ± 3.7	–	–	0.66 ± 0.20
17	3-Me	31 ± 13	75 ± 19	–	–	39.7 ± 14.6
18	4-Me	–	–	42	590	70.0 ± 2.5
19	3-OMe	–	–	70 ± 5	425 ± 159	53.3 ± 10.3
20	2,3-di-F	25 ± 12	26.3 ± 2.2	51	531	24.3 ± 7.8
21	2,4-di-F	72 ± 24	22.0 ± 5.6	42	550	3.2
22	2,5-di-F	137 ± 13	14.7 ± 8.4	–	–	1.5 ± 0.6
23	2,6-di-F	23 ± 5	32 ± 10	71	220	31.3 ± 6.5
24	3,4-di-F	143 ± 20	14.7 ± 6.0	–	–	3.6 ± 1.1
25	3,5-di-F	90 ± 13	47.7 ± 29.1	–	–	12.6 ± 2.6

[a] Values with standard errors (SEM) represent the mean value of at least three separate experiments with triplicate determinations, values without standard deviation represent a single experiment, and values with an asterisk represent the mean value of two experiments with standard deviation. [b] Agonist efficacies were compared to that of progesterone (100%), and antagonist efficacies were determined as a function (%) of maximal inhibition of progesterone at the EC_{50} value. [c] All EC_{50} and IC_{50} values were determined from full dose–response curves ranging from 10^{-12} to 10^{-5} M in CV-1 cell. [d] A hyphen indicates an efficacy of <20% and a potency of >10000 nM.

Table 2. Competitive Binding Data of 5-Benzylidene Analogues with hPR, hAR, hGR[a]

compd	hPR K_i (nM)	hAR K_i (nM)	hGR K_i (nM)
progesterone	3.5 ± 0.2	8.5 ± 3.1	30.5 ± 1.9
MPA	0.34 ± 0.04	2.9 ± 0.2	13.2 ± 1.8
6	4.9 ± 1.9	2660 ± 1370	299 ± 101
8	0.83 ± 0.04	798 ± 206	180 ± 76
16	0.66 ± 0.20	844 ± 90	564 ± 187
22	1.5 ± 0.6	887 ± 384	173 ± 56
24	3.6 ± 1.1	>1000	237

[a] Values with standard errors (SEM) represent the mean value of at least three separate experiments on receptor expressed in SF_{12} cells in a baculovirus expression system.

cal advantage over the 5-aryl skeleton (**1**) and offers opportunities for further optimization.

Experimental Section

General experimental methods have been previously described,[7] and reported yields are not optimized. ^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker AC400 spectrometer at 400 and 100 MHz, respectively. All *J* are in hertz.

(*R*/*S*)-5-Benzyl-1,2-dihydro-2,2,4-trimethyl-5*H*-chromeno-[3,4-*f*]quinoline (5). To a solution of lactone **3** (60 mg, 0.21 mmol) in dry THF (5 mL) at 0 °C was added a 2.0 M solution of benzylmagnesium chloride in THF (0.52 mL). After 15 h, TLC showed no starting material. The reaction mixture was poured into a saturated NH_4Cl solution (5 mL) and extracted with EtOAc (10 mL). The extract was washed with water (3 × 5 mL) and brine (3 × 5 mL), dried (Na_2SO_4), and concentrated. The crude product was purified by preparatory TLC to afford the lactol **4** as a yellow oil. To a solution of the lactol **4** (50 mg, 0.13 mmol) and CH_2Cl_2 (5 mL) at − 78 °C was added Et_3SiH (0.34 mL, 2.1 mmol) and TFA (0.16 mL, 2.1 mmol). The reaction mixture was allowed to warm to room temperature. After 4 h, TLC showed no starting material. The reaction mixture was quenched with saturated $NaHCO_3$ (3 mL) and then extracted with EtOAc (10 mL). The organic layer was washed with water (3 × 5 mL) and brine (3 × 5 mL), dried (Na_2SO_4), and concentrated. The crude product was purified by prepatory TLC to afford 22 mg (30%) of **5** as a yellow oil: ^{1}H NMR ($CDCl_3$) 7.66 (d, J = 8.0, H_{10}), 7.48 (d, J = 8.4, H_{11}), 7.31−7.20 (m, $H_{2'}$, $H_{3'}$, $H_{4'}$, $H_{5'}$, $H_{6'}$, H_8), 7.02 (t, J = 8.0, H_9), 6.89 (d, J = 8.0, H_7), 6.61 (d, J = 8.4, H_{12}), 6.13 (dd, J = 10.2, 3.4, H_5), 5.49 (s, H_3), 3.92 (br s, NH), 3.12 (dd, J = 14.6, 10.2, H_{5a}), 2.73 (dd, J = 14.6, 3.4, H_{5a}), 2.31 (s, 3 × H_{4a}), 1.29 (s, 3 × H_{2aa}), 1.19 (s, 3 × H_{2ab}). Anal. ($C_{26}H_{25}NO\cdot{}^1/_2H_2O$) C, H, N.

General Procedure for Preparing 5-Benzylidene Compounds 2 from Lactone 3. To a flame-dried flask equipped with a condenser, dropping funnel, and magnetic stir bar was charged magnesium turnings, a catalytic amount of 1,2-dibromoethane, and Et_2O (2 mL). To this mixture was charged one-fourth of a solution of the corresponding benzyl bromide or chloride (0.5−2.0 M). After initiation, the benzyl bromide or chloride solution was charged in at a rate sufficient to maintain a gentle reflux. The reaction was stirred for 10 min after the addition was complete. The Grignard solution (3−5 equiv) was then cannulated into a THF solution (0.2−1.0 M) of lactone **3**. The reaction mixture was stirrred for 45 min

Table 3. Antagonist Cross-Reactivities with hAR, hGR, hER, hMR[a]

	hAR		hGR		hMR		hER	
compd	eff (%)	IC_{50} (nM)[b]	eff (%)	IC_{50} (nM)[c]	eff (%)	IC_{50} (nM)[c]	eff (%)	IC_{50} (nM)[c]
prog	46 ± 7	37 ± 2	39 ± 8	>1000	83 ± 6	14 ± 4	–[d]	–
MPA	159 ± 10[e]	6.1 ± 1.0[e]	157 ± 22[e]	10 ± 1[e]	67 ± 10	1197 ± 852	46 ± 5[e]	924 ± 203[e]
6	81 ± 1	650 ± 390	74 ± 11	737 ± 319	72 ± 3	3000 ± 500	–	–
8	80 ± 6	795 ± 177	95 ± 4	458 ± 209	–	–	–	–
16	70 ± 5	1062 ± 410	76 ± 23	1050 ± 494	–	–	–	–
22	77 ± 3	1600 ± 100	98 ± 2	371 ± 98	–	–	–	–
24	70 ± 9	916 ± 275	80 ± 2*	1650 ± 212*	–	–	–	–

[a] Values with standard errors (SEM) represent the mean value of at least three separate experiments with triplicate determinations, and values with an asterisk represent the mean value of two experiments with standard deviation. [b] Antagonist efficacies were determined as a function (%) of maximal inhibition of an agonist. [c] All IC_{50} values were determined from full dose–response curves ranging from 10^{-12} to 10^{-5} M in CV-1 cell. [d] A hyphen indicates an efficacy of <20% and a potency of >10000 nM. [e] Agonist efficacy, EC_{50} (nM).

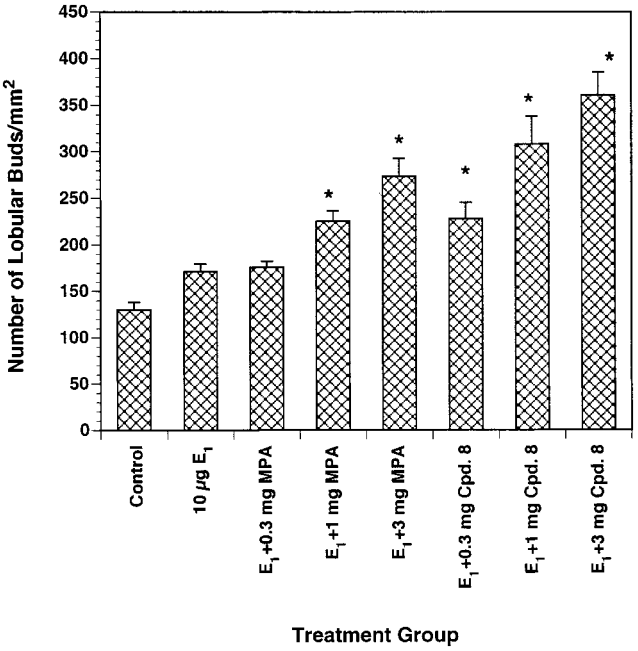


Figure 2. Stimulation of lobular alveolar bud formation in the ovariectomized rat by compound **8** or MPA in three different doses ($n = 4$ for all groups). All values represent the mean percent change ± SEM of lobular alveolar buds from animals treated with E alone (* indicates $p < 0.05$ vs E ANOVA).

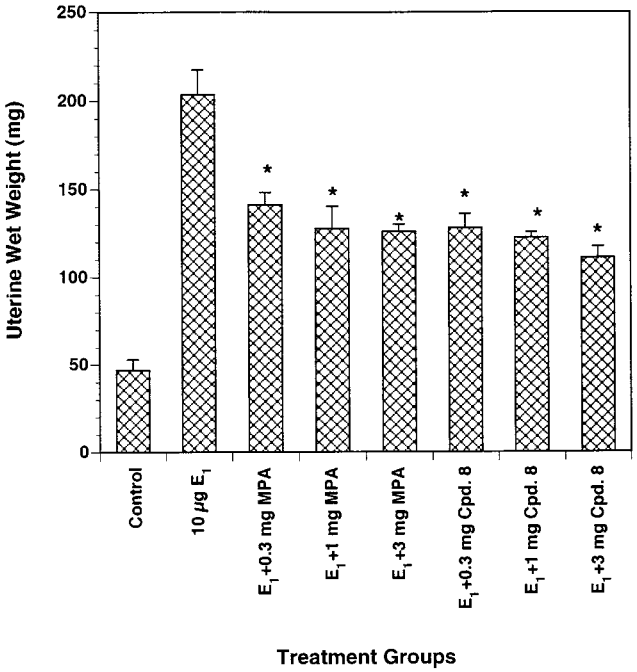


Figure 3. Inhibition of estrone-induced uterine wet weight in the ovariectomized rat by compound **8** or MPA in three different doses ($n = 4$ for all groups). All values represent the mean percent change ± SEM of uterine wet weight from animals treated with E alone (* indicates $p < 0.01$ vs E ANOVA).

and was poured into ice-cold 50% NH_4Cl and extracted with EtOAc (2×). The extracts were washed with brine (3×), dried (Na_2SO_4), and concentrated. A soluton (0.2–0.5 M) of the crude lactol in CH_2Cl_2 was treated with a catalytic amount of *p*-toluenesulfonic acid at room temperature for 3 h, quenched with saturated $NaHCO_3$, and extracted with EtOAc (2×). The extracts were washed with brine (3×), dried (Na_2SO_4), and concentrated. Flash chromatography (EtOAc–hexane 2–10% gradient) of the crude mixture afforded the final product as a yellow solid in good yield.

***(Z)*-5-Benzylidene-1,2-dihydro-2,2,4-trimethyl-5*H*-chromeno-[3,4-*f*]quinoline (6).** This compound was prepared by the general procedure from benzylmagnesium chloride (34 mL of a 1 M solution in Et_2O, 5 equiv) and lactone **3** (2.0 g, 6.9 mmol) to afford 1.7 g (68%) of **6** as a yellow solid: mp 49–50 °C; ^{1}H NMR (acetone-d_6) 7.82 (m, $H_{3'}$, $H_{7'}$, H_{10}), 7.64 (d, $J = 8.4$, H_{11}), 7.38 (m, H_7, H_8), 7.24–7.20 (m, $H_{4'}$, $H_{6'}$, H_9), 7.09–7.06 (m, $H_{5'}$), 6.84 (d, $J = 8.4$, H_{12}), 5.68 (s, H_{5a}), 5.55 (s, H_3), 2.11 (s, 3 × H_{4a}), 1.29 (br s, 6 × H_{2a}); ^{13}C NMR ($CDCl_3$) 152.2, 147.5, 145.7, 135.7, 132.2, 131.1, 129.0, 128.5, 127.8, 126.6, 122.6, 121.9, 121.6, 121.3, 121.1, 120.0, 116.4, 115.7, 115.2, 50.6, 21.3. Anal. ($C_{26}H_{23}NO$) C, H, N.

***(Z)*-5-(2-Fluorobenzylidene)-1,2-dihydro-2,2,4-trimethyl-5*H*-chromeno[3,4-*f*]quinoline (7).** This compound was prepared by the general procedure from 2-fluorobenzyl bromide (189 mg, 1.0 mmol) and **3** (20 mg, 0.069 mmol) in 36% yield as a bright yellow oil (10 mg): ^{1}H NMR (acetone-d_6) 8.39 (m, $H_{6'}$), 7.85 (d, $J = 7.4$, H_{10}), 7.67 (d, $J = 8.5$, H_{11}), 7.30–7.06 (m, H_7, H_8, H_9, $H_{3'}$, $H_{4'}$, $H_{5'}$), 6.86 (d, $J = 8.5$, H_{12}), 5.96 (s, H_{5a}), 5.90 (s, H_3), 5.55 (s, H_1), 2.13 (s, 3 × H_{4a}), and 1.32 (bs, 6 × H_{2a}). Anal. ($C_{26}H_{22}FNO$) C, H, N.

***(Z)*-5-(3-Fluorobenzylidene)-1,2-dihydro-2,2,4-trimethyl-5*H*-chromeno[3,4-*f*]quinoline (8).** This compound was prepared by the general procedure from 3-fluorobenzyl chloride (2.2 mL, 18 mmol, 10 equiv) and lactone **3** (0.52 g, 1.8 mmol) to afford 0.49 g (72%) of **8** as a yellow solid: mp 66–67 °C; ^{1}H NMR (acetone-d_6) 7.85 (d, $J = 7.9$, H_{10}), 7.66 (d, $J = 8.6$, H_{11}), 7.52 (d, $J = 7.6$, $H_{6'}$), 7.43–7.38 (m, $H_{5'}$), 7.11–7.07 (m, H_7, H_8), 7.02–6.97 (m, $H_{4'}$, H_9), 6.85 (d, $J = 8.6$, H_{12}), 5.70 (s, H_{5a}), 5.55 (s, H_3), 2.10 (s, 3 × H_{4a}), 1.29 (br s, 6 × H_{2a}); ^{13}C NMR ($CDCl_3$) 164.1, 161.7, 151.6, 148.4, 145.5, 137.6, 132.1, 130.7, 129.5, 127.7, 125.9, 124.5, 122.6, 121.7, 121.2, 120.9, 119.8, 116.1, 115.4, 115.1, 114.9, 114.06, 113.2, 112.9, 50.4, 21.0; IR (KBr) 3387 s, 2960 m, 2924 m, 1637 m, 1602 m, 1577 m, 1440 m. Anal. ($C_{26}H_{22}FNO$) C, H, N.

***(Z)*-5-(4-Fluorobenzylidene)-1,2-dihydro-2,2,4-trimethyl-5*H*-chromeno[3,4-*f*]quinoline (9).** This compound was prepared by the general procedure from 4-fluorobenzyl bromide (0.25 mL, 2.1 mmol, 30 equiv) and lactone **3** (20 mg, 0.07 mmol) to afford 22 mg (82%) of **9** as a yellow oil: ^{1}H NMR (acetone-d_6) 7.87–7.82 (m, $H_{2'}$, $H_{6'}$, H_{10}), 7.64 (d, $J = 8.4$, H_{11}), 7.22–

7.05 (m, $H_{3'}$, $H_{5'}$, H_7, H_8), 6.82 (d, $J = 8.4$, H_{12}), 5.85 (s, NH), 5.68 (s, H_{5a}), 5.54 (s, H_3), 2.10 (s, 3 × H_{4a}), 1.32 (br s, 6 × H_{2a}). Anal. ($C_{26}H_{22}FNO·H_2O$) C, H, N.

(*Z*)-5-(2-Bromobenzylidene)-1,2-dihydro-2,2,4-trimethyl-5*H*-chromeno[3,4-*f*]quinoline (10). This compound was prepared by the general procedure from 2-bromobenzyl bromide (0.25 g, 1.0 mmol) and lactone **3** (50 mg, 0.17 mmol) to afford 24 mg (32%) of **10** as a yellow oil: ^{1}H NMR (acetone-d_6) 8.44 (d, $J = 7.9$, H_{10}), 7.85 (d, $J = 7.9$, $H_{3'}$), 7.67 (d, $J = 8.2$, H_{11}), 7.64 (d, $J = 7.9$, $H_{6'}$), 7.45 (t, $J = 7.9$, $H_{4'}$), 7.23−7.07 (m, $H_{5'}$, H_7, H_8, H_9), 6.86 (d, $J = 8.2$, H_{12}), 6.20 (s, H_{5a}), 5.55 (s, H_3), 2.15 (s, 3 × H_{4a}), 1.29 (br s, 6 × H_{2a}). Anal. ($C_{26}H_{22}BrNO$) C, H, N.

(*Z*)-5-(3-Bromobenzylidene)-1,2-dihydro-2,2,4-trimethyl-5*H*-chromeno[3,4-*f*]quinoline (11). This compound was prepared by the general procedure from 3-bromobenzyl bromide (250 mg, 1.0 mmol) and **3** (15 mg, 0.05 mmol) in 98% yield as a bright yellow oil (21 mg): ^{1}H NMR (acetone-d_6) 8.03 (s, $H_{2'}$), 7.85 (d, $J = 7.9$, H_{10}), 7.78 (d, $J = 7.9$, $H_{4'}$), 7.66 (d, $J = 8.4$, H_{11}), 7.41−7.17 (m, H_7, H_8, $H_{5'}$, $H_{6'}$), 7.09 (t, $J = 7.9$, H_9), 6.85 (d, $J = 8.4$, H_{12}), 5.90 (bs, NH), 5.67 (s, H_{5a}), 5.55 (s, H_3), 2.10 (s, 3 × H_{4a}), 1.33 (bs, 6 × H_{2a}). Anal. ($C_{26}H_{22}BrNO$) C, H, N.

(*Z*)-5-(4-Bromobenzylidene)-1,2-dihydro-2,2,4-trimethyl-5*H*-chromeno[3,4-*f*]quinoline (12). This compound was prepared by the general procedure from 4-bromobenzyl bromide (250 mg, 1.0 mmol) and **3** (20 mg, 0.07 mmol) in 82% yield as a bright yellow oil (25 mg): ^{1}H NMR (acetone-d_6) 7.83 (d, $J = 8.4$, H_{10}), 7.77 (d, $J = 8.6$, $H_{3'}$, $H_{5'}$), 7.65 (d, $J = 8.3$, H_{11}), 7.55 (d, $J = 8.6$, $H_{2'}$, $H_{6'}$), 7.25 (t, $J = 8.4$, H_9), 7.12−7.09 (m, H_7, H_8), 6.84 (d, $J = 8.3$, H_{12}), 5.89 (bs, NH), 5.66 (s, H_{5a}), 5.55 (s, H_3), 2.09 (s, 3 × H_{4a}), 1.34 (bs, 6 × H_{2a}). Anal. ($C_{26}H_{22}$-BrNO) C, H, N.

(*Z*)-5-(2-Chlorobenzylidene)-1,2-dihydro-2,2,4-trimethyl-5*H*-chromeno[3,4-*f*]quinoline (13). This compound was prepared by the general procedure from 2-chlorobenzyl chloride (0.27 mL, 2.1 mmol, 30 equiv) and lactone **3** (20 mg, 0.07 mmol) to afford 7.8 mg (30%) of **13** as a yellow oil: ^{1}H NMR (acetone-d_6) 8.44 (d, $J = 7.9$, H_{10}), 7.85 (d, $J = 7.9$, $H_{3'}$), 7.67 (t, $J = 8.5$, H_{11}), 7.45−7.37 (m, $H_{6'}$), 7.25−7.21 (m, $H_{4'}$), 7.20−7.11 (m, $H_{5'}$, H_7, H_8, H_9), 6.86 (d, $J = 8.5$, H_{12}), 6.20 (s, H_{5a}), 5.55 (s, H_3), 2.15 (s, 3 × H_{4a}), 1.29 (br s, 6 × H_{2a}). Anal. ($C_{26}H_{22}ClNO$) C, H, N.

(*Z*)-5-(3-Chlorobenzylidene)-1,2-dihydro-2,2,4-trimethyl-5*H*-chromeno[3,4-*f*]quinoline (14). This compound was prepared by the general method from 3-methylbenzyl bromide (0.25 mL, 2.1 mmol, 30 equiv) and lactone **3** (20 mg, 0.07 mmol) to afford 22 mg (82%) of **14** as a yellow oil: ^{1}H NMR (acetone-d_6) 7.87−7.82 (m, $H_{2'}$, H_{10}, $H_{4'}$), 7.64 (d, $J = 8.4$, H_{11}), 7.22−7.05 (m, $H_{5'}$, $H_{6'}$, H_7, H_8, H_9), 6.82 (d, $J = 8.4$, H_{12}), 5.68 (s, H_{5a}), 5.54 (s, H_3), 2.10 (s, 3 × H_{4a}), 1.32 (br s, 6 × H_{2a}). Anal. ($C_{26}H_{22}ClNO$) C, H, N.

(*Z*)-5-(4-Chlorobenzylidene)-1,2-dihydro-2,2,4-trimethyl-5*H*-chromeno[3,4-*f*]quinoline (15). This compound was prepared by the general method from 4-chlorobenzyl bromide (0.34 g, 2.1 mmol, 30 equiv) and lactone **3** (20 mg, 0.07 mmol) to afford 10 mg (35%) of **15** as a yellow oil: ^{1}H NMR (acetone-d_6) 7.82 (m, H_{10}, $H_{3'}$, $H_{5'}$), 7.65 (d, $J = 8.5$, H_{11}), 7.39 (m, $H_{2'}$, $H_{6'}$, H_9), 7.11 (m, H_7, H_8), 6.84 (d, $J = 8.5$, H_{12}), 5.68 (s, H_{5a}), 5.55 (s, H_3), 2.09 (s, 3 × H_{4a}), 1.33 (br s, 6 × H_{2a}); ^{13}C NMR ($CDCl_3$) 147.8, 145.5, 134.0, 132.1, 131.8, 129.9, 128.4, 127.7, 122.5, 121.8, 121.3, 120.9, 116.1, 115.3, 114.1, 77.3, 77.2, 77.0, 76.7, 50.3, 21.0. Anal. ($C_{26}H_{22}ClNO·^3/_4H_2O$) C, H, N.

(*Z*)-1,2-Dihydro-5-(2-methylbenzylidene)-2,2,4-trimethyl-5*H*-chromeno[3,4-*f*]quinoline (16). This compound was prepared by the general procedure from α-chloro-*o*-xylene (0.20 mL, 1.6 mmol, 30 equiv) and lactone **3** (15 mg, 0.05 mmol) to afford 15 mg (76%) of **16** as a yellow oil: ^{1}H NMR (acetone-d_6) 8.22 (d, $J = 8.5$, H_{10}), 7.82 (d, $J = 8.5$, $H_{3'}$), 7.64 (d, $J = 8.5$, H_{11}), 7.26−7.04 (m, $H_{4'}$, $H_{5'}$, $H_{6'}$, H_7, H_8, H_9), 6.83 (d, $J = 8.5$, H_{12}), 5.94 (s, H_{5a}), 5.54 (s, H_3), 2.28 (s, 3 × $H_{2'a}$), 2.15 (s, 3 × H_{4a}), 1.25 (br s, 6 × H_{2a}); ^{13}C NMR ($CDCl_3$) 152.2, 147.3, 145.7, 136.2, 134.1, 132.0, 131.4, 130.3, 129.1, 127.8, 127.2, 126.7, 126.0, 122.5, 122.4, 121.8, 121.6, 121.3, 121.0, 120.2, 116.6, 115.2, 113.1, 60.6, 50.6, 21.4, 20.4; IR (CCl_4) 3377 s, 2958 m, 2924 m, 2860 m, 1633 m, 1589 m, 1579 m, 1568 m. Anal. ($C_{27}H_{25}NO$) C, H, N.

(*Z*)-1,2-Dihydro-5-(3-methylbenzylidene)-2,2,4-trimethyl-5*H*-chromeno[3,4-*f*]quinoline (17). This compound was prepared by the general procedure from 3-methylbenzyl bromide (0.35 mL, 2.6 mmol, 30 equiv) and lactone **3** (25 mg, 0.09 mmol) to afford 14 mg (40%) of **17** as a yellow oil: ^{1}H NMR (acetone-d_6) 7.83 (d, $J = 7.8$, H_{10}), 7.62−7.66 (m, H_7, H_{11}), 7.60 (s, $H_{2'}$), 7.26 (t, $J = 7.8$, $H_{4'}$), 7.21 (m, $H_{5'}$, H_8), 7.04−7.09 (m, $H_{6'}$, H_9), 6.82 (d, $J = 8.3$, H_{12}), 5.65 (s, H_{5a}), 5.54 (s, H_3), 2.34 (s, 3 × $H_{3'a}$), 2.11 (s, 3 × H_{4a}), 1.32 (br s, 6 × H_{2a}). Anal. ($C_{27}H_{25}NO·H_2O$) C, H, N.

(*Z*)-1,2-Dihydro-5-(4-methylbenzylidene)-2,2,4-trimethyl-5*H*-chromeno[3,4-*f*]quinoline (18). This compound was prepared by the general method from 4-methylbenzyl chloride (0.41 mL, 3.1 mmol, 30 equiv) and lactone **3** (30 mg, 0.10 mmol) to afford 19 mg (48%) of **18** as a yellow oil: ^{1}H NMR ($CDCl_3$) 7.86 (m, H_{10}, $H_{3'}$, $H_{5'}$), 7.50 (d, $J = 8.3$, H_{11}), 7.14−7.19 (m, $H_{2'}$, $H_{6'}$, H_7, H_9), 7.02 (t, $J = 8.2$, H_8), 6.65 (d, $J = 8.3$, H_{12}), 5.59 (s, H_{5a}), 5.52 (s, H_3), 4.41 (br s, NH), 2.36 (s, 3 × $H_{4'a}$), 2.12 (s, 3 × H_{4a}), 1.34 (br s, 6 × H_{2a}); ^{13}C NMR ($CDCl_3$) 152.1, 146.6, 145.5, 136.2, 132.7, 131.9, 130.9, 129.0, 128.7, 127.6, 126.7, 122.3, 121.7, 121.5, 121.2, 120.8, 119.7, 116.1, 115.6, 114.9, 77.3, 77.0, 76.7, 50.3, 21.3, 21.0. Anal. ($C_{27}H_{25}NO$) C, H, N.

(*Z*)-1,2-Dihydro-5-(3-methoxybenzylidene)-2,2,4-trimethyl-5*H*-chromeno[3,4-*f*]quinoline (19). This compound was prepared by the general method from 3-methoxybenzyl chloride (0.30 mL, 2.1 mmol, 30 equiv) and lactone **3** (20 mg, 0.07 mmol) to afford 9.0 mg (36%) of **19** as a yellow oil: ^{1}H NMR ($CDCl_3$) 7.70 (d, $J = 8.0$, H_{10}), 7.52 (d, $J = 8.3$, H_{11}), 7.49 (s, $H_{2'}$), 7.26 (m, H_7, $H_{4'}$), 7.18−7.01 (m, $H_{5'}$, $H_{6'}$), 6.80−6.77 (m, H_8, H_9), 6.88 (d, $J = 8.3$, H_{12}), 5.60 (s, H_{5a}), 5.52 (s, H_3), 4.13 (br s, NH), 3.89 (s, OCH_3), 2.12 (s, 3 × H_{4a}), 1.34 (br s, 6 × H_{2a}); ^{13}C NMR ($CDCl_3$)159.1, 152.8, 147.7, 145.5, 136.6, 132.0, 130.7, 129.2, 127.9, 126.2, 122.5, 122.0, 121.3, 120.0, 116.1, 115.2, 115.1, 113.3, 112.2, 55.2, 50.1, 21.0. Anal. ($C_{27}H_{25}NO_2$) C, H, N.

(*Z*)-5-(2,3-Difluorobenzylidene)-1, 2-dihydro-2,2,4-trimethyl-5*H*-chromeno[3,4-*f*]quinoline (20). This compound was prepared by the general procedure from 2,3-difluorobenzyl bromide (207 mg, 1.0 mmol) and **3** (30 mg, 0.10 mmol) in 40% yield as a bright yellow oil (16 mg): ^{1}H NMR (acetone-d_6) 8.18 (dd, $J = 8.0$ and 6.6, $H_{6'}$), 7.87 (d, $J = 7.5$, H_{10}), 7.69 (d, $J = 8.5$, H_{11}), 7.30−7.08 (m, H_7, H_8, H_9, $H_{4'}$, $H_{5'}$), 6.89 (d, $J = 8.5$, H_{12}), 5.94 (s, H_{5a}), 5.57 (s, H_3), 2.12 (s, 3 × H_{4a}), 1.31 (bs, 6 × H_{2a}). Anal. ($C_{26}H_{21}F_2NO$) C, H, N.

(*Z*)-5-(2,4-Difluorobenzylidene)-1,2-dihydro-2,2,4-trimethyl-5*H*-chromeno[3,4-*f*]quinoline (21). This compound was prepared by the general method from 2,4-difluorobenzyl bromide (0.27 mL, 2.1 mmol, 30 equiv) and lactone **3** (20 mg, 0.07 mmol) to afford 16 mg (56%) of **21** as a yellow oil in two steps: ^{1}H NMR (acetone-d_6) 8.43 (m, $H_{6'}$), 7.86 (d, $J = 8.5$, H_{10}), 7.67 (d, $J = 8.5$, H_{11}), 7.43−7.03 (m, $H_{3'}$, $H_{5'}$, H_7, H_8, H_9), 6.86 (d, $J = 8.5$, H_{12}), 5.88 (s, H_{5a}), 5.55 (s, H_3), 2.11 (s, 3 × H_{4a}), 1.29 (br s, 6 × H_{2a}). Anal. ($C_{26}H_{21}F_2NO$) C, H, N.

(*Z*)-5-(2,5-Difluorobenzylidene)-1, 2-dihydro-2,2,4-trimethyl-5*H*-chromeno[3,4-*f*]quinoline (22). This compound was prepared by the general procedure from 2,5-difluorobenzyl bromide (207 mg, 1.0 mmol) and **3** (15 mg, 0.05 mmol) in 82% yield as a bright yellow oil (17 mg): ^{1}H NMR (acetone-d_6) 8.12 (m, $H_{6'}$), 7.88 (d, $J = 8.3$, H_{10}), 7.69 (d, $J = 8.4$, H_{11}), 7.30−7.00 (m, H_7, H_8, H_9, $H_{3'}$, $H_{4'}$), 6.89 (d, $J = 8.4$, H_{12}), 5.93 (bs, NH), 5.94 (s, H_{5a}), 5.56 (s, H_3), 2.11 (s, 3 × H_{4a}), 1.32 (bs, 6 × H_{2a}); ^{13}C NMR (acetone-d_6) 160.7, 158.0, 151.9, 151.2, 147.7, 133.2, 133.1, 130.7, 128.5, 125.9, 123.9, 122.8, 122.0, 120.6, 119.7, 117.1, 116.9, 116.8, 116.7, 116.2, 115.69, 114.9, 114.6, 104.9, 50.8, 50.7, 21.4. Anal. ($C_{26}H_{21}F_2NO$) C, H, N.

(*Z*)-5-(2,6-Difluorobenzylidene)-1,2-dihydro-2,2,4-trimethyl-5*H*-chromeno[3,4-*f*]quinoline (23). This compound was prepared by the general method from α-bromo-2,6-difluorotoluene (0.43 g, 2.1 mmol) and lactone **3** (50 mg, 0.17 mmol) to afford 25 mg (37%) of **23** as a yellow oil: ^{1}H NMR

(acetone-d_6) 7.83 (d, $J = 8.5$, H_{10}), 7.68 (d, $J = 8.5$, H_{11}), 7.35−7.04 (m, H_8, H_9, $H_{3'}$, $H_{4'}$, $H_{5'}$), 6.90 (d, $J = 8.5$, H_7), 6.87 (d, $J = 8.5$, H_{12}), 5.61 (s, H_{5a}), 5.57 (s, H_3), 2.23 (s, 3 × H_{4a}), 1.32 (br s, 6 × H_{2a}). Anal. ($C_{26}H_{21}F_2NO$) C, H, N.

(*Z*)-5-(3,4-Difluorobenzylidene)-1,2-dihydro-2,2,4-trimethyl-5*H*-chromeno[3,4-*f*]quinoline (24). This compound was prepared by the general method from 3,4−difluorobenzyl bromide (0.27 mL, 2.1 mmol, 30 equiv) and lactone **3** (20 mg, 0.07 mmol) to afford 20 mg (70%) of **24** as a yellow oil: ^{1}H NMR (acetone-d_6) 7.83 (m, $H_{6'}$, H_{10}), 7.66 (d, $J = 8.5$, H_{11}), 7.55−7.10 (m, $H_{2'}$, H_7, H_8, H_9, $H_{5'}$), 6.85 (d, $J = 8.5$, H_{12}), 5.67 (s, H_{5a}), 5.55 (s, H_3), 2.08 (s, 3 × H_{4a}), 1.28 (br s, 6 × H_{2a}). Anal. ($C_{26}H_{21}F_2NO$) C, H, N.

(*Z*)-5-(3,5-Difluorobenzylidene)-1,2-dihydro-2,2,4-trimethyl-5*H*-chromeno[3,4-*f*]quinoline (25). This compound was prepared by the general procedure from α-bromo-3,5-difluorotoluene (0.27 mL, 2.1 mmol, 30 equiv) and lactone **3** (20 mg, 0.07 mmol) to afford 16 mg (56%) of **25** as a yellow oil: ^{1}H NMR (acetone-d_6) 7.87 (d, $J = 7.8$, H_{10}), 7.69 (d, $J = 8.5$, H_{11}), 7.43−7.09 (m, $H_{2'}$, $H_{4'}$, $H_{6'}$, H_7, H_9), 6.89−6.84 (m, H_8, H_{12}), 5.71 (s, H_{5a}), 5.56 (s, H_3), 2.09 (s, 3 × H_{4a}), 1.33 (br s, 6 × H_{2a}). Anal. ($C_{26}H_{21}F_2NO$) C, H, N.

Cotransfection Assays. The function and detailed preparation procedure of the cotransfection assays have been described previously.[10] The agonist activity was determined by examining the LUC expression (normalized response), and the efficacy readout was a relative value to the maximal LUC expression produced by a reference agonist, e.g., progesterone for hPR, dihydrotestosterone (DHT) for hAR, dexamethasone for hGR, aldosterone for hMR, estradiol for hER. All the cotransfection experiments were carried out in 96-well plates by automation (Beckman Biomomek automated workstation).

Receptor Binding Assays. The preparation of receptor binding assays for hPR-A, hGR, and hAR were described previously,[10] and the radioligands used in the competative binding assays are progesterone for hPR-A, DHT for hAR, and dexamethasone for hGR.

Material and Methods for Mammary Gland Morphology/Uterine Wet Weight Assay in the Ovariectomized Rat. The assay has been described previously[7] by a modification of literature methods.[11−13]

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Supporting Information Available: Data from the X-ray structure determination of compound **6** (4 pages). Ordering information is given on any current masthead page.

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