Discovery of Potent, Nonsteroidal, and Highly Selective Glucocorticoid Receptor Antagonists

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An approach to the computer-assisted, pharmacophore design of nonsteroidal templates for the glucocorticoid receptor (GR) that contained an element of pseudo-C2 symmetry was developed. The enatiomer of the initial design, **1Ra**, and not the designed molecule, **1S**, showed the desired ligand binding to the GR. The pseudo-C2 symmetry of the template allowed for rapid improvements in GR activity resulting in potent, selective, nonsteroidal GR antagonists, **CP-394531** and **CP-409069**.

Introduction

Glucocorticoid receptors (GRs), members of the steroid−thyroid−retinoid superfamily, are soluble, intracellular receptor proteins that act as ligand-regulated transcription factors controlling specific gene expression in most mammalian cells.[1−7] Glucocorticoids bind to and activate GRs, and they play a crucial role in the normal development and maintenance of basal and stress-related homeostasis.[8] The steroid nucleus of typical glucocorticoids is associated with a rich variety of hormonal activities and has inspired the development of an assortment of therapeutic agents.[9,10] Steroid hormones have evolved in nature to be short acting; therefore, the design of therapeutic agents based on the steroid skeleton can be plagued with several problems, including rapid clearance. Nonsteroidal ligands[11] have been developed for the estrogen (ER),[12−16] progesterone (PR),[17−20] and androgen receptor (AR).[21,22] However, there are relatively few literature reports of selective, nonsteroidal GR agonists or antagonists.[23] Our efforts have focused on the rational design of novel, potent, and selective nonsteroidal GR antagonists for the possible treatment of a variety of disorders, including diabetes,[24−27] obesity,[28,29] depression,[30−33] neurodegeneration,[34−37] glaucoma,[38,39] and Cushing's disease.[40,41]

A potentially general method to simplify the design of ligands for steroid receptors may be to begin with templates that contain a C2 or pseudo-C2 symmetric axis. For the estrogen receptor, pseudo-C2 symmetry may play a role in the binding orientation of the chosen ligands.[42,43] We recently developed an approach to pharmacophore design for nonsteroidal ligands for the GR that contained an element of pseudo-C2 symmetry. To control the possible involvement of this symmetry element, we prepared not only the "correct" enantiomer of our designed template **1S** but also its enantiomer **1R**. Interestingly, **1Rb** (K_i = 157 nM) and not **1S** (K_i > 10 μM) displayed the desired ligand binding to the GR. This result was rationalized by the pseudo-C2 symmetry of the template, and this concept led to rapid improvements in receptor-binding affinity using an analogous design strategy. This communication reports the design, synthesis, and evaluation of potent, selective, nonsteroidal GR antagonists, **CP-394531** and **CP-409069**.

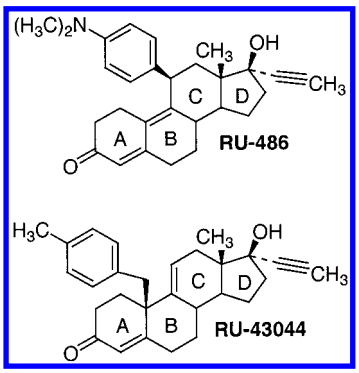

Figure 1. RU-486 and **RU-43044**.

Modeling

A three-dimensional pharmacophore model, based on steroidal antagonists **RU-43044** (selective) and **RU-486** (nonselective),[44−48] was developed for the design of selective nonsteroidal GR ligands (Figures 1 and 2). The initial tricyclic compounds (**1S**) dramatically simplified the steroid skeleton, kept the A and B rings and the angular benzyl pharmocophore of the steroid intact, and positioned a vector from the phenoxyl moiety of the C-ring that could occupy aspace similar to that of the third pharmacophore of interest (Figure 3). For most steroidal ligands, an A-ring is important for potent and specific binding to the receptor. A second pharmacophore, the C19-angular benzyl substituent of **RU-43044**, is important for GR selectivity and antagonism. From steroidal SAR,[49] the third pharmacophore, the alkyne on the D-ring of **RU-43044**, appears to be key for tight-binding compounds, because large differences in potency can be observed with relatively subtle changes in structure. Because the A-ring and the C19-angular benzyl substituent are important for GR binding, antagonism, and selectivity, the initial design (**1S**) focused on positioning these two pharmacophores in the proper

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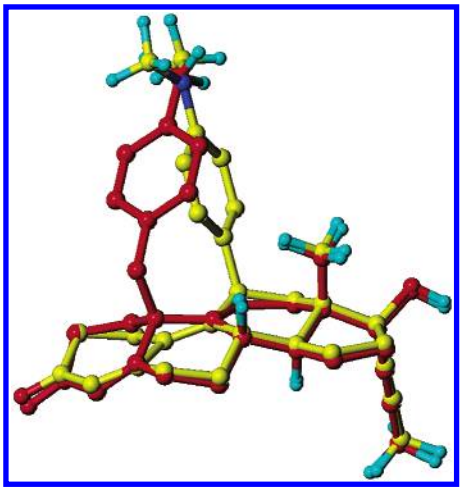


Figure 2. Overlap of **RU-486** (yellow) and **RU-43044** (red); the differences in the angular benzyl substituent of **RU-43044** and the angular phenyl substituent of **RU-486** are believed to impart GR selectivity to **RU-43044**.[44−48]

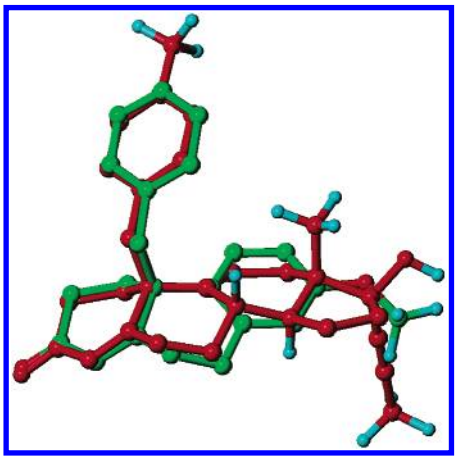


Figure 3. Overlap of **RU-43044** (red) and **1Sa** (green).

binding orientation. The third pharmacophore, the substituent attached to the D-ring, could then be used to increase the GR receptor affinity. This third pharmacophore could easily be varied by alkylation of the C-ring phenoxide. Finally, both enantiomers of **1** (**R** and **S**) were synthesized so that **1R** could serve as a control for the testing of **1S** which possesses the natural steroid configuration in the A- and B-rings.

Chemistry

Alkylation of the eneamine of 6-methoxy-2-tetralone[50] with benzyl bromide followed by hydrolysis and purification resulted in an 85% yield of 1-benzyl-6-methoxy-2-tetralone (**2**, Scheme 1).[51] Treatment of **2** with either (*R*)-(+)-α-methylbenylamine or (*S*)-(−)-α-methylbenzylamine with the azeotropic removal of water followed by treatment with methyl vinyl ketone and hydrolysis resulted in the bridged bicyclic ketols **3S** and **3R**, respectively. Treatment of **3S** and **3R** with sodium methoxide in methanol resulted in the initial targets of **1Sa** and **1Ra** with overall yields of 48 and 33%, respectively.[52−56] Compounds **1Sa** and **1Ra** were obtained in >98% ee after a single recrystallization.[57] Cleavage of the methyl ethers was accomplished by the treatment of **1Sa** and **1Ra** with tetrabutylammonium iodide and boron trichloride in dichloromethane to form **1Sb** and **1Rb** in 87 and 75% yields, respectively.[58] Compound **1Rb** was treated with lithium metal in liquid ammonia in dioxane and ether at −78 °C to form the trans Decalin **4** in 81% yield after purification.[59] Addition of either lithium chloroethyne and lithium propyne to **4** afforded **CP-394531** or **CP-409069** in 56 and 69% yields, respectively. The relative and absolute stereochemistry of **CP-394531** was confirmed by X-ray crystallography.[60]

Biology

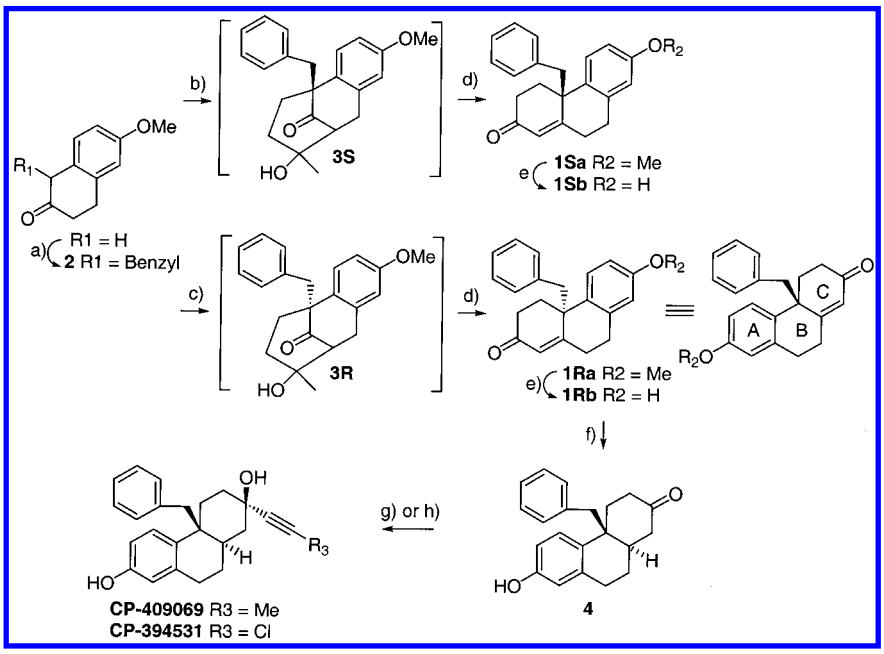
The human GR was overexpressed from baculovirus cells and isolated as a protein extract. Binding affinity to the human GR was determined by the displacement of 10 nM radiolabeled dexamethasone, a known GR agonist, by the test compounds. To assess binding selectivity, the binding affinity versus human PR, ERα, ERβ, and AR was determined by the displacement of the appropriate radiolabeled ligand by the test compounds (Table 1). Compounds **4**, **CP-395531**, and **CP-409069** displayed very potent and selective binding to the GR over the other steroid receptors that were assayed.

To assess agonism versus antagonism and the functional selectivity of GRs versus other closely related steroid receptors (e.g., PR and MR), human cell lines containing endogenous steroid receptors (SW1352-GR, T47D-GR, MR, and PR) were identified and characterized. Glucocorticoid receptor antagonist/agonist activity was determined in SW1353/MMTV-8 cells in the presence and absence of a half-maximal concentration of dexamethasone (40 nM). Selectivity for the GR versus the MR and PR was performed in transiently transfected T47D cells. In all cases, the compounds were tested alone for agonist activity and in the presence of the appropriate ligand for antagonist activity. None of the new, reported compounds displayed functional agonism above basal levels at concentrations up to 1 μM at the GR, MR, or PR under the assay conditions. **CP-409069** and **CP-394531** completely blocked (100%) the half-maximal agonistic effects of dexamethasone (40 nM) at concentrations ≥1 μM. Antagonism data for the GR, MR, and PR are reported in Table 2. **CP-395531** and **CP-409069** displayed very potent, complete, and selective antagonism to the GR over the other steroid receptors that were assayed.

Discussion

The enantiomeric phenol **1Rb** (K_i = 157 nM), included as a control, and not **1Sa** (K_i > 10 μM) displayed the desired ligand binding to the GR (Table 1). Stereoisomers **1S** and **1R** contain a pseudo-C2 symmetric axis. That is, **1Rb** may bind to the receptor with the phenol acting as the A-ring. From this orientation, the angular benzyl substituent can still occupy a space similar to that envisioned for it in the 3-D pharmacophore model (Figure 4). Addition of the third pharmacophore, intended to improve the GR potency, was now planned from a different site. By molecular modeling, an axial propyne moiety on the C-ring occupies a space similar to that of the propyne of **RU-43044** or **RU-486**. Moreover, an equatorial hydroxyl could participate in hydrogen-bonding interactions comparable to that of the C17 hydroxyl of **RU-43044** or **RU-486** (Figure 5). Reduction

Scheme 1[a]



[a] Reagents: (a) (i) pyrrolidine, toluene, Dean-Stark trap, reflux; (ii) benzyl bromide, dioxane, reflux; (iii) H_2O. (b) (i) (*R*)-(+)-α-Methylbenzylamine, methyl vinyl ketone, toluene, 40 °C; (ii) acetic acid. (c) (i) (*S*)-(−)-α-Methylbenzylamine, methyl vinyl ketone, toluene, 40 °C; (ii) acetic acid. (d) NaOMe, MeOH, 70 °C; (e) BCl_3, tetrabutylammonium iodide, CH_2Cl_2, −78 °C to rt. (f) Li, NH_3, dioxane/ether, −78 °C. (g) Propyne, LDA, THF. (h) *cis*-1,2-Dichloroethene, LDA, THF.

Table 1. Binding Affinities versus Human GR, PR, AR, ERα, and ERβ[a]

compound	GR	PR	AR	ERα	ERβ
RU-486	0.24 ± 0.02	15 ± 2.6	4.6 ± 0.41	44% at 10 μM	92% at 10 μM
RU-43044	0.10 ± 0.01	30% at 10 μM	90 ± 7.8	>10000[b]	>10000
1Sa	>10000	>10000	54 ± 1.1	>10000	>10000
1Sb	>10000	>10000	>10000	36% at 10 μM	24% at 10 μM
1Ra	>10000	>10000	690 ± 42	27% at 10 μM	>10000
1Rb	157 ± 13	>10000	180 ± 42	70% at 10 μM	39% at 10 μM
4	5.96 ± 0.49	>10000	1300 ± 220	45% at 10 μM	54% at 10 μM
CP-394531	0.10 ± 0.02	>10000	130 ± 26	62% at 10 μM	75% at 10 μM
CP-409069	0.17 ± 0.02	>10000	650 ± 140	50% at 10 μM	57% at 10 μM

[a] Reported as K_i's ± SEM ($n = 3$) in nM or % inhibition at dose. [b] >10000 = ≤20% inhibition at 10 μM.

Table 2. Functional Antagonism versus Human GR, MR, and PR[a]

compound	GR	MR	PR
RU-486	2.0 ± 0.7 (100% at 1 μM)	<10 (69.7 ± 3.6% at 10 nM)	11.8 ± 3.5 (100% at 1 μM)
RU-43044	12 ± 0.8 (100% at 1 μM)	>1000[b]	>1000
1Sa	>1000	>1000	>1000
1Sb	>1000	>1000	>1000
1Ra	>1000	>1000	>1000
1Rb	>1000	>1000	>1000
4	(35% at 1 μM)	>1000	>1000
CP-394531	4.1 ± 0.8 (100% at 1 μM)	>1000	>1000
CP-409069	10 ± 0.7 (100% at 1 μM)	>1000	>1000

[a] Reported as K_{if} ± SEM ($n = 3$) in nM (% inhibition at dose) of a half-maximal dexamethasone response.[69] [b] >1000 = no inhibition at 1 μM.

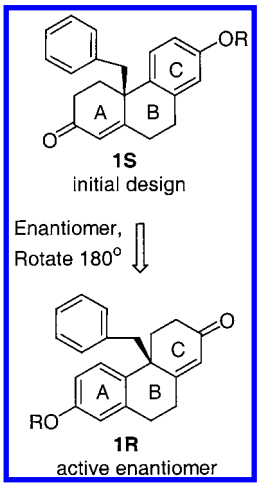


Figure 4. Computer-assisted designs.

of **1Rb** to **4** followed by a nucleophilic attack on the C-ring carbonyl of **4** with lithium propyne or lithium chloroethyne from the face opposite the angular benzyl moiety afforded **CP-409069** and **CP-394531**. This positions the third phamacophore in the appropriate relative orientation. As anticipated, the addition of this pharmacophore provided a dramatic increase in GR binding and antagonistic potency with exquisite selectivity. The GR binding potency of **CP-409069** and **CP-394531** increased approximately 1000-fold over the original lead (**1Rb**). **CP-409069** and **CP-394531** have GR binding and antagonism potencies similar to those of **RU-486** and selectivities similar to that of **RU-43044** (Tables 1 and 2).

The recognition of the pseudo-C2 symmetric axis helped to refine the 3-D pharmacophore model. The fact

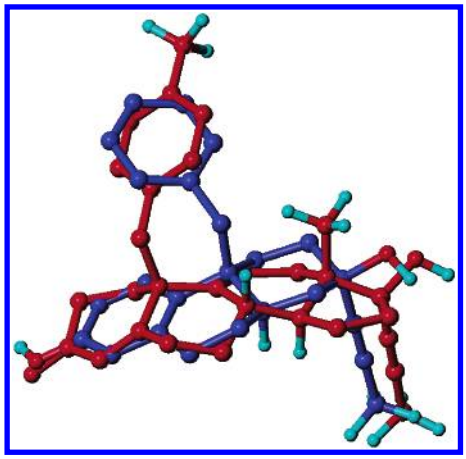


Figure 5. Overlap of **RU-43044** (red) and **CP-409069** (blue).

that **1S** did not bind to the GR at concentrations less than 10 μM and that **1Rb** had modest binding affinity to the GR (K_i = 157 nM) suggested that the nature of the C-ring of the tricyclic skeleton has a much greater effect on binding potency than we had initially anticipated. Saturation of the C-ring double bond of **1Rb** to the trans Decalin **4** increased GR binding potency by greater than an order of magnitude. Furthermore, **4** began to demonstrate antagonism in the GR whole-cell luciferase assay (35% block of a half-maximal dexamethasone activity, Table 2). This activity seemed to be selective for the GR versus the closely related MR and PR, and **4**, like **CP-394531** and **CP-409069**, did not display any GR, MR, or PR agonist activity. These results suggest that there are significant hydrophobic interactions between the saturated C-ring of our tricyclic template and the GR. Retrospectively, this result may be explained by at least two potential interactions with the GR. First, the aforementioned hydrophobic interaction of the saturated C-ring of **4**, **CP-394531**, and **CP-409069** may help to mimic the hydrophobic interaction of the C−D ring and its angular methyl group of the steroids **RU-486** and **RU-43044**. Second, the equatorial hydroxyl in the C-ring of **CP-394531** and **CP-409069** may fit into a similar hydrogen-bonding network with the GR as the hydroxyl moiety in the D-ring of **RU-486** and **RU-43044** (Figures 4 and 5).

Conclusion

In summary, a 3-D pharmacophore model for the design of selective GR antagonists that do not contain the steroid nucleus has been developed. The resultant templates contained a pseudo C2-symmetric axis that incorporated two of the three pharmacophores important for GR selectivity and functional handles to append the third pharmacophore to increase GR potency. This model assisted in the rapid design of **CP-394531** and **CP-409069**, potent, selective, nonsteroidal GR antagonists. Additional in vitro and in vivo studies with **CP-394531**, **CP-409069**, and related molecules are ongoing and will be presented in due course.[61]

Experimental Procedures

General Methods. Molecular modeling was performed on a Silicon Graphics workstation using SYBYL version 6.2 (Tripos, Inc., St. Louis, MO) with the TRIPOS force field. Molecular overlaps were executed manually. Melting points were determined with a capillary apparatus and are uncorrected. Nuclear magnetic resonance spectra (^{1}H-NMR at 300 or 400 MHz and ^{13}C-NMR at 75 MHz) were run using the residual solvent as an internal standard. Mass spectra determinations were performed by the Exploratory Medicinal Sciences Department of Pfizer Global Research and Development at Groton, CT. Elemental analyses were performed by Schwarzkopf Microanalytical Laboratory, Inc., Woodside, NY. Optical rotations were measured at 20 °C in MeOH. Analytical thin-layer chromatography was done on Kieselgel 60 F-254 plates precoated with 0.25 mm thick silica gel distributed by E. Merck. Column chromatography was performed on silica gel (Kieselgel 60, 230−400 mesh) from E. Merck. Reagents were purchased and used without further purification unless otherwise stated. Both (*S*)-(−)-α-methylbenzylamine and (*R*)-(+)-α-methylbenzylamine were freshly distilled from CaH at atmospheric pressure under nitrogen directly prior to use. Methyl vinyl ketone was placed over solid K_2CO_3 for 30 min, decanted, and distilled at 200 mmHg pressure. The colorless heart of the distillate was collected (40 °C) and used directly in the reaction. Human PR, ERα, and ERβ binding affinities were performed by Cerep (Rueil-Malmaison, France; www.cerep.com).

1-Benzyl-6-methoxy-3,4-dihydro-1*H*-naphthalen-2-one [247936-61-4] (2).[51] A solution of 51 g (0.289 mol) of 6-methoxy-2-tetralone and 24.2 mL (0.289 mol) of pyrrolidine in 1.5 L of toluene was heated to reflux under a Dean-Stark trap overnight. After removal of the azeotroped water, the reaction mixture was cooled to rt, concentrated to an oil, and dissolved in 725 mL of dioxane. To this solution was added 52 mL (0.434 mol) of benzyl bromide, and the resulting solution was heated to reflux overnight. Water (100 mL) was added to the solution, and heating to reflux of the resultant mixture was continued for an additional 2 h. The mixture was cooled to rt, poured into a solution of 1 N HCl, and extracted 3 times with EtOAc. The organic layers were washed with H_2O and saturated $NaHCO_3$ and then dried over Na_2SO_4, filtered, and evaporated to dryness. The crude product was purified by flash chromatography over SiO_2 using 10−15% EtOAc in hexanes as the gradient eluant to give 65.2 g of **2** as a yellow oil (85%): IR (neat) 2937, 1712, 1500 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$, δ) 2.41−2.59 (m, 3H), 2.76 (dt, 1H, J = 5.4, 15.5 Hz), 3.15−3.70 (m, 2H), 3.67 (t, 1H, J = 6.3 Hz), 3.77 (s, 3H), 6.67−6.70 (m, 2H), 6.81 (d, 1H, J = 8.1 Hz), 6.87−6.89 (m, 2H), 7.13−7.17 (m, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$, δ) 27.44, 38.19, 39.19, 54.13, 55.14, 112.11, 112.96, 126.30, 128.07, 128.26, 129.35, 129.53, 138.05, 138.20, 158.30, 212.41; MS *m*/*z* 267 (M + H)$^+$.

1(*S*)-Benzyl-6-methoxy-1(*R*)-(3-oxo-butyl)-3,4-dihydro-1*H*-naphthelen-2-one (3R). A solution of 62 g (0.23 mol) of 1-benzyl-6-methoxy-3,4-dihydro-1*H*-naphthalen-2-one (**2**) and 28 mL (0.23 mol) of freshly distilled (*S*)-(−)-α-methylbenzylamine in 100 mL of toluene was heated to reflux under a Dean-Stark trap overnight. After removal of the azeotroped water, the imine solution was cooled to 0 °C, and 21 mL (0.26 mol) of freshly distilled methyl vinyl ketone was added dropwise to the solution. The solution was stirred at 0 °C for 30 min and then heated to 40 °C overnight. The reaction solution was cooled to 0 °C. Acetic acid (17 mL) and H_2O (14 mL) were added, and the resultant solution was allowed to warm to rt for 2 h. The solution was poured into H_2O and extracted 3 times with EtOAc. The combined organic layers were washed with 1 N HCl, H_2O, and saturated $NaHCO_3$ and then dried over Na_2SO_4, filtered, and evaporated to dryness. The crude product was purified by chromatography over SiO_2 using 15−35% EtOAc in hexanes as the gradient eluant to give 48 g of **3R** as a yellow solid: ^{1}H-NMR (400 MHz, $CDCl_3$, δ) 1.38 (s, 3H), 1.40−1.51 (m, 2H), 1.64 (ddd, 1H, J = 2.1, 4.5, 13 Hz), 1.97 (broad s, 1H), 2.20 (dt, 1H, J = 4.5, 13 Hz), 2.59 (d, 1H, J = 6.6 Hz), 3.08 (d, 1H, J = 18 Hz), 3.16 (d, 1H, J = 16 Hz), 3.33 (dd, 1H, J = 6.6, 18 Hz), 3.62 (d, 1H, J = 16 Hz), 3.72 (s, 3H), 6.57 (d, 1H, J = 2.5 Hz), 6.67 (dd, 1H, J = 2.5, 8.8 Hz), 7.00−7.23 (m, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$, δ) 27.90, 32.79, 34.40, 38.43, 41.49, 53.51, 55.12, 58.47, 79.06, 112.05, 113.09,

125.37, 127.63, 127.69, 130.27, 132.21, 135.45, 138.65, 157.88, 213.49; MS *m*/*z* 337 $(M + H)^+$, 319 $(M - OH)^+$.

1(*R*)-Benzyl-6-methoxy-1(*R*)-(3-oxo-butyl)-3,4-dihydro-1*H*-naphthelen-2-one (3S). The compound **3S** was formed by the same procedure as 1(*S*)-benzyl-6-methoxy-1(*R*)-(3-oxo-butyl)-3,4-dihydro-1*H*-naphthelen-2-one (**3R**) using (*R*)-(+)-α-methylbenzylamine in the initial imine formation. Starting with 4.64 g 1-benzyl-6-methoxy-3,4-dihydro-1*H*-naphthalen-2-one (**2**) produced 3.58 g of **3S** as a yellow solid. All physical constants are the same as reported for **3R**.

4a(*S*)-Benzyl-7-methoxy-4,4a,9,10-tetrahydro-3*H*-phenanthren-2-one (1Ra). A solution of 48 g (143 mmol) of 1(*S*)-benzyl-6-methoxy-1(*R*)-(3-oxo-butyl)-3,4-dihydro-1*H*-naphthelen-2-one (**3R**) and 71 mL of 1 M sodium methoxide in 100 mL of methanol was stirred at rt for 15 min and then heated to 75 °C for 3 h. The solution was cooled to 0 °C, treated dropwise with 8.2 mL of acetic acid, and concentrated to an oil. The oil was dissolved in EtOAc, washed with saturated $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and evaporated to dryness. The crude product was purified by chromatography over SiO_2 using 15−35% EtOAc in hexanes as the gradient eluant to give 44 g of **1Ra** as an off-white powder. Recrystallization from EtOAc/hexane afforded 35 g of **1Ra** as a white crystalline solid (48% from 1-benzyl-6-methoxy-3,4-dihydro-1*H*-naphthalen-2-one, **2**): mp 101−102 °C; IR (neat) 1667, 1500 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$, δ) 1.83−1.90 (m, 1H), 2.02 (dt, 1H, *J* = 5.5, 14 Hz), 2.27 (dt, 1H, *J* = 4.3, 14 Hz), 2.44−2.51 (m, 2H), 2.64−2.79 (m, 3H), 3.14 (d, 1H, *J* = 13 Hz), 3.21 (d, 1H, *J* = 13 Hz), 3.78 (s, 3H), 5.96 (s, 1H), 6.54 (d, 1H, *J* = 2.6 Hz), 6.71 (d, 2H, *J* = 7.1 Hz), 6.77 (dd, 1H, *J* = 2.6, 8.7 Hz), 7.06−7.23 (m, 4H); ^{13}C-NMR (100 MHz, $CDCl_3$, δ) 30.71, 32.10, 34.62, 36.09, 43.62, 46.36, 55.20, 112.78, 112.84, 125.53, 126.68, 127.96, 128.12, 130.08, 133.01, 137.24, 137.28, 157.75, 169.16, 198.81; MS *m*/*z* 319 $(M + H)^+$; $[\alpha]^{20}_D$ −220° (*c* 0.0102, MeOH). Anal. ($C_{22}H_{22}O_2$) C, H, N.

4a(*R*)-Benzyl-7-methoxy-4,4a,9,10-tetrahydro-3*H*-phenanthren-2-one (1Sa). The compound **1Sa** was formed by the same procedure used for 4a(*S*)-benzyl-7-methoxy-4,4a,9,10-tetrahydro-3*H*-phenanthren-2-one (**1Ra**). Starting with 3.53 g of 1(*R*)-benzyl-6-methoxy-1(*R*)-(3-oxo-butyl)-3,4-dihydro-1*H*-naphthelen-2-one (**3S**) produced 2.78 g of **1Sa** as an off-white powder. Recrystallization from EtOAc/hexane afforded 2.15 g of **1Sa** as a white crystalline solid (33% from 1-benzyl-6-methoxy-3,4-dihydro-1*H*-naphthalen-2-one, **2**). All physical constants are the same as reported for 4a(*S*)-benzyl-7-methoxy-4,4a,9,10-tetrahydro-3*H*-phenanthren-2-one (**1Ra**) except optical rotation, $[\alpha]^{20}_D$ +225° (*c* 0.0114, MeOH). Anal. ($C_{22}H_{22}O_2$) C, H, N.

4a(*S*)-Benzyl-7-hydroxy-4,4a,9,10-tetrahydro-3*H*-phenanthren-2-one (1Rb). To a stirred solution of 40 g (0.126 mol) of 4a(*S*)-benzyl-7-methoxy-4,4a,9,10-tetrahydro-3*H*-phenanthren-2-one (**1Ra**) and 46.5 g (0.126 mol) of tetrabutylammonium iodide in 630 mL of dichloromethane was added 300 mL of 1 M boron trichloride in methylene chloride at −78 °C under an N_2 atmosphere. The resultant solution was allowed to warm to rt for 1.5 h and then poured into excess ice and stirred vigorously overnight. The mixture was extracted with dichloromethane (3 times), dried over Na_2SO_4, filtered, and concentrated to dryness. Purification by flash chromatography over SiO_2 using 20−60% EtOAc in hexanes as the gradient eluant afforded 33.3 g of an off-white powder (87%): ^{1}H-NMR (400 MHz, CD_3OD, δ) 1.81−2.00 (m, 2H), 2.26 (dt, 1H, *J* = 4.2, 13 Hz), 2.40 (dd, 1H, *J* = 4.5, 18 Hz), 2.53 (ddd, 1H, *J* = 1.7, 5.6, 14 Hz), 2.58−2.80 (m, 3H), 3.20 (d, 1H, *J* = 13 Hz), 3.26 (d, 1H, *J* = 13 Hz), 5.92 (s, 1H), 6.45 (d, 1H, *J* = 2.5 Hz), 6.67 (dd, 1H, *J* = 2.5, 8.5 Hz), 6.76 (d, 2H, *J* = 6.6 Hz), 7.05−7.14 (m, 4H); ^{13}C-NMR (100 MHz, CD_3OD, δ) 30.22, 32.03, 34.08, 36.04, 43.73, 45.97, 113.76, 113.91, 124.50, 126.25, 127.49, 127.94, 129.84, 131.86, 137.0, 137.71, 155.34, 171.73, 200.33; MS *m*/*z* 305 $(M + H)^+$; $[\alpha]^{20}_D$ −210° (*c* 0.0100, MeOH). Anal. ($C_{22}H_{22}O_2 \cdot {}^1/_3CH_3CO_2C_2H_5$) C, H, N.

4a(*R*)-Benzyl-7-hydroxy-4,4a,9,10-tetrahydro-3*H*-phenanthren-2-one (1Sb). The same procedure for 4a(*S*)-benzyl-7-hydroxy-4,4a,9,10-tetrahydro-3*H*-phenanthren-2-one (**1Rb**) was used to form compound **1Sb**. Starting with 1.8 g of 4a-(*R*)-benzyl-7-methoxy-4,4a,9,10-tetrahydro-3*H*-phenanthren-2-one (**1Sa**) produced 1.3 g of **1Sb** as a white solid (75%). All physical constants are the same as reported for **1Sb** except optical rotation, $[\alpha]^{20}_D$ +213° (*c* 0.0114, MeOH). Anal. ($C_{21}H_{20}O_2$) C, H, N.

4a(*S*)-Benzyl-7-hydroxy-3,4,4a,9,10,10a(*R*)-hexahydro-1*H*-phenanthren-2-one (4). Ammonia (1.5 L) was condensed into a round-bottom flask equipped with a dry-ice reflux condenser at −78 °C and a mechanical stirrer. To this flask was added 0.7 g (99 mmol) of lithium wire, and the solution turned dark blue. A solution of 10 g (32.8 mmol) of 4a(*S*)-benzyl-7-hydroxy-4,4a,9,10-tetrahydro-3*H*-phenanthren-2-one (**1Rb**) in 400 mL of 1:1 dioxane/ether was added to the mixture slowly in order to keep the mixture a dark blue. As the blue color dissipated, a small amount of lithium wire was added to the mixture to regenerate the blue color. The total amount of lithium added to the reaction mixture did not exceed 3.5 g (495 mmol). After the complete addition of **1Rb**, the reaction mixture was stirred for an additional 30 min, then 14 g of solid ammonium chloride was added, and an immediate dissipation of the blue color was observed. H_2O was added to the mixture, and it was extracted with EtOAc, dried over Na_2SO_4, filtered, and concentrated to dryness. The crude product was purified by flash chromatography over SiO_2 using 15−20% EtOAc in hexanes as the gradient eluant to afford 8.16 g of **4** as a white solid (81%): ^{1}H-NMR (400 MHz, CD_3OD, δ) 1.52 (dt, 1H, *J* = 4.5, 13 Hz), 1.64−1.71 (m, 1H), 1.90−2.15 (m, 2H), 2.27 (ddd, 1H, *J* = 2.5, 3.7, 15 Hz), 2.39 (dm, 1H, *J* = 15 Hz), 2.48 (ddd, 1H, *J* = 2.0, 6.5, 13 Hz), 2.72 (t, 1H, *J* = 14 Hz), 2.84 (d, 1H, *J* = 13 Hz), 2.89−3.01 (m, 3H), 3.22 (d, 1H, *J* = 13 Hz), 6.17 (d, 1H, *J* = 8.5 Hz), 6.24 (dd, 1H, *J* = 2.5, 8.5 Hz), 6.53 (d, 1H, *J* = 2.5 Hz), 6.65−6.68 (m, 1H), 7.04−7.13, (m, 3H); ^{13}C-NMR (100 MHz, CD_3OD, δ) 27.9, 33.7, 34.8, 36.0, 37.6, 39.4, 43.6, 44.0, 111.3, 114.6, 125.7, 127.0, 127.9, 130.5, 133.4, 136.8, 138.0, 155.1, 212.7; MS *m*/*z* 307 $(M + H)^+$; $[\alpha]^{20}_D$ −120° (*c* 0.0104, MeOH). Anal. ($C_{21}H_{22}O_2$) C, H, N.

4a(*S*)-Benzyl-2(*R*)-chloroethynyl-1,2,3,4,4a,9,10,10a(*R*)-octahydro-phenanthrene-2,7-diol (CP-394531). To a stirred solution of 95 mg of *cis*-dichloroethylene (0.98 mM) in 5 mL of THF at 0 °C was added 2.5 mL of 0.5 M lithium diisopropylamide in THF, and the resultant mixture was allowed to warm to rt for 30 min under a nitrogen atmosphere. A solution of 30 mg (0.098 mmol) of 4a(*S*)-benzyl-7-hydroxy-3,4,4a,9,10,10a(*R*)-hexahydro-1*H*-phenanthren-2-one (**4**) in 0.65 mL of THF was added dropwise, and the reaction mixture was stirred for an additional 2 h. Saturated aqueous ammonium chloride was added, and the mixture was extracted with EtOAc (3 times). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated to dryness. Initial purification by flash chromatography over SiO_2 using 20% EtOAc in hexanes as the eluant afforded 30 mg of a light brown solid. Further purification by flash chromatography over SiO_2 using 2−4% acetone in dichloromethane as a gradient eluant afforded 20 mg (56%) of **CP-394351**: mp 230−232 °C dec; ^{1}H-NMR (300 MHz, CD_3OD, δ) 1.40 (mt, 1H, *J* = 14 Hz), 1.64−1.70 (m, 1H), 1.80−2.13 (m, 7H), 2.59 (d, 1H, *J* = 13 Hz), 2.93−2.97 (m, 3H), 6.13 (d, 1H, *J* = 8.5 Hz), 6.25 (dd, 1H, *J* = 2.6, 8.5 Hz), 6.54−6.57 (m, 3H), 7.00−7.07 (m, 3H); ^{13}C-NMR (75 MHz, CD_3OD, δ) 25.5, 28.7, 31.8, 37.2, 40.52, 41.71, 43.43, 63.1, 70.7, 74.1, 112.4, 116.1, 126.8, 128.3, 138.9, 132.1, 136.2, 138.3, 139.5, 156.3; MS *m*/*z* 366 $(M + H)^+$, 349 $(M - OH)^+$. $[\alpha]^{20}_D$ −98° (*c* 0.0103, MeOH). Anal. ($C_{23}H_{23}ClO_2$) C, H, N.

4a(*S*)-Benzyl-2(*R*)-prop-1-ynyl-1,2,3,4,4a,9,10,10a(*R*)-octahydro-phenanthrene-2,7-diol (CP-409069). To a stirring solution of 30 mL of THF saturated with propyne gas at 0 °C was added 52 mL of 0.5 M lithium diisopropylamide in THF, and the resultant mixture was stirred under a nitrogen atomosphere for 20 min. A solution of 1.34 g (4.3 mmol) of 4a-(*S*)-benzyl-7-hydroxy-3,4,4a,9,10,10a(*R*)-hexahydro-1*H*-phenanthren-2-one (**4**) in 18 mL of THF was added dropwise, and the reaction mixture was warmed to rt and stirred for 1 h. Saturated aqueous ammonium chloride was added, and the mixture was extracted with EtOAc (3 times). The combined

organic layers were dried over Na_2SO_4, filtered, and concentrated to dryness. Purification by flash chromatography over SiO_2 using 2−4% acetone in dichloromethane as the eluant afforded 1.05 g (69%) of **CP-409069**: mp 227−229 °C dec); ^{1}H-NMR (400 MHz, CD_3OD, δ) 1.42 (mt, 1H, J = 14 Hz), 1.61 (ddd, 1H, J = 3.4, 4.1, 8.8 Hz), 1.72 (s, 3H), 1.73−1.82 (m, 2H), 1.84−2.10 (m, 5H), 2.55 (d, 1H, J = 13 Hz), 2.83−2.93 (m) and 2.94 (d, 3H, J = 13 Hz), 6.10 (d, 1H, J = 8.3 Hz), 6.23 (dd, 1H, J = 2.5, 8.4 Hz), 6.52−6.55 (m, 3H), 7.00−7.05 (m, 3H); ^{13}C-NMR (62 MHz, CD_3OD, δ) 2.5, 24.1, 27.3, 30.5, 35.8, 36.1, 39.1, 40.2, 42.4, 68.9, 79.5, 82.3, 110.9, 114.7, 125.4, 126.8, 127.5, 130.7, 135.1, 136.9, 138.3, 154.8; MS m/z 346 $(M + H)^+$, 329 $(M - OH)^+$; $[\alpha]^{20}_D$ −120° (c 0.0104, MeOH). Anal. ($C_{24}H_{26}O_2$) C, H, N.

Human Glucocorticoid Receptor (hGR) Binding Assay Protocol. Binding displacement assays were run using cytosolic preparations from Sf9 cells infected with the human glucocorticoid receptor (hGR).[62] Briefly, Sf9 cells were inoculated at a density of 3×10^5/mL and grown to ~2×10^6/mL in an SF900 II medium. Cells were infected at a multiplicity of infection (MOI) of 1, harvested 72 h postinfection, and batch centrifuged at 3500 rpm for 10 min. The cell pellets were stored at −75 °C. Cytosol was prepared by suspending the Sf9 cell pellets in 80 mL of lysing buffer (10 mM K_2HPO_4, pH 7.6, 100 μM EDTA, 5 mM dithiothreitol, 20 mM sodium molybdate, 200 μM AEBSF (lysing reagent from Calbiochem-Novabiochem Corporation, LaJolla, CA), and 80 μg/mL leupeptin), centrifuging at 100 000g for 30 min at 4 °C, and collecting the supernatant. Protein concentration was determined using an automated analyzer (Abbott CCX). Binding assays were carried out in 96-well v-bottom polypropylene plates with a final volume of 130 μL containing 200−600 μg of cytosolic protein, assay buffer (113 μL, containing 20 mM Tris, 1 μM EDTA, 5 mM DTT, 10 mM sodium molybdate), [^{3}H]dexamethasone (final concentration, 10 nM), and test compounds at concentrations ranging from 0.3 nM to 30 μM or test compound vehicle (to give total bound counts). Each concentration was run in duplicate. Nonspecific binding in duplicate wells was determined on each plate by the addition of excess unlabeled dexamethasone (final concentration, 11.5 μM). Cytosols were incubated for 18 h at 4 °C. Unbound radioactivity was removed by the addition of dextran-coated charcoal and centrifugation. One hundred microliters of the supernatant from each well was transferred to a 96-well PET plate with 200 μL of scintillation fluid and counted (1450 micro β counter from Perkin-Elmer Wallac, Gaithersburg, MD). The dose−response data for specifically bound counts were fitted using a sigmoidal equation to calculate IC_{50}, and K_i was calculated using the equation of Cheng and Prusoff.[63] The K_d for dexamethasone, determined by saturation binding, was 2.5 nM.

Human Androgen Receptor (hAR) Binding Assay Protocol. Binding displacement assays were run using whole cell preparations from Sf9 cells infected with the human androgen receptor (hAR). The human androgen receptor cDNA[64] was obtained from Dr. Chawnshang Chang (University of Chicago, Chicago, IL) and subcloned into the baculovirus transfer vector pBlueBac4.5 (Invitrogen, Carlsbad, CA). The transfer vector was cotransfected into Sf9 cells with Bac-N-Blue DNA (Invitrogen), and plaques containing recombinant virus were selected based on the expression of β-galactosidase. Appropriate recombination was confirmed by PCR, and the recombinant virus was subjected to an additional round of plaque purification. This virus (AR22) was expanded and titered. A MOI and time-course experiment was conducted by infecting Sf9 cells growing in spinner culture in Grace's Insect medium supplemented with 10% FCS and 0.1% F68 (Invitrogen) at 2×10^6 cells/mL with MOIs ranging from 0.1 to 10. Optimal expression of the AR was found by Western blot to be 48 h after infection with an MOI = 2. AR for binding assays was then produced by infecting 1 L Sf9 cultures with AR22 virus under these conditions of pelleting the cells by centrifugation 48 h after infection and resuspending and sonicating the cell pellet in binding buffer at pH 7.2 (5 mM dithiothreitol, 10 mM NaF, 100 μg/mL bacitracin, 50 mM HEPES, 1.5 mM EDTA, 10 mM Na_2MoO_4, 1 mM Pefabloc, 10% glycerol) at a concentration of 6.7×10^7 cells/mL. Following sonication, cell debris was pelleted in a microfuge, and cell extract was frozen in aliquots in liquid N_2 and stored at −80 °C. Binding assays were carried out in 96-well filter plates (Millipore MHVPN45) containing 45 μL of hydroxylapatite (Calbiochem 391947) in a final volume of 200 μL of binding buffer (previously described without Pefabloc) containing 5 μL of hAR extract, 1 nM [^{3}H]-dihydrotestosterone (Perkin-Elmer NET453), and the test compounds at concentrations ranging from 0.01 nM to 30 μM. Each concentration was run in triplicate. Nonspecific binding in triplicate wells was determined on each plate by the addition of excess unlabeled dihydrotestosterone (final concentration, 10 μM). The reaction mixture was incubated for 1 h at 4 °C. Unbound radioactivity was removed by filtration and three washes with buffer at pH 7.2 (50 mM HEPES, 10 mM $NaPO_4$, 0.1% Triton X-100, 100 mM KCl). Fifty microliters of scintillation fluid (Packard Microscint 20) was added to each well, and radioactive counts per minute were determined using a TopCount micro scintillation counter (Packard). The dose−response data for specifically bound counts were fitted using a sigmoidal equation to calculate IC_{50}, and K_i was calculated using the equation of Cheng and Prusoff.[63] The K_d for the radioligand is 0.4 nM.[65−68]

Whole Cell Functional Assays, GRE. SW1353 human chondrosarcoma cells expressing the endogenous glucocorticoid receptor were transfected with a plasmid containing the murine mammary tumor virus (MMTV) promoter linked to luciferase and conferring neomycin resistance (Clontech). Neomycin resistant (500 μg/mL) cell clones were isolated and characterized by generating dexamethasone response curves. Clone SW1353/MMTV-8, having a median response (data not shown), was used to determine the transcription activity of test compounds. Briefly, cells were transferred to 96-well microtiter plates in complete media (Dulbecco's Modified Eagle Media, 10% fetal bovine serum, gentamicin reagent, and 500 μg/mL neomycin), and after an overnight incubation, the medium was changed to a serum-free formulation (Dulbecco's Modified Eagle Media, 1 mg/L insulin, 0.5 mg/L ascorbate, 2 g/L lactalbumin hydrosylate) 4 h prior to treatment with various concentrations (10^{-10} to 10^{-6} M) of test compounds in the absence or presence of 40 nM dexamethasone (to determine agonist or antagonist activity, respectively). After 16 h, cell lysates were prepared, and luciferase activity was quantified using a LucLite reagent (Packard) and a luminometer. Agonist activity was expressed as the relative luciferase activity from cells treated with test compounds to those treated with dexamethasone. Antagonism was determined by the relative inhibition of luciferase activity from cells cotreated with the test compounds and a constant amount of dexamethasone (40 nM, that which produced a half-maximal response). The EC_{50} (concentration that produced 50% of the maximal response) for dexamethasone was calculated for the dose−response curves. The K_{if} was calculated using the modified equation of Cheng and Prusoff $K_{if} = IC_{50}/1 + ([Dex]/EC_{50}^{Dex})$.[63]

Whole Cell Functional Assays, MRE and PRE. T47D cells (from ATCC) containing endogenous human progesterone and mineralocorticoid receptors were transiently transfected with 3 × GRE-luciferase plasmid generated using Lipofectin plus (GIBCO-DRL, Gaithersburg, MD). Twentyfour hours post-transfection, cells were maintained in 10% charcoal-stripped serum and transferred to 96-well microtiter plates. The next day, cells were treated with various concentrations (10^{-12} to 10^{-5} M) of test compounds in the absence or presence of 10 nM progesterone and 10 nM aldosterone in triplicate for up to 24 h. Cell lysates were prepared, and luciferase activity was determined using a luminometer. Agonist activity was assessed by comparing the luciferease activity from cells treated with test compounds alone to cells treated with either progesterone or aldosterone. Antagonist activity was addressed by comparing the luciferease activity of an EC_{50} concentration of progesterone or aldosterone in the absence and presence of the test compound. The EC_{50} for dexamethasone was calculated for the dose−response curves. The K_{if} was calculated

using the modified equation of Cheng and Prusoff [$K_{if} = IC_{50}/(1 + [ligand]/EC_{50}^{ligand})$].[63]

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Supporting Information Available: X-ray crystal coordinates for **4** and **CP-394531**. This material is available free of charge via the Internet at http://pubs.acs.org.

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