Novel *N*-Arylpyrazolo[3,2-*c*]-Based Ligands for the Glucocorticoid Receptor: Receptor Binding and in Vivo Activity

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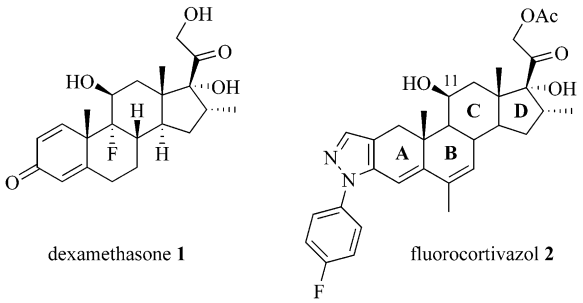
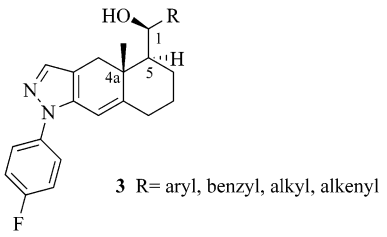
A novel series of selective ligands for the human glucocorticoid receptor (hGR) are described. Preliminary structure−activity relationships were focused on substitution at C-1 and indicated a preference for 3-, 4-, and 5-substituted aromatic and benzylic groups. The resulting analogues, e.g., **18** and **34**, exhibited excellent affinity for hGR (IC_{50} 1.9 nM and 2.8 nM**,** respectively) and an interesting partial agonist profile in functional assays of transactivation (tyrosine aminotransferase, TAT, and glutamine synthetase, GS) and transrepression (IL-6). The most potent compounds described in this study were the tertiary alcohol derivatives **21** and **25**. These candidates showed highly efficacious IL-6 inhibition versus dexamethasone. The thiophenyl analogue **25** was evaluated in vivo in the mouse LPS challenge model and showed an ED_{50} = 4.0 mg/kg, compared to 0.5 mg/kg for prednisolone in the same assay.

Introduction

Glucocorticoids (GCs), e.g., dexamethasone[1−4] **1** and fluorocortivazol[5,6] **2**, are among the most effective agents currently available for the treatment of inflammatory and allergic diseases. However, the desired immunosuppressive effects of glucocorticoids are accompanied by a number of side-effects, particularly when the GC is administered over a prolonged period of time. These include weight gain, muscle atrophy, development or aggravation of diabetes mellitus, onset of hypertension, steroid-induced osteoporosis, and suppression of the hypothalamic−pituitary adrenal axis.[2,3] Many of these effects may be attributed to the endocrine activity of glucocorticoids and are essentially identical to the syndromes of endogenous corticoid excess (Cushing's syndrome).[3] An unmet opportunity of glucocorticoid pharmacology, therefore, is the development of agents with markedly improved therapeutic ratios compared to currently available steroids, particularly upon systemic administration. This may be achieved by the identification of novel glucocorticoid selective ligands that elicit marked antiinflammatory effects but have an impaired effect on endocrine responses.[7−10] In a program aimed at discovering such novel GC ligands we have synthesized a series of compounds represented by the general structure **3**. The design of these lead structures was inspired by the following observations: (I) that most of the structural modifications of synthetic GCs including those that lead to dissociated profiles[7] occur in the C−D region of the steroidal skeleton, (II) the 11-OH group of a GC ligand is thought to confer steroid receptor selectivity, i.e., glucocorticoid receptor (GR) over progesterone (PR), mineralocorticoid (MR), estrogen (ER) etc.,[11] and (III) that incorporation of certain heterocycles appended to the A-ring of GCs, especially pyrazoles containing 2′-aryl substituents, e.g., fluorocortivazol **2**, lead to significant enhancements of in vivo antiinflammatory activity.[5,6] We surmised that the hybrid structures represented by **3** would provide the framework for the development of rapid structure−activity relationships in the C−D region of the steroid nucleus while at the same time maintaining affinity and selectivity for GR. Herein we describe our early results toward the synthesis and biological evaluation of these compounds.





The effects of GCs in target tissues are mediated by GR, the expression of which is ubiquitous. GR is a

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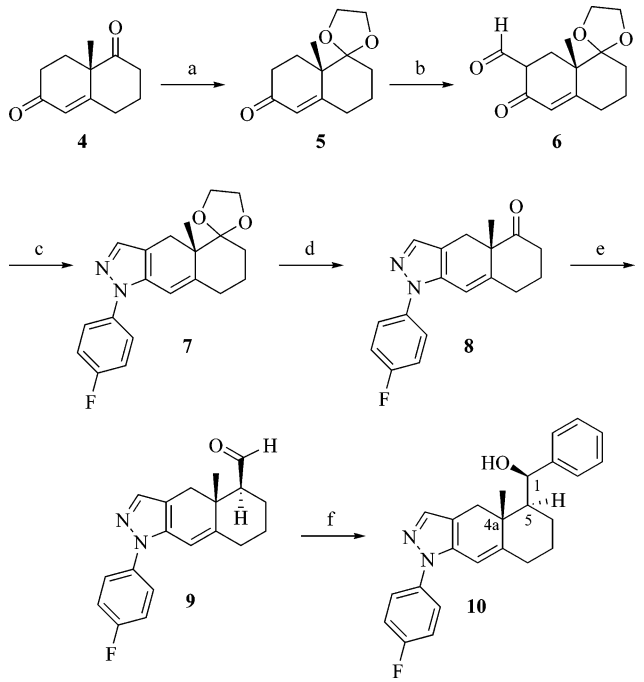
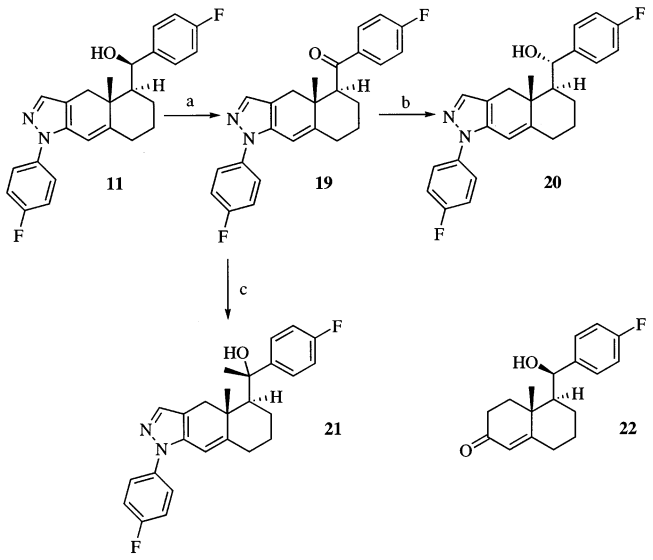
Scheme 1[a]



[a] Reagents: (a) $HOCH_2CH_2OH$, *p*-TsOH, 4 Å mol sieves, room temp, 30 min; (b) HCO_2Et, NaH, C_6H_6, MeOH, room temp, 3 h; (c) 4-fluorophenylhydrazine, NaOAc, HOAc, room temp, 24 h; (d) 6 N HCl, THF, 65 °C, 5 h; (e) $Ph_3PCH_2OCH_3Cl$, KHMDS, THF, room temp, 24 h, 1:1:1 THF:MeOH:4 N HCl; (f) PhLi, Et_2O, −78 °C, 2.5 h.

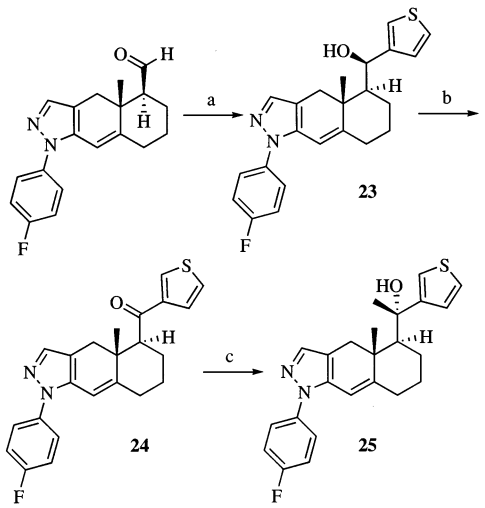
ligand-activated transcription factor that, following binding with ligand, is transported to the nucleus where it can regulate gene expression as a dimer or monomer.[12−14] In its dimeric form the GR−ligand complex binds to glucocorticoid response elements (GREs) in the promoter region of the glucocorticoid target genes leading to an increase in the transcription rate of the respective genes, a process referred to as transactivation (TA). As a monomer, the GR−ligand complex interacts directly with transcription factors such as activator protein-1 (AP-1)[15] or nuclear factor κB (NFκB)[16] which results in the repression of pro-inflammatory genes. This leads to the downregulation of numerous pro-inflammatory cytokines and other inflammatory mediators such as interleukin-1 (IL-1), IL-4, IL-6, collagenase, and tumor necrosis factor-α (TNF-α). There is increasing acceptance of the hypothesis[17,18] that the side effects of steroidal glucocorticoids such as dexamethasone and prednisolone are likely due to the transactivation of genes through binding of the GR−ligand dimer complex to DNA. The antiinflammatory effects are thought to result, in part, from the binding of monomeric GR−ligand complex to transcription factors or coactivators resulting in gene repression, i.e., transrepression (TR). Indeed, recent reports in the literature[7−10] have shown that dissociation of the various transcriptional activities of GR may lead to the development of drugs with improved therapeutic index (TI). We report herein the synthesis and biological characterization of novel, nonsteroidal GR selective ligands which demonstrate a differential partial agonist profile in cell-based assays of TA and TR.

Scheme 2[a]



[a] Reagents: (a) TPAP, NMO, CH_2Cl_2, 0 °C, 1 h; (b) $NaBH_4$, MeOH, 0 °C, 1 h; (c) MeLi, Et_2O, −40 °C, 2 h.

Scheme 3[a]



[a] Reagents: (a) 4-Bromothiophene, *t*-BuLi, Et_2O, −78 °C; (b) TPAP, NMO, CH_2Cl_2, 0 °C, 1 h; (c) MeLi, Et_2O, −40 °C, 2 h.

Chemistry

The target compounds were synthesized by the routes shown in Schemes 1−4. We were interested in developing a general approach which would allow the inclusion of a wide variety of substituents in the C- and D- ring environments. A typical example of this route is shown for the unsubstituted phenyl analogue **10**, Scheme 1. Commercially available Wieland−Mischer ketone **4** was selectively protected as the ketal **5** and then formylated to yield **6**. Formation of the 4-fluorophenylpyrazole proceeded smoothly to afford the ketone intermediate **8** following treatment with 6 N HCl. Conversion to the aldehyde **9** was best achieved by treatment with potassium hexamethyldisilazide and (methoxymethyl)triphenylphosphonium chloride followed by hydrolysis of the enol ether with a 1:1:1 mixture of THF:MeOH:4 N HCl[19] to provide the aldehyde **9** as a ca. 8:1 mixture of diastereomers. The key aldehyde intermediate **9** was treated with a wide variety of organolithium and Grignard reagents to provide an array of novel GR

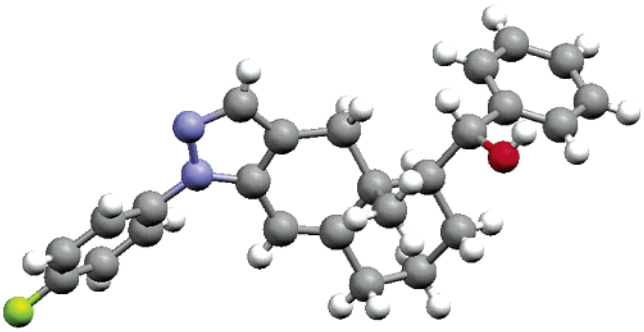


Figure 1. X-ray analysis of **10**. Relative stereochemistry at C-1 (red) is β (*R*), i.e., Steroidal.

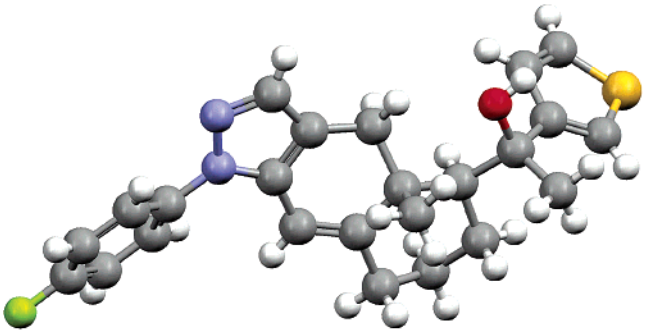


Figure 2. X-ray analysis of **25**.

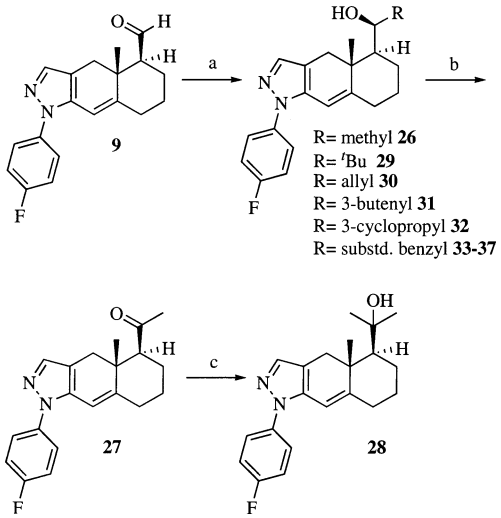
ligands for further biological evaluation. In most cases, a single major diastereomer was obtained upon addition to the aldehyde. Thus, for the phenyl analogue **10**, treatment of the aldehyde **9** with phenyllithium proceeded smoothly to afford **10** in 58% yield as a single diastereomer. The relative stereochemistry of **10** was unequivocally established by single-crystal X-ray analysis (Figure 1). Interestingly, the stereochemistry of the hydroxyl group at C-1 (equivalent to the 11-position of a steroidal nucleus) was found to be β (1*R*), i.e., steroidal. The tertiary alcohol derivatives, e.g., **21** and **25** were synthesized by the sequences outlined in Schemes 2 and 3. The relative stereochemistry at the newly formed tertiary center of **25** was established by X-ray analysis (Figure 2). Reduction of the intermediate ketone moiety with $NaBH_4$, i.e., **19** (path a, b; Scheme 2) proceeded diastereoselectively to afford the epimeric C-1 derivative, i.e., **20**. Alkyl, substituted alkyl, alkenyl, benzyl, and substituted benzyl analogues were synthesized according to similar procedures as summarized in Scheme 4.

Biological Evaluation

Each compound was first evaluated for its ability to specifically bind to the α-isoform of GR in a competition binding assay. The functional ability of each compound to activate or repress transcription was evaluated in a number of human and rodent based cellular assays.

Transrepression. The ability of the compounds to repress transcription was evaluated by IL-6-release assays using the human A549 lung epithelial cell line and freshly isolated mouse thioglycolate-elicited peritoneal exudate cells. Glucocorticoids have been shown to inhibit the production of IL-1 and TNF-α-induced IL-6 expression.[20] This inhibition has been shown to be GR-mediated at the transcriptional level and since conventional GCs are known to repress IL-6 production in this system,[21,22] monitoring IL-6 inhibition by this method provided a direct, immunologically relevant means for evaluating our novel GR ligands for transrepression activity.

Scheme 4[a]



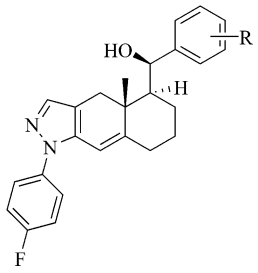
[a] Reagents:(a) RMgBr or RLi, 2 h, THF/Et_2O; (b) TPAP, NMO, CH_2Cl_2, 0 °C, 1 h; (c) MeLi, Et_2O, −78 °C, 2 h.

Transactivation. The transactivation potential of each compound was evaluated by its ability to induce tyrosine amino transferase (TAT) and glutamine synthetase (GS) activity in human HepG2 and skeletal muscle cells, respectively. TAT is a well characterized glucocorticoid responsive, liver specific gene, the product of which initiates tyrosine catabolism.[23] GS catalyses the conversion of glutamate to glutamine, an important regulator of skeletal muscle.[24−26] It was surmised that TAT and GS functional assays would afford representative markers for hepatic- and muscle-specific GR-transactivation. Induction of TAT and GS in these tissues leads to side effects characteristic of chronic glucocorticoid excess such as hyperglycemia and muscle wasting. These transactivation assays were used to guide SAR with the goal of minimizing transactivation and maximizing transrepression activity. The hGR, transactivation (TAT, GS), and transrepression (hIL-6, mIL-6) activities for a selection of compounds is provided in Tables 1−3.

Results and Discussion

We began our investigations by exploring the C-1 phenyl-substituted pyrazolo[3,2,*c*] platform. We felt that the phenyl group represented a convenient group to introduce synthetically and a good lipophilic surrogate for the C−D ring of a conventional steroidal glucocorticoid. The hGR binding and functional activities of these derivatives are presented in Table 1. All of the newly synthesized analogues displayed excellent affinity toward hGR and additionally many of the analogues displayed an interesting partial agonist profile in cell-based assays of TA and TR (Table 1). The best GR ligands in this series were those that contained small hydrophobic substituents in the ortho and para position of the aromatic ring, e.g., the *p*-fluorophenyl derivative **11**. Polar substituents such as acids, alcohols and

Table 1. Effect of Phenyl Ring Substitution on GR Activity



			TAT[b,c]		GS[c,d]		hIL-6[c,e]		mIL-6[c,f]	
compound	R	GR[a] IC_{50} (nM)	EC_{50} (nM)[b]	% dex	EC_{50} (nM)	% dex	EC_{50} (nM)	% dex	EC_{50} (nM)	% dex
prednisolone	–	13.8	211	81	32	97	4.5	102	5.7	95
10	H	8.4	n.r.[g]	9.3	n.r.[g]	21.4	2.9	61.3	110.8	77.8
11	4-F	6.2	n.r.[g]	25	n.r.[g]	16.4	9.7	67.5	21.6	74.0
12	2-F	7.2	n.r.[g]	10.8	n.r.[g]	0	2.7	45.0	57.4	68.1
13	3-OH	24.9	n.r.[g]	11.6	n.r.[g]	27	54.2	65.7	178.6	70.5
14	4-OH	9.5	n.r.[g]	12.8	n.r.[g]	0	19.5	76.5	77.2	72.1
15	4-Cl	9.0	n.r.[g]	32.7	n.r.[g]	30.4	1.9	77.5	60.1	86
16	3-OCH3,4-F	7.9	n.r.[g]	18.2	n.r.[g]	10	31.3	66.7	20.5	76.1
17	3-CH3,4-F	6.7	n.r.[g]	34	37.7	34	10.2	82.1	54.3	77.1
18	3-OCH_3,4,5-diF	1.9	520.8	36.2	n.r.[g]	24.7	14.4	76.6	15.2	82.9

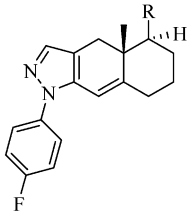
[a] Data reported is derived from duplicate wells and three independent experiments. Mean IC_{50} values were determined from 10-point, one-third log concentration−response curves and standard errors were ≤20%. [b] Human tyrosine amino transferase (TAT) assay in HepG2 cells. [c] Experiments were ran in duplicate. EC_{50} values were determined from eight-point, one-third log concentration−response curves and standard errors were ≤20%. [d] Human glutamine synthetase (GS) assay in skeletal muscle cells. [e] Human IL-6 assay in A549 lung carcinoma, cell line. [f] Mouse macrophage IL-6 assay in peritoneal exudate cells harvested by gavage from C57BI/6 mice. [g] n.r.: not reported, EC_{50} values not reported in cases were %dex ≤ 35%.

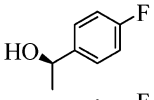
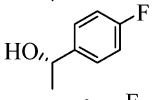
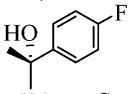
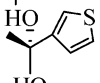
amines proved detrimental to both receptor binding and functional activity as did sterically bulky substituents.[27] Disubstituted derivatives, particularly those in the meta and para position, i.e., **16** and **17**, also displayed interesting profiles. The simultaneous presence of small hydrophobic substituents in the 4- and 5-positions (e.g., fluorine) in combination with alkoxy substituents in the 3-positions (e.g., 3-methoxy) produced the best GR ligand, i.e., **18**, in this series with the optimal dissociated window according to our cellular-based assays of TA and TR. Consistent with our ligand design hypothesis, the *p*-fluorophenyl analogue **22** lacking the pyrazolo[3,2-*c*] substituent was essentially inactive both in terms of receptor affinity and functional activity (hGR, IC_{50} 1168 nM). This is in line with previous observations wherein the inclusion of a pyrazolo[3,2-*c*] group appended to the A-ring of a steroidal nucleus led to significant boosts in activity and affinity for the glucocorticoid receptor.[28,29] We next turned our attention toward examining the stereochemical preference at the three stereogenic centers found in our lead compounds. The preferred stereochemical bias at C-4a and C-5 was found to be steroidal, i.e., β (4a*R*) and α (5*S*), respectively. The epimeric C-4a and C-5 analogues were found to be substantially less active.[27] The stereochemical preference at C-1 was less clear-cut. X-ray analysis of the parent phenyl analogue (Figure 1) had unequivocally established the C-1 stereochemistry as β (*R*; steroidal). We were able to invert the stereochemistry at this center by a two-step sequence outlined in Scheme 2. Quite surprisingly, as shown in Table 2 there appears to be only a slight stereochemical bias at C-1 toward isomer **11**, with the epimer **20** also retaining significant binding and functional affinity toward hGR. As shown in Table 2, a series of tertiary alcohol derivatives, e.g., **21**, **25**, were synthesized, and these analogues displayed a substantial boost in activity compared to their respective parent analogues. The tertiary alcohol derivatives clearly showed superior efficacy and potency when compared to the corresponding secondary alcohol derivatives. Unfortunately, the boost in activity was observed in cellular based assays of TA as well as TR and indeed this structural modification did not lead to improved dissociated GR ligands.

To broaden the scope of our investigations, we next turned our attention toward replacement of the phenyl ring. The activities of a series of substituted alkyl, alkenyl, and benzyl derivatives are shown in Tables 2, and 3, respectively. Much to our surprise, simple alkyl derivatives such as **26** and **28** (Table 2) also possessed significant affinity and functional activity toward GR. To our knowledge, derivatives such as the methyl-substituted analogues **26** and **28** are the simplest reported nonsteroidal GR agonists. In the alkenyl series, the 4-butenyl analogue **31** (Table 3) showed potent activity although as with other SAR observed within this class, the boost in potency was at the expense of dissociation. In contrast, the benzyl series of analogues generally exhibited a more dissociated profile with the 4-CH_3 analogue **35** exhibiting one of the optimal profiles observed for this structural class. To monitor steroid receptor cross-reactivity, counter screen binding assays were routinely performed on a subset of compounds against PR, MR, AR, and ERα. To our satisfaction, all of the newly synthesized analogues displayed excellent selectivity toward GR over other steroidal receptors (Table 5).

In vivo Evaluation. Unfortunately, the pharmokinetic profiles of the alkyl, alkenyl, and substituted benzyl analogues were poor across rodent species and, therefore precluded further evaluation of these analogues in vivo. In contrast, a number of the phenyl analogues displayed good levels of oral bioavailibility across rodent species. We examined a number of these

Table 2. C-1 Secondary and Tertiary Alcohol Derivatives



compound	R	GR[a] IC$_{50}$ (nM)	TAT[b,c] EC$_{50}$ (nM)	TAT[b,c] %dex	GS[d,c] EC$_{50}$ (nM)	GS[d,c] %dex	hIL-6[e,c] EC$_{50}$ (nM)	hIL-6[e,c] %dex	mIL-6[f,c] EC$_{50}$ (nM)	mIL-6[f,c] %dex
prednisolone	--	13.8	211	81	32	97	4.5	102	5.7	95
11		6.2	n.r.[g]	25	n.r.[g]	16.3	9.7	67.5	21.6	74.0
20		20.4	n.r.[g]	14.8	n.r.[g]	23[h]	15.5	48.5	6.5	69.6
21		2.5	57.2	78.7	129.9	57.9	2.2	102	1.4	92.3
25		0.8	35.6	69.1	14.2	63.5	1.0	96.8	5.2	94.2
26		8.8	n.r.[g]	0	n.r.[g]	6	14.3	52.9	n.r.[g]	72[i]
28		1.9	83.5	37.9	181.6	59.1	3.0	86.7	15.1	89.7

[a] Data reported is derived from duplicate wells and three independent experiments. Mean IC$_{50}$ values were determined from 10-point, one-third log concentration−response curves and standard errors were ≤20%. [b] Human tyrosine amino transferase (TAT) assay in HepG2 cells. [c] Experiments were ran in duplicate. EC$_{50}$ values were determined from eight-point, one-third log concentration−response curves and standard errors were ≤20%. [d] Human glutamine synthetase (GS) assay in skeletal muscle cells. [e] Human IL-6 assay in A549 lung carcinoma cell line. [f] Mouse macrophage IL-6 assay in peritoneal exudate cells harvested by gavage from C57BI/6 mice. [g] n.r.: not reported, EC$_{50}$ values not reported in cases were %dex ≤35%. [h] % inhibition at 3 μM. [i] % inhibition at 10 μM.

analogues in the mouse LPS challenge model of inflammation[30] to determine whether the functional activity observed in vitro was reflective of antiinflammatory activity in animals. Several of the analogues displayed good in vivo activity, and of the examples described, **25** was comparable to prednisolone. Upon oral administration of **25** in the mouse LPS challenge model of inflammation, a dose dependent inhibition of TNF-α was observed, similar to that for prednisolone (ED$_{50}$ = 4.0 mg/kg for **25** vs 0.5 mg/kg for prednisolone). Figure 3 depicts the dose−response curve for prednisolone versus **25** and **18**.

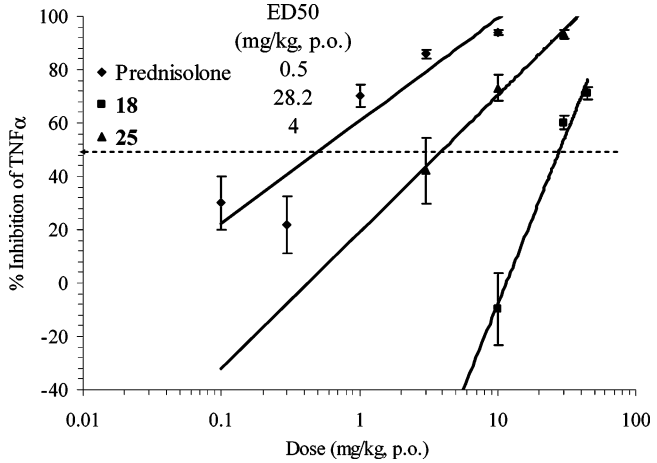
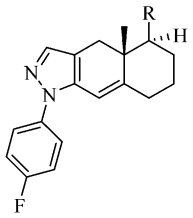


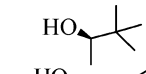
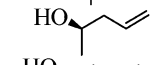
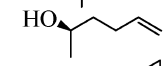
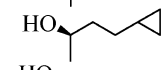
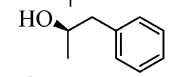
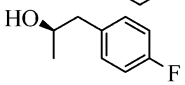
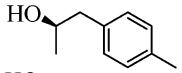
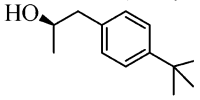
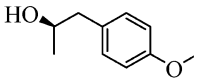
Figure 3. Prednisolone, **18**, and **25** dose dependently inhibit mouse LPS-induced TNFα production.

Conclusion

A novel series of potent and selective nonsteroidal ligands for the human glucocorticoid receptor have been discovered. The novel *N*-arylpyrazolo[3,2-*c*]-based platform offers several advantages over conventional steroidal scaffolds. Collectively, we can draw the following conclusions from our results. First, we have shown that a number of structural motifs can replace the conventional C- and D-rings of steroidal glucocorticoids, while still maintaining significant activity at the GR receptor. Preliminary SAR studies were focused on C-1 substitution and indicated a preference for 3-, 4-, and 5-substituted aromatic and benzylic groups. Second, we have confirmed that the *N*-arylpyrazolo[3,2-*c*] substituent is a significant and unique activity enhancing motif for GR. The 3-keto Δ^4 analogue **22** was ca. 200-fold less active in GR binding and functional assays of TA and TR. Third, our SAR studies have shown that by manipulating substituents in the C and D region of space it is possible to generate a number of analogues with varying degrees of dissociation between functional TA and TR activities. This result is currently under investigation and a more comprehensive discussion of this SAR will be published in separate reports detailing the

Table 3. Alkyl-, Alkenyl- and Benzyl-Substituted Analogues



compound	R	GR[a] IC$_{50}$ (nM)	TAT[b,c] EC$_{50}$ (nM)	TAT[b,c] %dex	GS[d,c] EC$_{50}$ (nM)	GS[d,c] %dex	hIL-6[e,c] EC$_{50}$ (nM)	hIL-6[e,c] %dex	mIL-6[f,c] EC$_{50}$ (nM)	mIL-6[f,c] %dex
prednisolone	--	13.8	211	81	32	97	4.5	102	5.7	95
29		77.3	n.r.[g]	0	n.r.[g]	0	n.r.[g]	0	n.r.[g]	0
30		3.9	660.9	33.3	66.5	39.5	9.3	82.9	36.2	83.1
31		1.1	255.7	67	n.r.[g]	0	4.4	91.7	5.7	86.5
32		1.5	332	45.5	66	44	8.6	84.8	26.3	79.6
33		4.2	n.r.[g]	22.6	n.r.[g]	15.5	13.9	65.4	24.7	75.2
34		2.8	1004	37.1	53.7	48.1	3.2	80.7	3.7	82.4
35		3.4	n.r.[g]	18.7	n.r.[g]	22.7	11.4	66.8	41.5	78.3
36		16.6	n.r.[g]	7.0	n.r.[g]	0	24.2	48.8	86.5	64.2
37		6.3	n.r.[g]	8.0	n.r.[g]	0	n.r.[g]	37[h]	66.6	72.9

[a] Data reported is derived from duplicate wells and three independent experiments. Mean IC$_{50}$ values were determined from 10-point, one-third log concentration−response curves and standard errors were ≤20%. [b] Human tyrosine amino transferase (TAT) assay in HepG2 cells. [c] Experiments were ran in duplicate. EC$_{50}$ values were determined from eight-point, one-third log concentration−response curves and standard errors were ≤20%. [d] Human glutamine synthetase (GS) assay in skeletal muscle cells. [e] Human IL-6 assay in A549 lung carcinoma cell line. [f] Mouse macrophage IL-6 assay in peritoneal exudate cells harvested by gavage from C57BI/6 mice. [g] n.r.: not reported, EC$_{50}$ values not reported in cases were %dex ≤ 35%. [h] % inhibition at 3 μM.

in vitro and in vivo effects of this novel class of selective glucocorticoid receptor ligands.

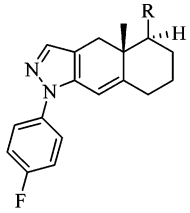
Experimental Procedures

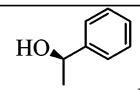
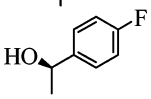
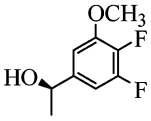
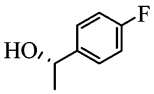
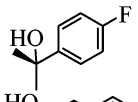
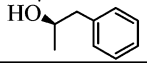
Chemistry. ^{1}H NMR spectra were recorded on a Varian InNova 500 MHz instrument in $CDCl_3$ or DMSO-d_6 solutions, unless otherwise stated. Analytical thin-layer chromatography (TLC) was carried out using Merck silica gel 60 F_{254} plates. All compounds were detected as single spots on TLC plates and visualized using UV light and phosphomolybdic acid stain. Low-resolution mass spectra (MS) were determined on a Micromass Platform LC by electrospray positive ionization. High-resolution mass spectra (HRMS) were determined on a 3 T Finnigan NewStar Fourier transform ion cyclotron resonance mass spectrometer (FTICR/MS) by electrospray ionization in positive ion mode. Elemental analyses were performed by Robertson Microlit Laboratories, Inc., Madison, NJ, and are within ±0.4% of the calculated values unless otherwise stated. Reagents were purchased commercially and used without further purification unless otherwise stated.

(8a′*S*)-8a′-Methyl-3′,4′,8′,8a′-tetrahydro-2′*H*-spiro[1,3-dioxolane-2,1′-naphthalen]-6′(7′*H*)-one (5). Molecular sieves (4 Å) (∼5 g) and *p*-toluenesulfonic acid (5.34 g, 28.05 mmol) were added to a solution of the Wieland−Miescher ketone (5 g, 28.05 mmol) in ethylene glycol (140 mL). After being stirred at room temperature for 23 min., the reaction was poured slowly into a 2:1 mixture of ice−water/sat. aqueous $NaHCO_3$ (150 mL). The reaction was extracted with EtOAc (4 × 100 mL), and the combined organic layers were washed with brine (100 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (0 to 40% EtOAc/hexanes) on silica gel to afford 5.77 g (93%) of **5** as a white solid. LCMS = 223 $(M + 1)^+$. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.83 (br d, J = 1.8 Hz, 1H), 4.43−3.94 (m, 4H), 2.49−2.40 (m, 3H), 2.39−2.27 (m, 2H), 1.95−1.88 (m, 1H), 1.84−1.78 (m, 1H), 1.76−1.64 (m, 3H), 1.37 (s, 3H).

(7′*Z*,8a′*S*)-7′-(Hydroxymethylene)-8a′-methyl-3′,4′,8′,8a′-tetrahydro-2′*H*-spiro[1,3-dioxolane-2,1′-naphthalen]-6′-(7′*H*)-one (6). Ethyl formate (7.36 mL, 86.48 mmol) and sodium hydride (60% suspension in mineral oil; 3.46 g, 86.48 mmol) were added to a cooled solution (−40 °C) of **5** in anhydrous benzene (200 mL). MeOH (450 μL) was added dropwise over 15 min, and the reaction allowed to warm to room temperature. After being stirred for 3 h, the reaction was cooled to 0 °C, and 50 mL of H_2O was added. The biphasic system was shaken, and the organic layer was washed with H_2O (3 × 50 mL). The combined aqueous layers were washed with diethyl ether (100 mL) and then acidified to pH 5.5−6

Table 4. Binding Affinities versus Human GR, PR, AR, and ER_α



cmpd	R	binding IC_{50} (uM)[a]				
		GR	PR	MR	AR	ERα
10		0.0084	0.66	>5[b]	>5	>1
11		0.0062	>1[c]	>5	>5	>5
18		0.0019	>1	>5	>5	>1
20		0.020	>1	>5	>5	>1
21		0.0025	>1	>5	>5	>1
31		0.0042	>1	>5	>5	>1

[a] Data reported is derived from duplicate wells and three independent experiments. Mean IC_{50} values were determined from 10-point, one-third log concentration−response curves and standard errors were ≤20%. [b] No inhibition at 5 μM. [c] No inhibition at 1 μM.

with sat. aqueous KH_2PO_4. The aqueous layer was extracted with EtOAc (5 × 200 mL). The combined extracts were dried over Na_2SO_4 and concentrated in vacuo to afford 5.04 g (93%) of **6** as an orange oil. LCMS = 251 $(M + 1)^+$. This material was used in the next reaction without further purification.

(4a*S*)-1-(4-Fluorephenyl)-4a-methyl-1,4,4a,6,7,8-hexahydrospiro[benzo[*f*]indazole-5,2′-[1,3]dioxolane] (7). Compound **6** (4.1 g, 16.4 mmol) was dissolved in glacial acetic acid (40 mL), and *p*-fluorophenylhydrazine hydrocloride (2.8 g, 17.22 mmol) and sodium acetate (1.41 g, 17.22 mmol) were added. After being stirred at room temperature for 2 h, the reaction was poured slowly into 10% $NaHCO_3$ (1 L) and extracted with EtOAc (6 × 500 mL). The combined extracts were washed with brine (500 mL), dried over $MgSO_4$, and concentrated in vacuo. The crude material was purified by flash chromatography (10% EtOAc/hexanes) on silica gel to afford 2.26 g (41%) of **7** as an orange solid. LCMS = 421 $(M + 1)^+$. ^1H NMR ($CDCl_3$, 500 MHz): δ 7.47−7.44 (m, 2H), 7.43 (s, 1H), 7.18−7.16 (d, *J* = 8.5 Hz, 1H), 7.16−7.14 (d, *J* = 8.7 Hz, 1H), 6.22 (br d, *J* = 2.2 Hz, 1H), 4.11−4.01 (m, 4H), 3.20−3.16 (d, *J* = 15.7 Hz, 1H), 2.54−2.51 (d, *J* = 16 Hz, 1H), 2.51−2.40 (m, 1H), 2.34−2.28 (m, 1H), 1.88−1.64 (m, 4H), 1.23 (s, 3H).

(4a*S*)-1-(4-Fluorophenyl)-4a-methyl-1,4,4a,6,7,8-hexahydro-5*H*-benzo[*f*]indazol-5-one (8). Compound **7** (2.26 g; 6.65 mmol) was dissolved in THF (65 mL), and 6 N HCl (4.43 mL, 26.6 mL) was added. The reaction was heated at 65 °C for 3.5 h and then poured slowly into 10% $NaHCO_3$ (150 mL). The mixture was extracted with EtOAc (4 × 250 mL), and the combined extracts were washed with brine (2 × 200 mL), dried over $MgSO_4$, and concentrated in vacuo to afford 1.97 g (100%) of **8** as a brown oil. LCMS = 297 $(M + 1)^+$. ^1H NMR ($CDCl_3$, 500 MHz): δ 7.50 (s, 1H), 7.49−7.45 (m, 2H), 7.20−7.16 (m, 2H), 6.31 (br d, *J* = 2 Hz, 1 H), 2.96−2.88 (m, 2H), 2.72−2.62 (m, 2H), 2.59−2.53 (m, 2H), 2.14−2.08 (m, 1H), 1.75−1,64 (qt, *J* = 13.1 Hz, *J* = 4.3 Hz, 1H), 1.27 (s, 3H).

(4aR,5*S*)-1-(4-Fluorophenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1*H*-benzo[*f*]indazole-5-carbaldehyde (9). A suspension of (methoxymethyl)triphenylphosponium chloride (4.17 g, 12.16 mmol) in THF (40 mL) was cooled to −40 °C. Potassium bis(trimethylsilyl)-amide (20.3 mL of a 0.5 M solution in toluene, 10.15 mmol) was added dropwise by syringe, and the reaction was allowed to warm to 0 °C and held at that temperature for 15 min. A solution of **8** (1.2 g, 4.05 mmol) in THF (12 mL) was added, and the reaction was allowed to warm to room temperature. After the reaction was stirred at room temperature for 24 h, 10 mL of a 1:1 solution of THF/MeOH was added to the reaction followed by 10 mL of 4 N HCl. The reaction became biphasic and stirring was continued at room temperature. After 36 h, the reaction was diluted with EtOAc (300 mL) and washed with H_2O, saturated $NaHCO_3$, and brine (50 mL each). The organic layer was dried over Na_2SO4, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (5 to 25% EtOAc/hexanes) on silica gel to afford 939.7 mg (75%) of the product **9** as a tan solid; 8:1 (β: α) mixture of aldehyde diastereomers. R_f = 0.19 (25% EtOAc/hexanes). LCMS = 311 $(M + 1)^+$. ^1H NMR (major isomer) ($CDCl_3$, 500 MHz) δ 9.91 (d, *J* = 1.8 Hz, 1H), 7.43−7.46 (m, 3H), 7.16 (t, *J* = 8.6 Hz, 2H), 6.17 (d, *J* = 1.9 Hz, 1H), 3.11 (d, *J* = 15.6 Hz, 1H), 2.91 (d, *J* = 15.6 Hz, 1H), 2.32−2.45 (m, 3H), 1.87−1.98 (m, 2H), 1.75 (m, 1H), 1.43 (m, 1H), 1.12 (s, 3H).

General Method for the Synthesis of Compounds 10−12 and 15−18. A solution of the appropriate aryl bromide (1.6 mmol) in Et_2O (16 mL) was cooled to −78 °C, and *t*-BuLi (3.2 mmol of a 1.7 M solution in pentanes) was added dropwise by syringe. The reaction was stirred at −78 °C for 20 min, and then aldehyde **9** (0.16 mmol) in THF (4 mL) was added by cannula. The reaction was stirred at −78 °C for 45 min. Isopropyl alcohol (1 mL) was added to quench the reaction at −78 °C, and the reaction was poured into saturated NH_4Cl. The mixture was extracted with EtOAc (100 mL), and the organic layer was washed with water and brine (25 mL each). The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by flash chromatography on silica gel (5 to 25% EtOAc/hexanes) gave the desired product. In some cases, small amounts of minor diastereomers were removed during the silica gel chromatography. In other cases, chiral HPLC was necessary to remove these minor diastereomers.

(*R*)-[(4a*R*,5*S*)-1-(4-Fluorophenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1*H*-benzo[*f*]indazol-5-yl](phenyl)methanol (10). From **9** using commercially available phenyllithium. Yield 58%. R_f = 0.30 (30% EtOAc/hexanes). LCMS = 389 $(M + 1)^+$. ^1H NMR ($CDCl_3$, 500 MHz): δ 7.50 (m, 3H), 7.38−7.36 (m, 4H), 7.29−7.26 (m, 2H), 6.14 (d, *J* = 2.0 Hz, 1H), 5.24 (d, *J* = 4.1 Hz, 1H), 3.24 (d, *J* = 15.1 Hz, 1H), 2.85 (d, *J* = 15.1 Hz, 1H), 2.45−2.43 (m, 2H), 2.31−2.06 (m, 2H), 1.82−1.62 (m, 3H), 1.25 (s, 3H), 1.20 (m, 1H). HRMS for $C_{25}H_{26}FN_2O$ $(M + 1)^+$: calcd, 389.2029; found, 389.2020.

(*R*)-(4-Fluorophenyl)[(4a*R*,5*S*)-1-(4-fluorophenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1*H*-benzo[*f*]indazol-5-yl]-methanol (11). From **9** using 1-bromo-4-fluorobenzene. Final purification on AD chiral column (20% IPA/heptanes), yield 55%. R_f = 0.16 (25% EtOAc/hexanes). ^1H NMR ($CDCl_3$, 500 MHz): δ 7.45 (m, 4H), 7.32 (dd, *J* = 9.5, 5.0 Hz, 2H), 7.15 (t, *J* = 8.5 Hz, 2H), 7.04 (t, *J* = 8.8 Hz, 2H), 6.12 (d, *J* = 2.1 Hz, 1H), 5.18 (s, 1H), 3.18 (d, *J* = 15.1 Hz, 1H), 2.75 (d, *J* = 15.1 Hz, 1H), 2.41 (m, 1H), 2.28 (bd, *J* = 15.1 Hz, 1H), 1.82 (m, 1H), 1.66−1.71 (m, 2H), 1.58 (m, 1H), 1.26 (s, 3H), 1.20 (m, 1H). Anal. ($C_{25}H_{24}F_2N_2O$) C, H, N.

(*R*)-(2-Fluorophenyl)[(4a*R*,5*S*)-1-(4-fluorophenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1*H*-benzo[*f*]indazol-5-yl]-methanol (12). From **9** using 1-bromo-2-fluorobenzene. Yield 70%. R_f = 0.19 (25% EtOAc/hexanes). ^1H NMR ($CDCl_3$, 500

MHz): δ 7.53 (td, J = 7.5, 1.3 Hz, 1H), 7.43−7.46 (m, 3 H), 7.24 (m, 1H), 7.12−7.18 (m, 3H), 7.02 (m, 1H), 6.10 (d, J = 2.3 Hz, 1H), 5.48 (s, 1H), 3.16 (d, J = 15.3 Hz, 1H), 2.83 (d, J = 15.3 Hz, 1H), 2.40 (m, 1H), 2.27 (bd, J = 15.1 Hz, 1H), 1.81 (m, 1H), 1.68−1.76 (m, 2H), 1.61 (m, 1H), 1.26 (s, 3H), 1.20 (m, 1H). HRMS for $C_{25}H_{25}F_2N_2O$ $(M + 1)^+$: calcd, 407.1935; found, 407.1896.

3-[(*R*)-[(4a*R*,5*S*)-1-(4-Fluorophenyl)-4a-methyl-4,4a,5,-6,7,8-hexahydro-1*H*-benzo[*f*]indazole-5-yl](hydroxy)methyl]-phenol (13). A solution of (3-bromophenoxy)(triisopropyl)-silane (220 mg, 0.667 mmol) in Et_2O (8 mL) was cooled to −78 °C, and *t*-BuLi (785 μL of a 1.7 M solution in pentanes, 1.335 mmol) was added dropwise by syringe. The yellow reaction was stirred for 30 min at − 78 °C, and then a solution of **9** (20.7 mg, 0.067 mmol) in THF (2 mL) was added by cannula. The reaction was stirred at −78 °C for 30 min. Isopropyl alcohol (1 mL) was added to quench the reaction at −78 °C, and the reaction was poured into saturated NH_4Cl. The mixture was extracted with EtOAc (100 mL), and the organic layer was washed with water and brine (25 mL each). The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by flash chromatography on silica gel (5 to 15% EtOAc/hexanes) gave 27.8 mg of desired product contaminated with small amounts of minor diastereomers. Further purification by chiral HPLC (AD column, 2% ethanol/heptanes) gave 14.0 mg (37%) of pure 3-[(R)-[(4a*R*,5*S*)-1-(4-fluorophenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1*H*-benzo[*f*]indazol-5-yl]{3-[(triisopropylsilyl)oxy]phenyl}methanol. R_f = 0.40 (25% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.44−7.47 (m, 3H), 7.14−7.21 (m, 3H), 6.89−6.90 (m, 2H), 6.77 (m, 1H), 6.11 (d, J = 1.9 Hz, 1H), 5.13 (d, J = 2.3 Hz, 1H), 3.16 (d, J = 15.1 Hz, 1H), 2.73 (d, J = 15.1 Hz, 1H), 2.41 (m, 1H), 2.8 (bd, J = 15.1 Hz, 1H), 1.61−1.82 (m, 4H), 1.16−1.30 (m, 7H) 1.10−1.11 (m, 18H).

The silyl ether intermediate (7.8 mg, 0.0139 mmol) was dissolved in THF (1 mL), cooled to 0 °C, and treated with TBAF (70 μL of a 1 M solution in THF, 0.0696 mmol). After 30 min, the reaction was quenched with 50 μL of HOAc. The reaction was diluted with EtOAc (25 mL) and washed with H_2O (5 mL) and brine (5 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by flash chromatography with 50% EtOAc/hexanes gave 2.0 mg (36%) of **13** as a white solid. R_f = 0.23 (40% EtOAc/hexanes). ^{1}H NMR (DMSO, 500 MHz): δ 9.32 (bs, 1H), 7.50−7.53 (m, 3 H), 7.32−7.36 (m, 2H), 7.07 (t, J = 7.8 Hz, 1H), 6.78 (s, 1H), 6.72 (d, J = 7.6 Hz, 1H), 6.56 (dd, J = 8.0, 2.0 Hz, 1 H), 6.16 (s, 1H), 4.95 (m, 2H), 3.16 (d, J = 13.6 Hz, 1H), 2.66 (d, J = 15.3 Hz, 1H), 2.26−2.33 (m, 1H), 1.60−1.72 (m, 2H), 1.51 (dd, J = 12.4, 2.5 Hz, 1H), 1.41 (bd, J = 13.5 Hz, 1H), 1.14 (s, 3H), 1.05 (m, 1H). HRMS for $C_{25}H_{26}FN_2O_2$ $(M + 1)^+$: calcd, 405.1978; found, 405.1957.

4-[(*R*)-[(4a*R*,5*S*)-1-(4-Fluorophenyl)-4a-methyl-4,4a,5,-6,7,8-hexahydro-1*H*-benzo[*f*] indazol-5-yl](hydroxy)methyl]phenol (14). Prepared in the same manner as **13** starting with (4-bromophenoxy)(triisopropyl)silane and **9**. Yield 28% (2 steps). R_f = 0.15 (40% EtOAc/hexanes). ^{1}H NMR (DMSO, 500 MHz): δ 9.14 (s, 1H), 7.50−7.52 (m, 3 H), 7.12 (d, J = 8.5 Hz, 2H), 6.69 (d, J = 8.5 Hz, 1H), 6.15 (s, 1H), 4.93 (d, J = 4.8 Hz, 1H), 4.84 (d, J = 4.2 Hz, 1H), 3.14 (d, J = 15.5 Hz, 1H), 2.67 (d, J = 15.5 Hz, 1H), 2.25−2.35 (m, 2H), 1.58−1.71 (m, 2H), 1.39−1.48 (m, 2H), 1.14 (s, 3H), 1.03 (m, 1H). HRMS for $C_{25}H_{26}FN_2O_2$ $(M + 1)^+$: calcd, 405.1978; found, 405.1969.

(*R*)-(4-Chlorophenyl)[(4a*R*,5*S*)-1-(4-fluorophenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1*H*-benzo[*f*]indazol-5-yl]-methanol (15). From **9** using 4-bromochlorobenzene. Final purification on AD chiral column (10% IPA/heptanes), yield 56%. R_f = 0.14 (25% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.44−7.46 (m, 3H), 7.29−7.33 (m, 2H), 7.27 (d, J = 9.2 Hz, 2H), 7.14−7.17 (m, 2H), 6.11 (d, J = 2.2 Hz, 1H), 5.17 (s, 1H), 3.17 (d, J = 15.1 Hz, 1H), 2.74 (d, J = 15.1 Hz, 1H), 2.40 (m, 1H), 2.28 (bd, J = 15.1 Hz, 1H), 1.89 (bs, 1H), 1.81 (m, 1H), 1.54−1.72 (m, 3H), 1.26 (s, 3H), 1.17 (m, 1H). Anal. ($C_{25}H_{24}ClFN_2O$) C, H, N.

(*R*)-(4-Fluoro-3-methoxyphenyl)[(4a*R*,5*S*)-1-(4-fluorophenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1*H*-benzo[*f*]-indazol-5-yl]methanol (16). From 9 using 4-bromo-1-fluoro-2-methoxybenzene. Final purification on AD chiral column (20% IPA/heptanes), yield 52%. R_f = 0.07 (25% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.44−7.46 (m, 3H), 7.14−7.17 (m, 2H), 7.05 (dd, J = 11.4, 8.4 Hz, 1H), 6.98 (dd, J = 7.8, 1.8 Hz, 1H), 6.84 (m, 1H), 6.12 (d, J = 2.2 Hz, 1H), 5.15 (s, 1H), 3.91 (s, 3H), 3.18 (d, J = 15 Hz, 1H), 2.75 (d, J = 15 Hz, 1H), 2.41 (m, 1H), 2.29 (bd, J = 15.0 Hz, 1H), 1.57−1.84 (m, 4H), 1.26 (s, 3H), 1.20 (m, 1H). Anal. ($C_{26}H_{26}F_2N_2O_2$) C, H, N.

(*R*)-(4-Fluoro-3-methylphenyl)[(4a*R*,5*S*)-1-(4-fluorophenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1*H*-benzo[*f*]indazol-5-yl]methanol (17). From **9** using 4-bromo-1-fluoro-2-methylbenzene. Final purification on AD chiral column (15% IPA/heptanes), yield 53%. R_f = 0.24 (25% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.43−7.45 (m, 3H), 7.10−7.16 (m, 4H), 6.97 (t, J = 8.8 Hz, 1H), 6.10 (d, J = 2.1 Hz, 1H), 5.11 (s, 1H), 3.15 (d, J = 15.1 Hz, 1H), 2.74 (d, J = 15.1 Hz, 1H), 2.40 (m, 1H), 2.28 (s, 3H), 2.27 (m, 1H), 1.81 (m, 1H), 1.65−1.72 (m, 2H), 1.59 (m, 1H), 1.25 (s, 3H), 1.19 (m, 1H). Anal. ($C_{26}H_{26}F_2N_2O$); C: calcd 74.26, found 73.00; H: calcd 6.23, found 6.29; N: calcd 6.66, found 6.45.

(*R*)-(3,4-Difluoro-5-methoxyphenyl)[(4a*R*,5*S*)-1-(4-fluorophenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1*H*-benzo[*f*]-indazol-5-yl]methanol (18). From **9** using 5-bromo-1,2-difluoro-3-methoxybenzene. Final purification by PTLC with 45/35/20 CH_2Cl_2/hexanes/Et_2O, yield 67%. R_f = 0.11 (25% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.44−7.47 (m, 3H), 7.14−7.18 (m, 2H), 6.73−6.79 (m, 2H), 6.13 (d, J = 2.3 Hz, 1H), 5.12 (d, J = 3.4 Hz, 1H), 3.92 (s, 3H), 3.18 (d, J = 14.9 Hz, 1H), 2.75 (d, J = 14.9 Hz, 1H), 2.41 (m, 1H), 2.29 (bd, J = 15.1 Hz, 1H), 1.83 (m, 1H), 1.78 (d, J = 3.9 Hz, 1H), 1.66−1.71 (m, 2H), 1.54 (m, 1H), 1.26 (s, 3H), 1.21 (m, 1H). Anal. ($C_{25}H_{25}F_2N_2O$) C, H, N.

(4-Fluorophenyl)[(4a*R*,5*S*)-1-(4-fluorophenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1*H*-benzo[*f*]indazol-5-yl]methanone (19). Compound **11** (23.0 mg, 0.057 mmol) was dissolved in CH_2Cl_2 (2 mL), cooled to 0 °C, and NMO (10 mg, 0.085 mmol) was added. After 5 min, TPAP (2 mg, 0.0057 mmol) was added. The reaction was stirred for 3 h at 0 °C and then loaded directly onto a column of silica gel. Elution with 100% CH_2Cl_2 followed by 25% EtOAc/hexanes afforded 19.2 mg (84%) of **19**. R_f = 0.34 (25% EtOAc/hexanes). LCMS = 405 $(M + 1)^+$.

(*S*)-(4-Fluorophenyl)[(4a*R*,5*S*)-1-(4-fluorophenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1*H*-benzo[*f*]indazol-5-yl]-methanol (20). Compound **19** (19.2 mg, 0.048 mmol) was dissolved in MeOH (2 mL) and cooled to 0 °C. $NaBH_4$ (10 mg, 0.238 mmol) was added and the reaction was stirred at 0 °C for 15 min. The reaction was quenched with saturated NH_4Cl (1 mL), diluted with EtOAc (25 mL), and washed with H_2O and brine (10 mL each). The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification of the residue by flash chromatography on silica gel (40% EtOAc/hexanes) followed by chiral HPLC (AD column with 12% IPA/heptanes) gave 12.6 mg (65%) of **20**. R_f = 0.16 (25% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.45 (dd, J = 9.0, 4.8 Hz, 2H), 7.40 (s, 1H), 7.32 (dd, J = 8.4, 5.4 Hz, 2H), 7.14 (t, J = 8.4 Hz, 2H), 7.04 (t, J = 8.4 Hz, 2H), 6.15 (s, 1H), 4.64 (d, J = 9.0 Hz, 1H), 3.63 (d, J = 16.2 Hz, 1H), 2.78 (d, J = 16.2 Hz, 1H), 2.27−2.29 (m, 2H), 2.07 (bs, 1H), 1.89 (m, 1H), 1.68 (m, 1H), 1.05−1.25 (m, 2H), 1.13 (s, 3H). Anal. ($C_{25}H_{24}F_2N_2O$); C: calcd 73.87, found 72.44; H: calcd 5.95, found 5.82; N: calcd 6.89, found 6.51. HRMS for $C_{25}H_{24}F_2N_2O$ $(M + 1)^+$: calcd, 407.1935; found, 407.1920.

(1*S*)-1-(4-Fluorophenyl)-1-[(4a*R*,5*S*)-1-(4-fluorophenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1*H*-benzo[*f*]indazol-5-yl]ethanol (21). Compound **19** (6.0 mg, 0.015 mmol) was dissolved in Et_2O (2 mL) and cooled to −40 °C. MeLi (93 μL of a 1.6 M solution in Et_2O, 0.15 mmol) was added, and the reaction was stirred at −40 °C for 45 min. The reaction was quenched with IPA (1 mL), poured into saturated NH_4Cl (10 mL), extracted with EtOAc (25 mL), and washed with H_2O

and brine (10 mL each). The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification of the residue by flash chromatography on silica gel (5 to 20% EtOAc/hexanes) gave 5.1 mg (82%) of **21** as a single diastereomer. R_f = 0.25 (25% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.42−7.48 (m, 4H), 7.28 (s, 1H), 7.13 (t, J = 8.4 Hz, 2H), 7.03 (t, J = 8.7 Hz, 2H), 6.09 (d, J = 1.8 Hz, 1H), 3.15 (d, J = 16.0 Hz, 1H), 2.61 (d, J = 16.0 Hz, 1H), 2.37 (m, 1H), 2.26 (bd, J = 14.6 Hz, 1H), 2.00 (dd, J = 12.0, 4.0 Hz, 1H), 1.78 (m, 1H), 1.69 (s, 1H), 1.67 (s, 3H), 1.53−1.64 (m, 2H), 1.26 (s, 3H), 1.20 (m, 1H). HRMS for $C_{26}H_{27}F_2N_2O$ (M + 1)$^+$: calcd, 421.2091; found, 421.2085.

(*R*)-(3-Thienyl)[(4a*R*,5*S*)-1-(4-fluorophenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1*H*-benzo[*f*]indazol-5-yl]methanol (23). From **9** using 3-bromothiophene. Yield 99%. R_f = 0.20 (25% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.51−7.41 (m, 2H), 7.34 (dd, J = 3.2, 3.0 Hz, 1H), 7.21−7.13 (m, 3H), 7.11 (dd, J = 5.1, 1.2 Hz, 1H), 3.16 (d, J = 15.3 Hz, 1H), 2.82 (d, J = 15.3 Hz, 1H), 2.41 (m, 1H), 2.26 (bd, J = 15.1 Hz, 1H), 1.81 (m, 1H), 1.68−1.77 (m, 2H), 1.61 (m, 1H), 1.28 (s, 3H). HRMS for $C_{23}H_{24}FN_2OS$ (M + 1)$^+$: calcd, 395.1593; found, 395.1592.

(*R*)-(3-Thienyl)[(4a*R*,5*S*)-1-(4-fluorophenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1*H*-benzo[*f*]indazol-5-yl]methanone (24). Prepared in the same manner as **19**. Yield 80%. R_f = 0.40 (25% EtOAc/hexanes). LCMS = 393 (M + 1) $^+$.

(1*S*)-1-(3-Thienyl)[(4a*R*,5*S*)-1-(4-fluorophenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1*H*-benzo[*f*]indazol-5-yl]ethanol (25). Prepared in the same manner as **21** to afford **25** as a single diastereomer. Yield 42%. R_f = 0.25 (30% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.48−7.45 (m, 2H), 7.34 (s, 1H), 7.30 (dd, J = 3.0, 2.9 Hz, 1H), 7.29 (s, 1H), 7.28−7.14 (m, 4H), 6.13 (brs, 1H), 3.29 (d, J = 16.0 Hz, 1H), 2.70 (d, J = 16.0 Hz, 1H), 2.39−2.28 (m, 2H), 2.08−2.05 (m, 2H), 1.76 (m, 1H), 1.81 (m, 1H), 1.65 (s, 3H), 1.52−1.63 (m, 2H), 1.25 (s, 3H), 1.05 (m, 1H). Anal. ($C_{24}H_{25}FN_2OS$) C, H, N.

(1*S*)-1-[(4a*R*,5*S*)-1-(4-Fluorophenyl)-4a-methyl-4,4a,-5,6,7,8-hexahydro-1*H*-benzo[*f*]indazol-5-yl]ethanol (26). From **9** using MeLi (1.6M in Et_2O) to afford **26** as a single diastereomer. Yield 76%. R_f = 0.15 (30% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.44−7.42 (m, 2H), 7.41 (s, 1H), 7.20−7.15 (m, 2H), 6.11 (s, 1H), 4.25 (brs, 1H), 3.01 (d, J = 15.4 Hz, 1H), 2.54 (d, J = 15.4 Hz, 1H), 2.41−2.30 (m, 2H), 1.98 (m, 1H), 1.82 (m, 1H), 1.61−1.31 (m, 4H), 1.21 (s, 3H), 1.12 (s, 3H). HRMS for $C_{20}H_{24}FN_2O$ (M + 1)$^+$: calcd, 327.1873; found, 327.1857.

1-[(4a*R*,5*S*)-1-(4-Fluorophenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1*H*-benzo[*f*]indazol-5-yl]ethanone (27). Prepared in the same manner as 19. Yield 60%. R_f = 0.30 (30% EtOAc/hexanes). LCMS = 326 (M + 1) $^+$.

2-[(4a*R*,5*S*)-1-(4-Fluorophenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1*H*-benzo[*f*]indazol-5-yl]propan-2-ol (28). Prepared in the same manner as **21**. Yield 56%. R_f = 0.20 (30% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.42−7.40 (m, 2H), 7.40 (s, 1H), 7.14 (dd apt t, J = 3.7 Hz, 2H), 6.13 (brd, J = 2.1 Hz, 1H), 3.59 (d, J = 15.8 Hz, 1H), 2.76 (d, J = 15.8 Hz, 1H), 2.41−2.23 (m, 2H), 1.89−1.79 (m, 2H), 1.65 (dd, J = 12.4, 3.1 Hz, 1H), 1.45−1.40 (m, 2H), 1.37 (s, 3H), 1.35 (s, 3H), 1.19 (s, 3H). HRMS for $C_{21}H_{26}FN_2O$ (M + 1)$^+$: calcd, 341.2029; found, 341.2035.

(1*R*)-1-[(4a*R*,5*S*)-1-(4-Fluorophenyl)-4a-methyl-4,4a,-5,6,7,8-hexahydro-1*H*-benzo[*f*]indazol-5-yl]-2,2-dimethylpropan-1-ol (29). Compound **9** (20.4 mg, 0.066 mmol) was dissolved in THF (5 mL) and cooled to −78 °C. *t*-BuLi (194 μL of a 1.7 M solution in pentanes, 0.33 mmol) was added, and the reaction was allowed to warm slowly to −40 °C and then poured into saturated NH_4Cl (10 mL). The mixture was extracted with EtOAc (25 mL). The organic layer was washed with H_2O and brine (10 mL each), dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification of the residue by flash chromatography on silica gel (5 to 20% EtOAc/hexanes) gave 7.0 mg (29%) of **29** as a single diastereomer. R_f = 0.19 (25% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.43−7.46 (m, 2H), 7.41 (s, 1H), 7.14 (t, J = 8.5 Hz, 2H), 6.11 (d, J = 1.6 Hz, 1H), 3.49 (s, 1H), 2.83 (d, J = 15.1 Hz, 1H), 2.45 (d, J = 15.1 Hz, 1H), 2.30−2.44 (m, 2H), 1.54−1.88 (m, 4H), 1.40 (m, 1H), 1.07 (s, 3H), 0.96 (s, 9H). Anal. ($C_{23}H_{29}FN_2O$) C, H, N.

(1*S*)-1-[(4a*R*,5*S*)-1-(4-Fluorophenyl)-4a-methyl-4,4a,-5,6,7,8-hexahydro-1*H*-benzo[*f*]indazol-5-yl]but-3-en-1-ol (30). From **9** using allylmagnesium bromide (1.0 M solution in Et_2O) to afford **30** as a single diastereomer. Yield 32%. R_f = 0.30 (20% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.44−7.40 (m, 2H), 7.40 (s, 1H), 7.14 (dd apt t, J = 3.5 Hz, 2H), 6.13 (brd, J = 2.0 Hz, 1H), 5.92 (m, 1H), 5.19 (dd, J = 16.2, 3.4 Hz, 1H), 5.05 (dd, J = 16.2, 3.4 Hz), 4.03 (m, 1H), 3.01 (d, J = 15.8 Hz, 1H), 2.76 (d, J = 15.8 Hz, 1H), 2.40−2.13 (m, 2H), 1.89−1.82 (m, 2H), 1.63 (m, 2H), 1.37 (s, 3H). HRMS for $C_{22}H_{26}FN_2O$ (M + 1)$^+$: calcd, 353.2029; found, 353.2020.

(1*S*)-1-[(4a*R*,5*S*)-1-(4-Fluorophenyl)-4a-methyl-4,4a,-5,6,7,8-hexahydro-1*H*-benzo[*f*]indazol-5-yl]pent-4-en-1-ol (31). Compound **9** (19.3 mg, 0.062 mmol) was dissolved in THF (2 mL) and cooled to 0 °C. 3-Butenylmagnesium bromide (620 μL of a 0.5 M solution in THF, 0.311 mmol) was added. The reaction was allowed to stir at 0 °C for 2 h and then poured into saturated NH_4Cl (10 mL). The mixture was extracted with EtOAc (25 mL). The organic layer was washed with H_2O and brine (10 mL each), dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification of the residue by flash chromatography on silica gel (5 to 20% EtOAc/hexanes) gave 17.0 mg (75%) of **31** as a single diastereomer. R_f = 0.21 (25% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.42−7.45 (m, 2H), 7.40 (s, 1H), 7.14 (t, J = 8.7 Hz, 2H), 6.09 (d, J = 2.3 Hz, 1H), 5.85 (m, 1H), 5.08 (dd, J = 17.2, 1.8 Hz, 1H), 5.01 (dd, J = 10.2, 1.6 Hz, 1H), 3.98 (dd, J = 8.2, 5.2 Hz, 1H), 2.95 (d, J = 15.3 Hz, 1H), 2.46 (d, J = 15.3 Hz, 1H), 2.39 (m, 1H), 2.30 (bd, J = 15.1 Hz, 1H), 2.10−2.24 (m, 2H), 1.91 (m, 1H), 1.59−1.75 (m, 3H), 1.45−1.52 (m, 2H), 1.38 (m, 1H), 1.12 (s, 3H). HRMS for $C_{23}H_{28}FN_2O$ (M + 1)$^+$: calcd, 367.2186; found, 367.2177.

(1*S*)-3-Cyclopropyl-1-[(4a*R*,5*S*)-1-(4-fluorophenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1*H*-benzo[*f*]indazol-5-yl]-propan-1-ol (32). A flask containing 4 mL of dichloroethane was cooled to 0 °C. Et_2Zn (1.24 mL of a 1 M solution in hexanes, 1.24 mmol) was added to the flask by syringe. CH_2I_2 (200 μL, 2.48 mmol) was added dropwise by syringe, and a white precipitate formed. After the reaction was stirred for 5 min, compound **31** (45.3 mg, 0.124 mmol) was added by cannula in dichloroethane (2 mL). The reaction was warmed to room temperature, stirred for 3 h, and then poured into 1 N HCl (5 mL). The mixture was extracted with EtOAc (40 mL). The organic layer was washed with H_2O, aq $NaHSO_3$, saturated $NaHCO_3$, and brine (15 mL each), dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification of the residue by flash chromatography on silica gel (5 to 20% EtOAc/hexanes) gave 28.3 mg (60%) of **32** as a single diastereomer. R_f = 0.30 (25% EtOAc/hexanes, two elutions). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.43−7.46 (m, 2H), 7.41 (s, 1H), 7.15 (t, J = 8.7 Hz, 2H), 6.10 (d, J = 2.1 Hz, 1H), 3.99 (m, 1H), 2.96 (d, J = 15.1 Hz, 1H), 2.47 (d, J = 15.1 Hz, 1H), 2.40 (m, 1H), 2.31 (bd, J = 15.1 Hz, 1H), 1.92 (m, 1H), 1.60−1.76 (m, 3H), 1.48−1.55 (m, 2H), 1.24−1.44 (m, 3H), 1.14 (s, 3H), 0.70 (m, 1H), 0.45 (m, 2H), 0.05 (m, 2H). HRMS for $C_{24}H_{30}FN_2O$ (M + 1)$^+$: calcd, 381.2342; found, 381.2331.

General Method for the Synthesis of Benzyl-Substituted Compounds 33−37. To a 0 °C solution of **9** (173.3 mg, 0.56 mmol) in THF (23 mL) was added the appropriate benzyl Grignard reagent (5 equiv of a commercially available solution in THF or Et_2O, 2.80 mmol). The reaction was stirred at 0 °C for 1 h and then poured into saturated NH_4Cl (15 mL). The mixture was extracted with EtOAc (100 mL), and the organic layer was washed with water and brine (25 mL each). The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by flash chromatography on silica gel (5 to 25% EtOAc/hexanes) gave the desired product. Small amounts of minor diastereomers were removed during the silica gel chromatography.

(1*S*)-1-[(4a*R*,5*S*)-1-(4-Fluorophenyl)-4a-methyl-4,4a,-5,6,7,8-hexahydro-1*H*-benzo[*f*] indazol-5-yl]-2-phenylethanol (33). From **9** and benzylmagnesium chloride (1 M solution in Et_2O). 74% yield. $R_f = 0.27$ (25% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.42−7.44 (m, 2H), 7.38 (s, 1H), 7.32−7.35 (m, 2H) 7.22−7.25 (m, 3H), 7.12−7.16 (m, 2H), 6.08 (d, $J = 2.3$ Hz, 1H), 4.19 (t, $J = 7$ Hz, 1H), 2.91 (d, $J = 13.3$ Hz, 1H), 2.90 (d, $J = 14.9$ Hz, 1H), 2.71 (dd, $J = 13.5$, 5.7 Hz, 1H), 2.41 (m, 1H), 2.26−2.32 (m, 2H), 1.96 (m, 1H), 1.83 (m, 1H), 1.72 (qd, $J = 12.5$, 3.2 Hz, 1H), 1.58 (dd, $J = 12.5$, 3.2 Hz, 1H), 1.39 (m, 1H), 1.12 (s, 3H). Anal. ($C_{26}H_{27}FN_2O$) C, H, N.

(1*S*)-2-(4-Fluorophenyl)-1-[(4a*R*,5*S*)-1-(4-fluorophenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1*H*-benzo[*f*]indazol-5-yl]ethanol (34). From **9** and 4-fluorobenzylmagnesium chloride (0.25 M solution in Et_2O from Rieke Metals, Inc.). 70% yield. $R_f = 0.11$ (25% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.42−7.45 (m, 2H), 7.39 (s, 1H), 7.18−7.21 (m, 2H) 7.13−7.16 (m, 2H), 7.01−7.04 (m, 2H), 6.09 (d, $J = 2.0$ Hz, 1H), 4.16 (m, 1H), 2.86−2.91 (m, 2H), 2.68 (dd, $J = 13.5$, 5.8 Hz, 1H), 2.41 (m, 1H), 2.26−2.37 (m, 2H), 1.95 (m, 1H), 1.80 (m, 1H), 1.71 (qd, $J = 13.0$, 3.2 Hz, 1H), 1.58 (m, 1H), 1.38 (m, 1H), 1.12 (s, 3H). HRMS for $C_{26}H_{27}F_2N_2O$ $(M + 1)^+$: calcd, 421.2091; found, 421.2089.

(1*S*)-1-[(4a*R*,5*S*)-1-(4-Fluorophenyl)-4a-methyl-4,4a,-5,6,7,8-hexahydro-1*H*-benzo[*f*]indazol-5-yl]-2-(4-methylphenyl)ethanol (35). From **9** and 4-methylbenzylmagnesium chloride (0.5 M solution in THF from Rieke Metals, Inc.). 47% yield. $R_f = 0.16$ (25% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.42−7.45 (m, 2H), 7.39 (s, 1H), 7.10−7.16 (m, 6H), 6.08 (d, $J = 2.0$ Hz, 1H), 4.16 (m, 1H), 2.90 (d, $J = 15.6$ Hz, 1H) 2.86 (dd, $J = 13.0$, 8.2 Hz, 1H), 2.67 (dd, $J = 13.5$, 5.7 Hz, 1H), 2.41 (m, 1H), 2.33 (s, 3H), 2.27−2.35 (m, 2H), 1.95 (m, 1H), 1.82 (m, 1H), 1.72 (qd, $J = 13.0$, 3.2 Hz, 1H), 1.58 (dd, $J = 12.3$, 3.2 Hz, 1H), 1.39 (m, 1H), 1.13 (s, 3H). HRMS for $C_{27}H_{30}FN_2O$ $(M + 1)^+$: calcd, 417.2342; found, 417.2332.

(1*S*)-2-(4-*tert*-Butylphenyl)-1-[(4a*R*,5*S*)-1-(4-fluorophenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1*H*-benzo[*f*]indazol-5-yl]ethanol (36). From **9** and 4-*tert*-butylbenzylmagnesium bromide (0.25M solution in THF). Yield 74%. $R_f = 0.16$ (25% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.42−7.45 (m, 2H), 7.35−7.38 (m, 3H), 7.13−7.18 (m, 4H), 6.09 (d, $J = 1.8$ Hz, 1H), 4.18 (m, 1H), 2.92 (d, $J = 15.3$ Hz, 1H) 2.86 (dd, $J = 13.5$, 8.7 Hz, 1H), 2.67 (dd, $J = 13.5$, 5.2 Hz, 1H), 2.42 (m, 1H), 2.29−2.36 (m, 2H), 1.95 (m, 1H), 1.83 (m, 1H), 1.74 (qd, $J = 13.5$, 3.0 Hz, 1H), 1.60 (dd, $J = 12.5$, 3.0 Hz, 1H), 1.41 (m, 1H), 1.32 (s, 9H), 1.12 (s, 3H). Anal. ($C_{30}H_{35}FN_2O$); C: calcd 78.57, found 77.55; H: calcd 7.69, found 7.71; N: calcd 6.11, found 5.83. HRMS for $C_{30}H_{35}FN_2O$ $(M + 1)^+$: calcd, 459.2812; found, 459.2811.

(1*S*)-1-[(4a*R*,5*S*)-1-(4-Fluorophenyl)-4a-methyl-4,4a,-5,6,7,8-hexahydro-1*H*-benzo[*f*]indazol-5-yl]-2-(4-methoxyphenyl)ethanol (37). From **9** and 4-methoxybenzylmagnesium chloride (0.25 M solution in THF from Rieke Metals, Inc.). Yield 37%. $R_f = 0.10$ (25% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.42−7.45 (m, 2H), 7.38 (s, 1H), 7.12−7.16 (m, 4H), 6.87 (d, $J = 8.5$ Hz, 2H), 6.08 (d, $J = 1.8$ Hz, 1H), 4.14 (m, 1H), 3.80 (s, 3H), 2.89 (d, $J = 15.3$ Hz, 1H) 2.84 (dd, $J = 13.5$, 8.5 Hz, 1H), 2.65 (dd, $J = 13.5$, 5.7 Hz, 1H), 2.40 (m, 1H), 2.27−2.31 (m, 2H), 1.94 (m, 1H), 1.81 (m, 1H), 1.71 (qd, $J = 13.5$, 3.2 Hz, 1H), 1.57 (dd, $J = 12.5$, 3.2 Hz, 1H), 1.39 (m, 1H), 1.12 (s, 3H). HRMS for $C_{27}H_{30}FN_2O_2$ $(M + 1)^+$: calcd, 433.2291; found, 433.2302.

Receptor Binding Assays. For hGRα and rMR ligand binding assays, protein was prepared from recombinant baculovirus expressed receptors. For hPR binding assays, hPR protein was made from the T-47D cell line. For all the receptors tested, frozen cell pellets were homogenized in ice cold KPO_4 buffer (10 mM KPO_4, 20 mM sodium molybdate, 1 mM EDTA, 5 mM DTT, and complete protease inhibitor tablets from Boehringer Mannheim) with a "B" plunger. The homogenates were centrifuged at 35 000*g* for 1 h at 4 °C in a JA-20 rotor. For GRα, the IC_{50}s were determined by incubating the cytosols at a final concentration of 2.5 nM [1,2,4,6,7-^{3}H]Dex in the presence of full log scale concentrations (10^{-11} to 10^{-6}) of cold Dex or the ligands at 4 °C for 24 h. Bound and free were separated by a gel filtration assay. The reaction plate was centrifuged at 1000*g* for 5 min, and the flow-through was collected in a second 96-well plate. Scintillation cocktail was added and counted in a double coincidence beta counter (Wallac). Assays for MR and PR were performed in a similar fashion with 2 nM [^{3}H]aldosterone and 5 nM [^{3}H]progesterone, respectively. The IC_{50}s were calculated assuming a single binding site sigmoidal dose−response model using the Prism software package from GraphPad.

Human Glutamine Synthetase Assay in Skeletal Muscle Cells.[26] A whole cell culture colorimetric assay was used to measure the γ-glutamyl transferase (GT) activity of GS. This assay used absorbance ($\lambda = 540$ nM) to measure GS-catalyzed conversion of L-glutamine to glutamyl-γ−hydroxamate, a salt which forms a purplish brown complex in the presence of trivalent iron.[31,32] To measure time-dependent dexamethasone induction of GS, a 96 well plate containing 40 000 cells/well (in glutamine free DMEM media containing 10% charcoal-stripped FBS and 100 units/mL penicillin) was treated with dexamethasone (100 nM) at different time points. Similarly, dose-dependent titrations of dexamethasone and prednisolone were carried out by treating the cells for 16−24 h with varying amounts of compound. At the end of the incubation period, the reaction plate was worked up by lysing the cells and measuring GS activity. The specific activity of GS is expressed as nmol/min/g of total protein. Absorbance values of the product glutamyl-γ-hydroxamate (γGH) formed in the enzyme assay were converted to nmoles of product by a calibration concentration curve obtained using commercially available γGH. The total protein in cell lysates prepared for the enzyme activity assay is measured using the Pierce Coomassie Plus Assay reagent kit based on the Bradford method of protein determination.[33] Measured protein concentration values were adjusted for cell number and volumes of dilution.

Tyrosine Amino Transferase Assay in Human HepG2 Cells. Glucocorticoid-induced TAT activity in HepG2 cells (ATCC HB-8065) was determined in a 96-well format by modification of the assay reported by Granner and Tomkins.[34] Nearly confluent, adherent cells in 96-well plates were fed fresh media (Minimum Essential Medium supplemented with 10% charcoal-treated fetal bovine serum) containing 10 μM forskolin and test compounds (0.5 μL of 400× stock in 100% DMSO; 0.25% DMSO final) were added immediately afterward. Following a 24 h incubation, the cells were lysed with 113 μL cell lysis buffer (Promega) and 30 μL of lysate added to assay cocktail (156 μL) containing 5.4 mM tyrosine, sodium salt, 10.8 mM α-ketoglutarate, and 0.06 mM pyridoxal-5′-phosphate prepared in 200 mM potassium phosphate buffer, pH 7.4. The 96-well assay plate was incubated for 3 h at 37 °C, the was assay terminated by addition of 14 μL of 10 N KOH, the plates were incubated an additional 30 min at 37 °C, and A_{340} was measured. EC_{50} values were determined from a sigmoidal dose−response fit using GraphPad Prism software. The reported % TAT activity was normalized to that of 10 μM dexamethasone ($EC_{50} = 20 + 9$ nM).

Human IL-6 Assay in A549 Lung Carcinoma Cells. One microliter of DMSO sample was mixed into 300 μL of prewarmed medium in flat-bottomed 96 deep-welled polypropylene plates. The growth medium on the plates with the cells was removed, and 100 μL of the sample solutions with the compounds/DMSO was added to the wells. The cells were incubated at 37 °C for 60 min. Media from each DMSO sample media plate was added to duplicate cellular plates. Five microliters of human TNFα was added to all wells (1 ng/mL final) except for the unstimulated controls. The media was mixed by shaking the plate for several seconds, and the cells were incubated overnight (16−20 h at 37 °C). The plates were read by standard ELISA assay (Duoset ELISA development kit).

Mouse Macrophage IL-6 Assay. Thioglycolate-elicited peritoneal exudate cells were harvested from rodents on day

3 in PBS/heparin. The cells were spun at 1200 rpm for 5 min, washed once in RPMI containing 10% FCS media, and resuspended at 5.6×10^5 cells/mL in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum and 1x pen/strep/glut. Cell suspension (180 μL/well) was plated out in 96-well flat bottom tissue culture plates. Typically, 1 μL of compound at 600 μM was added, for a final concentration of 3 μM, to the cell suspension and allowed to incubate for 1 h at 37 °C and 5% CO_2. Twenty mocroliters of 50 ng/mL solution of 10X LPS or TNF was added to the cell suspension and allowed to incubate at 37 °C, 5% CO_2 for 24 h. The supernatants were harvested and read in a Wallac Victor 2 1420 counter.

In Vivo Evaluation: Mouse LPS Challenge Assay. Female Balb/c mice (12−16 weeks old) from Taconic (Germantown, NY) were used in these studies. Mice were housed in a controlled environment, on a 12-h light/dark cycle, for at least 4 weeks prior to the studies, and were fed water and standard chow ad libitum. On the morning of the study, mice were dosed orally with the compounds prepared in 10% Tween 80 (Fisher Scientific, Fair Lawn, NJ) in water. Mice were challenged 1.5 h later by intraperitoneal injection of LPS (10 μg/mouse) and D-galactosamine (800 mg/kg, Sigma). Mice were euthanized 90 min later by CO_2 inhalation and heparinized blood collected by cardiac puncture. Plasma samples were either immediately analyzed by ELISA or frozen at −80 °C until used. TNFα was measured by standard ELISA methodology, using the DuoSet ELISA development kit from R&D Systems (Minneapolis, MN), according to the manufacturer's specifications. All experiments were approved by the Institutional Animal Care and Use Committee at Merck Research Laboratories.

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Supporting Information Available: Details of the X-ray crystallographic determinations of **10** and **25**. This material is available free of charge via the Internet at http://pubs.acs.org.

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