Nonsteroidal Selective Glucocorticoid Modulators: the Effect of C-5 Alkyl Substitution on the Transcriptional Activation/Repression Profile of 2,5-Dihydro-10-methoxy-2,2,4-trimethyl-1H-[1]benzopyrano[3,4-f]quinolines

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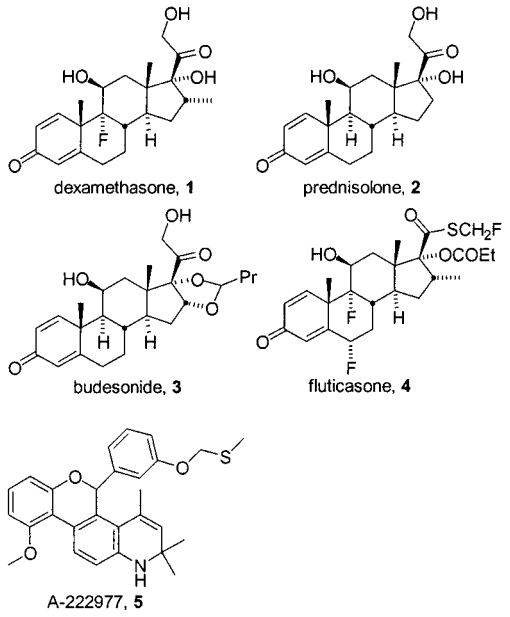
The preparation and characterization of a series of selective glucocorticoid receptor modulators are described. The preliminary structure−activity relationship of nonaromatic C-5 substitution on the tetracyclic quinoline core showed a preference for small lipophilic side chains. Proper substitution at this position maintained the transcriptional repression of proinflammatory transcription factors while diminishing the transcriptional activation activity of the ligand/glucocorticoid receptor complex. The optimal compounds described in this study were the allyl analogue **18** and cyclopentyl analogue **32**. These candidates showed slightly less potent, highly efficacious E-selectin repression with significantly reduced levels of glucocorticoid response element activation in reporter gene assays vs prednisolone. Allyl analogue **18** was evaluated in vivo. An oral dose of **18** showed an ED_{50} = 1.7 mg/kg as compared to 1.2 mg/kg for prednisolone in the Sephadex-induced pulmonary eosinophilia model and an ED_{50} = 15 mg/kg vs 4 mg/kg for prednisolone in the carrageenan-induced paw edema model.

Introduction

Oral glucocorticoids such as dexamethasone (Dex, **1**)[1] and prednisolone (Pred, **2**)[2] have long been considered some of the most potent antiinflammatory agents known, providing effective treatment for numerous musculoskeletal, respiratory, gastrointestinal, and dermatological diseases.[3] Unfortunately, a wide range of adverse events also accompanies the beneficial antiinflammatory and immunomodulating effects of glucocorticoid treatment. The frequency and severity of these deleterious effects tend to increase as dosage, length of therapy, and systemic exposure increases. This spectrum of side effects, including diabetogenesis, osteoporosis, lipid redistribution, and acute psychosis, has limited the more widespread therapeutic use of these agents. Over the last several decades, attempts to improve the therapeutic window of glucocorticoids have focused on methods of limiting systemic exposure. These include the development of topical or inhaled agents, such as budesonide (**3**),[4] or the development of "antedrugs"[5] such as fluticasone propionate (**4**)[6] that act at the site of administration but are transformed to inactive metabolites upon entry into systemic circulation.[7−14] It has only been in the last several years, however, that an understanding of the molecular mechanism by which glucocorticoids elicit their biological effects through the regulation of gene transcription via the glucocorticoid receptor (GR) has begun to evolve. Our goal is to develop structurally novel, small molecule GR modulators that mechanistically differentiate the antiinflammatory properties from the metabolic side effects of glucocorticoids.



The human glucocorticoid receptor (hGR) is a member of the intracellular hormone receptor superfamily that includes other steroid receptors such as the androgen, estrogen, mineralocorticoid, and progesterone receptors.[15−17] The endogenous glucocorticoids cortisol and cortisone are involved in a wide range of endocrine functions that include lipid, carbohydrate, and protein metabolism, stress response, and the maintenance of immunological and skeletal homeostasis.[18−20] The hGR resides predominantly in the cytosol held in an inactive

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form by a protein complex comprised of heat shock proteins (HSP 90, HSP 70) and immunophilin. Ligand binding to cytosolic hGR triggers dissociation from this chaperone protein complex and subsequent translocation to the nucleus where the GR/ligand complex (GRC) is then able to modulate gene transcription by one of several mechanisms.[21−23]

The first mode of action, transcriptional activation, occurs when the GRC homodimerizes and acts directly on DNA as a transcription factor.[24,25] The GRC homodimer recognizes and binds to chromosomal DNA at sequence-specific sites in the promoter region of regulated genes termed glucocorticoid response elements (GREs). The induction of gene transcription via the dimeric GRC has been associated with many of the undesired metabolic side effects of glucocorticoid therapy, such as glucocorticoid-induced osteoporosis,[26,27] glucose intolerance,[28] and fat redistribution.[19]

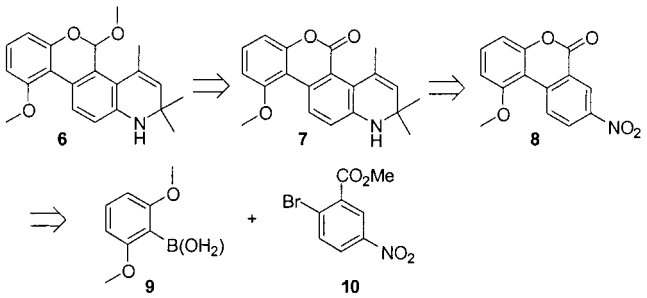
Another mechanism by which the GRC affects transcription is by direct interaction with other transcription factors[29] such as activator protein-1 (AP-1)[30−32] or nuclear factor κB (NFκB)[33,34] that results in the repression of the proinflammatory genes that they regulate. The gene products consequently down-regulated include numerous proinflammatory and matrix degrading substances such as interleukin-1 (IL-1), IL-4, IL-6, tumor necrosis factor-α (TNF-α), matrix metalloproteases, and collagenase.[34,35] This transcriptional repression is believed to be the basis of many of the antiinflammatory effects of clinical glucocorticoids.[36,37] Clinical glucocorticoid antiinflammatory agents elicit both powerful transcriptional repression and activation activities[38] giving rise to both their desired and their undesired effects, respectively. We surmised that the discovery of a nonsteroidal, small molecule ligand that selectively binds hGR and imparts an overall GRC conformation that would allow transcriptional repression activity while not engaging in GRE activation would afford a superior antiinflammatory agent with a reduced side effect profile.[33,37,39]

We have recently described the discovery and preliminary characterization of a series of 5-aryl-2,5-dihydro-10-methoxy-2,2,4-trimethyl-1H-[1]benzopyrano-[3,4-f]quinolones, such as A-222977 (**5**) as nonsteroidal, selective glucocorticoid modulators.[40] Several of these early analogues exhibited in vitro transcriptional repression and activation activity equivalent to that of commercial antiinflammatory steroids. We were ultimately unable to successfully uncouple the desired transcriptional repression from the undesired GRE activation in this series of compounds. It was clear, however, that the substitution pattern on the C-5 aryl group profoundly affected the functional activity of these compounds. We surmised that further modification at the C-5 position might allow us to dissociate the transcriptional activation properties from repression. Herein, we describe our initial investigation of the effects of C-5 aliphatic substitution of this series of compounds.

Chemistry

A detailed account of the synthesis of the 2,5-dihydro-10-methoxy-2,2,4-trimethyl-1H-[1]benzopyrano[3,4-f]-quinoline tetracyclic core has been described elsewhere.[40] Construction of the carbon framework relied on several key steps and is depicted retrosynthetically in Scheme 1. Suzuki coupling[41,42] of the sterically hindered boronic acid **9** and commercially available aryl halide **10** followed by lactone formation provided a rapid assembly of the B, C, D-ring scaffold. The A-ring was formed regioselectively in a single step by a modified Skraup annulation of the derived aniline.[43−45] Controlled reduction of coumarin **7** followed by acid-catalyzed methanolysis gave the key intermediate methyl acetal **6**.

Scheme 1



Methyl acetal **6** served as a versatile starting point for exploration of the C-5 structure−activity relationship (SAR).[46] Treatment of compound **6** with $BF_3{\cdot}Et_2O$ in a noncoordinating solvent such as dichloroethane or dichloromethane at low temperature produced a deep green activated complex that reacted with a variety of nucleophilic species including organomagnesium halides, organozincs, organostannanes, allylsilanes, and silyl ketene acetals.[47] The reaction progress was easily monitored by the colorimetric titration of the acetal−Lewis acid complex from a brilliant green to yellow color by dropwise addition of the nucleophilic species. The majority of analogues described was prepared in a single step from the activated Lewis acid complex of acetal **6**. Figure 1 shows method A that involves the addition of an ethereal solution of an alkylmagnesium halide to a preformed $BF_3{\cdot}Et_2O$−acetal complex in dichloroethane at −10 °C. This method was instrumental in the rapid construction of the C-5 alkyl SAR. Method B is a variation in which a dichloromethane solution of the desired nucleophile and methyl acetal **6** at −78 °C was treated with $BF_3{\cdot}Et_2O$ and warmed to 0 °C. The green complex formed as the temperature rose to −50 °C and was rapidly consumed upon further warming. This was the preferred method for the addition of organosilanes and organostannanes.

Treatment of acetal **6** with trimethylsilylcyanide or the *tert*-butyldimethylsilyl ketene acetal of methyl acetate[48] according to Method B gave the C-5 nitrile (**11**) and methyl acetate (**17**) derivatives in high yields, respectively. These intermediates served as convenient

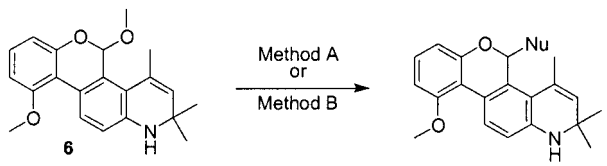
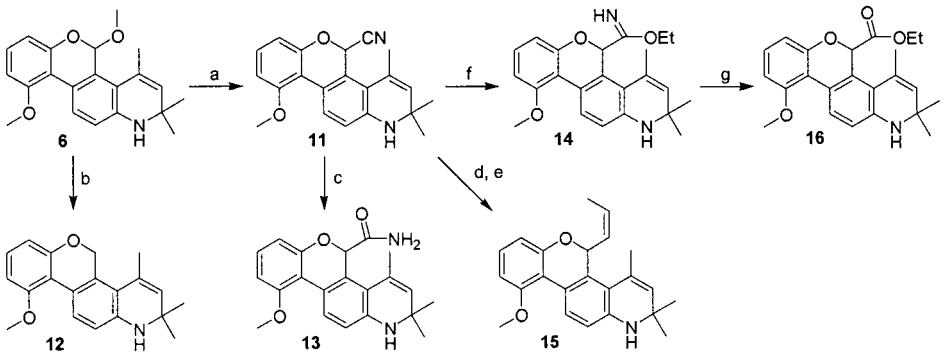


Figure 1. General methods for C-5 functionalization.

Scheme 2[a]



[a] Key: (a) TMSCN, CH_2Cl_2; then BF_3-OEt_2, −78 to 0 °C; 98%. (b) Et_3SiH, CH_2Cl_2; then BF_3-OEt_2, −78 to 0 °C; 99%. (c) KOH, aqueous ethylene glycol, 120 °C; 34%. (d) Dibal−H, CH_2Cl_2, −50 °C, 15 min. (e) $EtPPh_3Br$, *n*-BuLi, THF/Et_2O, −78 °C to room temperature; 49%, 2 steps. (f) HCl gas, EtOH/$CHCl_3$, 0 °C; 42%. (g) 5% aqueous HCl, EtOH, 0 °C to room temperature; 48%.

platforms for further functionalization. As outlined in Scheme 2, basic or acidic hydrolysis of the nitrile gave carboxamide and carboxylate analogues with direct attachment at C-5. Reduction of the nitrile with 1 equiv of Dibal-H in a noncoordinating solvent yielded an intermediate aldehyde that provided cis-fused C-5 vinyl analogues such as compound **15** upon Wittig olefination. Functional group manipulation of ester **17** as shown in Scheme 3 provided the homologated alcohol, acid, amides, and amines. Acetal **6** also efficiently reacted with Et_3SiH, allyltrimethylsilane, and (*E*)-1-propenyltributylstannane[49] using method B to give compounds **11**, **18**, and **19**, respectively.

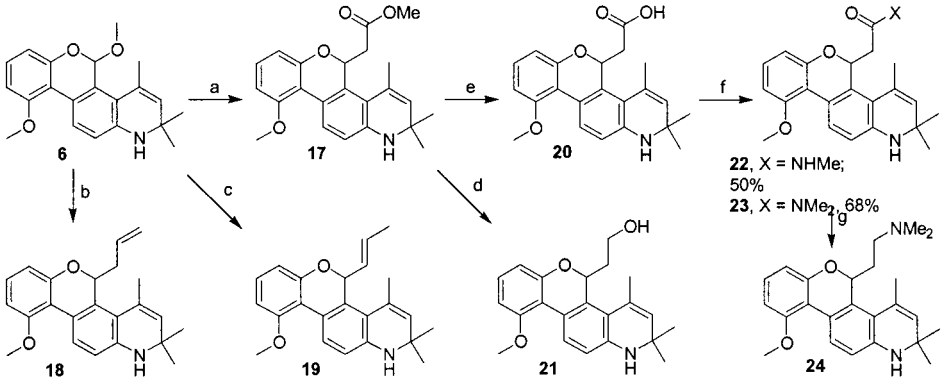
Biological Evaluation

Each compound was first evaluated for its ability to specifically bind the α-isoform of hGR in a competition-binding assay. Mindful of previous reports outlining the progesterone agonist activity of a similar 5-aryl-1,2-dihydrochromeno[3,4-f]quinoline series,[50,51] Progesterone receptor (PR) binding was monitored routinely as a measure of steroid receptor selectivity. The ability of each compound to functionally activate or repress transcription was evaluated in a cellular context using several cotransfection assays.[52,53]

In the GRE activation assay,[54] receptor plasmid contains hGR under constitutive control of the Rous sarcoma virus (RSV) long terminal repeat. The reporter plasmid contains the cDNA for firefly luciferase (LUC) under control of the mouse mammary tumor virus long terminal repeat, which is a conditional promoter containing multiple GREs. Dex and other hGR activators cause a concentration dependent increase in LUC response in the GRE cotransfection assay that can be reversed by known hGR antagonists. The maximal efficacies of compounds are reported as the percentage of the maximal response seen by Dex in the assay, and the EC_{50} values are calculated as concentration at half-maximal response.

The ability of the compounds to repress transcription was evaluated by the E-selectin cotransfection assay. The reporter plasmid in this instance uses a portion of the E-selectin promoter that contains both AP-1 and NFκB binding sites upstream of the LUC gene in HEPG2 cells. AP-1 and NFκB transcription factors are important in the up-regulation of numerous proinflammatory mediators such as IL-1, IL-6, TNF-α, matrix metalloproteases, and collagenase. Treatment of the cells with TNF-α or IL-1 induces expression of the E-selectin gene via AP-1 and NFκB, resulting in an increase in LUC expression. In the presence of repressors such as Dex, Pred, or synthetic ligands, a concentration dependent decrease in this LUC response is observed. These cotransfection assays were used to guide SAR with the goal of minimizing the GRE activation while maximizing E-selectin repression activity of these analogues. The hGR and hPR binding affinities along with the potencies and maximum ef-

Scheme 3[a]



[a] Key: (a) 1-*t*-Butilidimethylsiloxy-1-methoxyethylene, CH_2Cl_2; then BF_3-OEt_2, −78 to 0 °C; 81%. (b) Allyltrimethylsilane, CH_2Cl_2; then BF_3-OEt_2, −78 to 0 °C; 93%. (c) (*E*)-1-Propenyltrimethylstannane, CH_2Cl_2; then BF_3-OEt_2, −78 to 0 °C; 83%. (d) Dibal−H, THR, 0 °C; 87%. (e) LiOH, THF/MeOH/H_2O, 0 °C; 83%. (f) Amine, HOBT, NMO, DMF, room temperature. (g) AlH_3, Et_2O, room temperature; 54%.

Table 1. Analogues with C-5 Polar Groups Have Decreased Functional Activity[a]

		GR binding K_i (nM)	PR binding K_i (nM)	GRE activation[b] mean ± SEM		E-selectin repression[b] mean ± SEM	
Compd	R	mean ± SEM	mean ± SEM	pot (EC_{50}, nM)[c]	eff. (%dex)	pot (EC_{50}, nM)[c]	eff. (%dex)
Pred	---	2.4 ± 0.30*	-[d]	3.3 ± 0.51*	97 ± 6.6*	2.6 ± 1.1*	99 ± 0.94*
5		4.0 ± 0.84*	-	9.0 ± 0.97*	95 ± 4.0*	4.0 ± 0.89*	99 ± 2.1*
16		25 ± 14	966 ± 81	320 ± 170	33 ± 1.4	126 ± 38	64 ± 4.2
13		-	-	-	-	-	-
20		-	-	-	-	-	38
22		3200 ± 550	-	-	-	-	-
21		106 ± 50	-	-	-	-	-
23		511 ± 61	-	-	-	-	-
24		62 ± 10*	-	520	19 ± 2.0	306 ± 67	46 ± 7.0

[a] Values with standard deviation represent the mean value of two experiments with triplicate determinations; values with an asterisk represent the mean value of at least three separate experiments in triplicate with standard error (SEM); and values without standard deviation represent a single experiment in triplicate. [b] GRE activation efficacies are represented as the percentage of the maximal response of Dex. [c] All IC_{50} values were determined from full seven point, half-log concentration response curves. [d] A hyphen indicates a binding potency of >5 000 nM, a functional potency that was not calculated due to low efficacy, or a functional efficacy <20%.

ficacies in the GRE activation and E-selectin repression assays are provided in Tables 1−3 for a selection of compounds.

Results and Discussion

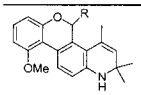
Structure−Activity Relationship. We began our investigation by probing the "C-5 pocket" with both hydrophobic and hydrophilic substituents to determine its polar surface area characteristics. In addition to defining the C-5 SAR, we hoped to improve the physiochemical properties of these very hydrophobic molecules by incorporating a hydrophilic or ionizable group at C-5. However, polar substituents such as acids (**20**), amides (**22** and **23**), alcohols (**21**), and amines (**24**) proved detrimental to both receptor binding and functional activity. Although several of these analogues display moderate receptor binding affinity, they show marked decreases in their abilities to elicit transcriptional activity. Selected examples are included in Table 1.

The C-5 unsubstituted dihydrochromene analogue **12** (R = H) also has significantly reduced receptor binding affinity and is devoid of functional activity. Lipophilic groups were systematically examined by progressive homologation. Incremental increases in chain length up to *n*-Pr, as shown in Table 2, conferred a gradual increase in GR binding affinity as well as activation and repression efficacies. Increasing steric bulk by branching, as with **29**, or increased chain length, as in compound **30**, showed a decreased GRE activation activity while maintaining E-selectin repression. Introduction of a more sterically bulky C-5 substituent, such as a benzyl group **31**, attenuated overall activity. Rigidification of the side chain by its inclusion in a ring, as with the cyclopentyl compound **32** or cyclohexyl compound **33** conveyed a further decrease in GRE activation with increasing ring size. A five member ring appeared optimal, providing a reduced level of GRE activation (38 nM, 34% eff) while maintaining E-selectin repression activity (18 nM, 92% eff). A direct comparison of C-5 *n*-propyl analogue **28** to C-5 allyl analogue **18** revealed the importance of unsaturation, contributing a 5-fold increase in E-selectin potency. Table 3 details a comparison of unsaturated C-5 substituents to determine the optimal position and degree of unsaturation in the C-5 side chain. Migration of the allyl olefin toward the core to give the C-5 vinyl analogues (**19** and **15**) resulted in reduced functional activity. One carbon homologation of the allyl group to homoallyl compound **34** or replacing allyl with a propargyl group as in compound **35** slightly lowered E-selectin repression potencies with no improvement in GRE activation. C-5 allyl compound **18** appeared optimal as a very potent, fully efficacious repressor with reduced levels of GRE activation potency and efficacy as compared to either prednisolone or the prototypic C-5 aromatic analogues such as **5**.

We next investigated the effect of the C-5 stereogenic center on the activity of these GR ligands. Separation of **18** by chiral high-performance liquid chromatography (HPLC) yielded its (−) and (+) enantiomers **36** and **37**, respectively. As in the C-5 aryl case, the (−) enantiomer is more potent and efficacious than its (+) counterpart in both receptor binding and functional assays (see Table 4). Although the (−) isomer has been correlated to the "S" absolute stereochemistry by X-ray crystallography in a closely analogous series of PR modulators,[55] we have not yet definitively assigned the absolute stereochemistry in the present case.

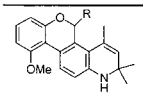
In Vivo Evaluation. We chose to evaluate analogue **18** in several in vivo models of inflammation. First, as a model of asthma, we used the Sephadex-induced lung eosinophil influx in Brown−Norway rats.[56] Animals were given intravenous injections containing Sephadex

Table 2. Optimization of C-5 Lipophilic Group[a]

		GR binding K_i (nM)	PR binding K_i (nM)	GRE activation[b] mean ± SEM		E-selectin repression[b] mean ± SEM	
Compd	R	mean ± SEM	mean ± SEM	pot (EC_{50}, nM)[c]	eff. (%dex)	pot (EC_{50}, nM)[c]	eff. (%dex)
Pred	---	2.4 ± 0.30*	-[d]	3.3 ± 0.51*	97 ± 6.6*	2.6 ± 1.1*	99 ± 0.94*
5		4.0 ± 0.84*	-	9.0 ± 0.97*	95 ± 4.0*	4.0 ± 0.89*	99 ± 2.1*
12		208 ± 75	-	-	-	-	-[d]
25		50 ± 3.6*	4300 ± 1700	-	-	15	26
26		14 ± 2.5	-	264 ± 1.3	62 ± 12	90 ± 58	78 ± 4.0
27		9.0 ± 4.7	-	146 ± 9.9	81 ± 3.0	39 ± 1.6	79 ± 2.0
28		5.0 ± 2.2	-	75 ± 0.40	92 ± 9.0	70 ± 18*	87 ± 6.0*
29		9.5 ± 4.2*	-	223 ± 38	61 ± 10	69 ± 11	87 ± 2.0
30		3.7 ± 1.2*	1700 ± 200*	405 ± 44*	35 ± 15*	122 ± 21	82 ± 0.70
31		8.1	-	-	11 ± 3.0	255	35 ± 49
32		2.1 ± .01	-	38 ± 10	34 ± 8.0	18 ± 5.4	92 ± 4.0
33		9.9 ± 1.4	-	-	6 ± 1.0	204 ± 21	73 ± 4.0
18		2.5 ± .46*	1800 ± 480*	33 ± 8.0*	68±38*	13 ± 6.3*	94 ± 2.0*

[a] Values with standard deviation represent the mean value of two experiments with triplicate determinations; values with an asterisk represent the mean value of at least three separate experiments in triplicate with standard error (SEM); and values without standard deviation represent a single experiment in triplicate. [b] GRE activation efficacies are represented as the percentage of the maximal response of Dex. [c] All IC_{50} values were determined from full seven point, half-log concentration response curves. [d] A hyphen indicates a binding potency of >5000 nM, a functional potency that was not calculated due to low efficacy, or a functional efficacy <20%.

Table 3. Effect of C-5 Unsaturation[a]

		GR binding K_i (nM)	PR binding K_i (nM)	GRE activation[b] mean ± SEM		E-selectin repression[b] mean ± SEM	
Compd	R	mean ± SEM	mean ± SEM	pot (EC_{50}, nM)[c]	eff. (%dex)	pot (EC_{50}, nM)[c]	eff. (%dex)
Pred	---	2.4 ± 0.30*	-[d]	3.3 ± 0.51*	97 ± 6.6*	2.6 ± 1.1*	99 ± 0.94*
5		4.0 ± 0.84*	-	9.0 ± 0.97*	95 ± 4.0*	4.0 ± 0.89*	99 ± 2.1*
19		9.0 ± 1.9*	2100 ± 72	400 ± 21	25 ± 0.70	144 ± 15	64 ± 6.0
15		18 ± 0.16	730 ± 220	460 ± 39	27 ± 0.70	168 ± 40	71 ± 4.0
18		2.5 ± .46*	1800 ± 480*	33 ± 8.0*	68 ± 38*	13 ± 6.3*	94 ± 2.0*
34		8.1 ± 0.04	-	78 ± 15	57 ± 13	22 ± 2.5	88 ± 3.0
35		2.7 ± 0.58*	760 ± 170	34 ± 18	88 ± 18	27 ± 4.0	90 ± 5.0

[a] Values with standard deviation represent the mean value of two experiments with triplicate determinations; values with an asterisk represent the mean value of at least three separate experiments in triplicate with standard error (SEM); and values without standard deviation represent a single experiment in triplicate. [b] GRE activation efficacies are represented as the percentage of the maximal response of Dex. [c] All IC_{50} values were determined from full seven point, half-log concentration response curves. [d] A hyphen indicates a binding potency of >5000 nM, a functional potency that was not calculated due to low efficacy, or a functional efficacy <20%.

beads that resulted in an acute inflammatory response in the lungs. The resulting lung eosinophilia was then quantified by a total and differential cell count in a BAL. The effectiveness of oral doses of our compound was determined vs prednisolone by the reduction in this cell influx as compared to vehicle control. Figure 2 outlines a dose response of **18** in this assay showing an ED_{50} = 1.7 mg/kg as compared to an ED_{50} = 1.2 mg/kg for prednisolone. We then studied the effectiveness of oral dosing of **18** in rat carrageenan-induced paw edema.[57,58] Sprague−Dawley rats were given injections of carrageenan in their hind paw that resulted in an acute inflammatory response. The resulting edema is quantified after 3 h by measuring the increase in volume of

Table 4. Activity of Optical Isomers of 18[a]

compd	sign of rotation	GR binding K_i (nM) mean ± SEM	PR binding K_i (nM) mean ± SEM	GRE activation[b] mean ± SEM pot (EC_{50}, nM)[c]	GRE activation[b] mean ± SEM eff (% Dex)	E-selectin repression mean ± SEM pot (EC_{50}, nM)[c]	E-selectin repression mean ± SEM eff (% Dex)
Pred		2.4 ± 0.30*	–[d]	3.3 ± 0.51*	97 ± 6.6*	2.6 ± 1.1*	99 ± 0.94*
18	(±)	2.5 ± 0.46*	1800 ± 480*	33 ± 8.0*	68 ± 38*	13 ± 6.3*	94 ± 2.0*
36	(−)	1.4 ± 0.62	1300 ± 430	14 ± 5.2	87 ± 16	6.9	92 ± 1.0
37	(+)	150 ± 40*	–[d]	520 ± 28	55 ± 6	450 ± 160	47 ± 9.0

[a] Values with standard deviation represent the mean value of two experiments with triplicate determinations; values with an asterisk represent the mean value of at least three separate experiments in triplicate with standard error (SEM); and values without standard deviation represent a single experiment in triplicate. [b] GRE activation efficacies are represented as the percentage of the maximal response of Dex. [c] All IC_{50} values were determined from full seven point, half-log concentration response curves. [d] A hyphen indicates a binding potency of >5000 nM, a functional potency that was not calculated due to low efficacy, or a functional efficacy <20%.

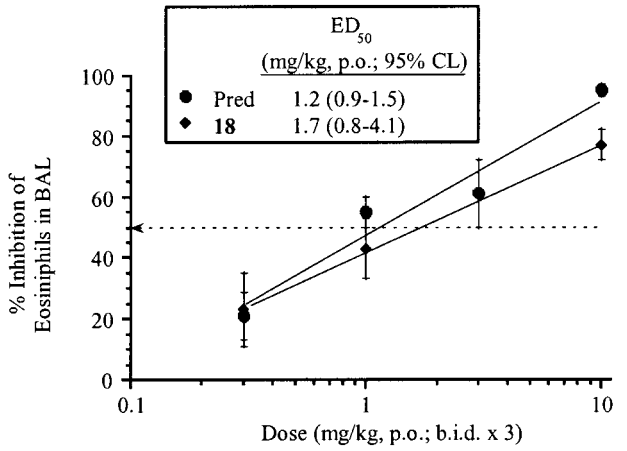


Figure 2. Compound **18** and prednisolone dose dependently inhibited Sephadex-induced lung eosinophilia in Brown–Norway rats.

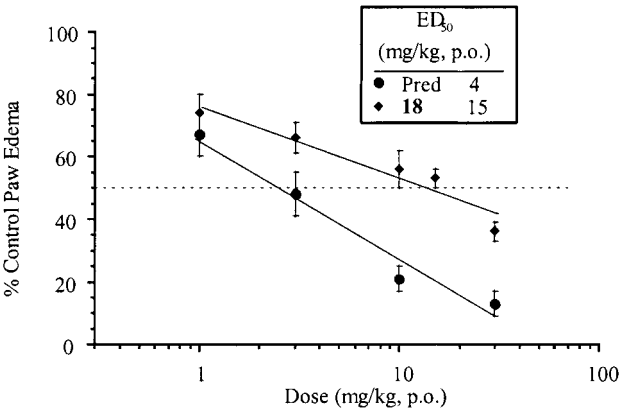


Figure 3. Prednisolone and **18** dose dependently inhibit rat carrageenan-induced paw edema.

the inflamed paw. As seen in Figure 3, the C-5 allyl analogue (**18**) dose dependently inhibited edema in this model with an ED_{50} = 15 mg/kg as compared to an ED_{50} = 4 mg/kg for prednisolone.

Conclusions

A novel series of C-5-substituted 10-methoxy-2,5-dihydro-2,2,4-trimethyl-1H-[1]benzopyrano[3,4-f]quinolines have been described that competitively and selectively bind hGR and impart varying degrees of gene transcription activity on the resulting receptor ligand complex. The C-5 substituent plays a crucial role not only in receptor binding but also in functional transcription activity of the GRC. The C-5 pocket prefers the binding of relatively small lipophilic groups, while polar groups render the analogues functionally inactive. Proper modification of the C-5 substituent has produced analogues that show the ability to differentiate the mechanistic pathways of repression of the proinflammatory transcription factors AP-1 and NFκB from the activation of GREs in cotransfection assays. The cyclopentyl and allyl analogues, **18** and **32**, respectively, were the optimal C-5 substituents in this study. Figure 4a shows the concentration response curves of the C-5 cyclopentyl compound **32** and C-5 allyl compound **18** as compared to prednisolone (**2**) for E-selectin repression. Both **18** and **32** are equipotent to prednisolone in hGR binding and are fully efficacious E-selectin repressors with a 5–10-fold decrease in potency. Figure 4b depicts the reduced propensity for GRE activation of the racemates **18** and **32** that elicited 68 and 34% of the level of GRE activation found for Dex, as compared to 99% for prednisolone. These compounds can be grouped into three classes based on their ability to activate GRE: full, partial, and low GRE activators. Structural modification at C-5 with the proper nonaromatic substituent appears promising in modulating GRE activation activity, while maintaining desired repression activity in vitro. The most potent and efficacious in vitro repressor reported here showed an oral ED_{50} value comparable to prednisolone in a rodent model of asthma and a standard model of inflammation. The level of reduction in GRE activation necessary to realize an improved metabolic side effect profile is not known. These compounds are currently being evaluated to determine if their altered GRE activation profiles correlate to fewer undesired side effects in in vivo models of inflammation. Work is also ongoing to develop a better mechanistic understanding of how these analogues interact with the hGR and how the resulting GRC interacts with the transcriptional machinery.

Experimental Section

General Methods. All reactions were carried out under inert atmosphere (N_2). Anhydrous solvents and reagents were obtained commercially and were used without further purification. All reported yields are of isolated products and are not optimized. Melting points were determined using a Thomas-Hoover melting point apparatus with a silicone oil bath and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were obtained on a Nicolet QE-300 (300 MHz), a General Electric GN-300 (300 MHz), or a Varian Unity 500 (500 MHz) instrument. Chemical shifts are reported as δ values (ppm) downfield relative to Me_4Si as an internal standard. Mass spectra determinations were performed by the Analytical Research Department, Abbott Laboratories; DCI/NH_3 indicates chemical ionization mode in the presence of ammonia. Elemental analyses were performed by Robertson Microlit Laboratories,

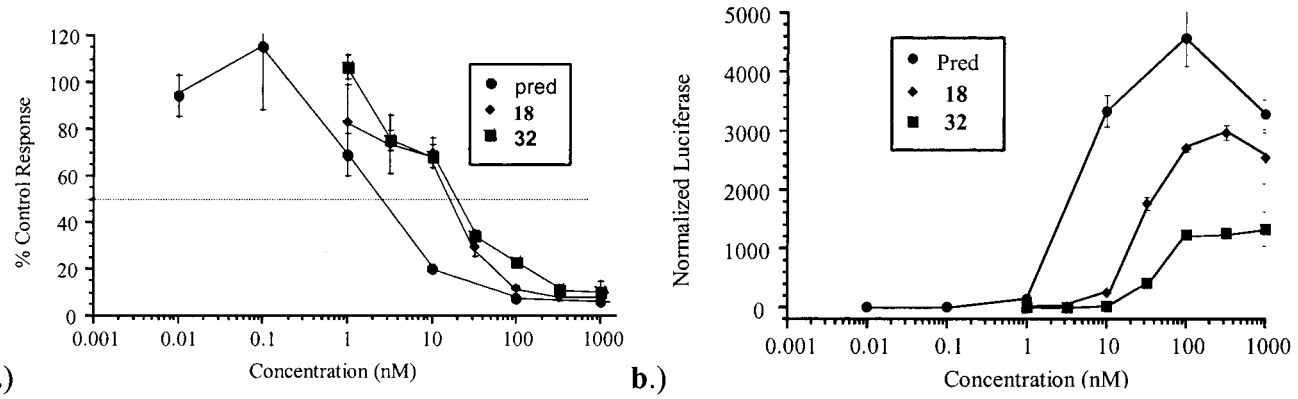


Figure 4. (a) Concentration response curve in E-selectin repression assay for prednisolone, **18**, and **32**. (b) Concentration response curve in GRE activation assay for prednisolone, **18**, and **32**. See Experimental Section for details.

Inc., Madison, NJ. Column chromatographies were carried out in flash mode on silica gel (Kieselgel 60, 230−400 mesh) from E. Merck.

2,5-Dihydro-10-methoxy-5-propyl-2,2,4-trimethyl-1H-[1]benzopyrano[3,4-f]quinoline (28). General Method A. A solution of **6** (0.030 g, 0.089 mmol) in 7 mL of dichloroethane was cooled to −10 °C and treated dropwise with $BF_3{\cdot}Et_2O$ (33 μL, 0.267 mmol). The resulting deep green solution was treated dropwise with propylmagnesium chloride (155 μL of a 2 M Et_2O solution, 0.311 mmol). At the end of the addition, the green color dissipated to give a slightly yellow solution. After the solution was stirred for 15 min at −10 °C, the reaction mixture was quenched by the addition of 5 mL of saturated aqueous $NaHCO_3$ followed by 10 mL of ethyl acetate and the layers separated. The aqueous phase was extracted with ethyl acetate, and the combined organic phases were washed with brine and dried over $MgSO_4$. Concentration gave an oily residue that was purified by silica gel chromatography eluting with 10 and then 20% ethyl acetate in hexanes to give the desired product (0.017 g, 55%) as a colorless foam: mp 55−57 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.94 (d, J = 8 Hz, 1H), 7.05 (t, J = 8 Hz, 1H), 6.69 (d, J = 8 Hz, 1H), 6.58 (d, J = 8 Hz, 1H), 6.54 (d, J = 8 Hz, 1H), 6.10 (d, J = 2 Hz, 1H), 5.70 (m, 1H), 5.44 (s, 1H), 3.85 (s, 3H), 2.16 (s, 3H), 1.70 (m, 1H), 1.43−1.31 (m, 3H), 1.16 (s, 3H), 1.14 (s, 3H), 0.83 (t, J = 7 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 156.1, 151.1, 145.4, 133.4, 132.8, 127.5, 127.1, 126.9, 116.2, 116.0, 113.0, 110.1, 105.3, 73.3, 55.5, 49.6, 34.3, 28.9, 28.8, 23.8, 18.5, 13.4. MS (DCI/NH_3) m/z: $(M + H)^+$ 350. Anal. Calcd for $C_{23}H_{27}NO_2$: C, H, N.

5-Benzyl-2,5-dihydro-10-methoxy-2,2,4-trimethyl-1H-[1]benzopyrano[3,4-f]quinoline (31). Compound **31** was prepared according to general method A using benzylmagnesium bromide (63%).

5-Cyclohexyl-2,5-dihydro-10-methoxy-2,2,4-trimethyl-1H-[1]benzopyrano[3,4-f]quinoline (33). Compound **33** was prepared according to general method A using cyclohexylmagnesium chloride (52%).

2,5-Dihydro-10-methoxy-5-methyl-2,2,4-trimethyl-1H-[1]benzopyrano[3,4-f]quinoline (25). Compound **25** was prepared according to general method A using methylmagnesium iodide (74%).

2,5-Dihydro-5-ethyl-10-methoxy-2,2,4-trimethyl-1H-[1]-benzopyrano[3,4-f]quinoline (26). Compound **26** was prepared according to general method A using ethylmagnesium chloride (73%).

2,5-Dihydro-10-methoxy-5-(2-propyl)-2,2,4-trimethyl-1H-[1]benzopyrano[3,4-f]quinoline (27). Compound **27** was prepared according to general method A using isopropylmagnesium chloride (62%).

2,5-Dihydro-10-methoxy-5-(2-methylpropyl)-2,2,4-trimethyl-1H-[1]benzopyrano[3,4-f]quinoline (28). Compound **28** was prepared according to general method A using isobutylmagnesium chloride (56%).

5-Butyl-2,5-dihydro-10-methoxy-2,2,4-trimethyl-1H-[1]-benzopyrano[3,4-f]quinoline (30). Compound **30** was prepared according to general method A using butylmagnesium chloride (83%).

5-Cyclopentyl-2,5-dihydro-10-methoxy-2,2,4-trimethyl-1H-[1]benzopyrano[3,4-f]quinoline (32). Compound **32** was prepared according to general method A using cyclopentylmagnesium chloride (63%).

2,5-Dihydro-10-methoxy-5-(3-butenyl)-2,2,4-trimethyl-1H-[1]benzopyrano[3,4-f]quinoline (34). Compound **34** was prepared according to general method A using 4-butenylmagnesium bromide (28%).

2,5-Dihydro-10-methoxy-5-(2-propynyl)-2,2,4-trimethyl-1H-[1]benzopyrano[3,4-f]quinoline (35). Compound **35** was prepared according to general method A using propargylmagnesium bromide and catalytic $HgCl_2$ (71%).[59]

2,5-Dihydro-10-methoxy-5-(2-propenyl)-2,2,4-trimethyl-1H-[1]benzopyrano[3,4-f]quinoline (18). General Method B. A mixture of **6** (13.52 g, 40.1 mmol) and allyl trimethylsilane (16.03 g, 0.140 mol) in 1 L of CH_2Cl_2 was cooled to −78 °C and treated with $BF_3{\cdot}Et_2O$ (17.2 mL, 0.140 mol) dropwise via syringe. The reaction mixture was allowed to warm to 0 °C in an ice bath. At approximately −50 °C, the solution turned a deep green color that faded upon warming. After 30 min at 0 °C, the yellow-brown mixture was poured into a rapidly stirring mixture of 500 mL of ethyl acetate and 1 L of saturated aqueous $NaHCO_3$ and stirred for 30 min and the layers separated. The aqueous phase was extracted with ethyl acetate, and the combined organic phases were washed with brine, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel chromatography eluting with 10 and then 20% ethyl acetate in hexanes to yield the desired product (13.00 g, 93%) as a colorless foam: mp 55−57 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.96 (d, J = 8 Hz, 1H), 7.07 (t, J = 8 Hz, 1H), 6.71 (d, J = 8 Hz, 1H), 6.60 (d, J = 8 Hz, 1H), 6.52 (d, J = 8 Hz, 1H), 6.12 (br s, 1H), 5.82 (m, 1H), 5.76 (dd, J = 3, 10 Hz, 1H), 5.44 (br s, 1H), 5.01 (m, 2H), 3.86 (s, 3H), 2.44 (m, 1H), 2.20 (m, 1H), 2.16 (s, 3H), 1.17 (s, 3H), 1.16 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 156.1, 150.8, 145.5, 134.2, 133.5, 132.0, 127.4, 127.1, 126.9, 124.6, 117.1, 116.2, 115.9, 113.2, 110.3, 105.4, 73.3, 55.6, 49.6, 36.4, 28.9 (2 × C), 23.9. MS (DCI/NH_3) m/z: $(M + H)^+$ 348. Anal. Calcd for $C_{23}H_{25}NO_2$: C, H, N.

The enantiomers of **18** were separated on a Chiralcel OJ 4.6 mm × 250 mm HPLC column eluting with 90:10 hexanes: ethanol at a flow rate of 1.0 mL/min to give **36**, (−) enantiomer, retention time = 6.06 min, $[\alpha]_D = -2.0°$ (c = 1.2, $CHCl_3$), and >99% ee by HPLC analysis and **37**, (+) enantiomer, retention time = 9.26 min, $[\alpha]_D = +1.9°$ (c = 1.1, $CHCl_3$), and 98% ee by HPLC analysis.

5-Cyano-2,5-dihydro-10-methoxy-2,2,4-trimethyl-1H-[1]benzopyrano[3,4-f]quinoline (11). Compound **11** was prepared according to general method B using trimethylsilyl cyanide (98%); mp 74−77 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.96 (d, J = 9 Hz, 1H), 7.20 (t, J = 8 Hz, 1H), 6.89 (d, J = 8

Hz, 1H), 6.84 (s, 1H), 6.74 (d, $J = 8$ Hz, 1H), 6.73 (d, $J = 9$ Hz, 1H), 6.46 (s, 1H), 5.51 (s, 1H), 3.90 (s, 3H), 2.22 (s, 3H), 1.29 (s, 3H), 1.09 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 156.3, 150.6, 145.9, 133.2, 127.7, 127.3, 126.8, 124.3, 117.8, 117.6, 116.0, 114.8, 112.6, 109.7, 107.3, 63.6, 55.8, 49.9, 29.8, 28.1, 22.8. MS (DCI/NH_3) *m/z*: $(M + H)^+$ 333. Anal. Calcd for $C_{21}H_{20}N_2O_2 \cdot 1/4H_2O$: C, H, N.

10-Methoxy-2,5,5-trihydro-2,2,4-trimethyl-1H-[1]benzopyrano[3,4-f]quinoline (12). Compound **12** was prepared according to general method B using triethylsilane (99%); mp 62−64 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.82 (d, $J = 8$ Hz, 1H), 7.05 (t, $J = 8$ Hz, 1H), 6.72 (dd, $J = 2$, 8 Hz, 1H), 6.58 (d, $J = 8$ Hz, 1H), 6.57 (dd, $J = 2$, 8 Hz, 1H), 6.13 (d, $J = 2$ Hz, 1H), 5.39 (t, $J = 2$ Hz, 1H), 5.10 (s, 2H), 3.84 (s, 3H), 2.02 (d, $J = 2$ Hz, 3H), 1.18 (s, 6H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 156.2, 154.4, 145.0, 131.4, 130.0, 128.1, 126.8, 126.7, 117.8, 117.0, 113.7, 112.6, 109.1, 105.7, 66.9, 55.6, 49.8, 28.8 (2 × C), 22.8. MS (DCI/NH_3) *m/z*: $(M + H)^+$ 308. Anal. Calcd for $C_{20}H_{21}NO_2 \cdot 0.1H_2O$: C, H, N.

Methyl 2,5-Dihydro-10-methoxy-2,2,4-trimethyl-1H-[1]-benzopyrano[3,4-f]quinoline-5-acetate (17). Compound **17** was prepared according to general method B using 1-*tert*-butyldimethylsilyloxy-1-methoxyethene (81%).[48] ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.01 (d, $J = 9$ Hz, 1H), 7.11 (t, $J = 8$ Hz, 1H), 6.78 (d, $J = 8$ Hz, 1H), 6.66 (d, $J = 9$ Hz, 1H), 6.53 (d, $J = 7$ Hz, 1H), 6.27 (d, $J = 8$ Hz, 1H), 6.22 (s, 2H), 5.52 (s, 1H), 3.93 (s, 3H), 3.67 (s, 3H), 2.70 (m, 1H), 2.64 (m, 1H), 2.27 (s, 3H), 1.22 (s, 6H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 170.0, 156.1, 150.4, 145.7, 133.7, 130.3, 127.2, 127.1, 116.1, 115.9, 113.5, 112.7, 110.3, 105.7, 70.8, 55.6, 51.5, 49.7, 37.3, 28.9, 28.8, 23.7. MS (DCI/NH_3) *m/z*: $(M + H)^+$ 380. Anal. Calcd for $C_{23}H_{25}O_4N \cdot 1/2H_2O$: C, H, N.

2,5-Dihydro-10-methoxy-5-[1-(*E*)-propenyl]-2,2,4-trimethyl-1H-[1]benzopyrano[3,4-f]quinoline (19). Compound **19** was prepared according to general method B using (*E*)-1-propenyltributyltin (83%).[49] ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.93 (d, $J = 8$ Hz, 1H), 7.01 (t, $J = 8$ Hz, 1H), 6.64 (d, $J = 8$ Hz, 1H), 7.61 (d, $J = 8$ Hz, 1H), 6.51 (d, $J = 8$ Hz, 1H), 6.10 (dd, $J = 2$, 4 Hz, 1H), 6.06 (d, $J = 2$ Hz, 1H), 5.58 (dd, $J = 2$, 5 Hz, 1H), 5.40 (m, 2H), 3.83 (s, 3H), 2.12 (s, 3H), 1.50 (t, $J = 6$ Hz, 3H), 1.20 (s, 3H), 1.12 (s, 3H). ^{13}C NMR (300 MHz, DMSO-d_6): δ 155.0, 152.0, 145.2, 132.0, 130.4, 130.3, 130.0, 127.8, 127.1, 126.8, 117.2, 116.9, 113.7, 113.2, 110.1, 105.2, 73.5, 55.5, 49.6, 29.4, 28.7, 23.4, 17.7. MS (FAB HRMS) *m/z*: calcd for $C_{23}H_{25}NO_2$, 347.1885; found, 347.1880.

10-Methoxy-2,5-dihydro-5-(2-ethyl-1-ol)-2,2,4-trimethyl-1H-[1]benzopyrano[3,4-f]quinoline (21). A THF solution of **17** (0.125 g, 0.330 mmol) was cooled to 0 °C and treated with a 1 M Dibal-H/toluene solution (1.0 mL, 1.0 mmol) dropwise via syringe. After 15 min, the reaction was quenched by the cautious addition of methanol (1.0 mL) and the reaction mixture was partitioned between CH_2Cl_2 and saturated aqueous potassium sodium tartrate. The organic layer was removed, washed with 1 N HCl, saturated aqueous bicarbonate, and brine, and dried over $MgSO_4$. The resulting crude product was purified by silica gel chromatography eluting with 10% ethyl acetate in CH_2Cl_2 to give **21** (0.092 g, 87%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.95 (d, $J = 8$ Hz, 1H), 7.05 (t, $J = 8$ Hz, 1H), 6.69 (d, $J = 8$ Hz, 1H), 6.59 (d, $J = 8$ Hz, 1H), 6.55 (d, $J = 8$ Hz, 1H), 610 (s, 1H), 5.95 (dd, $J = 2$, 10 Hz, 1H), 5.43 (s, 1H), 4.61 (t, $J = 6$ Hz, 1H), 3.84 (s, 3H), 3.52 (m, 1H), 2.20 (s, 3H), 1.80 (m, 1H), 1.50 (m, 1H), 1.19 (s, 3H), 1.16 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 156.2, 151.1, 145.5, 133.4, 132.8, 127.8, 127.1, 126.9, 116.2, 116.1, 113.4, 112.9, 110.2, 105.4, 70.3, 56.8, 55.6, 49.6, 35.3, 29.0, 28.7, 24.0. MS (DCI/NH3) *m/z*: $(M + H)^+$ 352. Anal. Calcd for $C_{22}H_{25}NO_3$: C, H, N.

10-Methoxy-2,5-dihydro-5-(2-ethanoicacid)-2,2,4-trimethyl-1H-[1]benzopyrano[3,4-f]quinoline (20). A solution of **17** (0.078 g, 0.21 mmol) and $LiOH \cdot H_2O$ (0.13 g, 2.0 mmol) in 10 mL of THF:CH_3OH:H_2O (1:1:1) was stirred overnight at room temperature. After the solution was adjusted to pH 4 with 2 N HCl, the reaction mixture was diluted with ethyl acetate and the layers separated. The aqueous phase was extracted with ethyl acetate, and the combined organics were washed with H_2O and brine, dried (Na_2SO_4), and concentrated. Purification by silica gel chromatography eluting with CH_2Cl_2:ethyl acetate:CH_3OH (10:10:2) gave the desired product (0.062 g, 83%); mp 140 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.3 (br s, 1H), 7.93 (d, $J = 8$ Hz, 1H), 7.04 (t, $J = 8$ Hz, 1H), 6.71 (d, $J = 8$ Hz, 1H), 6.59 (d, $J = 8$ Hz, 1H), 6.46 (d, $J = 8$ Hz, 1H), 6.16 (s, 1H), 5.45 (s, 1H), 3.86 (s, 3H), 2.60 (m, 1H), 2.43 (m, 1H), 2.20 (s, 3H), 1.16 (s, 6H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 156.1, 145.7, 133.6, 130.8, 127.4, 127.1, 115.9, 113.4, 112.9, 110.5, 105.6, 71.1, 55.6, 49.7, 37.5, 29.0, 28.8, 23.8. MS (FAB HRMS) *m/e*: calcd for $C_{22}H_{23}O_4N$, 365.1627; found, 365.1641. Anal. Calcd for $C_{22}H_{23}O_4N \cdot 3/4H_2O$: C, H, N.

***N*-Methyl-10-methoxy-2,5-dihydro-2,2,4-trimethyl-1H-[1]benzopyrano[3,4-f]quinoline-5-acetamide (22).** An 8 mL DMF solution of **20** (0.070 g, 0.19 mmol) was treated with 4-methylmorpholine (35 μL, 0.32 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.055 g, 0.28 mmol), and 1-hydroxybenzotriazole hydrate (0.044 g, 0.33 mmol) followed by methylamine (0.286 mL of a 2 M THF solution, 0.57 mmol). The reaction mixture was stirred for 16 h at room temperature and diluted with 10 mL of H_2O and 15 mL of ethyl acetate, and the layers separated. The organic phase was washed with H_2O, 2% HCl, saturated aqueous $NaHCO_3$, and brine and dried over Na_2SO_4. Purification by silica gel chromatography eluting with $CHCl_3$:CH_3OH (5:1) gave the desired product (0.036 g, 50%); mp > 200 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.95 (d, $J = 8$ Hz, 1H), 7.57 (d, $J = 5$ Hz, 1H), 7.03 (t, $J = 8$ Hz, 1H), 6.68 (d, $J = 8$ Hz, 1H), 6.58 (d, $J = 9$ Hz, 1H), 6.46 (d, $J = 7$ Hz, 1H), 6.24 (t, $J = 10$ Hz, 1H), 6.14 (s, 1H), 5.76 (s, 1H), 3.86 (s, 3H), 2.58 (m, 1H), 2.21 (s, 3H), 2.15 (m, 1H), 1.17 (s, 3H), 1.13 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 168.8, 156.2, 151.0, 145.6, 133.3, 131.4, 127.7, 127.0, 126.9, 116.2, 116.1, 113.2, 112.9, 110.4, 109.5, 105.4, 70.8, 55.5, 49.6, 38.5, 28.8, 28.6, 25.4, 23.8. MS (FAB HRMS) *m/z*: calcd for $C_{23}H_{26}O_3N_2$, 378.1943; found, 378.1952. Anal. Calcd for $C_{23}H_{26}O_3N_2 \cdot 1/4$ H2O: C, H, N.

***N,N*-Dimethyl-10-methoxy-2,5-dihydro-2,2,4-trimethyl-1H-[1]benzopyrano[3,4-f]quinoline-5-acetamide (23).** An 8 mL DMF solution of **20** (0.070 g, 0.19 mmol) was treated with 4-methylmorpholine (48 μL, 0.48 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.055 g, 0.28 mmol), and 1-hydroxybenzotriazole hydrate (0.039 g, 0.28 mmol) followed by dimethylamine (0.286 mL of a 2 M THF solution, 0.57 mmol). The reaction mixture was stirred for 16 h at room temperature and diluted with 10 mL of H_2O and 15 mL of ethyl acetate, and the layers separated. The organic phase was washed with H_2O, 2% HCl, saturated aqueous $NaHCO_3$, and brine and dried over Na_2SO_4. Purification by silica gel chromatography eluting with $CHCl_3$:CH_3OH (5:1) gave the desired product (0.052 g, 68%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.94 (d, $J = 8$ Hz, 1H), 7.04 (t, $J = 8$ Hz, 1H), 6.70 (d, $J = 8$ Hz, 1H), 6.59 (d, $J = 8$ Hz, 1H), 6.46 (d, $J = 8$ Hz, 1H), 6.26 (d, $J = 10$ Hz, 1H), 6.15 (s, 1H), 5.44 (s, 1H), 3.86 (s, 3H), 2.97−2.88 (m, 1H), 2.81 (s, 3H), 2.55 (s, 3H), 2.25 (s, 1H), 2.19 (s, 3H), 1.15 (s, 6H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 168.7, 156.1, 151.0, 145.6, 133.4, 131.5, 127.6, 127.0, 116.2, 116.1, 113.3, 113.0, 110.3, 105.5, 71.4, 55.6, 49.6, 36.6, 34.9, 28.8, 23.8. MS (FAB HRMS) *m/z*: calcd for $C_{24}H_{28}O_3N_2$, 392.2100; found, 392.2104. Anal. Calcd for $C_{24}H_{28}N_2O_3$: C, H, N.

10-Methoxy-2,5-dihydro-5-(2-*N*,*N*-dimethylethyl)-2,2,4-trimethyl-1H-[1]benzopyrano[3,4-f]quinoline (24). $LiAlH_4$ (3.00 g, 79.1 mmol) was suspended in 70 mL of Et_2O and treated dropwise via a dropping funnel at room temperature with a 30 mL ethereal solution of $ALCl_3$ (3.50 g, 26.2 mmol). The resulting mixture was stirred for 15 min to produce a 1.0 M solution of alane.

A 3 mL ethereal solution of **23** (0.031 g, 0.082 mmol) was added dropwise to a 1.0 mL aliquot of 1 M stock alane solution (1.0 mmol) at 0 °C and was allowed to warm to room temperature. The reaction mixture was stirred for 15 min and then quenched by the slow addition of 10.0 mL of H_2O followed by the dropwise addition of 15% NaOH until a thick white

paste formed. The resulting mixture was filtered through a pad of Celite rinsing with ethyl acetate and $CHCl_3$. The filtrate was diluted with ethyl acetate, washed with brine, dried (Na_2SO_4), and concentrated. Purification of the residue by silica gel chromatography eluting with CH_2Cl_2:ethyl acetate:CH_3OH (10:10:2) gave the desired product (0.016 g, 54%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.93 (d, J = 8 Hz, 1H), 7.03 (t, J = 8 Hz, 1H), 6.68 (d, J = 8 Hz, 1H), 6.54 (t, J = 8 Hz, 1H), 6.12 (s, 1H), 5.76 (m, 1H), 5.44 (s, 1H), 3.85 (s, 3H), 2.18 (s, 3H), 2.05 (s, 6H), 1.80−1.65 (m, 2H), 1.52−1.44 (m, 2H), 1.18 (s, 3H), 1.14 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 156.2, 151.1, 145.5, 133.4, 132.6, 127.7, 127.1, 127.0, 116.5, 116.1, 113.4, 113.0, 105.5, 72.0, 55.6, 55.3, 49.6, 45.3, 30.5, 28.9, 28.6, 23.6. MS (FAB HRMS) *m*/*z*: calcd for $C_{24}H_{30}O_2N_2$, 378.2307; found, 378.2307.

10-Methoxy-2,5-dihydro-5-carboxamide-2,2,4-trimethyl-1H-[1]benzopyrano[3,4-f]quinoline (13). A solution of **11** (0.035 g, 0.011 mmole) in 15 mL of ethylene glycol was treated with 0.225 mL of 4 N KOH and heated to 120 °C for 1 h. The reaction mixture was allowed to cool, quenched with 15 mL of H_2O, and adjusted to pH 4.0 with 5% aqueous HCl. The solution was extracted with ethyl acetate, and the combined extracts were washed with H_2O and brine and dried (Na_2SO_4). The concentrated residue was purified by silica gel chromatography eluting with CH_2Cl_2:ethyl acetate:CH_3OH (10:10:1) to give the desired product (0.013 g, 34%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.85 (d, J = 8 Hz, 1H), 7.36 (s, 1H), 7.27 (s, 1H), 7.03 (t, J = 8 Hz, 1H), 6.68−6.70 (m, 2H), 5.59 (d, J = 9 Hz, 1H), 6.10 (s, 1H), 6.00 (s, 1H), 5.41 (s, 1H), 3.83 (s, 3H), 2.08 (s, 3H), 1.25 (s, 3H), 1.10 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 170.9, 156.2, 151.9, 145.3, 132.6, 128.4, 127.7, 126.7, 126.6, 118.7, 116.7, 113.5, 113.4, 110.1, 105.9, 74.7, 55.6, 49.8, 29.9, 28.5, 22.7. MS (FAB HRMS) *m*/*z*: calcd for $C_{21}H_{23}O_3N_2$, 351.1709; found, 351.1714.

Ethyl 10-Methoxy-2,5-dihydro-2,2,4-trimethyl-1H-[1]-benzopyrano[3,4-f]quinoline-5-imidate Ester (14). A solution of **11** (0.075 g, 0.023 mmole) in 20 mL of 3:1 $CHCl_3$:EtOH was cooled to −10 °C and treated with dry HCl gas for 5 min. The bright yellow color of the solution dissipated. The solution was stirred for 10 min, and the flask was sealed and stored at −15 °C overnight. The mixture was concentrated, and the product was recrystallized from cold Et_2O to give the desired product (0.042 g 42%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.95 (d, J = 8 Hz, 1H), 7.31 (s, 1H), 7.05 (t, J = 8 Hz, 1H), 6.69 (t, J = 8 Hz, 2H), 6.61 (d, J = 8 Hz, 1H), 6.22 (s, 1H), 6.14 (s, 1H), 5.44 (s, 1H), 3.92−3.88 (m, 2H), 3.84 (s, 3H), 2.06 (s, 3H), 1.20 (s, 3H), 1.12 (s, 3H), 1.02 (t, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 171.1, 170.6, 156.5, 152.3, 143.4, 138.0, 133.4, 133.1, 128.9, 128.4, 127.7, 126.9, 126.7, 110.4, 110.2, 74.5, 73.8, 60.9, 55.7, 50.3, 44.9, 29.7, 28.2, 28.0, 26.5, 22.7, 22.4. MS (FAB HRMS) *m*/*z*: calcd for $C_{23}H_{26}O_3N_2$, 378.1943; found, 378.1938.

2,5-Dihydro-10-methoxy-5-[1-(*Z*)-propenyl]-2,2,4-trimethyl-1H-[1]benzopyrano[3,4-f]quinoline (15). A solution of **11** (0.63 g, 1.90 mmol) in 50 mL of CH_2Cl_2 was cooled to −42 °C and treated dropwise with 2.3 mL of 1 M Dibal-H/hexanes solution (2.30 mmol). After 15 min, the reaction mixture was treated with 5 mL of cold saturated aqueous NH_4Cl, allowed to warm to room temperature, and diluted with 150 mL of 2:1 ethyl acetate/saturated potassium sodium tartrate. After the mixture was stirred vigorously for 3 h, the layers were separated. The organic phase was washed with brine and dried (Na_2SO_4). Concentration gave 0.65 g of intermediate aldehyde as a yellow foam that was carried on without further purification.

Ethyltriphenylphosphonium iodide (0.374 g, 0.89 mmol) was suspended in 15 mL (5:3) of THF/Et_2O, cooled to −10 °C, and treated dropwise over 5 min with *n*-butyllithium (0.36 mL of a 2.5 M solution in hexanes, 0.89 mmol). The resulting yellow-orange solution was stirred for 30 min and treated dropwise with the aldehyde prepared above (0.100 g, 0.29 mmol) in 5 mL of THF. The reaction mixture was allowed to warm to room temperature, stirred overnight, quenched with saturated aqueous NH_4Cl, and diluted with ethyl ether. The mixture was filtered through a pad of Celite, the filtrate was partitioned between saturated $NaHCO_3$ and ethyl acetate, and the organic layer was washed with saturated $NaHCO_3$, H_2O, and brine and dried over Na_2SO_4. The residue was purified by silica gel chromatography eluting with 10% ethyl acetate in hexanes to give the desired compound (0.050 g, 49%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.90 (d, J = 8 Hz, 1H), 6.97 (t, J = 6 Hz, 1H), 6.67 (d, J = 8 Hz, 1H), 6.52 (d, J = 8 Hz, 1H), 6.48 (d, J = 12 Hz, 1H), 6.62 (d, J = 7 Hz, 1H), 6.10 (s, 1H), 5.63−5.59 (m, 1H), 5.41 (s, 2H), 3.83 (s, 3H), 2.08 (s, 3H), 1.79 (d, J = 7 Hz, 3H), 1.23 (s, 3H), 1.11 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 156.1, 152.4, 145.4, 132.4, 131.0, 130.2, 127.2, 127.0, 126.7, 116.9, 116.4, 113.7, 113.0, 109.9, 105.4, 69.4, 55.6, 49.7, 29.6, 28.3, 23.0, 13.8. MS (DCI/NH_3) *m*/*z*: $(M + H)^+$ 348. Anal. Calcd for $C_{23}H_{25}O_2N \cdot H_2O$: C, H, N.

Ethyl 10-Methoxy-2,5-dihydro-2,2,4-trimethyl-1H-[1]-benzopyrano[3,4-f]quinoline-5-carboxylate (16). To example **14** was added 2.0 mL of 5% HCl, 5.0 mL of H_2O, and enough ethanol to make the solution homogeneous. This was warmed at 35 °C for 1 h. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with ethyl acetate. The combined organic phases were washed with water and brine, dried (Na_2SO_4), and concentrated. The crude residue was purified by silica gel column chromatography eluting with a gradient from 15 to 67% ethyl acetate in hexanes to give 0.041 g (48%) of the desired product. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.90 (d, J = 9 Hz, 1H), 7.04 (t, J = 8 Hz, 1H), 6.64 (d, J = 8 Hz, 1H), 6.61 (m, 2H), 6.32 (s, 1H), 6.21 (s, 1H), 5.45 (s, 1H), 3.90 (m, 2H), 3.84 (s, 3H), 1.17 (s, 3H), 1.15 (s, 3H), 0.93 (t, J = 7 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.4, 156.2, 152.5, 145.4, 133.1, 127.6, 126.9, 126.0, 118.2, 117.7, 114.7, 109.8, 105.7, 73.0, 60.7, 55.6, 49.9, 28.9, 28.7, 22.8, 13.7. MS (DCI/NH_3) *m*/*z*: $(M + H)^+$ 380. Anal. Calcd for $C_{23}H_{25}O_4N \cdot 1/4H_2O$: C, H, N.

Receptor Binding Assays. Cytosol preparations of hGR-α isoform and human PR-A have been described previously.[60] Both receptor cDNAs were cloned into baculovirus expression vectors and expressed in insect SF21 cells. The GRX contains Thr-Met-Glu-Tyr-Met-Pro-Met-Glu-Asp on its N-terminus. [^{3}H]-Dex (specific activity 82−86 Ci/mmol) and [^{3}H] progesterone (specific activity 97−102 Ci/mmol) were purchased from Amersham Life Sciences (Arlington Heights, IL). Glass fiber type C multiscreen MAFC NOB plates were from Millipore (Burlington, MA). Hydroxyapatide Bio-Gel HTP gel was from Bio-Rad Laboratories (Hercules, CA). Tris(hydroxymethyl)-aminomethane (Tris), ethylenediaminetetraacetic acid (EDTA), glycerol, dithiothreitol (DTT), and sodium molybdate were obtained from Sigma Chemicals (St. Louis, MO).

Human GR-α and PR-A binding reactions were performed in Millipore multiscreen plates. For GR binding assays, [^{3}H]-Dex (∼35 000 dpm (∼0.9 nM)), GR-α cytosol (∼35 μg protein), test compounds, and binding buffer (10 mM Tris HCl, 1.5 mM EDTA, 10% glycerol, 1 mM DTT, 20 mM sodium molybdate, pH 7.6, at 4 °C) were mixed in a total volume of 200 μL and incubated at 4 °C overnight in a plate shaker. Specific binding was defined as the difference between binding of [^{3}H] Dex in the absence and in the presence of 1 μM unlabeled Dex. For PR binding assays, [^{3}H] progesterone (∼36 000 dpm (∼0.8 nM)) and PR-A cytosol (∼40 μg protein) were used. Specific binding was defined as the difference between binding of [^{3}H] progesterone in the absence and in the presence of 1 μM unlabeled progesterone. After an overnight incubation, 50 μL of hydroxyapatide (25% weight/volume) slurry was added to each well and plates were incubated for 15 min at 4 °C in a plate shaker. Plates were suctioned with a Millipore vacuum manifold, and each well was rinsed with 300 μL of ice-cold binding buffer. A total of 250 μL of Packard Microscint-20 was added to each well and shaken at room temperature for 20 min. The amount of radioactivity was determined with a Packard TopCount plate reader. IC_{50}, concentration of test compounds that inhibited 50% of specific binding, was determined from the Hill analysis of the binding curves. K_i of test compounds was determined using the Cheng−Prusoff equation.[61]

Cotransfection Assays. GRE Activation.[38,54] The cotransfection assay for GRE activation was carried out in CV-1 cells (African green monkey kidney fibroblasts), which were transiently transfected by the standard calcium phosphate coprecipitation procedure, with the plasmid containing RSV long terminal repeat. Activation activity was determined by quantifying the LUC expression (normalized response to β-gal), and the efficacy determinations were expressed relative to the maximal response produced by Dex. All cotransfection assays were automated in a 96 well plate format (Beckman Biomek automated workstation).

E-Selectin Repression. The E-selectin assay utilizes a reporter construct containing 600 bp of the promoter region fused to the LUC gene (E-sel-Luc). HepG2 cells are transfected with E-sel-Luc, an hGR expression vector driven by the RSV (RSV-hGR), and an RSV driven B-galactosidase expression vector as a transfection control. These cells are then treated with TNF-α (10 ng/mL) and IL-1 (10 ng/mL) in the absence or presence of compound. After incubation for 24 h, the cells are lysed and assayed for LUC activity.

In Vivo Assays. Sephadex-Induced Eosinophilia.[56] On the morning of day 1, male Brown−Norway rats (Charles River Laboratories), weighing between 160 and 180 g, were orally dosed with prednisolone (Sigma, St. Louis, MO), **18**, or vehicle (olive oil:carboxymethylcellulose 1:1, v/v) at a dosing volume of 2 mL/kg body weight. Immediately following the first oral dose, rats were injected intravenously in a tail vein with 1 mL of a suspension containing 0.5 mg/mL Sephadex G-200 in pyrogen free saline; negative control rats received 1 mL of pyrogen free saline. Rats were given the second dose of drug or vehicle in the afternoon on day 1. Dosing was continued twice a day for the next 2 days, and on the morning of day 4, the rats were given the seventh dose. Approximately 60 min after the last dose, rats were anesthetized with an intraperitoneal injection of 25% urethane (6 μL/g body weight). The tracheas were intubated, and the airways were lavaged with 2 × 5 mL of cold phosphate-buffered saline. The two BAL fluid aliquots were pooled, volumes were recorded, and total cell count was determined on a Z1 Coulter counter (Hialeah, FL) following erythrocyte lysis with Criterion (Riverdale, NJ). Hematology slides were prepared with 150 μL of BAL and stained with Wrights-Giemsa, and differential leukocyte numbers were determined from 100 cells based upon morphological criteria.

Carrageenan-Induced Paw Edema.[57,58] Male SD rats (150−160 g; Harlan, Indianapolis, IN) were fasted 18 h prior to experimentation. Lambda carrageenan (Sigma, St. Louis, MO; 0.1 mL of a 1% w/v solution in 0.9% pyrogen free saline) was administered subcutaneously in the right rear footpad with a 26-gauge needle attached to a 1 cm^3 syringe. Left and right hind paw volumes were measured 3 h after the challenge; left hind paws were used as negative controls for each rat. Compounds were orally administered 2 h prior to the carrageenan challenge using an olive oil:2% carboxymethylcellulose vehicle (1:1, v/v). Data are expressed as percent control edema in vehicle-dosed rats.

Supporting Information Available: Full characterization data is available for compounds prepared using general method A (compounds **25**−**28** and **30**−**35**). This information is available free of charge via the Internet at http://pubs.acs.org.

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