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Discovery of nonsteroidal glucocorticoid receptor ligands based on 6-indole-1,2,3,4-tetrahydroquinolines

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ABSTRACT

A series of nonsteroidal glucocorticoid receptor (GR) ligands based on a 6-indole-1,2,3,4-tetrahydroquinoline scaffold are reported. Structure–activity relationship (SAR) of the pendent indole group identified compound **20** exhibiting good GR binding affinity (K_i = 1.5 nM) and 100- to 1000-fold selectivity over MR, PR, and AR while showing activity in an E-selectin repression assay.

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Due to their effective anti-inflammatory and anti-proliferative properties, glucocorticoids (GCs) such as prednisolone **1** and dexamethasone **2** (Fig. 1) are widely prescribed to aid in the treatment of patients inflicted by inflammatory and autoimmune disorders.[1] However, a major drawback to the long-term use of GCs is their propensity to cause serious unwanted side effects, including myopathy, osteoporosis, hypertension, fat redistribution, and diabetes.[2] The desired therapeutic and undesired side effects are both controlled by GC binding to the glucocorticoid receptor (GR). Upon binding to the GR, the GR-ligand complex (GRC) translocates to the nucleus where it can either up-regulate or repress specific genes.[3] This transrepression (TR) of genes that encode for cytokines and other inflammatory mediators is thought to form the basis for the beneficial anti-inflammatory effects of GCs while direct transactivation (TA) leads to side effects. Compounds that display selectivity for TR over TA may provide new therapeutic agents with a reduced side effect profile, and hence continue to be an active area of research.[4–10]

In addition to TR/TA selectivity, there are also the challenges of discovering novel GR ligands that do not possess cross-reactivity with other nuclear hormone receptors (NHRs). For example, **1** and **2** are known to have mineralocorticoid receptor (MR) agonist activity, which may contribute to their hypertensive side effects. Interactions with other NHRs, such as the androgen receptor (AR) and progesterone receptor (PR) may lead to undesired activity on the prostate and uterus, respectively. Significant NHR cross-reactivity can be characteristic of new nonsteroidal GR ligands, thus optimizing receptor selectivity is of critical importance.[11]

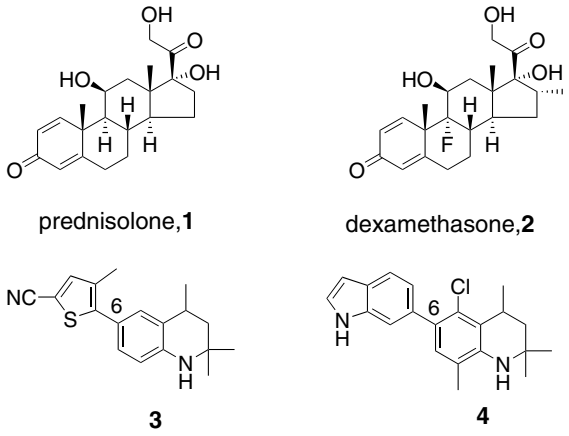

Figure 1. Steroidal glucocorticoids and 6-aryl tetrahydroquinolines.

The GR belongs to a superfamily of NHRs including estrogen (ER), MR, AR, and PR.[12] In our study of 6-thiophenylhydroquinolines as PR antagonists,[13] we reported tetrahydroquinoline **3** to be a PR-selective modulator possessing GR cross-reactivity. Subsequently, during our investigations into other NHRs, we discovered

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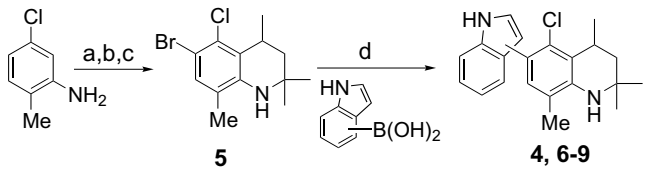
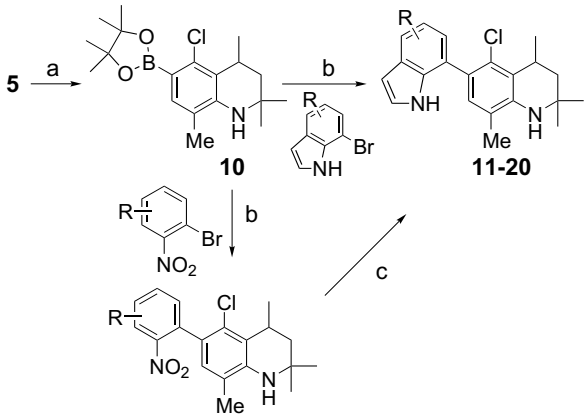


Scheme 1. Reagents and conditions: (a) Iodine, acetone, 120 °C, sealed tube; (b) Et_3SiH, 1,2-dichloroethane, trifluoroacetic acid, 80 °C; (c) *N*-bromosuccinimide, chloroform; (d) $PdCl_2$dppf, dioxane, 2 N Na_2CO_3, 100 °C.

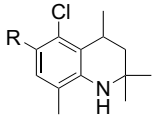


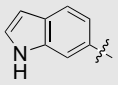
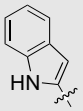
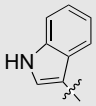
Scheme 2. Reagents and conditions: (a) 4,4,5,5-tetramethyl-1,3,2-dioxaborolane, $PdCl_2$dppf, Et_3N, dioxane 100 °C; (b) $PdCl_2$dppf, dioxane, 2 N Na_2CO_3, 100 °C; (c) vinylmagnesium bromide, THF, −40 °C.

indole **4** to possess modest GR binding affinity with diminished PR binding. We have previously demonstrated the feasibility of developing selective glucocorticoid receptor modulators (SGRMs) derived from an initial PR-selective 1*H*-[1]benzopyrano[3,4-*f*]quinoline core.[14] Herein, we disclose our initial developments of GR-selective ligands originating from a versatile PR tetrahydroquinoline template.

Due to the number of regioisomeric indole boronic acids that are readily available, we undertook a systematic SAR approach surrounding indole **4**. Starting from commercially available 5-chloro-2-methylaniline (Scheme 1), Skraup reaction[15] with catalytic iodine in acetone followed by cationic reduction of the 1,2-dihydroquinoline with triethylsilane and subsequent bromination with *N*-bromosuccinimide yielded racemic bromide **5**, which was subjected to a palladium-catalyzed Suzuki reaction with the appropriate boronic acid to generate C6-indoles **6–9** found in Table 1.

Table 1
In vitro data for regioisomeric C6-indoles[a]



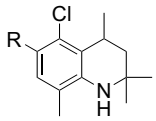
Compound[b]	R	GR binding K_i (nM)	PR binding K_i (nM)	GRE activation agonist mode		GRE activation antagonist mode		E-selectin repression assay	
				Eff. (%)	EC_{50} (nM)	Eff. (%)	IC_{50} (nM)	Eff. (%)	IC_{50} (nM)
1	Prednisolone	5.3 ± 0.3	—	130 ± 7	5.3 ± 3.6	—	—	100 ± 2	4.1 ± 0.8
3		43	8.4	—	—	97 ± 1	73 ± 14	—	—
4		460	2300	—	—	—	—	—	—
6		770	1700	—	—	—	—	—	—
7		24	450	—	—	60 ± 7	10 ± 2	—	—
8		4.4 ± 2.4	230 ± 60	34 ± 5	433 ± 20	62 ± 6	1.8 ± 1.2	—	—
9		0.6 ± 0.1	13 ± 1	110 ± 6	20 ± 3	—	—	90 ± 3	10 ± 3
	(-)-**9**	0.3	8.2 ± 1.3	89 ± 8	12 ± 2	—	—	94 ± 3	12 ± 8
	(+)-**9**	100	370	—	—	—	—	—	—

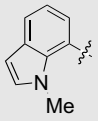
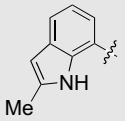
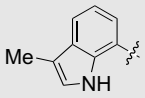
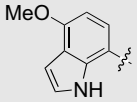
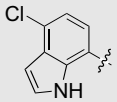
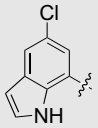
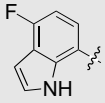
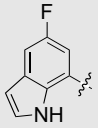
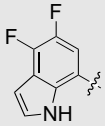
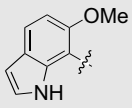
[a] EC_{50} and IC_{50} values determined from half-log concentration response curves. Agonist efficacies are represented as the percentage maximal response in comparison to dexamethasone (100%). Antagonist efficacies are represented as a percent of maximal inhibition of the response of an EC_{50} of dexamethasone. E-selectin repression efficacies are represented as a percent of maximal inhibition of the response induced by TNFα and IL-1β. Standard errors (SEM) represent the mean value of at least three separate experiments with triplicate determinations. If no SEM is noted, value is from a single determinant. M-dash (—), not active and denotes <20% efficacy or potency >1 μM.

[b] Except where noted, compounds were tested as racemates.

GR binding was determined using a radiolabeled dexamethasone competitive binding assay with baculovirus-expressed GR.[6] Aware that our tetrahydroquinoline 6-indole series originated from PR antagonists, we routinely monitored all new ligands for

Table 2
In vitro assay results for C6-indoles **11**–**20**[a]



Compound[b]	R	GR binding K_i (nM)	PR binding K_i (nM)	GRE activation agonist mode		GRE activation antagonist mode		E-selectin repression assay	
				Eff. (%)	EC_{50} (nM)	Eff. (%)	IC_{50} (nM)	Eff. (%)	IC_{50} (nM)
1	Prednisolone	5.3 ± 0.3	—	130 ± 7	5.3 ± 3.6	—	—	100 ± 2	4.1 ± 0.8
(−)-**9**		0.3	8.2 ± 1.3	89 ± 8	12 ± 2	—	—	94 ± 3	12 ± 8
11		79	650	63 ± 5	191 ± 70	—	—	68 ± 5	84
12		90	1400	57 ± 25	531 ± 20	—	—	—	—
13		11 ± 10	22	82 ± 6	7.5 ± 1.6	—	—	87 ± 11	5.3 ± 1.4
14		110	6600	—	—	88 ± 3	67 ± 20	—	—
15		131	520	—	—	78 ± 1	12 ± 4	—	—
16		24	650	—	—	94 ± 2	53 ± 12	—	—
17		1.3 ± 0.3	75 ± 18	190 ± 30	46 ± 9	—	—	79 ± 12	5 ± 1
18		0.8	15	88 ± 4	9.2 ± 3.4	—	—	103 ± 10	3.1 ± 2.4
19		1.8 ± 0.2	151 ± 30	127 ± 10	76 ± 11	—	—	106 ± 14	11 ± 3
20		1.5 ± 0.2	1400 ± 100	60 ± 7	48 ± 13	—	—	84 ± 9	33 ± 10

[a] See Table 1.
[b] Except where noted, compounds were tested as racemates.

Table 3
Cross-reactivity of selected C6-indoles[a]

Compound[b]	GR binding K_i (nM)	PR binding K_i (nM)	MR binding K_i (nM)	AR binding K_i (nM)
9	0.6 ± 0.1	13 ± 1	21	57 ± 7
17	1.3 ± 0.3	75 ± 18	32 ± 8	780
19	1.8 ± 0.2	151 ± 30	145 ± 50	50 ± 13
20	1.5 ± 0.2	1400 ± 100	155 ± 80	1500

[a] Standard errors (SEM) represent the mean value of at least three separate experiments with triplicate determinations. If no SEM is noted, value is from a single determinant.
[b] Except where noted, compounds were tested as racemates.

PR cross-reactivity. Evaluation of GR-mediated direct transcriptional activation was determined in a cotransfection (CTF) assay using a luciferase reporter containing a glucocorticoid response element (GRE).[16] The GRE activation assay determines both agonist and antagonist activity (when compounds are tested in the presence of an EC_{50} of dexamethasone) and provides an additional estimate of the ligand's affinity for GR. An E-selectin repression assay[17] was used in order to determine repression of transcriptional activation (transrepression) mediated by NFκB or AP-1, providing a measure of the potential anti-inflammatory properties of the compounds.

Testing results of indoles **4**, and **6–9** are summarized in Table 1. Regioconnectivity of the indole proved critical to both receptor selectivity and GR-mediated transcription. Attachment of the C6 indole at the 2′ (**6**) or 6′ (**4**) position showed modest GR binding affinity (K_i = 770 and 462 nM), with a slight preference for GR over PR. Improvements to both GR binding affinity and selectivity were accomplished by varying the regioconnectivity of the C6 indole via attachment to either the 3′, 4′, or 7′ positions. For example, **8** was found to be a partial antagonist in the GRE activation assay with >40-fold separation of GR over PR binding affinity. The 7′ indole **9** was shown to be a full GRE agonist with transrepression activity similar to that of **1** and good GR binding affinity (K_i = 0.6 nM); however, significant PR cross-reactivity (K_i = 13 nM) was observed.

Compound **9** exhibited full efficacy in the E-selectin repression assay suggesting that it may possess anti-inflammatory properties. Subsequently, **9** was separated via chiral HPLC and activity was found to reside in only one enantiomer.[18] Racemate **9** and the corresponding (−) enantiomer demonstrated similar GR binding affinity, GRE activation agonism, and E-selectin repression activity; while the (+) enantiomer was ∼150-fold less potent in GR binding affinity, and exhibited no GRE activation or E-selectin repression activity.

In an effort to improve the GR selectivity of **9** while maintaining its transrepression activity, we began exploring SAR around the pendent C6 indole. Racemic bromide **5** was converted into boronic ester **10**, which was utilized in one of two synthetic routes. When the appropriate 7-bromo indole was commercially available, **10** was subjected to a Suzuki reaction to directly yield compounds **11–20**. Alternatively, we employed a two-step procedure in which **10** was coupled to an aryl nitro group followed by a Bartoli reaction[19] to provide the substituted indoles (Scheme 2).

SAR of **9** (Table 2) showed that while introduction of a methyl group at N1′ (**11**) or 2′ (**12**) resulted in reduced GR binding and GRE activation, a 3′ methyl (**13**) was tolerated, albeit it showed no improvement in GR binding selectively. Larger substituents such as methoxy or chlorine at 4′ or 5′ (**14–16**) resulted in substantially reduced E-selectin activity compared to **9**. Compounds **14–16** also exhibited a switch in their GRE activation profile from agonist to antagonist. This agonist to antagonist switching is often difficult to predict. It has been noted previously that slight structural changes in GR ligands are known to affect agonist/antagonist activity.[10a,11c] Introduction of fluorine at 4′ or 5′ (**17–19**) resulted in efficacious GRE activation agonists. The 4′ fluorine analogs **17** and **19** showed improved GR selectivity (>40-fold) while maintaining comparable E-selectin repression activity to **9**. Introduction of a 6′ methoxy yielded one of the most selective ligands, **20**, appearing efficacious in E-selectin and showing ∼1000-fold separation in its binding affinity for GR over PR.

Indoles showing good activity in the E-selectin repression assay were examined for cross-reactivity against a panel of nuclear hormone receptors (Table 3). Whereas **9** showed only a modest GR selectivity profile, indole **20** exhibited high GR binding affinity (K_i = 1.5 nM) with good separation between PR (∼1000-fold) and AR (∼1000-fold), with MR showing less separation (∼100-fold).

In conclusion, we report our initial results of novel GR-selective ligands based on 6-indole-1,2,3,4-tetrahydroquinolines. Through SAR of the pendent C6 indole group, lead compound **4** was converted into GR-selective indole **20** possessing good E-selectin transrepression activity. These initial results suggest this bicyclic template may hold potential opportunities to develop GR ligands to aid in the treatment of inflammatory disorders. Further work in this series will be reported in due course.

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18. Enantiomers of compound **9** were separated on a Chiracel OJ column (2 cm × 25 cm ID) using 30% EtOH in hexanes as eluent at a flow rate of 6 mL/min. (−)-**9**, t_R = 39 min, $[\alpha]_D$ −20.7 (c = 0.27, EtOH, 25 °C), (+)-**9**, t_R = 48 min, $[\alpha]_D$ +19.3 (c = 0.27, EtOH, 25 °C). Absolute configuration of (−)-**9** and (+)-**9** has not been determined.
19. Substituted indoles were synthesized via the Bartoli reaction from the corresponding nitro compound. For example see: Dobbs, A. *J. Org. Chem.* **2001**, *66*, 638.