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Tetrahydroquinoline glucocorticoid receptor agonists: Discovery of a 3-hydroxyl for improving receptor selectivity

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ABSTRACT

We have previously disclosed a series of glucocorticoid receptor (GR) ligands derived from 6-indole-1,2,3,4-tetrahydroquinolines through structure–activity relationship (SAR) of the pendent C6-indole ring. In parallel with this effort, we now report SAR of the tetrahydroquinoline A-ring that identified the importance of a C3 hydroxyl in improving GR selectivity within a series of non-steroidal GR agonists.

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The glucocorticoid receptor (GR) belongs to the steroid receptor subfamily of nuclear hormone receptors¹ (NHRs) including the estrogen receptor (ER), mineralocorticoid receptor (MR), androgen receptor (AR), and progesterone receptor (PR). Glucocorticoids (GCs) such as prednisolone **1** and dexamethasone **2** (Fig. 1) are widely prescribed in the clinic to treat inflammatory and autoimmune disorders. Although effective therapeutic agents, the use of GCs must be managed against their propensity to induce a variety of side effects.² The transrepression (TR) of pro-inflammatory 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) of specific genes, such as those involved in gluconeogenesis, leads to their unwanted side effects.³ Discovery of GR ligands that selectively favor TR over TA has been the focus of considerable research effort and remains a difficult task.^{4–11}

In addition to TR/TA selectivity, the development of new non-steroidal GR-selective ligands possessing drug-like properties can be challenging.¹² We recently identified GR ligands based on 6-indole-1,2,3,4-tetrahydroquinolines, exemplified by **3**.¹³ Initial SAR in the series focused on the pendent C6-indole ring to improve GR selectivity while maintaining TR activity. Concurrent with this effort, we explored SAR of the tetrahydroquinoline A-ring of **3** that subsequently identified new GR-selective agonists possessing steroid-like TR activity.

GR binding was determined using a radiolabeled dexamethasone competitive binding assay with baculovirus-expressed GR.⁴ To assess the functional activity of the new ligands, GR-mediated TA was measured in a co-transfection (CTF) assay using a luciferase reporter containing a glucocorticoid response element (GRE).¹⁴ Potency (EC₅₀) was determined from half-log concentration–response curves and maximal efficacy was determined relative to dexamethasone. To evaluate TR activity of the new ligands, a CTF E-selectin repression assay in HepG2 cells was used in order to determine repression of transcriptional activation mediated by

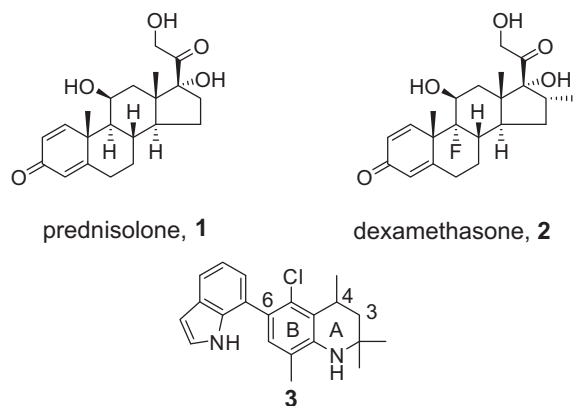
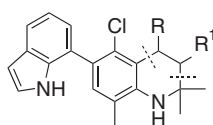


Figure 1. Steroidal glucocorticoids and 6-indole tetrahydroquinoline.

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Table 1
In vitro assay results^a

Compd ^b	R + R ¹	GR binding K _i (nM)	PR binding K _i (nM)	GRE activation		E-selectin repression assay		IL-6 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	97 ± 1	23 ± 2
3		0.6 ± 0.1	13 ± 1	110 ± 6	20 ± 3	92 ± 3	9.4 ± 2.9	67 ± 8	59 ± 23
7		5.8 ± 0.7	70	77 ± 9	235 ± 25	76 ± 11	13 ± 11	57	—
8		4.5 ± 2.2	14 ± 8	106 ± 10	95 ± 13	82 ± 7	4.7 ± 1.6	nt	nt
9		3.5 ± 0.7	25	223 ± 15	7.4 ± 0.5	123 ± 1	2.1 ± 1.9	92 ± 12	36 ± 14
10		6.0 ± 1.4	420	174 ± 36	215 ± 40	71 ± 5	18 ± 10	47 ± 7	64 ± 18
11		340	270	—	—	57 ± 8	—	nt	nt
12		1.7 ± 0.4	1200 ± 200	144 ± 6	7.1 ± 4.5	101 ± 3	1.1 ± 0.2	96 ± 5	16 ± 4
(–) 12		0.9 ± 0.1	2400 ± 700	133 ± 34	0.8 ± 0.3	79 ± 6	0.2 ± 0.03	103 ± 10	1.6 ± 0.5
(+) 12		85 ± 12	1400 ± 500	48 ± 18	483 ± 44	40 ± 7	—	56	100
13		16 ± 4	1300 ± 180	157 ± 15	105 ± 14	84 ± 4	14 ± 4	95 ± 18	37 ± 34
17		nt	nt	—	—	64 ± 6	—	—	—
19		436	3000	—	—	27 ± 5	—	49	—
20		416	1900	—	—	—	—	nt	nt
22		10 ± 3	1100 ± 150	71 ± 5	370 ± 35	69 ± 10	24 ± 8	40	—

^a EC₅₀ and IC₅₀ values determined from half-log concentration response curves. Agonist efficacies represent the percentage maximal response in comparison to dexamethasone (100%). E-selectin repression efficacies represent the percent of maximal inhibition of the response induced by TNFα and IL-1β. IL-6 repression efficacies represent the percent of maximal inhibition of the response induced by IL-1β. Standard errors (SEM) represent the mean value of at least two 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. nt, denotes not tested.

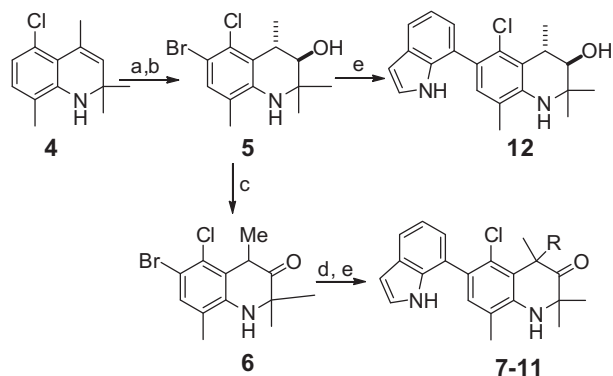
^b Except where noted, compounds were tested as racemates.

NFκB or AP-1¹⁵ while an IL-6 ELISA assay⁴ determined inflammatory cytokine repression in primary neonatal human dermal fibroblast (NHDF) cells.

Tetrahydroquinoline **3** (Table 1) is a GR agonist possessing high E-selectin and partial IL-6 repression efficacy. Although an effective ligand for GR, **3** is highly lipophilic (*c log P* = 6.9) and showed significant PR binding affinity (*K_i* = 13 nM). We sought to investigate the A-ring of **3** to minimize receptor cross-reactivity while in turn improving TR activity. In an effort to reduce the high lipophilicity of **3** the strategy of incorporating polar functionality into the A-ring was undertaken. Dihydroquinoline **4**¹³ (Scheme 1) served as a convenient starting point from which both the C3 and C4 positions of **3** could be examined. Toward this end, **4** was oxidized (borane–THF, hydrogen peroxide) followed by subsequent C6 bromination (*N*-bromosuccinimide) to yield intermediate

bromide **5**. Swern oxidation of **5** led to the formation of ketone **6** which could be alkylated with an appropriate electrophile (potassium *tert* butoxide, THF) followed by palladium-catalyzed Suzuki reaction using 1*H*-indol-7-ylboronic acid to provide C3 racemic ketone analogs **8–11**. Alternatively, Suzuki reaction of **5** or **6** generated C3 hydroxyl or ketone analog **12** and **7**, respectively.

The results of analogs **7–11** are summarized in Table 1. Introduction of a ketone at C3 (**7**) resulted in a less potent (~10-fold) and efficacious GR agonist with reduced GR binding affinity relative to **3**. Although, **7** offered an improved *c log P* (5.5) over **3**, it was inactive in the IL-6 repression assay. Incorporating substituents at the C4 position of **7** such as methyl (**8**), allyl (**9**), or prenyl (**10**) provided full agonists with single nanomolar binding affinity. GR agonist potency of **7** was enhanced by either a C4 methyl or allyl group, with **9** exhibiting an in vitro profile similar to prednisolone.



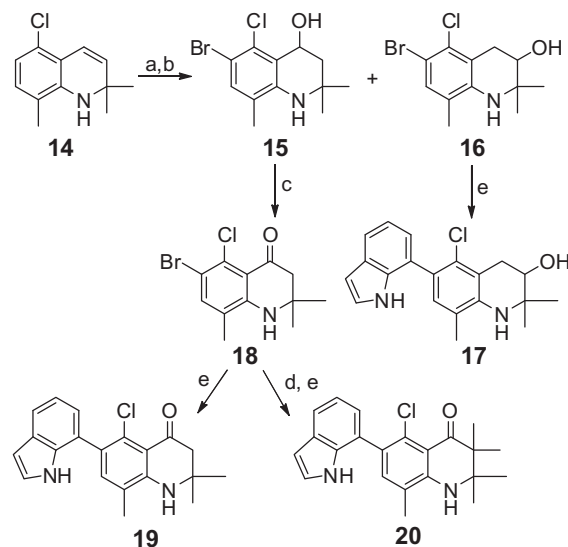
Scheme 1. Reagents and conditions: (a) borane–THF, THF 0 °C to room temperature then 2 M KOH, hydrogen peroxide; (b) *N*-bromosuccinimide, chloroform; (c) Swern oxidation; (d) potassium *tert*-butoxide, R-Br, THF; (e) 1*H*-indol-7-ylboronic acid, PdCl₂dppf, dioxane, 2 N Na₂CO₃, 100 °C.

lone in respect to its GR binding, TA and TR activity; however, significant PR cross-reactivity (K_i = 25 nM) was observed. Exchanging the allyl group of **9** for prenyl (**10**), served to diminished PR binding affinity (K_i = 420) and offered an improved GR/PR ratio, albeit, with a resulting loss in TR activity and increased lipophilicity. Benzyl analog (**11**) showed a complete loss of TA and TR activity and substantially reduced GR binding affinity (K_i = 340 nM) compared to **7**.

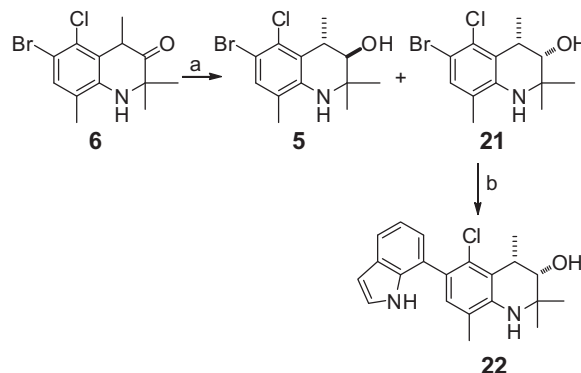
Introduction of a hydroxyl group at C3 provided alcohol **12** ($c \log P$ = 5.6) as a fully efficacious GR agonist with good binding affinity (K_i = 1.7 nM). With respect to **3**, alcohol **12** exhibited reduced (~100-fold) PR binding affinity and showed an improvement in repression of IL-6. Furthermore, this increase in both GR selectivity and TR activity is achieved with a reduction in lipophilicity. The C3 hydroxyl group of **12** may serve to impart unfavorable binding interactions within the PR, thereby, reducing unwanted PR cross-reactivity and improving GR selectivity. For example, compare ketones **7** and **8** with their respective alcohols **12** and **13**¹⁶ (Table 1). The PR binding affinity of the C3 ketones is reduced by conversion to their corresponding alcohols. Compound **12** was separated into its respective enantiomers via chiral HPLC.¹⁷ Racemate **12** and the corresponding (–) enantiomer demonstrated similar GR binding affinity, GR agonism, and TR activity; while the (+) enantiomer was ~50-fold less potent in GR binding affinity, and exhibited no TA or TR activity.

Since C3 ketones (**7–10**) were effective ligands for GR, we subsequently investigated whether a C4 ketone is similarly accepted. Hydroboration–oxidation of **14**¹⁸ (Scheme 2) followed by bromination provided racemic alcohols **15** and **16** as a readily separable mixture (1.5:1, respectively). Alcohol **15** was oxidized to the corresponding C4 intermediate ketone **18** and used to synthesize analogs **19** and **20**, whereas **16** was subjected to a Suzuki reaction using 1*H*-indol-7-ylboronic acid to provide **17**. Both ketones **19** and **20** showed modest GR binding affinity (K_i = 436 nM and 416 nM, respectively) with neither possessing any TA or TR activity (Table 1). Comparing **20** to **8** suggests a preference for the C3 ketone regioisomer.

An examination of **17**, which is devoid of GR activity and inactive in both TR assays with respect to **12**, suggests the importance of the C4 methyl for activity. The C3 hydroxyl and C4 methyl of **12** possess a *trans* relationship. To investigate whether this orientation was preferred, we synthesized the corresponding *cis* alcohol **22** from sodium borohydride reduction¹⁹ of ketone **6** followed by Suzuki reaction of racemic **21** as depicted in Scheme 3. While *cis* alcohol **22** maintained selectivity for GR (K_i = 10 nM) over PR (K_i = 1100 nM), reduced GR agonist activity and complete loss in repression of IL-6 was observed relative to *trans* alcohol **12**.



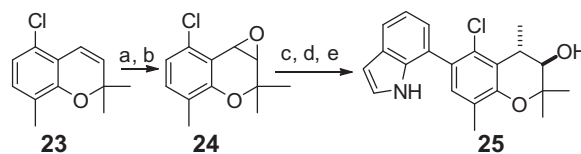
Scheme 2. Reagents and conditions: (a) borane–THF, THF 0 °C to room temperature then 2 M KOH, hydrogen peroxide; (b) *N*-bromosuccinimide, chloroform; (c) pyridinium chlorochromate, sodium acetate, CH₂Cl₂; (d) potassium *tert*-butoxide, iodomethane, THF; (e) 1*H*-indol-7-ylboronic acid PdCl₂dppf, dioxane, 2 N Na₂CO₃, 100 °C.



Scheme 3. Reagents and conditions: (a) sodium borohydride, methanol 23 °C; (b) 1*H*-indol-7-ylboronic acid, PdCl₂dppf, dioxane, 2 N Na₂CO₃, 100 °C.

To probe the importance of the N1 nitrogen in the tetrahydroquinoline A-ring, we synthesized racemic alcohol **25** from chromene **23**²⁰ as depicted in Scheme 4. In contrast to **12**, the tetrahydrochroman analog **25** showed no TA or TR activity (data not shown) but did maintain affinity for GR (K_i = 18 nM) over PR (K_i = 128 nM).

Selected new ligands were examined for their cross-reactivity against a panel of steroid hormone receptors (Table 2). In general, a significant improvement to the ligand's cross-reactivity profile was observed by the addition of a C3 hydroxyl group. The C3 hydroxyl did not enhance GR binding affinity relative to **3**, but



Scheme 4. Reagents and conditions: (a) *N*-bromosuccinimide, DMSO, water; (b) potassium hydroxide, diethyl ether; (c) methylolithium, diethyl ether; (d) bromine, sodium hydrogencarbonate, chloroform; (e) 1*H*-indol-7-ylboronic acid, PdCl₂dppf, dioxane, 2 N Na₂CO₃, 100 °C.

Table 2Cross-reactivity for selected new ligands^a

Compd ^b	GR binding K_i (nM)	PR binding K_i (nM)	MR binding K_i (nM)	AR binding K_i (nM)
3	0.6 ± 0.1	13 ± 1	21	57 ± 7
12	1.7 ± 0.4	1200 ± 200	800 ± 140	900 ± 200
13	16 ± 4	1300 ± 180	890	915
22	10 ± 3	1100 ± 150	1200 ± 100	400 ± 170

^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.

served to diminish off-target receptor (PR, MR, AR) binding, thus improving GR selectivity within the series. For example, **12** showed good GR selectivity over PR (~700-fold), MR (~470-fold), and AR (~530-fold).

In summary, we report our results based on A-ring SAR of 6-indole tetrahydroquinoline **3** which identified **12** as a selective non-steroidal GR agonist with TR activity (E-selectin and IL-6) similar to prednisolone. Polar functionality was successfully incorporated into the A-ring via addition of a C3 hydroxyl group, which resulted in commitment improvements in receptor cross-reactivity, IL-6 repression activity, and lipophilicity with respect to **3**. Further SAR of this bicyclic 6-indole tetrahydroquinoline series focusing on improvements in hepatic microsomal stability and subsequent demonstration of in vivo efficacy will follow.

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