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Discovery of orally available tetrahydroquinoline-based glucocorticoid receptor agonists

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ABSTRACT

A series of tetrahydroquinoline derivatives were synthesized and profiled for their ability to act as glucocorticoid receptor selective modulators. Structure–activity relationships of the tetrahydroquinoline B-ring lead to the discovery of orally available GR-selective agonists with high in vivo activity.

Glucocorticoids (GCs) such as prednisolone **1** and dexamethasone **2** (Fig. 1) are effective treatments for the signs and symptoms of inflammation.[1,2] However severe side-effects associated with chronic GC treatment, which include osteoporosis and hyperglycemia, can limit long-term use.[3] The glucocorticoid receptor (GR) is a member of the nuclear receptor superfamily that includes steroid hormone receptors androgen (AR), estrogen (ER), mineralocorticoid (MR) and progesterone (PR).[4] Upon ligand binding, GR is able to modulate gene transcription by directly binding to specific DNA sequences within genes. Such transcriptional activation (TA) is thought to be important for the regulation of glucose homeostasis in the liver and may be responsible for several side-effects associated with chronic GC use.[5,6] Alternatively, the receptor can directly repress transcription by modulating the activity of inflammatory mediators including NFκB and AP-1, the basis for the beneficial anti-inflammatory activity of GCs. Identifying GCs that are able to separate transrepression (TR) from transactivation (TA) is an active area of interest.[7–13]

We have previously disclosed a series of tetrahydroquinoline-based non-steroidal glucocorticoid receptor agonists.[14] Preliminary optimization of both the C-6 aryl group and tetrahydroquinoline A-ring lead to compound **3**, a non-steroidal GC agonist which was highly selective for GR and demonstrated in vitro activity similar to prednisolone (Fig. 1).

A liability of compound **3** that rendered it unsuitable for in vivo profiling was poor hepatic microsomal stability. Compound **3** underwent rapid metabolism upon incubation with both human (HLM) or rat liver microsomes (RLM) exhibiting a short half life of <5 min.[15] Here-in we describe SAR studies focused on the B-ring of **3** in an effort to improve microsomal stability while retaining high TR activity and GR selectivity. Such studies would help

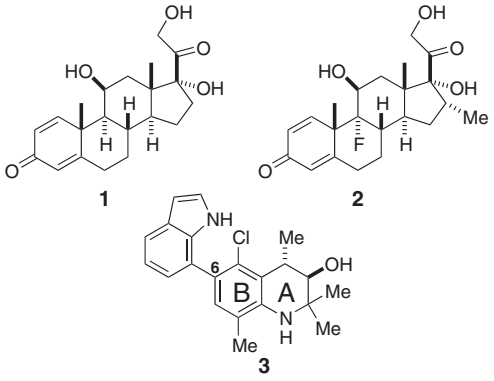

Figure 1. Steroidal and tetrahydroquinoline-based glucocorticoids.

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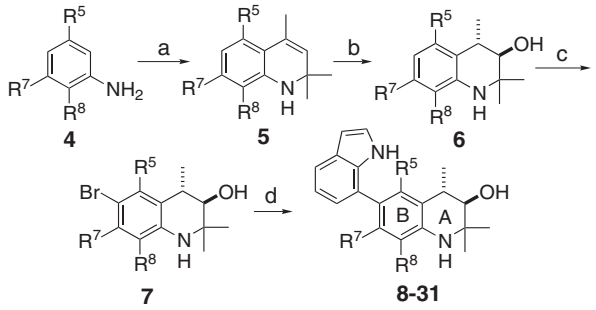


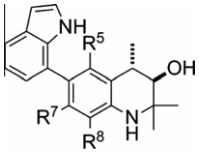
Scheme 1. Representative synthetic route. Reagents and conditions: (a) Iodine, acetone, 120 °C, sealed tube, 15 h; (b) BH_3-THF, THF, then KOH, H_2O_2; (c) NBS, $CHCl_3$; (d) $Pd(PPh_3)_4$, 1*H*-indol-7-ylboronic acid, 2:1 PhMe/EtOH, 2 N Na_2CO_3, 100 °C, 15 h.

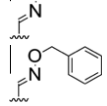
evaluate the suitability of the tetrahydroquinoline-based scaffold for further SAR investigation and to aid profiling of representative analogs in vivo.

The GR-mediated activity of the compounds was evaluated in a number of biological assays. GR binding was determined using a radiolabeled dexamethasone competitive binding assay with baculovirus-expressed GR.[16] Direct TA by GR was measured in a cotransfection (CTF) assay using an MMTV:luciferase reporter.[17] TR activity was determined using a CTF E-selectin[18] repression assay in HepG2 cells to determine repression of transcriptional activation mediated by NFκB or AP-1.[15] Compounds were also profiled in an IL-6 ELISA assay[12] to determine inflammatory cytokine repression in primary neonatal human dermal fibroblast (NHDF) cells as a further measurement of TR activity.

Compounds within the series were synthesized as depicted in Scheme 1. Skraup reaction[19] of substituted aniline **4** followed by hydroboration-oxidation gave racemic tetrahydroquinoline **6** as a single regioisomer. C-6 bromination followed by Suzuki coupling with 1*H*-indol-7-ylboronic acid gave the desired analogs **8–31**.[20] Starting from a variety of substituted anilines **4** we were able to access analogs with varied B-ring substitution patterns.

Table 1
In vitro assay results for selected glucocorticoid receptor modulators[a]



Compd	R^5	R^7	R^8	GR binding K_i (nM)	GRE activation agonist mode		E-Selectin repression		IL-6 repression	
					EC_{50} (nM)	Eff. (%)	IC_{50} (nM)	Eff. (%)	IC_{50} (nM)	Eff. (%)
1	Prednisolone			5.3 ± 0.3	5.3 ± 0.6	129 ± 6.5	4.1 ± 0.8	100 ± 1.4	23 ± 2.6	97 ± 0.7
3	Cl	H	Me	1.7 ± 0.4	7.1 ± 4.8	144 ± 5.9	1.1 ± 0.2	95 ± 6.8	7.8 ± 1.8	81 ± 2.7
8	H	H	Me	9.0	108 ± 34	161 ± 29	21 ± 13	83 ± 0.1	—	—
9	F	H	Me	2.3 ± 0.3	56 ± 33	123 ± 14	5.9 ± 2.1	88 ± 4.6	7.1 ± 1.1	75 ± 5.4
10	Me	H	Me	1.2 ± 0.2	0.9 ± 0.6	97 ± 11	4.7 ± 2.2	103 ± 2.1	9.3 ± 4.0	97 ± 1.1
11	Et	H	Me	11.7	16 ± 1.0	169 ± 25	15 ± 3.2	93 ± 0.1	—	—
12		H	Me	319	105 ± 45	160 ± 49	11 ± 1.1	82 ± 10	—	—
13		H	Me	6.9	0.4 ± 0.2	120 ± 4.6	1.7 ± 1.1	107 ± 1.8	4.2 ± 2.5	94 ± 3.0
14		H	Me	1.5	0.2 ± 0.1	130 ± 20	0.9 ± 0.4	106 ± 2.3	3.4 ± 1.1	95 ± 2.0
15		H	Me	6.0	34	122	5.5 ± 1.7	95 ± 4.7	3.8	68
16		H	Me	3.5	3.7 ± 1.7	127 ± 3.3	3.1 ± 1.2	94 ± 6.7	14.4	51
17		H	Me	19.6	40 ± 12	152 ± 29	—	—	—	—
18		H	Me	16.6	5.7 ± 0.9	120 ± 1.3	6.5 ± 0.5	95 ± 8.1	16 ± 15	78 ± 14
19	CN	H	Me	1.3	9.6 ± 3.1	173 ± 18	5.3 ± 0.8	96 ± 1.2	16 ± 2.2	64 ± 5.9
20		H	Me	10.4	112 ± 40	168 ± 62	7.8 ± 7.1	84 ± 0.9	—	—
21	H	Cl	Me	1.8	36 ± 17	134 ± 17	21 ± 19	96 ± 3.5	26 ± 9.4	82 ± 4.8
22	H	F	Me	0.9 ± 0.8	40 ± 29	162 ± 29	1.7 ± 0.3	97 ± 1.1	4.1 ± 1.7	64 ± 6.3
23	H	Me	Me	38.3	205 ± 92	165 ± 45	42 ± 19	79 ± 1.1	—	—
24	H	CF_3	Me	106	—	—	—	—	—	—
25	H	$CHCH_2$	Me	3.1	18 ± 0.6	110 ± 12	13 ± 4.2	91 ± 1.4	—	—
26	Cl	H	F	8.0	89 ± 17	126 ± 7.7	17 ± 1.6	86 ± 3.0	14	56
27	H	F	F	2.4	11 ± 4.6	193 ± 49	7.7 ± 1.3	95 ± 1.3	20 ± 0.1	44 ± 2.9
28	F	F	F	5.0	16 ± 10	156 ± 17	3.8 ± 1.0	89 ± 3.8	19 ± 3.1	68 ± 4.6
29	F	F	Cl	1.0	1.3 ± 0.1	139 ± 7.6	3.9 ± 1.3	97 ± 2.8	16 ± 3.0	52 ± 6.6
30	Cl	Cl	H	157	480 ± 0.4	100 ± 8.3	301 ± 38	72 ± 0.1	—	—
31	F	F	H	2.5 ± 1.0	0.6 ± 0.2	138 ± 16	1.5 ± 0.7	100 ± 2.7	11 ± 5.6	90 ± 2.3

[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%). E-selectin repression efficacies are represented as a 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 three separate experiments with triplicate determinations. If no SEM is noted, value is from a single determinant. A hyphen (—) = not active and denotes <20% efficacy or potency >1 μM.

Initially we assessed the importance of the C-5 chlorine atom of **3**. Analog **8** ($R^5 = R^7 = H$, $R^8 = Me$) exhibited greatly diminished activity in both the GRE activation and E-selectin assay (Table 1). Furthermore, the compound had no measurable IL-6 repression activity. In contrast R^5-substituted analogs **9** ($R^5 = F$), **10** ($R^5 = Me$) and **11** ($R^5 = Et$) demonstrated similar IL-6 repression activity to compound **3**. However both **10** ($R^5 = Me$) and **11** ($R^5 = Et$) lead to the generation of distinct interconverting atropisomers.[21] In an effort to eliminate atropisomers we turned our attention to less sterically demanding R^5 groups that reduced the propensity to restrict rotation around the biaryl axis. Skraup reaction product **5** ($R^5 = Cl$, $R^7 = H$, $R^8 = Me$) underwent aryl cyanation[22] followed by hydroboration–oxidation and subsequent nitrile reduction to yield aldehyde **6** ($R^5 = CHO$ $R^7 = H$, $R^8 = Me$) which was reacted with a number of hydroxylamines to probe steric tolerance at R^5. Oxime analog **12** was inactive in the IL-6 TR assay despite activity in the E-selectin assay. Methyl-(**13**) and ethyl-(**14**) oxime analogs were fully efficacious in both E-selectin and IL-6 repression assays with potencies superior to steroidal GC prednisolone. These compounds also avoided the added complication of atropisomerism. Bulkier *O*-substituted oximes such as *t*-Bu (**15**) or benzyl (**16**) were fully efficacious and potent in the E-selectin assay but showed reduced efficacy in the repression of IL-6 (68% and 51%, respectively). Aldehyde **7** ($R^5 = CHO$ $R^7 = H$, $R^8 = Me$) underwent reaction with a variety of phosphonium salts to introduce unsaturation at R^5.[23] Terminal alkene **17** showed no TR activity. Conjugated nitrile **18** had activity comparable to nitrile **19** whereas α,β-unsaturated ketone **20** was inactive in the IL-6 repression assay. Throughout the SAR study of the R^5 position microsomal stability was monitored and found to be poor. Only nitrile analog **19** exhibited a marginally longer half-life compared to compound **3** (Table 2). Furthermore, incremental improvements in compound potency upon increasing steric bulk at R^5 came at the expense of substantial increases in molecular weight and compound lipophilicity.

We turned our attention to examine substitution at the R^7 position of the tetrahydroquinoline core. Switching the position of the B-ring chlorine atom from R^5 to R^7 (**21**) lead to retention of high GR-mediated activity. Analog **22** ($R^7 = F$) was fully efficacious and potent in the E-selectin assay but had diminished activity in the repression of IL-6, consistent with previous analogs lacking substitution at R^5. Other R^7-substituted analogs **23** ($R^7 = Me$), **24** ($R^7 = CF_3$) and **25** ($R^7 = CH = CH_2$) had no activity in the IL-6 repression assay suggesting limited potential for further modification. Importantly however both **21** and **22** showed improvement in RLM and HLM (Table 2) compared to their R^5-substituted counterparts.

Replacement of R^8 methyl group with fluorine (**26**) lead to substantially reduced TR activity, particularly in the IL-6 repression assay. Microsomal stability half-life for compound **26** was also low (Table 2). R^8-F analogs **27** and **28** also showed reduced efficacy in the repression of IL-6. R^8-Cl analog **29** was fully efficacious in E-selectin but showed only partial efficacy (52%) in the IL-6 repression assay. Removal of the R^8-Me group altogether lead to regioisomeric products[24] of the modest yielding Skraup reaction requiring separation by preparative HPLC and was not pursued further.

Table 2
Rat and human microsomal stability data for select compounds[15]

Compd	RLM $t_{1/2}$ (min)	HLM $t_{1/2}$ (min)
3	3.3	4.7
19	13	13
21	25	21
22	16	17
26	3.8	15
31	30	48

One solution to this synthetic difficulty was to limit ourselves to symmetrical anilines. We speculated that substitution at R^7 in conjunction with R^5 should improve microsomal stability while maintaining high GR-mediated activity. Skraup reaction of 3,5-difluoro- or 3,5-dichloroaniline gave the corresponding dihydroquinoline product **5** ($R^5 = R^7 = F$ or Cl) in acceptable yield (35% and 44%, respectively) which were subsequently processed to the target compounds (Scheme 1). Compound **30** ($R^5 = R^7 = Cl$) showed very weak GR-mediated activity. In contrast **31** ($R^5 = R^7 = F$) was fully efficacious and potent in the E-selectin repression assay with activity in the IL-6 repression assay similar to prednisolone (Table 1). Importantly the stability of **31** was substantially improved (Table 2) over earlier analogs.

We had previously described how relatively minor structural changes within the scaffold can greatly alter hormone receptor selectivity, thus cross-reactivity was closely monitored throughout.[12] Several compounds described within this study bound with low nM potency to GR (Table 1). Generally alterations in the B-ring had modest effects on receptor selectivity. Compound **31** retained high receptor selectivity with >500 fold separation in binding to GR ($K_i = 2.5$ nM) over PR (2100 nM), MR (1520 nM) or AR (1430 nM). Analogs such as **21** ($R^7 = Cl$) showed more pronounced binding affinity to PR (190 nM) but separation in binding affinities remained >100 fold.

High TR activity and GR selectivity coupled with improved microsomal stability rendered **31** a suitable candidate for in vivo profiling. A single-dose PK study of **31** demonstrated systemic exposure upon oral administration in SD rats (30 mg/kg, AUC = 35 ± 3.3 μg h/mL, C_{max} 3.6 ± 0.6 μg/mL, apparent $t_{1/2} = 3.2 \pm 0.7$ h). Compound **31** was subsequently profiled in a carrageenan-induced paw edema (CPE) inflammation model.[25] Acute edema was induced by injection of pro-inflammatory agent carrageenan into the hind paw of a rat. Compound **31** was given orally and paw volume measured 3 h later as an indication of anti-inflammatory activity (Fig. 2). Compound **31** was fully efficacious and potent in this model showing reduction in paw swelling back to no edema levels with an ED_{50} of 6.6 mg/kg.

In conclusion SAR studies centered around the tetrahydroquinoline B-ring lead to the identification of non-steroidal GCs that maintained high TR activity and GR selectivity but improved hepatic microsomal stability. Substitution at R^5 was important for GR-mediated activity while derivatization at R^7 necessary for improved microsomal stability. In vivo characterization of representative analog **31** via oral administration demonstrated full efficacy in an acute model of inflammation with carrageenan-induced swelling fully reversed after 3 h. Future studies that characterize TR and TA activities based upon this chemical scaffold will follow.

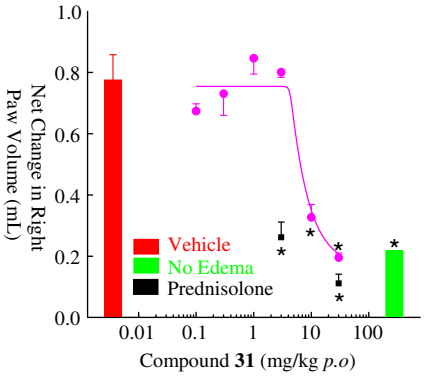


Figure 2. In vivo anti-inflammatory activity of compound **31** in a CPE model.*P <0.05 versus vehicle. Compound **31** ED_{50} 6.6 mg/kg (95% confidence level ED_{50} 4.7—9.2 mg/kg).

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15. Rat (CD-1, male) and human (mixed gender) liver microsomes were purchased from BD Gentest (Franklin Lakes, NJ). Liver microsomal incubations were conducted in triplicate. The incubation mixtures consisted of liver microsomes (0.5 mg microsomal protein/mL), test article (1 μM), $MgCl_2$ (3 mM), alamethicin (50 μg/mg microsomal protein), NADPH-generating system (1 mM NADP, 5 mM glucose-6-phosphate, 1 U/mL glucose-6-phosphate dehyrogenase), and UDPGA (5 mM) in a total volume of 0.5 mL potassium phosphate buffer (15 mM, pH 7.4). The metabolic reaction was initiated by the addition of cofactor (NADPH-generating system and UDPGA) and carried out in a 37 °C water bath. At 0, 5, 10, and 20 min after incubation, a 60 μL aliquot of the sample was withdrawn. The enzyme reaction was stopped by mixing the 60 μL aliquot with ice-cold acetonitrile (300 μL) including an analytical internal standard. The mixtures were centrifuged for 30 min at 3000 rpm at 4 °C, and analysis of the supernatant was performed by HPLC-MS/MS (API4000 or API4000-QTRAP (Applied Biosystems, Foster City, CA)). In vitro metabolic half-life was calculated using the percent remaining of test article versus the time elapsed.
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20. All compounds were tested as racemic mixtures of a single diastereoisomer. Previous studies have demonstrated that the activity within this series predominates in a single enantiomer. See Ref. [14].
21. Distinct atropisomers were observed by ^{1}H NMR (DMSO-d_6). Variable temperature ^{1}H NMR (DMSO-d_6) demonstrated that signals corresponding to each atropisomer broaden and coalesce to a single multiplicity at temperatures above 50 °C.
22. Conditions for the synthesis of compound **5** (R^5 = CN, R^7 = H, R^8 = Me); Chloride **5** (R^5 = Cl, R^7 = H, R^8 = Me), $Zn(CN)_2$ (0.5 equiv), $Pd(dba)_3$ (4 mol %), dppf (8 mol %) and Zn powder (25 mol %) were heated in dry DMA (c = 0.2 M) at 150 °C for 15 h. Aqueous work-up followed by purification by flash chromatography gave **5** in 58% yield.
23. Reagents for reaction of aldehyde **7** (R^5 = CHO, R^7 = H, R^8 = Me) with phosphonium ylides: Compound **7** (R^5 = $CHCH_2$, R^7 = H, R^8 = Me): $MePPh_3Br$, n-BuLi, THF, 0 °C, 0.5 h. Compound **7** (R^5 = $CHCH_2CN$, R^7 = H, R^8 = Me): $(OEt)_2P(O)CH_2CN$, NaH, THF, rt, 15 h. Compound **7** (R^5 = $CHCH_2C(O)Me$, R^7 = H, R^8 = Me): $(OEt)_2P(O)C(O)Me$, NaH, DME, 50 °C, 3 h.
24. Skraup reaction of 3-chloro-5-fluoroaniline gave a 32% yield of a 3:1 mixture of tetrahydroquinoline regioisomers favoring **5** (R^5 = F, R^7 = Cl, R^8 = H) over **5** (R^5 = Cl, R^7 = F, R^8 = H) which required separated by preparative HPLC.
25. Walz, D. T.; Martino, M. J.; Griffin, C. L.; Misher, A. *Arthritis Rheum.* **1970**, *42*, 2060.