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Nonsteroidal 2,3-dihydroquinoline glucocorticoid receptor agonists with reduced PEPCK activation

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ABSTRACT

Continuing studies based on dihydroquinoline glucocorticoid receptor agonists lead to the discovery of a series of C4-oxime analogs. Representative compounds exhibited potent transrepression activity with minimal transactivation of phosphoenolpyruvate caboxykinase (PEPCK), a key protein in the gluconeogenesis pathway. These compounds represent promising leads in identifying GR agonists with high anti-inflammatory activity and attenuated potential for glucose elevation.

Prednisolone **1** and dexamethasone **2** (Fig. 1) are widely prescribed synthetic glucocorticoids (GCs) for the treatment of numerous inflammatory and autoimmune disorders that include lupus and rheumatoid arthritis. However, GC-mediated side effects such as osteoporosis, hyperglycemia, adrenal suppression and hypertension can limit both higher-dose and long-term usage.[1] The glucocorticoid receptor (GR) is thought to be responsible for both the desired anti-inflammatory activity and the observed side-effects of GCs. Upon binding of the GC to GR, the GR–ligand complex translocates from the cytosol to the nucleus, where it can either activate or repress specific genes. Transcriptional repression (TR) of genes that encode for pro-inflammatory cytokines is thought to be responsible for the beneficial anti-inflammatory activity of GCs, while transcriptional activation (TA) can lead to unwanted side-effects. GCs that favor TR over TA may exhibit less side-effects while retaining their desired anti-inflammatory activity. A number of reports disclosing novel chemotypes have appeared recently in the literature and is testament to the high level of interested in this field.[2–10]

We recently disclosed a series of GC agonists based on 6-aryl-1,2,3,4-tetrahydroquinolines exemplified by compounds **3** and **4** (Fig. 1).[2] Compound **4** exhibited potent and efficacious transrepression activity in vitro which translated to in vivo anti-inflammatory activity comparable to prednisolone. It was our interest to further expand on the structure–activity relationship (SAR) of the quinoline 'A-ring' in an effort to both identify new chemotypes and compound profiles distinct from steroidal GCs, the results of which are reported in this Letter.

The GR-mediated activity was evaluated in a number of biological assays.[2,3] GR binding was determined using a radiolabeled dexamethasone competitive binding assay with baculovirus-expressed GR. TR activity was determined using a CTF E-selectin repression assay in HepG2 cells to determine repression of transcriptional activation mediated by NFκB or AP-1. Compounds were also profiled in an IL-6 ELISA assay to determine inflammatory cytokine repression in primary neonatal human dermal fibroblast (NHDF) cells as a further measurement of TR activity.[2,3]

The compounds were synthesized as depicted in Scheme 1. Commercially available 3,5-difluoroaniline was reacted with propargyl acetate **6** followed by Boc-protection of the quinoline ring to give **7** in good yield (71% over two steps). Hydroboration–oxidation followed by oxidation of the resultant alcohol gave **8** exclusively. Removal of the protecting group, C-6 bromination and subsequent Suzuki coupling with indole-7-boronic acid gave ketone **10**. Reaction with a variety of hydroxylamine derivatives gave desired analogs **11**–**22** as a single oxime regioisomer.[11]

4-Quinolone analog **10** showed no affinity for GR in the competitive binding assay. Further SAR lead to the identification of oxime **11** that, although had no TR activity, bound to GR with modest affinity (Table 1). Subsequent SAR studies found that *O*-methylation of the oxime lead to a compound (**12**) that exhibited reasonable activity in the E-selectin repression assay coupled with low nM binding affinity to GR. Increasing the size of the *O*-alkyl group

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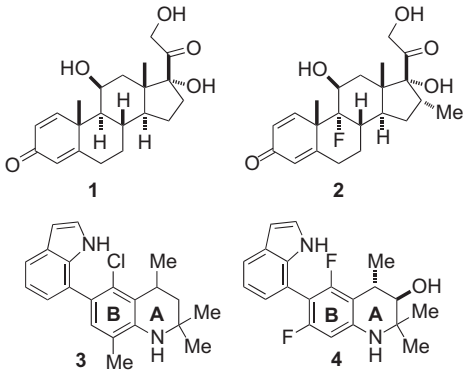
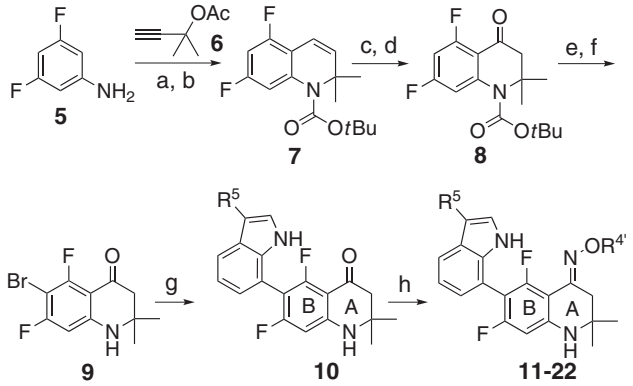


Figure 1. Steroidal glucocorticoids and representative 1,2,3,4-tetrahydroquinoline-derived GR agonists.



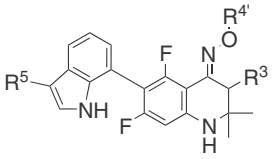
Scheme 1. Representative synthetic route. Reagents and conditions, (a) 6, Cu(I)Cl, THF, reflux, 15 h; (b) $(Boc)_2O$, *n*BuLi, Et_2O, −78 °C to rt; (c) BH_3-THF, THF, then KOH, H_2O_2; (d) PCC, CH_2Cl_2, 4 h; (e) NBS, DMF, 15 h; (f) 1:1 TFA:CH_2Cl_2, 1 h; (g) $Pd(PPh_3)_4$, indol-7-ylboronic acid derivative, 2:1 PhMe:EtOH, 2 N Na_2CO_3, 100 °C, 15 h; (h) NH_2OR^4, NaOAc, EtOH, 70 °C, 15 h.

from methyl to ethyl (**13**) resulted in an increase in E-selectin potency and efficacy. In addition the compound bound with high affinity to GR (K_i = 0.4 nM). However, both **12** and **13** were inactive in the IL-6 repression assay. Further branching of the alkyl group (**14**) lead to a two-fold improvement in E-selectin potency. Importantly, in contrast to earlier analogs, **14** also exhibited partial efficacy (57%) and reasonable potency (40 nM) in the IL-6 repression assay.

We have previously disclosed SAR investigations profiling the GR-mediated activity of a number of C-6 indole analogs.[2] Exploring a number of C-6 indole derivatives in combination with the C4-*t*-butyl oxime A-ring lead to the identification of **15** and **16** that incorporated either a methyl or chlorine at the 3-position of the indole ring. Both compounds **15** and **16** demonstrated improved efficacy and potency in the IL-6 repression assay. Isopropyl-oxime analog **17** exhibited similar TR activity to **15**. *O*-Phenyl or *O*-benzyl analogs **18** and **19** lead to a dramatic decrease in GR-mediated activity with weak activity in the E-selectin repression assay and no IL-6 repression activity. A number of functionalized oxime derivatives **20**–**22** were also synthesized and evaluated. Carboxylic acid **21** showed no TR activity while alcohol **22** bound tightly to GR but exhibited diminished TR potency compared to **15**.

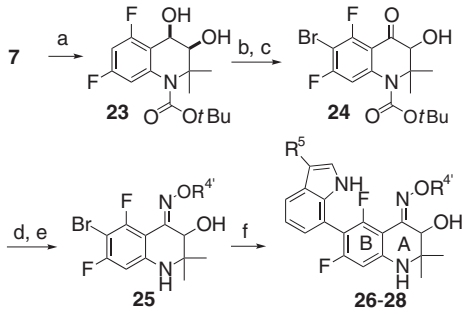
Previous studies had shown that incorporation of a C3-hydroxyl group within the A-ring lead to improved GR selectivity and

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



Compd	R^3	$R^{4'}$	R^5	GR binding K_i (nM)	E-Selectin repression		IL-6 repression	
					IC_{50} (nM)	eff. (%)	IC_{50} (nM)	eff. (%)
1			Prednisolone	5.3 ± 0.3	4.1 ± 0.8	100 ± 1.4	23 ± 2.6	97 ± 0.7
11	H	H	H	370	—	—	—	—
12	H	Me	H	7.0	42 ± 15	83 ± 1.3	—	—
13	H	Et	H	0.4	6.9 ± 2.1	91 ± 1.2	—	—
14	H	*t*-Bu	H	7.0	3.5 ± 1.1	93 ± 0.9	40 ± 5.3	57 ± 6.5
15	H	*t*-Bu	Me	2.2	5.6 ± 1.5	91 ± 1.6	13 ± 1.3	73 ± 5.5
16	H	*t*-Bu	Cl	2.0	4.0 ± 0.5	94 ± 1.2	19 ± 3.7	83 ± 5.6
17	H	*i*-Pr	Me	2.7	4.9 ± 1.0	90 ± 2.3	11 ± 4.3	77 ± 5.0
18	H	Ph	Me	31	51 ± 13	67 ± 9.5	—	—
19	H	Bn	Me	5.8	8.8 ± 3.1	74 ± 3.5	—	—
20	H		Me	1.5	22 ± 2.8	87 ± 2.7	—	—
21	H		Me	2700	—	—	—	—
22	H		Me	0.8	12 ± 4.9	91 ± 1.1	65	67
26	OH	Me	Me	3.8	3.4 ± 0.5	96 ± 2.0	17 ± 1.4	98 ± 7.9
27	OH	Et	Me	0.6	2.6 ± 0.4	98 ± 1.4	5.2	97
28	OH	Me	Cl	0.7	14 ± 7.2	100 ± 3.3	22 ± 7.6	99 ± 5.2

[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. 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. (—) = not active and denotes <20% efficacy or potency >1 μM. Compounds **26** and **27** tested as racemates.



Scheme 2. Representative synthetic route. Reagents and conditions, (a) $K_3Fe(CN)_6$, $CH_3SO_2NH_2$, OsO_4, pyridine, *t*-BuOH:H_2O, rt, 15 h; (b) NBS, DMF, 15 h; (c) IBX, EtOAc, reflux, 15 h; (d)) NH_2OR^4, NaOAc, EtOH, 70 °C, 15 h; (e) TFA:CH_2Cl_2, 15 h; (f) $Pd(PPh_3)_4$, indol-7-ylboronic acid derivative, 2:1 PhMe/EtOH, 2 N Na_2CO_3, 100 °C, 15 h.

compound hydrophilicity.[2] Initial efforts at hydroxylation alpha to C-3 ketone **10** were unsuccessful and a new synthetic route was sought. Sharpless dihydroxylation of quinoline **7** followed by C-6 bromination and selective oxidation of the benzylic alcohol gave **24** in good yield (Scheme 2). Oxime formation followed by removal of the Boc-group gave **25** which was coupled with the desired indole boronic acid to yield bicyclic analogs **26**–**28**.[12]

Alkyl-oxime analogs **26** and **27** bound tightly to GR and demonstrated high activity in the E-selectin assay. The compounds were also fully efficacious in IL-6 with efficacy similar to prednisolone. 3-Chloroindole analog **28** had a similar profile but was three-fold more potent in IL-6 repression. It is worth noting that incorporation of additional lipophilicity at the 4-position of the scaffold was not required to reach prednisolone-like TR activity upon reintroduction of the C3-hydroxyl group.

Unwanted off-target steroid hormone interactions can be characteristic of new nonsteroidal GR ligands and selected compounds of interest were evaluated (Table 2). Representative analogs bound tightly to GR with low to sub-nM potencies in a competitive binding assay. Unlike steroidal GCs no MR cross reactivity was observed for analogs within this series. Compound **15** showed high receptor selectivity with >100 fold increased affinity for GR over PR, MR and AR. Within this sub-series of analogs receptor selectivity is high in the absence of the C-3hydroxyl group. Analog **26** showed improved GR selectivity with >400 fold separation in binding potencies. Close analog **27** showed more pronounced binding to PR although separation in binding potencies was still >40 fold.

To further characterize the compounds from the series, selected compounds were evaluated in a transactivation assay using an MMTV:luciferase reporter, and in rat H4IIEC3 liver cells using a luciferase reporter containing the promoter of PEPCK. PEPCK is the rate-limiting step in hepatic gluconeogenesis and was measured as a further indicator of TA activity.[13] Compounds within this series of analogs demonstrated high efficacy and potency in the GRE activation assay irrespective of the nature of the A-ring (Table 3). Previously disclosed analog **4** also showed no separation on PEPCK activation with observed activity similar to steroidal GC prednisolone. However, oxime analogs **15** and **16** showed significantly reduced efficacy in the PEPCK assay compared to both **4** and prednisolone. *i*Pr-oxime **17** also exhibited low efficacy and reduced compound potency in the PEPCK assay. Analog **27** exhibited diminished activity in the PEPCK assay compared to prednisolone despite the compounds comparable TR activities. The molecular basis for the discrepancy in TA activities for analogs within this sub-series is unclear but suggests that low agonist activity in the GRE activation assay is not a prerequisite for attenuated activity on GC-mediated glucose end-points.

In conclusion we have discovered a new sub-series of nonsteroidal quinoline-derived A-ring analogues. Representative compounds have potent TR activity in both E-selectin and IL-6 repression assays with several analogs exhibiting TR activity equivalent to steroidal GC prednisolone. Compounds from this series show high GR selectivity and attenuated transcriptional activity in a PEPCK assay compared to prednisolone. These compounds represent promising leads in further identifying GR agonists with attenuated TA activities.

Table 2
[a]Competitive binding data for representative analogs with PR, MR, and AR

Cmpd	GR binding K_i (nM)	PR binding K_i (nM)	MR binding K_i (nM)	AR binding K_i (nM)
15	2.2	220	1600	1000
16	2.0	570	1040	90
17	2.7	380	2800	1500
22	0.8	950	2700	290
26	3.8	3800	1700	2800
27	0.6	27	>10,000	430

[a] Compounds **26** and **27** tested as racemates.

Table 3
[a]Comparison of TR and TA activities for representative analogs

Compd	IL-6 repression		GRE activation agonist mode		PEPCK activation	
	IC_{50} (nM)	Eff. (%)	EC_{50} (nM)	Eff. (%)	EC_{50} (nM)	Eff. (%)
Prednisolone, **1**	23 ± 2.6	97 ± 0.7	5.3 ± 0.6	129 ± 6.5	26 ± 6.9	84 ± 4.1
4	11 ± 5.6	90 ± 2.3	0.6 ± 0.2	138 ± 16	53 ± 3.7	75 ± 1.7
15	13 ± 1.3	73 ± 5.5	5.0 ± 0.7	97 ± 9.8	70 ± 12	28 ± 6.4
16	19 ± 3.7	83 ± 5.6	3.4 ± 0.4	97 ± 12	67	27
17	11 ± 4.3	77 ± 5.0	0.6 ± 0.2	138 ± 16	250 ± 13	35 ± 4.2
22	65	67	19 ± 7.4	130 ± 21	350	22
26	17 ± 1.4	98 ± 7.9	3.9 ± 0.9	150 ± 14	560 ± 19	79 ± 12
27	5.8 ± 0.4	97 ± 0.6	1.5 ± 0.2	138 ± 14	115 ± 7.3	61 ± 2.8

[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%). 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. (—) = not active and denotes <20% efficacy or potency >1 μM. Compounds **26** and **27** tested as racemates.

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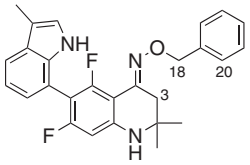
11. NMR studies confirmed the regioisomer isolated for representative analog **19** by observing NOE's between H3-H18/H18-H3, and H3-H20/H20-H3 protons.



12. Oxime analogs were isolated as a mixture of oxime regioisomers that were readily separated by preparative HPLC and individually characterized. The corresponding regioisomeric oxime-analogs of compounds **26**–**28** were found to be devoid of GR-mediated TR activity. Regioisomeric assignment based on analogy with compound **19**.
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