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Tetrahydroquinolin-3-yl carbamate glucocorticoid receptor agonists with reduced PEPCK activation

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ABSTRACT

Continuing studies on tetrahydroquinoline glucocorticoid receptor anti-inflammatory agents lead to the identification of several tetrahydroquinolin-3-yl carbamates that exhibited steroid-like activity in in vitro transrepression assays with reduced transactivation of phosphoenol pyruvate carboxykinase (PEPCK), a key enzyme in the gluconeogenesis pathway.

Synthetic glucocorticoids (GCs), such as prednisolone (**1**) and dexamethasone (**2**, Fig. 1), are widely prescribed to aid in the treatment of numerous inflammatory and autoimmune disorders, such as rheumatoid arthritis, lupus, Crohn's disease, and asthma. However, their desired anti-inflammatory effects must be balanced against such side-effects as osteoporosis, hyperglycemia, muscle wasting, behavioral changes, adrenal suppression, growth retardation, and hypertension.[1] Several of these side-effects often restricts a patient's long term treatment with GCs. Anti-inflammatory activity, as well as many of the side-effects of GCs, are mediated through the glucocorticoid receptor (GR), a ligand-dependent transcription factor which serves to either activate or repress gene transcription.[2] Transcriptional repression (TR) of genes that encode for pro-inflammatory cytokines and metalloproteinases is thought to be responsible for the anti-inflammatory effects of GCs,[2] while transcriptional activation (TA) of certain genes, for example those involved in gluconeogenesis, leads to unwanted side-effects.[3] The discovery of new GR ligands that favor TR over TA may serve to lessen GC-related side-effects while retaining beneficial anti-inflammatory activity. A number of accounts disclosing the development of several chemotypes as dissociated ligands have appeared.[4–11]

We have recently reported a series of GR agonists based on a 6-indole tetrahydroquinoline scaffold (**3** and **4**, Fig. 1).[12] SAR investigation of the tetrahydroquinoline A-ring discovered the importance of a C3-hydroxyl for improving receptor selectivity, while SAR within the B-ring resulted in improved microsomal stability, leading to demonstration of in vivo efficacy for the series. While fully efficacious and receptor selective GR agonists such as **4** were identified, it was our goal to further expand the SAR in an effort to ultimately identify selective glucocorticoid receptor modulators (SGRMs) from the series exhibiting a profile distinct from that of classical steroids in terms of TR/TA activity. Specifically,

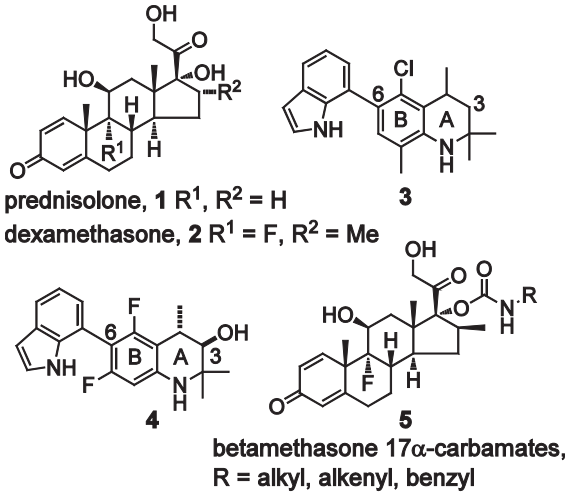

Figure 1. Glucocorticoid receptor ligands.

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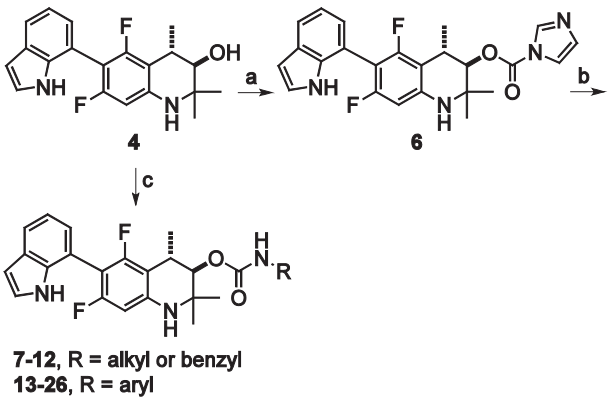
we sought to discover new nonsteroidal anti-inflammatory agents with a reduced impact on glucose elevation.

GR binding was determined using a radiolabeled dexamethasone competitive binding assay with baculovirus-expressed GR.[5] To assess the functional activity of the ligands, GR-mediated TA was measured in co-transfection (CTF) reporter assays in either CV-1 kidney cells, using a mouse mammary tumor virus (MMTV): luciferase reporter containing a glucocorticoid response element (GRE)[13] or in rat H4IIEC3 liver cells using a luceriferase reporter containing the promoter of phosphoenol pyruvate carboxykinase (PEPCK).[14] PEPCK is the rate-limiting step in gluconeogenesis, hence this assay was used to evaluate the potential hyperglycemic effect of the new ligands. Potency (EC_{50}) was determined from half-log concentration–response curves and maximal efficacy was determined relative to dexamethasone (**2**). To evaluate TR activity, a CTF assay in HepG2 cells using the promoter of the E-selectin gene was used in order to determine repression of transcriptional activation mediated by NFκB or AP-1[15] while an IL-6 ELISA assay[5] determined inflammatory cytokine repression in primary neonatal human dermal fibroblast (NHDF) cells.

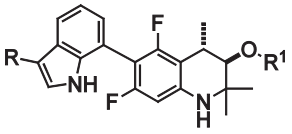


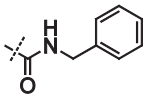
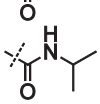
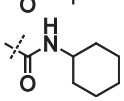
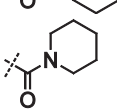
Scheme 1. General routes for carbamate synthesis. Reagents and conditions: (a) 1,1′-carbonyldiimidazole, dichloromethane; (b) RNH_2, toluene, reflux; (c) RNCO, toluene, 4-(dimethylamino)pyridine, reflux.

Tetrahydroquinolin-3-ol **4** is a fully efficacious GR agonist exhibiting in vitro TR activity similar to **1**. With respect to potential GC-related side-effects, specifically hyperglycemia, **4** exhibited comparable efficacy to **1** in activation of PEPCK. Therefore, in terms of TR (E-selectin and IL-6) versus TA (MMTV agonism and PEPCK), **4** is not selective. In an effort to develop SGRMs from our program, we sought to investigate whether the C3-hydroxyl of **4** could serve as a handle to further expand the tetrahydroquinoline A-ring pharmacophore. A report detailing the development of steroidal GR modulators derived from carbamates of the 17α-hydroxyl group of betamethasone **5** (Fig. 1) has appeared.[8a] We now disclose our findings which demonstrate that carbamates could be successfully incorporated onto the 6-indole tetrahydroquinolin-3-ol core resulting in ligands that show steroid-like binding affinity, MMTV agonism, and TR activity but with reduced TA of PEPCK.

C3-carbamates (**7**–**26**) were constructed as depicted in Scheme 1 using either of two representative routes. For example, **4** was reacted with 1,1′-carbonyldiimidazole in dichloromethane to yield

Table 1
In vitro assay results for carbamates **7–12**[a]



Compd[b]	R	R^1	GR K_i (nM)	GR activation		E-selectin repression		IL-6 repression		PEPCK activation	
				Eff. (%)	EC_{50} (nM)	Eff. (%)	IC_{50} (nM)	Eff. (%)	IC_{50} (nM)	Eff. (%)	EC_{50} (nM)
1			5.3 ± 0.3	130 ± 7	5.3 ± 3.6	100 ± 1.4	4.1 ± 0.8	97 ± 0.7	23 ± 2.6	84 ± 4	26 ± 7
4	H	H	2.5 ± 0.9	138 ± 16	0.6 ± 0.2	100 ± 2.2	1.5 ± 0.7	90 ± 3	11 ± 5.6	75 ± 2	98 ± 40
7	H		3.0	185 ± 30	39 ± 20	90 ± 1	10 ± 1	—	—	52 ± 9	199 ± 87
8	Cl		2.2	157 ± 20	21 ± 4	90 ± 7	12 ± 4	88	52	71 ± 7	463 ± 240
9	Cl		6.8	87 ± 8	17 ± 4	87 ± 2	44 ± 25	81 ± 5	40 ± 20	45 ± 4	454 ± 30
10	Cl		7.8	110 ± 18	89 ± 20	83 ± 3	45 ± 12	80 ± 4	17 ± 5	26 ± 4	361 ± 20
11	Cl		4.3 ± 2.7	89 ± 10	70 ± 53	90 ± 5	15 ± 3	89 ± 6	23 ± 10	38 ± 15	435 ± 100
12	H		132	—	—	—	—	nt	nt	nt	nt

[a] EC_{50} and IC_{50} 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] Compounds were tested as racemates.