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Novel heterocyclic glucocorticoids: in vitro profile and in vivo efficacy

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Abstract—A series of novel ligands for the glucocorticoid receptor containing two heterocycles were synthesized. These compounds were investigated for a dissociative profile using transrepression and transactivation assays. Several compounds were tested in vivo and showed the ability to reduce inflammation in a mouse.

Glucocorticoids (GCs), a class of steroids targeting the glucocorticoid receptor (GR), have a long history of use in the treatment of inflammatory disease. These powerful anti-inflammatory agents, which include prednisolone and dexamethasone, are quite valuable for the treatment of acute inflammation. However, the ability to manage chronic inflammatory diseases with GCs is hindered by side effects associated with long term use of these compounds. Prolonged systemic exposure to high doses of GCs results in numerous complications that are also evident in the syndrome of endogenous corticoid excess (Cushing's syndrome). The collection of unwanted side effects due to artificially high GC levels includes muscle wasting, osteoporosis, and the tendency to become hyperglycemic due to increased gluconeogenesis.[1,2]

For many years, scientists have been searching for GCs that avoid these side effects. Steroids are often promiscuous, but GCs with high specificity for GR have been designed, eliminating side effects that result from GC interactions with other steroid receptors, such as the mineralacorticoid receptor (MR) or estrogen receptor (ER). However, the 'Cushing's syndrome' side effects and the anti-inflammatory effects of GCs are both mediated through GR.[1] For this reason it initially seemed as though the discovery of a 'clean' GC with only the desired anti-inflammatory properties might not be possible. However, in the past decade, new results have suggested that the anti-inflammatory effects of GCs can be decoupled from their side effects.[2–9] A compound displaying this separation of properties would be termed 'dissociated'. GR is a nuclear hormone receptor that, upon binding a GC, undergoes a conformational change and translocates to the nucleus of the cell, where it plays a key role in mediating a number of critical cellular pathways.[3–9] Recent evidence suggests that many of the anti-inflammatory effects of steroids result from a GR–GC *monomer* downregulating inflammatory pathways by interacting directly with pro-inflammatory

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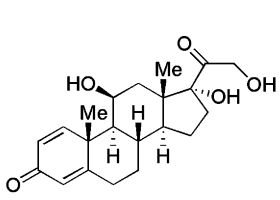
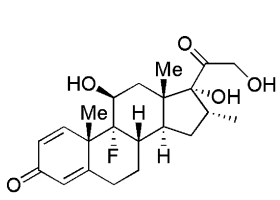
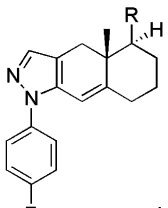
Table 1. Binding of GCs to GR; transrepression and transactivation in mouse and human cell lines



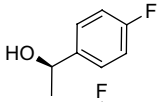
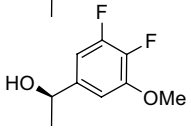
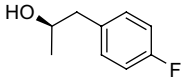
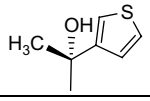
prednisilone



dexamethasone



compounds **1-4**

Compound	R	GR[a]	hIL-6[b]		hTAT[c]		mIL-6[d]		mGS[e]	
		IC_{50} (nM)	EC_{50} (nM)	%Dex	EC_{50} (nM)	%Dex	EC_{50} (nM)	%Dex	EC_{50} (nM)	%Dex
Prednisolone	—	13.8	4.5	102	24	82	5.7	95	3.4	89
1		5.3	9.7	67	nd	25	22	74	nd	31
2		1.9	14.4	77	521	36	15	83	nd	29
3		4.6	3.2	81	1004	37	3.7	82	17	50
4		0.8	0.96	97	36	69	5.2	89	8.3	69

nd = not determined; EC_{50} values were not determined in cases where %dex $\leqslant$ 35%. Compounds were tested at concentrations up to and including 10 μM in the mGS and hTAT assays.

[a] Binding to hGRα receptor determined by displacement of [^{3}H]dexamethasone.

[b] Human IL-6 assay in A549 lung carcinoma cell line.

[c] Human tyrosine amino transferase (TAT) assay in HepG2 cells.

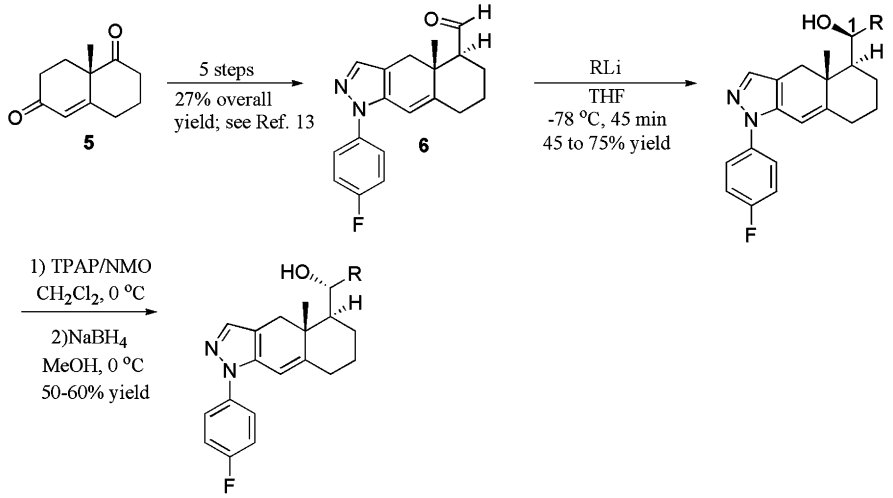
[d] Mouse IL-6 assay in peritoneal exudate cells harvested from C57BI/6 mice.

[e] Mouse glutamine synthetase (GS) assay in C2C12 cells.

transcription factors such as AP-1 and NF-κB.[10–12] These desired interactions are termed *transrepression* (TR). On the other hand, many side effects appear to be mediated by a GR–GC *dimer* binding to the DNA and upregulating certain metabolic genes, a process known as *transactivation* (TA).[3–9] A GC that favors the monomeric state of activated GR and/or GR–protein interactions relative to GR–DNA interactions should facilitate transrepression preferentially, and therefore display a dissociated profile in vivo.

Our labs previously disclosed compounds **1**–**4** (Table 1), which employed a novel scaffold consisting of a truncated steroid (A and B rings) with a *p*-fluorophenyl pyrazole appended to the A ring and a hydroxyl group and aromatic substituent replacing the C and D rings of the steroid.[13–15] These compounds were quite potent and highly specific for GR relative to other steroid receptors. To determine whether or not these GCs were dissociated, markers for TA and TR were assessed in both mouse and human cell lines.[16] Suppression of interleukin-6 (IL-6), a cytokine associated with inflammation, was used as an indicator of TR in both species.[13,17] For TA studies, tyrosine amino transferase (TAT), a hepatic gene known to be upregulated by glucocorticoids, was analyzed in human cell lines.[13,18,19] In mice, glutamine synthetase (GS), which is associated with protein metabolism in muscle, was used to evaluate TA.[13,20] Compounds bearing a phenyl moiety at C-1, such as **1**, appeared to be dissociated in our in vitro studies (Table 1). Unfortunately, for reasons that are unclear, **1** failed to reduce inflammation in a mouse model that analyzed levels of TNFα induced by LPS.[21] Many related compounds bearing a phenyl substituent were tested in mice, and all failed to show efficacy except for **2**, which gave a modest response, showing 70% of the anti-inflammatory response of prednisolone at a 10-fold higher dose. Benzyl compounds, represented by **3**, were potent and somewhat dissociated, but none showed any in vivo effect, presumably due to very poor PK (clearance [Cl_p] of 127.0 mL/min/kg in a rat). Only compound **4**, which was much more potent, showed good ability to reduce inflammation in a mouse; however, this compound was not dissociated. Thus, we needed to address the in vivo shortcomings of our molecules. Inspired by the activity in our animal model of compound **4**, which contained a thiophene, and the methoxy substituent on the phenyl ring of compound **2**, we chose to explore heterocyclic replacements for both the phenyl and benzyl rings. The results of that work are disclosed in this letter.

We decided to synthesize a small series of compounds containing heterocyclic substituents at the C-1 position (this corresponds to C-11 in a classical steroid). Our hope was that these compounds would display the in vitro dissociation exhibited by some of our earlier compounds, but have improved in vivo activity. The



Scheme 1. Synthesis of novel glucocorticoids.

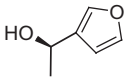
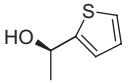
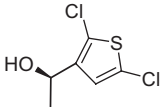
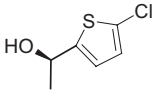
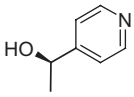
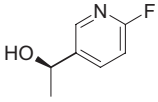
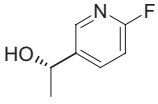
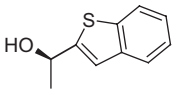
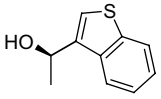
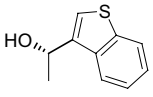
chemistry to synthesize these compounds has been described previously and is outlined in Scheme 1.[13] Briefly, the Wieland–Mischer ketone (**5**) can be converted in five steps and 27% overall yield to key intermediate **6**.[22] Addition of the appropriate heteroaryl lithium species gave one major product with the stereochemistry shown.[23] Minor diastereomers were removed by chromatography to give pure products **7**–**12** and **14**–**15** in good yield. In order to evaluate the importance of the hydroxyl stereochemistry, the C-1 stereocenter was inverted in two cases using an oxidation/reduction procedure to produce compounds **13** and **16**.

Furan, thiophenes, and pyridines were investigated as phenyl replacements (Table 2). All compounds bound tightly to GR ($\leqslant$23 nM). Furan **7** exhibited a dissociated profile, but showed weak TR in the human cell line, achieving only 57% of the activity of dexamethasone. Among the thiophenes, **8** and **9** were dissociated but not very potent, with <70% of dexamethasone's activity in both mouse and human cell lines. Compound **10** was the most interesting thiophene, with good TR activity (>80% of dexamethasone) in both mouse and human cell lines, and a nearly 2-fold TR/TA window in both species. The unsubstituted pyridine **11** was dissociated, but had modest activity. On the other hand, fluoro-pyridines **12** and **13** showed greater potency. As in the previously reported phenyl series, both diastereomers had activity, although differences in their profiles were clearly apparent. Compound **13** was quite active, but had a fairly small window of dissociation in both the mouse and human assays. In contrast, compound **12** showed an outstanding profile in the human cell line with very good TR activity (86% of dexamethasone), but no TA. Unfortunately, this was one of the few compounds prepared in which mouse and human cell lines did not correlate; **12** did not display dissociation in mouse.

Given the success of thiophenes as phenyl ring replacements and the impressive in vivo activity of compound **4**, benzothiophenes were examined as potential replacements for the metabolically liable benzyl moiety in the series of compounds that included **3**. A comparison of compounds **14** and **15** demonstrates that although attachment via either the C-2 or C-3 position of the benzothiophene ring leads to an active compound, C-3 (i.e., **15**) is preferred. Both **14** and **15** were dissociated, but **15** was more potent, both as a GR binder and in TR where it achieved >75% of dexamethasone's activity in both mouse and human cell lines. Importantly, **15** did not register a very strong response in the TA assays, where it showed <35% of dexamethasone's maximum activity in both species. Inversion of configuration at the hydroxyl center led to **16**, which was the most potent of the C-1 heterocycles in either the monocyclic or bicyclic series. Unfortunately, as has been illustrated in other cases (i.e., compound **4** and other examples previously reported), this very potent compound was not dissociated.

With promising in vitro results in hand, four compounds were tested in vivo in the mouse LPS-induced TNFα assay. For comparison, the established steroid prednisolone was included in the study. All compounds were administered orally, although the novel GCs were given at a higher dose (30 mg vs 3 mg) than prednisolone in this initial study. In the experiment (Table 3), the very potent but poorly dissociated compounds **13** and **16** were effective at suppressing inflammation, showing 80% and 120% reductions in inflammation respectively, when compared to the effect of prednisolone. Compound **12**, surprisingly, failed to show efficacy in vivo. On the other hand, the result obtained with compound **15** was particularly encouraging. This partially dissociated compound showed a 110% reduction in TNFα levels when compared to prednisolone. Due to the impressive in vitro profile and in vivo activity of compound **15**, a PK analysis was performed. **15** displayed modest PK parameters in a rat when dosed intravenously, with a normalized AUC of 0.82 μM h kg/mg, Cl_p of 46.1 mL/min/kg, and a half-life ($t_{1/2}$) of 2.6 h. However, we were pleased to find that **15** exhibited 31% oral bioavailability (F). The combination of in vitro, in vivo, and PK data make **15** a promising lead in the arena of dissociated GCs.

Table 2. Binding, transactivation, and transrepression data for novel GCs with heterocyclic substituents at C-1

Compound	R	GR[a]	hIL-6[b]		hTAT[c]		mIL-6[d]		mGS[e]	
		IC_{50} (nM)	EC_{50} (nM)	%Dex	EC_{50} (nM)	%Dex	EC_{50} (nM)	%Dex	EC_{50} (nM)	%Dex
7		2.9	2.7	57	nd	28	45	72	nd	13
8		7.1	37	54	nd	16	21	67	nd	14
9		10.2	31	65	nd	29	120	61	nd	12
10		7.6	13	82	1068	42	17	82	34	49
11		23.2	38	68	nd	0	215	65	nd	31
12		13.6	13	86	nd	0	12	73	260	69
13		9.1	21	76	4443	48	5	96	23	74
14		29.1	19	66	nd	22	42	66	1209	41
15		5.1	18	86	nd	21	54	78	nd	33
16		1.5	6	94	356	43	11	99	3	100

nd = Not determined; EC_{50} values were not determined in cases where %dex $\leqslant$ 35%. Compounds were tested at concentrations up to and including 10 μM in the mGS and hTAT assays.

[a] Binding to hGRα receptor determined by displacement of [^{3}H]dexamethasone.

[b] Human IL-6 assay in A549 lung carcinoma cell line.

[c] Human tyrosine amino transferase (TAT) assay in HepG2 cells.

[d] Mouse IL-6 assay in peritoneal exudate cells harvested from C57BI/6 mice.

[e] Mouse glutamine synthetase (GS) assay in C2C12 cells.

Table 3. In vivo efficacy of selected compounds

Compound (dose)	Absolute inhibition of TNFα (%)	Inhibition of TNFα relative to prednisolone (%)
Prednisolone (3 mpk)	71	100
12 (30 mpk)	ne	—
13 (30 mpk)	57	80
15 (30 mpk)	78	110
16 (30 mpk)	87	121

ne = Not effective at reducing TNFα levels.

We have successfully demonstrated the use of heterocycles as replacements for phenyl and benzyl rings on our novel GC platform. These compounds showed activity in mouse and human cell lines, although the most potent ones often failed to show significant dissociation, an apparent trend. The most impressive of these new compounds is **15**. This compound is moderately potent, shows efficacy in vivo, and is dissociated both in mouse and human cell lines relative to traditional steroids such as prednisolone and dexamethasone. In the current model species of mouse, experiments to study GC side effects are not well established.[15b] Thus, a future challenge for this program will be to design an experiment to determine whether the window of dissociation seen in **15** will lead to a decrease in side effects when compared to traditional steroids. The investigation of additional heterocycles at C-1 will also be undertaken.

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23. Heteroaryl-lithium species were generated either by lithium-halogen exchange of the corresponding bromide with *t*-BuLi or deprotonation of the appropriate heterocycle with *n*-BuLi.