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Novel ketal ligands for the glucocorticoid receptor: in vitro and in vivo activity

Cameron J. Smith,[a,*] Amjad Ali,[a] James M. Balkovec,[a] Donald W. Graham,[a] Milton L. Hammond,[a] Gool F. Patel,[a] Gregory P. Rouen,[a] Scott K. Smith,[a] James R. Tata,[a] Monica Einstein,[b] Lan Ge,[b] Georgianna S. Harris,[b] Theresa M. Kelly,[b] Paul Mazur,[b] Chris M. Thompson,[b] Chuanlin F. Wang,[b] Joanne M. Williamson,[b] Douglas K. Miller,[c] Shilpa Pandit,[c] Joseph C. Santoro,[c] Ayesha Sitlani,[c] Ting-ting D. Yamin,[c] Edward A. O'Neill,[d] Dennis M. Zaller,[d] Ester Carballo-Jane,[e] Michael J. Forrest[e] and Silvi Luell[e]

[a]*Department of Medicinal Chemistry, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA*
[b]*Department of Metabolic Disorders, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA*
[c]*Department of Cardiovascular Diseases, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA*
[d]*Department of Immunology, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA*
[e]*Department of Pharmacology, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA*

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Abstract—A novel series of selective ligands for the human glucocorticoid receptor is described. Structure–activity studies focused on variation of B-ring size, ketal ring size, and ketal substitution. These analogs were found to be potent and selective ligands for GR and have partial agonist profiles in functional assays for transactivation (TAT, GS) and transrepression (IL-6). Of these compounds, **27**, **28**, and **35** were evaluated further in a mouse LPS-induced TNF-α secretion model. Compound **28** had an ED_{50} of 14.1 mg/kg compared with 0.5 mg/kg for prednisolone in the same assay.

A variety of inflammatory diseases such as asthma, rheumatoid arthritis, Crohn's disease, and allergic rhinitis are treated by synthetic glucocorticoids such as dexamethasone (**1**) and prednisolone (**2**) (Fig. 1).[1,2] Associated with these diseases is an increased expression of pro-inflammatory cytokines such as interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α).[2] The production of the inflammatory cytokine IL-6 has been shown to be mediated by the glucocorticoid receptor (GR) at the transcriptional level.[2] Glucocorticoid use inhibits the production of inflammatory cytokines but unfortunately, is also associated with many side effects including osteoporosis, hyperglycemia, lipid redistribution, and muscle atrophy.[3] Some of these effects arise from tran-

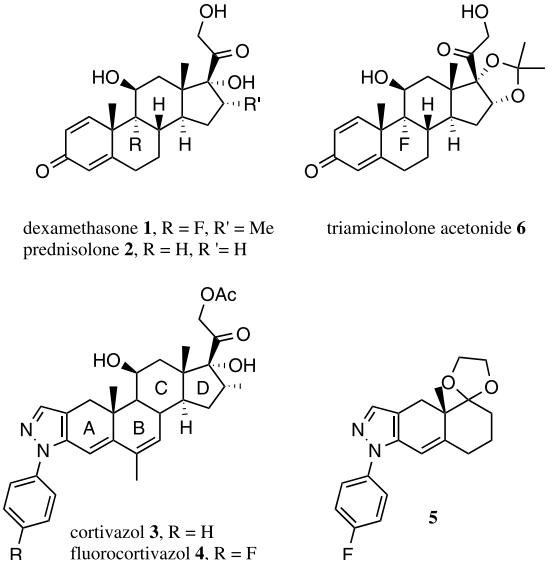

Figure 1.

Keywords: Glucocorticoid; Dissociation; Transrepression; Transactivation; Inflammation.
* Corresponding author. Tel.: +1 732 594 2529; fax: +1 732 594 9545; e-mail: cameron_smith@merck.com

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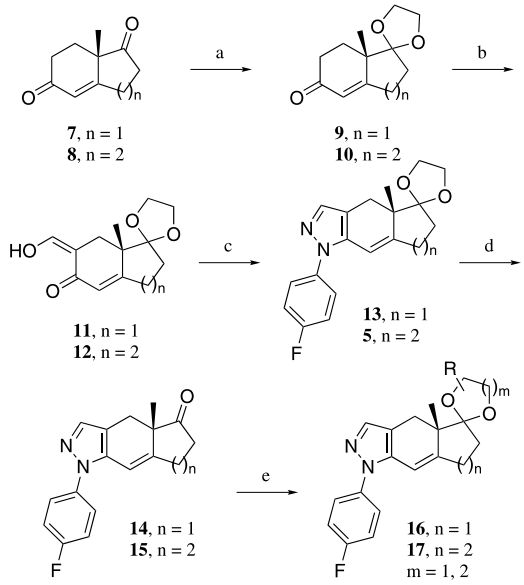
scription of genes such as tyrosine amino transferase (TAT), a metabolic gene involved in gluconeogenesis primarily found in the liver,[4] and glutamine synthetase (GS), an enzyme involved in the regulation of skeletal muscle that catalyzes the conversion of glutamate to glutamine.[3] Glucocorticoid-mediated induction of TAT and GS causes the side effects hyperglycemia and muscle wasting, respectively.[3] Consequently, it is of therapeutic interest to discover selective GR ligands that retain anti-inflammatory properties but have diminished side effect profiles, and a number of laboratories are active in this field.[5–14]

The mechanism[3,15,16] of glucocorticoid action is initiated by binding of the ligand to GR. The resulting GR/ligand complex can then dimerize and bind to DNA sequences known as glucocorticoid response elements (GREs) thus initiating the transcription of genes associated with the side effects of glucocorticoid use. Alternatively, as a monomer, the GR/ligand complex can bind to transcription factors such as nuclear factor κβ (NFκβ) and activator protein-1 (AP-1), thereby repressing their effect on transcription of pro-inflammatory cytokines like IL-6 and TNF-α. The development of anti-inflammatory drugs with reduced side effect profiles may be facilitated by the identification of compounds that repress transcription of inflammatory cytokines without activating GREs. Such compounds are often referred to as 'dissociated' compounds.[16]

The development of pyrazolosteroids such as cortivazol (**3**) and fluoro-cortivazol (**4**) evolved from the clinically successful steroids dexamethasone (**1**) and prednisolone (**2**) (Fig. 1).[17,18] These compounds have an *N*-aryl pyrazole ring fused to the A-ring of the steroid backbone. This structural moiety gave these compounds enhanced GR binding potency and selectivity over other nuclear hormone receptors and improved in vivo activity. Structural diversity between synthetic glucocorticoids occurs mainly in the region of space occupied by the C- and D-rings. In an effort to prepare compounds that bind selectively to GR and show improved anti-inflammatory properties in vivo, compounds were designed with an *N*-aryl pyrazole moiety fused to the conventional A- and B-rings of a steroid backbone, and structural diversity in the region of space usually occupied by the C- and D-rings.

We recently reported[19] a ketal intermediate (**5**) (Fig. 1) used in the synthesis of a series of GR ligands that was found to bind to GR with a reasonable affinity. Because clinically relevant glucocorticoids that contain a ketal functionality are known (e.g., triamicinolone acetonide (**6**) (Fig. 1)[20]), we wished to investigate this observation further. We decided to synthesize a series of ketals varying first the ketal substituents and later the ketal and B-ring sizes.

The key intermediate ketones required were synthesized as shown in Scheme 1 starting with either enantiopure Hajos–Parrish ketone (**7**, five-membered B-ring, $n = 1$)[21,22] or Wieland–Miescher ketone (**8**, six-membered B-ring, $n = 2$),[23,24] respectively. These ketones were



Scheme 1. Reagents and conditions: (a) $HOCH_2CH_2OH$, *p*-TsOH, 4 Å mol sieves, rt, 30 min, 93%; (b) HCO_2Et, NaH, C_6H_6, rt, 3 h, 93%; (c) 4-fluorophenyl hydrazine, NaOAc, HOAc, rt, 24 h, 41%; (d) 6 N HCl, THF, 65 °C, 5 h, 100%; (e) diol, *p*-TsOH, C_6H_6, reflux, 12–24 h, 50–95%.

selectively protected as ketals **9** and **10**[25] then formylated to give **11** and **12**, respectively. Condensation with 4-fluorophenylhydrazine then afforded *N*-arylpyrazole intermediates **13** and **5**. Hydrolysis of ketals **13** and **5** required 6 N aq HCl in THF at 65 °C to afford ketones **14** and **15**. Substituted ketals **16** and **17** were synthesized from the appropriate ketone **14** or **15** and substituted diol by heating at reflux with catalytic *p*-toluenesulfonic acid in benzene under a Dean–Stark apparatus. Diols were commercially available except where noted and were either racemic or enantiopure. If diastereoisomers were produced they were separated using flash chromatography and/or chiral HPLC and characterized using NMR spectroscopy (2D-NOESY studies).

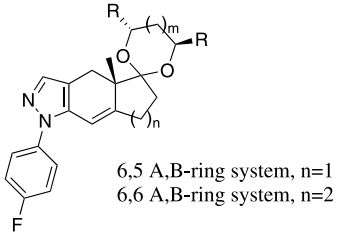
Initially, compounds were assayed for levels of binding potency in a competition binding assay with radiolabeled dexamethasone for the α-isoform of the human GR.[19] Steroid receptor selectivity was determined by counter-screening against other nuclear hormone receptors including progesterone (PR), mineralocorticoid (MR), and estrogen (ER-α and ER-β) receptors.[19] The functional activity of compounds in either activation or repression of transcription was evaluated using cell-based assays. Repression of transcription or 'transrepression' was determined by measuring IL-6 inhibition in both human A549 lung epithelial cells and mouse thioglycolate-elicited peritoneal exudate cells.[26–28] Activation of transcription or 'transactivation' was determined by measuring induction of TAT in human HepG2 cells,[4,29] and GS in both human skeletal muscle cells and mouse C2C12 cells.[30–32] The above cell-based assays were used to guide SAR development with the goal of minimizing transactivation and maximizing transrepression effects. Both human and mouse cell lines were used for in vitro screening

because the in vivo LPS-induced TNF-α secretion model[33] used to further characterize compounds that showed reasonable levels of potency and dissociation between transactivation and transrepression assays, was conducted in a mouse.

The first ketals investigated were synthesized using the 6,6-A,B-ring system ($n = 2$). Initially, symmetrically disubstituted 1,2- and 1,3-diols were chosen for ketal synthesis to eliminate the potential for formation of multiple ketal diastereoisomers, thus complicating purification and analysis. In general, GR was not tolerant of bulky, polar substituents and smaller lipophillic groups were preferred. A comparison of the GR binding potency and IL-6 repression for compounds **18**, **19**, **20**, and **21** showed that the optimal chain length was ethyl (Table 1). The addition of allylic double bonds (compound **22**) increased potency in both the binding and transrepression assays. Bis-propenyl ketal **23** was of comparable potency to **22** in the transrepression assays but increases in the levels of transactivation of TAT and GS were observed. Substituents such as phenyl (compound **24**) and increasing the ketal ring size to 6 (**25** vs **18**) were surprisingly well tolerated.

A selection of these compounds were synthesized in the 6,5-A,B-ring system ($n = 1$, Table 1). In general these compounds were found to have comparable GR binding and transactivation potency relative to those in the 6,6-A,B-ring system but were slightly more potent in the transrepression assays. Compounds **27** and **28** showed good levels of dissociation between the transactivation and transrepression assays. The bis-propenyl ketal (**29**) was about as potent as dexamethasone in the transrepression assays, but suffered from higher levels of transactivation of GS and TAT than other ketals in this

Table 1. SAR of disubstituted ketals

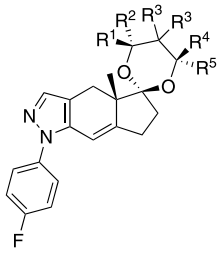


6,5 A,B-ring system, n=1
6,6 A,B-ring system, n=2

Compd	R	*m*	*n*	hGR[a]	hTAT[b,c]		hGS[d,c]		hIL-6[e,c]		mIL-6[f,c]	
				IC_{50} (nM)	EC_{50} (nM)	% dex	EC_{50} (nM)	% dex	EC_{50} (nM)	% dex	EC_{50} (nM)	% dex
2	—	—	—	13.8	211	81	32	97	4.5	102	5.7	95
5	H	0	2	14	n.d.[g]	5	n.d.[g]	2	n.d.[h]	7[h]	n.d.[h]	25[h]
18	Me	0	2	10	n.m.[i]	n.m.[i]	n.m.[i]	n.m.[i]	n.d.[h]	21[h]	n.d.[h]	47[h]
19[j]	Et	0	2	5.5	n.m.[i]	n.m.[i]	n.d.[g]	11	19	32	78	79
20[l]	*n*-Pr	0	2	8.9	n.d.[g]	8	29	58	n.d.[h]	10[h]	n.m.[i]	n.m.[i]
21[l]	*n*-Bu	0	2	22	n.d.[g]	1	n.d.[h]	54[h]	92	21	n.m.[i]	n.m.[i]
22	Vinyl	0	2	1.2	n.d.[g]	12	n.d.[g]	14	5.3	65	8.7	81
23[m]	1-Propenyl	0	2	2.5	n.d.[g]	16	n.d.[g]	25	7.8	67	37	79
24	Ph	0	2	10	n.m.[i]	n.m.[i]	n.d.[g]	6	n.d.[h]	23[h]	26	66
25	Me	1	2	8.3	n.m.[i]	n.m.[i]	n.d.[g]	0	n.d.[h]	27[h]	n.d.[h]	40[h]
13	H	0	1	73	n.d.[g]	3	n.d.[g]	0	28	69	n.d.[h]	17[h]
26	Me	0	1	24	n.d.[g]	6	n.d.[g]	3	n.d.[g]	30	n.d.[h]	40[h]
27[k]	Et	0	1	6.6	n.d.[g]	11	n.d.[g]	7	24	75	36	62
28	Vinyl	0	1	8.0	n.d.[g]	11	n.d.[g]	5	2.6	58	61	56
29[m]	1-Propenyl	0	1	2.7	164	74	17	45	6.5	93	7.6	111
30	CH_2OCH_3	0	1	26	n.d.[g]	3	n.d.[g]	23	8.3	62	10	69
31	Ph	0	1	10	n.d.[g]	19	10	0	10	67	60	85
32	Me	1	1	8.4	n.d.[g]	9	n.d.[g]	0	7.5	58	45	72

[a] Data reported is derived from duplicate wells and three independent experiments. Mean IC_{50} values were determined from 10-point, one-third log concentration response curves and standard errors were ⩽20%.
[b] Human tyrosine amino transferase (TAT) assay in HepG2 cells.
[c] Experiments were run in duplicate. EC_{50} values were determined from eight-point, one-third log concentration response curves and standard errors were ⩽20%.
[d] Human glutamine synthetase (GS) assay in skeletal muscle cells.
[e] Human IL-6 assay in A549 lung carcinoma cell line.
[f] Mouse macrophage IL-6 assay in peritoneal exudate cells harvested by gavage from C57B1/6 mice.
[g] n.d.: not determined; EC_{50} values not reported in cases where % dex ⩽ 35%.
[h] % single point inhibition at 3 μM, hence EC_{50} not determined.
[i] n.m.: not measured.
[j] Compound synthesized from **22** by catalytic hydrogenation over 10% Pd/C in EtOAc.
[k] Compound synthesized from **28** by catalytic hydrogenation over 10% Pd/C in EtOAc.
[l] Diol synthesized by Sharpless asymmetric dihydroxylation from the corresponding *trans*-olefin as in Ref. 34.
[m] Diol synthesized by method adapted from Refs. 35 and 36.

Table 2. SAR of six-membered ketals



Compd	R^1	R^2	R^3	R^4	R^5	hGR[a]	hTAT[b,c]		hGS[d,c]		hIL-6[e,c]		mIL-6[f,c]	
						IC_{50} (nM)	EC_{50} (nM)	% dex	EC_{50} (nM)	% dex	EC_{50} (nM)	% dex	EC_{50} (nM)	% dex
33	H	H	H	Me	H	18	n.d.[g]	4	n.d.[g]	0	0.48	46	n.d.[h]	38[h]
34	Me	H	H	H	H	21	n.d.[g]	4	n.d.[g]	15	0.53	35	n.d.[h]	20[h]
32	H	Me	H	Me	H	8.4	n.d.[g]	9	n.d.[g]	0	7.5	58	45	72
35	Me	H	H	H	Me	7.1	n.d.[g]	8	n.d.[g]	7	13	60	54	69
36	Me	H	H	Me	H	3.1	n.d.[g]	9	n.d.[g]	4	26	64	19	64
37	H	H	Me	H	H	28	n.d.[g]	8	n.d.[g]	6	12	73	n.d.[h]	35[h]
38	H	Me	H	Me	Me	2.2	n.d.[g]	2	n.d.[g]	0	12	44	n.d.[h]	39[h]
39	Me	Me	H	Me	H	2.1	n.d.[g]	5	n.d.[g]	15	25	59	18	77

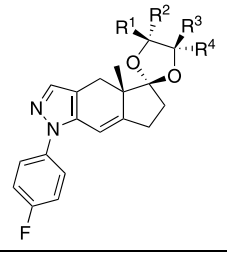
Legend as for Table 1.

series. Groups such as methoxymethyl (**30**) and phenyl (**31**) were also tolerated.

Increasing the ketal ring size to 6 in the 6,5-A,B-ring system was tolerated well ($m = 1$, $n = 1$, Table 1). Compound **32** retained good levels of dissociation and thus warranted the further investigation of similar six-membered ketals on the 6,5-A,B-ring system (Table 2). A series of six-membered methyl substituted ketals were synthesized both varying the number of methyl groups and substitution pattern and stereochemistry. These compounds were synthesized from either racemic or enantiopure 1,3-diols and where necessary, any ketal diastereoisomers produced were separated by chromatography and characterized by NMR spectroscopy (2D-NOESY studies). Levels of GR binding potency increased with the number of ketal methyl groups but functional activity appeared to be optimal with compounds containing two methyl groups (**32**, **35**, **36**, and **37**). A comparison of the asymmetrically substituted ketals **38** and **39** showed that although both compounds have similar GR binding potencies, transrepression activity was higher for ketal **39**, which has an extra methyl group projecting behind the plane of the molecule as drawn. This trend was not followed with a comparison of ketals **33** and **34**, as ketal **33**, with its solitary ketal methyl group projecting in front of the plane of the molecule was more potent in the transrepression assays.

The effect of number of substituents and stereochemistry was also investigated in the case of five-membered ketals and the 6,5-A,B-ring system (Table 3). These compounds were synthesized from either racemic or enantiopure 1,2-diols and the ketal diastereoisomers were

Table 3. SAR of monosubstituted five-membered ketals



Compd	R^1	R^2	R^3	R^4	hGR[a]	hTAT[b,c]		hGS[d,c]		hIL-6[e,c]		mIL-6[f,c]	
					IC_{50} (nM)	EC_{50} (nM)	% dex	EC_{50} (nM)	% dex	EC_{50} (nM)	% dex	EC_{50} (nM)	% dex
40	H	Vinyl	H	H	5.8	n.d.[g]	7	n.d.[g]	0	9.0	36	168	52
41	H	Ph	H	H	8.5	n.d.[g]	13	n.d.[g]	5	n.d.[h]	21[h]	91	70
42	Vinyl	H	H	H	5.6	n.d.[g]	7	n.d.[g]	0	25	47	183	57
43	Ph	H	H	H	12	n.d.[g]	2	n.d.[g]	7	n.d.[h]	2[h]	n.d.[h]	46[h]
44	H	H	Vinyl	H	38	n.d.[g]	4	n.d.[g]	0	n.d.[g]	32	n.d.[h]	27[h]
45	H	H	Ph	H	26	n.d.[g]	13	n.d.[g]	0	n.d.[h]	0[h]	n.d.[h]	42[h]
46	H	H	H	Vinyl	49	n.d.[g]	4	n.d.[g]	0	n.d.[h]	18[h]	n.d.[h]	35[h]
47	H	H	H	Ph	65	n.d.[g]	8	n.d.[g]	0	n.d.[h]	20[h]	n.d.[h]	49[h]

Legend as for Table 1.

separated by chiral HPLC and characterized by NMR spectroscopy (2D-NOESY studies). Although these compounds had far less functional activity than their disubstituted counterparts in Table 1, there was a definite trend in potency and functional activity where compounds with the R-group projecting back behind the plane of the compound as drawn (**40**–**43**), were more potent than compounds where the R-group is projected in front of the plane (**44**–**47**). It therefore appears that in the case of five-membered ketals, substitution at the carbon on the rear of the molecule has a greater contribution to potency than ketal substitution on the carbon on the front face of the molecule.

A subset of the ketals was counter-screened against other nuclear hormone receptors (PR, MR, and ER-α and ER-β) to determine cross-reactivity. As shown in Table 4, all of the compounds examined had excellent selectivity and affinity toward the glucocorticoid receptor.

A subset of the above compounds was evaluated in an LPS induced TNF-α secretion model[33] in mice to determine whether the functional activity observed in vitro was indicative of anti-inflammatory activity in animal systems (Table 5). The mouse in vitro data for IL-6 transrepression and GS transactivation is also included illustrating that these compounds are indeed dissociated in vitro in mouse cells. Also shown is the pharmacokinetic data for **28** and **35**. Compound **28** had a clearance of 114 mL/min/kg, a half life of 2.3 h and an oral bioavailability of 14%. In the LPS induced TNF-α secretion model, compounds **28**, **35**, and **27** at an oral dose of 30 mg/kg showed good in vivo inhibition of TNF-α secretion relative to that of dexamethasone, albeit at an 100-fold lower oral dose of 0.3 mg/kg. A dose-dependent inhibition of TNF-α secretion after oral dosing of compound **28** relative to that of prednisolone is shown in Figure 2. Compound **28** had an ED_{50} of 14.1 mg/kg compared with 0.5 mg/kg of prednisolone in the same assay.

In conclusion, a novel series of potent and selective nonsteroidal ligands has been discovered. The conventional C- and D-rings of steroidal glucocorticoids can be replaced with a variety of substituted ketals of differing ring size and the B-ring can be reduced in size to a

Table 4. Binding selectivity toward GR relative to other nuclear hormone receptors

Compd	hGR[a] (nM)	hPR[a] (μM)	rMR[a] (μM)	hERα[a] (μM)	hERβ[a] (μM)
13	73	>1	>10	>10	>10
5	14	>1	7.56	>10	>10
26	24	>1	>10	>10	>10
28	8.0	>1	>10	>10	>10
22	1.2	>1	7.40	>10	>10
35	7.1	n.m.[b]	>10	>10	>10

[a] Data reported is derived from duplicate wells and three independent experiments. Mean IC_{50} values were determined from ten-point, one-third log concentration response curves and standard errors were ⩽20%.
[b] n.m.: not measured.

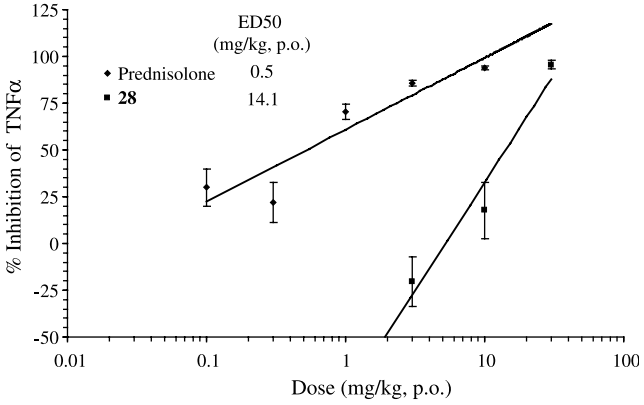


Figure 2. LPS induced TNF-α secretion model data for compound **28**.

Table 5. In vivo data

Compd	mGS[a,b]		mIL-6[c,b]		IV PK[d]				PO PK[e]		% Inhibition of TNF-α[f]
	EC_{50} (nM)	% dex	EC_{50} (nM)	% dex	Cl[g] (mL/min/kg)	V_{dss}[h] (L/kg)	$T_{1/2}$[i] (h)	AUCN[j] (μM h)	AUCN[j] (μM h)	F%[k]	
28	n.d.[l]	5	61	56	114.0	15.3	2.3	0.4	0.1	14.3	96.8
35	n.d.[l]	25	54	69	118.3	27.2	3.0	0.4	0.1	23.0	80.8
27	n.d.[l]	17	36	62	n.m.[m]	n.m.[m]	n.m.[m]	n.m.[m]	n.m.[m]	n.m.[m]	97.8

[a] Mouse glutamine synthetase (GS) assay in C2C12 cells.
[b] Experiments were run in duplicate. EC_{50} values were determined from eight-point, one-third log concentration response curves and standard errors were ⩽20%.
[c] Mouse macrophage IL-6 assay in peritoneal exudate cells harvested by gavage from C57B1/6 mice.
[d] IV pharmacokinetic study at a dose of 2 mg/kg.
[e] PO pharmacokinetic study at a dose of 10 mg/kg.
[f] Inhibition of TNF-α secretion reported for an oral dose of 30 mg/kg relative to that produced by dexamethasone at a dose of 0.3 mg/kg.
[g] Clearance.
[h] Volume of distribution.
[i] Half life.
[j] Normalized area under curve.
[k] Oral bioavailability.
[l] n.d.: not determined; EC_{50} values not reported in cases where %dex ⩽ 35%.
[m] n.m.: not measured.

five-membered ring. Symmetrically disubstituted ketals have better profiles than their corresponding monosubstituted analogs. These compounds retain comparable GR binding potency and steroid receptor selectivity in vitro to that of marketed glucocorticosteroids. The few compounds that showed elevated levels of transactivation also showed accompanying increases in levels of transrepression. Generally however, most of the compounds tested showed partial agonism in the transrepression assays and little to no agonism in the transactivation assays. Although anti-inflammatory potency in vivo is much reduced relative to marketed glucocorticoids, these compounds show improved degrees of dissociation between functional transactivation and transrepression activities in vitro. Currently, mouse models for characterizing the side effects of glucocorticoid use and consequently levels of dissociation in vivo are not well established.[6] Therefore, it remains to investigate whether these compounds will show a decrease in the usual side effects of glucocorticoids when compared with conventional steroids.

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