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ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 4158–4162
Bioorganic & Medicinal Chemistry Letters

5(*Z*)-Benzylidene-1,2-dihydro-9-hydroxy-10-methoxy-2,2,4-trimethyl-5*H*-1-aza-6-oxa-chrysenes as non-steroidal glucocorticoid receptor modulators

Robert J. Ardecky, Andrew R. Hudson,* Dean P. Phillips, John S. Tyhonas, Charlotte Deckhut, Thomas L. Lau, Yongkai Li, Esther A. Martinborough, Steven L. Roach, Robert I. Higuchi, Francisco J. Lopez, Keith B. Marschke, Jeffrey N. Miner, Donald S. Karanewsky, Andrés Negro-Vilar and Lin Zhi

Discovery Research, Ligand Pharmaceuticals, 10275 Science Center Drive, San Diego, CA 92121, USA

Received 23 March 2007; revised 15 May 2007; accepted 17 May 2007
Available online 23 May 2007

Abstract—A series of 5-benzylidene-1,2-dihydro-2,2,4-trimethyl-5*H*-1-aza-6-oxa-chrysenes was synthesized and profiled for their ability to act as selective glucocorticoid receptor modulators (SGRMs). The synthesis and structure–activity relationships for this series of compounds are presented.

Prednisolone[1] (prednisone) and dexamethasone[2] (Fig. 1) are widely prescribed glucocorticoids for the treatment of a number of inflammatory conditions. Their use, however, is restricted because of a range of severe side-effects including osteoporosis, diabetes, water retention, and psychosis.[3] Both the anti-inflammatory activity and many of the associated side-effects are mediated via binding of the glucocorticoid to the glucocorticoid receptor (GR). GR, a ligand-regulated transcription factor that binds cortisol, can activate and repress transcription.[4,5] The GR–ligand complex (GRC) binds directly to glucocorticoid response elements in the promoter region of regulated genes to activate gene expression. The GRC can also repress transcription inhibiting the activity of transcription factors such as NFκB and AP-1, key regulators of genes that encode cytokines and other inflammatory mediators.[5] Mutations to the GR have demonstrated that activation and repression are distinct functions of the receptor.[6] A glucocorticoid that favors repression over activation may exhibit less glucocorticoid-related side-effects while retaining its anti-inflammatory activity.

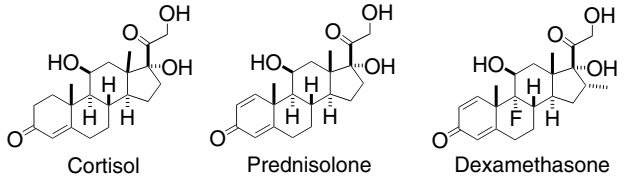

Figure 1. Steroidal glucocorticoids.

Other side-effects related to the administration of glucocorticoids may be attributed to their cross-reactivity with other nuclear hormone receptors, including the androgen receptor (AR), progesterone receptor (PR), and mineralocorticoid receptor (MR).[7] Thus identifying receptor- and gene-selective glucocorticoids with an improved therapeutic index continues to be an active area of research.[8–16]

Our SGRM program was based upon the observation that certain progesterone receptor agonists[17] displayed a significant degree of GR cross-reactivity (Fig. 2). We have previously disclosed AL-438,[9,10] a gene- and receptor-selective glucocorticoid that maintains steroid-like anti-inflammatory activity but with reduced negative effects on bone metabolism and glucose control at equivalent anti-inflammatory doses. It was later discovered

Keywords: Glucocorticoid; Hormone receptor; Cortisol; Inflammation; Prednisolone; Dexamethasone.
* Corresponding author. Tel.: +1 858 550 4432; fax: +1 858 550 7249; e-mail: ahudson@ligand.com

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doi:10.1016/j.bmcl.2007.05.062

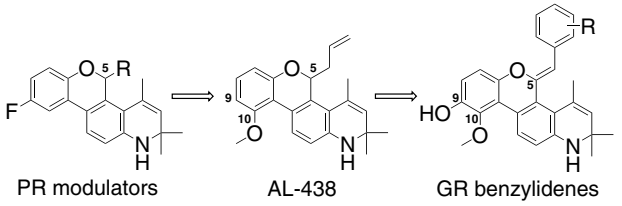
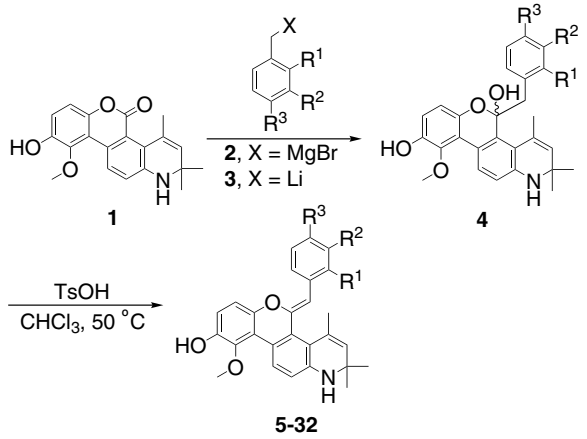


Figure 2.

that incorporation of a 9-OH substituent within this series improved compound potency.[11] Herein, we describe the synthesis and characterization of a number of C-5 benzylidene compounds based upon this scaffold that behave as glucocorticoid receptor modulators (Fig. 2).

We began by systematically exploring structure–activity relationships (SAR) around the C-5 phenyl group of the benzylidene motif utilizing readily available benzylic nucleophiles. We accomplished this by employing two different synthetic strategies (Scheme 1). Lactone **1**[11] was treated with benzyl Grignard **2**, generated from the corresponding bromide, to yield the intermediate lactol **4**. Alternatively we employed a lateral-lithiation strategy[18] whereby the desired tolyl derivative (with a neighboring directing group) was treated with a strong base to give the corresponding lithium anion **3**, which was added to the lactone in a similar manner. This second strategy served as a valuable alternative due to difficulties encountered in the preparation of certain benzyl Grignard reagents. In both strategies the corresponding lactol intermediate was subjected to acid-catalyzed dehydration to give the corresponding C-5 benzylidene product.[19,20]

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.[11] Direct transcriptional activation by GR was measured in a cotransfection[21] (CTF) assay using an MMTV:luciferase reporter. This GRE activation assay can determine agonist or antagonist activity (when compounds are tested in the presence of an EC_{50} of dexamethasone), and provide an estimate of the ligand's



Scheme 1. Synthesis of C-5 benzylidene analogs.

affinity for GR. The E-selectin assay was used to evaluate compounds for repression of transcription mediated by NFκB or AP-1 as an indicator of anti-inflammatory activity.[22]

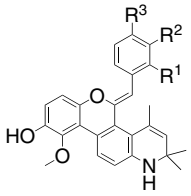
The SAR was found to be relatively broad with a number of substitution patterns well tolerated (Table 1). The majority of compounds bound with high affinity to GR. In general, compounds substituted at R^1 (**5**–**15**) exhibited a partial agonist/antagonist profile in the GRE activation assay but behaved as efficacious and potent agonists in the E-selectin repression assay. This type of profile is distinct from that of classical steroids and suggests that the compounds induce a differential gene regulation profile compared to ligands with a full agonist GRE activation profile. This is likely mediated by conformational changes in the GRC that result in differential recruitment of co-activators and co-repressor proteins through which the GR interacts to regulate gene transcription.[10] We explored a number of analogs bearing hydrophilic or ionizable groups at R^1. Several of these analogs demonstrated high activity in the E-selectin repression assay while maintaining a partial GRE agonist activation profile. Substitution at R^2 (**16**–**23**) translated to minimal GRE activation agonist activity while retaining excellent GR-repression activity. For example, compound **16** had no measurable GRE activation agonist activity but fully repressed transcription in the E-selectin assay. The majority of R^3 substituted analogs (**24**–**31**) exhibited an agonist GRE activation profile and thus had no obvious separation between activation and repression. Introduction of larger groups at R^3, such as phenyl analog **28**, showed reduced activity in the E-selectin repression assay.

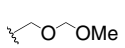
Activity toward other steroid hormone receptors was monitored within the series of compounds. The majority of these compounds were highly GR selective (Table 2). For example, compound **13** exhibited >1000-fold separation in its affinity to GR over other nuclear hormone receptors.

A number of C-5 heterocyclic analogs were also synthesized. Benzodioxane **33**, synthesized via the corresponding benzyl Grignard reagent, appeared efficacious and potent in E-selectin while retaining an antagonist GRE activation profile (Table 3). Analogs **34**–**36** were prepared via a lateral lithiation strategy (Scheme 2). Thienyl and furyl methylidenes **34** and **35** exhibited high GRE activation agonist activity, whereas pyridyl analog **36** behaved as a partial GRE activation agonist (Table 3). All heterocyclic analogs showed excellent E-selectin repression activity.

A number of these compounds were profiled in vivo. The carrageenan-induced paw edema assay (CPE) is a classical model of acute inflammation and has been widely used for profiling anti-inflammatory agents, including glucocorticoids.[23] Rats were dosed orally with the compound of interest (1, 3, 10, and 30 mg/kg, 5 rats per dose) and 1 h later carrageenan was injected into the right hindpaw, causing acute edema. The paw volume was measured 3 h later. All three representative

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



Compound	R^1	R^2	R^3	GR binding	GRE activation agonist mode		GRE activation antagonist mode		E-selectin repression assay	
				K_i (nM)	EC_{50} (nM)	Eff. (%)	IC_{50} (nM)	Eff. (%)	IC_{50} (nM)	Eff. (%)
	Prednisolone			5.3 ± 0.3	5.3 ± 3.6	130 ± 6.5	—	—	4.1 ± 0.8	100 ± 1.4
5	F	H	H	0.8 ± 0.3	0.6 ± 0.2	19 ± 3.4	1.3 ± 0.5	89 ± 2.0	1.4 ± 0.3	99 ± 1.1
6	Cl	H	H	15	18 ± 1.8	22 ± 9.1	24 ± 7.6	60 ± 3.5	18 ± 2.0	92 ± 3.1
7	CH_3	H	H	1.7 ± 0.5	3.4 ± 2.9	16 ± 1.2	0.4 ± 0.2	91 ± 0.5	1.0 ± 0.8	103 ± 1.8
8	Ph	H	H	6.7 ± 1.1	4.5 ± 1.8	13 ± 1.9	3.6 ± 1.5	92 ± 1.4	4.4 ± 2.0	101 ± 1.5
9		H	F	2.4 ± 0.9	5.1 ± 1.3	56 ± 4.4	11 ± 3.9	56 ± 4.4	3.4 ± 1.5	98 ± 1.0
10		H	F	3.1	79 ± 28	19 ± 2.4	85 ± 1.8	167 ± 88	27	98
11		H	F	0.9	5.5 ± 0.1	58 ± 9.3	—	—	1.9 ± 0.6	95 ± 1.3
12		H	F	94	108 ± 20	23 ± 1.5	6.6 ± 2.1	73 ± 2.0	22 ± 6.7	93 ± 2.4
13		H	F	0.5	16 ± 14	17 ± 4.9	89 ± 1.4	26 ± 25	1.0	105
14		H	F	1.5	356 ± 192	92 ± 4.5	0.1 ± 0.1	70 ± 13	17	59
15		H	F	0.6	192 ± 111	44 ± 3.6	0.4 ± 0.1	81 ± 4.6	4.1	107
16	H	CH_3	H	2.5 ± 0.5	—	—	0.2 ± 0.1	97 ± 0.4	0.9 ± 0.4	104 ± 1.9
17	H	CF_3	H	2.7 ± 0.1	—	—	2.5 ± 1.2	97 ± 0.2	4.1 ± 0.8	94 ± 0.8
18	H	Ph	H	7.2 ± 1.5	—	—	2.3 ± 1.3	91 ± 0.1	12 ± 4.4	102 ± 0.8
19	H	OH	H	1.4 ± 0.5	1.5 ± 0.2	32 ± 3.9	4.7 ± 1.3	65 ± 3.4	1.1 ± 0.3	101 ± 2.3
20	H	OCF_3	H	4.1 ± 0.9	—	—	1.9 ± 0.2	95 ± 0.1	4.7 ± 0.8	94 ± 2.5
21	H	OPh	H	4.6	—	—	1.9	94	74 ± 26	62 ± 7.2
22	H		H	3.1 ± 0.8	—	—	0.5 ± 0.1	95 ± 0.1	1.4 ± 0.9	101 ± 2.0
23	H		H	1.5 ± 0.1	—	—	0.7 ± 0.1	93 ± 1.9	3.6 ± 0.2	99 ± 1.1
24	H	H	Cl	1.7	43 ± 8.5	70 ± 34	—	—	12 ± 5.9	95 ± 5.5
25	H	H	CH_3	1.9 ± 0.3	38 ± 10	39 ± 10	12 ± 10	55 ± 8.1	15 ± 2.4	91 ± 1.0
26	H	H	Et	1.9 ± 0.8	207	54	—	—	17 ± 8.1	94 ± 5.4
27	H	H	*i*-Pr	2.9 ± 2.3	455 ± 69	37 ± 8.2	—	—	—	—
28	H	H	Ph	4.4	33	142	—	—	77	88
29	H	H	OH	1.1 ± 0.6	1.1 ± 0.8	15 ± 0.1	0.4 ± 0.1	88 ± 2.3	1.0 ± 0.5	96 ± 0.8
30	H	H	OCH_3	2.7 ± 1.1	133 ± 57	133 ± 15	—	—	10	70
31	H	H	OCF_3	4.3 ± 0.4	437 ± 25	24 ± 3.5	—	—	91	69
32	Me	CF_3	H	2.6 ± 0.5	—	—	2.6 ± 0.5	96 ± 0.4	15 ± 3.7	95 ± 3.1

[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%). Antagonist efficacies are represented as a percent of maximal inhibition of the response of an EC_{50} of dexamethasone. 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. A hyphen (—) denotes an efficacy <10% or potency >1000 nM.

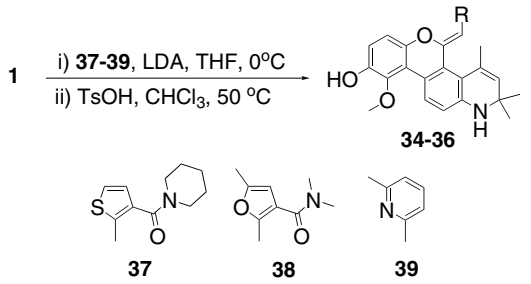
Table 2. [a]Binding cross-reactivity data for selected glucocorticoid receptor modulators

Compound	Binding K_i (nM)			
	GR	PR	AR	MR
13	0.5	970	700	1100
15	0.6	840	630	430
16	2.5 ± 0.5	400± 89	570 ± 130	ND
19	1.4 ± 0.5	750 ± 98	910 ± 17	290

[a] 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. ND, no data.

examples show good to excellent anti-inflammatory activity in this assay (Fig. 3). Compound **33** was fully efficacious at 30 mg/kg, compound **34** demonstrated efficacy equivalent to prednisolone at a lower dose of 10 mg/kg.

In conclusion, a number of C-5 benzylidene analogs were prepared and their SAR established. This series appears to mimic the efficacy of benchmark GCs in both in vitro and in vivo models of inflammation. Substitution at the 2- and 3-position of the C-5 benzylidene phenyl ring was well tolerated. A number of heterocycles were also explored and displayed high in vitro GR-mediated activity. Representative examples profiled in vivo demonstrated efficacy equal to that of prednisolone. The GR-mediated side-effects of compounds within this series will be reported in due course.



Scheme 2. Synthesis of compounds **34**–**36**.

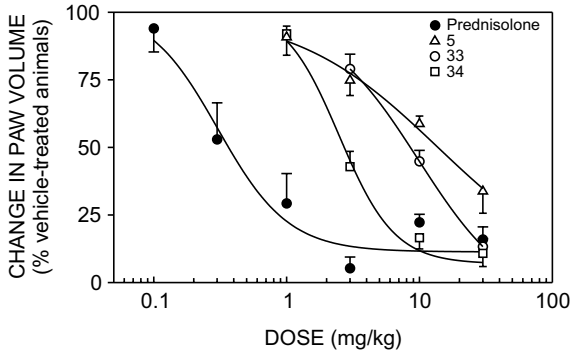
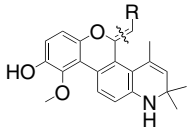
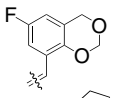
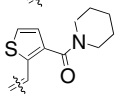
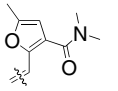
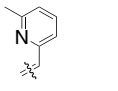


Figure 3. [a]In vivo anti-inflammatory effects of selected compounds. [a]Compounds orally administered using an olive oil vehicle.

Table 3. [a] In vitro assay results for selected heterocyclic glucocorticoid receptor modulators



Compound	R	GR binding	GRE activation agonist mode		GRE activation antagonist mode		E-selectin repression assay	
		K_i (nM)	EC_{50} (nM)	Eff. (%)	IC_{50} (nM)	Eff. (%)	IC_{50} (nM)	Eff. (%)
33		0.9 ± 0.2	0.2 ± 0.1	18 ± 0.2	0.8 ± 0.2	88 ± 1.6	0.7 ± 0.2	99 ± 5.0
34		1.4 ± 0.4	0.2 ± 0.1	93 ± 6.8	4.1 ± 1.6	70 ± 2.8	0.7 ± 0.3	102 ± 2.1
35		3.4 ± 0.6	1.5 ± 0.5	104 ± 23	4.9	40	2.4 ± 1.0	100 ± 0.8
36		1.6 ± 0.3	0.7 ± 0.2	24 ± 5.2	1.1 ± 0.2	84 ± 3.4	4.9 ± 2.5	99 ± 1.7

[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%). Antagonist efficacies are represented as a percent of maximal inhibition of an EC_{50} of dexamethasone. 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.

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19. The benzylidene analogs are light sensitive and should be handled under yellow light. The compounds were isolated as the *Z*-isomer only and were stable to the assay conditions described. Non-photolytic isomerization was not observed.
20. Representative experimental procedure—compound **17**. To a flame-dried 2-neck, 10 ml round-bottomed flask fitted with a reflux condenser were added magnesium turnings (28 mg, 2.0 mmol) and diethyl ether (3 ml). A solution of 3-trifluoromethylbenzyl bromide (478 mg, 2.0 mmol) in diethyl ether was added to the slurry of magnesium turnings. After 1 h, a solution of 9-hydroxy-10-methoxy-2,2,4-trimethyl-1,2-dihydro-5H-chromeno [3,4-f]quinoline-5-one **1** (30 mg, 0.09 mmol) in diethyl ether (1 ml) was added. After 18 h, the reaction was quenched with ammonium chloride (3 ml), extracted with ethyl acetate (2×10 ml), washed with brine (2×10 ml), dried over magnesium sulfate, filtered, and concentrated. The crude product was purified by precipitation from dichloromethane/hexanes and collected by filtration. The product was then dissolved in chloroform and treated with *p*-toluenesulfonic acid (1.7 mg, 0.009 mmol) and followed by TLC (0.1% triethylamine/dichloromethane). After 20 min, the solution was filtered on silica gel, washed with dichloromethane, and concentrated. The crude product was then purified by flash chromatography (0.1% triethylamine/dichloromethane) to afford benzylidene **17** as a yellow powder (12.8 mg, 30% over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (d, $J = 8.6$ Hz, 1H), 8.10 (s, 1H), 7.91–7.84 (m, 1H), 7.48–7.41 (m, 2H), 6.87 (d, $J = 10.8$ Hz, 1H), 6.85 (d, $J = 10.8$ Hz, 1H), 6.69 (d, $J = 8.6$ Hz, 1H), 5.65 (s, 1H), 5.56 (s, 1H), 5.53 (s, 1H), 4.21 (br s, 1H), 3.78 (s, 3H), 2.10 (s, 3H) and 1.37 (br s, 6H).
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