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Discovery and SAR study of novel dihydroquinoline-containing glucocorticoid receptor agonists

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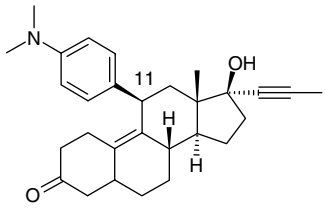
Abstract—We have recently reported the discovery of a novel class of glucocorticoid receptor (GR) antagonists, exemplified by **3**, containing a 1,2-dihydroquinoline molecular scaffold. Further SAR studies of these antagonists uncovered chemical modifications conveying agonist functional activity to this series. These agonists exhibit good GR binding affinity and are selective against other nuclear hormone receptors.

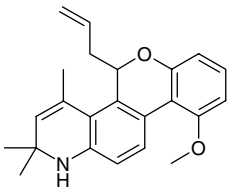
Synthetic glucocorticoids are widely used to treat many serious inflammatory and autoimmune disorders.[1] However, a major drawback in their clinical use is their association with a number of severe and, in some cases, life-threatening adverse events, such as enhanced bone resorption and muscle weakening. The discovery of glucocorticoid receptor (GR) agonists that are dissociated, that is, that exhibit a reduced incidence or a reduced severity of side effects while maintaining potent anti-inflammatory activity, is currently an area of intense research activity within the medicinal chemistry community.[2] There are also additional efforts underway to identify selective GR antagonists with the expectation that these may be useful in treating diabetes.[3]

The progesterone receptor (PR) antagonist Mifepristone (RU-486) **1** is a known GR antagonist effective at blocking gene-transcription mediated by endogenous glucocorticoids. The therapeutic utility of Mifepristone has been demonstrated for the treatment of Cushing's syndrome, diabetes, glaucoma, and depression.[4] Although structural similarity between Mifepristone and endogenous glucocorticoids is noticeable, it also shares structural features with a series of compounds from Abbott/Ligand, exemplified by **2**,[5] that reportedly demonstrate dissociated GR agonist properties in both cellular and in vivo systems. In addition, we have recently disclosed a new class of potent and selective GR ligands, such as **3**, that display antagonistic activity in a cellular system (Fig. 1).[6]

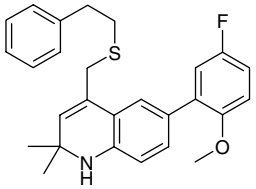
Literature precedents indicate that minor structural modifications of some GR ligands can cause a functional switch from agonist to antagonist activity.[7] Therefore, we decided to explore if our GR antagonists



1 Mifepristone (RU-486)



2 AL-438



3

Figure 1. Steroidal and nonsteroidal nuclear receptor ligands.

Keywords: Glucocorticoid receptor; Agonist; SAR.
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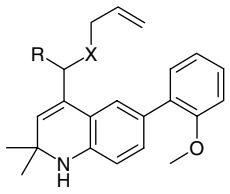
Table 1. Effects of α-methylation at the C-4 position on GR and PR binding affinity



Compound	X	R	GR[a] IC_{50} (nM)	PR[a] IC_{50} (nM)
12	S=O	H	>2000	>2000
13	S	H	360	460
14	S	Me	116	600
15	O	H	>2000	>2000
16	O	Me	116	480

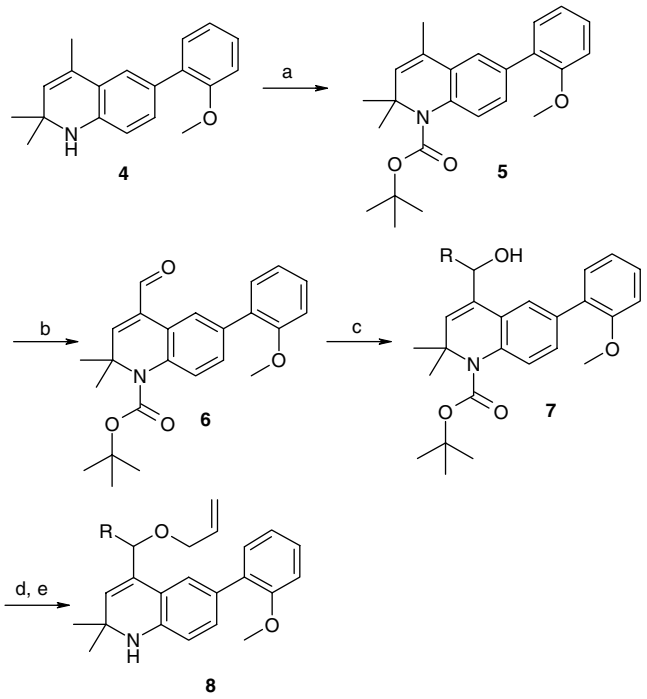
[a] Values are means of two experiments.

could also be converted to agonists via minor structural modifications. In addition, we attempted to improve the drug-like properties of our GR ligands represented by **3**. A primary concern was that thioether-containing compounds are likely to be extensively metabolized by flavin-containing monooxygenase (FMO) and cytochrome P450 enzymes.[8] These enzymes typically oxidize the thioether-containing xenobiotics to sulfoxide and/or sulfone metabolites, which often display high clearance in in vivo or in vitro systems. Efficacy was expected to be concomitantly lost since compound **12**, a sulfoxide analog of one of our antagonists, did not demonstrate appreciable GR binding affinity when tested at concentrations up 2000 nM (Table 1). We thus anticipated that improving the compounds metabolic stability would be beneficial for both their pharmacokinetic and pharmacodynamic profiles. This paper discloses our initial attempts at identifying a novel series of dissociated GR agonists that are structurally derived from our original series of GR antagonists exemplified by **3**.

For SAR study of the α-position on C4 substituent, we synthesized various derivatives, starting from compound **4**.[6] Protection of the secondary amine in **4** with a tBoc group followed by allylic oxidation with selenium dioxide yielded aldehyde **6** in good yield. Reactions of **6** with a variety of Grignard reagents at low temperature afforded the secondary alcohols **7** in modest to good yield. Further O-alkylation of **7** with allyl iodide, followed by removal of the tBoc group with trifluoroacetic acid, provided the desired allyl ether analogs **8**, in acceptable overall yield (Scheme 1).

To study the effects of oxygen substitution, we synthesized several ether analogs starting from compound **6**. The aldehyde **6** was treated with methyl magnesium bromide to afford the secondary alcohol **9** in good yield. This alcohol was then activated as a mesylate and treated with various alkoxides to afford the corresponding ether derivatives. These compounds were eventually deprotected under standard conditions to generate the analogs **11** (Scheme 2).

All the compounds were evaluated for binding in a panel of human nuclear hormone receptors, including GR,

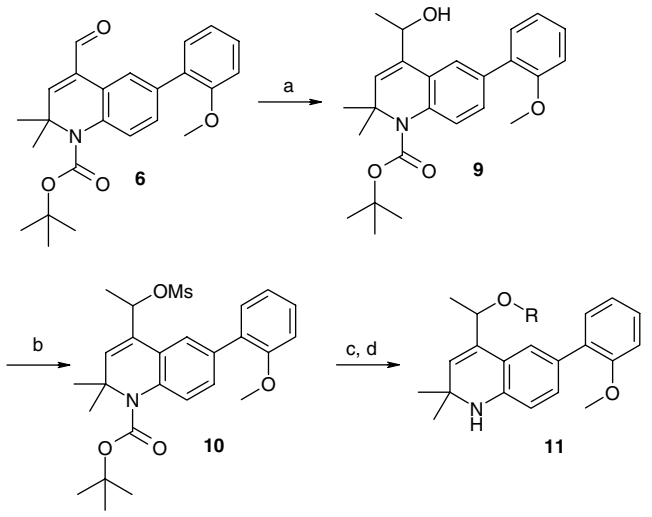


Scheme 1. Reagents and conditions: (a) Boc_2O, 1.6 M *n*-BuLi, THF, −78 °C → 0 °C → rt, 6 h, 91%; (b) selenium dioxide, 1,4-dioxane, reflux, 3 h, 94%; (c) Grignard reagent, THF, −78 °C, 30 min, 47–83%; (d) allyl iodide, 1.0 M sodium bis(trimethylsilyl)amide, DMSO, rt, 10 min, 51–88%; (e) TFA, dichloromethane, rt, 2 h, 60–90%.

PR, estrogen receptor (ER), and mineralo-corticoid receptor (MR) using a fluorescence polarization competitive binding assay.[9]

Introduction of a methyl group at the α-position of the 1,2-dihydroquinoline C4 substituent resulted in a significant increase in GR binding affinity for both the thioether- and the ether-containing analogs. For the thioether analogs, introduction of the methyl group at the α-position resulted in an approximately threefold increase in GR binding affinity, whereas more than a 15-fold increase in GR binding affinity was observed for the ether analogs. Reduction of the size of the heteroatom from sulfur to oxygen was not beneficial for GR binding affinity in the absence of the α-methyl substitution. However, increasing the size of C4 substituents in the presence of the α-methyl group restored GR binding affinity (Table 1). These data suggest that the size of the substituent at the C4 position is important to achieve potent GR binding affinity. This discovery encouraged us to prioritize the ether series for further exploration over the thioether analogs. We also felt that moving away from the thioether derivatives would also alleviate the potential metabolic liability associated with sulfur oxidation.

We then investigated the replacement of the α-methyl group in compound **16** with other substituents, and analyzed the impact of this modification on the resulting GR binding affinity. Increasing the size of the α-substituent from methyl (**16**; IC_{50} = 116 nM) to ethyl (**17**; IC_{50} = 355 nM) or *i*-propyl (**18**; IC_{50} = 710 nM) was

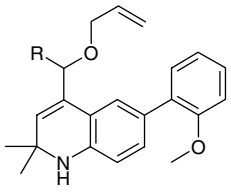


Scheme 2. Reagents and conditions: (a) MeMgBr ether solution, THF, 0 °C, 15 min, 87%; (b) MsCl, TEA, dichloromethane, 0 °C, 1 h, 99%; (c) primary alcohol, 60% NaH, DMF, 0 °C → rt, 4 h, 30–85%; (d) TFA, dichloromethane, rt, 2 h, 60–90%.

accompanied with an approximately threefold and sixfold loss of GR binding affinity, respectively. Compounds featuring a *n*-propyl (**19**) or larger substituents at the α-position lost a significant amount of GR binding affinity. The *n*-propyl substituted compound **19** and the phenyl substituted analog **21** were devoid of either GR or PR binding affinity when tested at concentrations up to 2000 nM. These results indicate that the size of the α-substituent is critical for GR binding affinity. Interestingly, the impact of the size of the α-substituents on PR binding affinity was not as significant as it was on GR binding affinity (Table 2).

Replacing the allyl group of compound **16** with other alkyl groups affected both the GR and the PR binding affinities. For example, replacing the methyl substituent in compound **24**, with an allyl group, as in compound **16**, resulted in about a ninefold increase in GR binding affinity. However, increasing the substituent size further resulted in a loss of GR binding affinity, as illustrated with the 2-butenyl, compound **25** (IC_{50} = 210 nM), the 2-pentenyl compound **27** (IC_{50} = 565 nM), and the 3-phenylallyl compound **28** (IC_{50} = 980 nM). Reduction of the double bond in compound **28** led to a threefold enhancement in GR binding affinity, while the phenethyl ether analogs (**30**–**33**) displayed GR binding affinities similar to those of compound **29** (IC_{50} = 185–400 nM). Regarding the PR binding affinity, no significant difference was observed between the methyl ether **24** (IC_{50} = 540 nM) and the allyl ether, **16** (IC_{50} = 480 nM). Larger oxygen substituents were also characterized by a significant loss of PR binding affinity. For example, compounds **29** and **30**, featuring a phenylpropyl and phenethyl substitution, respectively, showed IC_{50} > 2000 nM. Although an SAR emerged through oxygen substitution for both GR and PR binding affinity, it remained relatively flat for the GR binding affinity when

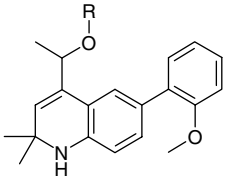
Table 2. GR and PR binding affinity with different α-substituents

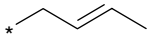
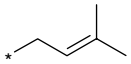
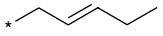
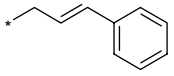
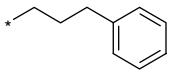
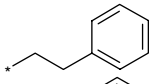
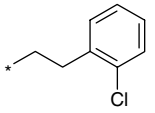
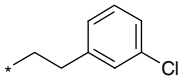
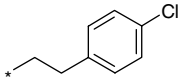
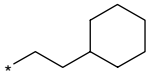


Compound	R	GR[a] IC_{50} (nM)	PR[a] IC_{50} (nM)
15	H	>2000	>2000
16	Methyl	116	480
17	Ethyl	355	330
18	*i*-Propyl	710	950
19	*n*-Propyl	>2000	>2000
20	Allyl	>2000	440
21	Phenyl	>2000	>2000
22	Benzyl	>2000	1200
23	Phenethyl	>2000	1000

[a] Values are means of two experiments.

Table 3. GR and PR binding affinity with different O-substituents



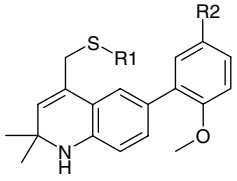
Compound	R	GR[a] IC_{50} (nM)	PR[a] IC_{50} (nM)
24	Me	1060	540
16	Allyl	116	480
25		210	1300
26		395	980
27		565	1600
28		980	>2000
29		260	>2000
30		405	>2000
31		290	>2000
32		275	1900
33		185	930
34		400	>2000

[a] Values are means of two experiments.

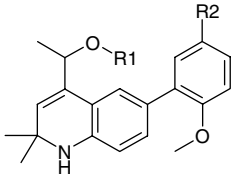
compared to the trends observed when modifying the α-substitution. These results suggest that α-substitution has a more significant impact on GR binding affinity than the oxygen substitution (Table 3).

Structural variations of the right-hand side phenyl ring were also examined. We have reported earlier that introduction of fluorine atom at the C5 position of this phenyl ring resulted in about a twofold increase in GR binding affinity (compounds **35** and **37**) in the thioether series.[6] A similar positive effect on GR binding affinity was also observed in the ether series. Especially in the allyl ether series, introduction of a fluorine atom as R2 produced a significant increase in GR binding affinity and afforded the most potent GR ligand, compound **38** (IC_{50} = 34 nM), in this series (Table 4).

Table 4. Effects of fluorine substitution at R2 on GR and PR binding affinity



Thioethers



Ethers

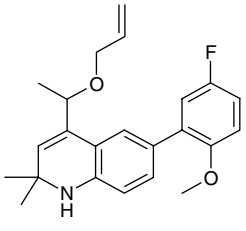
Compound	Series	R1	R2	GR[a] IC_{50} (nM)	PR[a] IC_{50} (nM)
13	Thioether	Allyl	H	360	460
35			F	190	1400
36	Thioether	Phenethyl	H	194	>2000
37			F	84	>2000
16	Ether	Allyl	H	116	480
38			F	34	451
39	Ether	Phenethyl	H	404	>2000
40			F	195	1300

[a] Values are means of two experiments.

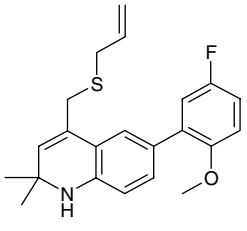
The enantiomers of the most potent GR ligand, compound **38**, were separated by chiral HPLC,[10] and profiled for binding affinities, functional activity, and in vitro metabolic stability. Compound **42**, (+)-isomer, and the corresponding racemate demonstrated similar GR binding affinities, whereas compound **41**, (−)-isomer, was about threefold less potent. No differences in selectivity profiles were observed between the racemate and either enantiomers. The in vitro metabolic stability of these compounds was assessed by measuring their half-lives in a human microsomal preparation.[11] Unfortunately, there was no significant difference between the ether and thioether series, as all compounds showed relatively short half-lives (⩽20 min). Finally, both enantiomers were evaluated for functional cellular activity using an IL-1 induced IL-6 assay in human foreskin fibroblasts.[12] Although both compounds **41** and **42** inhibited IL-6 production with IC_{50}'s of 260 and 170 nM, respectively, the magnitude of their inhibition of IL-6 production at the highest concentration (2000 nM) was modest. Indeed, compounds **41** and **42** displayed only 46% and 28% of dexamethasone's maximum effect, respectively. These data indicate that these ligands are partial GR agonists (Table 5).

In summary, a novel and potent series of GR partial agonists has been identified by structural modification of a GR antagonist lead. Structural modifications of substituents at the C4 and the C6 position impacted GR and PR binding affinities differently, and led to the discovery of potent and selective binders. The most potent GR ligand within the series (**38**) was resolved to its (+)-isomer **42**. Compound **42** represents a novel, potent, and selective GR partial agonist. Unfortunately, replacement of the thioether functionality with an ether substituent did not provide compounds metabolically more stable in human microsome preparations.

Table 5. GR, PR, ER, and MR binding affinity of compounds with the best substituents combinations at C4 and C6 position



16: Racemate
41: (-)-isomer
42: (+)-isomer



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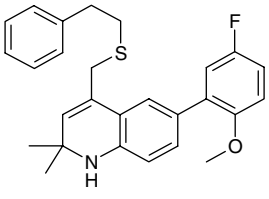

38

Compound	GR[a] IC_{50} (nM)	PR[a] IC_{50} (nM)	MR[a] IC_{50} (nM)	ER[a] IC_{50} (nM)	HLM $T_{1/2}$ (min)	IL-6 IC_{50} (nM)
16	34	450	970	>2000	6	Not tested
41	110	620	>2000	>2000	5	260 (46%)[b]
42	40	170	1100	>2000	6	170 (28%)[b]
35	190	>2000	>2000	>2000	12	Not active
37	84	>2000	>2000	>2000	17	Not active

[a] Values are means of two experiments.
[b] Values are maximum inhibition at 2000 nM percentage to dexamethasone inhibition.

Acknowledgment

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References and notes

1. Toogood, J. In *Glucocorticoids*; Goulding, N. J., Flowers, R. J., Eds.; Birkhauser: Boston, 2001; pp 161–174.
2. Rosen, J.; Marschke, K.; Deepa, R. *Curr. Opin. Drug Discov. Dev.* **2003**, *6*, 224.
3. Coghlan, M. J.; Elmore, S. W.; Kym, P. R.; Kort, M. E. *Ann. Rep. Med. Chem.* **2002**, *37*, 167.
4. (a) Chu, J. W.; Matthias, D. F.; Belanoff, J.; Schatzberg, A.; Hoffman, A. R.; Feldman, D. *J. Clin. Endocrinol. Metab.* **2001**, *86*, 3568; (b) Gettys, T. W.; Watson, P. M.; Taylor, I. L.; Collins, S. *Int. J. Obes.* **1997**, *21*, 865; (c) Phillips, C. I.; Green, K.; Gore, S. M.; Cullen, P. M.; Campbell, M. *Lancet* **1984**, *1*, 767; (d) Belanoff, J. K.; Flores, B. H.; Kalezhan, M.; Sund, B.; Schatzberg, A. F. *J. Clin. Psychopharmacol.* **2001**, *21*, 516.
5. (a) Elmore, S. W.; Coghlan, M. J.; Anderson, D. D.; Pratt, J. K.; Green, B. E.; Wang, A. X.; Stashko, M. A.; Lin, C. W.; Tyree, C. M.; Miner, J. N.; Jacobson, P. B.; Wilcox, D. M.; Lane, B. C. *J. Med. Chem.* **2001**, *44*, 4481; (b) Kym, P. R.; Kort, M. E.; Coghlan, M. J.; Moore, J. L.; Tang, R.; Ratajczyk, J. D.; Larson, S. W.; Elmore, J. D.; Pratt, J. K.; Stashko, M. A.; Douglass Falls, H.; Lin, C. W.; Nakane, M.; Miller, L.; Tyree, C. M.; Miner, J. N.; Jacobson, P. B.; Wilcox, D. M.; Nguyen, P.; Lane, B. C. *J. Med. Chem.* **2003**, *46*, 1016; (c) Coghlan, M. J.; Jacobson, P. B.; Lane, B.; Nakane, M.; Lin, C. W.; Elmore, S. W.; Kym, P. R.; Luly, J. R.; Carter, G. W.; Turner, R.; Tyree, C. M.; Hu, J.; Elgort, M.; Rosen, J.; Miner, J. N. *Mol. Endocrinol.* **2003**, *17*, 860.
6. Takahashi, H.; Bekkali, Y.; Capolino, A. J.; Gilmore, T.; Goldrick, S. E.; Nelson, R. M.; Trenzio, D.; Wang, J.; Zuvela-Jelaska, L.; Proudfoot, J.; Nabozny, G.; Thomson, D. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1549.
7. Zhi, L.; Ringgenberg, J. D.; Edwards, J. P.; Tegley, C. M.; West, S. J.; Pio, B.; Motamedi, M.; Jones, T. K.; Marschke, K. B.; Mais, D. E.; Schrader, W. T. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2075.
8. (a) Furnes, B.; Schlenk, D. *Drug Metab. Dispos.* **2005**, *33*, 214; (b) Usmani, K. A.; Karoly, E. D.; Hodgson, E.; Rose, R. L. *Drug Metab. Dispos.* **2004**, *32*, 333; (c) Furne, B.; Schlenk, D. *Toxicol. Sci.* **2004**, *78*, 196.
9. Fluorescence polarization competitive binding assays were performed to quantitate the ability of test compounds to displace ligands from GR, MR, ER, and PR in solution. Binding reactions were assembled in 96-well microplates. Baculovirus lysate containing either GR or MR was incubated with 5 nM tetramethyl-rhodamine conjugate of dexamethasone, and test compound dilutions in an assay buffer containing 10 mM TES, 50 mM KCl, 20 mM sodium molybdate, 1.5 mM EDTA, 0.04% w/v CHAPS, 10% v/v glycerol, 1 mM DTT, pH 7.4. For the PR assay, baculovirus lysate-containing PR was incubated with 5 nM tetramethyl-rhodamine conjugate of RU486, and the test compound dilutions. The ER binding assay was performed using the ER Competitor Assay kit from Panvera (Invitrogen part number P2614). This assay uses purified baculovirus-expressed human ER and fluorescein conjugate of a proprietary ER ligand (Fluormone™ ES2). IC_{50} values shown were means of a single experiment done in duplicate 11-point concentration–effect curves. Bekkkali, Y.; Gilmore, T.; Spero, D. M.; Takahashi, H.; Thomson, D. S.; Wang, J. *PCT Int. Appl.*, WO2004018429.
10. Enantiomers of compound **38** were separated by following conditions. Column: Chiralcel OD (25 cm × 2.5 cm ID) Mobile phase: 0.5% isopropanol in hexanes. Flow: 10 ml/min. Compound **41**: 94.4% ee (chiral HPLC), $[\alpha]_D$ −70° (c = 0.05, $CHCl_3$, 25 °C). Compound **42**: 94.5% ee (chiral HPLC), $[\alpha]_D$ +76° (c = 0.05, $CHCl_3$, 25 °C). Absolute configurations of compounds 41 and 42 have not been determined.
11. The assay was performed in 50 mM potassium phosphate buffer, pH 7.4, and 2.5 mM NADPH. Test samples were dissolved in acetonitrile for a final assay concentration of 1–10 μM. Human liver microsomes were diluted in assay buffer to a final assay concentration of 1 mg protein/ml. A volume of 25 μl compound solution and 50 μl microsome suspension were added to 825 μl assay buffer. The preparation is incubated for 5 min in a 37 °C water bath. The reaction was started by the addition of 100 μl NADPH. Volumes of 80 μl were removed from the incubation mix at 0, 3, 6, 10, 15, 20, 40, and 60 min after the start of the reaction and added to 160 μl acetonitrile. The samples were shaken for 20 s and then centrifuged for 3 min at 3000 rpm. A 200 μl volume of the supernatant is transferred to 0.25 mm glass fiber filter plates and centrifuged for 5 min at 3000 rpm. Injection volumes of 10 μl were typically added to Zorbax SB C8 HPLC columns with formic acid in water or acetonitrile at a flow rate of 1.5 ml/min. Percent loss of parent compound was calculated from the area under each time point to determine the half-life.
12. Human foreskin fibroblasts were stimulated with 1 ng/ml recombinant human IL-1 in the presence of test compound. After 24 h, the degree of GR agonist activity (transrepression) was determined by measuring IL-6 in the tissue culture media.