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Discovery of a novel non-steroidal GR antagonist with in vivo efficacy in the olanzapine-induced weight gain model in the rat

Hazel J. Hunt [a,*], Nicholas C. Ray [b], George Hynd [b], Jon Sutton [b], Mohammed Sajad [b], Elizabeth O'Connor [b], Shahadat Ahmed [b], Peter Lockey [b], Steve Daly [b], Gerry Buckley [b], Robin D. Clark [a], Robert Roe [a], Christine Blasey [a], Joe Belanoff [a]

[a] Corcept Therapeutics, 149 Commonwealth Drive, Menlo Park, CA 94025, USA
[b] Argenta, 8/9 Spire Green Centre, Flex Meadow, Harlow, Essex, CM19 5TR, UK

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ABSTRACT

We report the optimization of a series of non-steroidal GR antagonists that led to the identification of compound 7. This compound is efficacious when dosed orally in an olanzapine-induced weight gain model in rats.

A growing body of evidence suggests that glucocorticoid receptor (type II) antagonists, particularly mifepristone, can block the weight gain caused by antipsychotic medication. The effect of mifepristone has been demonstrated and replicated in both animal and human studies. In the rat, mifepristone has been shown to both prevent the onset of antipsychotic induced weight gain, and reverse weight gain after it is induced.[1] Subsequently, two randomized clinical trials in humans demonstrated that mifepristone significantly attenuated the weight gain caused by the antipsychotic medications olanzapine[2] and risperidone.[3] In the 28-day trial of weight gain induced by risperidone, mifepristone also reduced the adverse effects of risperidone on fasting insulin and triglycerides.[3]

Animal studies have shown that other, more specific glucocorticoid receptor antagonists, have effects that are similar to those observed in the mifepristone studies. CORT 108297, a potent glucocorticoid receptor antagonist without the progesterone receptor antagonist activity associated with mifepristone, was shown to effectively mitigate olanzapine-induced weight gain in rats.[4] More recently, rats receiving olanzapine plus either of the structural analogues CORT 112716 or CORT 113083 gained significantly less weight than rats receiving only olanzapine.[5] Differences in weight gain were not attributable to decreased food intake.

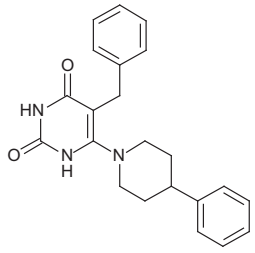

Figure 1. Structure of compound **1.**

We previously reported the discovery and optimization of a series of non-steroidal GR antagonists based on a disubstituted pyrimidinedione template [6], exemplified by compound **1** in Figure 1. Whilst **1** exhibited excellent affinity in a GR binding assay and good selectivity over other nuclear receptors, activity in a reporter gene assay was only modest. Since compound **1** represents a novel scaffold for GR antagonism with reduced molecular weight compared with many GR ligands, we considered that further optimization of this series was justified. In addition, compound **1** exhibited an acceptable in vitro ADME profile, with excellent stability in the human S9 system and no significant inhibition of the five major human CYP isoforms. Figure 2

Affinity for GR was determined as reported previously[6] by measuring displacement of [^{3}H]dexamethasone from recombinant

* Corresponding author.
E-mail address: hhunt@corcept.com (H.J. Hunt).

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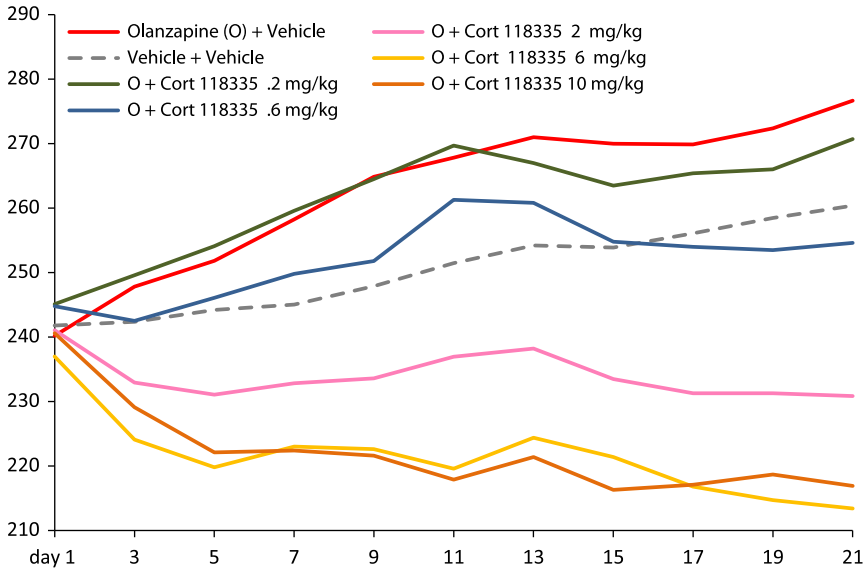


Figure 2. Change in Body weight (g) in Female Rats Receiving Olanzapine and CORT118335.

baculovirus derived human GR.[7] Functional activity was assessed in SW1353/MMTV-5 cells transfected with a plasmid encoding firefly luciferase located behind a glucocorticoid response element (GRE).[7] GR antagonist activity was measured as inhibition of dexamethasone induced luciferase expression. Selected compounds were tested for agonist activity by performing the assay in the absence of dexamethasone. Selectivity over the estrogen (ERα), androgen (AR) and progesterone (PR) receptors was determined using ligand binding assays[8], whilst selectivity over mineralocorticoid receptors (MR) was determined using a reporter gene assay, using T47D cells which express endogenous MR and PR.[7]

Our previous SAR investigation involved variation of the substituents on the pendant benzyl substituent and modification of the piperidine substituent. All of the original compounds incorporated a heterocyclic group attached through the nitrogen to the 4-position of the pyrimidinedione. We decided to replace the nitrogen by a carbon, that is, to use a cyclohexyl group in place of the piperidine, since we considered that this might affect the orientation of the phenyl substituent.

The original synthetic route used to prepare the cyclohexyl compounds is depicted in Scheme 1. Thus, conversion of 4-phenylcyclohexanone (i) to the corresponding vinyl boronate (iii) was achieved via a palladium-catalyzed cross-coupling reaction of bis(pinacolato)diboron with the readily prepared vinyl triflate (ii). Microwave assisted Suzuki cross-coupling of the vinyl boronate (iii) with 6-chloropyrimidinedione (iv) using an air-stable palladium catalyst[2] afforded the expected cyclohexenyl compound (v). Catalytic hydrogenation of the cyclohexene double bond yielded the target cyclohexyl compound as a 5:7 mixture of *trans* and *cis* isomers, **2** and **3**, respectively, which were separated by preparative reverse phase HPLC.

We were gratified to discover that the *trans* isomer **2** had excellent affinity for GR, with K_i = 4 nM. The *cis* isomer **3** was significantly less active, with K_i = 32 nM. Of greater interest was the improved potency of compound **2** in the reporter gene assay, with K_i = 44 nM compared to 93 nM for compound **1** (Table 1). Having determined that *trans* cyclohexyl was a good replacement for piperidine, we required a synthetic route that would allow more convenient access to *trans* compounds without the need for tedious separation from the corresponding *cis* isomer. The *trans* selective route is depicted in Scheme 2, and involved the reaction of *trans* 4-phenyl cyclohexane carboxylic acid (vi) with Meldrum's acid and transesterification, followed by alkylation of the resultant β-keto ethyl ester (vii) to provide (viii). Cyclization with thiourea provided intermediates (ix), and subsequent hydrolysis provided the desired pyrimidinediones (x).

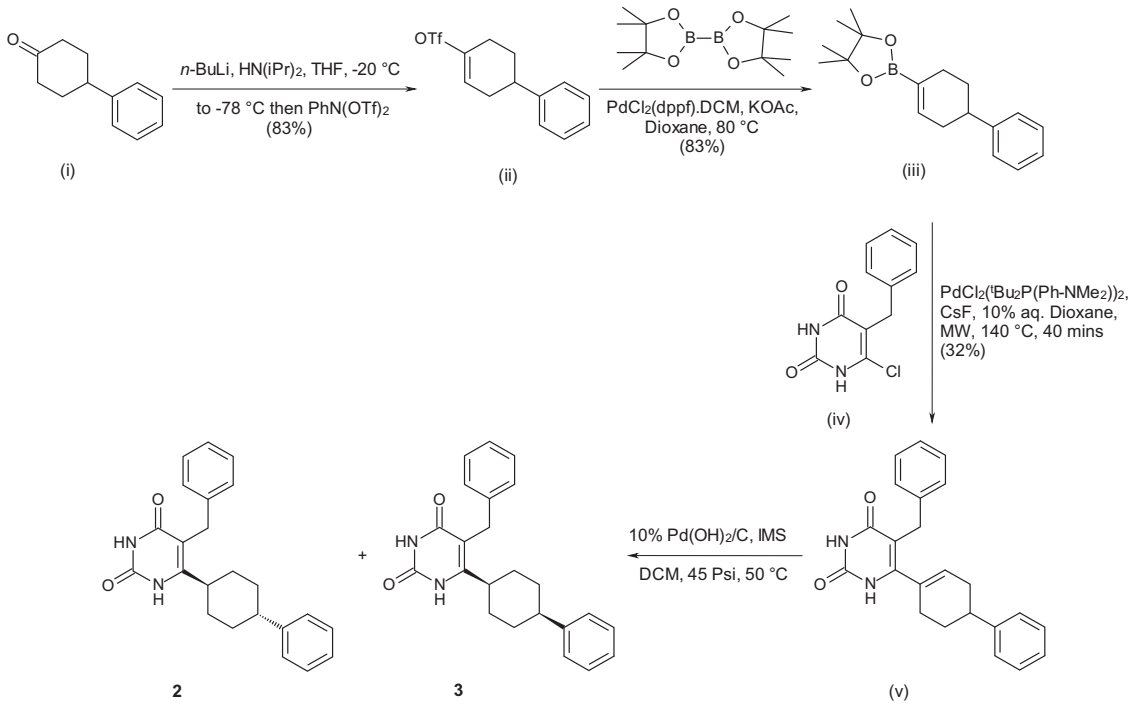
Previous investigation in the piperidine series indicated that a variety of small non-polar substituents were tolerated on the pendant benzyl group, and similar results were obtained in this cyclohexyl series. Compounds **4**–**7** and **8**–**13**, which incorporate halogens, alkyl, or haloalkyl substituents all have excellent affinity in the binding assay and good activity in the reporter gene assay. In contrast, compound **9**, which incorporates a more polar sulfone substituent, is significantly less active in the binding assay. Substitution is acceptable at every position, and di-substitution is well tolerated.

Next we investigated whether the benzyl substituent could be replaced by a heteroarylmethyl substituent, with the aim of reducing lipophilicity and improving aqueous solubility. The incorporation of 2-pyridyl (compound **14**), 2-pyrimidinyl (compound **15**) or 2-thiazolyl (compound **18**) resulted in a significant loss of GR binding affinity and activity in the reporter gene assay as shown in Table 2. Judicious substitution was able to restore some affinity for GR, see for example compounds **19**, **21**, **22** and **23**. The pyrazole compound **20** was inactive.

We undertook a limited exploration of substitution on the N-1 position (Table 3). We had shown previously[1] in the piperidine series that alkylation of N-1 was tolerated, whilst alkylation of N-3 was not. We prepared a small set of cylcohexyl compounds incorporating substitution on N-1, with a particular emphasis on analogues with solubilizing substituents. Unfortunately this endeavour was not successful; although *N*-methylation was tolerated, other substituents resulted in a significant loss of activity in the reporter gene assay.

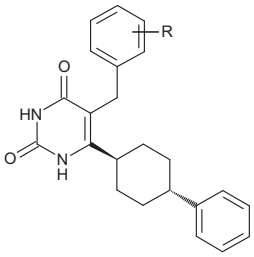
Finally, we confirmed the importance of the cyclohexyl ring by replacing it with a phenyl or a 4-substituted piperidine. Both of these compounds were inactive, data not shown.

Since we had identified a significant number of analogues with comparable GR binding affinity and activity in the reporter gene assay we carried out cassette dosing PK studies to prioritize compounds for further evaluation. Each cassette was comprised of 3 new compounds and a standard compound (with an excellent PK profile) from an unrelated series. The cassettes contained a total dose of 5 mg/kg and were dosed orally to normal Sprague Dawley



Scheme 1. Synthesis of cyclohexyl pyrimidinediones.

Table 1
GR binding and reporter gene assay data for substituted benzyl compounds

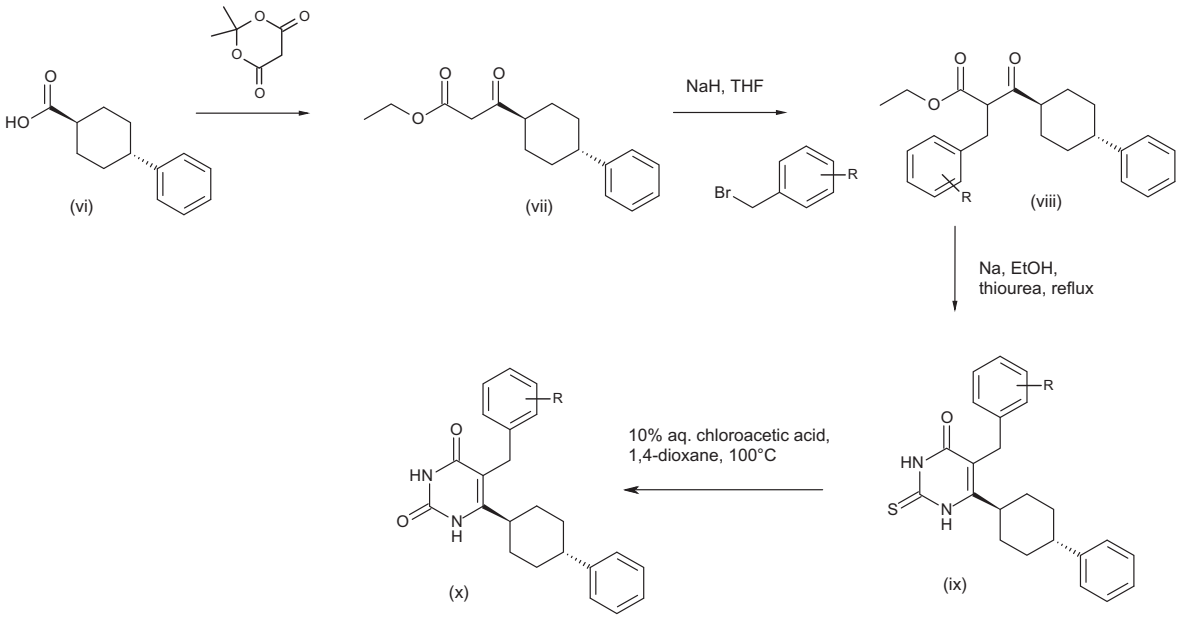


Compound	R	GR binding K_i (nM)	Reporter gene K_i (nM)
1		8	93
2	H	4	44
4	3-CH_3	3	21
5	2-CH_2CH_3	19	78
6	2-Cl	7	37
7	3-CF_3	8	24
8	2-SO_2CH_3	557	Not tested
9	2-OCH_3	9	56
10	2,5 Di-Cl	8	23
11	2,3 Di-Cl	5	25
12	2,4 Di-Cl	11	44
13	2,6 Di-Cl	11	67

rats. Based on the results of the cassette PK studies we selected compound **7** (**CORT118335**) for further progression. Whereas the majority of the other compounds evaluated in these cassette PK studies provided C_{max} well below 20 ng/ml, compound **7** achieved a C_{max} of 43 ng/ml, which was not much lower than the level achieved with the standard compound. CORT118335 exhibited excellent selectivity against PR, ER and AR, with no significant affinity in receptor binding assays. Whilst selectivity over MR was moderate (8 fold), as measured in reporter gene assays, we felt that it was appropriate for the therapeutic indications of interest to us, for example, Cushing's Syndrome. CORT118335 did not exhibit significant inhibition of the five major human CYPS (3A4, 2D6, 1A2, 2C9, 2C19) and was stable in human and rat microsomes. In a single compound iv/po PK study in rats an acceptable profile was obtained.[9] High plasma protein binding (>99.9% in rats and humans) did not appear to interfere with in vivo activity, vide infra.

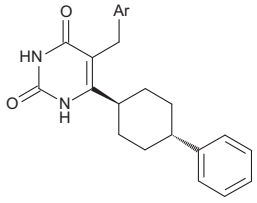
CORT118335 was tested in the previously described olanzapine-induced weight gain model in female rats. After a three week acclimatisation period, female Sprague Dawley rats were randomly assigned to receive twenty-one days dosing with one of the following six treatments: vehicle only, olanzapine (2.4 mg/kg/day) plus vehicle, or olanzapine (2.4 mg/kg/day) plus CORT118335 at 0.2, 0.6, 2, 6 or 10 mg/kg/day. All compounds were administered by oral gavage in divided doses twice daily. The dose of olanzapine was selected based on previous experience.[4,5]

Figure 1 shows the mean weights for each treatment group. Rats receiving no treatment (the vehicle group) gained significant weight throughout the study (mean change from baseline to day 28: 18.6 ± 11 g, $F = 47$, df = 10, p 0.0001). Rats receiving olanzapine plus vehicle gained approximately 2 times more weight than rats in the vehicle only group (mean change: +36.5 ± 10 grams), and this difference was statistically significant ($p = 0.0006$). Rats treated with olanzapine and a concomitant treatment of 0 .2 mg/kg of CORT118335 did not differ in weight change when compared to rats receiving only olanzapine (mean change: +25.6 ± 13 grams, p versus olanzapine = 0.78). Statistically significant differences in body weight were observed when comparing rats receiving higher doses of CORT118335 to rats receiving olanzapine. Weight change was significantly attenuated in the groups of rats receiving 0.6 mg/kg of CORT118335 (mean change: +9.8 ± 12 grams, p versus olanzapine = 0.034); 2 mg/kg (mean change: −10.2 ± 12 grams, p versus olanzapine <0.00001); 6 mg/kg (mean change: −23.6 ± 11 grams, p versus olanzapine <0.00001); and 10 mg/kg (mean change −23.7 ± 11 grams, p versus olanzapine <0.00001). Rats receiving olanzapine consumed more food than rats receiving vehicle, and this increased food consumption was prevented by CORT118335 (0.6 mg/kg and above). Although the mechanism by which GR antagonists prevent olanzapine-induced weight gain has not been



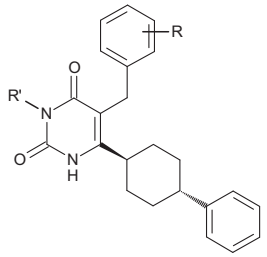
Scheme 2. Selective synthesis of *trans*-cyclohexylpyrimidinediones.

Table 2
Data for heteroarylmethyl compounds



Compound	Ar	GR binding K_i (nM)	Reporter gene K_i (nM)
14	2-Pyridyl	188	337
15	2-Pyrimidyl	400	>3000
16	3-Chloro-2-pyridyl	49	116
17	2-Methyl-4-pyridyl	>50	>3000
18	2-Thiazolyl	139	>300
19	4-Methyl-2-thiazole	28	119
20	2-Methyl-3-pyrazolyl	>1500	>3000
21	6-Methyl-2-pyridyl	22	57
22	6-Trifluoromethyl-2-pyridyl	18	47
23	4-Methyl-2-pyridyl	21	44

Table 3
Data for *N*-1alkylated compounds



Compound	R′	R	GR binding K_i (nM)	Reporter gene K_i (nM)
24	CH_3	H	24	57
25	CH_3	3-CH_3	19	39
26	$CH_2CH_2N(CH_3)_2$	3-CH_3	9	>300
27	CH_3	3-CF_3	16	162
28	CH_2CH_2OH	3-CF_3	98	>3000
29	$CH_2CH_2OCH_3$	3-CF_3	>50	Not tested

conclusively determined, we believe that olanzapine causes insulin insensitivity both peripherally and centrally, and that corticosterone blockade increases insulin sensitivity, peripherally and centrally. The observed results indicate a return to normal satiety and enhanced metabolism, both mediated by a return to normal insulin function. CORT118335 achieves impressive efficacy in this olanzapine-induced weight gain at low doses, with a minimal effective dose of 0.6 mg/kg/day.

In summary, we have identified a series of novel non-steroidal GR antagonists with excellent affinity for GR and good activity in a reporter gene assay. A practical synthetic route has been developed, that allows selective synthesis of the more active *trans* isomer. Compounds in this series exhibit excellent selectivity over PR, AR and ER and modest selectivity over MR. A representative from this series, CORT118335**,** has an acceptable in vitro and in vivo ADME profile and demonstrates impressive efficacy at a low dose in an olanzapine-induced weight gain model in rats.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.10.074. These data include MOL files and InChiKeys of the most important compounds described in this article.

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8. Estrogen receptor: [^{3}H]estradiol, Pan Vera 26467A Era; androgen receptor: [^{3}H]dihydrotestosterone, Pan Vera 24938 AR; progesterone receptor: [^{3}H]progesterone, Pan Vera 24900 PR.
9. CORT118335 was dosed at 1mg/kg iv and 5mg/kg po. The iv clearance was 22ml/min/kg with a half-life of 1.8 h. Bioavailability was 20%.