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1*H*-Pyrazolo[3,4-*g*]hexahydro-isoquinolines as potent GR antagonists with reduced hERG inhibition and an improved pharmacokinetic profile

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ABSTRACT

We report the further optimization of our series 1*H*-pyrazolo[3,4-*g*]hexahydro-isoquinoline sulfonamides as GR antagonists. By incorporating a heteroaryl ketone group at the ring junction, we have obtained compounds with excellent functional GR antagonism. Optimization of the sulfonamide substituent has provided compounds with a very desirable overall profile, including minimal hERG activity, good bioavailability and in vivo efficacy.

Glucocorticoids (GCs) regulate a plethora of biological effects, including metabolism, inflammation, immunity, skeletal growth, cardiovascular function, cognition, and physiological homeostasis. Excessive glucocorticoid activity leads to a large number of adverse effects, including glucose intolerance, diabetes, abnormal fat distribution, osteoporosis, skin atrophy, hypertension, impaired wound healing, depression, psychosis and impaired cognition. GCs exert their effects via the ubiquitously expressed glucocorticoid receptor (GR), a member of the nuclear hormone receptor family. GR normally resides in the cytoplasm as part of a multimeric complex that includes chaperone proteins such as HSP90. When a ligand binds, GR undergoes a conformational change, dissociates from the chaperone proteins, and the GR-ligand complex translocates to the nucleus. GR can act either as a monomer or homodimer, and has direct or indirect effects on a wide spectrum of transcriptional activities. Direct effects include binding to glucocorticoid response elements (GREs) in the promoter or intragenic region of GR responsive genes, which results in activation (positive GRE) or repression (negative GRE) of gene transcription. The interaction of GR with positive GREs results in the recruitment of co-activators, whilst binding to negative GREs results in the recruitment of co-repressors. GR can also bind to other transcription factors, such as NF-κB or AP-1, which themselves bind to DNA.[1,2]

Mifepristone (Korlym™) (Fig. 1), a potent GR antagonist, has been approved in the US for the treatment of patients suffering from endogenous Cushing's syndrome for whom surgery is not an option, or for whom surgery has failed. In addition to exhibiting potent GR antagonism, mifepristone is also a potent antagonist of the progesterone receptor (PR) and its approved use as an abortifacient drug depends on this activity. We are interested in the development of selective GR antagonists, devoid of PR antagonism, for the treatment of Cushing's syndrome and other disorders in which excess cortisol is implicated. We previously reported[3] the identification of a series of 1*H*-pyrazolo[3,4-*g*]hexahydro-isoquinolines **1**, shown in Figure 1. Various modifications of substituents Y and X were investigated, and numerous potent and selective GR antagonists were identified. Representative compounds were tested for oral bioavailability in rats and dogs, and most of them exhibited low bioavailability in rats but improved bioavailability in dogs. Unfortunately, the compounds with the highest bioavailability in rats were not the most potent GR antagonists in our in vitro assays.

One of the most promising compounds included in our previous publication was CORT108297, which was designated as compound **47** in that report. As shown in Figure 1, CORT108297 possesses an ethyl ether substituent at the ring junction and a trifluoromethylphenylsulfonamide. As reported previously, this

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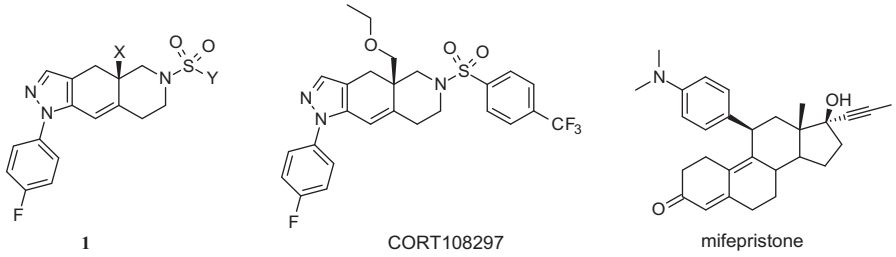


Figure 1. Glucocorticoid receptor antagonist structures.

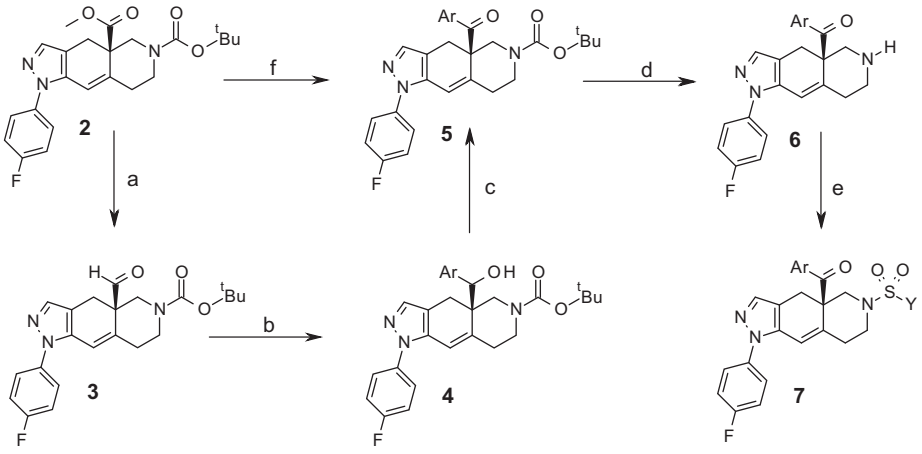
compound exhibited excellent potency (K_i = 6.8 nM) in a reporter gene assay. CORT108297 has demonstrated very encouraging in vivo results in several animal models, including the olanzapine induced weight gain model in rats,[4,5] the diet-induced obesity model in mice,[6] a rapid onset diabetes model in rats,[7] the 3xTg-AD mouse model of Alzheimer's disease,[8] the forced swim test in rats,[9] and the Wobbler mouse model of amyotrophic lateral sclerosis (ALS).[10] CORT108297 has also been shown to attenuate the deleterious effects of electroconvulsive therapy in rats.[11] A recent publication by Zalachoras et al.[12] describes differential targeting of brain stress circuits by CORT108297, and provides evidence that the compound acts as a GR agonist, rather than an antagonist, on some parameters.

We now wish to report further optimization of this series, leading to the identification of several compounds that combine excellent GR antagonism with an improved pharmacokinetic profile in rats, a reduction in hERG inhibition, and efficacy in vivo.

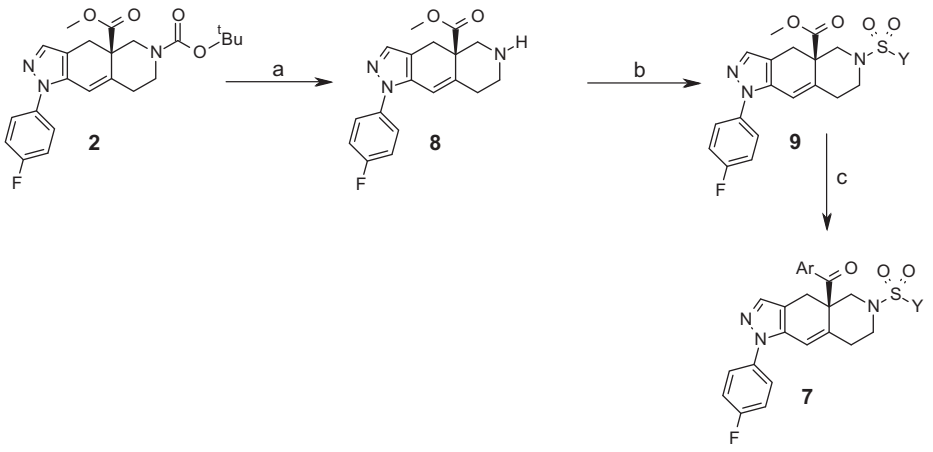
The heteroaryl ketones were synthesized from the common intermediate **2** as depicted in Scheme 1. Intermediate **2** was used to synthesize CORT108297 and other analogs, and its synthesis has been described previously.[3] Compound **2** was reduced to provide aldehyde **3**, and then addition of an appropriate heteroaryl lithium reagent resulted in the formation of the alcohols **4**, which could be readily oxidized to the required ketones **5**. Removal of the BOC protecting group under acidic conditions furnished the amines **6**, which were coupled with the appropriate sulfonyl chloride to provide the desired compounds **7**. Direct conversion of the ester **2** to the ketone **5** was accomplished for many analogs by using excess aryl lithium reagent. For some analogs, it was more convenient to install the sulfonamide before the ketone. In these cases, compound **2** was deprotected under acidic conditions and the resultant amine **8** was converted into the desired sulfonamide **9**. Addition of an appropriate heteroaryl lithium reagent then provided the required compounds **7**. This approach is depicted in Scheme 2.

The in vitro assays used to characterize the biological activities of the GR ligands included a fluorescence polarization (FP) GR binding assay[13] and a tyrosine amino transferase (TAT) induction assay in human HepG2 cells.[14] Further characterization included TAT induction assays in rat H4IIEC3 cells, and in primary hepatocytes derived from humans, rats, dogs and cynomolgus monkeys.[14] Dexamethasone induces TAT activity in various cells, including HepG2 cells, H4IIEC3 cells and hepatocytes. Compounds were tested for their ability to antagonize the effect of dexamethasone (measure of GR antagonism), and were also tested in the absence of dexamethasone for their ability to induce TAT activity (measure of GR agonism). hERG inhibition was evaluated in a manual patch clamp assay.

Our objectives in continuing the optimization of our series of GR antagonists were two-fold: we wanted to identify compounds with improved GR antagonism that were devoid of GR agonist effects, and we wanted to reduce the propensity for hERG inhibition. CORT108297 has an IC_{50} of 0.8 μM in our manual patch clamp assay. In the first stage of our optimization we maintained the trifluoromethylphenylsulfonamide present in CORT108297 and focused on varying the substituent at the ring junction. Based on assessment of our model of CORT108297 docked in the active site of GR (Fig. 2), we believed that increasing the size of this substituent would lead to improved antagonism, and abolish agonism. We believe that the substituent at the ring junction in our series of 1*H*-pyrazolo[3,4-*g*]hexahydro-isoquinolines performs the same role as the dimethylaminophenyl substituent in mifepristone. The substituted phenyl group in mifepristone has been reported to cause a shift in the position of helix 12[15] and thus prevent the formation of the conformation of GR required for agonism.



Scheme 1. Reagents and conditions: (a) DiBAL, CH_2Cl_2, −78 °C (32%); (b) ArBr, *n*BuLi, ether, −78° or ArH, *n*BuLi, ether, −78 °C (13–29%); (c) Dess–Martin periodinane, CH_2Cl_2, rt (quant.); (d) HCl, dioxane, rt or TFA, CH_2Cl_2, rt (quant.); (e) YSO_2Cl, $^iPr_2EtN$, CH_2Cl_2, rt (50–80%); (f) ArBr, *n*BuLi, ether, −78 °C (40–70%).



Scheme 2. Reagents and conditions: (a) HCl, dioxane, rt or TFA, CH_2Cl_2, rt (quant.); (b) YSO_2Cl, $^iPr_2EtN$, CH_2Cl_2, rt (40–90%); (c) ArBr, *n*BuLi, ether, −78 °C, or ArH, *n*BuLi, ether, −78 °C (40–80%).

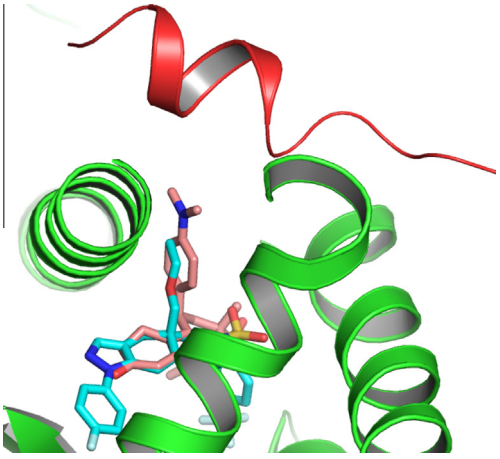
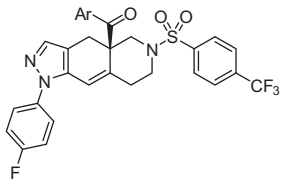


Figure 2. CORT108297 (blue) overlaid with mifepristone (pink) in the GR active site. Helix 12 shown in red. The GR structure comes from PDB 3H52, chain c. CORT108297 was placed onto mifepristone using an RMS fitting.

We chose to investigate the incorporation of a range of heteroaryl ketones, and the results are summarized in Table 1. Most of the analogs prepared exhibited excellent affinity for GR in our FP binding assay, but potency in the cell based TAT assay was more variable. Relatively lipophilic heteroaryl groups such as pyridyl (compound **10**) and thiazolyl (compound **13**) were superior to more polar heteroaryl groups such as imidazolyl (**11**) and pyrazinyl (**20**). The position of attachment of the heteroaryl group was also important; 2-pyridyl (**10**) was better than 3-pyridyl (**12**), and 2-thiazolyl (**13**) or 4-thiazolyl (**19**) were better than 5-thiazolyl (**21**). We selected the 2-pyridyl analog **10** for further profiling, including testing in TAT assays in the rat cell line H4IIEC3 (K_i = 4.2 nM) and in primary hepatocytes from several species. Compound **10** demonstrated full antagonism in human and monkey hepatocytes, partial antagonism in rat hepatocytes, and full agonism in dog hepatocytes. These results are summarized in Table 2. We previously observed similar differences between species for CORT108297[16], so agonism in the dog appears to be a feature of this series of compounds. As anticipated, based on results with earlier compounds in this series, Compound **10** was selective for GR over the related nuclear hormone receptors MR, PR, AR and ER (data not shown).

We had some concern that the inclusion of the ketone carbonyl group might lead to a poor pharmacokinetic profile, but evaluation

Table 1
GR binding and activity in HepG2 TAT assay for selected compounds



Compound	Ar	GR binding K_i (nM)	HepG2 TAT K_i (nM)
10	2-Pyridyl	0.16	14
11	2-Imidazolyl	0.47	52
12	3-Pyridyl	0.83	151
13	2-Thiazolyl	0.23	18
14	5-Methyl-2-oxadiaxolyl	1.6	129
15	4-Oxazolyl	0.16	63
16	2-Furanyl	0.17	31
17	2-Thienyl	0.41	27
18	1-Methyl-2-triazolyl	0.4	117
19	4-Thiazolyl	0.14	22
20	Pyrazinyl	0.21	98
21	5-Thiazolyl	0.33	260

Table 2
Cross species TAT assay results for compound **10**

Species	Antagonist K_i (nM)	Max	Agonist EC_{50} (nM)	E_{max} (%)
Human	100	110		
Rat	13	62	280	51
Dog	—	—	1,600	110
Monkey	765	100		

of the pharmacokinetic profile of the 2-pyridyl analog **10** dispersed this concern. We administered the compound orally, formulated in 10% DMSO in 0.5% aqueous methyl cellulose, at a dose of 5 mg/kg, and intravenously formulated in 10% DMSO, 45% PEG400 and 45% propylene glycol at a dose of 1 mg/kg. We were gratified to discover that this compound achieved good plasma levels following oral dosing, and had acceptable bioavailability (31%). For our initial in vivo study we used our previously described rat model of olanzapine-induced weight gain.[4] As previously reported[4], female rats administered olanzapine (3 mg/kg, bid) for 21 days gained more weight than rats given vehicle for the same period. When dosed orally twice a day for 21 days at a dose of 20 mg/kg (formulated

in 10% DMSO, 0.1% Tween 80, 89.9% hypromellose), compound **10** prevented the olanzapine-induced weight gain, as shown in Figure 3.[17] The body weight of the rats administered compound **10** and olanzapine differed significantly (p <0.05) from the body weight of rats given olanzapine alone from day 4 onwards. CORT108297 at the same dose in the same vehicle was included as a positive control, and was also effective from day 4 onwards.

The role of GR activation in the weight gain associated with the use of atypical antipsychotic drugs such as olanzapine has not been fully delineated. We wanted to demonstrate efficacy in an in vivo model that has been widely accepted as GR mediated. Repeated administration of cortisone to rats for six days results in elevated glucose and insulin levels, and provides a convenient short duration model in which to assess the efficacy of our GR antagonists. Cortisone is converted into cortisol in vivo by 11β-HSD1, and although cortisol is not the natural GR agonist in rats, it binds to the rat GR and acts similarly to the natural ligand, corticosterone. Mifepristone was included as a positive control, and the results are shown in Figure 4.[18] As anticipated, rats administered cortisone (30 mg/kg sc) once a day for six days exhibited higher glucose and insulin levels than control rats that did not receive cortisone. Mifepristone given orally twice a day at a dose of 30 mg/kg prevented the cortisone-induced elevation in glucose and insulin levels, and compound **10** at the same dose attenuated the increase in glucose, but did not have a significant effect on insulin levels. Compound **10** (**CORT113176**) has also been tested by Beaudry et al.[7] in their rapid onset diabetes model in rats, and encouraging results were obtained. Compound **10** improved fasting glycaemia and acute insulin response and attenuated peripheral insulin resistance. Vendruscolo et al.[19] have recently reported that **CORT113176** reduces alcohol intake in rats made dependent on alcohol.

Encouraged by these results, we next undertook optimization of the phenyl sulfonamide. For our initial exploration, we maintained the 2-pyridyl ketone substituent present in compound **10**. One of our objectives in this phase of the project was the reduction of inhibition of hERG activity. In a manual patch clamp assay, an IC_{50} of 5 μM was determined for compound **10**. Conscious that compounds such as **10** have a relatively high molecular weight, we focused predominantly on the inclusion of substituents that would not contribute excessive additional weight. We incorporated several small substituents, including fluoro, chloro, methyl, methoxy and cyano and also investigated the effect of *ortho*, *meta* or *para* substitution. Most of the compounds prepared possessed excellent affinity in the FP binding assay, and good GR antagonism in the dexamethasone induced TAT assay in human HepG2 cells. Details of selected analogs are provided in Table 3. We anticipated that reducing the lipophilicity of the compound, by suitable manipulation of the substituent R, would provide a reduction in hERG inhibition. We were surprised to discover that replacing the *para* trifluoromethyl substituent present in compound **10** with a *para* fluoro substituent resulted in increased hERG inhibition—compound **22** had an IC_{50} of 0.98 μM in a manual patch clamp assay. Another surprising discovery was the effect of the position of substitution on hERG inhibition. Moving the trifluoromethyl substituent from the *para* position to the *meta* position achieved a substantial reduction of hERG inhibition with compound **23** having an IC_{50} > 20 μM. Limited solubility in the medium used for the hERG assay precluded testing at a higher concentration. Compound **23** achieved only 29% inhibition at a concentration of 10 μM and 11% inhibition at a concentration of 1 μM. In order to determine whether this effect was general, we investigated other *meta* substituted compounds. Analogs incorporating *meta* methyl (compound **24**), *meta* chloro (compound **25**) or *meta* methoxy (compound **26**)

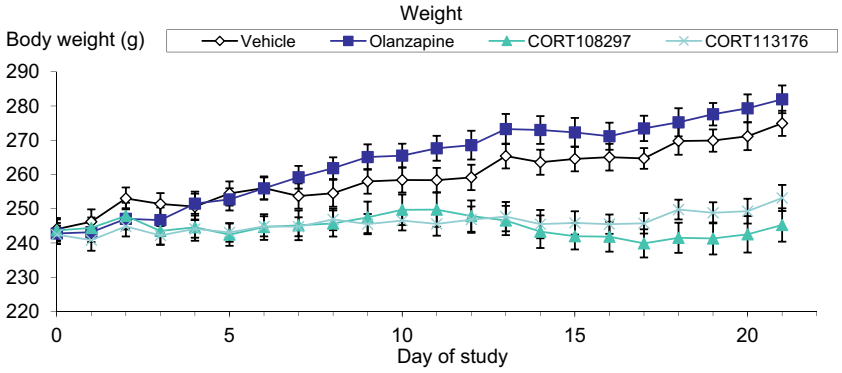


Figure 3. Effects of compound **10** (**CORT113176**) in the olanzapine-induced weight gain model.

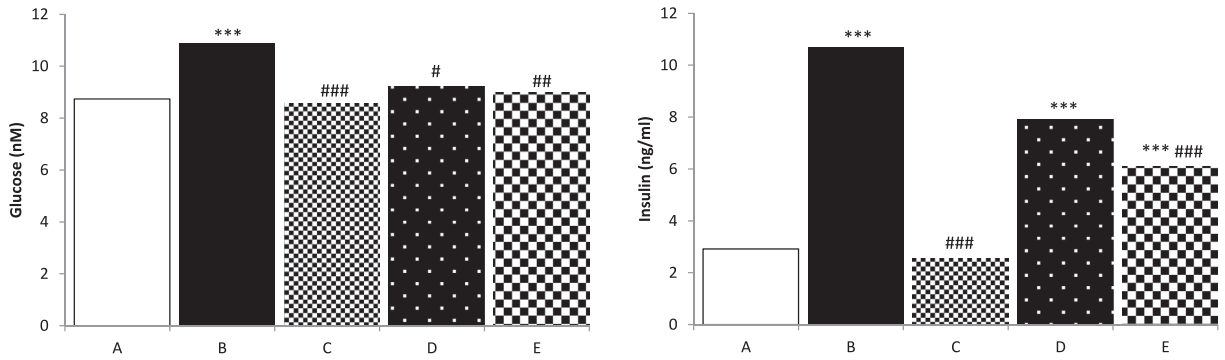
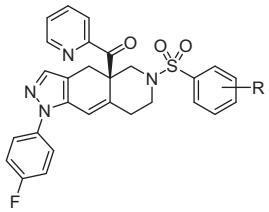


Figure 4. Effects of compounds **10** and **33** in the cortisone induced insulin elevation model. A = vehicle + vehicle; B = cortisone + vehicle; C = cortisone + mifepristone; D = cortisone + compound **10**; E = cortisone + compound **33**. $^{***}p$ <0.001 compared to group A; $^{###}p$ <0.001 compared to group B, $^{#}p$ <0.05 compared to group B.

Table 3
Variation of the phenyl sulfonamide



Compound	R	Substitution	GR binding K_i (nM)	HepG2 TAT K_i (nM)	hERG inhibition
10	CF_3	*p*	0.16	14	IC_{50} = 5 μM
22	F	*p*	0.13	16	IC_{50} = 0.98 μM
23	CF_3	*m*	0.1	14	IC_{50} >20 μM 11% @ 1 μM 29% @ 10 μM
24	CH_3	*m*	0.14	6.8	17% @ 1 μM 30% @ 10 μM
25	Cl	*m*	0.25	11	14% @ 1 μM 35% @ 10 μM
26	OCH_3	*m*	0.19	11.4	IC_{50} >19 μM 24% @ 1 μM 36% @ 10 μM
27	CH_3	*p*	0.22	4.5	34% @ 1 μM 59% @ 10 μM
28	CN	*m*	0.14	15	27% @ 1 μM 42% @ 10 μM
29	CH_3	*o*	0.21	18.5	26% @ 1 μM 45% @ 10 μM
30	CF_3 F	*m* *p*	0.73	9.7	16% @1 μM 31% @10 μM
31	F F	*m* *p*	0.22	7.5	10% @1 μM 35% @10 μM
32	CF_3 F	*p* *m*	0.29	11	8% @1 μM 17% @10 μM
33	Cl Cl	*m* *p*	0.64	14	IC_{50} ≫10 μM 7% @ 1 μM 16% @ 10 μM

substitution also exhibited reduced hERG inhibition, with 30%, 35% and 36% inhibition at 10 μM, respectively. In comparison, compound **27** with the methyl substituent in the *para* position showed 59% inhibition at 10 μM and 34% inhibition at 1 μM. The more polar cyano substituent did not provide much benefit, with *meta*-substituted compound **28** achieving 42% inhibition at 10 μM. *Ortho* substitution generally led to reduced GR antagonist potency, for example, compare compound **29** with an *ortho* methyl substituent to the corresponding *meta* and *para* substituted analogs **24** and **27**. There did not appear to be any advantage in terms of hERG inhibition, with compound **29** exhibiting 45% inhibition at 10 μM.

Encouraged by the reduced hERG inhibition exhibited by these compounds, we evaluated several *meta* substituted analogs in PK studies in rats. We were disappointed to discover that moving the trifluoromethyl substituent from the *para* (compound **10**) to the *meta* (compound **23**) position had a detrimental impact on the PK profile. The C_{max} achieved after administering a 5 mg/kg oral dose was decreased from 375 ng/ml to 155 ng/ml, and clearance after iv dosing was increased from 11 ml/min/kg to 40 ml/min/kg. Similarly, the *meta* chloro analog **25** suffered from a low C_{max} (112 ng/ml) after oral dosing and a high clearance (70 ml/min/kg) after iv dosing. Although we did not carry out any studies to determine the metabolic fate of these *meta* substituted compounds, we reasoned that the lack of a substituent at the *para* position contributed to the high clearance observed.

In an attempt to combine the advantageous effect of a *meta* substituent on hERG inhibition with the benefit of a *para* substituent on the PK profile, we synthesized several disubstituted compounds (see Table 3). We were pleased to discover that by adding a *meta* substituent to a *para* substituted compound we were able to mitigate hERG inhibition. For example, the *para* fluoro, *meta* trifluoromethyl analog **30** had significantly reduced hERG inhibition compared with compound **22** which lacked the *meta* substituent. Even the addition of a *meta* fluoro substituent was sufficient to reduce hERG inhibition, as shown by compound **31**. We were disappointed to discover that compound **30** did not exhibit an improved PK profile compared to compound **23**. There was no improvement in C_{max} after oral dosing (112 ng/ml for compound **30** compared to 155 ng/ml for compound **23**) and compound **30** actually suffered from increased clearance (103 ml/min/kg) after iv administration. The PK profile of compound **31** was more encouraging, with C_{max} 398 ng/ml after an oral dose of 5 mg/kg and clearance 44 ml/min/kg after an iv dose of 1 mg/kg. We discovered that one of the best combinations was a *meta* fluoro substituent and a *para* trifluoromethyl group, compound **32**. This combination provided minimal hERG inhibition and a good PK profile, with C_{max} 322 ng/ml after oral dosing and clearance 28.7 ml/min/kg after i.v. administration. The *meta*, *para* dichloro compound **33** also provided an excellent combination of minimal hERG inhibition (IC_{50} > 10 μM) and good oral bioavailability in rats (C_{max} 437 ng/ml after a 5 mg/kg dose). GR antagonism in the rat cell line H4IIEC3 (K_i = 8.5 nM) and primary rat hepatocytes (K_i 150 nM, 74% efficacy) was confirmed. Compound **33** was tested in the cortisone induced insulin resistance model in rats (30 mg/kg orally, twice a day). This compound achieved a statistically significant reduction in both glucose and insulin levels, and the results are included in Figure 3.

In summary, we have improved our original series of 1*H*-pyrazolo[3,4-*g*]hexahydro-isoquinoline GR antagonists by the incorporation of a heteroaryl ketone substituent, and the modification of the sulfonamide portion. Structure activity relationship studies have revealed that the inclusion of a *meta* substitution the phenyl sulfonamide provides compounds with a substantial reduction in hERG liability. The combination of *meta* and *para* substituents provides the optimum balance of hERG activity and oral bioavailability. Compound **33** has demonstrated promising in vivo activity in a rat model of insulin resistance. Further optimization of our series of 1*H*-pyrazolo[3,4-*g*]hexahydro-isoquinolines and the identification of our clinical candidate CORT125134 will be reported in due course.

Acknowledgements

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17. The rat olanzapine induced weight gain model was carried out by Saretius, UK.
18. The cortisone induced insulin resistance model was carried out by RenaSci, UK.
19. Vendruscolo, L. F.; Estey, D.; Goodell, V.; Macshane, L. G.; logrip, M. L.; Schlosburg, J. E.; McGinn, M. A.; Zamora-Martinez, E. R.; Belanoff, J. K.; Hunt, H. J.; Sanna, P. P.; George, O.; Koob, G. F.; Edwards, S.; Mason, B. J. *J. Clin. Invest.* **2015**, *125*, 3193.