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Discovery and optimisation of a selective non-steroidal glucocorticoid receptor antagonist

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ABSTRACT

High-throughput screening of 3.87 million compounds delivered a novel series of non-steroidal GR antagonists. Subsequent rounds of optimisation allowed progression from a non-selective ligand with a poor ADMET profile to an orally bioavailable, selective, stable, glucocorticoid receptor antagonist.

Nuclear receptors are a class of soluble intra-cellular receptor proteins which regulate the expression of specific genes in almost all mammalian cells. Binding of the endogenous ligand to the nuclear receptor results in a conformational change in the receptor which allows it to bind directly to specific DNA sequences and regulate transcription of the adjacent genes.[1] Hence, nuclear receptors and their endogenous ligands play key roles in a range of fundamental biological processes and as such are attractive targets for drug discovery.[2]

The glucocorticoid receptor (GR) is one member of this nuclear receptor super-family which has proved a fruitful target for drug discovery, resulting in a number of GR agonists being available as marketed drugs.[3] However, the therapeutic potential of GR antagonists remains less well developed. This is despite strong evidence linking the ability to block the action of the endogenous glucocorticoid steroid hormone cortisol **1** (Fig. 1), and the effect of this blockade on the pathology of disease states characterised by elevated cortisol levels (e.g. Cushing's syndrome,[4] hypertension,[5] diabetes,[6] glaucoma[7] and depression[7,8]).

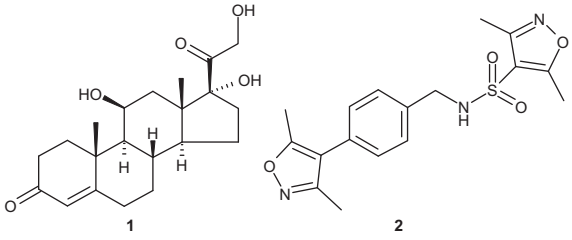

Figure 1. Structures of cortisol **1** and the HTS derived hit compound **2**.

The non-selective glucocorticoid receptor/progesterone receptor (PR) antagonist Mifepristone (RU-486) has been one of the most studied GR antagonists discovered to date, but its clinical use continues to be compromised largely due to the poly-pharmacology associated with is PR antagonist activity.[4,9] We were therefore interested in identifying more selective GR antagonists. Our main rational from the outset was that the lack of selectivity of the steroid derived ligands may be due, in part, to the steroid scaffold itself. Hence our initial goal was to identify a non-steroidal GR antagonist, of which several were known in the literature, and then

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determine if such a ligand would prove easier to optimise in terms of selectivity over related nuclear receptors.

Screening of 3.87 million compounds by Pharmacopeia, using a human GR fluorescence polarisation binding assay,[10] resulted in the identification of the heterobiaryl sulfonamide **2** (Fig. 1) as a potent GR antagonist (GR binding affinity[10] pEC_{50} 8.4; GR antagonism[11] pEC_{50} 7.3).

Further in vitro profiling of **2** revealed poor selectivity at the closely related progesterone receptor (PR) (PR bind pEC_{50} 8.1; fold selectivity GR vs PR ~2).[10] We therefore initiated a program aimed at optimising the in vitro profile of this series with a focus on improving selectivity over PR as our initial optimisation goal. The compounds detailed in Table 1 were prepared according to the general Schemes 1–4. Rapid analogue synthesis and in vitro profiling quickly allowed us to build up a picture of the key SAR for this novel heterobiaryl sulfonamide series exemplified by the progenitor compound **2**. Deletion of the terminal heteroaryl ring or variation of the biaryl connectivity to ortho or meta substitution was not tolerated, and the benzylic linker between biaryl unit and sulfonamide functional group was optimal, with longer or shorter chains resulting in loss of GR activity (data not shown).

Synthesis of **2** involved a two step sequence from commercially available starting materials and therefore allowed the rapid synthesis of a diverse set of close analogues. 4-Bromobenzylamine **3** was reacted with dimethylisoxazole sulfonyl chloride in the presence of base to provide the sulfonamide intermediate **4**. We then attempted to prepare a small one dimensional array by reacting **4** under standard Suzuki coupling conditions with 80 diverse aryl/heteroaryl boronic acid derivatives (Scheme 1). After extensive purification, the majority of compounds were isolated, albeit in low yield in many cases. Single point screening identified 35 actives (>50% binding inhibition at 10 μM) and these were profiled further. The phenyl analogue **9** showed weak GR binding, but introduction of an ortho substituent (Table 1, **10**–**12**) dramatically increased both the binding affinity for GR and also resulted in improved selectivity via loss of potency at PR.

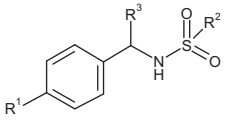
We then initiated a further round of synthesis to investigate the SAR of the sulfonamide region of compound **2**. We prepared the parent heterobiaryl benzylamine **5** (Scheme 2) along with three other biaryl cores from the first array which gave improved selectivity over PR (cores **6**–**8**). With these four biaryl benzylamines in hand we reacted each of these with 40 diverse sulfonyl chlorides in the presence of base (Scheme 2). The resulting crude products were then purified by RP-HPLC, fully characterised and screened for activity at GR and PR. This array of 160 analogues gave 93 actives (>50% inhibition, GR single point screening), 42 of which were active (<1 μM) in our in vitro functional assay of GR antagonism.[11] Alkyl sulfonamide derivatives were in general not tolerated (data not shown), although a wide range of heteroaryl and aryl derivatives such as **13** showed good potency at GR and retained selectivity over PR. Variation of the sulfonamide portion highlighted that the N–H was essential for potency and subsequent attempts to replace the sulfonamide functionality with bioisosteric replacements were not tolerated (data not shown).

Introduction of a chiral methyl group **14** delivered our first analogue with greater than 30 fold selectivity. The (*R*)-enantiomer displayed greater potency and selectivity compared to the corresponding (*S*)-enantiomer **15** and this preference for the (*R*)-stereochemistry has proved consistent for all subsequent analogues prepared to date. Chiral carbocyclic analogues were generally prepared via a ligand-free aqueous Suzuki coupling, followed by catch-and-release purification protocol (Scheme 3).[12]

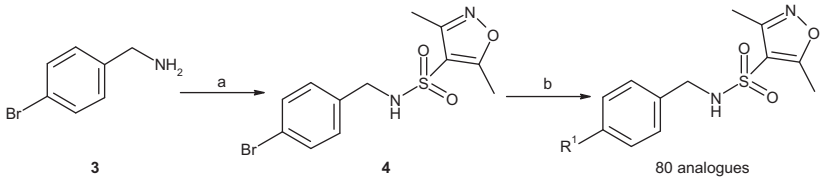
Whilst we had now demonstrated that good selectivity over PR was achievable within this chemical series, the majority of selective analogues also suffered from low aqueous solubility (<1 mg/L), poor microsome stability (Human and Rat intrinsic clearance, CL_i > 270 μL/min/mg protein) and low oral bioavailability (**14**, rat *F* = 1%). Initial efforts at improving metabolic stability focused on changes which would reduce the lipophilicity of the series (via discrete introduction of heteroatoms), whilst at the same time retaining the selectivity conferring features responsible for selectivity over PR.

In general, we found that heterocycles were tolerated in the terminal portion of the biaryl motif. In particular 3-pyridyl analogues such as **16** were almost equipotent with the progenitor compound **10**. The microsome clearance of **16** was found to be high (Human intrinsic clearance, CL_i = 237 μL/min/mg protein; Rat intrinsic clearance, CL_i = 251 μL/min/mg protein), with the major metabolite identified as the inactive O-dealkylated pyridone derivative (data not shown). Attempts to block metabolism via conversion to the corresponding difluoromethoxy derivative **17** gave a modest improvement in rodent microsome stability (intrinsic clearance, CL_i = 79 μL/min/mg protein), but measured human microsome stability was not improved (intrinsic clearance, CL_i > 270 μL/min/mg protein). Introduction of a nitrile group para to the site of metabolism **18** gave a dramatic improvement in

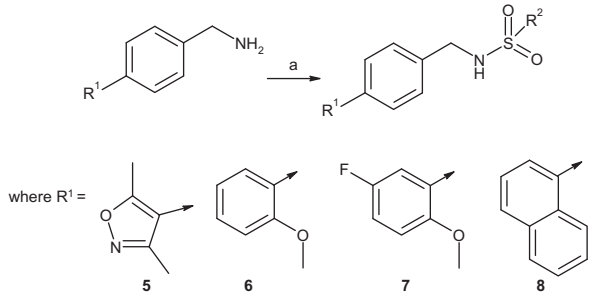
Table 1
Summary of the structure–activity relationships of the biaryl sulfonamide chemotype



Compd	R^1	R^2	R^3	Stereo chemistry	*C* log *P*	GR[10] pEC_{50}	PR[10] pEC_{50}	Binding selectivity PR/GR
2	2,5-Dimethylisoxazole	2,5-Dimethylisoxazole	H	—	2.15	8.4	8.1	2
9	Phenyl	2,5-Dimethylisoxazole	H	—	4.23	6.0	<5	—
10	2-Methoxyphenyl	2,5-Dimethylisoxazole	H	—	3.59	7.5	6.7	7
11	2-Methoxy-5-fluorophenyl	2,5-Dimethylisoxazole	H	—	3.87	7.9	6.9	10
12	Naphthalene	2,5-Dimethylisoxazole	H	—	5.41	8.1	6.7	23
13	2-Methoxy-5-fluorophenyl	2,5-Dichlorophenyl	H	—	5.75	8.7	7.3	29
14	2-Methoxy-5-fluorophenyl	2,5-Dimethylisoxazole	Me	*R*	4.18	8.3	6.7	38
15	2-Methoxy-5-fluorophenyl	2,5-Dimethylisoxazole	Me	*S*	4.18	7.6	6.6	9
16	2-Methoxy-3-pyridyl	2,5-Dimethylisoxazole	Me	*R*	3.30	7.6	6.1	34
17	2-Difluoromethoxy-3-pyridyl	2,5-Dimethylisoxazole	Me	*R*	3.75	8.0	6.2	52
18	2-Methoxy-5-cyano-3-pyridyl	2,5-Dimethylisoxazole	Me	*R*	2.88	8.2	7.1	12
19	2-Methoxy-5-fluorophenyl	1-Methyl-3-(trifluoromethyl)-1*H*-pyrazole	Me	*R*	3.79	8.4	6.1	198
20	2-Methoxy-3-pyridyl	1-Methyl-3-(trifluoromethyl)-1*H*-pyrazole	Me	*R*	2.91	7.9	<5	>800
21	2-Methoxy-5-cyano-3-pyridyl	1-Methyl-3-(trifluoromethyl)-1*H*-pyrazole	Me	*R*	2.48	7.9	<5	>800



Scheme 1. Reagents and conditions: (a) 3,5-dimethylisoxazole-4-sulfonyl chloride, DCM, DIEA, rt, quantitative; (b) boronic acid, $Pd(PPh_3)_4$, K_2CO_3, DMF, 80 °C.



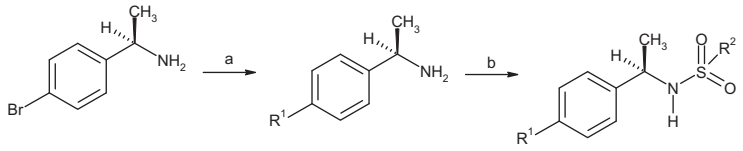
Scheme 2. Reagents and conditions: (a) R^2 sulfonyl chloride, DCM/DIEA, rt.

Table 2
Potency, selectivity and pharmacokinetic data for compound **21**

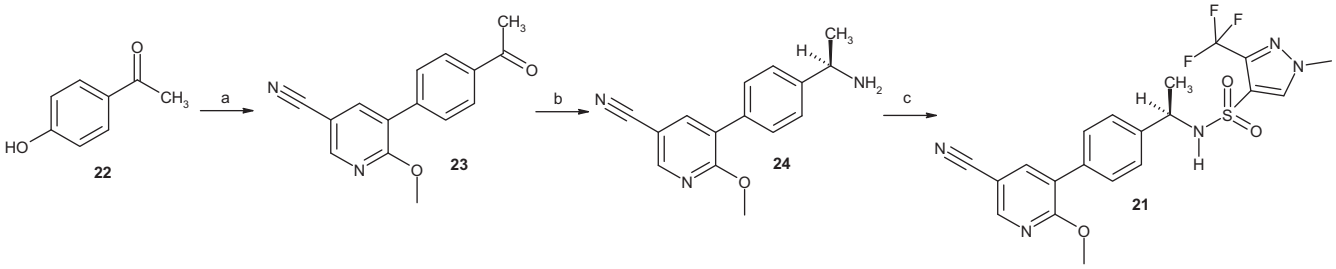
Parameter	Value
GR; PR (binding potency pEC_{50})	7.9; <5
Binding selectivity PR/GR	>800
Solubility in PBS, pH 7.4 (mg/L)	28.9
Human microsome intrinsic clearance (μL/min/mg protein)	<12
Rat microsome intrinsic clearance (μL/min/mg protein)	<12
GR; MR (antagonism[11] pEC_{50})	7; <5
$AUC_{po\ 0–7.5\ h}$ (ng/mL h)	8143
Rat *F* (%), Wistar, iv 2 mg/kg; po 10 mg/kg	42
CL_p (mL/min/kg)	9.8
V_{ss} (L/kg)	0.7

clearance (Human intrinsic clearance, CL_i = 36 μL/min/mg protein; Rat intrinsic clearance, CL_i = 19 μL/min/mg protein), although this change had an adverse effect on selectivity over PR. After re-visiting further heterocyclic variants of the sulfonamide region we were able to identify the 1-methyl-3-(trifluoromethyl)-1*H*-pyrazole sulfonamide derivative **19** with much improved selectivity which we rationalised to be due, in part, to this substituted heteroaryl ring (Table 1). Combining this new heterocyclic R^2 portion with the terminal biaryl 2-methoxy-3-pyridyl unit furnished analogue **20** which, although slightly less potent at GR and rapidly cleared, proved to be very selective over PR. Synthesis of the 5-nitrile analogue **21** (Scheme 4)[13] provided a potent, selective GR antagonist with an attractive in vivo PK profile (Table 2). For introducing heterocycles as the terminal portion of the biaryl motif, in situ generation of aryl triflates[14] followed by Suzuki coupling under microwave mediated conditions (Scheme 4)[13] gave improved yields in our hands. In addition, use of starting phenol **22** also allowed easy access to homochiral intermediates **24** via asymmetric synthesis of amines from ketone **23** using the method reported by Ellman.[15]

In summary, using an HTS campaign we have identified a novel, non-steroidal, non-selective GR antagonist. We have successfully optimised this heterobiaryl sulfonamide series to deliver a very selective antagonist of the glucocorticoid receptor, with excellent in vitro stability and acceptable oral bioavailability. Compound **21** was also shown to be efficacious in in vitro models of glucocorticoid receptor antagonism and will be the subject of a separate publication.



Scheme 3. Reagents and conditions: (a) aryl boronic acid, $Pd(OAc)_2$, water, microwave irradiation, 200 °C, 5 min followed by SCX purification; (b) aryl/heteroaryl sulfonyl chloride, DCM, DIEA, rt.[12]



Scheme 4. Reagents and conditions: (a) (i) *N*-phenyl-bis-(trifluoromethane-sulfonimide), K_2CO_3, THF, microwave irradiation 120 °C, 6 min; (ii) boronic acid, $Pd(PPh_3)_4$ (2 mol % w/w), NMP, microwave irradiation 120 °C, 10 min; (b) (i) *R*-(+)-*tert*-butanesulfinamide, DCM, titanium tetraethoxide, microwave irradiation 120 °C for 10 min; (ii) $NaBH_4$, 'wet' THF, MeOH; (c) 1-methyl-3-(trifluoromethyl)-1*H*-pyrazole-4-sulfonyl chloride, triethylamine, DCM, rt.[13]

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10. Binding affinity of GR antagonists for recombinant human GR and PR: compound affinity was determined via competition ligand binding experiments using GR and PR fluorescence polarisation (FP) binding assay kits (Panvera). This assay utilises the binding of a proprietary fluorescent ligand to partially purified recombinant baculovirus expressed human receptor. EC_{50} values were generated by analysis of the displacement curves by a minimum sum of squares method. All data are the means of 3–4 independent experiments.
11. The effect of GR antagonists on GR, PR and MR mediated functional responses in CHO cells expressing recombinant human receptor: CHO cells were stably transfected with human GR, PR or chimeric MR/GR (MR ligand binding domain, GR DNA binding domain) and the MMTV-LUC luciferase reporter plasmid. Antagonism of GR functional responses by test compounds was determined via inhibition of cortisol (20 nM)-stimulated luciferase activity. Antagonism of PR was determined by inhibition of progesterone (5 nM)-stimulated luciferase activity. Antagonism of MR was determined by inhibition of aldosterone (1 nM)-stimulated luciferase activity. EC_{50} values were generated by analysis of concentration–response curves by a minimum sum of squares method. All data are the means of 3–4 independent experiments.
12. Palladium acetate (56 mg, 0.25 mmol) was added to a mixture of 5-fluoro-2-methoxyphenylboronic acid (850 mg, 5.0 mmol) and (*R*)-1-(4-bromophenyl)ethylamine (500 mg, 2.5 mmol) in water (20 ml). This mixture was heated for 5 min at 200 °C in a Smithcreator microwave oven and then diluted with methanol (200 ml). The mixture was purified on a SCX column (20 g) using 2 M ammonia in methanol to elute the intermediate amine. Evaporation of solvents under reduced pressure gave (*R*)-1-(5′-fluoro-2′-methoxy-biphenyl-4-yl)-ethylamine as a gum (580 mg, 2.37 mmol, 96.6%). Triethylamine (41.1 μl, 0.295 mmol) and 1-methyl-3-(trifluoromethyl)-1*H*-pyrazole-4-sulfonyl chloride (24.5 mg, 0.0984 mmol) were added to a solution of (*R*)-1-(5′-fluoro-2′-methoxy-biphenyl-4-yl)-ethylamine (20 mg, 0.082 mmol) in dichloromethane (1 ml) and the resulting solution shaken at room temperature overnight. Purification by preparative LCMS and removal of solvent under reduced pressure gave **19** (8.5 mg, 40%): ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40, 7.16 (a/b, 4H), 7.05–6.88 (m, 3H), 4.89 (m, 1H), 4.60 (t, 1H), 3.80 (s, 3H), 3.72 (s, 3H), 1.52 (d, 3H) ppm; MS (ESI) *m*/*z*: 480 $[M+Na]^+$.
13. The phenol **22** (2.0 mmol), *N*-phenyl-bis-(trifluoromethane-sulfonimide) (2.0 mmol, 710 mg), K_2CO_3 (6.0 mmol, 830 mg), and 3.0 ml THF were heated in the microwave at 120 °C for 6 min. Boronic acid (2.0 mmol), $Pd(PPh_3)_4$ (2 mol % w/w) and NMP (1 ml) added the vessel resealed and heated for a further 10 min at 120 °C. The crude reaction mixture was partitioned between ethyl acetate (50 ml) and saturated aqueous $NaHCO_3$ (100 ml) washed with saturated aqueous $NaHCO_3$ (100 ml × 3), dried ($MgSO_4$) and evaporated to give the essentially pure biaryl ketone **23** in quantitative yield. The crude biaryl ketone (2 mmol) and *R*-(+)-*tert*-butanesulfinamide (2 equiv) was dissolved in DCM (3 ml) and to this was added titanium tetraethoxide (purity: 85–90%, 0.46 ml, 2 mmol). The reaction was then sealed and heated in the microwave at 120 °C for 10 min. The reaction was then cooled (to reduce internal pressure!), decapped and poured into a suspension of $NaBH_4$ (4 equiv) in wet THF (50 ml), MeOH (20 ml) was then added. The reaction was almost instantaneous and the mixture was then poured into brine (100 ml) with rapid stirring. The resulting suspension was then filtered through Celite, washed with ethyl acetate and the filtrate separated. The combined organic layers were then dried ($MgSO_4$) and evaporated to yield the crude product. The pure (*R*,*R*)-diastereomer was then isolated by chromatography (SiO_2, ethyl acetate) in 40–50% overall yield based on the original amount of phenol. (*S*)-*N*-((*R*)-1-(4-(5-Cyano-2-methoxypyridin-3-yl)phenyl)ethyl)-2-methylpropane-2-sulfinamide (22.60 mmol, 8.08 g) was dissolved in methanol (200 ml) and hydrogen chloride (67.8 mmol, 33.9 ml) (2 M in ether) added. Stirred at rt for 1 h then solvent removed. Solid residue dissolved in methanol and passed through 3 × 20 g SCX columns. Elution with 2 M ammonia in methanol followed by evaporation of volatiles gave **24** (5.43 g, 95%) as an oil. To an ice-cold solution of **24** (19.19 mmol, 4.86 g) in DCM (90 ml) was added triethylamine (67.2 mmol, 9.36 ml, 6.80 g) in one portion, followed over 15 min by a solution of 1-methyl-3-(trifluoromethyl)-1*H*-pyrazole-4-sulfonyl chloride (6.14 g) in DCM (30 ml). The reaction was stirred at ambient temperature for 6.5 h then washed with 2 N HCl (×2), Na_2CO_3, brine and dried over Na_2SO_4. Evaporation of volatiles and purification over silica (DCM/MeOH 99.5:0.5 then 99:1) gave a foam (8.8 g) that was recrystallised from ether/heptane to yield **21** as a colourless solid, (8.45 g, 95%): ^{1}H NMR (400 MHz, DMSO): δ 8.70 (s, 1H), 8.40 (s, 1H), 8.21 (s, 1H), 8.17 (s, 1H), 7.51, 7.35 (a/b, 4H), 4.45 (m, 1H), 3.99 (s, 3H), 3.83 (s, 3H), 1.36 (d, 3H) ppm; MS (ESI) *m*/*z*: 466 $[M+H]^+$.
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