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The identification a novel, selective, non-steroidal, functional glucocorticoid receptor antagonist

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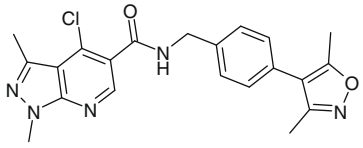
ABSTRACT

The identification of novel, potent, non-steroidal/small molecule functional GR antagonist GSK1564023A selective over PR is described. Associated structure–activity relationships and the process of optimisation of an initial HTS hit are also described.

The glucocorticoid receptor (GR) is a member of the nuclear receptor (NR) superfamily of ligand activated transcription factors. Activation of GR leads to either positive regulation of gene expression (via the trans-activation pathway) or negative regulation (via the trans-repression pathway).[1] GR agonists have found great utility as therapeutic agents, primarily as anti-inflammatory agents (e.g., dexamethasone).[1,2] In contrast, the therapeutic potential of antagonism of the GR receptor remains largely unexploited despite a strong therapeutic rationale in a wide range of disease states, including for example, diabetes, Cushing's syndrome and psychotic depression.[3]

The progesterone receptor (PR) antagonist Mifepristone (RU-486) also displays potent GR antagonism and has seen some clinical investigation/utility for its GR mediated effects, but its use remains compromised by the abortifacient properties associated with its PR pharmacology.[3] In common with RU-486 steroidal GR antagonists typically exhibit poor selectivity over other NR family members, and historically selective and non-steroidal GR antagonists have proved elusive. Recently however reports of non-steroidal selective GR antagonist have begun to emerge, for example, Argenta/Corcept report[4,5] a series pyrimidinediones exemplified by compound (**1**) and a series of aryl pyrazoles exemplified by compound (**2**) (Fig. 1). However non-steroids reported to date are typically highly lipophilic and often display disparities between binding affinity and functional activity. Against this background we report herein the identification of GSK1564023A, a potent and novel, small molecule functional GR antagonist, selective over PR.



GSK1564023A

GR MMTV	pIC_{50}	7.0
GR NFkB	pIC_{50}	< 4.9
PR	pIC_{50}	5.5
Mwt 410	clogP 1.9	PSA 86

A high throughput screen (HTS) was performed on the GSK compound library in MMTV/trans-activation functional antagonist format.[6] GSK325971A emerged from this HTS as an exemplar of a family of novel GR antagonists. This chloro-pyrazolopyrimidine template is an intermediate in the synthesis of PDE4 inhibitors,[7,8]

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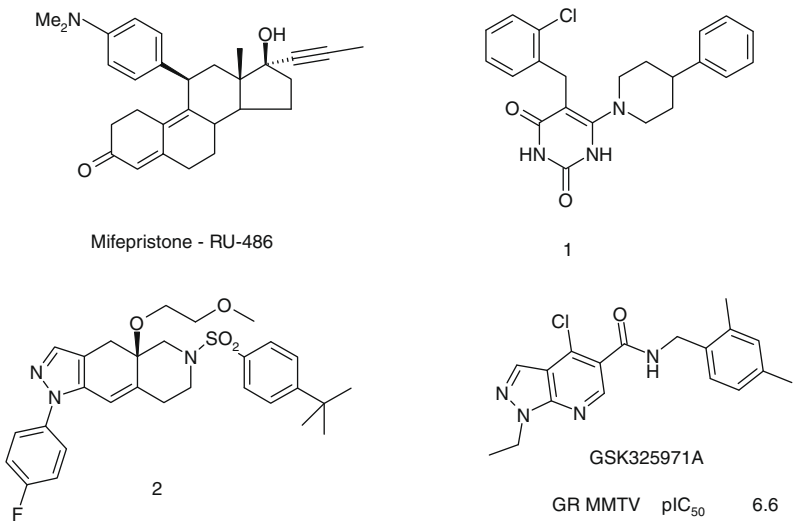


Figure 1. Structures of glucocorticoid receptor antagonists.

where at high temperatures the chlorine atom can be displaced by nucleophiles. Before further progression of GSK325971A and related compounds we thus took appropriate steps to rigorously confirm that the Cl atom was sufficiently stable for assessment in in vitro assays, and that displacement of the chlorine played no role in the GR Mechanism of Action (MoA).

MMTV[6] concentration response curves (CRC) of cortisol were generated in presence of increasing concentrations of GSK325971A (fig. 2). At lower concentrations of GSK325971A a parallel rightward shift of the CRC was observed, consistent with competitive antagonism of the cortisol response with a pK_B of 6.9. At higher concentrations some suppression of the maximum response was observed, indicative of a non-competitive component to the interaction, a slow dissociation rate or indeed potential cytotoxicity. The experiments were repeated with a 2 h pre-incubation of compound before cortisol addition, with identical results, indicative of no mechanistic covalent interaction with the potentially labile chlorine atom[9]. Moreover simple analytical experiments indicated GSK325971A to be unchanged in DMSO/water for 48h at ambient temperature[10].

In terms of its modest molecular footprint (Mw = 343, *c*Log *P* = 3.3, PSA = 60) GSK325971A represented an attractive startpoint for medicinal chemistry exploration. Once the MoA had been confirmed, and risk of Cl displacement discharged, an SAR study/optimisation programme based around the template was undertaken.

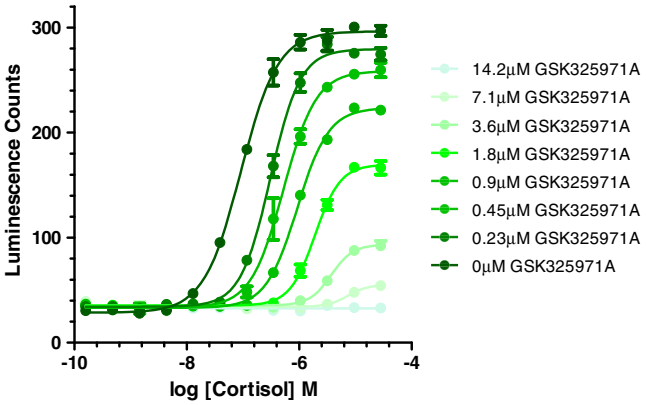


Figure 2. MMTV concentration–response curves of cortisol[6] raised to increasing concentrations of GSK325971A.

Analogues of GSK325971A were typically prepared according to the well precedented[8] chemistry of Scheme 1. Condensation of ketonitriles with an appropriate hydrazine affords the corresponding 1-substituted-5-aminopyrazole (**3**) Reaction of the 5-aminopyrazole with diethyl-ethoxymethylenemalonate, and subsequent ring closure and treatment with thionyl chloride afforded chloropyrazolopyrimidine (**4**) which were saponified and converted to desired amides (**5**) via standard coupling procedures.

The data of Table 1 illustrate aspects of the MMTV antagonist SAR around the GSK325971A template. Appropriate substitution of the benzyl group facilitates an increase in potency (e.g., 4-Cl,3-(trifluoromethyl)benzyl analogues **6** and **7**), and removal of the two methyl groups reduces potency (**15**). Replacement of the benzyl group with simple alkyl groups leads to inactivity (**19** and **20**) as does replacement of the benzyl phenyl group with some heterocycles (**17** and **18**). Simple replacement of the chlorine atom also leads to inactivity (**21** and **22**). Interestingly methyl substitution of the pyrazole 3-position has no significant effect on potency as illustrated by pairs of compounds **6** and **7**, and **8** and **10**.

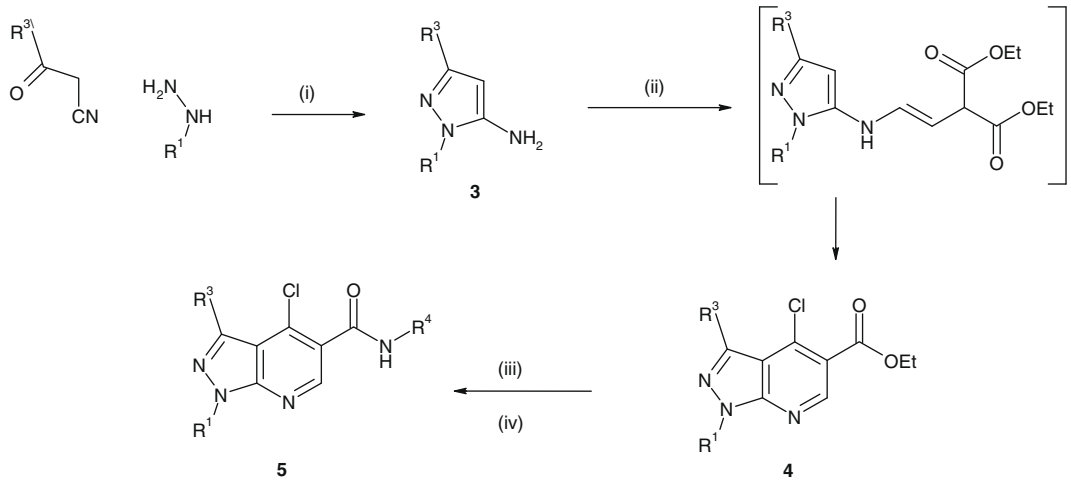
In analogues bearing the selectivity conferring isoxazolylbenzyl amide substituent (see below), MMTV potency proved surprisingly insensitive to changes to the pyrazole ring nitrogen substituent (Table 2) although all potencies were slightly reduced relative to *N*-methyl parent GSK1564023A itself.

Table 3 reports the NR selectivity of GSK325971A and other key analogues. Whilst GSK325971A itself shows significant activity in the NFκB assay.[11] reflecting agonism of the GR trans-repression pathway, GSK1564023A and compound **12** do not. No compounds demonstrate activity at the mineralocorticoid receptor[13] (MR), and moreover, in contrast to steroidal antagonist RU-486 (see above) GSK1564023A demonstrated a 30-fold selectivity over progesterone receptor (PR) antagonism, and **10** and **12** no significant PR activity.[12]

Given the origin of GSK325971A as an intermediate in the synthesis of PDE4 inhibitors, PDE4 activity[13] was also investigated. Whilst GSK325971A itself did show modest PDE4 inhibition this activity was absent in other analogues such as GSK1564023A.

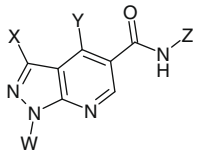
GSK1564023A displayed the most potent and selective pharmacology of the compounds in the series and was selected for more extensive profiling.

Whilst GSK1564023A showed no activation of the trans-repression pathway it demonstrated antagonism of an EC_{80} concentration of cortisol in the NFκB assay[11] with a pIC_{50} of 7.2. GSK1564023A is thus a non-dissociated antagonist, blocking both



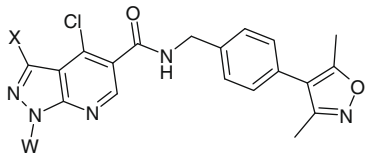
Scheme 1. Representative synthesis of GSK325971A analogues. Reagents and conditions: (i) EtOH, Reflux 60–100%; (ii) (a) diethyl-ethoxymethylene malonate, 160 °C, 5 h, 40–60%; (b) $SOCl_2$; (iii) KOH; (iv) R_4NH_2/DIC/Et_3N.

Table 1
Structure–activity relationships around GSK325971A template



Compound	Z	X	W	Y	MMTV[6] pIC_{50}
6	4-Cl,3-(Trifluoromethyl)benzyl	H	Et	Cl	7.2
7	4-Cl,3-(Trifluoromethyl)benzyl	Me	Me	Cl	7.2
GSK1564023A	4-(4-(3,5-Dimethyl oxazolyl)benzyl	Me	Me	Cl	7.0
8	3,5-Di-methylbenzyl	H	Et	Cl	6.8
9	2-(3-Methoxyphenyl)benzyl	Me	Me	Cl	6.7
10	3,5-Di-methylbenzyl	Me	Me	Cl	6.7
GSK325971A	2,4-Di-methylbenzyl	H	Et	Cl	6.7
11	4-Cl-benzyl	H	Et	Cl	6.6
12	Indan-2-yl	Et	Me	Cl	6.6
13	3-Me-Benzyl	H	Et	Cl	6.4
14	2-Methylbenzyl	H	Et	Cl	6.4
15	Benzyl	H	Me	Cl	6.0
16	2-(3-Methylphenyl) pyrrolidine	Me	Me	Cl	<5.2
17	Thiazol-5-yl methyl	H	Et	Cl	<5.2
18	Pyridyl-2-methyl	Me	Me	Cl	<5.2
19	n-Propyl	H	Et	Cl	<5.2
20	Methyl	H	Et	Cl	<5.2
21	4-(4-(3,5-Di-methyl oxazolyl)benzyl	Me	Et	Me	<5.2
22	4-MeO-Benzyl	Me	Me	OMe	<5.2

Table 2
Effects of modifications to pyrazole N-substituent



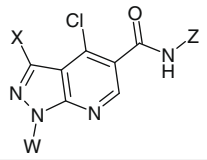
Compound	W	X	MMTV[6] pIC_{50}
23	*i*-Pr	Me	6.6
24	*n*-Pr	Me	6.6
25	Pyridinyl-2-methyl	Me	6.6
26	Benzyl	H	6.5
27	Phenyl	Me	6.5
28	Cyclopentyl	H	6.3
29	2-Methylphenyl	Me	6.1
30	4-Methyphenyl	Me	<5.2

the trans-activation and trans-repression GR pathways with comparable potency.

GSK1564023A demonstrates very good metabolic stability in in vitro assays (data not shown) but afforded no systemic exposure in rat following intravenous administration. Further investigation revealed that GSK1564023A is unstable in rat blood, and that the displaceable chlorine atom (see above) was the likely origin of this instability. As the compound has good aqueous stability (see above) it was inferred that instability was due to interaction with biological nucleophiles, but this was not investigated further.

Whilst blood instability of GSK1564023A precluded its further progression the compound and chemotype remain noteworthy in displaying a remarkable, if not unprecedented, pharmacological profile for a small molecule non-steroid. Moreover GSK1564023A and related compounds represent very useful tools for understanding pharmacophoric interactions with the GR protein and in the further design of novel GR ligands. Whilst arylpyrazoloazadecalin

Table 3
Selectivity of GSK325971 and analogues



Compound	Z	X	W	MMTV[6] pIC_{50}	MMTV[6] pEC_{50}	NFκB[11] Inhibition pIC_{50} (% max)	MR[13] pEC_{50}	MR[13] pIC_{50}	PR[12] pEC_{50}	PR[12] pIC_{50} (% max)	PDE4B[14] pIC_{50}
GSK1564023A	4-(4-(3,5-Dimethyl oxazolyl)benzyl	Me	Me	7.0	<5.2	<5.2	<4.7	<4.7	<5	5.5 (102%)	<5.6
9	2-(3-Methoxyphenyl)benzyl	Me	Me	6.7	<5.2	5.4 (62%)	<5	<5	<4.7	<5.3	4.7
12	Indan-2-yl	Et	H	6.6	<5.2	<5.2	<5	<5	<5	<5	4.9
GSK325971A	2,4-Di-methylbenzyl	Et	H	6.7	<5.2	5.8 (102%)	<5	<4.7	<5	5.9 (99%)	5.4

based GR antagonists[2] such as compound **2** show some common structural features with the GSK1564023A template (e.g., fused pyrazole), structural data (e.g., protein crystallography) would be required to rigorously investigate the significance of any commonality.

In summary, in GSK1564023A we have identified a novel small molecule functional GR antagonist. Whilst the compound class may not be progressable due to blood instability the structural features and remarkable pharmacology of this molecule/series provide valuable information to aid the quest for GR antagonists of clinical utility.

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6. Antagonism determined in human A549 lung epithelial fibroblasts expressing Renilla luciferase reporter gene construct under control of mouse mammary tumour virus (MMTV). MMTV sequences included glucocorticoid responsive elements (GRE) which confer glucocorticoid receptor (GR) dependence on luciferase gene expression. pIC_{50} values calculated from 11 point concentration–response curves (CRC) in 384 well format raised against EC_{80} of cortisol. pEC_{50} values recorded in presence of compound alone; All values typically from $n = 4$ experiments or greater.
7. Corresponding PDE4 inhibitors derived from displacement of the chlorine with amines showed no GR antagonism.
8. See for example: preparation of pyrazolo[3,4-*b*]pyridines as PDE4 inhibitors; PCT Int. Appl. WO 2005090354A1.
9. We concur with the comment of a reviewer that this experiment does not constitute definitive proof of a non-covalent MoA. However the MMTV reporter gene assay measures events which occur in the first 3–4 h after cortisol addition, thus 2 h pre-incubation is proportionately long enough for any covalent/pre-organisational mechanistic aspect to manifest itself. Moreover the high selectivity of compounds of this series over other NRs, and more importantly the SAR which demonstrate GR activity is ablated by simple structural changes (e.g., compounds **19** and **22**), are also strongly suggestive of specific MoA rather than simple Cl-displacement. It is planned that stabilisation of the chlorine substituent will form the subject of a subsequent publication.
10. Samples showed no significant decomposition whilst held in DMSO solution in the GSK compound bank over several years.
11. NFκB inhibition measured in a 384 format in human A549 lung epithelial cell line stably transfected with a plasmid containing an ELAM promotor sequence containing NFκB response elements, stimulated with TNFα and measuring SPAP fluorescence. pIC_{50} values were calculated from 11 point CRC's, all values typically from $n = 4$ experiments or greater.
12. Determined in 384 well format in African Green Monkey CV-1 cells stably expressing human progesterone nuclear receptor B isoform (PR-B). pIC_{50} values are calculated from 11 point concentration–response curves (CRC's) raised against EC_{80} of progesterone, and pEC_{50} values of compound alone, all values typically from $n = 4$ experiments or greater.
13. Determined in 384 well format in African Green Monkey CV-1 cells stably expressing human aldosterone-mineralocorticoid nuclear receptor (MR). pIC_{50} values are calculated from 11 point concentration–response curves (CRC's) raised against EC_{80} of aldosterone, and pEC_{50} values of compound alone, all values typically from $n = 4$ experiments or greater.
14. The inhibitory effect of compounds determined for human phosphodiesterase 4B (PDE4B) enzyme via ATP-luciferase luminescence readout. pIC_{50} values are calculated from 11 point concentration–response curves. All values typically from $n = 4$ experiments or greater.